Rapid diagnosis and drug resistance of Mycobacterium tuberculosis
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During the four years of research from which the articles for this thesis originated, several other experiments were carried out. A selection:

- A dual electrochemiluminescent probe PCR assay to detect bacteria belonging to the *Mycobacterium tuberculosis* complex in sputum samples, with IS6110 as the target sequence, was set up. Boom-extraction was used to extract nucleic acids from the sputum samples. Primers INS2 and pt18 (1) were used.

- Sputum samples from patients suffering from tuberculosis were collected from the GGGD in Amsterdam, The Netherlands. These samples were obtained at day 0, 3, 7, 14, and 28 after diagnosis and initiation of treatment. These samples were used for mRNA detection experiments (see below).

- An attempt was made to set up a real-time PCR assay, with Taqman probes, to detect mycobacterial mRNA in serial sputum samples from tuberculosis patients from the GGGD. The amount of mRNA correlates with the amount of viable mycobacteria in sputum; with this assay the treatment response of patients could be monitored: treatment failure is detected early and infectiousness can be assessed accurately. It was philosophized that with this assay, isolation measures for clinical patients could be ended at an earlier - yet still safe - moment than with the current protocols (see Chapter 2: General Introduction, paragraph 4.5). A gene encoding the abundant and constitutively expressed 85b protein was used as a target. Primers and probes were constructed using primer express software. An internal control was constructed by shuffling the target sequence of the specific probe. This sequence along with the flanking sequences of the PCR target fragment including the primer sequences was directly ordered from the manufacturer, amplified and cloned into a TA vector, which was subsequently transformed into *Escherichia coli*. Different protocols to extract nucleic acids from sputum samples were employed. However, we never succeeded in amplifying the mRNA from these samples in a satisfying manner.

- An internal control for the *katG315* PCR (see Chapter 6) was constructed by shuffling the target sequence of the specific probes. This shuffled sequence was ligated into the flanking sequences of the PCR target, cloned into a vector and expressed in *E. coli* DH5α.

References
