APPLICATION NOTE

DOPlify® v2 WGA kit comparison study.

DOPlify® v2 WGA kit and Takara® PicoPLEX® Single Cell WGA kit performed comparably when analyzed for whole chromosome copy number variations and targeted panel coverage

Introduction

Whole genome amplification (WGA) is used to obtain sufficient quantities of DNA from limited template samples and has become an essential first step for testing single and small numbers of cells for various molecular technologies. In this study, the Revvity DOPlify® v2 WGA kit was compared to the Takara® PicoPLEX® Single Cell WGA kit using 5-cell fibroblast samples. Samples were analyzed for whole chromosome copy number variations along with target coverage for panel applications.

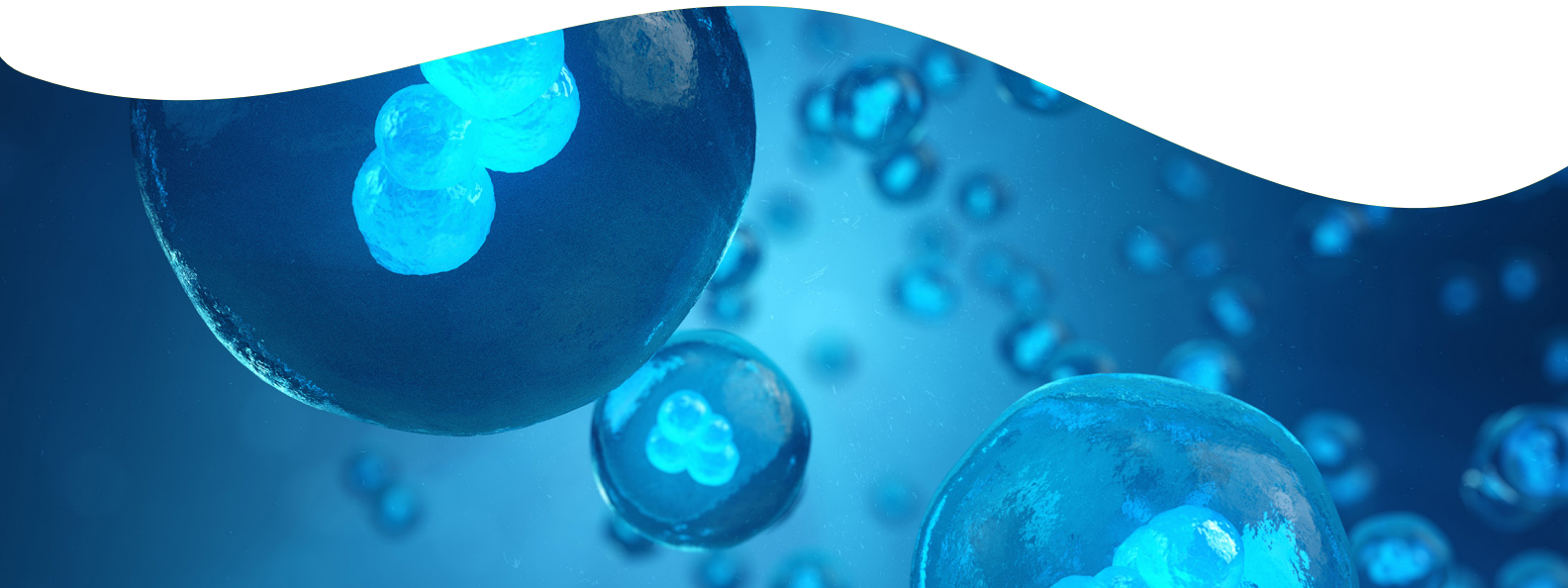
Methods

Cell isolation:

Fibroblast cell lines with karyotypes 47,XX,+18 (GM00143) and 48,XXY,+21 (GM04965) were purchased from Coriell Biorepository, grown to confluence then washed and manually grouped together in 5-cell aliquots before being transferred to a PCR tube for storage at -20 °C. The 5-cell model is representative of a trophectoderm biopsy.

