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Next-generation whole genome amplification method for CNV and SNV detection from single cells

- Highly reproducible whole genome amplification
- Accurate detection of single-nucleotide variants and copy number variations
- Fast, simple workflow can be completed in under three hours

Introduction

Due to the precious nature of challenging samples, such as liquid biopsies and FFPE tumor tissue, research aimed at identifying the best treatment regimen and molecular diagnoses of diseases through genetic analysis requires the preparation of amplified genomic material from small amounts of DNA or single cells. It is therefore critical for whole genome amplification (WGA) technologies to allow for accurate and reproducible detection of single-nucleotide variations (SNVs) and copy number variations (CNVs) in genomic material from limited samples with high fidelity and genome coverage. Additionally, these technologies should be flexible enough to be used on a variety of analytical platforms. To address these needs, we have developed the PicoPLEX Single Cell WGA Kit v3 (PicoPLEX WGA v3), a platform-agnostic whole genome amplification system. This kit uses optimized enzymes, primers, and protocols for exceptional sequencing coverage, uniformity, and accuracy in detecting SNVs, all while increasing the resolution for CNV detection relative to previous PicoPLEX WGA iterations. Importantly, the system maintains a simple workflow (Figure 1) and the unmatched cell-to-cell reproducibility that is a hallmark of our PicoPLEX technology.

In this technical note, we demonstrate CNV detection to 25.5-Mb resolution at a depth of 1 million read pairs in a single cell with validated copy number gains and losses. SNV detection and reproducibility are shown to be superior to competitive technologies.

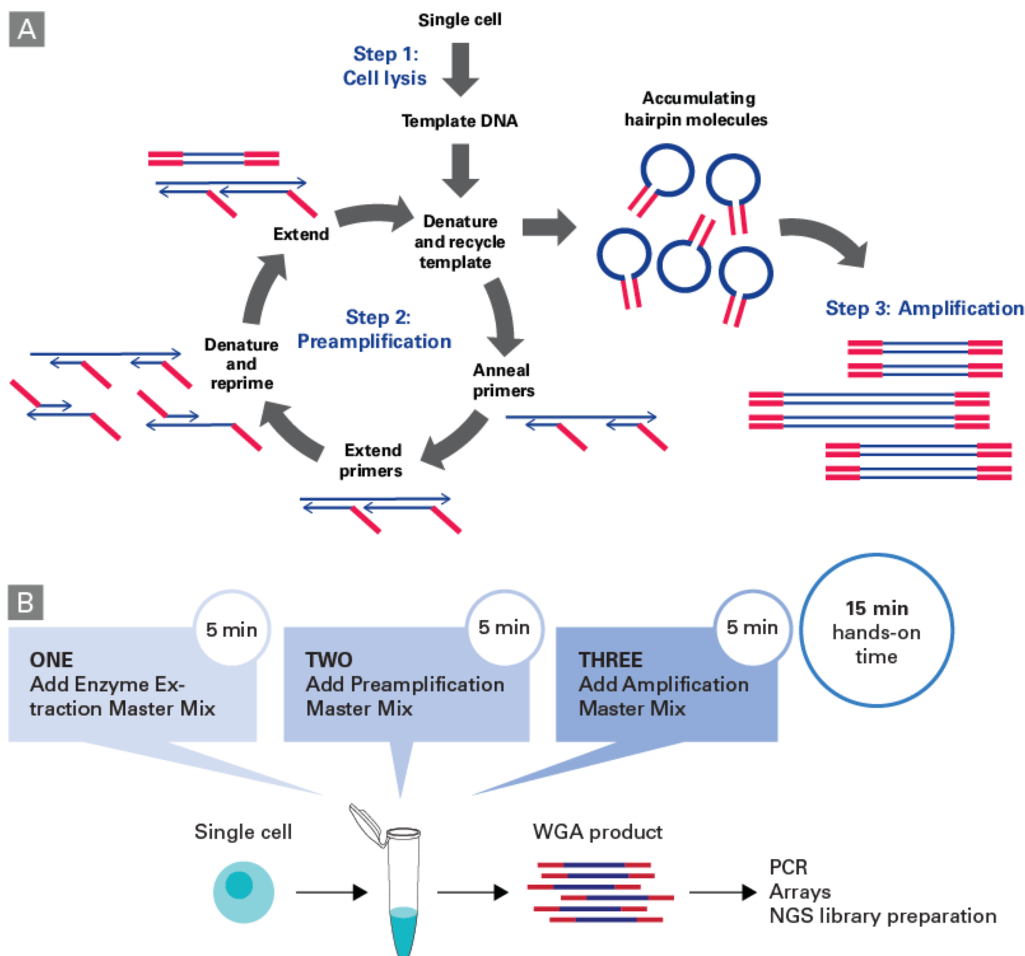

Figure 1. An overview of the principle of PicoPLEX technology and workflow schematic. Panel A. Step 1: A single cell is lysed, and the DNA is released free of proteins. Step 2: Multiple rounds of quasi-linear amplification of the single-cell gDNA is performed. The formation of hairpins prevents subsequent amplification of the products and promotes reutilization of the original template. Step 3: gDNA is amplified, and sample-barcoded adapters compatible with your NGS technology of choice are added. **Panel B.** Schematic of the PicoPLEX WGA v3 protocol showing a streamlined workflow with minimum hands-on time.

