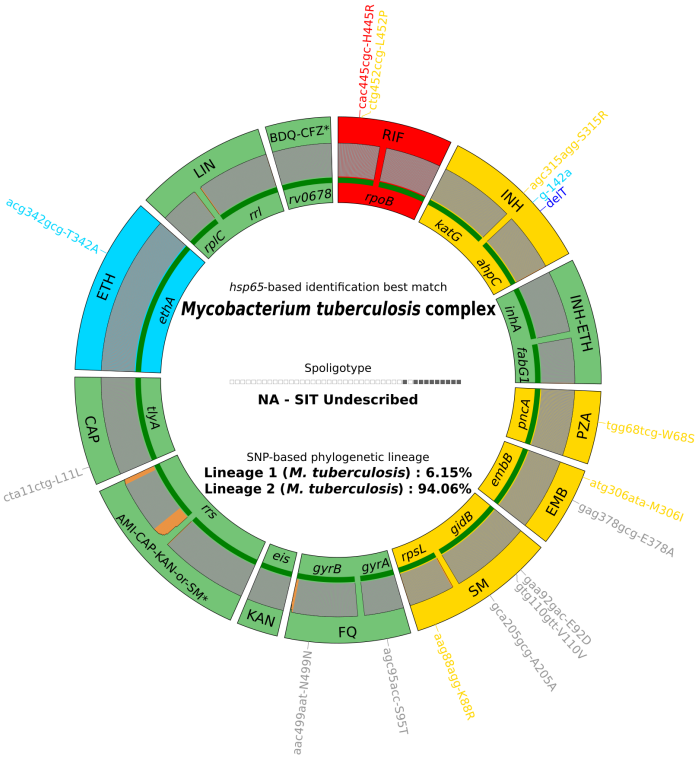
GenoScreen
Innovative Genomics

DEEPLEX® Myc-TB Report

SAMPLE ID: MIX

Date of submission	**May, 3 2022 14:31:18**
Analysis mode	**Deeplex Myc-TB V3.0 - Extended catalogue**
Quality	⊕⊕⊕
Experiment set	**Demo**
Sample source	
Validation	



Legend [1]

Sample controls and metrics[2]

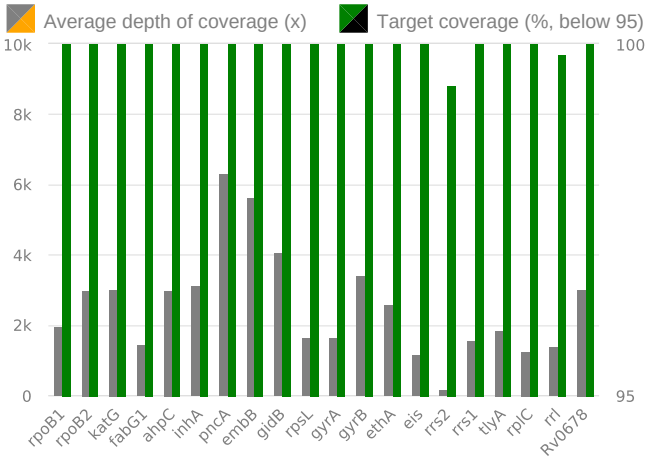
Sequencing Run:

Positive control*	**VALID**
Negative control*	**VALID**

Sample:

Internal control*	**VALID**
Composite reference coverage	**100.000%**
Median depth of coverage*	**3854x**

(min. rrs2: 161x; max. pncA: 6325x)



hsp65-based species identification

Av coverage depth (x)	Consensus length	% Identity	E-value	Best match
149.5	400.0	100.000	0.0	*Mycobacterium tuberculosis* complex

Drug resistance associated variants[3]

Gene	Genomic position	Codon change	% Variant	Dx-score	AA change	Drug*	Confidence	Resistance level	Reference
embB	4247431	atg306ata	5.970	36.00	M306I	EMB	Associated with resistance	Resistant	WHO 2021
katG	2155167	agc315agg	4.730	12.25	S315R	INH	n/a	Resistant	PUBMED
katG	2155167	agc315agg	4.730	12.25	S315R	INH	n/a	Resistant	PUBMED
pncA	2289039	tgg68tcg	5.020	104.00	W68S	PZA	n/a	Resistant	PUBMED
rpoB	761140	cac445cgc	94.770	625.00	H445R	RIF	Associated with resistance	Resistant	WHO 2021
rpoB	761161	ctg452ccg	5.080	34.25	L452P	RIF	Associated with resistance	Resistant	WHO 2021
rpsL	781822	aag88agg	5.430	29.50	K88R	STM	Associated with resistance	Resistant	WHO 2021

Uncharacterized and uncertain significance variants[3]

Uncharacterized variants designate sequence variants of as yet unknown association with drug resistance or drug susceptibility. Uncertain significance variants designate variants that could not be characterized yet as either drug resistant or drug susceptible according to the current WHO confidence grading classification.

