

PRODUCT CERTIFICATE



Product Name: NGS Prep Kit for Barcode Libraries in pScribe (CloneTracker XP™)
Catalog #: LNGS-300
Size: 6 - 48 samples (48 samples of 50 µg DNA each)
Shipment Contents: PCR Reagents Only (BOX 1 of 2) — Store at -20°C
PCR/Index/NGS Primers (BOX 2 of 2) — Store at -20°C
Shipping Conditions: Blue Ice or Dry Ice
Shelf Life: 1 year from date of receipt

Product Details

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 screened with pScribe and pScribeM vector-based Barcode Libraries, including the CloneTracker XP 1M/10M Barcode Libraries in pScribe and the 5M/50M Barcode Libraries in pScribeM. A total of 17 custom primers are used in the amplification, indexing, and NGS analysis of the samples.

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. For multiplexing up to 24 samples, Cellecta offers a Supplementary Primer Set of 12 additional index primers (Cat.# LNGS-300-SP).

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 (in 2 vials)
Taq DNA Polymerase Buffer	pink	10X	1,200 µl (in 2 vials)
dNTP Mix	white	50X (10 mM each)	200 µl
PCR-Grade Water	white	NA	7,320 µl (in 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	purple	100 µM	40 µl
Index-XP NGS Primer	purple	100 µM	40 µl

6-nt Collecta Indexes

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

Parameters for Next-Gen Sequencing (NGS) on the Illumina Platform

Illumina NextSeq (Single-Read Only)

What to Sequence	Program (orientation)	NGS Primer	Cartridge Well	Number of Cycles
Barcode	READ 1 (Reverse)	Seq-XP	#20	pScribe: 48 pScribeM: 65
6-nt Collecta Index	INDEX 1 (Reverse)	Index-XP	#22	6

Illumina HiSeq 2000/2500 (Single-Read Only)**

What to Sequence	Program (orientation)	NGS Primer	Cartridge Well	Number of Cycles
Barcode	READ 1 (Reverse)	Seq-XP	See HiSeq manual	pScribe: 48 pScribeM: 65
6-nt Collecta Index	INDEX 1 (Reverse)	Index-XP	See HiSeq manual	6

** Newer HiSeq models may require different parameters.

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.

Lot #s: 181030021 (Box 1: 180809006, Box 2: 181030020)
 190523024 (Box 1: 190305002, Box 2: 190523001)
 190906019 (Box 1: 190524001, Box 2: 190906018)

Additional Product Information can be found on the Collecta Website

User Manual: <https://manuals.collecta.com/ngs-prep-kit-for-sgrna-shrna-dna-barcode-libraries/>
 General Description: <https://collecta.com/collections/next-gen-sequencing-crispr-rnai-libraries>

Collecta NGS Library Prep Services for Screening Samples

Collecta also provides NGS Prep RNA purification, PCR amplification, NGS, and deconvolution services. For pricing, please inquire.

Catalog #	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

NGS Cassette Diagram (CloneTracker XP™ 1M/10M Barcode Libraries in pScribe)

Example NGS cassette diagram for an example CloneTracker XP barcode construct, from Cellecta's CloneTracker XP 10M Barcode Library in pScribe4-RFP-Puro.

NOTE: For the CloneTracker XP™ 5M/50M Barcode Libraries in pScribeM (e.g. pScribe4M, pScribe4HM), please see the diagrams on pages 5 and 6.

Vector Sequence in SnapGene or GenBank format: email tech@cellecta.com.



Second-Round PCR Amplicon Diagram (CloneTracker XP™ 1M/10M Barcode Libraries in pScribe)

