IMPROVING THE FUNCTIONALITY OF NON-INVASIVE PRENATAL SCREENING

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INTRODUCTION

Non-invasive prenatal screening is used to act as an aid in screening the risk of trisomy 13, 18, 21, Y and/or X in fetus using cell-free DNA from pregnant women. The screening method utilizes rolling circle amplification to form rolling circle products (RCPs) that are labelled with chromosome-spesific fluorescently labelled DNA probes. RCPs are imaged and counted with an automated microscopy scanner.

Several reagents are required to generate RCPs which makes stabilization challenging. Optimising the activity of enzymes can improve product performance and reliability of the results. By improving the performance, the incidence of false results decreases and more chromosomal abnormalities can be detected during pregnancy allowing a proper treatment plan to be made at the early stages.

MATERIALS AND METHODS

The study was performed by using a non-invasive prenatal screening system. The purpose of the study was to get acquainted with the operation of the raw materials, especially coenzyme, detergent and labelling buffer, and to improve the performance of the assay and the reliability of the results by optimizing reactions.

1. Low pH has been found to negatively affect ligase function decreasing signal level as a function of time. Alternative coenzymes at different pH values were tested in the reaction.

2. The pH of the detergent currently used in the reaction is temperature dependent, making stabilization challenging; Two alternative detergents were tested in the reaction.

    2.1. Hybridization buffer contains detergent and therefore it was also replaced and tested with alternative detergents.

3. Previous studies have shown that the background of the X chromosome is high in the assay, which reduces detection sensitivity. To address this problem, efforts were made to reduce the background by improving the specificity of detection oligo and RCP binding by changing the salt composition of the labelling buffer.

RESULTS

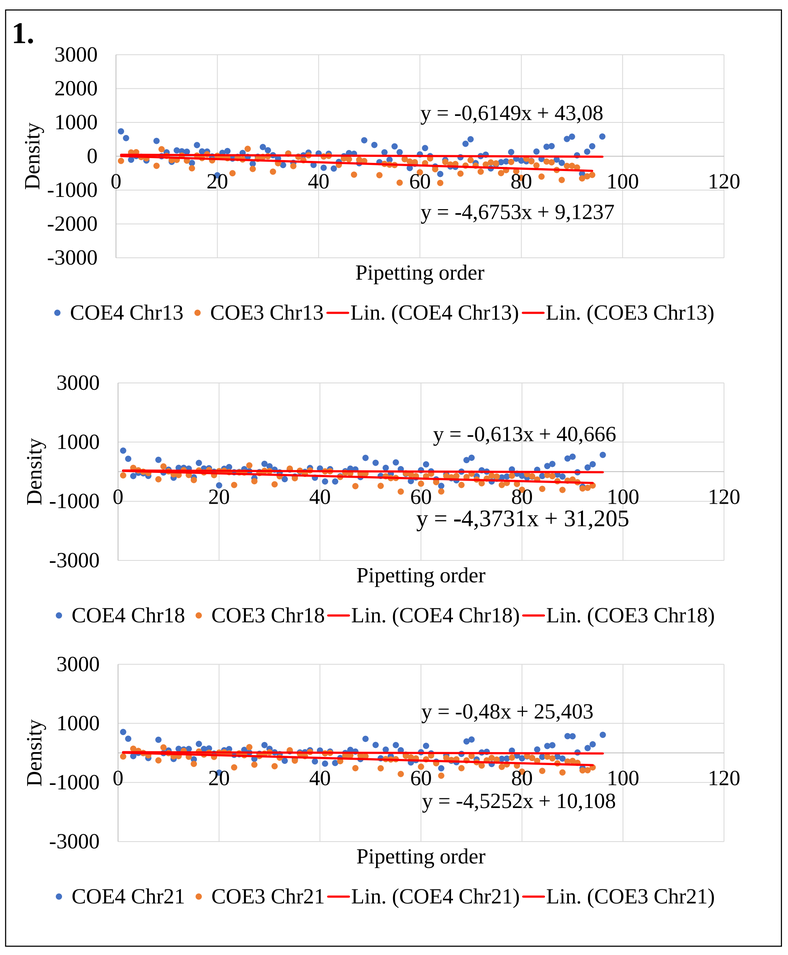

Figure 1. Effect of pipetting order on measured density values. Coenzyme 4 is the purest alternative coenzyme tested and coenzyme 3 is the coenzyme currently in use. The trend of coenzyme 3 decline significantly when compared to coenzyme 4.

