Int J Clin Exp Pathol 2018;11(8):3903-3914
www.ijcep.com /ISSN:1936-2625/IJCEP0079308

*Original Article*

# Genome-wide SNP and InDel mutations in Mycobacterium tuberculosis associated with rifampicin and isoniazid resistance

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Received May 9, 2018; Accepted June 19, 2018; Epub August 1, 2018; Published August 15, 2018

**Abstract:** Objective: Multiple resistances to isoniazid and rifampicin lead to the majority of death associated with *M. tuberculosis* infection. This study aimed to characterize the single nucleotide polymorphisms (SNPs) and insertion and deletion (InDel) mutations associated with isoniazid and rifampicin resistance. Methods: The *M. tuberculosis* strain H37Rv was cultured and treated with isoniazid or rifampicin for generations. Total DNA samples from different generations were extracted for construction of DNA library, and the SNP and InDel mutation in different samples were detected by whole genome sequencing. Bioinformatics analysis such as phylogenetic tree and heap map were also performed. Results: Totally 58 nonsynonymous SNP mutations, 64 synonymous SNP mutations, and 99 SNP mutations in intergenic regions were detected in *M. tuberculosis* strains treated with rifampicin or isoniazid. Seven InDel mutations were found in the intergenic regions, and also six frameshift InDel mutation and three non-frameshift InDel mutations were also characterized. The phylogenetic tree showed clustering of all samples into three main subgroups. A great number of known and newly identified genes associated with drug resistance were detected in *M. tuberculosis*, showing distinct mutation patterns. Conclusion: By whole genome sequencing, many genetic mutations in both known and new genes associated with isoniazid and rifampicin resistance were characterized in *M. tuberculosis*.

**Keywords:** Mycobacterium tuberculosis, rifampicin, isoniazid, drug resistance, SNP, InDel mutation

## Introduction

Mycobacterium tuberculosis (*M. tuberculosis*) is a highly harmful pathogenic bacterium that causes tuberculosis (TB) by severe infection of human lung tissues [1, 2]. Globally, TB caused by *M. tuberculosis* is still a major infectious killer, which was responsible for 1.7 million deaths in 2016 according to the World Health Organization report [3]. Even more serious, over 10 million new cases of TB each year were estimated on a global scale, especially in several countries such as India, Indonesia, and China [3]. Most *M. tuberculosis* infections exhibited no obvious symptoms, termed as latent tuberculosis. Approximately 10% latent cases could develop to active infections and severe tuberculosis, causing serous symptoms like chronic cough, bloody sputum and fever, which results into great damages to lung tissues and even death. The high infectivity and mortality if treated inappropriately made *Mycobacterium tuberculosis* infection a lasting public health problem of global significance.

Treatment of *Mycobacterium tuberculosis* infections largely depends on the combined application of first-line anti-TB drugs such as isoniazid (INH) and rifampicin (RIF), two most effective and widely administered drugs [1]. TB caused by susceptible stains could be completely cured by standard regimen containing INH and RIF within 6 months in most cases. However, multiple drug resistance (MDR)

against these two most effective anti-TB drugs has greatly hampered TB treatment efficacy, which causes most treatment failure and deaths from *Mycobacterium tuberculosis* infection [1, 2, 4-6]. Also, the following development of extensively drug resistant-TB (XDR-TB), which is resistant to INH, RIF, fluoroquinolone and injectable aminoglycosides, brought about greater challenges for tuberculosis treatment and new drug development [7-9]. The average cure rates of MDR- and XDR-TB were reported to be only 54% and 6.2% respectively worldwide [3]. Comprehensive understanding of the molecular mechanisms underlying rapidly developing drug resistances in *M. tuberculosis* strains is therefore a prerequisite of developing novel anti-tuberculosis drugs.

The evolution of drug resistance in TB strains is mediated by multi-faceted complex patterns [1]. For instance, key proteins responsible for maintenance of the cell wall integrity have been involved in the achievement of resistances to multiple anti-tuberculosis drugs in *Mycobacterium tuberculosis* [10]. Cell envelopes outside *Mycobacterium tuberculosis*, which mainly consist of peptidoglycan, arabinogalactan polysaccharide and long-chainmycolic acids, function as an important first line of defense against extracellular stress [11]. Enzymes controlling the dynamic metabolisms of cell wall lipids have been shown to be important regulators of cell wall functions and also associated with development of drug resistance in *Mycobacterium tuberculosis* [12]. In bacteria, those penicillin-binding D, D-transpeptidases catalyze the connection of glycan chains on stem peptides, and were inactivated by β-lactam antibiotics like carbapenems and amoxicillin [13]. Recent reports showed that expression of another class of transpeptidases in *Mycobacterium tuberculosis* could take the place of the above-mentioned D, D-transpeptidases, and thus contribute to the development of resistance against amoxicillin and carbapenems in *Mycobacterium tuberculosis* [14, 15]. In addition, a number of proteins involved in the regulation of cell wall integrity were found to mediators of drug resistance in *Mycobacterium tuberculosis* [15]. Also, the entry into a non-replicating dormant state with a shutdown of major metabolic activities has also been applied as a key drug-resistance strategy by resistant bacteria [16]. Moreover, alterations in channel proteins regulating cell wall permeability, efflux pumps that effectively transport many antibiotic drugs outside bacterial cells, modifications of intracellular sites targeted by antibiotics, and degradation and enzymatic modifications of antibiotics, were also involved in development of multiple drug resistance in *Mycobacterium tuberculosis* [1, 2].

At the molecular level, genetic mutations act as the principal mechanism underlying *Mycobacterium tuberculosis* resistance against major anti-TB agents including INH and RIF [5, 6]. The anti-TB activity of INH require activation by a catalase/peroxidase katG and the decrease of katG activity due to genetic mutations has been commonly observed in resistant TB strains showing strong insensitivity to INH treatment [17]. Recent research revealed that the resistance of TB against INH has been mainly mediated by genetic mutations in several genes including katG, inhA and fabG1 [18]. Similarly, the resistance against RIF in TB was also associated with genetic mutations in key genes mediating the therapeutic effects of RIF such as the RNA polymerase beta-subunit gene (rpoB) [19]. The disclosure of molecular mechanisms linked with TB resistance provided a basis for effective targeted treatment. However, due to the high complexity in drug resistance and strong adaptability of *Mycobacterium tuberculosis*, the molecular mechanism and genetic mutations associated with their resistance against INH and RIF remain far from being fully understood.

In the present study, *Mycobacterium tuberculosis* was treated with INH or RIF for over 26 generations, and the single nucleotide polymorphisms (SNPs) and Insertion and deletion (InDel) mutations were characterized by whole-genome sequencing. Findings will provide novel insights into the acquired resistances of TB against INH and RIF, which might help develop novel therapeutic agents for resistant strains.

