

Product Analysis Certificate

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



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

Shipment Contents: CloneTracker XP™ 1M Barcode-3' Library Pool 1 with RFP-Puro (Packaged)
Store at -80°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 titer of packaged libraries provided by Cellecta is functionally determined by transduction of 293T cells at low MOI and FACS of RFP-positive cells.

Biosafety Level: BSL-2
Storage: -80°C
Shelf Life: 1 year from date of receipt
Shipping Conditions: Dry Ice

Product Information (Cellecta Website):

User Manual: <https://www.cellecta.com/resources/product-manuals-and-certificates/>
Vector Info (Sequence, cassette, etc): Please contact Cellecta Technical Support at tech@cellecta.com.

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Contents:

Part #	Description
BCXP1M3RP-01-V	CloneTracker XP™ 1M Barcode-3' Library Pool 1 with RFP-Puro (packaged) in pScribe4-EFS-TagRFP-2A-Puro-bc-3LTR Packaged, $>1 \times 10^8$ TU: 1.06×10^8 TU , 5.03×10^8 TU/ml (210 µl total: 50 µl × 4 vials, 5 µl × 2 vials) Lot# 180809001; Store at -80°C

Library Barcode Sequence Files (sent with PAC):

30nt Barcodes (BC30): [Cellecta-SEQ-CloneTracker-XP-Barcode-Libraries-100K_x_BC30.xlsx](#)

14nt Barcodes (BC14): [Cellecta-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 barcodes (BC14):

Collecta ID	BC14 Sense (as read from FSeq- BC14-XP NGS Primer)	BC14 Antisense (as read from RSeq- BC30-XP NGS Primer)
bc14-001	GTTTAGATATACAC	GTGTATATCTAAAC
bc14-002	TAACTTCGCCTGCT	AGCAGGCGAAGTTA
bc14-003	ATCCCCAAAGAGGA	TCCTCTTTGGGGAT
bc14-004	TCATATCAGCCGTC	GACGGCTGATATGA
bc14-005	AGGTGTCCGGTATG	CATACCGGACACCT
bc14-006	ATATGTTCTGGCAT	ATGCCAGAACATAT
bc14-007	ACACGCAGGAAACT	AGTTTCCTGCGTGT
bc14-008	GAGTTGTAAGAGAC	GTCTCTTACAACCTC
bc14-009	AGCAGAAAAGTTCG	CGAACTTTTCTGCT
bc14-010	ATTATCTGCGCCT	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):	48×10^6
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%

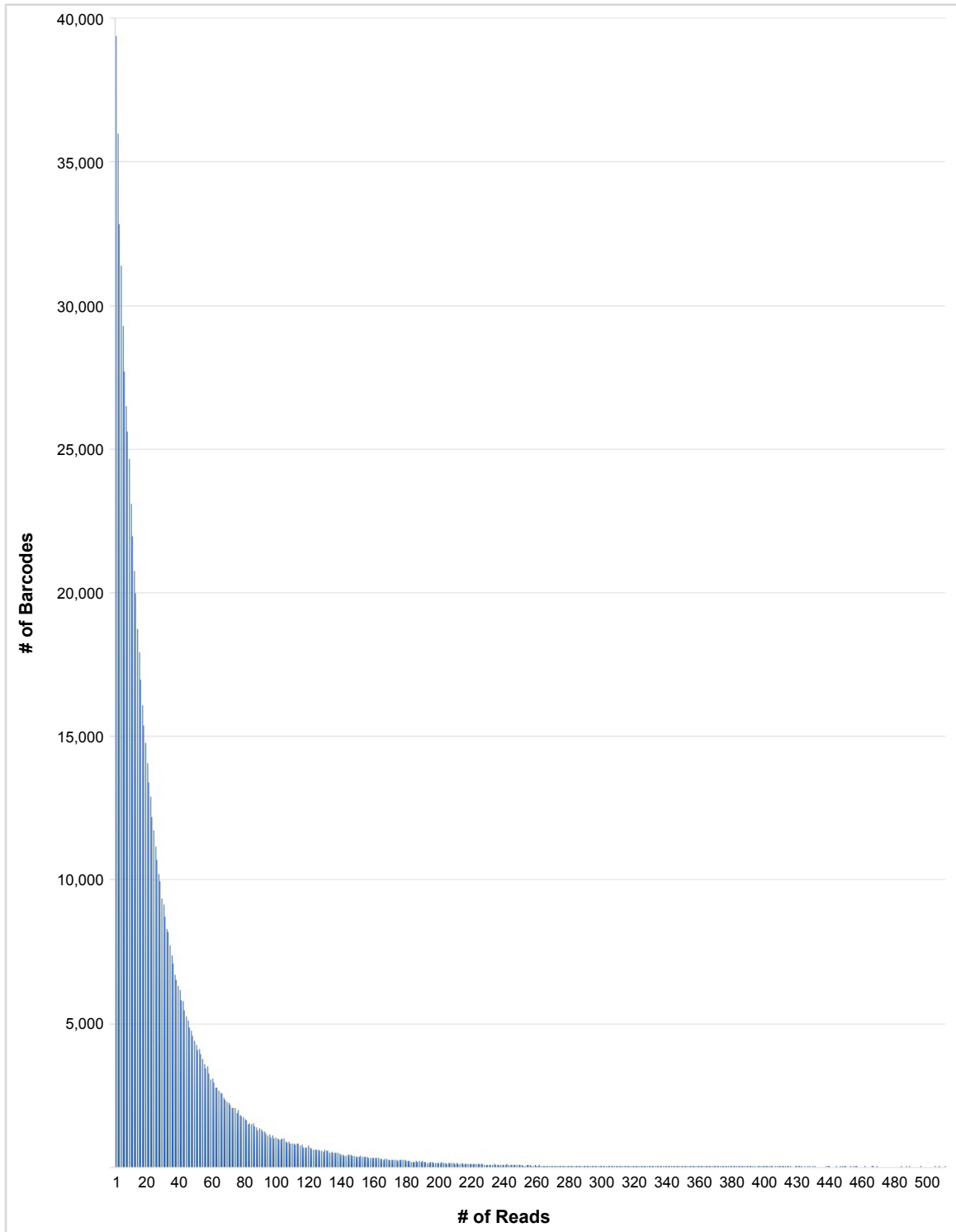
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NGS Barcode Representation Data

