Development of <u>o</u>ligonucleotide <u>l</u>igation <u>a</u>ssay (**OLA**) and lateral flow test to detect <u>m</u>ulti-<u>d</u>rug <u>r</u>esistant <u>t</u>u<u>b</u>erculosis (**MDR-TB**) for Kenyan population

Brian Tran, Kyla Yamashita, Inyoung Seo, Whitney Nakata, Ronald Odero, Barry Lutz, <u>Nuttada Panpradist</u>

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Development of oligonucleotide ligation assay (OLA) and lateral flow test to detect multi-drug resistant tuberculosis (MDR-TB) for Kenyan population

Brian Tran, Kyla Yamashita, Inyoung Seo, Whitney Nakata, Ronald Odero, Barry Lutz, Nuttada Panpradist

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2023 Biomedical Engineering Society Meeting, Seattle, Washington
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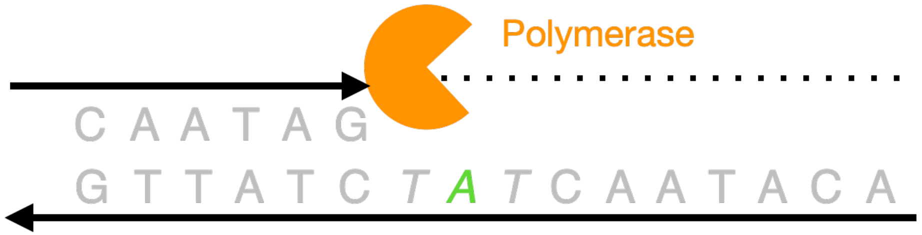
Fund: 2022 Seattle Tuberculosis Research Advancement Center (SEATRAC) New Investigator Award (PI: Panpradist)

Outline

- **Platform technology**: What is OLA? OLA-Simple?
- **Bi-directional collaboration with local Kenyan researchers** to develop OLA and lateral flow test for MDR-TB
  - *Need identification*
  - *Pipeline for implementation*
  - *Tech knowledge transfer plan*

OLA = Oligonucleotide ligation assay; OLA-Simple = simplified OLA; MDR-TB = multi-drug resistance TB

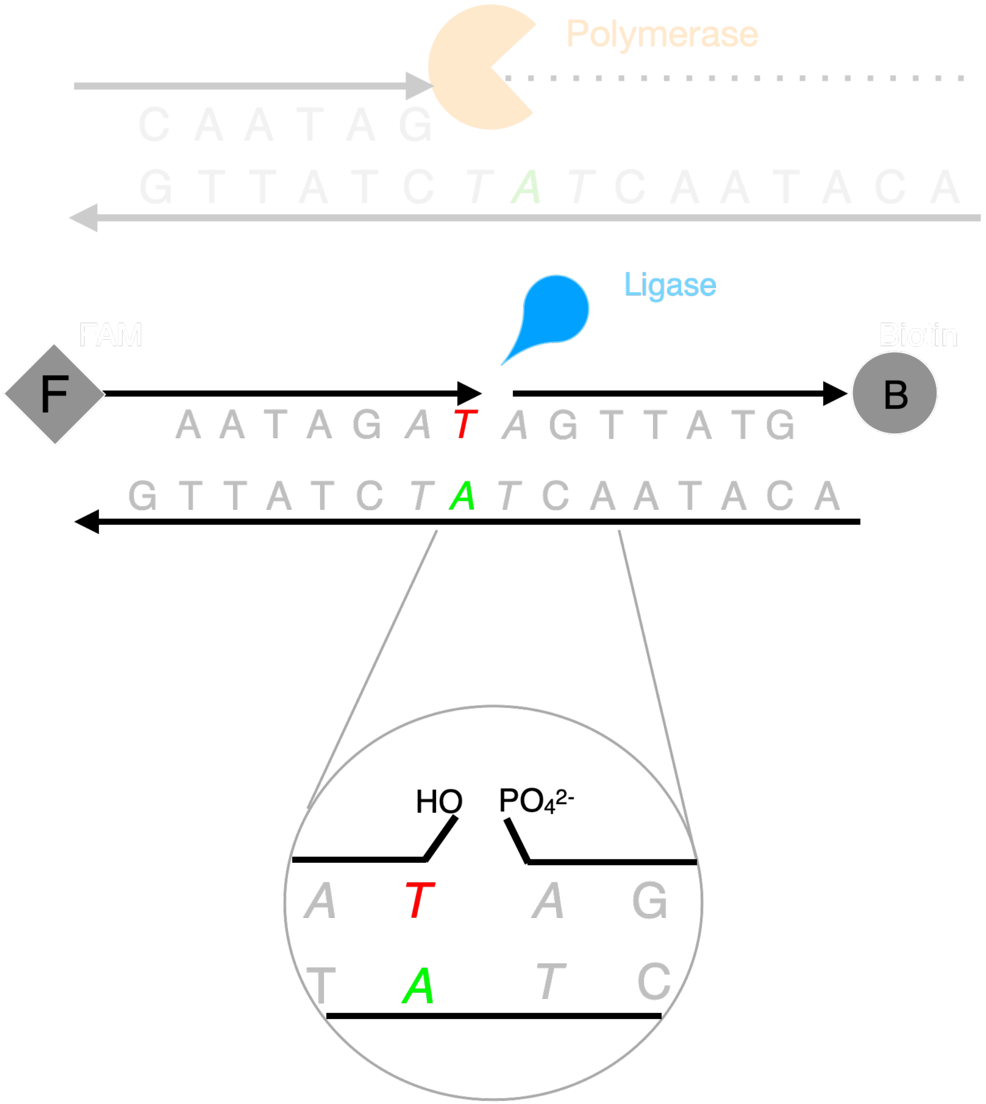
What is OLA?



