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Unique Genomic Solutions

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PG-Seq™- an accurate complete NGS solution for PGS

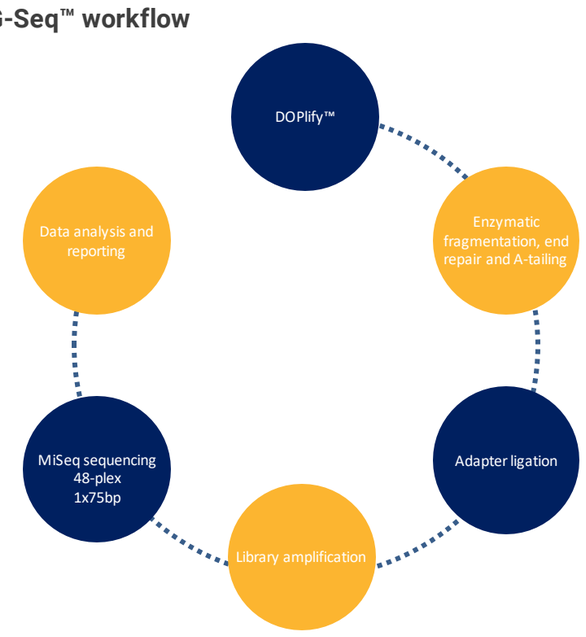
PG-Seq™ for Pre-implantation Genetic Screening developed by RHS Ltd offers a novel complete, cost effective workflow. The product includes DOPlify™ lysis and whole genome amplification reagents, latest generation NGS library preparation reagents and easy to use data analysis software all compatible with the Illumina MiSeq sequencer. The product has been developed for the analysis of up to 48 embryo biopsies in a single NGS run, twice the capacity of VeriSeq, with no change to equipment required.

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

Methods – Cell lines included in the validation data set were purchased from the Coriell Cell Repository; 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 with a 31Mb gain on chromosome 3 and a 7Mb gain on chromosome 21 (GM09552 Figure 1), 7Mb loss and 31Mb gain both on chromosome 8 (GM14485) and a 16Mb loss on chromosome 13 (GM07312) were also included.

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

PG-Seq™ workflow



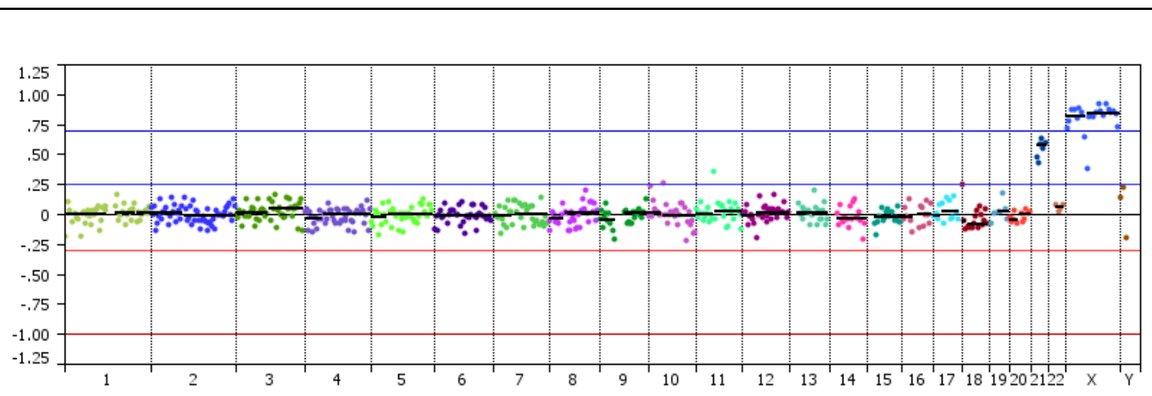
NGS libraries were prepared using enzymatic fragmentation prior to indexing. A total of 48 samples were subsequently multiplexed and sequenced on a MiSeq platform according to standard 1x75bp protocol (Illumina). The sequencing data was analysed using RHS software and results were compared to the known karyotype of the cell line.

Results
Average total reads per sample was 600,000 with an average mapping rate to hg19 of >98%.

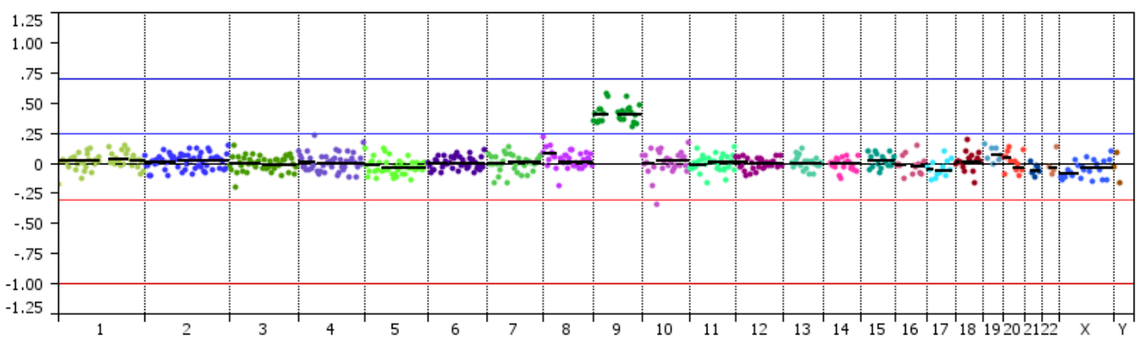
✓ Aneuploidy detection

Of the 5-cell samples analysed, 94% provided a correct aneuploidy result corresponding to the cell line karyotype. Of the single cells analysed, 89% provided the correct aneuploidy result corresponding to the cell line karyotype. The total number of chromosomes analysed for single cells was 9,023; the results were 99.64% correct. The total number of chromosomes analysed for 5-cell samples was 11,713; including the mosaic results in the correct result reporting, the PG-Seq™ results were 99.91% correct. Mosaicism has been observed when these cell lines have been karyotyped (data available in the RHS PG-Seq™ and EmbryoCellect® Validation Application Notes).

	Total	Correct	Mosaic	FP	FPS	FN	FNS	FP + FN	Chaotic
Single cell	192	89% (171)	-	2.6% (5)	5.7% (11)	0.5% (1)	1.0% (2)	0.5% (1)	0.5% (1)
5-cell	250	94% (235)	1.6% (4)	1.2% (3)	3.2% (8)	0% (0)	1.2% (3)	0% (0)	-



Sample: GM0496548,XXY,+21



Sample: GM0928647,XY,+9

✓ Subchromosomal CNV detection

Segmental aberrations of 7-31Mb were detected with 98.3% 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.

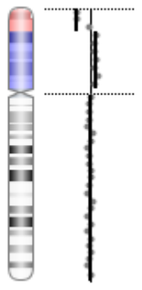


Figure 1 – GM09552 cell line chromosome 8; 7Mb loss and 31Mb gain.

Conclusions

- PG-Seq™ provides high sensitivity and accuracy for ploidy detection from single and 5-cell samples
- Software analysis detects 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

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