



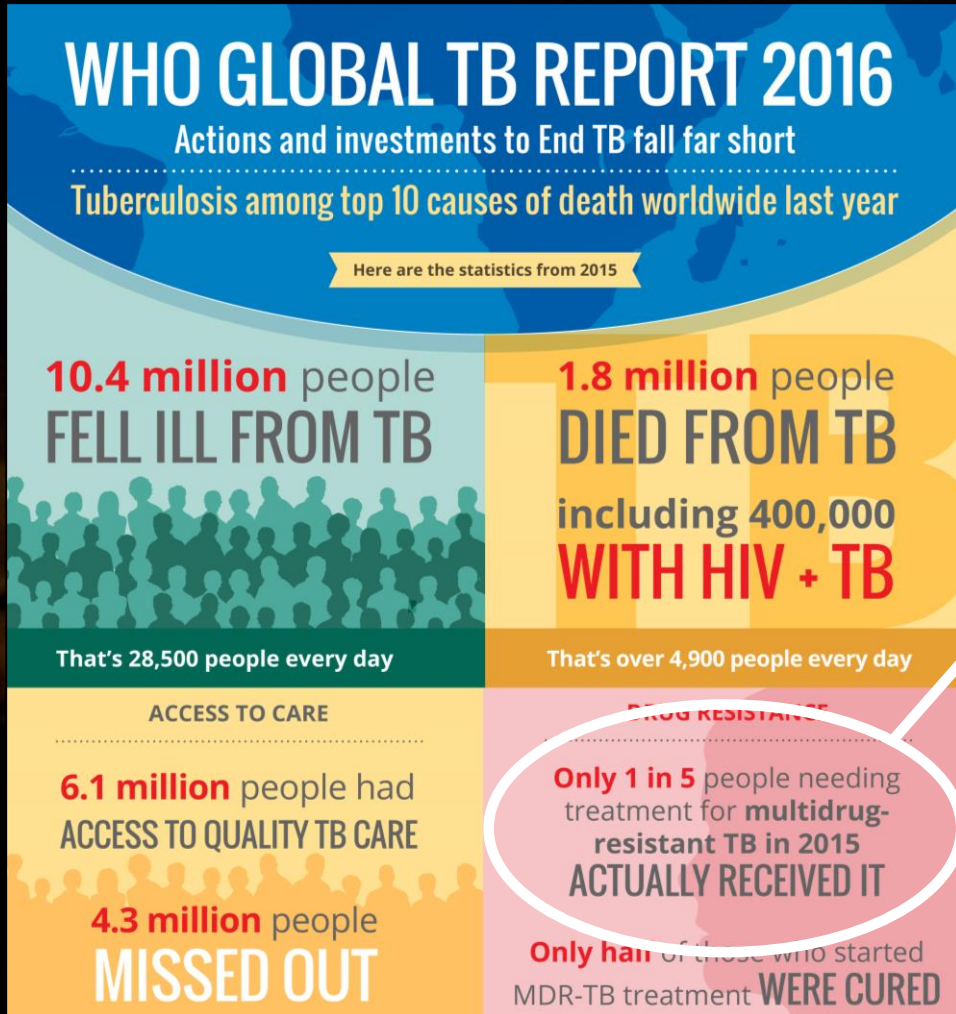
Programmatic Implementation of NGS for TB & Future Plans for the ReSeqTB Knowledgebase

