TECHNICAL NOTE

# PG-Seq™ Rapid kit v2 assessment summary.


## Authors

Kimberly Warren

Revvity, Inc.



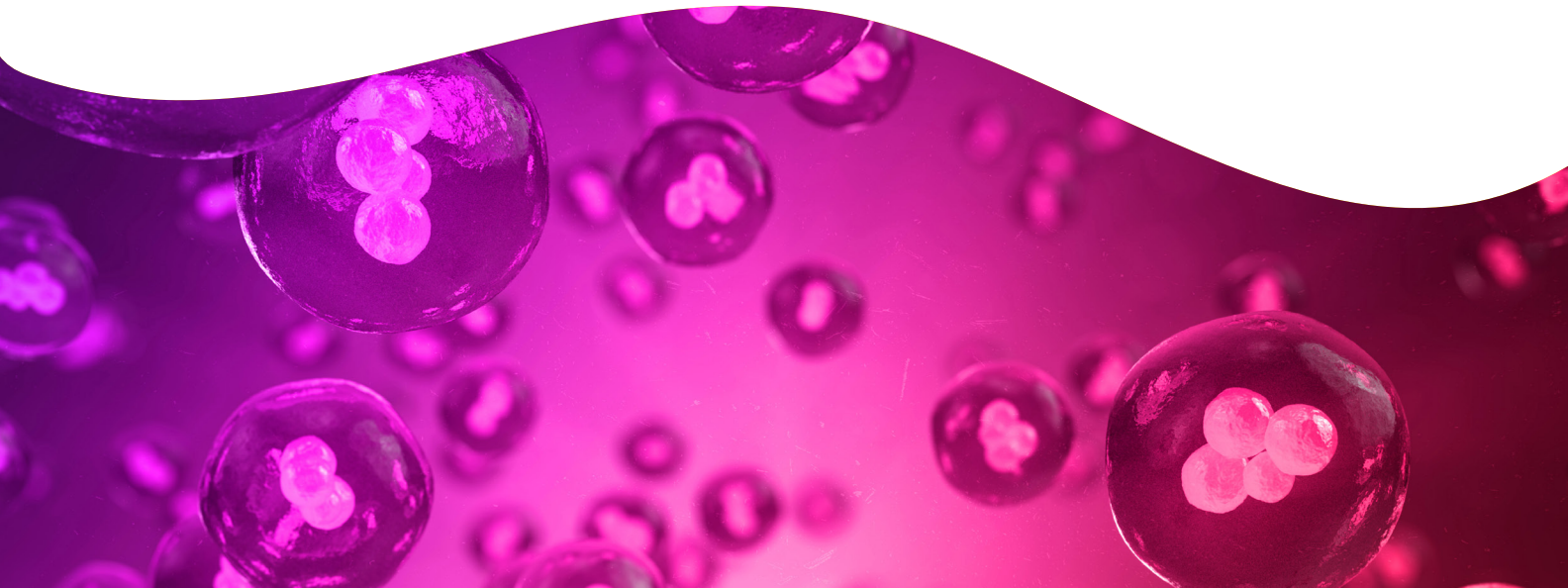

## Introduction

PG-Seq™ Rapid kit v2 was developed to offer a streamlined workflow and improved whole genome coverage to facilitate an improved user experience and allow compatibility for optional downstream applications.

### Methods

Cell lines of the following karyotypes; 47,XX,+18 (GM00143), 47,XY,+15 (GM07189), 48,XXY,+21 (GM04965) and genomic DNA 48,XY,+2,+21 (GM03576), 47,XXX (NA04626), 46,XX,del(13)(pter>q14.1::q21.2>qter) (NA07312) and 47,XY,+der(21)t(3;21)(p24.1;q21) (NA09552) were purchased from Coriell Biorepository (USA). Peripheral lymphocytes from a male were also isolated from peripheral blood mononuclear cells and were designated 46,XY. Cells were washed in droplets of PBS and manually grouped together in 5-cell aliquots before being transferred to a PCR tube and stored at -20°C. Genomic DNA was quantified and serially diluted in 10mM Tris-HCl pH 8 to a final concentration of 30pg/µL.

