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

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Abstract

Preparation of amplified genomic material from small amounts of DNA or single cells is extremely important in aiding research involving genetic analyses of clinical samples aimed at identifying the best treatment regimen and molecular diagnosis of diseases such as cancer. Technologies that allow for accurate and reproducible detection of single nucleotide variations (SNVs) and copy number variations (CNVs) in genomic material from limited samples need to do so with high fidelity and high genome coverage. Additionally, these technologies should be flexible enough to be used in a variety of analytical platforms. To address these needs, we have developed the PicoPLEX® VEGA II System (PicoPLEX WGAv2), a platform-agnostic whole genome amplification system, and the PicoPLEX Gold Single Cell DNA-Seq Kit (PicoPLEX Gold), a complete cells-to-library solution for Illumina® sequencers. These systems use 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 versions. Both systems maintain the technology’s simple workflow and unmatched cell-to-cell reproducibility that is a hallmark of the PicoPLEX technology.

In this study, we demonstrate CNV detection to 5.5-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.

1 PicoPLEX Gold technology—principle and workflow

Figure 1. Overview of the principle of PicoPLEX technology and workflow schematic. Panel A. Step 1: A single cell is lysed and the DNA is extracted from the lysate. Step 2: Multiple rounds of quasi-linear amplification of the single-cell gDNA is performed. The formation of hairpins prevents subsequent amplification.

Figure 2. High-fidelity detection of single nucleotide variants.

Conclusions

• The newly launched PicoPLEX Gold and PicoPLEX WGAv2 kits show improved performance for the detection of CNVs and SNVs.
• Comparison of the PicoPLEX Gold and QIAGEN REPLi-g library prep systems shows better reproducibility and superior CNV and SNV detection with the PicoPLEX Gold kit.
• Comparison of the PicoPLEX WGAv2 kit to QIAGEN REPLi-g and PerkinElmer DOPlify systems shows superior reproducibility and accuracy for SNV detection with the PicoPLEX WGAv2 kit.
• Both PicoPLEX Gold and PicoPLEX WGAv2 kits demonstrate the capability to detect segmental aneuploidies to a resolution of 20 kb with as few as 1 million reads.