

DRUG RESISTANCE

A blue-bordered slide with a dark blue background. The text 'DRUG RESISTANCE' is written in yellow, bold, capital letters in the bottom right corner.

- Conventional Drug Susceptibility Testing**
- **Growth based**
 - Time consuming (week to month)
 - Laborious
 - Infrastructure
 - **Liquid or solid media**
 - Equivalent concentrations?
 - **Critical concentration**
 - **Minimum inhibitory concentration**
- A blue-bordered slide with a dark blue background. The title 'Conventional Drug Susceptibility Testing' is in yellow. Below it is a list of four items, each with a square bullet point. The first item has a sub-list of three items with square bullets. The text is white.

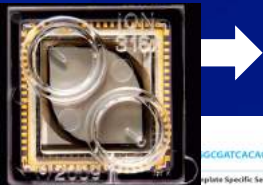
Drug Resistant Survey

- **Based on phenotypic assays**
 - Some countries perform routine DST
 - Resource-limited areas (once every 3-5 years)
 - Lack infrastructure
- **Can we use molecular assays?**
 - Do we have the knowledge
 - What tools are needed

rpoB Surveillance

- **Rifamycin resistance**
 - Role of mutations
- **Frequency of mutations**
 - Population level
 - Assay development
- **Importance of data**
 - Calculate sensitivity and specificity of assays
 - Silent mutations
 - How much phenotypic resistance is missed
 - What is the affect on patient outcome

Fusion Primers for Ion Torrent PGM



5' - CCTCTCATCCCTG GGGATCACACCGCAGAC - 3'

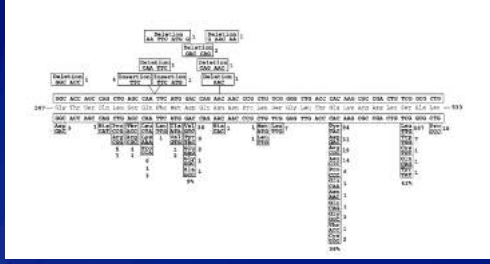
Ion Adapter Sequence Template Specific Sequence

Reverse Primer

5' - CCTCTCATGGGCAGTCGGTGATACTCCAGCCCGGCACGCTCACGT - 3'

Ion Adapter Sequence Template Specific Sequence

Rifampin Resistant Determining Region (RRDR) of *rpoB*



Sequence alignment showing mutations in the RRDR of *rpoB*. The reference sequence is shown at the top, and various mutant sequences are listed below. Mutations are indicated by colored letters (red for G, yellow for A, blue for C, green for T) and asterisks for insertions. The alignment covers approximately 100 amino acid positions.

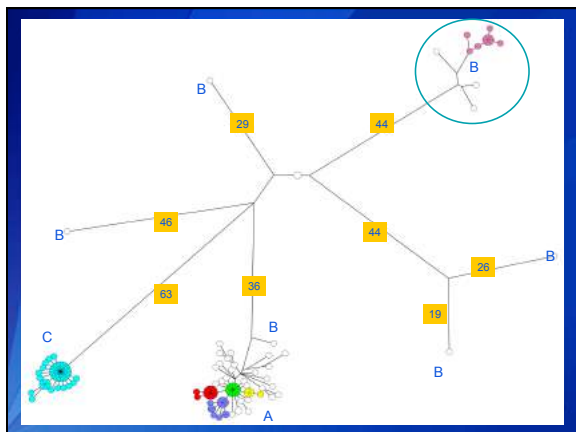
***rpoB* Surveillance**

- **14 months**
 - >10,000 isolates
- **Mutations**
 - 411 isolates (35 unique mutations)
 - Ser531Leu (184 isolates)
 - Silent mutations (94 isolates)

RESEARCH


Areas to Address

- ❑ Identify new mechanisms of drug resistance
 - Existing and new drugs
- ❑ Identify preexisting resistance
 - Repurposed and new drugs
- ❑ Microevolution in the patient
- ❑ Compensatory mutations



