NGS Target Enrichment

NEBNEXT® DIRECT™ CUSTOM READY PANELS

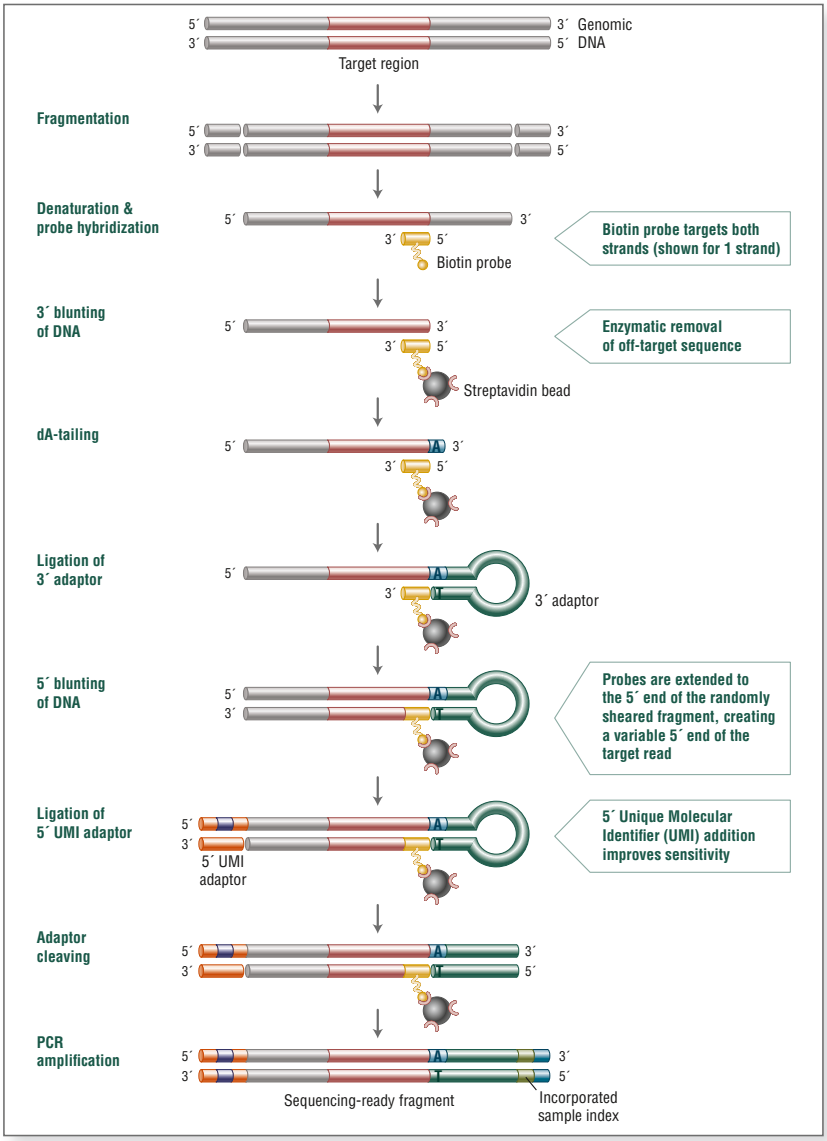
NEW ENGLAND BioLabs®

be **INSPIRED**
drive **DISCOVERY**
stay **GENUINE**

NEBNext Direct Custom Ready Panels for NGS target enrichment

NEBNext Direct combines a unique hybridization-based technology for highly specific target enrichment of genomic regions of interest with a superior library preparation workflow for Illumina® sequencing. This innovative approach to target enrichment balances the speed and precision of multiplexed PCR-based approaches with the content scalability typical of hybridization-based methods. The flexibility of NEBNext Direct allows a single workflow for assays ranging from single gene tests to comprehensive panels including several hundred genes. Regardless of sample type or assay content, NEBNext Direct allows you to enrich your targets with precision.

