

The logo for Florida Health is positioned on the left side of the slide. It features the words "Florida" and "HEALTH" in white, stacked vertically. The background of the logo is a vertical strip showing a sunset or sunrise with orange and yellow clouds against a blue sky.

Implementation of a CLIA-compliant Targeted Next Generation Sequencing Assay for the Detection of *Mycobacterium tuberculosis* Antimicrobial Resistance Mutations

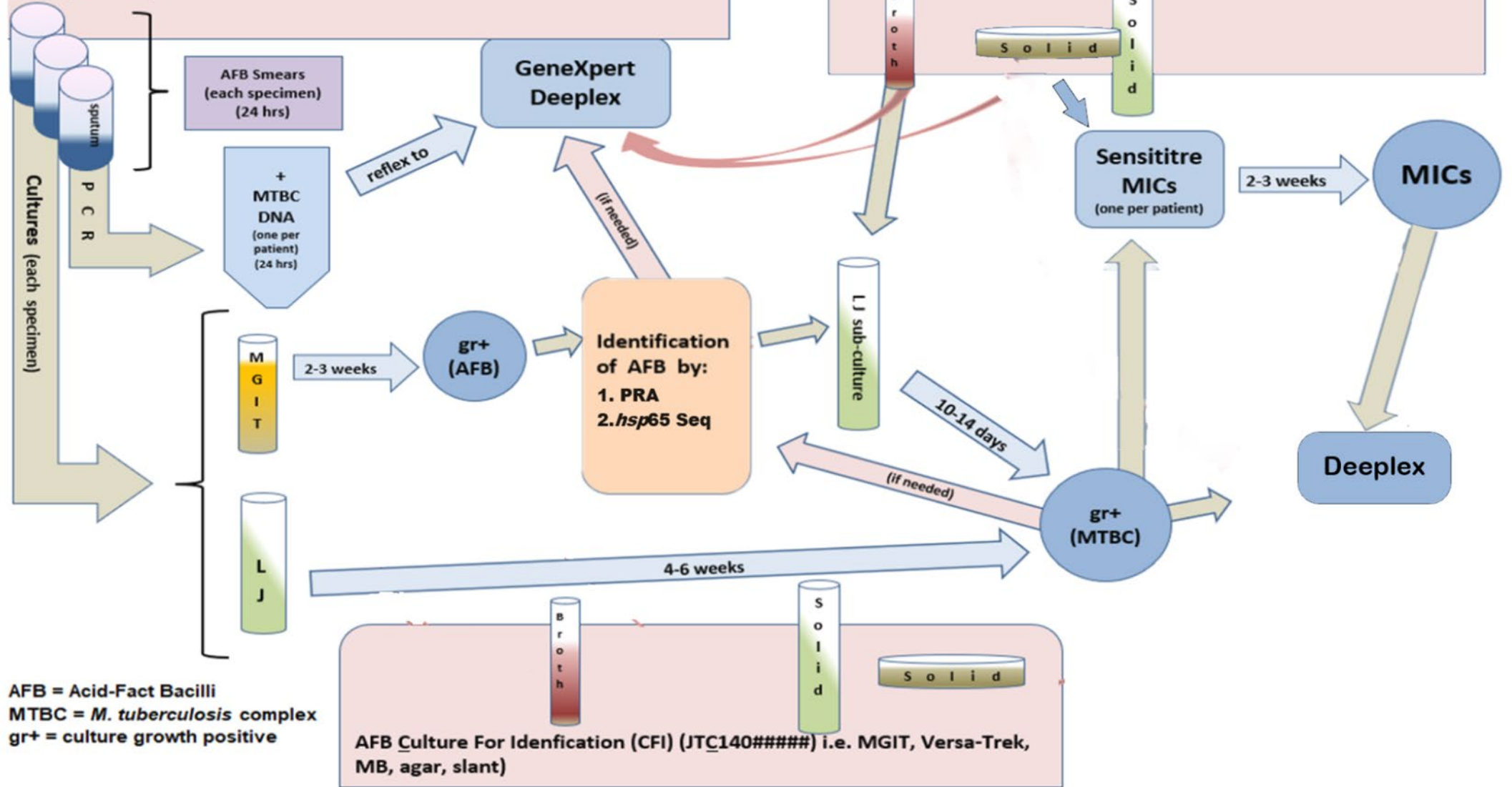
Next Generation Sequencing Applications in Infectious Disease Testing