C	G	C133998C	NR_123111.1		C	T	C166385G	acc
C	T	C134001T	Rv0609C		B	A	C167263A	fabG4
C	T	C134002T	Rv0310C		T	C	C1167205A	pkcS7
C	T	C247984T	Rv0207C		C	G	C138807G	pkcS9
T	C	T462962C	PE_PGHS		T	C	T408707G	5' UTR
C	G	C477186G	Rv0398C		C	T	C227212G	upd17
C	A	C480678A	myoD1.1		G	C	C283333C	Rv2332C
A	G	A48997A	ubtE		T	C	T261314T	48 bp
C	T	C761147T	ppgB		G	A	C276147A	upd17
G	C	G765710C	ppgC		G	A	C280855A	Rv2681
C	G	C799139C	Rv0698		G	A	C812648A	Rv2810C
G	A	G80488A	Rv0813C		G	A	C812743G	ecrA16
C	A	C82680A	IS		C	T	C83313C	ispH
C	G	C102843C	hsp		C	C	A337793A	ppk26
T	G	T109453T	PE_PGHS		A	C	A380180A	ppk27
G	A	G133351A	Rv0997		A	G	A381630A	Rv3055
C	T	C130883C			C	T	T445543T	Rv3088
C	T	C131140C	Rv1104		G	C	C460803T	Rv3280C
A	C	A136478A	hsp68		C	T	T476618T	ppk26
C	T	C136579C	Rv1390C		G	A	C137612G	ppk26
C	T	C136931A	hsp68		C	A	A377777A	48 bp
A	C	A137038A	pppA.3		A	G	A382661A	5' UTR
G	A	G138388G	hspA		C	A	C403728A	PE_PGHS
G	A	G142108A	Rv1272C		T	A	T407248T	SD
					G	C	C483616G	Rv3613
					G	C	C483616G	topA
					A	G	A51147A	hspH


Identify New Mechanisms of Resistance



L203L

A silent mutation in *mabA* confers isoniazid resistance on *Mycobacterium tuberculosis*.
[Ando H, Miyoshi-Akiyama T, Watanabe S, Kirikae T.](#)

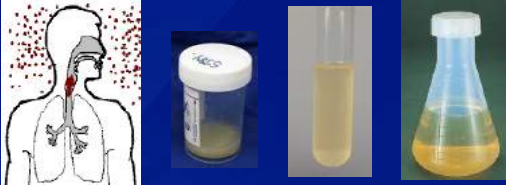
***inhA* Transcript Levels**



Heatmap showing *inhA* transcript levels across various *M. tuberculosis* strains. The heatmap is divided into two main sections, each with sub-sections labeled *mabD*, *mabE*, and *mabF*.

CLINICAL MANAGEMENT

Starting Material



Sputum
Dx Culture
Subculture

WGS versus Targeted Approach

- **WGS**
 - Need a culture
 - High molecular weight DNA (1—5 ug)
 - Analyze about 90—99% of genome
 - Low to medium throughput
- **Targeted loci**
 - Could possibly start with processed sputum
 - PCR based
 - Lower quality and quantity of DNA
 - Only analyze the areas amplified
 - High throughput

Sensitivity and Specificity of Loci

Drug	Gene(s)	Sensitivity (%)	Specificity (%)
RIF	<i>rpoB</i>	97.1	93.6
INH	<i>inhA, katG</i>	90.6	100
EMB	<i>embB</i>	78.6	93.1
FQ	<i>gyrA</i>	81.6	97.7
KAN	<i>rrs, eis</i>	86.5	96.1
AMK	<i>rrs</i>	90.0	98.8
CAP	<i>rrs, tlyA</i>	60.9	87.3
MDR	<i>rpoB, inhA, katG</i>	90.8	94.7

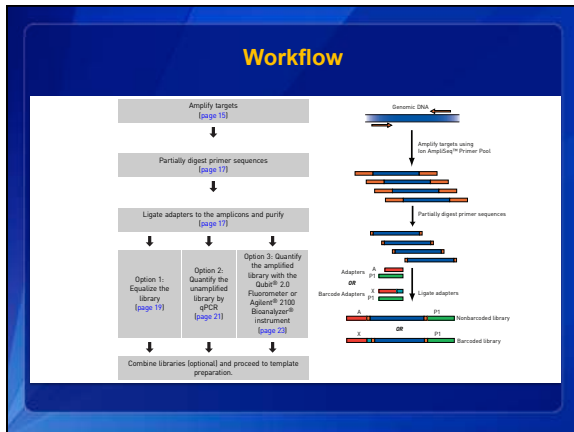
Retrospective DR Study and MDDR Service Sensitivity and Specificity of Loci