DOPlify® v2 WGA:

Cell Lysis and WGA were performed according to the standard protocol (summarized in figure 1).



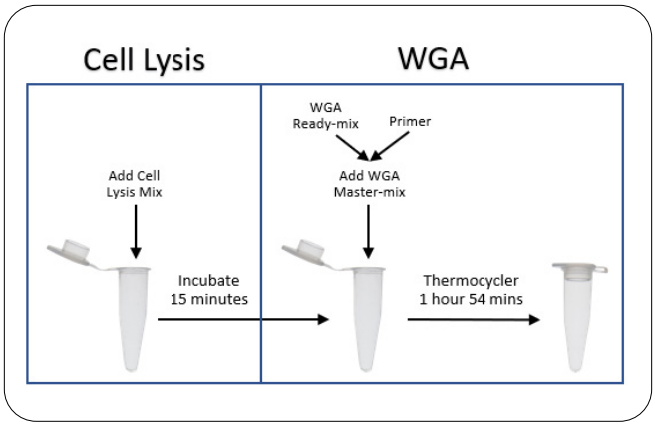


Figure 1: DOPlify® WGA v2 workflow

Takara® PicoPLEX® WGA:

Cell Lysis, Pre-Amplification and Amplification were performed according to the instructions contained within the Takara® PicoPLEX® Single Cell WGA kit manual (summarized in figure 2). The number of PCR cycles in the Amplification step was adjusted to 11 to match the number of total PCR cycles with DOPlify® v2 (DOPlify® v2 WGA: 8+15, Takara® PicoPLEX® WGA: 12+11).

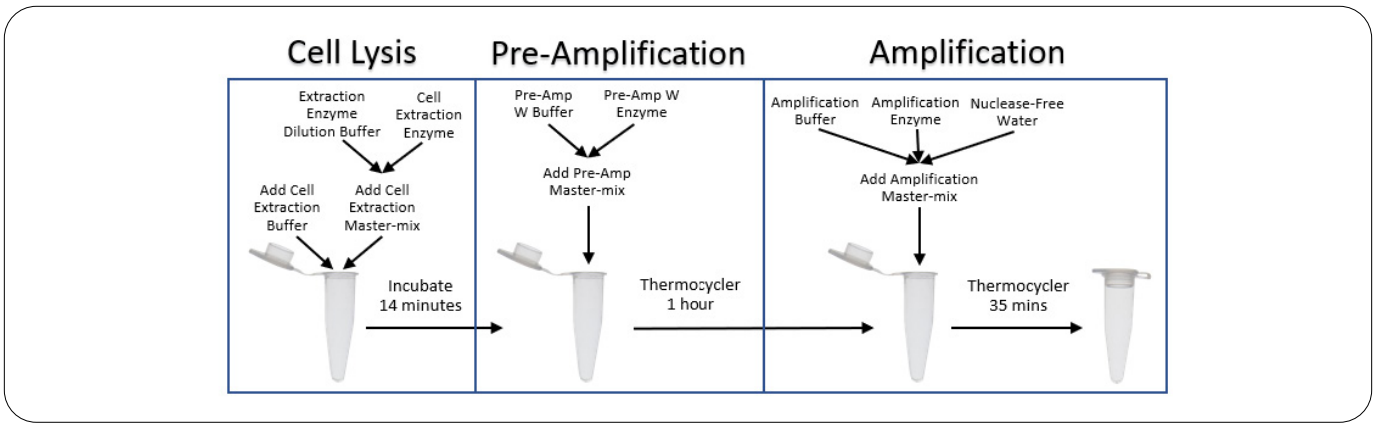


Figure 2: Takara® PicoPLEX® Single Cell WGA workflow

Takara® PicoPLEX® WGA:

DOPlify® v2 and Takara® PicoPLEX® products were purified with a 1X SPRI bead clean up. Approximately 200-400ng of purified WGA products and 200ng of unamplified genomic DNA from the same cell line had libraries prepared using the NEXTFLEX® Rapid XP V2 DNA-Seq kit. Final libraries were pooled and sequenced using the Illumina® MiniSeq® Instrument with a High Output Reagent Kit (75 cycles) with single 1x75 reads. Resultant fastq files were aligned to hg19 using BWA-MEM, downsampled to 400,000 total reads then processed through the PG-Find™ v3 software using the default settings.