## Materials and methods

### *Bacterial culture and antibiotics treatments*

The *M. tuberculosis* strain H37Rv used in this study as wild type was purchased from the Sample Bank of the Reference Laboratory of Guangdong Province. For analysis of gene mutation and expressional alterations, *M. tub-*

*erculosis* H37Rv strain was cultured at 37°C in Löwenstein-Jensen (LJ) medium containing glycerol, asparagine, potato starch, coagulated eggs, mineral salt solution, potassium dihydrogen phosphate, magnesium sulfate and sodium citrate. Monoclines of M. tuberculosis H37Rv strain were chosen as the Generation 0 (G0) group by amplification culture. For generation of resistant strains, the *M. tuberculosis* G0 strains cultured and survived in Löwenstein-Jensen (LJ) medium containing $10^{-4}$ isoniazid (Cat. No 75182; Sigma-Aldrich) and rifampicin (Cat. No R3501; Sigma-Aldrich) for four weeks were defined as Generation 1 (G1) group. The following generations of isoniazid- or rifampicin-resistant M. tuberculosis strains were prepared by continuing the above-mentioned culture protocol, according to the World Health Organization (WHO)'s criteria for isoniazid (20 μg/mL) and rifampicin (4000 μg/mL) resistances in *M. tuberculosis*. Drug resistance of obtained resistant strains was verified by drug-susceptibility test using corresponding concentrations of antibiotics. The isoniazid- and rifampicin-resistant strains were selected as previously described [20, 21]. Following successfully preparation of resistant strains, the total genomic DNA samples and RNA samples from specific generations of wild-type, isoniazid-resistant and rifampicin-resistant strains were extracted for subsequent whole-genome and expressional profiles analysis respectively.

*Total DNA extraction*

The whole extraction procedures were carried out a in fume hood by standard protocol. *M. tuberculosis* of different groups were collected by centrifuge at 6000 g for 15 min, and the precipitants were then mixed with 300 ml CTAB (Cetyl trimethyl ammonium bromide) lysis buffer which containing 2% CTAB, 1.5 M NaCl, 0.1 M Tris-HCl (pH 8.0), 20 mM EDTA and 0.4% b-mercaptoethanol. The bacteria were lysed by grinding in liquid nitrogen, and the resulting powder was then mixed with 20 μl proteinase K solution (20 mg/ml), incubated at 55°C for 30 min with intermittent mixture for 3-5 times, and then mixed with equal volume of phenol: chloroform: isoamyl alcohol solution (25:24:1) followed by a centrifuge at 12000 rpm for 5 min at 4°C. The supernatant were then mixed with 0.8 volume of isopropanol solution for precipitation of DNA sample, followed by extraction with a solution of chloroform: isoamyl alcohol solution (24:1). After being mixed with 3 mol/L NaAC (pH 5.2) and absolute ethanol, the DNA samples were collected by centrifuge at 12000 rpm for 10 min at 4°C, washed with 70% ethanol solution, and finally dissolved in $ddH_2O$ for the following analysis. The quality of DNA samples was determined by spectrophotometry and 1% agarose gel electrophoresis.

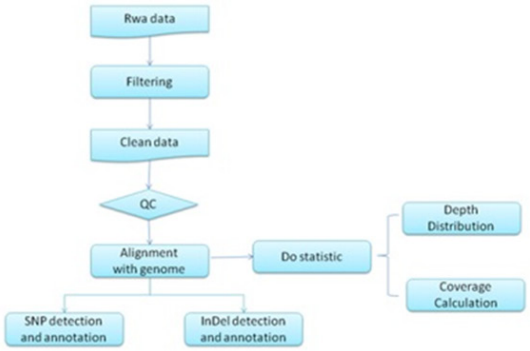
*DNA library construction and whole genome sequencing*

For establishment of *M. tuberculosis* whole genome DNA library, the DNA samples were fragmented by incubation with dsDNA fragmentase at 37°C for 15 min, which was terminated by adding 0.5 M EDTA solution. DNA fragments were then purified with VAHTSTM DNA Clean Beads, and the DNA terminals were repaired using Endprep Enzyme by incubation at 20°C for 30 min and 65°C for 30 min. Then, DNA fragments were ligated with DNA Adapter using Quick T4 DNA Ligase by incubation at 20°C for 15 min, and purified using VAHTSTM DNA Clean Beads. The Adapter-ligated DNA fragments were then amplified by Polymerase Chain Reaction (PCR) using 2X Super Canace High-Fidelity Mix, in combination with Hieff NGS Universal PCR Primer and Hieff NGS Index Primer. PCR products were then purified using VAHTSTM DNA Clean Beads again, and stored in 17.5 μl NF-$H_2O$ at -20°C for following genome sequencing. For analysis of SNP and insertion/deletion mutation in different *M. tuberculosis* strains, the whole genome sequencing was performed on a Pacific Biosciences RSII DNA sequencing system Pacific Biosciences, USA). Raw data from DNA sequencing were the processed by filtering, data cleaning, quality control, alignment against the *M. tuberculosis* genome database, SNP and insertion/deletion detection and annotation as described in previous studies [22-24].

*Bioinformatics and statistical analysis*

For clear display of gene mutations in different groups, the SNP mutation numbers were summarized using the Venn diagrams. Subclasses of *M. tuberculosis* were characterized by EXPANDS analysis based on SNP and InDel mutation patterns, from which a phylogenetic tree was built by calculating absolute distance matrices via Kullback-Leibler divergence meth-

A




B

| Mutation type | SNP number | InDel number |
|---|---|---|
| 5'-UTR | 0 | 0 |
| Nonsynonymous SNPs | 58 | — |
| Synonymous SNPs | 64 | — |
| 3'-UTR | 0 | 0 |
| Intergenic region | 99 | 7 |
| Frameshift InDels | — | 6 |
| Non-frameshift InDels | — | 3 |

C

| | C1 | C5 | C10 | C18 | C26 | I1 | I5 | I10 | I18 | I26 | R1 | R5 | R10 | R26 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SNVs | 65 | 38 | 51 | 57 | 45 | 58 | 41 | 54 | 58 | 47 | 46 | 61 | 66 | 71 |
| InDels | 5 | 6 | 5 | 8 | 4 | 5 | 8 | 4 | 7 | 7 | 7 | 7 | 7 | 5 |
| Total | 70 | 44 | 56 | 65 | 49 | 63 | 49 | 58 | 65 | 54 | 53 | 68 | 73 | 76 |

**Figure 1.** Whole genome sequencing of mutations in *M. tuberculosis*. A. The procedure of whole genome sequencing in *M. tuberculosis* strains. B. Total numbers of SNP and InDel mutations detected in strains treated with rifampicin and isoniazid. C. Statistical analysis of SNP and InDel mutation in strains treated with rifampicin and isoniazid for different generations. C1-C26 indicates the control groups, I1-I26 indicates *M. tuberculosis* treated with isoniazid, and R1-R26 indicates *M. tuberculosis* treated with rifampicin. SNP: single nucleotide polymorphisms; InDel: Insertion and deletion mutations.

od as described previously [24]. The mutation profiles between distinct groups were also presented using heat maps.