March 26, 2025

Conflicts of Interest

None

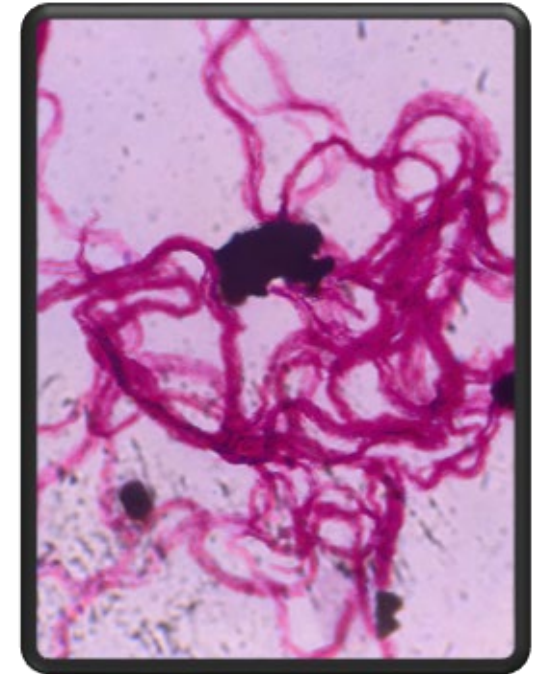
Patient Clinical Specimens (JTT140#####) i.e. sputum, BW, CSF, urine, tissue, etc.



Florida TB Laboratory Workload

Monday-Saturday Full-Time

- 2021 Numbers:
 - 13,521 clinical specimens: Received, processed, smear and culture.
 - 2,639 CFI–cultures for identification
 - 321 cultures for drug susceptibility testing (DST).



Mycobacterium tuberculosis under the microscope, retrieved from TB Staff.

Deeplex® Myc-TB Principle

- The Deeplex® Myc-TB assay utilizes targeted next generation sequencing (NGS) technology directly on clinical samples.
- The assay relies on a single 24-plex amplicon mix to amplify mycobacterium genes that are associated with:
 - Species Identification
 - Genotyping
 - Prediction of Drug Resistance
- Samples are then pooled and run on a single bench-top NGS platform.



Deeplex® Myc-TB assay kit retrieved from
www.Genoscreen.com

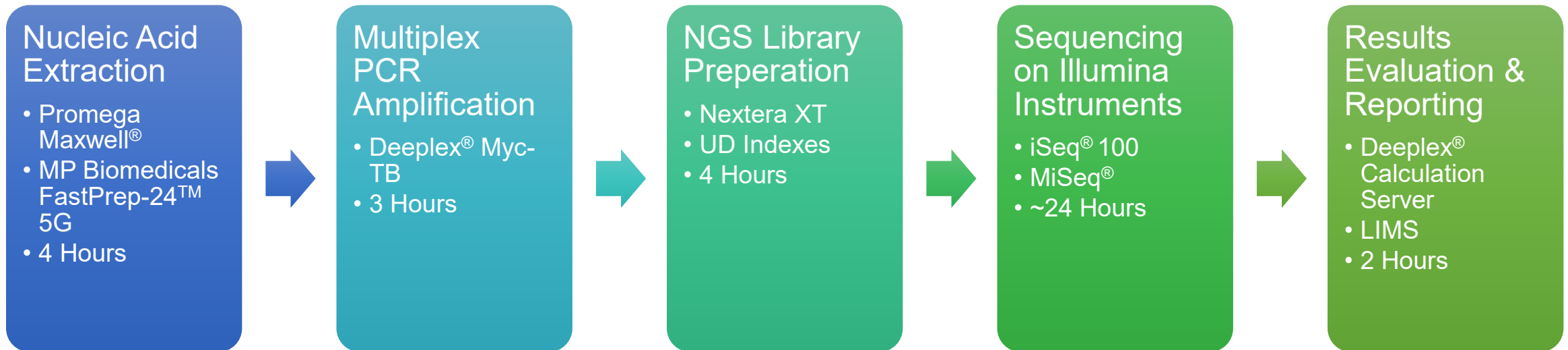
Deeplex® Myc-TB Principle

- 18 main MTBC gene targets that are associated with first- and second-line drugs.
- Can detect mutant/heteroresistant subpopulations representing as low as 3% of total reads.
- Works on many complex clinical matrices.
- TB ID using *hsp65*
- MTBC strain genotyping
 - Spoligotyping target
 - SNP's (Single Nucleotide Polymorphisms)

Table 2. Deeplex® Myc-TB drug resistance-associated targets. (* full genes)

