

Product Analysis Certificate

Product: CloneTracker XP™ 1M Barcode-3' Library Pool 1 with RFP-Puro (Plasmid)
Part #: BCXP1M3RP-01-P



CloneTracker XP™ 1M Barcode-3' Library Pool 1 with RFP-Puro

Shipment Contents: CloneTracker XP™ 1M Barcode-3' Library Pool 1 with RFP-Puro (Plasmid)
Store at -20°C

Description:

Cellecta's CloneTracker XP™ 1M Barcode-3' Library with RFP-Puro is a pooled barcode library that enables the tracking of individual clones derived from a population of cells using either RNA-Seq or PCR/NGS from genomic DNA. The library contains 1 million lentiviral constructs, each expressing a unique barcode sequence. When a cell population is transduced with this barcode library, the barcodes integrate into the genomic DNA of the cells. The result is a founder population where almost every cell has a different DNA-sequenceable barcode that is both integrated into its DNA and expressed on an RNA transcript. Since the barcode is stably integrated, it is passed onto any cell progeny when genomic DNA is replicated. This feature enables identification of all the progeny derived from each individual cell.

When used in conjunction with single-cell RNA-Seq, the expressed barcode may be used to identify expression profiles and activated genes in different cells so that distinct clonal populations of cells from a single progenitor, as well as sub-populations of cells with distinct pathway activations or expression profiles, can be readily identified.

The CloneTracker XP Barcode Library is constructed in a third-generation lentiviral vector that expresses TagRFP (Evrogen) and Puro resistance gene under an EF1a core (EFS) promoter. The specially-designed, optimized barcodes facilitate Next-Gen Sequencing (NGS) data analysis and barcode identification. Using the Illumina NextSeq or HiSeq NGS platform, barcode sequences can be identified either by RNA-Seq or PCR/NGS from genomic DNA and converted to lists with enumerated data.

The library can be packaged into VSV-G pseudotyped viral particles using most commercially-available second or third-generation packaging mixes or Cellecta's second-generation psPAX2/ pMD2.G packaging plasmid mix (Ready-to-use Packaging Plasmid Mix, Cat.# CPCP-K2A, 250 µg).

Fluorescent Marker: TagRFP (Evrogen)
Biosafety Level: BSL-2
Storage: -20°C
Shelf Life: 2 years from date of receipt
Shipping Conditions: Blue or Dry Ice

Product Information

The user manual and vector map/sequence are available on the CloneTracker XP product page:
<https://www.cellecta.com/clonetracker-xp-expressed-barcode-libraries/>

The barcode library sequences file with NGS QC is provided by email upon shipment.
Please contact Cellecta at orders@cellecta.com if you have not received it.

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Contents:

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BCXP1M3RP-01-P	CloneTracker XP™ 1M Barcode-3' Library Pool 1 with RFP-Puro (plasmid) in pScribe4-EFS-TagRFP-2A-Puro-bc-3LTR Plasmid, 200 µg: 200 µg , 1.0 µg/µl (200 µl × 1 tube) Lot# 180817002; Store at -20°C

Library Barcode Sequence Files (sent with PAC):

30nt Barcodes (BC30): Collecta-SEQ-CloneTracker-XP-Barcode-Libraries-100K_x_BC30.xlsx

14nt Barcodes (BC14): Collecta-SEQ-CloneTracker-XP-Barcode-Libraries-100_x_BC14.xlsx

NOTE: The **CloneTracker XP™ 1M Barcode-3' Library Pool 1 with RFP-Puro** uses the following 10 BC14 barcodes paired with the 100,000 BC30 barcodes:

Collecta ID	BC14 Sense	BC14 Antisense (as read from Seq-XP NGS Primer)
bc14-001	GTTTAGATATACAC	GTGTATATCTAAAC
bc14-002	TAAC TCGCCTGCT	AGCAGGCGAAGTTA
bc14-003	ATCCCCAAAGAGGA	TCCTCTTTGGGGAT
bc14-004	TCATATCAGCCGTC	GACGGCTGATATGA
bc14-005	AGGTGTCCGGTATG	CATACCGGACACCT
bc14-006	ATATGTTCTGGCAT	ATGCCAGAACATAT
bc14-007	ACACGCAGGAAACT	AGTTTCCTGCGTGT
bc14-008	GAGTTGTAAGAGAC	GTCTCTTACAACCT
bc14-009	AGCAGAAAAGTTCG	CGAACTTTCTGCT
bc14-010	ATTATTCTGCGCCT	AGGCGCAGAATAAT

