

NEBNext Direct[®] Custom Ready Panels Ordering Guide

OVERVIEW

What are NEBNext Direct Custom Ready Panels?

NEBNext Direct Custom Ready Panels allow users to select from an extensive list of genes to create customized target enrichment panels for Illumina[®] sequencing. Modular bait sets for each gene have been designed, synthesized and optimized for exceptional specificity and target coverage uniformity, and bait sets of these genes can be mixed and matched into customized panels with rapid turnaround time.

What is the minimum reaction commitment for NEBNext Direct Custom Ready Panels?

NEBNext Direct Custom Ready Panels do not have a minimum reaction commitment. Kits are available in 8, 24 and 96 reaction sizes.

What sequencers are NEBNext Direct Custom Ready Panels compatible with?

NEBNext Direct Custom Ready Panels are compatible with the full range of Illumina[®] sequencing instrumentation.

GENE PANELS

What genes can be included in NEBNext Direct Custom Ready Panels?

The genes available through the NEBNext Direct Custom Ready offering will be continually updated, and currently include those associated with a variety of translational research areas, including cancer, neurological disorders, cardiological disease, autism, severe combined immunodeficiency, cystic fibrosis and the recommended genes for incidental findings by the American College of Medical Genetics. The full list of genes currently available can be found at www.neb.com/CustomReadyPanelForm.

How many genes can I include in a NEBNext Direct Custom Ready Panel, and are there any limitations as to how genes can be combined?

NEBNext Direct Custom Ready Panels can include anywhere from a single specific gene up to 1.5 megabases of total target territory. There are no limitations on genes that can be combined together in a Custom Ready Panel.

What if my gene or region of interest is not on the list?

For the inclusion of genes or other regions of interest that are not available through the NEBNext Direct Custom Ready menu, please contact NEBsolutions@neb.com. Please note that the addition of content beyond what is currently available through NEBNext Direct Custom Ready Panels will impact the pricing and turnaround time of the panels, and minimum reaction quantities may apply.

INPUT

What is the input DNA requirement?

NEBNext Direct Custom Ready Panels are compatible with DNA inputs ranging from 10 ng to 1 µg of genomic DNA. For panels where sequencing data will be used for somatic variant calling, we recommend using 100 ng of DNA or greater.

COVERAGE

What genomic regions are covered for each gene?

Each gene contains baits covering the full coding regions (all exons) for each gene selected, as defined by RefSeq. Baits are designed with variable padding of 0 to 60 bases into the intronic regions.

Can I select specific exons from each gene?

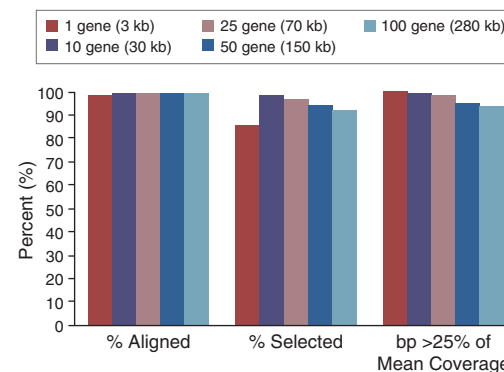
It is possible to select specific exons from each gene, however these requests will be treated as a custom panel, and will impact the turnaround time and pricing of the panel. Please contact nebnextdirect@neb.com for any inquiries.

What is the specificity and coverage uniformity of a NEBNext Direct Custom Ready Panel?

Bait sets for each gene included in the panel have undergone a rigorous development and optimization process to maximize specificity and target coverage uniformity. Because each individual panel contains a unique subset of genes, official specifications for specificity, coverage uniformity, and other performance metrics can not be provided. However, panels typically demonstrate specificity with >85% of the reads mapping to targets and coverage uniformity with >95% of the targeted bases having coverage >25% of the mean target coverage of the panel. Please see the data below for typical specificity and uniformity metrics that can be achieved using NEBNext Direct Custom Ready Panels.

NEBNext Direct Custom Ready Panels demonstrate optimum performance across a wide range of panel

Key target enrichment metrics demonstrate consistent performance across a range of panel sizes. 100 ng of DNA was tested against panels of 1, 10, 25, 50 and 100 genes, and sequenced using Illumina paired-end 150 bp sequencing. Larger panels included all genes present in smaller panels.

