RHS
Unique Genomic Solutions

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

Innovative embryo identification using PG-Seq™

Mitochondria are maternally inherited. The mitochondrial genome (mtDNA) contains single nucleotide variants (SNVs). PG-Seq™ with DOPlify™ WGA provides superior amplification of the mitochondrial genome, with 100% of the mitochondrial sequence available from 48 sample multiplexing on a MiSeq.

Using a SNV panel, the mtDNA sequence provides a novel opportunity for DNA-based confirmation of maternal origin of an embryo biopsy and sibling embryo identification, which could be used for sample tracking within an IVF or genetic service provider laboratory.

Aim – To demonstrate the use of PG-Seq™ with RHS' Embryo ID panel to achieve accurate and economical PGS with embryo identification using low pass NGS.

Methods – Initial evaluation of a novel SNV panel was performed using five different aneuploid cell lines (Coriell Institute, USA). Multiple 5-cell aliquots were manually sorted from the cultures.

Samples were whole genome amplified and sequenced according to the standard PG-Seq™ protocol (RHS, Australia). NGS libraries were prepared and 48 samples were subsequently pooled and sequenced on a MiSeq platform according to a standard 1x75bp protocol (Illumina, USA).

The sequencing data was bioinformatically aligned to hg19 then following PG-Seq™ data analysis, the mtDNA was evaluated using the RHS Embryo ID panel. Each cell line was scored to generate a sample signature.

To demonstrate the clinical usefulness of the Embryo ID panel to provide embryo signatures, Day 5 embryo biopsy PG-Seq™ data from the same patients' embryos generated from multiple IVF cycles (FertilitySA, Australia) were scored to generate embryo biopsy signatures.

Results

14 SNV sites were identified across the 16,569bp mtDNA genome and included in the RHS Embryo ID signature panel. These sites are spread across the mitochondrial genome.

Due to the ability for PG-Seq™ to provide sequence data across the entire mitochondrial genome, a unique Embryo ID signature was achieved for each cell line and patient.

Results (continued)

The RHS Embryo ID panel was 100% concordant across multiple individual 5-cell samples derived from the same cell line.

Cell line	Karyotype	RHS Embryo ID signature	Concordance
Ref hg19		GTCGTTGGGTTAAT	
GM04435	48,XY,+16,+21	------AA------	100% (12/12)
GM09552	47,XY (gain of 31Mb on 3, 7Mb on 21)	---AG-A---C---	100% (5/5)
GM00143	47,XX,+18	--T--C--------	100% (5/5)
GM07189	47,XY,+15	A--A--A---C---	100% (5/5)
GM04965	48,XXY,+21	-C------A--G--	100% (5/5)

RHS Embryo ID panel signatures of embryo biopsies derived from different embryos from the same patient in a single cycle and across embryos from different cycles from the same patient were 100% concordant across all 14 SNV sites.

Patient ID	Cycle ID	Embryo Biopsied	RHS Embryo ID signature
Ref hg19			GTCGTTGGGTTAAT
Patient A	Cycle 1	E3	-C-A---------C
		E5	-C-A---------C
		E8	-C-A---------C
	Cycle 2	E1	-C-A---------C
		E4	-C-A---------C
Patient B	Cycle 1	E9	---A--A---C-G-
		E11	---A--A---C-G-
	Cycle 2	E6	---A--A---C-G-
		E8	---A--A---C-G-
	Cycle 3	E10	---A--A---C-G-
Patient C	Cycle 1	E5	-C-A----------
		E9	-C-A----------
	Cycle 2	E2	-C-A----------
		E14	-C-A----------
		E15	-C-A----------

Conclusions

A low pass PGS NGS protocol using PG-Seq™ in combination with the RHS Embryo ID panel;

- Readily generates a unique embryo biopsy signature of maternal origin
- Allows confirmation of sibling embryos
- Can be matched to maternal DNA to offer a definitive identification protocol

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