Gold nanoprobes methodology for diagnosis of Multi-Drug Resistant Tuberculosis

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Abstract

Tuberculosis (TB), still one of the leading human infectious diseases, reported 8.7 million new cases in 2011 alone. Also, the increasing rate of multidrug-resistant tuberculosis (MDRTB) and its treatment difficulties pose a serious public health problem especially in developing countries [1]. Resistance to isoniazid and rifampicin, first line antibiotics, is commonly associated with point mutations in *inhA*, *katG*, and *rpoB* genes of *Mycobacterium tuberculosis* (Mtb), the main etiologic agent of human TB [2]. Therefore, the development of cheap, fast and simple molecular methods to access susceptibility profiles would have a huge impact in the capacity of early diagnosis and treatment of MDRTB patients.

Gold nanoparticles functionalized with thiol-modified oligonucleotides (Au-nanoprobes) have shown the potential to provide a rapid and sensitive detection method for Mtb and of single base mutations associated with antibiotic resistance [3], namely the characterization of the 3 most relevant codons in *rpoB* gene associated to rifampicin resistance [4].

Here we extend the Au-nanoprobe approach towards discriminating specific mutations within *inhA* and *katG* genes in PCR amplified DNA from clinical samples. Using a multiplex PCR reaction for *inhA* and *rpoB* genes, it is possible to assess both *loci* in parallel, and might extend the Au-nanoprobe method's potential to MDRTB molecular characterization at a point-of-need. We have also optimized the detection procedure using loop mediated isothermal amplification (LAMP) so as to avoid standard PCR, and applied it to discriminate between *rpoB531WT* and *rpoBS531L* mutations.

References

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Figure 1:

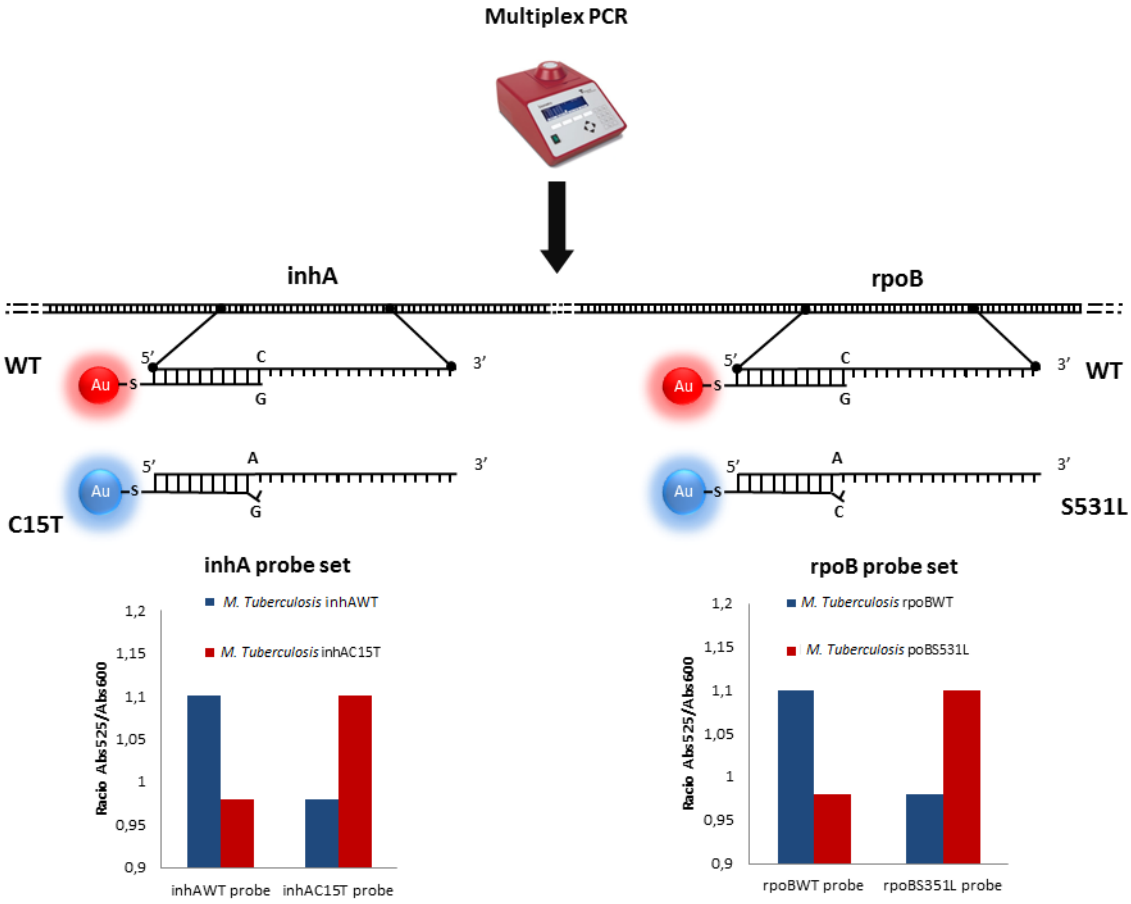

Figure 1: After a first round of Multiplex PCR with *inhA* and *rpoB* sets of primers the Au-nanoprobe assay is performed for mutation detection. Aggregation is measured by ratio of non-aggregated particles (SPR intensity at 526nm) versus aggregated particles (SPR intensity at 600nm) with the two sets of probes (*inhA* and *rpoB*). Two Au-nanoprobes within the same set are used to create redundancy in the system. The graphs shown in the figure are illustrative and do not contain real data.

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TO WHOM IT MAY CONCERN

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I hereby declare that Mr Pedro Pedrosa has been developing his MSc research project under my supervision at Nanotheranostics Group at Centre for Research in Human Molecular Genetics. The submitted abstract is part of the results attained thus far and, as Thesis Adviser, I fully support the submission for communication.

If you require any further information, please do not hesitate and contact me.

Sincerely,

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