



Product Name: NGS Prep Kit for Barcode Libraries in pScribe (CloneTracker XP™)

Catalog #: LNGS-300

Kit Lot #: 190103007

Number of Samples: 6 - 48 samples (48 samples of 50 µg DNA each)

Shipment Contents: PCR Reagents Only (BOX 1 of 2) — Store at -20°C
Lot# 181127005

PCR/Index/NGS Primers (BOX 2 of 2) — Store at -20°C
Lot# 181030020

Description:

Cellecta's NGS Prep Kit for Barcode Libraries in pScribe provides the protocol and reagents for PCR amplification and NGS sequencing of barcode inserts from genomic DNA isolated from cell populations transduced with pScribe vector-based Barcode Libraries, including the CloneTracker XP Lentiviral Expressed Barcode Library. A total of 17 custom primers are used in the amplification, indexing, and NGS analysis of the samples. Please contact Cellecta if you are unsure that the kit is compatible with the library you are using.

The NGS Prep Kit provides sufficient reagents to prepare 48 samples of 50 µg of genomic DNA for NGS. Enough indexes are also provided for multiplexing up to 12 samples on a single Illumina flow cell or lane.

Shipping Conditions: Blue Ice

Shelf Life: 1 year from date of receipt

Product Information (Cellecta Website):

User Manual

Web: <https://www.cellecta.com/manuals/ngs-prep-kit-for-sgrna-shrna-dna-barcode-libraries/>

PDF: <https://www.cellecta.com/wp-content/uploads/Cellecta-Manual-Library-NGS-Prep-Kits.pdf>

Quality Control

Each lot of the NGS Prep Kit for Barcode Libraries in pScribe (CloneTracker XP™) is quality tested for functionality by following the protocols in the User Manual and the information in this Product Insert.



Kit Contents

PCR Reagents Only (BOX 1 of 2) — Store at -20°C

Box 1 Component	Cap Color	Concentration	Volume
Taq DNA Polymerase	pink	50X	200 µl (2 vials)
Taq DNA Polymerase Buffer	pink	10X	1,200 µl (2 vials)
dNTP Mix	white	50X (10 mM each)	200 µl
PCR-Grade Water	white	NA	7,320 µl (4 vials)

PCR/Index/NGS Primers (BOX 2 of 2) — Store at -20°C

Box 2 Component	Cap Color	Concentration	Volume
Forward-XP Primer	blue	10 µM	150 µl
Reverse-XP Primer	blue	10 µM	150 µl
NFwd-XP Primer	green	20X (10 µM)	240 µl
NRev-XP Index Primer A	white	20X (10 µM)	20 µl
NRev-XP Index Primer B	white	20X (10 µM)	20 µl
NRev-XP Index Primer C	white	20X (10 µM)	20 µl
NRev-XP Index Primer D	white	20X (10 µM)	20 µl
NRev-XP Index Primer E	white	20X (10 µM)	20 µl
NRev-XP Index Primer F	white	20X (10 µM)	20 µl
NRev-XP Index Primer G	white	20X (10 µM)	20 µl
NRev-XP Index Primer H	white	20X (10 µM)	20 µl
NRev-XP Index Primer I	white	20X (10 µM)	20 µl
NRev-XP Index Primer J	white	20X (10 µM)	20 µl
NRev-XP Index Primer K	white	20X (10 µM)	20 µl
NRev-XP Index Primer L	white	20X (10 µM)	20 µl
Seq-XP NGS Primer	blue	100 µM	40 µl
Index-XP NGS Primer	blue	100 µM	40 µl

Sequencing on the Illumina NextSeq®

Program for Single-Read (SR) or Paired-End (PE) Sequencing:

Program	NGS Primer	Cartridge Well	Number of Cycles
BC30+4nt+BC14: Read 1 (Reverse orientation)	Seq-XP	#20	48
6-nt Index: Index 1 (Reverse orientation)	Index-XP	#22	6

Sequencing on the Illumina HiSeq® 2000/2500

Program for Single-Read (SR) or Paired-End (PE) Sequencing:

Program	NGS Primer	Cartridge Well	Number of Cycles
BC30+4nt+BC14: Read 1 (Reverse orientation)	Seq-XP	See <i>HiSeq manual</i>	48
6-nt Index: Index 1 (Reverse orientation)	Index-XP	See <i>HiSeq manual</i>	6



Index Sequences

Index Primer	Index Sequence
NRev-XP Index Primer A	TACGAC
NRev-XP Index Primer B	CTGATG
NRev-XP Index Primer C	GCATCA
NRev-XP Index Primer D	AGTCGT
NRev-XP Index Primer E	TCGCAT
NRev-XP Index Primer F	CATAGC
NRev-XP Index Primer G	AGCGTA
NRev-XP Index Primer H	GTAGGC
NRev-XP Index Primer I	TTCAAG
NRev-XP Index Primer J	GGATTC
NRev-XP Index Primer K	CCTGGA
NRev-XP Index Primer L	AAGCCT

NGS Cassette Diagram (CloneTracker XP™ Library in pScribe)

Example NGS cassette diagram for an example CloneTracker XP barcode construct in pScribe4, from Cellecta's CloneTracker XP Barcode Library.





Second-Round PCR Amplicon Diagram (CloneTracker XP™ Library in pScribe)

Example Second-Round (Nested) PCR amplicon diagram for a CloneTracker XP barcode construct in pScribe4, from Cellecta's CloneTracker XP™ Barcode Library. The **NFwd-XP Primer** contains the minimal sequence of P7 (5' end, sequence in orange) and **NRev-XP Index Primer A** contains the minimal sequence of P5 (3' end, sequence in orange) required for compatibility with all Illumina flow cells. The **Index-XP NGS Primer** binding site is created by the **NRev-XP Index Primer A**.

- Size of Second-Round (Nested) PCR amplicon: **227 bp**
- Starting library concentration (see NGS Sample Purification step): **10 nM**



Primer Sequences

Available Upon Request

NGS Prep of Screening Samples

Cellecta also provides NGS Prep DNA purification, PCR amplification, NGS, deconvolution, and enumeration services. For pricing, please inquire.

Cat.#	Description	Quantity
CANA-SQD	NGS of DNA from Genetic Screen	per DNA sample
CANA-100SQD	NGS of DNA from Genetic Screen, >100M Reads	per DNA sample
CANA-DNA	DNA Isolation from Cell Pellets for Sequencing	per sample
CANA-DNAT	DNA Isolation from Tissues for Sequencing	per sample



Technical Support

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Visit <https://www.cellecta.com/ngs-analysis-kits-for-pooled-libraries/> for more information, or contact your local distributor.



Terms and Conditions

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