ABSTRACT

Efficient utilization of targeted gene panels for oncology research is challenged by the wide variation in gene constituents specific to a given study. While focused gene panels efficiently provide the necessary depth of coverage for low frequency variant detection, the high costs and design challenges associated with de novo panel design present challenges.

The NEBNext Direct® technology utilizes a novel approach to selectively enrich nucleic acid targets ranging from a single gene to several hundred genes, without sacrificing specificity. The approach rapidly hybridizes both strands of genomic DNA with biotinylated probes prior to streptavidin bead capture, enzymatic removal of off-target sequence, and conversion of captured molecules into sequence-ready libraries. This results in a unique read coverage profile that confirms in unique read coverage across a given target. Unlike alternative hybridization methods, the approach does not necessitate upfront library preparation, and instead converts the captured molecules into dual-indexed illumina compatible barcodes containing an 8 basepair sample ID and a 12bp Unique Molecule Index (UMI). The UMI individualy tags each molecule prior to the final PCR amplification of the library, enabling identification of PCR duplicate molecules. The result is a 1-day protocol that enables the preparation of sequence-ready libraries from purified genomic DNA specific to the gene content included in the panel.

We have designed and developed baits specific to the full exonic content of ~850 genes with clinical significance across a variety of disease areas. These are designed, balanced, and pooled on a per gene basis, and can be combined into custom panels, allowing rapid turnaround of specific custom gene subsets. Here, we present the ability to rapidly deploy custom gene panels across a variety of panel sizes and content, while maintaining high specificity, uniformity of coverage across target content, and sensitivity to detect nucleic acid variants to drive translational research applications.

445 genes Custom Ready Genes associated with cancer were selected at random. Batch pools were created from 1, 10, 25, 50, 100, and 445 genes, respectively, to construct the NEBNext Direct panel. Each of the 445+ genes were used in the capture at a range of 250 ng to 4.5 mg of DNA using the NEBNext Direct method. 100 ng of DNA, representing a blend of 24 well-characterized HapMap panels. Following enrichment, resulting libraries were sequenced on a MiSeq using 2 x 150 bp sequencing chemistry.

4. Results: Sensitivity to detect variants

Greatest than 99% of the reads aligned as pair and 85%-98% of the reads mapped to the target, depending on the panel.

5. Results: Uniformity and specificity vs. panel size

Coverage for all exom bases for the targeted genes was determined. Small regions from a few of the genes were unable to be targeted due to repetitive sequences, resulting in some decrease in coverage with the larger panels.