Cell lysis, WGA PCR 1 and Indexing PCR 2 was performed using 5-cell samples and 30pg dilutions of genomic DNA, according to the instructions in the PG-Seq™ Rapid kit v2 user manual. Following Cell lysis and WGA PCR 1, the samples were visualized via LabChip® GX II Touch™ HT nucleic acid analyzer (figure 1) to determine WGA success. After Indexing PCR 2, samples underwent the clean-up and size selection protocol, either individually or as a pool of samples. Final library pools were analyzed for fragment size on the LabChip® GX II



Touch™ HT nucleic acid analyzer (figure 2). Sequencing was performed on an Illumina® MiSeq® instrument or Illumina® MiniSeq® instrument with 1x75bp single index reads, aiming for approximately 500,000 reads per sample or the equivalent of 48 samples per run on the Illumina® MiSeq® v3 or MiniSeq® High Output flow cell. FASTQ files were aligned to hg19 using the Burrows-Wheels Aligner (BWA) application on Basespace (Illumina, USA) and the resultant BAM files were down-sampled to 400,000 reads before analysis with the PG-Find™ v3 software. The PG-Find™ self-reference algorithm was utilized with the default settings, and one adjustment of the significance threshold from 1.0E-7 to 1.0E-6 to facilitate improved detection of smaller CNVs.

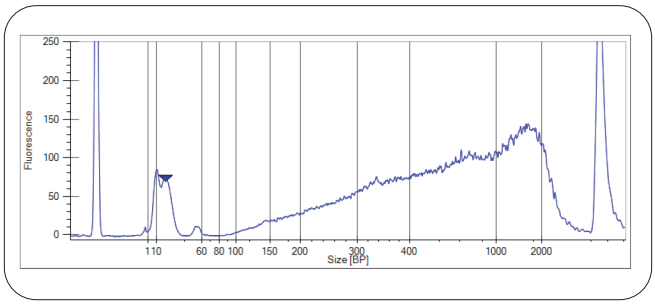



Figure 1. LabChip® GX Touch™ II HT nucleic acid analyzer trace of typical products generated from the PG-Seq™ Rapid kit v2 WGA PCR 1.

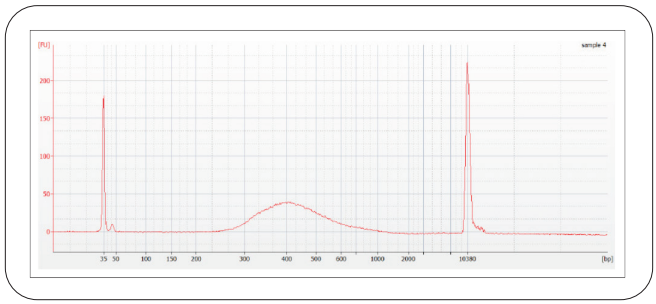


Figure 2. Bioanalyzer trace of a final library pool after clean-up and size selection, confirming that the DNA is a suitable size for Illumina® sequencing.

## Results

A total of 100 samples were amplified and analyzed with the PG-Seq™ Rapid kit v2. All samples were successfully amplified; however, two samples failed QC (high noise) and were excluded from the analysis. Of the 98 samples that amplified and passed QC metrics, 95 (97.0%) generated the expected karyotype and 3 (3.0%) generated the correct karyotype, but with a slightly lowered copy number ratio and were designated mosaic. Median copy number ratios for the chromosomes expected to be aneuploid but determined as mosaic were all greater than 2.63. From previous cell line karyotyping performed (data reported previously), it is known that mosaicism is present in some cell lines, and it is not unexpected to see a mosaic result. There were no false positives or false negatives detected. Copy number and breakpoint detection remained accurate for sub-chromosomal CNV samples, with the breakpoints determined by the software concordant with that determined by karyotyping. A per cell line summary is provided in table 1 and an example of the copy number profile of each cell line is shown in figure 3-10.

