Functionality of a cell free DNA-based noninvasive prenatal test – optimization of raw materials for analyzing chromosomal abnormalities

Sanni Sihvonen[1] and Ph.D. Thomas Nyholm[2]
[1]Department of Biochemistry, University of Turku, [2]PerkinElmer, Turku
BIOCHEMISTRY

Introduction

Non-invasive prenatal screening methods (NIPT) for detecting chromosomal abnormalities in fetuses use cell free DNA (cfDNA) extracted from mother's plasma samples, and thus reduces the risk of miscarriage caused by invasive sample taking methods. Perkin Elmer's Vanadis NIPT® Assay (Fig.1) uses *rolling circle amplification* (RCA) –based technology in amplifying the target cfDNA, indicative for abnormalities, instead of more time consuming and robust PCR- or NGS-based amplification.

The target cfDNA is processed via highly specific and complex enzymatic pathways prior and during the amplification. One of the key players in this processing are different oligonucleotides which are utilized to guide and support the correct formation of labelled DNA products (RCPs).

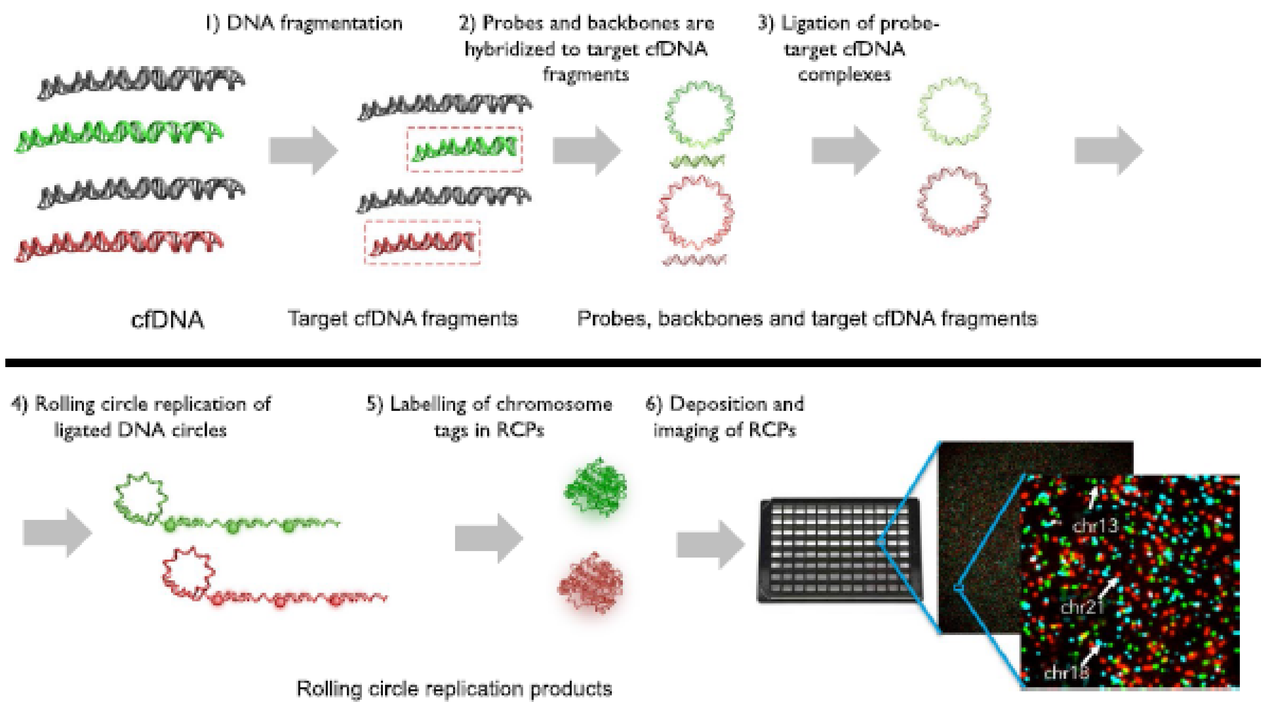

Fig. 1 Vanadis® NIPT Assay method.
(Modified figure Dahl et. Al. 2018)

