NEBNext Direct® Genotyping Solution

HIGH-THROUGHPUT TARGETED GENOTYPING

NEW ENGLAND BioLabs®

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Your Genotyping Solution –

NEBNext Direct Genotyping Solution – High-throughput targeted genotyping for Illumina® Sequencing

The NEBNext Direct Genotyping Solution combines highly multiplexed, capture-based enrichment with maximum efficiency next generation sequencing to deliver cost-effective, high-throughput genotyping for a wide variety of applications. Applicable for ranges spanning 100-5,000 markers, pre-capture multiplexing of up to 96 samples combined with dual indexed sequencing allows over 3.8 million genotypes in a single Illumina sequencing run.

Features:

- Single-day workflow
- 96-plex pre-capture sample multiplexing of hundreds to thousands of markers
- Bait design and sample multiplexing to maximize sequencer efficiency
- High specificity and coverage uniformity

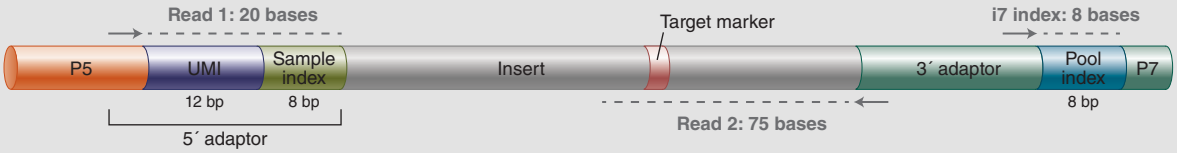
NGS-based targeted genotyping for a wide range of applications

PLANT	ANIMAL	HUMAN
Marker assisted selection / breeding	Mouse Genotyping	Biobanking
Quantitative Trait Locus (QTL) Screening	Livestock Breeding	NGS Sample Tracking

Sample Indexing and Multiplexing

With 96 pre-capture sample indexes and standard 8 post-capture pool indexes available, up to 96 samples can be combined for a single capture, and 768 samples can be pooled into a single Illumina sequencing run. An expanded post-capture pool index strategy is available upon request to process a maximum of 9216 samples all at a time. Additionally, a 12 bp Unique Molecular Identifier (UMI) is added prior to sample pooling and enrichment, allowing for accurate assessment of input coverage and improving the accuracy of genotyping calls. Finally, sequencing cycle numbers are optimized to sequence only the necessary target region, indexes and UMI required for marker genotyping. The NEBNext Direct Genotyping Solution is compatible with the full range of Illumina sequencers.

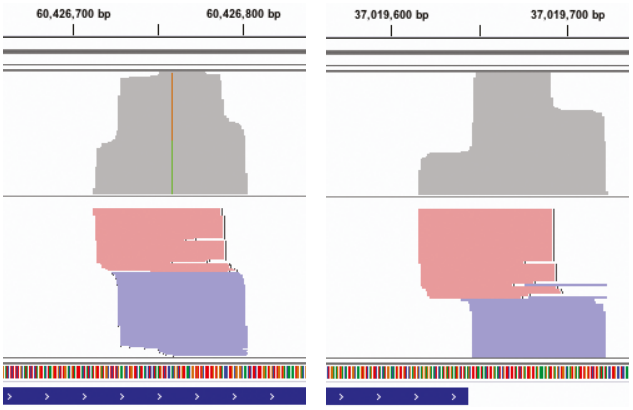
NEBNext Direct Genotyping Solution Sequencing Read Structure



Optimized Bait Design

The NEBNext Direct Genotyping Solution employs a purpose-built bait designer that has been optimized to provide both highly specific capture of target loci and maximized sequencer efficiency. By designing baits independently to each target DNA strand with proximity to the target loci, shorter sequence reads can be utilized for genotyping calls. Further, by removing upstream off-target sequence, individual baits can be unambiguously linked to their corresponding sequencing read, presenting opportunities for bait optimization on a per target level and resulting in extremely uniform coverage levels across markers.

Marker coverage across DNA strands



Two examples of the coverage of targeted markers within a single sample from the 96-plex enrichment as visualized in the Integrative Genome Browser (IGV)[1,2]. Reads shown are de-duplicated using UMIs. Baits target both strands of the input DNA, as indicated by the red and blue aligning reads.

1. Robinson, J.T., et al (2011) *Nat Biotech.*, 29, 24-26.
2. Thorvaldsdottir, H., et al (2013) Briefings in Bioinformatics. 14, 178-192.