High sensitivity

polymerase chain reaction (PCR) to amplify 100 to billion copies

OLA: a sensitive and specific method to detect known point-mutations

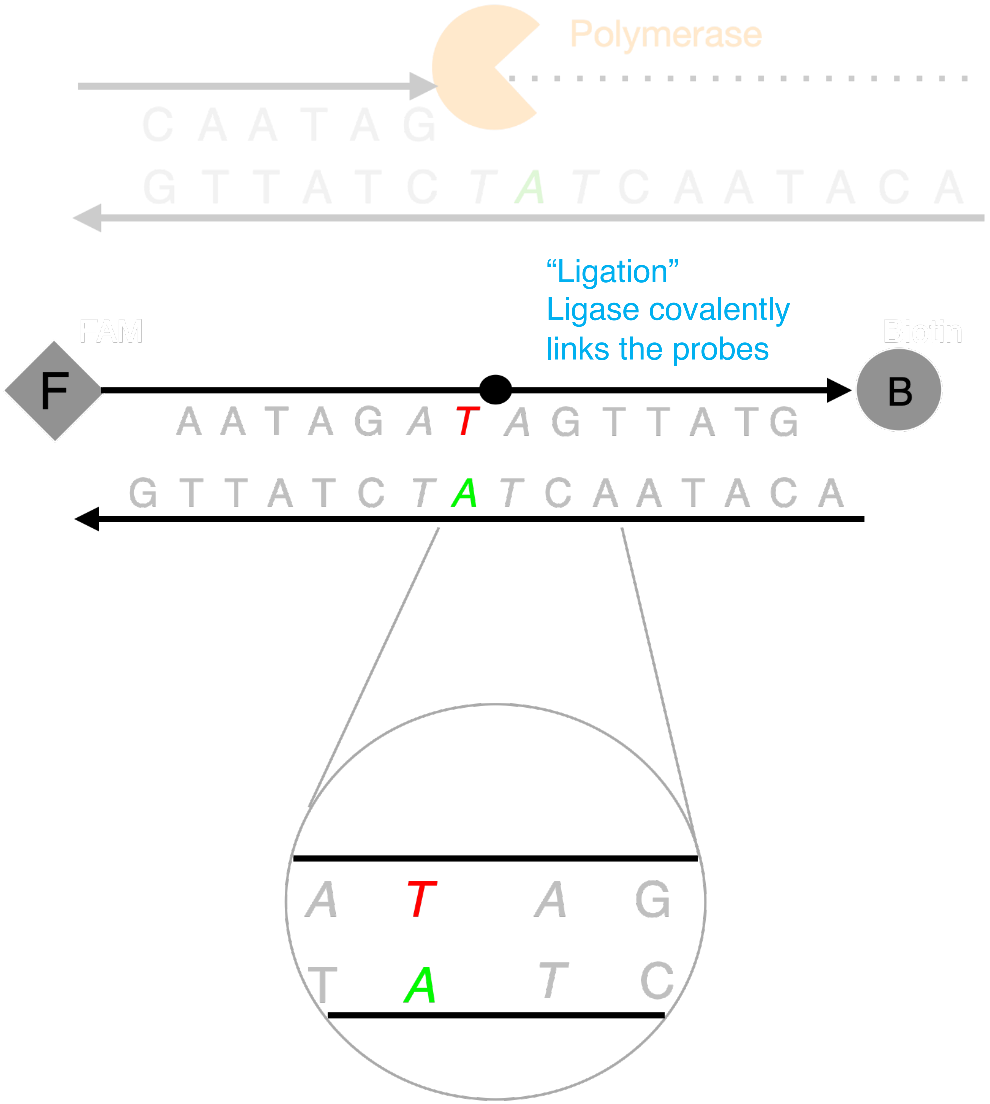


High sensitivity
polymerase chain reaction (PCR) to amplify 100 to billion copies

High specificity via ligase detection reaction (LDR)
Ligation of probes does occur when the bases are matched

* Point-mutations are often referred to as "SNP" which stands for single nucleotide polymorphisms