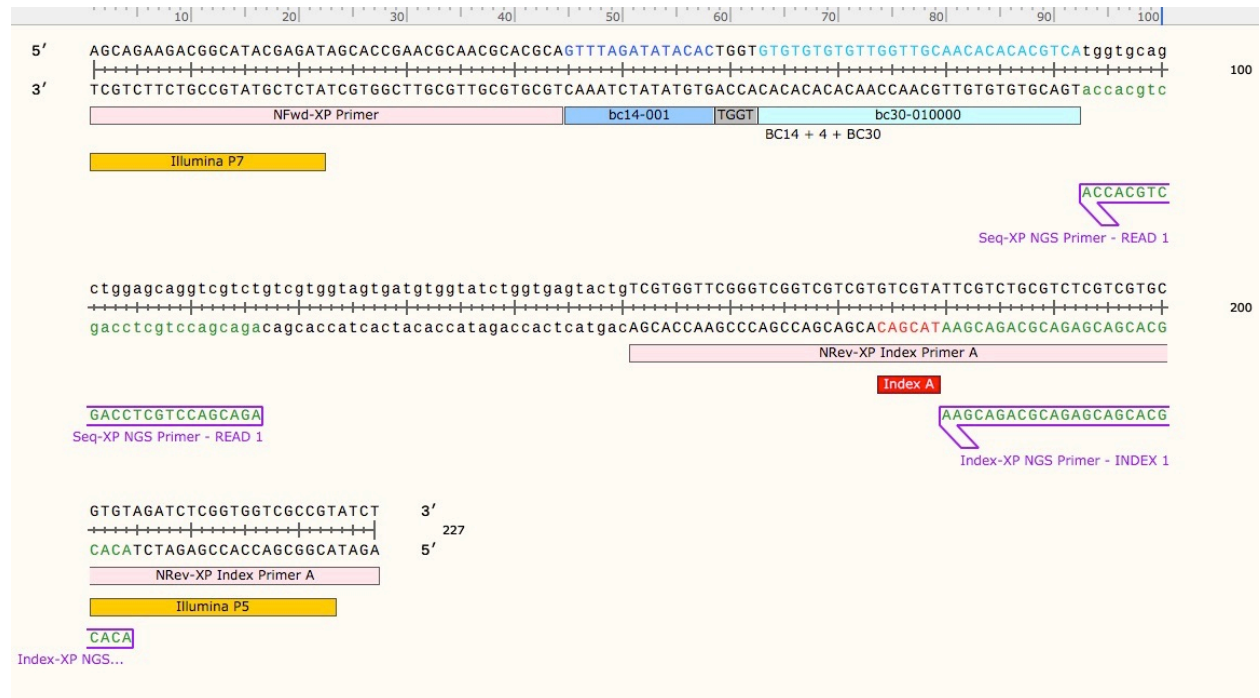
Example Second-Round (Nested) PCR amplicon diagram for a CloneTracker XP barcode construct, from Collecta's CloneTracker XP™ 10M Barcode Library in pScribe4-RFP-Puro. The NFwd-XP Primer contains the minimal sequence of P7 (5' end, sequence in orange) and NRev-XP Index Primer A contains the minimal sequence of P5 (3' end, sequence in orange) required for compatibility with all Illumina flow cells.

NOTE: For the CloneTracker XP™ 5M/50M Barcode Libraries in pScribeM (e.g. pScribe4M, pScribe4HM), please see the diagrams on pages 5 and 6.

The 48-nt barcode insert, comprising a 14-nt barcode + 4-nt spacer + 30-nt barcode (BC14 + 4 + BC30), is sequenced using the **Seq-XP NGS Primer** in Read 1, in the reverse orientation. The **Index-XP NGS Primer** binding site is created by NRev-XP Index Primer A after First Round PCR for reading the 6-nt Collecta Index in Index 1, also in the reverse orientation.

- Size of Second-Round (Nested) PCR amplicon: **227 bp**
- Starting library concentration (see [NGS Sample Purification](#) step in User Manual): **10 nM (1.59 ng/µl)**

Amplicon Sequence in SnapGene or GenBank format: email tech@collecta.com.



