Application Forum

Targeted resequencing of the EGFR and MET genes using the Fluidigm Access Array System and the Roche GS Junior System

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Introduction

GS Junior System users can streamline their targeted resequencing workflow by automating amplicon preparation with the Fluidigm Access Array System (Figure 1). The Access Array System enables high throughput targeted resequencing by combining PCR-based targeting capability with integrated amplicon tagging requiring minimal manual manipulations. The Access Array System facilitates parallel amplification of 48 amplicons across 48 unique samples thus providing up to 2,304 PCR reactions per array. The targeting process focuses the sequencing bandwidth and allows processing of up to 48 samples at a time on a single GS Junior run. The Access Array system applies an amplicon tailing strategy to incorporate sequencing adaptors and Multiplex Identifiers (MIDs, unique barcode sequences) that uniquely identify each sample so that all 48 samples can be sequenced in the same sequencing run. After sequencing, each MID tag is recognized and separated by the sequence data analysis software provided by with the GS Junior allowing for independent analysis of each sample. PCR products from the Access Array system already contain the necessary sequencing adaptors, so the PCR products can be purified and proceed through emulsion PCR (emPCR) amplification and sequencing without additional library preparation.

Methods

DNA from twelve NCI-60 cell-line samples were amplified on the Access Array System using the 48 EGFR-MET exon-specific primer sets (Fluidigm). Samples were uniquely identified with 12 different MID primer sets from the 454 GS FLX Titanium Barcode Library (Fluidigm) following the schematic of Figure 2. The samples and primer sets were processed using the Access Array System according to the manufacturer's instructions.

After amplification, each sample pool (48 amplicons) was analyzed using an Agilent BioAnalyzer. Amplicons from twelve samples were pooled together by volume (1

μl each) and then purified using Solid Phase Reversible Immobilization (SPRI) (AMPure XP; Beckman Genomics product #A63880). Finally, the pooled library was sequenced in a single GS Junior Instrument run according to the protocol described in the GS Junior Sequencing Methods Manual and the resulting sequences were analyzed using the GS AVA software.

Note: To ensure high-quality sequencing, it is critical that any spurious PCR products (<200 bp) are completely removed prior to emPCR amplification. Additional amplicon sequencing tips are available in Application Note No. 001-2010 Amplicon Sequencing Tips for GS FLX Titanium Reagents at www.454.com.

Results

A total of 300,678 reads were generated in a single GS Junior sequencing run. For each of the 48 samples sequenced, the

Figure 1. The Fluidigm instrument and the GS Junior System.

A Primer Loading on the Fluidigm Access Array System

Figure 2. Schematic of region-specific and MID primer workflow on the Fluidigm Access Array.

average depth of coverage was approximately 216x. The Access Array system provided PCR products resulting in 99.7% of amplicons within 1-fold coverage of the mean and 95% within 0.5x of the mean. The AVA software called variants across all samples that were consistent with the published genotypes for these loci. Sequencing all 48 samples in a single GS Junior run will provide roughly 50x coverage per amplicon which is sufficient for statistical confidence in heterozygote calling.

Conclusions

The high quality and uniformity of amplicons prepared using the Access Array System provides sample consistency

Figure 3. Distribution of reads per amplicon on one GS Junior run.

ideal for multiplexing strategies on the GS Junior System in targeted resequencing projects. The combination of the GS Junior System and the Access Array System offers a high-throughput amplicon resequencing solution within reach of the average research laboratory.

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