Drug	Gene(s)	Sensitivity (%)	Specificity (%)
RIF	<i>rpoB</i>	97.1	97.4
INH	<i>inhA, katG</i>	86.0	99.1
EMB	<i>embB</i>	78.8	94.3
FQ	<i>gyrA</i>	79.0	99.6
KAN	<i>rrs, eis</i>	86.7	99.6
AMK	<i>rrs</i>	90.9	98.4
CAP	<i>rrs, tlyA</i>	55.2	91.0

- Molecular Detection of Drug Resistance**
- **Original assay**
 - 8 single PCR reactions
 - Sanger DNA sequencing
 - 16 sequencing reactions
 - **High throughput assay**
 - NGS – Ion Torrent PGM
 - Multiplex PCR
 - Barcoded – 96 samples per assay

Targeted Loci

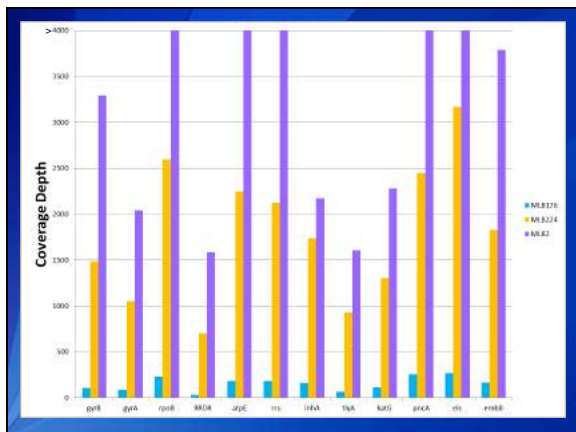
DRUG	Gene	Region
Rifampin	<i>rpoB</i>	176 and RRDR
Isoniazid	<i>katG</i>	315
	<i>inhA</i>	Promoter
Ethambutol	<i>embB</i>	ERDR
Pyrazinamide	<i>pncA</i>	Promoter and ORF
Fluoroquinolones	<i>gyrA</i>	QRDR
	<i>gyrB</i>	QRDR
Kanamycin	<i>eis</i>	Promoter
	<i>rrs</i>	1401
Amikacin	<i>rrs</i>	1401
Capreomycin	<i>rrs</i>	1401
	<i>tlyA</i>	Promoter and ORF



- ### Pilot Study
- ❑ 8 and 48 samples
 - ❑ Use crude DNA preps
 - ❑ Compare Ion Torrent data to Sanger sequence

Number of Reads Per Sample

Sample	Number of Reads
MLB2	100,039
MLB18	55,909
MLB36	40,246
MLB138	5,715
MLB149	51,144
MLB176	4,525
MLB207	98,238
MLB224	50,865