Tim Rodwell, Rebecca Colman, Anita Suresh & Claudia Denking

A TB Patient Dies Every 18 Seconds



A TB Patient Dies Every 18 Seconds



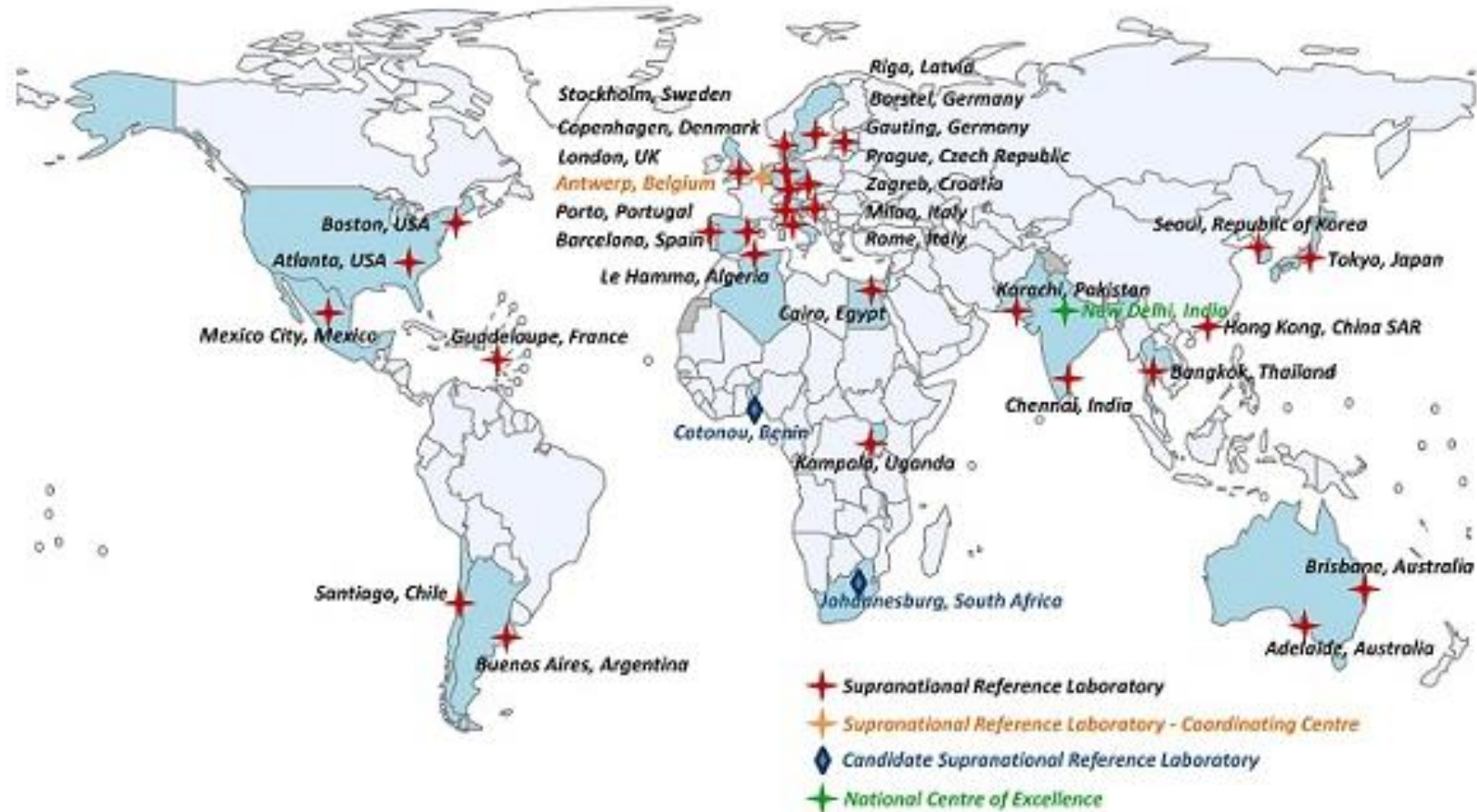
~20% of 580,000
MDR patients were
diagnosed 2015

<10% of MDR pts
got 2nd line DST



Next Generation Sequencing – Part of the Solution

Goal: Culture-Free, NGS for Rapid DST in TB Reference Laboratories by 2020





Next Generation Sequencing – Part of the Solution

PROS

- High Throughput
- 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

- Can be expensive if not implemented or used correctly
- Complex (it's a method not an assay or solution)



NGS for TB RDST – Min 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 <50 USD (library prep + seq + analysis)
- LOD: 5,000 Mtb genomes (Scanty AFB ~10⁴ genomes)
 - Optimal: 100 genomes
- Heteroresistance detection: 500-R/4500-S (10%)
 - Optimal: 1-R/99-S