Genomic targets	Drugs
<i>rpoB</i>	rifampicin
<i>inhA</i>	isoniazid, ethionamide
<i>fabG1</i>	isoniazid, ethionamide
<i>katG</i>	isoniazid
<i>ahpC</i>	isoniazid
<i>pncA</i> *	pyrazinamide
<i>embB</i>	ethambutol
<i>gidB</i> *	streptomycin
<i>rpsL</i> *	streptomycin
<i>rrs</i> *	streptomycin, amikacin, kanamycin, capreomycin
<i>eis</i>	kanamycin
<i>tlyA</i> *	capreomycin
<i>gyrA</i>	fluoroquinolones
<i>gyrB</i>	fluoroquinolones
<i>ethA</i> *	ethionamide
<i>rrl</i>	linezolid
<i>rplC</i>	linezolid
<i>rv0678</i> *	bedaquiline, clofazimine

Deeplex[®] Myc-TB Workflow



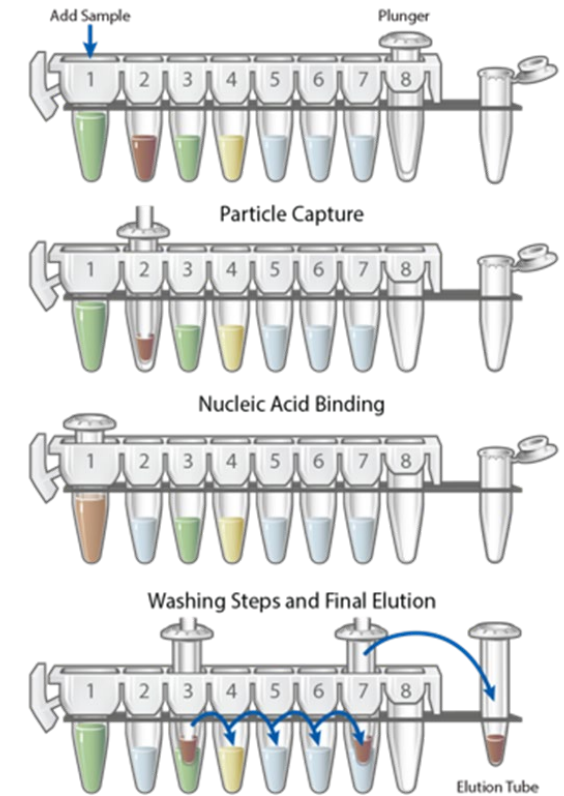
-Deeplex Myc-TB Workflow retrieved from TB Staff.

Deeplex[®] Myc-TB Implementation

- Clinical Laboratory Improvement Amendments (CLIA) compliant assay.
 - 17 unique AMR genes are reported clinically.
 - *gidB* is excluded.
- Every TB positive patient gets a Deeplex[®] Myc-TB test.
 - **Primary Test:** Clinical sample (RT-PCR Ct ≤ 34)
 - If a patient's clinical samples have a high Ct value (Ct > 34) we will BOLO an isolate.
 - **Secondary Test:** Isolate of *Mycobacterium tuberculosis* grown internally or submitted externally from other providers.

Modified Maxwell[®] Extraction

- RSC Genomic DNA Kit (Maxwell[®] Reagent Kit)
 - Modified to include FastPrep-24[™] 5G bead beating.
 - Developed cooperatively and specifically for this application.
 - Minimum hands-on time.
 - Minimum transfers to reduce cross-contamination.
- Maxwell[®] RSC 16 (AS4500) Instrument
 - Size (BSL-3)
 - Resin-based extraction
 - Pre-filled cartridges



Images of Promega Maxwell[®] – 16 Instrument including a reagent diagram retrieved from Promega Staff.

Illumina Sequencing Platforms



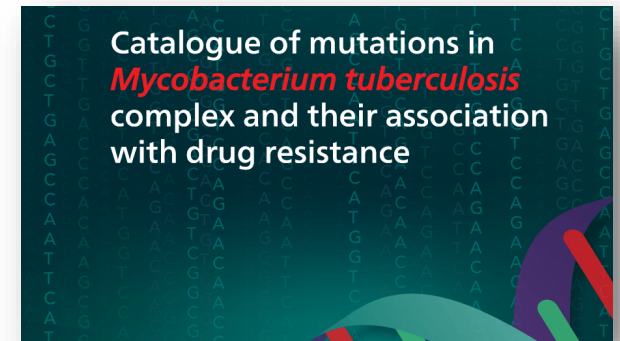
Platforms	Specifications	Run time (2x150pb)	Number of samples	Number of tests (including controls)
Iseq® 100	1,2Gb	19h	13	16
MiniSeq®	Mid Output (2,1Gb)	17h	21	24
	High Output (6,6Gb)	24h	66/69	72
MiSeq®	Nano kit (300Mb)	17h	1	4
	Micro kit (1,2Gb)	19h	13	16
	Full kit (4,5Gb)	24h	45	48
NextSeq®	Mid Output (32,5Gb)	26h	372	384
	High Output (100Gb)	29h	Not enough indices available	1333 (theoretical)

Images of Illumina iSeq and MiSeq Instruments retrieved from www.illumina.com.

