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Development and optimization of a diagnostic method for drug resistant tuberculosis based on padlock probes and rolling circle amplification

A master thesis position (or longer student project) is now available at the department of Immunology, Genetics and Pathology at Uppsala University in collaboration with the Karolinska Institute and the Swedish Institute for Communicable Disease Control (SMI), involving method development in molecular biology for tuberculosis (TB) diagnostics.

A padlock probe is a linear oligonucleotide comprising two target-specific sequences at both ends and a linker segment containing sequences for amplification and identification. The probe is designed to bring the two ends into juxtaposition upon hybridization to the target sequence. This conformation allows the oligonucleotide to be circularized with great target specificity by a DNA ligase. Signal amplification can be achieved by rolling circle amplification (RCA) of the reacted padlock probes. The concatemers of DNA circles produced by RCA can then be digested, ligated into new circles and subjected to another round of RCA, known as circle-to-circle amplification (C2CA).

Mycobacterium tuberculosis, the causative agent of TB, has infected approximately a third of the world population. The spread of multi-drug resistant TB (MDR-TB) and the emergence of extensively-drug resistant TB (XDR-TB) pose new challenges for prevention, treatment and control of this deadly disease. In order to quickly identify MDR- and XDR-TB it is necessary to use molecular diagnostic methods. These can identify a drug-resistant strain in a day or two, while conventional phenotypic diagnostic methods require weeks to months. The development of new and improved methods for fast detection of drug-resistant TB strains is crucial to meet the threat of drug resistant TB, as they would greatly facilitate the choice of appropriate patient treatment.

The aim of this project is to develop a highly multiplex molecular detection method for XDR-TB. Padlock probes will be designed to target the most common mutations linked to resistance, their corresponding wildtype sequences as well as sequences specific for the *M. tuberculosis* complex. C2CA will be used to amplify ligated padlocks. For a quick and simple read-out format, monomerized RCA products will be hybridized to a low-density array and visualized by a horseradish peroxidase enzymatic reaction. The project is already underway, but ahead lays the tasks of padlock probe evaluation, multiplexibility tests, redesign of suboptimal padlock probes, real specimen testing and finalization of the assay.

The work will take place in the group of Prof. Mats Nilsson at the department of Immunology, Genetics and Pathology at the Rudbeck Laboratory, Uppsala University.

Competence: You should have experience in lab work, specifically in molecular biology. You are thorough and have good analytical skills, possess cooperative skills and a positive personality.

For more information, or to submit an application (letter and CV), please contact:

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