Whole Genome vs Targeted NGS for RDST

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

Whole Genome Sequencing

■ Strengths

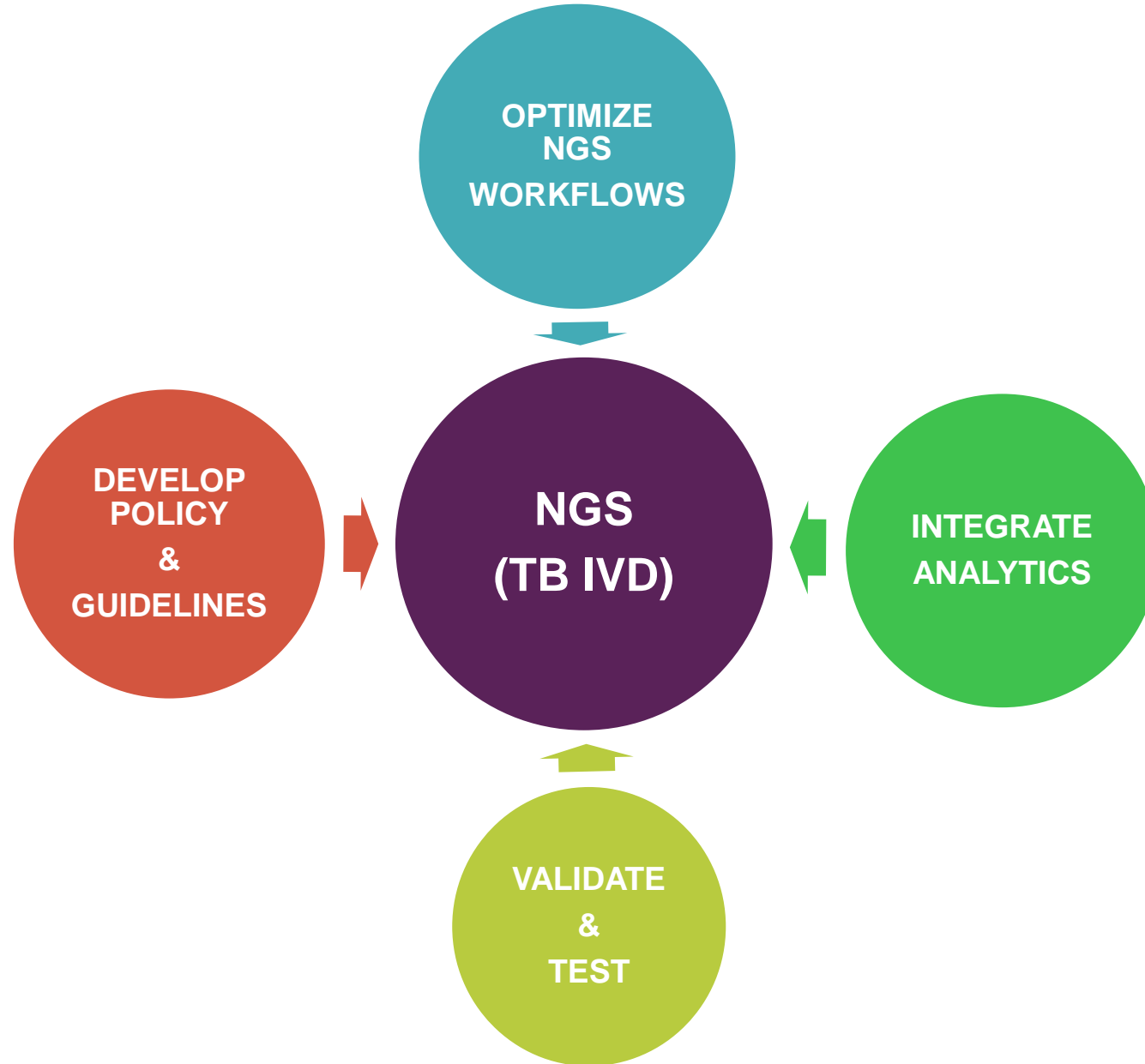
- Full genome sequenced
- Comprehensive solution

