

Supplementary Information

1. Unified variant pipeline (UVP)

The UVP incorporates a suite of the most current bioinformatics analysis tools. It is designed to be hosted on a UNIX computing platform and is scripted in Python programming language. A detailed schematic of the pipeline is depicted in Figure S1.

In broad outline, there are four major steps implemented in the pipeline:

1. Input data validation & QC
2. Sequence reads mapping & refinement
3. Variant calling
4. Functional annotation & lineage analysis

For each of these steps, one or more bioinformatic tools are employed to perform analysis on the data to generate an output file which is subsequently passed on to the next analysis step of the pipeline.

1.1 Input data validation & QC

Current Input data for the pipeline will be in fastq format from illumina NGS technology platform. There is a need to ascertain that data accepted in the pipeline meets this basic requirement before it is analyzed.

The file format is validated using the FastQValidator [1] Version 1.0.5. FastQValidator is a C++ program that checks that the four mandatory lines found in fastq format files are present in the input data file. It also ensures these lines are formatted correctly with unique read IDs and that there is similarity in the length of the raw sequence line and the quality string line.

The thresholds set for all the QC steps were adopted by consensus agreement at the ReSeqTB Expert panel meeting in Geneva Switzerland on the 27th and 28th of July 2015. The average genome coverage is estimated using a custom script embedded in the pipeline script. The QC threshold set for the acceptable minimum average genome coverage is 10X. This implies that for an input fastq file to be acceptable, there should be an average of 10 sequence reads overlapping each nucleotide base position of the TB genome. The need for this minimum level of coverage is to establish prior confidence in the base calls from the sequencing platform before other downstream analysis.

The validated input data is assessed for species specificity using Kraken [3] Version 0.10.5. Kraken is a tool developed in C++ and Perl programming languages and is used to assign

taxonomic labels to short sequence reads. Kraken details the percentage of sequence reads within each sample, that maps to particular taxonomic groupings. We set a threshold of 90% as the minimum acceptable proportion of sequence reads in the input data that has to map to the MTBC group of Kraken's database. This threshold was affirmed after running more than 1000 isolates through the pipeline and the minimum observed average proportion of reads mapping to MTBC for actual MTBC isolates never falling below 90%.

If the file passes the Kraken threshold, the input fastq data is trimmed using the Prinseq-lite.pl [2] Version 0.20.4 tool. The QC threshold is set at Q20, sequence reads with an average sequence quality of less than Q20 are trimmed. This implies a 99% accuracy in the average base calls for each acceptable sequence read within each sequencing project.

1.2 Sequence reads mapping & refinement

The MTB genome H37Rv (NCBI Accession: NC_000962.3) is the reference genome used for mapping sequencing reads in the pipeline. The Burrows Wheeler Alignment (BWA) [4] Version 0.7.12 was used to map the reads to the reference genome. BWA is a C++ bioinformatics tool designed for mapping relatively low divergent sequences against increasingly large reference genomes. The Maximal Exact Matches (MEM) algorithm of BWA which is fast and suitable for the long Illumina sequence reads was implemented for the mapping in the pipeline using default settings.

The quality of the mapping is reported using the Mapping quality QC Tool, Qualimap [5] Version 2.1.1. Qualimap outputs detailed reports on the sequence alignment using the 'bamqc' option of the tool. The report includes the mapping quality of the alignments, the genome coverage, nucleotide distribution and the mean and median insert size. Qualimap also outputs visualization files that could highlight any problems with the mapping process.

Next, the mapped data is refined to eliminate false positive variant calls using the bioinformatics tool suite Picard Tools [6] Version 1.134. This is achieved using the 'MarkDuplicates' and the 'AddOrReplaceReadGroups' options of the tool. Subsequently, the reads are also re-aligned proximal to insertions and deletions (Indels) to compensate for the stricter calling of gaps by mappers. The Genome Analysis Toolkit (GATK) [7] Version 3.4.0 is applied to make this re-alignment, using the 'IndelRealigner' option within GATK. Both Picard Tools and GATK are implemented in the JAVA programming platform.

1.3 Variant Calling

Variants are called using Samtools [9] Version 1.2, a C++ program that includes a suite of bioinformatics tools used to process and analyze NGS data. The 'mpileup' command is used to generate variant and genotype calls from the preprocessed genomic data. The QC Thresholds supplied to this command include: Q20 Minimum base quality, Q20 Minimum mapping quality, 10X Minimum Read depth and a maximum 3 SNPs within any 10 nucleotide base region for SNP

clusters. This implies a minimum 99% accuracy in calling genetic variants, at the minimum of 10 sequence read coverage for each base position. This threshold also eliminates SNPs that occur in clusters within a short span of nucleotide bases, which are often spurious and artefacts of the sequencing platform. The output is represented in a variant call format (VCF) file, which is filtered to remove repeat and problematic loci in the *M. tuberculosis* genome (see Appendix 1). The VCF file contains information on all the variant positions, the reference and alternate base positions as well as details on the quality and genotyping information for each sample.

