



Massachusetts Department of Public Health

TB SEQUENCING IN MASSACHUSETTS

Laboratory Aspects of TB

July 13, 2022

Tracy Stiles

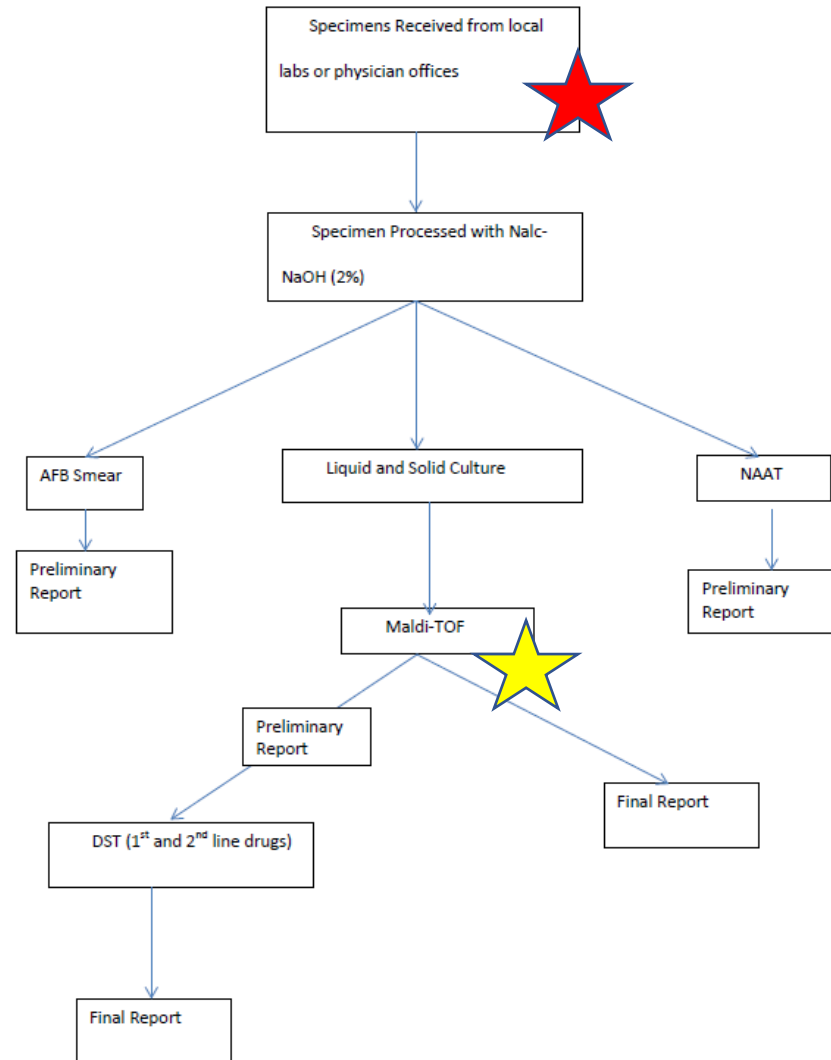
Microbiology Division Director, Massachusetts State Public Health

