APPLICATION NOTE

Analysis of embryo biopsies using the Illumina MiSeq and MiSeq i100 sequencing platforms.

Introduction

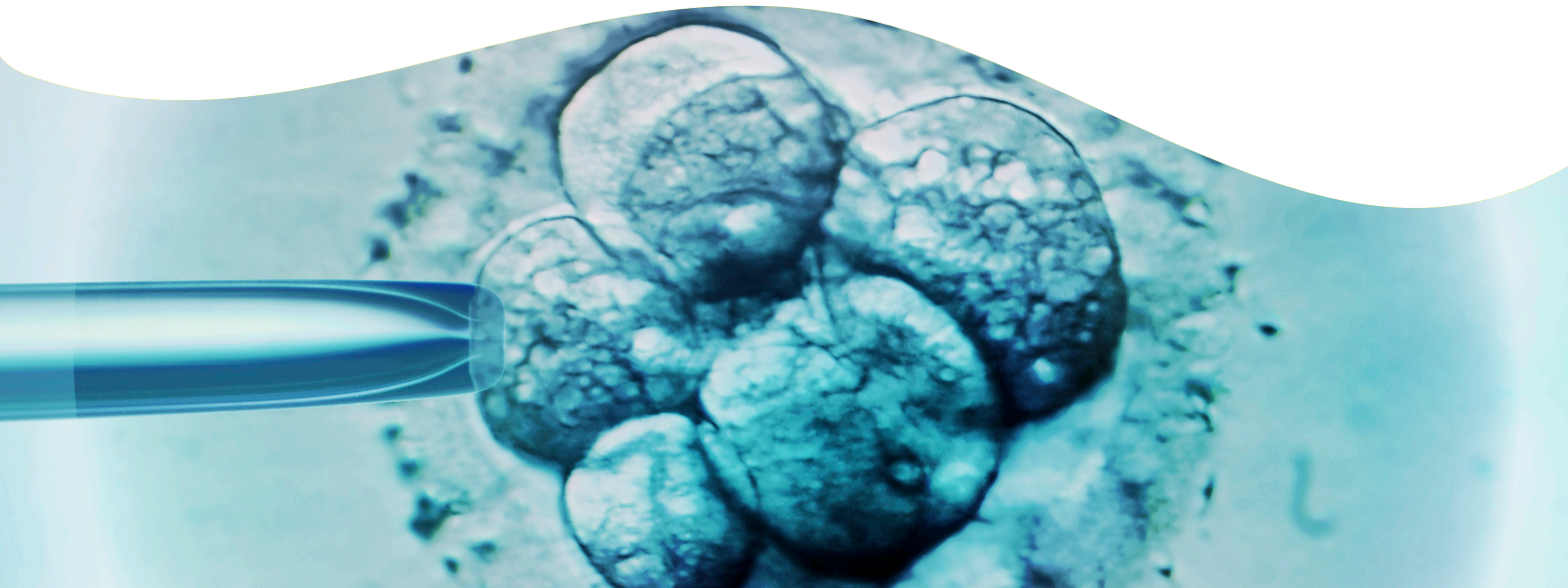
The PG-Seq™ Rapid kit v2 has been developed to analyze picogram quantities of DNA (single/multi-cells or low template DNA) from an embryo biopsy for preimplantation genetic testing. The kit utilizes whole genome amplification (WGA) and next generation sequencing (NGS) technology to test for all 24 chromosomes for whole chromosome aneuploidy and sub-chromosomal abnormalities. PG-Seq Rapid kit v2 is fully compatible with Element Biosciences and all Illumina® sequencers, including Illumina MiSeq® i100 which is the objective of the following study.

Methods

Individual embryo biopsy samples were processed using the PG-Seq Rapid v2 kit workflow. Each library was split in two aliquots and sequencing was performed using a 75 cycles output kit on the Illumina MiSeq® and MiSeq® i100 instrument. After demultiplexing, BAM files were uploaded and analyzed with the PG-Find™ analysis software. Results were compared to determine performance of the Illumina MiSeq® i100 vs MiSeq®.

Results

A total of 7 samples (listed below as sample 1 to 7) were sequencing in parallel with Illumina MiSeq® and MiSeq® i100 instruments. Analysis was performed using the PG-Find software with 10Mb resolution.