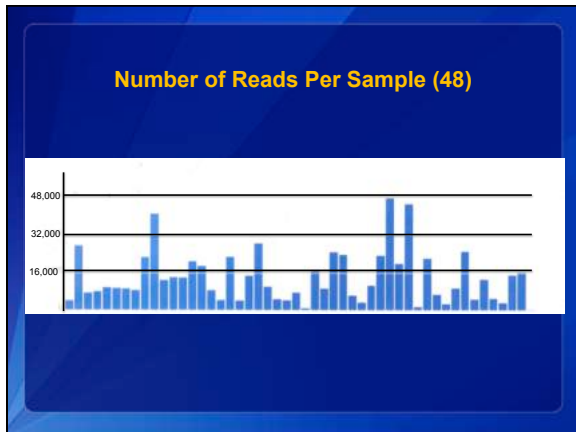


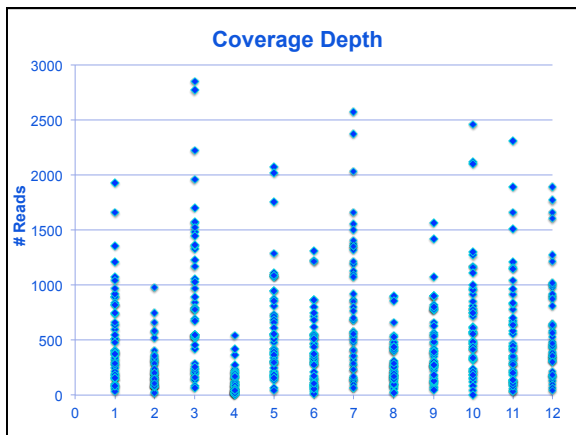
SNP Report

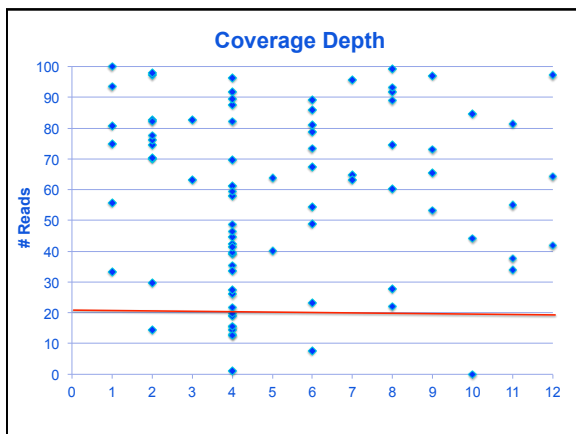
MID	Ref Pos	Type	Ref Base	Called Base	SNP %	Feature Name	Amino Ac	Depth
MLB2	4247730	SNP	G	C	97.70%	embB	G406A	1196
MLB224	4247578	SNP	G	A	99.20%	embB	L355L	1658
MLB224	4247646	SNP	A	C	95.90%	embB	E378A	560
MLB138	7585	SNP	G	C	90.90%	gyrA	S95T	220
MLB149	7585	SNP	G	C	90.30%	gyrA	S95T	944
MLB176	7585	SNP	G	C	72.00%	gyrA	S95T	100
MLB176	7582	SNP	A	G	84.00%	gyrA	D94G	100
MLB18	7585	SNP	G	C	90.00%	gyrA	S95T	964
MLB2	7585	SNP	G	C	89.80%	gyrA	S95T	2538
MLB224	7585	SNP	G	C	91.80%	gyrA	S95T	1279
MLB36	7585	SNP	G	C	89.20%	gyrA	S95T	869
MLB36	7570	SNP	C	T	99.50%	gyrA	A90V	830
MLB18	2155168	SNP	C	G	99.60%	katG	S315T	1308
MLB2	2155168	SNP	C	G	99.60%	katG	S315T	2475
MLB36	2155168	SNP	C	G	99.30%	katG	S315T	957
MLB18	2288827	SNP	C	T	78.30%	pncA	V139M	2702
MLB2	2288935	SNP	A	G	99.80%	pncA	Y103H	8046
MLB224	2288733	SNP	G	A	98.70%	pncA	A170V	1475
MLB36	2288826	SNP	A	G	78.90%	pncA	V139A	1882
MLB176	761161	SNP	T	C	60.00%	rpoB	L452P	25
MLB18	761155	SNP	C	T	99.10%	rpoB	S450L	900
MLB2	761155	SNP	C	T	99.50%	rpoB	S450L	1577

SNP Report

MLB36	761155	SNP	C	T	99.10%	rpoB	S450L	680
MLB2	1473246	SNP	A	G	99.80%	rrs		5051
MLB207	1473183	SNP	A	G	35.00%	rrs		20
MLB36	1473246	SNP	A	G	99.80%	rrs		1658
MLB138	1917972	SNP	A	G	93.10%	tlyA	L111L	159
MLB149	1917972	SNP	A	G	96.40%	tlyA	L111L	863
MLB176	1917972	SNP	A	G	97.40%	tlyA	L111L	76
MLB176	1918523	SNP	G	A	98.90%	tlyA	G195D	89
MLB18	1917972	SNP	A	G	97.00%	tlyA	L111L	943
MLB2	1917972	SNP	A	G	96.60%	tlyA	L111L	1511
MLB207	1917972	SNP	A	G	97.40%	tlyA	L111L	1337
MLB224	1917972	SNP	A	G	96.30%	tlyA	L111L	934
MLB36	1917972	SNP	A	G	96.50%	tlyA	L111L	818