Laboratory

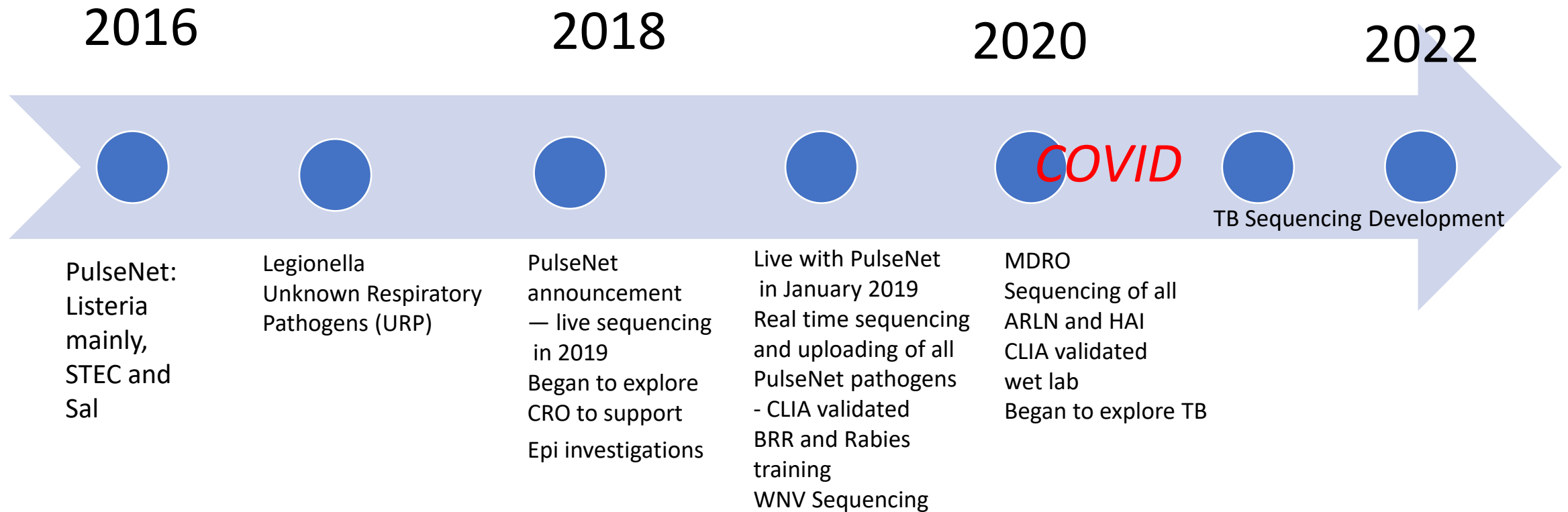
TB testing in Massachusetts

- Only full-service TB lab in Massachusetts
- 17,000 specimens annually
- 10,000 patients
- 150-250 new cases of TB annually
- 1 MDR in 2021
- 2022: 1 MDR and 1 pre-XDR

TB Algorithm



Sequencing Timeline



Sequencing Volumes to date

	2016	2017	2018	2019	2020	2021	2022 (YTD)	Total
PulseNet	447	951	1017	1462	951	1149	433	6410
GenomeTrakr	0	12	0	2	34	231	118	397
AR (CRO)	0	0	10	54	206	248	99	617
HAI (GAS/MRSA)	0	0	0	9	17	25	30	81
TB	0	0	0	0	0	37	3	40
SARS-CoV-2	0	0	0	0	674	7481	2428	10583
Monkeypox	0	0	0	0	0	0	3	3
Rabies	0	0	0	0	24	31	0	55
Other*	29	7	3	10	13	289	4	355
Total	447	963	1027	1527	1906	9202	3114	18186

* *Legionella*, *Burkholderia cepacia* complex, *Achromobacter*, *Yersinia*, *Neisseria meningitidis*, *Neisseria gonorrhea*

Wet Lab Procedure

*Validation Pending...

BSL-3/TB Lab

BSL-2/Sequencing Lab

Heat Inactivation

Solid: 1000uL LC-MS grade water
Half-loop (10uL loop) growth
Swirl, Vortex, Spin
100°C +/- 5°C for 30 minutes

Liquid: 1.2mL from the bottom
Spin >13000rpm for 3 minutes
Remove sup, resuspend pellet
1000uL LC-MS grade water
Vortex, Spin
100°C +/- 5°C for 30 minutes

Extraction

Spin >13000rpm/15 minutes
Remove sup
200uL Instagene Matrix
56°/30 minutes
Add 3mm glass beads-Vortex
100°C/20 minutes
Vortex → FastPrep24
Vortex, Spin >13000/15 min
Pull off 200uL, freeze at -20°C
Quantify (Qubit/Nanodrop)

Library Prep

Nextera XT (4-5 hours)
tagmentation,
amplification,
normalization,
pooling

Experimenting with Extractions

A (“small” loop, X), B (“medium” loop, 2X), C (“large” loop, 3X)