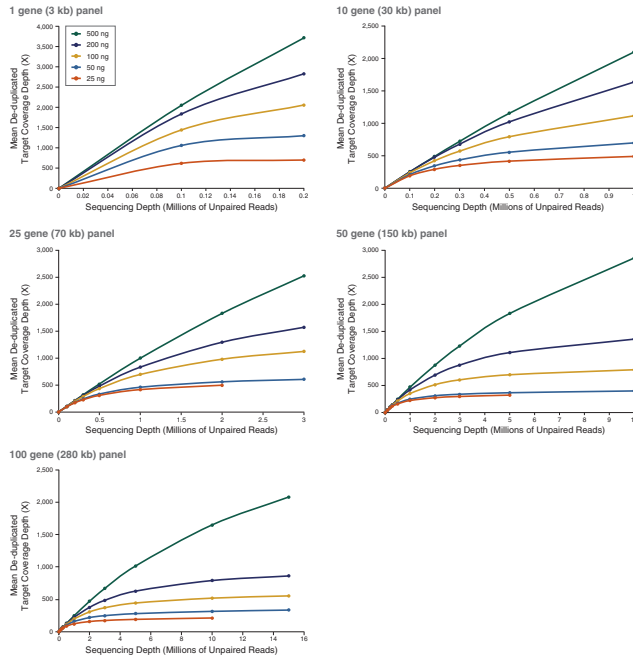


How much coverage can I expect from my panel?

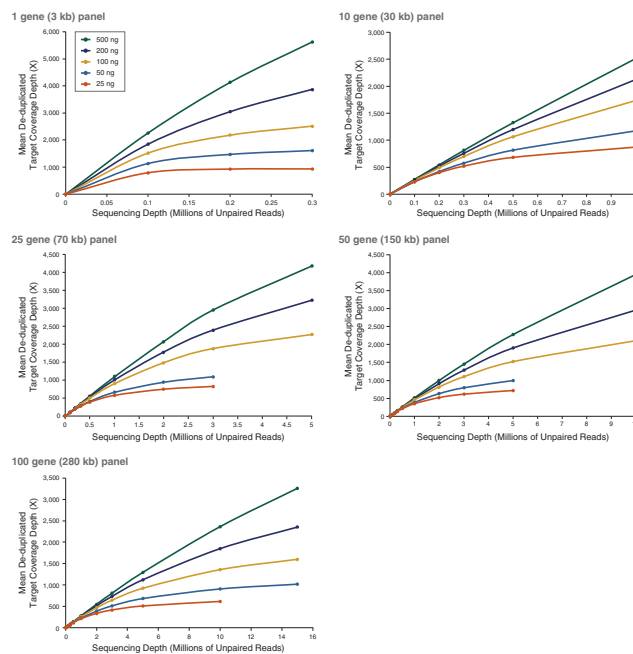
Target coverage is dependent on the input DNA amount, the total target territory of the panel, and the depth of sequencing. Data shown below demonstrates the typical performance of NEBNext Direct Custom Ready Panels. However, coverage for each individual panel may vary based on the specific genes that are requested.

Mean target depth achieved after PCR duplicate filtering using 10-500 ng of DNA and panels containing 1-100 genes

1.5-hour hybridization



16-hour hybridization



ANALYSIS

What are the recommended sequencing read lengths?

We recommend paired end 150 base sequencing to adequately cover the targets. In addition, 8 bases of i7 index sequencing, to read the sample index and 12 bases of i5 index sequencing, to read the unique molecule ID.

What are the recommendations for analyzing data from NEBNext Direct Custom Ready Panels?

We have developed and optimized a pipeline for internal processing of data directly from FASTQ files using open-source bioinformatics tools. Details on this pipeline can be found at: <https://github.com/DirectedGenomics/DemoPipeline>. Additionally, NEB has an agreement with Bluebee, who have hosted the demo pipeline on their platform, where NEB can provide activation codes for a limited number of samples to run through the pipeline. In order to request activation codes, please email nebnextdirect@neb.com. Please note that these pipelines are intended for evaluation use only, and that any production pipeline should be implemented and verified appropriately, with parameters optimized for the intended use.

PRODUCT DETAILS

Do NEBNext Direct Custom Ready Panels undergo verification prior to shipment?

Yes. For each NEBNext Direct Custom Ready Panel that is purchased, an NEBNext Direct library is created and sequenced internally to ensure that the expected target regions are included and that specificity and target coverage uniformity are optimal. A performance report will be emailed to you along with shipment of the panel.

How much does a NEBNext Direct Custom Ready Panel cost?

Tiered pricing is offered based on the total target territory included in the panel. Volume-based pricing is also available. Please contact your local New England Biolabs representative for official pricing, or visit www.neb.com/CustomReadyPanelForm.

How do I order an NEBNext Direct Custom Ready Panel?

Panels can be designed and ordered by visiting www.neb.com/CustomReadyPanelForm, launching the web tool, and selecting the genes of interest. Once a design has been submitted, you will receive target bed files for the coordinates included in the panel and a quotation for the panel that includes pricing and a unique part number for order placement.

How long will it take to receive my NEBNext Direct Custom Ready Panel?

Because the development and optimization of the baits has already been performed, panels are typically shipped within two weeks of order receipt.

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