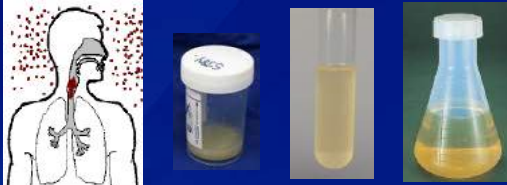


Summary and Next Steps

- **Pilot project**
 - 8 samples – completed and 100% agreement with Sanger data
 - 48 samples – analyzing data
- **Quality and quantity of DNA**
- **Determine the minimal coverage and number of reads**
- **Scale up to 96 samples**
- **Test processed specimens**

METAGENOMICS

Starting Material



Sputum Dx Culture Subculture

Synthetic Dilution Series Strategy

- Pure gDNA dilution to extinction
 - Mtb, STEC, C diff
- gDNA/gRNA dilution to extinction in complex background
 - Mtb/Sputum
 - STEC/Stool
 - Influenza/A549 RNA
 - OC43 Coronavirus/A549 RNA
 - Targeted enrichment study
 - Background depletion study

Mtb Sputum Synthetic Sets

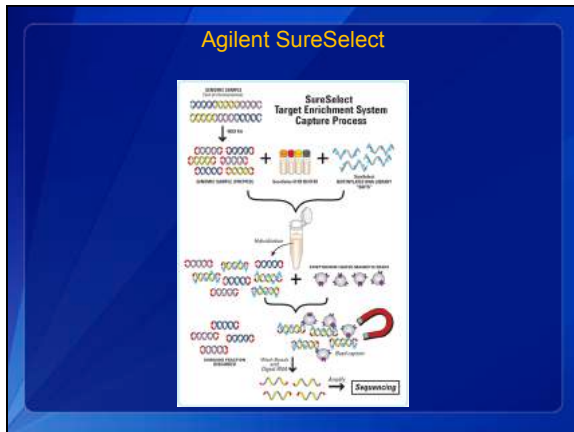
- Create seven sets of dilutions (100 x 50 μ L ea.) in a constant background of Sputum gDNA (25 ng μ L⁻¹)

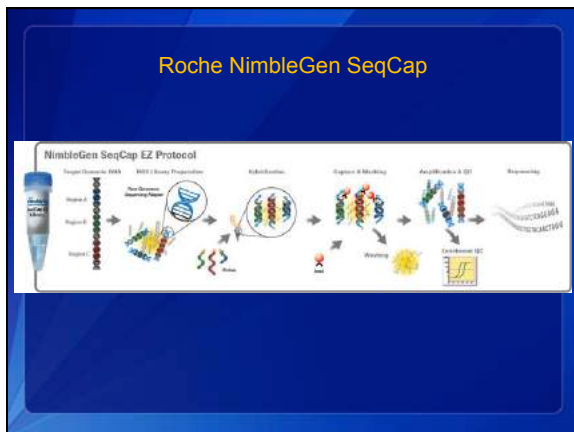
Mtb IS6110 RT-PCR

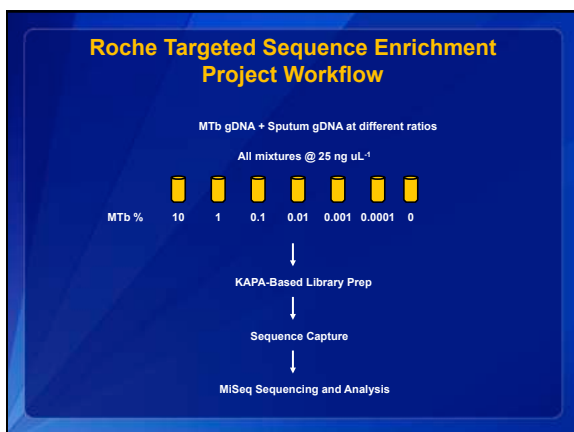
- Dilution 0 – Unspiked Sputum Ct >40
- Dilution 1 – 10% Mtb Ct 13.0 \pm 0.16
- Dilution 2 – 1% Mtb Ct 16.7 \pm 0.12
- Dilution 3 – 0.1% Mtb Ct 20.1 \pm 0.05
- Dilution 4 – 0.01% Mtb Ct 23.5 \pm 0.14
- Dilution 5 – 0.001% Mtb Ct 27.1 \pm 0.15
- Dilution 6 – 0.0001% Mtb Ct 30.4 \pm 0.12

