AnyGenomics Lab

QIAact Actionable Insights Tumor Panel For the GeneReader NGS System (RUO)

Report Date Apr 9, 2018
Report Status -

Patient Information

Patient Name
Date of Birth
Ethnicity
Sex
Accession 20180322144720_8160085015705246905180014 0_37116_16A2_BC7_2018-03-22

Client Information

Client
Client ID
Physician
Pathologist

Specimen

Specimen Type
Specimen ID
Collection Date
Accession Date Mar 26, 2018
Primary Tumor Site Brain
Diagnosis Glioblastoma
Diagnosis Stage

Result: No variants detected

0 Clinically Significant Variants Reported	0 Approved Therapy	0 Potential Clinical Trials

Genes Tested

KRAS *NRAS* *KIT* *BRAF* *PDGFRA* *ALK* *EGFR* *ERBB2* *PIK3CA* *ERBB3* *ESR1* *RAF1*

Methods and Limitations

EXAMPLE Statement including sample type (FFPE, etc), method of extraction, amplification reactions, panel targeted regions, sequencing technology, etc. Additionally, a description of the data analysis software(s), genome of reference and the sensitivity of the methods should be described.

QIAGEN Clinical Insight (QCI™) software includes the following underlying databases, data reference sets and tools; QIAGEN Clinical Insight-Interpret (5.2.20180316), Ingenuity Knowledge Base (Pandora 180405.003), CADD (v1.3), CentoMD (4.1), EVS (ESP6500SI-V2), Allele Frequency Community (2018-01-17), JASPAR (2013-11), Ingenuity Knowledge Base Snapshot Timestamp (2018-04-05 17:54:03.0), Vista Enhancer hg18 (2012-07), Vista Enhancer hg19 (2012-07), OMIM (May 26, 2017), gnomAD (2.0.1), Clinical Trials (Pandora 180405.003), BSIFT (2016-02-23), TCGA (2013-09-05), PolyPhen-2 (v2.2.2), 1000 Genome Frequency (phase3v5b), Clinvar (2018-01-03), DGV (2016-05-15), COSMIC (v83), ExAC (0.3.1), HGMD (2017.4),

QIAact Actionable Insights Tumor Panel For the GeneReader NGS System (RUO)

PhyloP hg18 (2009-11), PhyloP hg19 (2009-11), DbSNP (150(2017-07-10)), TargetScan (6.2), SIFT4G (2016-02-23)

Laboratory Statement

This section can be customized to provide additional information regarding laboratory methods etc.

AnyGenomics Lab

QIAact Actionable Insights Tumor Panel For the GeneReader NGS System (RUO)

Report Date Mar 26, 2018
Report Status -

Patient Information

Patient Name
Date of Birth
Ethnicity
Sex
Accession 20180322144720_8160085015705246905180014 0_39261_16_BC9_2018-03-22

Client Information

Client
Client ID
Physician
Pathologist

Specimen

Specimen Type
Specimen ID
Collection Date
Accession Date Mar 26, 2018
Primary Tumor Site Brain
Diagnosis Glioblastoma
Diagnosis Stage

Interpretation

1 Clinically Significant Variant Reported | 0 Approved Therapy | 0 Potential Clinical Trials

Summary of Clinically Significant Variants

Variants Reported	Approved Therapies for Same Cancer	Approved Therapies for Other Cancers	Therapies Associated with Resistance	Potential Clinical Trials
KIT p.P627L				

Variant Details

Gene	Exon #	Nucleotide Change	Amino Acid Change	Effect on Protein
KIT	13	NM_000222.2: c.1880C>T	p.P627L	loss of function

KIT is an oncogene involved in cell proliferation and survival through activation of RAS/RAF/MAPK and PI3K/AKT/MTOR pathways [2]. Amplification, gain-of-function mutations, and protein overexpression cause KIT activation [3, 4, 1].

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Genes Tested

KRAS *NRAS* *KIT* *BRAF* *PDGFRA* *ALK* *EGFR* *ERBB2* *PIK3CA* *ERBB3* *ESR1* *RAF1*

Methods and Limitations

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Laboratory Statement

This section can be customized to provide additional information regarding laboratory methods etc.

Selected Citations

1. Carvajal RD, Antonescu CR, Wolchok JD, Chapman PB, Roman RA, Teitcher J, Panageas KS, Busam KJ, Chmielowski B, Lutzky J, Pavlick AC, Fusco A, Cane L, Takebe N, Vemula S, Bouvier N, Bastian BC, Schwartz GK (2011) KIT as a therapeutic target in metastatic melanoma. JAMA. 2011 Jun 08;305(22):2327-34 (PMID: 21642685)
2. Corless CL, Barnett CM, Heinrich MC (2011) Gastrointestinal stromal tumours: origin and molecular oncology. Nat Rev Cancer. 2011 Nov 17;11(12):865-78 (PMID: 22089421)
3. Heinrich MC, Corless CL, Demetri GD, Blanke CD, von Mehren M, Joensuu H, McGreevey LS, Chen CJ, Van den Abbeele AD, Druker BJ, Kiese B, Eisenberg B, Roberts PJ, Singer S, Fletcher CD, Silberman S, Dimitrijevic S, Fletcher JA (2003) Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. J Clin Oncol. 2003 Dec 01;21(23):4342-9 (PMID: 14645423)
4. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, Kawano K, Hanada M, Kurata A, Takeda M, Muhammad Tunio G, Matsuzawa Y, Kanakura Y, Shinomura Y, Kitamura Y (1998) Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science. 1998 Jan 23;279(5350):577-80 (PMID: 9438854)

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Client Information

Client
Client ID
Physician
Pathologist

Specimen

Specimen Type
Specimen ID
Collection Date
Accession Date Mar 26, 2018
Primary Tumor Site Brain
Diagnosis Glioblastoma
Diagnosis Stage

Interpretation

1 Clinically Significant Variant Reported | 0 Approved Therapy | 0 Potential Clinical Trials

Summary of Clinically Significant Variants

Variants Reported	Approved Therapies for Same Cancer	Approved Therapies for Other Cancers	Therapies Associated with Resistance	Potential Clinical Trials
EGFR p.R836R				

Variant Details

Gene	Exon #	Nucleotide Change	Amino Acid Change	Effect on Protein
EGFR	21	NM_005228.4: c.2508C>T	p.R836R	normal function

EGFR is an oncogene involved in cell growth and differentiation through activation of the PI3K/AKT/MTOR and RAS/RAF/MAPK pathways [4]. Amplification, gain-of-function mutations, and protein overexpression cause EGFR activation [2, 8, 3, 5]. EGFR mutations are reported to be mutually exclusive with ALK rearrangements and KRAS mutations in non-small cell lung cancer [1, 6, 7].

QIAact Actionable Insights Tumor Panel For the GeneReader NGS System (RUO)

Genes Tested

KRAS *NRAS* *KIT* *BRAF* *PDGFRA* *ALK* *EGFR* *ERBB2* *PIK3CA* *ERBB3* *ESR1* *RAF1*

Methods and Limitations

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Laboratory Statement

This section can be customized to provide additional information regarding laboratory methods etc.

Selected Citations

1. Gainor JF, Varghese AM, Ou SH, Kabraji S, Awad MM, Katayama R, Pawlak A, Mino-Kenudson M, Yeap BY, Riely GJ, Iafrate AJ, Arcila ME, Ladanyi M, Engelman JA, Dias-Santagata D, Shaw AT (2013) ALK rearrangements are mutually exclusive with mutations in EGFR or KRAS: an analysis of 1,683 patients with non-small cell lung cancer. Clin Cancer Res. 2013 Aug 01;19(15):4273-81. Epub 2013 May 31 (PMID: 23729361)
2. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med. 2004 May 20;350(21):2129-39. Epub 2004 Apr 29 (PMID: 15118073)
3. Rusch V, Klimstra D, Venkatraman E, Pisters PW, Langenfeld J, Dmitrovsky E (1997) Overexpression of the epidermal growth factor receptor and its ligand transforming growth factor alpha is frequent in resectable non-small cell lung cancer but does not predict tumor progression. Clin Cancer Res. 1997 Apr;3(4):515-22 (PMID: 9815714)
4. Scaltriti M, Baselga J (2006) The epidermal growth factor receptor pathway: a model for targeted therapy. Clin Cancer Res. 2006 Sep 15;12(18):5268-72 (PMID: 17000658)
5. Selvaggi G, Novello S, Torri V, Leonardo E, De Giuli P, Borasio P, Mossetti C, Ardissone F, Lausi P, Scagliotti GV (2004) Epidermal growth factor receptor overexpression correlates with a poor prognosis in completely resected non-small-cell lung cancer. Ann Oncol. 2004 Jan;15(1):28-32 (PMID: 14679115)
6. Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, Wistuba II, Fong KM, Lee H, Toyooka S, Shimizu N, Fujisawa T, Feng Z, Roth JA, Herz J, Minna JD, Gazdar AF (2005) Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst. 2005 Mar 02;97(5):339-46 (PMID: 15741570)
7. Shigematsu H, Takahashi T, Nomura M, Majmudar K, Suzuki M, Lee H, Wistuba II, Fong KM, Toyooka S, Shimizu N, Fujisawa T, Minna JD, Gazdar AF (2005) Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. Cancer

Selected Citations

Res. 2005 Mar 01;65(5):1642-6 (PMID: 15753357)

8. Yoshida K, Tsuda T, Matsumura T, Tsujino T, Hattori T, Ito H, Tahara E (1989) Amplification of epidermal growth factor receptor (EGFR) gene and oncogenes in human gastric carcinomas. Virchows Arch B Cell Pathol Incl Mol Pathol. 1989;57(5):285-90 (PMID: 2570489)

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Client Information

Client
Client ID
Physician
Pathologist

Specimen

Specimen Type
Specimen ID
Collection Date
Accession Date Mar 26, 2018
Primary Tumor Site Brain
Diagnosis Glioblastoma
Diagnosis Stage

Interpretation

3 Clinically Significant Variants Reported | 0 Approved Therapy | 0 Potential Clinical Trials

Summary of Clinically Significant Variants

Variants Reported	Approved Therapies for Same Cancer	Approved Therapies for Other Cancers	Therapies Associated with Resistance	Potential Clinical Trials
PDGFRA p.G79D				
KIT p.P627L				
EGFR p.A289T				

Variant Details

Gene	Exon #	Nucleotide Change	Amino Acid Change	Effect on Protein
EGFR	7	NM_005228.4: c.865G>A	p.A289T	loss of function

EGFR is an oncogene involved in cell growth and differentiation through activation of the PI3K/AKT/MTOR and RAS/RAF/MAPK pathways [11]. Amplification, gain-of-function mutations, and protein overexpression cause EGFR activation [8, 15, 10, 12]. EGFR mutations are reported to be mutually exclusive with ALK rearrangements and KRAS mutations in non-small cell lung cancer [4, 13, 14].

QIAact Actionable Insights Tumor Panel For the GeneReader NGS System (RUO)

Variant Details

Gene	Exon #	Nucleotide Change	Amino Acid Change	Effect on Protein
KIT	13	NM_000222.2: c.1880C>T	p.P627L	**loss of function**

KIT is an oncogene involved in cell proliferation and survival through activation of RAS/RAF/MAPK and PI3K/AKT/MTOR pathways [3]. Amplification, gain-of-function mutations, and protein overexpression cause KIT activation [5, 7, 1].

PDGFRA	3	NM_006206.5: c.236G>A	p.G79D	**normal function**

PDGFRA is an oncogene involved in cell proliferation and survival through activation of RAS/RAF/MAPK and PI3K/AKT/MTOR pathways [3]. Gain-of-function mutations, amplification, and fusions cause PDGFRA activation [2, 6, 9].

Genes Tested

KRAS *NRAS* *KIT* *BRAF* *PDGFRA* *ALK* *EGFR* *ERBB2* *PIK3CA* *ERBB3* *ESR1* *RAF1*

Methods and Limitations

EXAMPLE Statement including sample type (FFPE, etc), method of extraction, amplification reactions, panel targeted regions, sequencing technology, etc. Additionally, a description of the data analysis software(s), genome of reference and the sensitivity of the methods should be described.

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Laboratory Statement

This section can be customized to provide additional information regarding laboratory methods etc.

Selected Citations

1. Carvajal RD, Antonescu CR, Wolchok JD, Chapman PB, Roman RA, Teitcher J, Panageas KS, Busam KJ, Chmielowski B,

Selected Citations

Lutzky J, Pavlick AC, Fusco A, Cane L, Takebe N, Vemula S, Bouvier N, Bastian BC, Schwartz GK (2011) KIT as a therapeutic target in metastatic melanoma. JAMA. 2011 Jun 08;305(22):2327-34 (PMID: 21642685)

2. Cools J, DeAngelo DJ, Gotlib J, Stover EH, Legare RD, Cortes J, Kutok J, Clark J, Galinsky I, Griffin JD, Cross NC, Tefferi A, Malone J, Alam R, Schrier SL, Schmid J, Rose M, Vandenberghe P, Verhoef G, Boogaerts M, Wlodarska I, Kantarjian H, Marynen P, Coutre SE, Stone R, Gilliland DG (2003) A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N Engl J Med. 2003 Mar 27;348(13):1201-14 (PMID: 12660384)
3. Corless CL, Barnett CM, Heinrich MC (2011) Gastrointestinal stromal tumours: origin and molecular oncology. Nat Rev Cancer. 2011 Nov 17;11(12):865-78 (PMID: 22089421)
4. Gainor JF, Varghese AM, Ou SH, Kabraji S, Awad MM, Katayama R, Pawlak A, Mino-Kenudson M, Yeap BY, Riely GJ, Iafrate AJ, Arcila ME, Ladanyi M, Engelman JA, Dias-Santagata D, Shaw AT (2013) ALK rearrangements are mutually exclusive with mutations in EGFR or KRAS: an analysis of 1,683 patients with non-small cell lung cancer. Clin Cancer Res. 2013 Aug 01;19(15):4273-81. Epub 2013 May 31 (PMID: 23729361)
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7. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, Kawano K, Hanada M, Kurata A, Takeda M, Muhammad Tunio G, Matsuzawa Y, Kanakura Y, Shinomura Y, Kitamura Y (1998) Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science. 1998 Jan 23;279(5350):577-80 (PMID: 9438854)
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9. Ramos AH, Dutt A, Mermel C, Perner S, Cho J, Lafargue CJ, Johnson LA, Stiedl AC, Tanaka KE, Bass AJ, Barretina J, Weir BA, Beroukhim R, Thomas RK, Minna JD, Chirieac LR, Lindeman NI, Giordano T, Beer DG, Wagner P, Wistuba II, Rubin MA, Meyerson M (2009) Amplification of chromosomal segment 4q12 in non-small cell lung cancer. Cancer Biol Ther. 2009 Nov;8(21):2042-50. Epub 2009 Nov 7 (PMID: 19755855)
10. Rusch V, Klimstra D, Venkatraman E, Pisters PW, Langenfeld J, Dmitrovsky E (1997) Overexpression of the epidermal growth factor receptor and its ligand transforming growth factor alpha is frequent in resectable non-small cell lung cancer but does not predict tumor progression. Clin Cancer Res. 1997 Apr;3(4):515-22 (PMID: 9815714)
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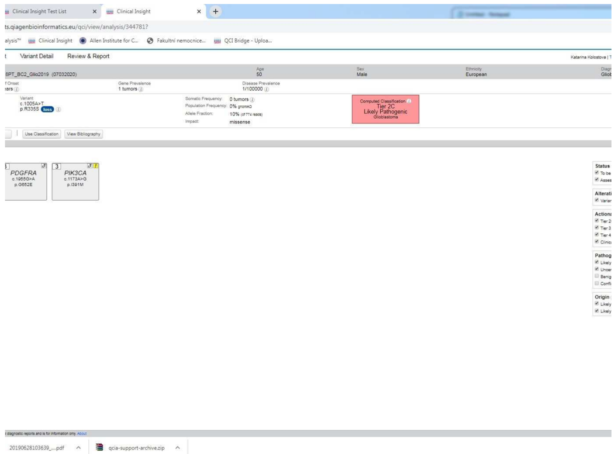
Selected Citations

14. Shigematsu H, Takahashi T, Nomura M, Majmudar K, Suzuki M, Lee H, Wistuba II, Fong KM, Toyooka S, Shimizu N, Fujisawa T, Minna JD, Gazdar AF (2005) Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. Cancer Res. 2005 Mar 01;65(5):1642-6 (PMID: 15753357)

15. Yoshida K, Tsuda T, Matsumura T, Tsujino T, Hattori T, Ito H, Tahara E (1989) Amplification of epidermal growth factor receptor (EGFR) gene and oncogenes in human gastric carcinomas. Virchows Arch B Cell Pathol Incl Mol Pathol. 1989;57(5):285-90 (PMID: 2570489)


Clinical Insight Test List
Clinical Insight
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Clinical Insight
Allen Institute for C...
Fakultní nemocnice...
QCI Bridge - Uploa...
Variant Detail
Review & Report
Katarina Kolostova
8PT_BC2_Glio2019 (07032020)
Age
50
Sex
Male
Ethnicity
European
Gene Prevalence
1 tumors
Disease Prevalence
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Variant
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p.R335S
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Population Frequency: 0% gnomAD
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Computed Classification
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Use Classification
View Bibliography
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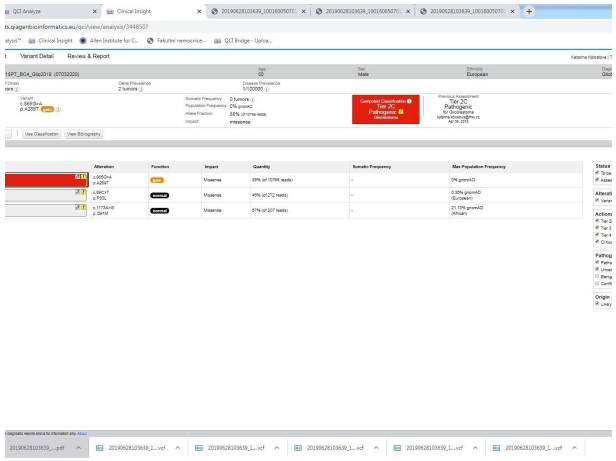
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Fakultní nemocnice...
QCI Bridge - Uploa...
Variant Detail
Review & Report
Use Classification
View Bibliography
Alteration
Function
Impact
Quantity
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Accession Date Mar 23, 2018
Primary Tumor Site Brain
Diagnosis Glioblastoma
Diagnosis Stage

Interpretation

1 Clinically Significant Variant Reported | 0 Approved Therapy | 0 Potential Clinical Trials

Summary of Clinically Significant Variants

Variants Reported	Approved Therapies for Same Cancer	Approved Therapies for Other Cancers	Therapies Associated with Resistance	Potential Clinical Trials
PIK3CA p.D350G				

Variant Details

Gene	Exon #	Nucleotide Change	Amino Acid Change	Effect on Protein
PIK3CA	5	NM_006218.3: c.1049A>G	p.D350G	gain of function

PIK3CA is an oncogene involved in cell growth, apoptosis, transformation, motility, and adhesion through activation of the PI3K/AKT/MTOR pathway [3, 5, 8]. Gain-of-function mutations, amplification, and protein overexpression cause PI3K activation [7, 2, 4, 9]. PIK3CA mutations are reported to be mutually exclusive with PTEN mutations [6, 1].

QIAact Actionable Insights Tumor Panel For the GeneReader NGS System (RUO)

Genes Tested

KRAS *NRAS* *KIT* *BRAF* *PDGFRA* *ALK* *EGFR* *ERBB2* *PIK3CA* *ERBB3* *ESR1* *RAF1*

Methods and Limitations

EXAMPLE Statement including sample type (FFPE, etc), method of extraction, amplification reactions, panel targeted regions, sequencing technology, etc. Additionally, a description of the data analysis software(s), genome of reference and the sensitivity of the methods should be described.

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Laboratory Statement

This section can be customized to provide additional information regarding laboratory methods etc.

Selected Citations

1. Broderick DK, Di C, Parrett TJ, Samuels YR, Cummins JM, McLendon RE, Fults DW, Velculescu VE, Bigner DD, Yan H (2004) Mutations of PIK3CA in anaplastic oligodendrogliomas, high-grade astrocytomas, and medulloblastomas. Cancer Res. 2004 Aug 01;64(15):5048-50 (PMID: 15289301)
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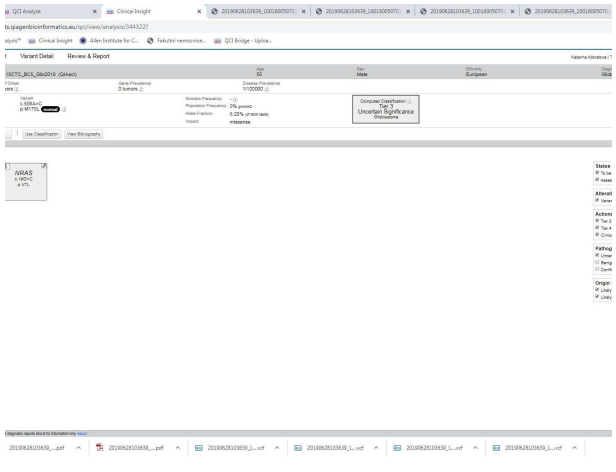
QIAact Actionable Insights Tumor Panel For the GeneReader NGS System (RUO)

Selected Citations

H, Toyooka S, Date H, Lam WL, Minna JD, Gazdar AF (2008) PIK3CA mutations and copy number gains in human lung cancers. Cancer Res. 2008 Sep 01;68(17):6913-21 (PMID: 18757405)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Computed Classification
Tier 3
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NRAS
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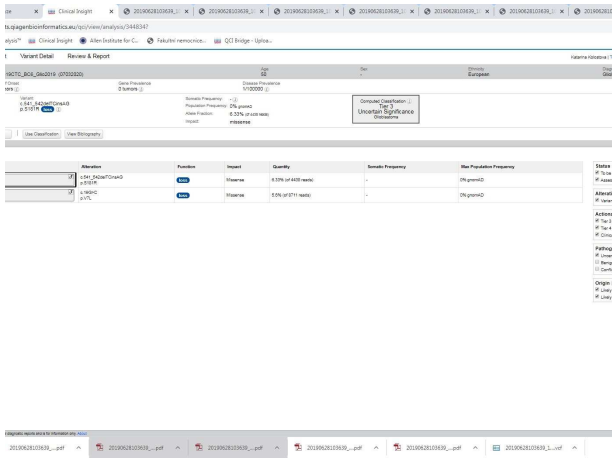
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Variant Detail
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Tier 3
Uncertain Significance
Distinctome
Use Classification
View Bibliography
Alteration
Function
Impact
Quantity
Somatic Frequency
Max Population Frequency
Missense
6.53% (of 4435 reads)
0% gnomAD
Missense
5.6% (of 5711 reads)
0% gnomAD
Diagnostic reports are a for information only. About
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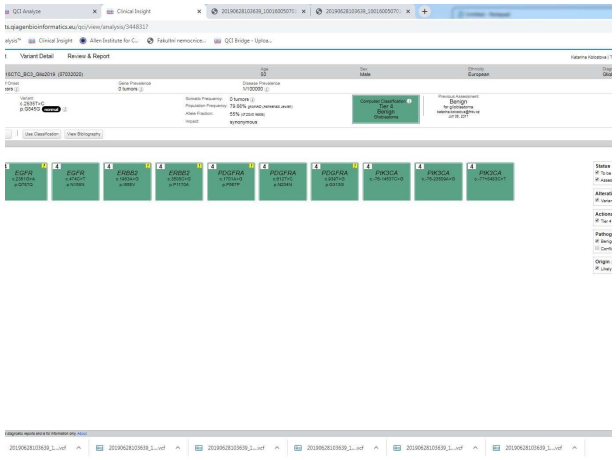
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Clinical Insight
Variant Detail
Review & Report
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ERBB2
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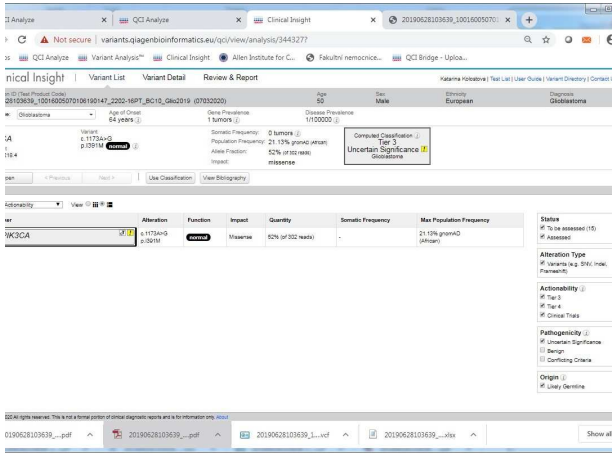
PIK3CA
PIK3CA
PIK3CA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QCI Analyze
Variant Analysis
Clinical Insight
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nical Insight
Variant List
Variant Detail
Review & Report
Katarina Holcatova | Test List | User Guide | Variant Directory | Contact
Age
50
Sex
Male
Ethnicity
European
Diagnosis
Glioblastoma
Age of Onset
64 years
Gene Prevalence
1 tumors
Disease Prevalence
1/100000
Somatic Frequency:
0 tumors
Population Frequency:
21.13% gnomAD (African)
Allele Fraction:
52% (of 302 reads)
Impact:
missense
Computed Classification
Tier 3
Uncertain Significance
Glioblastoma
Use Classification
View Bibliography
Alteration
Function
Impact
Quantity
Somatic Frequency
Max Population Frequency
Missense
52% (of 302 reads)
21.13% gnomAD (African)
Status
To be assessed (15)
Assessed
Alteration Type
Actionability
Tier 3
Tier 4
Clinical Trials
Pathogenicity
Uncertain Significance
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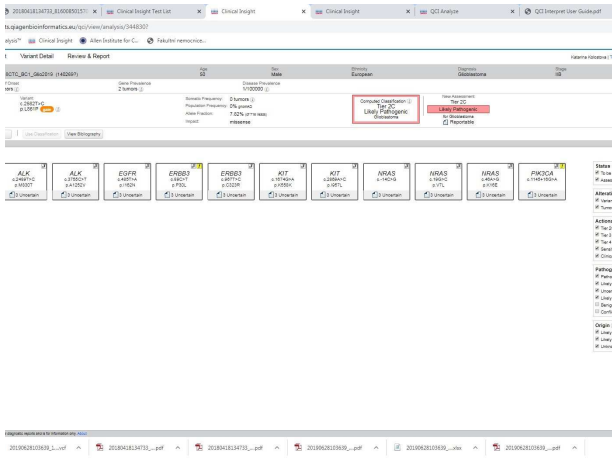
Conflicting Criteria
Origin
Likely Germline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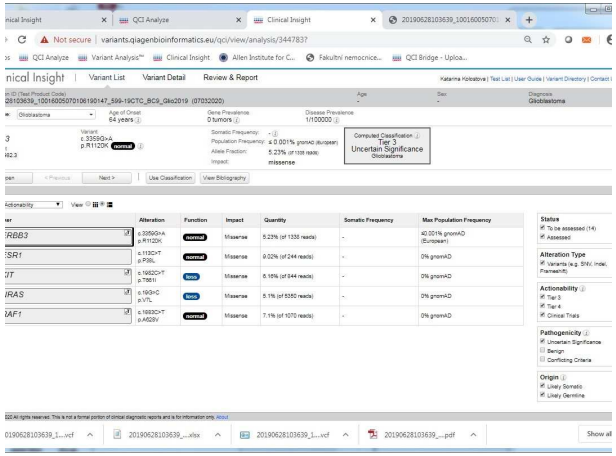
Review & Report
Tier 2C
Likely Pathogenic
Oligodendroglioma
ALK
EGFR
ERBB3
KIT
NRAS
PIK3CA
3 Uncertain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Glioblastoma
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Max Population Frequency
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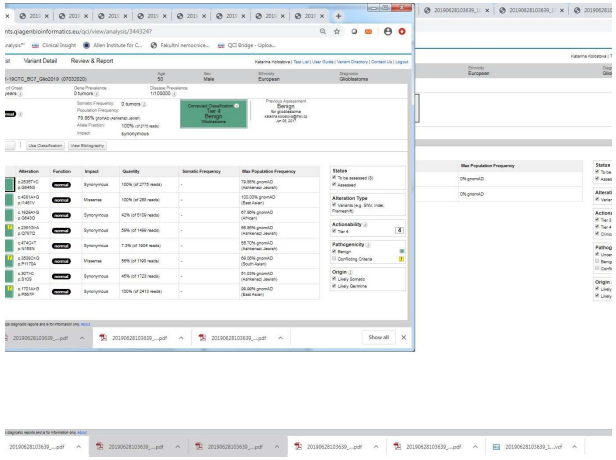

Variant Detail
Review & Report
Show all

YOUR LAB

Your Lab
1700 Lincoln Blvd, Suite 20, Redwood City, CA 94063
labx.com / (650) 484 4040
Additional Information

Test Performed: **Somatic Panel**

Report Date Mar 7, 2020
Status -

Patient		Client		Specimen	
Patient Name		Client		Accession ID	20190628103639_100160050701061 90147_78-18PT_BC2_Glio2019
Date of Birth		Client ID			
Age	50	Physician			
Sex	Male	Pathologist		Specimen Collection	
Ethnicity	European			Accession	Mar 7, 2020
Diagnosis	Glioblastoma			Primary Tumor Site	Brain

Result: Negative

Genes Tested

Test information such as gene name and hot spot region can be included in this section.

Methods and Limitations

EXAMPLE Statement including sample type (FFPE, etc), method of extraction, amplification reactions, panel targeted regions, sequencing technology, etc. Additionally, a description of the data analysis software(s), genome of reference and the sensitivity of the methods should be described.

QIAGEN Clinical Insight (QCI™) is a variant analysis, interpretation and decision support tool for research and clinical labs analyzing human genetics data and is not intended to be used for diagnostic purposes. QCI Interpret software includes the following underlying databases, data reference sets and tools; QIAGEN Clinical Insight-Interpret (5.6.20200226), Ingenuity Knowledge Base (K-release), CADD (v1.4), Allele Frequency Community (2019-09-25), EVS (ESP6500SI-V2), Refseq Gene Model (2019-02-05), JASPAR (2013-11), Ingenuity Knowledge Base Snapshot Timestamp (2020-02-21 12:37:01.0), Vista Enhancer hg18 (2012-07), Vista Enhancer hg19 (2012-07), Clinical Trials (K-release), PolyPhen-2 (v2.2.2), 1000 Genome Frequency (phase3v5b), ExAC (0.3.1), iva (Nov 19 12:28 iva-1.0.1200.jar), PhyloP hg18 (2009-11), PhyloP hg19 (2009-11), DbSNP (151), TargetScan (7.2), GENCODE (Release 29), CentoMD (5.3), OMIM (May 26, 2017), gnomAD (2.1.1), BSIFT (2016-02-23), TCGA (2013-09-05), Clinvar (2019-06-05), DGV (2016-05-15), COSMIC (v89), HGMD (2019.2), SIFT4G (2016-02-23)

Clinical Significance of Variants Based on AMP / ASCO / CAP Guidelines*

Strong Significance	**Tier 1A**	• Biomarker predicts response or resistance to an FDA or EMA approved therapy, according to drug label or professional guidelines for this diagnosis • Biomarker included in professional guidelines is prognostic or diagnostic for this diagnosis
	Tier 1B	• Biomarker predicts response or resistance to a therapy for this diagnosis based on well-powered studies • Biomarker is prognostic or diagnostic for this diagnosis based on well-powered studies
Potential Significance	**Tier 2C**	• Biomarker is associated with response or resistance to an FDA or EMA approved therapy, according to drug label or professional guidelines but only for different diagnosis • Biomarker is an inclusion criterion for an active clinical trial • Biomarker is prognostic or diagnostic based on multiple small studies
	Tier 2D	• Biomarker shows plausible response or resistance based on case or preclinical studies • Biomarker may assist in disease diagnosis or prognosis based on small studies
Uncertain Significance	**Tier 3**	• Biomarker has uncertain clinical significance and not known to be likely benign or benign

**Adapted from PMID:27993330 jmd.amjpathol.org/article/S1525-1578(16)30223-9/pdf

Analysis Report

20190628103639_10016005070106190147_78-18CTC_BC1_Glio2019

Table of contents

1 Summary

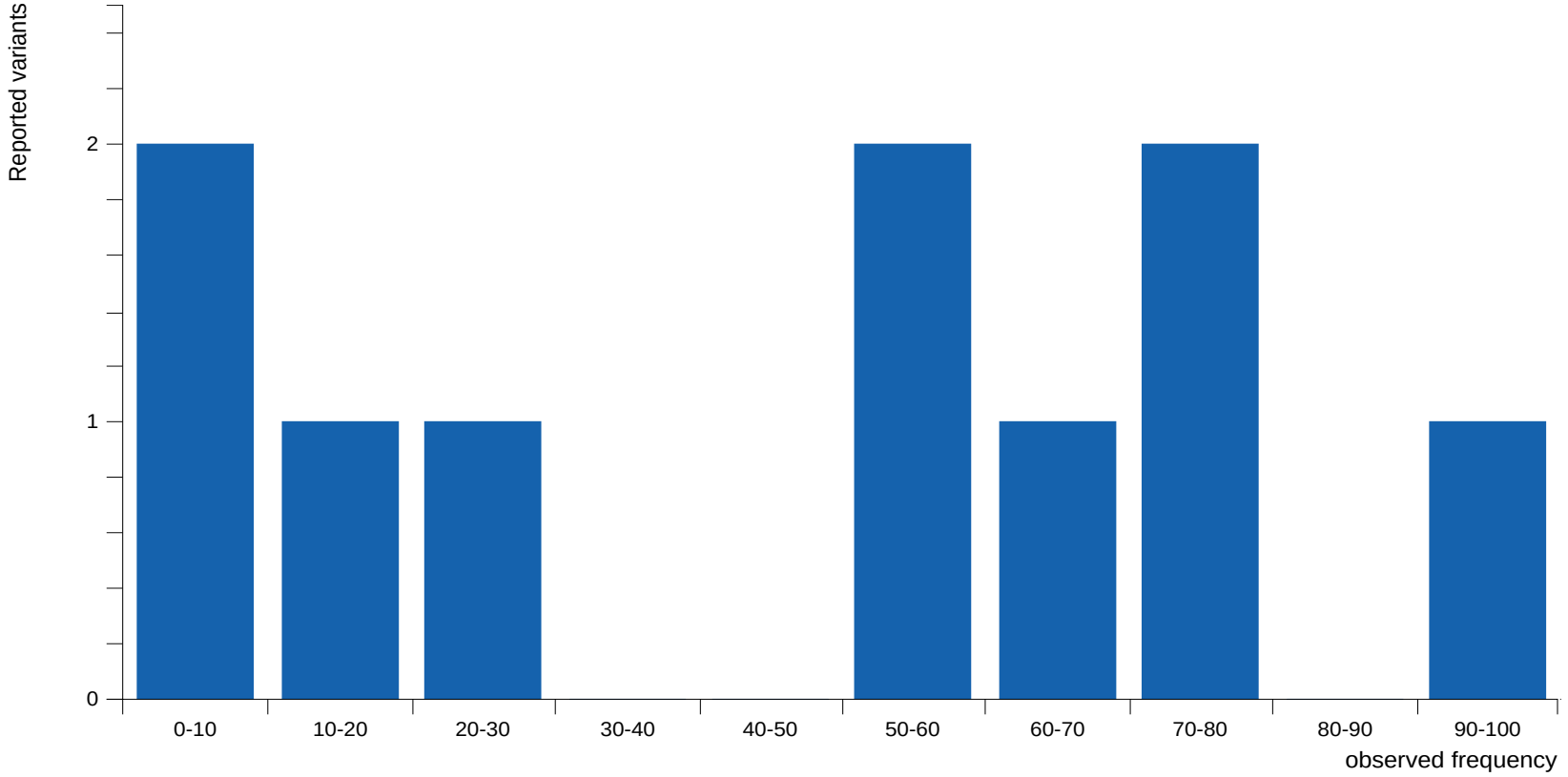
Report created	Sat Jun 29 18:08:15 CEST 2019
Sample ID	20190628103639_10016005070106190147_78-18CTC_BC1_Glio2019
Analysis workflow	AIT FFPE v4.5: QIAact Actionable Insights Tumor Panel on FFPE
Analyst	root
Reported variants	20
Analysis results	56 Untested variants

1.1 Comments

No comments

1.2 Distribution of observed frequencies for reported variants

Includes variants initially listed in variant table 'Reported variants'.



2 Quality control

Quality control for the sample analysis. Includes information on the input data, read mapping, and coverage information per gene.

2.1 Fastq

Fastq	20190628103639_1001600507010619014 7_78-18CTC_BC1_Glio2019
Reads	1,610,398
Nucleotides*	149,563,442
Average read length*	92.87
Reads with average quality ≥ 25	97.52%

** Including sample barcode*

Recommendations:

Reads with average quality ≥ 25 should be ≥ 80.00%

2.2 Secondary analysis summary

Reads mapped	1,059,106 (65.77%)
Reads in target regions	433,350 (40.92%)
Percentage of base positions in regions of interest with coverage ≥ 500x	85.68%
Percentage of base positions in regions of interest with coverage ≥ 200x	95.48%

Recommendations:

Percentage of base positions in regions of interest with coverage ≥ 500x should be ≥ 90.00%

Percentage of base positions in regions of interest with coverage ≥ 200x should be ≥ 95.00%

2.3 Coverage

Name	ROI	Bases	≥500x	≥200x	0x	Median	VOI	VOI <500x	VOI <200x
NRAS	6	27	100.00%	100.00%	0.00%	4,008	41	0	0
ALK	22	47	74.47%	95.74%	0.00%	1,341	40	5	2
RAF1	2	2	100.00%	100.00%	0.00%	1,569	2	0	0
PIK3CA	81	131	85.50%	98.47%	0.00%	1,924	165	24	2

Name	ROI	Bases	≥500x	≥200x	0x	Median	VOI	VOI <500x	VOI <200x
PDGFRA	21	64	100.00%	100.00%	0.00%	1,657	46	0	0
KIT	49	140	98.57%	100.00%	0.00%	1,180	235	2	0
ESR1	6	7	71.43%	71.43%	0.00%	988	11	2	2
EGFR	96	208	62.98%	86.06%	0.00%	788	443	197	41
BRAF	29	72	100.00%	100.00%	0.00%	3,169	153	0	0
KRAS	21	55	100.00%	100.00%	0.00%	2,209	148	0	0
ERBB3	8	8	100.00%	100.00%	0.00%	1,439	10	0	0
ERBB2	16	35	94.29%	97.14%	0.00%	2,256	61	1	1

ROI: Number of Regions of Interest, i.e. reportable regions that overlap with the gene.

Bases: Total number of base positions in Regions of Interest that overlap with the gene.

≥500x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is equal to or above the significant coverage threshold.

≥200x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is equal to or above the minimum coverage threshold.

0x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is zero.

Median: Median coverage of base positions in the Regions of Interest that overlap with the gene.

VOI: Total number of Variants of Interest, whether detected or not, that overlap with the gene. The list of Variants of Interest is defined by the analysis pipeline.

VOI <500x: Number of Variants of Interest in the gene for which coverage is below the significant coverage threshold.

VOI <200x: Number of Variants of Interest in the gene for which coverage is below the minimum coverage threshold.

2.4 Detected variants

Number of detected variants per gene. Variants for which coverage is above the minimum coverage threshold.

Name	In total	VOI	- non-syn	- syn	Non-VOI	- non-syn	- syn
NRAS	3	0	0	0	3	2	1
ALK	6	2	0	2	4	2	2
RAF1	0	0	0	0	0	0	0
PIK3CA	19	2	1	1	17	3	14
PDGFRA	4	1	0	1	3	1	2
KIT	5	0	0	0	5	1	4
ESR1	6	2	0	2	4	0	4
EGFR	11	1	0	1	10	5	5
BRAF	6	1	0	1	5	0	5

Name	In total	VOI	- non-syn	- syn	Non-VOI	- non-syn	- syn
KRAS	8	0	0	0	8	2	6
ERBB3	3	0	0	0	3	2	1
ERBB2	3	1	1	0	2	1	1

In total: Total number of variants detected within the gene. Variants initially listed in variant tables 3.1 and 3.2.

VOI: Number of detected Variants of Interest detected within the gene. The list of Variants of Interest is defined by the analysis pipeline.

- non-syn: Number of detected, gene-specific Variants of Interest that are non-synonymous.

- syn: Number of detected, gene-specific Variants of Interest that are synonymous.

Non-VOI: Number of detected variants that are not found within the analysis pipeline-defined list of Variants of Interest.

- non-syn: Number of gene-specific, non-VOIs that are non-synonymous.

- syn: Number of gene-specific, non-VOIs that are synonymous.

3 Variants

Variants detected within regions of interest with more than significant coverage are found in 3.1 and variants with more than minimum coverage are found in 3.2.
Variants of interest that could not be tested due to insufficient coverage are listed in table 3.3.
The coverage thresholds and minimum frequency cutoffs configured for the analysis workflow are listed in the History section.
Setting a variant review state to "Confirmed by review" moves it to 3.1, "Artifact" moves it to 3.2.
Only the variants in table 3.1 are exported as VCF and uploaded to QCI Interpret.

3.1 Reported variants

Variants that will be exported to VCF and uploaded to QCI Interpret. Initially contains: Variants detected within regions of interest with more than significant coverage and frequency above the cutoff set for the analysis workflow. These variants are assigned the initial review state "Valid".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
NRAS	c.46A>G	p.Lys16Glu	SNV	4.12%	28.51	0.03	4,883	No	No	Confirmed by review	
NRAS	c.19G>C	p.Val7Leu	SNV	13.20%	29.25	0.00	4,167	No	No	Confirmed by review	
NRAS	c.-14C>G		SNV	5.47%	32.10	3.81E-3	1,973	No	No	Confirmed by review	
ALK	c.4338C>T		SNV	72.26%	29.67	1.00	1,114	Yes	Yes	Valid	
ALK	c.3755C>T	p.Ala1252Val	SNV	8.01%	28.69	4.96E-3	362	No	No	Confirmed by review	
ALK	c.2489T>C	p.Met830Thr	SNV	6.94%	31.68	0.97	317	No	No	Confirmed by review	
PIK3CA	c.-77+8483C>T		SNV	57.40%	33.80	1.00	1,202	Yes	Yes	Valid	
PIK3CA	c.1145+16G>A		SNV	6.51%	34.18	5.89E-5	676	No	No	Confirmed by review	
PIK3CA	c.1173A>G	p.Ile391Met	SNV	59.19%	32.04	1.00	1,524	Yes	Yes	Valid	
PDGFRA	c.1701A>G		SNV	99.90%	29.73	1.00	959	Yes	Yes	Valid	
KIT	c.1674G>A		SNV	17.00%	32.08	2.03E-12	941	Yes	No	Valid	
KIT	c.1680T>C		SNV	25.45%	31.55	1.00	1,100	Yes	No	Valid	
KIT	c.2869A>C	p.Ile957Leu	SNV	6.69%	29.24	1.00	1,017	No	No	Confirmed by review	
ESR1	c.30T>C		SNV	61.34%	29.64	1.00	988	Yes	Yes	Valid	
ESR1	c.1782G>A		SNV	7.78%	32.26	5.20E-4	3,497	Yes	Yes	Valid	
EGFR	c.485T>A	p.Ile162Asn	SNV	9.66%	30.68	0.36	621	No	No	Confirmed by review	
EGFR	c.2582T>C	p.Leu861Pro	SNV	7.82%	29.71	0.34	716	Yes	No	Valid	
BRAF	c.1929A>G		SNV	73.35%	27.90	1.00	2,597	Yes	Yes	Valid	

ERBB3	c.89C>T	p.Pro30Leu	SNV	36.42%	35.11	1.00	313	No	No	Confirmed by review	
ERBB3	c.967T>C	p.Cys323Arg	SNV	5.03%	32.55	1.00	437	No	No	Confirmed by review	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
NRAS	c.46A>G	mis-sense	No	201	81	120	200	115258736	1
NRAS	c.19G>C	mis-sense	No	550	0	550	200	115258763	1
NRAS	c.-14C>G		No	108	0	108	200	115258795	1
ALK	c.4338C>T		No	805	348	457	200	29416615	2
ALK	c.3755C>T	mis-sense	No	29	0	29	200	29432733	2
ALK	c.2489T>C	mis-sense	No	22	9	13	200	29455313	2
PIK3CA	c.-77+8483C>T		No	690	149	541	200	178874874	3
PIK3CA	c.1145+16G>A		No	44	0	44		178922392	3
PIK3CA	c.1173A>G	mis-sense	No	902	513	389	200	178927410	3
PDGFRA	c.1701A>G		No	958	369	589	200	55141055	4
KIT	c.1674G>A		No	160	156	4	200	55593608	4
KIT	c.1680T>C		No	280	161	119	200	55593614	4
KIT	c.2869A>C	mis-sense	No	68	41	27	200	55604661	4
ESR1	c.30T>C		No	606	284	322	200	152129077	6
ESR1	c.1782G>A		No	272	110	162	200	152420095	6
EGFR	c.485T>A	mis-sense	No	60	0	60	200	55214359	7
EGFR	c.2582T>C	mis-sense	No	56	0	56	200	55259524	7
BRAF	c.1929A>G		No	1,905	771	1,134	200	140449150	7
ERBB3	c.89C>T	mis-sense	No	114	114	0	200	56477541	12
ERBB3	c.967T>C	mis-sense	No	22	0	22	93	56482419	12

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

Comment: Remark added by user during variant review.

3.2 Variants available for review

Detected variants that will not be exported to the VCF and uploaded to QCI Interpret. Initially contains: Variants with more than minimum coverage and frequency above the cutoff set for the analysis workflow. Depending on workflow configuration, this table may include variants outside of regions of interest including those with coverage above significant coverage threshold. These variants are assigned the initial review state "Review".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
ALK	c.3804T>C		SNV	5.16%	30.93	0.73	1,047	No	No	Review	
ALK	c.3216G>A		SNV	37.17%	31.73	0.99	460	No	No	Review	
ALK	c.2535T>C		SNV	71.90%	29.90	1.00	395	Yes	Yes	Review	

PIK3CA	c.-77+8455A>G		SNV	5.04%	23.10	0.06	1,588	No	No	Review	
PIK3CA	c.-76-18864C>A		SNV	34.21%	32.55	3.53E-4	874	No	No	Review	
PIK3CA	c.353-65delA		Deletion	4.56%	33.95	1.00	417	No	No	Review	
PIK3CA	c.1005A>G		SNV	10.57%	26.97	0.72	350	No	No	Review	
PIK3CA	c.1060-17C>A		SNV	53.38%	32.26	1.00	3,080	No	No	Review	
PIK3CA	c.1145+9G>A		SNV	8.36%	34.51	6.50E-7	610	No	No	Review	
PIK3CA	c.1145+14T>A		SNV	7.62%	31.85	8.06E-6	682	No	No	Review	
PIK3CA	c.1145+16_1145+17delGAinsTC		MNV	7.69%	30.12	1.16E-5	676	No	No	Review	
PIK3CA	c.1145+19T>A		SNV	7.52%	33.65	2.92E-5	718	No	No	Review	
PIK3CA	c.1145+19T>C		SNV	5.01%	29.25	7.73E-4	718	No	No	Review	
PIK3CA	c.1145+24_1145+25insA		Insertion	8.38%	29.19	3.33E-6	573	No	No	Review	
PIK3CA	c.1145+54A>G		SNV	20.94%	33.76	1.00	320	No	No	Review	
PIK3CA	c.1664+14T>C		SNV	8.84%	30.21	2.30E-3	475	No	No	Review	
PIK3CA	c.2686C>A	p.Leu896Met	SNV	5.07%	33.48	0.54	2,405	No	No	Review	
PIK3CA	c.2930T>C	p.Phe977Ser	SNV	4.86%	29.75	1.00	247	No	No	Review	
PIK3CA	c.3004T>C	p.Phe1002Leu	SNV	17.33%	28.36	0.98	727	No	No	Review	
PDGFRA	c.1406T>C	p.Val469Ala	SNV	4.25%	25.03	9.90E-6	1,743	No	No	Review	
PDGFRA	c.2440-50_2440-49insA		Insertion	98.43%	32.64	1.00	575	No	No	Review	
PDGFRA	c.3222T>C		SNV	99.74%	31.41	1.00	1,163	No	No	Review	
KIT	c.1338T>A		SNV	5.60%	33.85	1.00	232	No	No	Review	
KIT	c.2454G>A		SNV	8.32%	36.99	0.81	913	No	No	Review	
ESR1	c.-17G>A		SNV	19.98%	28.43	1.00	1,061	No	No	Review	
ESR1	c.975G>C		SNV	29.46%	30.99	0.00	2,668	No	No	Review	
ESR1	c.1369+13826C>T		SNV	57.95%	30.47	1.00	2,307	No	No	Review	
ESR1	c.*33C>T		SNV	21.55%	34.03	0.98	2,933	No	No	Review	
EGFR	c.645T>A	p.Cys215*	SNV	4.42%	32.43	4.94E-5	633	No	No	Review	
EGFR	c.890-4C>G		SNV	50.91%	32.42	9.43E-3	4,213	No	No	Review	
EGFR	c.1498+22A>T		SNV	44.22%	33.42	1.00	2,691	No	No	Review	
EGFR	c.1726delC	p.Pro576fs	Deletion	6.58%	25.14	1.00	745	No	No	Review	
EGFR	c.2069A>T	p.Glu690Val	SNV	12.27%	30.35	0.95	1,687	No	No	Review	
EGFR	c.2283+46A>G		SNV	5.31%	30.00	1.00	546	No	No	Review	

EGFR	c.2361G>A		SNV	98.94%	31.05	1.00	472	Yes	Yes	Review	
EGFR	c.2470-26T>C		SNV	4.95%	30.79	0.96	586	No	No	Review	
EGFR	c.2709T>C		SNV	98.73%	29.49	1.00	1,964	No	No	Review	
BRAF	c.1860+71_1860+72insC		Insertion	100.00%	32.43	1.00	203	No	No	Review	
BRAF	c.1860+67A>C		SNV	18.93%	36.82	1.00	317	No	No	Review	
BRAF	c.1860+66A>C		SNV	90.91%	36.41	1.00	1,320	No	No	Review	
BRAF	c.1860+3A>G		SNV	5.95%	35.46	3.52E-3	991	No	No	Review	
BRAF	c.1742-38A>T		SNV	4.67%	37.16	0.00	407	No	No	Review	
KRAS	c.541_542delTCinsAG	p.Ser181Arg	MNV	58.24%	31.44	0.00	4,085	No	No	Review	
KRAS	c.534A>G		SNV	58.09%	31.90	0.00	4,109	No	No	Review	
KRAS	c.525A>G		SNV	55.34%	31.56	0.00	4,163	No	No	Review	
KRAS	c.522T>C		SNV	53.60%	35.35	0.00	4,155	No	No	Review	
KRAS	c.519T>C		SNV	56.93%	31.10	0.00	4,137	No	No	Review	
KRAS	c.516A>G		SNV	51.76%	29.42	0.00	4,177	No	No	Review	
KRAS	c.508A>C	p.Met170Leu	SNV	9.95%	30.40	0.00	4,082	No	No	Review	
KRAS	c.237T>C		SNV	5.07%	29.86	0.31	1,301	No	No	Review	
ERBB3	c.3348G>A		SNV	99.93%	33.03	1.00	1,468	No	No	Review	
ERBB2	c.2271A>G		SNV	4.79%	31.05	6.78E-5	1,191	No	No	Review	
ERBB2	c.2281C>T	p.Pro761Ser	SNV	11.44%	32.60	0.00	944	No	No	Review	
ERBB2	c.3508C>G	p.Pro1170Ala	SNV	97.86%	24.09	1.00	420	Yes	Yes	Review	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
ALK	c.3804T>C		No	54	17	37	200	29432684	2
ALK	c.3216G>A		No	171	36	135	200	29446351	2
ALK	c.2535T>C		No	284	118	166	200	29455267	2
PIK3CA	c.-77+8455A>G		No	80	29	51	200	178874846	3
PIK3CA	c.-76-18864C>A		No	299	72	227	200	178897674	3
PIK3CA	c.353-65delA		Yes	19	19	0	200	178917413	3
PIK3CA	c.1005A>G		No	37	17	20	200	178921523	3

PIK3CA	c.1060-17C>A		No	1,644	862	782	200	178922274	3
PIK3CA	c.1145+9G>A		No	51	0	51	200	178922385	3
PIK3CA	c.1145+14T>A		No	52	0	52	200	178922390	3
PIK3CA	c.1145+16_1145+17delGAinsTC		No	52	0	52	200	178922392..178922393	3
PIK3CA	c.1145+19T>A		No	54	0	54	200	178922395	3
PIK3CA	c.1145+19T>C		No	36	0	36	200	178922395	3
PIK3CA	c.1145+24_1145+25insA		No	48	0	48	200	178922400^178922401	3
PIK3CA	c.1145+54A>G		No	67	0	67	200	178922430	3
PIK3CA	c.1664+14T>C		No	42	0	42	200	178936136	3
PIK3CA	c.2686C>A	mis-sense	No	122	73	49	200	178947811	3
PIK3CA	c.2930T>C	mis-sense	No	12	4	8	18	178948158	3
PIK3CA	c.3004T>C	mis-sense	No	126	64	62	200	178951949	3
PDGFRA	c.1406T>C	mis-sense	No	74	35	39	200	55139745	4
PDGFRA	c.2440-50_2440-49insA		No	566	200	366	200	55151958^55151959	4
PDGFRA	c.3222T>C		No	1,160	581	579	200	55161391	4
KIT	c.1338T>A		No	13	0	13	200	55589856	4
KIT	c.2454G>A		No	76	0	76	200	55599328	4
ESR1	c.-17G>A		No	212	109	103	200	152129031	6
ESR1	c.975G>C		No	786	106	680	200	152265522	6
ESR1	c.1369+13826C>T		No	1,337	626	711	200	152396085	6
ESR1	c.*33C>T		No	632	368	264	200	152420134	6
EGFR	c.645T>A	non-sense	No	28	12	16	200	55220255	7
EGFR	c.890-4C>G		No	2,145	2,145	0	200	55223519	7
EGFR	c.1498+22A>T		No	1,190	521	669	200	55228053	7
EGFR	c.1726delC	frame-shift	No	49	28	21	200	55232976	7
EGFR	c.2069A>T	mis-sense	No	207	124	83	200	55241621	7
EGFR	c.2283+46A>G		No	29	0	29	90	55242559	7
EGFR	c.2361G>A		No	467	254	213	200	55249063	7
EGFR	c.2470-26T>C		No	29	29	0	78	55259386	7
EGFR	c.2709T>C		No	1,939	644	1,295	200	55266417	7
BRAF	c.1860+71_1860+72insC		No	203	203	0	200	140453003^140453004	7
BRAF	c.1860+67A>C		No	60	60	0	200	140453008	7

BRAF	c.1860+66A>C		No	1,200	1,200	0	200	140453009	7
BRAF	c.1860+3A>G		No	59	31	28	200	140453072	7
BRAF	c.1742-38A>T		No	19	0	19	200	140453231	7
KRAS	c.541_542delTCinsAG	mis-sense	No	2,379	2,379	0	200	25362754..25362755	12
KRAS	c.534A>G		No	2,387	2,385	2	200	25362762	12
KRAS	c.525A>G		No	2,304	2,299	5	200	25362771	12
KRAS	c.522T>C		No	2,227	2,227	0	200	25362774	12
KRAS	c.519T>C		No	2,355	2,280	75	200	25362777	12
KRAS	c.516A>G		No	2,162	2,158	4	200	25362780	12
KRAS	c.508A>C	mis-sense	No	406	406	0	200	25362788	12
KRAS	c.237T>C		No	66	49	17	200	25380221	12
ERBB3	c.3348G>A		No	1,467	631	836	200	56494991	12
ERBB2	c.2271A>G		No	57	1	56	200	37880227	17
ERBB2	c.2281C>T	mis-sense	No	108	108	0	200	37880237	17
ERBB2	c.3508C>G	mis-sense	No	411	63	348	200	37884037	17

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

Comment: Remark added by user during variant review.

3.3 Untested variants

Variants of interest that could not be tested due to insufficient coverage. These variants are assigned the initial review state "Untested".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
ALK	c.3271G>A	p.Asp1091Asn	SNV	0.00%			131	Yes	Yes	Untested	
ALK	c.3036G>A		SNV	0.00%			124	Yes	Yes	Untested	
PIK3CA	c.3036A>G		SNV	0.58%	31.00		172	Yes	Yes	Untested	
PIK3CA	c.3044C>T	p.Ser1015Phe	SNV	0.00%			172	Yes	Yes	Untested	

ESR1	c.1609T>A	p.Tyr537Asn	SNV	0.00%			87	Yes	Yes	Untested	
ESR1	c.1610A>C	p.Tyr537Ser	SNV	0.00%			87	Yes	Yes	Untested	
EGFR	c.2117T>C	p.Ile706Thr	SNV	0.00%			81	Yes	Yes	Untested	
EGFR	c.2125G>A	p.Glu709Lys	SNV	0.00%			146	Yes	Yes	Untested	
EGFR	c.2126A>C	p.Glu709Ala	SNV	0.00%			146	Yes	Yes	Untested	
EGFR	c.2126A>G	p.Glu709Gly	SNV	0.00%			146	Yes	Yes	Untested	
EGFR	c.2126A>T	p.Glu709Val	SNV	0.00%			146	Yes	Yes	Untested	
EGFR	c.2127_2129delAAC	p.Glu709_Thr710delinsAsp	Deletion	0.00%			146	Yes	Yes	Untested	
EGFR	c.2127_2130delAACTinsC	p.Glu709_Thr710delinsAsp	Replacement	0.00%			145	Yes	Yes	Untested	
EGFR	c.2131G>A	p.Glu711Lys	SNV	0.00%			145	Yes	Yes	Untested	
EGFR	c.2142G>C	p.Lys714Asn	SNV	0.00%			139	Yes	Yes	Untested	
EGFR	c.2142G>T	p.Lys714Asn	SNV	0.00%			139	Yes	Yes	Untested	
EGFR	c.2152C>T		SNV	0.00%			188	Yes	Yes	Untested	
EGFR	c.2154G>A		SNV	0.53%	29.00		189	Yes	Yes	Untested	
EGFR	c.2154G>C		SNV	0.00%			189	Yes	Yes	Untested	
EGFR	c.2154G>T		SNV	0.00%			189	Yes	Yes	Untested	
EGFR	c.2154_2155delGGinsTT	p.Gly719Cys	MNV	0.00%			189	Yes	Yes	Untested	
EGFR	c.2155G>A	p.Gly719Ser	SNV	0.00%			189	Yes	Yes	Untested	
EGFR	c.2155G>T	p.Gly719Cys	SNV	0.00%			189	Yes	Yes	Untested	
EGFR	c.2155_2157delGGCinsTAA	p.Gly719*	MNV	0.00%			189	Yes	Yes	Untested	
EGFR	c.2155_2157delGGCinsTAG	p.Gly719*	MNV	0.00%			189	Yes	Yes	Untested	
EGFR	c.2155_2157delGGCinsTGA	p.Gly719*	MNV	0.00%			189	Yes	Yes	Untested	
EGFR	c.2156G>A	p.Gly719Asp	SNV	1.06%	20.50		189	Yes	Yes	Untested	
EGFR	c.2156G>C	p.Gly719Ala	SNV	0.53%	8.00		189	Yes	Yes	Untested	
EGFR	c.2158T>C	p.Ser720Pro	SNV	0.00%			191	Yes	Yes	Untested	
EGFR	c.2159C>T	p.Ser720Phe	SNV	0.00%			191	Yes	Yes	Untested	
EGFR	c.2161G>A	p.Gly721Ser	SNV	0.00%			176	Yes	Yes	Untested	
EGFR	c.2162G>C	p.Gly721Ala	SNV	0.00%			173	Yes	Yes	Untested	
EGFR	c.2170G>A	p.Gly724Ser	SNV	0.57%	35.00		176	Yes	Yes	Untested	
EGFR	c.2174C>T	p.Thr725Met	SNV	0.00%			175	Yes	Yes	Untested	
EGFR	c.2184+19G>A		SNV	0.00%			68	Yes	Yes	Untested	

EGFR	c.2289_2290in sTTCCAAGAAGCA	p.Ala763_Tyr764insPheGlnGluAla	Insertion	0.00%			198	Yes	Yes	Untested	
EGFR	c.2289_2290in sTTCCAAGAAGCG	p.Ala763_Tyr764insPheGlnGluAla	Insertion	0.00%			198	Yes	Yes	Untested	
EGFR	c.2289_2290in sTTCCAAGAAGCT	p.Ala763_Tyr764insPheGlnGluAla	Insertion	0.00%			198	Yes	Yes	Untested	
EGFR	c.2289_2290in sTTCCAAGAGGCA	p.Ala763_Tyr764insPheGlnGluAla	Insertion	0.00%			198	Yes	Yes	Untested	
EGFR	c.2289_2290in sTTCCAAGAGGCG	p.Ala763_Tyr764insPheGlnGluAla	Insertion	0.00%			198	Yes	Yes	Untested	
EGFR	c.2289_2290in sTTCCAAGAGGCT	p.Ala763_Tyr764insPheGlnGluAla	Insertion	0.00%			198	Yes	Yes	Untested	
EGFR	c.2289_2290in sTTCCAGGAGGCA	p.Ala763_Tyr764insPheGlnGluAla	Insertion	0.00%			198	Yes	Yes	Untested	
EGFR	c.2293G>A	p.Val765Met	SNV	0.00%			113	Yes	Yes	Untested	
EGFR	c.2296_2297insTGGCCAGCG	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			81	Yes	Yes	Untested	
EGFR	c.2296_2297insTGGCAAGCG	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			81	Yes	Yes	Untested	
EGFR	c.2296_2297insTGGCATCTG	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			81	Yes	Yes	Untested	
EGFR	c.2298_2299insGCAAGCGTA	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			80	Yes	Yes	Untested	
EGFR	c.2298_2299insGCAAGCGTC	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			80	Yes	Yes	Untested	
EGFR	c.2298_2299insGCAAGCGTT	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			80	Yes	Yes	Untested	
EGFR	c.2298_2299insGCATCGGTA	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			80	Yes	Yes	Untested	
EGFR	c.2298_2299insGCATCGGTC	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			80	Yes	Yes	Untested	
EGFR	c.2298_2299insGCATCTGTA	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			80	Yes	Yes	Untested	
EGFR	c.2298_2299insGCATCTGTC	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			80	Yes	Yes	Untested	
EGFR	c.2298_2299insGCATCTGTT	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			80	Yes	Yes	Untested	
EGFR	c.2596G>A	p.Glu866Lys	SNV	0.00%			46	Yes	Yes	Untested	

ERBB2	c.2198C>T	p.Thr733Ile	SNV	0.00%			145	Yes	Yes	Untested	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
ALK	c.3271G>A	mis-sense		0	0	0		29446296	2
ALK	c.3036G>A			0	0	0		29449819	2
PIK3CA	c.3036A>G			1	1	0		178951981	3
PIK3CA	c.3044C>T	mis-sense		0	0	0		178951989	3
ESR1	c.1609T>A	mis-sense		0	0	0		152419922	6
ESR1	c.1610A>C	mis-sense		0	0	0		152419923	6
EGFR	c.2117T>C	mis-sense		0	0	0		55241669	7
EGFR	c.2125G>A	mis-sense		0	0	0		55241677	7
EGFR	c.2126A>C	mis-sense		0	0	0		55241678	7
EGFR	c.2126A>G	mis-sense		0	0	0		55241678	7
EGFR	c.2126A>T	mis-sense		0	0	0		55241678	7
EGFR	c.2127_2129delAAC			0	0	0		55241679..55241681	7
EGFR	c.2127_2130delAACTinsC			0	0	0		55241679..55241682	7
EGFR	c.2131G>A	mis-sense		0	0	0		55241683	7
EGFR	c.2142G>C	mis-sense		0	0	0		55241694	7
EGFR	c.2142G>T	mis-sense		0	0	0		55241694	7
EGFR	c.2152C>T			0	0	0		55241704	7
EGFR	c.2154G>A			1	1	0		55241706	7
EGFR	c.2154G>C			0	0	0		55241706	7
EGFR	c.2154G>T			0	0	0		55241706	7
EGFR	c.2154_2155delGGinsTT	mis-sense		0	0	0		55241706..55241707	7
EGFR	c.2155G>A	mis-sense		0	0	0		55241707	7
EGFR	c.2155G>T	mis-sense		0	0	0		55241707	7
EGFR	c.2155_2157delGGCinsTAA	non-sense		0	0	0		55241707..55241709	7
EGFR	c.2155_2157delGGCinsTAG	non-sense		0	0	0		55241707..55241709	7
EGFR	c.2155_2157delGGCinsTGA	non-sense		0	0	0		55241707..55241709	7

EGFR	c.2156G>A	mis-sense		2	0	2		55241708	7
EGFR	c.2156G>C	mis-sense		1	0	1		55241708	7
EGFR	c.2158T>C	mis-sense		0	0	0		55241710	7
EGFR	c.2159C>T	mis-sense		0	0	0		55241711	7
EGFR	c.2161G>A	mis-sense		0	0	0		55241713	7
EGFR	c.2162G>C	mis-sense		0	0	0		55241714	7
EGFR	c.2170G>A	mis-sense		1	0	1		55241722	7
EGFR	c.2174C>T	mis-sense		0	0	0		55241726	7
EGFR	c.2184+19G>A			0	0	0		55241755	7
EGFR	c.2289_2290insTTCCAAGAAGCA			0	0	0		55248991^55248992	7
EGFR	c.2289_2290insTTCCAAGAAGCG			0	0	0		55248991^55248992	7
EGFR	c.2289_2290insTTCCAAGAAGCT			0	0	0		55248991^55248992	7
EGFR	c.2289_2290insTTCCAAGAGGCA			0	0	0		55248991^55248992	7
EGFR	c.2289_2290insTTCCAAGAGGCG			0	0	0		55248991^55248992	7
EGFR	c.2289_2290insTTCCAAGAGGCT			0	0	0		55248991^55248992	7
EGFR	c.2289_2290insTTCCAGGAGGCA			0	0	0		55248991^55248992	7
EGFR	c.2293G>A	mis-sense		0	0	0		55248995	7
EGFR	c.2296_2297insTGGCCAGCG			0	0	0		55248998^55248999	7
EGFR	c.2296_2297insTGGCAAGCG			0	0	0		55248998^55248999	7
EGFR	c.2296_2297insTGGCATCTG			0	0	0		55248998^55248999	7
EGFR	c.2298_2299insGCAAGCGTA			0	0	0		55249000^55249001	7
EGFR	c.2298_2299insGCAAGCGTC			0	0	0		55249000^55249001	7
EGFR	c.2298_2299insGCAAGCGTT			0	0	0		55249000^55249001	7
EGFR	c.2298_2299insGCATCGGTA			0	0	0		55249000^55249001	7
EGFR	c.2298_2299insGCATCGGTC			0	0	0		55249000^55249001	7
EGFR	c.2298_2299insGCATCTGTA			0	0	0		55249000^55249001	7
EGFR	c.2298_2299insGCATCTGTC			0	0	0		55249000^55249001	7
EGFR	c.2298_2299insGCATCTGTT			0	0	0		55249000^55249001	7
EGFR	c.2596G>A	mis-sense		0	0	0		55259538	7
ERBB2	c.2198C>T	mis-sense		0	0	0		37879903	17

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

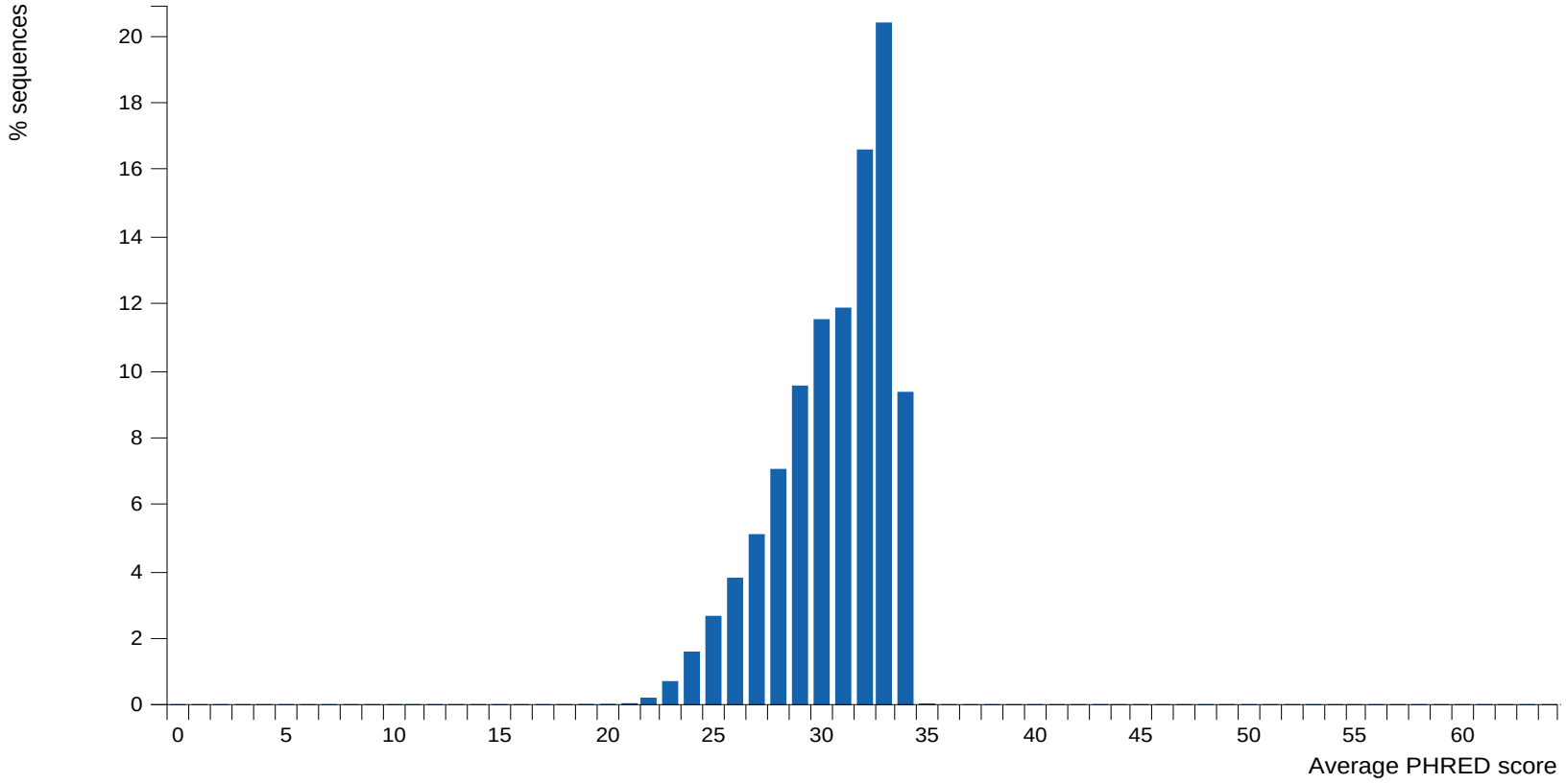
Comment: Remark added by user during variant review.

4 Detailed QC

Quality control metrics for detailed inspection. These metrics can indicate possible problems in the upstream workflow or data analysis. Quality control are divided in metrics on the incoming reads from input data, and metrics per base positions in these reads. Lastly section 4.3 and 4.4 display metrics on how well the positions in the region of interest are covered

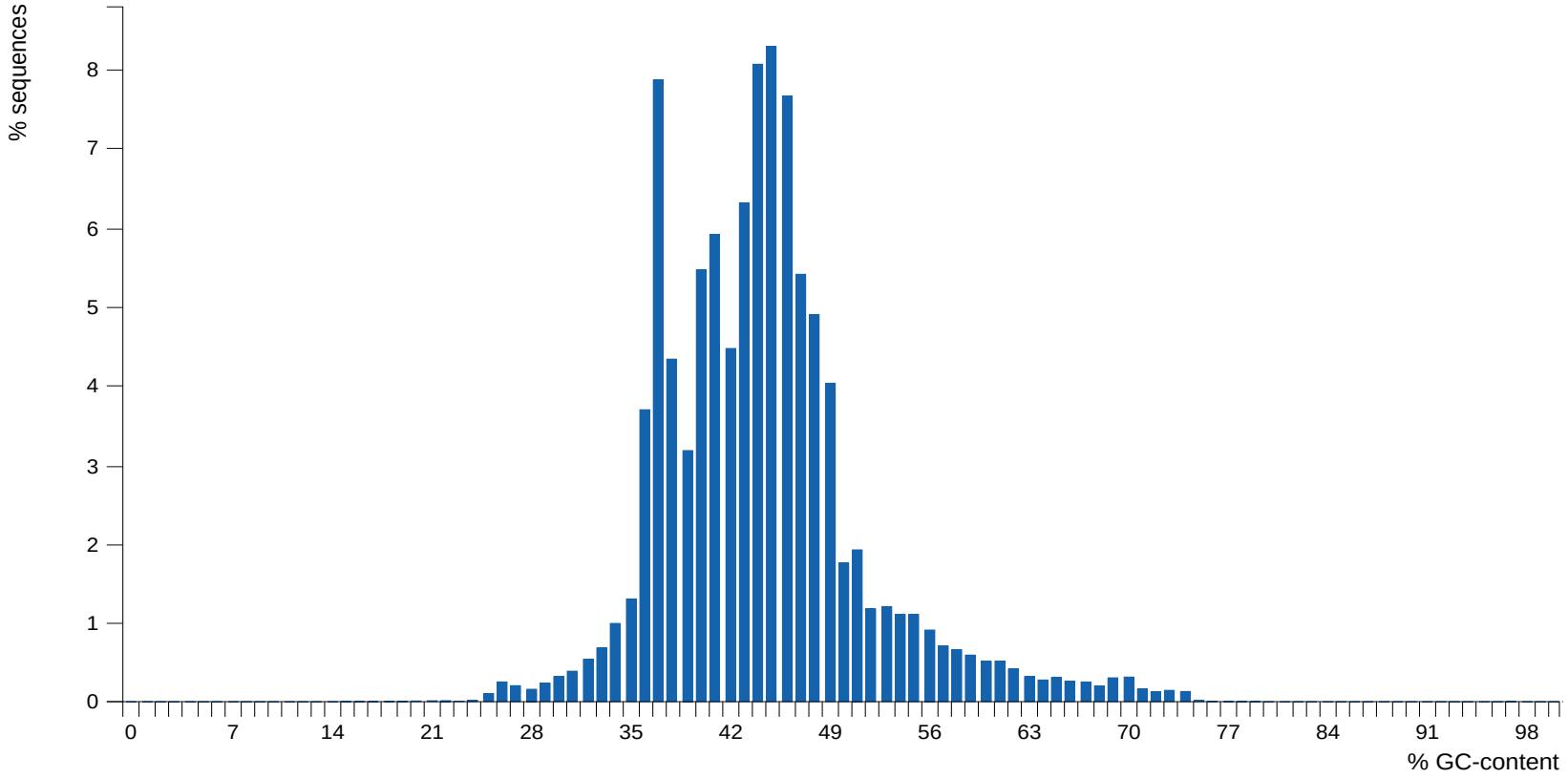
4.1 QC for reads

4.1.1 Average base quality of reads



Distribution of average sequence quality scores. The quality of a sequence is calculated as the arithmetic mean of its base qualities.
x: PHRED-score
y: number of sequences observed at that qual. score normalized to the total number of sequences

4.1.2 GC content of reads



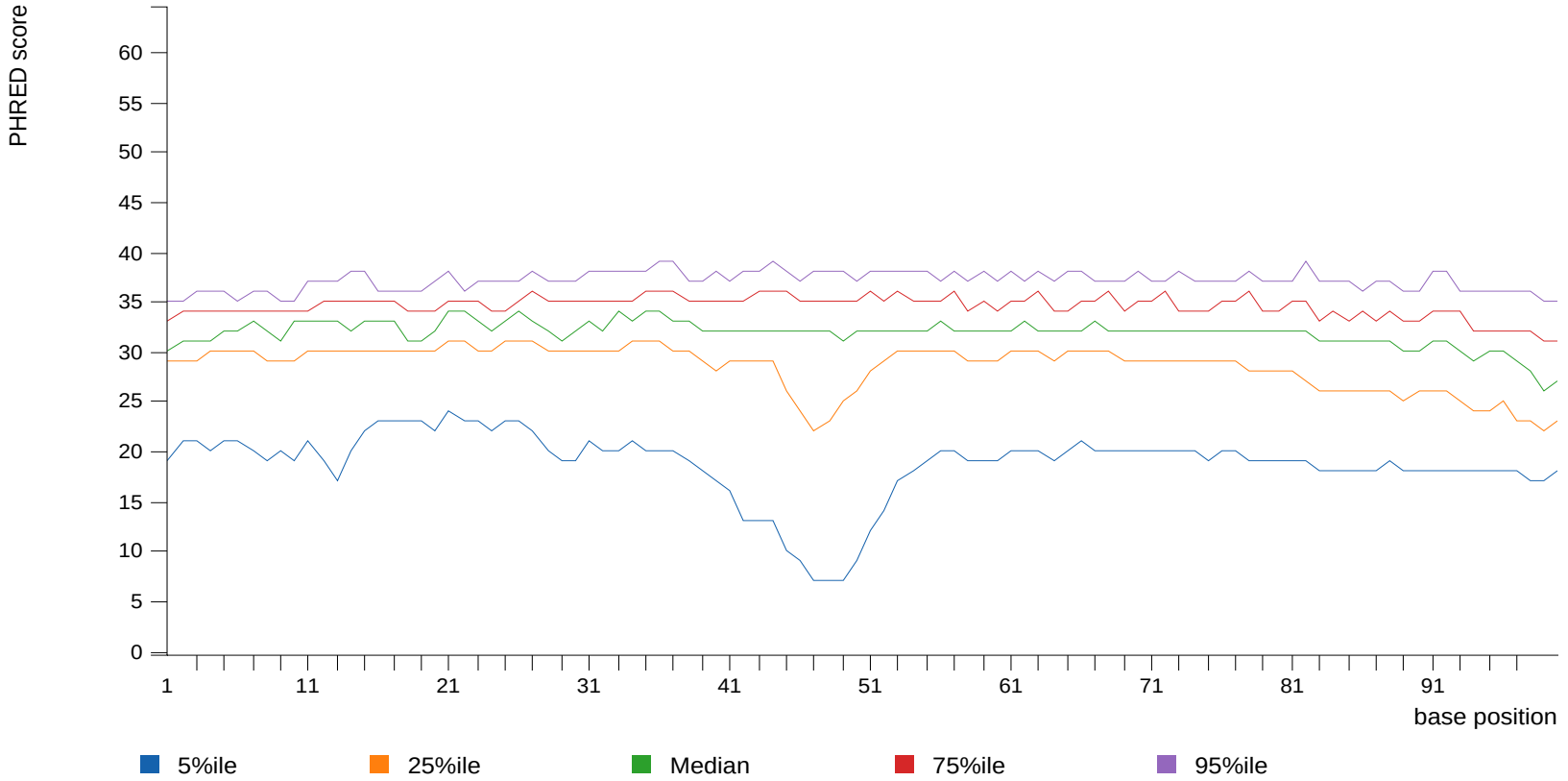
Distribution of GC-contents. The GC-content of a sequence is calculated as the number of GC-bases compared to all bases (including ambiguous bases).
x: relative GC-content of a sequence in percent
y: number of sequences featuring particular GC-percentages normalized to the total number of sequences

4.1.3 Ambiguous base content of reads

No ambiguous bases detected.