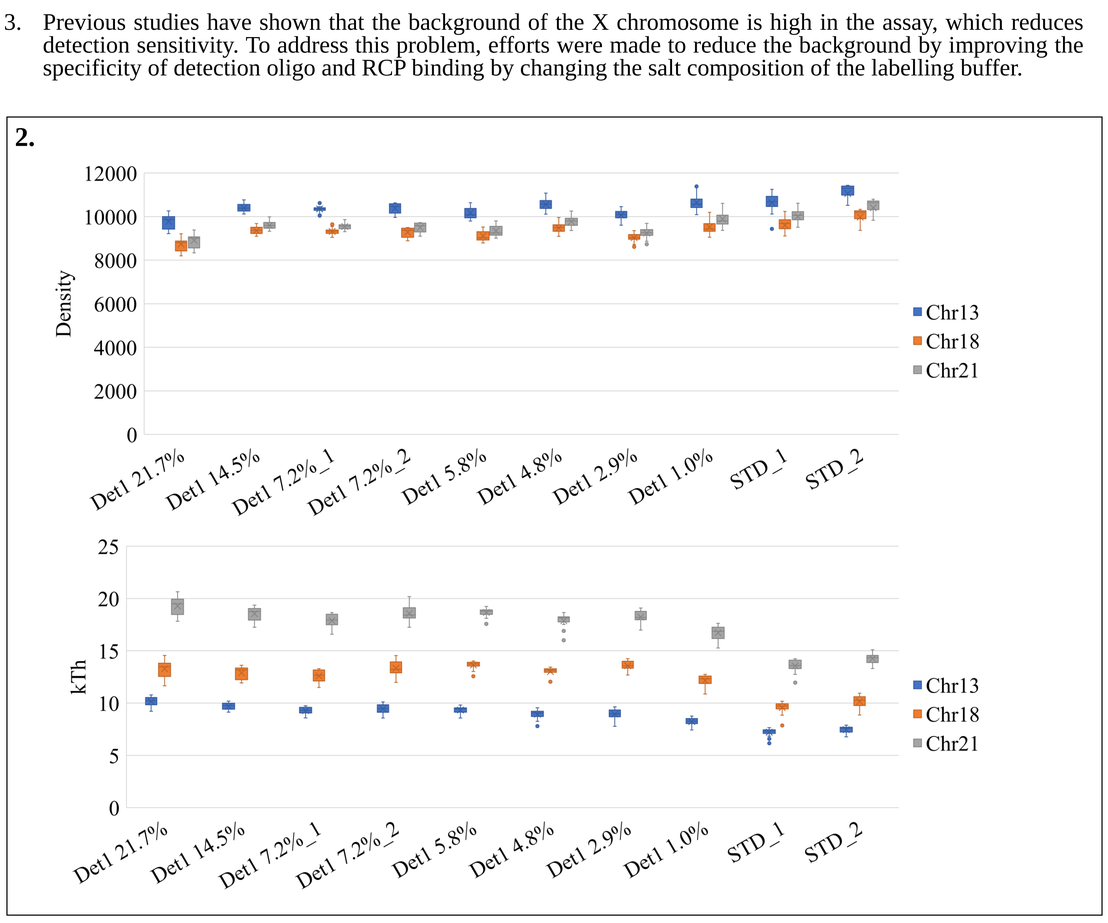

Figure 2. Effect of alternative detergent 1 on density and kTh values. Density describes the number of generated RCPs and kTh describes the separtion of RCPs from the background. Detergent 1 decreases the density but increases the kTh.

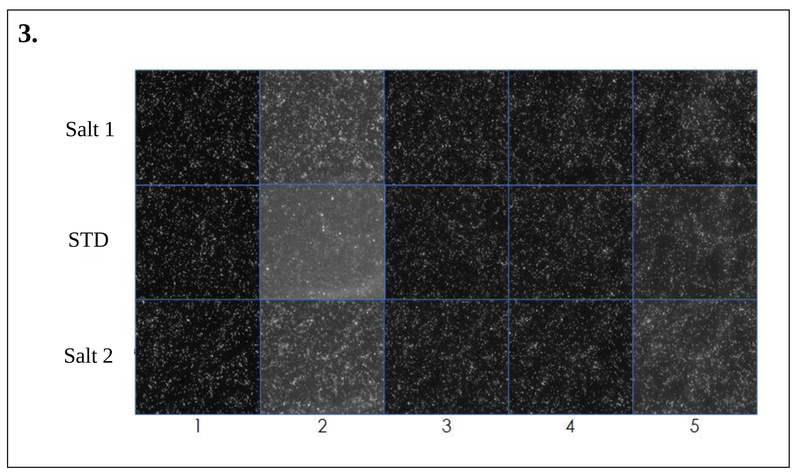

Figure 3. The effect of labelling buffer salt composition on backround. The background changes. The most significant changes can be observed in Y (2) and X (5) channels.
*1 = Chr21 (647nm), 2 = ChrY (720nm), 3 = Chr13 (590nm), 4 = Chr18 (550nm), 5 = ChrX (488nm)