NEBNext Direct target enrichment workflow



Advantages of Technology

- Save time with a 1-day workflow that combines enrichment with library preparation
- Generate a higher percentage of your sequencing reads aligning to your targets
- Eliminate the need to over-sequence, reducing cost per sample
- Obtain uniform sequencing of all targets, regardless of gene content
- Generate high quality libraries with limited input amounts and degraded DNA samples, including FFPE and ctDNA
- Distinguish molecular duplicates, reducing false positive variants and improving sensitivity

Customer Feedback:

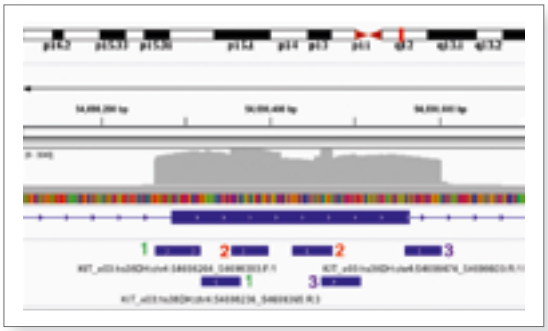
> *The kit and its technology are easy to use and easy to automate, allowing us to get up and running quickly. The protocol itself is fast and efficient to obtain deep coverage of targets, giving homogeneous results for FFPE and frozen tumors, therefore opening doors for customized panels.*
>
> *Francis Rousseau, Ph.D., Director of Genomics for IntegraGen SA*

> *NEBNext Direct enrichment technology is by far the fastest and most automation friendly protocol available today. I can have samples on the sequencer in 6 hours starting from genomic DNA.(...)*
>
> *Eric C. Olivares, Founder, SEQanswers.com*

Sequence only what you need!

Employing the unique NEBNext Direct hybridization-based enrichment method, NEBNext Direct Custom Ready Panels allow rapid customization of targeted gene panels. Select from a list of genes for which baits have been carefully designed and optimized to produce complete coverage of the full coding regions. High quality panels can be designed by you and rapidly delivered from any combination of genes. NEBNext Direct Custom Ready Panels provide the content you want with the performance you need.

Unique coverage profile of NEBNext Direct:
Even coverage across target with less padding than other hybridization based approaches.

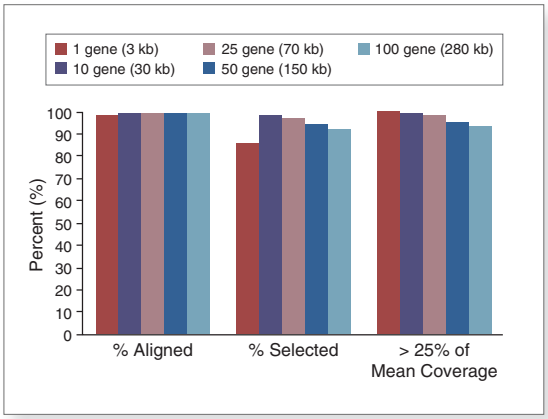


IGV image showing coverage profile for exon 3 from the gene KIT, targeted using NEBNext Direct. Coverage profile demonstrates the ability to optimize individual baits to produce even coverage across longer exons. 100 ng of DNA was used as input for NEBNext Direct enrichment. Libraries were sequenced using Illumina 2 x 150 basepair sequencing

Advantages of Custom Ready Panels

- Built your own panel by choosing from single to hundreds of readily-available genes
- Panel content scalable from a single gene up to 1.5 MB
- No minimum order quantities: Pack sizes with 8, 24 and 96 reactions available
- Extremely fast delivery time
- Experience unmatched specificity and coverage uniformity
- Eliminate synthesis and optimization steps for faster turnaround
- Improve sensitivity with Unique Molecule Indexes (UMI)

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



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.

See more performance data on the back cover and at
www.NEBNextDirect.com

Customer Feedback:

> *NEB was fantastic while developing our panel or updating an existing one. The protocol is simple and fast and the results have been superb.*
>
> *Luca Magnani, Ph.D, CRUK Fellow, Imperial Centre for Translational and Experimental Medicine*

> *NEBNext Direct Custom Ready Panels have allowed my research to focus on the specific genes we need to explore. In addition to the convenience of easily selecting genes for focused panels, NEBNext Direct enrichment has provided the necessary reliability and depth of coverage to enable robust somatic variant calling.*
>
> *Guang Peng, M.D., PhD, MD Anderson Cancer Center*

See more testimonials on www.NEBNextDirect.com

Currently available NEBNext Direct custom ready genes:

Select from a list of genes and design your panel of interest. As all baits for the listed genes have already been carefully designed and optimized to give complete coverage of the full coding regions, your custom panel is rapidly delivered.