1.4 Functional Annotation & Lineage Analysis

The VCF file serves as an input file for the Functional Annotation Tool SnpEff [10] Version 4.1. SnpEff is implemented in the JAVA programming language and annotates each variant position in the VCF file using the same reference genome that was used to map the sequence reads in the first place. The H37Rv reference annotation file used for functional annotation was built into the application's data base using the GenBank file for NC_000962.3 and following the developer's instruction for creating custom databases within the tool.

The custom python script `parse_annotation.py` reformats SnpEff output to a format that is easily mapped to the ReSeqTB database.

The custom python script `lineage_parser.py` performs a lineage analysis, to determine the lineage and sub-lineage of each analyzed strain. The informative SNPs which this script uses to make the lineage inferences is based on a set of 62 diagnostic SNPs defined by Coll *et al* [8].

References

1. FastqValidator – Genome Analysis
[<http://genome.sph.umich.edu/wiki/FastQValidator>]
2. Schmieder R and Edwards R: Quality control and preprocessing of metagenomic datasets. *Bioinformatics* 2011, 27:863-864
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4. Li H, Aligning Sequence Reads, Clone Sequences and Assembly Contigs with BWA-MEM. arXiv e-print, 2013
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6. Picard Tools – [<http://broadinstitute.github.io/picard/>]
7. A framework for variation discovery and genotyping using next-generation DNA sequencing data DePristo M, Banks E, Poplin R, Garimella K, Maguire J, Hartl C, Philippakis A, del Angel G, Rivas MA, Hanna M, McKenna A, Fennell T, Kernytsky A, Sivachenko A, Cibulskis K, Gabriel S, Altshuler D, Daly M, 2011 *NATURE GENETICS* 43:491-498
8. Coll F, McNerney R, Guerra-Assuncao J, Glynn J, Perdigao J, Viveiros M, Portugal I, Pain A, Martin N, Clark T. A robust SNP barcode for typing *Mycobacterium tuberculosis* complex strains; *Nature Communications* 2014 Sep 1, 5: 4814
9. Li H A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics*. 2011 Nov 1; 27(21):2987-93. Epub 2011 Sep 8.
10. Cingolani P, Platts A, Wang le L, Coon M, Nguyen T, Wang L, Land SJ, Lu X, Ruden DM A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3. *Fly* (Austin). 2012 Apr-Jun;6(2):80-92

