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DOPlify® A new generation of whole genome amplification

Optimisation of whole genome amplification specifically for non-invasive PGT-A using spent embryo culture media

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® (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.

Aim – To identify an optimal protocol to amplify DNA in spent embryo culture media that maximises WGA DNA yield and NGS results.

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. Pooling was used to provide a consistent test sample to analyse across a range of protocol variants to overcome sample to sample variability. A total of 32 Whole Genome Amplification (WGA) protocol variants were tested in triplicate. A modified, reformulated single step DOPlify® was used to accommodate the larger media sample input volume into the WGA and the composition of the culture media following embryo culture. The same pooled media samples were amplified in parallel using the standard DOPlify® protocol. WGA DNA yield and PCR amplicon size distribution was assessed following gel electrophoresis and HS Qubit quantification (Thermo Fisher). Samples were then sequenced on a MiSeq (Illumina) according to the standard PG-Seq™ 48 sample protocol. The sequencing data for each sample was bioinformatically aligned to hg19 and the PG-Seq™ NGS metrics and software quality scores and %GC content for each protocol were compared.

	Standard DOPlify®	Modified Protocol
Lysis	Standard	Solution A + Heat
Sample volume (µl)	5	4
WGA volume (µl)	25	40
WGA formulation	Standard	Modified
PCR Program	Standard	Standard
Sample number	8	7

Results

✓ WGA DNA yield per culture media sample increased significantly following the amplification of pooled media samples using a modified, reformulated version of DOPlify® 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

[a] T-test P < 0.05

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

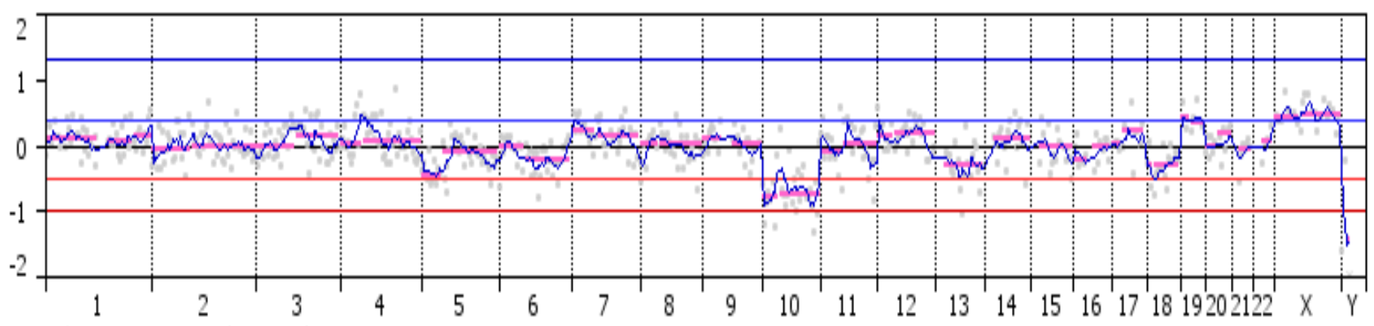

Figure 1. Sample result, 45,XX,-10

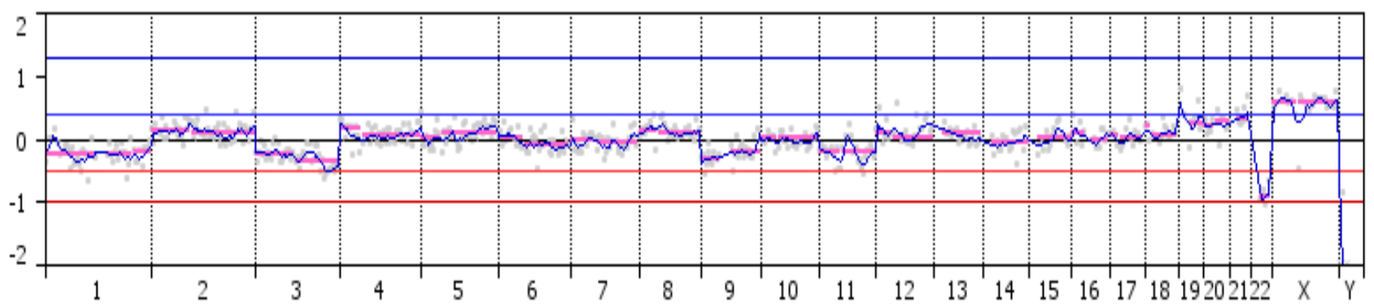

Figure 2. Sample result, 45,XX-22

✓ 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 %.

Conclusions

Reformulation of DOPlify®:

- Improves DNA amplification yield from spent embryo culture media;
- Is suitable for a wide range of culture media droplet sizes and media manufacturers;
- Is robust and automation ready using a single step protocol; and
- provides an opportunity for combined non-invasive PGT-A and PGT-M in a single amplification.

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