# In situ detection of RNA using circle probes and rolling circle DNA synthesis

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ABSTRACT

This PhD dissertation has been made at the Institute of Pathology, University Hospital of Aarhus. The aim was to develop an in situ detection method using circular in situ hybridization probes (circle probes) in combination with target primed rolling circle DNA synthesis for detection of RNA. This has resulted in two new circle probe designs, the turtle probe and the slicer probe.

Initially we designed the turtle probe and used it for detection of non-polyadenylated RNA species (rRNA, EBER1 and hTR). The probes were hybridized to the 3'-part of the target RNA, ligated to form a closed DNA circle, and then the rolling circle DNA synthesis was performed primed from the 3'-end of the RNA. Rolling circle DNA synthesis results in a tandem repeat with a sequence complementary to the probe and can easily be detected by FISH. Because the rolling circle DNA synthesis was primed from the 3'-end of the RNA, the rolling circle product was covalently anchored to the RNA ensuring prefect signal localization. Thus, ISH with circle probes and target primed rolling circle DNA synthesis requires both hybridization of the probe and the presence of a 3'-end in the target RNA, to prime the rolling circle reaction and is therefore less prone to non-specific signals where probe molecules stick or hybridize non-specifically in the specimen.

However, a different approach is needed if the target RNA is polyadenylated or if the existing 3'-end is not an option, e.g. if the detected sequences is located in the midst of an RNA molecule. For this we designed the slicer probe, which is a circle probe containing a DNA enzyme. DNA enzymes are DNA sequences capable of performing specific chemical reactions using divalent metal ions as cofactors. Inclusion of a DNA enzyme in the footprint of the probe enables the probe to cleave the target RNA, thereby generating a new 3'-end at the hybridization site. This new 3'-end can be used for priming the rolling circle DNA synthesis and the rolling circle product detected by FISH.