RHS
Unique Genomic Solutions

rhsc.com.au

PG-Seq™ A novel complete NGS solution

Clinical embryo sample tracking and identification using the mitochondrial genome

The mitochondrial genome contains single nucleotide variants (SNVs) that can be used to differentiate individuals, and are routinely used for population genetic studies and ancestry. Mitochondrial DNA (mtDNA) is maternally inherited, providing a novel opportunity for DNA-based confirmation of maternal origin of embryo biopsies and sibling embryo identification. The mitochondrial genome is sequenced during Preimplantation Genetic Testing for Aneuploidy (PGT-A) by Next Generation Sequencing (NGS) and the depth and breadth of coverage obtained from PG-Seq™ readily allows SNV analysis, even from a 48 sample NGS run. This readily available information could be used for sample tracking within an IVF or genetic service provider laboratory.

Aim – To demonstrate the use of the RHS Embryo ID panel to achieve accurate and economical embryo identification as part of routine PGT-A using PG-Seq™.

Methods – A large putative panel of mtDNA SNVs was collated from published literature and PG-Seq™ clinical data. SNVs associated with disease-related markers or in regions of known lower depth of coverage were excluded. A panel comprising 48 SNVs was compiled for evaluation. Three PG-Seq™ scenarios were modelled, wherein patients had 1, 2 or 4 embryos analysed. The performance of the panel was tested across 10,000 in-silico PG-Seq™ runs in each scenario using randomly selected mtDNA genomes from a globally-diverse database of 377 individuals (http://www.mtdb.igp.uu.se/) (Figure 1). Following analysis of a clinical dataset of mtDNA genomes from 52 patients (Figure 2), a population-specific panel comprising another 23 SNVs was added.

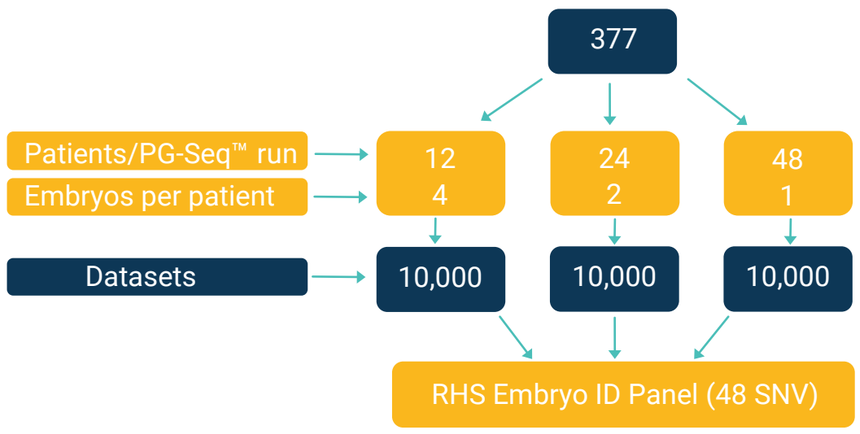

Figure 1. – Global mtDNA PG-Seq™ scenario.

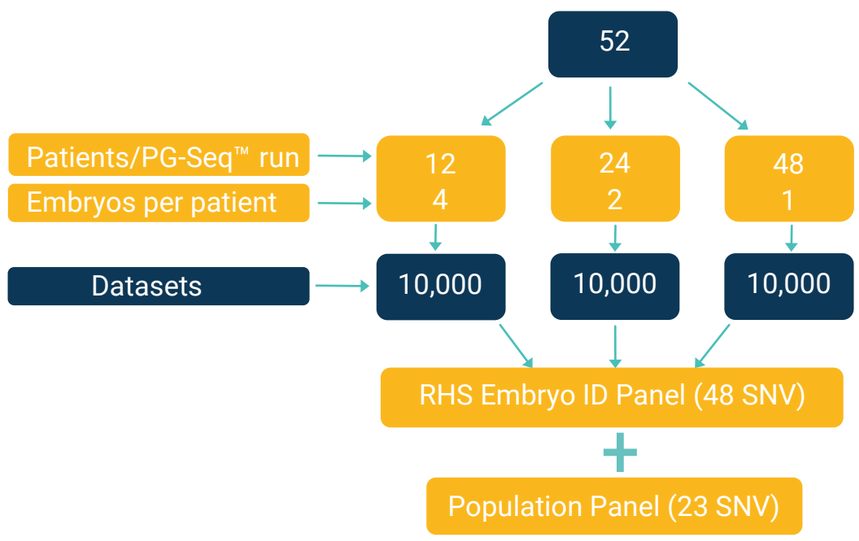

Figure 2. – Clinical mtDNA PG-Seq™ scenario.