Quality Control

Individual Clone Sequencing Data

Library:	CloneTracker XP™ 1M Barcode-3' Library with RFP-Puro
Plasmid Lot #:	180817002
Library Complexity (number of clones):	48 x 10 ⁶
Number of good clones picked:	22
Correct Structure:	>95%
Number of clones with at least one mutation, deletion, or insertion:	0
Mutation / Deletion / Insertion Rate:	0.00%
Estimated % of Inserts without any mutations, deletions, or insertions in <u>barcode</u> portion (Based on Clone QC):	>95%
Estimated % of Inserts without any mutations, deletions, or insertions in <u>barcode</u> portion (Based on NGS QC):	>95%

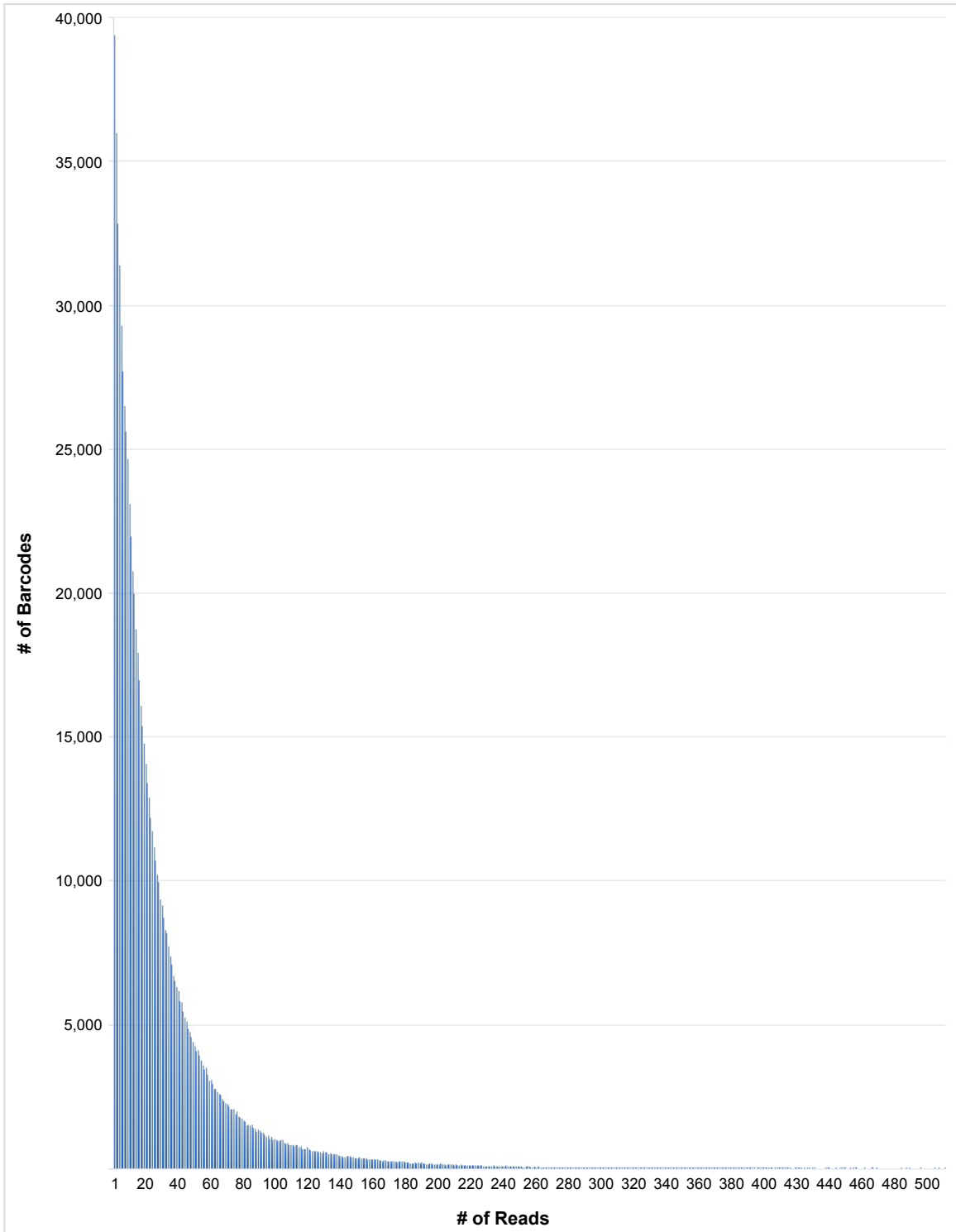
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Next-Gen Sequencing (NGS) Data

Histogram, Barcode Representation (27M total reads)
Number of barcodes (y) with x # of reads