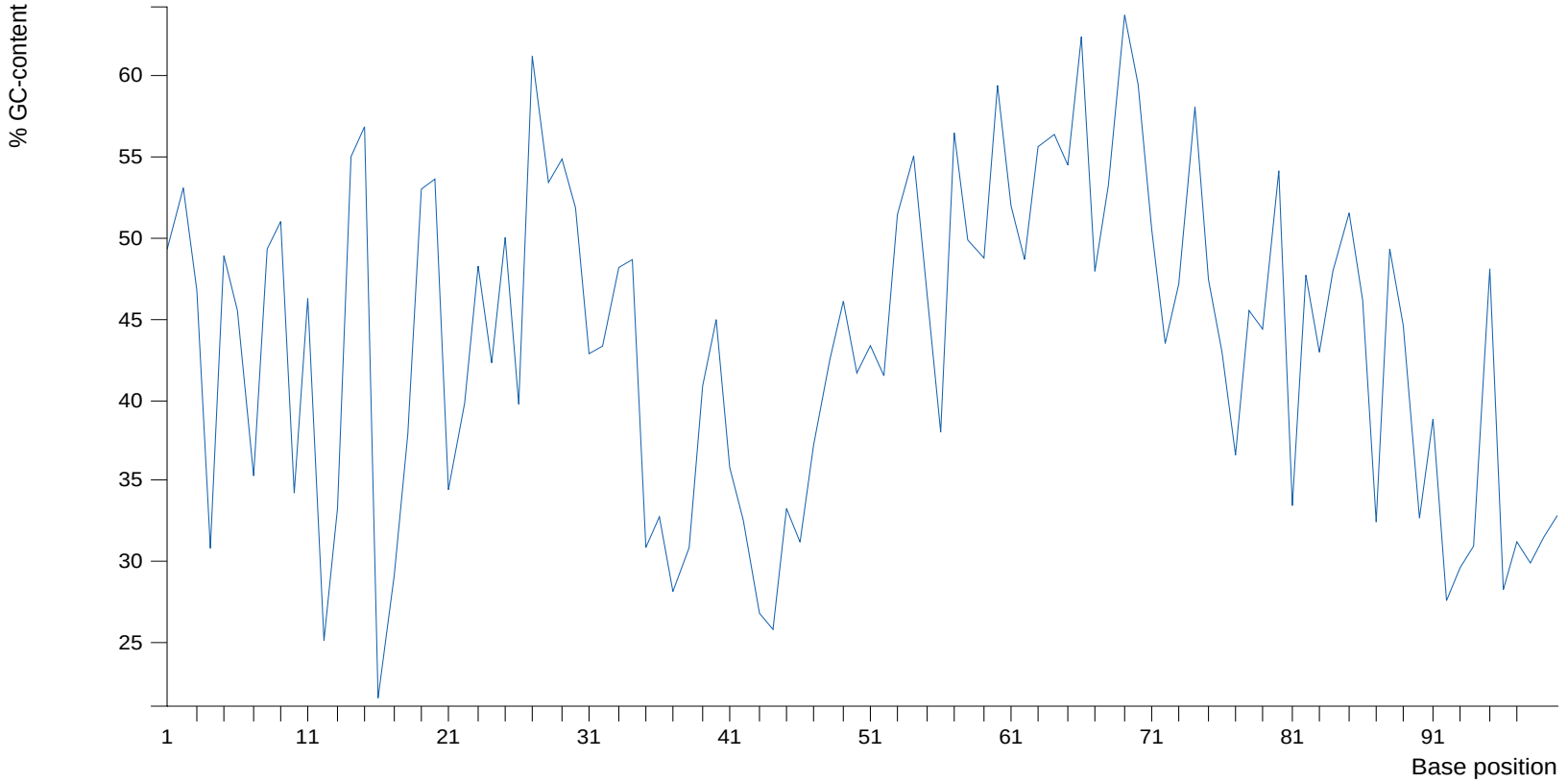
4.2 QC for bases

4.2.1 Quality score per base position



Base-quality distribution along the base positions.
x: base position
y: median & percentiles of quality scores observed at that base position

4.2.2 GC content per base position



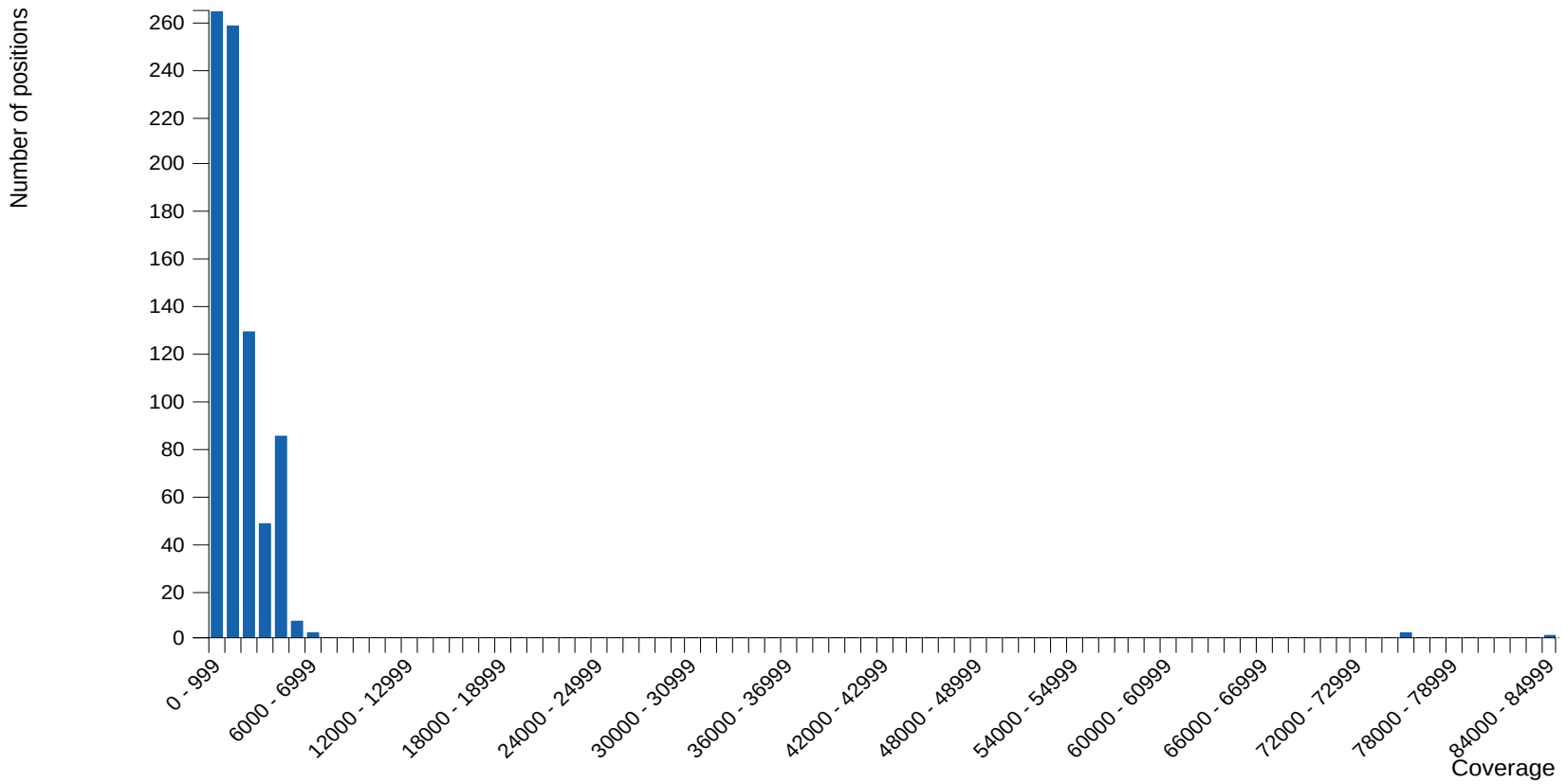
Combined coverage of G- and C-bases.
x: base position
y: number of G- and C-bases observed at current position normalized to the total number of bases observed at that position

4.2.3 Ambiguous base content per base position

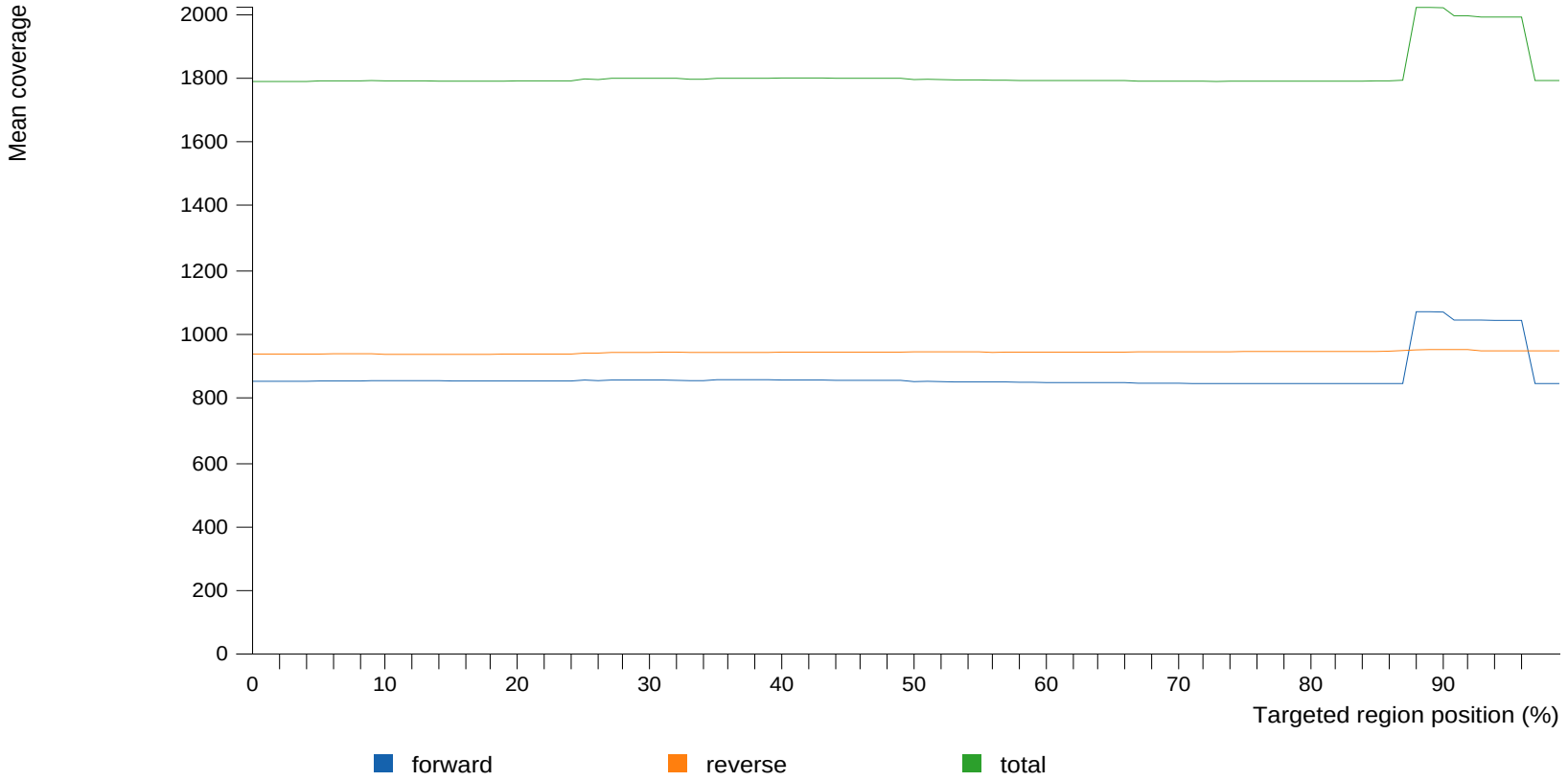
No ambiguous bases detected

4.3 Coverage of Regions of Interest positions

Coverage distribution



4.4 Mean coverage of relative positions in regions of interest



5 History

5.1 Log Entries

Type	Time	User	Details
State change	Sat Mar 07 16:46:56 CET 2020	root	Completed
Variant change	Sat Mar 07 16:46:11 CET 2020	root	The variant ERBB3: c.967T>C was changed from Review to Confirmed by review. Comment: (no comment)
Variant change	Sat Mar 07 16:45:53 CET 2020	root	The variant ERBB3: c.89C>T was changed from Review to Confirmed by review. Comment: (no comment)
Variant change	Sat Mar 07 16:45:22 CET 2020	root	The variant EGFR: c.485T>A was changed from Review to Confirmed by review. Comment: (no comment)
Variant change	Sat Mar 07 16:44:52 CET 2020	root	The variant KIT: c.2869A>C was changed from Review to Confirmed by review. Comment: (no comment)
Variant change	Sat Mar 07 16:44:31 CET 2020	root	The variant PIK3CA: c.1145+16G>A was changed from Review to Confirmed by review. Comment: (no comment)
Variant change	Sat Mar 07 16:43:53 CET 2020	root	The variant ALK: c.2489T>C was changed from Review to Confirmed by review. Comment: (no comment)
Variant change	Sat Mar 07 16:43:44 CET 2020	root	The variant ALK: c.3755C>T was changed from Review to Confirmed by review. Comment: (no comment)
Variant change	Sat Mar 07 16:43:26 CET 2020	root	The variant NRAS: c.19G>C was changed from Review to Confirmed by review. Comment: (no comment)
Variant change	Sat Mar 07 16:43:16 CET 2020	root	The variant NRAS: c.-14C>G was changed from Review to Confirmed by review. Comment: (no comment)
Variant change	Sat Mar 07 16:43:02 CET 2020	root	The variant NRAS: c.46A>G was changed from Review to Confirmed by review. Comment: (no comment)
State change	Sat Jun 29 18:08:25 CEST 2019	system	Ready for Review
State change	Sat Jun 29 17:53:26 CEST 2019	root	In Progress

5.2 Execution Information

QCIA version	QCI Analyze 1.4.5
Analysis start time	Sat Jun 29 17:53:26 CEST 2019
Analysis workflow	AIT FFPE 4.5
Analysis description	QIAact Actionable Insights Tumor Panel on FFPE

5.3 Transcripts

Table listing the genes, transcript IDs and protein IDs used in the analysis.

Gene Name	Transcript ID	Protein ID
NRAS	NM_002524.4	NP_002515.1
ALK	NM_004304.4	NP_004295.2
RAF1	NM_002880.3	NP_002871.1
PIK3CA	NM_006218.2	NP_006209.2
PDGFRA	NM_006206.4	NP_006197.1
KIT	NM_000222.2	NP_000213.1
ESR1	NM_001122742.1	NP_001116214.1
EGFR	NM_005228.3	NP_005219.2
BRAF	NM_004333.4	NP_004324.2
KRAS	NM_004985.3	NP_004976.2
ERBB3	NM_001982.3	NP_001973.2
ERBB2	NM_004448.2	NP_004439.2

5.4 Open Parameters

Workflow parameters that are editable by the administrator

Reported variants

Significant coverage threshold	500
SNV/MNV frequency threshold in %	4.00
Insertions, deletions and replacements frequency threshold in %	4.00

Variants available for review

Minimum coverage threshold	200

SNV/MNV frequency threshold in %	4.00
Insertions, deletions and replacements frequency threshold in %	4.00
Detect variants outside regions of interest	Yes

5.5 Locked Parameters

Adapter trimming

Trim adapter list	GRadapter_160913
Ambiguous trim	false
Ambiguous limit	2
Quality trim	false
Quality limit	0.05
Use colorspace	false
Also search on reversed sequence	false
Remove 5' terminal nucleotides	false
Number of 5' terminal nucleotides	1
Maximum number of nucleotides in reads	1000
Minimum number of nucleotides in reads	15
Discard short reads	false
Remove 3' terminal nucleotides	false
Number of 3' terminal nucleotides	1
Discard long reads	false

Map Reads to Reference

References	Homo_sapiens_sequence_hg19
Masking mode	No masking
Masking track	Not set
Match score	1
Mismatch cost	2

Cost of insertions and deletions	Affine gap cost
Insertion cost	3
Deletion cost	3
Insertion open cost	6
Insertion extend cost	1
Deletion open cost	6
Deletion extend cost	1
Length fraction	0.5
Similarity fraction	0.8
Global alignment	false
Color space alignment	false
Color error cost	3
Auto-detect paired distances	false
Non-specific match handling	Map randomly

InDels and Structural Variants

P-Value threshold	1.0E-4
Maximum number of mismatches	3
Ignore broken pairs	true
Minimum relative consensus coverage	0.0
Minimum quality score	0
Filter variants	true
Minimum number of reads	2
Restrict calling to target regions	ATPv2_TargetRegions_170302_ver1.1

Local Realignment (Short Unaligned End version)

Realign unaligned ends	true
Multi-pass realignment	2
Local bound for unaligned ends of size one	0.75

Local bound for unaligned ends of size two	0.75
Force realignment to guidance-variants	false
Maximum guidance-variant length	100

Trim Primers and their Dimers of Mapped Reads

Primer track	101x_GR_primers_15_10_15_V1.0
Reference	Homo_sapiens_sequence_hg19
Minimum primer overlap length	9
Allow dangling 3' end base	true
Minimal primer overlap fraction	0.7
Only keep reads that have hit a primer	true
Additional bases to trim	1

Remove Pseudogene Reads

Genes track	AITv2_PseudoGenes_170912_ver1.0
Gene and pseudogene links	KRAS -> KRASP1
Required unaligned ends %	2.0

Low Frequency Variant Detection

Required significance (%)	0.01
Ignore positions with coverage above	1000000000
Restrict calling to target regions	ATPv2_TargetRegions_170302_ver1.1
Ignore broken pairs	true
Ignore non-specific matches	Reads
Minimum read length	20
Minimum coverage	Parameter editable by administrator
Minimum count	8

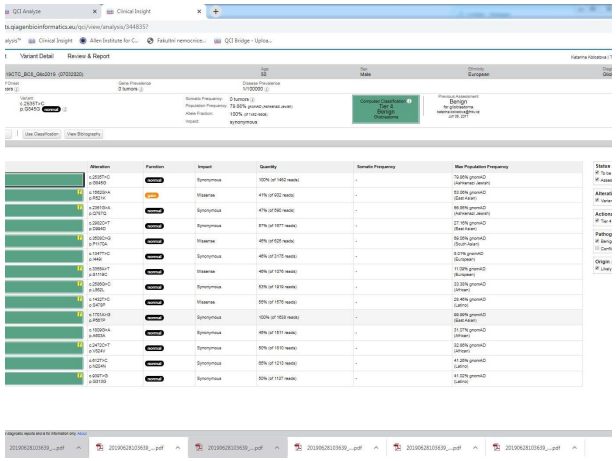
Minimum frequency (%)	Parameter editable by administrator
Base quality filter	false
Neighborhood radius	5
Minimum central quality	5
Minimum neighborhood quality	5
Read direction filter	false
Direction frequency (%)	5.0
Relative read direction filter	false
Significance (%)	1.0E-5
Read position filter	false
Significance (%)	1.0
Remove pyro-error variants	false
In homopolymer regions with minimum length	3
With frequency below	0.8

Remove False Positives

Minimum frequency (%)	Parameter editable by administrator
Minimum forward/reverse balance	0.05
Minimum average base quality	22.0
Variant frequency	true
Forward/reverse balance	false
Average base quality	true

Annotate Variants With Primers

Minimum coverage count	400
Minimum variant percentage	1.0
Minimum variant read count	2


QCI Analyze
Clinical Insight
Variant Detail
Review & Report
Use Classification
View Bibliography
Alteration
Function
Impact
Quantity
Somatic Frequency
Max Population Frequency

YOUR LAB

Your Lab
1700 Lincoln Blvd, Suite 20, Redwood City, CA 94063
labx.com / (650) 484 4040
Additional Information

Test Performed: **Somatic Panel**

Report Date Mar 7, 2020
Status -

Patient		Client		Specimen	
Patient Name		Client		Accession ID	20190628103639_100160050701061 90147_115-18CTC_BC3_Glio2019
Date of Birth		Client ID			
Age	50	Physician			
Sex	Male	Pathologist		Specimen Collection	
Ethnicity	European			Accession	Mar 7, 2020
Diagnosis	Glioblastoma			Primary Tumor Site	Brain

Result: Negative

Genes Tested

Test information such as gene name and hot spot region can be included in this section.

Methods and Limitations

EXAMPLE Statement including sample type (FFPE, etc), method of extraction, amplification reactions, panel targeted regions, sequencing technology, etc. Additionally, a description of the data analysis software(s), genome of reference and the sensitivity of the methods should be described.

QIAGEN Clinical Insight (QCI™) is a variant analysis, interpretation and decision support tool for research and clinical labs analyzing human genetics data and is not intended to be used for diagnostic purposes. QCI Interpret software includes the following underlying databases, data reference sets and tools; QIAGEN Clinical Insight-Interpret (5.6.20200226), Ingenuity Knowledge Base (K-release), CADD (v1.4), Allele Frequency Community (2019-09-25), EVS (ESP6500SI-V2), Refseq Gene Model (2019-02-05), JASPAR (2013-11), Ingenuity Knowledge Base Snapshot Timestamp (2020-02-21 12:37:01.0), Vista Enhancer hg18 (2012-07), Vista Enhancer hg19 (2012-07), Clinical Trials (K-release), PolyPhen-2 (v2.2.2), 1000 Genome Frequency (phase3v5b), ExAC (0.3.1), iva (Nov 19 12:28 iva-1.0.1200.jar), PhyloP hg18 (2009-11), PhyloP hg19 (2009-11), DbSNP (151), TargetScan (7.2), GENCODE (Release 29), CentoMD (5.3), OMIM (May 26, 2017), gnomAD (2.1.1), BSIFT (2016-02-23), TCGA (2013-09-05), Clinvar (2019-06-05), DGV (2016-05-15), COSMIC (v89), HGMD (2019.2), SIFT4G (2016-02-23)

Clinical Significance of Variants Based on AMP / ASCO / CAP Guidelines*

Strong Significance	**Tier 1A**	• Biomarker predicts response or resistance to an FDA or EMA approved therapy, according to drug label or professional guidelines for this diagnosis • Biomarker included in professional guidelines is prognostic or diagnostic for this diagnosis
	Tier 1B	• Biomarker predicts response or resistance to a therapy for this diagnosis based on well-powered studies • Biomarker is prognostic or diagnostic for this diagnosis based on well-powered studies
Potential Significance	**Tier 2C**	• Biomarker is associated with response or resistance to an FDA or EMA approved therapy, according to drug label or professional guidelines but only for different diagnosis • Biomarker is an inclusion criterion for an active clinical trial • Biomarker is prognostic or diagnostic based on multiple small studies
	Tier 2D	• Biomarker shows plausible response or resistance based on case or preclinical studies • Biomarker may assist in disease diagnosis or prognosis based on small studies
Uncertain Significance	**Tier 3**	• Biomarker has uncertain clinical significance and not known to be likely benign or benign

**Adapted from PMID:27993330 jmd.amjpathol.org/article/S1525-1578(16)30223-9/pdf

GeneReader Flow Cell Report

Run Name	Glio2019	**Instrument**	1605018
Flow Cell Name	Glio2019		
Lot	0160050701	**Id**	0147
Number of Cycles	107		
Mode	Single Flow Cell Run / Standard		
Read Design	Default(V2)		
Note			
Start Time	6/28/2019 10:56 AM	**End Time**	6/29/2019 11:36 AM
Analysis End Time	6/29/2019 2:25 PM		

Sample Information

Sample Id	Adapter Q	Adapter Q Sequence	GenePanel
115-18CTC	BC3	TTAGGC	
115-18PT	BC4	TGACCA	
2202-16PT	BC10	TAGCTT	
374-19CTC	BC6	GCCAAT	
411-19CTC	BC7	CAGATC	
475-19CTC	BC8	ACTTGA	
599-19CTC	BC9	GATCAG	
78-18CTC	BC1	ATCACG	
78-18PT	BC2	CGATGT	
868-18CTC	BC5	ACAGTG	
unindexed	unindexed	NNNNNN	

Reagent Kits

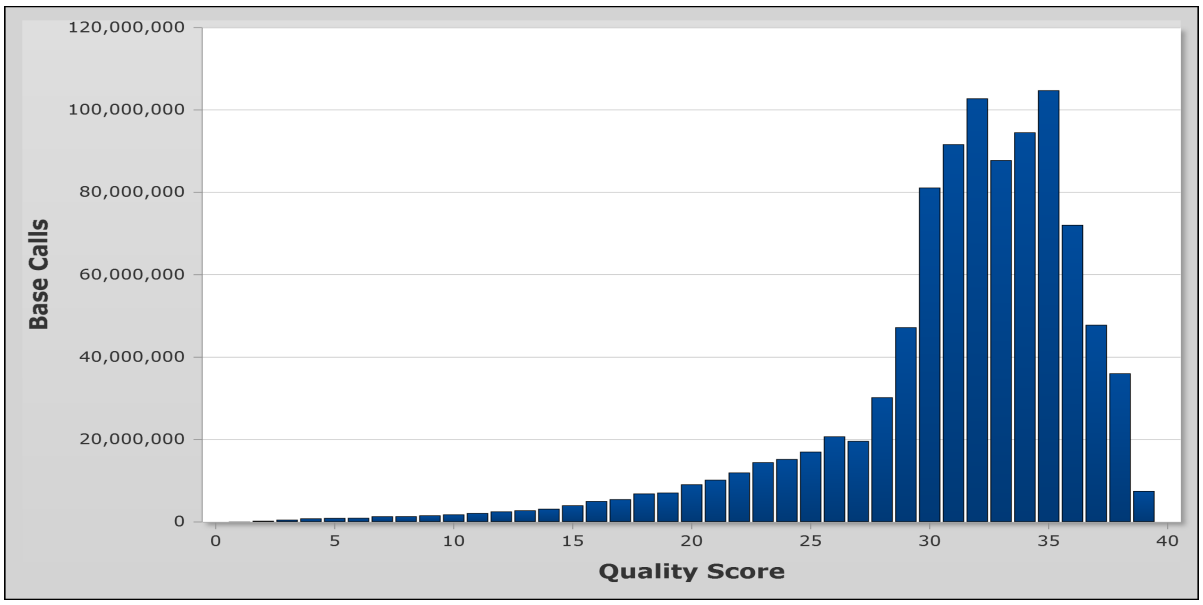
Sequencing Kit	Lot	Material	Exp date
GeneRead UMI Advanced Sequencing Q Buffers (3)	0160046262	9904401	6/30/2019
GeneRead UMI Advanced Sequencing Q Add-Ons (3)	0160021450	9904501	11/30/2019

Wash Kits

Wash Kit	Lot	Material	Exp date
Wash Buffer 9	0160016166	8170091	1/31/2020
Wash Buffer 11	0160018902	8130064	2/29/2020

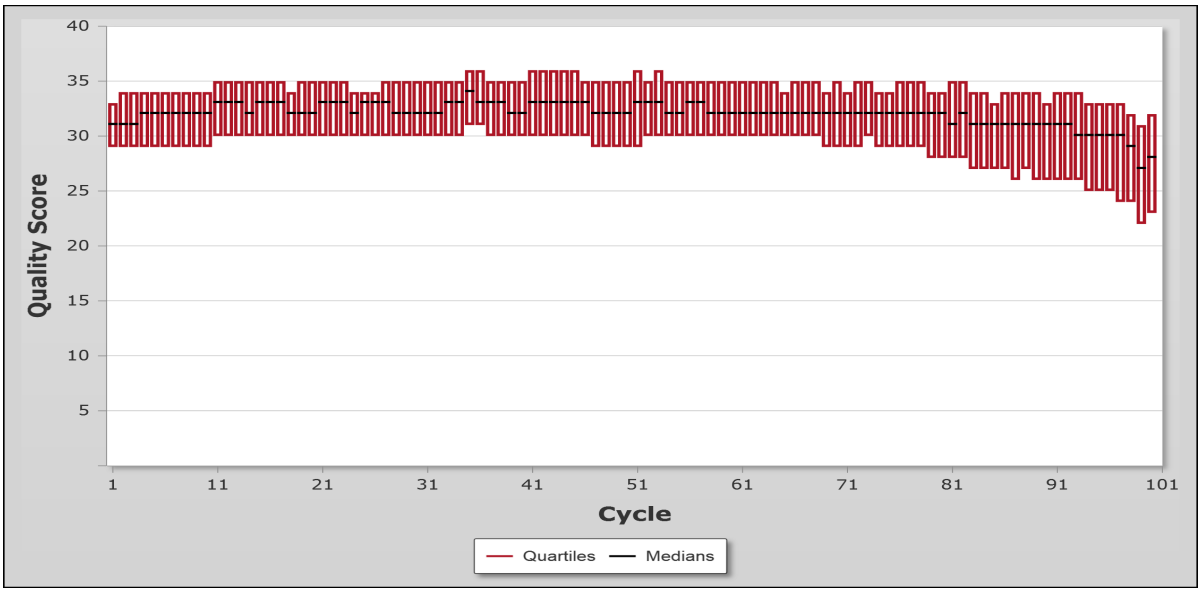
Software Version	GeneReader Software 1.6.1.1

Quality Score Distribution



Reads Past Filtering	10,962,957
Reads Past Demultiplexing	10,452,635
Yield [bases]	975,025,487
Yield Past Demultiplexing	934,777,120

Sequence Quality Score



Reads

Sample Id	Sample Validity	Reads Past Filtering	Yield [bases]	FastQ file
115-18CTC	Valid	578,940	53,794,209	20190628103639_10016005070106190147_115-18CTC_BC3_Glio2019.fastq
115-18PT	Valid	545,677	51,371,782	20190628103639_10016005070106190147_115-18PT_BC4_Glio2019.fastq
2202-16PT	Valid	371,240	35,045,054	20190628103639_10016005070106190147_2202-16PT_BC10_Glio2019.fastq
374-19CTC	Valid	1,404,621	126,802,842	20190628103639_10016005070106190147_374-19CTC_BC6_Glio2019.fastq
411-19CTC	Valid	917,733	83,988,179	20190628103639_10016005070106190147_411-19CTC_BC7_Glio2019.fastq
475-19CTC	Valid	523,930	47,869,315	20190628103639_10016005070106190147_475-19CTC_BC8_Glio2019.fastq
599-19CTC	Valid	1,274,597	113,174,360	20190628103639_10016005070106190147_599-19CTC_BC9_Glio2019.fastq
78-18CTC	Valid	1,610,398	139,901,054	20190628103639_10016005070106190147_78-18CTC_BC1_Glio2019.fastq
78-18PT	Valid	1,835,948	157,943,243	20190628103639_10016005070106190147_78-18PT_BC2_Glio2019.fastq
868-18CTC	Valid	1,389,551	124,887,082	20190628103639_10016005070106190147_868-18CTC_BC5_Glio2019.fastq
unindexed	Valid	510,322	40,248,367	20190628103639_10016005070106190147_unindexed_Glio2019.fastq

YOUR LAB

Your Lab
1700 Lincoln Blvd, Suite 20, Redwood City, CA 94063
labx.com / (650) 484 4040
Additional Information

Test Performed: **Somatic Panel**

Report Date Mar 7, 2020
Status -

Patient		Client		Specimen	
Patient Name		Client		Accession ID	20190628103639_100160050701061 90147_78-18CTC_BC1_Glio2019
Date of Birth		Client ID			
Age	50	Physician			
Sex	Male	Pathologist		Specimen Collection	
Ethnicity	European			Accession	Feb 23, 2020
Diagnosis	Glioblastoma			Primary Tumor Site	Brain
Diagnosis Stage	IIB				

Result: Positive

1
Clinically Significant Variants

2
Therapies with Potential Clinical Benefit

10
Clinical Trials

Biomarker Findings

	Approved Therapies in Glioblastoma	Approved Therapies in Other Indications	Clinical Trials
Tumor Mutation Burden: TMB-high (1 Mutations /Megabase) **Tier 2C**	-	ipilimumab/nivolumab nivolumab	2

Actionable Variants With Associated Therapies

		Approved Therapies			
Gene / Variant	Allelic Fraction	Glioblastoma	Other Indications	Associated With Resistance	Clinical Trials
EGFR c.2582T>C p.L861P g.55259524T>C **Tier 2C** Likely Pathogenic	7.82% (of 716 reads)	-	-	-	8

Variants of Unknown Clinical Significance

Gene / Variant	Allelic Fraction	Function	Classification	Assessment
ALK c.3755C>T p.A1252V g.29432733G>A	8.01% (of 362 reads)	gain	**Tier 3**	**Uncertain Significance**

Gene / Variant	Allelic Fraction	Function	Classification	Assessment
ALK c.2489T>C p.M830T g.29455313A>G	6.94% (of 317 reads)	loss	**Tier 3**	**Uncertain Significance**
EGFR c.485T>A p.I162N g.55214359T>A	9.66% (of 621 reads)	loss	**Tier 3**	**Uncertain Significance**
ERBB3 c.89C>T p.P30L g.56477541C>T	36.0% (of 313 reads)	normal	**Tier 3**	**Uncertain Significance**
ERBB3 c.967T>C p.C323R g.56482419T>C	5.03% (of 437 reads)	loss	**Tier 3**	**Uncertain Significance**
KIT c.1674G>A p.K558K g.55593608G>A	17.0% (of 941 reads)	normal	**Tier 3**	**Uncertain Significance**
KIT c.2869A>C p.I957L g.55604661A>C	6.69% (of 1017 reads)	normal	**Tier 3**	**Uncertain Significance**
NRAS c.46A>G p.K16E g.115258736T>C	4.12% (of 4883 reads)	loss	**Tier 3**	**Uncertain Significance**
NRAS c.19G>C p.V7L g.115258763C>G	13.0% (of 4167 reads)	loss	**Tier 3**	**Uncertain Significance**
NRAS c.-14C>G g.115258795G>C	5.47% (of 1973 reads)	normal	**Tier 3**	**Uncertain Significance**
PIK3CA c.1145+16G>A g.178922392G>A	6.51% (of 676 reads)	normal	**Tier 3**	**Uncertain Significance**

Therapeutic Implications for Other Indications

Therapies for Other Indications	Gene / Variant	Response	Therapies Description
ipilimumab/ nivolumab	***TMB-high*** **Tier 2C** **Pathogenic**	Sensitive	Nivolumab, a PD-1 blocking antibody, in combination with ipilimumab, a CTLA-4 blocking antibody, is FDA- and EMA-approved for treating patients with unresectable or metastatic melanoma; and intermediate or poor risk, previously untreated advanced renal cell carcinoma; nivolumab, in combination with ipilimumab, is FDA-approved for treating adult and pediatric (12 years and older) patients with microsatellite instability-high or mismatch repair deficient metastatic colorectal cancer that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan.
nivolumab	***TMB-high*** **Tier 2C** **Pathogenic**	Sensitive	Nivolumab, a PD-1 blocking antibody, is FDA- and EMA-approved for treating patients with unresectable or metastatic melanoma, as a single agent or in combination with ipilimumab; melanoma with lymph node involvement or metastatic disease who have undergone complete resection, in the adjuvant setting; metastatic non-small cell lung cancer with progression on or after platinum-based chemotherapy (patients with EGFR or ALK genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving nivolumab); patients with advanced renal cell carcinoma who have received prior anti-angiogenic therapy; intermediate or poor risk, previously untreated advanced renal cell carcinoma, in combination with ipilimumab; adult patients with classical Hodgkin lymphoma that has relapsed or progressed after autologous hematopoietic stem cell transplantation and brentuximab vedotin (HSCT), or has relapsed or progressed after 3 or more lines of systemic therapy that includes autologous HSCT; recurrent or metastatic squamous cell carcinoma of the head and neck with disease progression on or after a platinum-based therapy; patients with locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy or have disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy; nivolumab is also FDA-approved for treating adult and pediatric (12 years and older) patients with microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer that has progressed following

Therapies for Other Indications	Gene / Variant	Response	Therapies Description
			treatment with a fluoropyrimidine, oxaliplatin, and irinotecan, as a single agent or in combination with ipilimumab; patients with hepatocellular carcinoma who have been previously treated with sorafenib; and metastatic small cell lung cancer with progression after platinum-based chemotherapy and at least one other line of therapy.

Available Clinical Trials

Gene / Variant	Trial Title Trial ID	Treatments	Trial Phase	Location / Contact
TMB-high **Tier 2C** **Pathogenic**	Phase 2 Investigation of MEDI4736 (Durvalumab) and Tremelimumab Combination in Somatically Hypermutated Recurrent Solid Tumors NCT03911557	durvalumab ticilimumab	Phase 2	United States: KY Heather Heath; heather.flynn@uky.edu ; 859-323-6720;
TMB-high **Tier 2C** **Pathogenic**	Phase 2 Study of MK-3475 (an Antibody That Blocks Negative Signals to T Cells) in Patients With Microsatellite Unstable (MSI) Tumors NCT04098068	pembrolizumab	Phase 2	United States: MA, MD, NY, OH, OR, PA Ellen Lilly-Foreman, RN; lillyel@jhmi.edu; 443-287-4961;
EGFR p.L861P g.55259524T>C **Tier 2C** **Likely Pathogenic**	INdividualized Screening Trial of Innovative Glioblastoma Therapy (INSIGhT) NCT02977780	temozolomide neratinib	Phase 2	United States: AL, MA, MN, NY, OH, PA, RI, TX, UT, VA Patrick Y Wen, MD; patrick_wen@dfci.harvard.edu; 617-632-2166;
EGFR p.L861P g.55259524T>C **Tier 2C** **Likely Pathogenic**	Creating Metabolic Vulnerabilities in Patients With EGFR Activated Recurrent Glioblastoma by Inhibiting EGFR With Osimertinib NCT03732352	osimertinib	Phase 2	United States: CA See clinicaltrials.gov for contact information.
EGFR p.L861P g.55259524T>C **Tier 2C** **Likely Pathogenic**	A Phase 1 Study for the Evaluation of Excretion (Mass Balance) and Pharmacokinetics of 14C-Labeled Poziotinib in Cancer Patients Suitable for Treatment With Poziotinib NCT03804515	poziotinib	Phase 1	United States: PA Shanta Chawla, MD; shanta.chawla@sppirx.com; 949-743-9218;
EGFR p.L861P g.55259524T>C	A Phase 1/2 Study of the Safety, Pharmacokinetics, and Anti-Tumor Activity of the Oral EGFR/HER2 Inhibitor	AP32788	Phase 1 /Phase 2	

Gene / Variant	Trial Title Trial ID	Treatments	Trial Phase	Location / Contact
Tier 2C **Likely Pathogenic**	TAK-788 (AP32788) in Non-Small Cell Lung Cancer NCT02716116			United States: AZ, CA, CO, FL, GA, IL, KS, MA, MI, MO, NC, NJ, NY, OR, TN, TX, VA, WA Takeda Study Registration Call Center; globaloncologymedinfo@takeda.com; +1-866-835-2233;
EGFR p.L861P g.55259524T>C **Tier 2C** **Likely Pathogenic**	A Phase 1/1b Open-label, Dose-escalation and Dose-expansion Study of TPST-1120 as a Single Agent or in Combination With Systemic Anti-Cancer Therapies in Subjects With Advanced Solid Tumors NCT03829436	cetuximab TPST-1120	Phase 1	United States: NC, PA, TN Tempest Clinical Trial Support; TPST-1120-001enrollment@tempesttx.com; (415) 798-8589 x119;
EGFR p.L861P g.55259524T>C **Tier 2C** **Likely Pathogenic**	A Phase 1 Study to Evaluate the Safety, Tolerability, and Immunogenicity of EGFR (Vectibix® Sequence)-Targeted EnGeneIC Dream Vectors Containing Doxorubicin (EGFR(V)-EDV-Dox) in Subjects With Recurrent Glioblastoma Multiforme (GBM) NCT02766699	doxorubicin-loaded EGFR-targeting nanocells	Phase 1	United States: NY Kelly Szajna, RN BSN; kszajna1@jhmi.edu; 410-502-4081;
EGFR p.L861P g.55259524T>C **Tier 2C** **Likely Pathogenic**	Phase I/II, Open-Label Study Evaluating the Efficacy and Pharmacokinetics of Panitumumab-IRDye800 as an Optical Imaging Agent to Detect Neoplasms During Neurosurgical Procedures NCT03510208	panitumumab-IRDye800 panitumumab	Phase 1 /Phase 2	United States: CA Stefania Chirita; schirita@stanford.edu ; 650-723-1423;
EGFR p.L861P g.55259524T>C **Tier 2C** **Likely Pathogenic**	An Open-label, Phase 2 Study of Neratinib in Patients With Solid Tumors With Somatic Human Epidermal Growth Factor Receptor (EGFR, HER2, HER3) Mutations or EGFR Gene Amplification. NCT01953926	neratinib	Phase 2	United States: AL, AZ, CA, DE, FL, GA, IL, LA, MA, MN, MO, NY, OH, PA, SC, TN, TX, WI Puma Biotechnology Clinical Operations Senior Director; ClinicalTrials@pumabiotechnology.com; (424) 248-6500;

Individual Variant Interpretations

Biomarker	***TMB-high***	**Interpretation**

Classification **Tier 2C**
Assessment **Pathogenic**

High tumor mutational burden (TMB-high) indicates the presence of an elevated number of somatic non-synonymous mutations in the coding regions of a tumor cell genome, expressed as mutations per megabase (mut/Mb) of sequenced tumor DNA [PMID:28481359, PMID:30505709, PMID:30505710]. Tumor mutational burden can be assessed by whole-exome sequencing or by targeted next-generation sequencing using large gene panels [PMID:30505710, PMID:26439694, PMID:29337640]. However, optimal thresholds for classification of TMB values into high, intermediate, and low categories are not yet standardized across different methods and panels [PMID:30664300]. Somatic hypermutation or high TMB levels may be associated with abnormal genome maintenance due to defective DNA mismatch repair (dMMR)/high frequency microsatellite instability (MSI-high), mutations in the exonuclease domain of DNA polymerase eta (POLE), or mutations in APOBEC family members [PMID:23945592, PMID:26181000]. Alternatively, high TMB may occur as a result of exposure to environmental carcinogens like tobacco smoke and ultraviolet radiation [PMID:23945592, PMID:29056344]. While the majority of MSI-high tumors are also TMB-high, typically only a subset of TMB-high tumors are MSI-high [PMID:28420421]. TMB values vary broadly across different tumor types and may also fall within a broad range for tumors of the same type, as exemplified by colorectal, bladder, endometrial cancers, and melanoma [PMID:29262275, PMID:28481359, PMID:28420421]. TMB correlates with the neoantigen load carried by tumor cells, with a higher TMB expected to result in increased neoantigen presentation by MHC proteins on the tumor cell surface, enhancing the probability of tumor cell recognition and cytolysis by tumor-infiltrating lymphocytes (TIL) [PMID:25409260, PMID:26359337]. Preliminary evidence suggests that high TMB may be a prognostic factor associated with improved patient survival [PMID:26997480, PMID:30865548]. TMB is an emerging predictive and prognostic biomarker, with accumulating clinical evidence supporting an association of high TMB with improved response to immune checkpoint inhibitors such as nivolumab, ipilimumab, pembrolizumab, and atezolizumab in cancer types including non-small cell lung cancer, melanoma, and urothelial carcinoma [PMID:30395155, PMID:28835386, PMID:26359337, PMID:25765070, PMID:26952546, PMID:29658848]. PD-L1 expression and TMB status have been demonstrated to be independent biomarkers with value for predicting immunotherapy response [PMID:30895946, PMID:29657128, PMID:29337640, PMID:29658845].

Gene ***EGFR***
Variation -
Exon 21
Nucleotide NM_005228.5:
g.55259524T>C
c.2582T>C
Amino Acid p.L861P
Function gain
Allelic Fraction 7.82% (of 716 reads)
Classification **Tier 2C**
Assessment **Likely Pathogenic**

Interpretation
EGFR is an oncogene involved in cell growth and differentiation through activation of the PI3K/AKT/MTOR and RAS/RAF/MAPK pathways [39]. Amplification, gain-of-function mutations, and protein overexpression cause EGFR activation [28, 53, 36, 41]. EGFR mutations are reported to be mutually exclusive with ALK rearrangements and KRAS mutations in non-small cell lung cancer [16, 42, 43].

Gene ***ALK***
Variation -

Interpretation
ALK is an oncogene involved in cell proliferation and survival through

Exon	25
Nucleotide	NM_004304.5: g.29432733G>A c.3755C>T
Amino Acid	p.A1252V
Function	gain
Allelic Fraction	8.01% (of 362 reads)
Classification	**Tier 3**
Assessment	**Uncertain Significance**

activation of several pathways, including the RAS/RAF/MEK and PI3K/AKT /MTOR pathways [35]. Gain-of-function mutations and fusions cause ALK activation [11, 2, 23].

Gene	***ALK***
Variation	-
Exon	15
Nucleotide	NM_004304.5: g.29455313A>G c.2489T>C
Amino Acid	p.M830T
Function	loss
Allelic Fraction	6.94% (of 317 reads)
Classification	**Tier 3**
Assessment	**Uncertain Significance**

Interpretation
ALK is an oncogene involved in cell proliferation and survival through activation of several pathways, including the RAS/RAF/MEK and PI3K/AKT /MTOR pathways [35]. Gain-of-function mutations and fusions cause ALK activation [11, 2, 23].

Gene	***EGFR***
Variation	-
Exon	4
Nucleotide	NM_005228.5: g.55214359T>A c.485T>A
Amino Acid	p.I162N
Function	loss
Allelic Fraction	9.66% (of 621 reads)
Classification	**Tier 3**
Assessment	**Uncertain Significance**

Interpretation
EGFR is an oncogene involved in cell growth and differentiation through activation of the PI3K/AKT/MTOR and RAS/RAF/MAPK pathways [39]. Amplification, gain-of-function mutations, and protein overexpression cause EGFR activation [28, 53, 36, 41]. EGFR mutations are reported to be mutually exclusive with ALK rearrangements and KRAS mutations in non-small cell lung cancer [16, 42, 43].

Gene	***ERBB3***
Variation	-
Exon	2
Nucleotide	NM_001982.3: g.56477541C>T c.89C>T
Amino Acid	p.P30L
Function	normal
Allelic Fraction	36.0% (of 313 reads)
Classification	**Tier 3**
Assessment	**Uncertain Significance**

Interpretation
ERBB3 is a receptor tyrosine kinase involved in cell growth and proliferation through activation of the RAS/RAF/MAPK and PI3K/AKT /MTOR pathways [55, 46]. Amplification, gain-of-function mutations and protein overexpression cause ERBB3 activation [27, 29, 49].

Gene	***ERBB3***
Variation	-
Exon	8
Nucleotide	NM_001982.3: g.56482419T>C

Interpretation
ERBB3 is a receptor tyrosine kinase involved in cell growth and proliferation through activation of the RAS/RAF/MAPK and PI3K/AKT /MTOR pathways [55, 46]. Amplification, gain-of-function mutations and protein overexpression cause ERBB3 activation [27, 29, 49].

	c.967T>C
Amino Acid	p.C323R
Function	loss
Allelic Fraction	5.03% (of 437 reads)
Classification	**Tier 3**
Assessment	**Uncertain Significance**

Gene	***KIT***
Variation	-
Exon	11
Nucleotide	NM_000222.2: g.55593608G>A c.1674G>A
Amino Acid	p.K558K
Function	normal
Allelic Fraction	17.0% (of 941 reads)
Classification	**Tier 3**
Assessment	**Uncertain Significance**

Interpretation
KIT is an oncogene involved in cell proliferation and survival through activation of RAS/RAF/MAPK and PI3K/AKT/MTOR pathways [13]. Amplification, gain-of-function mutations, and protein overexpression cause KIT activation [18, 22, 8].

Gene	***KIT***
Variation	-
Exon	21
Nucleotide	NM_000222.2: g.55604661A>C c.2869A>C
Amino Acid	p.I957L
Function	normal
Allelic Fraction	6.69% (of 1017 reads)
Classification	**Tier 3**
Assessment	**Uncertain Significance**

Interpretation
KIT is an oncogene involved in cell proliferation and survival through activation of RAS/RAF/MAPK and PI3K/AKT/MTOR pathways [13]. Amplification, gain-of-function mutations, and protein overexpression cause KIT activation [18, 22, 8].

Gene	***NRAS***
Variation	-
Exon	2
Nucleotide	NM_002524.5: g.115258736T>C c.46A>G
Amino Acid	p.K16E
Function	loss
Allelic Fraction	4.12% (of 4883 reads)
Classification	**Tier 3**
Assessment	**Uncertain Significance**

Interpretation
NRAS is an oncogene involved in cell survival and proliferation through activation of RAS/RAF/MAPK and PI3K/AKT/MTOR pathways [31]. Gain-of-function mutations cause NRAS activation [40].

Gene	***NRAS***
Variation	-
Exon	2
Nucleotide	NM_002524.5: g.115258763C>G c.19G>C
Amino Acid	p.V7L
Function	loss

Interpretation
NRAS is an oncogene involved in cell survival and proliferation through activation of RAS/RAF/MAPK and PI3K/AKT/MTOR pathways [31]. Gain-of-function mutations cause NRAS activation [40].

Allelic Fraction	13.0% (of 4167 reads)
Classification	**Tier 3**
Assessment	**Uncertain Significance**

Gene	***NRAS***
Variation	-
Exon	2
Nucleotide	NM_002524.5: g.115258795G>C c.-14C>G
Amino Acid	
Function	normal
Allelic Fraction	5.47% (of 1973 reads)
Classification	**Tier 3**
Assessment	**Uncertain Significance**

Interpretation
NRAS is an oncogene involved in cell survival and proliferation through activation of RAS/RAF/MAPK and PI3K/AKT/MTOR pathways [31]. Gain-of-function mutations cause NRAS activation [40].

Gene	***PIK3CA***
Variation	-
Exon	6
Nucleotide	NM_006218.4: g.178922392G>A c.1145+16G>A
Amino Acid	
Function	normal
Allelic Fraction	6.51% (of 676 reads)
Classification	**Tier 3**
Assessment	**Uncertain Significance**

Interpretation
PIK3CA is an oncogene involved in cell growth, apoptosis, transformation, motility, and adhesion through activation of the PI3K/AKT/MTOR pathway [7, 15, 48]. Gain-of-function mutations, amplification, and protein overexpression cause PI3K activation [38, 6, 12, 50]. PIK3CA mutations are reported to be mutually exclusive with PTEN mutations [37, 3].

Genes Tested

Test information such as gene name and hot spot region can be included in this section.

Methods and Limitations

EXAMPLE Statement including sample type (FFPE, etc), method of extraction, amplification reactions, panel targeted regions, sequencing technology, etc. Additionally, a description of the data analysis software(s), genome of reference and the sensitivity of the methods should be described.

QIAGEN Clinical Insight (QCI™) is a variant analysis, interpretation and decision support tool for research and clinical labs analyzing human genetics data and is not intended to be used for diagnostic purposes. QCI Interpret software includes the following underlying databases, data reference sets and tools; QIAGEN Clinical Insight-Interpret (5.6.20200226), Ingenuity Knowledge Base (K-release), CADD (v1.4), Allele Frequency Community (2019-09-25), EVS (ESP6500SI-V2), Refseq Gene Model (2019-02-05), JASPAR (2013-11), Ingenuity Knowledge Base Snapshot Timestamp (2020-02-21 12:37:01.0), Vista Enhancer hg18 (2012-07), Vista Enhancer hg19 (2012-07), Clinical Trials (K-release), PolyPhen-2 (v2.2.2), 1000 Genome Frequency (phase3v5b), ExAC (0.3.1), iva (Nov 19 12:28 iva-1.0.1200.jar), PhyloP hg18 (2009-11), PhyloP hg19 (2009-11), DbSNP (151), TargetScan (7.2), GENCODE (Release 29), CentoMD (5.3), OMIM (May 26, 2017), gnomAD (2.1.1), BSIFT (2016-02-23), TCGA (2013-09-05), Clinvar (2019-06-05), DGV (2016-05-15), COSMIC (v89), HGMD (2019.2), SIFT4G (2016-02-23)

Clinical Significance of Variants Based on AMP / ASCO / CAP Guidelines*

Strong Significance	**Tier 1A**	• Biomarker predicts response or resistance to an FDA or EMA approved therapy, according to drug label or professional guidelines for this diagnosis • Biomarker included in professional guidelines is prognostic or diagnostic for this diagnosis
	Tier 1B	• Biomarker predicts response or resistance to a therapy for this diagnosis based on well-powered studies • Biomarker is prognostic or diagnostic for this diagnosis based on well-powered studies
Potential Significance	**Tier 2C**	• Biomarker is associated with response or resistance to an FDA or EMA approved therapy, according to drug label or professional guidelines but only for different diagnosis • Biomarker is an inclusion criterion for an active clinical trial • Biomarker is prognostic or diagnostic based on multiple small studies
	Tier 2D	• Biomarker shows plausible response or resistance based on case or preclinical studies • Biomarker may assist in disease diagnosis or prognosis based on small studies
Uncertain Significance	**Tier 3**	• Biomarker has uncertain clinical significance and not known to be likely benign or benign

**Adapted from PMID:27993330 jmd.amjpathol.org/article/S1525-1578(16)30223-9/pdf

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Analysis Report

20190628103639_10016005070106190147_115-18PT_BC4_Glio2019

Table of contents

1 Summary

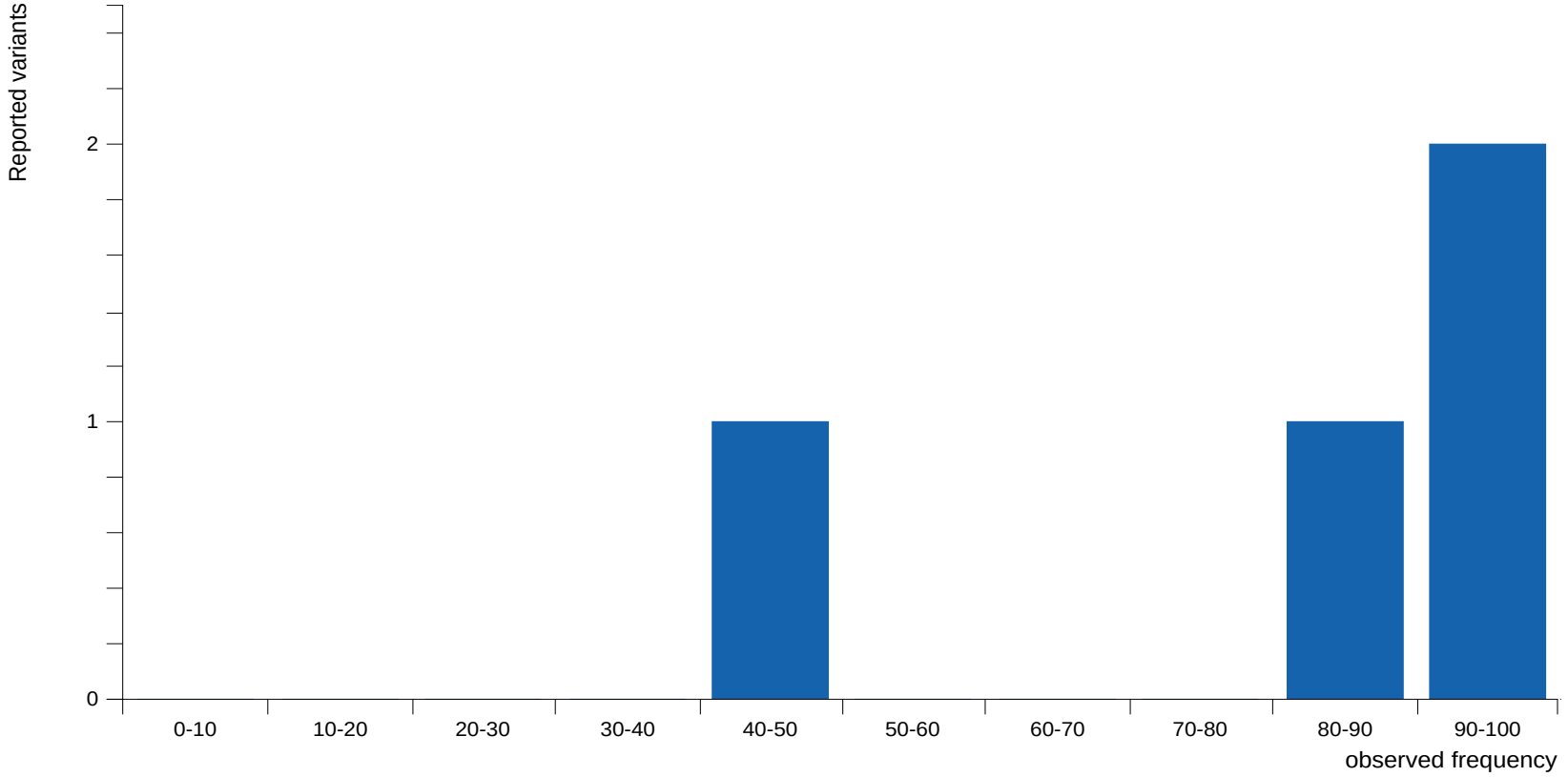
Report created	Sat Jun 29 18:08:23 CEST 2019
Sample ID	20190628103639_10016005070106190147_115-18PT_BC4_Glio2019
Analysis workflow	AIT FFPE v4.5: QIAact Actionable Insights Tumor Panel on FFPE
Analyst	root
Reported variants	4
Analysis results	107 Untested variants

1.1 Comments

No comments

1.2 Distribution of observed frequencies for reported variants

Includes variants initially listed in variant table 'Reported variants'.



2 Quality control

Quality control for the sample analysis. Includes information on the input data, read mapping, and coverage information per gene.

2.1 Fastq

Fastq	20190628103639_10016005070106190147_115-18PT_BC4_Glio2019
Reads	545,677
Nucleotides*	54,645,844
Average read length*	100.14
Reads with average quality ≥ 25	99.42%

** Including sample barcode*

Recommendations:

Reads with average quality ≥ 25 should be ≥ 80.00%

2.2 Secondary analysis summary

Reads mapped	540,026 (98.96%)
Reads in target regions	515,947 (95.54%)
Percentage of base positions in regions of interest with coverage ≥ 500x	47.86%
Percentage of base positions in regions of interest with coverage ≥ 200x	87.19%

Recommendations:

Percentage of base positions in regions of interest with coverage ≥ 500x should be ≥ 90.00%

Percentage of base positions in regions of interest with coverage ≥ 200x should be ≥ 95.00%

2.3 Coverage

Name	ROI	Bases	≥500x	≥200x	0x	Median	VOI	VOI <500x	VOI <200x
NRAS	6	27	77.78%	100.00%	0.00%	730	41	1	0
ALK	22	47	70.21%	80.85%	0.00%	595	40	7	3
RAF1	2	2	100.00%	100.00%	0.00%	698	2	0	0
PIK3CA	81	131	16.79%	71.76%	0.00%	271	165	127	41

Name	ROI	Bases	≥500x	≥200x	0x	Median	VOI	VOI <500x	VOI <200x
PDGFRA	21	64	54.69%	96.88%	0.00%	615	46	22	2
KIT	49	140	15.00%	83.57%	0.00%	288	235	208	19
ESR1	6	7	28.57%	28.57%	0.00%	124	11	6	6
EGFR	96	208	100.00%	100.00%	0.00%	10,158	443	0	0
BRAF	29	72	5.56%	87.50%	0.00%	315	153	144	12
KRAS	21	55	1.82%	74.55%	0.00%	305	148	147	19
ERBB3	8	8	50.00%	100.00%	0.00%	472	10	6	0
ERBB2	16	35	80.00%	91.43%	0.00%	824	61	7	2

ROI: Number of Regions of Interest, i.e. reportable regions that overlap with the gene.

Bases: Total number of base positions in Regions of Interest that overlap with the gene.

≥500x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is equal to or above the significant coverage threshold.

≥200x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is equal to or above the minimum coverage threshold.

0x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is zero.

Median: Median coverage of base positions in the Regions of Interest that overlap with the gene.

VOI: Total number of Variants of Interest, whether detected or not, that overlap with the gene. The list of Variants of Interest is defined by the analysis pipeline.

VOI <500x: Number of Variants of Interest in the gene for which coverage is below the significant coverage threshold.

VOI <200x: Number of Variants of Interest in the gene for which coverage is below the minimum coverage threshold.

2.4 Detected variants

Number of detected variants per gene. Variants for which coverage is above the minimum coverage threshold.

Name	In total	VOI	- non-syn	- syn	Non-VOI	- non-syn	- syn
NRAS	0	0	0	0	0	0	0
ALK	2	2	0	2	0	0	0
RAF1	0	0	0	0	0	0	0
PIK3CA	3	1	1	0	2	0	2
PDGFRA	2	1	0	1	1	0	1
KIT	0	0	0	0	0	0	0
ESR1	2	1	0	1	1	0	1
EGFR	4	2	1	1	2	0	2
BRAF	1	1	0	1	0	0	0

Name	In total	VOI	- non-syn	- syn	Non-VOI	- non-syn	- syn
KRAS	0	0	0	0	0	0	0
ERBB3	2	0	0	0	2	1	1
ERBB2	0	0	0	0	0	0	0

In total: Total number of variants detected within the gene. Variants initially listed in variant tables 3.1 and 3.2.

VOI: Number of detected Variants of Interest detected within the gene. The list of Variants of Interest is defined by the analysis pipeline.

- non-syn: Number of detected, gene-specific Variants of Interest that are non-synonymous.

- syn: Number of detected, gene-specific Variants of Interest that are synonymous.

Non-VOI: Number of detected variants that are not found within the analysis pipeline-defined list of Variants of Interest.

- non-syn: Number of gene-specific, non-VOIs that are non-synonymous.

- syn: Number of gene-specific, non-VOIs that are synonymous.

3 Variants

Variants detected within regions of interest with more than significant coverage are found in 3.1 and variants with more than minimum coverage are found in 3.2.
Variants of interest that could not be tested due to insufficient coverage are listed in table 3.3.
The coverage thresholds and minimum frequency cutoffs configured for the analysis workflow are listed in the History section.
Setting a variant review state to "Confirmed by review" moves it to 3.1, "Artifact" moves it to 3.2.
Only the variants in table 3.1 are exported as VCF and uploaded to QCI Interpret.

3.1 Reported variants

Variants that will be exported to VCF and uploaded to QCI Interpret. Initially contains: Variants detected within regions of interest with more than significant coverage and frequency above the cutoff set for the analysis workflow. These variants are assigned the initial review state "Valid".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
ALK	c.2535T>C		SNV	47.93%	31.61	1.00	726	Yes	Yes	Valid	
ESR1	c.1782G>A		SNV	96.52%	33.10	1.00	892	Yes	Yes	Valid	
EGFR	c.865G>A	p.Ala289Thr	SNV	88.30%	25.82	1.00	10,766	Yes	Yes	Valid	
EGFR	c.2361G>A		SNV	99.66%	31.24	1.00	10,664	Yes	Yes	Valid	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
ALK	c.2535T>C		No	348	171	177	200	29455267	2
ESR1	c.1782G>A		No	861	423	438	200	152420095	6
EGFR	c.865G>A	mis-sense	No	9,506	2,757	6,749	200	55221821	7
EGFR	c.2361G>A		No	10,628	5,391	5,237	200	55249063	7

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

Comment: Remark added by user during variant review.

3.2 Variants available for review

Detected variants that will not be exported to the VCF and uploaded to QCI Interpret. Initially contains: Variants with more than minimum coverage and frequency above the cutoff set for the analysis workflow. Depending on workflow configuration, this table may include variants outside of regions of interest including those with coverage above significant coverage threshold. These variants are assigned the initial review state "Review".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
ALK	c.4338C>T		SNV	54.24%	29.81	1.00	472	Yes	Yes	Review	
PIK3CA	c.-76-18864C>A		SNV	45.45%	32.87	1.00	231	No	No	Review	
PIK3CA	c.1060-17C>A		SNV	34.48%	32.74	1.00	203	No	No	Review	

PIK3CA	c.1173A>G	p.Ile391Met	SNV	57.00%	31.92	1.00	207	Yes	Yes	Review	
PDGFRA	c.1701A>G		SNV	99.75%	30.04	1.00	402	Yes	Yes	Review	
PDGFRA	c.3222T>C		SNV	99.67%	31.34	1.00	600	No	No	Review	
ESR1	c.975G>C		SNV	94.71%	31.08	1.00	359	No	No	Review	
EGFR	c.1498+22A>T		SNV	98.53%	33.23	1.00	7,759	No	No	Review	
EGFR	c.2709T>C		SNV	98.80%	29.83	1.00	10,625	No	No	Review	
BRAF	c.1929A>G		SNV	52.29%	27.43	1.00	436	Yes	Yes	Review	
ERBB3	c.89C>T	p.Pro30Leu	SNV	46.32%	35.05	1.00	272	No	No	Review	
ERBB3	c.3348G>A		SNV	99.15%	32.53	1.00	473	No	No	Review	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
ALK	c.4338C>T		No	256	102	154	200	29416615	2
PIK3CA	c.-76-18864C>A		No	105	26	79	200	178897674	3
PIK3CA	c.1060-17C>A		No	70	43	27	200	178922274	3
PIK3CA	c.1173A>G	mis-sense	No	118	57	61	200	178927410	3
PDGFRA	c.1701A>G		No	401	163	238	200	55141055	4
PDGFRA	c.3222T>C		No	598	304	294	200	55161391	4
ESR1	c.975G>C		No	340	134	206	200	152265522	6
EGFR	c.1498+22A>T		No	7,645	3,230	4,415	200	55228053	7
EGFR	c.2709T>C		No	10,497	5,315	5,182	200	55266417	7
BRAF	c.1929A>G		No	228	98	130	200	140449150	7
ERBB3	c.89C>T	mis-sense	No	126	126	0	200	56477541	12
ERBB3	c.3348G>A		No	469	220	249	200	56494991	12

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

Comment: Remark added by user during variant review.