Methods

1. 100 mM oligonucleotide in 1 x TE-buffer purification

- Preparative purification with RP-HPLC-DAD
- Varying purification volumes to find the optimal gradient and parameters for maximum volume

2. Analysis of purification level

- Analytical RP-HPLC-DAD for each collected fragment
- UHPLC-MS-analysis

3. Functionality tests using different sample setups on Vanadis NIPT® Assay (Fig.1)

- Control samples of originally good purity level oligonucleotides and bad purity level used
- Purified oligonucleotide samples with different purity levels and with different impurities tested

Conclusions

→ Preliminary studies suggest that a correlation between the purity-level of the oligonucleotides and the functionality exists. The type of impurity does matter, and thus should be taken in consideration when analyzing the re-purification quality.

→ Further studies on oligonucleotide purification are needed in order to achieve the optimal level of functionality.

References: Dahl F., Ericsson O., Karlberg O., Karlsson F., Howell M., Persson F., Roos F., Stenberg J., Ahola T., Alftrén I., Andersson B., Barkenäs E., Brandner B., Dahlberg J., Elfman S., Eriksson M., Forsgren P.O., Francois N., Gousseva A., Hakamali F., Janfalk-Carlsson Å., Johansson H., Lundgren J., Mohsenchian A., Olausson L., Olofsson S., Qureshi A., Skarpås B., Sävneby A., Åström E., Öhman O., Westgren M., Kopp-Kallner H., Fianu-Jonasson A., Syngelaki A. & Nicolaides K. (2018) Imaging single DNA molecules for high precision NIPT. Sci Rep. 8:4549

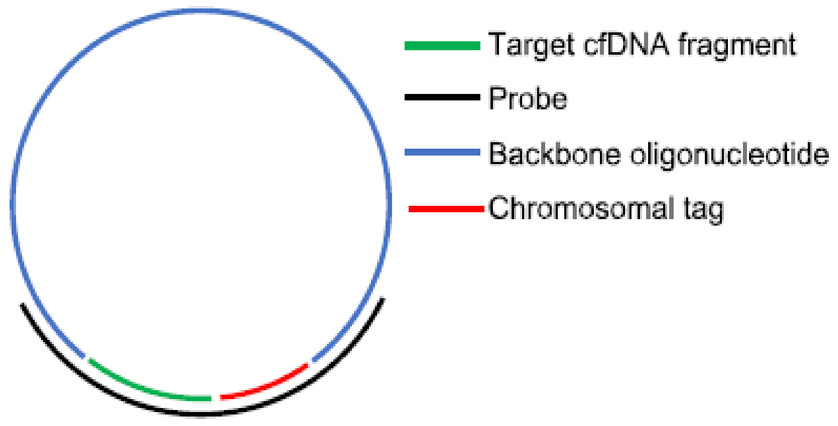

Fig. 2 Formation of cfDNA-complex to be amplified in RCA-reaction. Target cfDNA fragments are hybridized with backbone oligonucleotide and probes, with assistance of other small oligonucleotides. Thus, the whole process of cfDNA-complex formation is highly dependent of the correct and effective functionality of the different oligonucleotides.
(Modified figure Dahl et. Al. 2018)

Aims

- To analyze the impurities of backbone oligonucleotide and investigate correlation between the impurities and the functionality of the oligonucleotide
- To optimize the purification and purity level of the oligonucleotide for better functionality

