Oligonucleotide Ligation Assay *Multi-Drug Resistant TuBerculosis*

A Rapid, High-throughput Melt-based Optimization of OLA to Detect MDR-TB

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Why MDR-TB?

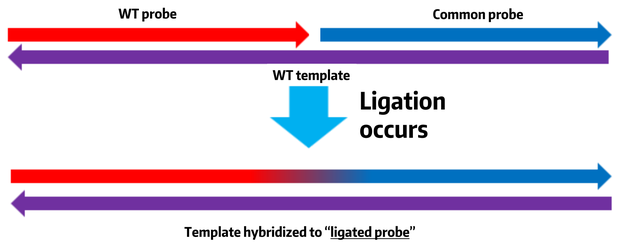
- Tuberculosis (TB) is the **2nd leading cause of death** by infectious disease [1]
- Over **50%** of reported TB cases exhibit **resistance to the two primary TB drugs: rifampicin (RIF) or isoniazid (INH)** [2]
- Multi-drug resistant TB (MDR-TB) is resistant to both RIF and INH.
- **MDR-TB cases** account for **3.6%** of new TB cases and **18%** of previously treated cases and **rising** [1]
- Detecting MDR-TB in patients helps **clinicians select proper treatment**, improving outcome and reducing transmission
- Many MDR-TB are associated with **single nucleotide polymorphism (SNP) biomarkers** [2]

Why OLA?

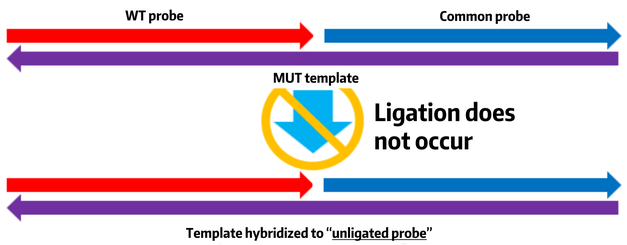
Oligonucleotide ligation assay (OLA):

- ✓ Highly sensitive & specific
- ✓ Robust against nearby polymorphisms [3]
- ✓ Demonstrated success with HIV drug resistance detection [4]
- ✓ A simplified kit, "OLA-Simple" could be scalable for industrial mass production [4]

When bases at SNP are complementary to probes



When bases at SNP are NOT complementary to probes



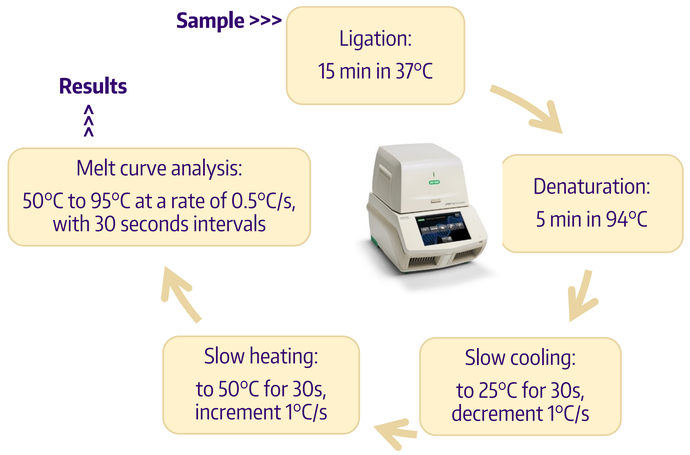
Our solution

- Adapt OLA to detect MDR-TB
- Develop a melt-based analysis that accelerates OLA (**10h** → **2h**)
- Reduce cost of OLA probe optimization (**$500 USD** → **$50 USD**)
- Select only best probe candidates for point-of-care "OLA-Simple"

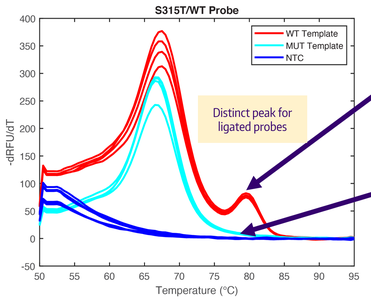
Materials & Methods

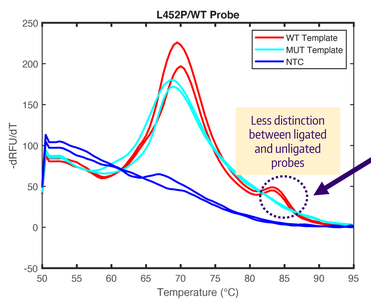
10 SNPs are associated with RIF and INH resistance									
INH			RIF						
inhA		*katG*	*rpoB*						
c-777t	g-154a	S315T	H455L	L452P	S450L/W	S450F	H445Y/D	D435Y	D435V

- ***In-silico* design:** wild-type (**WT**), mutant (**MUT**), and common (**COM**) probes corresponding to each SNP based on consensus sequence of *rpoB*, *katG*, *inhA* from European Nucleotide Archive.
- ***In-vitro* ligation reaction set-up:**
  - Ampligase thermostable ligase, NAD, DTT, salts, EvaGreen (intercalating dye), synthetic single-stranded TB DNA targets (templates) and ligation probes
- **Ligation yield calculation**:
  - Area under the curve (**AUC**), -dRFU/dT, trapezoidal approximation
  - Slope of -dRFU/dT near melting temperature



Results, Discussion, Conclusion



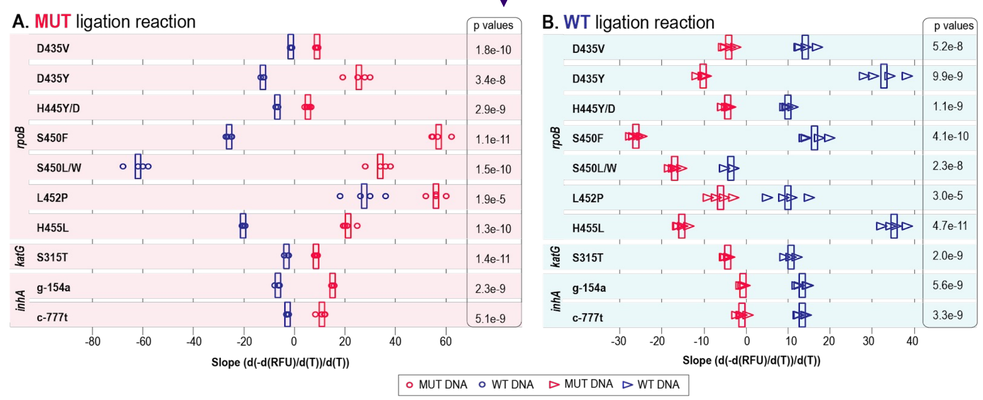


Our assay specifically detected MDR-TB SNPs.

- **Melt peaks** of ligated probes observed in reactions with "Matched" probes and templates (e.g., WT probe with WT template)
- **No melt peaks** of ligated probes observed in "Mismatched" probes and templates (e.g., MUT probe with WT template)

Slope-based approach yielded a better distinction.

- **AUC approach**: successfully distinguished melt peaks of ligated probes from unligated probes for all SNPs **except L452P**
  - We observed unusually high baseline in WT probe + MUT template reaction
- **Slope-based approach** differentiated melt peaks between ligated and unligated probes for all SNPs.



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[3] IA Beck et. al. (2002). Journal of Clinical Microbiology.

[4] N Panpradist et. al. (2019). Lancet Ebiomedicine.

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