Targeted coverage analysis:

Purified WGA products from both the DOPlify® v2 and Takara® PicoPLEX® kits along with unamplified genomic DNA from the same cell line were used as template for a molecular inversion probe (MIP) targeted panel. The panel covers approximately 904k bases, consisting of 656 target CDS regions along with 1972 haplotyping SNPs.

Final products from the MIP panel were sequenced on an Illumina® MiniSeq® instrument with a High Output Reagent Kit (300 cycles) using paired end 2x150bp reads. Fastq files were processed through a custom developed pipeline which performs trimming and alignment, analyzes MIP quality control, coverage over the target locations, variants and SNP zygositiy.

Results

Chromosome copy number analysis

Karyotype results obtained from the 5-cell WGA samples for both DOPlify® v2 and Takara® PicoPLEX® samples showed most results were concordant with the expected karyotype of the cell lines. DOPlify® v2 and Takara® PicoPLEX® samples both had 8/10 samples correct, with 2 samples appearing mosaic for the expected trisomy chromosome. Example whole genome profile plots are displayed below in figures 3A-H and 4A-H for Takara® PicoPLEX® and DOPlify® v2 WGA samples, respectively.

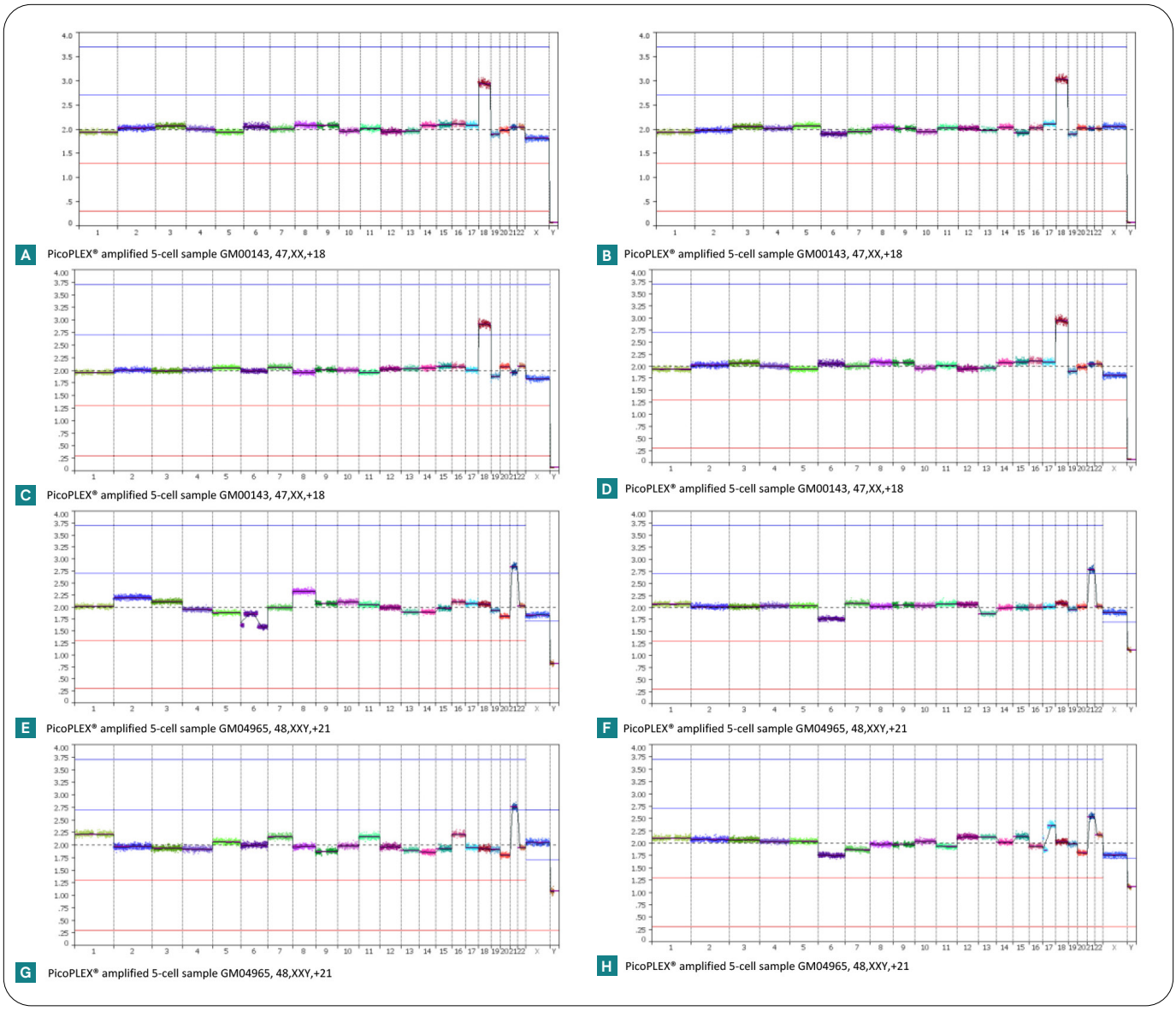


Figure 3: Whole Genome Graphs showing the copy number profile for Takara® PicoPLEX® amplified 5-cell samples.