Deeplex® sequencing platforms and their respective metrics Adapted from Deeplex® Myc-TB User Manual, 2020, retrieved from <https://emea.support.illumina.com/content/dam/illumina-support/documents/documentation/>.

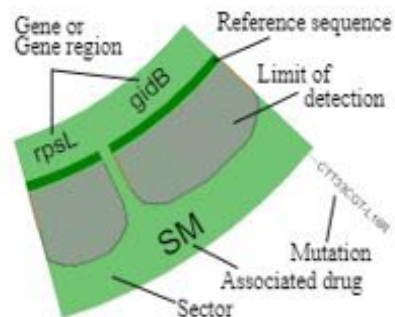
Deeplex[®] Analysis Pipeline

- Cloud-based server – transaction based (1 credit=1 sample)
- **Reference:** Catalogue of mutations in *Mycobacterium tuberculosis* complex and their association with drug resistance, 2nd ed
 - World Health Organization
- Deeplex[®] analysis options:
 - Extended Catalogue
 - Known Mutations



drug	gene	mutation	variant	effect	genomic_position	INITIAL CONFIDENCE GRADING	initial_confidence_maximum	algorithm_pass	Present_SOLO_SR	Present_SOLO_R	Present_SOLO_S	Present_R	Present_S	Absent_R	Absent_S
Rifampicin	rpoB	c.1773G>A	rpoB_synonymous_variant	(A)	11502	Uncertain significance	1	NA	NA	NA	0	2	11502	23705	
Rifampicin	rpoB	c.1773G>C	rpoB_synonymous_variant	(f A)	11501	Uncertain significance	NA	NA	NA	NA	1	0	11501	23707	
Rifampicin	rpoB	c.1788C>T	rpoB_synonymous_variant	(A)	11502	Uncertain significance	NA	NA	NA	NA	NA	NA	NA	NA	
Rifampicin	rpoB	c.-179C>T	rpoB_upstream_gene_variant	()	11476	Uncertain significance	0	0	0	0	26	0	11476	23707	
Rifampicin	rpoB	c.1800C>A	rpoB_synonymous_variant	(A)	11502	Uncertain significance	1	NA	NA	NA	0	1	11502	23706	
Rifampicin	rpoB	c.1806G>C	rpoB_synonymous_variant	(A)	11502	Uncertain significance	1	NA	NA	NA	0	1	11502	23706	
Rifampicin	rpoB	c.1806G>T	rpoB_synonymous_variant	(f A)	11501	Uncertain significance	NA	NA	NA	NA	1	0	11501	23707	
Rifampicin	rpoB	c.1809A>C	rpoB_synonymous_variant	(f A)	11501	Uncertain significance	NA	NA	NA	NA	1	0	11501	23707	
Rifampicin	rpoB	c.1809A>G	rpoB_synonymous_variant	(A)	11502	Uncertain significance	1	NA	NA	NA	0	2	11502	23705	

Reading Deeplex® Reports



Limit of detection:

- 1% ≤ limit of detection ≤ 3%
- 3% < limit of detection ≤ 80%

Reference sequence:

- Target covered over 95%
- Target covered below 95%

Sectors:



Drug resistance-associated variant or indel detected in target, with percent subpopulation according to colouring



Non-synonymous uncharacterized variant or indel detected in target, with percent subpopulation according to colouring

No resistance-associated or uncharacterized variant or indel detected in target

Targets with suboptimal/low coverage

Nontuberculosis Mycobacterium identified

Mutations (text):



Drug resistance-associated variant or indel detected in target, with percent subpopulation according to colouring



Non-synonymous uncharacterized variant or indel detected in target, with percent subpopulation according to colouring

Not associated with drug resistance (synonymous, phylogenetic or benign non-synonymous)

Antibiotics abbreviations:

RIF: Rifampicin

INH: Isoniazid

PZA: Pyrazinamide

EMB: Ethambutol

SM: Streptomycin

FQ: Fluoroquinolones

KAN: Kanamycin

AMI: Amikacin

CAP: Capreomycin

ETH: Ethionamide

LIN: Linezolid

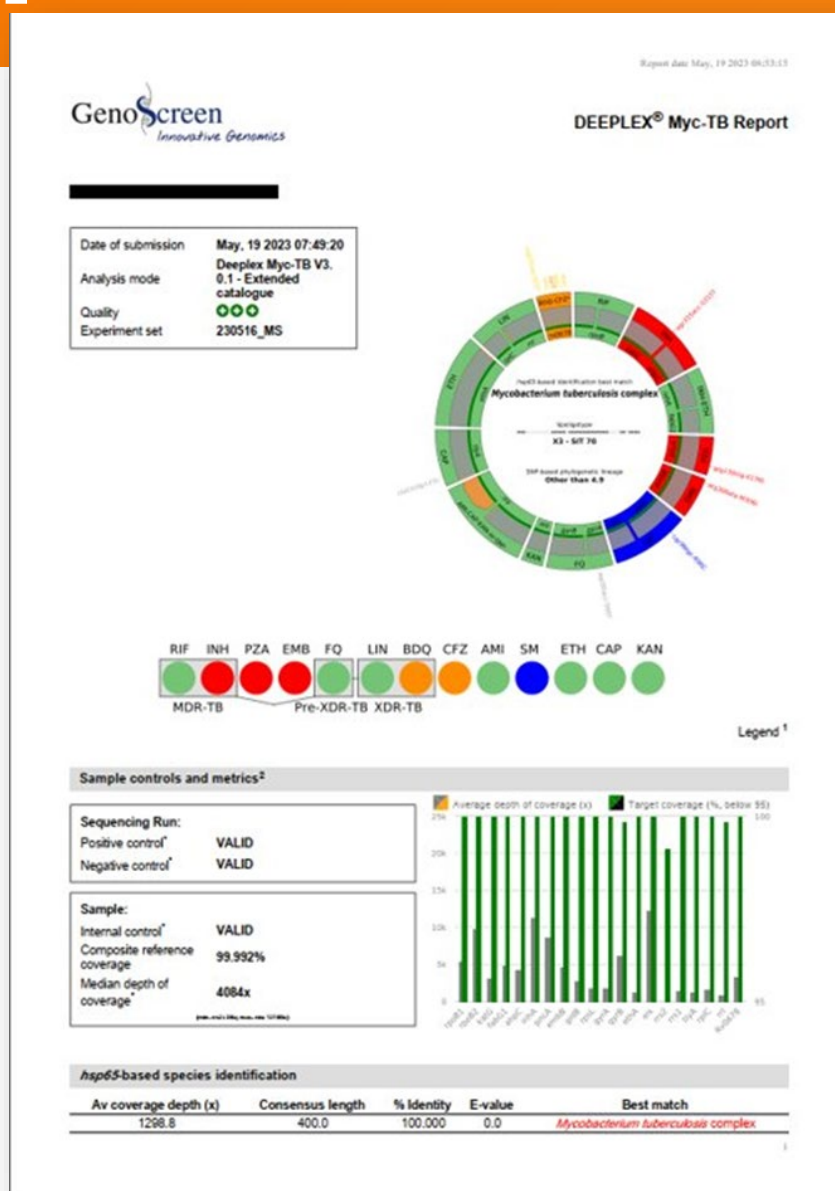
BDQ: Bedaquiline

CFZ: Clofazimin

Fluoroquinolones (FQ) includes Levofloxacin (LEV), Ofloxacin (OFX) and Moxifloxacin (MOX).

Reading a Deeplex® Report Adapted from Deeplex® Myc-TB User Manual, 2020, retrieved from <https://emea.support.illumina.com/content/dam/illumina-support/documents/documentation/>.

Deeplex® Detailed Report



Drug resistance associated variants³

Gene	Genomic position	Codon change	% Variant	Dx-score	AA change	Drug *	Confidence	Resistance level	Reference
<i>embB</i>	4247431	atg308ata	99.860	357.75	M308I	EMB	Associated with resistance	Resistant	WHO 2021
<i>katG</i>	2155168	agc315acc	100.000	142.50	S315T	INH	Associated with resistance	Resistant	WHO 2021
<i>pncA</i>	2288827	gtg139ctg	99.930	1883.25	V139L	PZA	Associated with resistance - Interim	Resistant	WHO 2021
<i>rv0678</i>	779053	cag22tag	5.040	41.50	Q22*	n/a	n/a	Resistant	n/a
<i>rv0678</i>	779265	delT	43.990	0.00	frameshift	n/a	n/a	Resistant	n/a
<i>rv0678</i>	779315	delG	9.010	0.00	frameshift	n/a	n/a	Resistant	n/a
<i>rv0678</i>	779447	delC	16.690	0.00	frameshift	n/a	n/a	Resistant	n/a
<i>rv0678</i>	779181	insG	6.920	0.00	frameshift	n/a	n/a	Resistant	n/a

Uncharacterized and uncertain significance variants³

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
<i>glaB</i>	4407917	cgc98tgc	99.770	860.50	R98C	STM	Uncertain significance	WHO 2021

Spoligotype

Octal code/ Binary code	Av. coverage depth (x)	SIT	SITVIT occurrence	Clade
700076777780671 111000000000111110111111111111100001101111	2776.5	70	100	X3