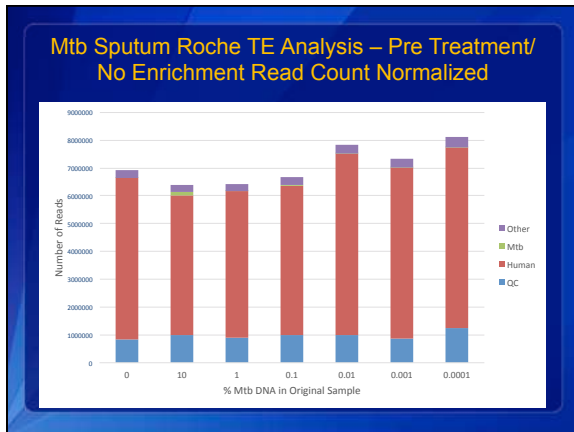
DNA Targeted Sequence Enrichment Strategy

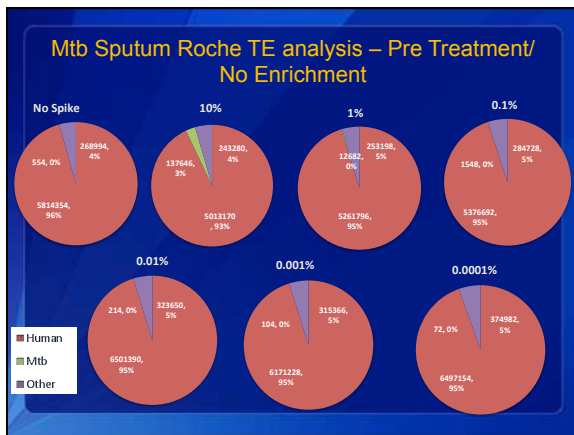
- Commercial and lab developed kits
 - Agilent SureSelect
 - Roche NimbleGen SeqCap
 - NuGEN Select
 - Custom assays
- Three initial designs for each approach
 - Mtb H37Rv
 - STEC O157:H7 Sakai
 - Influenza H3N2 A/Texas/50/2012