Extraction Procedure	Concentration	Nanodrop Reading	Qubit Reading
Target Ranges		1.75-2.05	>1ng/uL
DNeasy Gram positive protocol -Solid Culture	A	1.7	3.53 ng/ul
	B	1.76	25.5 ng/ul
	C	1.69	44.6 ng/ul
FastPrep24/InstaGene Protocol - Solid Culture	A	1.19	2.64 ng/ul
	B	1.87	16.8 ng/ul
	C	1.74	31.4 ng/ul
FastPrep24/InstaGene Protocol - 7H9 Broth	A	1.86	4.03 ng/ul
	B	1.76	7.90 ng/ul
	C	2.21	19.8 ng/ul

Sequencing Capacity

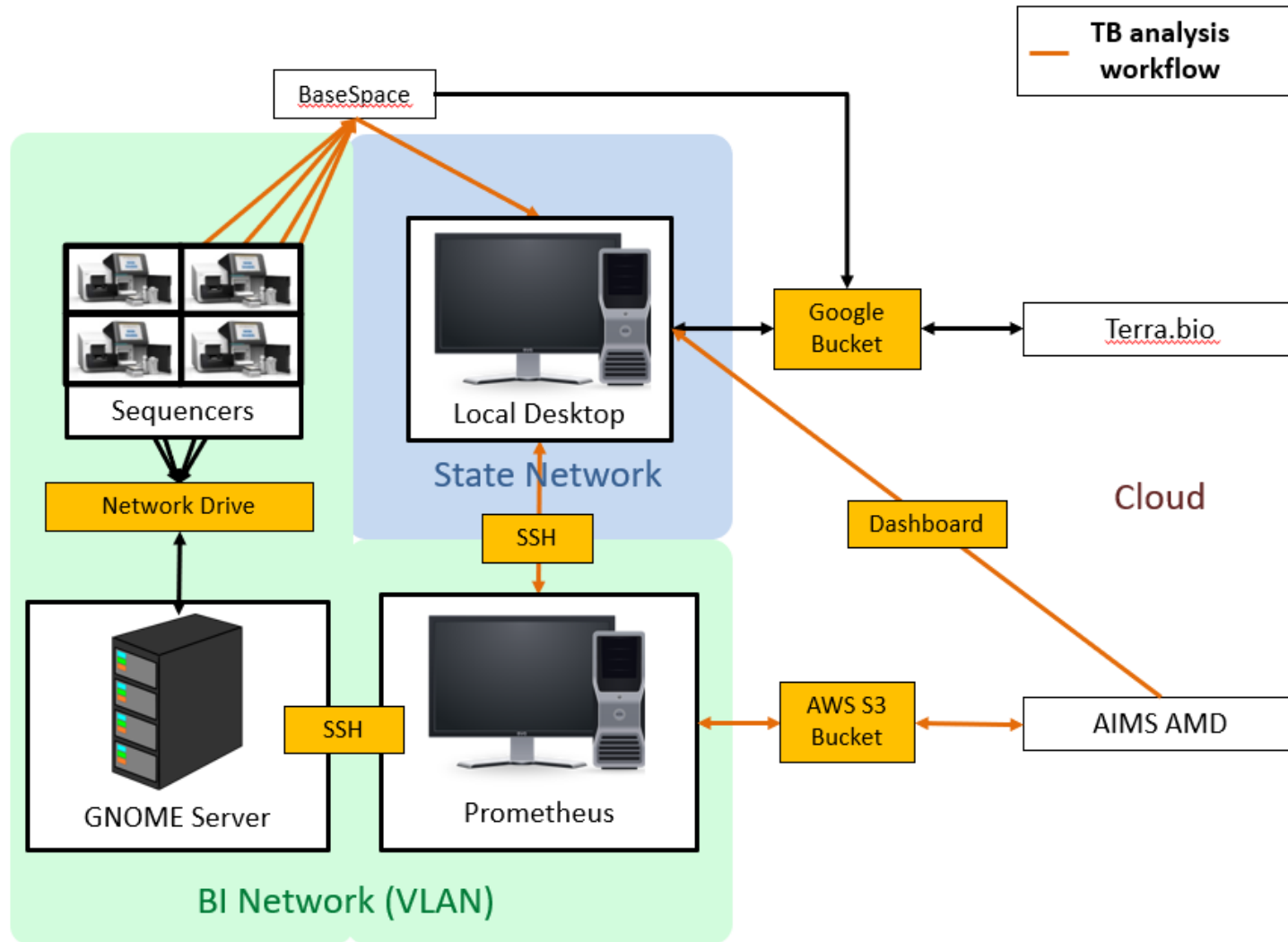
4 Illumina MiSeq
1 Illumina NextSeq 550
1 Illumina iSeq
1 Clear Labs instrument
1 ONP MinION