Samples showed similar averaging total reads and percentage of aligning reads (Table 1). Percentage of mapped reads was >95% for both platforms, although MiSeq® showed in the samples studied ~2% higher mapping rate than MiSeq® i100. We think this is a consequence of the differences in the number of fluorescent channels (4 in the MiSeq® and 2 in the MiSeq® i100) and the scheme for base calling in the two platforms, which can influence read alignment.

Table 1: Sample metrics from PG-Find software.

Sample	Sequencer	Total reads	Mapped reads	% Mapped
1	MiSeq i100	525.317	504.447	96.0272
1	MiSeq	457.385	451.226	98.6534
2	MiSeq i100	463.817	446.169	96.1951
2	MiSeq	409.422	403.446	98.5404
3	MiSeq i100	587.949	565.306	96.1488
3	MiSeq	501.676	494.497	98.5690
4	MiSeq i100	497.64	480.153	96.4860
4	MiSeq	446.158	441.448	98.9443
5	MiSeq i100	539.04	518.936	96.2704
5	MiSeq	491.712	486.331	98.9057
6	MiSeq i100	413.468	397.31	96.0921
6	MiSeq	368.435	364.09	98.8207
7	MiSeq i100	457.849	439.24	95.9356
7	MiSeq	429.275	424.646	98.9217
Average MiSeq i100		497.87	478.79	96.17
Average MiSeq		443.44	437.95	98.77

Copy number and breakpoint detection remained reliable for sub-chromosomal CNV samples, with the breakpoints determined by the software concordant between samples from the Illumina and MiSeq®. Detailed plots of the copy number profile of each sample are shown in figure 1-14.