OLA: a sensitive and specific method to detect known point-mutations



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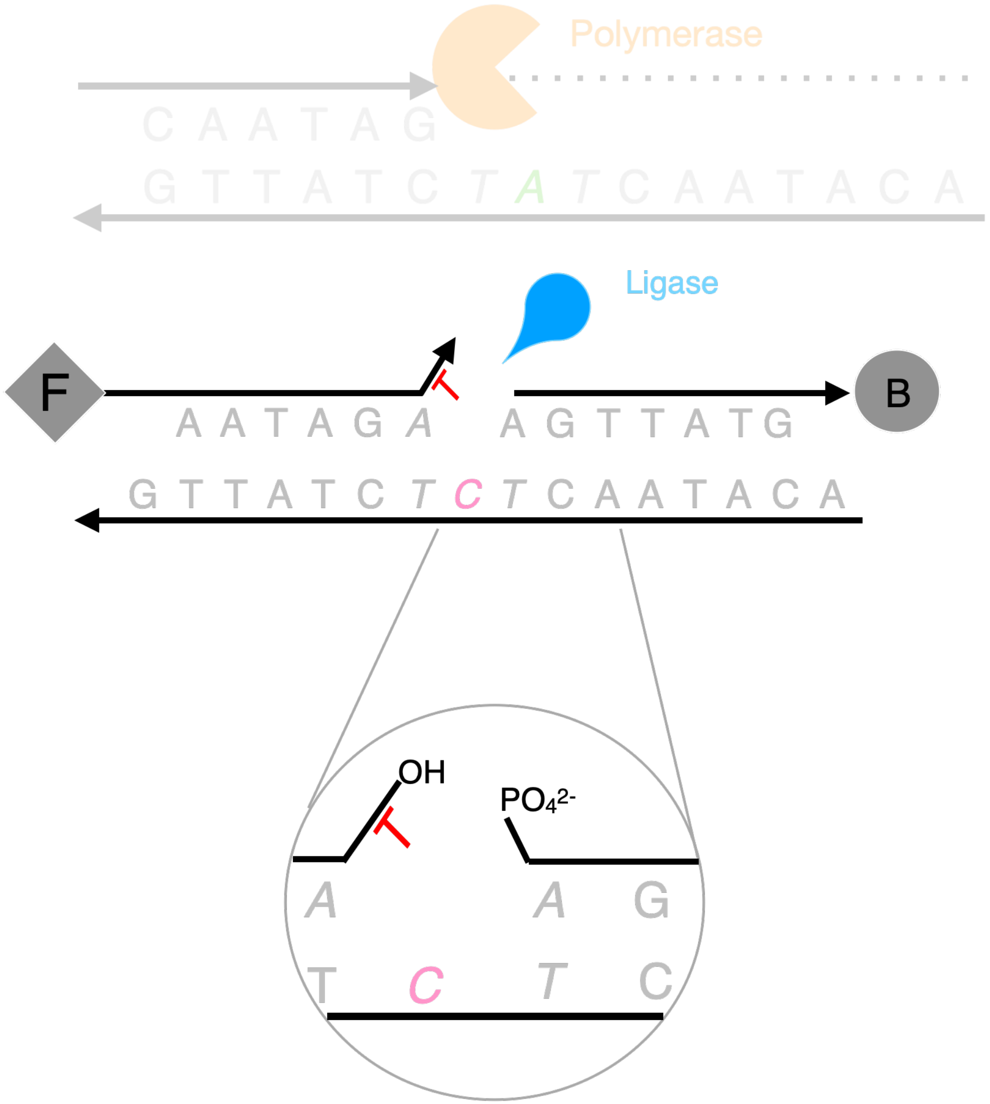

High sensitivity
polymerase chain reaction (PCR) to amplify 100 to billion copies

High specificity via ligase detection reaction (LDR)
Ligation of probes does NOT occur when the bases are MISmatched

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OLA: a sensitive and specific method to detect known point-mutations

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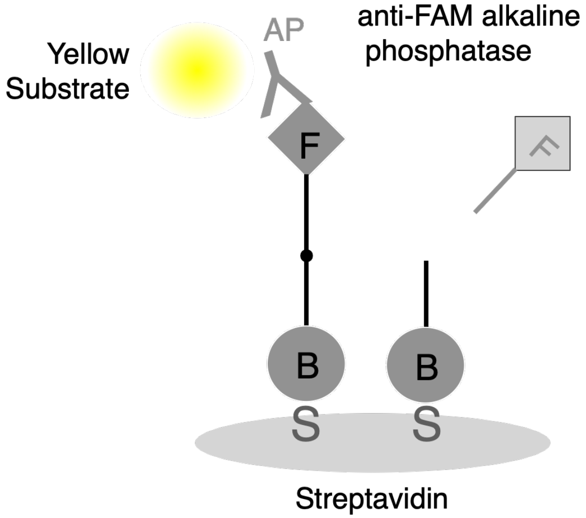
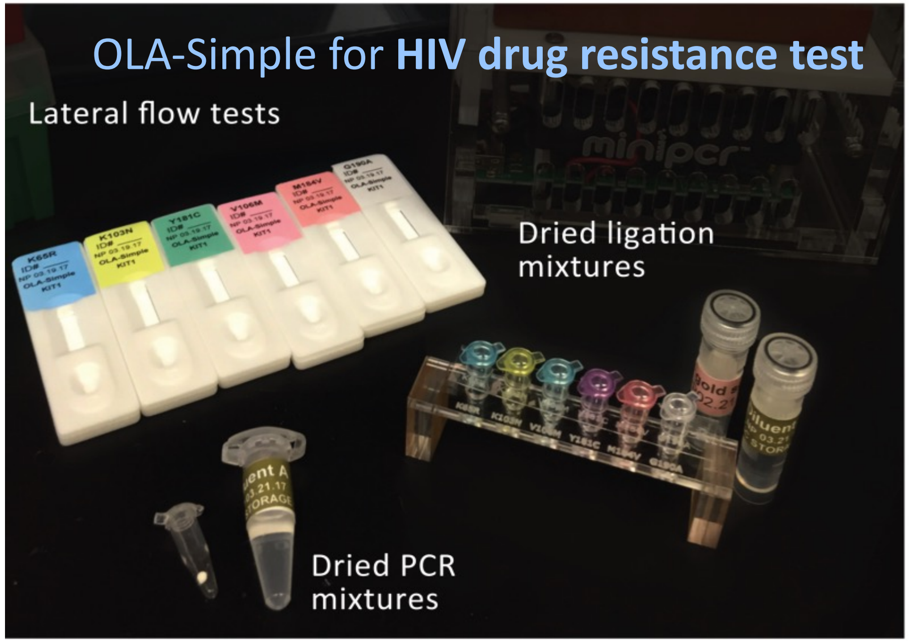