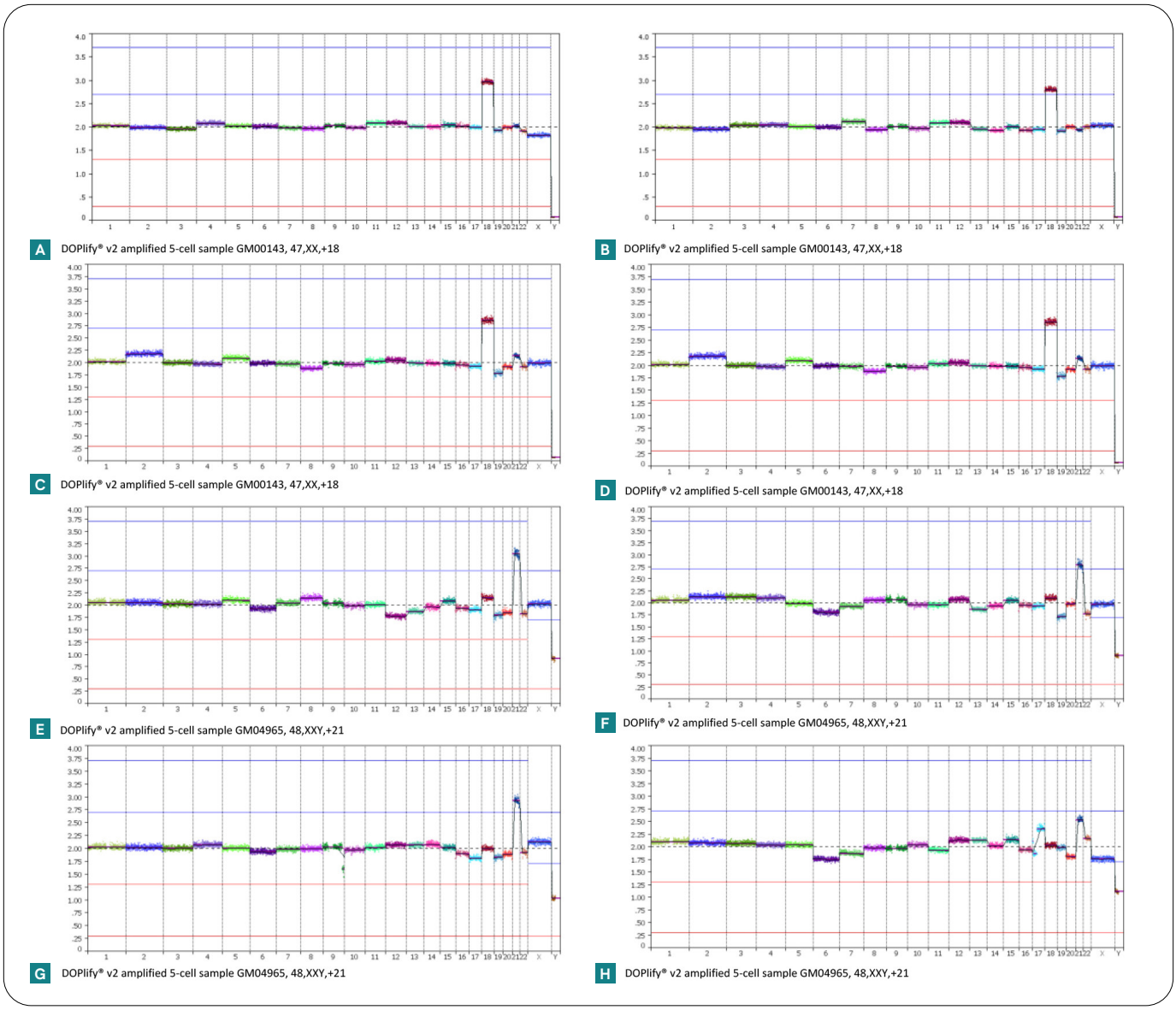


Figure 4: Whole Genome Graphs showing the copy number profile for DOPlify® v2 amplified 5-cell samples.

The PG-Find™ v3 software generates a metric called "PG-Find Quality" which assesses bin to bin variation of the genome segments, similar to a standard deviation. A low value quality score indicates less noise and a more reliable result while a value greater than 0.2 is likely to be unreliable. The DOPlify® v2 and Takara® PicoPLEX® WGA samples performed similarly, displaying excellent quality scores with no significant difference detected (p=0.6485, t-test) (see figure 5).

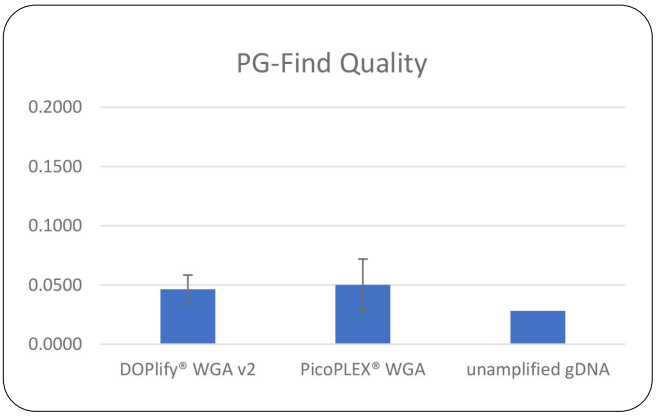


Figure 5: Quality score comparison of different WGA technologies

Targeted panel results:

Coverage over the CDS target regions was calculated and the number of regions with coverage depth greater than 20X was determined. The difference in percentage of CDS target regions covered with at least 20X depth was found to be not statistically when comparing DOPlify® to Takara® PicoPLEX® data (p=0.2789, t-test), with an average of 81.9% of regions covered for DOPlify® v2, 84.7% for Takara® PicoPLEX® data and 98.9% for the unamplified genomic DNA control (figure 6). The fraction of all target bases achieving X coverage is displayed in figure 7.