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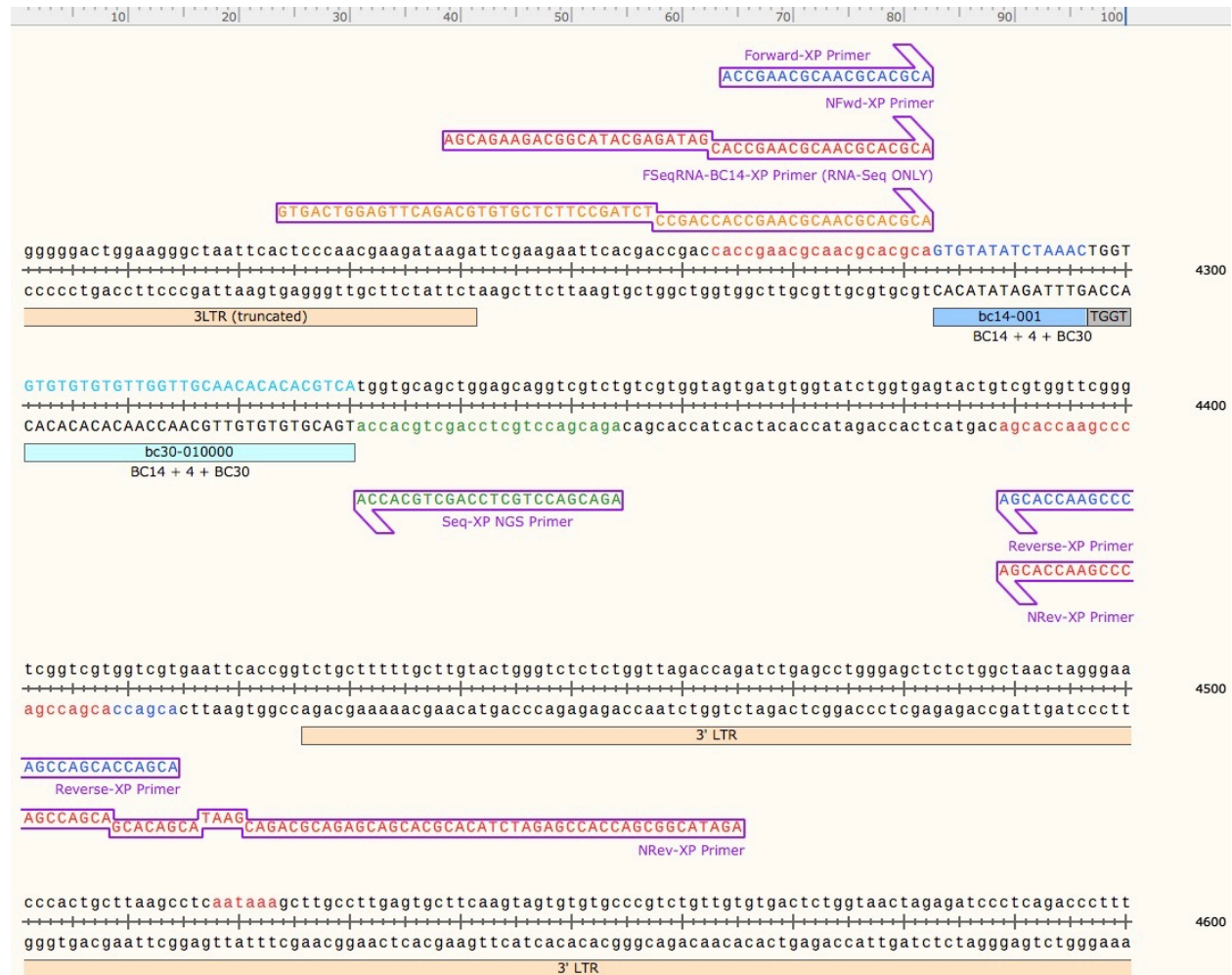
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NGS Barcode Cassette Diagram (in pScribe4 Vector)

Example NGS cassette diagram for the bc14-001_bc30-010000 construct in pScribe4-EFS-TagRFP-2A-Puro-bc-3LTR, from the CloneTracker XP™ 10M Barcode-3' Library with RFP-Puro. RNA-Seq primer **FSeqRNA-BC14-XP** (not included) is also shown and is required in order to read CloneTracker XP barcode sequences on most single-cell Drop-Seq systems.