Table 1: Samples and results

| Cell Line ID | Expected Karyotype | Replicate Number | CNV Result |
|---|---|---|---|
| GM00143 5-cell | 47,XX,+18 | 14 | 13 correct, 1 mosaic |
| GM07189 5-cell | 47,XY,+15 | 42 | 40 correct, 2 mosaic |
| GM04965 5-cell | 48,XXY,+21 | 10 | 9 correct, 1 failed QC |
| NA07312 30pg | 46,XX,del(13)<br>(pter>q14.1::q21.2>qter | 6 | 6 correct |
| NA09552 30pg | 47,XY,+der(21)t(3;21)(p24.1;q21) | 6 | 6 correct |
| NA03576 30pg | 48,XY,+2,+21 | 6 | 6 correct |
| NA04626 30pg | 47,XXX | 6 | 6 correct |
| 46,XY 5-cell | 46,XY | 10 | 9 correct, 1 failed QC |

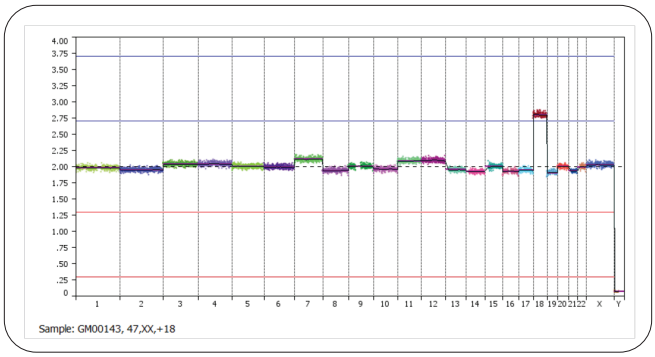



Figure 3: 5 cell sample GM00143 47,XX,+18

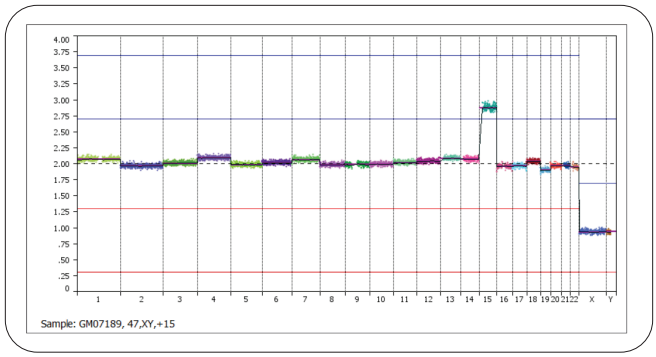