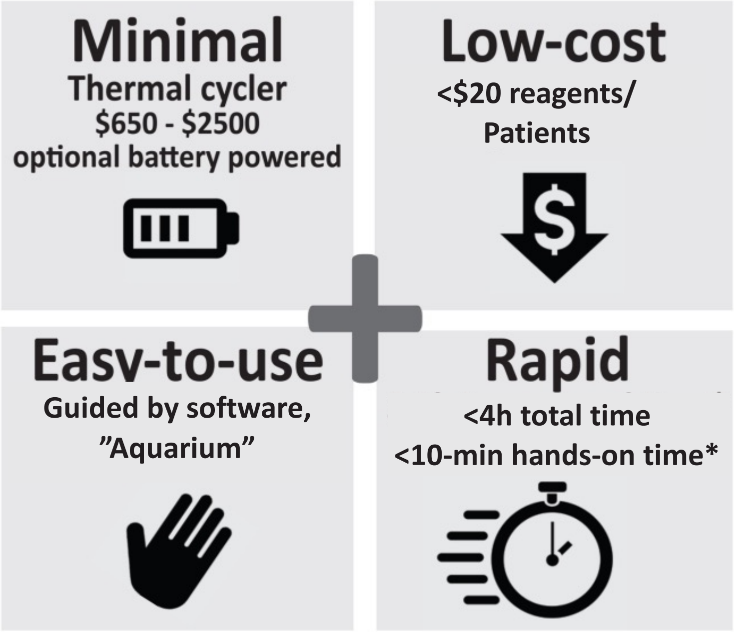
Enzyme Linked ImmunoSorbent Assay (ELISA)
Only ligated probes are detected

* Point-mutations are often referred to as “SNP” which stands for single nucleotide polymorphisms

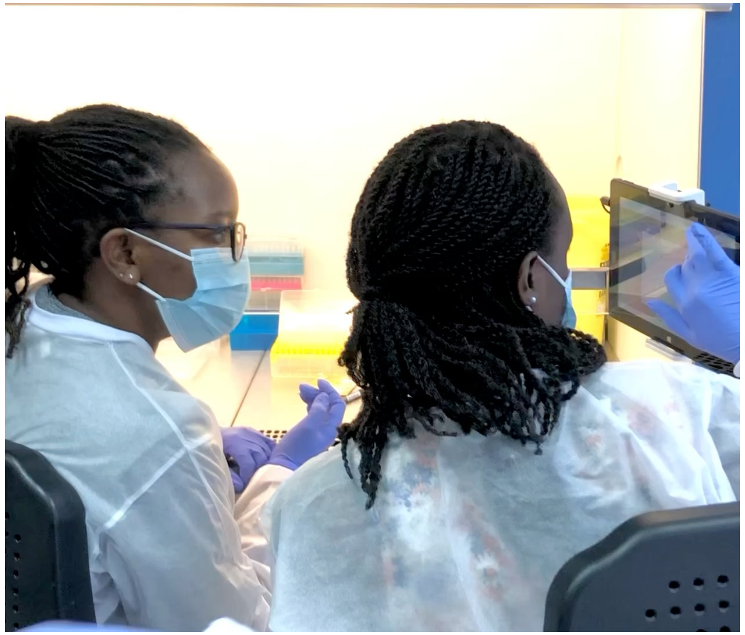
OLA-Simple: a simplified OLA platform for point-mutation detection

- *Ready-to-go dried mixtures* – easy assay set up
- *Lateral flow tests* – visual readout
- *Interactive software “Aquarium”* – 1st-time users showed 97% accuracy operating OLA-Simple [1,2]
    - **Near point-of-care -** simple enough that a hospital lab can perform.





