PG-Seq™ Kit - A novel complete NGS solution

Validation of using the mitochondrial genome for embryo identification

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 the PG-Seq™ kit readily allows SNV analysis, even from a 48 sample NGS run. This 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 the PG-Seq™ kit.

Methods – A large putative panel of mtDNA SNVs was collated from published literature and PG-Seq™ 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 1, 2 or 4 sibling embryos were analysed per individual. 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 dataset of embryo mtDNA genomes from 52 individuals (Figure 2), a population-specific panel comprising another 23 SNVs was added.

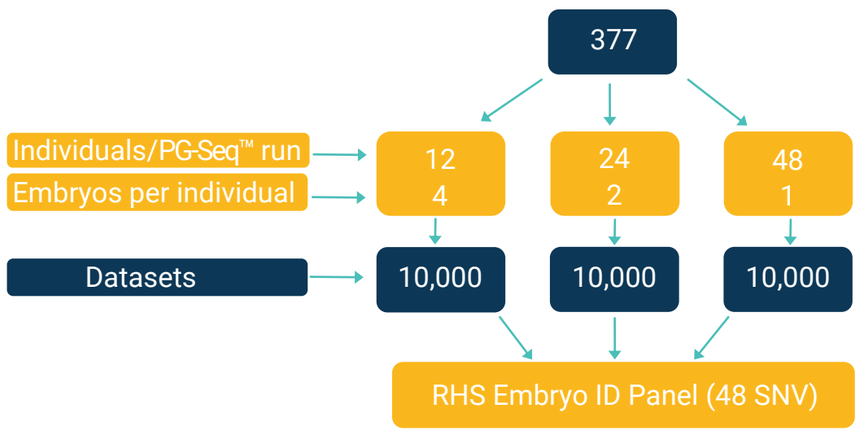

Figure 1. – Global mtDNA PG-Seq™ scenario.

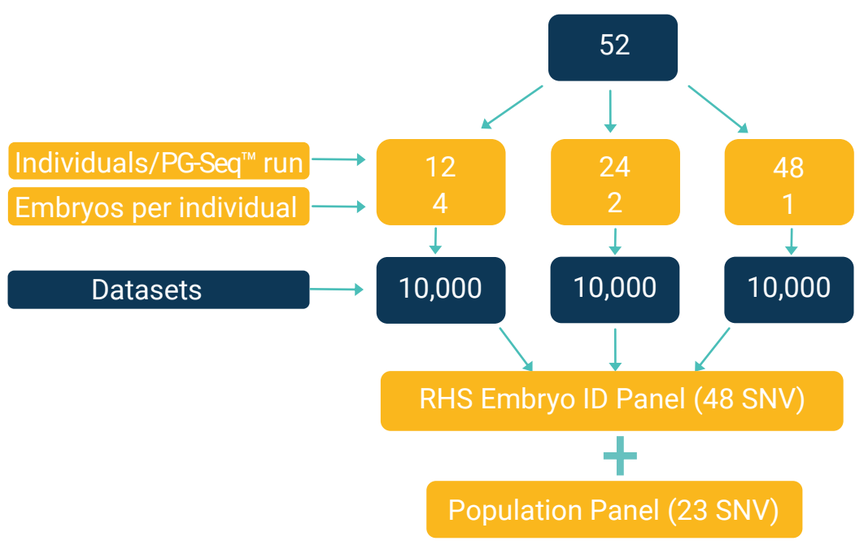

Figure 2. – Embryo mtDNA PG-Seq™ scenario

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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 individuals, on average the SNV panel differentiated 91.5%, 84.6% or 75.0% of embryos, respectively.

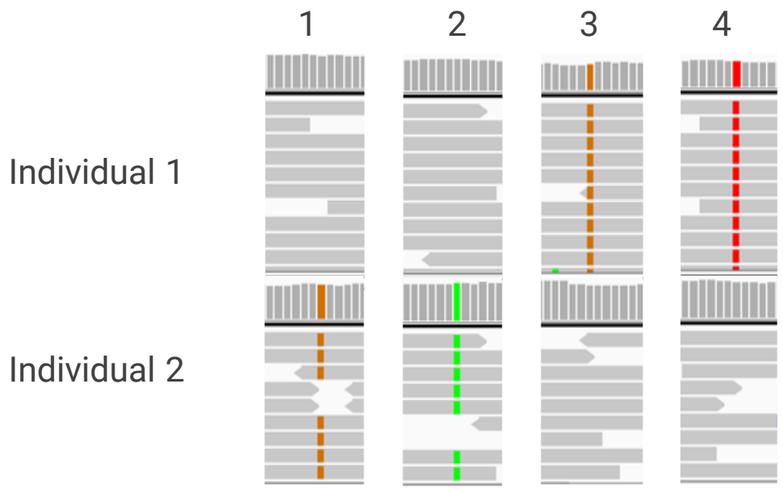

Figure 3. – Integrative Genomics Viewer (IGV) screenshot of PG-Seq™ mtDNA sequencing data aligned for 2 individuals showing heterozygosity across 4 SNV positions.

ID	Cycle ID	Embryo Biopsied	RHS Embryo ID signature
Individual 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----------
Individual 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 individuals showing heterozygosity across RHS Embryo ID SNV positions. Embryo samples from the same individual display the same SNV profile.

For the embryo 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 individuals, the SNV panel differentiated on average 99.3%, 98.3% or 96.3% of embryos respectively (Figure 4 and Table 1).

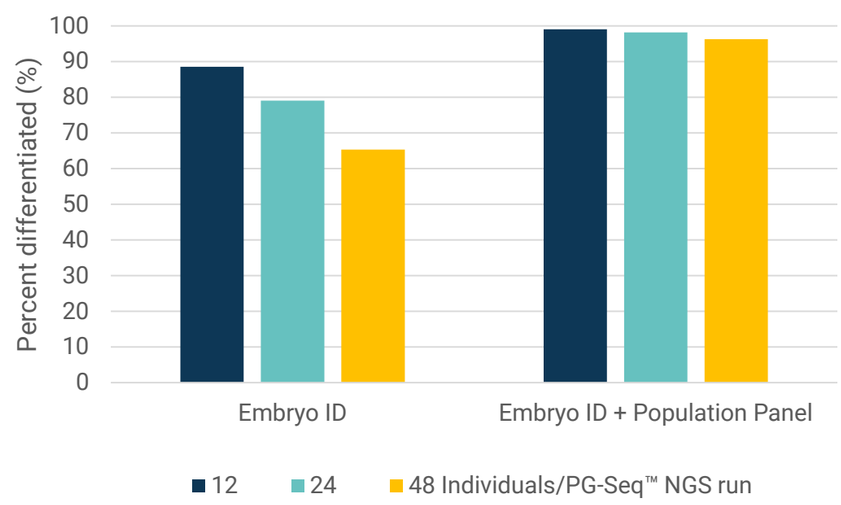

Figure 4. – Discriminatory power of the RHS Embryo ID SNV Panel was improved following incorporation of a population-specific panel.

If embryo signatures matched between individuals, the rest of the mtDNA genome can still be used to further differentiate samples. It should be noted that maternally-related women will share mtDNA genomes. In this case, it will not be possible to distinguish between embryos from these individuals 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.