Figure 4: 5 cell sample GM07189 47,XY,+15

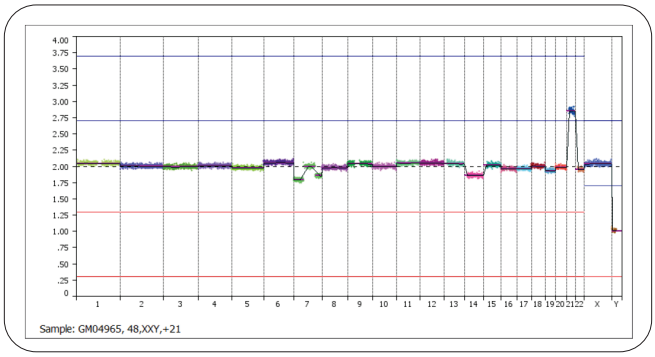



Figure 5: 5 cell sample GM04965 48,XXY,+21

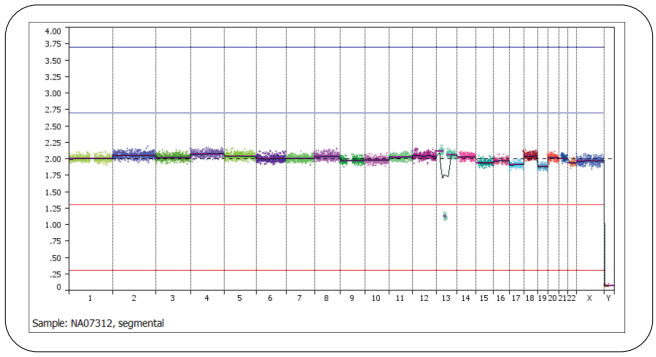



Figure 6a: 30pg genomic DNA sample NA07312 46,XX,del(13) (pter>q14.1::q21.2>qter)

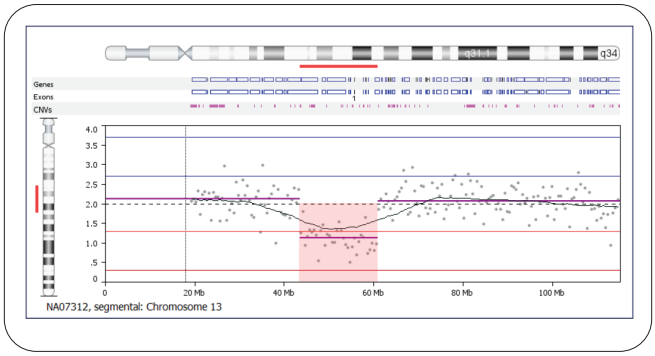



Figure 6b: 30pg genomic DNA sample GM07312, 46,XX,del(13) (pter>q14.1::q21.2>qter), closer view of the 18Mb loss on chromosome 13

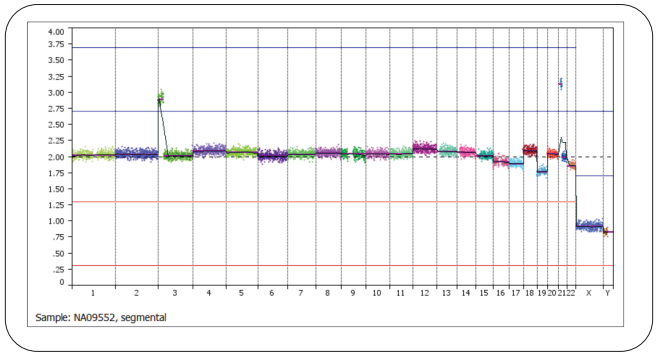



Figure 7a: 30pg genomic DNA sample NA09552 47,XY,+der(21) t(3;21) (p24.1;q21).

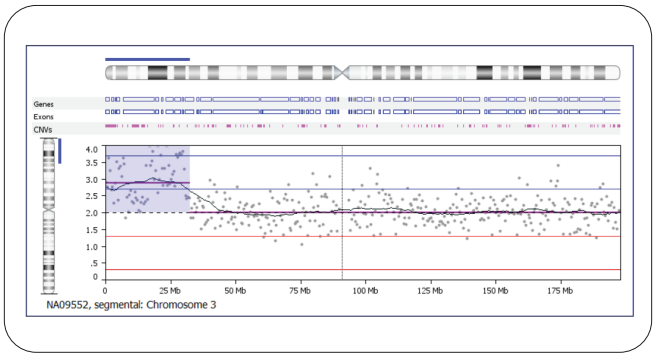



Figure 7b: 30pg genomic DNA sample NA09552 47,XY,+der(21) t(3;21) (p24.1;q21), closer view of the 31Mb gain on chromosome 3.