3.3 Untested variants

Variants of interest that could not be tested due to insufficient coverage. These variants are assigned the initial review state "Untested".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
ALK	c.3469T>A	p.Ser1157Thr	SNV	0.00%			160	Yes	Yes	Untested	
ALK	c.3457C>N	p.Pro1153Xaa	SNV	0.00%			185	Yes	Yes	Untested	
ALK	c.3452C>T	p.Thr1151Met	SNV	0.00%			186	Yes	Yes	Untested	
PIK3CA	c.-77+8483C>T		SNV	54.70%	33.61	1.00	181	Yes	Yes	Untested	

PIK3CA	c.112C>T	p.Arg38Cys	SNV	0.00%			112	Yes	Yes	Untested	
PIK3CA	c.113G>A	p.Arg38His	SNV	0.00%			112	Yes	Yes	Untested	
PIK3CA	c.115G>A	p.Glu39Lys	SNV	0.00%			130	Yes	Yes	Untested	
PIK3CA	c.241G>A	p.Glu81Lys	SNV	0.00%			129	Yes	Yes	Untested	
PIK3CA	c.263G>A	p.Arg88Gln	SNV	0.00%			194	Yes	Yes	Untested	
PIK3CA	c.277C>T	p.Arg93Trp	SNV	0.00%			190	Yes	Yes	Untested	
PIK3CA	c.278G>A	p.Arg93Gln	SNV	0.53%	34.00		190	Yes	Yes	Untested	
PIK3CA	c.353G>A	p.Gly118Asp	SNV	0.00%			129	Yes	Yes	Untested	
PIK3CA	c.519T>C		SNV	0.00%			125	Yes	Yes	Untested	
PIK3CA	c.519T>G		SNV	0.00%			125	Yes	Yes	Untested	
PIK3CA	c.1030G>A	p.Val344Met	SNV	0.00%			100	Yes	Yes	Untested	
PIK3CA	c.1031T>G	p.Val344Gly	SNV	0.00%			100	Yes	Yes	Untested	
PIK3CA	c.1035T>A	p.Asn345Lys	SNV	1.00%	6.00		100	Yes	Yes	Untested	
PIK3CA	c.1035T>G	p.Asn345Lys	SNV	0.00%			100	Yes	Yes	Untested	
PIK3CA	c.1048G>A	p.Asp350Asn	SNV	0.00%			120	Yes	Yes	Untested	
PIK3CA	c.1049A>G	p.Asp350Gly	SNV	0.00%			120	Yes	Yes	Untested	
PIK3CA	c.1132T>C	p.Cys378Arg	SNV	0.00%			59	Yes	Yes	Untested	
PIK3CA	c.1134T>C		SNV	0.00%			57	Yes	Yes	Untested	
PIK3CA	c.1357G>A	p.Glu453Lys	SNV	0.00%			78	Yes	Yes	Untested	
PIK3CA	c.1357G>C	p.Glu453Gln	SNV	0.00%			78	Yes	Yes	Untested	
PIK3CA	c.1370A>G	p.Asn457Ser	SNV	0.00%			78	Yes	Yes	Untested	
PIK3CA	c.1412C>T	p.Pro471Leu	SNV	0.00%			76	Yes	Yes	Untested	
PIK3CA	c.2908G>A	p.Glu970Lys	SNV	0.00%			147	Yes	Yes	Untested	
PIK3CA	c.2945A>G	p.Glu982Gly	SNV	0.00%			164	Yes	Yes	Untested	
PIK3CA	c.3001C>A	p.Leu1001Ile	SNV	0.00%			199	Yes	Yes	Untested	
PIK3CA	c.3012G>A	p.Met1004Ile	SNV	0.00%			126	Yes	Yes	Untested	
PIK3CA	c.3012G>C	p.Met1004Ile	SNV	0.00%			126	Yes	Yes	Untested	
PIK3CA	c.3012G>T	p.Met1004Ile	SNV	0.00%			126	Yes	Yes	Untested	
PIK3CA	c.3019G>C	p.Gly1007Arg	SNV	0.00%			115	Yes	Yes	Untested	
PIK3CA	c.3022T>C	p.Ser1008Pro	SNV	0.00%			115	Yes	Yes	Untested	
PIK3CA	c.3026G>A	p.Gly1009Glu	SNV	0.00%			145	Yes	Yes	Untested	
PIK3CA	c.3033A>G		SNV	0.69%	31.00		145	Yes	Yes	Untested	

PIK3CA	c.3036A>G		SNV	0.00%			86	Yes	Yes	Untested	
PIK3CA	c.3044C>T	p.Ser1015Phe	SNV	1.16%	37.00		86	Yes	Yes	Untested	
PIK3CA	c.3049G>C	p.Asp1017His	SNV	0.00%			180	Yes	Yes	Untested	
PIK3CA	c.3050A>G	p.Asp1017Gly	SNV	1.67%	29.67		180	Yes	Yes	Untested	
PIK3CA	c.3194A>T	p.His1065Leu	SNV	0.00%			197	Yes	Yes	Untested	
PIK3CA	c.3197C>T	p.Ala1066Val	SNV	0.51%	29.00		198	Yes	Yes	Untested	
PIK3CA	c.3204_3205insA	p.*1069fs	Insertion	0.00%			197	Yes	Yes	Untested	
PIK3CA	c.3204_3205insTTCT	p.*1069fs	Insertion	0.00%			197	Yes	Yes	Untested	
PIK3CA	c.3207A>G	p.*1069Trp	SNV	0.51%	32.00		198	Yes	Yes	Untested	
PDGFRA	c.612T>C		SNV	0.00%			117	Yes	Yes	Untested	
PDGFRA	c.939T>G		SNV	0.00%			173	Yes	Yes	Untested	
KIT	c.1621A>C	p.Met541Leu	SNV	0.00%			48	Yes	Yes	Untested	
KIT	c.1621A>T	p.Met541Leu	SNV	0.00%			48	Yes	Yes	Untested	
KIT	c.1638A>G		SNV	0.00%			30	Yes	Yes	Untested	
KIT	c.1648_1662delAAACCCATGTATGAA	p.Lys550_Glu554del	Deletion	0.00%			93	Yes	Yes	Untested	
KIT	c.1648_1665delAAACCCATGTATGAAGTA	p.Lys550_Val555del	Deletion	0.00%			93	Yes	Yes	Untested	
KIT	c.1649_1660delAACCCATGTATG	p.Pro551_Glu554del	Deletion	0.00%			93	Yes	Yes	Untested	
KIT	c.1649_1666delAACCCATGTATGAAGTAC	p.Pro551_Gln556del	Deletion	0.00%			93	Yes	Yes	Untested	
KIT	c.1718C>T	p.Pro573Leu	SNV	0.00%			167	Yes	Yes	Untested	
KIT	c.1726C>T	p.Leu576Phe	SNV	0.00%			172	Yes	Yes	Untested	
KIT	c.1726_1728delCTT	p.Leu576del	Deletion	0.00%			171	Yes	Yes	Untested	
KIT	c.1727T>C	p.Leu576Pro	SNV	0.58%	29.00		172	Yes	Yes	Untested	
KIT	c.1728_1736delTCCTTATGA	p.Pro577_Asp579del	Deletion	0.00%			171	Yes	Yes	Untested	
KIT	c.1729C>T	p.Pro577Ser	SNV	0.00%			172	Yes	Yes	Untested	
KIT	c.1731T>C		SNV	0.57%	30.00		175	Yes	Yes	Untested	
KIT	c.1733_1735delATG	p.Asp579del	Deletion	0.00%			172	Yes	Yes	Untested	
KIT	c.2362-77G>A		SNV	0.00%			18	Yes	Yes	Untested	
KIT	c.2387G>A	p.Arg796Lys	SNV	0.59%	37.00		170	Yes	Yes	Untested	
KIT	c.2474T>C	p.Val825Ala	SNV	0.00%			130	Yes	Yes	Untested	

KIT	c.2484+43T>A		SNV	0.00%			93	Yes	Yes	Untested	
ESR1	c.30T>C		SNV	7.92%	30.38	0.15	101	Yes	Yes	Untested	
ESR1	c.30T>A		SNV	0.00%			101	Yes	Yes	Untested	
ESR1	c.30T>G		SNV	0.00%			101	Yes	Yes	Untested	
ESR1	c.1369+13777T>G		SNV	0.00%			124	Yes	Yes	Untested	
ESR1	c.1609T>A	p.Tyr537Asn	SNV	0.00%			49	Yes	Yes	Untested	
ESR1	c.1610A>C	p.Tyr537Ser	SNV	0.00%			49	Yes	Yes	Untested	
ESR1	c.1613A>G	p.Asp538Gly	SNV	0.00%			156	Yes	Yes	Untested	
BRAF	c.1853T>G	p.Leu618Trp	SNV	0.00%			148	Yes	Yes	Untested	
BRAF	c.1853T>C	p.Leu618Ser	SNV	0.00%			148	Yes	Yes	Untested	
BRAF	c.1847C>T	p.Ser616Phe	SNV	0.00%			149	Yes	Yes	Untested	
BRAF	c.1846T>C	p.Ser616Pro	SNV	0.00%			149	Yes	Yes	Untested	
BRAF	c.1843G>C	p.Gly615Arg	SNV	0.00%			148	Yes	Yes	Untested	
BRAF	c.1843G>A	p.Gly615Arg	SNV	0.00%			148	Yes	Yes	Untested	
BRAF	c.1840T>C	p.Ser614Pro	SNV	0.67%	10.00		149	Yes	Yes	Untested	
BRAF	c.1750C>T	p.Leu584Phe	SNV	1.56%	25.00		192	Yes	Yes	Untested	
BRAF	c.1749T>C		SNV	0.52%	32.00		192	Yes	Yes	Untested	
BRAF	c.1746A>G	p.Ile582Met	SNV	0.00%			191	Yes	Yes	Untested	
BRAF	c.1742A>T	p.Asn581Ile	SNV	0.00%			191	Yes	Yes	Untested	
BRAF	c.1742A>G	p.Asn581Ser	SNV	0.00%			191	Yes	Yes	Untested	
KRAS	c.491G>A	p.Arg164Gln	SNV	0.00%			132	Yes	Yes	Untested	
KRAS	c.439A>G	p.Lys147Glu	SNV	0.00%			86	Yes	Yes	Untested	
KRAS	c.437C>T	p.Ala146Val	SNV	0.00%			85	Yes	Yes	Untested	
KRAS	c.436G>C	p.Ala146Pro	SNV	0.00%			85	Yes	Yes	Untested	
KRAS	c.436G>A	p.Ala146Thr	SNV	0.00%			85	Yes	Yes	Untested	
KRAS	c.352T>A	p.Cys118Ser	SNV	0.00%			187	Yes	Yes	Untested	
KRAS	c.351A>T	p.Lys117Asn	SNV	0.00%			187	Yes	Yes	Untested	
KRAS	c.351A>C	p.Lys117Asn	SNV	0.00%			187	Yes	Yes	Untested	
KRAS	c.104C>T	p.Thr35Ile	SNV	0.00%			152	Yes	Yes	Untested	
KRAS	c.27T>G		SNV	0.00%			143	Yes	Yes	Untested	
KRAS	c.27T>C		SNV	0.00%			143	Yes	Yes	Untested	
KRAS	c.27T>A		SNV	0.00%			143	Yes	Yes	Untested	

KRAS	c.24A>T		SNV	0.00%			142	Yes	Yes	Untested	
KRAS	c.24A>G		SNV	0.00%			142	Yes	Yes	Untested	
KRAS	c.24A>C		SNV	0.00%			142	Yes	Yes	Untested	
KRAS	c.20T>C	p.Val7Ala	SNV	0.00%			158	Yes	Yes	Untested	
KRAS	c.15A>T	p.Lys5Asn	SNV	0.00%			81	Yes	Yes	Untested	
KRAS	c.15A>C	p.Lys5Asn	SNV	1.23%	12.00		81	Yes	Yes	Untested	
KRAS	c.13A>G	p.Lys5Glu	SNV	0.00%			100	Yes	Yes	Untested	
ERBB2	c.2198C>T	p.Thr733Ile	SNV	0.00%			126	Yes	Yes	Untested	
ERBB2	c.2524G>A	p.Val842Ile	SNV	0.00%			158	Yes	Yes	Untested	
ERBB2	c.3508C>G	p.Pro1170Ala	SNV	97.25%	25.19	1.00	182	Yes	Yes	Untested	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
ALK	c.3469T>A	mis-sense		0	0	0		29445256	2
ALK	c.3457C>N	mis-sense		0	0	0		29445268	2
ALK	c.3452C>T	mis-sense		0	0	0		29445273	2
PIK3CA	c.-77+8483C>T		No	99	14	85	200	178874874	3
PIK3CA	c.112C>T	mis-sense		0	0	0		178916725	3
PIK3CA	c.113G>A	mis-sense		0	0	0		178916726	3
PIK3CA	c.115G>A	mis-sense		0	0	0		178916728	3
PIK3CA	c.241G>A	mis-sense		0	0	0		178916854	3
PIK3CA	c.263G>A	mis-sense		0	0	0		178916876	3
PIK3CA	c.277C>T	mis-sense		0	0	0		178916890	3
PIK3CA	c.278G>A	mis-sense		1	0	1		178916891	3
PIK3CA	c.353G>A	mis-sense		0	0	0		178917478	3
PIK3CA	c.519T>C			0	0	0		178917644	3
PIK3CA	c.519T>G			0	0	0		178917644	3
PIK3CA	c.1030G>A	mis-sense		0	0	0		178921548	3
PIK3CA	c.1031T>G	mis-sense		0	0	0		178921549	3
PIK3CA	c.1035T>A	mis-sense		1	0	1		178921553	3

PIK3CA	c.1035T>G	mis-sense		0	0	0		178921553	3
PIK3CA	c.1048G>A	mis-sense		0	0	0		178921566	3
PIK3CA	c.1049A>G	mis-sense		0	0	0		178921567	3
PIK3CA	c.1132T>C	mis-sense		0	0	0		178922363	3
PIK3CA	c.1134T>C			0	0	0		178922365	3
PIK3CA	c.1357G>A	mis-sense		0	0	0		178928079	3
PIK3CA	c.1357G>C	mis-sense		0	0	0		178928079	3
PIK3CA	c.1370A>G	mis-sense		0	0	0		178928092	3
PIK3CA	c.1412C>T	mis-sense		0	0	0		178928226	3
PIK3CA	c.2908G>A	mis-sense		0	0	0		178948136	3
PIK3CA	c.2945A>G	mis-sense		0	0	0		178951890	3
PIK3CA	c.3001C>A	mis-sense		0	0	0		178951946	3
PIK3CA	c.3012G>A	mis-sense		0	0	0		178951957	3
PIK3CA	c.3012G>C	mis-sense		0	0	0		178951957	3
PIK3CA	c.3012G>T	mis-sense		0	0	0		178951957	3
PIK3CA	c.3019G>C	mis-sense		0	0	0		178951964	3
PIK3CA	c.3022T>C	mis-sense		0	0	0		178951967	3
PIK3CA	c.3026G>A	mis-sense		0	0	0		178951971	3
PIK3CA	c.3033A>G			1	1	0		178951978	3
PIK3CA	c.3036A>G			0	0	0		178951981	3
PIK3CA	c.3044C>T	mis-sense		1	1	0		178951989	3
PIK3CA	c.3049G>C	mis-sense		0	0	0		178951994	3
PIK3CA	c.3050A>G	mis-sense		3	3	0		178951995	3
PIK3CA	c.3194A>T	mis-sense		0	0	0		178952139	3
PIK3CA	c.3197C>T	mis-sense		1	1	0		178952142	3
PIK3CA	c.3204_3205insA	frame-shift		0	0	0		178952149^178952150	3
PIK3CA	c.3204_3205insTTCT	frame-shift		0	0	0		178952149^178952150	3
PIK3CA	c.3207A>G			1	0	1		178952152	3
PDGFRA	c.612T>C			0	0	0		55130078	4
PDGFRA	c.939T>G			0	0	0		55133726	4
KIT	c.1621A>C	mis-sense		0	0	0		55593464	4
KIT	c.1621A>T	mis-sense		0	0	0		55593464	4

KIT	c.1638A>G			0	0	0		55593481	4
KIT	c.1648_1662delAAACCCATGTATGAA			0	0	0		55593582..55593596	4
KIT	c.1648_1665delAAACCCATGTATGAAGTA			0	0	0		55593582..55593599	4
KIT	c.1649_1660delAACCCATGTATG			0	0	0		55593583..55593594	4
KIT	c.1649_1666delAACCCATGTATGAAGTAC			0	0	0		55593583..55593600	4
KIT	c.1718C>T	mis-sense		0	0	0		55593652	4
KIT	c.1726C>T	mis-sense		0	0	0		55593660	4
KIT	c.1726_1728delCTT			0	0	0		55593660..55593662	4
KIT	c.1727T>C	mis-sense		1	1	0		55593661	4
KIT	c.1728_1736delTCCTTATGA			0	0	0		55593662..55593670	4
KIT	c.1729C>T	mis-sense		0	0	0		55593663	4
KIT	c.1731T>C			1	0	1		55593665	4
KIT	c.1733_1735delATG			0	0	0		55593667..55593669	4
KIT	c.2362-77G>A			0	0	0		55599159	4
KIT	c.2387G>A	mis-sense		1	1	0		55599261	4
KIT	c.2474T>C	mis-sense		0	0	0		55599348	4
KIT	c.2484+43T>A			0	0	0		55599401	4
ESR1	c.30T>C		No	8	1	7	11	152129077	6
ESR1	c.30T>A			0	0	0		152129077	6
ESR1	c.30T>G			0	0	0		152129077	6
ESR1	c.1369+13777T>G			0	0	0		152396036	6
ESR1	c.1609T>A	mis-sense		0	0	0		152419922	6
ESR1	c.1610A>C	mis-sense		0	0	0		152419923	6
ESR1	c.1613A>G	mis-sense		0	0	0		152419926	6
BRAF	c.1853T>G	mis-sense		0	0	0		140453082	7
BRAF	c.1853T>C	mis-sense		0	0	0		140453082	7
BRAF	c.1847C>T	mis-sense		0	0	0		140453088	7
BRAF	c.1846T>C	mis-sense		0	0	0		140453089	7
BRAF	c.1843G>C	mis-sense		0	0	0		140453092	7
BRAF	c.1843G>A	mis-sense		0	0	0		140453092	7

BRAF	c.1840T>C	mis-sense		1	0	1		140453095	7
BRAF	c.1750C>T	mis-sense		3	2	1		140453185	7
BRAF	c.1749T>C			1	0	1		140453186	7
BRAF	c.1746A>G	mis-sense		0	0	0		140453189	7
BRAF	c.1742A>T	mis-sense		0	0	0		140453193	7
BRAF	c.1742A>G	mis-sense		0	0	0		140453193	7
KRAS	c.491G>A	mis-sense		0	0	0		25362805	12
KRAS	c.439A>G	mis-sense		0	0	0		25378559	12
KRAS	c.437C>T	mis-sense		0	0	0		25378561	12
KRAS	c.436G>C	mis-sense		0	0	0		25378562	12
KRAS	c.436G>A	mis-sense		0	0	0		25378562	12
KRAS	c.352T>A	mis-sense		0	0	0		25378646	12
KRAS	c.351A>T	mis-sense		0	0	0		25378647	12
KRAS	c.351A>C	mis-sense		0	0	0		25378647	12
KRAS	c.104C>T	mis-sense		0	0	0		25398215	12
KRAS	c.27T>G			0	0	0		25398292	12
KRAS	c.27T>C			0	0	0		25398292	12
KRAS	c.27T>A			0	0	0		25398292	12
KRAS	c.24A>T			0	0	0		25398295	12
KRAS	c.24A>G			0	0	0		25398295	12
KRAS	c.24A>C			0	0	0		25398295	12
KRAS	c.20T>C	mis-sense		0	0	0		25398299	12
KRAS	c.15A>T	mis-sense		0	0	0		25398304	12
KRAS	c.15A>C	mis-sense		1	0	1		25398304	12
KRAS	c.13A>G	mis-sense		0	0	0		25398306	12
ERBB2	c.2198C>T	mis-sense		0	0	0		37879903	17
ERBB2	c.2524G>A	mis-sense		0	0	0		37881332	17
ERBB2	c.3508C>G	mis-sense	No	177	28	149	200	37884037	17

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

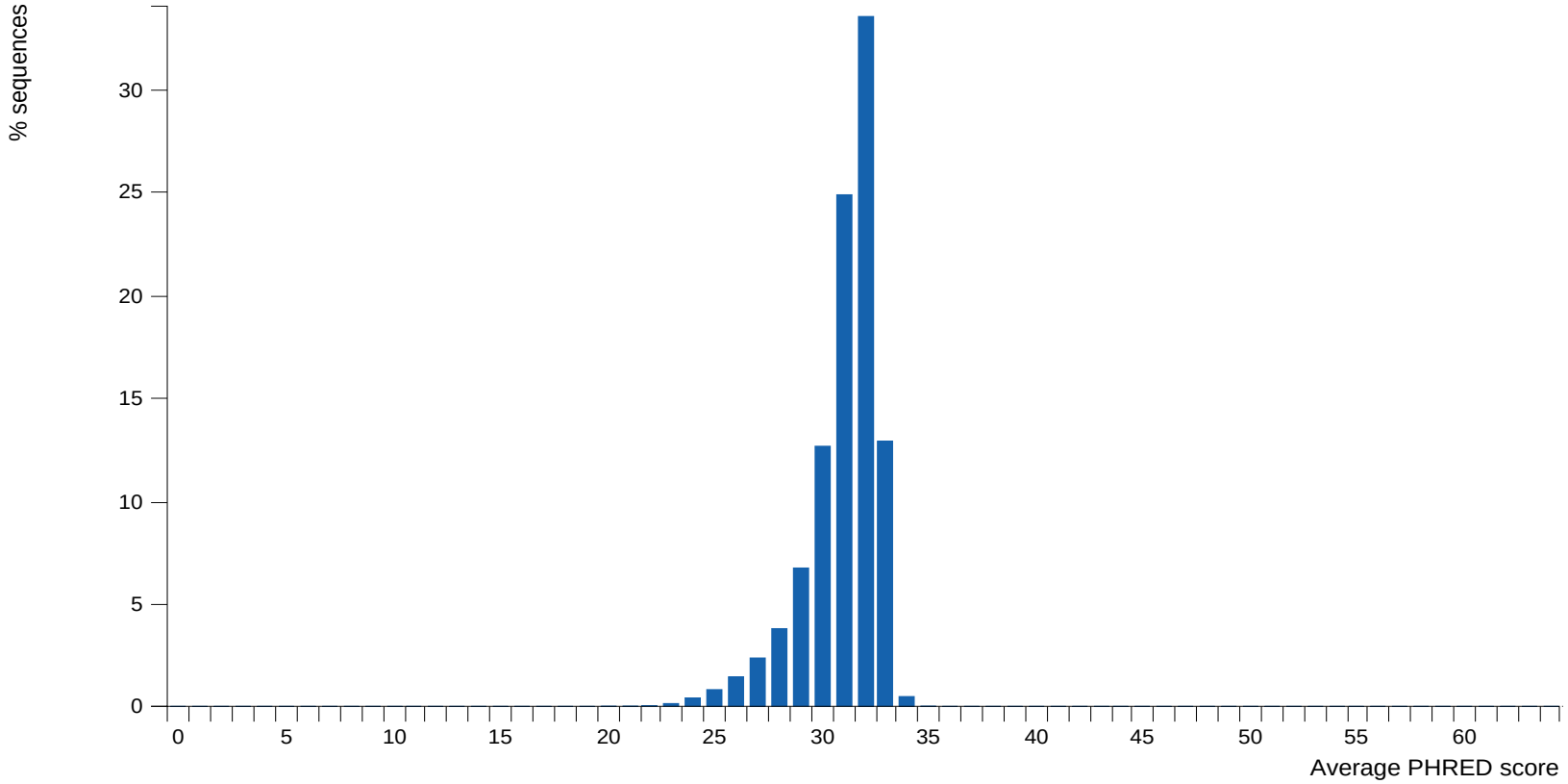
Comment: Remark added by user during variant review.

4 Detailed QC

Quality control metrics for detailed inspection. These metrics can indicate possible problems in the upstream workflow or data analysis. Quality control are divided in metrics on the incoming reads from input data, and metrics per base positions in these reads. Lastly section 4.3 and 4.4 display metrics on how well the positions in the region of interest are covered

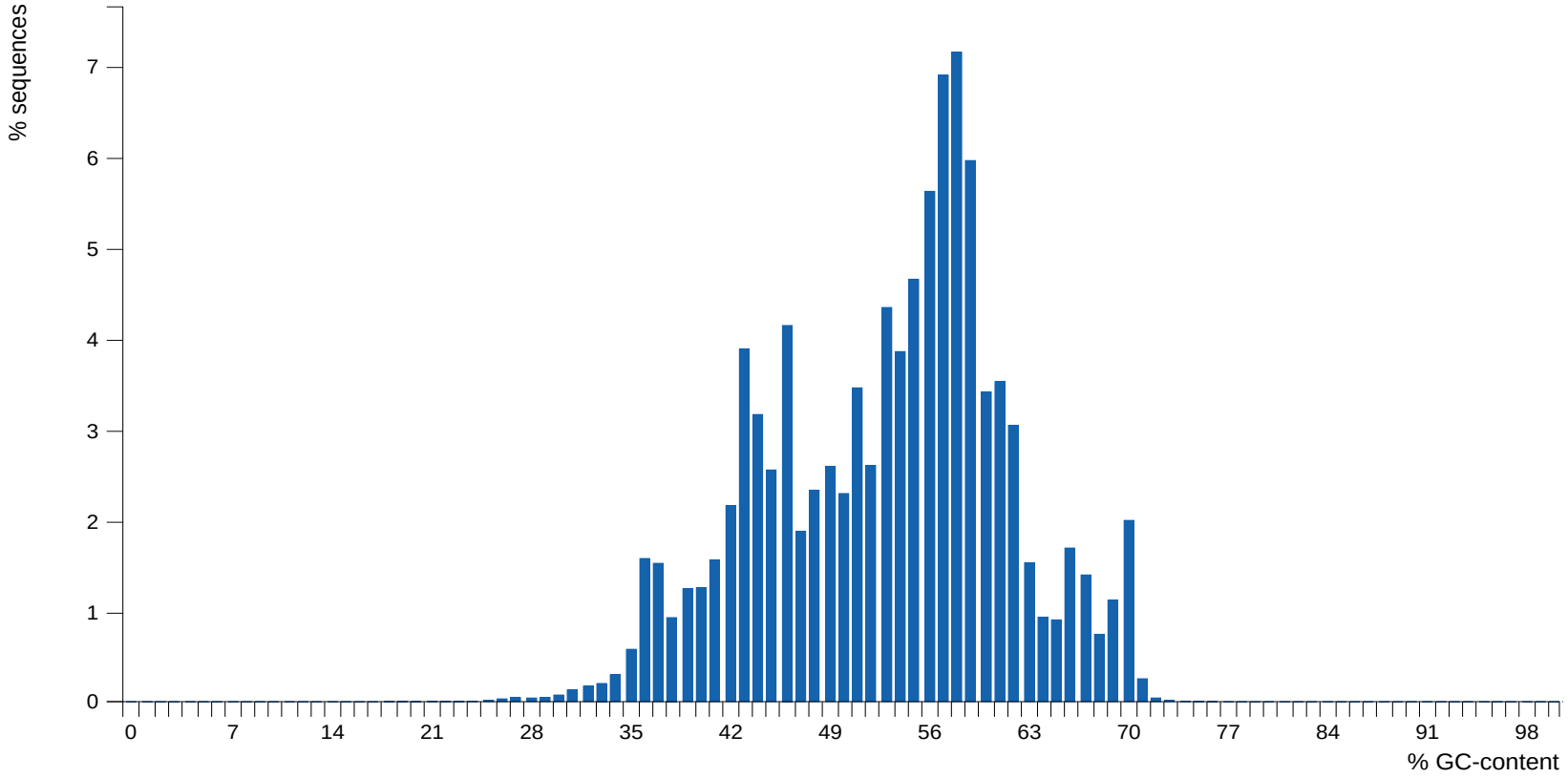
4.1 QC for reads

4.1.1 Average base quality of reads



Distribution of average sequence quality scores. The quality of a sequence is calculated as the arithmetic mean of its base qualities.
x: PHRED-score
y: number of sequences observed at that qual. score normalized to the total number of sequences

4.1.2 GC content of reads



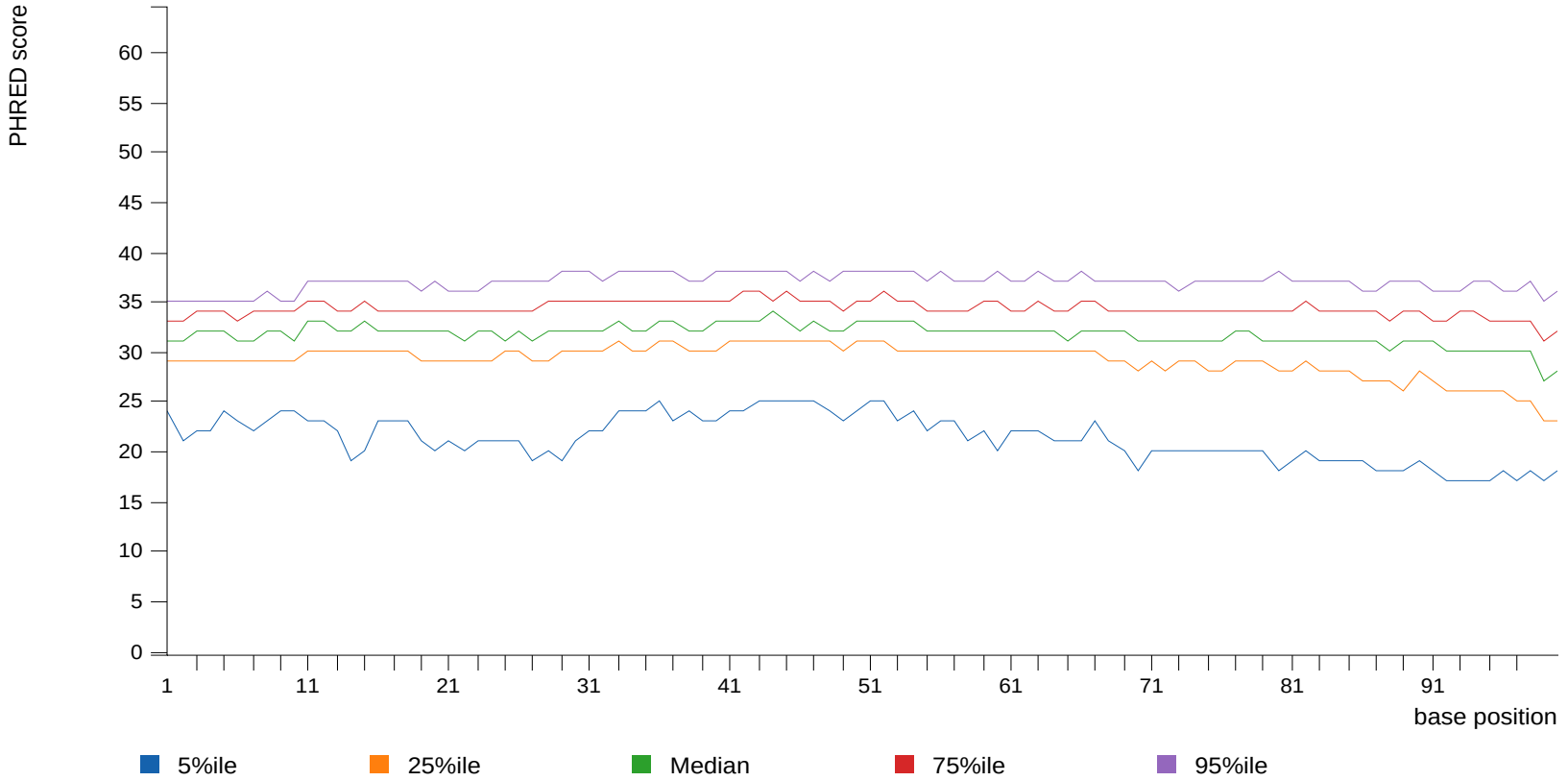
Distribution of GC-contents. The GC-content of a sequence is calculated as the number of GC-bases compared to all bases (including ambiguous bases).
x: relative GC-content of a sequence in percent
y: number of sequences featuring particular GC-percentages normalized to the total number of sequences

4.1.3 Ambiguous base content of reads

No ambiguous bases detected.

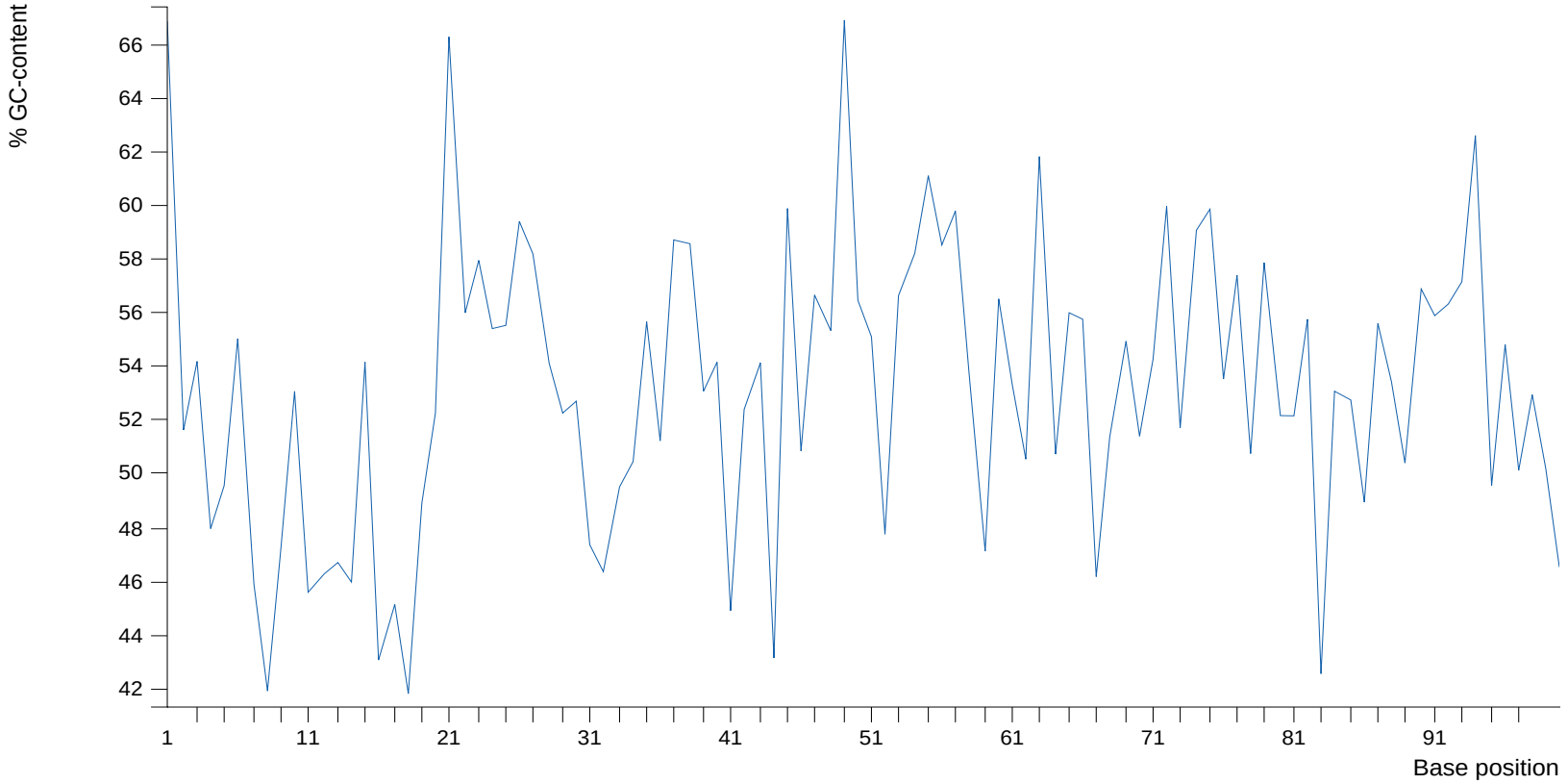
4.2 QC for bases

4.2.1 Quality score per base position



Base-quality distribution along the base positions.
x: base position
y: median & percentiles of quality scores observed at that base position

4.2.2 GC content per base position



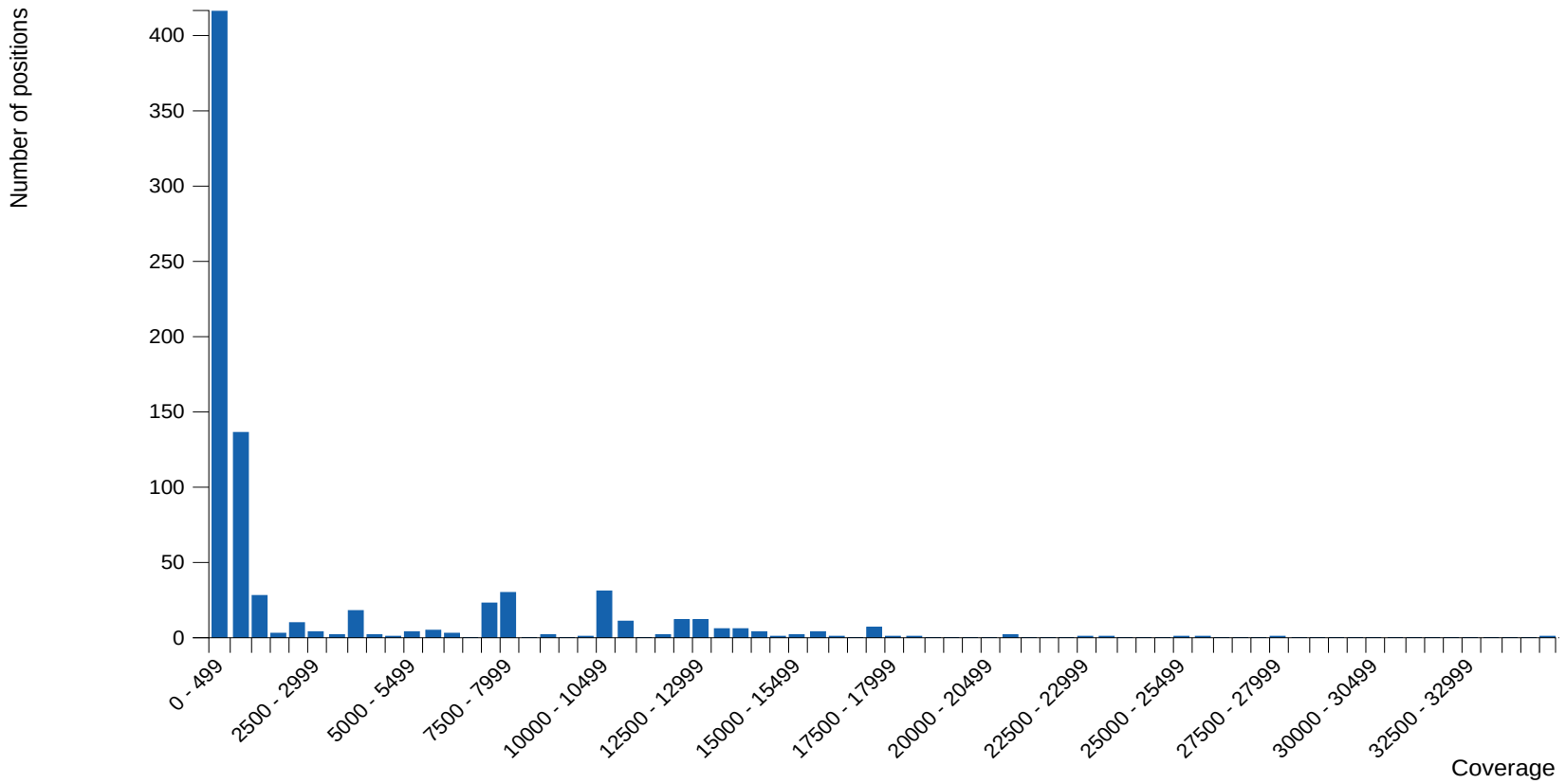
Combined coverage of G- and C-bases.
x: base position
y: number of G- and C-bases observed at current position normalized to the total number of bases observed at that position

4.2.3 Ambiguous base content per base position

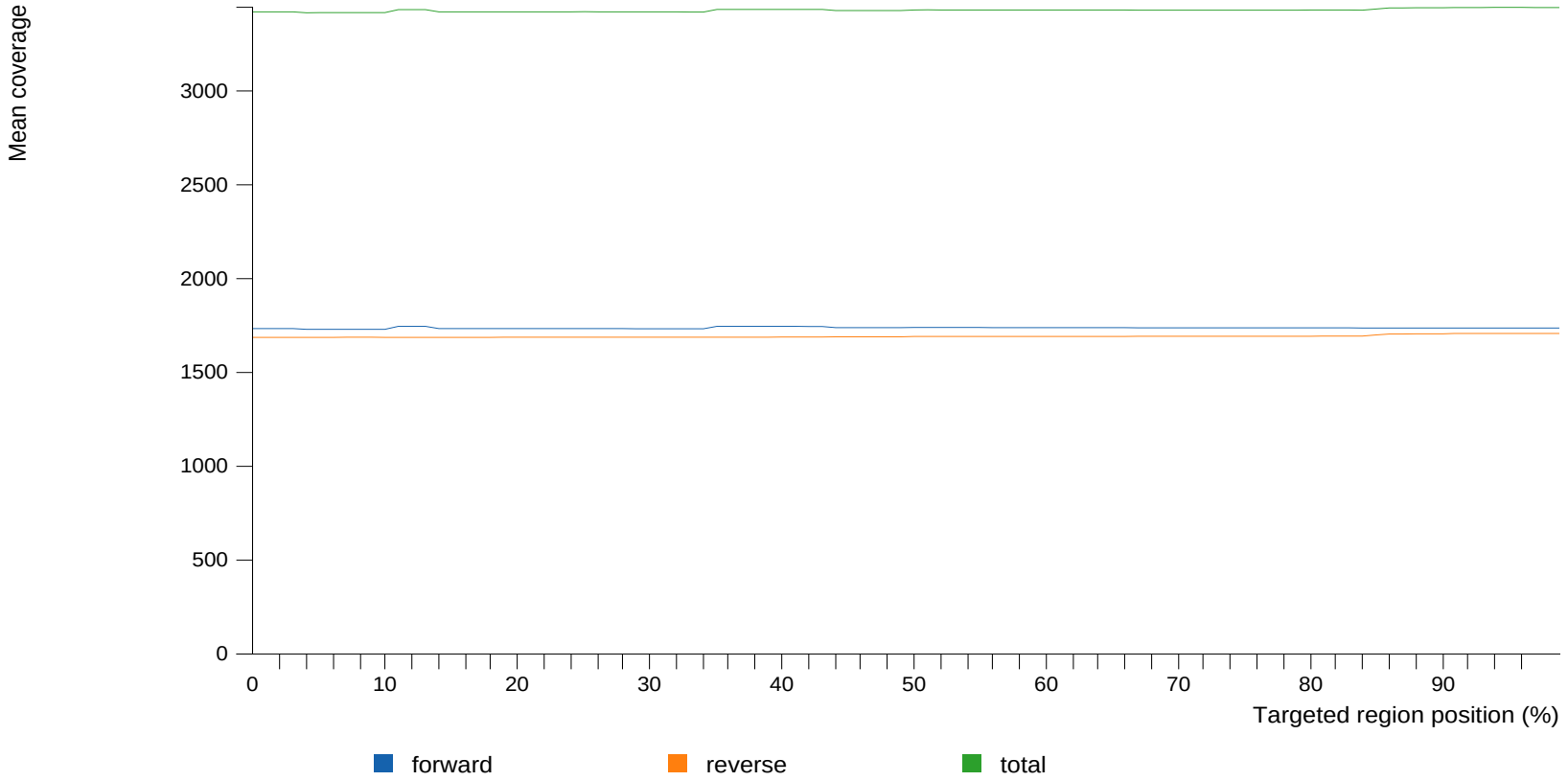
No ambiguous bases detected

4.3 Coverage of Regions of Interest positions

Coverage distribution



4.4 Mean coverage of relative positions in regions of interest



5 History

5.1 Log Entries

Type	Time	User	Details
State change	Sat Jun 29 18:08:32 CEST 2019	system	Ready for Review
State change	Sat Jun 29 17:54:17 CEST 2019	root	In Progress

5.2 Execution Information

QCIA version	QCI Analyze 1.4.5
Analysis start time	Sat Jun 29 17:54:17 CEST 2019
Analysis workflow	AIT FFPE 4.5
Analysis description	QIAact Actionable Insights Tumor Panel on FFPE

5.3 Transcripts

Table listing the genes, transcript IDs and protein IDs used in the analysis.

Gene Name	Transcript ID	Protein ID
NRAS	NM_002524.4	NP_002515.1
ALK	NM_004304.4	NP_004295.2
RAF1	NM_002880.3	NP_002871.1
PIK3CA	NM_006218.2	NP_006209.2
PDGFRA	NM_006206.4	NP_006197.1
KIT	NM_000222.2	NP_000213.1
ESR1	NM_001122742.1	NP_001116214.1
EGFR	NM_005228.3	NP_005219.2
BRAF	NM_004333.4	NP_004324.2
KRAS	NM_004985.3	NP_004976.2
ERBB3	NM_001982.3	NP_001973.2

Gene Name	Transcript ID	Protein ID
ERBB2	NM_004448.2	NP_004439.2

5.4 Open Parameters

Workflow parameters that are editable by the administrator

Reported variants

Significant coverage threshold	500
SNV/MNV frequency threshold in %	4.00
Insertions, deletions and replacements frequency threshold in %	4.00

Variants available for review

Minimum coverage threshold	200
SNV/MNV frequency threshold in %	4.00
Insertions, deletions and replacements frequency threshold in %	4.00
Detect variants outside regions of interest	Yes

5.5 Locked Parameters

Adapter trimming

Trim adapter list	GRadapter_160913
Ambiguous trim	false
Ambiguous limit	2
Quality trim	false
Quality limit	0.05
Use colorspace	false

Also search on reversed sequence	false
Remove 5' terminal nucleotides	false
Number of 5' terminal nucleotides	1
Maximum number of nucleotides in reads	1000
Minimum number of nucleotides in reads	15
Discard short reads	false
Remove 3' terminal nucleotides	false
Number of 3' terminal nucleotides	1
Discard long reads	false

Map Reads to Reference

References	Homo_sapiens_sequence_hg19
Masking mode	No masking
Masking track	Not set
Match score	1
Mismatch cost	2
Cost of insertions and deletions	Affine gap cost
Insertion cost	3
Deletion cost	3
Insertion open cost	6
Insertion extend cost	1
Deletion open cost	6
Deletion extend cost	1
Length fraction	0.5
Similarity fraction	0.8
Global alignment	false
Color space alignment	false
Color error cost	3
Auto-detect paired distances	false
Non-specific match handling	Map randomly

InDels and Structural Variants

P-Value threshold	1.0E-4
Maximum number of mismatches	3
Ignore broken pairs	true
Minimum relative consensus coverage	0.0
Minimum quality score	0
Filter variants	true
Minimum number of reads	2
Restrict calling to target regions	ATPv2_TargetRegions_170302_ver1.1

Local Realignment (Short Unaligned End version)

Realign unaligned ends	true
Multi-pass realignment	2
Local bound for unaligned ends of size one	0.75
Local bound for unaligned ends of size two	0.75
Force realignment to guidance-variants	false
Maximum guidance-variant length	100

Trim Primers and their Dimers of Mapped Reads

Primer track	101x_GR_primers_15_10_15_V1.0
Reference	Homo_sapiens_sequence_hg19
Minimum primer overlap length	9
Allow dangling 3' end base	true
Minimal primer overlap fraction	0.7
Only keep reads that have hit a primer	true
Additional bases to trim	1

Remove Pseudogene Reads

Genes track	AITv2_PseudoGenes_170912_ver1.0
Gene and pseudogene links	KRAS -> KRASP1
Required unaligned ends %	2.0

Low Frequency Variant Detection

Required significance (%)	0.01
Ignore positions with coverage above	1000000000
Restrict calling to target regions	ATPv2_TargetRegions_170302_ver1.1
Ignore broken pairs	true
Ignore non-specific matches	Reads
Minimum read length	20
Minimum coverage	Parameter editable by administrator
Minimum count	8
Minimum frequency (%)	Parameter editable by administrator
Base quality filter	false
Neighborhood radius	5
Minimum central quality	5
Minimum neighborhood quality	5
Read direction filter	false
Direction frequency (%)	5.0
Relative read direction filter	false
Significance (%)	1.0E-5
Read position filter	false
Significance (%)	1.0
Remove pyro-error variants	false
In homopolymer regions with minimum length	3
With frequency below	0.8

Remove False Positives

Minimum frequency (%)	Parameter editable by administrator
Minimum forward/reverse balance	0.05
Minimum average base quality	22.0
Variant frequency	true
Forward/reverse balance	false
Average base quality	true

Annotate Variants With Primers

Minimum coverage count	400
Minimum variant percentage	1.0
Minimum variant read count	2

YOUR LAB

Your Lab
1700 Lincoln Blvd, Suite 20, Redwood City, CA 94063
labx.com / (650) 484 4040
Additional Information

Test Performed: **Somatic Panel**

Report Date Mar 7, 2020
Status -

Patient		Client		Specimen	
Patient Name		Client		Accession ID	20190628103639_100160050701061 90147_115-18PT_BC4_Glio2019
Date of Birth		Client ID			
Age	50	Physician			
Sex	Male	Pathologist		Specimen Collection	
Ethnicity	European			Accession	Mar 7, 2020
Diagnosis	Glioblastoma			Primary Tumor Site	Brain

Result: Negative

Genes Tested

Test information such as gene name and hot spot region can be included in this section.

Methods and Limitations

EXAMPLE Statement including sample type (FFPE, etc), method of extraction, amplification reactions, panel targeted regions, sequencing technology, etc. Additionally, a description of the data analysis software(s), genome of reference and the sensitivity of the methods should be described.

QIAGEN Clinical Insight (QCI™) is a variant analysis, interpretation and decision support tool for research and clinical labs analyzing human genetics data and is not intended to be used for diagnostic purposes. QCI Interpret software includes the following underlying databases, data reference sets and tools; QIAGEN Clinical Insight-Interpret (5.6.20200226), Ingenuity Knowledge Base (K-release), CADD (v1.4), Allele Frequency Community (2019-09-25), EVS (ESP6500SI-V2), Refseq Gene Model (2019-02-05), JASPAR (2013-11), Ingenuity Knowledge Base Snapshot Timestamp (2020-02-21 12:37:01.0), Vista Enhancer hg18 (2012-07), Vista Enhancer hg19 (2012-07), Clinical Trials (K-release), PolyPhen-2 (v2.2.2), 1000 Genome Frequency (phase3v5b), ExAC (0.3.1), iva (Nov 19 12:28 iva-1.0.1200.jar), PhyloP hg18 (2009-11), PhyloP hg19 (2009-11), DbSNP (151), TargetScan (7.2), GENCODE (Release 29), CentoMD (5.3), OMIM (May 26, 2017), gnomAD (2.1.1), BSIFT (2016-02-23), TCGA (2013-09-05), Clinvar (2019-06-05), DGV (2016-05-15), COSMIC (v89), HGMD (2019.2), SIFT4G (2016-02-23)

Clinical Significance of Variants Based on AMP / ASCO / CAP Guidelines*

Strong Significance	**Tier 1A**	• Biomarker predicts response or resistance to an FDA or EMA approved therapy, according to drug label or professional guidelines for this diagnosis • Biomarker included in professional guidelines is prognostic or diagnostic for this diagnosis
	Tier 1B	• Biomarker predicts response or resistance to a therapy for this diagnosis based on well-powered studies • Biomarker is prognostic or diagnostic for this diagnosis based on well-powered studies
Potential Significance	**Tier 2C**	• Biomarker is associated with response or resistance to an FDA or EMA approved therapy, according to drug label or professional guidelines but only for different diagnosis • Biomarker is an inclusion criterion for an active clinical trial • Biomarker is prognostic or diagnostic based on multiple small studies
	Tier 2D	• Biomarker shows plausible response or resistance based on case or preclinical studies • Biomarker may assist in disease diagnosis or prognosis based on small studies
Uncertain Significance	**Tier 3**	• Biomarker has uncertain clinical significance and not known to be likely benign or benign

**Adapted from PMID:27993330 jmd.amjpathol.org/article/S1525-1578(16)30223-9/pdf

YOUR LAB

Your Lab
1700 Lincoln Blvd, Suite 20, Redwood City, CA 94063
labx.com / (650) 484 4040
Additional Information

Test Performed: **Somatic Panel**

Report Date Mar 7, 2020
Status -

Patient		Client		Specimen	
Patient Name		Client		Accession ID	20190628103639_100160050701061 90147_374-19CTC_BC6_Glio2019
Date of Birth		Client ID			
Age	50	Physician			
Sex		Pathologist			
Ethnicity	European			Specimen Collection	
Diagnosis	Glioblastoma			Accession	Mar 7, 2020
				Primary Tumor Site	Brain

Result: Negative

Genes Tested

Test information such as gene name and hot spot region can be included in this section.

Methods and Limitations

EXAMPLE Statement including sample type (FFPE, etc), method of extraction, amplification reactions, panel targeted regions, sequencing technology, etc. Additionally, a description of the data analysis software(s), genome of reference and the sensitivity of the methods should be described.

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Clinical Significance of Variants Based on AMP / ASCO / CAP Guidelines*

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Potential Significance	**Tier 2C**	• Biomarker is associated with response or resistance to an FDA or EMA approved therapy, according to drug label or professional guidelines but only for different diagnosis • Biomarker is an inclusion criterion for an active clinical trial • Biomarker is prognostic or diagnostic based on multiple small studies
	Tier 2D	• Biomarker shows plausible response or resistance based on case or preclinical studies • Biomarker may assist in disease diagnosis or prognosis based on small studies
Uncertain Significance	**Tier 3**	• Biomarker has uncertain clinical significance and not known to be likely benign or benign

**Adapted from PMID:27993330 jmd.amjpathol.org/article/S1525-1578(16)30223-9/pdf

Analysis Report

20190628103639_10016005070106190147_78-18PT_BC2_Glio2019

Table of contents

1 Summary

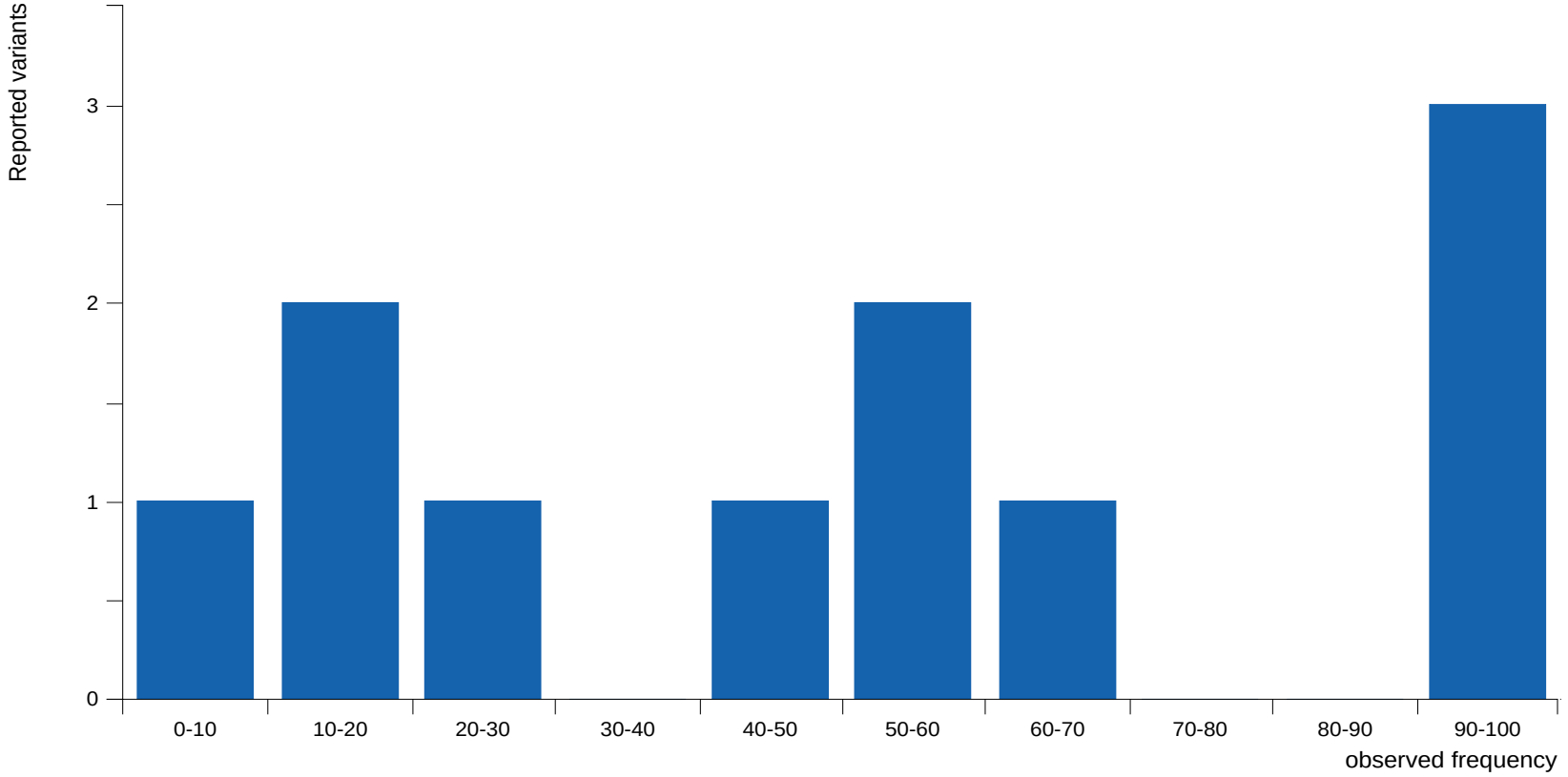
Report created	Sat Jun 29 18:08:23 CEST 2019
Sample ID	20190628103639_10016005070106190147_78-18PT_BC2_Glio2019
Analysis workflow	AIT FFPE v4.5: QIAact Actionable Insights Tumor Panel on FFPE
Analyst	root
Reported variants	11
Analysis results	4 Untested variants

1.1 Comments

No comments

1.2 Distribution of observed frequencies for reported variants

Includes variants initially listed in variant table 'Reported variants'.



2 Quality control

Quality control for the sample analysis. Includes information on the input data, read mapping, and coverage information per gene.

2.1 Fastq

Fastq	20190628103639_10016005070106190147_78-18PT_BC2_Glio2019
Reads	1,835,948
Nucleotides*	168,958,931
Average read length*	92.03
Reads with average quality ≥ 25	96.27%

** Including sample barcode*

Recommendations:

Reads with average quality ≥ 25 should be ≥ 80.00%

2.2 Secondary analysis summary

Reads mapped	1,185,513 (64.57%)
Reads in target regions	457,869 (38.62%)
Percentage of base positions in regions of interest with coverage ≥ 500x	93.47%
Percentage of base positions in regions of interest with coverage ≥ 200x	99.50%

Recommendations:

Percentage of base positions in regions of interest with coverage ≥ 500x should be ≥ 90.00%

Percentage of base positions in regions of interest with coverage ≥ 200x should be ≥ 95.00%

2.3 Coverage

Name	ROI	Bases	≥500x	≥200x	0x	Median	VOI	VOI <500x	VOI <200x
NRAS	6	27	100.00%	100.00%	0.00%	4,470	41	0	0
ALK	22	47	53.19%	100.00%	0.00%	627	40	8	0
RAF1	2	2	100.00%	100.00%	0.00%	2,534	2	0	0
PIK3CA	81	131	98.47%	99.24%	0.00%	2,559	165	2	1

Name	ROI	Bases	≥500x	≥200x	0x	Median	VOI	VOI <500x	VOI <200x
PDGFRA	21	64	100.00%	100.00%	0.00%	2,584	46	0	0
KIT	49	140	100.00%	100.00%	0.00%	1,752	235	0	0
ESR1	6	7	85.71%	100.00%	0.00%	1,146	11	2	0
EGFR	96	208	87.02%	98.56%	0.00%	1,267	443	34	3
BRAF	29	72	100.00%	100.00%	0.00%	1,849	153	0	0
KRAS	21	55	100.00%	100.00%	0.00%	2,498	148	0	0
ERBB3	8	8	100.00%	100.00%	0.00%	2,453	10	0	0
ERBB2	16	35	100.00%	100.00%	0.00%	3,086	61	0	0

ROI: Number of Regions of Interest, i.e. reportable regions that overlap with the gene.

Bases: Total number of base positions in Regions of Interest that overlap with the gene.

≥500x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is equal to or above the significant coverage threshold.

≥200x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is equal to or above the minimum coverage threshold.

0x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is zero.

Median: Median coverage of base positions in the Regions of Interest that overlap with the gene.

VOI: Total number of Variants of Interest, whether detected or not, that overlap with the gene. The list of Variants of Interest is defined by the analysis pipeline.

VOI <500x: Number of Variants of Interest in the gene for which coverage is below the significant coverage threshold.

VOI <200x: Number of Variants of Interest in the gene for which coverage is below the minimum coverage threshold.

2.4 Detected variants

Number of detected variants per gene. Variants for which coverage is above the minimum coverage threshold.

Name	In total	VOI	- non-syn	- syn	Non-VOI	- non-syn	- syn
NRAS	4	0	0	0	4	3	1
ALK	6	3	1	2	3	1	2
RAF1	1	0	0	0	1	1	0
PIK3CA	14	2	1	1	12	3	9
PDGFRA	5	1	0	1	4	2	2
KIT	3	0	0	0	3	2	1
ESR1	5	2	0	2	3	1	2
EGFR	6	2	1	1	4	0	4
BRAF	4	1	0	1	3	0	3

Name	In total	VOI	- non-syn	- syn	Non-VOI	- non-syn	- syn
KRAS	9	0	0	0	9	3	6
ERBB3	4	0	0	0	4	2	2
ERBB2	2	1	1	0	1	1	0

In total: Total number of variants detected within the gene. Variants initially listed in variant tables 3.1 and 3.2.

VOI: Number of detected Variants of Interest detected within the gene. The list of Variants of Interest is defined by the analysis pipeline.

- non-syn: Number of detected, gene-specific Variants of Interest that are non-synonymous.

- syn: Number of detected, gene-specific Variants of Interest that are synonymous.

Non-VOI: Number of detected variants that are not found within the analysis pipeline-defined list of Variants of Interest.

- non-syn: Number of gene-specific, non-VOIs that are non-synonymous.

- syn: Number of gene-specific, non-VOIs that are synonymous.

3 Variants

Variants detected within regions of interest with more than significant coverage are found in 3.1 and variants with more than minimum coverage are found in 3.2.
Variants of interest that could not be tested due to insufficient coverage are listed in table 3.3.
The coverage thresholds and minimum frequency cutoffs configured for the analysis workflow are listed in the History section.
Setting a variant review state to "Confirmed by review" moves it to 3.1, "Artifact" moves it to 3.2.
Only the variants in table 3.1 are exported as VCF and uploaded to QCI Interpret.

3.1 Reported variants

Variants that will be exported to VCF and uploaded to QCI Interpret. Initially contains: Variants detected within regions of interest with more than significant coverage and frequency above the cutoff set for the analysis workflow. These variants are assigned the initial review state "Valid".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
ALK	c.4472A>G	p.Lys1491Arg	SNV	9.10%	29.46	1.00	1,836	Yes	Yes	Valid	
ALK	c.4338C>T		SNV	63.71%	29.53	1.00	970	Yes	Yes	Valid	
ALK	c.2535T>C		SNV	14.09%	29.44	5.15E-5	1,732	Yes	Yes	Valid	
PIK3CA	c.-77+8483C>T		SNV	23.21%	34.36	1.54E-11	1,021	Yes	Yes	Valid	
PIK3CA	c.1173A>G	p.Ile391Met	SNV	55.22%	30.29	0.88	2,501	Yes	Yes	Valid	
PDGFRA	c.1701A>G		SNV	99.82%	30.10	1.00	2,244	Yes	Yes	Valid	
ESR1	c.1782G>A		SNV	45.34%	33.03	1.00	2,069	Yes	Yes	Valid	
EGFR	c.1562G>A	p.Arg521Lys	SNV	16.79%	35.12	0.28	1,090	Yes	Yes	Valid	
EGFR	c.2361G>A		SNV	99.71%	31.39	1.00	1,380	Yes	Yes	Valid	
BRAF	c.1929A>G		SNV	50.90%	26.83	1.00	2,933	Yes	Yes	Valid	
ERBB2	c.3508C>G	p.Pro1170Ala	SNV	98.77%	25.17	1.00	1,624	Yes	Yes	Valid	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
ALK	c.4472A>G	mis-sense	No	167	95	72	200	29416481	2
ALK	c.4338C>T		No	618	350	268	200	29416615	2

ALK	c.2535T>C		No	244	78	166	200	29455267	2
PIK3CA	c.-77+8483C>T		No	237	0	237	200	178874874	3
PIK3CA	c.1173A>G	mis-sense	No	1,381	266	1,115	200	178927410	3
PDGFRA	c.1701A>G		No	2,240	770	1,470	200	55141055	4
ESR1	c.1782G>A		No	938	489	449	200	152420095	6
EGFR	c.1562G>A	mis-sense	No	183	37	146	200	55229255	7
EGFR	c.2361G>A		No	1,376	841	535	200	55249063	7
BRAF	c.1929A>G		No	1,493	514	979	200	140449150	7
ERBB2	c.3508C>G	mis-sense	No	1,604	72	1,532	200	37884037	17

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

Comment: Remark added by user during variant review.

3.2 Variants available for review

Detected variants that will not be exported to the VCF and uploaded to QCI Interpret. Initially contains: Variants with more than minimum coverage and frequency above the cutoff set for the analysis workflow. Depending on workflow configuration, this table may include variants outside of regions of interest including those with coverage above significant coverage threshold. These variants are assigned the initial review state "Review".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
NRAS	c.296A>T	p.Gln99Leu	SNV	5.14%	28.10	0.93	797	No	No	Review	
NRAS	c.236T>C	p.Leu79Pro	SNV	4.46%	28.21	0.89	1,817	No	No	Review	
NRAS	c.19G>C	p.Val7Leu	SNV	20.98%	29.33	0.00	4,505	No	No	Review	
NRAS	c.-14C>G		SNV	5.08%	32.23	1.09E-6	1,260	No	No	Review	
ALK	c.3451-22C>T		SNV	5.80%	28.12	0.92	586	No	No	Review	
ALK	c.3451-26_3451-24delTCC		Deletion	10.37%	26.50	0.61	598	No	No	Review	
ALK	c.2566T>C	p.Phe856Leu	SNV	5.12%	29.62	0.98	1,348	No	No	Review	
RAF1	c.1858G>A	p.Ala620Thr	SNV	5.08%	31.76	1.00	649	No	No	Review	
PIK3CA	c.-77+8519A>T		SNV	29.64%	34.35	0.92	253	No	No	Review	
PIK3CA	c.-76-18864C>A		SNV	64.38%	30.44	0.04	786	No	No	Review	
PIK3CA	c.97A>G	p.Thr33Ala	SNV	8.01%	26.48	0.03	1,173	No	No	Review	
PIK3CA	c.1005A>T	p.Arg335Ser	SNV	9.82%	25.54	0.62	774	No	No	Review	
PIK3CA	c.1060-17C>A		SNV	83.48%	32.38	1.00	1,489	No	No	Review	
PIK3CA	c.1134T>A	p.Cys378*	SNV	7.09%	29.70	3.70E-3	282	Yes	No	Review	
PIK3CA	c.1145+9G>A		SNV	15.06%	35.96	4.82E-3	332	No	No	Review	
PIK3CA	c.1145+14T>A		SNV	21.64%	34.16	8.59E-4	439	No	No	Review	
PIK3CA	c.1145+16_1145+17delGAinsTC		MNV	22.15%	30.00	8.23E-4	438	No	No	Review	
PIK3CA	c.1145+19T>A		SNV	19.64%	34.11	2.45E-3	499	No	No	Review	
PIK3CA	c.1145+24_1145+25insA		Insertion	6.06%	29.39	0.13	297	No	No	Review	
PIK3CA	c.1145+54A>G		SNV	100.00%	33.20	1.00	229	No	No	Review	
PDGFRA	c.1955G>A	p.Gly652Glu	SNV	6.68%	31.81	1.25E-4	2,694	No	No	Review	
PDGFRA	c.2104A>G	p.Lys702Glu	SNV	6.03%	30.54	1.00	398	No	No	Review	

PDGFRA	c.2440-50_2440-49insA		Insertion	99.60%	33.00	1.00	1,252	No	No	Review	
PDGFRA	c.3222T>C		SNV	99.40%	31.51	1.00	1,661	No	No	Review	
KIT	c.1234A>G	p.Lys412Glu	SNV	5.01%	28.79	1.00	1,738	No	No	Review	
KIT	c.2484+23A>G		SNV	10.44%	29.08	0.05	1,744	No	No	Review	
KIT	c.2837G>A	p.Arg946Gln	SNV	15.04%	30.72	0.49	1,170	No	No	Review	
ESR1	c.30T>C		SNV	48.07%	29.69	0.23	285	Yes	Yes	Review	
ESR1	c.1369+13826C>T		SNV	37.42%	30.79	0.63	2,079	No	No	Review	
ESR1	c.1676C>T	p.Ser559Phe	SNV	4.84%	35.38	1.00	929	No	No	Review	
ESR1	c.1692C>T		SNV	8.59%	33.97	1.35E-4	675	No	No	Review	
EGFR	c.890-4C>G		SNV	43.86%	32.42	0.22	2,127	No	No	Review	
EGFR	c.1498+22A>T		SNV	48.68%	33.28	1.00	1,518	No	No	Review	
EGFR	c.2460G>A		SNV	10.02%	32.57	1.00	539	No	No	Review	
EGFR	c.2709T>C		SNV	98.34%	30.25	1.00	1,929	No	No	Review	
BRAF	c.1860+67A>C		SNV	13.73%	36.46	1.00	255	No	No	Review	
BRAF	c.1860+66A>C		SNV	78.10%	36.51	1.00	1,009	No	No	Review	
BRAF	c.1742-38A>T		SNV	14.85%	36.82	0.00	559	No	No	Review	
KRAS	c.541_542delTCinsAG	p.Ser181Arg	MNV	19.28%	31.47	0.00	3,092	No	No	Review	
KRAS	c.534A>G		SNV	19.77%	31.82	0.00	3,146	No	No	Review	
KRAS	c.525A>G		SNV	17.87%	31.26	0.00	3,246	No	No	Review	
KRAS	c.522T>C		SNV	16.88%	35.33	0.00	3,317	No	No	Review	
KRAS	c.519T>C		SNV	16.36%	30.97	0.00	3,307	No	No	Review	
KRAS	c.516A>G		SNV	16.00%	29.39	0.00	3,418	No	No	Review	
KRAS	c.508A>C	p.Met170Leu	SNV	5.64%	29.89	0.00	3,367	No	No	Review	
KRAS	c.291-10delT		Deletion	4.52%	31.00	1.00	642	No	No	Review	
KRAS	c.220A>G	p.Thr74Ala	SNV	5.15%	25.80	0.93	1,146	No	No	Review	
ERBB3	c.89C>T	p.Pro30Leu	SNV	97.72%	35.05	1.00	395	No	No	Review	
ERBB3	c.340A>G	p.Lys114Glu	SNV	4.32%	22.63	0.40	1,133	No	No	Review	
ERBB3	c.936A>G		SNV	4.01%	33.73	0.10	649	No	No	Review	
ERBB3	c.3348G>A		SNV	99.50%	32.70	1.00	2,210	No	No	Review	
ERBB2	c.2953C>T	p.Arg985Cys	SNV	5.66%	28.87	0.55	1,360	No	No	Review	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
NRAS	c.296A>T	mis-sense	No	41	26	15	200	115252344	1
NRAS	c.236T>C	mis-sense	No	81	43	38	200	115256475	1
NRAS	c.19G>C	mis-sense	No	945	0	945	200	115258763	1
NRAS	c.-14C>G		No	64	0	64	200	115258795	1
ALK	c.3451-22C>T		No	34	22	12	200	29445296	2
ALK	c.3451-26_3451-24delTCC		No	62	42	20	200	29445298..29445300	2
ALK	c.2566T>C	mis-sense	No	69	39	30	200	29455236	2
RAF1	c.1858G>A	mis-sense	No	33	0	33	200	12626102	3
PIK3CA	c.-77+8519A>T		No	75	60	15	200	178874910	3
PIK3CA	c.-76-18864C>A		No	506	223	283	200	178897674	3
PIK3CA	c.97A>G	mis-sense	No	94	44	50	200	178916710	3
PIK3CA	c.1005A>T	mis-sense	No	76	16	60	200	178921523	3
PIK3CA	c.1060-17C>A		No	1,243	743	500	200	178922274	3
PIK3CA	c.1134T>A	non-sense	No	20	0	20	200	178922365	3
PIK3CA	c.1145+9G>A		No	50	0	50	200	178922385	3
PIK3CA	c.1145+14T>A		No	95	0	95	200	178922390	3
PIK3CA	c.1145+16_1145+17delGAinsTC		No	97	0	97	200	178922392..178922393	3
PIK3CA	c.1145+19T>A		No	98	0	98	200	178922395	3
PIK3CA	c.1145+24_1145+25insA		No	18	0	18	200	178922400^178922401	3
PIK3CA	c.1145+54A>G		No	229	0	229	200	178922430	3
PDGFRA	c.1955G>A	mis-sense	No	180	149	31	200	55144126	4
PDGFRA	c.2104A>G	mis-sense	No	24	0	24	146	55144630	4
PDGFRA	c.2440-50_2440-49insA		No	1,247	478	769	200	55151958^55151959	4
PDGFRA	c.3222T>C		No	1,651	683	968	200	55161391	4
KIT	c.1234A>G	mis-sense	No	87	42	45	200	55589752	4
KIT	c.2484+23A>G		No	182	100	82	200	55599381	4
KIT	c.2837G>A	mis-sense	No	176	111	65	200	55604629	4
ESR1	c.30T>C		No	137	29	108	200	152129077	6

ESR1	c.1369+13826C>T		No	778	401	377	200	152396085	6
ESR1	c.1676C>T	mis-sense	No	45	26	19	200	152419989	6
ESR1	c.1692C>T		No	58	6	52	200	152420005	6
EGFR	c.890-4C>G		No	933	933	0	200	55223519	7
EGFR	c.1498+22A>T		No	739	360	379	200	55228053	7
EGFR	c.2460G>A		No	54	28	26	200	55249162	7
EGFR	c.2709T>C		No	1,897	1,521	376	200	55266417	7
BRAF	c.1860+67A>C		No	35	35	0	200	140453008	7
BRAF	c.1860+66A>C		No	788	788	0	200	140453009	7
BRAF	c.1742-38A>T		No	83	0	83	200	140453231	7
KRAS	c.541_542delTCinsAG	mis-sense	No	596	596	0	200	25362754..25362755	12
KRAS	c.534A>G		No	622	619	3	200	25362762	12
KRAS	c.525A>G		No	580	578	2	200	25362771	12
KRAS	c.522T>C		No	560	559	1	200	25362774	12
KRAS	c.519T>C		No	541	539	2	200	25362777	12
KRAS	c.516A>G		No	547	540	7	200	25362780	12
KRAS	c.508A>C	mis-sense	No	190	186	4	200	25362788	12
KRAS	c.291-10delT		Yes	29	0	29	200	25378717	12
KRAS	c.220A>G	mis-sense	No	59	27	32	200	25380238	12
ERBB3	c.89C>T	mis-sense	No	386	386	0	200	56477541	12
ERBB3	c.340A>G	mis-sense	No	49	2	47	200	56478884	12
ERBB3	c.936A>G		No	26	9	17	200	56482388	12
ERBB3	c.3348G>A		No	2,199	993	1,206	200	56494991	12
ERBB2	c.2953C>T	mis-sense	No	77	27	50	200	37882895	17

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

Comment: Remark added by user during variant review.

3.3 Untested variants

Variants of interest that could not be tested due to insufficient coverage. These variants are assigned the initial review state "Untested".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
PIK3CA	c.1132T>C	p.Cys378Arg	SNV	1.04%	20.50		192	Yes	Yes	Untested	
EGFR	c.2117T>C	p.Ile706Thr	SNV	0.67%	31.00		149	Yes	Yes	Untested	
EGFR	c.2184+19G>A		SNV	0.00%			90	Yes	Yes	Untested	
EGFR	c.2596G>A	p.Glu866Lys	SNV	0.00%			162	Yes	Yes	Untested	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
PIK3CA	c.1132T>C	mis-sense		2	2	0		178922363	3
EGFR	c.2117T>C	mis-sense		1	0	1		55241669	7
EGFR	c.2184+19G>A			0	0	0		55241755	7
EGFR	c.2596G>A	mis-sense		0	0	0		55259538	7

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

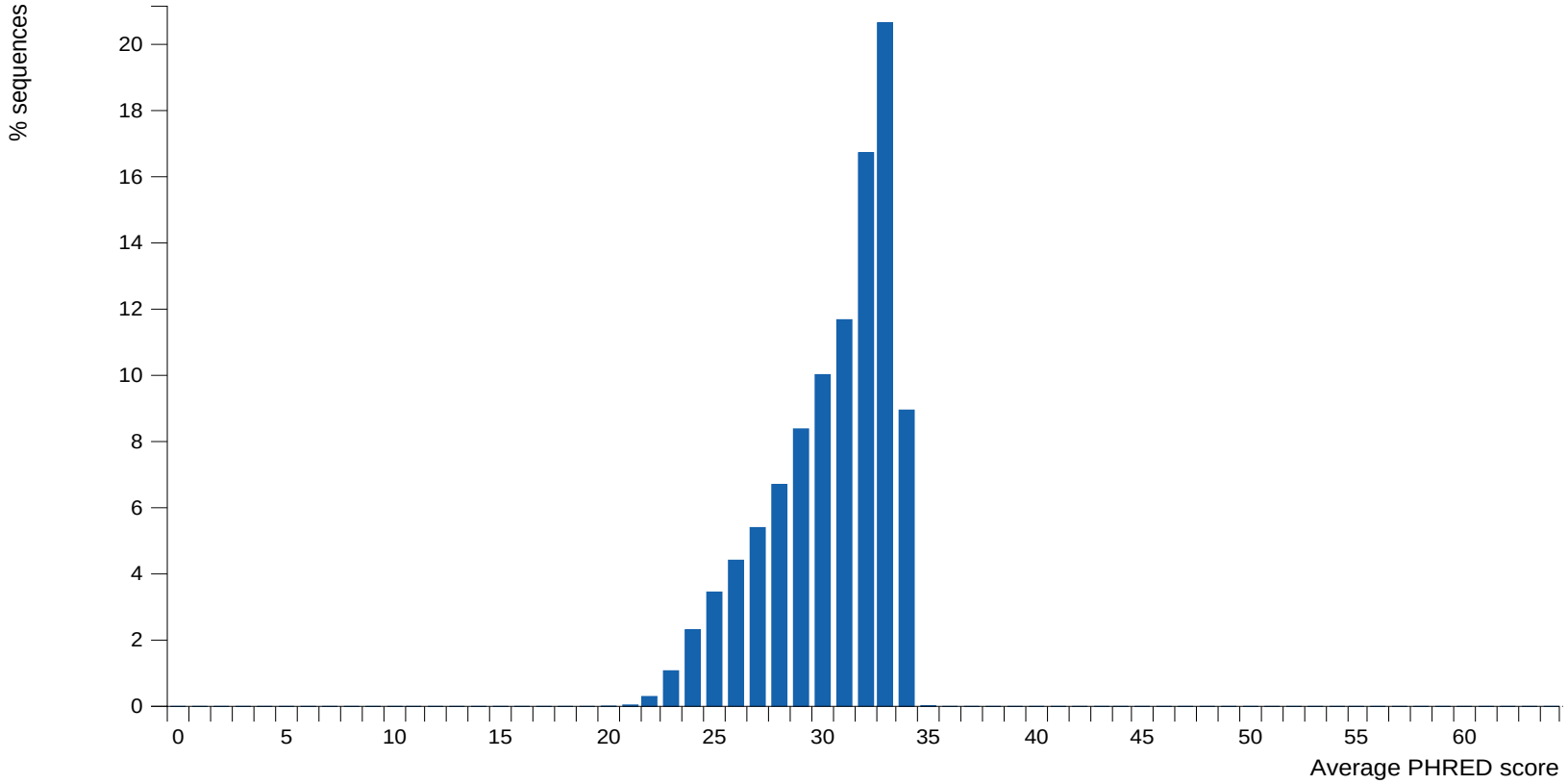
Comment: Remark added by user during variant review.

4 Detailed QC

Quality control metrics for detailed inspection. These metrics can indicate possible problems in the upstream workflow or data analysis. Quality control are divided in metrics on the incoming reads from input data, and metrics per base positions in these reads. Lastly section 4.3 and 4.4 display metrics on how well the positions in the region of interest are covered

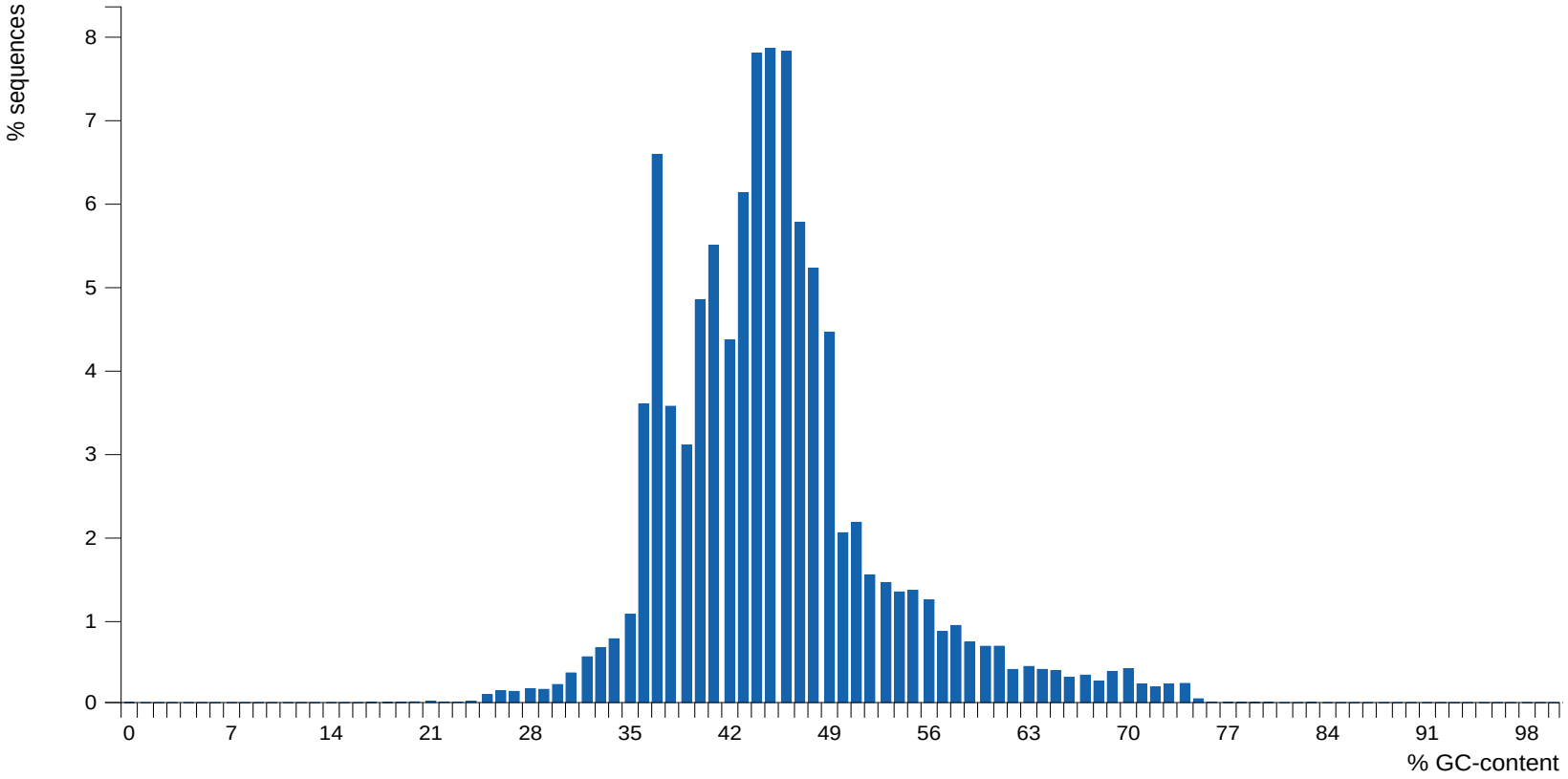
4.1 QC for reads

4.1.1 Average base quality of reads



Distribution of average sequence quality scores. The quality of a sequence is calculated as the arithmetic mean of its base qualities.
x: PHRED-score
y: number of sequences observed at that qual. score normalized to the total number of sequences

4.1.2 GC content of reads



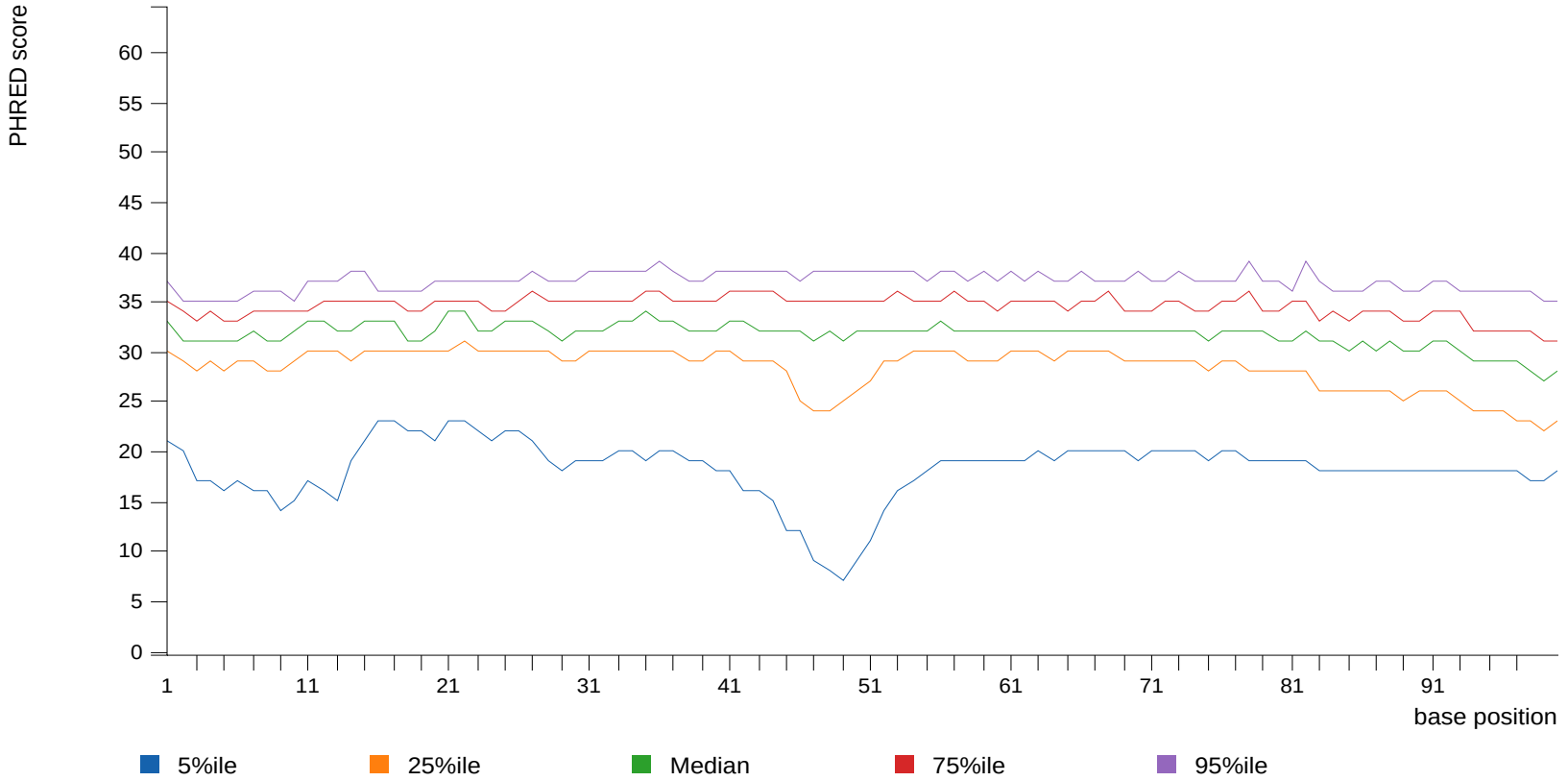
Distribution of GC-contents. The GC-content of a sequence is calculated as the number of GC-bases compared to all bases (including ambiguous bases).
x: relative GC-content of a sequence in percent
y: number of sequences featuring particular GC-percentages normalized to the total number of sequences

4.1.3 Ambiguous base content of reads

No ambiguous bases detected.