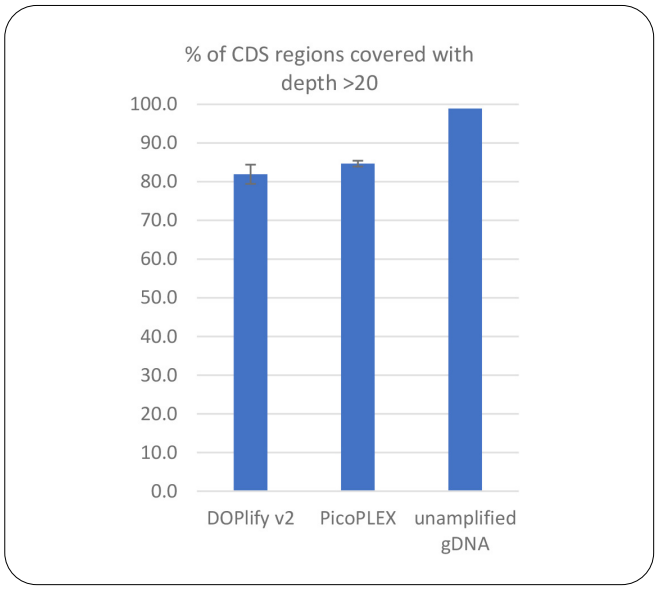


Figure 6: The average percentage of CDS regions that were covered with minimum depth of 20X for DOPlify® v2 WGA samples, PicoPLEX® WGA samples and unamplified genomic DNA.

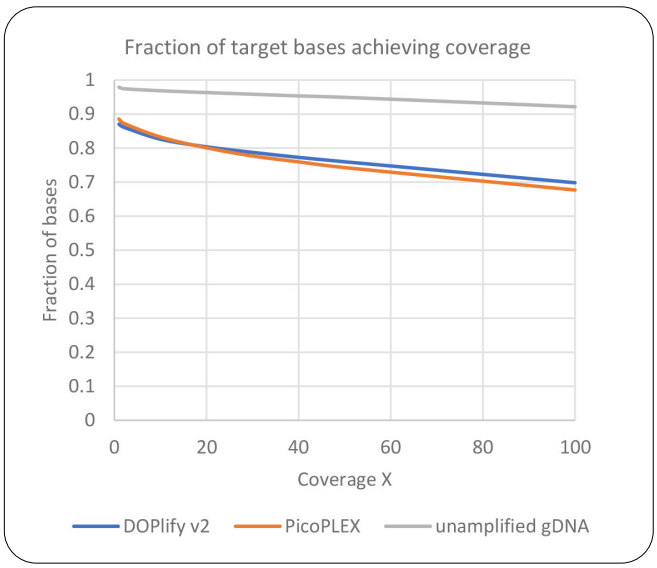


Figure 7: The average fraction of bases achieving specified coverage for DOPlify® v2 WGA samples, PicoPLEX® WGA samples and unamplified genomic DNA.

SNPs designed for haplotyping were assessed for coverage depth and zygosity with results compared to the control unamplified genomic DNA sample. Each SNP result was assigned a term based on the coverage depth and allele ratio, as described in table 1.

Table 1: Description of SNP assigned terms.

Term	Meaning
Zero Count	Zero coverage over the SNP location.
Low Depth	Less than 20X coverage over the SNP location.
Homozygous	Coverage >20X and the allele ratio of bases detected between 0-0.05 or 0.95-1.
Heterozygous	Coverage >20X and the allele ratio of bases detected between 0.15-0.85.
Ambiguous	Coverage >20X and the allele ratio of bases detected between 0.05-0.15 or 0.85-0.95.

The result for each SNP analyzed in the WGA samples was compared to the unamplified genomic DNA control and the average percentage of matching and non-matching results were calculated and shown in figure 8.