* excluding sampe prep step



My PhD thesis 2021 (PI: Barry Lutz) [1] N Panpradist, et.al. 2019. Ebiomedicine; [2] N Panpradist and J Vrana et al. 2021 PLOS Global Health

Ronald and Preston:

“Can we make an OLA-Simple for MDR-TB detection?”`

Beginning of MDR-TB project – during my visit in Kenya

Outline

- **Platform technology**: What is **OLA**? **OLA-Simple**?
- **Bi-directional collaboration with local Kenya researchers** to develop OLA and lateral flow test for MDR-TB
  - Need assessment
  - Pipeline for implementation
  - Tech knowledge transfer plan

OLA-Simple for MTB-DR?

Need assessment

- Why is MTB-DR test needed?
- Where is the gap in the existing methods?
- What does OLA-Simple offer?

Implementation plan

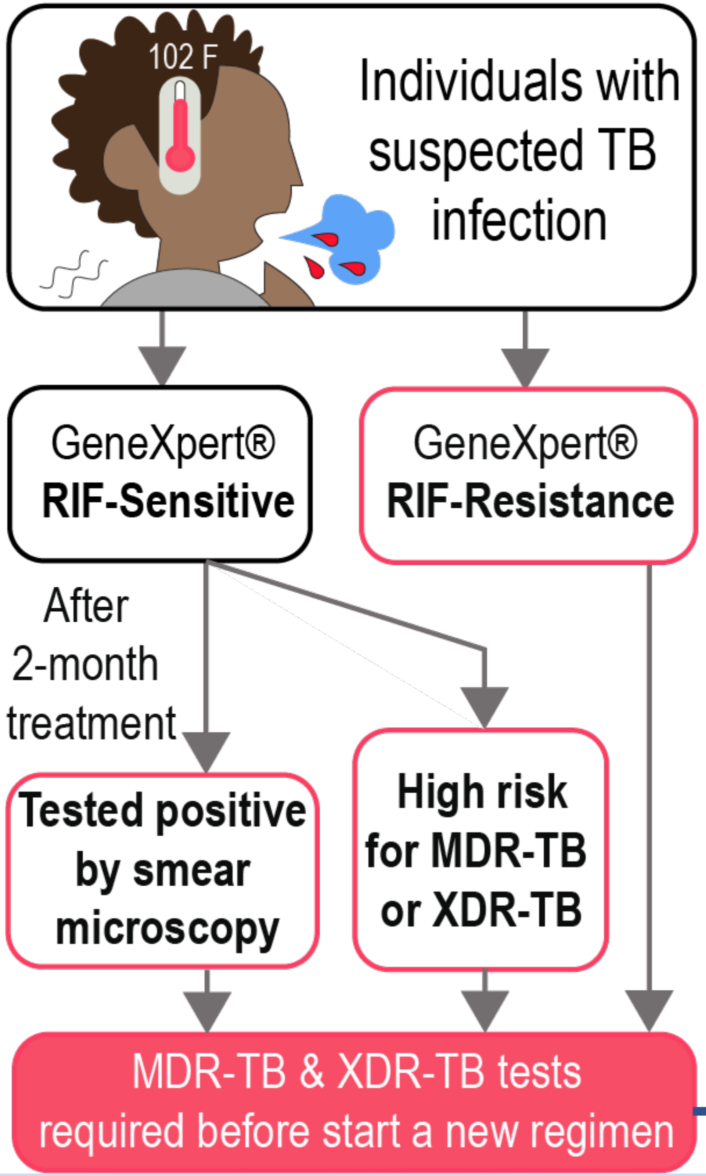
Tech knowledge Transfer / R&D

Need assessment: clinical need for MDR-TB test

- Tuberculosis (TB) is the **2nd leading cause of death by infectious disease worldwide, _10% of children deaths in Kenya_.**
- Multi-drug resistant TB (MDR-TB) is resistant to both **rifampicin (RIF) or isoniazid (INH) – _in Kenya about 2% in the untreated; 10% in the previously treated population_.**
- MDR-TB test results inform **clinicians to select proper treatment**, improving treatment outcome and reducing transmission
- **Technical compatibility** – a set of **point-mutations** determined by WHO to be associated with MDR-TB.

Gavi, 2023;

Need assessment: where is the gap for MDR-TB test in Kenya?