Appendix

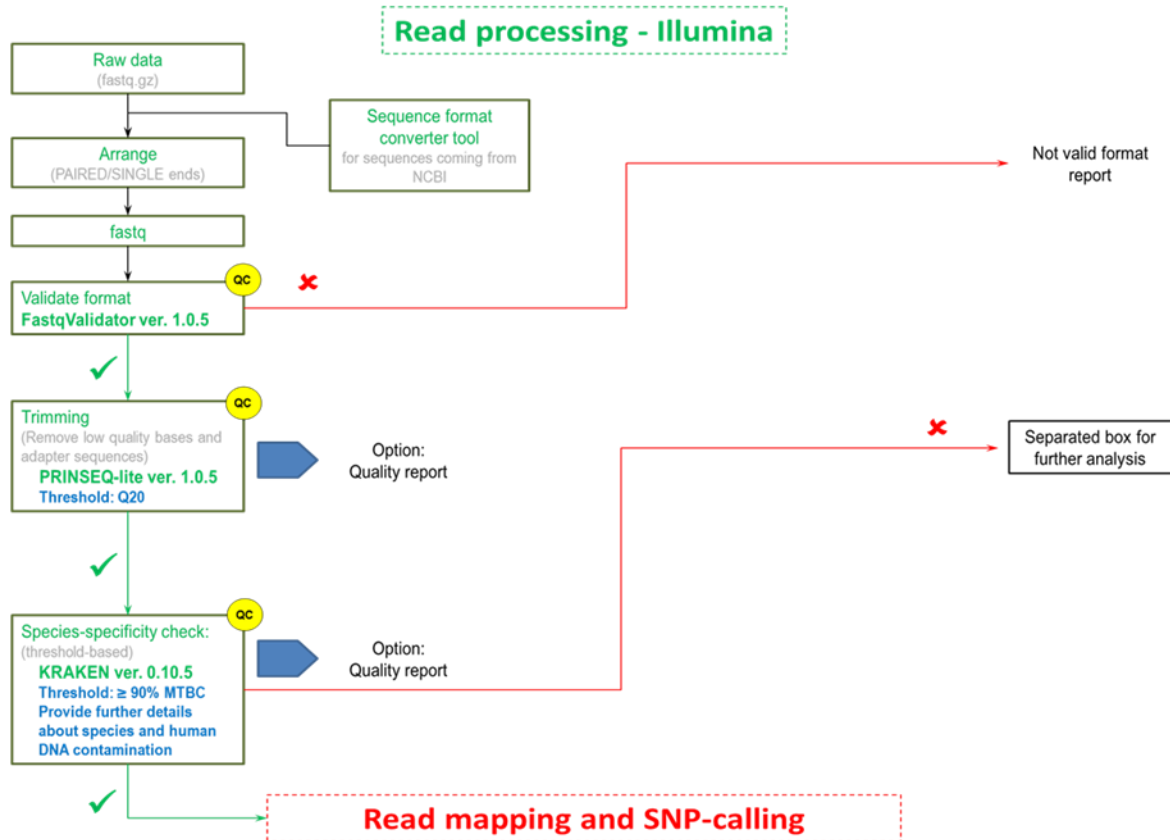


Figure S1A. Unified variant pipeline QC and read processing stage

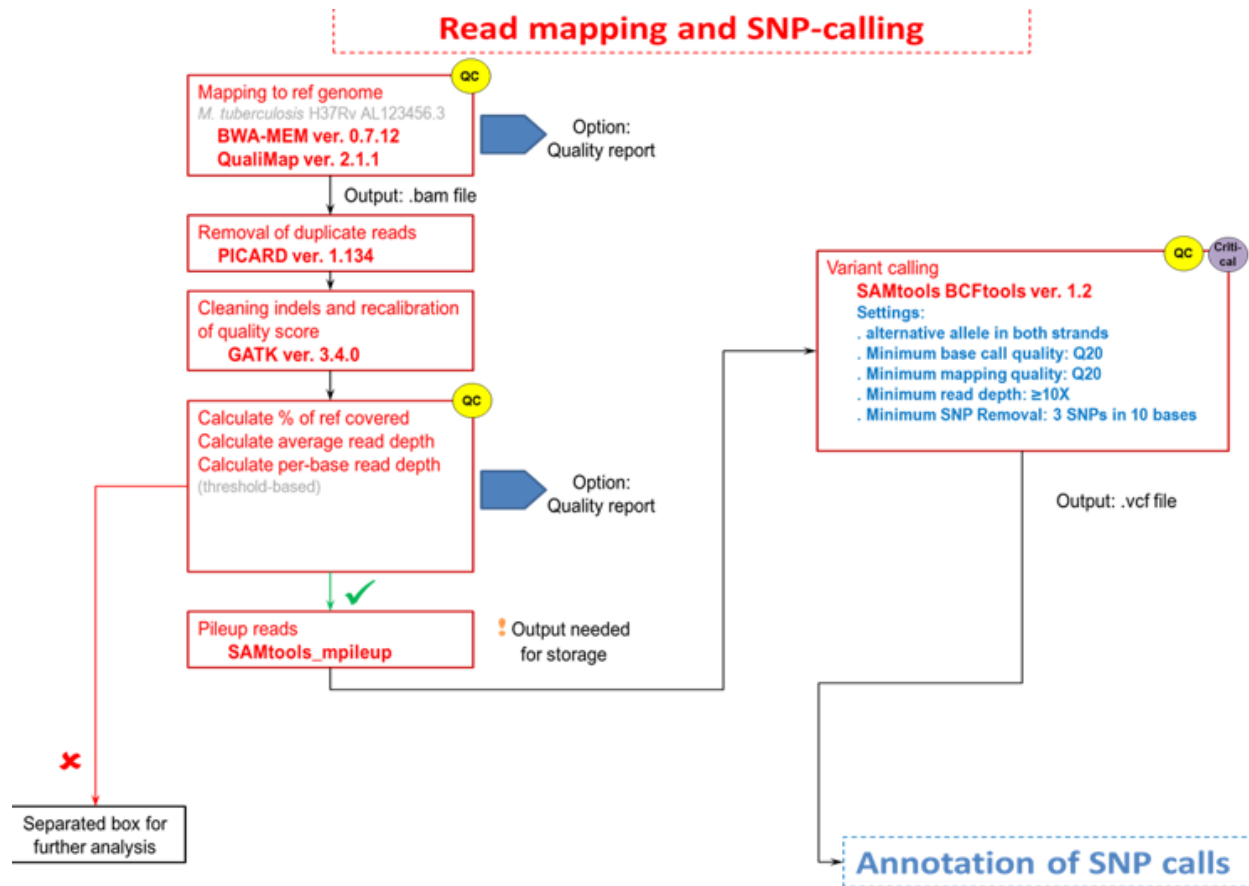


Figure S1B. Unified variant pipeline mapping and variant calling stage

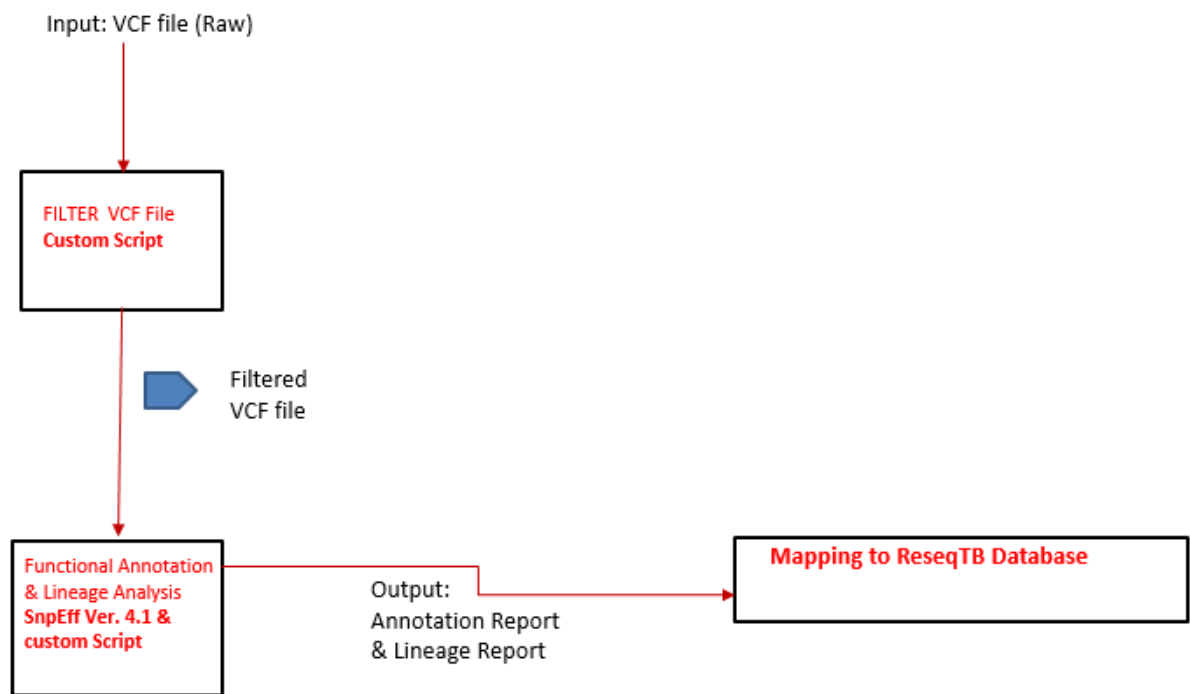


Figure S1C. Unified variant pipeline functional annotation and lineage classification stage

Table S1 List of Excluded loci

Chrom	ChromStart	ChromEnd	locus tag	Comment
NC_000962	33582	33794	Rv0031	remnant of A transposase
NC_000962	103710	104663	Rv0094c	50bp_duplicated
NC_000962	104805	105215	Rv0095c	50bp_duplicated
NC_000962	105324	106715	Rv0096	PPE family protein
NC_000962	131382	132872	Rv0109	PE-PGRS family protein
NC_000962	149533	150996	Rv0124	PE-PGRS family protein
NC_000962	177543	179309	Rv0151c	PE family protein
NC_000962	179319	180896	Rv0152c	PE family protein
NC_000962	187433	188839	Rv0159c	PE family protein
NC_000962	188931	190439	Rv0160c	PE family protein
NC_000962	307877	309547	Rv0256c	PPE family protein
NC_000962	309699	310073	Rv0257	50bp_duplicated
NC_000962	332708	333136	Rv0277c	50bp_duplicated
NC_000962	333437	336310	Rv0278c	PE-PGRS family protein
NC_000962	336560	339073	Rv0279c	PE-PGRS family protein
NC_000962	339364	340974	Rv0280	PPE family protein