## Results

### *Mutation spectrum under rifampicin and isoniazid treatment*

For general analysis of single nucleotide polymorphisms (SNPs) and Insertion and deletion (InDel) mutations associated with rifampicin and isoniazid resistances in *M. tuberculosis*, the total DNA samples from bacteria treated with rifampicin or isoniazid for different generations were extracted for DNA library construction and whole genome sequencing as described above (**Figure 1A**). Basically, 58 nonsynonymous SNP mutations, 64 synonymous SNP mutations, and 99 SNP mutation in intergenic regions were detected by the whole genome sequencing of *M. tuberculosis* strains treated with rifampicin or isoniazid for different generations, as well as the control groups (**Figure 1B**, **1C** and Supplementary Table 1). Totally, seven InDel mutations were found in the intergenic regions, and also six frameshift InDel mutations and three non-frameshift InDel mutations were found in these groups of samples (**Figure 1B**, **1C** and Supplementary Table 1). More strikingly, we showed that the number of SNP mutation in *M. tuberculosis* strains treated with rifampicin increased in proportion to the elongation of treatment duration (**Figure 1C**). Detailed information of all SNP and InDel mutations can be found in the attached table (Supplementary Table 1). These results demonstrated that SNP and InDel mutation might relate to rifampicin and isoniazid resistance of *M. tuberculosis*.

### *SNP mutation induced by rifampicin and isoniazid treatment*

The whole genome sequencing disclosed much more SNP mutations in *M. tuberculosis* treated with rifampicin and isoniazid, compared with the InDel mutations (**Figure 1B**). For more properties of SNP mutations found in *M. tuberculosis* treated with rifampicin and isoniazid, the numbers of SNP mutations in the control, rifampicin, and isoniazid groups were summarized using Venn diagrams. We found that 31 SNP mutations were detected in the control groups of *M. tuberculosis* among different generations (**Figure 2A**). Thirty-two SNP mutations were shared by *M. tuberculosis* treated with isoniazid for distinct generations (**Figure 2A**). Totally, 45 SNPs were shared by different generations of *M. tuberculosis* treated with rifampi-

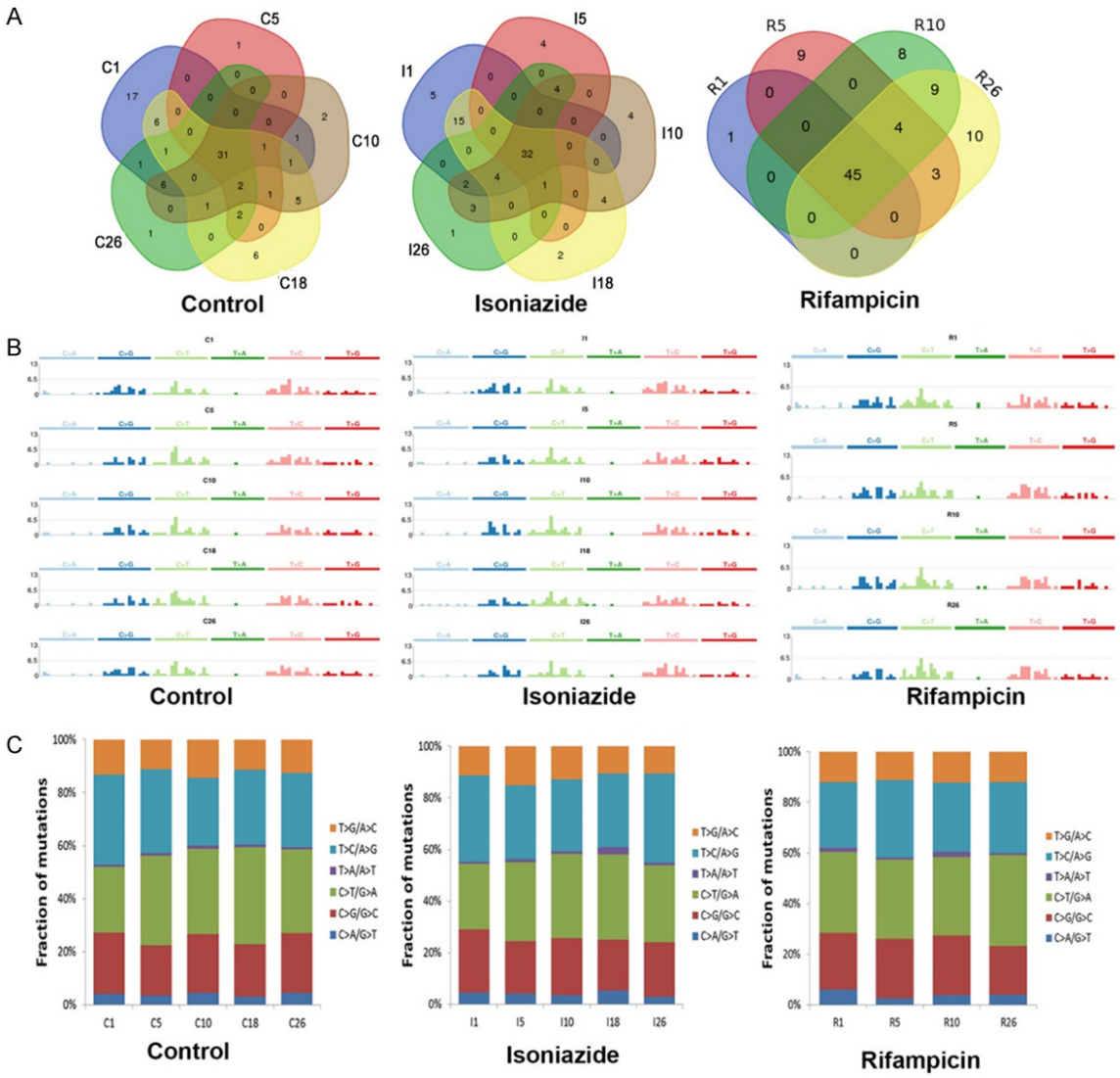



**Figure 2.** SNP mutations in *M. tuberculosis* treated with rifampicin and isoniazid. A. Venn diagrams presenting the SNP mutation numbers shared by different bacteria groups. Totally 31, 32 and 45 SNP mutations were shared by different generations in the control, isoniazid and rifampicin groups respectively. B. Detailed information of SNP mutations in groups treated with rifampicin and isoniazid. The majority of SNPs were C-to-G, C-to-T and T-to-C mutations. C. SNP mutation spectrums in the control, isoniazid and rifampicin groups at different generations. C1-C26 indicates the control groups, I1-I26 indicates *M. tuberculosis* treated with isoniazid, and R1-R26 indicates *M. tuberculosis* treated with rifampicin.

cin (**Figure 2A**), which were much higher that the control and isoniazid groups. By analyzing the details of SNP mutation spectrums, we demonstrated that C-to-G, C-to-T and T-to-C mutations were predominantly distributed in genomes of *M. tuberculosis* strains treated with rifampicin and isoniazid (**Figure 2B**). More importantly, we showed here that T-to-C mutations decreased in the control group with increase of culture generations, while the number of C-to-T numbers in the control group significantly increased (**Figure 2C**). However, we observed that the number of T-to-C mutations in M. tuberculosis treated with rifampicin and isoniazid showed no significant decrease, suggesting that rifampicin and isoniazid could induce T-to-C mutations in M. tuberculosis genome which might be responsible for their resistance to these anti-TB drugs.