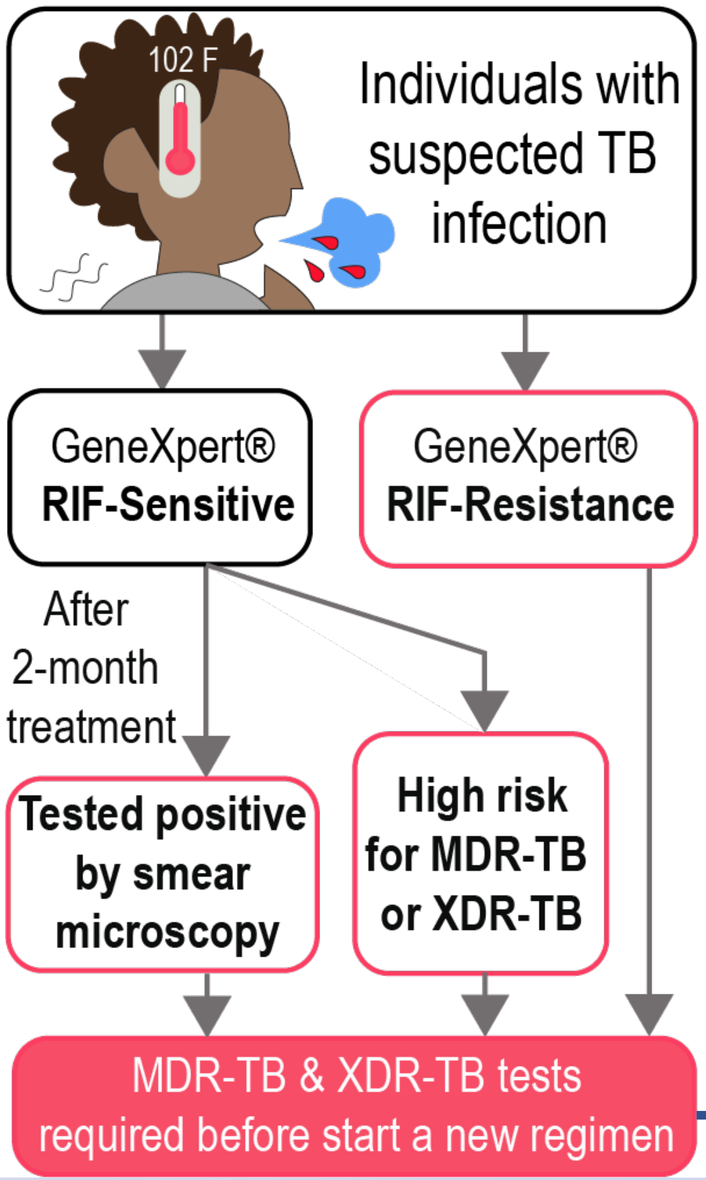


	Xpert® MTB/RIF	FL LPA
1) Coverage		
INH resistance	No	Yes
RIF resistance	Yes	Yes
2) Equipment		
Thermal cycler*	>$12,900	>$3000
Sequencer	No	No
Ultrasonic bath	No	Yes
3) Consumables		
Waste	Toxic	Non-toxic
Cost / sample**	$77.9	$12
4) Usability		
Turn around	<2h	72h
Training	Minimal	Extensive

(First-line Line Probe Assay)

* The smallest module cost, ** if not subsidized

Need assessment: where is the gap for MDR-TB test in Kenya?



	Xpert® MTB/RIF	FL LPA	OLA-Simple (proposed)
1) Coverage			
INH resistance	No	Yes	**Yes**
RIF resistance	Yes	Yes	**Yes**
2) Equipment			
Thermal cycler*	>$12,900	>$3000	**>$500**
Sequencer	No	No	**No**
Ultrasonic bath	No	Yes	**No**
3) Consumables			**No**
Waste	Toxic	Non-toxic	**Non-toxic**
Cost / sample**	$77.9	$12	**$10**
4) Usability			
Turn around	<2h	72h	**3h**
Training	Minimal	Extensive	**Minimal**

* The smallest module cost, ** if not subsidized

OLA-Simple for MTB-DR?

Need assessment

- Why MTB-DR test is needed?
- Where is the gap in the existing methods?
- What does OLA-Simple offer?

Implementation plan

- What is the regulatory pathway?
- How did the test kits get manufactured at scale?
- Who will pay for it?

Tech knowledge Transfer / R&D

Implementation plan and local manufacturing partner:

- Reference lab (running FL LPA routinely) – access to **specimen panels with known status** (enriched for mutation)
- KEMRI in Nairobi has experienced developing an antigen-based **lateral flow test**
- KEMRI identifies **regulatory pathway**.
- Government currently covers the cost of MDR-TB test (Xpert and LPA) – our team is connected **national TB program**.

FL LPA – first line line probe assay

OLA-Simple for MTB-DR?

Need assessment

- Why MTB-DR test is needed?
- Where is the gap in the existing methods?
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Implementation plan

- What is the regulatory pathway?
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- Who will pay for it?

Tech knowledge Transfer / R&D

- How can we reduce the R & D cost?
- How can we maximize engagement with local researchers?