■ Weaknesses

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



Implementing NGS for Surveillance & Dx of DR TB





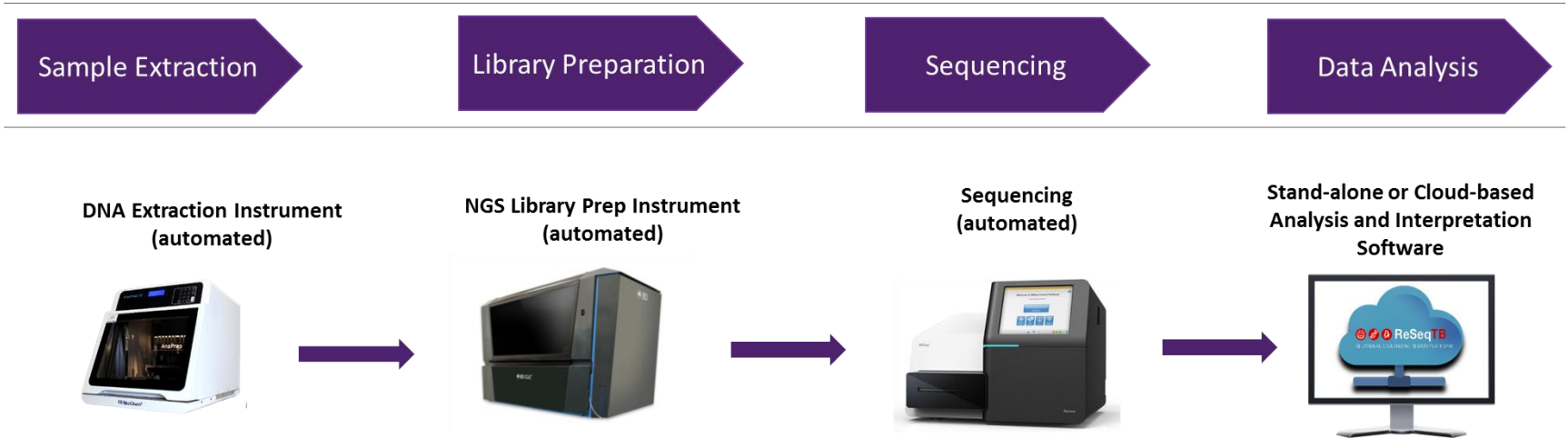
Implementing NGS for Surveillance & Dx of DR TB





Implementing NGS for Clinical Diagnosis of DR TB

OPTIMIZE NGS
WORKFLOWS



Ongoing & Recently Completed Studies

■ H2H Evaluations - DNA Extraction

- Landscape of 18 instruments – chose top 4 (Genolution, Molbio, Diasorin & Claremontbio)
- H2H evaluation completed using FIND standard samples – data analysis ongoing

■ H2H Evaluations – Targeted NGS Assays for Clinical Dx of DR TB

- 4 Cx-free, end-to-end TB NGS solutions
- 3 sequencing technologies (Illumina, Thermo Fisher, Qiagen)
- Analytical studies using FIND standards, synthetic mixtures and clinical samples

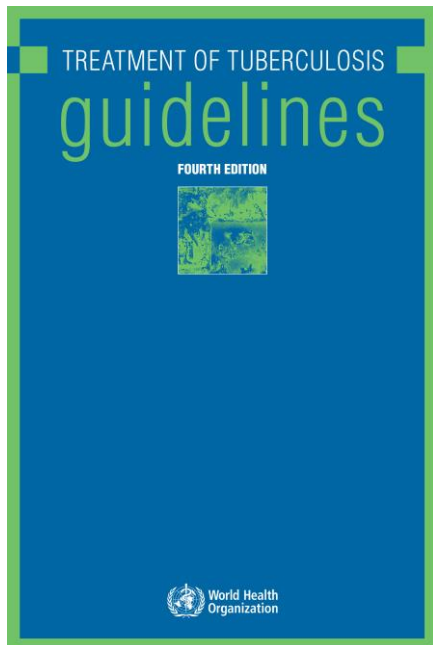


Implementing NGS for Clinical Diagnosis of DR TB

DEVELOP
POLICY
&
GUIDELINES

Ongoing Policy & Guidelines Development

- Manual: Use of Sequencing Technologies For The Detection of Mutations Associated with Drug Resistance in *Mycobacterium Tuberculosis* Complex
- Guideline: Use of Sequencing as a Reference Standard
- Guideline: Use of Sequencing for Clinical Diagnosis of DR TB
- Target Product Profiles (TPP)
 - DNA Extraction for NGS (drafted)
 - NGS Assays for Dx of DR TB (drafted and reviewed)
 - Respondents from 45 countries (NTPs, Industry, NGOs, researchers, clinicians, laboratorians)
 - >50% agreement on all characteristics, >75% agreement on majority of characteristics
 - NGS Analysis (not started)