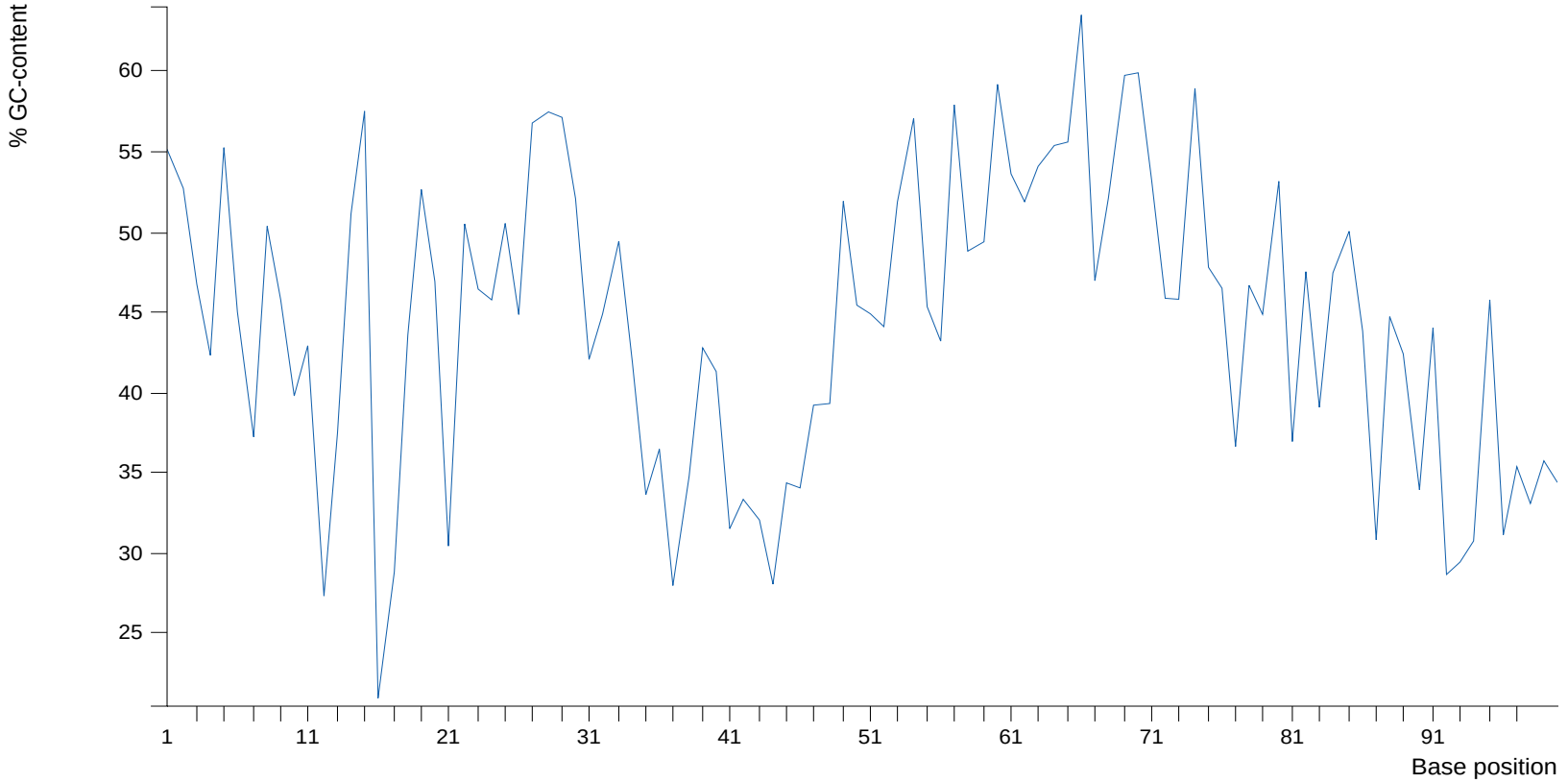
4.2 QC for bases

4.2.1 Quality score per base position



Base-quality distribution along the base positions.
x: base position
y: median & percentiles of quality scores observed at that base position

4.2.2 GC content per base position



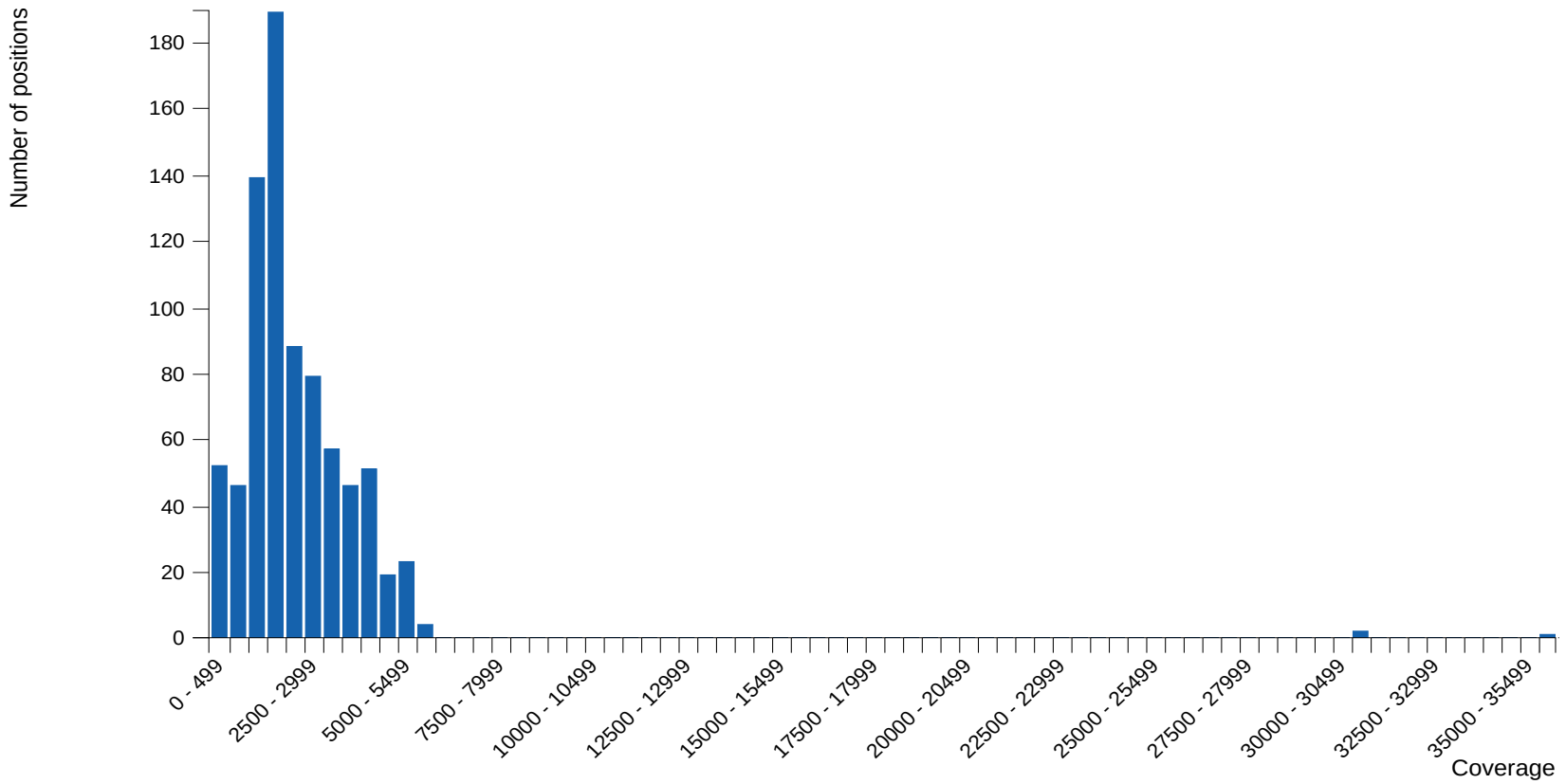
Combined coverage of G- and C-bases.
x: base position
y: number of G- and C-bases observed at current position normalized to the total number of bases observed at that position

4.2.3 Ambiguous base content per base position

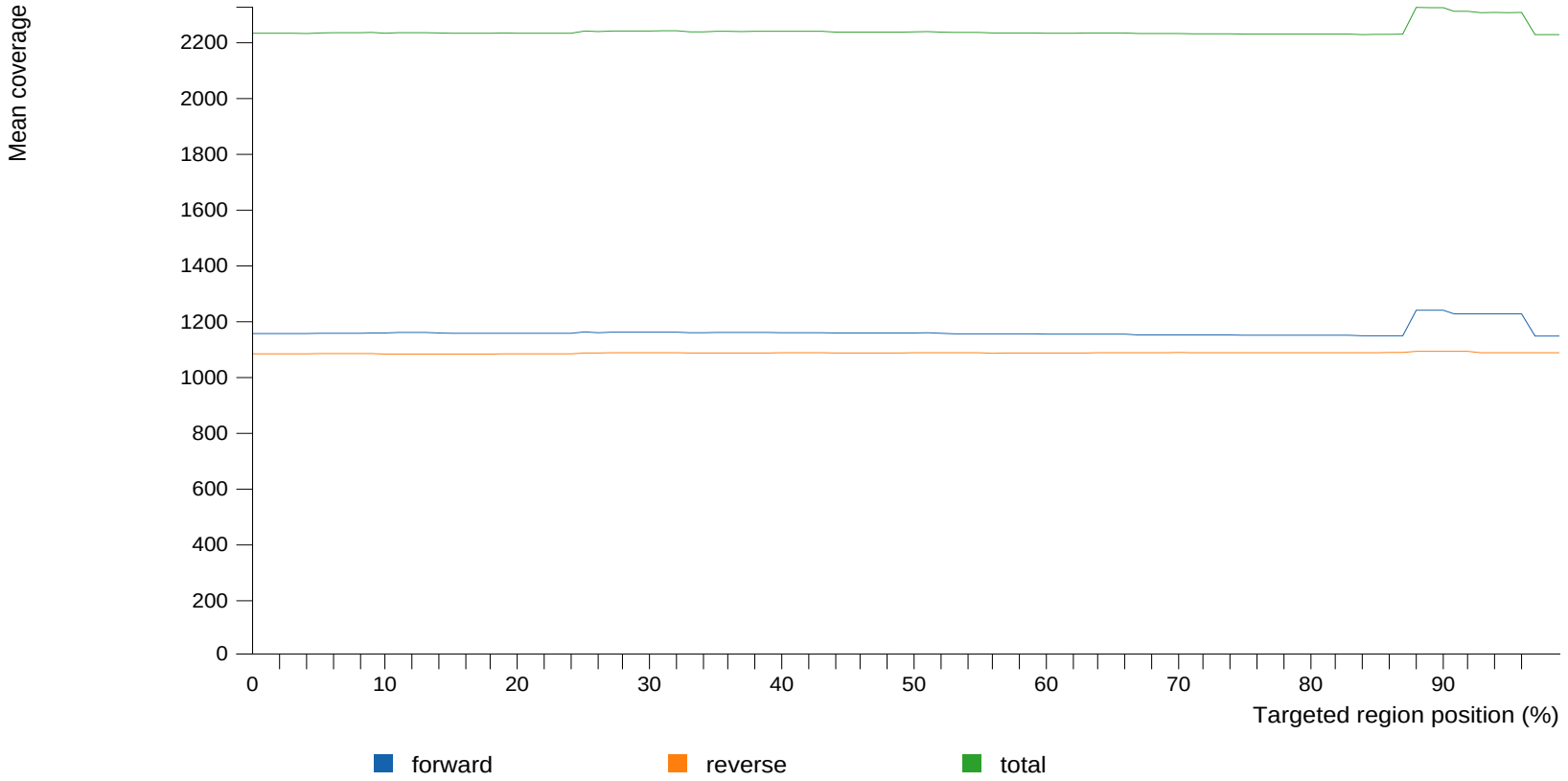
No ambiguous bases detected

4.3 Coverage of Regions of Interest positions

Coverage distribution



4.4 Mean coverage of relative positions in regions of interest



5 History

5.1 Log Entries

Type	Time	User	Details
State change	Sat Jun 29 18:08:36 CEST 2019	system	Ready for Review
State change	Sat Jun 29 17:53:46 CEST 2019	root	In Progress

5.2 Execution Information

QCIA version	QCI Analyze 1.4.5
Analysis start time	Sat Jun 29 17:53:46 CEST 2019
Analysis workflow	AIT FFPE 4.5
Analysis description	QIAact Actionable Insights Tumor Panel on FFPE

5.3 Transcripts

Table listing the genes, transcript IDs and protein IDs used in the analysis.

Gene Name	Transcript ID	Protein ID
NRAS	NM_002524.4	NP_002515.1
ALK	NM_004304.4	NP_004295.2
RAF1	NM_002880.3	NP_002871.1
PIK3CA	NM_006218.2	NP_006209.2
PDGFRA	NM_006206.4	NP_006197.1
KIT	NM_000222.2	NP_000213.1
ESR1	NM_001122742.1	NP_001116214.1
EGFR	NM_005228.3	NP_005219.2
BRAF	NM_004333.4	NP_004324.2
KRAS	NM_004985.3	NP_004976.2
ERBB3	NM_001982.3	NP_001973.2

Gene Name	Transcript ID	Protein ID
ERBB2	NM_004448.2	NP_004439.2

5.4 Open Parameters

Workflow parameters that are editable by the administrator

Reported variants

Significant coverage threshold	500
SNV/MNV frequency threshold in %	4.00
Insertions, deletions and replacements frequency threshold in %	4.00

Variants available for review

Minimum coverage threshold	200
SNV/MNV frequency threshold in %	4.00
Insertions, deletions and replacements frequency threshold in %	4.00
Detect variants outside regions of interest	Yes

5.5 Locked Parameters

Adapter trimming

Trim adapter list	GRadapter_160913
Ambiguous trim	false
Ambiguous limit	2
Quality trim	false
Quality limit	0.05
Use colorspace	false

Also search on reversed sequence	false
Remove 5' terminal nucleotides	false
Number of 5' terminal nucleotides	1
Maximum number of nucleotides in reads	1000
Minimum number of nucleotides in reads	15
Discard short reads	false
Remove 3' terminal nucleotides	false
Number of 3' terminal nucleotides	1
Discard long reads	false

Map Reads to Reference

References	Homo_sapiens_sequence_hg19
Masking mode	No masking
Masking track	Not set
Match score	1
Mismatch cost	2
Cost of insertions and deletions	Affine gap cost
Insertion cost	3
Deletion cost	3
Insertion open cost	6
Insertion extend cost	1
Deletion open cost	6
Deletion extend cost	1
Length fraction	0.5
Similarity fraction	0.8
Global alignment	false
Color space alignment	false
Color error cost	3
Auto-detect paired distances	false
Non-specific match handling	Map randomly

InDels and Structural Variants

P-Value threshold	1.0E-4
Maximum number of mismatches	3
Ignore broken pairs	true
Minimum relative consensus coverage	0.0
Minimum quality score	0
Filter variants	true
Minimum number of reads	2
Restrict calling to target regions	ATPv2_TargetRegions_170302_ver1.1

Local Realignment (Short Unaligned End version)

Realign unaligned ends	true
Multi-pass realignment	2
Local bound for unaligned ends of size one	0.75
Local bound for unaligned ends of size two	0.75
Force realignment to guidance-variants	false
Maximum guidance-variant length	100

Trim Primers and their Dimers of Mapped Reads

Primer track	101x_GR_primers_15_10_15_V1.0
Reference	Homo_sapiens_sequence_hg19
Minimum primer overlap length	9
Allow dangling 3' end base	true
Minimal primer overlap fraction	0.7
Only keep reads that have hit a primer	true
Additional bases to trim	1

Remove Pseudogene Reads

Genes track	AITv2_PseudoGenes_170912_ver1.0
Gene and pseudogene links	KRAS -> KRASP1
Required unaligned ends %	2.0

Low Frequency Variant Detection

Required significance (%)	0.01
Ignore positions with coverage above	1000000000
Restrict calling to target regions	ATPv2_TargetRegions_170302_ver1.1
Ignore broken pairs	true
Ignore non-specific matches	Reads
Minimum read length	20
Minimum coverage	Parameter editable by administrator
Minimum count	8
Minimum frequency (%)	Parameter editable by administrator
Base quality filter	false
Neighborhood radius	5
Minimum central quality	5
Minimum neighborhood quality	5
Read direction filter	false
Direction frequency (%)	5.0
Relative read direction filter	false
Significance (%)	1.0E-5
Read position filter	false
Significance (%)	1.0
Remove pyro-error variants	false
In homopolymer regions with minimum length	3
With frequency below	0.8

Remove False Positives

Minimum frequency (%)	Parameter editable by administrator
Minimum forward/reverse balance	0.05
Minimum average base quality	22.0
Variant frequency	true
Forward/reverse balance	false
Average base quality	true

Annotate Variants With Primers

Minimum coverage count	400
Minimum variant percentage	1.0
Minimum variant read count	2

YOUR LAB

Your Lab
1700 Lincoln Blvd, Suite 20, Redwood City, CA 94063
labx.com / (650) 484 4040
Additional Information

Test Performed: **Somatic Panel**

Report Date Mar 7, 2020
Status -

Patient		Client		Specimen	
Patient Name		Client		Accession ID	20190628103639_100160050701061 90147_411-19CTC_BC7_Glio2019
Date of Birth		Client ID			
Age	50	Physician			
Sex	Male	Pathologist			
Ethnicity	European			Specimen Collection	
Diagnosis	Glioblastoma			Accession	Mar 7, 2020
				Primary Tumor Site	Brain

Result: Negative

Genes Tested

Test information such as gene name and hot spot region can be included in this section.

Methods and Limitations

EXAMPLE Statement including sample type (FFPE, etc), method of extraction, amplification reactions, panel targeted regions, sequencing technology, etc. Additionally, a description of the data analysis software(s), genome of reference and the sensitivity of the methods should be described.

QIAGEN Clinical Insight (QCI™) is a variant analysis, interpretation and decision support tool for research and clinical labs analyzing human genetics data and is not intended to be used for diagnostic purposes. QCI Interpret software includes the following underlying databases, data reference sets and tools; QIAGEN Clinical Insight-Interpret (5.6.20200226), Ingenuity Knowledge Base (K-release), CADD (v1.4), Allele Frequency Community (2019-09-25), EVS (ESP6500SI-V2), Refseq Gene Model (2019-02-05), JASPAR (2013-11), Ingenuity Knowledge Base Snapshot Timestamp (2020-02-21 12:37:01.0), Vista Enhancer hg18 (2012-07), Vista Enhancer hg19 (2012-07), Clinical Trials (K-release), PolyPhen-2 (v2.2.2), 1000 Genome Frequency (phase3v5b), ExAC (0.3.1), iva (Nov 19 12:28 iva-1.0.1200.jar), PhyloP hg18 (2009-11), PhyloP hg19 (2009-11), DbSNP (151), TargetScan (7.2), GENCODE (Release 29), CentoMD (5.3), OMIM (May 26, 2017), gnomAD (2.1.1), BSIFT (2016-02-23), TCGA (2013-09-05), Clinvar (2019-06-05), DGV (2016-05-15), COSMIC (v89), HGMD (2019.2), SIFT4G (2016-02-23)

Clinical Significance of Variants Based on AMP / ASCO / CAP Guidelines*

Strong Significance	**Tier 1A**	• Biomarker predicts response or resistance to an FDA or EMA approved therapy, according to drug label or professional guidelines for this diagnosis • Biomarker included in professional guidelines is prognostic or diagnostic for this diagnosis
	Tier 1B	• Biomarker predicts response or resistance to a therapy for this diagnosis based on well-powered studies • Biomarker is prognostic or diagnostic for this diagnosis based on well-powered studies
Potential Significance	**Tier 2C**	• Biomarker is associated with response or resistance to an FDA or EMA approved therapy, according to drug label or professional guidelines but only for different diagnosis • Biomarker is an inclusion criterion for an active clinical trial • Biomarker is prognostic or diagnostic based on multiple small studies
	Tier 2D	• Biomarker shows plausible response or resistance based on case or preclinical studies • Biomarker may assist in disease diagnosis or prognosis based on small studies
Uncertain Significance	**Tier 3**	• Biomarker has uncertain clinical significance and not known to be likely benign or benign

**Adapted from PMID:27993330 jmd.amjpathol.org/article/S1525-1578(16)30223-9/pdf

Analysis Report

20190628103639_1001600507010619014 7_374-19CTC_BC6_Glio2019

Table of contents

1 Summary

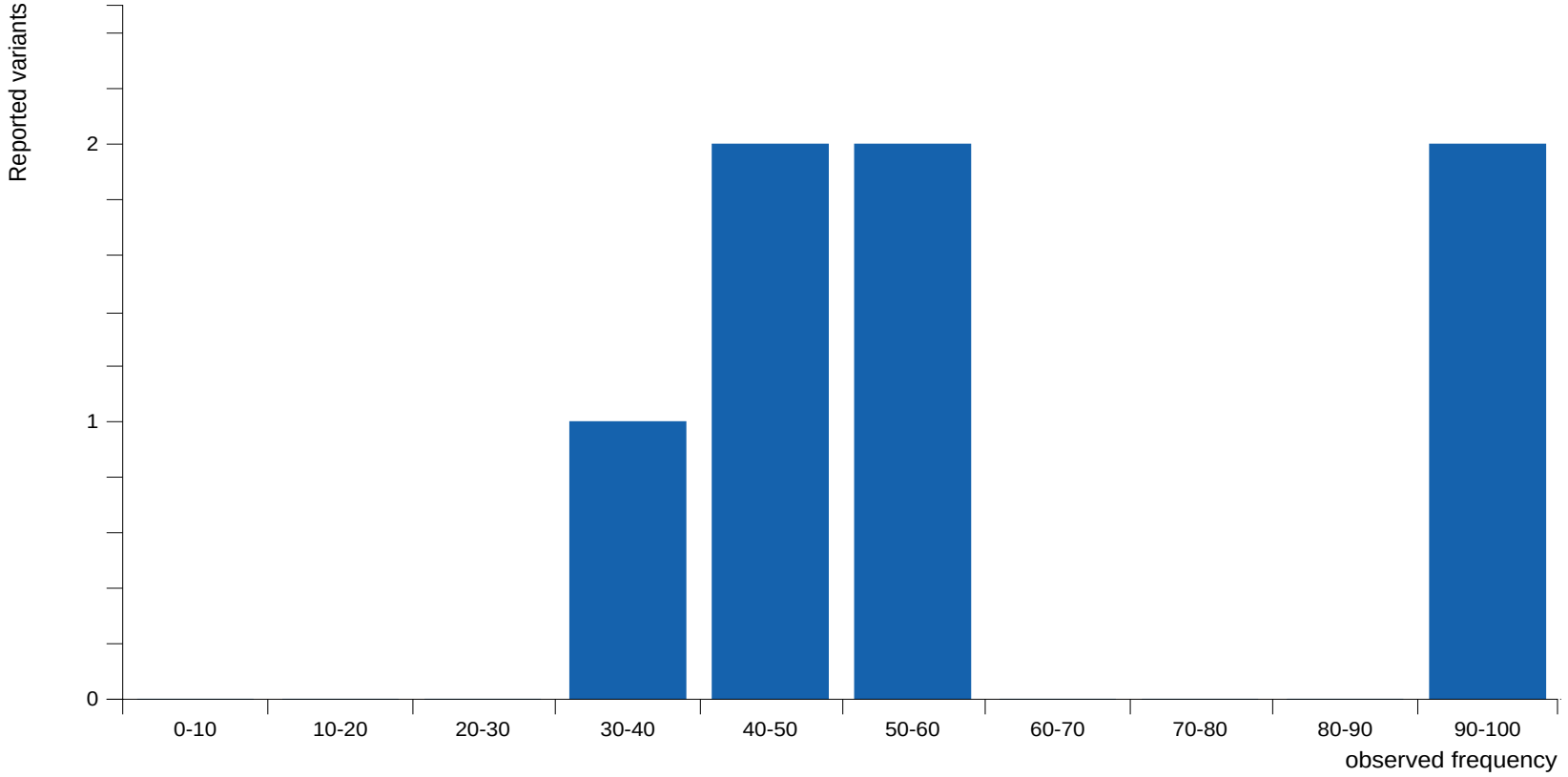
Report created	Sat Jun 29 18:10:36 CEST 2019
Sample ID	20190628103639_10016005070106190147_374-19CTC_BC6_Glio2019
Analysis workflow	AIT FFPE v4.5: QIAact Actionable Insights Tumor Panel on FFPE
Analyst	root
Reported variants	7
Analysis results	1 Untested variants

1.1 Comments

No comments

1.2 Distribution of observed frequencies for reported variants

Includes variants initially listed in variant table 'Reported variants'.



2 Quality control

Quality control for the sample analysis. Includes information on the input data, read mapping, and coverage information per gene.

2.1 Fastq

Fastq	20190628103639_10016005070106190147_374-19CTC_BC6_Glio2019
Reads	1,404,621
Nucleotides*	135,230,568
Average read length*	96.28
Reads with average quality ≥ 25	98.55%

** Including sample barcode*

Recommendations:

Reads with average quality ≥ 25 should be ≥ 80.00%

2.2 Secondary analysis summary

Reads mapped	1,208,967 (86.07%)
Reads in target regions	660,609 (54.64%)
Percentage of base positions in regions of interest with coverage ≥ 500x	99.50%
Percentage of base positions in regions of interest with coverage ≥ 200x	99.87%

Recommendations:

Percentage of base positions in regions of interest with coverage ≥ 500x should be ≥ 90.00%

Percentage of base positions in regions of interest with coverage ≥ 200x should be ≥ 95.00%

2.3 Coverage

Name	ROI	Bases	≥500x	≥200x	0x	Median	VOI	VOI <500x	VOI <200x
NRAS	6	27	100.00%	100.00%	0.00%	5,091	41	0	0
ALK	22	47	100.00%	100.00%	0.00%	1,993	40	0	0
RAF1	2	2	100.00%	100.00%	0.00%	3,687	2	0	0
PIK3CA	81	131	100.00%	100.00%	0.00%	2,852	165	0	0

Name	ROI	Bases	≥500x	≥200x	0x	Median	VOI	VOI <500x	VOI <200x
PDGFRA	21	64	100.00%	100.00%	0.00%	4,054	46	0	0
KIT	49	140	100.00%	100.00%	0.00%	4,291	235	0	0
ESR1	6	7	100.00%	100.00%	0.00%	1,940	11	0	0
EGFR	96	208	98.08%	99.52%	0.00%	2,138	443	4	1
BRAF	29	72	100.00%	100.00%	0.00%	3,327	153	0	0
KRAS	21	55	100.00%	100.00%	0.00%	4,240	148	0	0
ERBB3	8	8	100.00%	100.00%	0.00%	2,727	10	0	0
ERBB2	16	35	100.00%	100.00%	0.00%	4,198	61	0	0

ROI: Number of Regions of Interest, i.e. reportable regions that overlap with the gene.

Bases: Total number of base positions in Regions of Interest that overlap with the gene.

≥500x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is equal to or above the significant coverage threshold.

≥200x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is equal to or above the minimum coverage threshold.

0x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is zero.

Median: Median coverage of base positions in the Regions of Interest that overlap with the gene.

VOI: Total number of Variants of Interest, whether detected or not, that overlap with the gene. The list of Variants of Interest is defined by the analysis pipeline.

VOI <500x: Number of Variants of Interest in the gene for which coverage is below the significant coverage threshold.

VOI <200x: Number of Variants of Interest in the gene for which coverage is below the minimum coverage threshold.

2.4 Detected variants

Number of detected variants per gene. Variants for which coverage is above the minimum coverage threshold.

Name	In total	VOI	- non-syn	- syn	Non-VOI	- non-syn	- syn
NRAS	1	0	0	0	1	1	0
ALK	2	2	1	1	0	0	0
RAF1	0	0	0	0	0	0	0
PIK3CA	7	0	0	0	7	0	7
PDGFRA	3	1	0	1	2	0	2
KIT	0	0	0	0	0	0	0
ESR1	2	1	0	1	1	0	1
EGFR	7	3	1	2	4	0	4
BRAF	3	0	0	0	3	0	3

Name	In total	VOI	- non-syn	- syn	Non-VOI	- non-syn	- syn
KRAS	6	0	0	0	6	1	5
ERBB3	1	0	0	0	1	0	1
ERBB2	0	0	0	0	0	0	0

In total: Total number of variants detected within the gene. Variants initially listed in variant tables 3.1 and 3.2.

VOI: Number of detected Variants of Interest detected within the gene. The list of Variants of Interest is defined by the analysis pipeline.

- non-syn: Number of detected, gene-specific Variants of Interest that are non-synonymous.

- syn: Number of detected, gene-specific Variants of Interest that are synonymous.

Non-VOI: Number of detected variants that are not found within the analysis pipeline-defined list of Variants of Interest.

- non-syn: Number of gene-specific, non-VOIs that are non-synonymous.

- syn: Number of gene-specific, non-VOIs that are synonymous.

3 Variants

Variants detected within regions of interest with more than significant coverage are found in 3.1 and variants with more than minimum coverage are found in 3.2.
Variants of interest that could not be tested due to insufficient coverage are listed in table 3.3.
The coverage thresholds and minimum frequency cutoffs configured for the analysis workflow are listed in the History section.
Setting a variant review state to "Confirmed by review" moves it to 3.1, "Artifact" moves it to 3.2.
Only the variants in table 3.1 are exported as VCF and uploaded to QCI Interpret.

3.1 Reported variants

Variants that will be exported to VCF and uploaded to QCI Interpret. Initially contains: Variants detected within regions of interest with more than significant coverage and frequency above the cutoff set for the analysis workflow. These variants are assigned the initial review state "Valid".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
ALK	c.4472A>G	p.Lys1491Arg	SNV	53.13%	29.50	1.00	4,615	Yes	Yes	Valid	
ALK	c.4338C>T		SNV	45.19%	29.68	1.00	3,005	Yes	Yes	Valid	
PDGFRA	c.1701A>G		SNV	97.69%	29.95	1.00	4,151	Yes	Yes	Valid	
ESR1	c.30T>C		SNV	40.67%	29.08	1.00	1,940	Yes	Yes	Valid	
EGFR	c.474C>T		SNV	55.23%	31.54	1.00	3,038	Yes	Yes	Valid	
EGFR	c.1562G>A	p.Arg521Lys	SNV	36.98%	35.15	0.85	1,455	Yes	Yes	Valid	
EGFR	c.2361G>A		SNV	98.44%	31.58	1.00	769	Yes	Yes	Valid	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
ALK	c.4472A>G	mis-sense	No	2,452	1,301	1,151	200	29416481	2
ALK	c.4338C>T		No	1,358	902	456	200	29416615	2
PDGFRA	c.1701A>G		No	4,055	1,341	2,714	200	55141055	4
ESR1	c.30T>C		No	789	490	299	200	152129077	6
EGFR	c.474C>T		No	1,678	632	1,046	200	55214348	7
EGFR	c.1562G>A	mis-sense	No	538	103	435	200	55229255	7

EGFR	c.2361G>A		No	757	425	332	200	55249063	7

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

Comment: Remark added by user during variant review.

3.2 Variants available for review

Detected variants that will not be exported to the VCF and uploaded to QCI Interpret. Initially contains: Variants with more than minimum coverage and frequency above the cutoff set for the analysis workflow. Depending on workflow configuration, this table may include variants outside of regions of interest including those with coverage above significant coverage threshold. These variants are assigned the initial review state "Review".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
NRAS	c.19G>C	p.Val7Leu	SNV	5.60%	29.35	0.00	8,711	No	No	Review	

PIK3CA	c.353-65delA		Deletion	4.61%	32.29	1.00	608	No	No	Review	
PIK3CA	c.1060-17C>A		SNV	56.52%	32.24	1.00	2,095	No	No	Review	
PIK3CA	c.1145+9G>A		SNV	6.75%	35.03	5.06E-4	919	No	No	Review	
PIK3CA	c.1145+14T>A		SNV	9.34%	32.30	5.73E-5	1,006	No	No	Review	
PIK3CA	c.1145+16_1145+17delGAinsTC		MNV	9.57%	29.53	1.06E-4	993	No	No	Review	
PIK3CA	c.1145+19T>A		SNV	8.87%	33.37	2.85E-4	1,094	No	No	Review	
PIK3CA	c.1145+54A>G		SNV	61.66%	33.27	1.00	699	No	No	Review	
PDGFRA	c.2440-50_2440-49insA		Insertion	99.78%	33.35	1.00	2,226	No	No	Review	
PDGFRA	c.3222T>C		SNV	99.83%	31.38	1.00	4,051	No	No	Review	
ESR1	c.975G>C		SNV	99.55%	31.19	1.00	2,883	No	No	Review	
EGFR	c.890-4C>G		SNV	24.41%	32.38	0.09	2,130	No	No	Review	
EGFR	c.1498+22A>T		SNV	99.57%	32.81	1.00	4,402	No	No	Review	
EGFR	c.2284-60T>C		SNV	50.13%	32.89	1.00	377	No	No	Review	
EGFR	c.2709T>C		SNV	97.72%	30.20	1.00	3,371	No	No	Review	
BRAF	c.1860+67A>C		SNV	20.50%	37.02	1.00	200	No	No	Review	
BRAF	c.1860+66A>C		SNV	82.26%	36.52	1.00	885	No	No	Review	
BRAF	c.1742-38A>T		SNV	5.51%	36.89	0.00	799	No	No	Review	
KRAS	c.541_542delTCinsAG	p.Ser181Arg	MNV	6.33%	31.58	0.00	4,438	No	No	Review	
KRAS	c.534A>G		SNV	6.32%	31.75	0.00	4,560	No	No	Review	
KRAS	c.525A>G		SNV	5.60%	31.04	0.00	4,926	No	No	Review	
KRAS	c.522T>C		SNV	5.11%	35.07	0.00	5,105	No	No	Review	
KRAS	c.519T>C		SNV	52.47%	31.68	1.00	5,100	No	No	Review	
KRAS	c.516A>G		SNV	4.93%	29.34	0.00	5,297	No	No	Review	
ERBB3	c.3348G>A		SNV	63.36%	32.80	1.00	2,290	No	No	Review	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
NRAS	c.19G>C	mis-sense	No	488	0	488	200	115258763	1
PIK3CA	c.353-65delA		Yes	28	28	0	200	178917413	3

PIK3CA	c.1060-17C>A		No	1,184	664	520	200	178922274	3
PIK3CA	c.1145+9G>A		No	62	0	62	200	178922385	3
PIK3CA	c.1145+14T>A		No	94	0	94	200	178922390	3
PIK3CA	c.1145+16_1145+17delGAinsTC		No	95	0	95	200	178922392..178922393	3
PIK3CA	c.1145+19T>A		No	97	0	97	200	178922395	3
PIK3CA	c.1145+54A>G		No	431	0	431	200	178922430	3
PDGFRA	c.2440-50_2440-49insA		No	2,221	1,186	1,035	200	55151958^55151959	4
PDGFRA	c.3222T>C		No	4,044	1,636	2,408	200	55161391	4
ESR1	c.975G>C		No	2,870	1,526	1,344	200	152265522	6
EGFR	c.890-4C>G		No	520	520	0	200	55223519	7
EGFR	c.1498+22A>T		No	4,383	2,125	2,258	200	55228053	7
EGFR	c.2284-60T>C		No	189	189	0	200	55248926	7
EGFR	c.2709T>C		No	3,294	2,687	607	200	55266417	7
BRAF	c.1860+67A>C		No	41	41	0	200	140453008	7
BRAF	c.1860+66A>C		No	728	728	0	200	140453009	7
BRAF	c.1742-38A>T		No	44	0	44	200	140453231	7
KRAS	c.541_542delTCinsAG	mis-sense	No	281	281	0	200	25362754..25362755	12
KRAS	c.534A>G		No	288	283	5	200	25362762	12
KRAS	c.525A>G		No	276	271	5	200	25362771	12
KRAS	c.522T>C		No	261	260	1	200	25362774	12
KRAS	c.519T>C		No	2,676	1,351	1,325	200	25362777	12
KRAS	c.516A>G		No	261	250	11	200	25362780	12
ERBB3	c.3348G>A		No	1,451	618	833	200	56494991	12

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

Comment: Remark added by user during variant review.

3.3 Untested variants

Variants of interest that could not be tested due to insufficient coverage. These variants are assigned the initial review state "Untested".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
EGFR	c.2184+19G>A		SNV	2.04%	6.00		49	Yes	Yes	Untested	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
EGFR	c.2184+19G>A			1	0	1		55241755	7

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

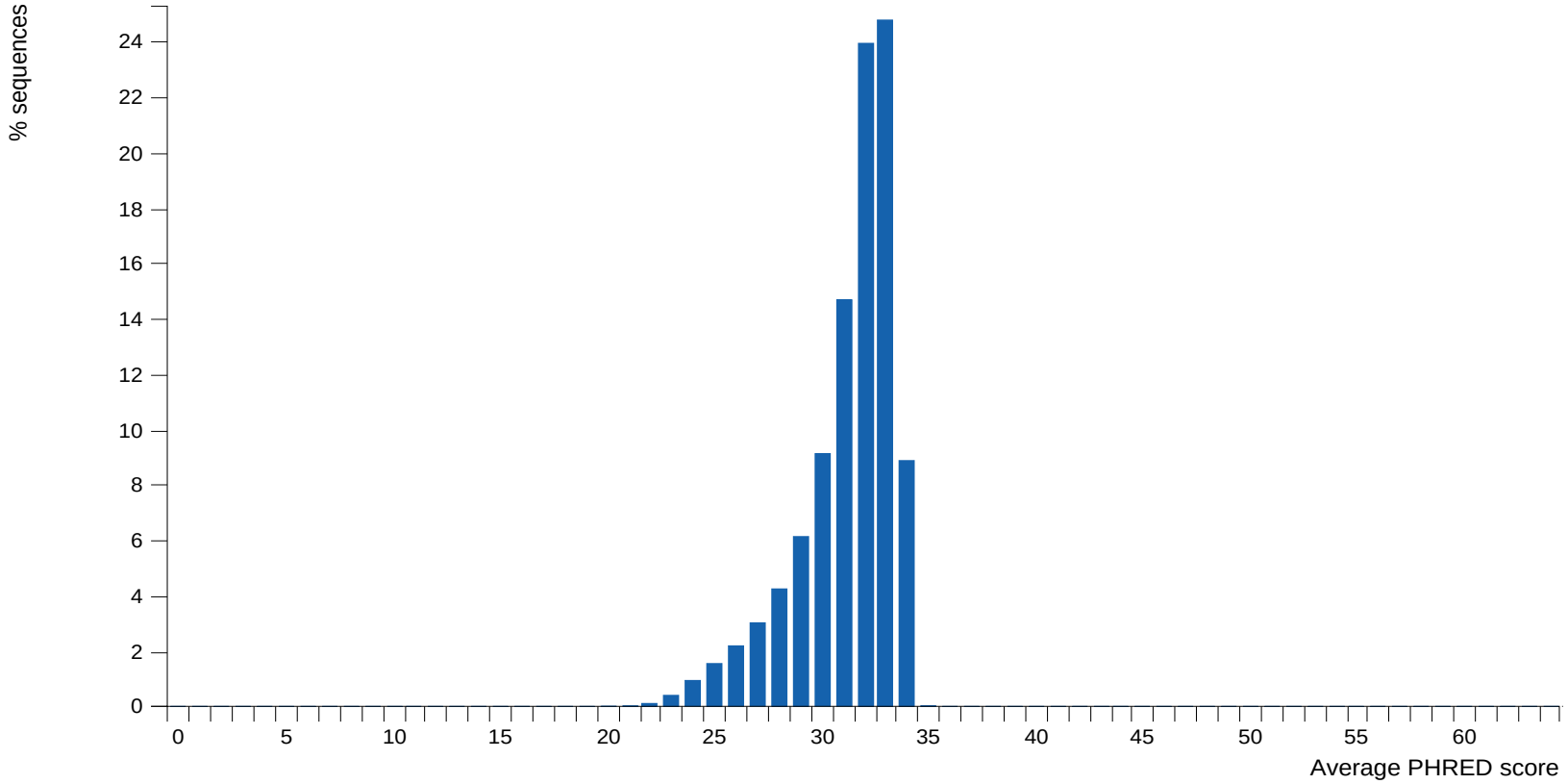
Comment: Remark added by user during variant review.

4 Detailed QC

Quality control metrics for detailed inspection. These metrics can indicate possible problems in the upstream workflow or data analysis. Quality control are divided in metrics on the incoming reads from input data, and metrics per base positions in these reads. Lastly section 4.3 and 4.4 display metrics on how well the positions in the region of interest are covered

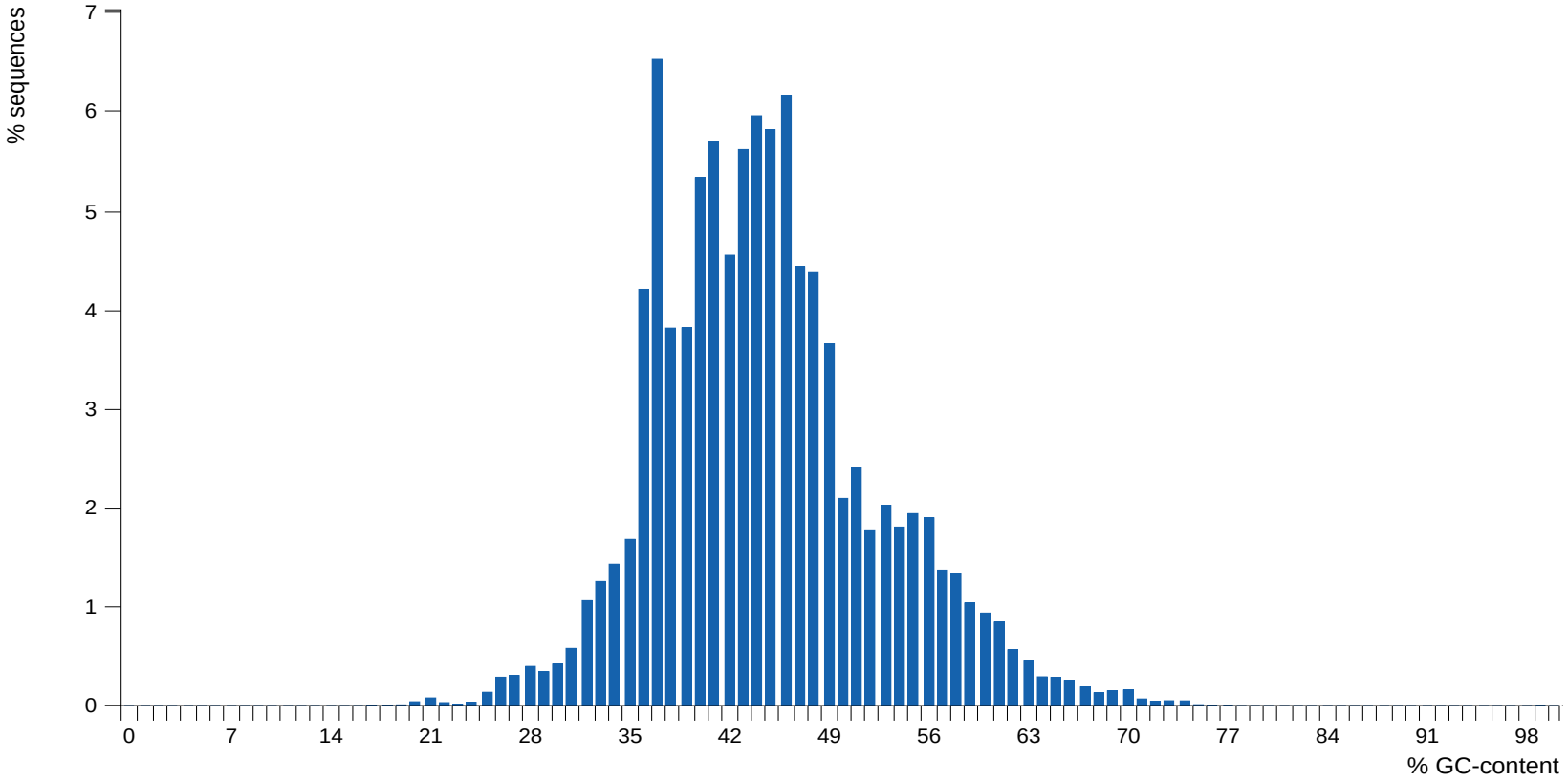
4.1 QC for reads

4.1.1 Average base quality of reads



Distribution of average sequence quality scores. The quality of a sequence is calculated as the arithmetic mean of its base qualities.
x: PHRED-score
y: number of sequences observed at that qual. score normalized to the total number of sequences

4.1.2 GC content of reads



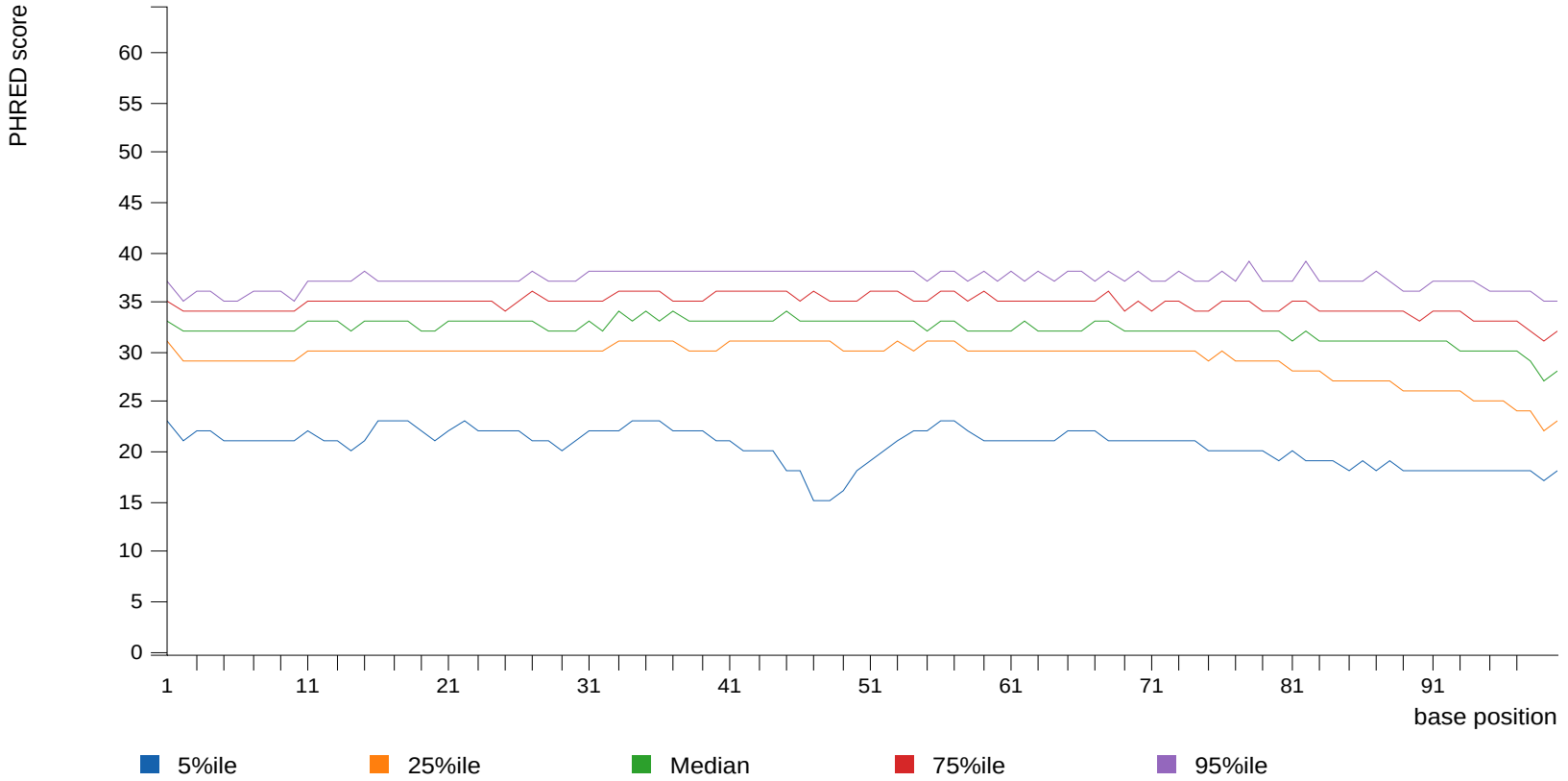
Distribution of GC-contents. The GC-content of a sequence is calculated as the number of GC-bases compared to all bases (including ambiguous bases).
x: relative GC-content of a sequence in percent
y: number of sequences featuring particular GC-percentages normalized to the total number of sequences

4.1.3 Ambiguous base content of reads

No ambiguous bases detected.

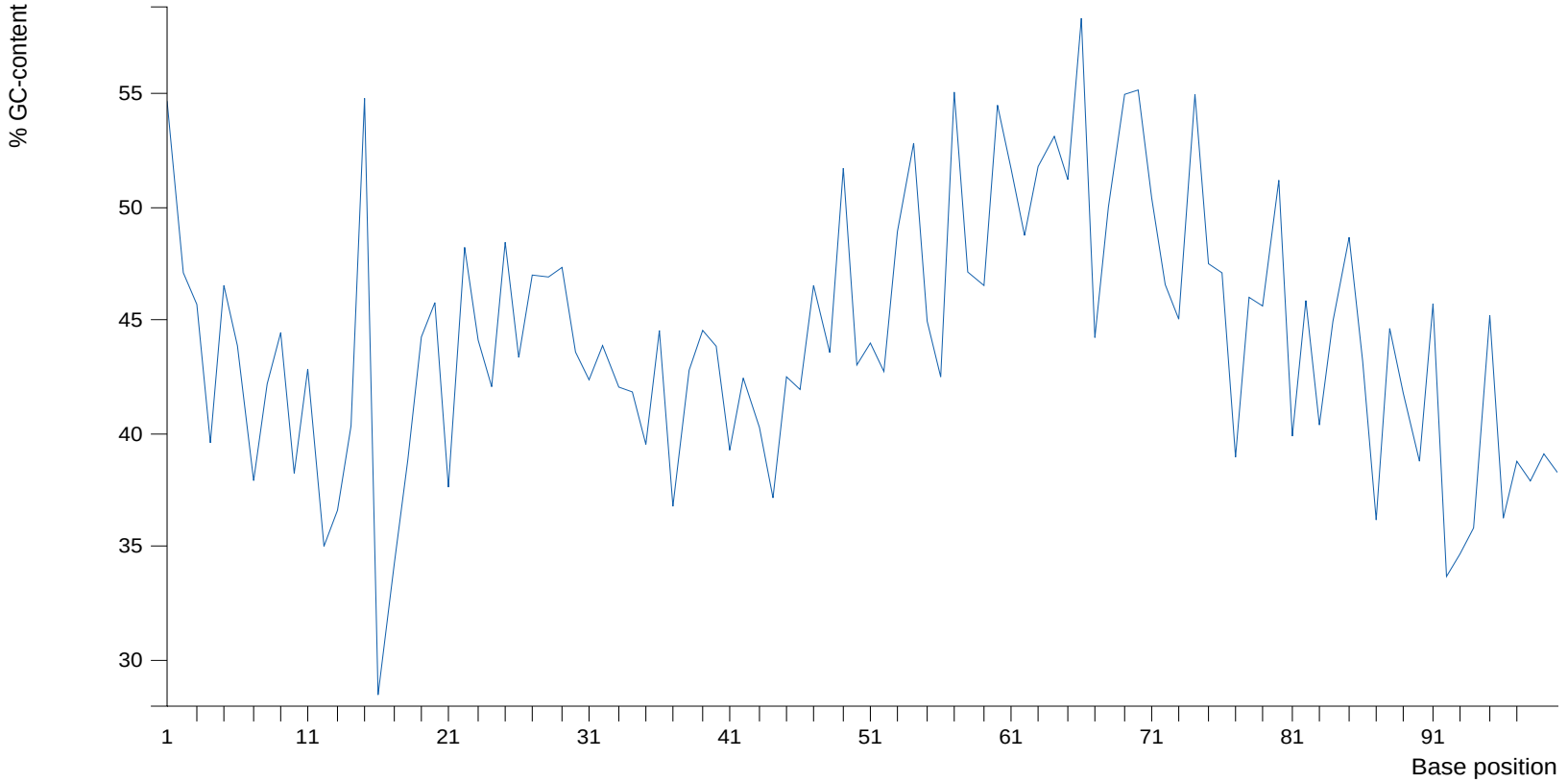
4.2 QC for bases

4.2.1 Quality score per base position



Base-quality distribution along the base positions.
x: base position
y: median & percentiles of quality scores observed at that base position

4.2.2 GC content per base position



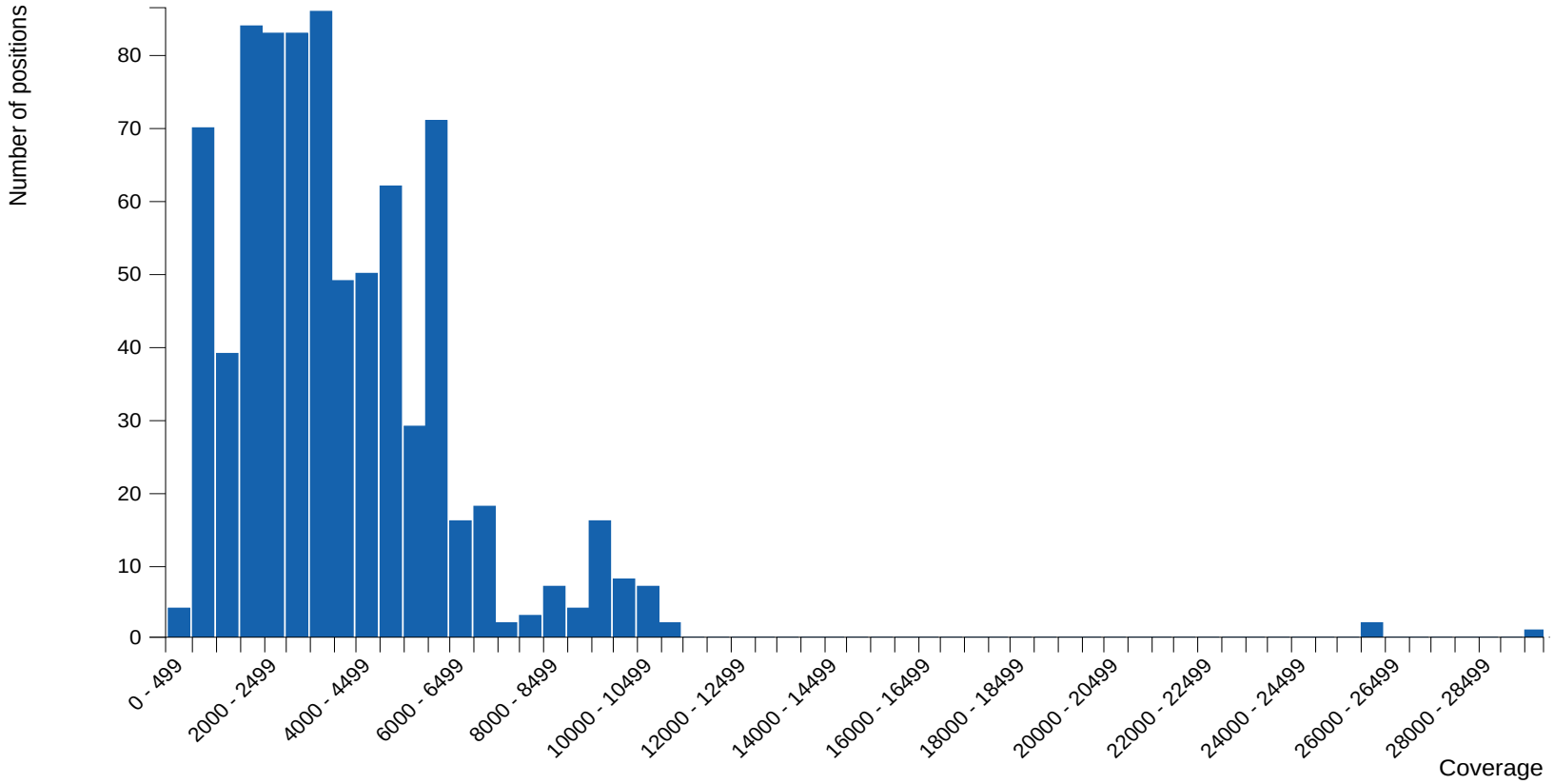
Combined coverage of G- and C-bases.
x: base position
y: number of G- and C-bases observed at current position normalized to the total number of bases observed at that position

4.2.3 Ambiguous base content per base position

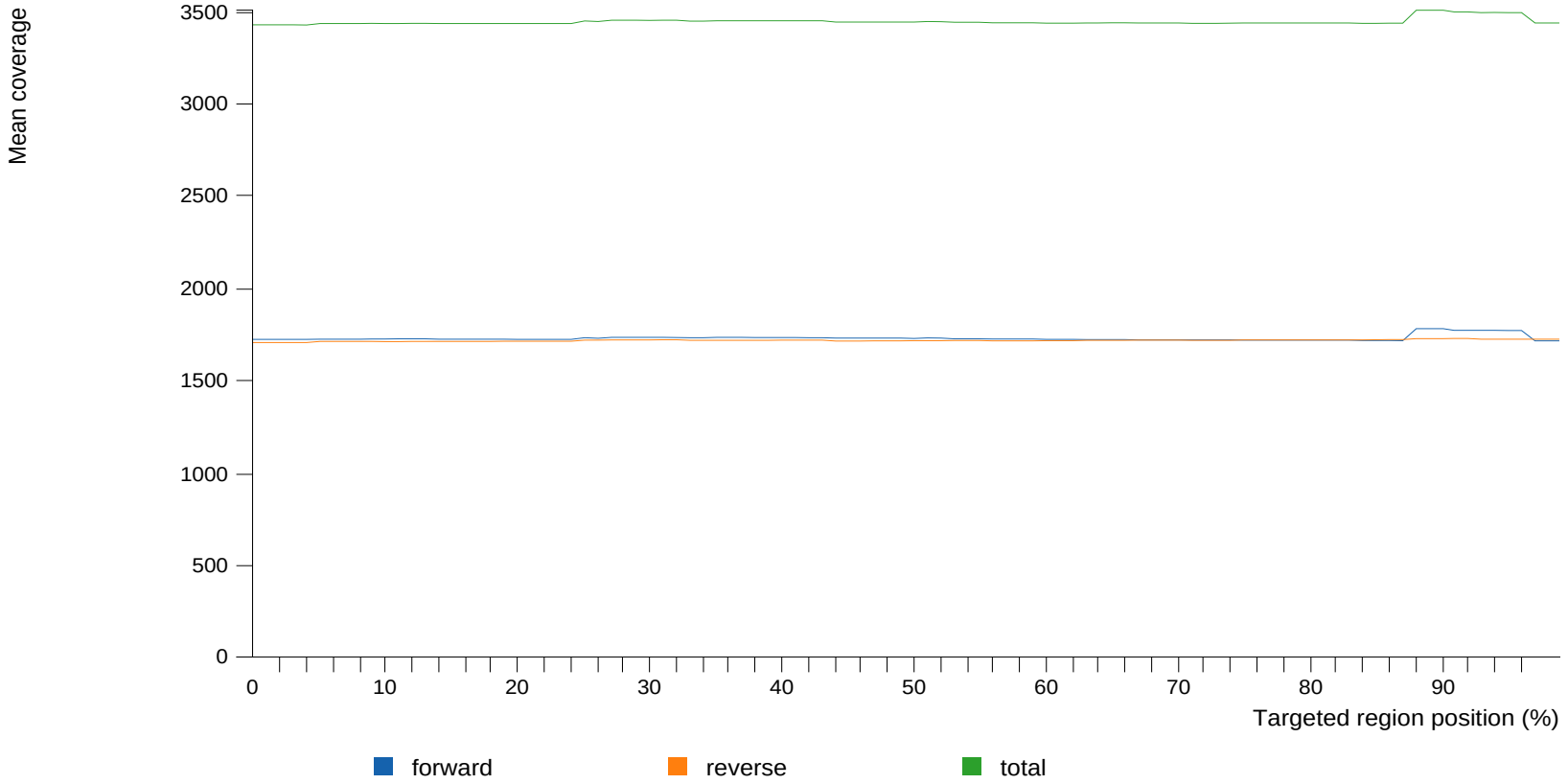
No ambiguous bases detected

4.3 Coverage of Regions of Interest positions

Coverage distribution



4.4 Mean coverage of relative positions in regions of interest



5 History

5.1 Log Entries

Type	Time	User	Details
State change	Sat Jun 29 18:10:45 CEST 2019	system	Ready for Review
State change	Sat Jun 29 17:54:35 CEST 2019	root	In Progress

5.2 Execution Information

QCIA version	QCI Analyze 1.4.5
Analysis start time	Sat Jun 29 17:54:35 CEST 2019
Analysis workflow	AIT FFPE 4.5
Analysis description	QIAact Actionable Insights Tumor Panel on FFPE

5.3 Transcripts

Table listing the genes, transcript IDs and protein IDs used in the analysis.

Gene Name	Transcript ID	Protein ID
NRAS	NM_002524.4	NP_002515.1
ALK	NM_004304.4	NP_004295.2
RAF1	NM_002880.3	NP_002871.1
PIK3CA	NM_006218.2	NP_006209.2
PDGFRA	NM_006206.4	NP_006197.1
KIT	NM_000222.2	NP_000213.1
ESR1	NM_001122742.1	NP_001116214.1
EGFR	NM_005228.3	NP_005219.2
BRAF	NM_004333.4	NP_004324.2
KRAS	NM_004985.3	NP_004976.2
ERBB3	NM_001982.3	NP_001973.2

Gene Name	Transcript ID	Protein ID
ERBB2	NM_004448.2	NP_004439.2

5.4 Open Parameters

Workflow parameters that are editable by the administrator

Reported variants

Significant coverage threshold	500
SNV/MNV frequency threshold in %	4.00
Insertions, deletions and replacements frequency threshold in %	4.00

Variants available for review

Minimum coverage threshold	200
SNV/MNV frequency threshold in %	4.00
Insertions, deletions and replacements frequency threshold in %	4.00
Detect variants outside regions of interest	Yes

5.5 Locked Parameters

Adapter trimming

Trim adapter list	GRadapter_160913
Ambiguous trim	false
Ambiguous limit	2
Quality trim	false
Quality limit	0.05
Use colorspace	false

Also search on reversed sequence	false
Remove 5' terminal nucleotides	false
Number of 5' terminal nucleotides	1
Maximum number of nucleotides in reads	1000
Minimum number of nucleotides in reads	15
Discard short reads	false
Remove 3' terminal nucleotides	false
Number of 3' terminal nucleotides	1
Discard long reads	false

Map Reads to Reference

References	Homo_sapiens_sequence_hg19
Masking mode	No masking
Masking track	Not set
Match score	1
Mismatch cost	2
Cost of insertions and deletions	Affine gap cost
Insertion cost	3
Deletion cost	3
Insertion open cost	6
Insertion extend cost	1
Deletion open cost	6
Deletion extend cost	1
Length fraction	0.5
Similarity fraction	0.8
Global alignment	false
Color space alignment	false
Color error cost	3
Auto-detect paired distances	false
Non-specific match handling	Map randomly

InDels and Structural Variants

P-Value threshold	1.0E-4
Maximum number of mismatches	3
Ignore broken pairs	true
Minimum relative consensus coverage	0.0
Minimum quality score	0
Filter variants	true
Minimum number of reads	2
Restrict calling to target regions	ATPv2_TargetRegions_170302_ver1.1

Local Realignment (Short Unaligned End version)

Realign unaligned ends	true
Multi-pass realignment	2
Local bound for unaligned ends of size one	0.75
Local bound for unaligned ends of size two	0.75
Force realignment to guidance-variants	false
Maximum guidance-variant length	100

Trim Primers and their Dimers of Mapped Reads

Primer track	101x_GR_primers_15_10_15_V1.0
Reference	Homo_sapiens_sequence_hg19
Minimum primer overlap length	9
Allow dangling 3' end base	true
Minimal primer overlap fraction	0.7
Only keep reads that have hit a primer	true
Additional bases to trim	1

Remove Pseudogene Reads

Genes track	AITv2_PseudoGenes_170912_ver1.0
Gene and pseudogene links	KRAS -> KRASP1
Required unaligned ends %	2.0

Low Frequency Variant Detection

Required significance (%)	0.01
Ignore positions with coverage above	1000000000
Restrict calling to target regions	ATPv2_TargetRegions_170302_ver1.1
Ignore broken pairs	true
Ignore non-specific matches	Reads
Minimum read length	20
Minimum coverage	Parameter editable by administrator
Minimum count	8
Minimum frequency (%)	Parameter editable by administrator
Base quality filter	false
Neighborhood radius	5
Minimum central quality	5
Minimum neighborhood quality	5
Read direction filter	false
Direction frequency (%)	5.0
Relative read direction filter	false
Significance (%)	1.0E-5
Read position filter	false
Significance (%)	1.0
Remove pyro-error variants	false
In homopolymer regions with minimum length	3
With frequency below	0.8

Remove False Positives

Minimum frequency (%)	Parameter editable by administrator
Minimum forward/reverse balance	0.05
Minimum average base quality	22.0
Variant frequency	true
Forward/reverse balance	false
Average base quality	true

Annotate Variants With Primers

Minimum coverage count	400
Minimum variant percentage	1.0
Minimum variant read count	2

YOUR LAB

Your Lab
1700 Lincoln Blvd, Suite 20, Redwood City, CA 94063
labx.com / (650) 484 4040
Additional Information

Test Performed: **Somatic Panel**

Report Date Mar 7, 2020
Status -

Patient		Client		Specimen	
Patient Name		Client		Accession ID	20190628103639_100160050701061 90147_475-19CTC_BC8_Glio2019
Date of Birth		Client ID			
Age	50	Physician			
Sex	Male	Pathologist		Specimen Collection	
Ethnicity	European			Accession	Mar 7, 2020
Diagnosis	Glioblastoma			Primary Tumor Site	Brain

Result: Negative

Genes Tested

Test information such as gene name and hot spot region can be included in this section.

Methods and Limitations

EXAMPLE Statement including sample type (FFPE, etc), method of extraction, amplification reactions, panel targeted regions, sequencing technology, etc. Additionally, a description of the data analysis software(s), genome of reference and the sensitivity of the methods should be described.

QIAGEN Clinical Insight (QCI™) is a variant analysis, interpretation and decision support tool for research and clinical labs analyzing human genetics data and is not intended to be used for diagnostic purposes. QCI Interpret software includes the following underlying databases, data reference sets and tools; QIAGEN Clinical Insight-Interpret (5.6.20200226), Ingenuity Knowledge Base (K-release), CADD (v1.4), Allele Frequency Community (2019-09-25), EVS (ESP6500SI-V2), Refseq Gene Model (2019-02-05), JASPAR (2013-11), Ingenuity Knowledge Base Snapshot Timestamp (2020-02-21 12:37:01.0), Vista Enhancer hg18 (2012-07), Vista Enhancer hg19 (2012-07), Clinical Trials (K-release), PolyPhen-2 (v2.2.2), 1000 Genome Frequency (phase3v5b), ExAC (0.3.1), iva (Nov 19 12:28 iva-1.0.1200.jar), PhyloP hg18 (2009-11), PhyloP hg19 (2009-11), DbSNP (151), TargetScan (7.2), GENCODE (Release 29), CentoMD (5.3), OMIM (May 26, 2017), gnomAD (2.1.1), BSIFT (2016-02-23), TCGA (2013-09-05), Clinvar (2019-06-05), DGV (2016-05-15), COSMIC (v89), HGMD (2019.2), SIFT4G (2016-02-23)

Clinical Significance of Variants Based on AMP / ASCO / CAP Guidelines*

Strong Significance	**Tier 1A**	• Biomarker predicts response or resistance to an FDA or EMA approved therapy, according to drug label or professional guidelines for this diagnosis • Biomarker included in professional guidelines is prognostic or diagnostic for this diagnosis
	Tier 1B	• Biomarker predicts response or resistance to a therapy for this diagnosis based on well-powered studies • Biomarker is prognostic or diagnostic for this diagnosis based on well-powered studies
Potential Significance	**Tier 2C**	• Biomarker is associated with response or resistance to an FDA or EMA approved therapy, according to drug label or professional guidelines but only for different diagnosis • Biomarker is an inclusion criterion for an active clinical trial • Biomarker is prognostic or diagnostic based on multiple small studies
	Tier 2D	• Biomarker shows plausible response or resistance based on case or preclinical studies • Biomarker may assist in disease diagnosis or prognosis based on small studies
Uncertain Significance	**Tier 3**	• Biomarker has uncertain clinical significance and not known to be likely benign or benign

**Adapted from PMID:27993330 jmd.amjpathol.org/article/S1525-1578(16)30223-9/pdf

Analysis Report

20190628103639_1001600507010619014 7_115-18CTC_BC3_Glio2019

Table of contents

1 Summary

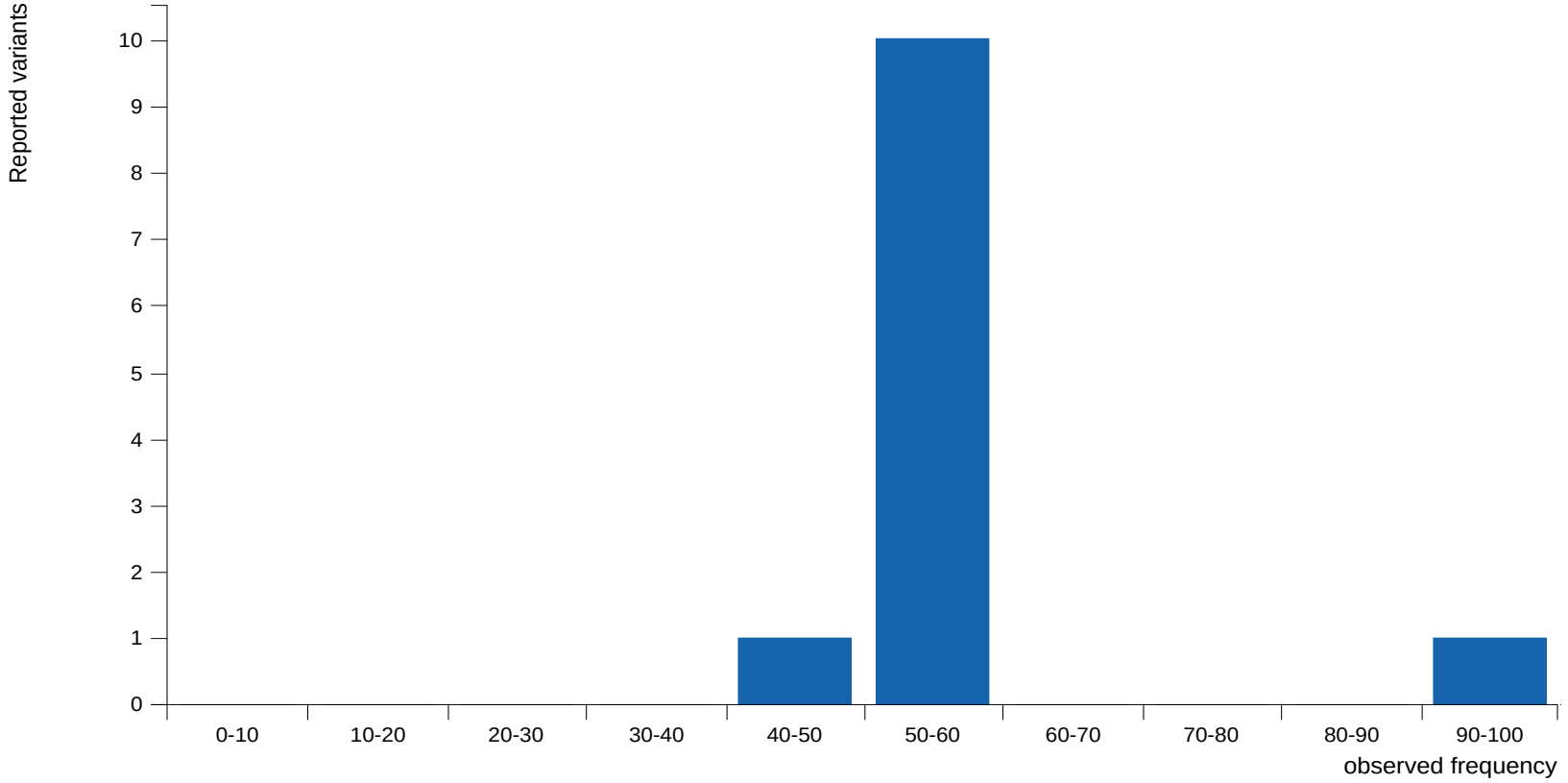
Report created	Sat Jun 29 18:08:07 CEST 2019
Sample ID	20190628103639_10016005070106190147_115-18CTC_BC3_Glio2019
Analysis workflow	AIT FFPE v4.5: QIAact Actionable Insights Tumor Panel on FFPE
Analyst	root
Reported variants	12
Analysis results	2 Untested variants

1.1 Comments

No comments

1.2 Distribution of observed frequencies for reported variants

Includes variants initially listed in variant table 'Reported variants'.



2 Quality control

Quality control for the sample analysis. Includes information on the input data, read mapping, and coverage information per gene.

2.1 Fastq

Fastq	20190628103639_10016005070106190147_115-18CTC_BC3_Glio2019
Reads	578,940
Nucleotides*	57,267,849
Average read length*	98.92
Reads with average quality ≥ 25	99.09%

** Including sample barcode*

Recommendations:

Reads with average quality ≥ 25 should be ≥ 80.00%

2.2 Secondary analysis summary

Reads mapped	545,334 (94.20%)
Reads in target regions	419,869 (76.99%)
Percentage of base positions in regions of interest with coverage ≥ 500x	97.11%
Percentage of base positions in regions of interest with coverage ≥ 200x	99.75%

Recommendations:

Percentage of base positions in regions of interest with coverage ≥ 500x should be ≥ 90.00%

Percentage of base positions in regions of interest with coverage ≥ 200x should be ≥ 95.00%

2.3 Coverage

Name	ROI	Bases	≥500x	≥200x	0x	Median	VOI	VOI <500x	VOI <200x
NRAS	6	27	100.00%	100.00%	0.00%	3,773	41	0	0
ALK	22	47	100.00%	100.00%	0.00%	1,556	40	0	0
RAF1	2	2	100.00%	100.00%	0.00%	1,886	2	0	0
PIK3CA	81	131	98.47%	100.00%	0.00%	2,488	165	2	0

Name	ROI	Bases	≥500x	≥200x	0x	Median	VOI	VOI <500x	VOI <200x
PDGFRA	21	64	100.00%	100.00%	0.00%	2,412	46	0	0
KIT	49	140	100.00%	100.00%	0.00%	2,648	235	0	0
ESR1	6	7	100.00%	100.00%	0.00%	1,639	11	0	0
EGFR	96	208	90.38%	99.04%	0.00%	1,398	443	21	2
BRAF	29	72	100.00%	100.00%	0.00%	2,853	153	0	0
KRAS	21	55	100.00%	100.00%	0.00%	3,200	148	0	0
ERBB3	8	8	100.00%	100.00%	0.00%	2,456	10	0	0
ERBB2	16	35	97.14%	100.00%	0.00%	3,652	61	1	0

ROI: Number of Regions of Interest, i.e. reportable regions that overlap with the gene.

Bases: Total number of base positions in Regions of Interest that overlap with the gene.

≥500x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is equal to or above the significant coverage threshold.

≥200x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is equal to or above the minimum coverage threshold.

0x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is zero.

Median: Median coverage of base positions in the Regions of Interest that overlap with the gene.

VOI: Total number of Variants of Interest, whether detected or not, that overlap with the gene. The list of Variants of Interest is defined by the analysis pipeline.

VOI <500x: Number of Variants of Interest in the gene for which coverage is below the significant coverage threshold.

VOI <200x: Number of Variants of Interest in the gene for which coverage is below the minimum coverage threshold.

2.4 Detected variants

Number of detected variants per gene. Variants for which coverage is above the minimum coverage threshold.

Name	In total	VOI	- non-syn	- syn	Non-VOI	- non-syn	- syn
NRAS	0	0	0	0	0	0	0
ALK	2	2	0	2	0	0	0
RAF1	0	0	0	0	0	0	0
PIK3CA	7	3	0	3	4	0	4
PDGFRA	5	3	0	3	2	0	2
KIT	0	0	0	0	0	0	0
ESR1	1	0	0	0	1	0	1
EGFR	6	2	0	2	4	0	4
BRAF	1	0	0	0	1	0	1

Name	In total	VOI	- non-syn	- syn	Non-VOI	- non-syn	- syn
KRAS	2	0	0	0	2	0	2
ERBB3	1	0	0	0	1	1	0
ERBB2	2	2	2	0	0	0	0

In total: Total number of variants detected within the gene. Variants initially listed in variant tables 3.1 and 3.2.