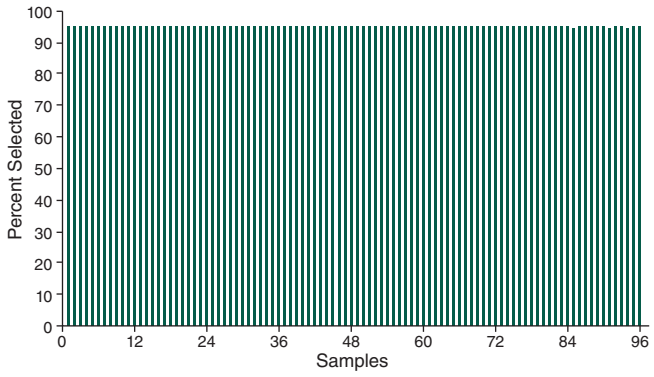
Performance

The NEBNext Direct Genotyping Solution produces reliable and accurate genotyping calls using fewer sequencing reads. This is achieved through the combination of highly specific and highly consistent enrichment across targeted loci, reducing excess sequencing data due to off-target sequence, while ensuring more markers can be included in analyses.

Highly specific enrichment across 96 pooled samples

Pre-capture pooling of 96 samples provides additional advantages, both in the significant reduction in pipetting steps, reducing plastic waste and consumables cost, as well as in the benefits from leveraging higher-throughput, lower cost sequencing platforms and configurations.

Specificity of enrichment of NEBNext Direct Genotyping Solution across 96 pooled samples

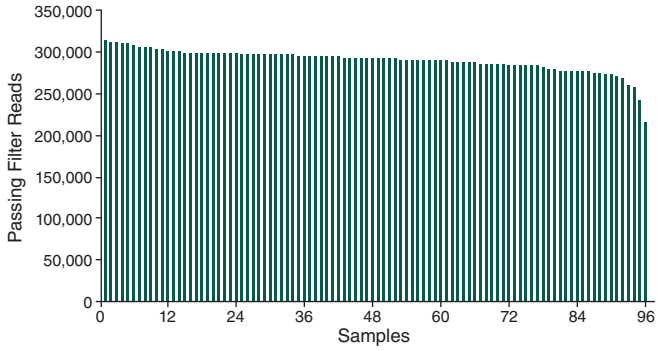


The percent of passing filter reads mapping to targeted regions demonstrates high specificity across 96 multiplexed samples using the NEBNext Direct Genotyping Solution. 25 ng of purified tomato DNA was used as input for each sample. Samples were index-tagged and pooled prior to hybridization and Libraries were sequenced on an Illumina MiSeq® with 20 cycles of Read 1 to sequence the 12 base UMI and 8 base sample index, and 75 cycles of Read 2 to sequence the targets.

Consistent coverage across 96 samples

Capture-based enrichment has distinct advantages over amplification-based methods in producing highly even coverage across targeted loci. Even coverage across the targeted loci reduces the total amount of sequencing data required, and maximizes the number of markers included in analyses.

Passing filter reads across 96 pooled samples

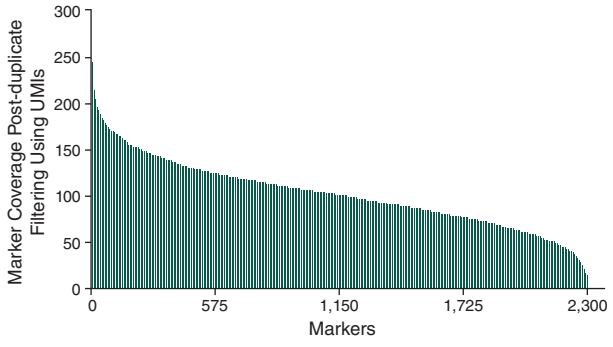


Passing filter reads across 96 tomato DNA samples that were enriched using a genotyping panel consisting of 2,309 publicly available SolCAP markers and the NEBNext Direct Genotyping Solution. 25 ng of purified tomato DNA was used for each sample. Samples were index-tagged and pooled prior to hybridization and libraries were sequenced on an Illumina MiSeq with 20 cycles of Read 1 to sequence the 12 base UMI and 8 base sample index, and 75 cycles of Read 2 to sequence the targets.

Superior coverage uniformity

NEBNext Direct produces highly consistent, highly specific enrichment across samples, with >90% of sequencing reads mapping to the targeted regions. High specificity is achieved through the unique combination of capture-based enrichment conditions optimized for genotyping applications with proprietary enzymatic removal of off-target sequence.

