RHS Unique Genomic Solutions

repromed Fertility Specialists.

PG-Seq™ A novel complete NGS solution for PGS and PGD

Non-invasive PGS using cell free DNA from spent embryo culture media

Cell-free DNA (cfDNA) has been observed in spent embryo culture media, which creates an exciting possibility for the development of non-invasive preimplantation genetic screening (PGS). Non-invasive PGS offers several advantages. Most notably this includes removing the requirement for embryologists to have biopsy training and experience. It also provides the option to perform PGS on embryos that are not able to be biopsied due to embryo conditions such as stage of hatching or positioning of the inner cell mass. In the lead up to clinical evaluation, it is critical to confirm the ability to reliably whole genome amplify (WGA) the limited cfDNA in spent embryo culture media, and determine the concordance, accuracy and sensitivity with biopsy-based PGS.

Aim – To confirm robust, accurate and reliable whole genome amplification and subsequent chromosomal analysis of cfDNA from spent embryo culture media for non-invasive PGS.

Methods – Embryos were cultured and spent media was collected using an optimised method (Repromed, Australia). PGS on spent culture media was performed using the PG-Seq™ protocol with minor modifications (RHS Ltd, Australia). NGS indexed libraries were pooled and a total of 48 samples were multiplexed on a MiSeq sequencer (Illumina). The data was analysed using the PG-Seq™ software for chromosomal aneuploidy. Results were then compared to the matched Day 5 embryo biopsy PGS result which was generated using the VeriSeq protocol (Illumina). Samples failing the PG-Seq™ software quality control score were excluded from the study.

Results

cfDNA released into spent culture media was successfully whole genome amplified using PG-Seq™ (figure 1).

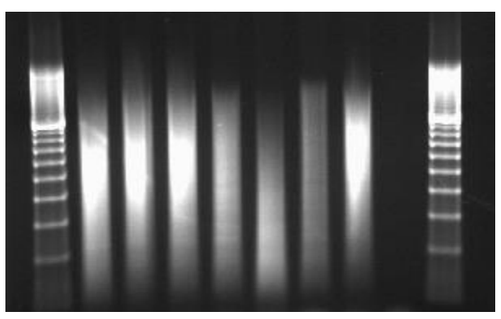


Figure 1: Whole genome amplified cfDNA from culture media samples (lane 1 & 10, DNA Ladder DMW-100M, lane 2-8 cfDNA and lane 9 no template control (NTC).

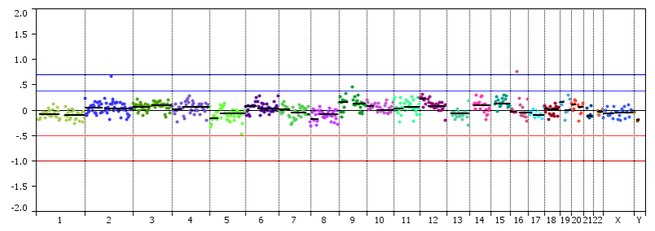
Results (continued)

The results using cfDNA from spent culture media and the matched euploid or aneuploid Day 5 embryo biopsy were 95% (21/22) concordant for chromosomal content. There was one false positive result where both the media and the biopsy gave an aneuploid result but the media detected an additional trisomy (table 1).

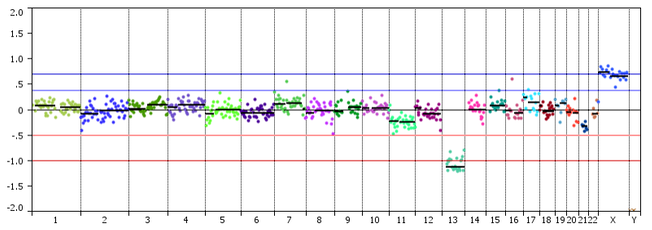
	Sample ID	Media Result (PG-Seq™)	Day 5 Biopsy Result (VeriSeq)
Euploid concordant	1	46,XY	46,XY
	2	46,XY	46,XY
	3	46,XY	46,XY
	4	46,XX	46,XX
	5	46,XY	46,XY
	6	46,XY	46,XY
	7	46,XX	46,XX
	8	46,XX	46,XX
	9	46,XY	46,XY
	10	46,XX	46,XX
Aneuploid concordant	11	45,XX,-13	45,XX,-13
	12	46,XY,-13,+20	45,XY,-13
	13	46,XX,+15,-22	47,XX,+15,-22
	14	47,XX,+16	47,XX, +16
	15	47,XY,+18	47,XY,+18
	16	45,XY,-18	45,XY,-18
	17	47,XY,+19	47,XY,+19
	18	47,XY,+22	47,XY,+22
	19	49,XY,+14,+18,+21	49,XY,+14,+18,+21
	20	47,XX,+15,-20,+22	47,XX,+15,-20,+22
	21	45,XX,-14	45,XX,-14
Non concordant	22	46,XX,+6,-18	46,XX (mosaic+19)

Results for cfDNA from spent culture media using PG-Seq™ were easy to interpret.

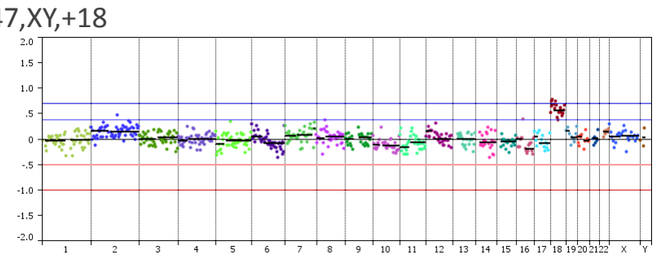
46,XY



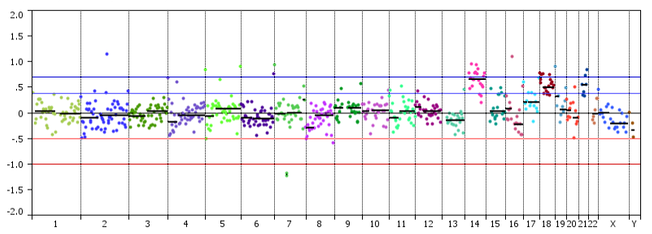
45,XX,-13



47,XY,+18



49,XY,+14,+18,+21



Conclusions

An optimised embryo culturing protocol enables the robust collection of cfDNA from embryos that is suited to non-invasive PGS.

PG-Seq™ reliably amplifies the limited cfDNA collected allowing:

- Accurate aneuploidy analysis by NGS
- A cost efficient and scalable method for non-invasive PGS

Non-invasive PGS using PG-Seq™ eliminates the need for embryo biopsy, significantly expanding the accessibility of PGS.

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