R & D pipeline for OLA-Simple MDR-TB

1. *In-silico* design of probes

2. New high-throughput screening method based on melt analysis

3. Transfer OLA into OLA-Simple format using labeled probes

- Consensus sequence of *rpoB, katG, inhA* from European Nucleotide Archive.
- Wild-type (**WT**), mutant (**MUT**), and common (**COM**) probes corresponding to each mutation

10 mutations are associated with RIF and INH resistance									
INH			RIF						
inhA		*katG*	*rpoB*						
c-777t	g-154a	S315T	H455L	L452P	S450L/W	S450F	H445Y/D	D435Y	D435V

Inyoung Seo, Brian Tran, Barry R Lutz, Nuttada Panpradist. A Rapid, High-throughput Melt-based Optimization of OLA to Detect MDR-TB. BMES. 2023

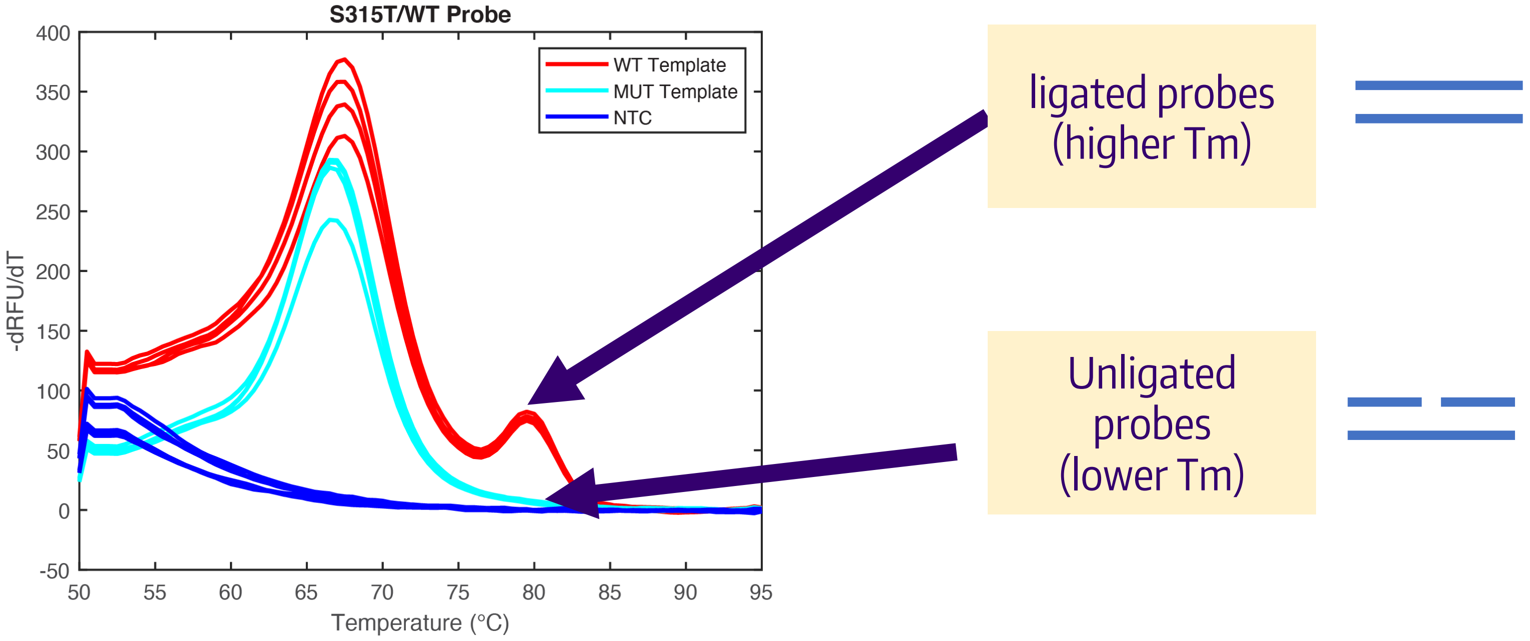
R & D pipeline for OLA-Simple MDR-TB

1. *In-silico* design of probes

2. New high-throughput screening method based on melt analysis

3. Transfer OLA into OLA-Simple format using labeled probes

- Unlabeled probes + template ($50 USD/SNP)
- High-throughput screening using intercalating dye and ligation mixture.



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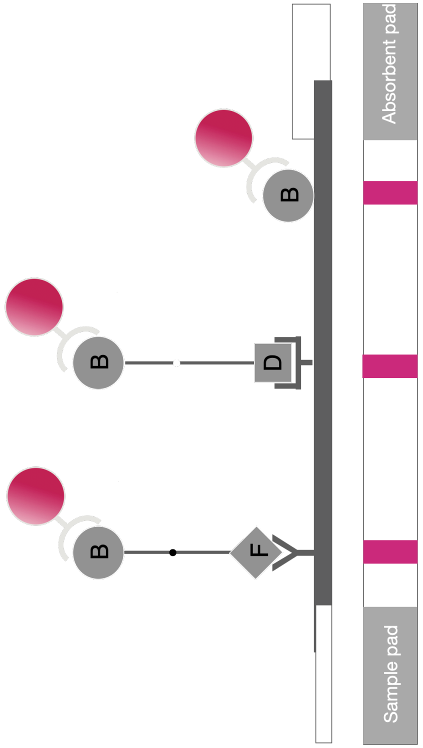
R & D pipeline for OLA-Simple MDR-TB