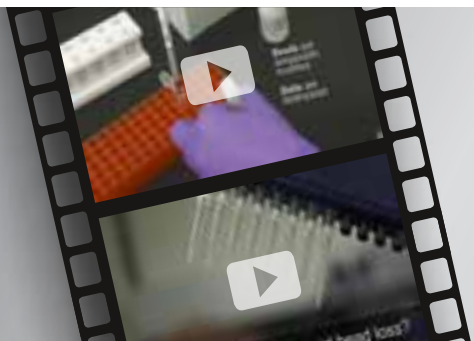
NEBNext Direct Custom Ready Panels are scalable from a single gene up to 1.5 MB of content and can be ordered with no minumum ordering quantity in pack sizes with 8, 24 or 96 reactions!

AARS	ASXL1	BSCL2	CDK12	CUL1	EGFR	FANCA	FOXP1	HDAC4	IRF1
ABCC9	ATL1	BTG1	CDK4	CUL3	EGLN1	FANCB	FRS2	HDAC7	IRF2
ABL1	ATM	BTG2	CDK6	CUX1	EGR2	FANCC	FUBP1	HGF	IRF4
ABL2	ATP2A1	BTK	CDK8	CXCR4	ELAC1	FANCD2	FZR1	HINT1	IRF8
ABRAXAS1	ATP7A	BTLA	CDKN1A	CYLD	ELAC2	FANCE	GAA	HIST1H1C	IRS2
ACD	ATP7B	BTNL2	CDKN1B	DAG1	ELP1	FANCF	GABRA6	HIST1H1D	ISPD
ACTA1	ATR	BUB1B	CDKN1C	DAXX	ELP2	FANCG	GADD45B	HIST1H1E	ITGA7
ACTA2	ATRX	CACNA1C	CDKN2A	DCLRE1C	EMD	FANCI	GALNT12	HIST1H2AC	JAK1
ACTB	AURKA	CACNA1S	CDKN2B	DDB2	EMSY	FANCL	GAN	HIST1H2AG	JAK2
ACTC1	AURKB	CACNA2D1	CDKN2C	DDR2	ENG	FANCM	GARS	HIST1H2AL	JAK3
ACTN2	AXIN1	CACNB2	CEBPA	DDX3X	EP300	FAS	GATA1	HIST1H2AM	JARID2
ACVR1B	AXIN2	CAD	CEP112	DDX41	EP400	FBN1	GATA2	HIST1H2BC	JPH2
ADA	AXL	CALR	CEP57	DES	EPCAM	FBXO11	GATA3	HIST1H2BJ	JUN
ADGRA2	B2M	CALR3	CFL2	DHX29	EPHA3	FBXO31	GATA4	HIST1H2BK	JUP
ADGRB3	B3GALNT2	CAPN3	CFTR **	DICER1	EPHA5	FBXO7	GATA6	HIST1H2BO	KAT6A
AIP	B3GNT2	CARD11	CHD1	DIS3L2	EPHA7	FBXW7	GATAD1	HIST1H3B	KBTBD13
AK2	BAG3	CASQ2	CHD2	DKC1	EPHB1	FGD4	GDAP1	HNF1A	KCNE1
AKAP9	BAP1	CASR	CHD4	DMD	ERBB2	FGF10	GDNF	HNF1B	KCNE2