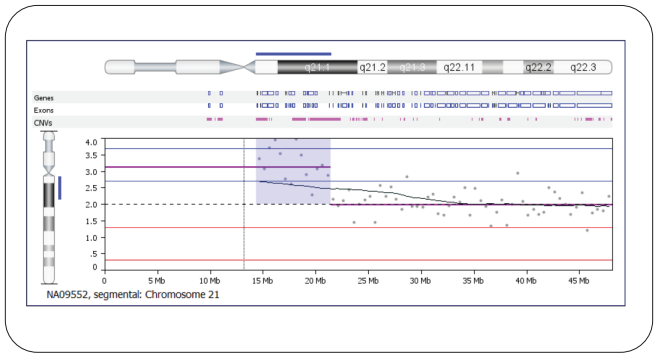



Figure 7c: 30pg genomic DNA sample NA09552 47,XY,+der(21) t(3;21) (p24.1;q21).

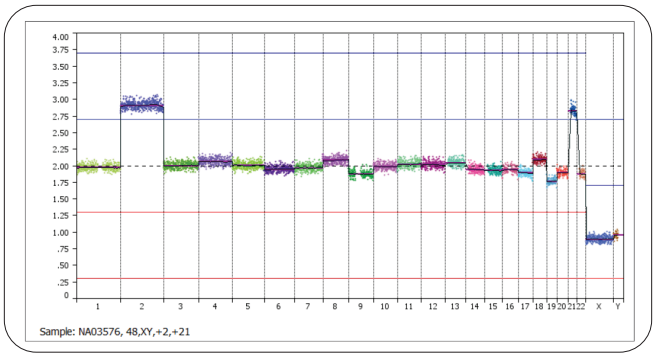



Figure 8: 30pg genomic DNA sample NA03576 48,XY,+2,+21

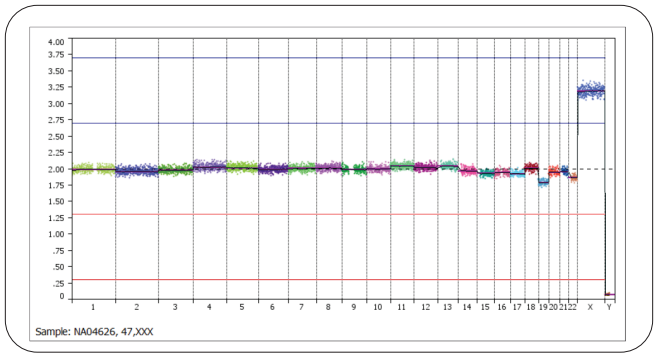



Figure 9: 30pg genomic DNA sample NA04626 47,XXX

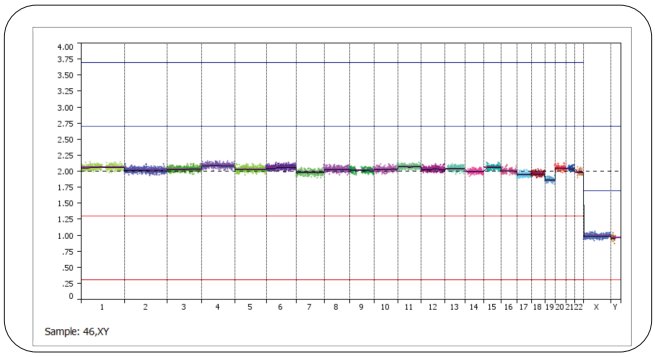



Figure 10: 5 cell sample 46,XY

## Conclusions

The PG-Seq™ Rapid kit v2 offers analysis of whole chromosome and sub chromosomal copy number changes down to 7 Mb in size. The data presented in this study was generated with 5-cell aliquots and diluted genomic DNA extracted from cell lines with known karyotypes as a model of trophectoderm biopsy. The amplification is reliable, flexible, easy to use and in this study showed 100% amplification success and 98% of samples generating a result that passed quality control metrics. Correct results were displayed in 97% of samples and this performance was achieved by multiplexing 48 samples in a single Illumina® sequencing run, generating approximately 400,000-500,000 reads per sample.

**Revvity, Inc.**
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com