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Abstract 1164: Parallel preparation of targeted resequencing libraries from 480 genomic regions using multiplex PCR on the Access Array system FREE

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Abstract

Next generation sequencing platforms have dramatically reduced sequencing costs. However, it currently remains too expensive to routinely resequence entire human genomes in order to discover genetic variants or somatic mutations underlying tumorigenesis. Therefore, a need exists for multiplexed, targeted amplification methods that allow for the analysis of multiple genomic regions in large cohorts. Available targeted enrichment technologies are either aimed at the capture of regions of interest from a single sample, exhibit uneven representation or require significant amounts of input material. The novel microfluidic platform, the Access Array system, combines 48 samples with 48 primer sets resulting in 2,304 simultaneously occurring PCR amplifications requiring as little as 50ng DNA per sample. PCR products generated on the Access Array system can be used for sequencing on all next-generation sequencing platforms, including 454 GS FLX and Illumina GAII. To increase coverage and throughput, PCR reactions can be multiplexed within Access Array chips generating up to 480 amplicons per sample.

As proof-of-principal experiments, we have carried out multiplexed amplifications of a set of commonly mutated cancer gene exons across 48 genomic DNA samples. In initial experiments, 580 primer pairs were validated in individual PCR reactions in 96-well plates. Each primer pair was designed to include 5' sequences that allow for the incorporation of 454 and Illumina adapters necessary for subsequent emPCR and cluster generation, respectively. 480 primer pairs that produced a single band of the correct size, as determined on a Caliper LabChip system, were selected for multiplex PCR experiments. Primer pairs yielding amplicons with a similar size were combined in groups of 10 sets, resulting in 48 primer pools of 10 primer pairs each. Multiplex PCR was carried out on Access Array chips, followed by harvesting of the 48 amplicon pools. Each pool was diluted and then subjected to a second round of PCR in standard 96-well plates with barcoded universal primers corresponding to the 454 and Illumina sequences. The resulting products are 48 uniquely barcoded amplicon pools, each comprising 480 amplicons derived from one sample, that are ready for sequencing. We will present sequencing data generated on both 454 and Illumina systems.

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