VOI: Number of detected Variants of Interest detected within the gene. The list of Variants of Interest is defined by the analysis pipeline.

- non-syn: Number of detected, gene-specific Variants of Interest that are non-synonymous.

- syn: Number of detected, gene-specific Variants of Interest that are synonymous.

Non-VOI: Number of detected variants that are not found within the analysis pipeline-defined list of Variants of Interest.

- non-syn: Number of gene-specific, non-VOIs that are non-synonymous.

- syn: Number of gene-specific, non-VOIs that are synonymous.

3 Variants

Variants detected within regions of interest with more than significant coverage are found in 3.1 and variants with more than minimum coverage are found in 3.2.
Variants of interest that could not be tested due to insufficient coverage are listed in table 3.3.
The coverage thresholds and minimum frequency cutoffs configured for the analysis workflow are listed in the History section.
Setting a variant review state to "Confirmed by review" moves it to 3.1, "Artifact" moves it to 3.2.
Only the variants in table 3.1 are exported as VCF and uploaded to QCI Interpret.

3.1 Reported variants

Variants that will be exported to VCF and uploaded to QCI Interpret. Initially contains: Variants detected within regions of interest with more than significant coverage and frequency above the cutoff set for the analysis workflow. These variants are assigned the initial review state "Valid".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
ALK	c.4338C>T		SNV	55.11%	29.66	1.00	1,838	Yes	Yes	Valid	
ALK	c.2535T>C		SNV	55.29%	31.05	1.00	2,040	Yes	Yes	Valid	
PIK3CA	c.-77+8483C>T		SNV	53.43%	33.63	0.95	1,445	Yes	Yes	Valid	
PIK3CA	c.-76-23509A>G		SNV	52.86%	34.36	1.00	3,485	Yes	Yes	Valid	
PIK3CA	c.-76-14537C>G		SNV	45.30%	32.08	0.76	1,733	Yes	Yes	Valid	
PDGFRA	c.612T>C		SNV	56.70%	30.33	1.00	1,986	Yes	Yes	Valid	
PDGFRA	c.939T>G		SNV	50.61%	29.92	0.92	1,474	Yes	Yes	Valid	
PDGFRA	c.1701A>G		SNV	99.85%	30.09	1.00	1,967	Yes	Yes	Valid	
EGFR	c.474C>T		SNV	54.83%	31.65	1.00	2,424	Yes	Yes	Valid	
EGFR	c.2361G>A		SNV	52.73%	31.58	1.00	768	Yes	Yes	Valid	
ERBB2	c.1963A>G	p.Ile655Val	SNV	52.93%	31.34	1.00	2,505	Yes	Yes	Valid	
ERBB2	c.3508C>G	p.Pro1170Ala	SNV	52.04%	24.99	0.99	1,249	Yes	Yes	Valid	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
ALK	c.4338C>T		No	1,013	611	402	200	29416615	2

ALK	c.2535T>C		No	1,128	494	634	200	29455267	2
PIK3CA	c.-77+8483C>T		No	772	214	558	200	178874874	3
PIK3CA	c.-76-23509A>G		No	1,842	1,218	624	200	178893029	3
PIK3CA	c.-76-14537C>G		No	785	250	535	200	178902001	3
PDGFRA	c.612T>C		No	1,126	296	830	200	55130078	4
PDGFRA	c.939T>G		No	746	68	678	200	55133726	4
PDGFRA	c.1701A>G		No	1,964	695	1,269	200	55141055	4
EGFR	c.474C>T		No	1,329	482	847	200	55214348	7
EGFR	c.2361G>A		No	405	228	177	200	55249063	7
ERBB2	c.1963A>G	mis-sense	No	1,326	562	764	200	37879588	17
ERBB2	c.3508C>G	mis-sense	No	650	41	609	200	37884037	17

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

Comment: Remark added by user during variant review.

3.2 Variants available for review

Detected variants that will not be exported to the VCF and uploaded to QCI Interpret. Initially contains: Variants with more than minimum coverage and frequency above the cutoff set for the analysis workflow. Depending on workflow configuration, this table may include variants outside of regions of interest including those with coverage above significant coverage threshold. These variants are assigned the initial review state "Review".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
PIK3CA	c.-76-18864C>A		SNV	53.72%	32.39	1.00	2,193	No	No	Review	
PIK3CA	c.353-65delA		Deletion	4.58%	33.54	1.00	524	No	No	Review	
PIK3CA	c.1060-17C>A		SNV	99.73%	32.56	1.00	1,863	No	No	Review	
PIK3CA	c.1145+54A>G		SNV	98.42%	33.12	1.00	379	No	No	Review	
PDGFRA	c.2440-50_2440-49insA		Insertion	99.60%	33.06	1.00	1,001	No	No	Review	
PDGFRA	c.3222T>C		SNV	99.61%	31.54	1.00	2,281	No	No	Review	
ESR1	c.975G>C		SNV	99.48%	31.11	1.00	1,737	No	No	Review	
EGFR	c.890-4C>G		SNV	8.44%	32.56	0.61	1,173	No	No	Review	
EGFR	c.1498+22A>T		SNV	99.46%	33.14	1.00	2,049	No	No	Review	
EGFR	c.2047C>T		SNV	30.54%	29.49	1.66E-3	645	No	No	Review	
EGFR	c.2709T>C		SNV	97.73%	30.26	1.00	2,506	No	No	Review	
BRAF	c.1860+66A>C		SNV	32.30%	36.50	1.00	387	No	No	Review	
KRAS	c.534A>G		SNV	4.00%	31.86	2.72E-12	2,523	No	No	Review	
KRAS	c.291-10delT		Deletion	4.15%	30.22	1.00	554	No	No	Review	
ERBB3	c.1397A>G	p.His466Arg	SNV	47.26%	30.92	1.00	1,462	No	No	Review	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
PIK3CA	c.-76-18864C>A		No	1,178	328	850	200	178897674	3
PIK3CA	c.353-65delA		Yes	24	24	0	200	178917413	3
PIK3CA	c.1060-17C>A		No	1,858	1,187	671	200	178922274	3

PIK3CA	c.1145+54A>G		No	373	0	373	200	178922430	3
PDGFRA	c.2440-50_2440-49insA		No	997	552	445	200	55151958^55151959	4
PDGFRA	c.3222T>C		No	2,272	855	1,417	200	55161391	4
ESR1	c.975G>C		No	1,728	964	764	200	152265522	6
EGFR	c.890-4C>G		No	99	99	0	200	55223519	7
EGFR	c.1498+22A>T		No	2,038	953	1,085	200	55228053	7
EGFR	c.2047C>T		No	197	150	47	200	55240803	7
EGFR	c.2709T>C		No	2,449	1,968	481	200	55266417	7
BRAF	c.1860+66A>C		No	125	125	0	200	140453009	7
KRAS	c.534A>G		No	101	98	3	200	25362762	12
KRAS	c.291-10delT		Yes	23	0	23	200	25378717	12
ERBB3	c.1397A>G	mis-sense	No	691	289	402	200	56487251	12

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

Comment: Remark added by user during variant review.

3.3 Untested variants

Variants of interest that could not be tested due to insufficient coverage. These variants are assigned the initial review state "Untested".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
EGFR	c.2184+19G>A		SNV	0.00%			82	Yes	Yes	Untested	
EGFR	c.2596G>A	p.Glu866Lys	SNV	0.00%			186	Yes	Yes	Untested	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
EGFR	c.2184+19G>A			0	0	0		55241755	7
EGFR	c.2596G>A	mis-sense		0	0	0		55259538	7

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

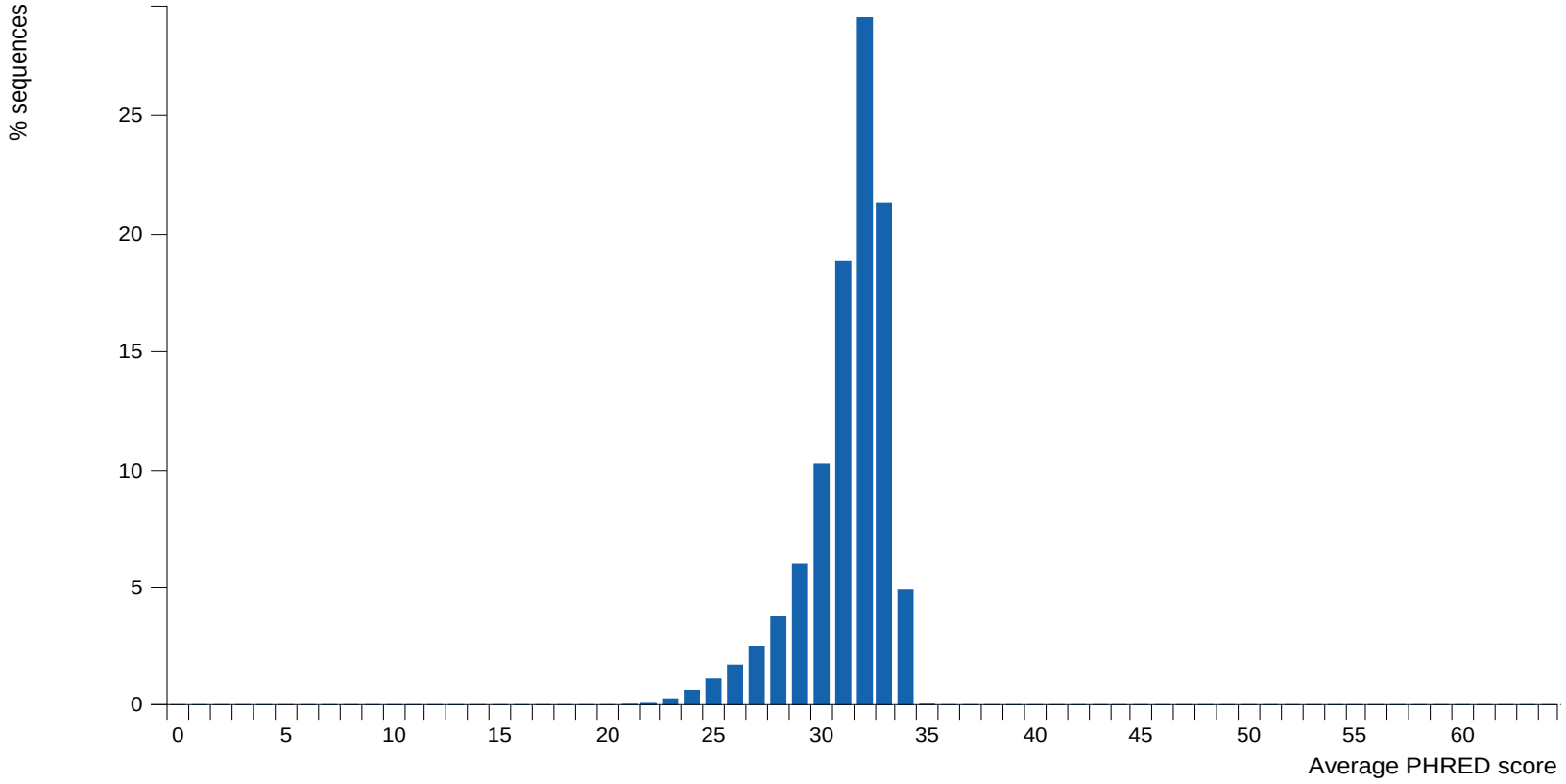
Comment: Remark added by user during variant review.

4 Detailed QC

Quality control metrics for detailed inspection. These metrics can indicate possible problems in the upstream workflow or data analysis. Quality control are divided in metrics on the incoming reads from input data, and metrics per base positions in these reads. Lastly section 4.3 and 4.4 display metrics on how well the positions in the region of interest are covered

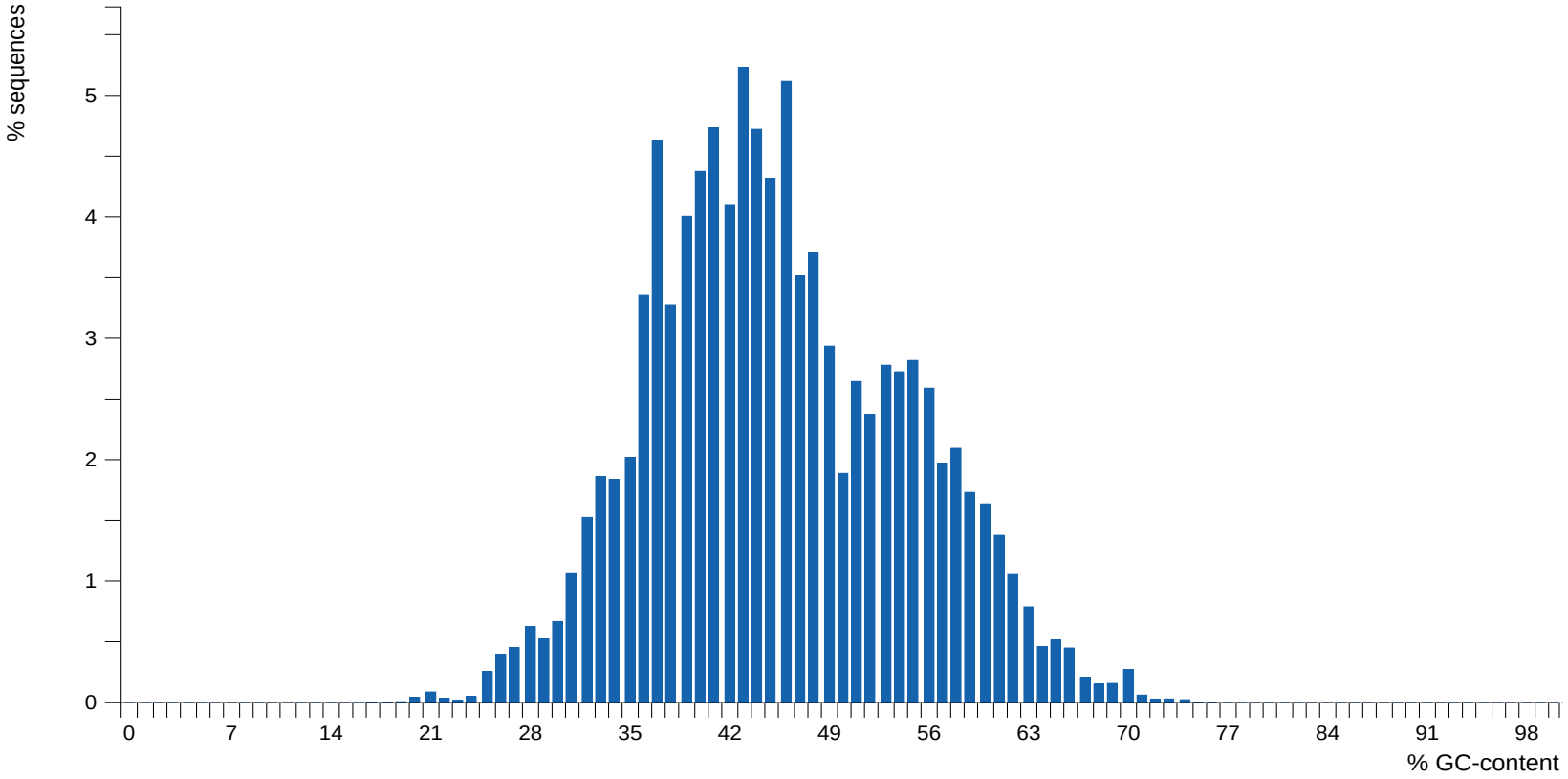
4.1 QC for reads

4.1.1 Average base quality of reads



Distribution of average sequence quality scores. The quality of a sequence is calculated as the arithmetic mean of its base qualities.
x: PHRED-score
y: number of sequences observed at that qual. score normalized to the total number of sequences

4.1.2 GC content of reads



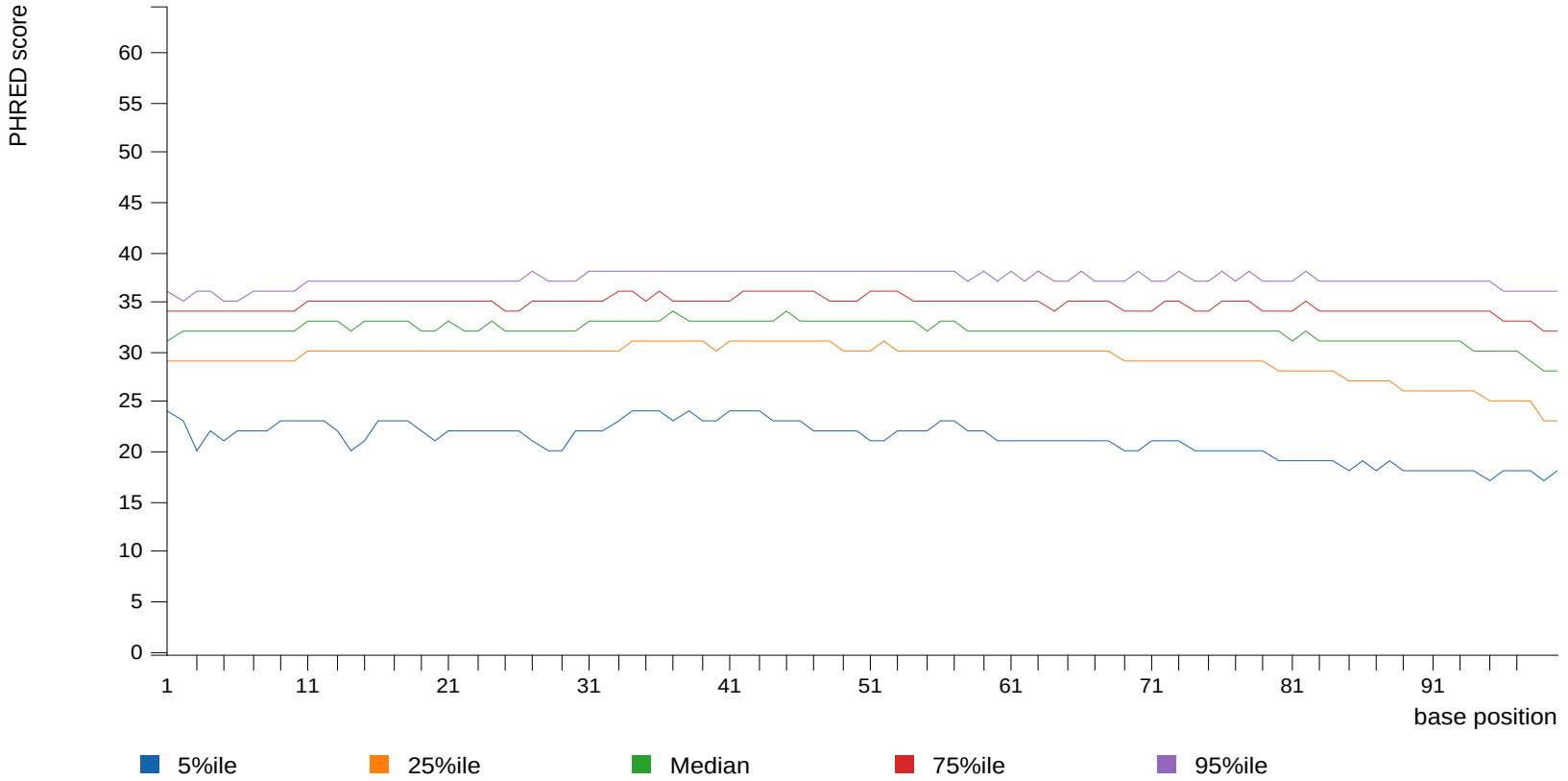
Distribution of GC-contents. The GC-content of a sequence is calculated as the number of GC-bases compared to all bases (including ambiguous bases).
x: relative GC-content of a sequence in percent
y: number of sequences featuring particular GC-percentages normalized to the total number of sequences

4.1.3 Ambiguous base content of reads

No ambiguous bases detected.

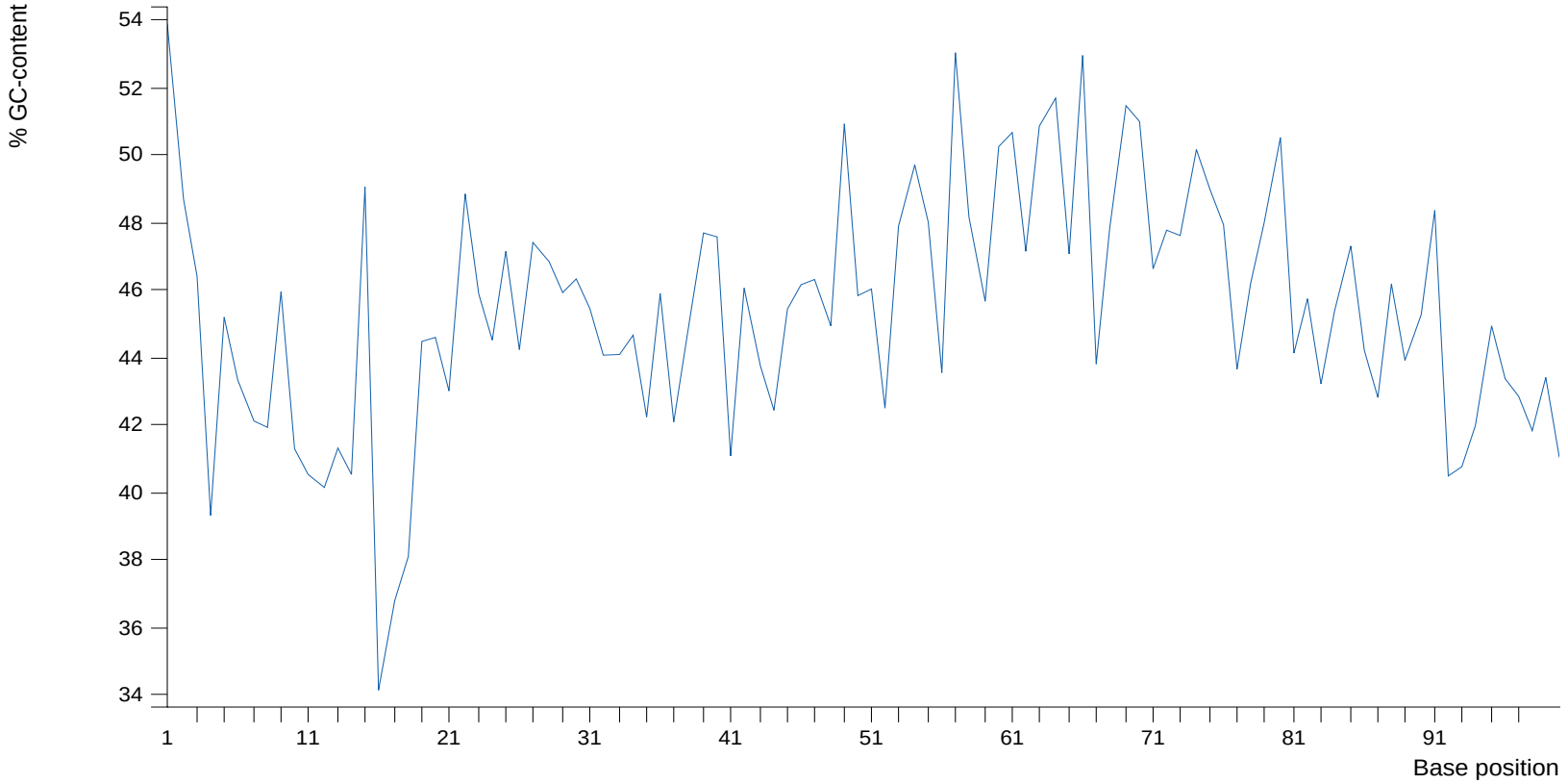
4.2 QC for bases

4.2.1 Quality score per base position



Base-quality distribution along the base positions.
x: base position
y: median & percentiles of quality scores observed at that base position

4.2.2 GC content per base position



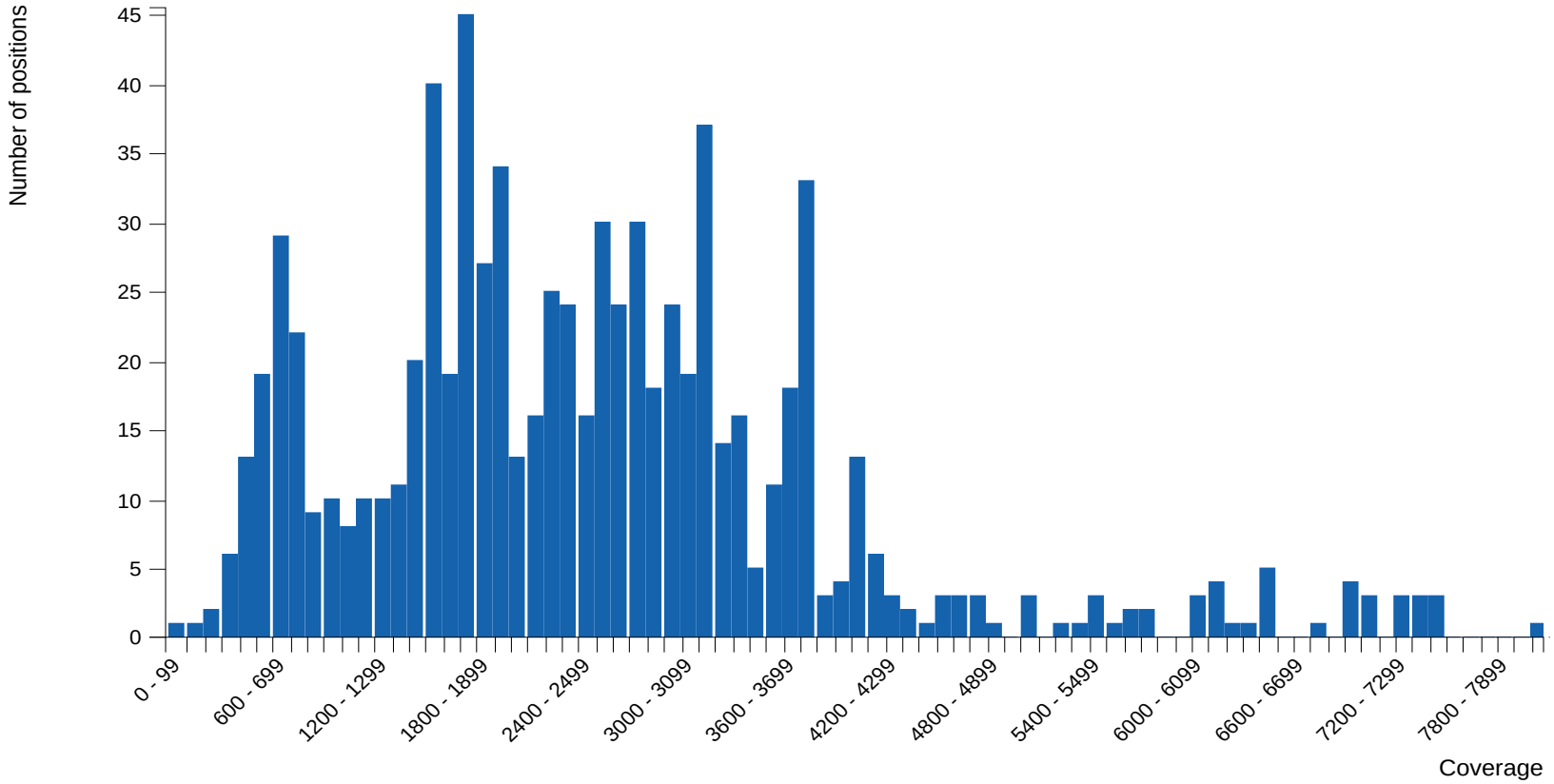
Combined coverage of G- and C-bases.
x: base position
y: number of G- and C-bases observed at current position normalized to the total number of bases observed at that position

4.2.3 Ambiguous base content per base position

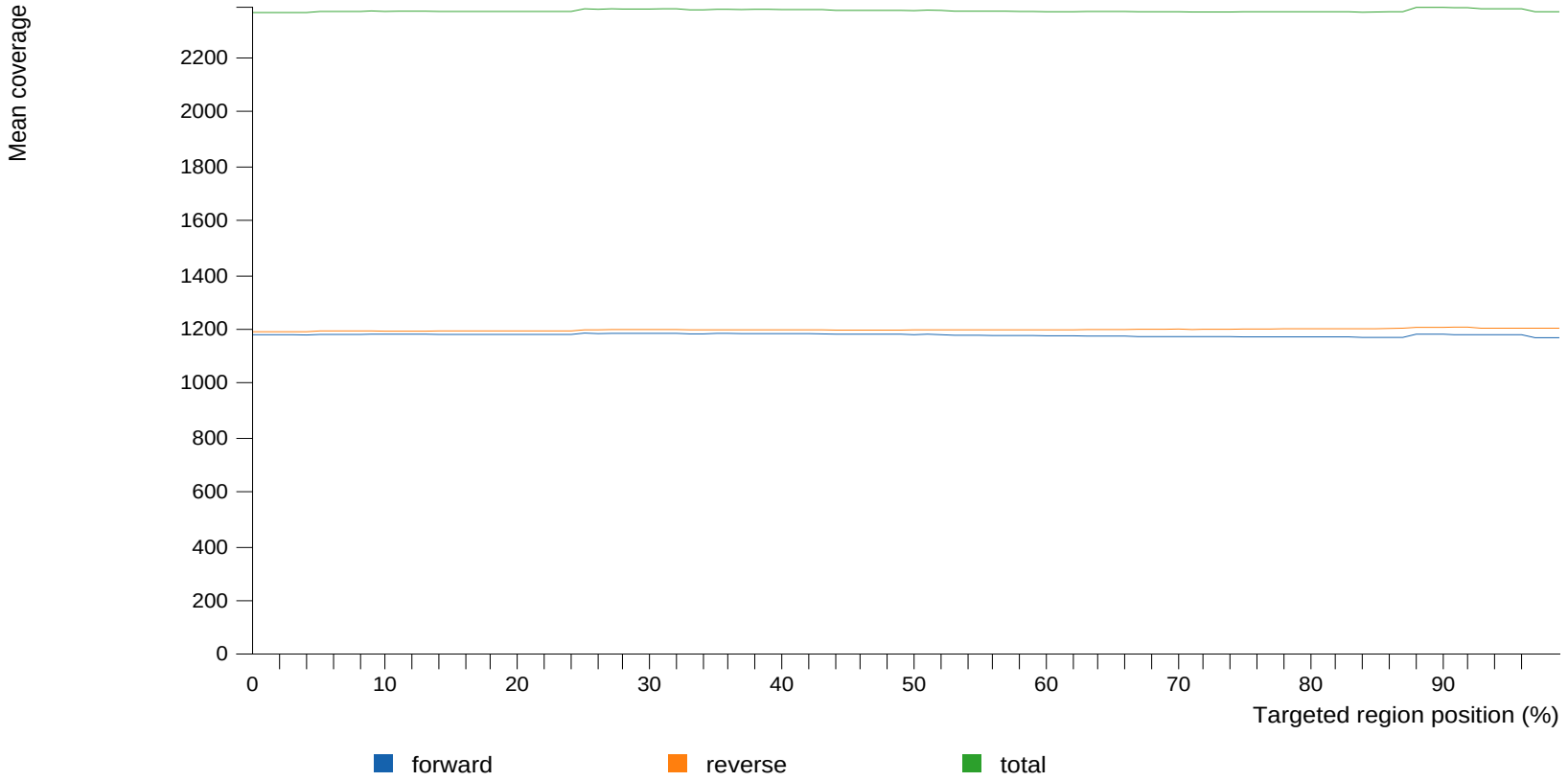
No ambiguous bases detected

4.3 Coverage of Regions of Interest positions

Coverage distribution



4.4 Mean coverage of relative positions in regions of interest



5 History

5.1 Log Entries

Type	Time	User	Details
State change	Sat Jun 29 18:08:19 CEST 2019	system	Ready for Review
State change	Sat Jun 29 17:54:03 CEST 2019	root	In Progress

5.2 Execution Information

QCIA version	QCI Analyze 1.4.5
Analysis start time	Sat Jun 29 17:54:03 CEST 2019
Analysis workflow	AIT FFPE 4.5
Analysis description	QIAact Actionable Insights Tumor Panel on FFPE

5.3 Transcripts

Table listing the genes, transcript IDs and protein IDs used in the analysis.

Gene Name	Transcript ID	Protein ID
NRAS	NM_002524.4	NP_002515.1
ALK	NM_004304.4	NP_004295.2
RAF1	NM_002880.3	NP_002871.1
PIK3CA	NM_006218.2	NP_006209.2
PDGFRA	NM_006206.4	NP_006197.1
KIT	NM_000222.2	NP_000213.1
ESR1	NM_001122742.1	NP_001116214.1
EGFR	NM_005228.3	NP_005219.2
BRAF	NM_004333.4	NP_004324.2
KRAS	NM_004985.3	NP_004976.2
ERBB3	NM_001982.3	NP_001973.2

Gene Name	Transcript ID	Protein ID
ERBB2	NM_004448.2	NP_004439.2

5.4 Open Parameters

Workflow parameters that are editable by the administrator

Reported variants

Significant coverage threshold	500
SNV/MNV frequency threshold in %	4.00
Insertions, deletions and replacements frequency threshold in %	4.00

Variants available for review

Minimum coverage threshold	200
SNV/MNV frequency threshold in %	4.00
Insertions, deletions and replacements frequency threshold in %	4.00
Detect variants outside regions of interest	Yes

5.5 Locked Parameters

Adapter trimming

Trim adapter list	GRadapter_160913
Ambiguous trim	false
Ambiguous limit	2
Quality trim	false
Quality limit	0.05
Use colorspace	false

Also search on reversed sequence	false
Remove 5' terminal nucleotides	false
Number of 5' terminal nucleotides	1
Maximum number of nucleotides in reads	1000
Minimum number of nucleotides in reads	15
Discard short reads	false
Remove 3' terminal nucleotides	false
Number of 3' terminal nucleotides	1
Discard long reads	false

Map Reads to Reference

References	Homo_sapiens_sequence_hg19
Masking mode	No masking
Masking track	Not set
Match score	1
Mismatch cost	2
Cost of insertions and deletions	Affine gap cost
Insertion cost	3
Deletion cost	3
Insertion open cost	6
Insertion extend cost	1
Deletion open cost	6
Deletion extend cost	1
Length fraction	0.5
Similarity fraction	0.8
Global alignment	false
Color space alignment	false
Color error cost	3
Auto-detect paired distances	false
Non-specific match handling	Map randomly

InDels and Structural Variants

P-Value threshold	1.0E-4
Maximum number of mismatches	3
Ignore broken pairs	true
Minimum relative consensus coverage	0.0
Minimum quality score	0
Filter variants	true
Minimum number of reads	2
Restrict calling to target regions	ATPv2_TargetRegions_170302_ver1.1

Local Realignment (Short Unaligned End version)

Realign unaligned ends	true
Multi-pass realignment	2
Local bound for unaligned ends of size one	0.75
Local bound for unaligned ends of size two	0.75
Force realignment to guidance-variants	false
Maximum guidance-variant length	100

Trim Primers and their Dimers of Mapped Reads

Primer track	101x_GR_primers_15_10_15_V1.0
Reference	Homo_sapiens_sequence_hg19
Minimum primer overlap length	9
Allow dangling 3' end base	true
Minimal primer overlap fraction	0.7
Only keep reads that have hit a primer	true
Additional bases to trim	1

Remove Pseudogene Reads

Genes track	AITv2_PseudoGenes_170912_ver1.0
Gene and pseudogene links	KRAS -> KRASP1
Required unaligned ends %	2.0

Low Frequency Variant Detection

Required significance (%)	0.01
Ignore positions with coverage above	1000000000
Restrict calling to target regions	ATPv2_TargetRegions_170302_ver1.1
Ignore broken pairs	true
Ignore non-specific matches	Reads
Minimum read length	20
Minimum coverage	Parameter editable by administrator
Minimum count	8
Minimum frequency (%)	Parameter editable by administrator
Base quality filter	false
Neighborhood radius	5
Minimum central quality	5
Minimum neighborhood quality	5
Read direction filter	false
Direction frequency (%)	5.0
Relative read direction filter	false
Significance (%)	1.0E-5
Read position filter	false
Significance (%)	1.0
Remove pyro-error variants	false
In homopolymer regions with minimum length	3
With frequency below	0.8

Remove False Positives

Minimum frequency (%)	Parameter editable by administrator
Minimum forward/reverse balance	0.05
Minimum average base quality	22.0
Variant frequency	true
Forward/reverse balance	false
Average base quality	true

Annotate Variants With Primers

Minimum coverage count	400
Minimum variant percentage	1.0
Minimum variant read count	2

Analysis Report

20190628103639_1001600507010619014 7_599-19CTC_BC9_Glio2019

Table of contents

1 Summary

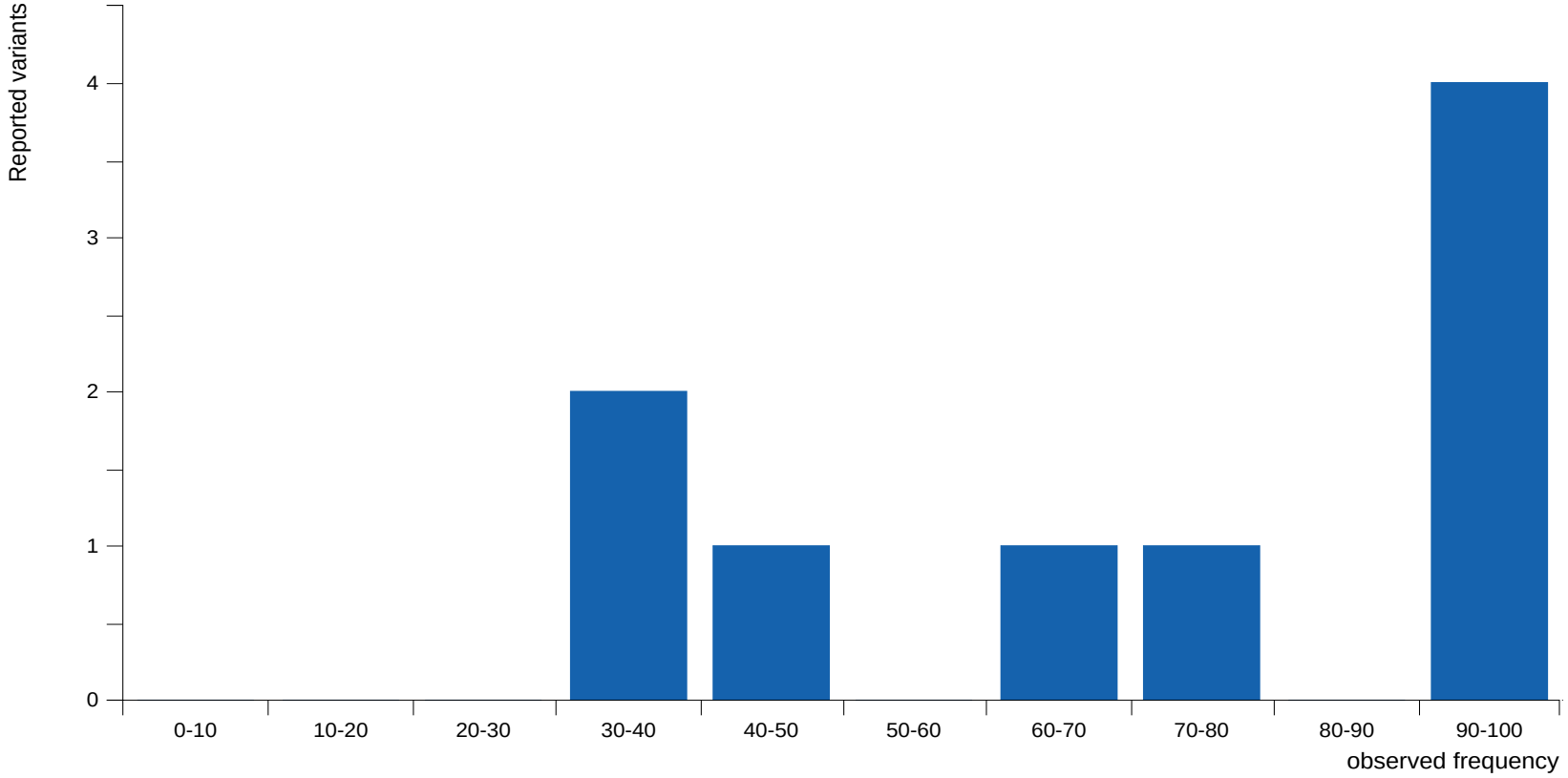
Report created	Fri Oct 25 17:39:03 CEST 2019
Sample ID	20190628103639_10016005070106190147_599-19CTC_BC9_Glio2019
Analysis workflow	AIT FFPE v4.5: QIAact Actionable Insights Tumor Panel on FFPE
Analyst	root
Reported variants	9
Analysis results	1 Untested variants

1.1 Comments

No comments

1.2 Distribution of observed frequencies for reported variants

Includes variants initially listed in variant table 'Reported variants'.



2 Quality control

Quality control for the sample analysis. Includes information on the input data, read mapping, and coverage information per gene.

2.1 Fastq

Fastq	20190628103639_10016005070106190147_599-19CTC_BC9_Glio2019
Reads	1,274,597
Nucleotides*	120,821,942
Average read length*	94.79
Reads with average quality ≥ 25	97.94%

** Including sample barcode*

Recommendations:

Reads with average quality ≥ 25 should be ≥ 80.00%

2.2 Secondary analysis summary

Reads mapped	998,472 (78.34%)
Reads in target regions	460,762 (46.15%)
Percentage of base positions in regions of interest with coverage ≥ 500x	98.74%
Percentage of base positions in regions of interest with coverage ≥ 200x	99.87%

Recommendations:

Percentage of base positions in regions of interest with coverage ≥ 500x should be ≥ 90.00%

Percentage of base positions in regions of interest with coverage ≥ 200x should be ≥ 95.00%

2.3 Coverage

Name	ROI	Bases	≥500x	≥200x	0x	Median	VOI	VOI <500x	VOI <200x
NRAS	6	27	100.00%	100.00%	0.00%	4,695	41	0	0
ALK	22	47	100.00%	100.00%	0.00%	1,872	40	0	0
RAF1	2	2	100.00%	100.00%	0.00%	2,338	2	0	0
PIK3CA	81	131	98.47%	100.00%	0.00%	2,238	165	2	0

Name	ROI	Bases	≥500x	≥200x	0x	Median	VOI	VOI <500x	VOI <200x
PDGFRA	21	64	100.00%	100.00%	0.00%	2,166	46	0	0
KIT	49	140	100.00%	100.00%	0.00%	2,809	235	0	0
ESR1	6	7	100.00%	100.00%	0.00%	2,050	11	0	0
EGFR	96	208	96.63%	99.52%	0.00%	1,223	443	7	1
BRAF	29	72	100.00%	100.00%	0.00%	3,212	153	0	0
KRAS	21	55	100.00%	100.00%	0.00%	3,204	148	0	0
ERBB3	8	8	100.00%	100.00%	0.00%	1,786	10	0	0
ERBB2	16	35	97.14%	100.00%	0.00%	2,860	61	1	0

ROI: Number of Regions of Interest, i.e. reportable regions that overlap with the gene.

Bases: Total number of base positions in Regions of Interest that overlap with the gene.

≥500x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is equal to or above the significant coverage threshold.

≥200x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is equal to or above the minimum coverage threshold.

0x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is zero.

Median: Median coverage of base positions in the Regions of Interest that overlap with the gene.

VOI: Total number of Variants of Interest, whether detected or not, that overlap with the gene. The list of Variants of Interest is defined by the analysis pipeline.

VOI <500x: Number of Variants of Interest in the gene for which coverage is below the significant coverage threshold.

VOI <200x: Number of Variants of Interest in the gene for which coverage is below the minimum coverage threshold.

2.4 Detected variants

Number of detected variants per gene. Variants for which coverage is above the minimum coverage threshold.

Name	In total	VOI	- non-syn	- syn	Non-VOI	- non-syn	- syn
NRAS	1	0	0	0	1	1	0
ALK	4	2	0	2	2	1	1
RAF1	1	0	0	0	1	1	0
PIK3CA	8	1	0	1	7	0	7
PDGFRA	4	1	0	1	3	0	3
KIT	2	1	0	1	1	1	0
ESR1	3	1	0	1	2	1	1
EGFR	4	1	0	1	3	2	1
BRAF	1	0	0	0	1	0	1

Name	In total	VOI	- non-syn	- syn	Non-VOI	- non-syn	- syn
KRAS	7	1	0	1	6	1	5
ERBB3	2	0	0	0	2	1	1
ERBB2	1	1	1	0	0	0	0

In total: Total number of variants detected within the gene. Variants initially listed in variant tables 3.1 and 3.2.

VOI: Number of detected Variants of Interest detected within the gene. The list of Variants of Interest is defined by the analysis pipeline.

- non-syn: Number of detected, gene-specific Variants of Interest that are non-synonymous.

- syn: Number of detected, gene-specific Variants of Interest that are synonymous.

Non-VOI: Number of detected variants that are not found within the analysis pipeline-defined list of Variants of Interest.

- non-syn: Number of gene-specific, non-VOIs that are non-synonymous.

- syn: Number of gene-specific, non-VOIs that are synonymous.

3 Variants

Variants detected within regions of interest with more than significant coverage are found in 3.1 and variants with more than minimum coverage are found in 3.2.
Variants of interest that could not be tested due to insufficient coverage are listed in table 3.3.
The coverage thresholds and minimum frequency cutoffs configured for the analysis workflow are listed in the History section.
Setting a variant review state to "Confirmed by review" moves it to 3.1, "Artifact" moves it to 3.2.
Only the variants in table 3.1 are exported as VCF and uploaded to QCI Interpret.

3.1 Reported variants

Variants that will be exported to VCF and uploaded to QCI Interpret. Initially contains: Variants detected within regions of interest with more than significant coverage and frequency above the cutoff set for the analysis workflow. These variants are assigned the initial review state "Valid".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
ALK	c.3036G>A		SNV	41.88%	34.18	0.99	1,022	Yes	Yes	Valid	
ALK	c.2535T>C		SNV	77.68%	30.72	1.00	1,586	Yes	Yes	Valid	
PIK3CA	c.-76-14537C>G		SNV	32.47%	31.87	0.82	1,925	Yes	Yes	Valid	
PDGFRA	c.1701A>G		SNV	99.73%	30.00	1.00	2,605	Yes	Yes	Valid	
KIT	c.2362-77G>A		SNV	39.74%	34.62	0.62	702	Yes	Yes	Valid	
ESR1	c.30T>C		SNV	95.97%	29.30	1.00	968	Yes	Yes	Valid	
EGFR	c.474C>T		SNV	99.62%	31.67	1.00	2,091	Yes	Yes	Valid	
KRAS	c.*2505T>G		SNV	66.82%	33.76	1.00	2,601	Yes	Yes	Valid	
ERBB2	c.3508C>G	p.Pro1170Ala	SNV	94.75%	24.96	1.00	895	Yes	Yes	Valid	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
ALK	c.3036G>A		No	428	3	425	200	29449819	2
ALK	c.2535T>C		No	1,232	565	667	200	29455267	2
PIK3CA	c.-76-14537C>G		No	625	282	343	200	178902001	3
PDGFRA	c.1701A>G		No	2,598	951	1,647	200	55141055	4

KIT	c.2362-77G>A		No	279	181	98	200	55599159	4
ESR1	c.30T>C		No	929	523	406	200	152129077	6
EGFR	c.474C>T		No	2,083	695	1,388	200	55214348	7
KRAS	c.*2505T>G		No	1,738	953	785	200	25360224	12
ERBB2	c.3508C>G	mis-sense	No	848	108	740	200	37884037	17

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

Comment: Remark added by user during variant review.

3.2 Variants available for review

Detected variants that will not be exported to the VCF and uploaded to QCI Interpret. Initially contains: Variants with more than minimum coverage and frequency above the cutoff set for the analysis workflow. Depending on workflow configuration, this table may include variants outside of regions of interest including those with coverage above significant coverage threshold. These variants are assigned the initial review state "Review".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
NRAS	c.19G>C	p.Val7Leu	SNV	5.10%	29.22	0.00	5,350	No	No	Review	
ALK	c.4381A>G	p.Ile1461Val	SNV	100.00%	30.49	1.00	259	No	No	Review	
ALK	c.3306C>T		SNV	8.69%	32.98	1.00	495	No	No	Review	
RAF1	c.1883C>T	p.Ala628Val	SNV	7.10%	29.66	1.00	1,070	No	No	Review	
PIK3CA	c.1060-17C>A		SNV	65.04%	32.93	1.00	2,125	No	No	Review	
PIK3CA	c.1145+9G>A		SNV	5.64%	35.40	2.34E-3	851	No	No	Review	
PIK3CA	c.1145+14T>A		SNV	7.02%	32.85	8.50E-4	926	No	No	Review	
PIK3CA	c.1145+16_1145+17delGAinsTC		MNV	6.99%	29.82	1.36E-3	916	No	No	Review	
PIK3CA	c.1145+19T>A		SNV	6.80%	34.71	2.32E-3	970	No	No	Review	
PIK3CA	c.1145+54A>G		SNV	45.47%	33.13	1.00	640	No	No	Review	
PIK3CA	c.1404+13_1404+18delTTGTCAinsACTGTT		MNV	12.20%	31.38	0.86	295	No	No	Review	
PDGFRA	c.1654-40C>T		SNV	5.37%	33.53	1.00	1,416	No	No	Review	
PDGFRA	c.2440-50_2440-49insA		Insertion	98.97%	33.23	1.00	773	No	No	Review	
PDGFRA	c.3222T>C		SNV	99.61%	31.44	1.00	2,069	No	No	Review	
KIT	c.1982C>T	p.Thr661Ile	SNV	6.16%	30.75	1.00	844	No	No	Review	
ESR1	c.113C>T	p.Pro38Leu	SNV	9.02%	27.18	1.00	244	No	No	Review	
ESR1	c.975G>C		SNV	99.15%	31.37	1.00	1,534	No	No	Review	
EGFR	c.716G>A	p.Gly239Asp	SNV	7.02%	30.50	1.00	285	No	No	Review	
EGFR	c.890-4C>G		SNV	24.94%	32.36	0.39	1,937	No	No	Review	
EGFR	c.1573T>C	p.Ser525Pro	SNV	4.17%	28.46	0.96	575	No	No	Review	
BRAF	c.1860+66A>C		SNV	83.25%	36.49	1.00	967	No	No	Review	

KRAS	c.541_542delTCinsAG	p.Ser181Arg	MNV	8.05%	31.54	0.00	3,181	No	No	Review	
KRAS	c.534A>G		SNV	8.03%	31.43	0.00	3,288	No	No	Review	
KRAS	c.525A>G		SNV	6.68%	31.18	0.00	3,519	No	No	Review	
KRAS	c.522T>C		SNV	6.56%	35.36	0.00	3,659	No	No	Review	
KRAS	c.519T>C		SNV	6.46%	31.15	0.00	3,670	No	No	Review	
KRAS	c.516A>G		SNV	6.25%	29.54	0.00	3,857	No	No	Review	
ERBB3	c.948G>A		SNV	22.64%	26.47	0.05	349	No	No	Review	
ERBB3	c.3359G>A	p.Arg1120Lys	SNV	5.23%	34.36	1.00	1,338	No	No	Review	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
NRAS	c.19G>C	mis-sense	No	273	0	273	200	115258763	1
ALK	c.4381A>G	mis-sense	No	259	259	0	200	29416572	2
ALK	c.3306C>T		No	43	24	19	200	29446261	2
RAF1	c.1883C>T	mis-sense	No	76	39	37	200	12626077	3
PIK3CA	c.1060-17C>A		No	1,382	940	442	200	178922274	3
PIK3CA	c.1145+9G>A		No	48	0	48	200	178922385	3
PIK3CA	c.1145+14T>A		No	65	0	65	200	178922390	3
PIK3CA	c.1145+16_1145+17delGAinsTC		No	64	0	64	200	178922392..178922393	3
PIK3CA	c.1145+19T>A		No	66	0	66	200	178922395	3
PIK3CA	c.1145+54A>G		No	291	0	291	200	178922430	3
PIK3CA	c.1404+13_1404+18delTTGTCA insACTGTT		No	36	29	7	200	178928139..178928144	3
PDGFRA	c.1654-40C>T		No	76	54	22	200	55140968	4
PDGFRA	c.2440-50_2440-49insA		No	765	296	469	200	55151958^55151959	4
PDGFRA	c.3222T>C		No	2,061	733	1,328	200	55161391	4
KIT	c.1982C>T	mis-sense	No	52	0	52	200	55594279	4
ESR1	c.113C>T	mis-sense	No	22	0	22	200	152129160	6
ESR1	c.975G>C		No	1,521	838	683	200	152265522	6
EGFR	c.716G>A	mis-sense	No	20	0	20	200	55220326	7

EGFR	c.890-4C>G		No	483	483	0	200	55223519	7
EGFR	c.1573T>C	mis-sense	No	24	0	24	69	55229266	7
BRAF	c.1860+66A>C		No	805	805	0	200	140453009	7
KRAS	c.541_542delTCinsAG	mis-sense	No	256	256	0	200	25362754..25362755	12
KRAS	c.534A>G		No	264	259	5	200	25362762	12
KRAS	c.525A>G		No	235	229	6	200	25362771	12
KRAS	c.522T>C		No	240	237	3	200	25362774	12
KRAS	c.519T>C		No	237	236	1	200	25362777	12
KRAS	c.516A>G		No	241	236	5	200	25362780	12
ERBB3	c.948G>A		No	79	27	52	200	56482400	12
ERBB3	c.3359G>A	mis-sense	No	70	29	41	200	56495002	12

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

Comment: Remark added by user during variant review.

3.3 Untested variants

Variants of interest that could not be tested due to insufficient coverage. These variants are assigned the initial review state "Untested".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
EGFR	c.2184+19G>A		SNV	0.00%			114	Yes	Yes	Untested	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
EGFR	c.2184+19G>A			0	0	0		55241755	7

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

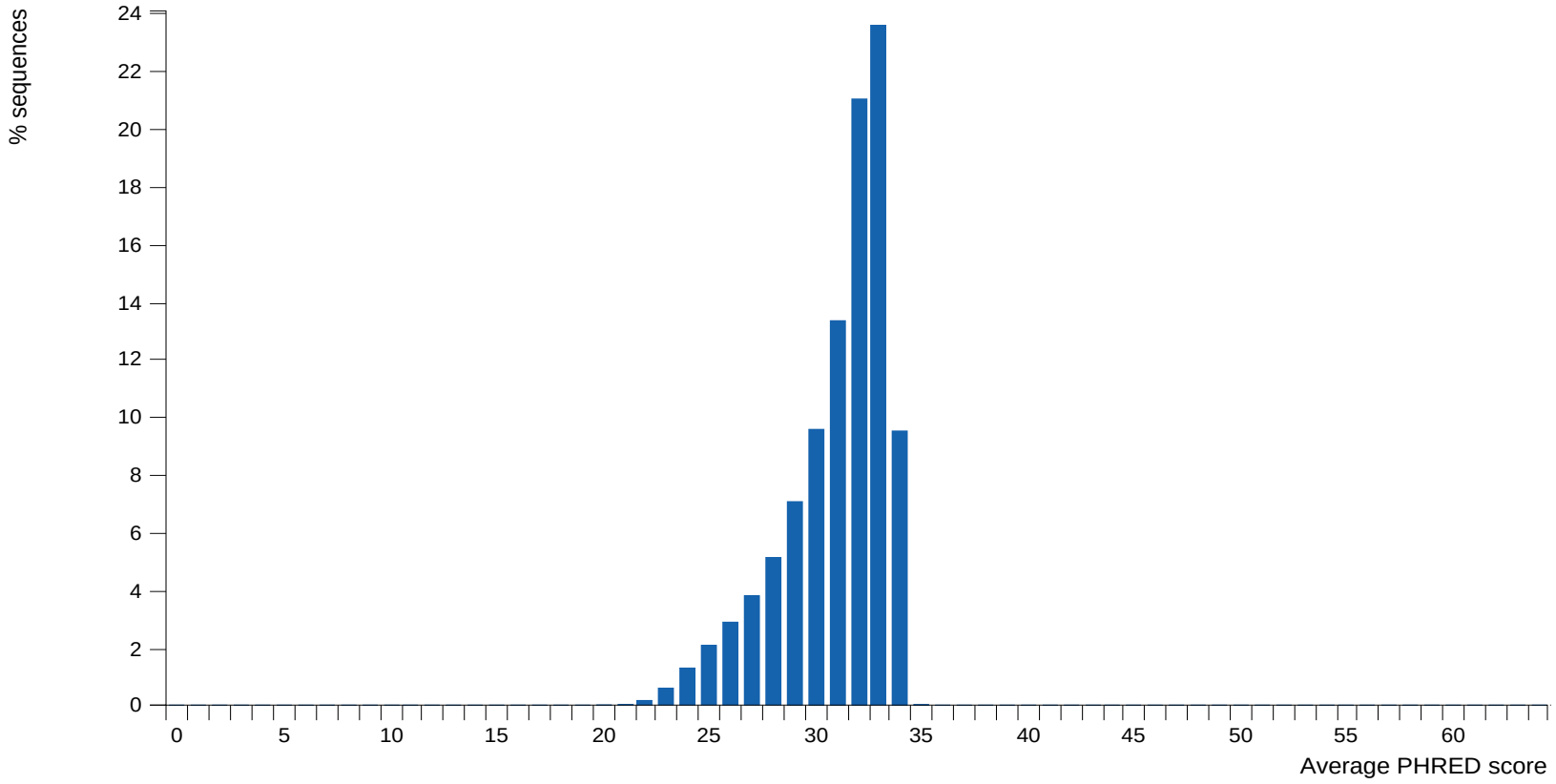
Comment: Remark added by user during variant review.

4 Detailed QC

Quality control metrics for detailed inspection. These metrics can indicate possible problems in the upstream workflow or data analysis. Quality control are divided in metrics on the incoming reads from input data, and metrics per base positions in these reads. Lastly section 4.3 and 4.4 display metrics on how well the positions in the region of interest are covered

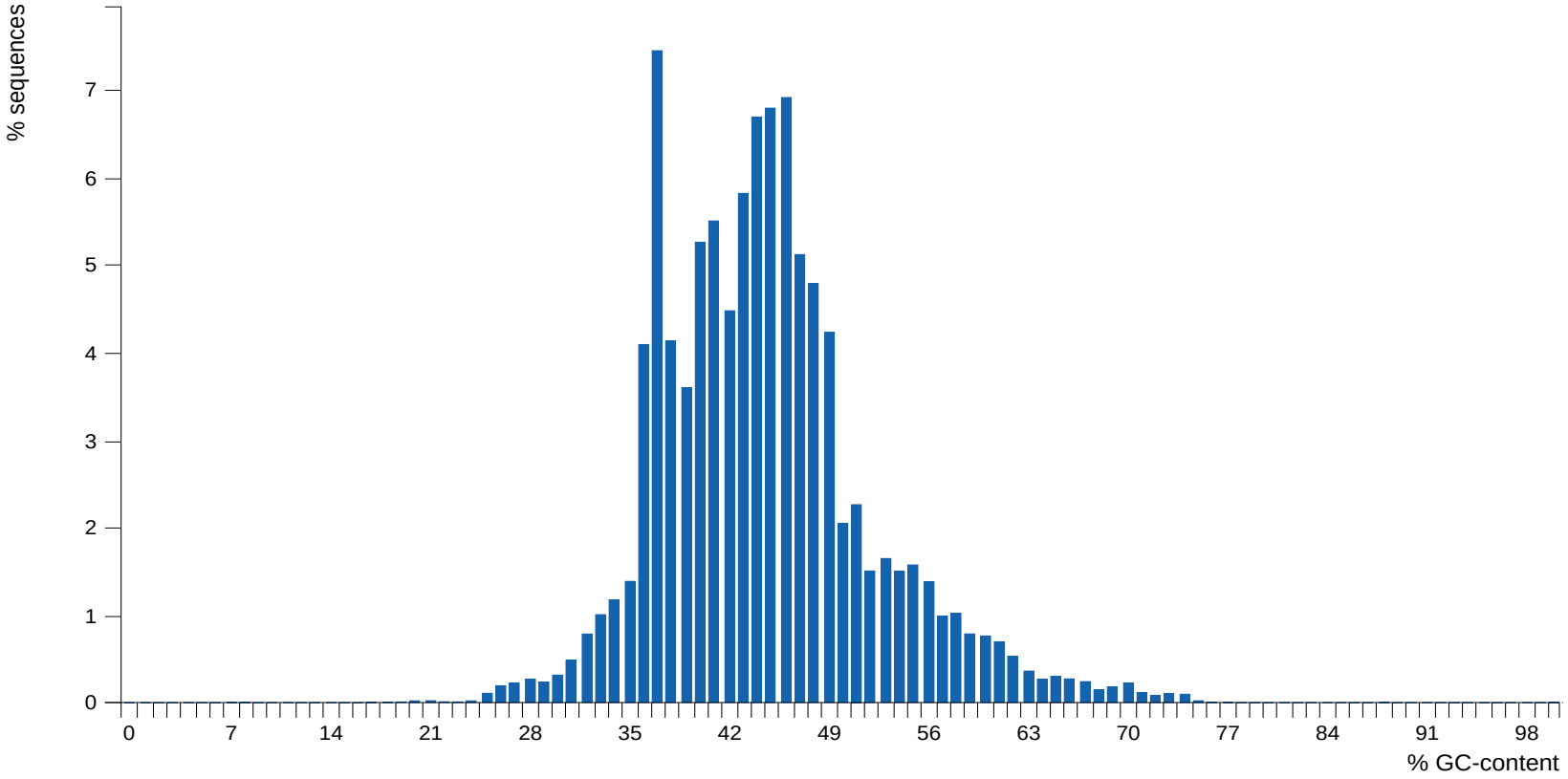
4.1 QC for reads

4.1.1 Average base quality of reads



Distribution of average sequence quality scores. The quality of a sequence is calculated as the arithmetic mean of its base qualities.
x: PHRED-score
y: number of sequences observed at that qual. score normalized to the total number of sequences

4.1.2 GC content of reads



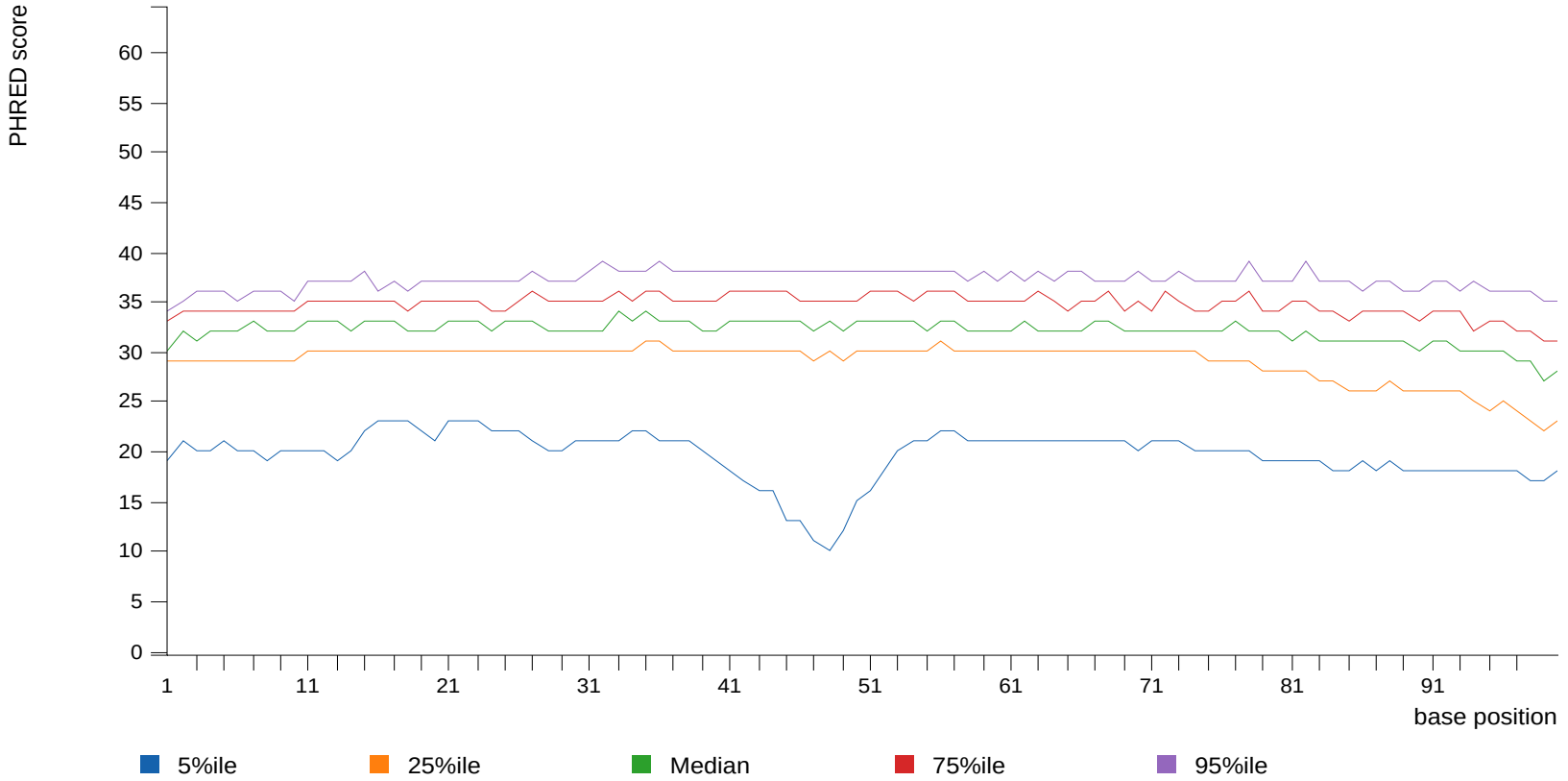
Distribution of GC-contents. The GC-content of a sequence is calculated as the number of GC-bases compared to all bases (including ambiguous bases).
x: relative GC-content of a sequence in percent
y: number of sequences featuring particular GC-percentages normalized to the total number of sequences

4.1.3 Ambiguous base content of reads

No ambiguous bases detected.

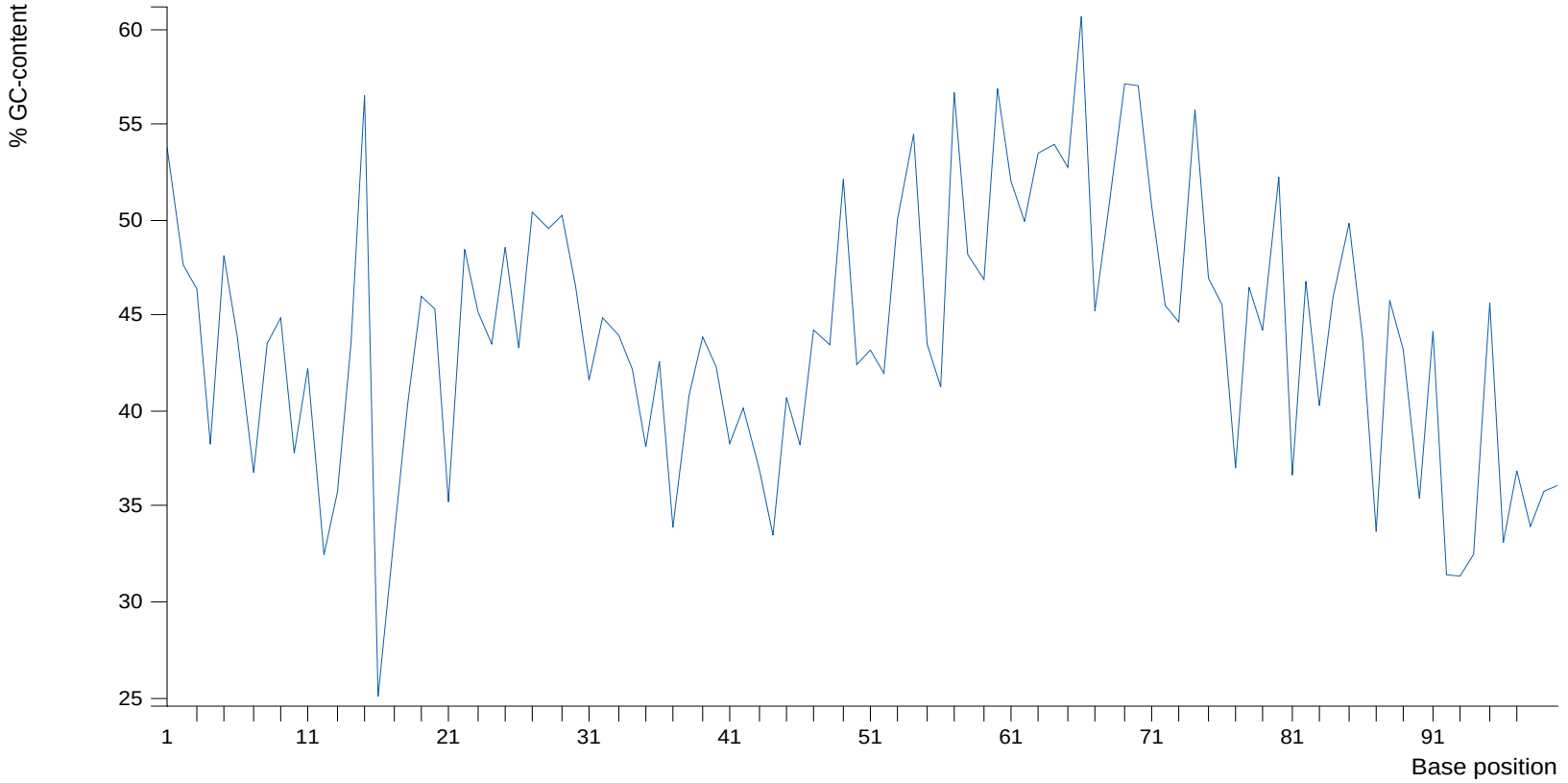
4.2 QC for bases

4.2.1 Quality score per base position



Base-quality distribution along the base positions.
x: base position
y: median & percentiles of quality scores observed at that base position

4.2.2 GC content per base position



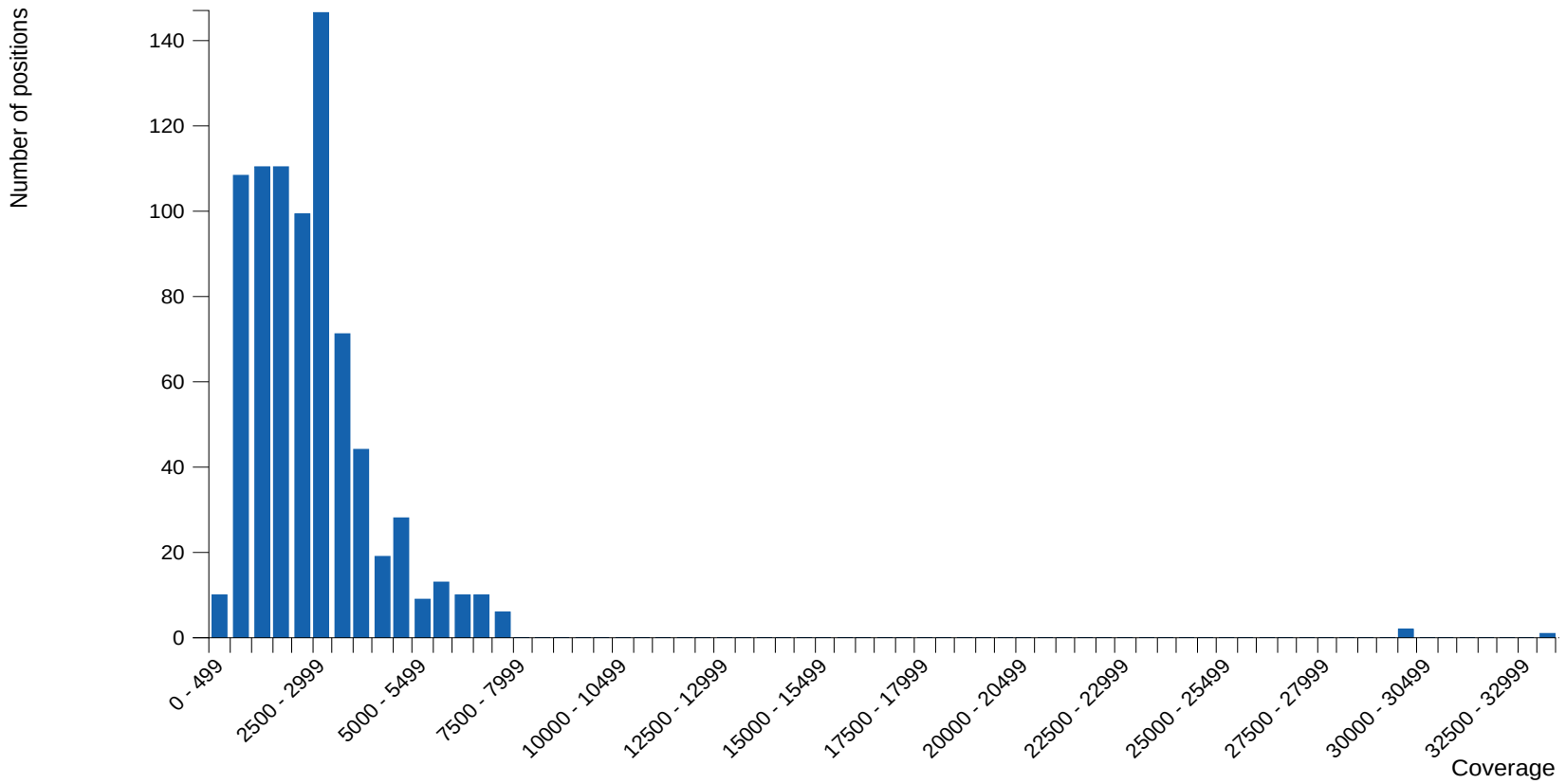
Combined coverage of G- and C-bases.
x: base position
y: number of G- and C-bases observed at current position normalized to the total number of bases observed at that position

4.2.3 Ambiguous base content per base position

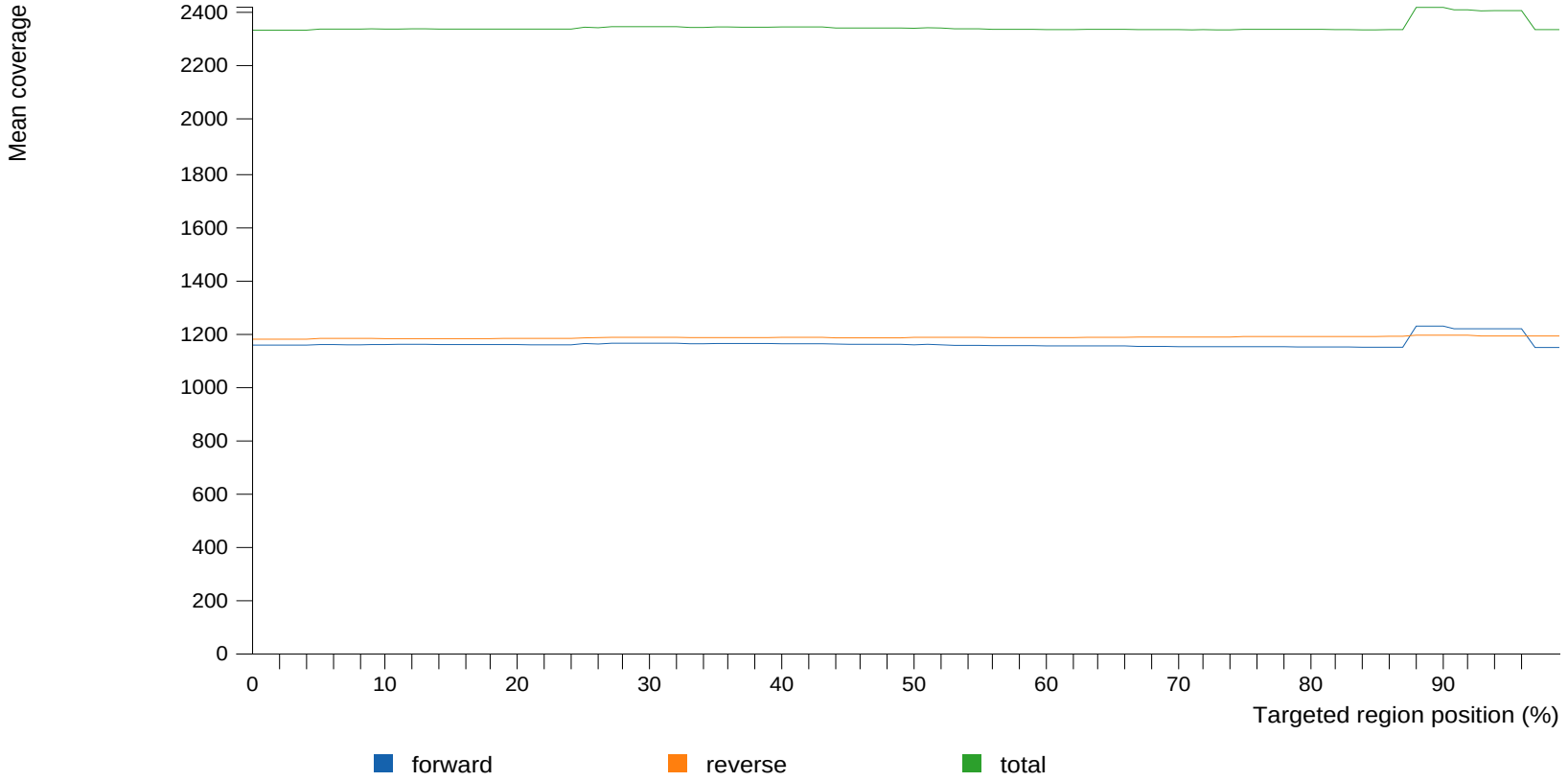
No ambiguous bases detected

4.3 Coverage of Regions of Interest positions

Coverage distribution



4.4 Mean coverage of relative positions in regions of interest



5 History

5.1 Log Entries

Type	Time	User	Details
State change	Fri Oct 25 17:39:14 CEST 2019	system	Ready for Review
State change	Fri Oct 25 17:16:00 CEST 2019	root	In Progress

5.2 Execution Information

QCIA version	QCI Analyze 1.4.5
Analysis start time	Fri Oct 25 17:16:00 CEST 2019
Analysis workflow	AIT FFPE 4.5
Analysis description	QIAact Actionable Insights Tumor Panel on FFPE

5.3 Transcripts

Table listing the genes, transcript IDs and protein IDs used in the analysis.