SNP-based phylogenetic lineage

Other than 4.9

Potential mixed infection

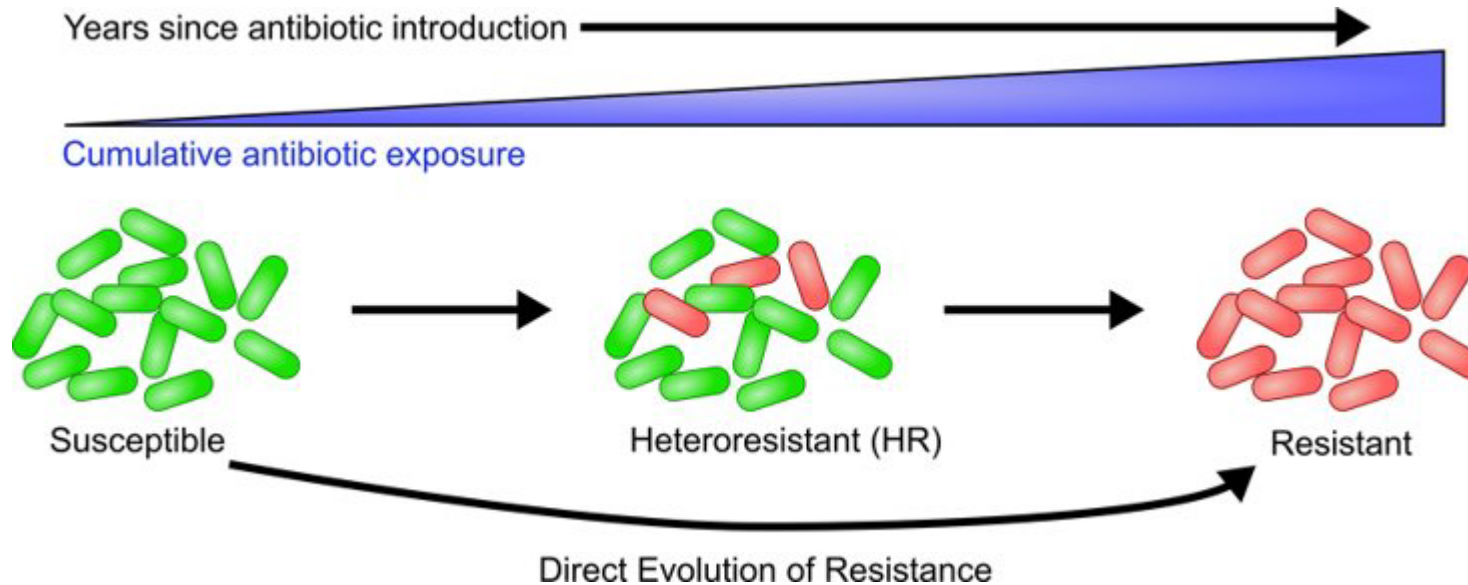
Mixed infection is signaled by a phylogenetic variant detected at less than 97%, indicating the simultaneous presence of 1 strain harboring this variant present at this percentage and another strain sharing the same sequence as the reference at this position, present at approx. 100% minus this percentage.

Not detected.

Sample Deeplex® Report, Adapted from Deeplex® Myc-TB User Manual, 2020, retrieved from <https://emea.support.illumina.com/content/dam/illumina-support/documents/documentation/>.

