Methods for the analysis of bio-molecules using rolling circle DNA synthesis

Jakob Schwalbe Lohmann, MSc

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Official opponents: Thomas Ledet, Niels Tommerup, and Eigil Kjeldsen.

Tutors: Jørn Koch, and Stephen Hamilton-Dutoit.

Correspondence: Jakob Lohmann, Laboratory of Molecular Pathology, Institute of Pathology, Nørrebrogade 44, Aarhus University Hospital, 8000 Aarhus C, Denmark.

E-mail: jlohm@as.aaa.dk

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ABSTRACT

This PhD project was mainly carried out in the Laboratory of Molecular Pathology, Institute of Pathology, Aarhus University Hospital, and funded by the EU-framework 6 project Moltools (www.Moltools.org), which was set to develop new molecular tools. In this particular thesis we have aimed at: I Develop new methods for the detection of DNA in situ, II Improve the quality of the DNA probes used and III Develop new methods for the detection of the activity of DNA modifying enzymes. All of these projects used rolling circle replication (RCR) of short circular DNA oligonucleotides as the basic tool. In this technique a DNA circle is replicated resulting in a long single stranded DNA sequence consisting of tandem repeats of the complementary sequence of the original circle. This product can e.g. be detected by in situ hybridization of short fluorescently labeled oligonucleotides.

Under I we have been able to detect short repetitive genomic DNA sequences in situ on condensed metaphase chromosomes as well as the *apolipoprotein(a)* gene using padlock probes and target primed RCR. Similar results had previously only been obtained on mitochondrial DNA with the setup we have used. This work was published in BMC Molecular Biology.

The quality of long chemically synthesized oligonucleotides is normally not impressive. Under II we have developed a method for the enzymatic production of DNA oligonucleotides using so-called "suicide cassettes", which is a hairpin structure containing binding and cleavage sites for both a nicking enzyme and a restriction enzyme. Hairpin-containing oligonucleotides were circularized and amplified by RCR and nicked with a nicking enzyme. This results in that the tandem repeat is turned into monomers, which can be re-circularized and again amplified by RCR. These steps can be performed numerous times, allowing for a massive amplification. In the final step the hairpin can be removed by cleavage with the restriction enzyme Mly I, which cleaves blunt end outside its recognition sequence, allowing for the release of the DNA sequence contained within the suicide cassette. This work was published in BMC Biotechnology.

RCR has previously been used as an amplification step for the detection of DNA, RNA and protein. Using the molecular design derived under II we introduce under III a new method for the detection of protein activity of DNA modifying enzymes. The method is based on enzymatic circularization of DNA oligonucleotides, which can be detected by RCR. We have detected activity of topoisomerase I and Fen1 using purified enzymes. This method may be a useful tool in determining the activity of DNA modifying enzymes or enzymatic pathways. This work has been submitted, but not yet published.