Illumina MiSeq
500 cycle v2 kits
Tb genome 4.4 Mbp
16 per cartridge
1-2 cartridges/week when live

Goal: Mixed runs on the NextSeq

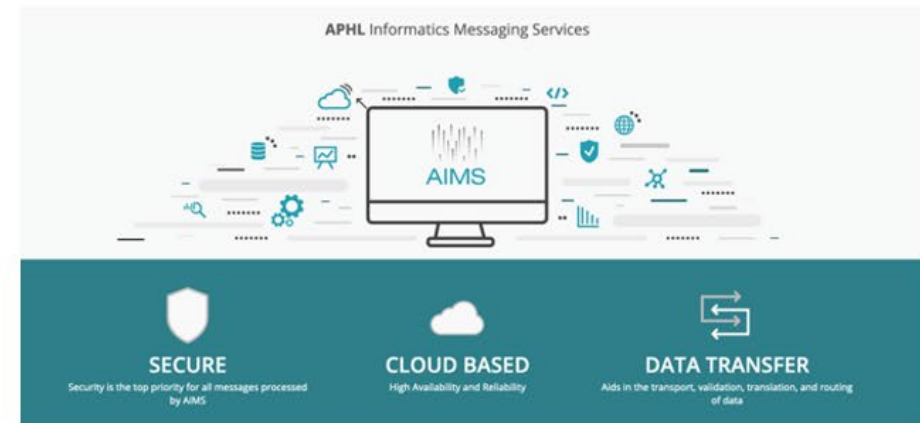


Bioinformatic Network



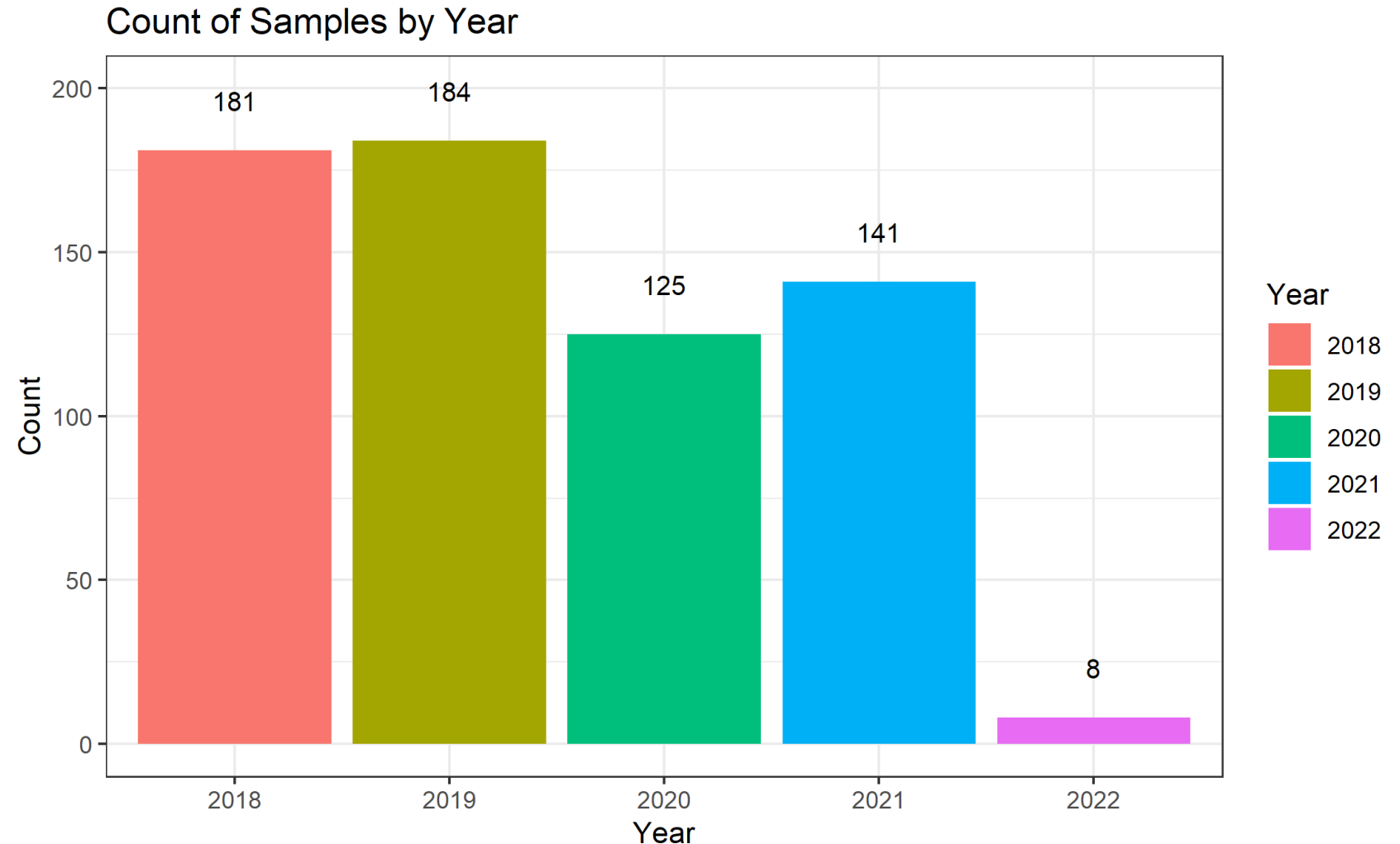
APHL AIMS Platform Pilot of Wadsworth TB Pipeline

- Project in work 2019- 2022
- WC shared pipeline
- 3 pilot state labs now have access- FL, MA, NC
- **Available soon at Datapult**