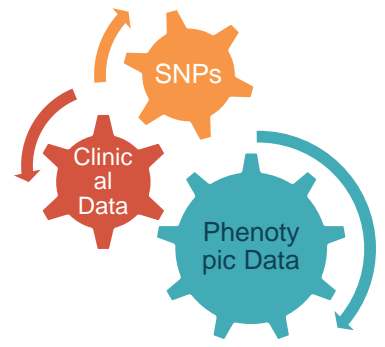




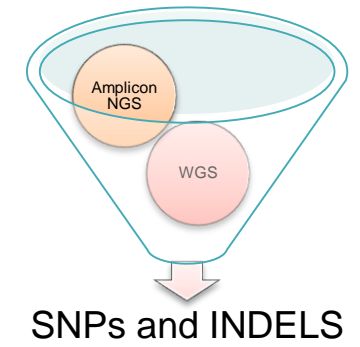
Implementing NGS for Clinical Diagnosis of DR TB

INTEGRATE ANALYTICS

The ReSeqTB Knowledgebase



1. A standardized and validated NGS variant detection pipeline
2. A regulatory-grade, curated database of genotypic, phenotypic and clinical data
3. A data analysis tool for reporting clinically relevant mutations



MYCOBACTERIUM TUBERCULOSIS
WHOLE GENOME SEQUENCING REPORT

NOT FOR DIAGNOSTIC USE

Patient Name	JOHN DOE	Patient ID	12345678910
Birth Date	2000-01-01	Location	SOMEPLACE
Sample Type	SPUTUM	Sample Date	2016-12-25
Sample Source	PULMONARY	Collection Method	INDUCED SPUTUM
Sample ID	A12345678	Barcode	
Reporting Lab	LAB NAME	Report Date/Time	2017-01-01, 15:36
Requested By	REQUESTER NAME	Requester Contact	REQUESTER@EMAIL.COM

Summary
The specimen was positive for *Mycobacterium tuberculosis*. It is resistant to isoniazid, rifampin, ofloxacin, moxifloxacin, amikacin, and capreomycin.

Organism
The specimen was positive for *Mycobacterium tuberculosis*, lineage 2.2.1 (East-Asian Beijing).

Drug Susceptibility
Resistance is reported when a high likelihood (>5, see page 2) resistance-conferring mutation is detected in the *esr* promoter, *catB*, *gyrA*, *gyrB*, *rpoB*, *hspR*, *hspX*, *hspY*, *hspZ*, *hspV*, and *hspU*. No mutation detected does not exclude the possibility of resistance.

Interpretation	Drug	Resistance Gene (Mutation, Alternate Allele %)
Resistant	Isoniazid	<i>katG</i> (Ser315Thr, 100%)
Resistant	Rifampin	<i>rpoB</i> (Ser531Leu, 100%) *Rifabutin resistance likely
First Line	Ethambutol	No mutation detected
Sensitive	Pyrazinamide	No mutation detected
	Ofloxacin	<i>gyrA</i> (Asp90Val)
	Moxifloxacin	<i>gyrA</i> (Asp90Val, 1.4%) *At least low-level resistance predicted
Resistant	Amikacin	<i>rrs</i> (A1401G) (nucleotide)
Second Line	Capreomycin	<i>rrs</i> (A1401G) (nucleotide)
Sensitive	Kanamycin	Expert consultation advised
	Ethionamide	No mutation detected



Implementing NGS for Clinical Diagnosis of DR TB

INTEGRATE
ANALYTICS

Legend

- Data in platform or being curated
- Data/samples transferred or DCA completed
- Contribution in discussion