AKT1	BARD1	CAV3	CHEK1	DNAJB2	ERBB3	FGF14	GID4	HOXB13	KCNE3
AKT2	BCL10	CBFB	CHEK2	DNAJB6	ERBB4	FGF19	GJB1	HRAS	KCNH2
AKT3	BCL11B	CBL	CHKB	DNM2	ERCC1	FGF23	GLA	HSD3B1	KCNJ2
ALK	BCL2	CCND1	CIC	DNMT1	ERCC2	FGF3	GLI1	HSP90AA1	KCNJ5
ALMS1	BCL2L1	CCND2	CIITA	DNMT3A	ERCC3	FGF4	GMPPB	HSPB1	KCNJ8
AMER1	BCL2L2	CCND3	CKS1B *	DNMT3B	ERCC4	FGF6	GNA11	HSPB8	KCNQ1
ANK2	BCL6	CCNE1	CLCN1	DOCK8	ERCC5	FGFR1	GNA12	ICK	KDM2B
ANKRD1	BCL7A	CCT6B	CNTN1	DOT1L	ERG	FGFR2	GNA13	ID3	KDM4B
ANO5	BCOR	CD22	COL3A1	DPM1	ERRFI1	FGFR3	GNAQ	IDH1	KDM4C
APC	BCORL1	CD247	COL6A1	DPM2	ESR1	FGFR4	GNAS	IDH2	KDM5A
APH1A	BICD2	CD274	COL6A3	DPM3	ETNK1	FH	GNB1	IGF1R	KDM5C
APOA4	BIN1	CD36	CORO1A	DPYD	ETS1	FHIT	GNE	IGF2	KDM6A
APOA5	BIRC3	CD3D	CPS1	DSC2	ETV1	FHL1	GPC3	IGHMBP2	
APOB	BIRC6	CD3E	CREBBP	DSG2	ETV4	FIG4	GPD1L	IKBKE	
APOC2	BLM	CD58	CRKL	DSP	ETV5	FKRP	GREM1	IKZF1	
AR	BMPR1A	CD70	CRLF2	DTNA	ETV6	FKTN	GRIN2A	IKZF2	
ARAF	BRAF	CD79A	CRYAB	DTX1	EWSR1	FLCN	GRM3	IKZF3	
ARFRP1	BRCA1	CD79B	CSF1R	DUSP2	EXO1	FLNC	GRM8	IL2RG	
ARHGAP26	BRCA2	CD82	CSF3R	DUSP9	EXOSC6	FLT1	GSK3B	IL7R	
ARID1A	BRD2	CD83	CSRP3	DVL3	EXT1	FLT3	GTSE1	ILK	
ARID1B	BRD3	CDC73	CTCF	DYNC1H1	EXT2	FLT4	H3F3A *	INF2	
ARID2	BRD4	CDH1	CTNNA1	EBF1	EZH2	FOXL2	H3F3B	INHBA	
ASIP	BRIP1	CDH2	CTNNB1	ECT2L	FAF1	FOXN1	HCN4	INPP4B	
ASMTL	BRSK1	CDH4	CTRC	EED	FAM46C	FOXO1	HDAC1	INPP5D	

