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Because diagnosis matters

Standardizing NGS for Culture-free Drug Susceptibility Testing in Low and Middle Income Countries

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Why do we need NGS for TB Dx?

In 2016 WHO recommended that all patients with pulmonary MDR/RR-TB that is not resistant to second-line drugs should get short course TB Tx.[1]

580,000 new MDR/RR-TB cases in 2015[1]

<50,000 MDR/RR pts received SL DST in 2015[1]

1. WHO Global TB Report 2016

Next Generation Sequencing – Part of the Solution

PROS

- Comprehensive
- Flexible (open platform)
- Scalable (flexible throughput)
- Rapid (days vs weeks relative to phenotypic DST)
- Multi-use (RDST, surveillance, Tx monitoring, transmission mapping & non-TB uses)

CONS

- Expensive
- Complex (it's a method not an assay or solution)

Whole Genome vs Targeted NGS for RDST

Whole Genome Sequencing

Strengths

- Full genome sequenced
- Comprehensive solution

Weaknesses

- Slow
- Can't yet get Mtb WGS direct from sputum consistently or cost-effectively
- expensive
- complicated bioinformatics

Targeted Next Gen Sequencing

Strengths

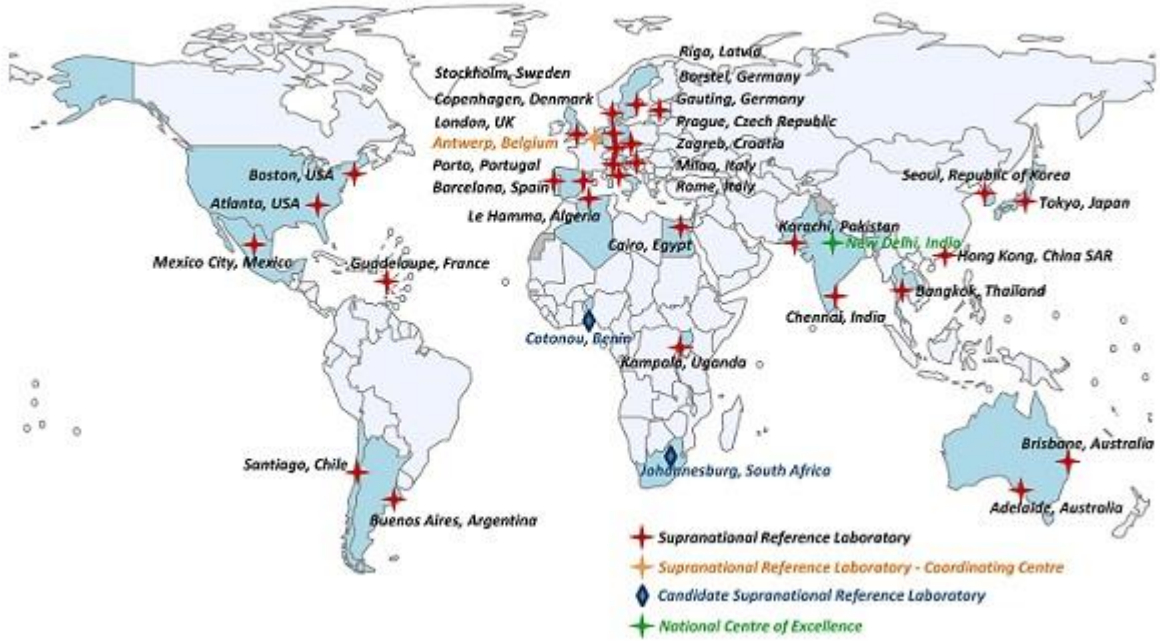
- Sequence DNA direct from sputum
- Up to 200 gene targets
- Faster
- Simpler
- Less expensive than WGS

Weaknesses

- Need some pre-knowledge of targets
- Less information than WGS

Goal

A Culture-Free, end-to-end NGS Solution for RDST in Reference Laboratories in LMICs