NC_000962	349624	349932	Rv0285	PE family protein
NC_000962	349935	351476	Rv0286	PPE family protein
NC_000962	361334	363109	Rv0297	PE-PGRS family protein
NC_000962	366150	372764	Rv0304c	PPE family protein
NC_000962	372820	375711	Rv0305c	PPE family protein
NC_000962	399535	400050	Rv0335c	PE family protein
NC_000962	400192	401703	Rv0336	50bp_duplicated
NC_000962	423639	424019	Rv0353	50bp_duplicated
NC_000962	424269	424694	Rv0354c	PPE family protein
NC_000962	424777	434679	Rv0355c	PPE family protein
NC_000962	466672	467406	Rv0387c	PPE family protein
NC_000962	467459	468001	Rv0388c	PPE family protein
NC_000962	472781	474106	Rv0393	50bp_duplicated
NC_000962	475816	476184	Rv0397	50bp_duplicated
NC_000962	530751	532214	Rv0442c	PPE family protein
NC_000962	543174	544730	Rv0453	PPE family protein
NC_000962	576787	577338	Rv0487	50bp_duplicated
NC_000962	579349	580581	Rv0490	50bp_duplicated
NC_000962	606551	608062	Rv0515	50bp_duplicated
NC_000962	622793	624577	Rv0532	PE-PGRS family protein
NC_000962	630040	631686	Rv0538	50bp_duplicated
NC_000962	671996	675916	Rv0578c	PE-PGRS family protein
NC_000962	701406	702014	Rv0605	repeat region
NC_000962	701406	702014	Rv0605	resolvase
NC_000962	831776	832303	Rv0740	50bp_duplicated
NC_000962	832534	832848	Rv0741	transposase

