

# Genotyping of drug resistance in *Mycobacterium tuberculosis* using diagnostic microarrays

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Tuberculosis is a disease of worldwide concern. Antimicrobial resistance in *Mycobacterium tuberculosis* is an increasing challenge and, in contrast to other bacteria, not caused by the acquisition of certain genes, but by acquisition of single point mutations in genes which are present in all strains. Unfortunately, culturing and subsequent growth inhibition assays are still time demanding preventing fast detection and treatment. Genotyping methods as PCR followed by sequencing are an alternative. Here the bottlenecks are processing time, overall costs and lack of parallelisation. Hybridisation of such PCR products against highly discriminatory oligonucleotide probes is an alternative approach which could solve these problems. We developed an assay using a diagnostic oligonucleotide microarray and covering probes for relevant mutations in genes *rpoB*, *katG*, *embA*, and *embB*, for the *embC/embA*-intergenic region, and the *mabA/inhA* promoter. PCR is employed to amplify these genes and to incorporate biotin labels during elongation. These labeled amplicons are hybridised to the array which are inserted into ArrayStrips, and hybridisation is visualised using dye precipitation triggered by streptavidin-peroxidase complexes bound to the biotin labels and the ArrayMate reading device. The procedure is currently tested using DNA from previously characterized strains for which conventional susceptibility test results and relevant sequences are available.

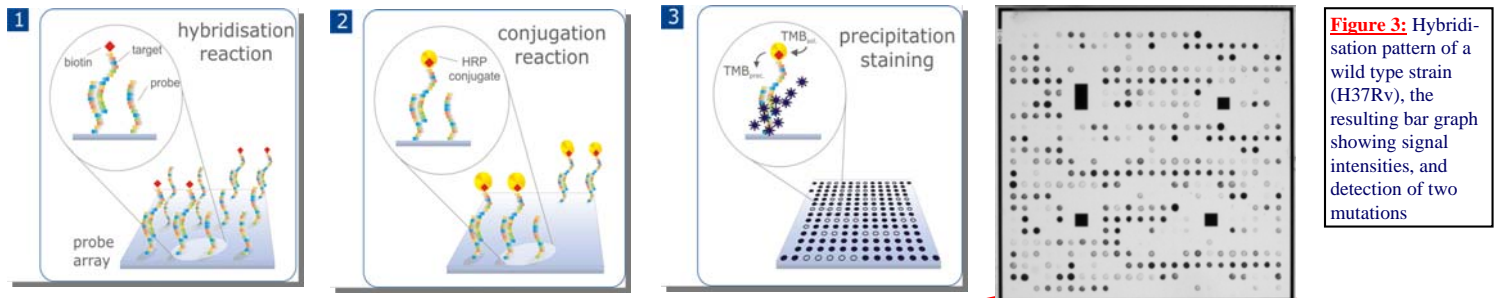
**Introduction:** Antimicrobial resistance in *Mycobacterium tuberculosis* is an increasingly important problem. Starting from patient samples like sputum, genotyping of *Mycobacterium tuberculosis* can be faster than culture-based methods, and thus such an approach might be helpful to guide therapy. Since resistance in *M. tuberculosis* is not caused by the acquisition of dedicated genes, but by single point mutations in ubiquitous genes, PCR followed by sequencing or hybridisation against a set of specific and discriminating oligonucleotide probes which are specific relevant mutations is the method of choice. Diagnostic microarray technology offers a fast and economic approach by allowing parallel and automated assay performance and data interpretation.