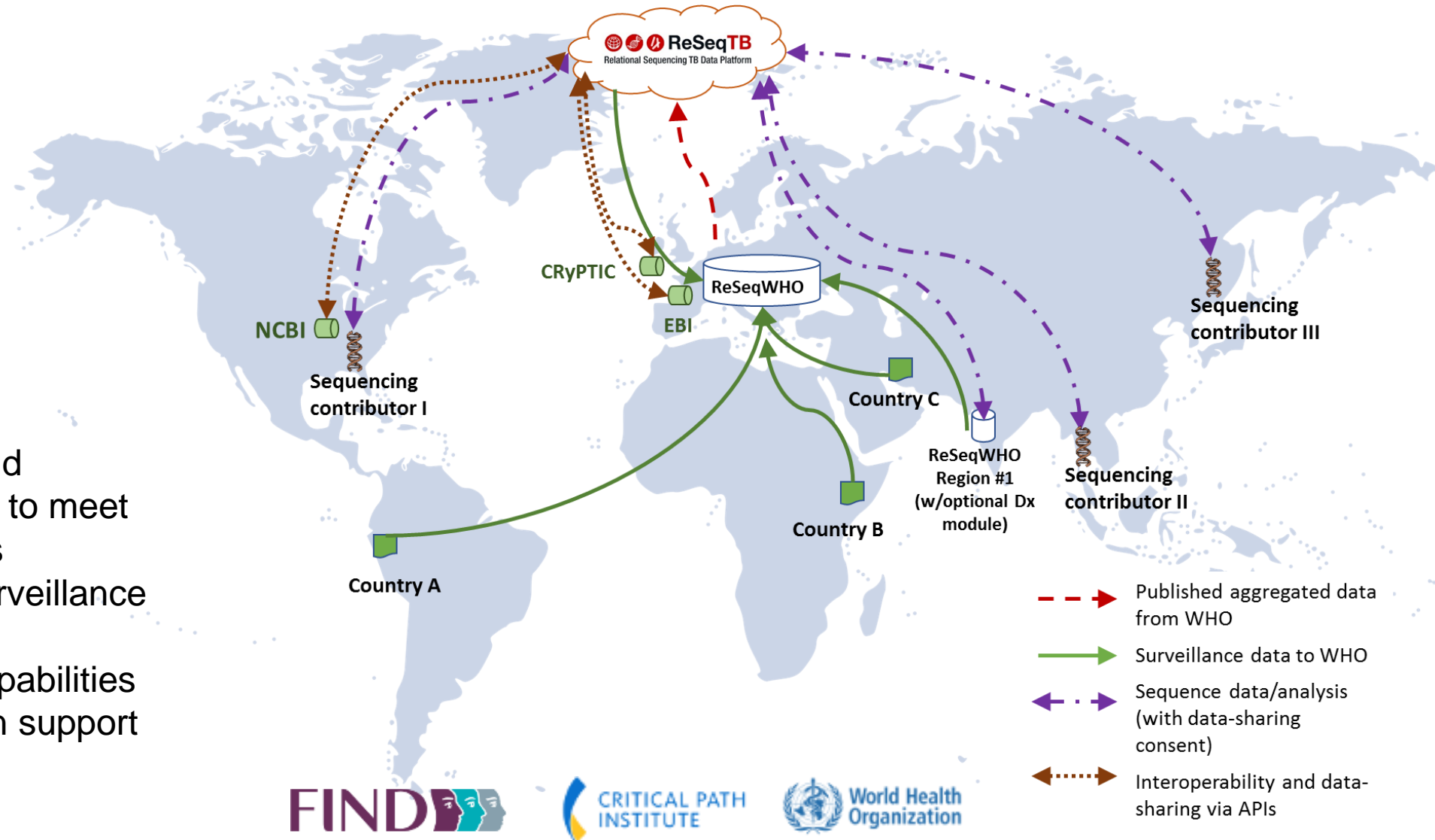


Future Strategy for ReSeqTB – FIND, C-path & WHO

INTEGRATE ANALYTICS

ReSeq 2.0

- Move ReSeqTB to Cloud
- Strengthen architecture to meet regulatory requirements
- Transfer to WHO for surveillance network (ReSeqWHO)
- Build interoperability capabilities
- Build treatment decision support tools