NC_000962	832981	833508	Rv0742	PE-PGRS family protein
NC_000962	835701	838052	Rv0746	PE-PGRS family protein
NC_000962	838451	840856	Rv0747	PE-PGRS family protein
NC_000962	842033	842278	Rv0750	50bp_duplicated
NC_000962	846159	847913	Rv0754	PE-PGRS family protein
NC_000962	850527	850342	Rv0755A	transposase
NC_000962	848103	850040	Rv0755c	PPE family protein
NC_000962	889072	889398	Rv0795	transposase IS6110
NC_000962	889347	890333	Rv0796	transposase IS6110
NC_000962	890388	891482	Rv0797	50bp_duplicated
NC_000962	908181	908483	Rv0814c	50bp_duplicated
NC_000962	916477	917646	Rv0823c	50bp_duplicated
NC_000962	921575	921865	Rv0829	50bp_duplicated
NC_000962	924951	925364	Rv0832	PE-PGRS family protein
NC_000962	925361	927610	Rv0833	PE-PGRS family protein
NC_000962	927837	930485	Rv0834c	PE-PGRS family protein
NC_000962	947312	947644	Rv0850	transposase
NC_000962	964312	965535	Rv0867c	50bp_duplicated
NC_000962	968424	970244	Rv0872c	PE-PGRS family protein
NC_000962	976872	978203	Rv0878c	PPE family protein
NC_000962	1020058	1021329	Rv0915c	PPE family protein
NC_000962	1021344	1021643	Rv0916c	PE family protein
NC_000962	1025497	1026816	Rv0920c	transposase
NC_000962	1027104	1027685	Rv0921	resolvase
NC_000962	1027685	1029337	Rv0922	transposase
NC_000962	1090373	1093144	Rv0977	PE-PGRS family protein
NC_000962	1093361	1094356	Rv0978c	PE-PGRS family protein
NC_000962	1095078	1096451	Rv0980c	PE-PGRS family protein
NC_000962	1158918	1159307	Rv1034c	transposase
NC_000962	1159375	1160061	Rv1035c	transposase
NC_000962	1160095	1160433	Rv1036c	truncated IS1560 transposase
NC_000962	1160544	1160828	Rv1037c	50bp_duplicated
NC_000962	1160855	1161151	Rv1038c	50bp_duplicated
NC_000962	1161297	1162472	Rv1039c	PPE family protein
NC_000962	1162549	1163376	Rv1040c	PE family protein
NC_000962	1164572	1165435	Rv1041c	IS like-2 transposase
NC_000962	1165092	1165499	Rv1042c	IS like-2 transposase
NC_000962	1169423	1170670	Rv1047	transposase
NC_000962	1188421	1190424	Rv1067c	PE-PGRS family protein
NC_000962	1190757	1192148	Rv1068c	PE-PGRS family protein
NC_000962	1211560	1213863	Rv1087	PE-PGRS family protein
NC_000962	1214513	1214947	Rv1088	PE family protein
NC_000962	1214769	1215131	Rv1089	PE family protein
NC_000962	1216469	1219030	Rv1091	PE-PGRS family protein
NC_000962	1251617	1252972	Rv1128c	repeat_region
NC_000962	1262272	1264128	Rv1135c	PPE family protein
NC_000962	1276300	1277748	Rv1148c	50bp_duplicated
NC_000962	1277893	1278300	Rv1149	transposase