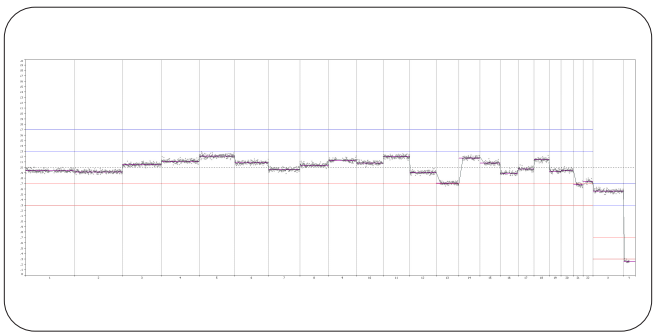


Figure 1: Sample 1 MiSeq i100 sample 46,XX,mosaicism Chr.13

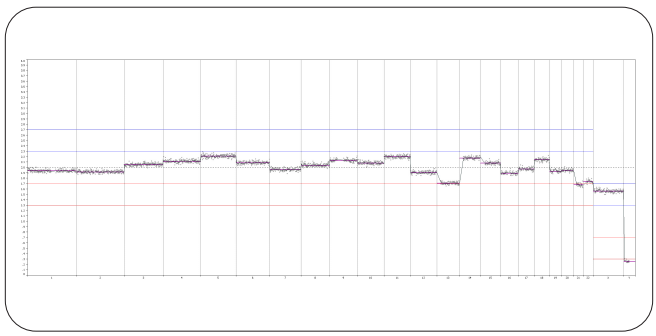


Figure 2: Sample 1 MiSeq sample 46,XX,mosaicism Chr.13

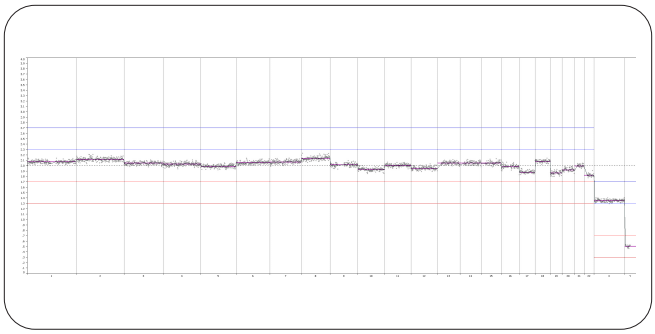


Figure 3: Sample 2 MiSeq i100 sample 46,XY, Euploid

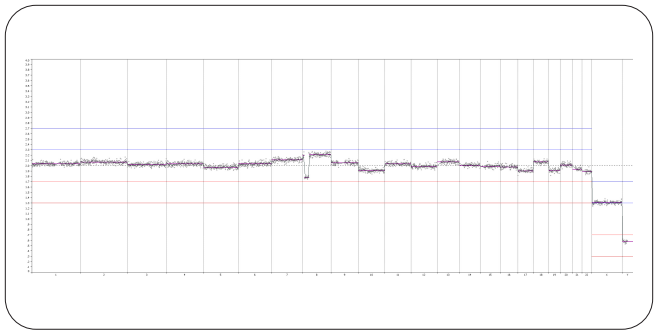


Figure 4: Sample 2 MiSeq sample 46,XY, Euploid

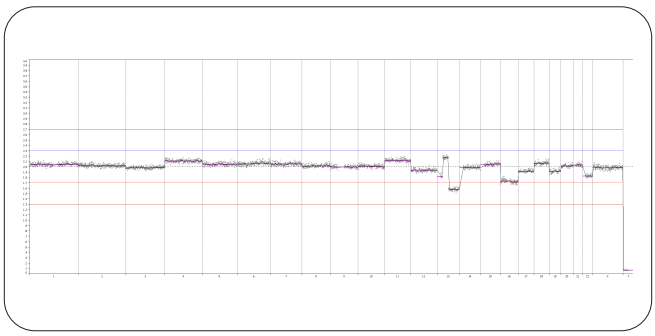


Figure 5: Sample 3 MiSeq i100 sample 46,XX,Euploid

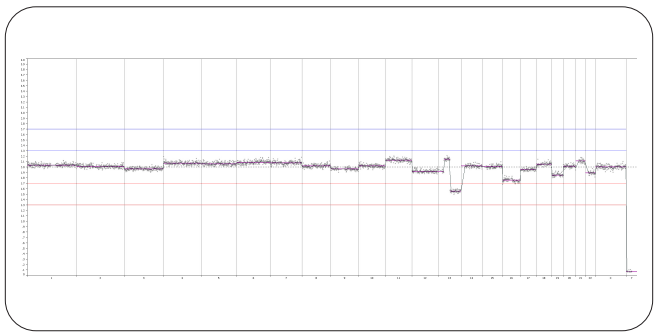


Figure 6: Sample 3 MiSeq sample 46,XX,Euploid

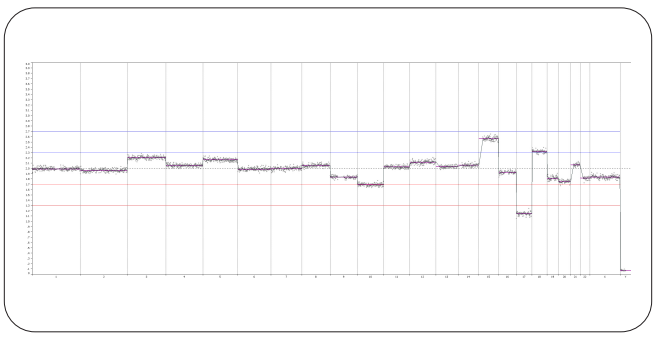


Figure 7: Sample 4 MiSeq i100 sample 46,XX,-17,2 mosaicism Chr.10,15,18

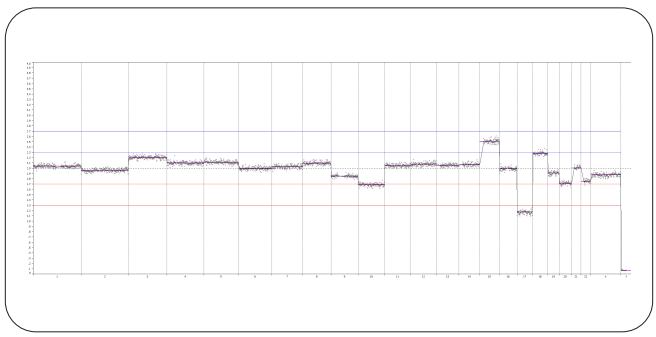


Figure 8: Sample 4 MiSeq sample 46,XX,-17,2