Gene	Genomic position	Codon change	% Variant	Dx-score	AA change	Drug*	Category	Reference
ethA	4326450	acg342gcg	7.450	62.50	T342A	ETH	Uncertain significance	WHO 2021
ahpC	2726051	g-142a	6.010	8.25	n/a	n/a	Uncharacterised	n/a
ahpC	2726149	delT	94.630	0.00	deletion	n/a	Uncharacterised	n/a

Spoligotype

Octal code/ Binary code	Av. coverage depth (x)	SIT	SITVIT occurence	Clade
000000000013771	404.9	n/a	n/a	n/a
00000000000000000000000000000000010111111111				

SNP-based phylogenetic lineage

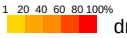
Lineage 1 (*M. tuberculosis*) : 6.15% Lineage 2 (*M. tuberculosis*) : 94.06%

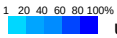
Potential mixed infection

Mixed infection is signaled by a phylogenetic variant detected at less than 97%, indicating the simultaneous presence of 1 strain harboring this variant present at this percentage and another strain sharing the same sequence as the reference at this position, present at approx. 100% minus this percentage.

Gene	Genomic position	Codon change	% Variant	AA change	Lineage	Reference
embB	4247646	gag378gcg	6.680	E378A	1, 5, 6, 7, M. bovis, M. canettii, M. caprae, M. microti, M. pinipedii	PUBMED
gidB	4407927	gaa92gac	94.060	E92D	2	PUBMED
gidB	4407873	gtg110gtt	5.620	V110V	1	PUBMED

Comment: the spoligotype should be inspected for potential biphasic distribution of coverage depth across spacers, also suggestive of mixed profile.

[1] Deeplex map: the circular map is divided in 13 “sectors” associated with 13 drugs or drug classes. Sectors are colour-coded as follows: 

drug resistance-associated variants (or indels) detected in gene target with percent subpopulation according to colouring; 

uncharacterized variants (or indels) detected in gene target with percent subpopulation according to colouring; no variant or indel detected in gene target or only variants or indels unrelated to drug resistance; suboptimal gene target coverage; nontuberculous *Mycobacterium* (NTM) identified.
Detected variants or indels are specified by codon, amino acid or nucleotide changes (deletions shown as *) on the outermost part, using the same colour codes as above for drug resistance-associated and uncharacterized categories, or in grey ( ) for variants or indels unrelated to drug resistance. Target reference sequences are coloured according to gene target coverage as follows: coverage >95%, coverage <95%. Limit of detection histogram for each target is colour-coded as follows: 1% ≤ limit of detection ≤ 3%, 3% < limit of detection ≤ 80%.

Resistotype: First-line drugs: rifampicin (RIF), isoniazid (INH), pyrazinamide (PZA), ethambutol (EMB). Second-line injectables: Streptomycin (SM), kanamycin (KAN), amikacin (AMI), capreomycin (CAP); fluoroquinolone class (FQ) includes levofloxacin (LEV), ofloxacin (OFX) and moxifloxacin (MOX); ethionamide (ETH); linezolid (LIN); bedaquiline (BDQ); clofazimine (CFZ). MDR-TB, multidrug-resistant TB defined as resistance to at least rifampicin and isoniazid. XDR-TB, extensively drug-resistant TB defined as MDR-TB plus resistance to at least one fluoroquinolone and a second-line injectable.

[2] Positive control: valid if identified as *Mycobacterium tuberculosis* complex with spoligotype SIT 482, lineage *Mycobacterium bovis* BCG and 9 expected mutations detected at >99% and no other mutations detected at >5%; Negative control: composite reference coverage breadth <40% (far below minimal coverage considered for identification and resistance prediction); Internal control: valid if average coverage depth >100x and coverage breadth >95% on internal control, and average coverage depth <100x over the other targets; Composite reference coverage: coverage breadth over the concatenated reference sequences associated with drug resistance; Median depth of coverage: median of average read depths among reference sequences associated with drug resistance; (min x, max x): minimal/maximal average coverage depth among the targets

[3] **Dx-score** designates the excess of coverage depth at the variant position, relative to the minimal coverage depth required to detect the observed percent of variant. Minimal value is 1. * Antibiotics highlighted in graded colours according to percent of drug resistance associated or uncharacterized variant detected (see colour grades above).

[4]OFX is not used for the treatment of *Mycobacterium tuberculosis* although potential resistance to OFX is tested, as part of the Fluoroquinolones

Disclaimer

Resistance is reported when a documented resistance-conferring mutation is detected in targets of interest*. **The absence of detected mutations does not exclude the possibility of resistance.** Low-frequency hereteroresistance below the limit of detection by sequencing may affect typing results. The interpretation provided is based on the current understanding of genotype-phenotype relationships. All results reference the *M. tuberculosis* mutation numbering system, which differs from *Escherichia coli* numbering system. *Resistance-conferring mutations as documented in the "Catalogue of mutations in *Mycobacterium tuberculosis* complex and their association with drug resistance" (WHO, 2021), the ReSeqTB Data Platform, Miotto P. et al. Eur Resp J 2017, Miotto P. et al. mBio 2014, PhyResSE (Feurriegel et al. J. Clin. Microbiol. 2015), Walker et al. Lancet. Infect. Dis. 2015. Additional expert rules, (as yet) not endorsed by WHO, are used for *tlyA* and *rv0678*, where all premature stop codon and frameshift-causing indels, or complete gene deletion are assumed to result in a loss of function phenotype and are consequently associated with drug resistance.