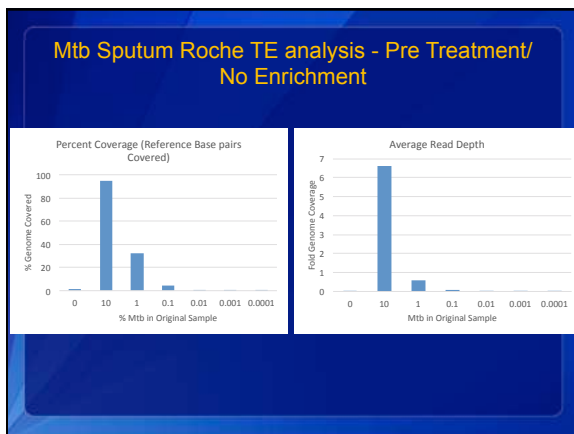


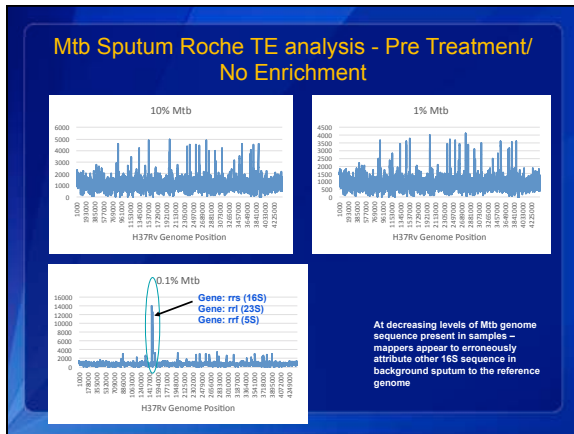


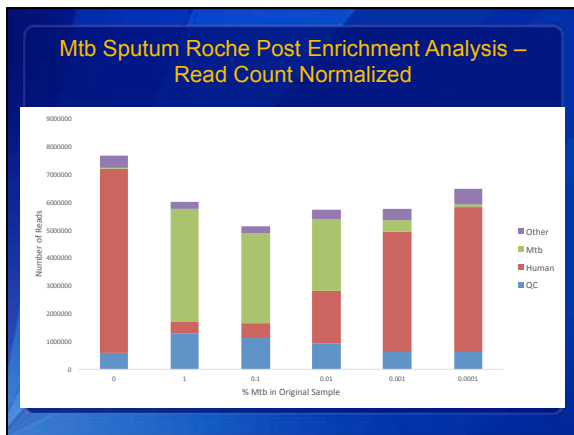


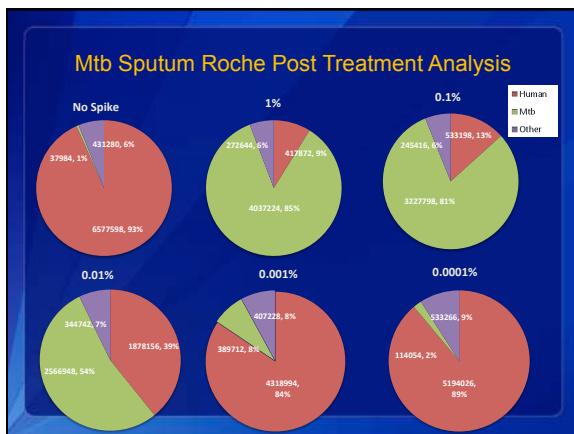


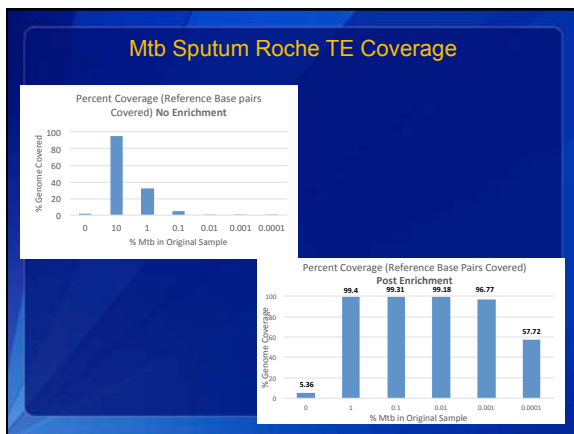
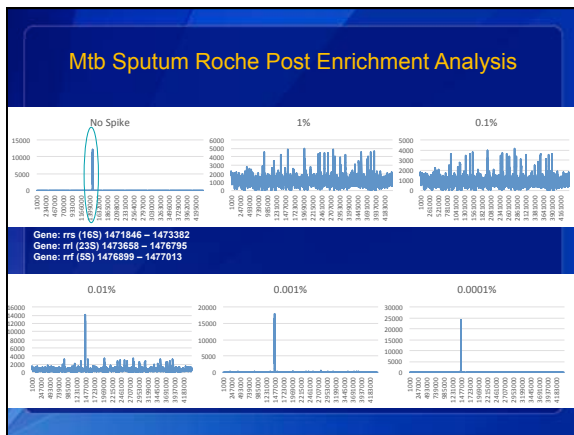
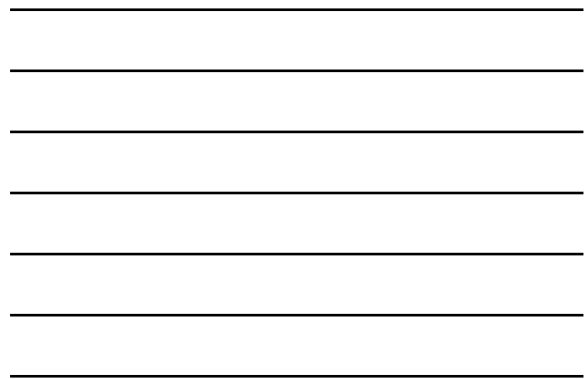
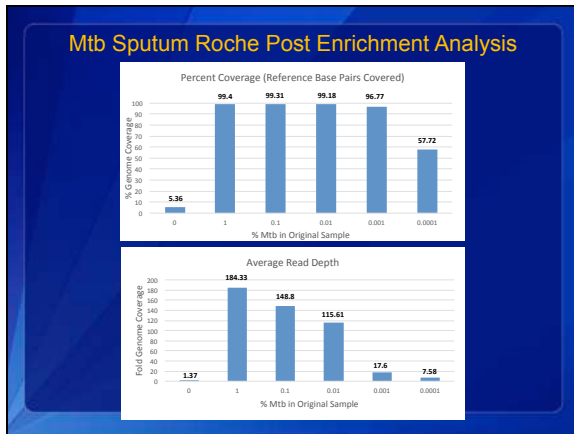