Steven Myers[a], Michael Barry[b] and Melinda Jasper[a]
[a]RHS Ltd, Australia and [b]FertilitySA, Australia

RHS Ltd, 40-46 West Thebarton Road, Thebarton
South Australia 5031, Australia | **rhsc.com.au**

Results

Detection of SNVs within the mtDNA genome was used to create unique maternal origin signatures (Figure 3). Using the global mtDNA genome database and modelling embryos from 12, 24 or 48 patients, on average the SNV panel differentiated 91.5%, 84.6% or 75.0% of embryos, respectively.

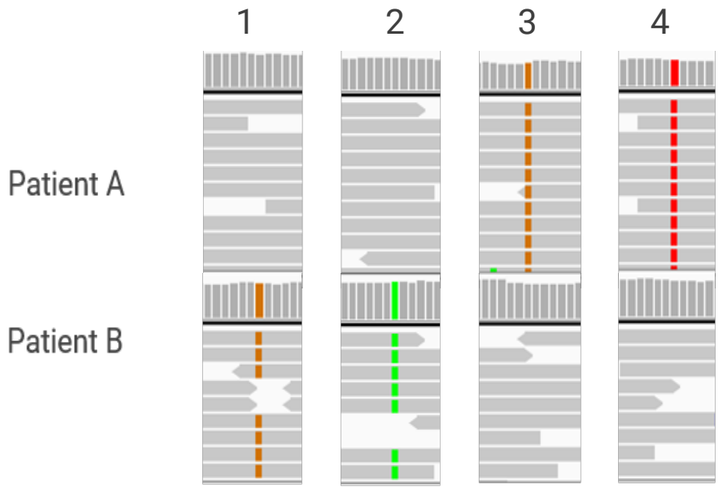

Figure 3. – Integrative Genomics Viewer (IGV) screenshot of PG-Seq™ mtDNA sequencing data aligned for 2 patients showing heterozygosity across 4 SNV positions.

Patient ID	Cycle ID	Embryo Biopsied	RHS Embryo ID signature
Patient 1	Cycle 1	1	---------C-G----G------T-------A---------------
		2	---------C-G----G------T-------A---------------
	Cycle 2	1	---------C-G----G------T-------A---------------
		2	---------C-G----G------T-------A---------------
Patient 2	Cycle 1	1	---------T-A----A------C-------G---------------
		2	---------T-A----A------C-------G---------------
	Cycle 2	1	---------T-A----A------C-------G---------------
		2	---------T-A----A------C-------G---------------
	Cycle 3	1	---------T-A----A------C-------G---------------

Table 1. – mtDNA sequencing data aligned for 2 patients showing heterozygosity across RHS Embryo ID SNV positions. Embryo samples from the same individual display the same SNV profile.

For the clinical PG-Seq™ NGS files analysed using the RHS Embryo ID SNV panel and an additional population-specific panel, with embryos from 12, 24 or 48 patients, the SNV panel differentiated on average 99.3%, 98.3% or 96.3% of embryos respectively (Figure 4 and Table 1).

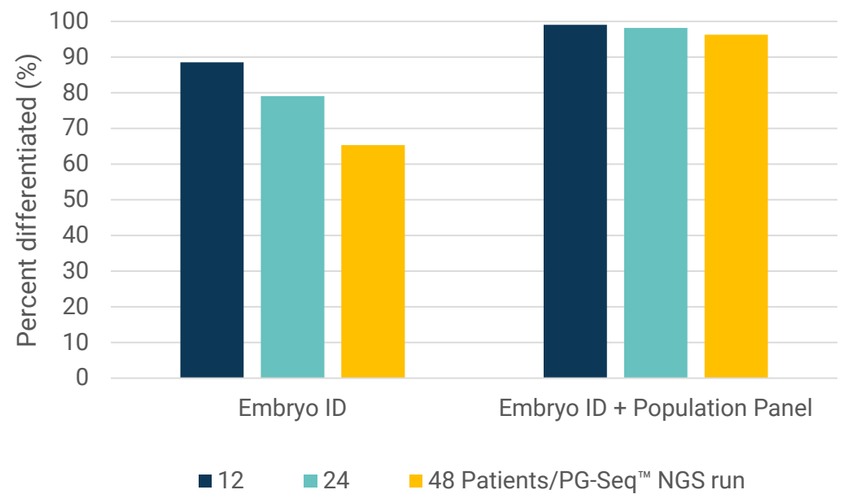

Figure 4. – Discriminatory power of the RHS Embryo ID SNV Panel was improved for clinical samples following incorporation of a population-specific panel.

In a clinical setting, if embryo signatures matched between patients, the rest of the mtDNA genome can still be used to further differentiate samples. It should be noted that maternally-related patients will share mtDNA genomes. In this case, it will not be possible to distinguish between embryos from these patients using the mitochondrial genome.

Conclusions

PG-Seq™ and the RHS Embryo ID SNV Panel:

- readily generates a unique embryo signature
- potentially improves PGT-A practice by providing a DNA-based confirmation of maternal origin & sibling embryo identification

The RHS Embryo ID SNV Panel will be incorporated into a future release of PG-Seq™ software.