Expressed barcode structure (sense): BC14-TGGT-BC30-3LTR-Poly(A)signal



Primer*	Sequence	Purpose
Forward-XP Primer	ACCGAACGCAACGCACGCA	1st Round PCR
Reverse-XP Primer	ACGACCAGCACCAGCCGCAACCACGA	1st Round PCR
NFwd-XP Primer	AGCAGAAGACGGCATAACGAGATAGCACCAGCAACGCACGCA	2nd Round PCR
NRev-XP Primer	AGATACGGCGACCACCGAGATCTACACGCACGACGAGACGCAATACGACACGACGACC GACCCGCAACCACGA	2nd Round PCR

* Primers and reagents for multiplexing up to 12 samples available in Collecta's NGS Prep Kit (See Page 5)

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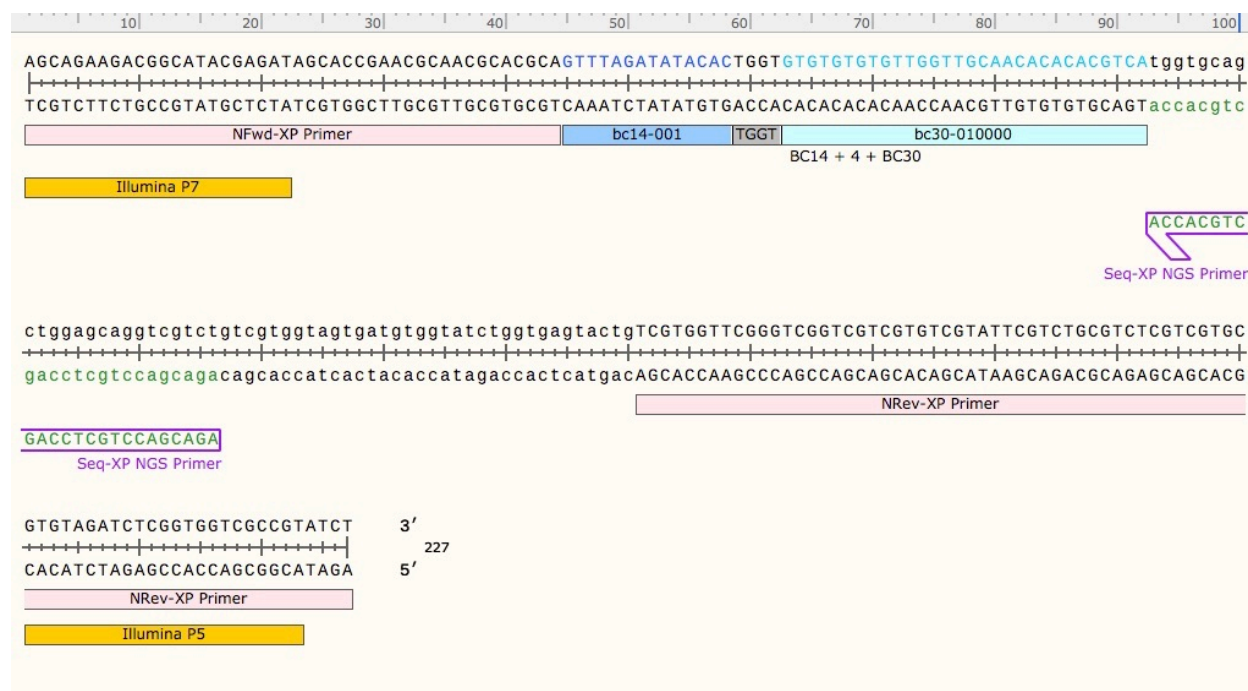
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Diagram of PCR Amplicon

Example Second-Round (Nested) PCR amplicon diagram for a barcode-3' library construct in pScribe4, from the CloneTracker XP™ 1M Barcode-3' Library. The **NFwd-XP Primer** contains the minimal sequence of P7 (5' end, sequence in orange) and **NRev-XP Primer** contains the minimal sequence of P5 (3' end, sequence in orange) required for compatibility with Illumina flow cells.

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



NGS Primer*	Sequence	Purpose
Seq-XP NGS Primer	AGACGACCTGCTCCAGCTGCACCA	NGS of barcode (reverse)

* Primers and reagents for multiplexing up to 12 samples available in Collecta's NGS Prep Kit (See below)

NGS Prep of Screening Samples

Collecta provides the following NGS products and services. For pricing, please inquire.

Cat.#	Description	Quantity
LNGS-300	NGS Prep Kit for Barcode Libraries in pScribe (CloneTracker XP)	Kit for 6-48 samples (48 preps of 50 µg each, 12 multiplex)
LNGS-300-SP	Supplementary Primer Set for LNGS-300 (12 Additional Index Primers)	48 Reactions (12 Index Primers x 4 reactions each)
CANA-SQD	NGS of DNA from Genetic Screen	Service (per DNA sample)

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