** This gene has strong sequence homology to one or more other locations in the genome which can interfere with read mapping. Special care should be taken when interpreting sequencing results for this gene.*

*** CFTR targets include non-coding, CF-causing variants from the website CFTR2.org.*

KDR	MDH2	NEBL	PEX2	PTPN1	RPL35A	SMAD3	TCF12	UBA1
KEAP1	MDM2	NEFL	PHF21A	PTPN11	RPL5	SMAD4	TCF3	UGT1A1
KEL	MDM4	NEXN	PHF6	PTPN2	RPS10 *	SMARCA1	TCL1A	UTP6
KIF1A	MED12	NF1	PHIP	PTPN6	RPS19	SMARCA4	TERC	VCL
KIF1B	MEF2B	NF2	PHKA1	PTPRC	RPS24	SMARCB1	TERF2IP	VCP
KIF5A	MEF2C	NFE2L2	PHOX2B	PTPRD	RPS26 *	SMARCE1	TERT	VEGFA
KIT	MEGF10	NFIB	PICK1	PTPRO	RPS7 *	SMC1A	TET2	VHL
KLHL40	MEN1	NFKBIA	PIGA	PYGM	RPTOR	SMC3	TFG	VRK1
KLHL6	MET	NGF	PIK3C2B	QKI	RSPO2	SMO	TGFBR1	WAS
KMT2A	MFN2	NHEJ1	PIK3CA	RAB35	RUNX1	SMOX	TGFBR2	WDR90
KMT2B	MGA	NHP2	PIK3CB	RAB7A	RUNX1T1	SNCAIP	TINF2	WISP3
KMT2C	MIB1	NKX2-1	PIK3CG	RAC1	RYR1	SNTA1	TLL2	WNK1
KMT2D	MITF	NOD1	PIK3R1	RAC2	RYR2	SNW1	TMEM127	WRN
KRAS	MKI67	NOP10	PIK3R2	RAD21	S1PR2	SOCS1	TMEM30A	WT1
LAMA2	MLH1	NOTCH1	PIM1	RAD50	SBDS	SOCS2	TMEM43	XBP1
LAMA4	MLH3	NOTCH2	PKHD1	RAD51	SBF2	SOCS3	TMEM5	XPA
LAMP2	MORC3	NOTCH3	PKP2	RAD51C	SCN1B	SOS1	TMPO	XPC
LARGE1	MPL	NPM1 *	PLCG2	RAD51D	SCN3B	SOX10	TMPRSS2	XPO1
LDB1	MPZ	NRAS	PLEC	RAF1	SCN4A	SOX2	TNFAIP3	XRCC2
LDB3	MRE11	NSD1	PLEKHG5	RAG1	SCN4B	SOX9	TNFRSF11A	XRCC3
LDLR	MRPL36	NSD2	PLN	RAG2	SCN5A	SPEN	TNFRSF14	YARS
LEF1	MSH2	NT5C2	PMS1	RANGRF	SCN9A	SPINK1	TNFRSF17	YLPM1
LIG4	MSH3	NTHL1	PMS2	RARA	SDHA	SPOP	TNNC1	YTHDC1
LITAF	MSH6	NTRK1	PNP	RARB	SDHAF2	SPRED1	TNNI3	YY1AP1
LMNA	MSR1	NTRK2	POLD1	RASAL1	SDHB	SPTA1	TNNT1	ZAP70
LMO1	MTM1	NTRK3	POLE	RASGEF1A	SDHC	SPTLC2	TNNT2	ZBTB2
LRP1B	MTMR2	NUP93	POLH	RASSF1	SDHD	SRC	TNPO3	ZBTB33
LRRFIP2	MTOR	NUP98	POMGNT1	RB1	SELENON	SRCAP	TOP1	ZFHX3
LRRK2	MUTYH	ORAI1	POMT1	RBM10	SERP2	SRSF2	TOP2A	ZMYM3
LRSAM1	MXI1	OTC	POMT2	RBM20	SET	STAG2	TP53	ZNF217
LTN1	MYBPC3	P2RY8	POT1	RECQL4	SETBP1	STAT3	TP63	ZNF24
LYN	MYC	PAG1	PPM1D	REEP1	SETD2	STAT4	TPM1	ZNF318
LZTR1	MYCL	PAK3	PPP2R1A	RELN	SF1	STAT5A	TPM2	ZNF703
MAF	MYCN	PALB2	PRDM1	RET	SF3A1	STAT5B	TPM3	ZNRF3
MAFB	MYD88	PALLD	PREX2	RETREG1	SF3B1	STAT6	TRAF2	ZRSR2
MAGED1	MYH10	PASK	PRF1	RFFL	SGCA	STIM1	TRAF3	
MAGI1	MYH11	PAX5	PRKAG2	RFX7	SGCB	STK11	TRAF5	
MAGI2	MYH7	PBRM1	PRKAR1A	RHBDF2	SGCD	STXBP5	TRIM32	
MALT1	MYL2	PBX1	PRKCI	RHEB	SGCG	SUFU	TRPV4	
MAP2K1	MYL3	PC	PRKDC	RHOA	SGK1	SUZ12	TRPV5	
MAP2K2	MYLK	PCBP1	PRKN	RICTOR	SH2B3	SYK	TSC1	
MAP2K4	MYLK2	PCLO	PRPF40B	RINT1	SH3TC2	SYNE1	TSC2	
MAP3K1	MYO18A	PCSK9	PRPF8	RIT1	SHOC2	TAF1	TSHR	
MAP3K14	MYOT	PDCD1	PRPS1	RNASEL	SIL1	TAF4	TTR	
MAP3K6	MYOZ2	PDCD11	PRSS1	RNF2	SLC12A6	TANC2	TUSC3	
MAP3K7	MYPN	PDCD1LG2	PRSS8	RNF38	SLC52A2	TAZ	TYK2	
MAPK1	NBN	PDGFRA	PRX	RNF43	SLIT2	TBL1XR1	TYR	
MAX	NCOR2	PDGFRB	PTCH1	ROS1	SLTM	TBX1	TYRP1	
MC1R	NCSTN	PDK1	PTCH2	RPL11	SLX4	TBX3	U2AF1 *	
MCL1	NDRG1	PDLIM3	PTEN	RPL26	SMAD2	TCAP	U2AF2	