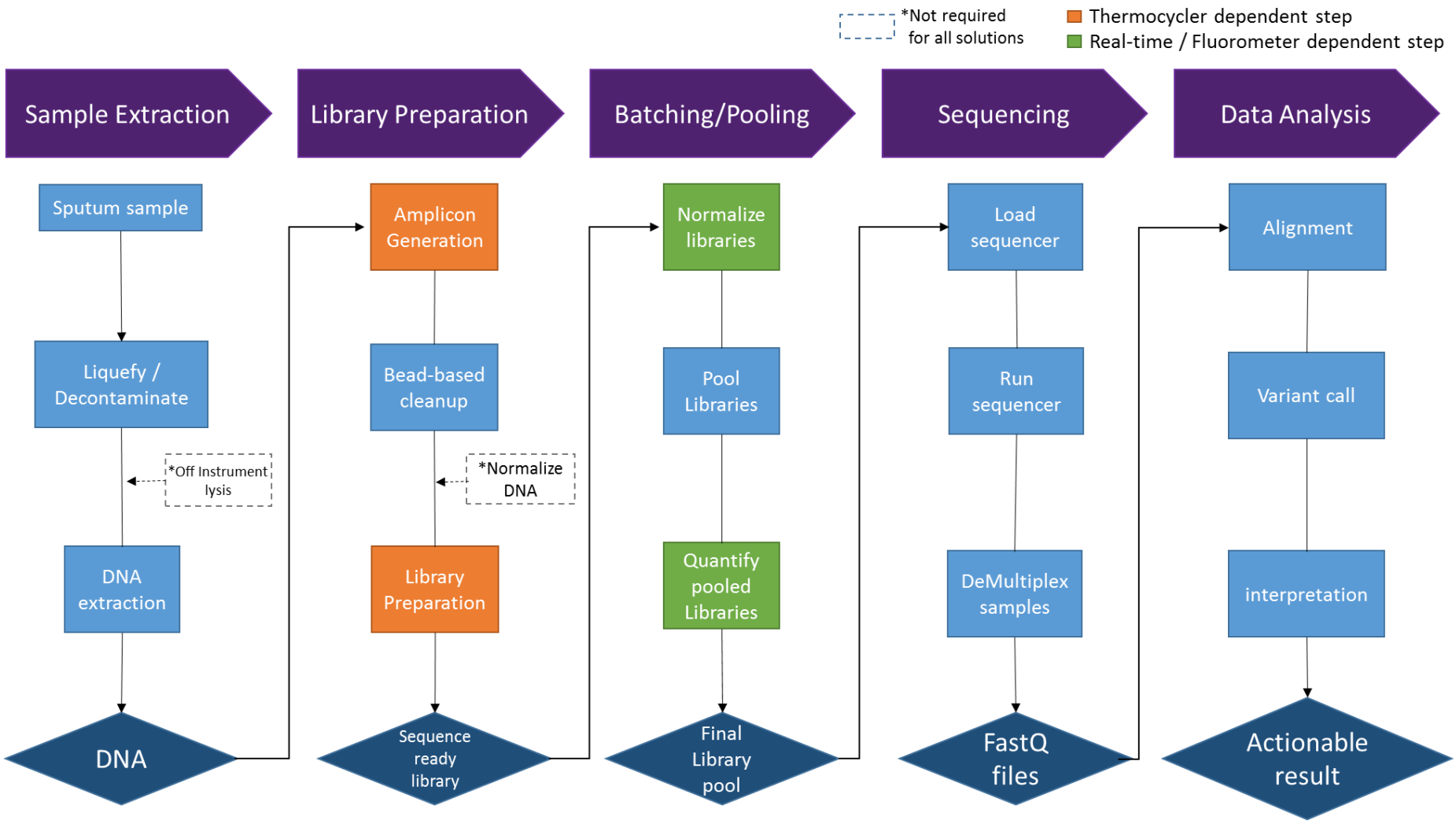
Source: http://www.stoptb.org/wg/gli/srln.asp

Minimum Acceptable Characteristics*

- Direct from sputum sample (culture-free, no BSL3)
- Detect primary 1st and 2nd line drug resistance mutations (XDR+PZA)
  - Open design – expandable to new mutations (e.g. Delamanid, Bedaquiline) and additional features (surveillance, Tx monitoring)
- Cost should be <30 USD (library prep + seq + analysis)
- LOD: 5000 Mtb genomes/reaction (0.01 ng DNA)
  - Optimal: 100 genomes
- Heteroresistance detection: 500-R/4500-S (<1:10 ratio)
  - Optimal: 1-R/99-S

*Draft FIND TPP

A Generic Targeted Amplicon NGS Workflow



Converting an NGS Method into an End-to-end Solution for RDST

- Simplifying and automating the workflow
- Reducing the number of instruments, consumables and reagents needed
- Decreasing costs
- Improving reliability and interoperability of component instruments and reagents

Proposed Simplified, Standard Solution for Targeted NGS RDST

Sample Extraction → Library Preparation → Batching/Pooling → Sequencing → Data Analysis

DNA Extraction Instrument (automated)

NGS Library Prep Instrument (automated)

Illumina MiSeqDx (automated)

Stand-alone or Cloud-based Analysis and Interpretation Software

Sample Instrument images for demonstration purposes only

Integrated NGS Solution – Gen I

	Simplified PCR	Comprehensive Nextera
Instrumentation required	3-4 instruments	3-5 instruments
Approx. total instrumentation costs[1]	~$150,000	~$150,000
Approx total reagent/consumable cost/ sample[2]	~$37 USD	~$90 USD
Approx. time to result[2]	~29 hrs	~62 hrs
Number of steps in assay	4	<10
Drug Resistance Detected	XDR+PZA	XDR+PZA+expanded
Gene targets interrogated	partial genes	Full genes
Mycobacterial species identification	no	yes
Individual MTBC lineages/major genotypes differentiated	no	yes

[1]Rough FIND estimate based on retail Pricing
[2]Rough FIND estimate of potential time and cost only. Based on retail PCR and Nextera reagents and consumables likely to be needed

Next Steps

- Conduct comparative performance analyses of top 5 sample extraction instruments and top two target amplicon NGS assays (Q1-2 ’17)
- Integrate instruments and SOPs into Gen I end-to-end NGS solution (Q3 ’17)
- Verify and validate prototype integrated solution performance in at least 1 SNRL Q4 ’17
- Evaluate final version in 3 NRLS Q1-3 ’18
- Submit for CE IVD/WHO endorsement Q4 ‘18

Conclusions

- A validated end-to-end, targeted amplicon NGS RDST solution for DR-TB by end 2018
- Simplified, mostly automated benchtop workflow
- Suitable for Cx-free RDST and surveillance
- Instrumentation costs <USD 150,000
- Reagents and consumable costs <40/sample
- Time to result in 1-2 days

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