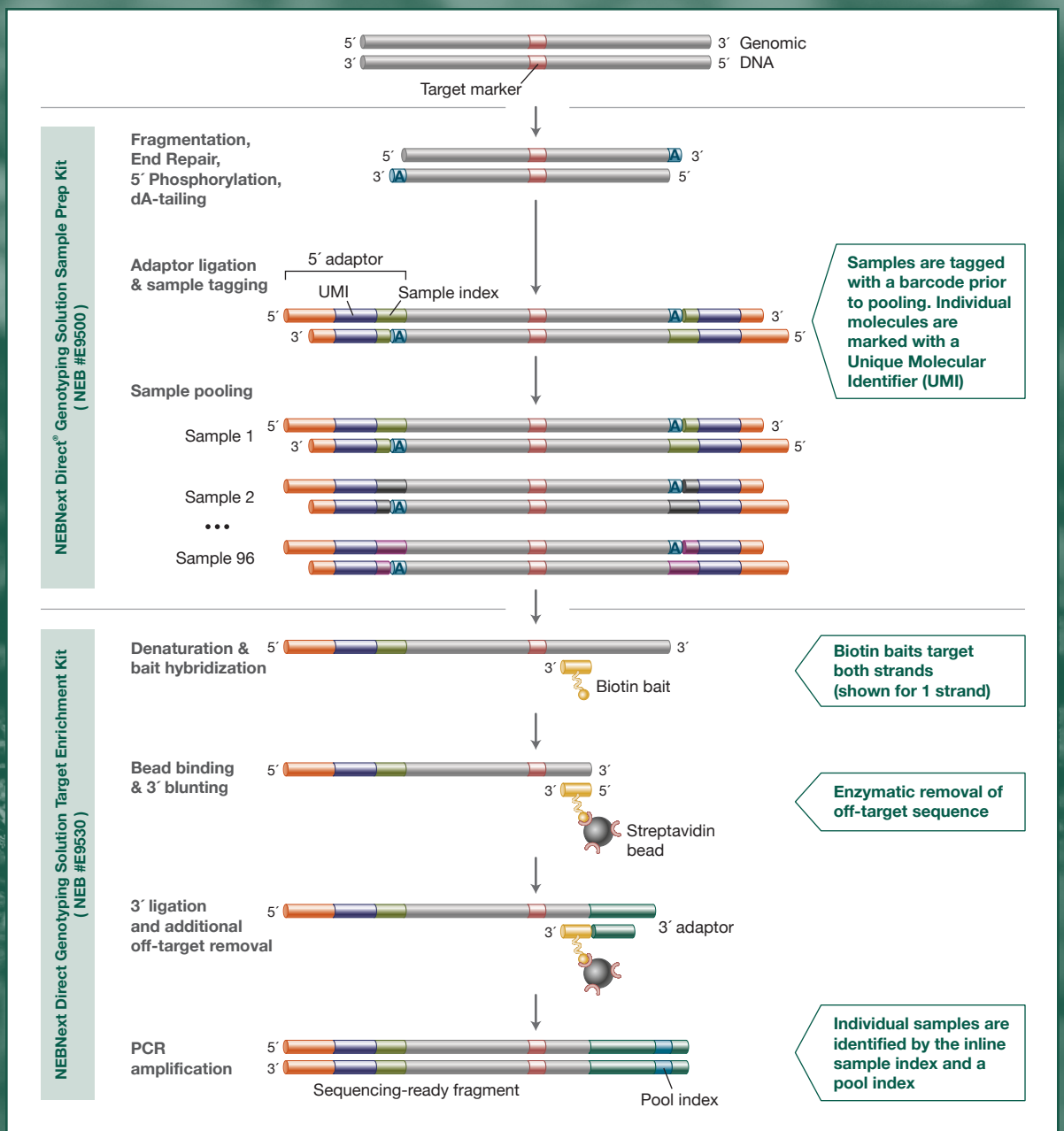
Mean Coverage across 2309 markers within a single sample



Histogram of coverage across each of the 2,309 SolCAP markers demonstrates evenness of enrichment across targets and coverage levels sufficient for genotyping calls. These data represent enrichment of a single tomato sample pooled with 95 others prior to hybridization. 25 ng of purified tomato DNA was used for each sample. Samples were index-tagged and pooled prior to hybridization and libraries were sequenced on an Illumina MiSeq with 20 cycles of Read 1 to sequence the 12 base UMI and 8 base sample index, and 75 cycles of Read 2 to sequence the targets.

NEBNext Direct

The NEBNext Direct Genotyping Solution begins with 25-100 ng of purified genomic DNA. The DNA molecules are enzymatically fragmented and 5´ tagged with an Illumina-compatible P5 adaptors, incorporating both an inline sample index to tag each sample prior to pooling and an inline Unique Molecular Identifier (UMI) to mark each unique DNA fragment within the samples. Up to 96 samples are subsequently pooled together prior to hybridization-based enrichment using biotinylated baits and captured on streptavidin beads. For the remainder of the protocol, up to 96 samples are processed as a single pool through ligation of a 3´ adaptor, removal of off-target sequence and final PCR, which amplifies the material and adds a second pool index to produce the final sequencing-ready fragment.



Combine the following NEBNext Direct Genotyping Solution components for optimal flexibility

NEB will develop and balance bait sets using purified genomic DNA from your source material. This allows us to optimize performance for your specific application, with the quality of DNA that is typically obtained. Individual baits are balanced according to empirical data, providing high performance with your samples.

PRODUCT	NEB #	SIZE
Sample Prep Kit	E9500	96 reactions
Target Enrichment Kit	E9530	8 reactions

An expanded post-capture pool index strategy is available upon request to process a maximum of 9216 samples in a single Illumina sequencing run.

To discuss your application, please visit **www.neb.com/forms/genotyping**, or email **NEBNextDirect@neb.com**.

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