



REMOVAL OF AMPLICONS IN TARGETED NGS CCS: A PATHWAY TO DISCOVERY OF NOVEL EMBRYO VIABILITY BIOMARKERS

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Abstract:

OBJECTIVE: Trophoectoderm (TE) biopsy followed by targeted next generation sequencing for comprehensive chromosome screening (tNGS CCS) is increasingly used in ART. As more than 35% of transferred euploid embryos fail to achieve sustained implantation, simultaneous assessment of additional embryonic parameters in TE samples could be pursued to help increase implantation rates. However, due to the small sample size of TE biopsies (5-10 cells), tNGS CCS requires a pre-amplification step using polymerase chain reaction (PCR), and generates $>500 \times 10^9$ amplicon copies for each sample, compared to only 5-10 copies of the original DNA, making it impossible to assess further genetic/epigenetic parameters from the same sample. In this study, we hypothesized that labeled-primers could allow the removal of PCR amplicons from the TE sample without impairing the diagnostic accuracy of tNGS CCS.

DESIGN: Experimental study.

MATERIALS AND METHODS: Three experimental approaches were adopted to purify amplicons generated during pre-amplification PCR. 1) Forward and reverse primers labeled with biotin at 5'-end were synthesized (IDT) and used for pre-amplification for tNGS CCS; 2) Synthesized biotin-labeled primers were purified using high-performance liquid chromatography (HPLC) prior to use; 3) Biotin-labeled primers purified with HPLC were enriched with Dynabeads Streptavidin (Thermo Fisher) to further eliminate the biotin-unlabeled primers prior to pre-amplification. To determine the efficiency of each purification methodology, 5-cell samples isolated from human fibroblast cell line (Coriell 00323) were lysed and PCR-amplified using differentially labeled primers with an established pre-amplification protocol. tNGS CCS was then performed and the efficiency of aneuploidy detection was assessed using euploid (Coriell 00323) and aneuploid (Coriell 04435) human fibroblast cell lines. Efficiency of amplicon removal was determined by D1k ScreenTape (Agilent Tech. Inc) generating an amplicon-specific concentration for each sample.

RESULTS: 1) Using biotin-labeled primers for pre-amplification, $> 70\%$ of amplicons were removed; 2) Pre-purification of biotin-labeled primers with HPLC resulted in removal of $>90\%$ of amplicons; 3) Enrichment of HPLC-purified biotin-labeled primers using Dynabeads Streptavidin resulted in further purification, achieving removal of $>97\%$ of amplicons ($p < 0.001$). Use of labeled primers did not affect CCS efficiency, achieving 100% CCS diagnostic consistency for the 5-cell samples, similar to that obtained with unlabeled controls.

CONCLUSIONS: Our findings demonstrate that the use of biotin-labeled primers combined with a multi-step purification protocol allows the removal of $>97\%$ of amplicons without affecting the diagnostic efficiency of tNGS CCS. The protocol reported here may allow identification of novel genetic, epigenetic, or transcriptomic embryo viability parameters.