*Evolutionary relationship between different M. tuberculosis groups*

To analyze the evolutionary relationships between all *M. tuberculosis* sequenced after being treated with rifampicin and isoniazid for

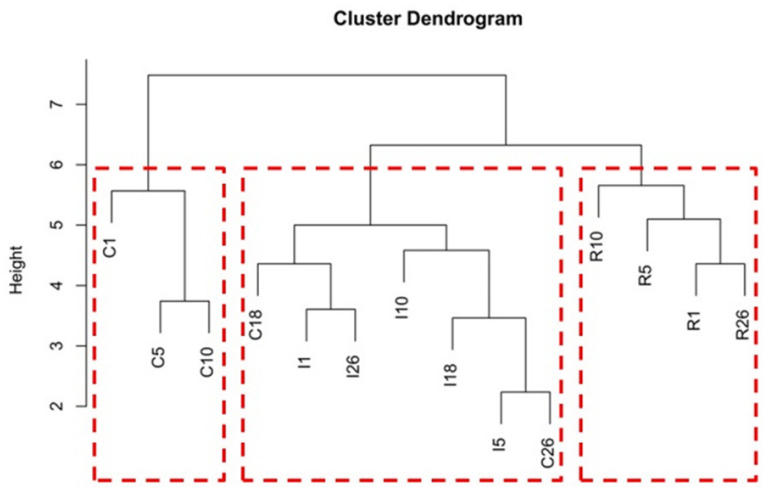



**Figure 3.** Clustering of *M. tuberculosis* based on mutations. Subgroups of *M. tuberculosis* treated with rifampicin and isoniazid for distinct generations were clustered using a phylogenic tree. Generally, three subgroups were detected among all samples by relationships based on SNP and InDel mutations. C1, C5 and C10 shares close evolutionary relationship. I1, I5, I10, I18 and I26 were evolutionarily closer, also to C18 and C26. R1, R5, R10 and R26 were clustered into the same subgroup. C1-C26 indicates the control groups, I1-I26 indicates *M. tuberculosis* treated with isoniazid, and R1-R26 indicates *M. tuberculosis* treated with rifampicin. SNP: single nucleotide polymorphisms; InDel: Insertion and deletion mutations.

different generations, a phylogenic tree was drawn based on the SNP and InDel mutation spectrums detected by whole genome sequencing (**Figure 3**). As expected, the majority of *M. tuberculosis* samples from the control, rifampicin and isoniazid groups were clustered into different subgroups. Specifically, three samples from the control group C1, C5 and C10 were clustered into the same subgroup, showing their close evolutionary relationship (**Figure 3**). All samples from the isoniazid groups I1, I5, I10, I18 and I26 were found to be evolutionarily closer, compared with the control groups (**Figure 3**). Similarly, these four samples from the rifampicin group R1, R5, R10 and R26 were clustered into another subgroup (**Figure 3**). The evolutionary relationships revealed by the phylogenic tree confirmed the reliability of our whole genome sequencing results, and also showed the significantly different mutation patterns caused by isoniazid and rifampicin treatments in *M. tuberculosis*. These results also suggested that the resistances to isoniazid and rifampicin might be mediated by different genetic mechanisms. Of note, the C18 and C26 samples have closer relationship with the isoniazid groups (**Figure 3**), suggesting that naturally occurring mutation might also function in the development of resistance to isoniazid.

*Frequently mutated genes induced by rifampicin and isoniazid treatment*

For further information of the genes and related biological processes associated with rifampicin and isoniazid resistance in M. tuberculosis, these frequently mutated genes with SNP or InDel mutations under rifampicin and isoniazid treatment for distinct generations were shown with heat maps (**Figures 4** and **5**). Totally 65 genes were detected as mutated genes by whole genome sequencing from the control group and the rifampicin and isoniazid-treated *M. tuberculosis groups* (**Figures 4** and **5**). Among them, 29 genes showed SNP or InDel mutations in all groups of *M. tuberculosis* samples tested by genome sequencing, including acg, bkdC, dnaG, ercc3, fadE34, fdxA, lprQ, moaX, nrp, multiple members of the PE_PGRS family, etc. (**Figure 4**). These results showed that gene mutations are common event in *M. tuberculosis* genome, which could be utilized as an effective method for the development of resistance to drugs. More importantly, we also identified several genes that were specifically mutated under rifampicin or isoniazid treatment, or even at specific generations after drug treatments. For instance, mutations of kAtG, pckA, crp, glgP, ugpB, lldD2, PPE34 and rplT genes were only detected in the genomes of *M. tuberculosis* treated with isoniazid (**Figure 4**). However, another group of genes like rpoB, esxO, PE_PGRS15, ansP2, glpK, hemA, PPE51 and trpB showed detectable SNP or InDel mutations only in M. tuberculosis groups under the treatment of rifampicin (**Figure 4**). The significantly different mutation pattern between genomes of rifampicin and isoniazid-treated *M. tuberculosis* showed that these two anti-TB drugs might target different biologic processes and signaling pathways during the development of drug resistance. Also, several genes were only mutated in the control groups, such as hemC,

