Detecting antimicrobial-resistant *Mycobacterium tuberculosis* directly from respiratory specimens using targeted Next Generation Sequencing

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### M. tuberculosis Drug Susceptibility Testing
- Mycobacterium tuberculosis (MTB) infections are treated with combination antimicrobial therapies. Multi- and extensively-drug resistant infections require altered antimicrobial regimens; inappropriate therapy can result in treatment failure and promote further resistance
- Phenotypic drug susceptibility testing requires many weeks to months to complete due to MTB's slow growth rate
- Sequencing-based assays predict antimicrobial resistance (AR) faster than phenotypic methods

**Sequencing-based drug susceptibility testing in NYS**

**Objective:** Design and validate a multiplexed, targeted Next Generation Sequencing (tNGS) assay that can predict MTB AR directly from respiratory specimens

**Targeted NGS (tNGS) Assay Design**
- Primers designed to amplify 13 full-length genes or loci with mutations known to confer resistance to first-line, or second-line antimicrobials
- PCRs optimized against a heat-killed MTB isolate (H37Rv)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Loci</th>
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<tbody>
<tr>
<td>Isoniazid</td>
<td>katG1, katG2, mabA, inhA, oxyR, aphC</td>
</tr>
<tr>
<td>Rifampin</td>
<td>rpoB*</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>pncA</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>embB</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>gyrA, gyrB</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>rrs, rpsL</td>
</tr>
</tbody>
</table>
| Kanamycin | aac,
| Amikacin, Capreomycin | mcr |

* Current pyrosequencing targets

### tNGS workflow in the NYS public health lab
- **Library Preparation Time:** ~ 2 days
- **Sequencing time:** 20 - 120 min, depending on run size
- **Reagent Cost:** ~$52/sample (extraction, amplification, and sequencing)
- **Flow Cell Cost:** variable, ~$25/sample

**tNGS accurately predicts drug susceptibility profiles**

### Comparison with other direct methods
- INGS is multiplexed and covers more loci (13 targets, 9 drugs) than pyrosequencing (3-5 targets, RIF/INH/FPQ only)
- INGS works well on smear-positive samples with Ct-value < 34, pyrosequencing failure is more sporadic (possibly due to PCR inhibition, different extraction methods)

### Conclusions
- **Targeted Next Generation Sequencing (tNGS)**
  - Is a viable way to predict MTB AR directly from respiratory specimens
  - Correctly identifies high confidence mutations within 13 targets and predicts susceptibility for 9 antimicrobials (both first- and second-line)
  - Generates AR predictions faster than culture-based WGS assays and has a higher success rate than other direct methods (pyrosequencing)

### Next Steps
- Continue prospective sequencing of incoming MTB-positive specimens
- Submit INGS for approval as a clinical diagnostic assay for initial AR predictions ahead of culture-based WGS results
- Implement an additional PCR pool targeting loci involved in resistance to linezolid, pretomanid, and bedaquiline/clofazimine

### Works Cited