NC_000962	1278269	1278820	Rv1150	Possible fragment of transposase
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NC_000962	2430159	2431145	Rv2167c	transposase
NC_000962	2431094	2431420	Rv2168c	transposase
NC_000962	2439282	2439947	Rv2177c	transposase
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NC_000962	2530836	2531897	Rv2258c	50bp_duplicated
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NC_000962	2600731	2601879	Rv2328	PE family protein
NC_000962	2617667	2618908	Rv2340c	PE-PGRS family protein
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NC_000962	2626223	2626519	Rv2347c	50bp_duplicated
NC_000962	2632923	2634098	Rv2352c	PPE family protein
NC_000962	2634528	2635592	Rv2353c	PPE family protein
NC_000962	2635628	2635954	Rv2354	transposase
NC_000962	2635903	2636889	Rv2355	transposase
NC_000962	2637688	2639535	Rv2356c	PPE family protein
NC_000962	2651753	2651938	Rv2371	PE-PGRS family protein
NC_000962	2692799	2693884	Rv2396	PE-PGRS family protein
NC_000962	2706017	2706736	Rv2408	PE family protein
NC_000962	2720776	2721777	Rv2424c	transposase
NC_000962	2727336	2727920	Rv2430c	PPE family protein
NC_000962	2727967	2728266	Rv2431c	PE family protein
NC_000962	2762531	2763175	Rv2460c	repeat_region
NC_000962	2763172	2763774	Rv2461c	repeat_region
NC_000962	2784657	2785643	Rv2479c	transposase
NC_000962	2785592	2785918	Rv2480c	transposase
NC_000962	2795301	2797385	Rv2487c	PE-PGRS family protein
NC_000962	2800846	2801145	Rv2489c	repeat_region
NC_000962	2801254	2806236	Rv2490c	PE-PGRS family protein
NC_000962	2828556	2829803	Rv2512c	IS1081 transposase
NC_000962	2835785	2837263	Rv2519	PE family protein
NC_000962	2866468	2867127	Rv2543	50bp_duplicated
NC_000962	2867124	2867786	Rv2544	50bp_duplicated
NC_000962	2921551	2923182	Rv2591	PE-PGRS family protein
NC_000962	2935046	2936788	Rv2608	PPE family protein
NC_000962	2943600	2944985	Rv2615c	PE-PGRS family protein
NC_000962	2960105	2962441	Rv2634c	PE-PGRS family protein

NC_000962	2972160	2972486	Rv2648	transposase IS6110
NC_000962	2972435	2973421	Rv2649	transposase IS6110
NC_000962	2973795	2975234	Rv2650c	phiRv2 prophage protein
NC_000962	2975242	2975775	Rv2651c	phiRv2 prophage protease
NC_000962	2975928	2976554	Rv2652c	phiRv2 prophage protein
NC_000962	2976586	2976909	Rv2653c	phiRv2 prophage protein
NC_000962	2976989	2977234	Rv2654c	phiRv2 prophage protein
NC_000962	2977231	2978658	Rv2655c	phiRv2 prophage protein
NC_000962	2978660	2979052	Rv2656c	phiRv2 prophage protein
NC_000962	2979049	2979309	Rv2657c	phiRv2 prophage protein
NC_000962	2979691	2980818	Rv2659c	phiRv2 prophage integrase
NC_000962	2982699	2982980	Rv2665	50bp_duplicated
NC_000962	2983071	2983874	Rv2666	truncated IS1081 transposase
NC_000962	2989291	2990592	Rv2673	50bp_duplicated
NC_000962	2996105	2996737	Rv2680	50bp_duplicated
NC_000962	3005845	3007062	Rv2689c	50bp_duplicated
NC_000962	3007236	3009209	Rv2690c	repeat_region
NC_000962	3053914	3055491	Rv2741	PE-PGRS family protein
NC_000962	3076894	3078078	Rv2768c	PPE family protein
NC_000962	3078158	3078985	Rv2769c	PE family protein
NC_000962	3079309	3080457	Rv2770c	PPE family protein
NC_000962	3082352	3082756	Rv2774c	50bp_duplicated
NC_000962	3100202	3101581	Rv2791c	transposase
NC_000962	3101581	3102162	Rv2792c	resolvase
NC_000962	3112867	3113271	Rv2805	50bp_duplicated
NC_000962	3113658	3114812	Rv2807	50bp_duplicated
NC_000962	3115741	3116142	Rv2810c	transposase
NC_000962	3116818	3118227	Rv2812	transposase
NC_000962	3120566	3121552	Rv2814c	transposase
NC_000962	3121501	3121827	Rv2815c	transposase
NC_000962	3132892	3133539	Rv2825c	50bp_duplicated
NC_000962	3135788	3136333	Rv2828c	50bp_duplicated
NC_000962	3162268	3164115	Rv2853	PE-PGRS family protein
NC_000962	3170720	3171646	Rv2859c	50bp_duplicated
NC_000962	3191644	3192201	Rv2882c	50bp_duplicated
NC_000962	3194166	3195548	Rv2885c	transposase
NC_000962	3195545	3196432	Rv2886c	resolvase
NC_000962	3200794	3202020	Rv2892c	PPE family protein
NC_000962	3245445	3251075	Rv2931	50bp_duplicated
NC_000962	3251072	3255688	Rv2932	50bp_duplicated
NC_000962	3288464	3289705	Rv2943	IS1533 transposase
NC_000962	3289705	3290235	Rv2943A	transposase
NC_000962	3289790	3290506	Rv2944	IS1533 transposase
NC_000962	3313283	3313672	Rv2961	transposase
NC_000962	3332787	3333788	Rv2977c	50bp_duplicated
NC_000962	3333785	3335164	Rv2978c	transposase
NC_000962	3335164	3335748	Rv2979c	resolvase
NC_000962	3335960	3336505	Rv2980	50bp_duplicated