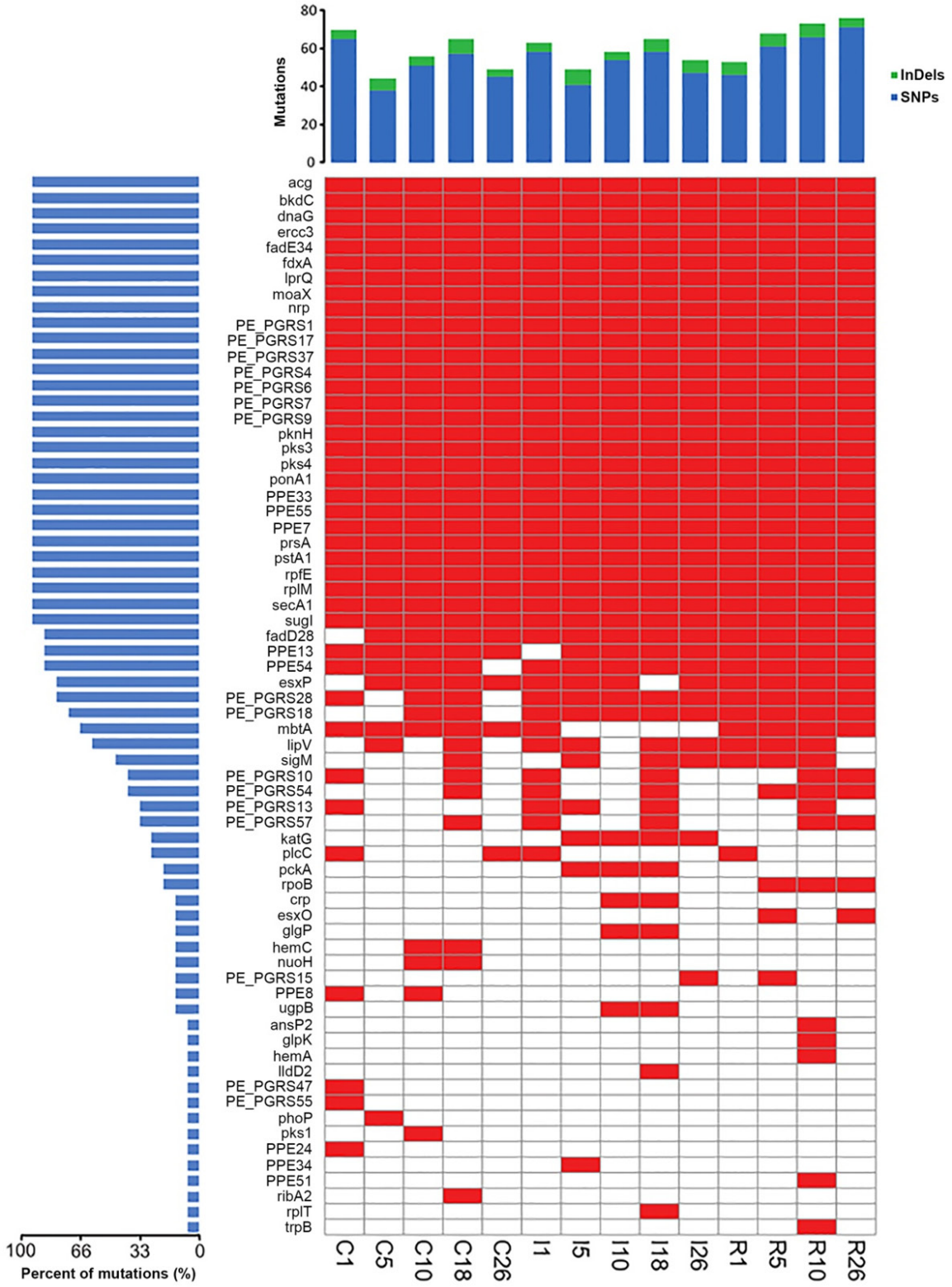



**Figure 4.** Frequently mutated genes in rifampicin and isoniazid-treated *M. tuberculosis*. Genes with significant mutations by whole genome sequencing were listed in original grouping order. The SNP and InDel mutations in all 65 genes detected by sequencing were marked with red rectangles. The percentages of mutations among all groups and the ratios of SNP and InDel mutations are listed on the left and top positions respectively. C1-C26 indicates the control groups, I1-I26 indicates *M. tuberculosis* treated with isoniazid, and R1-R26 indicates *M. tuberculosis* treated with rifampicin. SNP: single nucleotide polymorphisms; InDel: Insertion and deletion mutations.

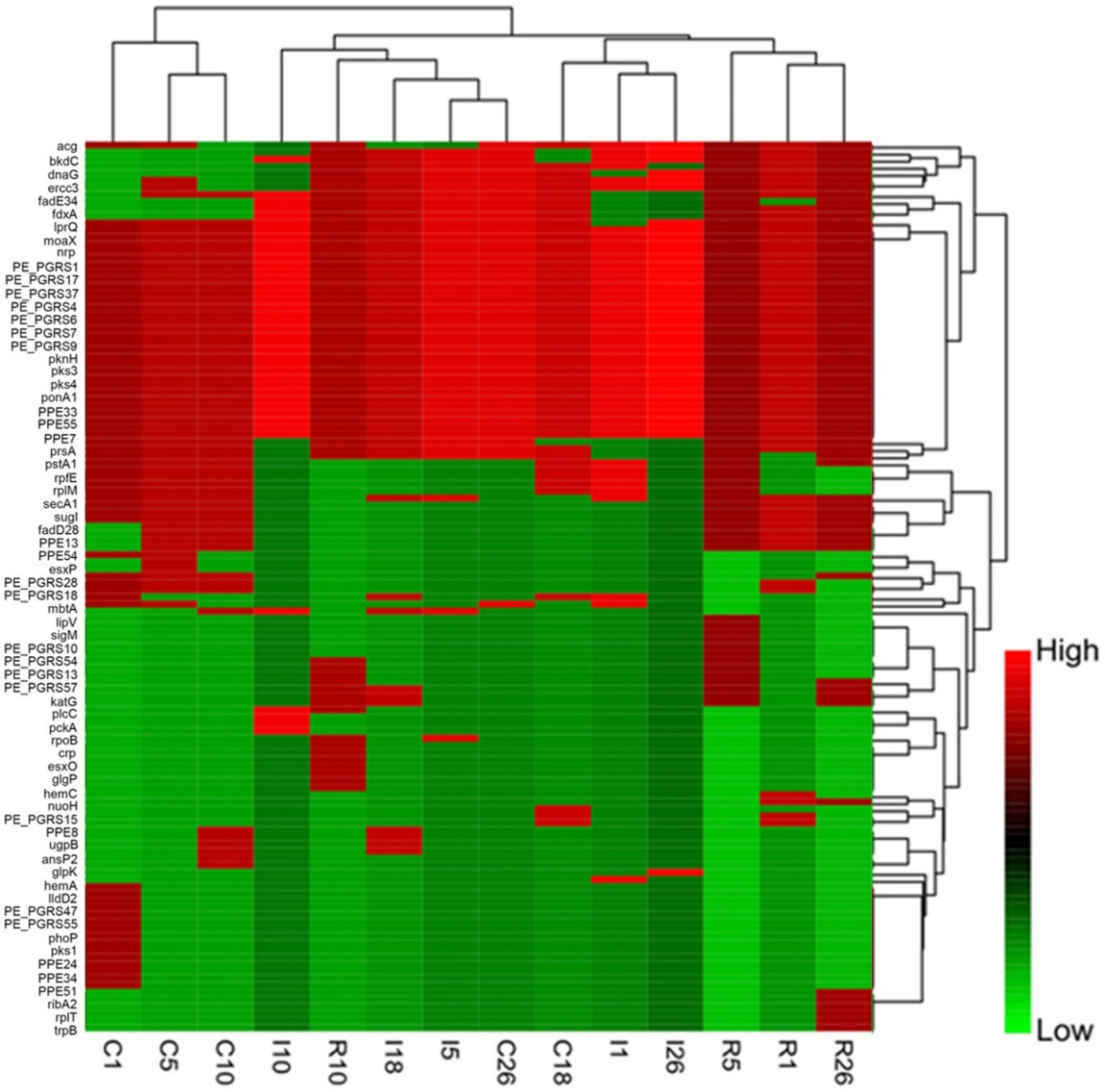



**Figure 5.** A heat map of gene mutations in rifampicin and isoniazid-treated *M. tuberculosis*. Totally, 65 mutated genes were listed in a heat map in clustered grouping order. Genes with the SNP and InDel mutations shown in whole genome sequencing were marked in red, and gene with no mutations were shown in green. I1-I26 indicates *M. tuberculosis* treated with isoniazid, and R1-R26 indicates M. tuberculosis treated with rifampicin. SNP: single nucleotide polymorphisms; InDel: Insertion and deletion mutations.

nuoH, PE_PGRS47, PE_PGRS55, phoP, pks1, PPE24 and ribA2, showing the possible inhibitory effects of rifampicin and isoniazid on mutations of specific genes. Furthermore, the presentation of mutated genes by clustering also revealed multiple gene and biological processes influenced by rifampicin and isoniazid treatments (**Figure 5**). These observations showed that resistance to rifampicin and isoniazid in M. tuberculosis involve alteration of various biologic functions and signaling processes.