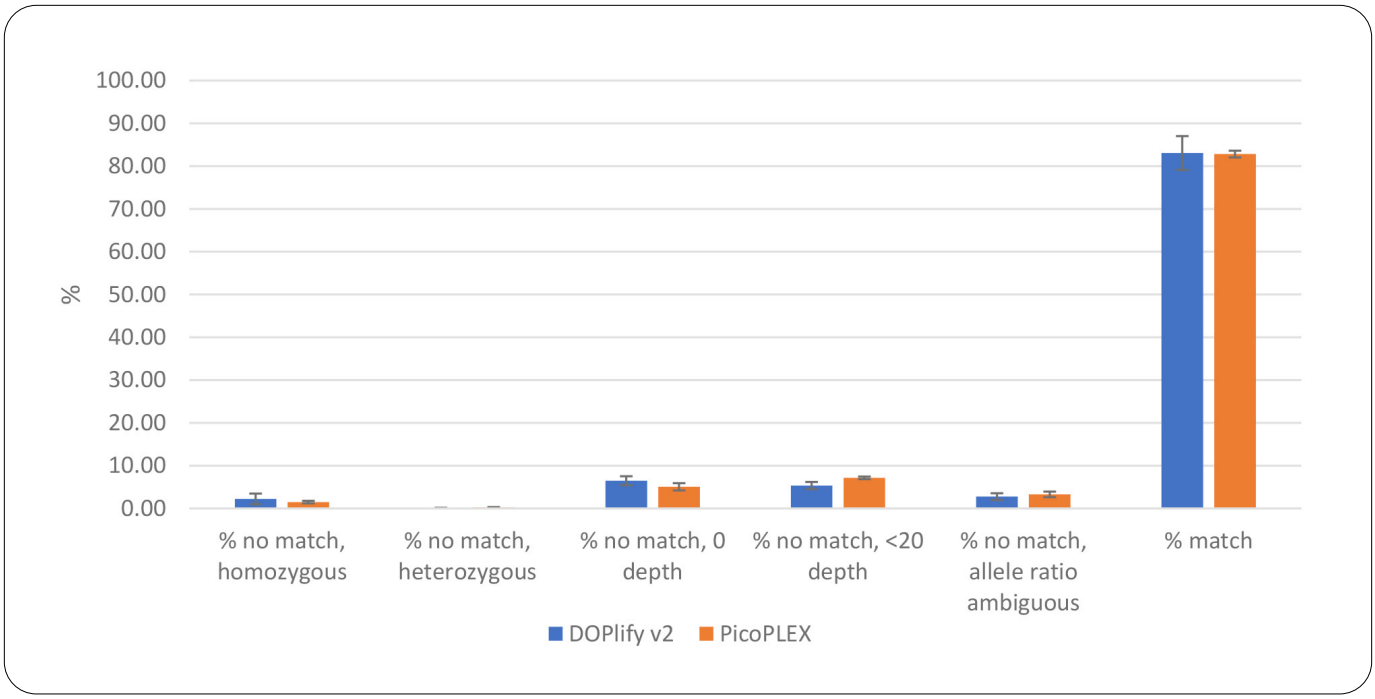


Figure 8: SNP Zygosity concordance of DOPlify® v2 WGA and PicoPLEX® WGA samples to unamplified genomic DNA.

On average, 83.1% of SNP calls for DOPlify® v2 WGA samples were concordant to the unpurified genomic DNA. Takara® PicoPLEX® WGA samples were on average 82.81% concordant. In the cases where the DOPlify® v2 WGA and Takara® PicoPLEX® WGA SNP result did not match the genomic DNA control, the respective results determined 2.23% and 1.49% were due to the call being homozygous, 6.47% and 5.06% were due to SNPs having zero coverage depth, 5.34% and 7.14% due to SNP depth less than 20X and 2.76% and 3.29% due to ambiguous allele ratio designations. Overall, the two whole genome amplification technologies performed similarly, with less than 1% difference in the number of SNP calls matching the control unamplified genomic DNA.

Conclusion

The DOPlify® v2 WGA kit and Takara® PicoPLEX® Single Cell WGA kit performed comparably when analyzed for whole chromosome copy number variations and targeted panel coverage when using 5-cell samples as input.

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