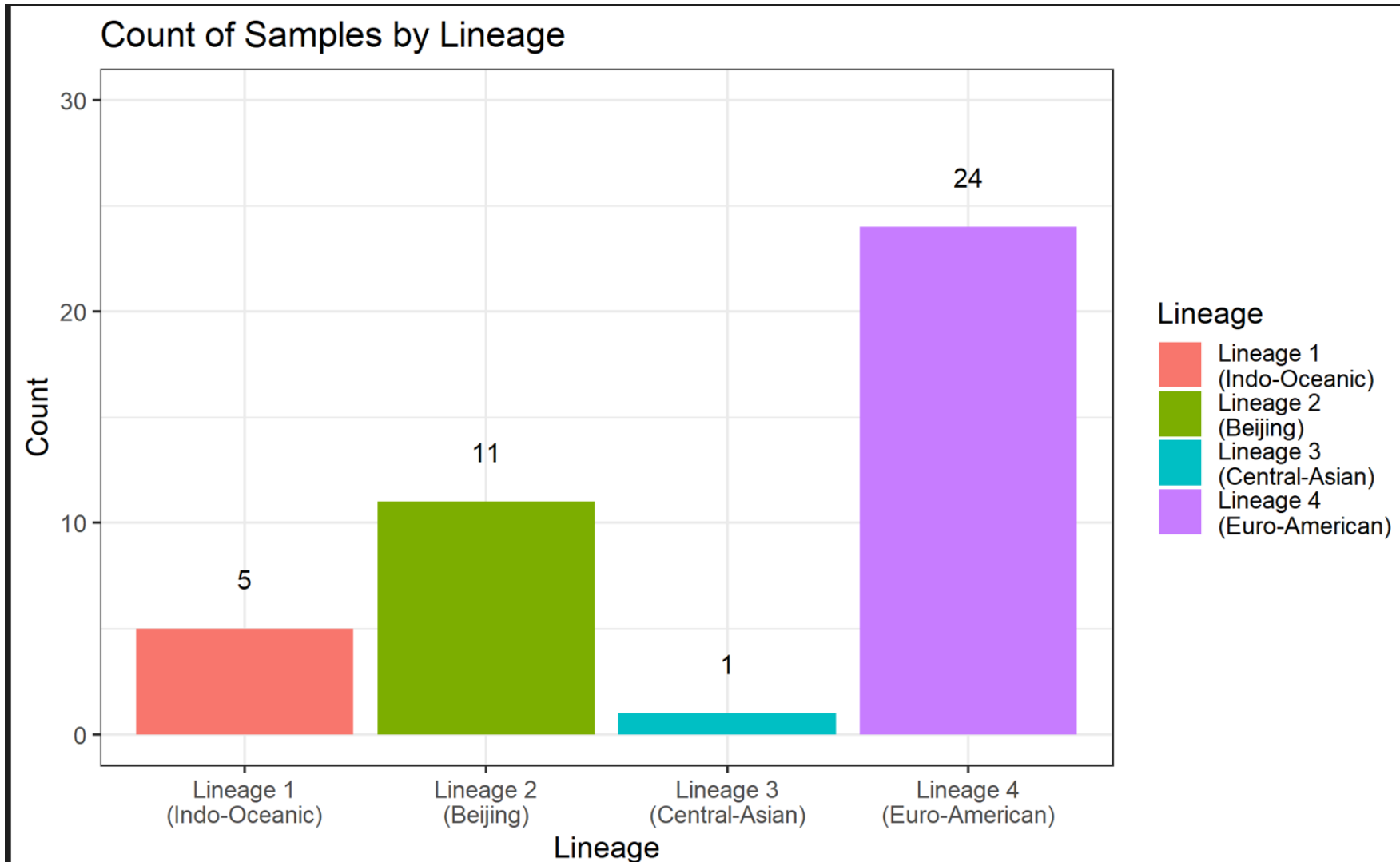


Piloting the AIMS (NY) Pipeline

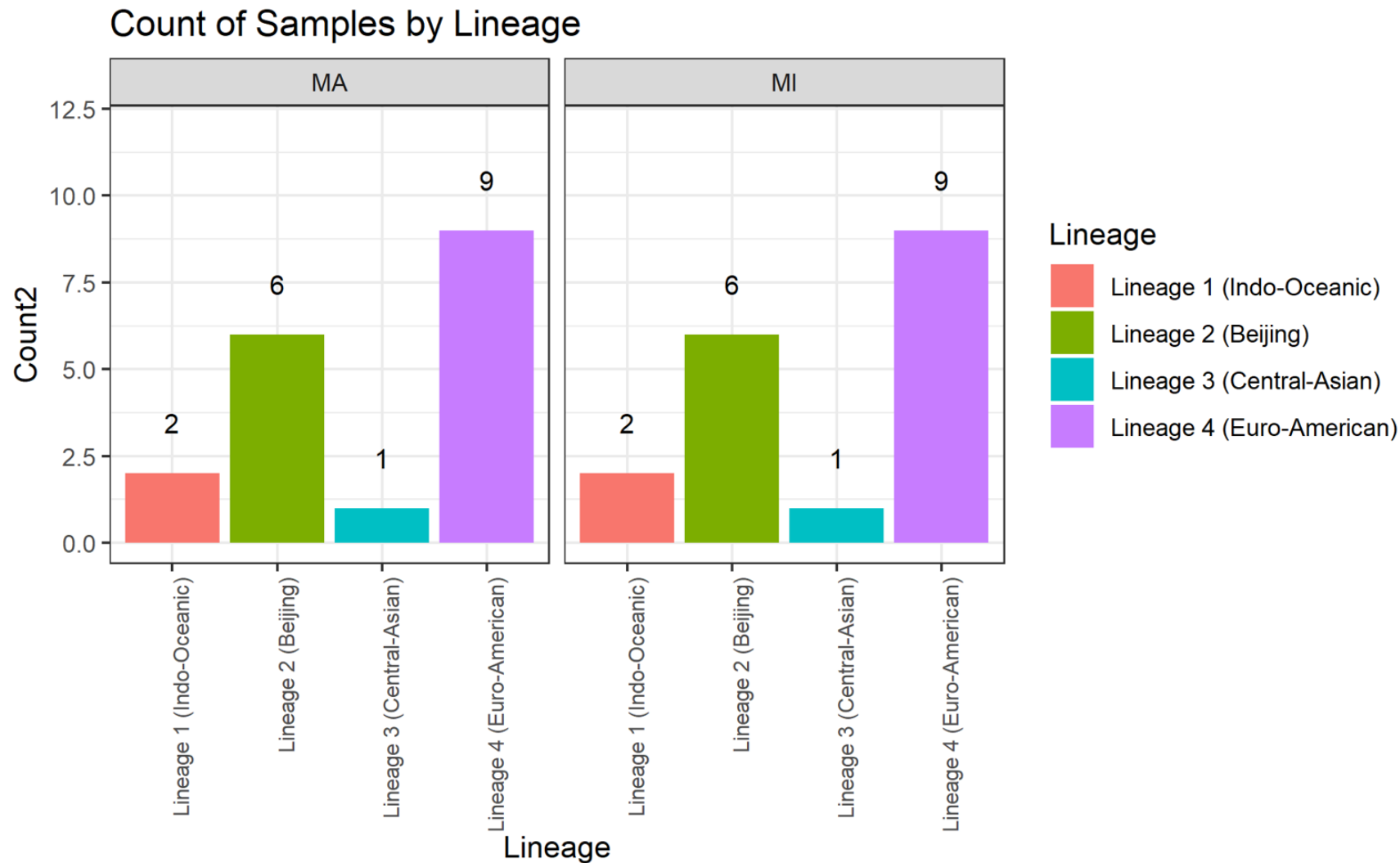
- 615 Sequences have been analyzed
 - 574 raw data files from MI
 - 41 sequenced in MA and analyzed in parallel
 - MI: NextSeq/MA: MiSeq
 - Early issues with fastq files generated from the NextSeq since resolved



Piloting the AIMS (NY) Pipeline: lineage determination



Analysis Comparison-Lineage



Genotypic vs Phenotypic Susceptibility

- 8/41 contained molecular resistance genes
- 4 failed coverage at a target site
 - unable to determine resistance
- 1 discordant result

