Webinar Q&A: Challenges and Opportunities for Next Generation Sequencing Target Enrichment

Q: What is the size of the panels optimal for NEBNext Direct?

A: NEBNext Direct target enrichment is optimal for panels ranging from a single target, up to about 500 kilobases in total target territory.

Q: How are the UMIs incorporated and sequenced?

A: The Unique Molecule Index, or UMI, is incorporated in the 5' adaptor, and is sequenced in the i5 position (Index 2 read) using Illumina dual barcode indexing.

Q: How long is the workflow?

A: The workflow takes between 6.5 and 7 hrs, from genomic DNA through generation of a sequencer-ready library.

Q: Are there options for customization?

A: Yes. New England Biolabs offers customization of the baits for custom panels, based off genomic coordinates. We also offer the option to customize a panel based off of ~450 genes for which baits have been designed, synthesized, and balanced.

Q: Could you use a much shorter bait (around 6 bp) to get a RAD-seq like dataset?

A: No. A bait this short would not likely have enough specificity to pull down the region of interest.

Q: Any experience with GC rich regions?

A: Yes. The NEBNext Direct Cancer HotSpot Panel covers regions ranging from 25-75% GC content, with normalized coverage of the 75% GC regions showing coverage within 30% of the mean target coverage.

Q: How do you deal with pseudogenes?

A: Pseudogenes can cause issues because although these regions can be captured, they often cannot be unambiguously aligned. One of the ways we can overcome this challenge is by padding the target further into the intronic region, providing target coverage into regions that allow for uniambiguous mapping.

Q: Can it be used with cfDNA?

A: NEBNext Direct can be used with human cell-free DNA to discover variants as low as 2% Variant allele frequency. For applications requiring detection of variants with lower allele frequency, it may be necessary to increase the DNA input and apply bioinformatics filtering to remove false positive variants with the Unique Molecule Indexes.

Q: How does the approach deal with fragmented/degraded DNA?

A: The approach works well with fragmented or degraded DNA. Because the technology targets both strands independently, and uses a single, short (50-60 nucleotide) bait, there is a higher probability of capturing fragmented or degraded DNA than alternative approaches that require longer baits, or pairs of PCR primers to anneal.

Q: Would you expect NEBNext Direct to work with DNA amplified by multiple displacement amplification (a form of WGA)?

A: In principle, the NEBNext Direct approach works well with multiple displacement amplification. One concern would be that the WGA may present some biases, which could adversely effect the uniformity of coverage across targets.

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