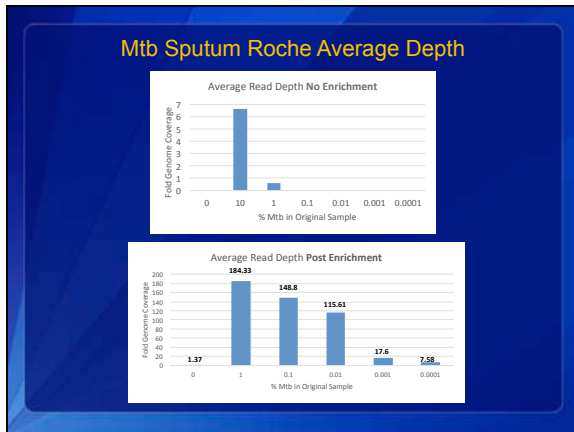


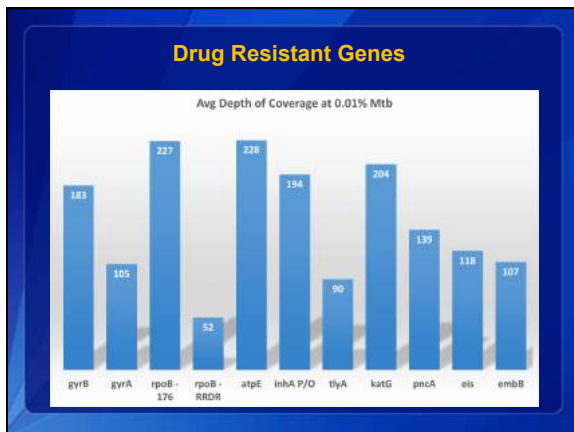


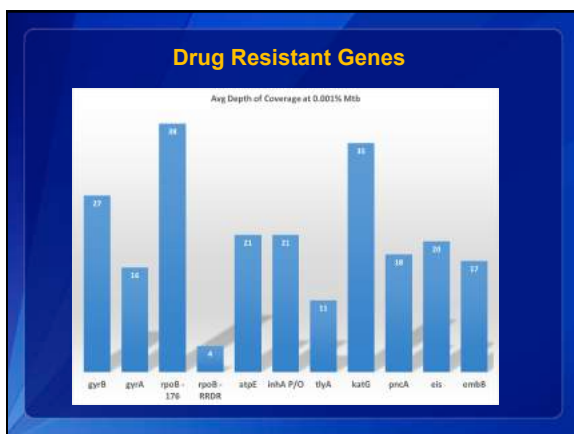


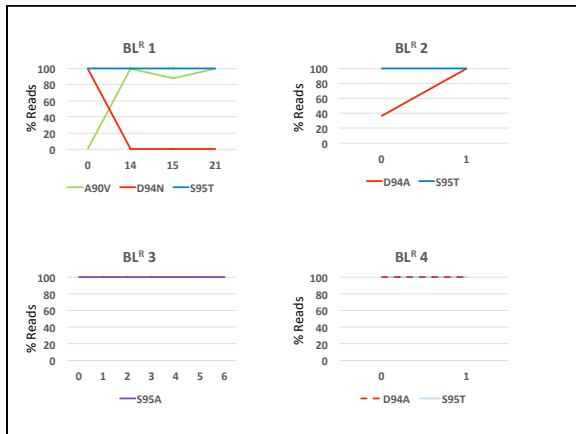


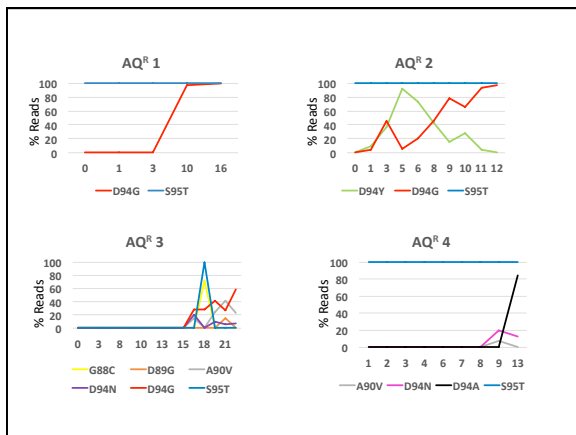












Molecular Epidemiology

Drug Resistance

Metagenomics

Acknowledgements

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