Results

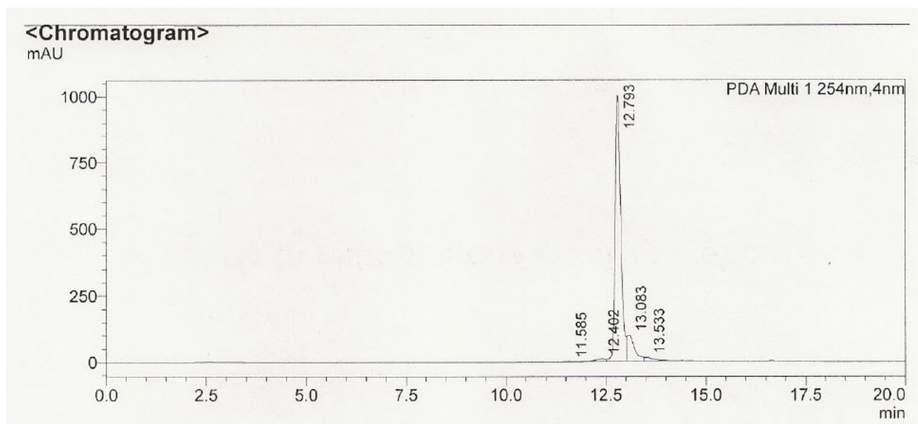

Fig. 3 HPLC-analysis of a non-purified Backbone oligonucleotide batch which showed decreased functionality in the functionality tests. Impurities present before and after the main peak.

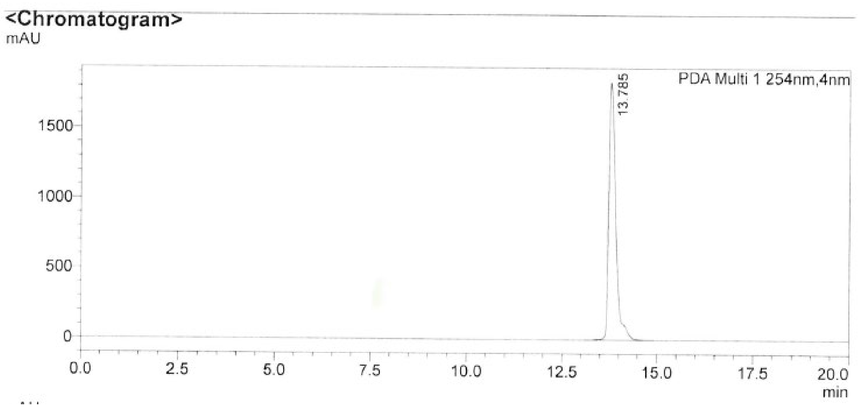

Fig. 4 HPLC-analysis of the bad Backbone oligonucleotide batch after preparative purification and fraction combination. Analytical HPLC-analysis suggests, that the combined oligonucleotide fragments were purified from the impurities present in Fig 3.

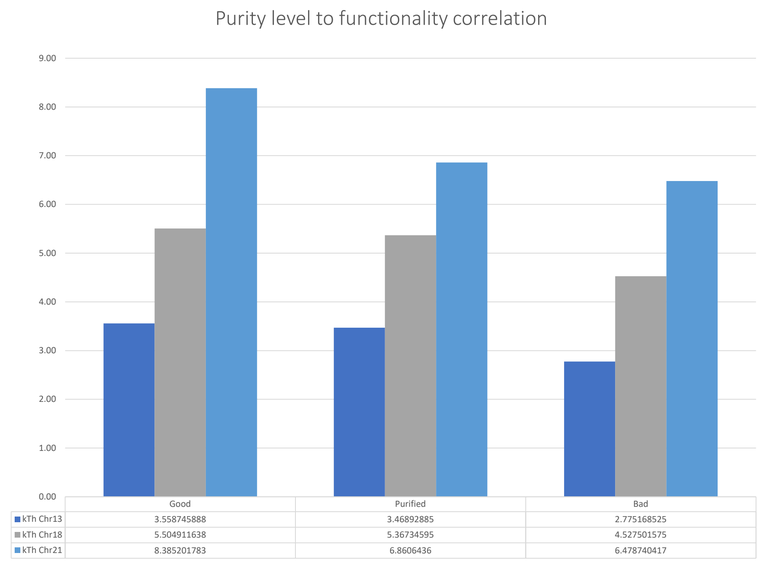

Fig. 5 Correlation between the purity level of the oligonucleotides to kTh-factors representing the level of functionality.