## Discussion

Clinical treatment of major severe human diseases such as cancer, malaria, and other infectious diseases has always been greatly impeded by rapid-developing resistances to first-line drugs, especially multiple drug resistance (MDR) [25-27]. Recent progress in pathogenic mechanism studies showed that genetic mutations have played critical parts in the development of main drug resistances [28]. Isoniazid (INH) and rifampicin (RIF) are the major anti-TB

drugs in clinics, but their efficacies have been greatly reduced by the rapid development of multiple drug resistance [29]. Although previous investigations indicated that genetic mutations such as single nucleotide variation (SNV) and other mutation types such as deletion and insertion were involved in drug resistance of *M. tuberculosis*, the underlying mechanisms still remains far from being understood [5, 6]. Due to rapid development of large-scale DNA sequencing technology, the analysis of whole genome of pathogens has been proven as an effective research strategy for investigation of the gene mutations underlying multiple drug resistance. For instance, the study of resistances to anti-malaria drugs through whole genome sequencing revealed key molecular events responsible for acquirement of resistance in *Plasmodium vivax* [25]. The primary advantage of whole genome sequencing for drug resistance study is its capability for characterizing large number of genetic mutations associated with resistance development by comparison of different samples [30]. For more understanding of the mechanisms of resistance to INH and RIF, we carried out a comprehensive investigation of genetic mutations by whole genome sequencing in *M. tuberculosis*, with a focus on the SNP and InDel mutations.

To characterize the major gene mutation associated with resistances to isoniazid and rifampicin, *M. tuberculosis* was treated with isoniazid or rifampicin for successive generations, and SNP and InDel mutations were characterized by whole genome sequencing for comparison with the control group. We identified a large number of synonymous and nonsynonymous SNP mutations in *M. tuberculosis* under treatment of isoniazid and rifampicin, as well as many InDel mutations. These SNP and InDel mutations occurring with isoniazid and rifampicin treatment persuasively confirmed the great involvement of DNA sequence alteration in development of multiple drug resistance to anti-TB drugs. Genetic mutations in *M. tuberculosis* have been previously shown to be associated with geographical origin, clinical stages, and drug resistance [31]. In the present study, we disclosed various novel SNP and InDel mutations in *M. tuberculosis* treated with isoniazid and rifampicin, which might provide new insights into the molecular events responsible for the acquirement of isoniazid and rifampicin resistance. Also, different groups of samples tested in our study were clustered into three main subgroups by clustering based on SNP and InDel mutations, indicating that the resistance to isoniazid and rifampicin resistances may be induced by distinct molecular processes, except for common events shared by both types of drug resistance. The concurrence of similarities and differences in genetic mutations between isoniazid and rifampicin-treated *M. tuberculosis* are consistent with previous investigations aiming to disclose the underlying mechanisms of these drug resistances [32, 33]. These genetic mutations induced by isoniazid and rifampicin would act as a basis for development of new anti-TB drugs with improved treatment efficacy, and also for screening of susceptible *M. tuberculosis* strains and directing the design of most suitable treatment regiments in clinics.

New perspectives on the molecular processes of drug resistance in *M. tuberculosis* were provided by genetic mutations revealed in our genome sequencing. The cAMP receptor protein (CRP) functions as a key regulator of gene transcription that controls cell growth in *M. tuberculosis* [34, 35]. We showed here an A-to-G mutation in the crp gene of *M. tuberculosis* treated with isoniazid which would predictively cause a I-to-V mutation in its primary protein sequences, but not in *M. tuberculosis* treated with rifampicin or the control groups. The mutation of crp gene exclusively in isoniazid-treated *M. tuberculosis* suggested that the alteration of gene expression by specific transcription regulators such as crp might act as important promoting events underlying the development of resistance to isoniazid. Likewise, a group of other genes were found in this study mutated only in isoniazid-treated group, including katG, pckA, crp, glgP, ugpB, lldD2, PPE34 and rplT genes. Of note, the mutation of bifunctional enzyme catalase–peroxidase (katG) has been established by extensive research as one of the major player linked with isoniazid resistance [18, 36-38]. Similarly, another well-known drug resistance-related gene RNA polymerase beta-subunit (rpoB) was also identified in our assay as mutated gene induced by rifampicin treatment. The involvement of the rpoB gene, which encodes a subunit of the DNA-dependent RNA polymerase, with rifampicin resistance has also been exten-

sively proven by previous investigations [36, 38-40]. The detection of these known genes associated with drug resistance such as katG and rpoB enhanced the reliability of our genome analysis. Moreover, we also detected a group of new genes with SNP or InDel mutations under the treatment of isoniazid and rifampicin, such as early secretory antigenic target 0 (esx0), a member of the 6-kDa antigenic target that regulates bacillary survival through genomic instability modulation [41]. Further investigation of the function and mechanisms of these newly characterized genes would bring about comprehensive understanding of the multiple drug resistance development.

In summary, we performed a whole genome sequencing of *M. tuberculosis* treated with isoniazid and rifampicin for distinctive generations, and a large number of SNP and InDel mutations were identified. This characterization of new genetic mutations in isoniazid and rifampicin-treated *M. tuberculosis* provided useful information for novel drug development and tuberculosis treatment.

## Acknowledgements

This work was supported by the Science and Technology Planning Project of Guangdong Province (2014A020212238); Science and Technology Planning Project of Guangdong Province (2016A020216021); National Science and Technology Major Project of the Ministry of Science and Technology of China (2014-ZX10003001-002).

## Disclosure of conflict of interest

None.

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**Supplementary Table 1.** Mutations of coding gene in each sample