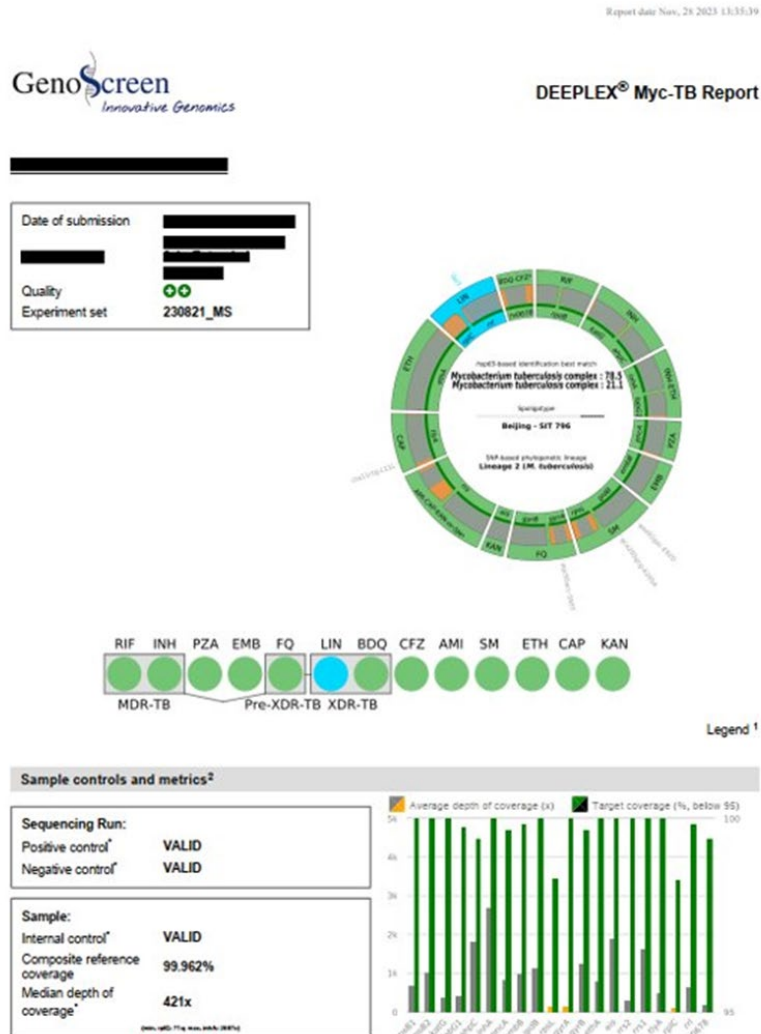
Heteroresistance

A phenomenon where subpopulations of seemingly isogenic bacteria exhibit a range of susceptibilities to a particular antibiotic.



Proposed model for the evolution of resistance through heteroresistance retrieved from <https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.3001346>

Heteroresistance Example



hsp65-based species identification				
Av coverage depth (x)	Consensus length	% Identity	E-value	Best match
105.4	400.0	99.241 / 87.342	0.0 / 6.2e-142	<i>Mycobacterium tuberculosis</i> complex : 78.5 / <i>Mycobacterium tuberculosis</i> complex : 21.1

Drug resistance associated variants³

No drug resistance associated variants detected in any gene target.

Uncharacterized and uncertain significance variants³

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
<i>mt</i>	1476113	delT	3.980	0.00	deletion	n/a	Uncharacterised	n/a

Spoligotype

[illegible]

SNP-based phylogenetic lineage

Lineage 2 (*M. tuberculosis*)

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.

Not detected.

-Figure 41-42. Deeplex® report example showing heteroresistance adapted from Deeplex® Myc-TB User Manual, 2020, retrieved from <https://emea.support.illumina.com/content/dam/illumina-support/documents/documentation/>.

Results Visualization

Sequencing result acceptability	Composite target coverage breadth %	Average coverage depth (x)	Species best match (main population (%)/min population (%))	Resistotype												
				RIF	INH	PZA	EMB	SM	FQ	KAN	AMI	CAP	ETH	LIN	BDQ	CFZ
+++	100.0	2115.2	Mycobacterium tuberculosis complex	0	0	0	0	0	0	0	0	0	0	0	0	0
+++	100.0	2620.2	Mycobacterium tuberculosis complex	0	0	0	0	0	0	0	0	0	0	0	0	0
+++	100.0	2633.8	Mycobacterium tuberculosis complex	0	0	1	0	0	0	0	0	0	0	0	0	0
+	99.6	495.8	Mycobacterium tuberculosis complex	0	0	0	0	0	0	0	0	0	0	0	0	0
+++	100.0	2352.7	Mycobacterium tuberculosis complex	0	0	0	0	0	1	0	0	0	0	0	0	0
++	100.0	1748.6	Mycobacterium tuberculosis complex	0	1	0	0	1	0	1	1	1	0	0	1	1
-	90.9	126.3	Mycobacterium tuberculosis complex	1	0	0	0	1	1	0	0	1		0	0	0
++	100.0	2207.8	Mycobacterium tuberculosis complex	0	0	0	0	0	0	0	0	0	0	0	0	0
+++	100.0	1971.1	Mycobacterium tuberculosis complex	0	0	0	0	0	0	0	0	0	0	0	0	0
++	100.0	2426.1	Mycobacterium tuberculosis complex	0	0	0	0	1	0	0	0	0	0	0	0	0
++	100.0	2277.6	Mycobacterium tuberculosis complex	0	0	0	0	0	0	0	0	0	0	0	0	0
-	98.3	80.5	Mycobacterium tuberculosis complex	0		0	0		0						0	0
++	99.9	911.0	Mycobacterium tuberculosis complex	0	0	0	0	0	0	0	0	0	0	0	0	0

-Figure 44. Deeplex® results visualized on the calculation server adapted from the Deeplex® web app.

