As laboratories expand their next generation sequencing (NGS) capabilities, it is increasingly possible to use molecular drug susceptibility testing (mDST) to predict *Mycobacterium tuberculosis* (MTB) drug resistance (DR) by identifying key mutations in the MTB genome known to be associated with DR.

This document focuses on resistance associated with changes at the genetic level (i.e., mutations), though other factors can also result in observed resistance, such as intrinsic resistance and expression of efflux pumps.

**Mutations that confer DR can be simple...**

Sometimes resistance to a drug is associated with a single gene or area of a gene.

**Drug Resistance Terminology**

**DST:** Drug susceptibility test; there are two categories of tests:

- **Phenotypic DST (pDST):** Growth-based antimicrobial susceptibility (e.g., MGIT, agar proportion)
- **Molecular DST (mDST):** Detection of genetic mutations associated with DR

**Discordance:** Lack of agreement between laboratory results (e.g., when two pDSTs or a pDST and mDST produce discrepant results)

**Heteroresistance:** Coexistence of organisms susceptible and resistant to the same MTB drug in a patient

**Intrinsic Resistance:** Innate ability of a particular species to resist a certain antibiotic or family of antibiotics

**Low-level Resistance:** Organisms resistant to low-level drug concentrations

**Genetic Terminology**

**Amino Acid:** Building blocks of protein

**Codon:** Three consecutive nucleotides that code for an amino acid

**Gene:** DNA sequence that encodes specific traits

**Locus/Loci:** Fixed position on chromosome where a particular gene, genetic marker or mutation is located

**Numbering System:** Specific location in TB gene or loci where a mutation is located

**Promoter:** Region within the genome that initiates the expression of a gene; promoters do not code for protein

**Indel:** Insertion/deletion

**Silent Mutation:** Change to nucleotide sequence that does not result in a change to the amino acid

**SNP:** Single nucleotide polymorphism, a variation at a single nucleotide position; the most common type of mutation causing DR in MTB

**Variant or Mutation:** Alteration in the nucleotide sequence of an organism that may or may not have a phenotypic effect

**WT:** Wild Type; standard genetic sequence to which mutations are compared (e.g., H37Rv is a commonly used WT reference strain for MTB)

**Heteroresistance is important to detect.**

If heteroresistance is undetected in a patient infected with a mixed population of fluoroquinolone (FQ) susceptible and FQ resistant MTB, FQ treatment may not effectively clear the FQ-resistant subpopulation.

NGS-based methods can be used to detect heteroresistance in MTB.
Overview of Drug Resistance Mutations in MTB

Decades of research have helped define many genetic mutations associated with DR. Understanding the most common of these mutations is essential for effectively leveraging the power of mDST.

Rifamycins (RIF) & rpoB Gene
- Rifamycins include: rifampin (rifampicin), rifabutin and rifapentine.
- ~95% of DR mutations are found in the 81-basepair RIF Resistance Determining Region (RRDR).
- Although less common, mutations outside of the RRDR can confer DR.
- A subset of mutations confer low-level resistance and may not be detected by pDST.

Ethambutol (EMB) & embB Gene
- SNPs in embA, embC and the embC-embA promoter region have also been associated with EMB DR.
- Not all EMB DR associated mutations are known and discordance between methods can be observed.

Fluoroquinolone (FQ) & gyrA Gene
- FQs used for MTB treatment include moxifloxacin, levofloxacin and ofloxacin.
- Mutations within the quinolone-resistance-determining region (QRDR) of DNA gyrase subunit gyrA are most frequently linked to DR; mutations in the QRDR of gyrB can also be linked to DR.
- Heteroresistance is common and can cause discordant results.

Isoniazid (INH) & katG Gene
- katG mutations are responsible for ~85% of observed INH DR.
- Most frequent katG mutation is Ser315Thr, though other katG variants also cause resistance.
- Mutations in the promoter and genes for inhA and fab-G1(mabA) may also be associated with INH DR.
- Silent fabG1(mabA) mutation Leu203Leu is also known to be associated with INH DR.

Ethionamide (ETH) & inhA and ethA Genes
- INH and ETH are structural analogs and cross-resistance is common.
- Mutations in ethA are known to be associated with ETH DR.
- inhA promoter mutation C-15T is often associated with low-level INH and ETH resistance.
- Mutations in fabG1(mabA) are also associated with ETH DR.

Second Line Injectable Drugs & rrs, eis and tlyA Genes
Second line injectable drugs include amikacin, kanamycin and capreomycin.

Pyrazinamide (PZA) & pncA Gene/Promoter
~85% of pncA genetic variants are associated with phenotypic PZA DR.

New TB Drugs
The following drugs and genes have known DR associations:
- Bedaquiline & Rv0678 and atpE
- Linezolid & atpE, rplC and rrl
- Delamanid and pretomanid & fgd1, ddn, fabI, fabB, fbiC and fbiD
- Clofazimine & pepQ, Rv0678, mmpL5 and mmp

Note that newer drugs have less robust DR data, so continued tracking is essential.