| Chromo-some | Start | End | Ref | Alt | Gene affected | CDS mutation | Amino acid change | Mutation type | C1 | C5 | C10 | C18 | C26 | I1 | I5 | I10 | I18 | I26 | R1 | R5 | R10 | R26 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NC_000962.3 | 55553 | 55553 | C | T | PonA1 | C.C1891T | P.P631S | Missense | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 55553 | 55555 | CCG | - | PonA1 | C.1891_1893del | P.631_631del | In-frameshift deletion | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 116000 | 116000 | T | G | Nrp | C.T6000G | P.V2000V | Synonymous SNV | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 132417 | 132417 | C | G | PE_PGRS1 | C.C1036G | P.R346G | Missense | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 253126 | 253126 | A | G | PckA | C.A1345G | P.T449A | Missense | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 253322 | 253322 | G | A | PckA | C.G1541A | P.G514D | Missense | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 336681 | 336684 | GGGA | - | PE_PGRS4 | C.2390_2393del | P.797_798del | Frameshift deletion | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 336698 | 336698 | C | G | PE_PGRS4 | C.G2376C | P.G792G | Synonymous SNV | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| NC_000962.3 | 336701 | 336701 | A | G | PE_PGRS4 | C.T2373C | P.G791G | Synonymous SNV | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| NC_000962.3 | 336707 | 336707 | G | A | PE_PGRS4 | C.C2367T | P.D789D | Synonymous SNV | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| NC_000962.3 | 336708 | 336708 | T | C | PE_PGRS4 | C.A2366G | P.D789G | Missense | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| NC_000962.3 | 336710 | 336710 | A | G | PE_PGRS4 | C.T2364C | P.A788A | Synonymous SNV | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 |
| NC_000962.3 | 337959 | 337959 | A | C | PE_PGRS4 | C.T1115G | P.I372S | Missense | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 338020 | 338020 | A | C | PE_PGRS4 | C.T1054G | P.C352G | Missense | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 338100 | 338100 | T | C | PE_PGRS4 | C.A974G | P.N325S | Missense | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 338453 | 338453 | A | G | PE_PGRS4 | C.T621C | P.A207A | Synonymous SNV | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 416551 | 416551 | G | A | AnsP2 | C.C415T | P.L139L | Synonymous SNV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| NC_000962.3 | 424322 | 424322 | - | C | PPE7 | C.373dupG | P.G125fs | Frameshift insertion | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 427360 | 427374 | GAGGTTTG CACTGCC | - | PPE8 | C.7306_7320del | P.2436_2440del | In-frameshift deletion | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 571745 | 571745 | G | C | IprQ | C.G36C | P.L12F | Missense | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 601503 | 601503 | T | G | HemA | C.T1063G | P.L355V | Missense | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| NC_000962.3 | 602261 | 602261 | G | A | HemC | C.G405A | P.L135L | Synonymous SNV | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 623472 | 623472 | A | G | PE_PGRS6 | C.A680G | P.D227G | Missense | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 623508 | 623508 | C | G | PE_PGRS6 | C.C716G | P.A239G | Missense | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 672491 | 672491 | C | G | PE_PGRS7 | C.G3426C | P.G1142G | Synonymous SNV | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 761140 | 761140 | A | G | RpoB | c.A1334G | P.H445R | Missense | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 |
| NC_000962.3 | 836272 | 836272 | A | G | PE_PGRS9 | C.A572G | P.E191G | Missense | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 836291 | 836291 | A | G | PE_PGRS9 | C.A591G | P.G197G | Synonymous SNV | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 836426 | 836426 | A | C | PE_PGRS9 | C.A726C | P.L242L | Synonymous SNV | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 836454 | 836454 | A | G | PE_PGRS9 | C.A754G | P.T252A | Missense | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 |
| NC_000962.3 | 836538 | 836538 | A | G | PE_PGRS9 | C.A838G | P.N280D | Missense | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 836658 | 836658 | A | G | PE_PGRS9 | C.A958G | P.T320A | Missense | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 837033 | 837033 | A | G | PE_PGRS9 | C.A1333G | P.T445A | Missense | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 838990 | 838990 | C | G | PE_PGRS10 | C.C540G | P.A180A | Synonymous SNV | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 839123 | 839123 | A | G | PE_PGRS10 | C.A673G | P.R225G | Missense | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 |
| NC_000962.3 | 839129 | 839129 | C | G | PE_PGRS10 | C.C679G | P.R227G | Missense | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 |
| NC_000962.3 | 839194 | 839194 | A | G | PE_PGRS10 | C.A744G | P.T248T | Synonymous SNV | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 |
| NC_000962.3 | 839334 | 839334 | A | G | PE_PGRS10 | C.A884G | P.K295R | Missense | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NC_000962.3 | 839348 | 839348 | A | G | PE_PGRS10 | C.A898G | P.S300G | Missense | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 840496 | 840496 | C | G | PE_PGRS10 | C.C2046G | P.G682G | Synonymous SNV | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 851821 | 851821 | G | A | PhoP | C.G214A | P.V72M | Missense | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 927110 | 927110 | A | G | PE_PGRS13 | C.A1750G | P.S584G | Missense | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| NC_000962.3 | 927462 | 927462 | A | G | PE_PGRS13 | C.A2102G | P.D701G | Missense | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 958922 | 958922 | C | A | Ercc3 | C.G1230T | P.A410A | Synonymous SNV | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 969689 | 969689 | A | C | PE_PGRS15 | C.T556G | P.W186G | Missense | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 |
| NC_000962.3 | 976906 | 976906 | - | G | PPE13 | C.1298dupC | P.A433fs | Frameshift insertion | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 1093406 | 1093406 | A | G | PE_PGRS17 | C.T951C | P.V317V | Synonymous SNV | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 1093907 | 1093907 | T | C | PE_PGRS17 | C.A450G | P.G150G | Synonymous SNV | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 1096205 | 1096205 | T | C | PE_PGRS18 | C.A247G | P.S83G | Missense | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 1135897 | 1135897 | A | G | PrsA | C.T585C | P.V195V | Synonymous SNV | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 1315884 | 1315884 | G | A | Pks4 | C.G651A | P.A217A | Synonymous SNV | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 1414021 | 1414021 | C | T | PknH | C.G1820A | P.R607Q | Missense | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 1495992 | 1495992 | G | C | GlgP | C.G1429C | P.V477L | Missense | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 1590925 | 1590925 | C | G | ribA2 | C.C529G | P.H177D | Missense | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 1636991 | 1636991 | T | C | PE_PGRS28 | C.A1239G | P.G413G | Synonymous SNV | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 1636996 | 1636996 | G | C | PE_PGRS28 | C.C1234G | P.R412G | Missense | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 1637006 | 1637006 | G | A | PE_PGRS28 | C.C1224T | P.V408V | Synonymous SNV | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 1637009 | 1637009 | G | A | PE_PGRS28 | C.C1221T | P.G407G | Synonymous SNV | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 1637012 | 1637012 | A | G | PE_PGRS28 | C.T1218C | P.G406G | Synonymous SNV | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 1637015 | 1637015 | A | G | PE_PGRS28 | C.T1215C | P.A405A | Synonymous SNV | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 1637018 | 1637018 | G | C | PE_PGRS28 | C.C1212G | P.G404G | Synonymous SNV | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 1812088 | 1812088 | C | T | TrpB | C.C962T | P.P321L | Missense | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| NC_000962.3 | 1853360 | 1853360 | G | A | RplT | C.G177A | P.K59K | Synonymous SNV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 1982064 | 1982064 | G | C | PPE24 | C.C2712G | P.P904P | Synonymous SNV | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 1982067 | 1982067 | G | C | PPE24 | C.C2709G | P.L903L | Synonymous SNV | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 1982178 | 1982178 | C | G | PPE24 | C.G2598C | P.L866L | Synonymous SNV | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 2051746 | 2051746 | T | C | PPE33 | C.T465C | P.A155A | Synonymous SNV | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 2122769 | 2122769 | G | C | lldD2 | C.C383G | P.A128G | Missense | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 2163790 | 2163790 | A | C | PPE34 | C.T3522G | P.P1174P | Synonymous SNV | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 2256291 | 2256291 | G | A | FdxA | C.C138T | P.C46C | Synonymous SNV | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 2256341 | 2256341 | C | T | FdxA | C.G88A | P.A30T | Missense | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| NC_000962.3 | 2279804 | 2279804 | C | G | Acg | C.C676G | P.P226A | Missense | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 2387733 | 2387733 | T | C | PE_PGRS37 | C.A240G | P.E80E | Synonymous SNV | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 2622150 | 2622150 | G | A | DnaG | C.C303T | P.T101T | Synonymous SNV | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 2626056 | 2626056 | A | G | EsxO | C.T117C | P.G39G | Synonymous SNV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| NC_000962.3 | 2626062 | 2626062 | G | A | EsxO | C.C111T | P.A37A | Synonymous SNV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| NC_000962.3 | 2626077 | 2626077 | A | C | EsxO | C.T96G | P.V32V | Synonymous SNV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| NC_000962.3 | 2626083 | 2626083 | G | C | EsxO | C.C90G | P.A30A | Synonymous SNV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| NC_000962.3 | 2626095 | 2626095 | C | A | EsxO | C.G78T | P.A26A | Synonymous SNV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NC_000962.3 | 2626103 | 2626103 | G | A | EsxO | C.C70T | P.L24L | Synonymous SNV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| NC_000962.3 | 2626105 | 2626105 | A | G | EsxO | C.T68C | P.L23S | Missense | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| NC_000962.3 | 2626106 | 2626106 | A | C | EsxO | C.T67G | P.L23V | Missense | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| NC_000962.3 | 2626108 | 2626108 | C | G | EsxO | C.G65C | P.G22A | Missense | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| NC_000962.3 | 2626110 | 2626110 | G | C | EsxO | C.C63G | P.A21A | Synonymous SNV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| NC_000962.3 | 2626131 | 2626131 | G | C | EsxO | C.C42G | P.G14G | Synonymous SNV | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| NC_000962.3 | 2626140 | 2626140 | G | A | EsxO | C.C33T | P.D11D | Synonymous SNV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| NC_000962.3 | 2626143 | 2626143 | G | C | EsxO | C.C30G | P.V10V | Synonymous SNV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| NC_000962.3 | 2626161 | 2626161 | G | A | EsxO | C.C12T | P.N4N | Synonymous SNV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| NC_000962.3 | 2626167 | 2626167 | G | C | EsxO | C.C6G | P.T2T | Synonymous SNV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| NC_000962.3 | 2626244 | 2626244 | G | C | EsxO | C.C276G | P.S92S | Synonymous SNV | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 2626247 | 2626247 | G | C | EsxO | C.C273G | P.A91A | Synonymous SNV | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 2626271 | 2626271 | G | A | EsxP | C.C249T | P.N83N | Synonymous SNV | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 2626274 | 2626274 | G | C | EsxP | C.C246G | P.A82A | Synonymous SNV | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 2626280 | 2626280 | G | A | EsxP | C.C240T | P.R80R | Synonymous SNV | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 2626283 | 2626283 | A | G | EsxP | C.T237C | P.V79V | Synonymous SNV | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 2626288 | 2626288 | G | A | EsxP | C.C232T | P.L78L | Synonymous SNV | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 2626295 | 2626295 | A | C | EsxP | C.T225G | P.R75R | Synonymous SNV | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 2626304 | 2626304 | G | A | EsxP | C.C216T | P.H72H | Synonymous SNV | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 2626319 | 2626319 | G | A | EsxP | C.C201T | P.I67I | Synonymous SNV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 |
| NC_000962.3 | 2626322 | 2626322 | G | A | EsxP | C.C198T | P.N66N | Synonymous SNV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 |
| NC_000962.3 | 2626328 | 2626328 | A | G | EsxP | C.T192C | P.F64F | Synonymous SNV | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 |
| NC_000962.3 | 2627314 | 2627314 | T | C | PlcC | C.A1385G | P.Q462R | Missense | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| NC_000962.3 | 2676058 | 2676058 | C | T | MbtA | C.C123T | P.T41T | Synonymous SNV | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 2751804 | 2751804 | C | T | RpfE | C.G377A | P.R126Q | Missense | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 2809621 | 2809621 | T | C | bkdC | C.A319G | P.T107A | Missense | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 3054724 | 3054724 | A | G | PE_PGRS47 | C.A811G | P.S271G | Missense | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 3139725 | 3139725 | C | T | UgpB | C.G760A | P.A254T | Missense | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 3284585 | 3284585 | - | A | FadD28 | C.1251dupA | P.K417fs | Frameshift insertion | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 3293439 | 3293439 | C | T | Pks1 | C.G2915A | P.G972D | Missense | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 3501846 | 3501846 | G | A | PPE51 | C.G53A | P.G18D | Missense | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| NC_000962.3 | 3520295 | 3520295 | C | T | NuoH | C.C1014T | P.R338R | Synonymous SNV | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 3580639 | 3580639 | T | - | LipV | C.2delT | P.L1fs | Frameshift deletion | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 |
| NC_000962.3 | 3618969 | 3618969 | C | T | SecA1 | C.G1563A | P.E521E | Synonymous SNV | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 3709355 | 3709357 | GTT | - | MoaX | C.358_360del | P.120_120del | In-frameshift deletion | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 3718357 | 3718357 | C | T | SugI | C.C1268T | P.P423L | Missense | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 3732370 | 3732370 | A | G | PPE54 | C.T4566C | P.N1522N | Synonymous SNV | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 3732719 | 3732719 | C | G | PPE54 | C.G4217C | P.G1406A | Missense | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 3743965 | 3743965 | C | G | PPE55 | C.G9220C | P.G3074R | Missense | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 3862336 | 3862336 | C | T | RplM | C.G55A | P.D19N | Missense | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 3934699 | 3934699 | G | A | PE_PGRS54 | C.G3695A | P.S1232N | Missense | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| NC_000962.3 | 3934733 | 3934733 | G | C | PE_PGRS54 | C.G3729C | P.G1243G | Synonymous SNV | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 |