Results

Accurate detection of targeted SNVs using the PicoPLEX WGA v3 system

SNV detection by PicoPLEX WGA v3 chemistry was compared to SNVs observed in a bulk gDNA control and two other commonly used kits, DOPlify (Perkin Elmer) and REPLI-g (Qiagen). Whole genome amplification products from single- or five-cell samples of a GM12878 cell line (Coriell Institute) were prepared in replicates, and SNVs detected were reported as numbers and percentages. Although the REPLI-g system produced sufficient yield, only one of the five-cell samples contained enough amplicon material to sequence, and, therefore, no data is available for the other samples. An intersection of Genome In a Bottle (GIB) variants to hg19 (human genome assembly GRCh37, Ensembl) indicates that a total of 78 variants are expected to be present for the GM12879 cell line. Due to the amplicon design, paired-read lengths of 75 bp were too short to capture 4 out of the 78 SNVs; therefore, the total number of capturable SNVs was cut down to 74. VarDict was used to interpret SNVs from BAM files using the following criteria: depth of SNV position ≥10 reads (10X coverage), allele frequency ≥20%.

PicoPLEX WGA v3 showed higher call rates and fewer allele dropouts than the other two kits tested.

The PicoPLEX WGA v3 system is more accurate at detecting SNVs when compared to DOPlify and REPLI-g technologies

Depth of SNV position ≥10 Allele frequency ≥20%		PicoPLEX WGA v3 1 cell		PicoPLEX WGA v3 5 cells		DOPlify 1 cell		DOPlify 5 cells		REPLI-g 1 cell		REPLI-g 5 cells	
	Bulk	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2
Number of SNVs called	74	57	67	69	67	34	57	62	67	Failed	Failed	40	Failed
Number of false positives		3	1	0	1	5	1	0	7	Failed	Failed	0	Failed
Average false positives		0.02%		0.005%		0.03%		0.035%		Failed		0	Failed
Call rate		78%	92%	95%	92%	47%	78%	85%	92%	Failed	Failed	55%	Failed
Average call rate		85%		93%		62%		88%		Failed		55%	Failed
Missed		17	7	5	7	40	17	12	7	Failed	Failed	34	Failed
Average locus dropouts		16.2%		8.1%		38.5%		12.8%		Failed		45.9%	Failed
Number of heterozygous SNVs called	45	45	38	45	45	36	31	38	41	Failed	Failed	32	Failed
Average allele dropouts		7.8%		0.0%		25.6%		12.2%		Failed		71.1%	Failed

Accurate detection of segmental aneuploidies with low-pass sequencing

The performance of PicoPLEX Single Cell WGA Kit v3 was examined by preparing samples with known segmental aneuploidies. These include cell line GM22601, known to contain a 25.5 Mb deletion in chromosome 4 that is implicated in Wolf-Hirschhorn Syndrome, and GM05067, known to contain an amplification of a 44.7 Mb region in chromosome 9. Single-cell preparations using PicoPLEX WGA v3, followed by library preparation and Illumina sequencing, showed detection of both aberrations, along with good global genome representation for GM12878, a standard euploid cell line. These data demonstrate excellent resolution in the detection of segmental aneuploidies, in addition to the reliable detection of chromosomal aneuploidies (data not shown).

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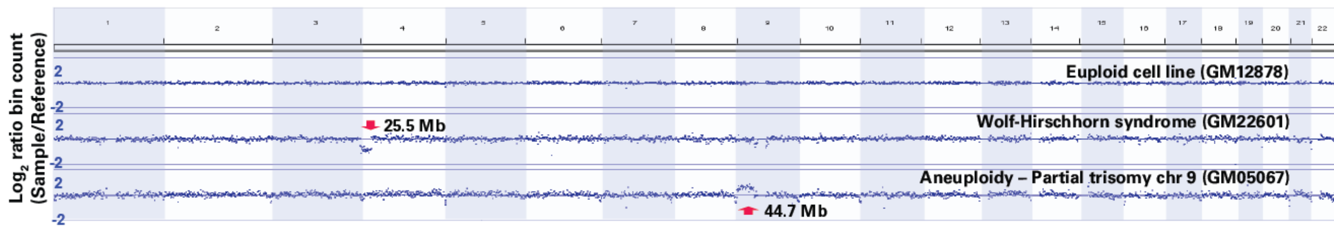

Figure 2. Characterized CNVs detected using PicoPLEX WGA v3. Single cells from various cell lines (GM22601, GM05067, and GM12878) were amplified using a prototype of the PicoPLEX Single Cell WGA Kit v3. 1 ng of the purified product was used as input for a Nextera® XT kit and sequenced on an Illumina MiSeq® platform at a read length of 2 x 75 bp. Fastq files were trimmed to remove adapters and then aligned to the human genome assembly GRCh37. Only autosomes are reported. For both panels, alignment was normalized to 1 million reads, and the number of reads per bin of 1 Mb was calculated using bedtools 2.25.0. The log2 ratio of the bin counts (Sample/Reference) was plotted using the Integrative Genomics Viewer.