NC_000962	3378415	3378329	Rv3018A	PE family protein
NC_000962	3376939	3378243	Rv3018c	PPE family protein
NC_000962	3379376	3380452	Rv3021c	PPE family protein
NC_000962	3380993	3380679	Rv3022A	PE family protein
NC_000962	3380440	3380682	Rv3022c	PPE family protein
NC_000962	3381375	3382622	Rv3023c	transposase
NC_000962	3465778	3467091	Rv3097c	PE-PGRS family protein
NC_000962	3481451	3482698	Rv3115	transposase
NC_000962	3490476	3491651	Rv3125c	PPE family protein
NC_000962	3501334	3501732	Rv3135	PPE family protein
NC_000962	3501794	3502936	Rv3136	PPE family protein
NC_000962	3510088	3511317	Rv3144c	PPE family protein
NC_000962	3527391	3529163	Rv3159c	PPE family protein
NC_000962	3551281	3551607	Rv3184	transposase
NC_000962	3551556	3552542	Rv3185	transposase
NC_000962	3552764	3553090	Rv3186	transposase
NC_000962	3553039	3554025	Rv3187	transposase
NC_000962	3557311	3558345	Rv3191c	transposase
NC_000962	3663689	3664222	Rv3281	50bp_duplicated
NC_000962	3710433	3710759	Rv3325	transposase
NC_000962	3710708	3711694	Rv3326	transposase
NC_000962	3711749	3713461	Rv3327	transposase
NC_000962	3729364	3736935	Rv3343c	PPE family protein
NC_000962	3736984	3738438	Rv3344c	PE-PGRS family protein
NC_000962	3738158	3742774	Rv3345c	PE-PGRS family protein
NC_000962	3743198	3743455	Rv3346c	50bp_duplicated
NC_000962	3743711	3753184	Rv3347c	PPE family protein
NC_000962	3753765	3754256	Rv3348	transposase
NC_000962	3754293	3755033	Rv3349c	transposase
NC_000962	3755952	3767102	Rv3350c	PPE family protein
NC_000962	3769514	3769807	Rv3355c	50bp_duplicated
NC_000962	3778568	3780334	Rv3367	PE-PGRS family protein
NC_000962	3795100	3796086	Rv3380c	transposase
NC_000962	3796035	3796361	Rv3381c	transposase
NC_000962	3800092	3800796	Rv3386	transposase
NC_000962	3800786	3801463	Rv3387	transposase
NC_000962	3801653	3803848	Rv3388	PE-PGRS family protein
NC_000962	3841714	3842076	Rv3424c	50bp_duplicated
NC_000962	3842239	3842769	Rv3425	PPE family protein
NC_000962	3843036	3843734	Rv3426	PPE family protein
NC_000962	3843885	3844640	Rv3427c	transposase
NC_000962	3844738	3845970	Rv3428c	transposase
NC_000962	3847165	3847701	Rv3429	PPE family protein
NC_000962	3847642	3848805	Rv3430c	transposase
NC_000962	3849294	3850139	Rv3431c	repeat_region
NC_000962	3883525	3884193	Rv3466	repeat_region
NC_000962	3883964	3884917	Rv3467	repeat_region
NC_000962	3890830	3891156	Rv3474	transposase IS6110

