DOPlify® A new generation of whole genome amplification

Strategies to achieve combined non-invasive PGT-M & PGT-A on spent culture media using target sequence enrichment

The clinical use of non-invasive preimplantation genetic testing for aneuploidy (PGT-A) requires concordance of the spent embryo culture media result to the embryo biopsy result and the ability to distinguish maternal contamination from the embryonic DNA, especially for a euploid female result. Although concordance of spent embryo culture media and trophectoderm biopsy has been reported at as high as 95% following the collection of samples at Day 5-7 using DOPlify® kit (Lane et al, 2017), the ability to test media collected earlier in culture requires an increased level of sensitivity. Additionally, there are a number of known PCR inhibitors in culture media, including salts and proteins, which need to be overcome. Optimisation of a Whole Genome Amplification (WGA) reaction along with specific primers in a single PCR reaction allows both PGT-A and targeted higher resolution Next Generation Sequencing (NGS) providing a strategy to combine monogenic disease detection (PGT-M) with PGT-A.

Aim – To identify an optimal protocol to amplify DNA in spent embryo culture media that maximises WGA DNA yield and NGS results, and demonstrate the development of a combined PGT-M and PGT-A workflow for spent embryo culture media.

Methods – Spent embryo culture media was collected by clinics with ethics approval from single embryo culture droplets and pooled prior to storage at -20°C. Media was WGA using standard DOPlify® kit WGA protocol and to accommodate the biochemical composition of culture media and the presence of known PCR inhibitors, samples were also amplified using a re-formulated version of the DOPlify® kit. Furthermore, the TSE protocol (PerkinElmer) was added with the inclusion of sequence specific PCR primers for Haemoglobin subunit beta (HBB) for β-thalassemia mutation detection. Enrichment of the target sequence during WGA was confirmed using 5-cell aliquots and multiplex PCR and the specific PCR products generated were pooled back in with the original WGA with TSE DNA prior to sequencing using a single index. Sequencing was performed according to the standard PG-Seq™ 48 sample protocol on a MiSeq® instrument (Illumina) and data was bioinformatically aligned to hg19, analysed using PG-Seq™ software and viewed using Integrative Genomics Viewer (IGV).

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Results

✓ WGA DNA yield per culture media sample increased significantly following the amplification of pooled media samples using a modified, reformulated version of DOPlify® kit with NGS output measures becoming comparable to biopsy results.

	Culture Media Standard DOPlify®	Culture Media Modified Protocol	Biopsy Standard DOPlify®
Culture media WGA DNA yield (ng/µl)	3.7 ± 2.9[a]	13.3 ± 8.1[a]	> 20
Total reads per sample	390,000 ± 180,000	530,000 ± 140,000	> 300,000
% Reads mapping to hg19	86 ± 10	95 ± 5	> 95
% mtDNA	0.16 ± 0.19	0.08 ± 0.04	2.06 ± 1.23
% GC content	41	40	41
PG-Seq™ Software QC score	0.12 ± 0.10	0.07 ± 0.03	< 0.03

✓ Increased yield and improved NGS QC results have been obtained for individual culture droplets ranging in size from 10-60 µl of media and using media from a range of manufacturers.

✓ Mitochondrial DNA was successfully amplified from spent culture media; 0.08 + 0.04%, although with fewer reads per sample than typically seen for Day 5 biopsy samples; 2.06 + 1.23%.

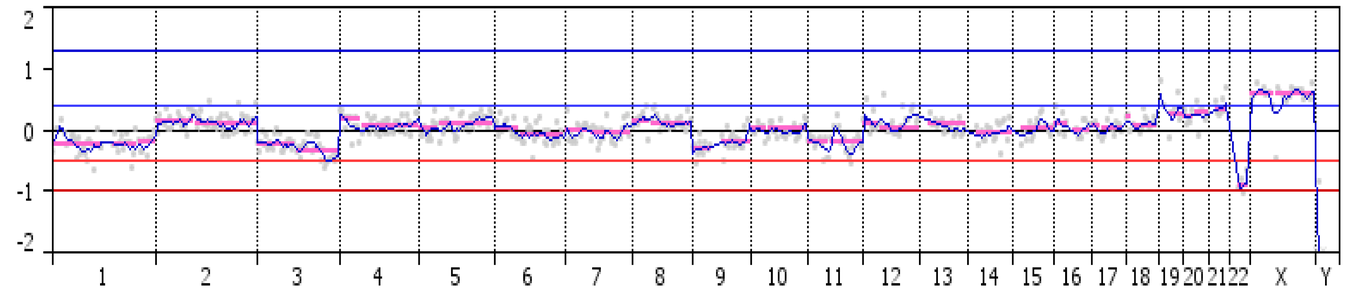
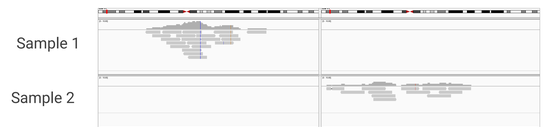


Figure 1. Spent embryo culture media sample result, 45,XX-22.

✓ Enrichment of the target sequences was confirmed by semi-quantitative sequence-specific PCR and NGS for all 5-cell aliquots and some pooled spent embryo culture media samples (n=2/3) compared to control samples (WGA only).

Figure 2. Integrative Genomics Viewer (IGV) screenshot of HBB for pooled spent embryo culture media samples amplified using a reformulated DOPlify® WGA kit + TSE.



✓ Coverage and read depth of sequences could be further optimised through the dilution of multiplex PCR products pooled with the WGA and TSE DNA prior to sequencing. Testing of individual spent embryo culture media samples is still required.

Conclusions

Reformulation of the DOPlify® kit:

- improves DNA amplification yield from spent embryo culture media;
- is suitable for a wide range of culture media droplet sizes and media manufacturers; and
- provides an opportunity for combined non-invasive PGT-M and PGT-A in a single amplification.

Reference - Lane et al (2017) Fertility and Sterility, Volume 108, Issue 3, e61.