New to NGS Target Enrichment?

Watch our tutorial videos on NEBNextDirect.com incl.:

- Webinar "*Challenges and Opportunities for NGS target enrichment*"
- NEBNext Direct Workflow overview
- NEB TV – Episode 11 about target enrichment in clinical applications

FAQ

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.

Configure & order your custom panel easily online:

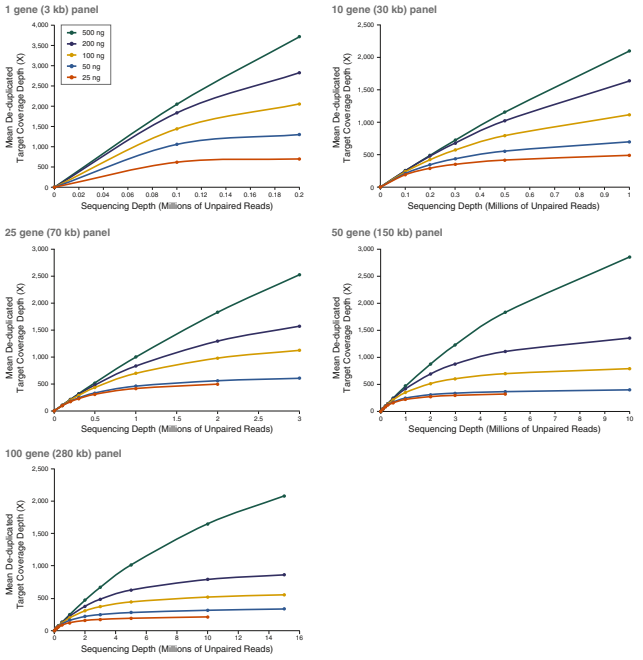
www.neb.com/CustomReadyPanelForm

How much coverage can I expect from my panel?

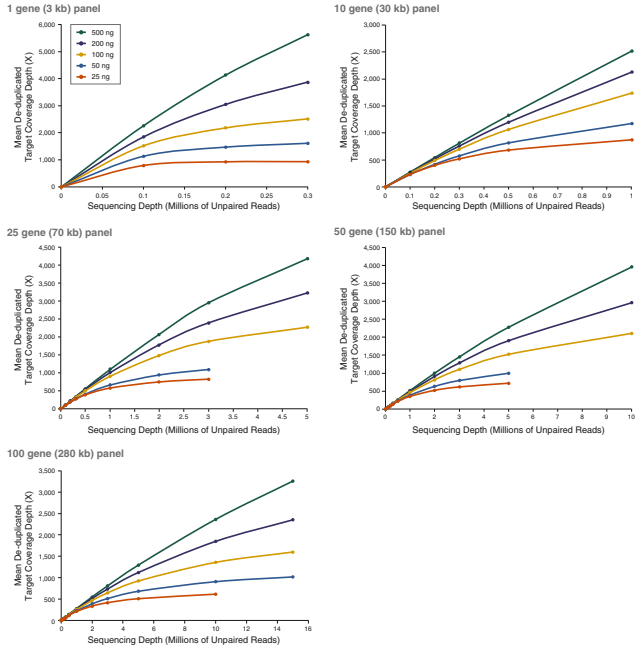
Target coverage is dependent on the input DNA amount, the total target territory of the panel, and the depth of sequencing. Data shown below demonstrates the typical performance of NEBNext Direct Custom Ready Panels. However, coverage for each individual panel may vary based on the specific genes that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