NC_000962	3891105	3892091	Rv3475	transposase IS6110
NC_000962	3894093	3894389	Rv3477	PE family protein
NC_000962	3894426	3895607	Rv3478	PE family protein
NC_000962	3926569	3930714	Rv3507	PE-PGRS family protein
NC_000962	3931005	3936710	Rv3508	PE-PGRS family protein
NC_000962	3939617	3941761	Rv3511	PE-PGRS family protein
NC_000962	3941724	3944963	Rv3512	PE-PGRS family protein
NC_000962	3945092	3945748	Rv3513c	50bp_duplicated
NC_000962	3945794	3950263	Rv3514	PE-PGRS family protein
NC_000962	3950824	3952470	Rv3515c	50bp_duplicated
NC_000962	3969343	3970563	Rv3532	PPE family protein
NC_000962	3970705	3972453	Rv3533c	PPE family protein
NC_000962	3978059	3979498	Rv3539	PPE family protein
NC_000962	3997980	3999638	Rv3558	PPE family protein
NC_000962	4031404	4033158	Rv3590c	PE-PGRS family protein
NC_000962	4036731	4038050	Rv3595c	PE-PGRS family protein
NC_000962	4052950	4053603	Rv3611	50bp_duplicated
NC_000962	4059984	4060268	Rv3619c	50bp_duplicated
NC_000962	4060295	4060591	Rv3620c	50bp_duplicated
NC_000962	4060648	4061889	Rv3621c	PPE family protein
NC_000962	4061899	4062198	Rv3622c	PE family protein
NC_000962	4075752	4076099	Rv3636	transposase
NC_000962	4076484	4076984	Rv3637	transposase
NC_000962	4076984	4077730	Rv3638	transposase
NC_000962	4077884	4078450	Rv3639c	50bp_duplicated
NC_000962	4078520	4079749	Rv3640c	transposase
NC_000962	4091233	4091517	Rv3650	PE family protein
NC_000962	4119795	4120955	Rv3680	50bp_duplicated
NC_000962	4153740	4155674	Rv3710	50bp_duplicated
NC_000962	4189285	4190232	Rv3738c	PPE family protein
NC_000962	4190284	4190517	Rv3739c	PPE family protein
NC_000962	4196171	4196506	Rv3746c	PE family protein
NC_000962	4252993	4254327	Rv3798	transposase
NC_000962	4276571	4278085	Rv3812	PE-PGRS family protein
NC_000962	4299812	4301566	Rv3826	50bp_duplicated
NC_000962	4301563	4302789	Rv3827c	transposase
NC_000962	4302786	4303397	Rv3828c	resolvase
NC_000962	4318775	4319266	Rv3844	transposase
NC_000962	4351075	4352181	Rv3873	PPE family protein
NC_000962	4353010	4355010	Rv3876	50bp_duplicated
NC_000962	4374484	4375683	Rv3892c	PPE family protein
NC_000962	4375762	4375995	Rv3893c	PE family protein
NC_000962	1306002	1306201	IG1195_Rv1174c-Rv1175c	
NC_000962	154130	154231	IG127_Rv0126-Rv0127	
NC_000962	1907321	1907593	IG1711_Rv1682-Rv1683	
NC_000962	23182	23269	IG18_Rv0018c-Rv0019c	
NC_000962	3318816	3318900	IG3012_Rv2965c-Rv2966c	
NC_000962	3319468	3319662	IG3013_Rv2966c-Rv2967c	

NC_000962	616832	616845	IG533_Rv0525-Rv0526
NC_000962	642812	642888	IG559_Rv0551c-Rv0552
NC_000962	706930	706947	IG622_Rv0612-Rv0613c
NC_000962	80194	80623	IG71_Rv0071-Rv0072
NC_000962	863159	863255	IG784_Rv0769-Rv0770
NC_000962	960152	960341	IG877_Rv0861c-Rv0862c

Table S2: List of high confidence resistance mutation loci

Chrom	chromStart	chromEnd	locus tag
NC_000962	5240	7267	Rv0005
NC_000962	7302	9818	Rv0006
NC_000962	490783	491793	Rv0407
NC_000962	575348	576790	Rv0486
NC_000962	731930	732406	Rv0635
NC_000962	759807	763325	Rv0667
NC_000962	775586	778480	Rv0676c
NC_000962	778477	778905	Rv0677c
NC_000962	778990	779487	Rv0678
NC_000962	781560	781934	Rv0682
NC_000962	800809	801462	Rv0701
NC_000962	1302931	1305501	Rv1137
NC_000962	1406081	1407340	Rv1258c
NC_000962	1461045	1461290	Rv1305
NC_000962	1471846	1473382	rrs
NC_000962	1473658	1476495	rrl
NC_000962	1673339	1673439	fabG11/inhA promoter
NC_000962	1673440	1674183	Rv1483
NC_000962	1674202	1675011	Rv1484
NC_000962	1917940	1918746	Rv1694
NC_000962	1929786	1931456	Rv1704c
NC_000962	2153889	2156111	Rv1908c
NC_000962	2156149	2156592	Rv1909c
NC_000962	2231680	2232219	Rv1988
NC_000962	2288681	2289241	Rv2043c
NC_000962	2289242	2289281	pncA promoter
NC_000962	2714124	2715332	Rv2416c
NC_000962	2715333	2715383	eis promoter
NC_000962	2726193	2726780	Rv2428
NC_000962	2725992	2726912	ahpC promoter
NC_000962	2746135	2747598	Rv2447c
NC_000962	2986839	2987615	Rv2671
NC_000962	3073130	3073609	Rv2763c
NC_000962	3073680	3074471	Rv2764c
NC_000962	3568401	3568679	Rv3197A
NC_000962	3568680	3569108	whiB7 promoter
NC_000962	3640543	3641538	Rv3261
NC_000962	3641535	3642881	Rv3262
NC_000962	3986844	3987299	Rv3547
NC_000962	4237932	4239863	Rv3792
NC_000962	4239863	4243147	Rv3793
NC_000962	4243233	4246517	Rv3794

NC_000962	4246514	4249810	Rv3795
NC_000962	4268925	4269833	Rv3806c
NC_000962	4326004	4327473	Rv3854c
NC_000962	4407528	4408202	Rv3919c