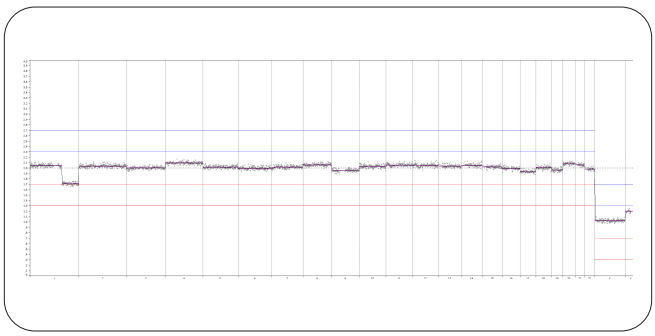


Figure 9: Sample 5 MiSeq i100 sample 46,XY,Euploid

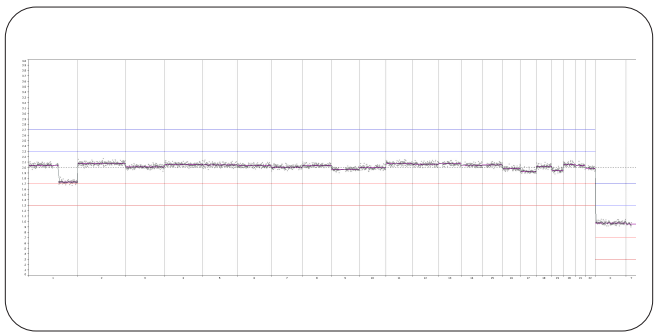


Figure 10: Sample 5 MiSeq sample 46,XY,Euploid

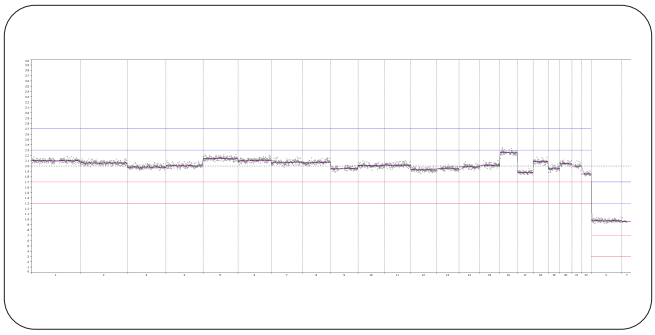


Figure 11: Sample 6 MiSeq i100 sample 46,XY,Euploid

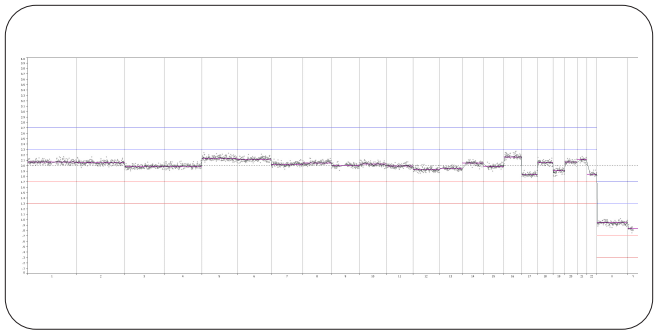


Figure 12: Sample 6 MiSeq sample 46,XY,Euploid

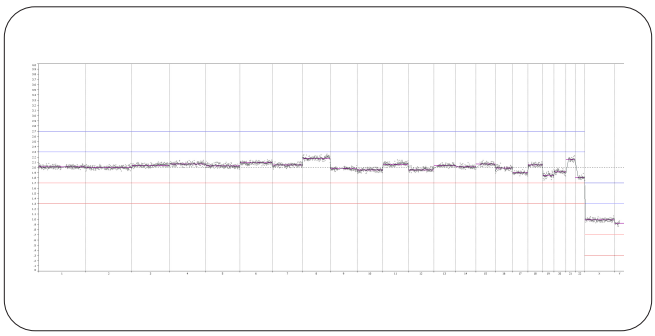


Figure 13: Sample 7 MiSeq i100 sample 46,XY,Euploid

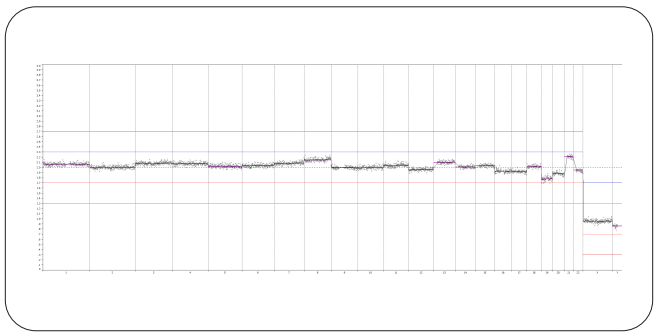


Figure 14: Sample 7 MiSeq sample 46,XY,Euploid

Conclusions

Embryo biopsies processed with the PG-Seq Rapid v2 kit using the Illumina MiSeq® i100 can successfully be analyzed.
No significant differences were observed in the results between the Illumina MiSeq® and MiSeq® i100 sequencing platforms.