Gene Name	Transcript ID	Protein ID
NRAS	NM_002524.4	NP_002515.1
ALK	NM_004304.4	NP_004295.2
RAF1	NM_002880.3	NP_002871.1
PIK3CA	NM_006218.2	NP_006209.2
PDGFRA	NM_006206.4	NP_006197.1
KIT	NM_000222.2	NP_000213.1
ESR1	NM_001122742.1	NP_001116214.1
EGFR	NM_005228.3	NP_005219.2
BRAF	NM_004333.4	NP_004324.2
KRAS	NM_004985.3	NP_004976.2
ERBB3	NM_001982.3	NP_001973.2

Gene Name	Transcript ID	Protein ID
ERBB2	NM_004448.2	NP_004439.2

5.4 Open Parameters

Workflow parameters that are editable by the administrator

Reported variants

Significant coverage threshold	500
SNV/MNV frequency threshold in %	4.00
Insertions, deletions and replacements frequency threshold in %	4.00

Variants available for review

Minimum coverage threshold	200
SNV/MNV frequency threshold in %	4.00
Insertions, deletions and replacements frequency threshold in %	4.00
Detect variants outside regions of interest	Yes

5.5 Locked Parameters

Adapter trimming

Trim adapter list	GRadapter_160913
Ambiguous trim	false
Ambiguous limit	2
Quality trim	false
Quality limit	0.05
Use colorspace	false

Also search on reversed sequence	false
Remove 5' terminal nucleotides	false
Number of 5' terminal nucleotides	1
Maximum number of nucleotides in reads	1000
Minimum number of nucleotides in reads	15
Discard short reads	false
Remove 3' terminal nucleotides	false
Number of 3' terminal nucleotides	1
Discard long reads	false

Map Reads to Reference

References	Homo_sapiens_sequence_hg19
Masking mode	No masking
Masking track	Not set
Match score	1
Mismatch cost	2
Cost of insertions and deletions	Affine gap cost
Insertion cost	3
Deletion cost	3
Insertion open cost	6
Insertion extend cost	1
Deletion open cost	6
Deletion extend cost	1
Length fraction	0.5
Similarity fraction	0.8
Global alignment	false
Color space alignment	false
Color error cost	3
Auto-detect paired distances	false
Non-specific match handling	Map randomly

InDels and Structural Variants

P-Value threshold	1.0E-4
Maximum number of mismatches	3
Ignore broken pairs	true
Minimum relative consensus coverage	0.0
Minimum quality score	0
Filter variants	true
Minimum number of reads	2
Restrict calling to target regions	ATPv2_TargetRegions_170302_ver1.1

Local Realignment (Short Unaligned End version)

Realign unaligned ends	true
Multi-pass realignment	2
Local bound for unaligned ends of size one	0.75
Local bound for unaligned ends of size two	0.75
Force realignment to guidance-variants	false
Maximum guidance-variant length	100

Trim Primers and their Dimers of Mapped Reads

Primer track	101x_GR_primers_15_10_15_V1.0
Reference	Homo_sapiens_sequence_hg19
Minimum primer overlap length	9
Allow dangling 3' end base	true
Minimal primer overlap fraction	0.7
Only keep reads that have hit a primer	true
Additional bases to trim	1

Remove Pseudogene Reads

Genes track	AITv2_PseudoGenes_170912_ver1.0
Gene and pseudogene links	KRAS -> KRASP1
Required unaligned ends %	2.0

Low Frequency Variant Detection

Required significance (%)	0.01
Ignore positions with coverage above	1000000000
Restrict calling to target regions	ATPv2_TargetRegions_170302_ver1.1
Ignore broken pairs	true
Ignore non-specific matches	Reads
Minimum read length	20
Minimum coverage	Parameter editable by administrator
Minimum count	8
Minimum frequency (%)	Parameter editable by administrator
Base quality filter	false
Neighborhood radius	5
Minimum central quality	5
Minimum neighborhood quality	5
Read direction filter	false
Direction frequency (%)	5.0
Relative read direction filter	false
Significance (%)	1.0E-5
Read position filter	false
Significance (%)	1.0
Remove pyro-error variants	false
In homopolymer regions with minimum length	3
With frequency below	0.8

Remove False Positives

Minimum frequency (%)	Parameter editable by administrator
Minimum forward/reverse balance	0.05
Minimum average base quality	22.0
Variant frequency	true
Forward/reverse balance	false
Average base quality	true

Annotate Variants With Primers

Minimum coverage count	400
Minimum variant percentage	1.0
Minimum variant read count	2

YOUR LAB

Your Lab
1700 Lincoln Blvd, Suite 20, Redwood City, CA 94063
labx.com / (650) 484 4040
Additional Information

Test Performed: **Somatic Panel**

Report Date Mar 7, 2020
Status -

Specimen Information

Date of Birth	
Age	50
Sex	Male
Ethnicity	European
Diagnosis	Glioblastoma
Accession ID	20190628103639_1001600507010 6190147_868-18CTC_BC5_Glio2019
Specimen Collection	
Accession	Mar 7, 2020
Primary Tumor Site	Brain

Result: Negative

Genes Tested

Variants Tested

NRAS, ALK, RAF1, PIK3CA, PDGFRA, KIT, ESR1, EGFR, BRAF, KRAS, ERBB3, ERBB2

CNV Gain Tested

NRAS, ALK, RAF1, PIK3CA, PDGFRA, KIT, ESR1, EGFR, BRAF, KRAS, ERBB3, ERBB2

Methods and Limitations

EXAMPLE Statement including sample type (FFPE, etc), method of extraction, amplification reactions, panel targeted regions, sequencing technology, etc. Additionally, a description of the data analysis software(s), genome of reference and the sensitivity of the methods should be described.

QIAGEN Clinical Insight (QCI™) Interpret software includes the following underlying databases, data reference sets and tools; QIAGEN Clinical Insight-Interpret (5.6.20200226), Ingenuity Knowledge Base (K-release), CADD (v1.4), Allele Frequency Community (2019-09-25), EVS (ESP6500SI-V2), Refseq Gene Model (2019-02-05), JASPAR (2013-11), Ingenuity Knowledge Base Snapshot Timestamp (2020-02-21 12:37:01.0), Vista Enhancer hg18 (2012-07), Vista Enhancer hg19 (2012-07), Clinical Trials (K-release), PolyPhen-2 (v2.2.2), 1000 Genome Frequency (phase3v5b), ExAC (0.3.1), iva (Nov 19 12:28 iva-1.0.1200.jar), PhyloP hg18 (2009-11), PhyloP hg19 (2009-11), DbSNP (151), TargetScan (7.2), GENCODE (Release 29), CentoMD (5.3), OMIM (May 26, 2017), gnomAD (2.1.1), BSIFT (2016-02-23), TCGA (2013-09-05), Clinvar (2019-06-05), DGV (2016-05-15), COSMIC (v89), HGMD (2019.2), SIFT4G (2016-02-23)

Clinical Significance of Variants Based on AMP / ASCO / CAP Guidelines*

Strong Significance	**Tier 1A**	• Biomarker predicts response or resistance to an FDA or EMA approved therapy, according to drug label or professional guidelines for this diagnosis • Biomarker included in professional guidelines is prognostic or diagnostic for this diagnosis
	Tier 1B	• Biomarker predicts response or resistance to a therapy for this diagnosis based on well-powered studies • Biomarker is prognostic or diagnostic for this diagnosis based on well-powered studies
Potential Significance	**Tier 2C**	• Biomarker is associated with response or resistance to an FDA or EMA approved therapy, according to drug label or professional guidelines but only for different diagnosis • Biomarker is an inclusion criterion for an active clinical trial • Biomarker is prognostic or diagnostic based on multiple small studies
	Tier 2D	• Biomarker shows plausible response or resistance based on case or preclinical studies • Biomarker may assist in disease diagnosis or prognosis based on small studies
Uncertain Significance	**Tier 3**	• Biomarker has uncertain clinical significance and not known to be likely benign or benign

**Adapted from PMID:27993330 jmd.amjpathol.org/article/S1525-1578(16)30223-9/pdf

YOUR LAB

Your Lab
1700 Lincoln Blvd, Suite 20, Redwood City, CA 94063
labx.com / (650) 484 4040
Additional Information

Test Performed: **Somatic Panel**

Report Date Mar 7, 2020
Status -

Patient		Client		Specimen	
Patient Name		Client		Accession ID	20190628103639_100160050701061 90147_599-19CTC_BC9_Glio2019
Date of Birth		Client ID			
Age		Physician			
Sex		Pathologist		Specimen Collection	
Ethnicity				Accession	Mar 7, 2020
Diagnosis	Glioblastoma			Primary Tumor Site	Brain

Result: Inconclusive

Variants of Unknown Clinical Significance

Gene / Variant	Allelic Fraction	Function	Classification	Assessment
KIT c.1982C>T p.T661I g.55594279C>T	6.16% (of 844 reads)	loss	**Tier 3**	**Uncertain Significance**
NRAS c.19G>C p.V7L g.115258763C>G	5.1% (of 5350 reads)	loss	**Tier 3**	**Uncertain Significance**

Individual Variant Interpretations

Gene	***KIT***
Variation	-
Exon	13
Nucleotide	NM_000222.2: g.55594279C>T c.1982C>T
Amino Acid	p.T661I
Function	loss
Allelic Fraction	6.16% (of 844 reads)
Classification	**Tier 3**
Assessment	**Uncertain Significance**

Interpretation
KIT is an oncogene involved in cell proliferation and survival through activation of RAS/RAF/MAPK and PI3K/AKT/MTOR pathways [2]. Amplification, gain-of-function mutations, and protein overexpression cause KIT activation [3, 4, 1].

Gene	***NRAS***
Variation	-
Exon	2
Nucleotide	NM_002524.5: g.115258763C>G c.19G>C
Amino Acid	p.V7L
Function	loss
Allelic Fraction	5.1% (of 5350 reads)

Interpretation
NRAS is an oncogene involved in cell survival and proliferation through activation of RAS/RAF/MAPK and PI3K/AKT/MTOR pathways [5]. Gain-of-function mutations cause NRAS activation [6].

Classification Assessment **Tier 3 Uncertain Significance**

Genes Tested

Test information such as gene name and hot spot region can be included in this section.

Methods and Limitations

EXAMPLE Statement including sample type (FFPE, etc), method of extraction, amplification reactions, panel targeted regions, sequencing technology, etc. Additionally, a description of the data analysis software(s), genome of reference and the sensitivity of the methods should be described.

QIAGEN Clinical Insight (QCI™) is a variant analysis, interpretation and decision support tool for research and clinical labs analyzing human genetics data and is not intended to be used for diagnostic purposes. QCI Interpret software includes the following underlying databases, data reference sets and tools; QIAGEN Clinical Insight-Interpret (5.6.20200226), Ingenuity Knowledge Base (K-release), CADD (v1.4), Allele Frequency Community (2019-09-25), EVS (ESP6500SI-V2), Refseq Gene Model (2019-02-05), JASPAR (2013-11), Ingenuity Knowledge Base Snapshot Timestamp (2020-02-21 12:37:01.0), Vista Enhancer hg18 (2012-07), Vista Enhancer hg19 (2012-07), Clinical Trials (K-release), PolyPhen-2 (v2.2.2), 1000 Genome Frequency (phase3v5b), ExAC (0.3.1), iva (Nov 19 12:28 iva-1.0.1200.jar), PhyloP hg18 (2009-11), PhyloP hg19 (2009-11), DbSNP (151), TargetScan (7.2), GENCODE (Release 29), CentoMD (5.3), OMIM (May 26, 2017), gnomAD (2.1.1), BSIFT (2016-02-23), TCGA (2013-09-05), Clinvar (2019-06-05), DGV (2016-05-15), COSMIC (v89), HGMD (2019.2), SIFT4G (2016-02-23)

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Potential Significance	**Tier 2C**	• Biomarker is associated with response or resistance to an FDA or EMA approved therapy, according to drug label or professional guidelines but only for different diagnosis • Biomarker is an inclusion criterion for an active clinical trial • Biomarker is prognostic or diagnostic based on multiple small studies
	Tier 2D	• Biomarker shows plausible response or resistance based on case or preclinical studies • Biomarker may assist in disease diagnosis or prognosis based on small studies
Uncertain Significance	**Tier 3**	• Biomarker has uncertain clinical significance and not known to be likely benign or benign

**Adapted from PMID:27993330 jmd.amjpathol.org/article/S1525-1578(16)30223-9/pdf

Selected Citations

1. Carvajal RD, Antonescu CR, Wolchok JD, Chapman PB, Roman RA, Teitcher J, Panageas KS, Busam KJ, Chmielowski B, Lutzky J, Pavlick AC, Fusco A, Cane L, Takebe N, Vemula S, Bouvier N, Bastian BC, Schwartz GK (2011) KIT as a therapeutic target in metastatic melanoma. JAMA. 2011 Jun 08;305(22):2327-34 (PMID: 21642685)

2. Corless CL, Barnett CM, Heinrich MC (2011) Gastrointestinal stromal tumours: origin and molecular oncology. Nat Rev Cancer. 2011 Nov 17;11(12):865-78 (PMID: 22089421)

3. Heinrich MC, Corless CL, Demetri GD, Blanke CD, von Mehren M, Joensuu H, McGreevey LS, Chen CJ, Van den Abbeele AD, Druker BJ, Kiese B, Eisenberg B, Roberts PJ, Singer S, Fletcher CD, Silberman S, Dimitrijevic S, Fletcher JA (2003) Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. J Clin Oncol. 2003 Dec 01;21(23):4342-9 (PMID: 14645423)

4. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, Kawano K, Hanada M, Kurata A, Takeda M, Muhammad Tunio G, Matsuzawa Y, Kanakura Y, Shinomura Y, Kitamura Y (1998) Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science. 1998 Jan 23;279(5350):577-80 (PMID: 9438854)

5. Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D (2011) RAS oncogenes: weaving a tumorigenic web. Nat Rev Cancer. 2011 Oct 13;11(11):761-74 (PMID: 21993244)

6. Schubbert S, Shannon K, Bollag G (2007) Hyperactive Ras in developmental disorders and cancer. Nat Rev Cancer. 2007 Apr;7(4):295-308 (PMID: 17384584)

Analysis Report

20190628103639_1001600507010619014 7_411-19CTC_BC7_Glio2019

Table of contents

1 Summary

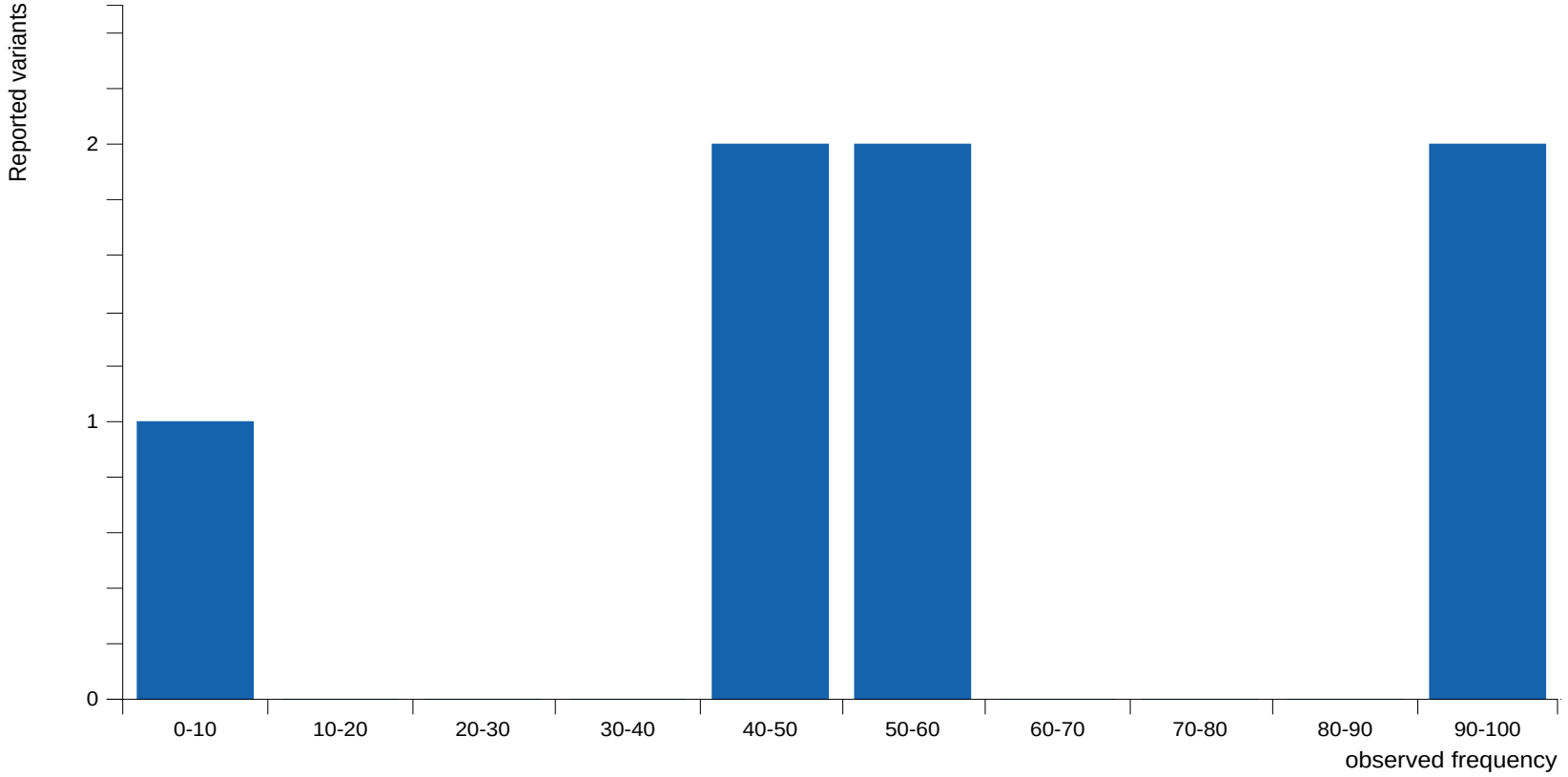
Report created	Sat Jun 29 18:12:56 CEST 2019
Sample ID	20190628103639_10016005070106190147_411-19CTC_BC7_Glio2019
Analysis workflow	AIT FFPE v4.5: QIAact Actionable Insights Tumor Panel on FFPE
Analyst	root
Reported variants	7
Analysis results	1 Untested variants

1.1 Comments

No comments

1.2 Distribution of observed frequencies for reported variants

Includes variants initially listed in variant table 'Reported variants'.



2 Quality control

Quality control for the sample analysis. Includes information on the input data, read mapping, and coverage information per gene.

2.1 Fastq

Fastq	20190628103639_10016005070106190147_411-19CTC_BC7_Glio2019
Reads	917,733
Nucleotides*	89,494,577
Average read length*	97.52
Reads with average quality ≥ 25	98.90%

** Including sample barcode*

Recommendations:

Reads with average quality ≥ 25 should be ≥ 80.00%

2.2 Secondary analysis summary

Reads mapped	830,229 (90.47%)
Reads in target regions	547,749 (65.98%)
Percentage of base positions in regions of interest with coverage ≥ 500x	96.73%
Percentage of base positions in regions of interest with coverage ≥ 200x	99.87%

Recommendations:

Percentage of base positions in regions of interest with coverage ≥ 500x should be ≥ 90.00%

Percentage of base positions in regions of interest with coverage ≥ 200x should be ≥ 95.00%

2.3 Coverage

Name	ROI	Bases	≥500x	≥200x	0x	Median	VOI	VOI <500x	VOI <200x
NRAS	6	27	100.00%	100.00%	0.00%	5,871	41	0	0
ALK	22	47	100.00%	100.00%	0.00%	1,860	40	0	0
RAF1	2	2	100.00%	100.00%	0.00%	4,212	2	0	0
PIK3CA	81	131	100.00%	100.00%	0.00%	2,849	165	0	0

Name	ROI	Bases	≥500x	≥200x	0x	Median	VOI	VOI <500x	VOI <200x
PDGFRA	21	64	100.00%	100.00%	0.00%	2,537	46	0	0
KIT	49	140	100.00%	100.00%	0.00%	3,324	235	0	0
ESR1	6	7	100.00%	100.00%	0.00%	1,723	11	0	0
EGFR	96	208	87.98%	99.52%	0.00%	1,593	443	32	1
BRAF	29	72	100.00%	100.00%	0.00%	3,764	153	0	0
KRAS	21	55	100.00%	100.00%	0.00%	4,449	148	0	0
ERBB3	8	8	100.00%	100.00%	0.00%	3,146	10	0	0
ERBB2	16	35	97.14%	100.00%	0.00%	4,244	61	1	0

ROI: Number of Regions of Interest, i.e. reportable regions that overlap with the gene.

Bases: Total number of base positions in Regions of Interest that overlap with the gene.

≥500x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is equal to or above the significant coverage threshold.

≥200x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is equal to or above the minimum coverage threshold.

0x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is zero.

Median: Median coverage of base positions in the Regions of Interest that overlap with the gene.

VOI: Total number of Variants of Interest, whether detected or not, that overlap with the gene. The list of Variants of Interest is defined by the analysis pipeline.

VOI <500x: Number of Variants of Interest in the gene for which coverage is below the significant coverage threshold.

VOI <200x: Number of Variants of Interest in the gene for which coverage is below the minimum coverage threshold.

2.4 Detected variants

Number of detected variants per gene. Variants for which coverage is above the minimum coverage threshold.

Name	In total	VOI	- non-syn	- syn	Non-VOI	- non-syn	- syn
NRAS	0	0	0	0	0	0	0
ALK	2	1	0	1	1	1	0
RAF1	0	0	0	0	0	0	0
PIK3CA	7	0	0	0	7	0	7
PDGFRA	3	1	0	1	2	0	2
KIT	0	0	0	0	0	0	0
ESR1	3	1	0	1	2	1	1
EGFR	7	2	0	2	5	0	5
BRAF	5	1	0	1	4	0	4

Name	In total	VOI	- non-syn	- syn	Non-VOI	- non-syn	- syn
KRAS	7	0	0	0	7	1	6
ERBB3	1	0	0	0	1	0	1
ERBB2	1	1	1	0	0	0	0

In total: Total number of variants detected within the gene. Variants initially listed in variant tables 3.1 and 3.2.

VOI: Number of detected Variants of Interest detected within the gene. The list of Variants of Interest is defined by the analysis pipeline.

- non-syn: Number of detected, gene-specific Variants of Interest that are non-synonymous.

- syn: Number of detected, gene-specific Variants of Interest that are synonymous.

Non-VOI: Number of detected variants that are not found within the analysis pipeline-defined list of Variants of Interest.

- non-syn: Number of gene-specific, non-VOIs that are non-synonymous.

- syn: Number of gene-specific, non-VOIs that are synonymous.

3 Variants

Variants detected within regions of interest with more than significant coverage are found in 3.1 and variants with more than minimum coverage are found in 3.2.
Variants of interest that could not be tested due to insufficient coverage are listed in table 3.3.
The coverage thresholds and minimum frequency cutoffs configured for the analysis workflow are listed in the History section.
Setting a variant review state to "Confirmed by review" moves it to 3.1, "Artifact" moves it to 3.2.
Only the variants in table 3.1 are exported as VCF and uploaded to QCI Interpret.

3.1 Reported variants

Variants that will be exported to VCF and uploaded to QCI Interpret. Initially contains: Variants detected within regions of interest with more than significant coverage and frequency above the cutoff set for the analysis workflow. These variants are assigned the initial review state "Valid".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
ALK	c.2535T>C		SNV	99.78%	30.99	1.00	2,775	Yes	Yes	Valid	
PDGFRA	c.1701A>G		SNV	99.83%	30.14	1.00	2,413	Yes	Yes	Valid	
ESR1	c.30T>C		SNV	44.52%	29.14	1.00	1,723	Yes	Yes	Valid	
EGFR	c.474C>T		SNV	7.30%	32.37	1.00	1,904	Yes	Yes	Valid	
EGFR	c.2361G>A		SNV	58.84%	31.59	1.00	1,499	Yes	Yes	Valid	
BRAF	c.1929A>G		SNV	41.59%	27.15	1.00	5,109	Yes	Yes	Valid	
ERBB2	c.3508C>G	p.Pro1170Ala	SNV	55.59%	24.88	0.28	1,198	Yes	Yes	Valid	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
ALK	c.2535T>C		No	2,769	1,255	1,514	200	29455267	2
PDGFRA	c.1701A>G		No	2,409	864	1,545	200	55141055	4
ESR1	c.30T>C		No	767	483	284	200	152129077	6
EGFR	c.474C>T		No	139	48	91	200	55214348	7
EGFR	c.2361G>A		No	882	517	365	200	55249063	7
BRAF	c.1929A>G		No	2,125	503	1,622	200	140449150	7

ERBB2	c.3508C>G	mis-sense	No	666	30	636	200	37884037	17

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

Comment: Remark added by user during variant review.

3.2 Variants available for review

Detected variants that will not be exported to the VCF and uploaded to QCI Interpret. Initially contains: Variants with more than minimum coverage and frequency above the cutoff set for the analysis workflow. Depending on workflow configuration, this table may include variants outside of regions of interest including those with coverage above significant coverage threshold. These variants are assigned the initial review state "Review".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
ALK	c.4381A>G	p.Ile1461Val	SNV	99.63%	31.02	1.00	268	No	No	Review	

PIK3CA	c.353-65delA		Deletion	4.24%	31.83	1.00	566	No	No	Review	
PIK3CA	c.1060-17C>A		SNV	53.15%	32.27	1.00	2,924	No	No	Review	
PIK3CA	c.1145+9G>A		SNV	4.47%	35.46	1.43E-3	626	No	No	Review	
PIK3CA	c.1145+14T>A		SNV	6.02%	33.33	3.91E-4	664	No	No	Review	
PIK3CA	c.1145+16_1145+17delGAinsTC		MNV	6.29%	29.77	5.42E-4	652	No	No	Review	
PIK3CA	c.1145+19T>A		SNV	6.08%	33.95	1.24E-3	707	No	No	Review	
PIK3CA	c.1145+54A>G		SNV	48.51%	33.11	1.00	402	No	No	Review	
PDGFRA	c.2440-50_2440-49insA		Insertion	94.23%	33.36	1.00	1,507	No	No	Review	
PDGFRA	c.3222T>C		SNV	99.80%	31.42	1.00	2,962	No	No	Review	
ESR1	c.16C>T	p.His6Tyr	SNV	43.24%	29.60	1.00	1,760	No	No	Review	
ESR1	c.975G>C		SNV	29.81%	30.45	0.00	1,674	No	No	Review	
EGFR	c.890-4C>G		SNV	11.46%	32.31	0.36	2,199	No	No	Review	
EGFR	c.1498+22A>T		SNV	41.71%	33.23	1.00	3,095	No	No	Review	
EGFR	c.1839C>T		SNV	59.24%	32.87	1.00	2,122	No	No	Review	
EGFR	c.2284-60T>C		SNV	7.57%	31.87	1.00	502	No	No	Review	
EGFR	c.2709T>C		SNV	61.20%	29.97	1.00	1,871	No	No	Review	
BRAF	c.1860+67A>C		SNV	14.73%	35.70	1.00	224	No	No	Review	
BRAF	c.1860+66A>C		SNV	70.93%	36.54	1.00	664	No	No	Review	
BRAF	c.1742-38A>T		SNV	4.15%	37.12	0.00	579	No	No	Review	
BRAF	c.980+27G>A		SNV	42.80%	28.51	1.00	771	No	No	Review	
KRAS	c.541_542delTCinsAG	p.Ser181Arg	MNV	6.39%	31.43	0.00	2,628	No	No	Review	
KRAS	c.534A>G		SNV	6.48%	31.64	0.00	2,686	No	No	Review	
KRAS	c.525A>G		SNV	5.99%	31.02	0.00	2,821	No	No	Review	
KRAS	c.522T>C		SNV	5.52%	35.43	0.00	2,937	No	No	Review	
KRAS	c.519T>C		SNV	5.47%	30.86	0.00	2,941	No	No	Review	
KRAS	c.516A>G		SNV	5.08%	29.48	0.00	3,052	No	No	Review	
KRAS	c.291-10delT		Deletion	4.32%	30.75	1.00	741	No	No	Review	
ERBB3	c.3348G>A		SNV	96.83%	32.50	1.00	1,608	No	No	Review	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
ALK	c.4381A>G	mis-sense	No	267	267	0	200	29416572	2
PIK3CA	c.353-65delA		Yes	24	24	0	200	178917413	3
PIK3CA	c.1060-17C>A		No	1,554	881	673	200	178922274	3
PIK3CA	c.1145+9G>A		No	28	0	28	200	178922385	3
PIK3CA	c.1145+14T>A		No	40	0	40	200	178922390	3
PIK3CA	c.1145+16_1145+17delGAinsTC		No	41	0	41	200	178922392..178922393	3
PIK3CA	c.1145+19T>A		No	43	0	43	200	178922395	3
PIK3CA	c.1145+54A>G		No	195	0	195	200	178922430	3
PDGFRA	c.2440-50_2440-49insA		No	1,420	749	671	200	55151958^55151959	4
PDGFRA	c.3222T>C		No	2,956	1,135	1,821	200	55161391	4
ESR1	c.16C>T	mis-sense	No	761	489	272	200	152129063	6
ESR1	c.975G>C		No	499	78	421	200	152265522	6
EGFR	c.890-4C>G		No	252	252	0	200	55223519	7
EGFR	c.1498+22A>T		No	1,291	680	611	200	55228053	7
EGFR	c.1839C>T		No	1,257	640	617	200	55233089	7
EGFR	c.2284-60T>C		No	38	38	0	200	55248926	7
EGFR	c.2709T>C		No	1,145	671	474	200	55266417	7
BRAF	c.1860+67A>C		No	33	33	0	200	140453008	7
BRAF	c.1860+66A>C		No	471	471	0	200	140453009	7
BRAF	c.1742-38A>T		No	24	0	24	200	140453231	7
BRAF	c.980+27G>A		No	330	330	0	200	140500135	7
KRAS	c.541_542delTCinsAG	mis-sense	No	168	168	0	200	25362754..25362755	12
KRAS	c.534A>G		No	174	172	2	200	25362762	12
KRAS	c.525A>G		No	169	165	4	200	25362771	12
KRAS	c.522T>C		No	162	161	1	200	25362774	12
KRAS	c.519T>C		No	161	160	1	200	25362777	12
KRAS	c.516A>G		No	155	154	1	200	25362780	12
KRAS	c.291-10delT		Yes	32	0	32	200	25378717	12

ERBB3	c.3348G>A		No	1,557	777	780	200	56494991	12

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

Comment: Remark added by user during variant review.

3.3 Untested variants

Variants of interest that could not be tested due to insufficient coverage. These variants are assigned the initial review state "Untested".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
EGFR	c.2184+19G>A		SNV	1.39%	12.00		72	Yes	Yes	Untested	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
EGFR	c.2184+19G>A			1	0	1		55241755	7

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

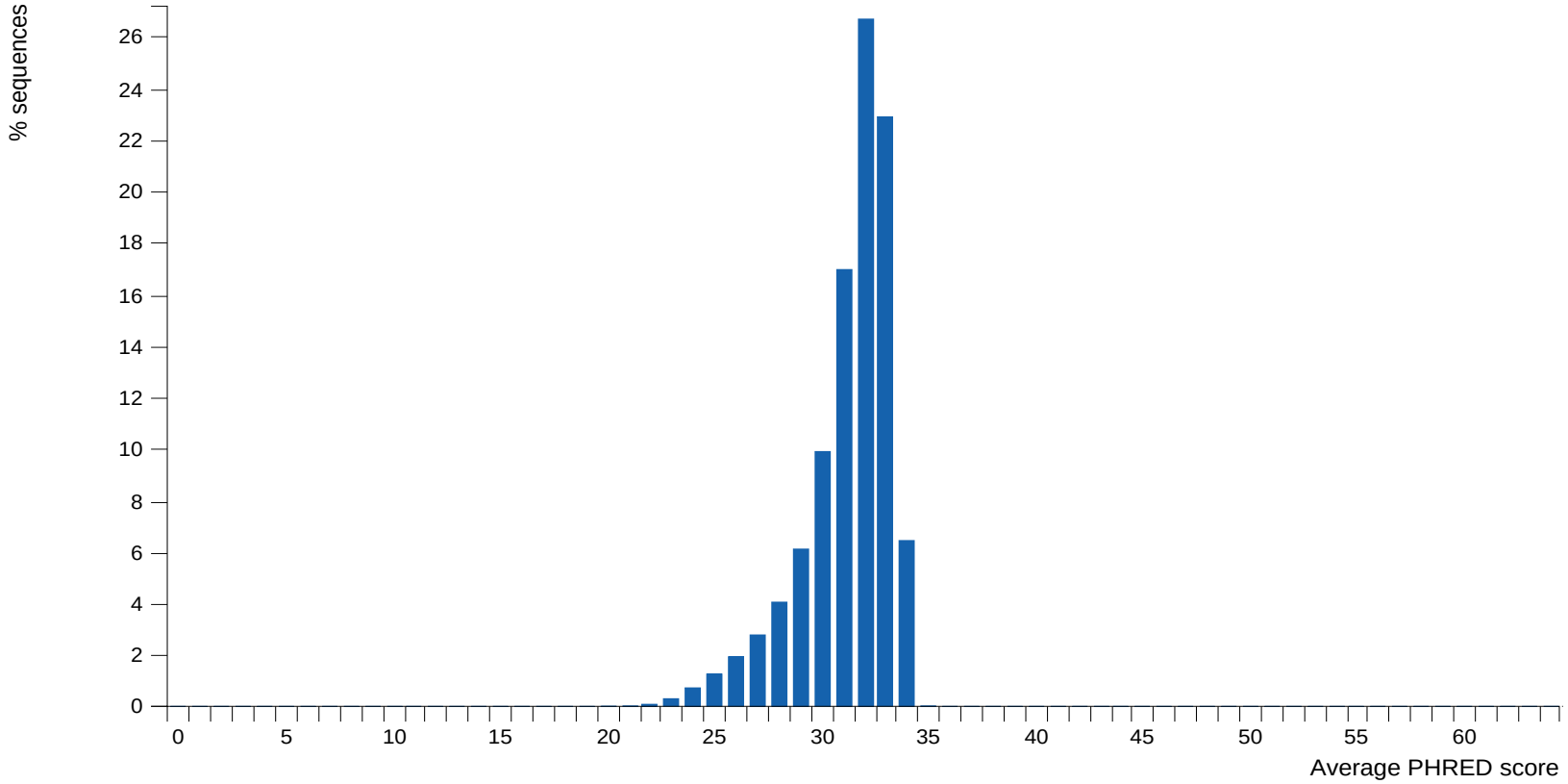
Comment: Remark added by user during variant review.

4 Detailed QC

Quality control metrics for detailed inspection. These metrics can indicate possible problems in the upstream workflow or data analysis. Quality control are divided in metrics on the incoming reads from input data, and metrics per base positions in these reads. Lastly section 4.3 and 4.4 display metrics on how well the positions in the region of interest are covered

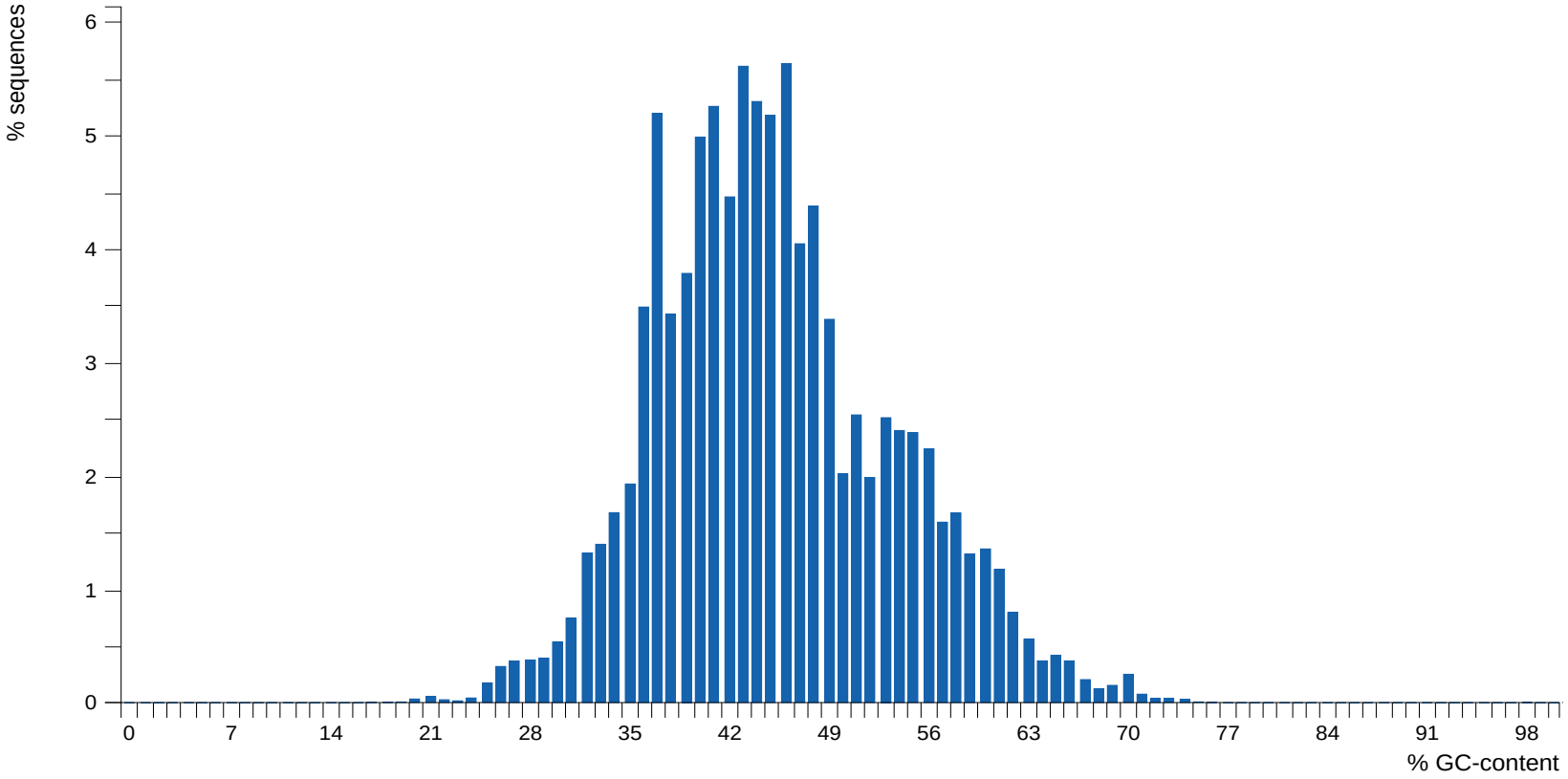
4.1 QC for reads

4.1.1 Average base quality of reads



Distribution of average sequence quality scores. The quality of a sequence is calculated as the arithmetic mean of its base qualities.
x: PHRED-score
y: number of sequences observed at that qual. score normalized to the total number of sequences

4.1.2 GC content of reads



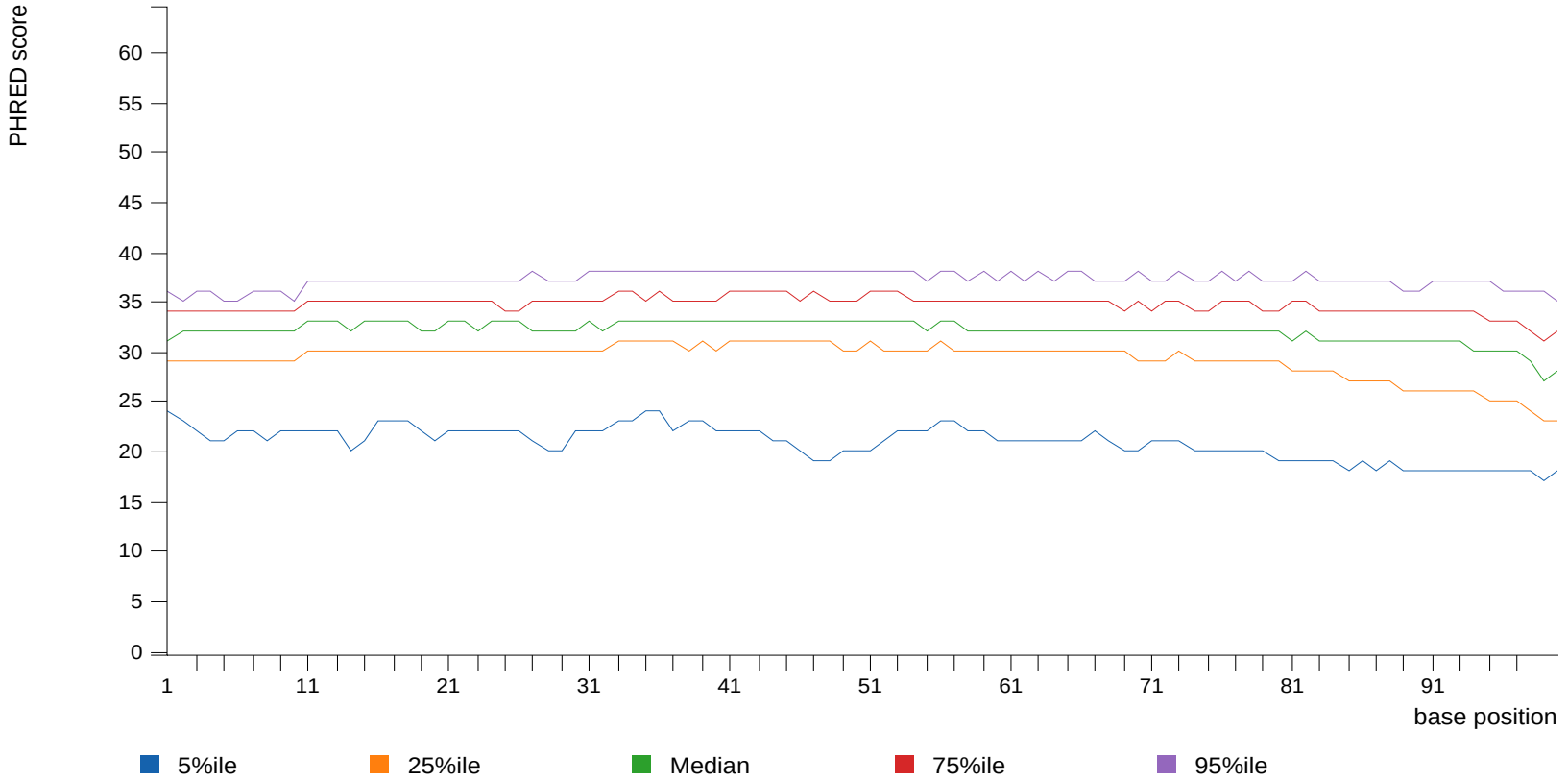
Distribution of GC-contents. The GC-content of a sequence is calculated as the number of GC-bases compared to all bases (including ambiguous bases).
x: relative GC-content of a sequence in percent
y: number of sequences featuring particular GC-percentages normalized to the total number of sequences

4.1.3 Ambiguous base content of reads

No ambiguous bases detected.

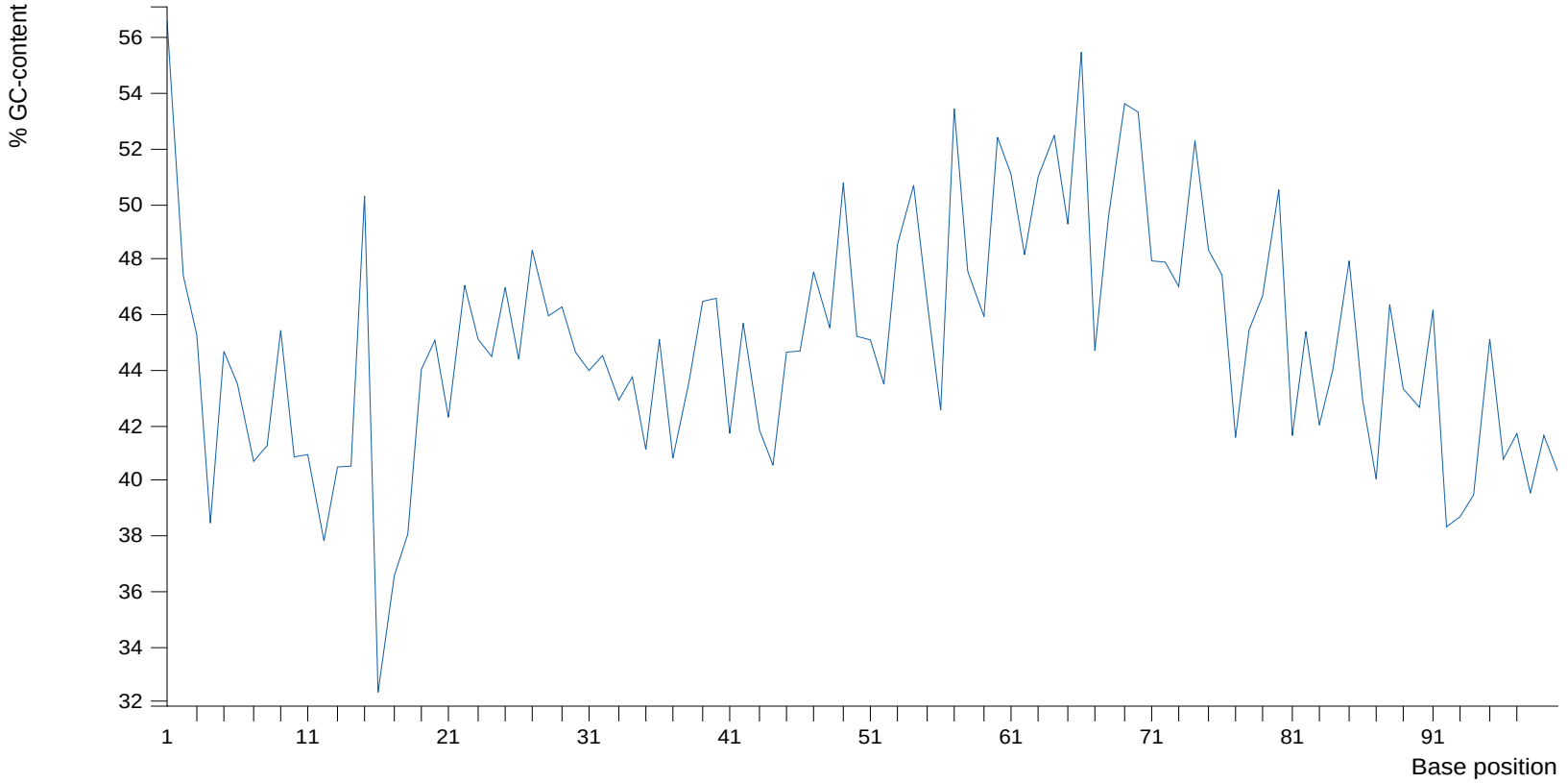
4.2 QC for bases

4.2.1 Quality score per base position



Base-quality distribution along the base positions.
x: base position
y: median & percentiles of quality scores observed at that base position

4.2.2 GC content per base position



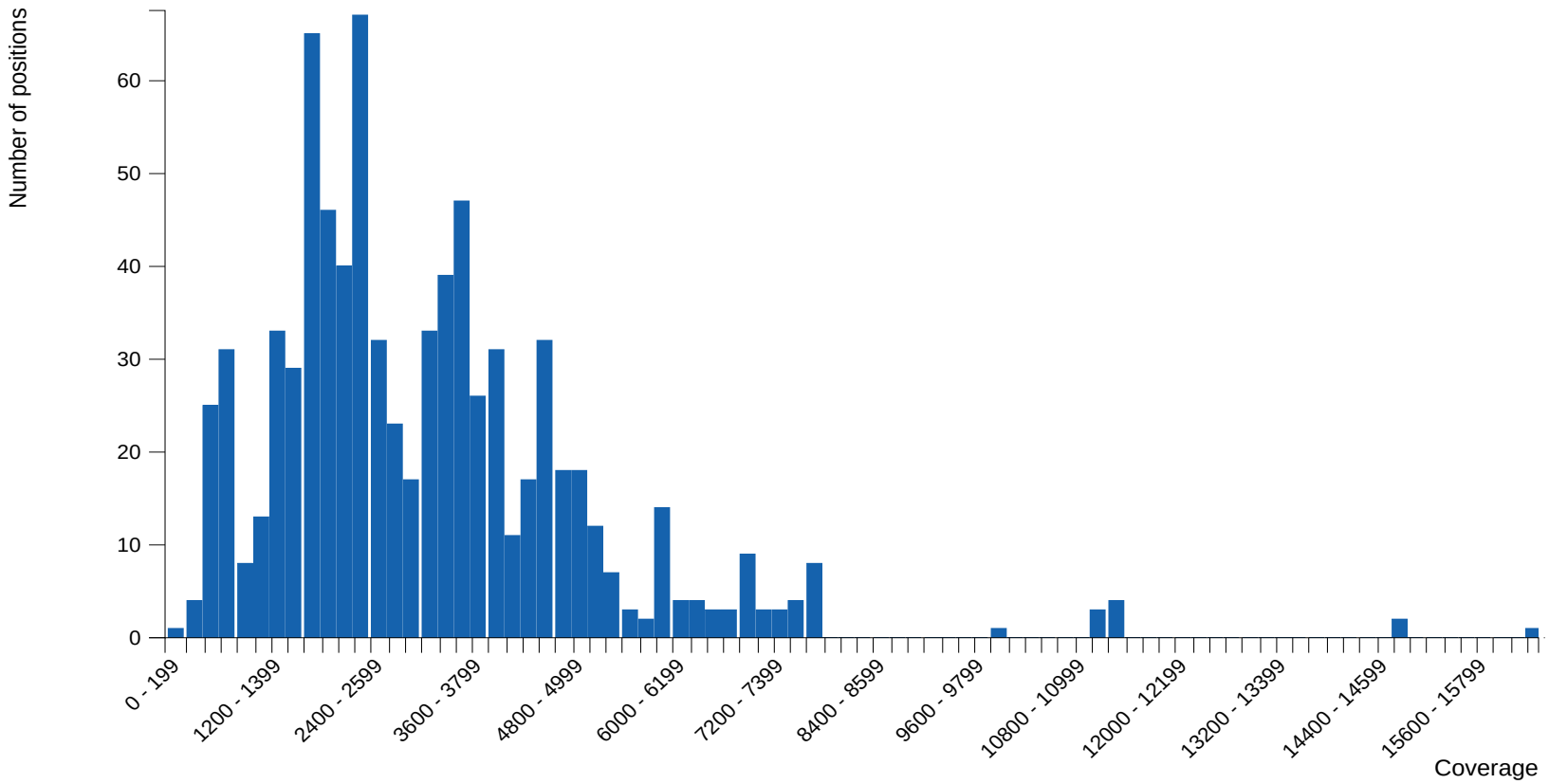
Combined coverage of G- and C-bases.
x: base position
y: number of G- and C-bases observed at current position normalized to the total number of bases observed at that position

4.2.3 Ambiguous base content per base position

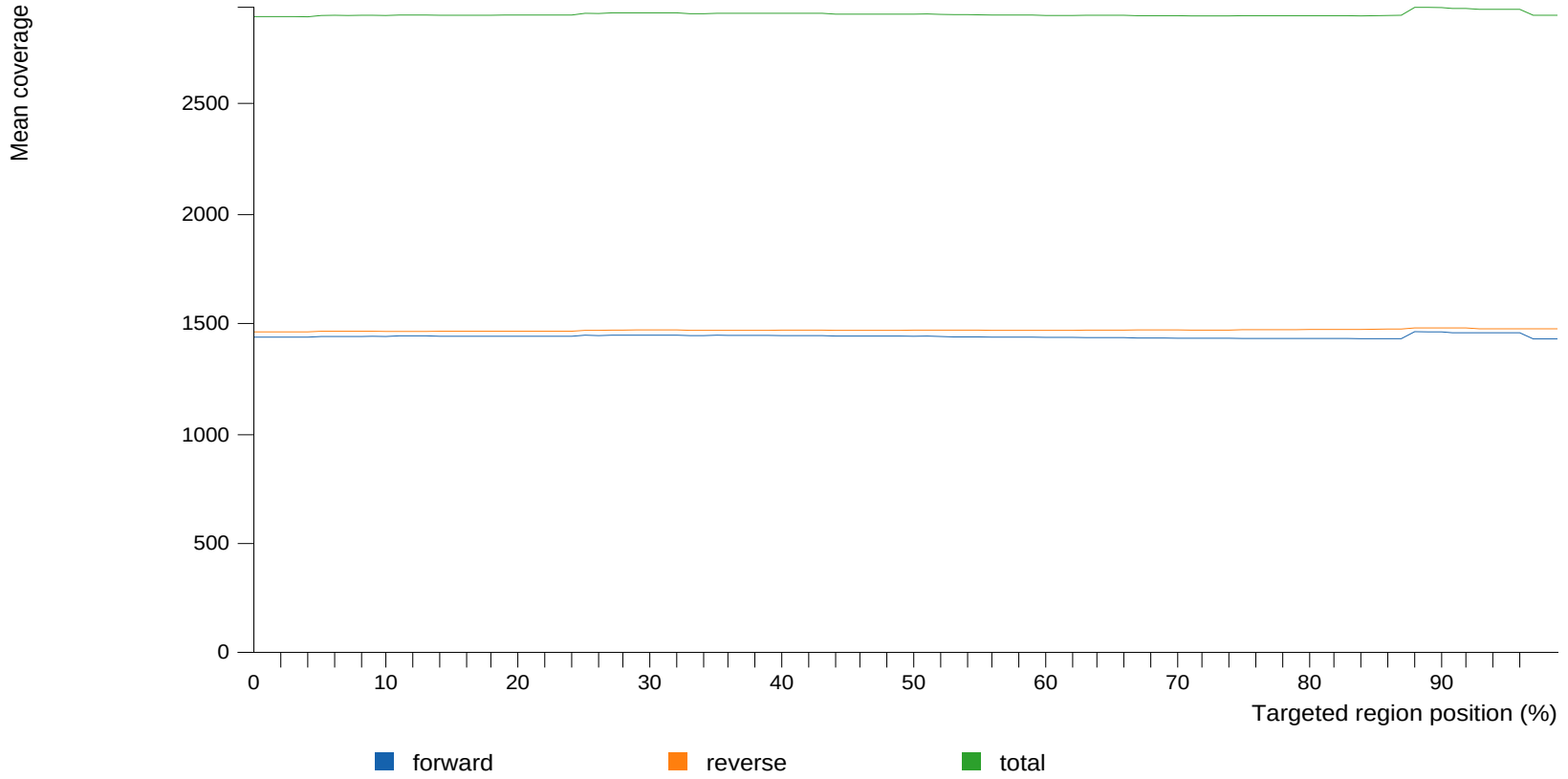
No ambiguous bases detected

4.3 Coverage of Regions of Interest positions

Coverage distribution



4.4 Mean coverage of relative positions in regions of interest



5 History

5.1 Log Entries

Type	Time	User	Details
State change	Sat Jun 29 18:13:05 CEST 2019	system	Ready for Review
State change	Sat Jun 29 17:55:03 CEST 2019	root	In Progress

5.2 Execution Information

QCIA version	QCI Analyze 1.4.5
Analysis start time	Sat Jun 29 17:55:03 CEST 2019
Analysis workflow	AIT FFPE 4.5
Analysis description	QIAact Actionable Insights Tumor Panel on FFPE

5.3 Transcripts

Table listing the genes, transcript IDs and protein IDs used in the analysis.

Gene Name	Transcript ID	Protein ID
NRAS	NM_002524.4	NP_002515.1
ALK	NM_004304.4	NP_004295.2
RAF1	NM_002880.3	NP_002871.1
PIK3CA	NM_006218.2	NP_006209.2
PDGFRA	NM_006206.4	NP_006197.1
KIT	NM_000222.2	NP_000213.1
ESR1	NM_001122742.1	NP_001116214.1
EGFR	NM_005228.3	NP_005219.2
BRAF	NM_004333.4	NP_004324.2
KRAS	NM_004985.3	NP_004976.2
ERBB3	NM_001982.3	NP_001973.2

Gene Name	Transcript ID	Protein ID
ERBB2	NM_004448.2	NP_004439.2

5.4 Open Parameters

Workflow parameters that are editable by the administrator

Reported variants

Significant coverage threshold	500
SNV/MNV frequency threshold in %	4.00
Insertions, deletions and replacements frequency threshold in %	4.00

Variants available for review

Minimum coverage threshold	200
SNV/MNV frequency threshold in %	4.00
Insertions, deletions and replacements frequency threshold in %	4.00
Detect variants outside regions of interest	Yes

5.5 Locked Parameters

Adapter trimming

Trim adapter list	GRadapter_160913
Ambiguous trim	false
Ambiguous limit	2
Quality trim	false
Quality limit	0.05
Use colorspace	false

Also search on reversed sequence	false
Remove 5' terminal nucleotides	false
Number of 5' terminal nucleotides	1
Maximum number of nucleotides in reads	1000
Minimum number of nucleotides in reads	15
Discard short reads	false
Remove 3' terminal nucleotides	false
Number of 3' terminal nucleotides	1
Discard long reads	false

Map Reads to Reference

References	Homo_sapiens_sequence_hg19
Masking mode	No masking
Masking track	Not set
Match score	1
Mismatch cost	2
Cost of insertions and deletions	Affine gap cost
Insertion cost	3
Deletion cost	3
Insertion open cost	6
Insertion extend cost	1
Deletion open cost	6
Deletion extend cost	1
Length fraction	0.5
Similarity fraction	0.8
Global alignment	false
Color space alignment	false
Color error cost	3
Auto-detect paired distances	false
Non-specific match handling	Map randomly

InDels and Structural Variants

P-Value threshold	1.0E-4
Maximum number of mismatches	3
Ignore broken pairs	true
Minimum relative consensus coverage	0.0
Minimum quality score	0
Filter variants	true
Minimum number of reads	2
Restrict calling to target regions	ATPv2_TargetRegions_170302_ver1.1

Local Realignment (Short Unaligned End version)

Realign unaligned ends	true
Multi-pass realignment	2
Local bound for unaligned ends of size one	0.75
Local bound for unaligned ends of size two	0.75
Force realignment to guidance-variants	false
Maximum guidance-variant length	100

Trim Primers and their Dimers of Mapped Reads

Primer track	101x_GR_primers_15_10_15_V1.0
Reference	Homo_sapiens_sequence_hg19
Minimum primer overlap length	9
Allow dangling 3' end base	true
Minimal primer overlap fraction	0.7
Only keep reads that have hit a primer	true
Additional bases to trim	1

Remove Pseudogene Reads

Genes track	AITv2_PseudoGenes_170912_ver1.0
Gene and pseudogene links	KRAS -> KRASP1
Required unaligned ends %	2.0

Low Frequency Variant Detection

Required significance (%)	0.01
Ignore positions with coverage above	1000000000
Restrict calling to target regions	ATPv2_TargetRegions_170302_ver1.1
Ignore broken pairs	true
Ignore non-specific matches	Reads
Minimum read length	20
Minimum coverage	Parameter editable by administrator
Minimum count	8
Minimum frequency (%)	Parameter editable by administrator
Base quality filter	false
Neighborhood radius	5
Minimum central quality	5
Minimum neighborhood quality	5
Read direction filter	false
Direction frequency (%)	5.0
Relative read direction filter	false
Significance (%)	1.0E-5
Read position filter	false
Significance (%)	1.0
Remove pyro-error variants	false
In homopolymer regions with minimum length	3
With frequency below	0.8

Remove False Positives

Minimum frequency (%)	Parameter editable by administrator
Minimum forward/reverse balance	0.05
Minimum average base quality	22.0
Variant frequency	true
Forward/reverse balance	false
Average base quality	true

Annotate Variants With Primers

Minimum coverage count	400
Minimum variant percentage	1.0
Minimum variant read count	2

AnyGenomics Lab

QIAact Actionable Insights Tumor Panel For the GeneReader NGS System (RUO)

Report Date Mar 26, 2018
Report Status -

Patient Information

Patient Name
Date of Birth
Ethnicity
Sex
Accession 20180322144720_8160085015705246905180014 0_48241_16A1_BC12_2018-03-22

Client Information

Client
Client ID
Physician
Pathologist

Specimen

Specimen Type
Specimen ID
Collection Date
Accession Date Mar 26, 2018
Primary Tumor Site Brain
Diagnosis Glioblastoma
Diagnosis Stage

Interpretation

2 Clinically Significant Variants Reported | 0 Approved Therapy | 0 Potential Clinical Trials

Summary of Clinically Significant Variants

Variants Reported	Approved Therapies for Same Cancer	Approved Therapies for Other Cancers	Therapies Associated with Resistance	Potential Clinical Trials
PDGFRA p.L221F				
EGFR p.R836R				

Variant Details

Gene	Exon #	Nucleotide Change	Amino Acid Change	Effect on Protein
EGFR	21	NM_005228.4: c.2508C>T	p.R836R	normal function

EGFR is an oncogene involved in cell growth and differentiation through activation of the PI3K/AKT/MTOR and RAS/RAF/MAPK pathways [8]. Amplification, gain-of-function mutations, and protein overexpression cause EGFR activation [5, 12, 7, 9]. EGFR mutations are reported to be mutually exclusive with ALK rearrangements and KRAS mutations in non-small cell lung cancer [3, 10, 11].

QIAact Actionable Insights Tumor Panel For the GeneReader NGS System (RUO)

Variant Details

Gene	Exon #	Nucleotide Change	Amino Acid Change	Effect on Protein
PDGFRA	5	NM_006206.5: c.661C>T	p.L221F	**normal function**

PDGFRA is an oncogene involved in cell proliferation and survival through activation of RAS/RAF/MAPK and PI3K/AKT/MTOR pathways [2]. Gain-of-function mutations, amplification, and fusions cause PDGFRA activation [1, 4, 6].

Genes Tested

KRAS *NRAS* *KIT* *BRAF* *PDGFRA* *ALK* *EGFR* *ERBB2* *PIK3CA* *ERBB3* *ESR1* *RAF1*

Methods and Limitations

EXAMPLE Statement including sample type (FFPE, etc), method of extraction, amplification reactions, panel targeted regions, sequencing technology, etc. Additionally, a description of the data analysis software(s), genome of reference and the sensitivity of the methods should be described.

QIAGEN Clinical Insight (QCI™) software includes the following underlying databases, data reference sets and tools; QIAGEN Clinical Insight-Interpret (5.2.20180316), Ingenuity Knowledge Base (Pandora 180329.003), CADD (v1.3), CentoMD (4.1), EVS (ESP6500SI-V2), Allele Frequency Community (2018-01-17), JASPAR (2013-11), Ingenuity Knowledge Base Snapshot Timestamp (2018-03-29 18:20:56.0), Vista Enhancer hg18 (2012-07), Vista Enhancer hg19 (2012-07), OMIM (May 26, 2017), gnomAD (2.0.1), Clinical Trials (Pandora 180329.003), BSIFT (2016-02-23), TCGA (2013-09-05), PolyPhen-2 (v2.2.2), 1000 Genome Frequency (phase3v5b), Clinvar (2018-01-03), DGV (2016-05-15), COSMIC (v83), ExAC (0.3.1), HGMD (2017.4), PhyloP hg18 (2009-11), PhyloP hg19 (2009-11), DbSNP (150(2017-07-10)), TargetScan (6.2), SIFT4G (2016-02-23)

Laboratory Statement

This section can be customized to provide additional information regarding laboratory methods etc.

Selected Citations

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2. Corless CL, Barnett CM, Heinrich MC (2011) Gastrointestinal stromal tumours: origin and molecular oncology. Nat Rev

QIAact Actionable Insights Tumor Panel For the GeneReader NGS System (RUO)

Selected Citations

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8. Scaltriti M, Baselga J (2006) The epidermal growth factor receptor pathway: a model for targeted therapy. Clin Cancer Res. 2006 Sep 15;12(18):5268-72 (PMID: 17000658)
9. Selvaggi G, Novello S, Torri V, Leonardo E, De Giuli P, Borasio P, Mossetti C, Ardissone F, Lausi P, Scagliotti GV (2004) Epidermal growth factor receptor overexpression correlates with a poor prognosis in completely resected non-small-cell lung cancer. Ann Oncol. 2004 Jan;15(1):28-32 (PMID: 14679115)
10. Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, Wistuba II, Fong KM, Lee H, Toyooka S, Shimizu N, Fujisawa T, Feng Z, Roth JA, Herz J, Minna JD, Gazdar AF (2005) Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst. 2005 Mar 02;97(5):339-46 (PMID: 15741570)
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12. Yoshida K, Tsuda T, Matsumura T, Tsujino T, Hattori T, Ito H, Tahara E (1989) Amplification of epidermal growth factor receptor (EGFR) gene and oncogenes in human gastric carcinomas. Virchows Arch B Cell Pathol Incl Mol Pathol. 1989;57(5):285-90 (PMID: 2570489)

Analysis Report

20190628103639_10016005070106190147_2202-16PT_BC10_Glio2019

Table of contents

1 Summary

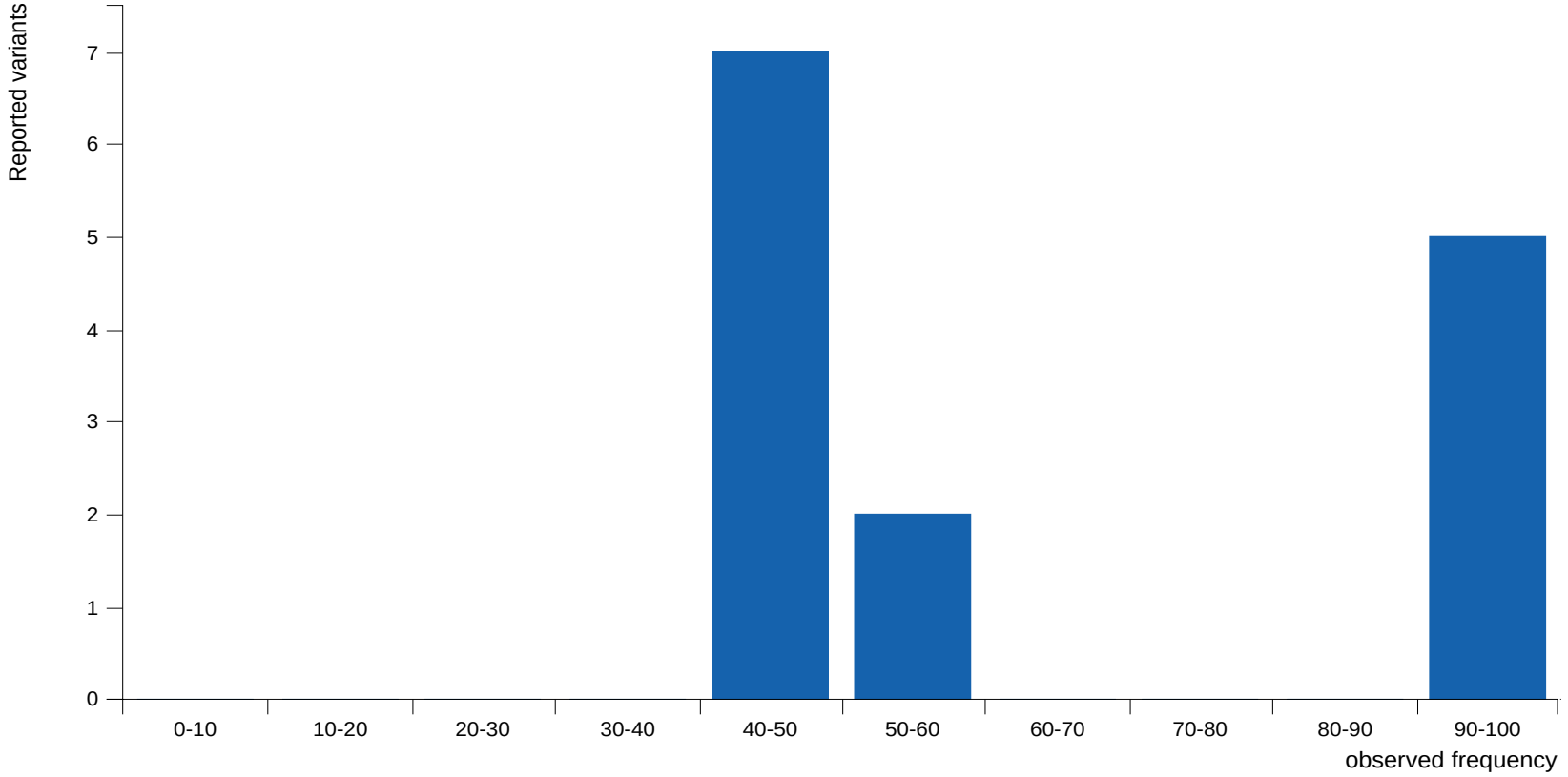
Report created	Fri Oct 25 17:36:45 CEST 2019
Sample ID	20190628103639_10016005070106190147_2202-16PT_BC10_Glio2019
Analysis workflow	AIT FFPE v4.5: QIAact Actionable Insights Tumor Panel on FFPE
Analyst	root
Reported variants	14
Analysis results	42 Untested variants

1.1 Comments

No comments

1.2 Distribution of observed frequencies for reported variants

Includes variants initially listed in variant table 'Reported variants'.



2 Quality control

Quality control for the sample analysis. Includes information on the input data, read mapping, and coverage information per gene.

2.1 Fastq

Fastq	20190628103639_1001600507010619014 7_2202-16PT_BC10_Glio2019
Reads	371,240
Nucleotides*	37,272,494
Average read length*	100.40
Reads with average quality ≥ 25	99.40%

** Including sample barcode*

Recommendations:

Reads with average quality ≥ 25 should be ≥ 80.00%

2.2 Secondary analysis summary

Reads mapped	364,531 (98.19%)
Reads in target regions	345,250 (94.71%)
Percentage of base positions in regions of interest with coverage ≥ 500x	73.24%
Percentage of base positions in regions of interest with coverage ≥ 200x	95.85%

Recommendations:

Percentage of base positions in regions of interest with coverage ≥ 500x should be ≥ 90.00%

Percentage of base positions in regions of interest with coverage ≥ 200x should be ≥ 95.00%

2.3 Coverage

Name	ROI	Bases	≥500x	≥200x	0x	Median	VOI	VOI <500x	VOI <200x
NRAS	6	27	100.00%	100.00%	0.00%	969	41	0	0
ALK	22	47	100.00%	100.00%	0.00%	3,837	40	0	0
RAF1	2	2	100.00%	100.00%	0.00%	1,704	2	0	0
PIK3CA	81	131	57.25%	90.08%	0.00%	527	165	62	16

Name	ROI	Bases	≥500x	≥200x	0x	Median	VOI	VOI <500x	VOI <200x
PDGFRA	21	64	96.88%	100.00%	0.00%	2,660	46	0	0
KIT	49	140	59.29%	99.29%	0.00%	595	235	133	1
ESR1	6	7	57.14%	100.00%	0.00%	2,046	11	2	0
EGFR	96	208	97.60%	99.52%	0.00%	1,597	443	5	1
BRAF	29	72	50.00%	93.06%	0.00%	498	153	54	7
KRAS	21	55	1.82%	76.36%	0.00%	337	148	147	17
ERBB3	8	8	100.00%	100.00%	0.00%	2,677	10	0	0
ERBB2	16	35	100.00%	100.00%	0.00%	11,532	61	0	0

ROI: Number of Regions of Interest, i.e. reportable regions that overlap with the gene.

Bases: Total number of base positions in Regions of Interest that overlap with the gene.

≥500x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is equal to or above the significant coverage threshold.

≥200x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is equal to or above the minimum coverage threshold.

0x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is zero.

Median: Median coverage of base positions in the Regions of Interest that overlap with the gene.

VOI: Total number of Variants of Interest, whether detected or not, that overlap with the gene. The list of Variants of Interest is defined by the analysis pipeline.

VOI <500x: Number of Variants of Interest in the gene for which coverage is below the significant coverage threshold.

VOI <200x: Number of Variants of Interest in the gene for which coverage is below the minimum coverage threshold.

2.4 Detected variants

Number of detected variants per gene. Variants for which coverage is above the minimum coverage threshold.

Name	In total	VOI	- non-syn	- syn	Non-VOI	- non-syn	- syn
NRAS	0	0	0	0	0	0	0
ALK	3	2	0	2	1	1	0
RAF1	0	0	0	0	0	0	0
PIK3CA	4	2	1	1	2	0	2
PDGFRA	8	6	1	5	2	0	2
KIT	0	0	0	0	0	0	0
ESR1	5	3	0	3	2	0	2
EGFR	8	3	1	2	5	0	5
BRAF	1	1	0	1	0	0	0

Name	In total	VOI	- non-syn	- syn	Non-VOI	- non-syn	- syn
KRAS	0	0	0	0	0	0	0
ERBB3	1	0	0	0	1	0	1
ERBB2	2	2	2	0	0	0	0

In total: Total number of variants detected within the gene. Variants initially listed in variant tables 3.1 and 3.2.

VOI: Number of detected Variants of Interest detected within the gene. The list of Variants of Interest is defined by the analysis pipeline.

- non-syn: Number of detected, gene-specific Variants of Interest that are non-synonymous.

- syn: Number of detected, gene-specific Variants of Interest that are synonymous.

Non-VOI: Number of detected variants that are not found within the analysis pipeline-defined list of Variants of Interest.

- non-syn: Number of gene-specific, non-VOIs that are non-synonymous.

- syn: Number of gene-specific, non-VOIs that are synonymous.

3 Variants

Variants detected within regions of interest with more than significant coverage are found in 3.1 and variants with more than minimum coverage are found in 3.2.
Variants of interest that could not be tested due to insufficient coverage are listed in table 3.3.
The coverage thresholds and minimum frequency cutoffs configured for the analysis workflow are listed in the History section.
Setting a variant review state to "Confirmed by review" moves it to 3.1, "Artifact" moves it to 3.2.
Only the variants in table 3.1 are exported as VCF and uploaded to QCI Interpret.

3.1 Reported variants

Variants that will be exported to VCF and uploaded to QCI Interpret. Initially contains: Variants detected within regions of interest with more than significant coverage and frequency above the cutoff set for the analysis workflow. These variants are assigned the initial review state "Valid".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
ALK	c.4338C>T		SNV	51.36%	29.41	1.00	4,488	Yes	Yes	Valid	
ALK	c.2535T>C		SNV	99.83%	31.07	1.00	4,209	Yes	Yes	Valid	
PDGFRA	c.1432T>C	p.Ser478Pro	SNV	43.94%	25.12	1.00	2,319	Yes	Yes	Valid	
PDGFRA	c.1701A>G		SNV	99.24%	29.98	1.00	1,183	Yes	Yes	Valid	
PDGFRA	c.1809G>A		SNV	44.14%	33.89	1.00	2,558	Yes	Yes	Valid	
PDGFRA	c.2472C>T		SNV	46.54%	33.62	1.00	3,328	Yes	Yes	Valid	
ESR1	c.30T>C		SNV	49.93%	29.00	1.00	5,342	Yes	Yes	Valid	
ESR1	c.1782G>A		SNV	47.55%	33.17	1.00	3,066	Yes	Yes	Valid	
EGFR	c.474C>T		SNV	99.58%	31.39	1.00	2,849	Yes	Yes	Valid	
EGFR	c.1562G>A	p.Arg521Lys	SNV	40.83%	35.07	1.00	6,368	Yes	Yes	Valid	
EGFR	c.2361G>A		SNV	99.84%	31.58	1.00	2,455	Yes	Yes	Valid	
BRAF	c.1929A>G		SNV	44.07%	26.29	1.00	717	Yes	Yes	Valid	
ERBB2	c.1963A>G	p.Ile655Val	SNV	50.01%	31.72	1.00	9,879	Yes	Yes	Valid	
ERBB2	c.3508C>G	p.Pro1170Ala	SNV	99.09%	25.24	1.00	2,304	Yes	Yes	Valid	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
ALK	c.4338C>T		No	2,305	1,494	811	200	29416615	2
ALK	c.2535T>C		No	4,202	1,872	2,330	200	29455267	2
PDGFRA	c.1432T>C	mis-sense	No	1,019	294	725	200	55139771	4
PDGFRA	c.1701A>G		No	1,174	499	675	200	55141055	4
PDGFRA	c.1809G>A		No	1,129	463	666	200	55143577	4
PDGFRA	c.2472C>T		No	1,549	1,165	384	200	55152040	4
ESR1	c.30T>C		No	2,667	1,878	789	200	152129077	6
ESR1	c.1782G>A		No	1,458	731	727	200	152420095	6
EGFR	c.474C>T		No	2,837	1,219	1,618	200	55214348	7
EGFR	c.1562G>A	mis-sense	No	2,600	603	1,997	200	55229255	7
EGFR	c.2361G>A		No	2,451	1,432	1,019	200	55249063	7
BRAF	c.1929A>G		No	316	97	219	200	140449150	7
ERBB2	c.1963A>G	mis-sense	No	4,940	1,772	3,168	200	37879588	17
ERBB2	c.3508C>G	mis-sense	No	2,283	291	1,992	200	37884037	17

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

Comment: Remark added by user during variant review.