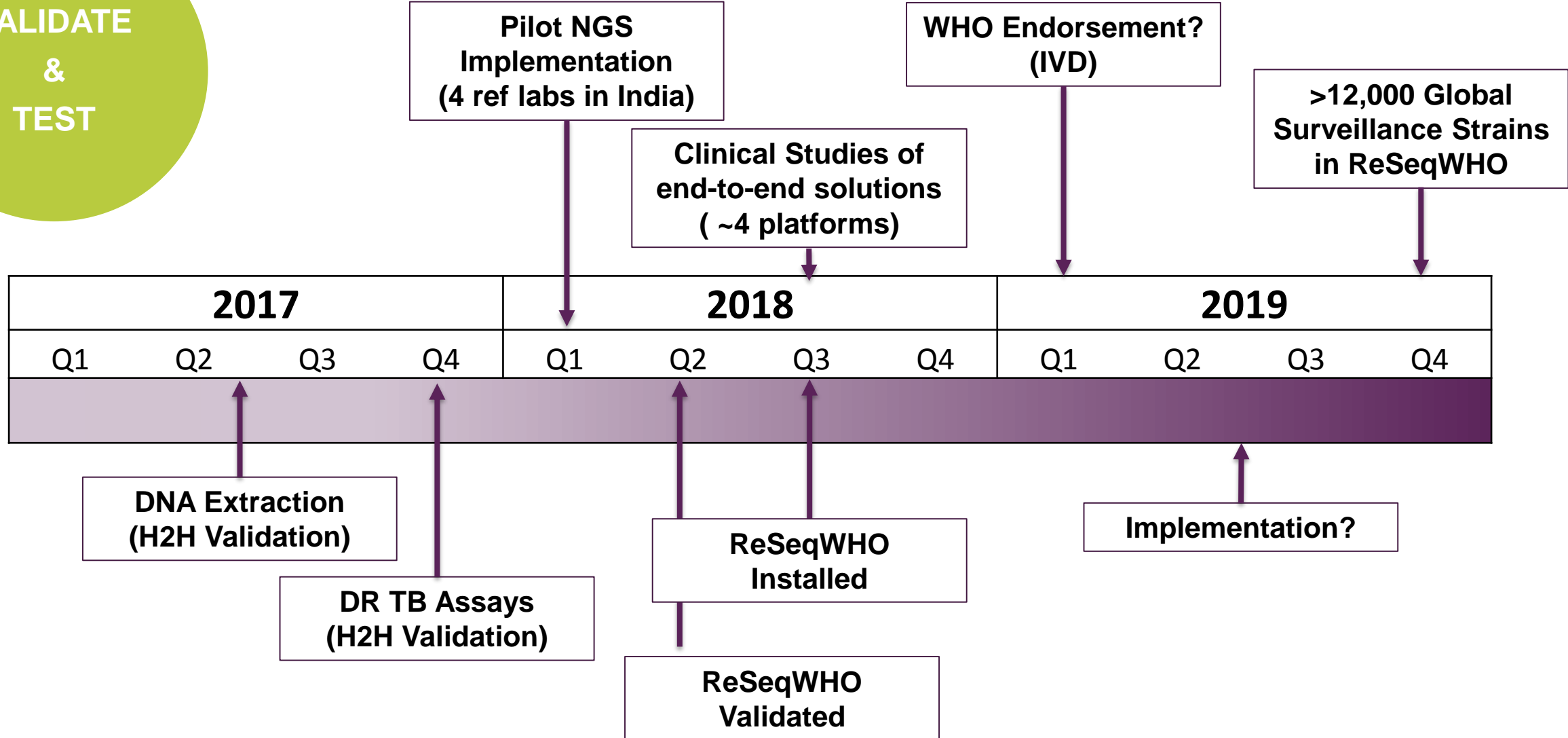


- ▶ Published aggregated data from WHO
- ▶ Surveillance data to WHO
- ▶ Sequence data/analysis (with data-sharing consent)
- ⋯▶ Interoperability and data-sharing via APIs





Timelines for Validation, Testing and Implementation



Conclusions

Implementation of Cx-free, Targeted NGS

- Feasible, cost-effective and pragmatic
- Rapid and comprehensive DST Solution
- Field is moving quickly and we need to move fast but methodically
- Industry interest is gaining momentum
- The 2020 plan for implementation is both ambitious and not aggressive enough




Thank you



BILL & MELINDA
GATES foundation

 **The Global Fund**
To Fight AIDS, Tuberculosis and Malaria

 **Australian Government**
AusAID

 Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Swiss Development and Cooperation:
State Secretariat for Education, Research and Innovation

 REPUBLIQUE
ET CANTON
DE GENEVE

 **UKaid**
from the British people

 **CDC**
CENTER FOR DISEASE
CONTROL AND PREVENTION

 **World Health
Organization**

 **Stop TB Partnership**
New Diagnostics Working Group

DFID Department for
International
Development

 Development Cooperation
Ministry of Foreign Affairs
Netherlands Enterprise Agency

SPONSORED BY THE

 Federal Ministry of
Education
and Research
KFW



Stop TB Partnership
TB REACH

UNITAID

 **CRITICAL PATH
INSTITUTE**

ReSeqTB Expert Consortium