## Material and methods:

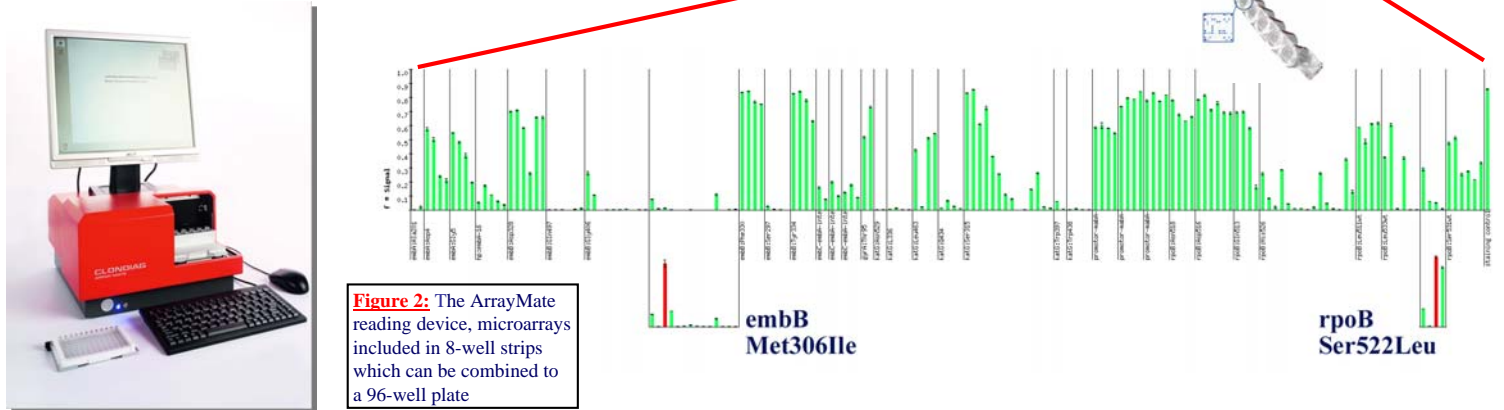
**Microarray:** Oligonucleotide (primers and probes) sequences were designed using CLONDIAG ArrayDesign software from published resistance-associated mutations (<http://www.tbdreamdb.com/Other.html>) in genes *rpoB*, *katG*, *embA*, and *embB*, the *embC/embA*-intergenic region, and the *mabA/inhA* promoter. Primers were 5' Biotin labelled and designed to bind in consensus sequences covering the regions with relevant mutations. 3'-aminomodified oligonucleotides were purchased (Metabion) and spotted on arrays which were inserted into ArrayStrip™ reaction vials (CLONDIAG).

***Mycobacterium tuberculosis* isolates:** Reference DNA was either kindly provided from the German National Reference Center for Mycobacteria (Borstel) or it was commercially available (H37Rv). For DNA preparation in samples Qiagen QiAmp DNA Mini Kit was used.

**Labelling, Amplification and Hybridisation:** PCR was performed separately or as a multiplex reaction using 5' Biotin labelled primers. Subsequently, all labelled PCR products of a given isolate / DNA were hybridised against the DNA-microarray. Then, horseradish peroxidase coupled streptavidin was conjugated which catalyses afterwards the local precipitation of dye (Seramun Green) resulting in the formation of spots on the array which can be scanned and analysed (ArrayMate System CLONDIAG). Signal intensities of the spots and their local backgrounds were determined. Intensities for the probes recognising different alleles of a given target were compared. The probe yielding the strongest signal was regarded as the probe recognising the sequence being actually present.



**Figure 1:** The principle of the hybridisation, conjugation and local precipitation on a microarray.



**Figure 2:** The ArrayMate reading device, microarrays included in 8-well strips which can be combined to a 96-well plate

**Results and discussion:** Using previously characterised material the following loci were possible to discriminate between wild type and mutant strains so far: *rpoB*:Ser531, *rpoB*:Ser522, *rpoB*:Leu533, *rpoB*:Leu511, *rpoB*:His526, *rpoB*:Gln513, *rpoB*:Asp516, *rpoB*:Asn518, promoter-*mabA-inhA*:upstream 8, promoter-*mabA-inhA*:upstream 15, promoter-*mabA-inhA*:upstream 147, *katG*:Trp438, *katG*:Trp397, *katG*:Ser315, *katG*:Leu463, *katG*:L336, *gyrA*:Thr95, *embB*:Tyr334, *embB*:Glu497, *embB*:Ser297, *embB*:Phe330, *embB*:Met306, *embB*:Gly406, *embB*:Asp328, *embA*:Gly5 and *embA*:Asp4. The microarray-based test is able to facilitate a reliable genotypic prediction of phenotypic susceptibility of resistance. Furthermore, a high number of isolates can be tested fast and economically, and possible new targets can be included rapidly. This provides a feasible option for the detection of resistance mutations without further sequencing.

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