3.2 Variants available for review

Detected variants that will not be exported to the VCF and uploaded to QCI Interpret. Initially contains: Variants with more than minimum coverage and frequency above the cutoff set for the analysis workflow. Depending on workflow configuration, this table may include variants outside of regions of interest including those with coverage above significant coverage threshold. These variants are assigned the initial review state "Review".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
ALK	c.4381A>G	p.Ile1461Val	SNV	99.80%	30.90	1.00	512	No	No	Review	
PIK3CA	c.-77+8483C>T		SNV	53.33%	33.24	0.92	225	Yes	Yes	Review	
PIK3CA	c.-76-18864C>A		SNV	44.30%	33.38	0.74	307	No	No	Review	

PIK3CA	c.1060-17C>A		SNV	50.18%	31.73	1.00	277	No	No	Review	
PIK3CA	c.1173A>G	p.Ile391Met	SNV	51.66%	30.91	1.00	302	Yes	Yes	Review	
PDGFRA	c.612T>C		SNV	39.76%	30.43	1.00	249	Yes	Yes	Review	
PDGFRA	c.939T>G		SNV	48.32%	29.41	1.00	327	Yes	Yes	Review	
PDGFRA	c.2440-50_2440-49insA		Insertion	50.00%	33.38	1.00	796	No	No	Review	
PDGFRA	c.3222T>C		SNV	99.33%	31.39	1.00	3,122	No	No	Review	
ESR1	c.975G>C		SNV	99.64%	31.05	1.00	822	No	No	Review	
ESR1	c.1369+13754T>C		SNV	42.86%	32.53	1.00	231	No	No	Review	
ESR1	c.1369+13777T>G		SNV	44.99%	29.80	1.00	469	Yes	Yes	Review	
EGFR	c.1498+22A>T		SNV	100.00%	32.90	1.00	354	No	No	Review	
EGFR	c.2284-60T>C		SNV	52.32%	32.94	1.00	2,372	No	No	Review	
EGFR	c.2703G>T		SNV	4.19%	29.74	5.63E-8	454	No	No	Review	
EGFR	c.2709T>A		SNV	5.66%	29.31	3.63E-7	459	No	No	Review	
EGFR	c.2709T>C		SNV	94.12%	30.29	0.99	459	No	No	Review	
ERBB3	c.3348G>A		SNV	55.44%	32.05	1.00	2,437	No	No	Review	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
ALK	c.4381A>G	mis-sense	No	511	511	0	200	29416572	2
PIK3CA	c.-77+8483C>T		No	120	19	101	200	178874874	3
PIK3CA	c.-76-18864C>A		No	136	30	106	200	178897674	3
PIK3CA	c.1060-17C>A		No	139	70	69	200	178922274	3
PIK3CA	c.1173A>G	mis-sense	No	156	51	105	200	178927410	3
PDGFRA	c.612T>C		No	99	15	84	200	55130078	4
PDGFRA	c.939T>G		No	158	23	135	200	55133726	4
PDGFRA	c.2440-50_2440-49insA		No	398	178	220	200	55151958^55151959	4
PDGFRA	c.3222T>C		No	3,101	1,239	1,862	200	55161391	4
ESR1	c.975G>C		No	819	435	384	200	152265522	6
ESR1	c.1369+13754T>C		No	99	59	40	200	152396013	6
ESR1	c.1369+13777T>G		No	211	163	48	200	152396036	6

EGFR	c.1498+22A>T		No	354	185	169	200	55228053	7
EGFR	c.2284-60T>C		No	1,241	1,241	0	200	55248926	7
EGFR	c.2703G>T		No	19	1	18	200	55266411	7
EGFR	c.2709T>A		No	26	4	22	200	55266417	7
EGFR	c.2709T>C		No	432	318	114	200	55266417	7
ERBB3	c.3348G>A		No	1,351	720	631	200	56494991	12

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

Comment: Remark added by user during variant review.

3.3 Untested variants

Variants of interest that could not be tested due to insufficient coverage. These variants are assigned the initial review state "Untested".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
PIK3CA	c.353G>A	p.Gly118Asp	SNV	0.00%			159	Yes	Yes	Untested	
PIK3CA	c.519T>C		SNV	0.76%	30.00		131	Yes	Yes	Untested	
PIK3CA	c.519T>G		SNV	0.00%			131	Yes	Yes	Untested	
PIK3CA	c.1030G>A	p.Val344Met	SNV	0.00%			141	Yes	Yes	Untested	
PIK3CA	c.1031T>G	p.Val344Gly	SNV	0.00%			141	Yes	Yes	Untested	
PIK3CA	c.1035T>A	p.Asn345Lys	SNV	0.00%			142	Yes	Yes	Untested	
PIK3CA	c.1035T>G	p.Asn345Lys	SNV	0.00%			142	Yes	Yes	Untested	
PIK3CA	c.1048G>A	p.Asp350Asn	SNV	0.00%			168	Yes	Yes	Untested	
PIK3CA	c.1049A>G	p.Asp350Gly	SNV	0.00%			168	Yes	Yes	Untested	
PIK3CA	c.1132T>C	p.Cys378Arg	SNV	0.00%			89	Yes	Yes	Untested	
PIK3CA	c.1134T>C		SNV	0.00%			88	Yes	Yes	Untested	
PIK3CA	c.1357G>A	p.Glu453Lys	SNV	0.00%			146	Yes	Yes	Untested	
PIK3CA	c.1357G>C	p.Glu453Gln	SNV	0.00%			146	Yes	Yes	Untested	
PIK3CA	c.1370A>G	p.Asn457Ser	SNV	2.05%	28.33		146	Yes	Yes	Untested	
PIK3CA	c.3036A>G		SNV	0.54%	31.00		185	Yes	Yes	Untested	
PIK3CA	c.3044C>T	p.Ser1015Phe	SNV	0.00%			185	Yes	Yes	Untested	
KIT	c.1638A>G		SNV	0.00%			124	Yes	Yes	Untested	
EGFR	c.2184+19G>A		SNV	0.00%			61	Yes	Yes	Untested	
BRAF	c.1853T>G	p.Leu618Trp	SNV	0.00%			191	Yes	Yes	Untested	
BRAF	c.1853T>C	p.Leu618Ser	SNV	0.00%			191	Yes	Yes	Untested	
BRAF	c.1847C>T	p.Ser616Phe	SNV	0.00%			184	Yes	Yes	Untested	
BRAF	c.1846T>C	p.Ser616Pro	SNV	0.56%	33.00		179	Yes	Yes	Untested	
BRAF	c.1843G>C	p.Gly615Arg	SNV	0.00%			163	Yes	Yes	Untested	
BRAF	c.1843G>A	p.Gly615Arg	SNV	1.23%	31.00		163	Yes	Yes	Untested	

BRAF	c.1840T>C	p.Ser614Pro	SNV	1.21%	32.00		165	Yes	Yes	Untested	
KRAS	c.491G>A	p.Arg164Gln	SNV	0.00%			189	Yes	Yes	Untested	
KRAS	c.439A>G	p.Lys147Glu	SNV	0.71%	30.00		141	Yes	Yes	Untested	
KRAS	c.437C>T	p.Ala146Val	SNV	0.00%			141	Yes	Yes	Untested	
KRAS	c.436G>C	p.Ala146Pro	SNV	0.00%			141	Yes	Yes	Untested	
KRAS	c.436G>A	p.Ala146Thr	SNV	0.00%			141	Yes	Yes	Untested	
KRAS	c.352T>A	p.Cys118Ser	SNV	0.76%	36.00		131	Yes	Yes	Untested	
KRAS	c.351A>T	p.Lys117Asn	SNV	0.00%			131	Yes	Yes	Untested	
KRAS	c.351A>C	p.Lys117Asn	SNV	0.00%			131	Yes	Yes	Untested	
KRAS	c.104C>T	p.Thr35Ile	SNV	0.00%			159	Yes	Yes	Untested	
KRAS	c.27T>G		SNV	0.00%			147	Yes	Yes	Untested	
KRAS	c.27T>C		SNV	0.00%			147	Yes	Yes	Untested	
KRAS	c.27T>A		SNV	0.00%			147	Yes	Yes	Untested	
KRAS	c.24A>T		SNV	0.00%			148	Yes	Yes	Untested	
KRAS	c.24A>G		SNV	0.68%	38.00		148	Yes	Yes	Untested	
KRAS	c.24A>C		SNV	0.00%			148	Yes	Yes	Untested	
KRAS	c.15A>T	p.Lys5Asn	SNV	0.00%			182	Yes	Yes	Untested	
KRAS	c.15A>C	p.Lys5Asn	SNV	0.55%	14.00		182	Yes	Yes	Untested	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
PIK3CA	c.353G>A	mis-sense		0	0	0		178917478	3
PIK3CA	c.519T>C			1	1	0		178917644	3
PIK3CA	c.519T>G			0	0	0		178917644	3
PIK3CA	c.1030G>A	mis-sense		0	0	0		178921548	3
PIK3CA	c.1031T>G	mis-sense		0	0	0		178921549	3
PIK3CA	c.1035T>A	mis-sense		0	0	0		178921553	3
PIK3CA	c.1035T>G	mis-sense		0	0	0		178921553	3
PIK3CA	c.1048G>A	mis-sense		0	0	0		178921566	3
PIK3CA	c.1049A>G	mis-sense		0	0	0		178921567	3

PIK3CA	c.1132T>C	mis-sense		0	0	0		178922363	3
PIK3CA	c.1134T>C			0	0	0		178922365	3
PIK3CA	c.1357G>A	mis-sense		0	0	0		178928079	3
PIK3CA	c.1357G>C	mis-sense		0	0	0		178928079	3
PIK3CA	c.1370A>G	mis-sense		3	2	1		178928092	3
PIK3CA	c.3036A>G			1	1	0		178951981	3
PIK3CA	c.3044C>T	mis-sense		0	0	0		178951989	3
KIT	c.1638A>G			0	0	0		55593481	4
EGFR	c.2184+19G>A			0	0	0		55241755	7
BRAF	c.1853T>G	mis-sense		0	0	0		140453082	7
BRAF	c.1853T>C	mis-sense		0	0	0		140453082	7
BRAF	c.1847C>T	mis-sense		0	0	0		140453088	7
BRAF	c.1846T>C	mis-sense		1	0	1		140453089	7
BRAF	c.1843G>C	mis-sense		0	0	0		140453092	7
BRAF	c.1843G>A	mis-sense		2	0	2		140453092	7
BRAF	c.1840T>C	mis-sense		2	1	1		140453095	7
KRAS	c.491G>A	mis-sense		0	0	0		25362805	12
KRAS	c.439A>G	mis-sense		1	1	0		25378559	12
KRAS	c.437C>T	mis-sense		0	0	0		25378561	12
KRAS	c.436G>C	mis-sense		0	0	0		25378562	12
KRAS	c.436G>A	mis-sense		0	0	0		25378562	12
KRAS	c.352T>A	mis-sense		1	1	0		25378646	12
KRAS	c.351A>T	mis-sense		0	0	0		25378647	12
KRAS	c.351A>C	mis-sense		0	0	0		25378647	12
KRAS	c.104C>T	mis-sense		0	0	0		25398215	12
KRAS	c.27T>G			0	0	0		25398292	12
KRAS	c.27T>C			0	0	0		25398292	12
KRAS	c.27T>A			0	0	0		25398292	12
KRAS	c.24A>T			0	0	0		25398295	12
KRAS	c.24A>G			1	0	1		25398295	12
KRAS	c.24A>C			0	0	0		25398295	12
KRAS	c.15A>T	mis-sense		0	0	0		25398304	12

KRAS	c.15A>C	mis-sense		1	0	1		25398304	12

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

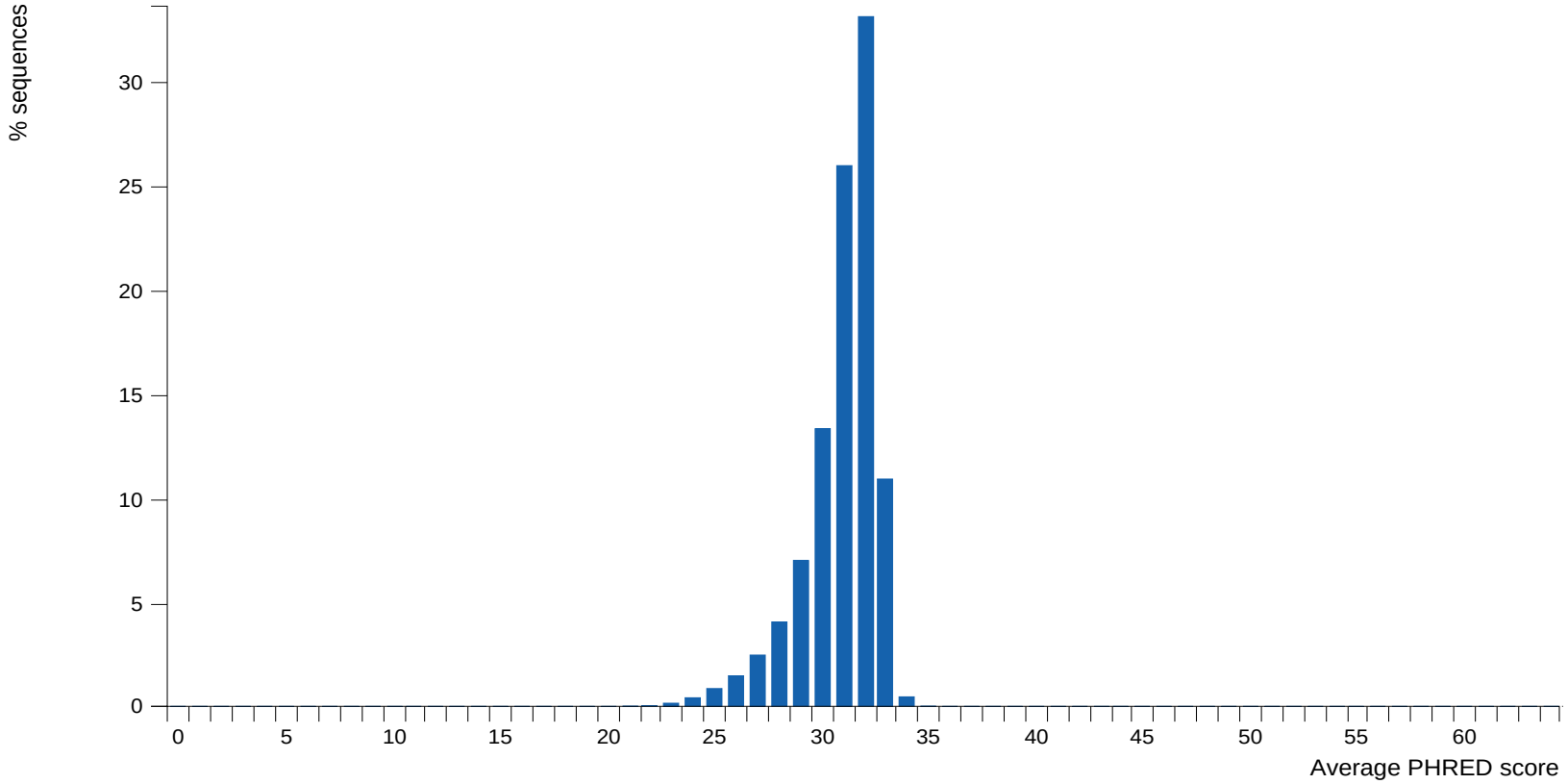
Comment: Remark added by user during variant review.

4 Detailed QC

Quality control metrics for detailed inspection. These metrics can indicate possible problems in the upstream workflow or data analysis. Quality control are divided in metrics on the incoming reads from input data, and metrics per base positions in these reads. Lastly section 4.3 and 4.4 display metrics on how well the positions in the region of interest are covered

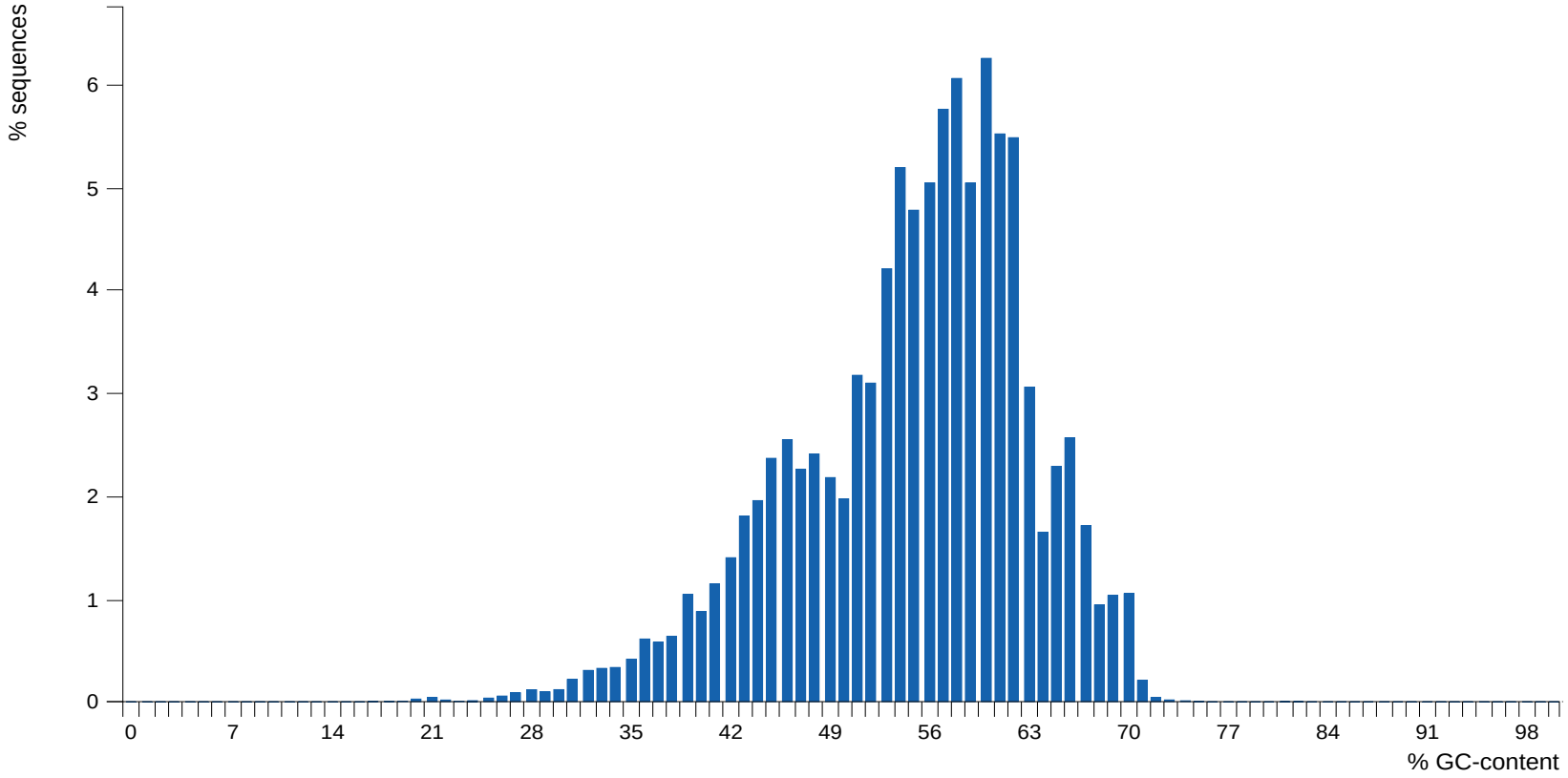
4.1 QC for reads

4.1.1 Average base quality of reads



Distribution of average sequence quality scores. The quality of a sequence is calculated as the arithmetic mean of its base qualities.
x: PHRED-score
y: number of sequences observed at that qual. score normalized to the total number of sequences

4.1.2 GC content of reads



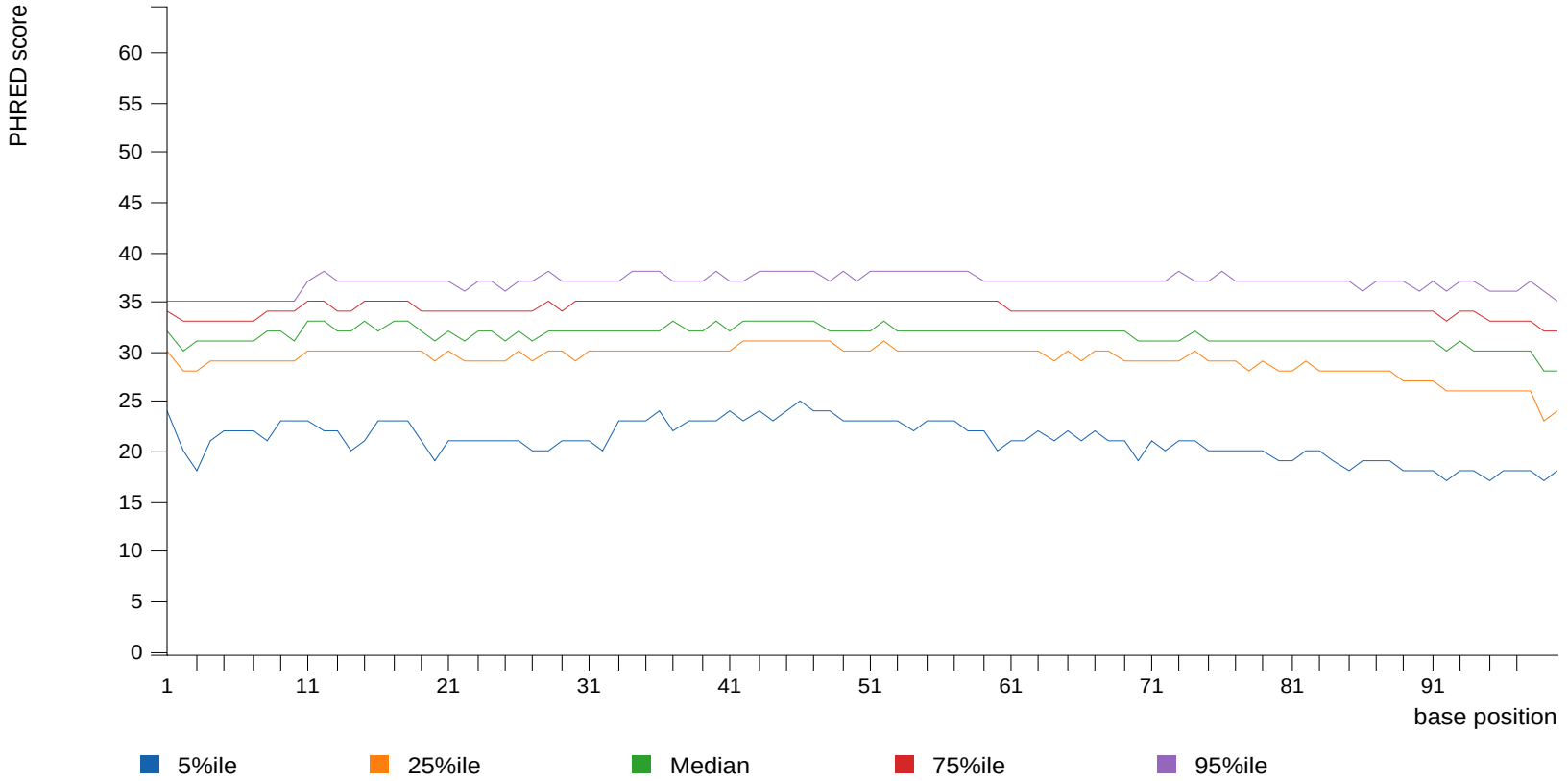
Distribution of GC-contents. The GC-content of a sequence is calculated as the number of GC-bases compared to all bases (including ambiguous bases).
x: relative GC-content of a sequence in percent
y: number of sequences featuring particular GC-percentages normalized to the total number of sequences

4.1.3 Ambiguous base content of reads

No ambiguous bases detected.

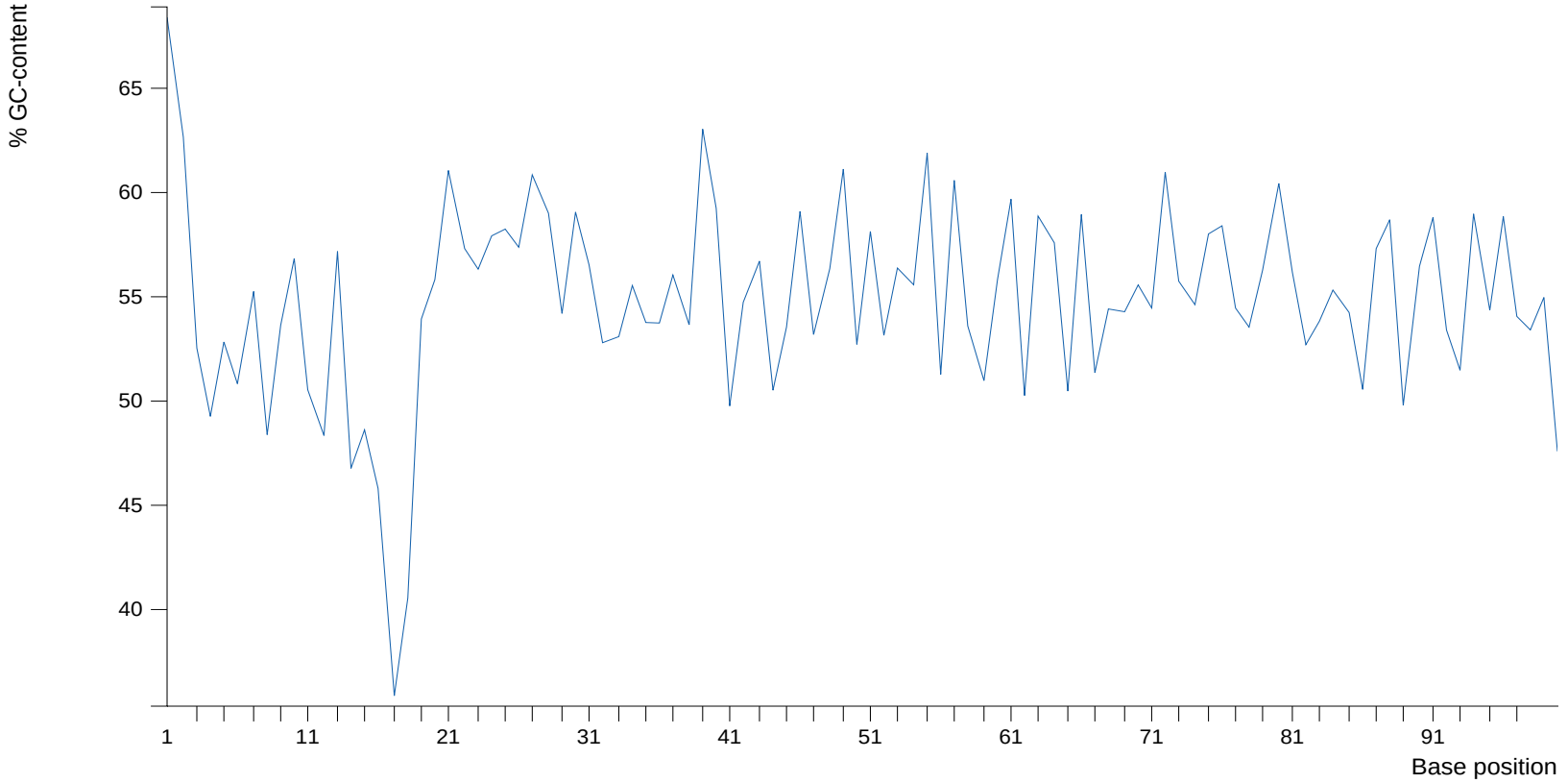
4.2 QC for bases

4.2.1 Quality score per base position



Base-quality distribution along the base positions.
x: base position
y: median & percentiles of quality scores observed at that base position

4.2.2 GC content per base position



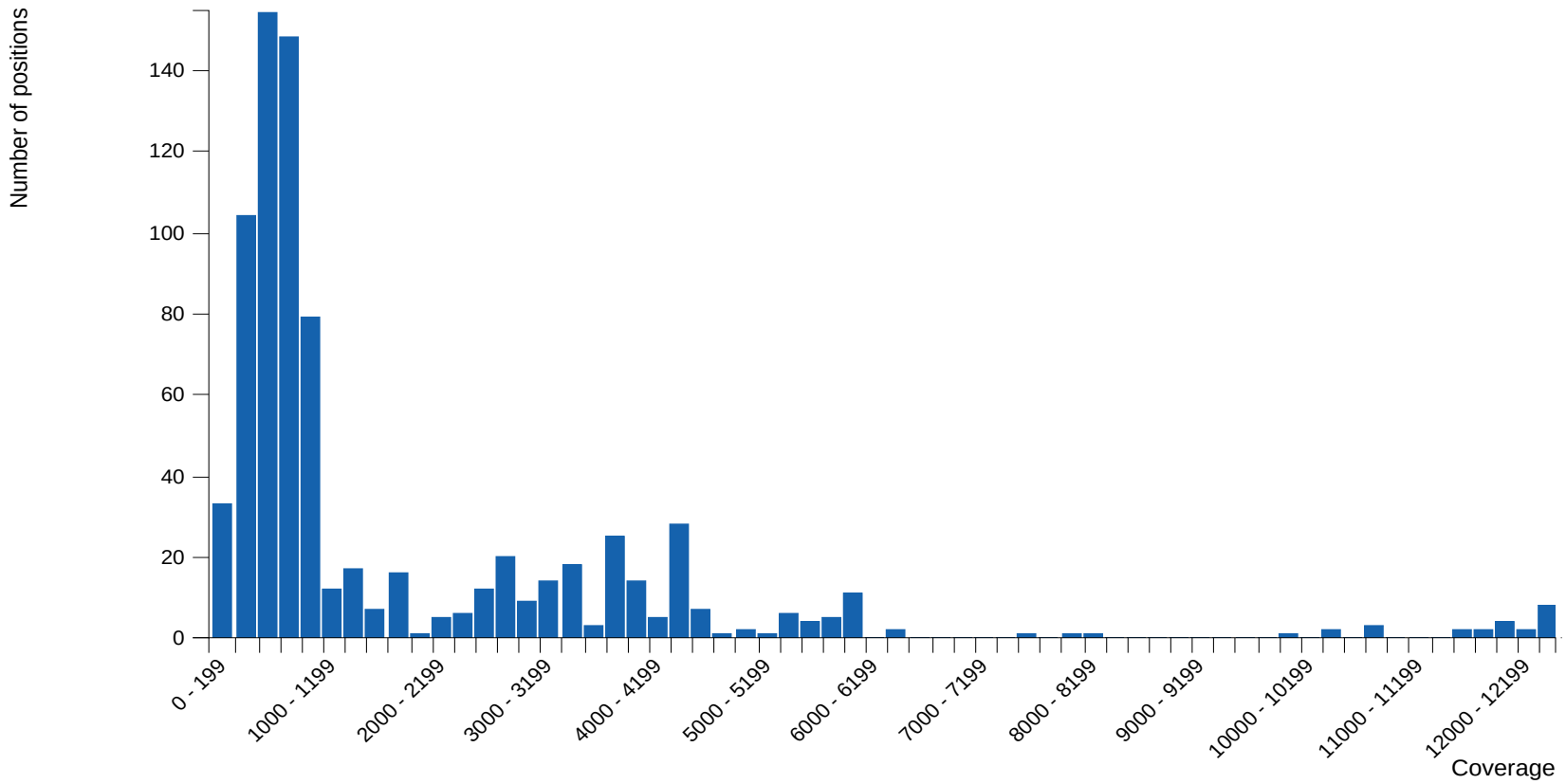
Combined coverage of G- and C-bases.
x: base position
y: number of G- and C-bases observed at current position normalized to the total number of bases observed at that position

4.2.3 Ambiguous base content per base position

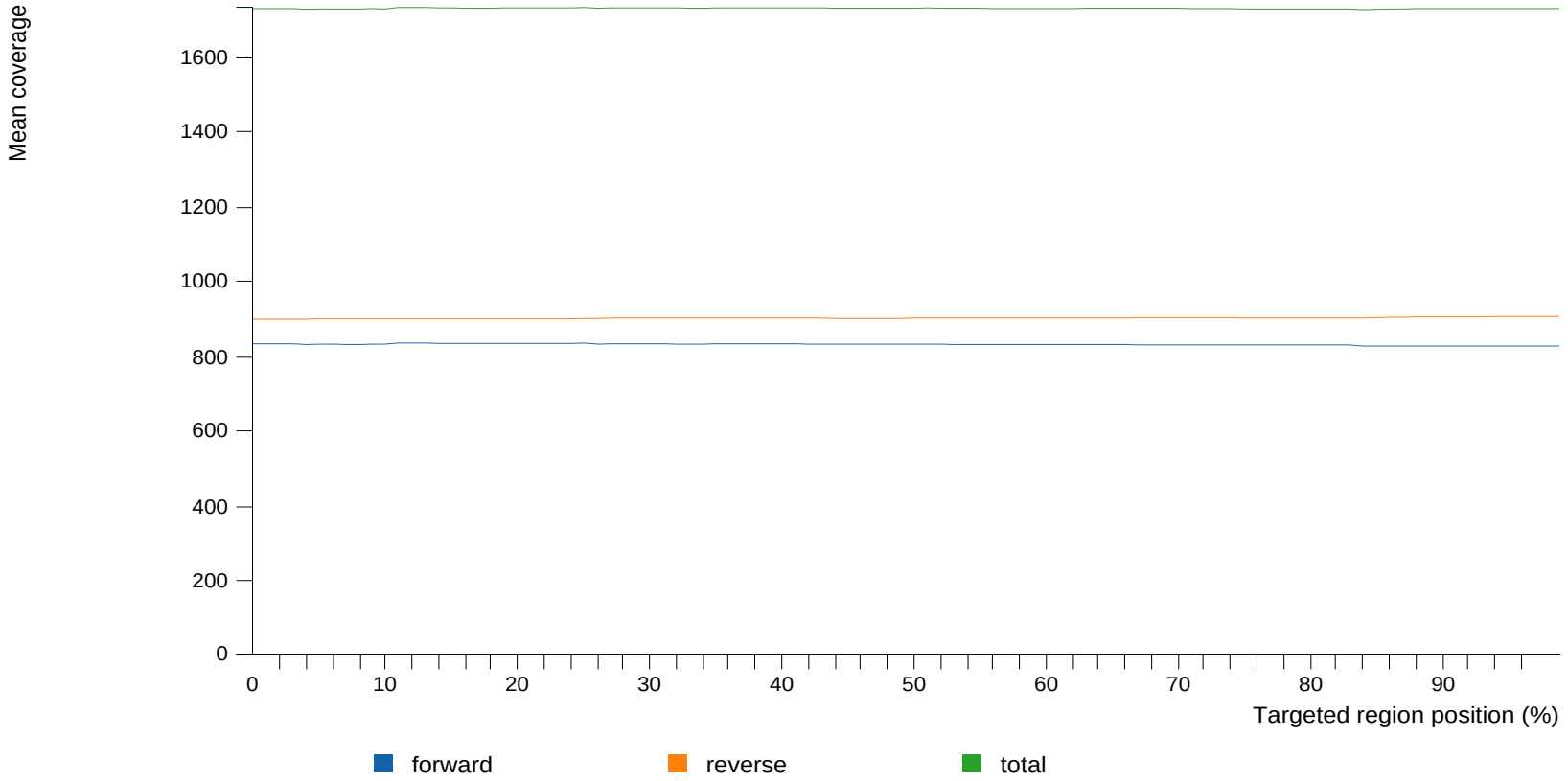
No ambiguous bases detected

4.3 Coverage of Regions of Interest positions

Coverage distribution



4.4 Mean coverage of relative positions in regions of interest



5 History

5.1 Log Entries

Type	Time	User	Details
State change	Fri Oct 25 17:36:53 CEST 2019	system	Ready for Review
State change	Fri Oct 25 17:16:06 CEST 2019	root	In Progress

5.2 Execution Information

QCIA version	QCI Analyze 1.4.5
Analysis start time	Fri Oct 25 17:16:06 CEST 2019
Analysis workflow	AIT FFPE 4.5
Analysis description	QIAact Actionable Insights Tumor Panel on FFPE

5.3 Transcripts

Table listing the genes, transcript IDs and protein IDs used in the analysis.

Gene Name	Transcript ID	Protein ID
NRAS	NM_002524.4	NP_002515.1
ALK	NM_004304.4	NP_004295.2
RAF1	NM_002880.3	NP_002871.1
PIK3CA	NM_006218.2	NP_006209.2
PDGFRA	NM_006206.4	NP_006197.1
KIT	NM_000222.2	NP_000213.1
ESR1	NM_001122742.1	NP_001116214.1
EGFR	NM_005228.3	NP_005219.2
BRAF	NM_004333.4	NP_004324.2
KRAS	NM_004985.3	NP_004976.2
ERBB3	NM_001982.3	NP_001973.2

Gene Name	Transcript ID	Protein ID
ERBB2	NM_004448.2	NP_004439.2

5.4 Open Parameters

Workflow parameters that are editable by the administrator

Reported variants

Significant coverage threshold	500
SNV/MNV frequency threshold in %	4.00
Insertions, deletions and replacements frequency threshold in %	4.00

Variants available for review

Minimum coverage threshold	200
SNV/MNV frequency threshold in %	4.00
Insertions, deletions and replacements frequency threshold in %	4.00
Detect variants outside regions of interest	Yes

5.5 Locked Parameters

Adapter trimming

Trim adapter list	GRadapter_160913
Ambiguous trim	false
Ambiguous limit	2
Quality trim	false
Quality limit	0.05
Use colorspace	false

Also search on reversed sequence	false
Remove 5' terminal nucleotides	false
Number of 5' terminal nucleotides	1
Maximum number of nucleotides in reads	1000
Minimum number of nucleotides in reads	15
Discard short reads	false
Remove 3' terminal nucleotides	false
Number of 3' terminal nucleotides	1
Discard long reads	false

Map Reads to Reference

References	Homo_sapiens_sequence_hg19
Masking mode	No masking
Masking track	Not set
Match score	1
Mismatch cost	2
Cost of insertions and deletions	Affine gap cost
Insertion cost	3
Deletion cost	3
Insertion open cost	6
Insertion extend cost	1
Deletion open cost	6
Deletion extend cost	1
Length fraction	0.5
Similarity fraction	0.8
Global alignment	false
Color space alignment	false
Color error cost	3
Auto-detect paired distances	false
Non-specific match handling	Map randomly

InDels and Structural Variants

P-Value threshold	1.0E-4
Maximum number of mismatches	3
Ignore broken pairs	true
Minimum relative consensus coverage	0.0
Minimum quality score	0
Filter variants	true
Minimum number of reads	2
Restrict calling to target regions	ATPv2_TargetRegions_170302_ver1.1

Local Realignment (Short Unaligned End version)

Realign unaligned ends	true
Multi-pass realignment	2
Local bound for unaligned ends of size one	0.75
Local bound for unaligned ends of size two	0.75
Force realignment to guidance-variants	false
Maximum guidance-variant length	100

Trim Primers and their Dimers of Mapped Reads

Primer track	101x_GR_primers_15_10_15_V1.0
Reference	Homo_sapiens_sequence_hg19
Minimum primer overlap length	9
Allow dangling 3' end base	true
Minimal primer overlap fraction	0.7
Only keep reads that have hit a primer	true
Additional bases to trim	1

Remove Pseudogene Reads

Genes track	AITv2_PseudoGenes_170912_ver1.0
Gene and pseudogene links	KRAS -> KRASP1
Required unaligned ends %	2.0

Low Frequency Variant Detection

Required significance (%)	0.01
Ignore positions with coverage above	1000000000
Restrict calling to target regions	ATPv2_TargetRegions_170302_ver1.1
Ignore broken pairs	true
Ignore non-specific matches	Reads
Minimum read length	20
Minimum coverage	Parameter editable by administrator
Minimum count	8
Minimum frequency (%)	Parameter editable by administrator
Base quality filter	false
Neighborhood radius	5
Minimum central quality	5
Minimum neighborhood quality	5
Read direction filter	false
Direction frequency (%)	5.0
Relative read direction filter	false
Significance (%)	1.0E-5
Read position filter	false
Significance (%)	1.0
Remove pyro-error variants	false
In homopolymer regions with minimum length	3
With frequency below	0.8

Remove False Positives

Minimum frequency (%)	Parameter editable by administrator
Minimum forward/reverse balance	0.05
Minimum average base quality	22.0
Variant frequency	true
Forward/reverse balance	false
Average base quality	true

Annotate Variants With Primers

Minimum coverage count	400
Minimum variant percentage	1.0
Minimum variant read count	2

AnyGenomics Lab

QIAact Actionable Insights Tumor Panel For the GeneReader NGS System (RUO)

Report Date Mar 26, 2018
Report Status -

Patient Information

Patient Name
Date of Birth
Ethnicity
Sex
Accession 20180322144720_8160085015705246905180014012359_14_BC4_2018-03-22

Client Information

Client
Client ID
Physician
Pathologist

Specimen

Specimen Type
Specimen ID
Collection Date
Accession Date Mar 23, 2018
Primary Tumor Site Brain
Diagnosis Glioblastoma
Diagnosis Stage

Interpretation

2 Clinically Significant Variants Reported | 0 Approved Therapy | 1 Potential Clinical Trial

Summary of Clinically Significant Variants

Variants Reported	Approved Therapies for Same Cancer	Approved Therapies for Other Cancers	Therapies Associated with Resistance	Potential Clinical Trials
EGFR p.E709K				1 potential trial
KIT p.D579D				

Variant Details

Gene	Exon #	Nucleotide Change	Amino Acid Change	Effect on Protein
EGFR	18	NM_005228.4: c.2125G>A	p.E709K	gain of function

EGFR is an oncogene involved in cell growth and differentiation through activation of the PI3K/AKT/MTOR and RAS/RAF/MAPK pathways [8]. Amplification, gain-of-function mutations, and protein overexpression cause EGFR activation [6, 12, 7, 9]. EGFR mutations are reported to be mutually exclusive with ALK rearrangements and KRAS mutations in non-small cell lung cancer [3, 10, 11].

QIAact Actionable Insights Tumor Panel For the GeneReader NGS System (RUO)

Variant Details

Gene	Exon #	Nucleotide Change	Amino Acid Change	Effect on Protein
KIT	11	NM_000222.2: c.1737T>C	p.D579D	**normal function**

KIT is an oncogene involved in cell proliferation and survival through activation of RAS/RAF/MAPK and PI3K/AKT/MTOR pathways [2]. Amplification, gain-of-function mutations, and protein overexpression cause KIT activation [4, 5, 1].

Clinical Trials

Potential clinical trial(s) related to EGFR

Study Title	AN OPEN-LABEL, PHASE 2 STUDY OF NERATINIB IN PATIENTS WITH SOLID TUMORS WITH SOMATIC HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR, HER2, HER3) MUTATIONS OR EGFR GENE AMPLIFICATION
Treatment	neratinib
Identifier	NCT01953926
Location(s)	United States: CA, FL, LA, MA, MO, NY, PA, TN, TX
Contact	Puma Biotechnology Clinical Operations Senior Director; ClinicalTrials@pumabiotechnology.com; (424) 248-6500

Genes Tested

KRAS *NRAS* *KIT* *BRAF* *PDGFRA* *ALK* *EGFR* *ERBB2* *PIK3CA* *ERBB3* *ESR1* *RAF1*

Methods and Limitations

EXAMPLE Statement including sample type (FFPE, etc), method of extraction, amplification reactions, panel targeted regions, sequencing technology, etc. Additionally, a description of the data analysis software(s), genome of reference and the sensitivity of the methods should be described.

QIAGEN Clinical Insight (QCI™) software includes the following underlying databases, data reference sets and tools; QIAGEN Clinical Insight-Interpret (5.2.20180316), Ingenuity Knowledge Base (Pandora 180329.003), CADD (v1.3), CentoMD (4.1), EVS (ESP6500SI-V2), Allele Frequency Community (2018-01-17), JASPAR (2013-11), Ingenuity Knowledge Base Snapshot Timestamp (2018-03-29 18:20:56.0), Vista Enhancer hg18 (2012-07), Vista Enhancer hg19 (2012-07), OMIM (May 26, 2017), gnomAD (2.0.1), Clinical Trials (Pandora 180329.003), BSIFT (2016-02-23), TCGA (2013-09-05), PolyPhen-2 (v2.2.2), 1000 Genome Frequency (phase3v5b), Clinvar (2018-01-03), DGV (2016-05-15), COSMIC (v83), ExAC (0.3.1), HGMD (2017.4),

QIAact Actionable Insights Tumor Panel For the GeneReader NGS System (RUO)

PhyloP hg18 (2009-11), PhyloP hg19 (2009-11), DbSNP (150(2017-07-10)), TargetScan (6.2), SIFT4G (2016-02-23)

Laboratory Statement

This section can be customized to provide additional information regarding laboratory methods etc.

Selected Citations

1. Carvajal RD, Antonescu CR, Wolchok JD, Chapman PB, Roman RA, Teitcher J, Panageas KS, Busam KJ, Chmielowski B, Lutzky J, Pavlick AC, Fusco A, Cane L, Takebe N, Vemula S, Bouvier N, Bastian BC, Schwartz GK (2011) KIT as a therapeutic target in metastatic melanoma. JAMA. 2011 Jun 08;305(22):2327-34 (PMID: 21642685)
2. Corless CL, Barnett CM, Heinrich MC (2011) Gastrointestinal stromal tumours: origin and molecular oncology. Nat Rev Cancer. 2011 Nov 17;11(12):865-78 (PMID: 22089421)
3. Gainor JF, Varghese AM, Ou SH, Kabraji S, Awad MM, Katayama R, Pawlak A, Mino-Kenudson M, Yeap BY, Riely GJ, Iafrate AJ, Arcila ME, Ladanyi M, Engelman JA, Dias-Santagata D, Shaw AT (2013) ALK rearrangements are mutually exclusive with mutations in EGFR or KRAS: an analysis of 1,683 patients with non-small cell lung cancer. Clin Cancer Res. 2013 Aug 01;19(15):4273-81. Epub 2013 May 31 (PMID: 23729361)
4. Heinrich MC, Corless CL, Demetri GD, Blanke CD, von Mehren M, Joensuu H, McGreevey LS, Chen CJ, Van den Abbeele AD, Druker BJ, Kiese B, Eisenberg B, Roberts PJ, Singer S, Fletcher CD, Silberman S, Dimitrijevic S, Fletcher JA (2003) Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. J Clin Oncol. 2003 Dec 01;21(23):4342-9 (PMID: 14645423)
5. Hirota S, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, Kawano K, Hanada M, Kurata A, Takeda M, Muhammad Tunio G, Matsuzawa Y, Kanakura Y, Shinomura Y, Kitamura Y (1998) Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science. 1998 Jan 23;279(5350):577-80 (PMID: 9438854)
6. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med. 2004 May 20;350(21):2129-39. Epub 2004 Apr 29 (PMID: 15118073)
7. Rusch V, Klimstra D, Venkatraman E, Pisters PW, Langenfeld J, Dmitrovsky E (1997) Overexpression of the epidermal growth factor receptor and its ligand transforming growth factor alpha is frequent in resectable non-small cell lung cancer but does not predict tumor progression. Clin Cancer Res. 1997 Apr;3(4):515-22 (PMID: 9815714)
8. Scaltriti M, Baselga J (2006) The epidermal growth factor receptor pathway: a model for targeted therapy. Clin Cancer Res. 2006 Sep 15;12(18):5268-72 (PMID: 17000658)
9. Selvaggi G, Novello S, Torri V, Leonardo E, De Giuli P, Borasio P, Mossetti C, Ardissone F, Lausi P, Scagliotti GV (2004) Epidermal growth factor receptor overexpression correlates with a poor prognosis in completely resected non-small-cell lung cancer. Ann Oncol. 2004 Jan;15(1):28-32 (PMID: 14679115)
10. Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, Wistuba II, Fong KM, Lee H, Toyooka S, Shimizu N, Fujisawa T, Feng Z, Roth JA, Herz J, Minna JD, Gazdar AF (2005) Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst. 2005 Mar 02;97(5):339-46 (PMID: 15741570)
11. Shigematsu H, Takahashi T, Nomura M, Majmudar K, Suzuki M, Lee H, Wistuba II, Fong KM, Toyooka S, Shimizu N, Fujisawa T, Minna JD, Gazdar AF (2005) Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. Cancer Res. 2005 Mar 01;65(5):1642-6 (PMID: 15753357)
12. Yoshida K, Tsuda T, Matsumura T, Tsujino T, Hattori T, Ito H, Tahara E (1989) Amplification of epidermal growth factor

QIAact Actionable Insights Tumor Panel For the GeneReader NGS System (RUO)

Selected Citations

receptor (EGFR) gene and oncogenes in human gastric carcinomas. Virchows Arch B Cell Pathol Incl Mol Pathol. 1989;57(5):285-90 (PMID: 2570489)

Analysis Report

20190628103639_1001600507010619014 7_475-19CTC_BC8_Glio2019

Table of contents

1 Summary

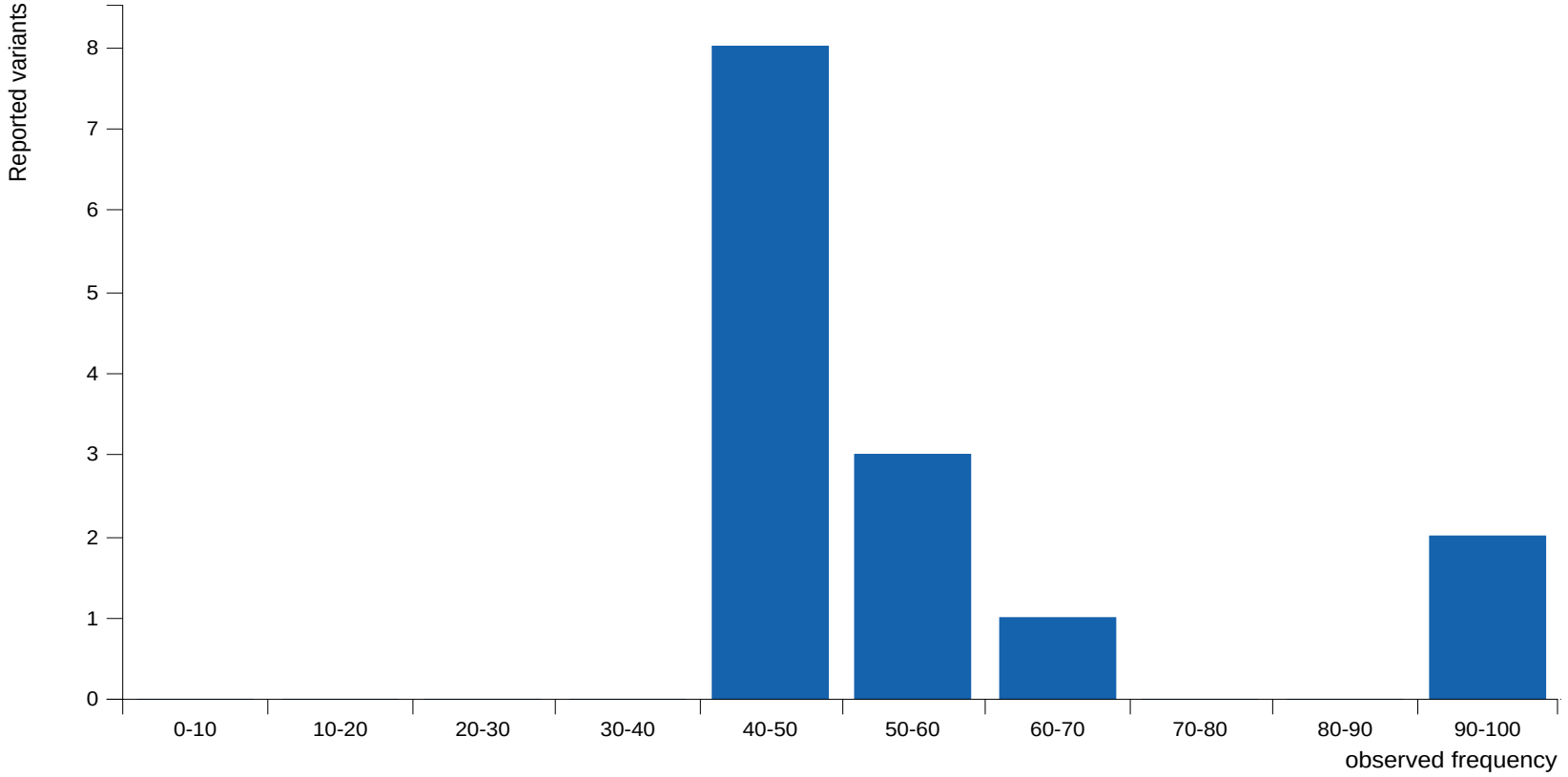
Report created	Sat Jun 29 18:11:23 CEST 2019
Sample ID	20190628103639_10016005070106190147_475-19CTC_BC8_Glio2019
Analysis workflow	AIT FFPE v4.5: QIAact Actionable Insights Tumor Panel on FFPE
Analyst	root
Reported variants	14
Analysis results	3 Untested variants

1.1 Comments

No comments

1.2 Distribution of observed frequencies for reported variants

Includes variants initially listed in variant table 'Reported variants'.



2 Quality control

Quality control for the sample analysis. Includes information on the input data, read mapping, and coverage information per gene.

2.1 Fastq

Fastq	20190628103639_10016005070106190147_475-19CTC_BC8_Glio2019
Reads	523,930
Nucleotides*	51,012,895
Average read length*	97.37
Reads with average quality ≥ 25	98.89%

** Including sample barcode*

Recommendations:

Reads with average quality ≥ 25 should be ≥ 80.00%

2.2 Secondary analysis summary

Reads mapped	473,966 (90.46%)
Reads in target regions	306,517 (64.67%)
Percentage of base positions in regions of interest with coverage ≥ 500x	93.97%
Percentage of base positions in regions of interest with coverage ≥ 200x	99.62%

Recommendations:

Percentage of base positions in regions of interest with coverage ≥ 500x should be ≥ 90.00%

Percentage of base positions in regions of interest with coverage ≥ 200x should be ≥ 95.00%

2.3 Coverage

Name	ROI	Bases	≥500x	≥200x	0x	Median	VOI	VOI <500x	VOI <200x
NRAS	6	27	100.00%	100.00%	0.00%	2,797	41	0	0
ALK	22	47	91.49%	100.00%	0.00%	1,196	40	1	0
RAF1	2	2	100.00%	100.00%	0.00%	1,819	2	0	0
PIK3CA	81	131	96.95%	100.00%	0.00%	1,535	165	4	0

Name	ROI	Bases	≥500x	≥200x	0x	Median	VOI	VOI <500x	VOI <200x
PDGFRA	21	64	100.00%	100.00%	0.00%	1,722	46	0	0
KIT	49	140	99.29%	100.00%	0.00%	1,882	235	1	0
ESR1	6	7	100.00%	100.00%	0.00%	890	11	0	0
EGFR	96	208	82.21%	98.56%	0.00%	906	443	48	3
BRAF	29	72	100.00%	100.00%	0.00%	1,727	153	0	0
KRAS	21	55	100.00%	100.00%	0.00%	2,147	148	0	0
ERBB3	8	8	100.00%	100.00%	0.00%	1,451	10	0	0
ERBB2	16	35	94.29%	100.00%	0.00%	2,722	61	2	0

ROI: Number of Regions of Interest, i.e. reportable regions that overlap with the gene.

Bases: Total number of base positions in Regions of Interest that overlap with the gene.

≥500x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is equal to or above the significant coverage threshold.

≥200x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is equal to or above the minimum coverage threshold.

0x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is zero.

Median: Median coverage of base positions in the Regions of Interest that overlap with the gene.

VOI: Total number of Variants of Interest, whether detected or not, that overlap with the gene. The list of Variants of Interest is defined by the analysis pipeline.

VOI <500x: Number of Variants of Interest in the gene for which coverage is below the significant coverage threshold.

VOI <200x: Number of Variants of Interest in the gene for which coverage is below the minimum coverage threshold.

2.4 Detected variants

Number of detected variants per gene. Variants for which coverage is above the minimum coverage threshold.

Name	In total	VOI	- non-syn	- syn	Non-VOI	- non-syn	- syn
NRAS	0	0	0	0	0	0	0
ALK	1	1	0	1	0	0	0
RAF1	0	0	0	0	0	0	0
PIK3CA	0	0	0	0	0	0	0
PDGFRA	8	6	1	5	2	0	2
KIT	1	1	0	1	0	0	0
ESR1	1	0	0	0	1	0	1
EGFR	9	3	1	2	6	0	6
BRAF	1	0	0	0	1	0	1

Name	In total	VOI	- non-syn	- syn	Non-VOI	- non-syn	- syn
KRAS	6	0	0	0	6	1	5
ERBB3	3	2	1	1	1	0	1
ERBB2	1	1	1	0	0	0	0

In total: Total number of variants detected within the gene. Variants initially listed in variant tables 3.1 and 3.2.

VOI: Number of detected Variants of Interest detected within the gene. The list of Variants of Interest is defined by the analysis pipeline.

- non-syn: Number of detected, gene-specific Variants of Interest that are non-synonymous.

- syn: Number of detected, gene-specific Variants of Interest that are synonymous.

Non-VOI: Number of detected variants that are not found within the analysis pipeline-defined list of Variants of Interest.

- non-syn: Number of gene-specific, non-VOIs that are non-synonymous.

- syn: Number of gene-specific, non-VOIs that are synonymous.

3 Variants

Variants detected within regions of interest with more than significant coverage are found in 3.1 and variants with more than minimum coverage are found in 3.2.
Variants of interest that could not be tested due to insufficient coverage are listed in table 3.3.
The coverage thresholds and minimum frequency cutoffs configured for the analysis workflow are listed in the History section.
Setting a variant review state to "Confirmed by review" moves it to 3.1, "Artifact" moves it to 3.2.
Only the variants in table 3.1 are exported as VCF and uploaded to QCI Interpret.

3.1 Reported variants

Variants that will be exported to VCF and uploaded to QCI Interpret. Initially contains: Variants detected within regions of interest with more than significant coverage and frequency above the cutoff set for the analysis workflow. These variants are assigned the initial review state "Valid".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
ALK	c.2535T>C		SNV	99.93%	31.46	1.00	1,462	Yes	Yes	Valid	
PDGFRA	c.612T>C		SNV	64.88%	30.44	0.99	1,213	Yes	Yes	Valid	
PDGFRA	c.939T>G		SNV	49.52%	29.66	1.00	1,137	Yes	Yes	Valid	
PDGFRA	c.1432T>C	p.Ser478Pro	SNV	54.95%	25.05	1.00	1,576	Yes	Yes	Valid	
PDGFRA	c.1701A>G		SNV	99.57%	30.13	1.00	1,638	Yes	Yes	Valid	
PDGFRA	c.1809G>A		SNV	45.00%	33.78	1.00	1,511	Yes	Yes	Valid	
PDGFRA	c.2472C>T		SNV	50.00%	34.06	1.00	1,810	Yes	Yes	Valid	
KIT	c.2586G>C		SNV	52.84%	29.38	0.99	1,919	Yes	Yes	Valid	
EGFR	c.1562G>A	p.Arg521Lys	SNV	40.91%	34.62	1.00	902	Yes	Yes	Valid	
EGFR	c.2361G>A		SNV	47.46%	31.20	1.00	590	Yes	Yes	Valid	
EGFR	c.2982C>T		SNV	57.30%	35.33	1.00	1,677	Yes	Yes	Valid	
ERBB3	c.1347T>C		SNV	45.67%	35.70	1.00	3,175	Yes	Yes	Valid	
ERBB3	c.3355A>T	p.Ser1119Cys	SNV	46.10%	29.57	1.00	1,076	Yes	Yes	Valid	
ERBB2	c.3508C>G	p.Pro1170Ala	SNV	45.44%	24.96	0.76	625	Yes	Yes	Valid	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
ALK	c.2535T>C		No	1,461	747	714	200	29455267	2
PDGFRA	c.612T>C		No	787	168	619	200	55130078	4
PDGFRA	c.939T>G		No	563	71	492	200	55133726	4
PDGFRA	c.1432T>C	mis-sense	No	866	231	635	200	55139771	4
PDGFRA	c.1701A>G		No	1,631	647	984	200	55141055	4
PDGFRA	c.1809G>A		No	680	273	407	200	55143577	4
PDGFRA	c.2472C>T		No	905	704	201	200	55152040	4
KIT	c.2586G>C		No	1,014	315	699	200	55602765	4
EGFR	c.1562G>A	mis-sense	No	369	133	236	200	55229255	7
EGFR	c.2361G>A		No	280	149	131	200	55249063	7
EGFR	c.2982C>T		No	961	437	524	200	55268916	7
ERBB3	c.1347T>C		No	1,450	765	685	200	56487201	12
ERBB3	c.3355A>T	mis-sense	No	496	217	279	200	56494998	12
ERBB2	c.3508C>G	mis-sense	No	284	28	256	200	37884037	17

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

Comment: Remark added by user during variant review.

3.2 Variants available for review

Detected variants that will not be exported to the VCF and uploaded to QCI Interpret. Initially contains: Variants with more than minimum coverage and frequency above the cutoff set for the analysis workflow. Depending on workflow configuration, this table may include variants outside of regions of interest including those with coverage above significant coverage threshold. These variants are assigned the initial review state "Review".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
PDGFRA	c.2440-50_2440-49insA		Insertion	50.95%	33.80	1.00	952	No	No	Review	
PDGFRA	c.3222T>C		SNV	99.67%	31.41	1.00	2,094	No	No	Review	
ESR1	c.975G>C		SNV	99.38%	30.92	1.00	1,280	No	No	Review	

EGFR	c.890-4C>G		SNV	10.89%	32.24	0.63	1,598	No	No	Review	
EGFR	c.1498+22A>T		SNV	99.65%	33.06	1.00	1,694	No	No	Review	
EGFR	c.1839C>T		SNV	42.10%	33.07	1.00	1,107	No	No	Review	
EGFR	c.1881-858_1881-857delGA		Deletion	51.70%	30.98	1.00	499	No	No	Review	
EGFR	c.2284-60T>C		SNV	49.76%	32.73	1.00	211	No	No	Review	
EGFR	c.2709T>C		SNV	96.88%	30.00	1.00	1,090	No	No	Review	
BRAF	c.1860+66A>C		SNV	68.91%	36.41	1.00	402	No	No	Review	
KRAS	c.541_542delTCinsAG	p.Ser181Arg	MNV	7.25%	31.12	9.33E-15	1,545	No	No	Review	
KRAS	c.534A>G		SNV	7.31%	31.38	1.67E-15	1,586	No	No	Review	
KRAS	c.525A>G		SNV	6.59%	30.80	6.00E-15	1,714	No	No	Review	
KRAS	c.522T>C		SNV	5.82%	35.11	0.00	1,786	No	No	Review	
KRAS	c.519T>C		SNV	51.17%	31.41	1.00	1,790	No	No	Review	
KRAS	c.516A>G		SNV	5.89%	29.49	0.00	1,885	No	No	Review	
ERBB3	c.3348G>A		SNV	53.28%	32.86	1.00	1,083	No	No	Review	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
PDGFRA	c.2440-50_2440-49insA		No	485	238	247	200	55151958^55151959	4
PDGFRA	c.3222T>C		No	2,087	1,019	1,068	200	55161391	4
ESR1	c.975G>C		No	1,272	473	799	200	152265522	6
EGFR	c.890-4C>G		No	174	174	0	200	55223519	7
EGFR	c.1498+22A>T		No	1,688	815	873	200	55228053	7
EGFR	c.1839C>T		No	466	216	250	200	55233089	7
EGFR	c.1881-858_1881-857delGA		No	258	258	0	200	55238010..55238011	7
EGFR	c.2284-60T>C		No	105	105	0	200	55248926	7
EGFR	c.2709T>C		No	1,056	643	413	200	55266417	7
BRAF	c.1860+66A>C		No	277	277	0	200	140453009	7
KRAS	c.541_542delTCinsAG	mis-sense	No	112	112	0	200	25362754..25362755	12
KRAS	c.534A>G		No	116	115	1	200	25362762	12
KRAS	c.525A>G		No	113	107	6	200	25362771	12

KRAS	c.522T>C		No	104	103	1	200	25362774	12
KRAS	c.519T>C		No	916	448	468	200	25362777	12
KRAS	c.516A>G		No	111	105	6	200	25362780	12
ERBB3	c.3348G>A		No	577	257	320	200	56494991	12

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

Comment: Remark added by user during variant review.

3.3 Untested variants

Variants of interest that could not be tested due to insufficient coverage. These variants are assigned the initial review state "Untested".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment

EGFR	c.2117T>C	p.Ile706Thr	SNV	0.57%	22.00		175	Yes	Yes	Untested	
EGFR	c.2184+19G>A		SNV	0.00%			48	Yes	Yes	Untested	
EGFR	c.2596G>A	p.Glu866Lys	SNV	0.00%			182	Yes	Yes	Untested	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
EGFR	c.2117T>C	mis-sense		1	0	1		55241669	7
EGFR	c.2184+19G>A			0	0	0		55241755	7
EGFR	c.2596G>A	mis-sense		0	0	0		55259538	7

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

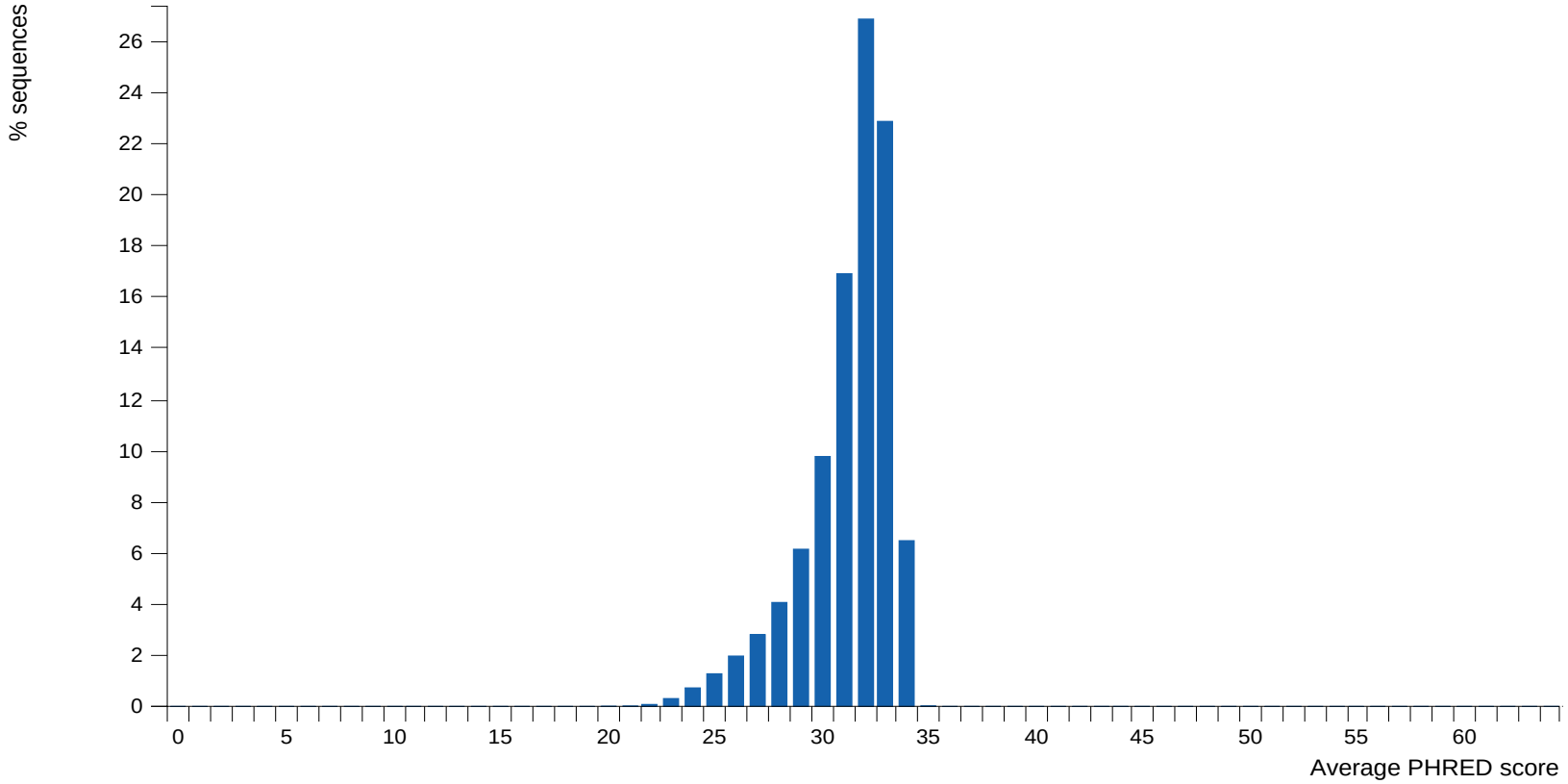
Comment: Remark added by user during variant review.

4 Detailed QC

Quality control metrics for detailed inspection. These metrics can indicate possible problems in the upstream workflow or data analysis. Quality control are divided in metrics on the incoming reads from input data, and metrics per base positions in these reads. Lastly section 4.3 and 4.4 display metrics on how well the positions in the region of interest are covered

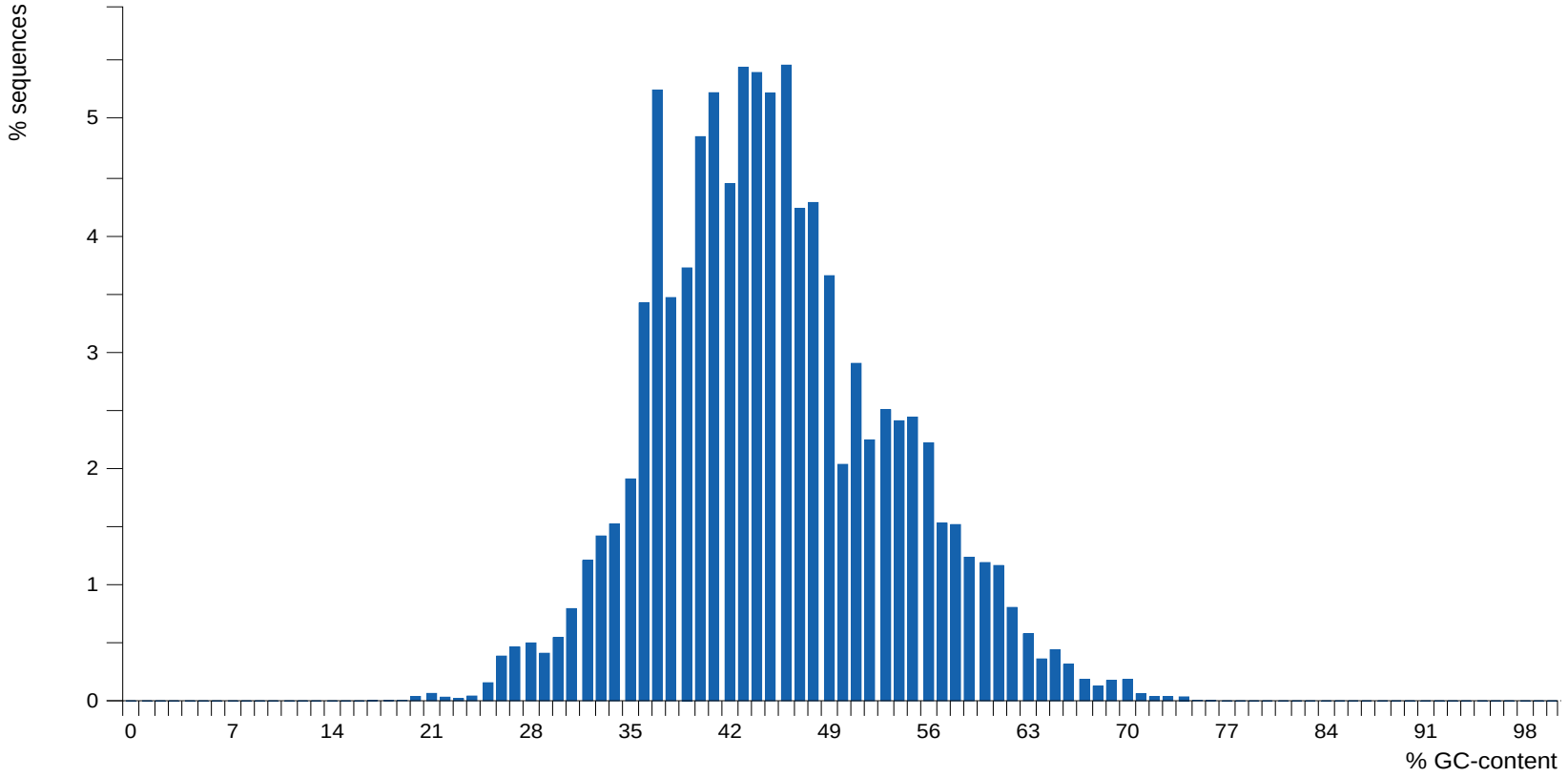
4.1 QC for reads

4.1.1 Average base quality of reads



Distribution of average sequence quality scores. The quality of a sequence is calculated as the arithmetic mean of its base qualities.
x: PHRED-score
y: number of sequences observed at that qual. score normalized to the total number of sequences

4.1.2 GC content of reads



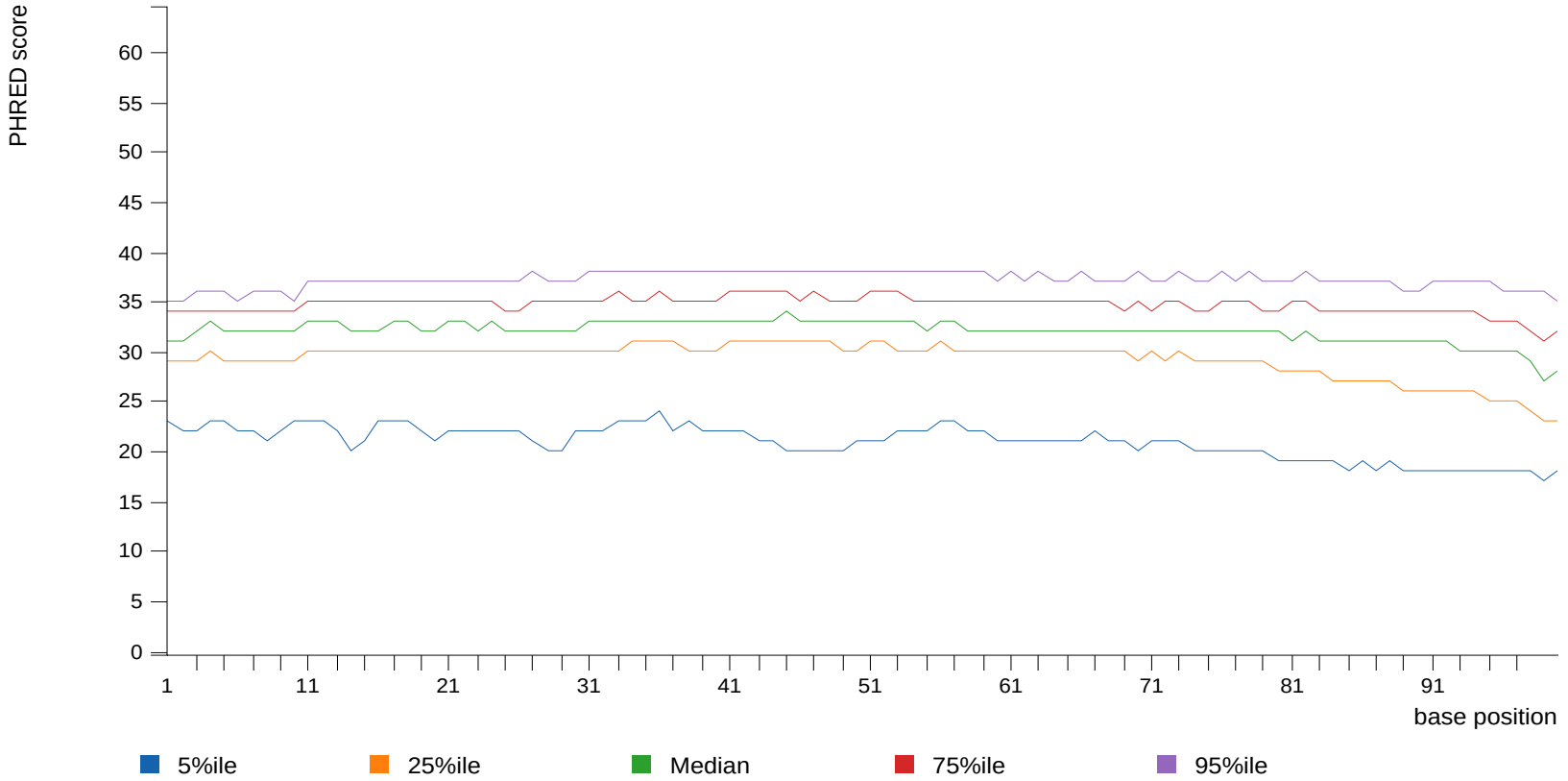
Distribution of GC-contents. The GC-content of a sequence is calculated as the number of GC-bases compared to all bases (including ambiguous bases).
x: relative GC-content of a sequence in percent
y: number of sequences featuring particular GC-percentages normalized to the total number of sequences

4.1.3 Ambiguous base content of reads

No ambiguous bases detected.