	WGS Resistance (MA)	Conventional DST	WGS Resistance (MA)
1	Isoniazid=RESISTANT, Ethionamide=RESISTANT	Isoniazid, ethionamide R	Isoniazid, ethionamide R
2	Isoniazid=RESISTANT, Streptomycin=Not Determined, Kanamycin/Amikacin=Not Determined	Isoniazid R	Isoniazid R
3	Streptomycin=RESISTANT	Strep R	
4	Streptomycin=Not Determined	pan S	
5	Isoniazid=RESISTANT, Ethionamide=RESISTANT	Isoniazid, ethionamide R	
6	Streptomycin=RESISTANT	pan S	
7	Streptomycin=Not Determined	pan S	
8	Streptomycin=Not Determined	pan S	

Next Steps

Wet Lab	Analysis	QC	Communication
Optimize and validate extraction and library prep from primary clinical specimens	Pipeline is command line heavy; make user friendly; parse out the summary section	Finalize QC metrics and run acceptance criteria	What goes into the LIMS
V2 chemistry vs v3 chemistry	CLIA Validation lineage and MDDR	Compare against theiaprok and reference free pipeline	Do we report to providers or epi? And How?
CLIA Validation	How do we integrate real time surveillance?	Version control of pipelines	Concurrent testing for genotypic and phenotypic AST; messaging to providers and TB Control



150 YEARS
OF ADVANCING
PUBLIC
HEALTH

Massachusetts Department of Public Health

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Questions?