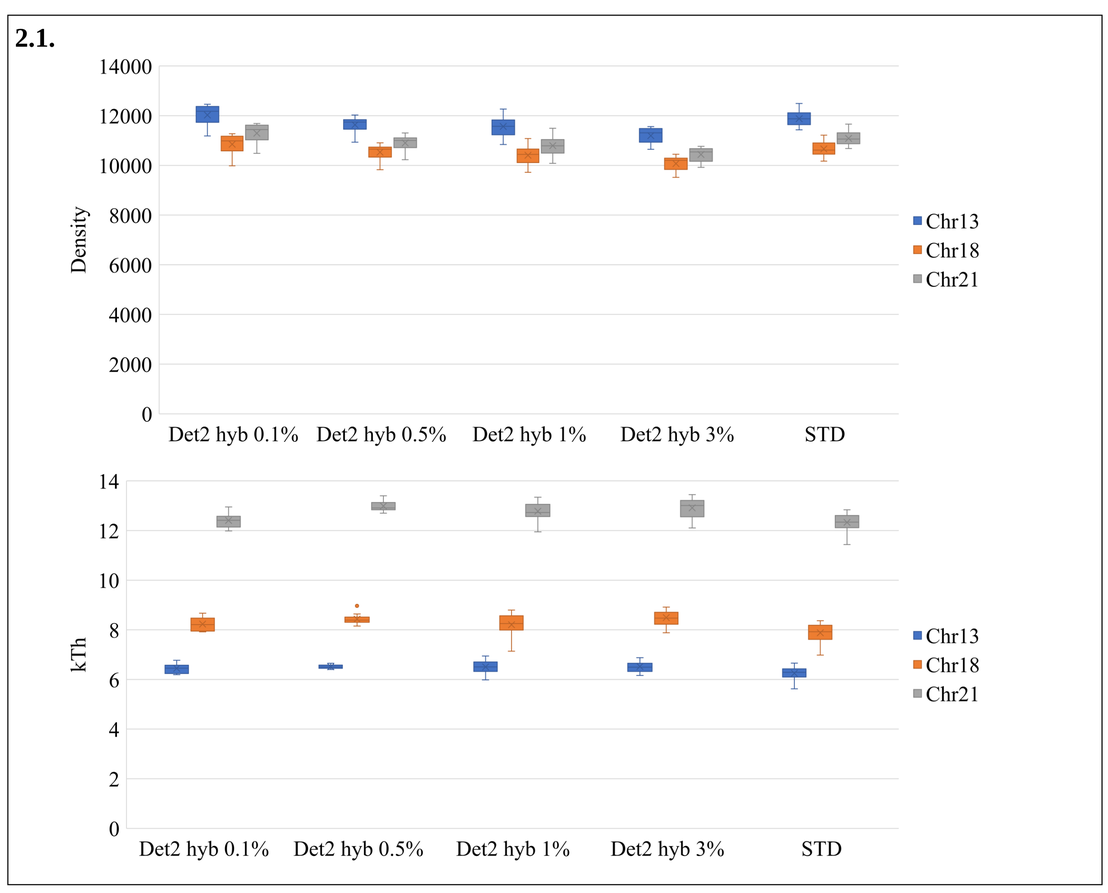

Figure 2.1. Effect of hybridization buffer on density and kTh values when detergent is replaced by detergent 2. Detergent 2 decreases the density and increases the kTh.

CONCLUSIONS

1. Alternative coenzymes were tested for the reaction and their effects on the signal level were significant; The signal level remained constant throughout the plate when the purest coenzyme was used in assay and the pH of its storage solution was raised.

2. Detergent 1 performed better in the reaction than the detergent currently in use. Detergent 1 gave higher kTh values, but reduced the amount of RCP obtained. 2.1. Detergent 2 performed better in hybridization buffer. Detergents did not form a precipitate after the freeze-thaw cycle. The pH of the detergents, such as the detergent currently used, was temperature dependent.

3. The salt composition of the labelling buffer has effect on the signal to background ratio.

The obtained research results seem promising and based on them the performance as well as the reliability of the assay can be improved. However, further research and testing is required to verify the results obtained.

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