RHS
Unique Genomic Solutions

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Introducing PG-Seq™, a complete NGS solution for PGS

PG-Seq™ for Pre-implantation Genetic Screening offers a novel complete, cost effective workflow. Suitable for the analysis of up to 48 embryo biopsies in a single NGS run, the workflow includes DOPlify™, library preparation reagents and data analysis software.

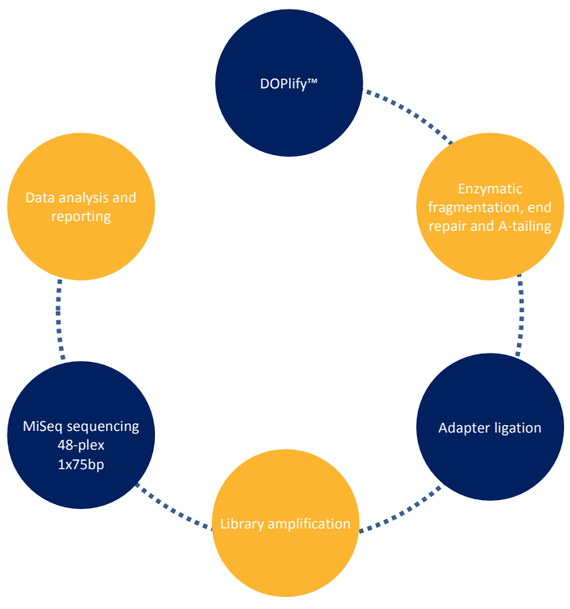
Prior to clinical validation, it is critical to validate the performance of PG-Seq™ using cell lines of known identity.

Aim – To assess the performance of PG-Seq™ using euploid, single and double trisomy and segmental aneuploidy single cells and 5-cells.

Methods – Cell lines included in this early release validation data set include 47,XY+9, 47,XY+13, 47,XY+15, 47,XX+18, 47,XX+21, 47,XY+22, 48,XY,+16,+21, 48,XXY,+21, 46,XX and 46,XY. Cell line aberrations of a 31Mb gain on chromosome 3 and a 7Mb gain on 21; GM09552, 7Mb loss and 31Mb gain on chromosome 8; GM14485 and a 16Mb loss on 13; GM07312 were also included.

Cell lysis and whole genome amplification of single cell and 5-cell samples was performed according to the PG-Seq™ protocol incorporating DOPlify™. Amplification success was confirmed by gel electrophoresis.

PG-Seq™ workflow



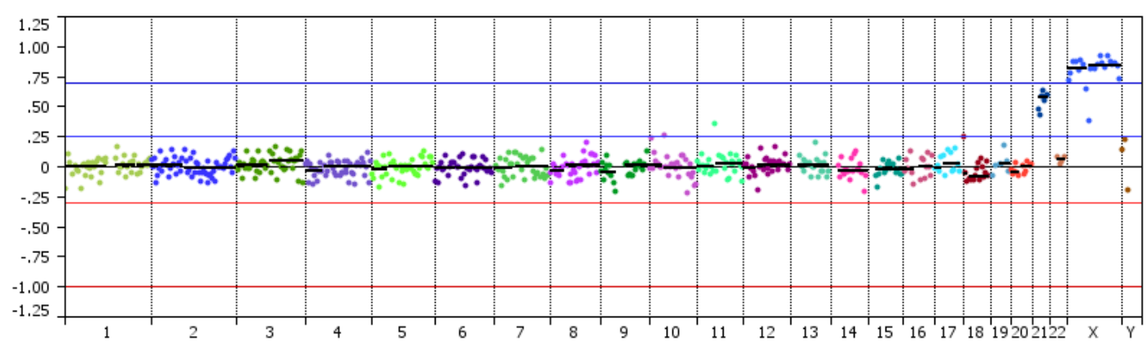
NGS libraries were prepared using enzymatic fragmentation prior to indexing. A total of 48 samples were subsequently multiplexed and sequenced on a MiSeq platform according to standard 1x75bp protocol (Illumina). The sequencing data was analysed using RHS software and results were compared to the known karyotype of the cell line. Average total reads per sample was 600,000 with an average mapping rate to hg19 of >98%.

✓ Aneuploidy detection

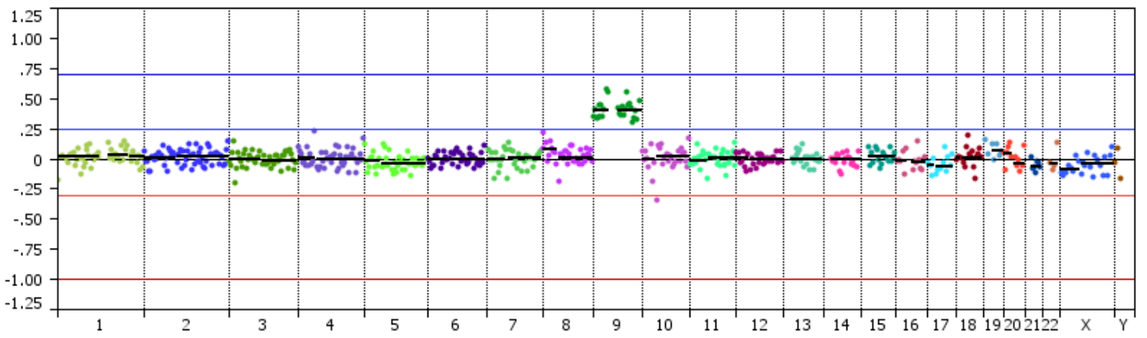
Of the single cells analysed, 94% provided the correct aneuploidy result corresponding to the cell line karyotype. Of the 5-cell samples analysed, 99% provided a correct aneuploidy result corresponding to the cell line karyotype. There was for the 5-cell data set, 1 false negative result for the loss of chromosome Y, but this sample was still positive for the known aneuploidy.

The total number of chromosomes analysed for single cells was 4,857; the results were 99.88% correct. The total number of chromosomes analysed for 5-cell samples was 4,403; the PG-Seq™ results were 99.99% correct.

	Total	Correct	FP	FN	FP + FN
Single cell	103	94% (97)	5% (5)	0% (0)	1% (1)
5-cell	94	99% (93)	0% (0)	1% (1)	0% (0)



Sample: GM04965 48,XXY,+21



Sample: GM09286 47,XY,+9

✓ Subchromosomal CNV detection

Segmental aberrations of 7-31Mb were detected with 100% sensitivity and specificity in 5-cell samples.

✓ Mitochondrial DNA content assessment

The average mtDNA content of aneuploid samples was 0.34% compared to 0.55% for euploid 5-cell samples.

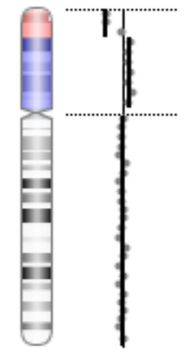


Figure 1 – GM09552 cell line chromosome 8; 7Mb loss and 31Mb gain.

Conclusions

- PG-Seq™ provides high sensitivity and accuracy for ploidy detection from single and 5-cell samples
- Software analysis determines sample aneuploidy, sub chromosomal copy number aberrations and mtDNA content
- Preparation and analysis of 48 samples can be completed comfortably in 24 hours
- The PG-Seq™ workflow is also compatible with the RHS Targeted Sequence Enrichment protocol for combined PGS + PGD

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