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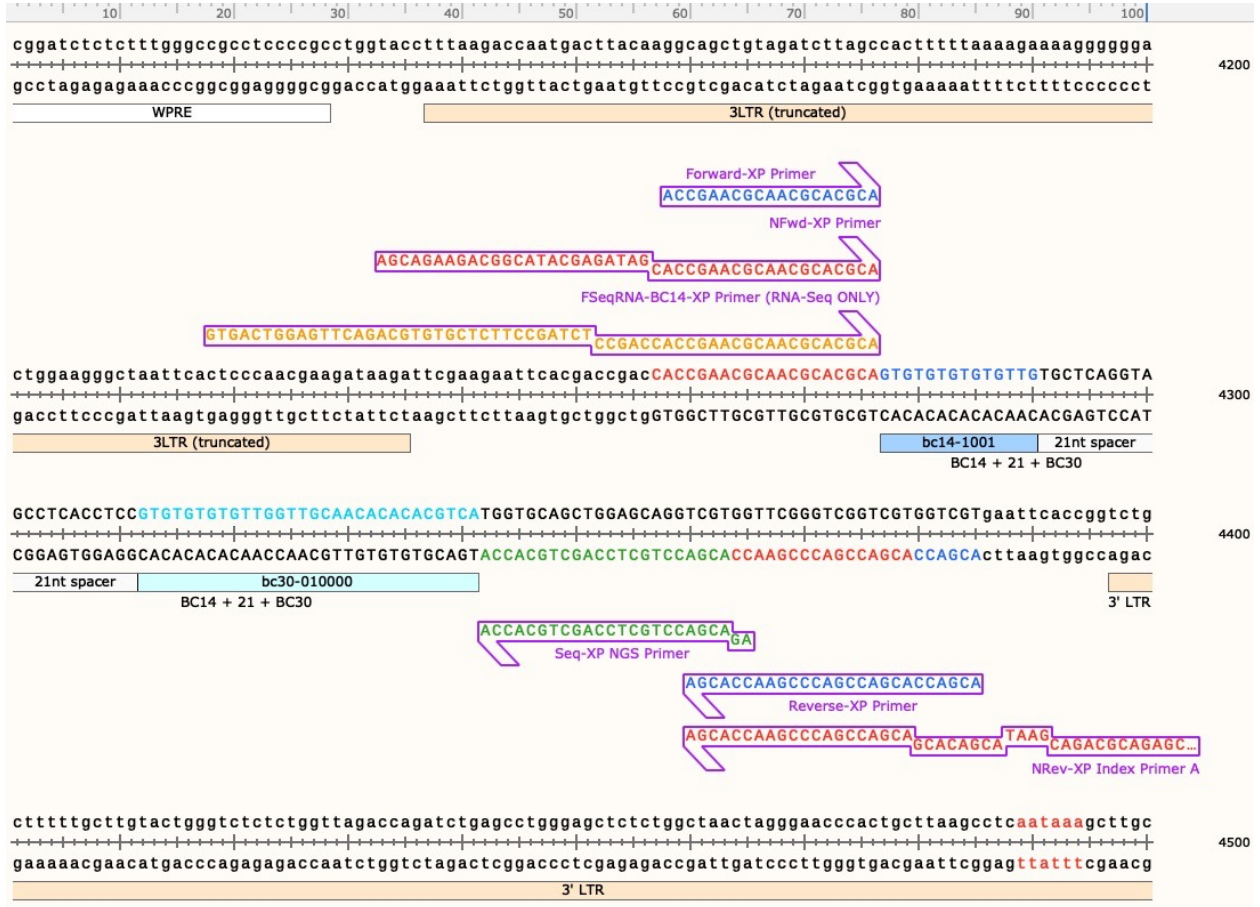


NGS Cassette Diagram (CloneTracker XP™ 5M/50M Barcode Library in pScribeM)

Example NGS cassette diagram for an example CloneTracker XP barcode construct, from Cellecta's CloneTracker XP™ 50M Barcode Library in pScribe4M-RFP-Puro.

NOTE: For the CloneTracker XP™ 1M/10M Barcode Libraries in pScribe vectors (e.g. pScribe4, pScribe5, pScribe5.2, and pScribe6), please see the diagrams on pages 3 and 4.

Vector Sequence in SnapGene or GenBank format: email tech@cellecta.com.



Second-Round PCR Amplicon Diagram (CloneTracker XP™ 5M/50M Barcode Libraries in pScribeM)

Example Second-Round (Nested) PCR amplicon diagram for a CloneTracker XP barcode construct, from Collecta's CloneTracker XP™ 50M Barcode Library in pScribe4M-RFP-Puro. 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.

NOTE: For the CloneTracker XP™ 1M/10M Barcode Libraries in pScribe vectors (e.g. pScribe4, pScribe5, pScribe5.2, and pScribe6), please see the diagrams on pages 3 and 4.

The 65-nt barcode insert, comprising a 14-nt barcode + 21-nt spacer + 30-nt barcode (BC14 + 21 + BC30), is sequenced using the **Seq-XP NGS Primer** in Read 1, in the reverse orientation. The **Index-XP NGS Primer** binding site is created by NRev-XP Index Primer A after First Round PCR for reading the 6-nt Collecta Index in Index 1, also in the reverse orientation.

- Size of Second-Round (Nested) PCR amplicon: **204 bp**
- Starting library concentration (see [NGS Sample Purification](#) step in User Manual): **10 nM (1.42 ng/µl)**

Amplicon Sequence in SnapGene or GenBank format: email tech@collecta.com.



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