Best-in-class reproducibility

While there are many options for whole genome amplification, none are as reproducible as PicoPLEX technology. We compared the reproducibility of the GM12878 single-cell preparations (see table above) using PicoPLEX WGA v3 and two competitors (DOPlify and REPLI-g, Figure 3). As expected, PicoPLEX demonstrated significantly better reproducibility than the competitor systems. This reproducibility is critical when samples are limiting, and an experimenter does not have the luxury of performing multiple preparations to obtain a reliable result.

Figure 3. Whole genome coverage reproducibility of the prototype PicoPLEX Single Cell WGA Kit v3 in comparison to DOPlify and REPLI-g (MDA) kits. WGA products were prepared from single-cell samples of GM12878 in replicates, using a prototype of PicoPLEX WGA v3, DOPlify, and REPLI-g (MDA) kits. 1 ng of amplified product was used as input for a Nextera XT kit, and the resulting libraries were sequenced on an Illumina MiSeq platform using a read length of 2 x 75 bp. After read alignment to human genome assembly GRCh37 and normalization to 1 million reads (0.5 million read pairs), the number of reads per bin of 1 Mb was calculated using bedtools 2.25.0. Total reads in each window from two single-cell libraries were plotted, with Pearson and Spearman correlations calculated and indicated on each graph. **Panel A.** Samples containing outliers. *Due to the different biases of the technologies compared, the graphs have different scales since no correlation points have been excluded. **Panel B.** Samples with outliers excluded from calculations. Results from the PicoPLEX WGA v3 kit show high reproducibility and robust coverage compared to the DOPlify and REPLI-g kits, which provides a clear advantage for the detection of structural variants (CNVs).

Conclusions

In summary, PicoPLEX WGA v3 enables preparation of amplified DNA, in under three hours, that is highly dependable and results in accurate measurement of single-nucleotide variants and copy number variations. When compared to the QIAGEN REPLI-g and PerkinElmer DOPlify systems, PicoPLEX WGA v3 shows superior mutation (SNV) detection and reproducibility. In addition, detection of segmental aneuploidies at a

resolution of 25.5 Mb are demonstrated. The improvements in PicoPLEX WGA v3 make this an excellent choice for a variety of single-cell and low-input applications, including research detecting aneuploidies in embryo biopsies, characterizing the heterogeneity and tumor evolution of cancer tissues, and profiling circulating tumor and immune cells.

Methods

Sample preparation

GM12878 cells were sourced from the Coriell Institute, stained with CD81-FITC antibody, and flow sorted using a BD FACSJazz instrument. WGA products were prepared from single-cell samples of GM12878 in replicates, using a prototype of PicoPLEX WGA v3. 1 ng of amplified product was used as input for a Nextera XT kit, and the resulting libraries were sequenced on an Illumina MiSeq platform using a read length of 2 x 75 bp.

Bioinformatic analysis

FASTQ reads were trimmed to remove the primer sequence from the 5' end of the read. Trimmed reads were aligned using BWA (default parameters). Single-nucleotide variants were generated using GATK (according to its best-practices guidelines, found at https://software.broadinstitute.org/gatk/best-practices/) and filtered at a minimum depth of 10X, with a minimum quality score of 75. Allele drop-out rates were calculated as described in Leung et al. 2015. CNVs were generated using CNV-seq (Xie and Tammi, 2009). Normalized counts in 50 kb bins from H929 cells were compared to GM12878 cells (euploid reference) to detect CNVs.

References

Leung, M. L, *et al*. SNES: single nucleus exome sequencing. *Gen. Biol*. **16**, 55 (2015).

Xie, C. & Tammi, M. T. CNV-seq, a new method to detect copy number variation using high-throughput sequencing. *BMC Bioinformatics* **10,** 80 (2009).

Related Products

Cat. #	Product	Size	License	Quantity	Details
R300718	PicoPLEX® Single Cell WGA Kit v3	24 Rxns		*	

The PicoPLEX Single Cell WGA Kit v3 uses a single-tube protocol developed specifically for the reproducible amplification of genomic DNA (gDNA) starting from 1–10 cells or equivalent picogram quantities of isolated gDNA. Cell lysis and whole genome preamplification is followed by amplification with very low background to yield over 2 µg of product in under 3 hours.

Documents | Components

Cat. #	Product	Size	License	Quantity	Details
R300722	PicoPLEX® Single Cell WGA Kit v3	96 Rxns		*	
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