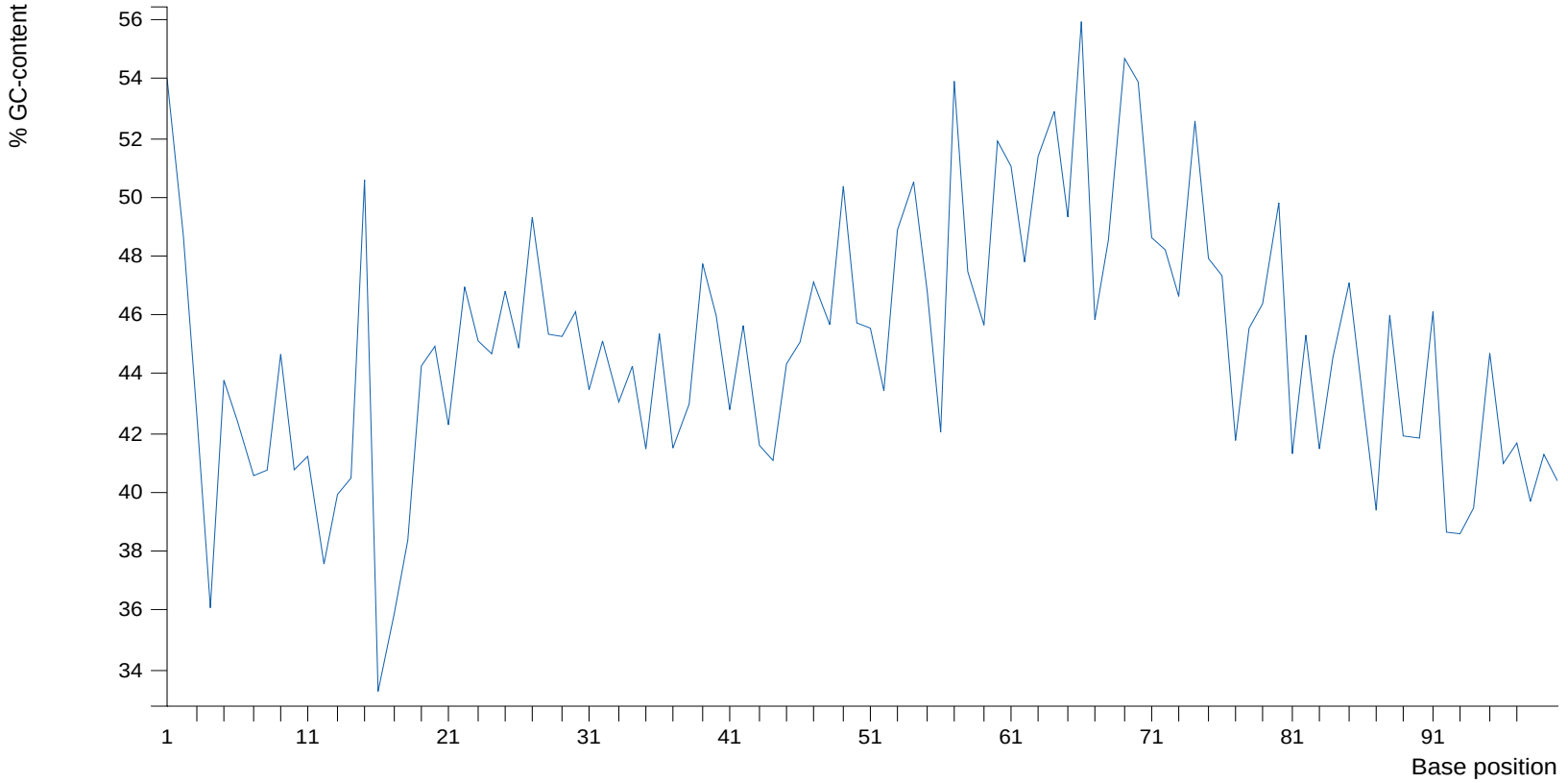
4.2 QC for bases

4.2.1 Quality score per base position



Base-quality distribution along the base positions.
x: base position
y: median & percentiles of quality scores observed at that base position

4.2.2 GC content per base position



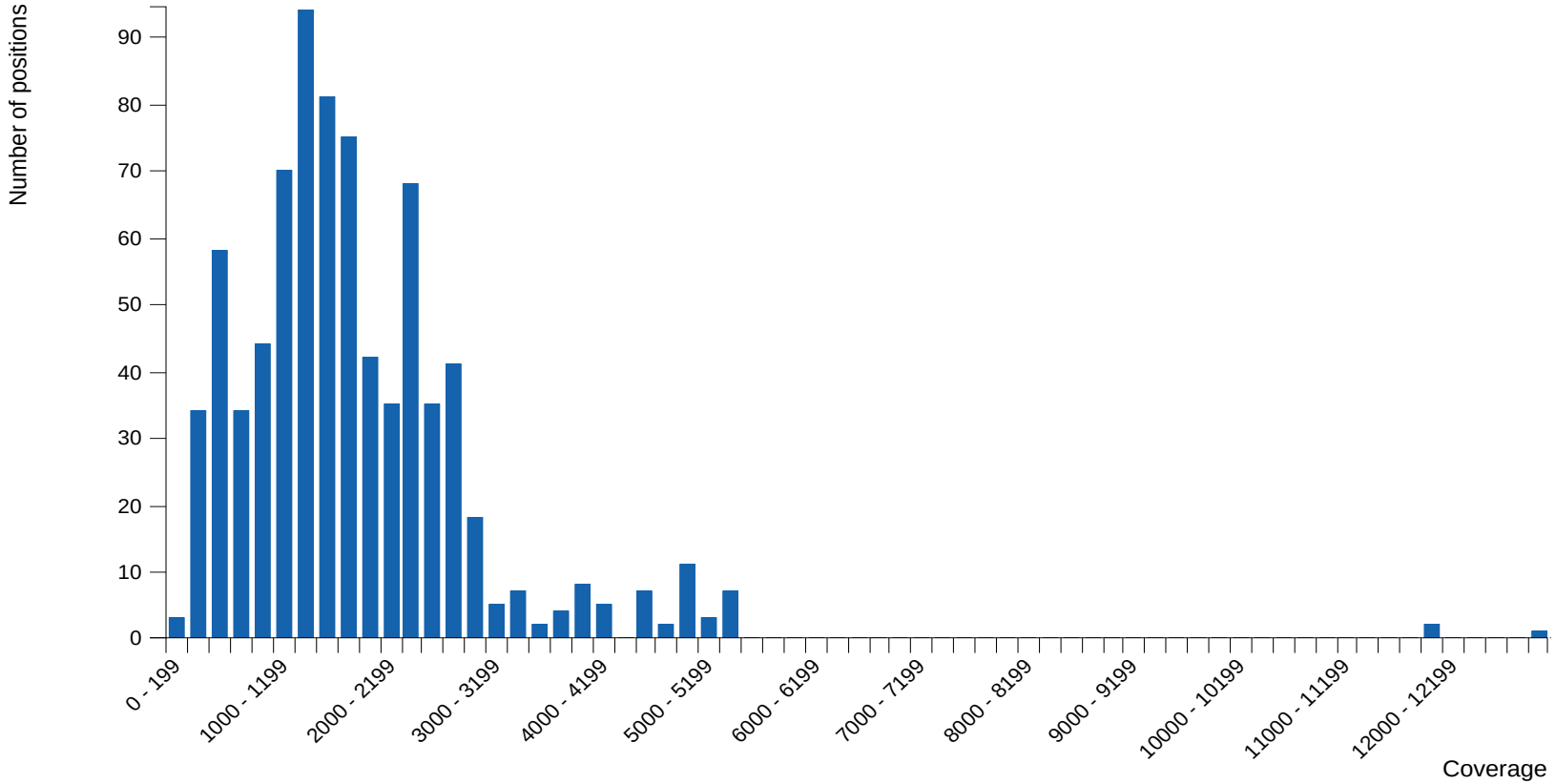
Combined coverage of G- and C-bases.
x: base position
y: number of G- and C-bases observed at current position normalized to the total number of bases observed at that position

4.2.3 Ambiguous base content per base position

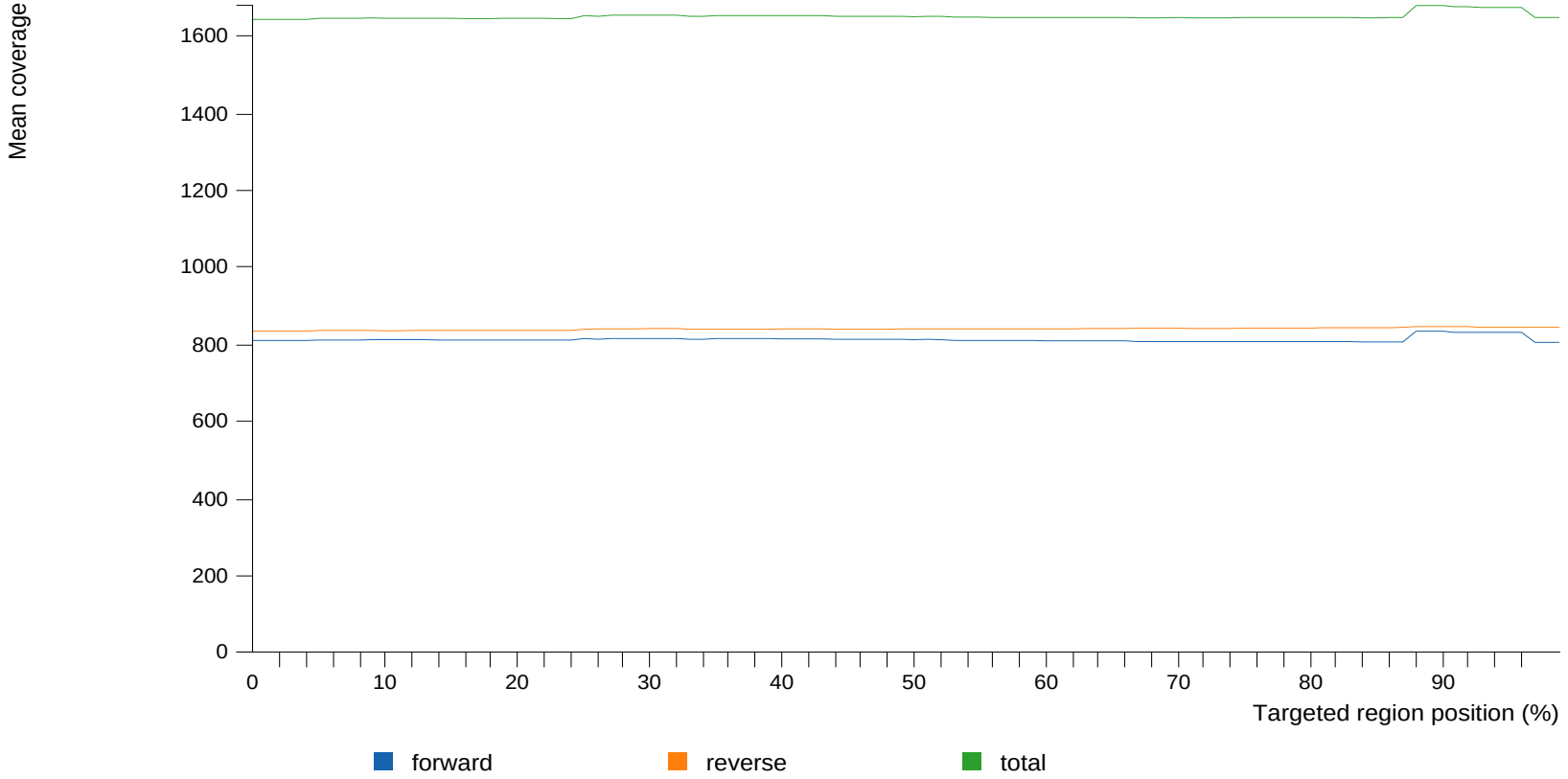
No ambiguous bases detected

4.3 Coverage of Regions of Interest positions

Coverage distribution



4.4 Mean coverage of relative positions in regions of interest



5 History

5.1 Log Entries

Type	Time	User	Details
State change	Sat Jun 29 18:11:32 CEST 2019	system	Ready for Review
State change	Sat Jun 29 17:56:44 CEST 2019	root	In Progress

5.2 Execution Information

QCIA version	QCI Analyze 1.4.5
Analysis start time	Sat Jun 29 17:56:44 CEST 2019
Analysis workflow	AIT FFPE 4.5
Analysis description	QIAact Actionable Insights Tumor Panel on FFPE

5.3 Transcripts

Table listing the genes, transcript IDs and protein IDs used in the analysis.

Gene Name	Transcript ID	Protein ID
NRAS	NM_002524.4	NP_002515.1
ALK	NM_004304.4	NP_004295.2
RAF1	NM_002880.3	NP_002871.1
PIK3CA	NM_006218.2	NP_006209.2
PDGFRA	NM_006206.4	NP_006197.1
KIT	NM_000222.2	NP_000213.1
ESR1	NM_001122742.1	NP_001116214.1
EGFR	NM_005228.3	NP_005219.2
BRAF	NM_004333.4	NP_004324.2
KRAS	NM_004985.3	NP_004976.2
ERBB3	NM_001982.3	NP_001973.2

Gene Name	Transcript ID	Protein ID
ERBB2	NM_004448.2	NP_004439.2

5.4 Open Parameters

Workflow parameters that are editable by the administrator

Reported variants

Significant coverage threshold	500
SNV/MNV frequency threshold in %	4.00
Insertions, deletions and replacements frequency threshold in %	4.00

Variants available for review

Minimum coverage threshold	200
SNV/MNV frequency threshold in %	4.00
Insertions, deletions and replacements frequency threshold in %	4.00
Detect variants outside regions of interest	Yes

5.5 Locked Parameters

Adapter trimming

Trim adapter list	GRadapter_160913
Ambiguous trim	false
Ambiguous limit	2
Quality trim	false
Quality limit	0.05
Use colorspace	false

Also search on reversed sequence	false
Remove 5' terminal nucleotides	false
Number of 5' terminal nucleotides	1
Maximum number of nucleotides in reads	1000
Minimum number of nucleotides in reads	15
Discard short reads	false
Remove 3' terminal nucleotides	false
Number of 3' terminal nucleotides	1
Discard long reads	false

Map Reads to Reference

References	Homo_sapiens_sequence_hg19
Masking mode	No masking
Masking track	Not set
Match score	1
Mismatch cost	2
Cost of insertions and deletions	Affine gap cost
Insertion cost	3
Deletion cost	3
Insertion open cost	6
Insertion extend cost	1
Deletion open cost	6
Deletion extend cost	1
Length fraction	0.5
Similarity fraction	0.8
Global alignment	false
Color space alignment	false
Color error cost	3
Auto-detect paired distances	false
Non-specific match handling	Map randomly

InDels and Structural Variants

P-Value threshold	1.0E-4
Maximum number of mismatches	3
Ignore broken pairs	true
Minimum relative consensus coverage	0.0
Minimum quality score	0
Filter variants	true
Minimum number of reads	2
Restrict calling to target regions	ATPv2_TargetRegions_170302_ver1.1

Local Realignment (Short Unaligned End version)

Realign unaligned ends	true
Multi-pass realignment	2
Local bound for unaligned ends of size one	0.75
Local bound for unaligned ends of size two	0.75
Force realignment to guidance-variants	false
Maximum guidance-variant length	100

Trim Primers and their Dimers of Mapped Reads

Primer track	101x_GR_primers_15_10_15_V1.0
Reference	Homo_sapiens_sequence_hg19
Minimum primer overlap length	9
Allow dangling 3' end base	true
Minimal primer overlap fraction	0.7
Only keep reads that have hit a primer	true
Additional bases to trim	1

Remove Pseudogene Reads

Genes track	AITv2_PseudoGenes_170912_ver1.0
Gene and pseudogene links	KRAS -> KRASP1
Required unaligned ends %	2.0

Low Frequency Variant Detection

Required significance (%)	0.01
Ignore positions with coverage above	1000000000
Restrict calling to target regions	ATPv2_TargetRegions_170302_ver1.1
Ignore broken pairs	true
Ignore non-specific matches	Reads
Minimum read length	20
Minimum coverage	Parameter editable by administrator
Minimum count	8
Minimum frequency (%)	Parameter editable by administrator
Base quality filter	false
Neighborhood radius	5
Minimum central quality	5
Minimum neighborhood quality	5
Read direction filter	false
Direction frequency (%)	5.0
Relative read direction filter	false
Significance (%)	1.0E-5
Read position filter	false
Significance (%)	1.0
Remove pyro-error variants	false
In homopolymer regions with minimum length	3
With frequency below	0.8

Remove False Positives

Minimum frequency (%)	Parameter editable by administrator
Minimum forward/reverse balance	0.05
Minimum average base quality	22.0
Variant frequency	true
Forward/reverse balance	false
Average base quality	true

Annotate Variants With Primers

Minimum coverage count	400
Minimum variant percentage	1.0
Minimum variant read count	2

AnyGenomics Lab

QIAact Actionable Insights Tumor Panel For the GeneReader NGS System (RUO)

Report Date Mar 26, 2018
Report Status -

Patient Information

Patient Name
Date of Birth
Ethnicity
Sex
Accession 20180322144720_8160085015705246905180014 0_34050_16A2_BC5_2018-03-22

Client Information

Client
Client ID
Physician
Pathologist

Specimen

Specimen Type
Specimen ID
Collection Date
Accession Date Mar 23, 2018
Primary Tumor Site Brain
Diagnosis Glioblastoma
Diagnosis Stage

Interpretation

1 Clinically Significant Variant Reported | 0 Approved Therapy | 0 Potential Clinical Trials

Summary of Clinically Significant Variants

Variants Reported	Approved Therapies for Same Cancer	Approved Therapies for Other Cancers	Therapies Associated with Resistance	Potential Clinical Trials
PIK3CA p.T1025A				

Variant Details

Gene	Exon #	Nucleotide Change	Amino Acid Change	Effect on Protein
PIK3CA	21	NM_006218.3: c.3073A>G	p.T1025A	gain of function

PIK3CA is an oncogene involved in cell growth, apoptosis, transformation, motility, and adhesion through activation of the PI3K/AKT/MTOR pathway [3, 5, 8]. Gain-of-function mutations, amplification, and protein overexpression cause PI3K activation [7, 2, 4, 9]. PIK3CA mutations are reported to be mutually exclusive with PTEN mutations [6, 1].

QIAact Actionable Insights Tumor Panel For the GeneReader NGS System (RUO)

Genes Tested

KRAS *NRAS* *KIT* *BRAF* *PDGFRA* *ALK* *EGFR* *ERBB2* *PIK3CA* *ERBB3* *ESR1* *RAF1*

Methods and Limitations

EXAMPLE Statement including sample type (FFPE, etc), method of extraction, amplification reactions, panel targeted regions, sequencing technology, etc. Additionally, a description of the data analysis software(s), genome of reference and the sensitivity of the methods should be described.

QIAGEN Clinical Insight (QCI™) software includes the following underlying databases, data reference sets and tools; QIAGEN Clinical Insight-Interpret (5.2.20180316), Ingenuity Knowledge Base (Pandora 180329.003), CADD (v1.3), CentoMD (4.1), EVS (ESP6500SI-V2), Allele Frequency Community (2018-01-17), JASPAR (2013-11), Ingenuity Knowledge Base Snapshot Timestamp (2018-03-29 18:20:56.0), Vista Enhancer hg18 (2012-07), Vista Enhancer hg19 (2012-07), OMIM (May 26, 2017), gnomAD (2.0.1), Clinical Trials (Pandora 180329.003), BSIFT (2016-02-23), TCGA (2013-09-05), PolyPhen-2 (v2.2.2), 1000 Genome Frequency (phase3v5b), Clinvar (2018-01-03), DGV (2016-05-15), COSMIC (v83), ExAC (0.3.1), HGMD (2017.4), PhyloP hg18 (2009-11), PhyloP hg19 (2009-11), DbSNP (150(2017-07-10)), TargetScan (6.2), SIFT4G (2016-02-23)

Laboratory Statement

This section can be customized to provide additional information regarding laboratory methods etc.

Selected Citations

1. Broderick DK, Di C, Parrett TJ, Samuels YR, Cummins JM, McLendon RE, Fults DW, Velculescu VE, Bigner DD, Yan H (2004) Mutations of PIK3CA in anaplastic oligodendrogliomas, high-grade astrocytomas, and medulloblastomas. Cancer Res. 2004 Aug 01;64(15):5048-50 (PMID: 15289301)
2. Cancer Genome Atlas Research Network (2011) Integrated genomic analyses of ovarian carcinoma. Nature. 2011 Jun 29;474(7353):609-15 (PMID: 21720365)
3. Cantley LC (2002) The phosphoinositide 3-kinase pathway. Science. 2002 May 31;296(5573):1655-7 (PMID: 12040186)
4. Cizkova M, Susini A, Vacher S, Cizeron-Clairac G, Andrieu C, Driouch K, Fourme E, Lidereau R, Bièche I (2012) PIK3CA mutation impact on survival in breast cancer patients and in ER, PR and ERBB2-based subgroups. Breast Cancer Res. 2012 Feb 13;14(1):R28 (PMID: 22330809)
5. Fruman DA, Meyers RE, Cantley LC (1998) Phosphoinositide kinases. Annu Rev Biochem. 1998;67:481-507 (PMID: 9759495)
6. Saal LH, Holm K, Maurer M, Memeo L, Su T, Wang X, Yu JS, Malmström PO, Mansukhani M, Enoksson J, Hibshoosh H, Borg A, Parsons R (2005) PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. Cancer Res. 2005 Apr 01;65(7):2554-9 (PMID: 15805248)
7. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE (2004) High frequency of mutations of the PIK3CA gene in human cancers. Science. 2004 Apr 23;304(5670):554. Epub 2004 Mar 11 (PMID: 15016963)
8. Volinia S, Hiles I, Ormondroyd E, Nizetic D, Antonacci R, Rocchi M, Waterfield MD (1994) Molecular cloning, cDNA sequence, and chromosomal localization of the human phosphatidylinositol 3-kinase p110 alpha (PIK3CA) gene. Genomics. 1994 Dec;24(3):472-7 (PMID: 7713498)

QIAact Actionable Insights Tumor Panel For the GeneReader NGS System (RUO)

Selected Citations

9. Yamamoto H, Shigematsu H, Nomura M, Lockwood WW, Sato M, Okumura N, Soh J, Suzuki M, Wistuba II, Fong KM, Lee H, Toyooka S, Date H, Lam WL, Minna JD, Gazdar AF (2008) PIK3CA mutations and copy number gains in human lung cancers. Cancer Res. 2008 Sep 01;68(17):6913-21 (PMID: 18757405)

AnyGenomics Lab

QIAact Actionable Insights Tumor Panel For the GeneReader NGS System (RUO)

Report Date Mar 23, 2018
Report Status -

Patient Information

Patient Name
Date of Birth
Ethnicity
Sex
Accession 20180322144720_8160085015705246905180014 0_32760_16A2_BC3_2018-03-22

Client Information

Client
Client ID
Physician
Pathologist

Specimen

Specimen Type
Specimen ID
Collection Date
Accession Date Mar 23, 2018
Primary Tumor Site Brain
Diagnosis Glioblastoma
Diagnosis Stage

Result: No variants detected

0 Clinically Significant Variants Reported	0 Approved Therapy	0 Potential Clinical Trials

Genes Tested

KRAS *NRAS* *KIT* *BRAF* *PDGFRA* *ALK* *EGFR* *ERBB2* *PIK3CA* *ERBB3* *ESR1* *RAF1*

Methods and Limitations

EXAMPLE Statement including sample type (FFPE, etc), method of extraction, amplification reactions, panel targeted regions, sequencing technology, etc. Additionally, a description of the data analysis software(s), genome of reference and the sensitivity of the methods should be described.

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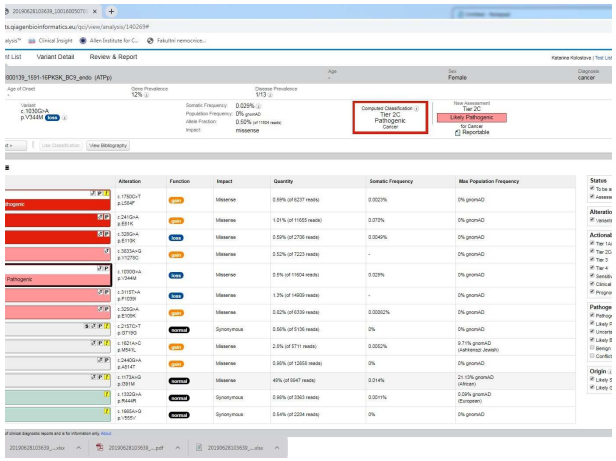
QIAact Actionable Insights Tumor Panel For the GeneReader NGS System (RUO)

PhyloP hg18 (2009-11), PhyloP hg19 (2009-11), DbSNP (150(2017-07-10)), TargetScan (6.2), SIFT4G (2016-02-23)

Laboratory Statement

This section can be customized to provide additional information regarding laboratory methods etc.



Analysis Report

20190628103639_1001600507010619014 7_868-18CTC_BC5_Glio2019

Table of contents

1 Summary

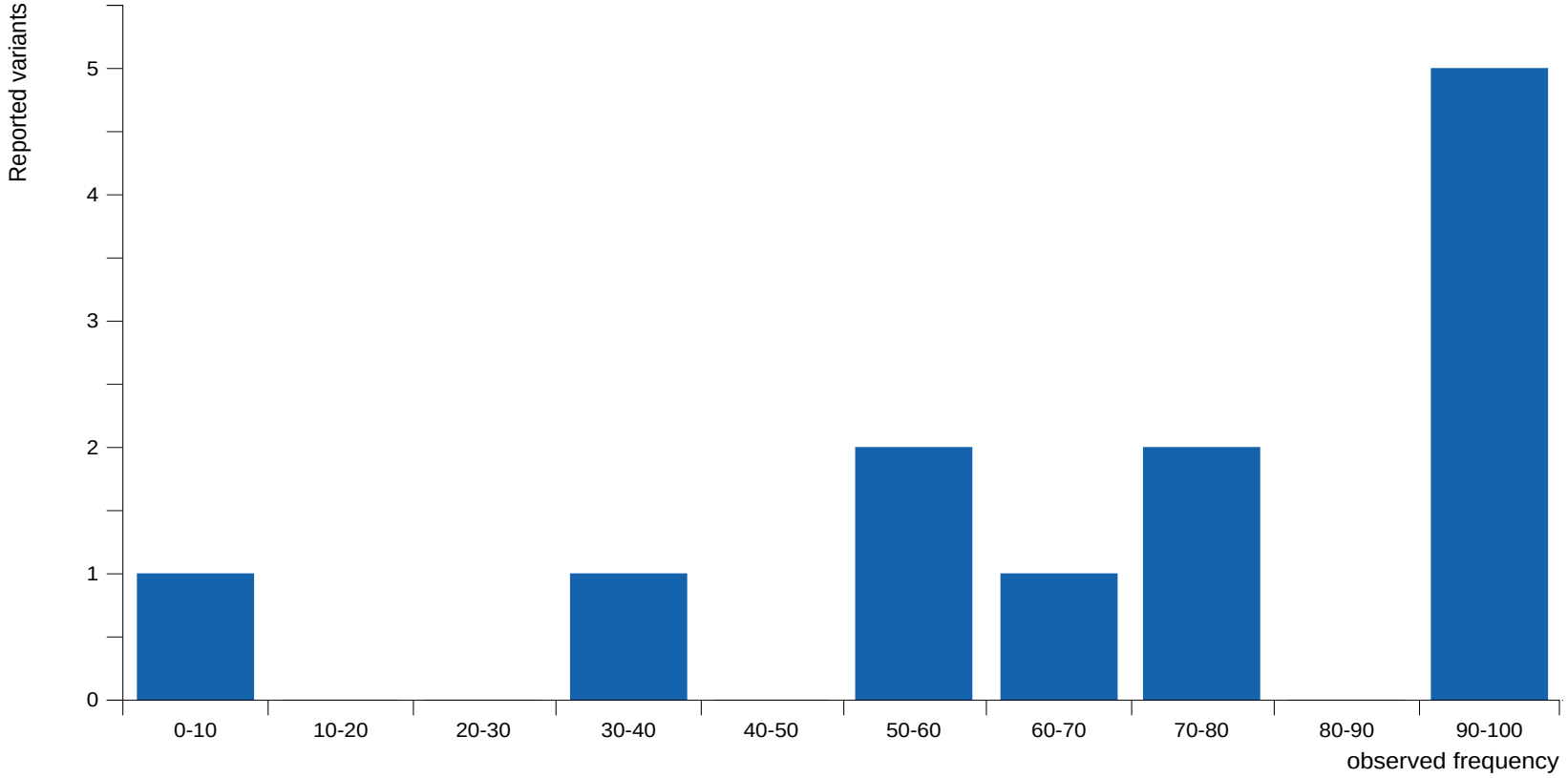
Report created	Fri Oct 25 17:37:56 CEST 2019
Sample ID	20190628103639_1001600507010619014 7_868-18CTC_BC5_Glio2019
Analysis workflow	AIT FFPE v4.5: QIAact Actionable Insights Tumor Panel on FFPE
Analyst	root
Reported variants	12
Analysis results	13 Untested variants

1.1 Comments

No comments

1.2 Distribution of observed frequencies for reported variants

Includes variants initially listed in variant table 'Reported variants'.



2 Quality control

Quality control for the sample analysis. Includes information on the input data, read mapping, and coverage information per gene.

2.1 Fastq

Fastq	20190628103639_10016005070106190147_868-18CTC_BC5_Glio2019
Reads	1,389,551
Nucleotides*	133,224,388
Average read length*	95.88
Reads with average quality ≥ 25	98.70%

** Including sample barcode*

Recommendations:

Reads with average quality ≥ 25 should be ≥ 80.00%

2.2 Secondary analysis summary

Reads mapped	1,182,489 (85.10%)
Reads in target regions	527,435 (44.60%)
Percentage of base positions in regions of interest with coverage ≥ 500x	95.35%
Percentage of base positions in regions of interest with coverage ≥ 200x	99.50%

Recommendations:

Percentage of base positions in regions of interest with coverage ≥ 500x should be ≥ 90.00%

Percentage of base positions in regions of interest with coverage ≥ 200x should be ≥ 95.00%

2.3 Coverage

Name	ROI	Bases	≥500x	≥200x	0x	Median	VOI	VOI <500x	VOI <200x
NRAS	6	27	100.00%	100.00%	0.00%	5,394	41	0	0
ALK	22	47	100.00%	100.00%	0.00%	1,948	40	0	0
RAF1	2	2	100.00%	100.00%	0.00%	3,008	2	0	0
PIK3CA	81	131	100.00%	100.00%	0.00%	1,965	165	0	0

Name	ROI	Bases	≥500x	≥200x	0x	Median	VOI	VOI <500x	VOI <200x
PDGFRA	21	64	100.00%	100.00%	0.00%	3,011	46	0	0
KIT	49	140	98.57%	100.00%	0.00%	2,093	235	3	0
ESR1	6	7	100.00%	100.00%	0.00%	1,088	11	0	0
EGFR	96	208	83.65%	98.08%	0.00%	1,175	443	45	2
BRAF	29	72	100.00%	100.00%	0.00%	3,345	153	0	0
KRAS	21	55	100.00%	100.00%	0.00%	3,705	148	0	0
ERBB3	8	8	100.00%	100.00%	0.00%	2,459	10	0	0
ERBB2	16	35	97.14%	100.00%	0.00%	2,948	61	1	0

ROI: Number of Regions of Interest, i.e. reportable regions that overlap with the gene.

Bases: Total number of base positions in Regions of Interest that overlap with the gene.

≥500x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is equal to or above the significant coverage threshold.

≥200x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is equal to or above the minimum coverage threshold.

0x: Percentage of base positions in Regions of Interest that overlap with the gene for which coverage is zero.

Median: Median coverage of base positions in the Regions of Interest that overlap with the gene.

VOI: Total number of Variants of Interest, whether detected or not, that overlap with the gene. The list of Variants of Interest is defined by the analysis pipeline.

VOI <500x: Number of Variants of Interest in the gene for which coverage is below the significant coverage threshold.

VOI <200x: Number of Variants of Interest in the gene for which coverage is below the minimum coverage threshold.

2.4 Detected variants

Number of detected variants per gene. Variants for which coverage is above the minimum coverage threshold.

Name	In total	VOI	- non-syn	- syn	Non-VOI	- non-syn	- syn
NRAS	2	0	0	0	2	1	1
ALK	2	1	0	1	1	1	0
RAF1	0	0	0	0	0	0	0
PIK3CA	14	2	0	2	12	1	11
PDGFRA	3	1	0	1	2	0	2
KIT	0	0	0	0	0	0	0
ESR1	7	2	0	2	5	0	5
EGFR	9	3	1	2	6	0	6
BRAF	5	0	0	0	5	0	5

Name	In total	VOI	- non-syn	- syn	Non-VOI	- non-syn	- syn
KRAS	8	1	0	1	7	2	5
ERBB3	1	0	0	0	1	1	0
ERBB2	4	2	2	0	2	2	0

In total: Total number of variants detected within the gene. Variants initially listed in variant tables 3.1 and 3.2.

VOI: Number of detected Variants of Interest detected within the gene. The list of Variants of Interest is defined by the analysis pipeline.

- non-syn: Number of detected, gene-specific Variants of Interest that are non-synonymous.

- syn: Number of detected, gene-specific Variants of Interest that are synonymous.

Non-VOI: Number of detected variants that are not found within the analysis pipeline-defined list of Variants of Interest.

- non-syn: Number of gene-specific, non-VOIs that are non-synonymous.

- syn: Number of gene-specific, non-VOIs that are synonymous.

3 Variants

Variants detected within regions of interest with more than significant coverage are found in 3.1 and variants with more than minimum coverage are found in 3.2.
Variants of interest that could not be tested due to insufficient coverage are listed in table 3.3.
The coverage thresholds and minimum frequency cutoffs configured for the analysis workflow are listed in the History section.
Setting a variant review state to "Confirmed by review" moves it to 3.1, "Artifact" moves it to 3.2.
Only the variants in table 3.1 are exported as VCF and uploaded to QCI Interpret.

3.1 Reported variants

Variants that will be exported to VCF and uploaded to QCI Interpret. Initially contains: Variants detected within regions of interest with more than significant coverage and frequency above the cutoff set for the analysis workflow. These variants are assigned the initial review state "Valid".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
ALK	c.2535T>C		SNV	99.88%	31.08	1.00	1,719	Yes	Yes	Valid	
PIK3CA	c.-77+8483C>T		SNV	35.08%	33.70	0.46	995	Yes	Yes	Valid	
PIK3CA	c.-76-23509A>G		SNV	59.82%	33.86	1.00	5,090	Yes	Yes	Valid	
PDGFRA	c.1701A>G		SNV	100.00%	30.27	1.00	2,600	Yes	Yes	Valid	
ESR1	c.30T>C		SNV	99.16%	29.24	1.00	952	Yes	Yes	Valid	
ESR1	c.1369+13777T>G		SNV	63.68%	29.49	0.25	2,547	Yes	Yes	Valid	
EGFR	c.474C>T		SNV	70.90%	31.89	1.00	2,780	Yes	Yes	Valid	
EGFR	c.1562G>A	p.Arg521Lys	SNV	6.00%	34.54	0.06	1,449	Yes	Yes	Valid	
EGFR	c.2361G>A		SNV	55.50%	31.84	1.00	1,182	Yes	Yes	Valid	
KRAS	c.*2505T>G		SNV	70.52%	33.65	1.00	3,029	Yes	Yes	Valid	
ERBB2	c.1963A>G	p.Ile655Val	SNV	97.32%	31.62	1.00	2,161	Yes	Yes	Valid	
ERBB2	c.3508C>G	p.Pro1170Ala	SNV	98.64%	24.38	1.00	514	Yes	Yes	Valid	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
ALK	c.2535T>C		No	1,717	857	860	200	29455267	2

PIK3CA	c.-77+8483C>T		No	349	85	264	200	178874874	3
PIK3CA	c.-76-23509A>G		No	3,045	1,497	1,548	200	178893029	3
PDGFRA	c.1701A>G		No	2,600	1,075	1,525	200	55141055	4
ESR1	c.30T>C		No	944	529	415	200	152129077	6
ESR1	c.1369+13777T>G		No	1,622	1,148	474	200	152396036	6
EGFR	c.474C>T		No	1,971	647	1,324	200	55214348	7
EGFR	c.1562G>A	mis-sense	No	87	38	49	200	55229255	7
EGFR	c.2361G>A		No	656	399	257	200	55249063	7
KRAS	c.*2505T>G		No	2,136	1,122	1,014	200	25360224	12
ERBB2	c.1963A>G	mis-sense	No	2,103	1,245	858	200	37879588	17
ERBB2	c.3508C>G	mis-sense	No	507	70	437	200	37884037	17

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

Comment: Remark added by user during variant review.

3.2 Variants available for review

Detected variants that will not be exported to the VCF and uploaded to QCI Interpret. Initially contains: Variants with more than minimum coverage and frequency above the cutoff set for the analysis workflow. Depending on workflow configuration, this table may include variants outside of regions of interest including those with coverage above significant coverage threshold. These variants are assigned the initial review state "Review".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
NRAS	c.19G>C	p.Val7Leu	SNV	7.31%	29.26	0.00	5,840	No	No	Review	
NRAS	c.-14C>G		SNV	4.77%	32.23	6.40E-6	3,104	No	No	Review	
ALK	c.4381A>G	p.Ile1461Val	SNV	98.08%	30.72	1.00	208	No	No	Review	
PIK3CA	c.-77+8545A>C		SNV	10.56%	34.34	1.00	303	No	No	Review	
PIK3CA	c.-76-18864C>A		SNV	47.18%	33.27	0.35	1,473	No	No	Review	
PIK3CA	c.178C>T	p.Gln60*	SNV	6.67%	34.96	1.00	420	No	No	Review	
PIK3CA	c.1060-17C>A		SNV	55.24%	32.33	1.00	2,156	No	No	Review	
PIK3CA	c.1145+9G>A		SNV	13.64%	35.76	3.20E-10	557	No	No	Review	
PIK3CA	c.1145+14T>A		SNV	11.23%	34.12	1.01E-7	659	No	No	Review	
PIK3CA	c.1145+16G>A		SNV	5.20%	35.09	4.53E-4	654	No	No	Review	
PIK3CA	c.1145+16_1145+17delGAinsTC		MNV	11.62%	30.45	9.63E-8	654	No	No	Review	
PIK3CA	c.1145+19T>A		SNV	10.55%	33.01	1.67E-6	777	No	No	Review	
PIK3CA	c.1145+24_1145+25insA		Insertion	7.92%	28.05	1.32E-6	480	No	No	Review	
PIK3CA	c.1145+54A>G		SNV	35.19%	32.95	1.00	233	No	No	Review	
PIK3CA	c.1145+56G>T		SNV	6.44%	33.60	1.00	233	No	No	Review	
PDGFRA	c.2440-50_2440-49insA		Insertion	99.63%	33.28	1.00	1,086	No	No	Review	
PDGFRA	c.3222T>C		SNV	99.36%	31.48	1.00	2,979	No	No	Review	
ESR1	c.-14C>T		SNV	4.27%	34.05	0.99	867	No	No	Review	
ESR1	c.975G>C		SNV	75.04%	31.16	1.00	1,699	No	No	Review	
ESR1	c.1369+13754T>C		SNV	79.39%	32.68	1.00	1,087	No	No	Review	
ESR1	c.1369+13849T>C		SNV	54.11%	33.03	0.04	1,887	No	No	Review	
ESR1	c.1587G>A		SNV	5.64%	32.91	0.96	780	No	No	Review	

EGFR	c.890-6_890-5insCGCA		Insertion	31.55%	30.31	1.00	4,336	No	No	Review	
EGFR	c.1498+22A>T		SNV	98.57%	33.32	1.00	1,749	No	No	Review	
EGFR	c.1842C>T		SNV	7.12%	27.59	1.00	1,855	No	No	Review	
EGFR	c.2061+31C>T		SNV	8.29%	25.00	1.00	434	No	No	Review	
EGFR	c.2626-53C>T		SNV	5.94%	30.49	1.00	1,195	No	No	Review	
EGFR	c.2709T>C		SNV	98.75%	29.65	1.00	1,518	No	No	Review	
BRAF	c.1860+72A>C		SNV	100.00%	30.62	1.00	209	No	No	Review	
BRAF	c.1860+71_1860+72insC		Insertion	25.09%	35.49	1.00	279	No	No	Review	
BRAF	c.1860+67A>C		SNV	7.83%	35.98	1.00	626	No	No	Review	
BRAF	c.1860+66A>C		SNV	81.57%	36.60	1.00	1,894	No	No	Review	
BRAF	c.1742-38A>T		SNV	7.13%	36.16	0.00	617	No	No	Review	
KRAS	c.541_542delTCinsAG	p.Ser181Arg	MNV	27.61%	31.54	0.00	3,626	No	No	Review	
KRAS	c.534A>G		SNV	27.30%	31.89	0.00	3,670	No	No	Review	
KRAS	c.525A>G		SNV	24.80%	31.54	0.00	3,806	No	No	Review	
KRAS	c.522T>C		SNV	23.44%	35.45	0.00	3,904	No	No	Review	
KRAS	c.519T>C		SNV	23.13%	31.18	0.00	3,900	No	No	Review	
KRAS	c.516A>G		SNV	22.12%	29.47	0.00	4,005	No	No	Review	
KRAS	c.508A>C	p.Met170Leu	SNV	8.20%	30.10	0.00	3,938	No	No	Review	
ERBB3	c.241C>T	p.Arg81*	SNV	4.64%	34.70	0.58	2,457	No	No	Review	
ERBB2	c.536T>C	p.Leu179Pro	SNV	4.03%	28.23	1.00	869	No	No	Review	
ERBB2	c.554A>G	p.Asp185Gly	SNV	4.01%	28.46	0.05	1,196	No	No	Review	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
NRAS	c.19G>C	mis-sense	No	427	0	427	200	115258763	1
NRAS	c.-14C>G		No	148	0	148	200	115258795	1
ALK	c.4381A>G	mis-sense	No	204	204	0	200	29416572	2
PIK3CA	c.-77+8545A>C		No	32	0	32	200	178874936	3
PIK3CA	c.-76-18864C>A		No	695	134	561	200	178897674	3
PIK3CA	c.178C>T	non-sense	No	28	0	28	200	178916791	3

PIK3CA	c.1060-17C>A		No	1,191	663	528	200	178922274	3
PIK3CA	c.1145+9G>A		No	76	0	76	200	178922385	3
PIK3CA	c.1145+14T>A		No	74	0	74	200	178922390	3
PIK3CA	c.1145+16G>A		No	34	0	34		178922392	3
PIK3CA	c.1145+16_1145+17delGAinsTC		No	76	0	76	200	178922392..178922393	3
PIK3CA	c.1145+19T>A		No	82	0	82	200	178922395	3
PIK3CA	c.1145+24_1145+25insA		No	38	0	38	200	178922400^178922401	3
PIK3CA	c.1145+54A>G		No	82	0	82	200	178922430	3
PIK3CA	c.1145+56G>T		No	15	0	15	200	178922432	3
PDGFRA	c.2440-50_2440-49insA		No	1,082	534	548	200	55151958^55151959	4
PDGFRA	c.3222T>C		No	2,960	1,355	1,605	200	55161391	4
ESR1	c.-14C>T		No	37	21	16	200	152129034	6
ESR1	c.975G>C		No	1,275	571	704	200	152265522	6
ESR1	c.1369+13754T>C		No	863	448	415	200	152396013	6
ESR1	c.1369+13849T>C		No	1,021	120	901	200	152396108	6
ESR1	c.1587G>A		No	44	29	15	200	152419900	6
EGFR	c.890-6_890-5insCGCA		No	1,368	1,368	0	200	55223517^55223518	7
EGFR	c.1498+22A>T		No	1,724	728	996	200	55228053	7
EGFR	c.1842C>T		No	132	62	70	200	55233092	7
EGFR	c.2061+31C>T		No	36	0	36	200	55240848	7
EGFR	c.2626-53C>T		No	71	71	0	200	55260406	7
EGFR	c.2709T>C		No	1,499	630	869	200	55266417	7
BRAF	c.1860+72A>C		No	209	209	0	200	140453003	7
BRAF	c.1860+71_1860+72insC		No	70	70	0	200	140453003^140453004	7
BRAF	c.1860+67A>C		No	49	49	0	200	140453008	7
BRAF	c.1860+66A>C		No	1,545	1,545	0	200	140453009	7
BRAF	c.1742-38A>T		No	44	0	44	200	140453231	7
KRAS	c.541_542delTCinsAG	mis-sense	No	1,001	1,001	0	200	25362754..25362755	12
KRAS	c.534A>G		No	1,002	1,000	2	200	25362762	12
KRAS	c.525A>G		No	944	943	1	200	25362771	12
KRAS	c.522T>C		No	915	915	0	200	25362774	12
KRAS	c.519T>C		No	902	899	3	200	25362777	12

KRAS	c.516A>G		No	886	882	4	200	25362780	12
KRAS	c.508A>C	mis-sense	No	323	318	5	200	25362788	12
ERBB3	c.241C>T	non-sense	No	114	114	0	200	56478785	12
ERBB2	c.536T>C	mis-sense	No	35	35	0	86	37865667	17
ERBB2	c.554A>G	mis-sense	No	48	26	22	157	37865685	17

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

Comment: Remark added by user during variant review.

3.3 Untested variants

Variants of interest that could not be tested due to insufficient coverage. These variants are assigned the initial review state "Untested".

Variants, primary review annotations.

This table lists variants with the primary review information. Secondary review information can be found in the next table below this. Use the gene and c.variant information to locate the same variant in each table.

Gene	c. variant	p. variant	Type	%	Avg Q	F/R test	Coverage	ROI	VOI	Review	Comment
EGFR	c.2184+19G>A		SNV	0.00%			149	Yes	Yes	Untested	
EGFR	c.2296_2297insTGGCCAGCG	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			142	Yes	Yes	Untested	
EGFR	c.2296_2297insTGGCAAGCG	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			142	Yes	Yes	Untested	
EGFR	c.2296_2297insTGGCATCTG	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			142	Yes	Yes	Untested	
EGFR	c.2298_2299insGCAAGCGTA	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			138	Yes	Yes	Untested	
EGFR	c.2298_2299insGCAAGCGTC	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			138	Yes	Yes	Untested	
EGFR	c.2298_2299insGCAAGCGTT	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			138	Yes	Yes	Untested	
EGFR	c.2298_2299insGCATCGGTA	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			138	Yes	Yes	Untested	
EGFR	c.2298_2299insGCATCGGTC	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			138	Yes	Yes	Untested	
EGFR	c.2298_2299insGCATCTGTA	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			138	Yes	Yes	Untested	
EGFR	c.2298_2299insGCATCTGTC	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			138	Yes	Yes	Untested	
EGFR	c.2298_2299insGCATCTGTT	p.Val769_Asp770insAlaSerVal	Insertion	0.00%			138	Yes	Yes	Untested	
EGFR	c.2596G>A	p.Glu866Lys	SNV	0.00%			127	Yes	Yes	Untested	

Variants, secondary review information

This table lists variants with the secondary review information

Gene	c. variant	Impact	Repeat	Count	F Count	R Count	Qual	Region	Chr
EGFR	c.2184+19G>A			0	0	0		55241755	7
EGFR	c.2296_2297insTGGCCAGCG			0	0	0		55248998^55248999	7
EGFR	c.2296_2297insTGGCAAGCG			0	0	0		55248998^55248999	7
EGFR	c.2296_2297insTGGCATCTG			0	0	0		55248998^55248999	7
EGFR	c.2298_2299insGCAAGCGTA			0	0	0		55249000^55249001	7
EGFR	c.2298_2299insGCAAGCGTC			0	0	0		55249000^55249001	7
EGFR	c.2298_2299insGCAAGCGTT			0	0	0		55249000^55249001	7

EGFR	c.2298_2299insGCATCGGTA			0	0	0		55249000^55249001	7
EGFR	c.2298_2299insGCATCGGTC			0	0	0		55249000^55249001	7
EGFR	c.2298_2299insGCATCTGTA			0	0	0		55249000^55249001	7
EGFR	c.2298_2299insGCATCTGTC			0	0	0		55249000^55249001	7
EGFR	c.2298_2299insGCATCTGTT			0	0	0		55249000^55249001	7
EGFR	c.2596G>A	mis-sense		0	0	0		55259538	7

Gene: Name of affected gene.

Type: Variant type.

c. variant: Coding DNA sequence variant nomenclature based on Human Genome Variation Society recommendations.

p. variant: Protein sequence variant nomenclature based on Human Genome Variation Society recommendations.

Impact: Translational impact of variant.

%: Detected variant frequency.

Avg Q: Average quality score of the bases supporting the variant.

F/R test: Value reflecting the relative forward/reverse read balance; is forward/reverse ratio of reads supporting variant similar to ratio of all reads covering the position (1: well-balanced, 0: un-balanced).

Repeat: Variant is located in a low-complexity region.

Count: Number of fragments with the detected variant.

F Count: Number of forward reads with the detected variant.

R Count: Number of reverse reads with the detected variant.

Coverage: The number of fragments covering the variant position.

Qual: Value reflecting the significance of the variant (200: highly significant, 0: in-significant).

Region: Position of the variant relative to the reference sequence.

Chr: Affected chromosome.

ROI: In Regions of Interest.

VOI: Variant of interest, as specified for the analysis workflow.

Review: Status of variant review.

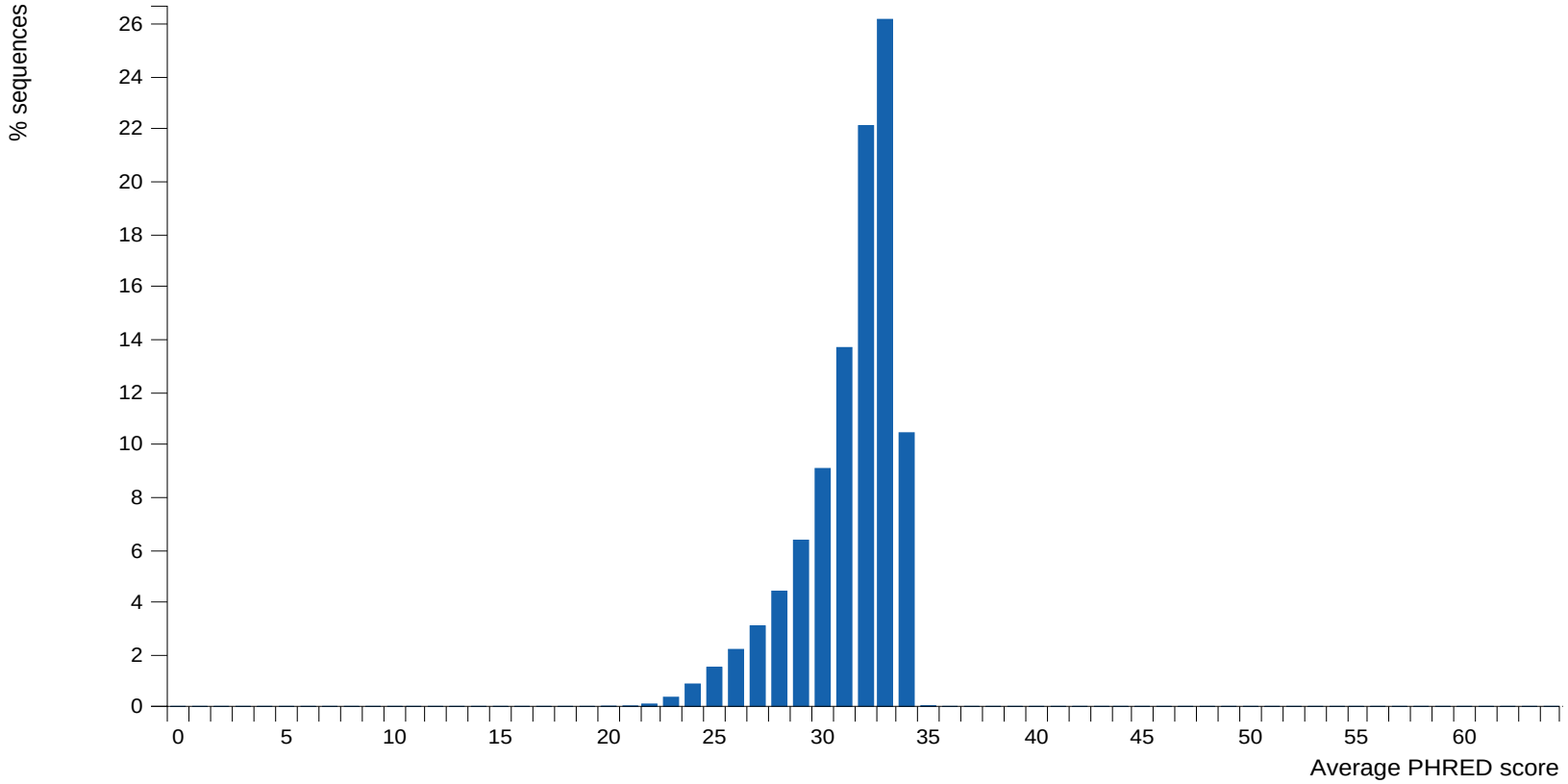
Comment: Remark added by user during variant review.

4 Detailed QC

Quality control metrics for detailed inspection. These metrics can indicate possible problems in the upstream workflow or data analysis. Quality control are divided in metrics on the incoming reads from input data, and metrics per base positions in these reads. Lastly section 4.3 and 4.4 display metrics on how well the positions in the region of interest are covered

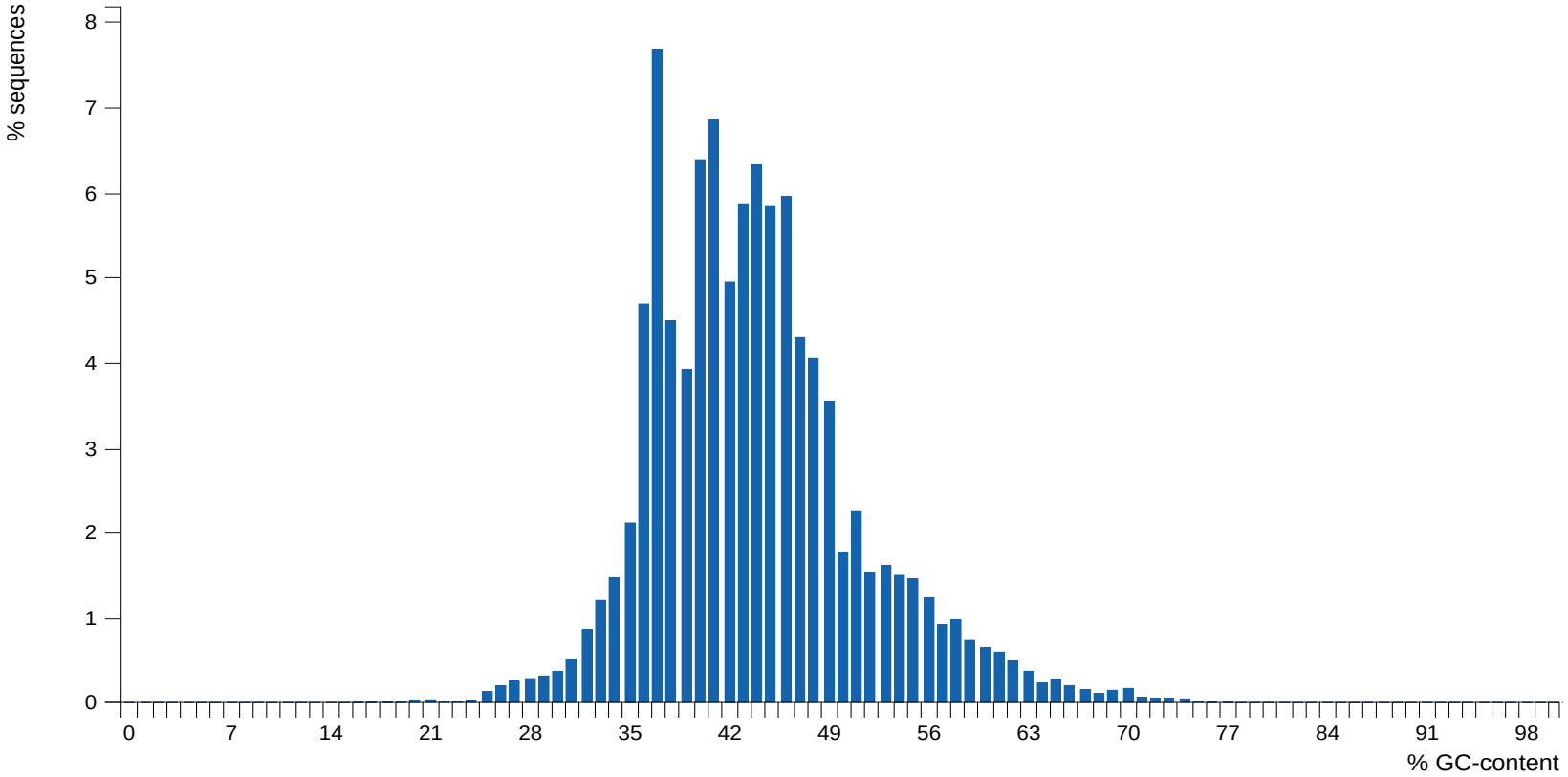
4.1 QC for reads

4.1.1 Average base quality of reads



Distribution of average sequence quality scores. The quality of a sequence is calculated as the arithmetic mean of its base qualities.
x: PHRED-score
y: number of sequences observed at that qual. score normalized to the total number of sequences

4.1.2 GC content of reads



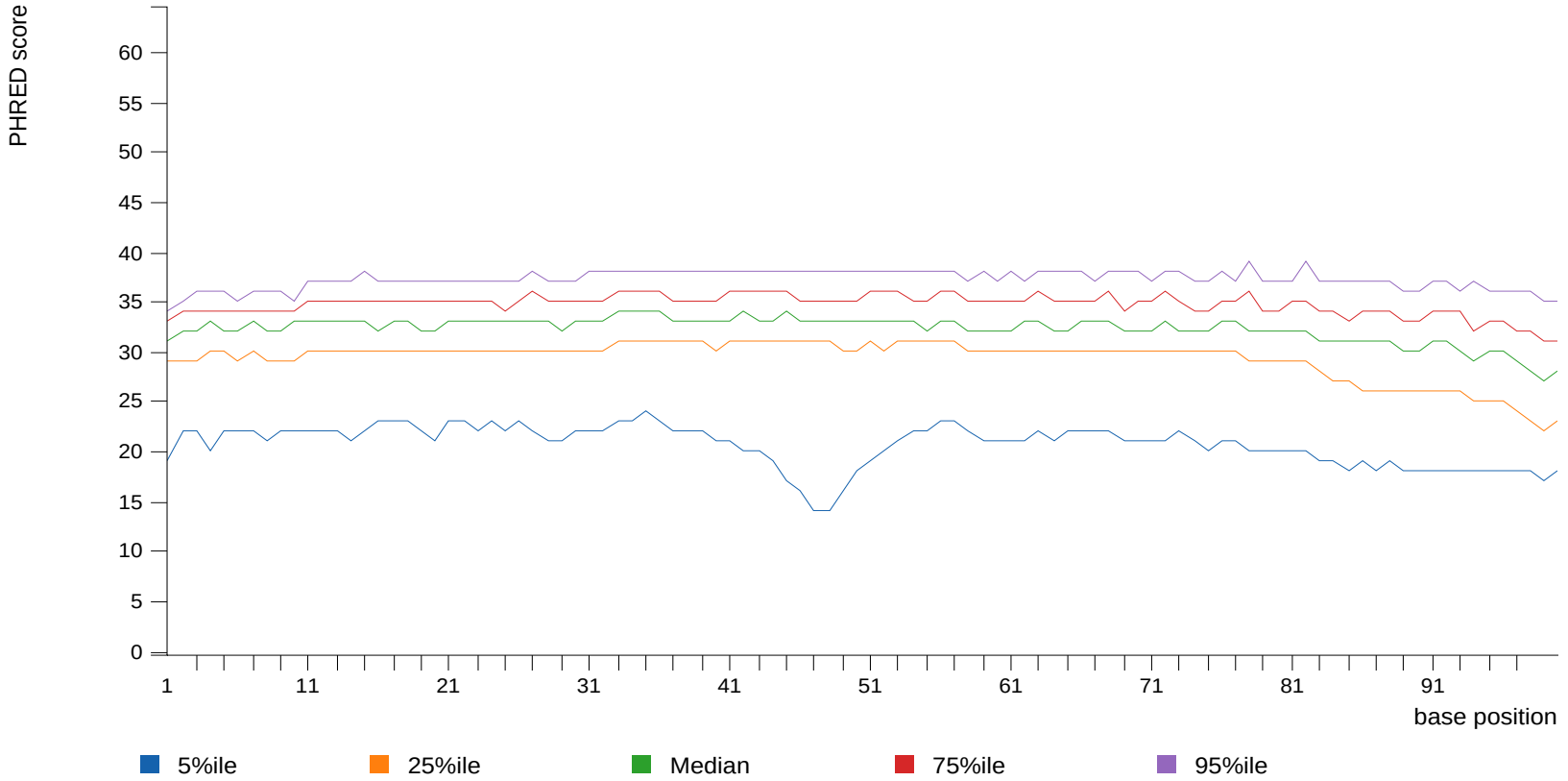
Distribution of GC-contents. The GC-content of a sequence is calculated as the number of GC-bases compared to all bases (including ambiguous bases).
x: relative GC-content of a sequence in percent
y: number of sequences featuring particular GC-percentages normalized to the total number of sequences

4.1.3 Ambiguous base content of reads

No ambiguous bases detected.

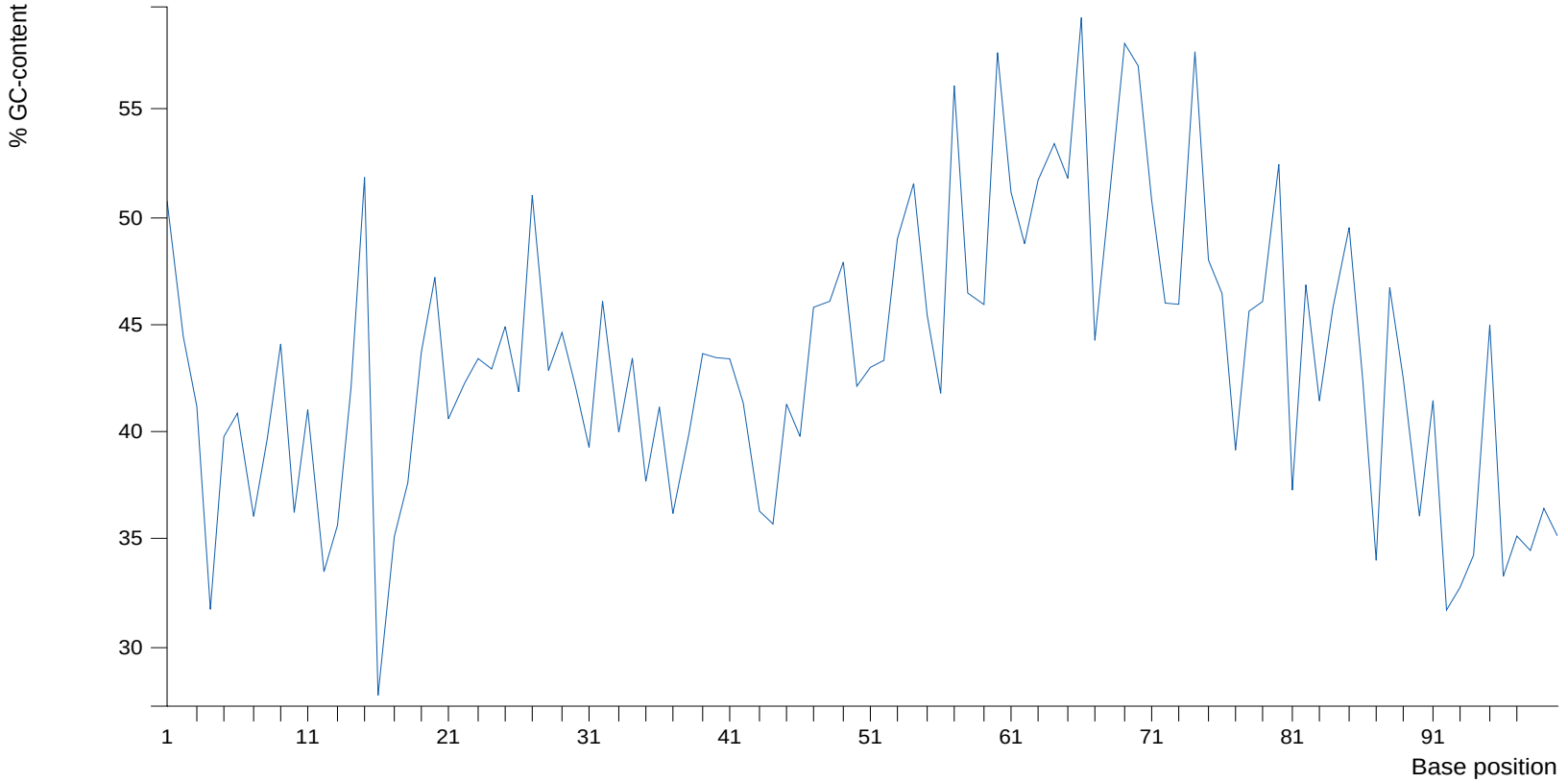
4.2 QC for bases

4.2.1 Quality score per base position



Base-quality distribution along the base positions.
x: base position
y: median & percentiles of quality scores observed at that base position

4.2.2 GC content per base position



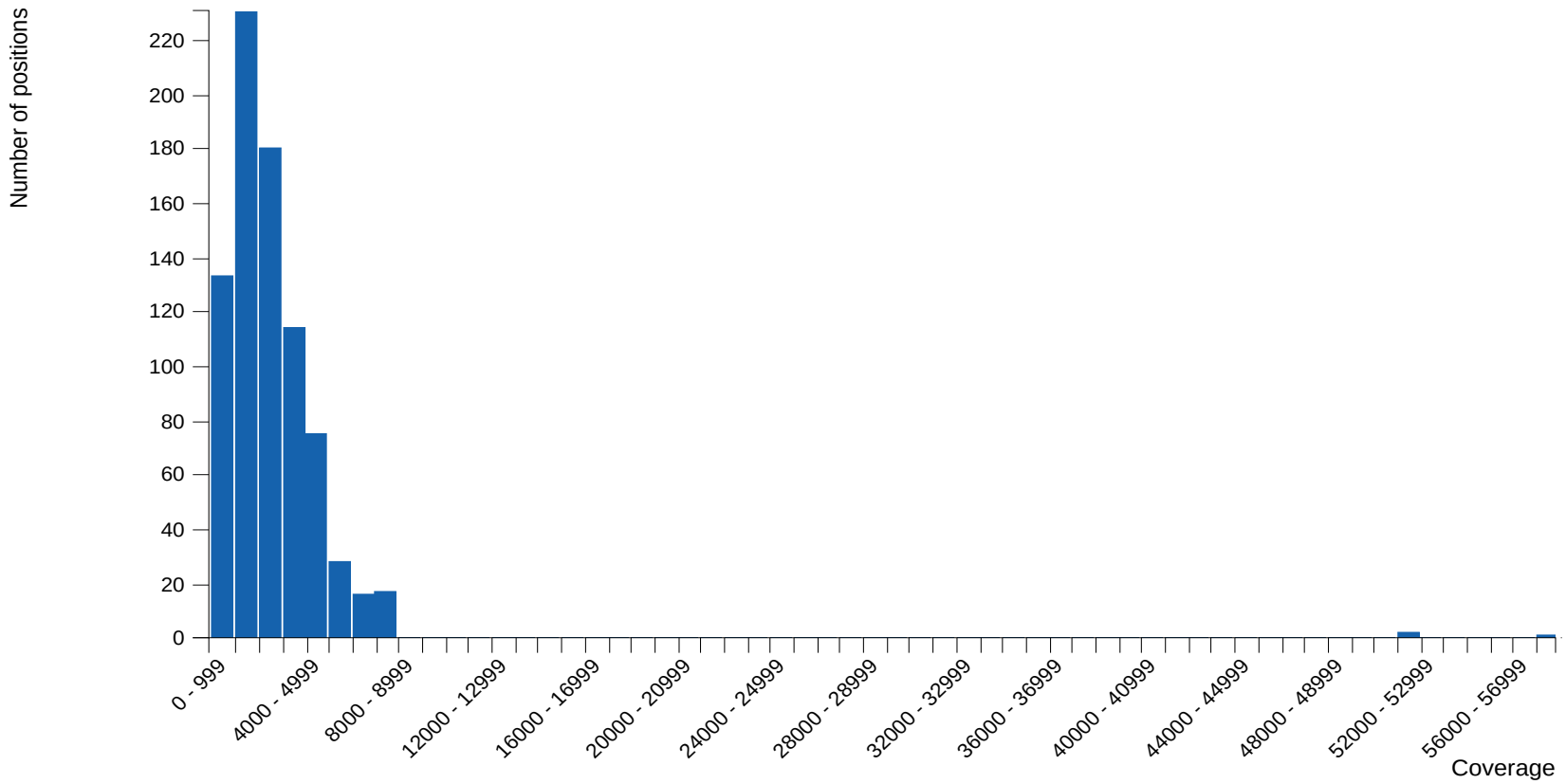
Combined coverage of G- and C-bases.
x: base position
y: number of G- and C-bases observed at current position normalized to the total number of bases observed at that position

4.2.3 Ambiguous base content per base position

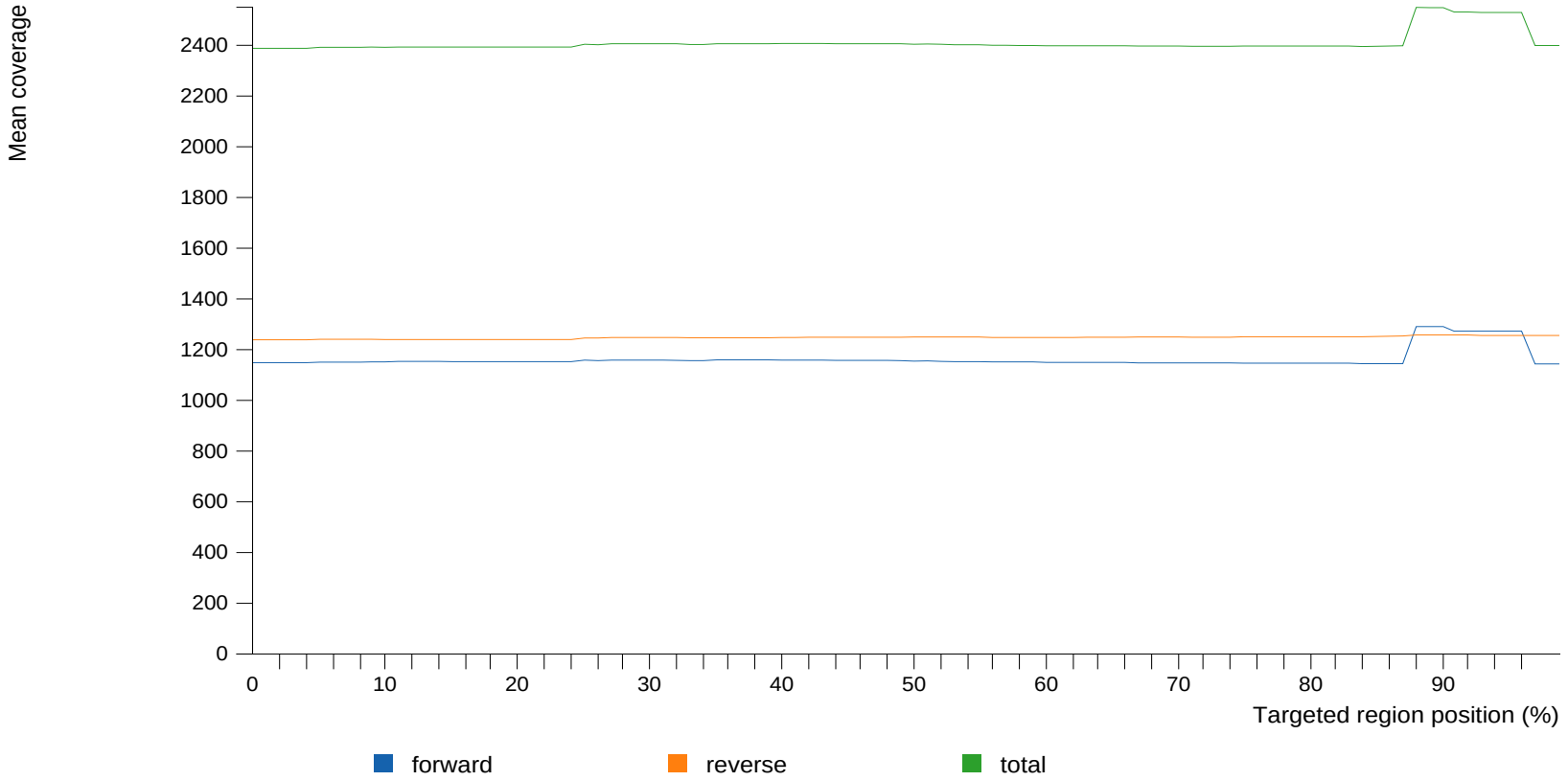
No ambiguous bases detected

4.3 Coverage of Regions of Interest positions

Coverage distribution



4.4 Mean coverage of relative positions in regions of interest



5 History

5.1 Log Entries

Type	Time	User	Details
State change	Fri Oct 25 17:38:06 CEST 2019	system	Ready for Review
State change	Fri Oct 25 17:14:57 CEST 2019	root	In Progress

5.2 Execution Information

QCIA version	QCI Analyze 1.4.5
Analysis start time	Fri Oct 25 17:14:57 CEST 2019
Analysis workflow	AIT FFPE 4.5
Analysis description	QIAact Actionable Insights Tumor Panel on FFPE

5.3 Transcripts

Table listing the genes, transcript IDs and protein IDs used in the analysis.

Gene Name	Transcript ID	Protein ID
NRAS	NM_002524.4	NP_002515.1
ALK	NM_004304.4	NP_004295.2
RAF1	NM_002880.3	NP_002871.1
PIK3CA	NM_006218.2	NP_006209.2
PDGFRA	NM_006206.4	NP_006197.1
KIT	NM_000222.2	NP_000213.1
ESR1	NM_001122742.1	NP_001116214.1
EGFR	NM_005228.3	NP_005219.2
BRAF	NM_004333.4	NP_004324.2
KRAS	NM_004985.3	NP_004976.2
ERBB3	NM_001982.3	NP_001973.2

Gene Name	Transcript ID	Protein ID
ERBB2	NM_004448.2	NP_004439.2

5.4 Open Parameters

Workflow parameters that are editable by the administrator

Reported variants

Significant coverage threshold	500
SNV/MNV frequency threshold in %	4.00
Insertions, deletions and replacements frequency threshold in %	4.00

Variants available for review

Minimum coverage threshold	200
SNV/MNV frequency threshold in %	4.00
Insertions, deletions and replacements frequency threshold in %	4.00
Detect variants outside regions of interest	Yes

5.5 Locked Parameters

Adapter trimming

Trim adapter list	GRadapter_160913
Ambiguous trim	false
Ambiguous limit	2
Quality trim	false
Quality limit	0.05
Use colorspace	false

Also search on reversed sequence	false
Remove 5' terminal nucleotides	false
Number of 5' terminal nucleotides	1
Maximum number of nucleotides in reads	1000
Minimum number of nucleotides in reads	15
Discard short reads	false
Remove 3' terminal nucleotides	false
Number of 3' terminal nucleotides	1
Discard long reads	false

Map Reads to Reference

References	Homo_sapiens_sequence_hg19
Masking mode	No masking
Masking track	Not set
Match score	1
Mismatch cost	2
Cost of insertions and deletions	Affine gap cost
Insertion cost	3
Deletion cost	3
Insertion open cost	6
Insertion extend cost	1
Deletion open cost	6
Deletion extend cost	1
Length fraction	0.5
Similarity fraction	0.8
Global alignment	false
Color space alignment	false
Color error cost	3
Auto-detect paired distances	false
Non-specific match handling	Map randomly

InDels and Structural Variants

P-Value threshold	1.0E-4
Maximum number of mismatches	3
Ignore broken pairs	true
Minimum relative consensus coverage	0.0
Minimum quality score	0
Filter variants	true
Minimum number of reads	2
Restrict calling to target regions	ATPv2_TargetRegions_170302_ver1.1

Local Realignment (Short Unaligned End version)

Realign unaligned ends	true
Multi-pass realignment	2
Local bound for unaligned ends of size one	0.75
Local bound for unaligned ends of size two	0.75
Force realignment to guidance-variants	false
Maximum guidance-variant length	100

Trim Primers and their Dimers of Mapped Reads

Primer track	101x_GR_primers_15_10_15_V1.0
Reference	Homo_sapiens_sequence_hg19
Minimum primer overlap length	9
Allow dangling 3' end base	true
Minimal primer overlap fraction	0.7
Only keep reads that have hit a primer	true
Additional bases to trim	1

Remove Pseudogene Reads

Genes track	AITv2_PseudoGenes_170912_ver1.0
Gene and pseudogene links	KRAS -> KRASP1
Required unaligned ends %	2.0

Low Frequency Variant Detection

Required significance (%)	0.01
Ignore positions with coverage above	1000000000
Restrict calling to target regions	ATPv2_TargetRegions_170302_ver1.1
Ignore broken pairs	true
Ignore non-specific matches	Reads
Minimum read length	20
Minimum coverage	Parameter editable by administrator
Minimum count	8
Minimum frequency (%)	Parameter editable by administrator
Base quality filter	false
Neighborhood radius	5
Minimum central quality	5
Minimum neighborhood quality	5
Read direction filter	false
Direction frequency (%)	5.0
Relative read direction filter	false
Significance (%)	1.0E-5
Read position filter	false
Significance (%)	1.0
Remove pyro-error variants	false
In homopolymer regions with minimum length	3
With frequency below	0.8

Remove False Positives

Minimum frequency (%)	Parameter editable by administrator
Minimum forward/reverse balance	0.05
Minimum average base quality	22.0
Variant frequency	true
Forward/reverse balance	false
Average base quality	true

Annotate Variants With Primers

Minimum coverage count	400
Minimum variant percentage	1.0
Minimum variant read count	2