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| NC_000962.3 | 3934734 | 3934734 | G | A | PE_PGRS54 | C.G3730A | P.A1244T | Missense | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 |
| NC_000962.3 | 3934878 | 3934878 | G | A | PE_PGRS54 | C.G3874A | P.D1292N | Missense | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| NC_000962.3 | 3934879 | 3934879 | A | T | PE_PGRS54 | C.A3875T | P.D1292V | Missense | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| NC_000962.3 | 3940802 | 3940802 | A | G | PE_PGRS55 | C.A1186G | P.N396D | Missense | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 3949000 | 3949000 | G | C | PE_PGRS57 | C.G3207C | P.G1069G | Synonymous SNV | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 |
| NC_000962.3 | 3949001 | 3949001 | G | A | PE_PGRS57 | C.G3208A | P.A1070T | Missense | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 |
| NC_000962.3 | 4014438 | 4014438 | C | G | FadE34 | C.G1775C | P.G592A | Missense | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NC_000962.3 | 4116901 | 4116901 | A | G | Crp | C.A424G | P.I142V | Missense | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| NC_000962.3 | 4139700 | 4139700 | G | A | GlpK | C.C56T | P.A19V | Missense | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| NC_000962.3 | 4400663 | 4400663 | C | - | SigM | C.478delC | P.R160fs | Frameshift deletion | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 |