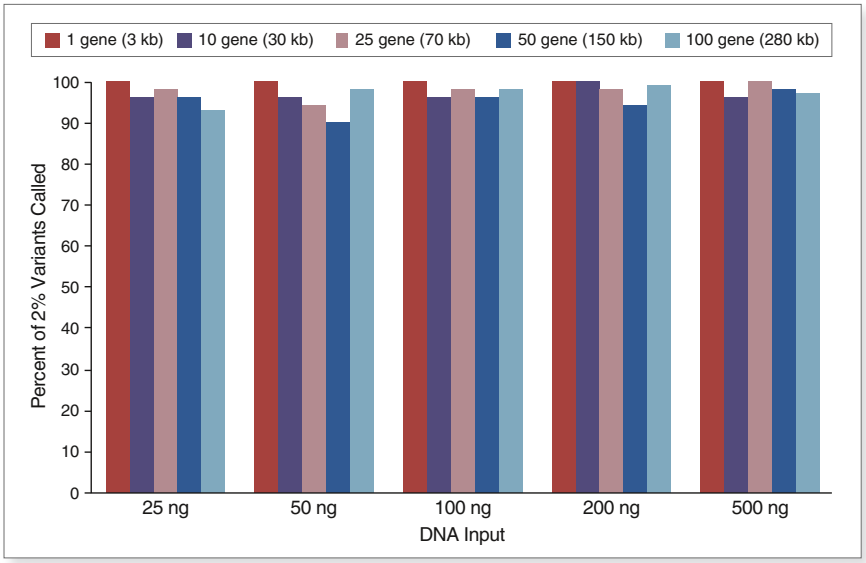
How do I order an NEBNext Direct Custom Ready Panel?

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?

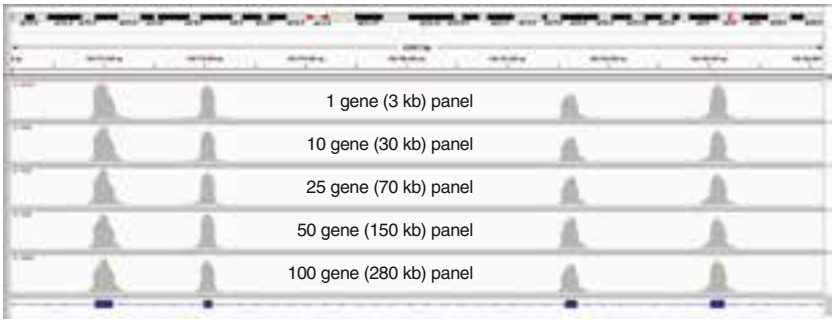
Because the development and optimization of the baits has already been performed, panels are typically shipped within two weeks of order receipt.

Sensitivity in detection of variants across panel size and DNA input amount



24 HapMap samples were blended to create a range of variant allele frequencies (VAF) down to 2%. 25, 50, 100, 200 and 500 ng of this blended DNA was enriched using NEBNext Direct Custom Ready Panels of 1, 10, 50, and 100 genes. Larger panels were inclusive of the genes in smaller panels. Resulting libraries were sequenced using 2 x 150 bp Illumina sequencing and variants were called using Mutect and Vardict variant calling algorithms.

NEBNext Direct Custom Ready Panels demonstrate retention of target behavior across panel sizes



IGV image of coverage profile for 4 BRAF exons included in panels of 1, 10, 25, 50 and 100 genes, demonstrate consistent target behavior with the addition of gene targets. 100 ng of DNA was used as input for NEBNext Direct enrichment using the 5 panels, including the BRAF gene. Libraries were sequenced using Illumina 2 x 150 basepair sequencing.

ORDERING INFORMATION

PRODUCTS	NEB #	SIZE
NEBNext Direct Custom Ready Panel	E6631S/L/X	8/24/96 rxns

ALSO AVAILABLE	NEB #	SIZE
NEBNext Direct Cancer HotSpot Panel	E7000S/L/X	8/24/96 rxns
NEBNext Direct BRCA1/BRCA2 Panel	E6627S/L/X	8/24/96 rxns

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