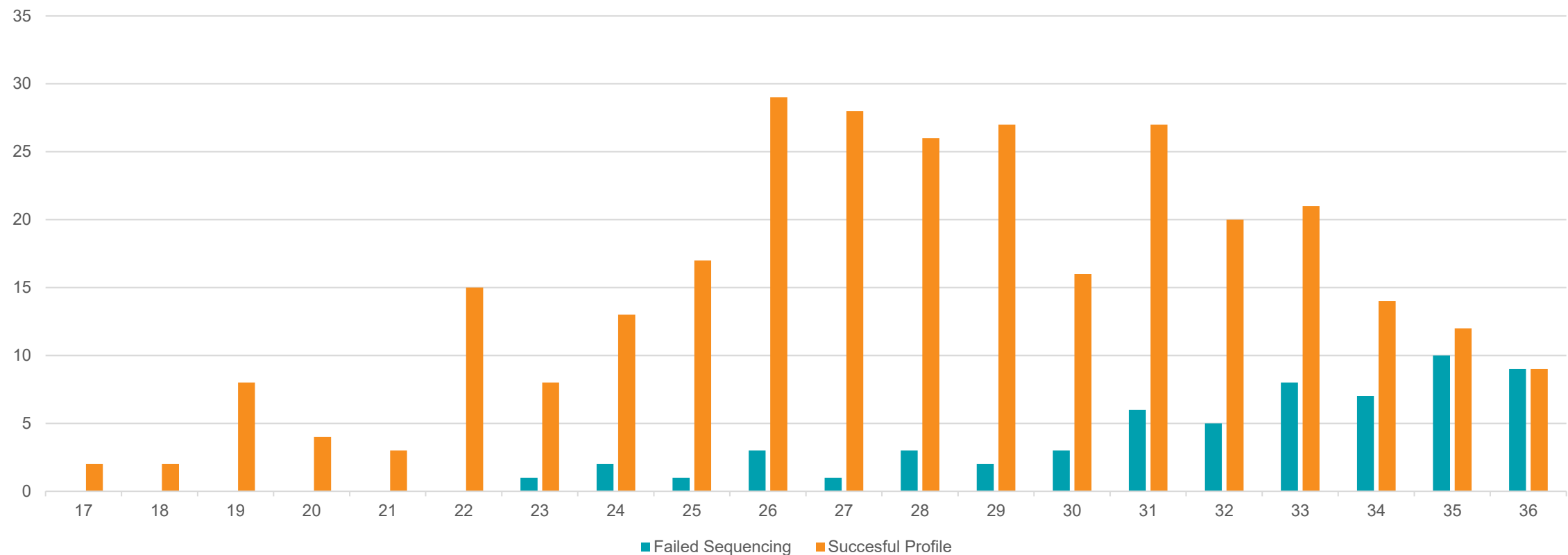
Deplex[®] Myc-TB Cost Analysis

	Samples/Kit*	Cost/Kit	Cost/Sample
Deplex [®] Reagent Kit	39	\$2,800.00	\$71.80
iSeq [®] 100 Reagent Kit	13	\$582.11	\$44.78
Nextera XT Kit	78	\$3361.1	\$43.10
UD Indexes v2	78	\$480.7	\$6.20
Qubit 1xHSdsDNA	250	\$436.6	\$1.75
Maxwell [®] Extraction Kit	48	\$405	\$8.44
Total/Sample			\$176.07

**Does not include the necessary controls for a thirteen-sample run.*

Deeplex[®] Success: 2024 Snapshot

RT-PCR CT Value vs Total Sequenced



Deeplex[®] Myc-TB XT – In Development

- Early 2026?
- Increased sensitivity across some/all targets.
- Adoption of Illumina DNA Prep streamlines the process.
- Adding multiple targets for second-line treatments:
 - Delamanid
 - Pretomanid

Future Thoughts



- Affordability and operability will improve over time:
 - Microfluidics library prep
 - Full automation
- Database with clinical feedback:
 - Is a mutation causing resistance?
- Global Health:
 - Responsibility to disseminate life-saving technologies.
- Clinicians and Laboratorians harmonized:
 - Florida TB Control

Contact Information



TB Staff by Phil Lee, authorized by TB Staff.

Matthew D. Schimenti MPH, M(ASCP)

Bureau of Public Health Laboratories – TB Control
Division of Disease Control and Health Protection

Florida Department of Health

1217 N Pearl Street, Jacksonville, FL 32202

Matthew.Schimenti@Flhealth.gov

904-791-1556 (Phone)

- Genoscreen. July 2020 – v3. Deeplex® Myc-TB User Manual
- Illumina. May 2019. Document # 15031942v05. Nextera XT DNA Library Prep
- Promega Corporation. 10/22. TM708. Maxwell® RSC Genomic DNA Kit