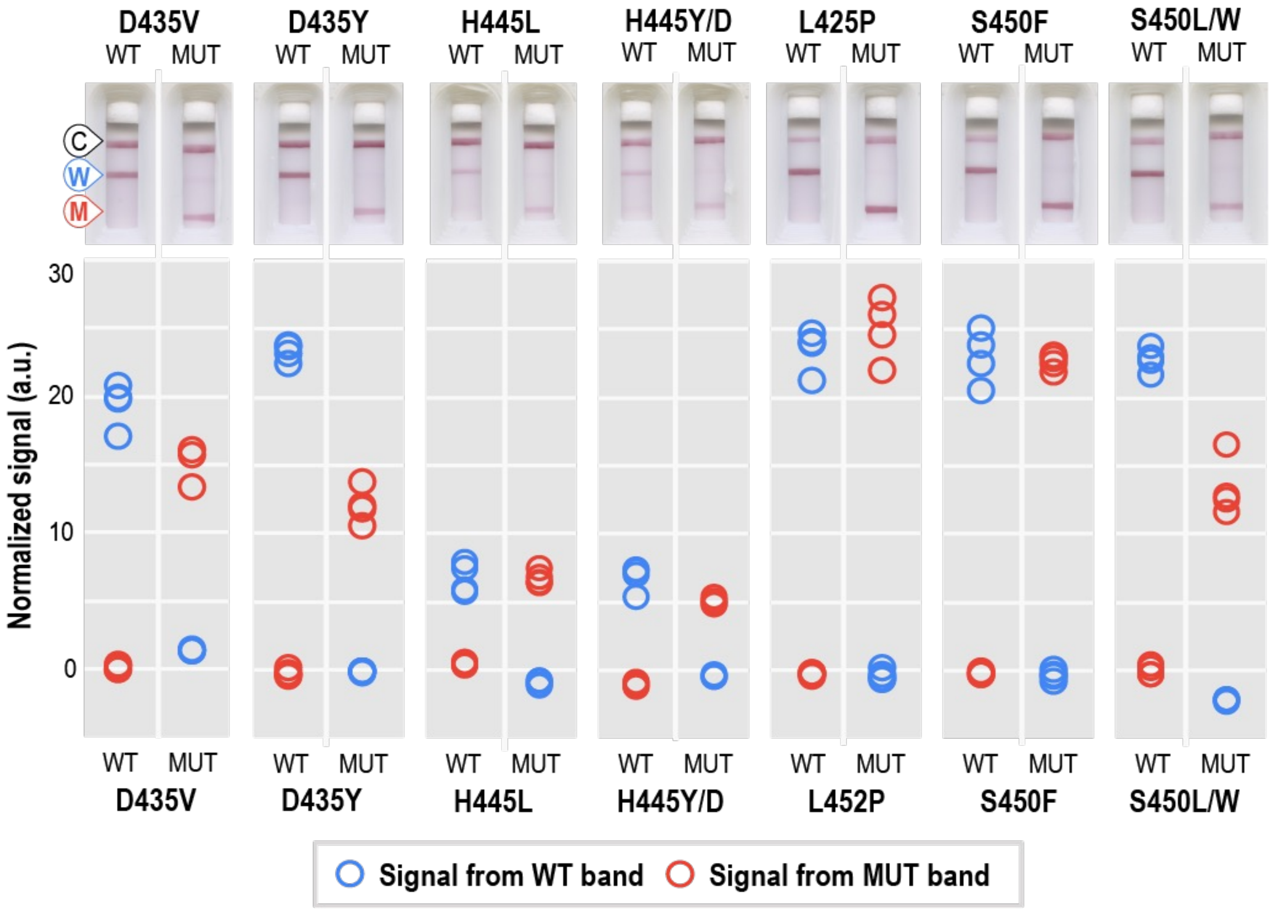
1. *In-silico* design of probes

2. New high-throughput screening method based on melt analysis

3. Transfer OLA into OLA-Simple format using labeled probes

- Labeled probes for RIF mutations
- Lateral flow test with corresponding antibody captures & BSA control line
- Anti-biotin gold nanoparticles
- Strand displacement oligo to eliminate probe-template duplex





Method described in N Panpradist, et.al. 2019. Lancet Ebiomedicine

Key messages:

- **Bi-directional collaboration with local researchers** could be a pathway to **accelerate:**
  - **Tech development** (i.e., right product for the context),
  - **Implementation** (i.e., plan for scale up and regulatory)
  - **User uptake of medical technology** (i.e., engaging with national program)

- **Transferring technical knowledge and skills to local researchers** will **increase equity in medical research and promote decolonization**.
  - Local researchers are very coachable. They just lack educational opportunities.
  - By building technical capability and giving credits (through authorship), the LMICs will have more autonomy and (to me that is the pathway for sustainable diagnostics).

Thank: Amazing research team members

BMES2023: Total 10 posters/talks   **Download here** →

Thank: UW collaborators from

1) Global WACh at Hans Rosling Building Pop Health
2) **Lutz Lab at Bioengineering**
3) Wasser Lab at Conservation Biology
4) Bohringer Lab at Electrical and Computer Engineering
5) Frenkel Lab at Seattle Children's Research Institute
6) Hladik Lab at Fred Hutch
7) MISL Lab at Paul G Allen Computer Science
8) Klavins Lab at MOLES/NanoES