Histogram, Raw Counts (27 million reads)
Number of barcodes (y) with x # of reads (1-510)



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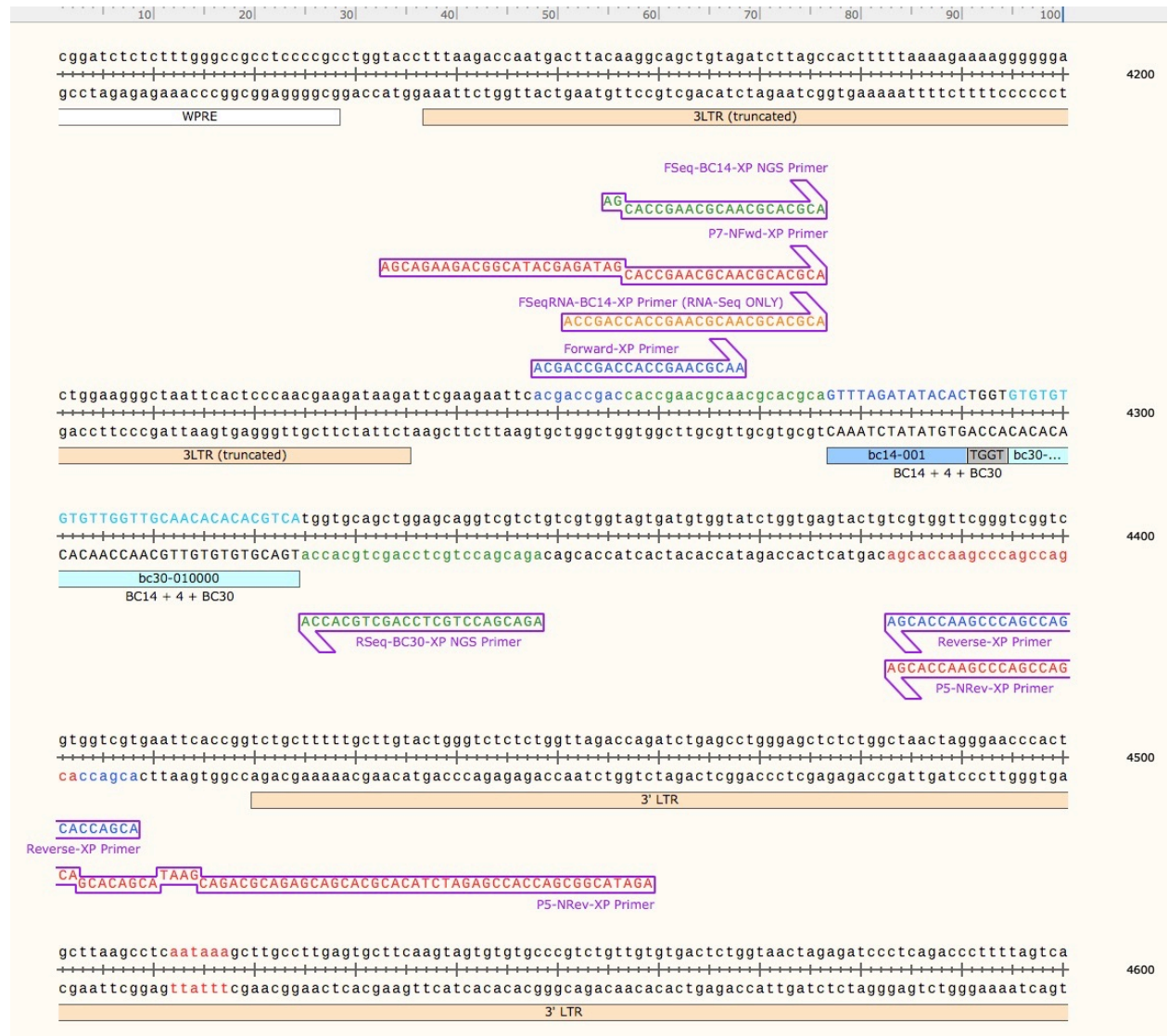
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NGS Barcode Cassette Diagram (in pScribe Vector)

Example NGS cassette diagram for the bc14-001_bc30-010000 construct in pScribe4-EFS-TagRFP-2A-Puro-bc-3LTR, from the CloneTracker XP™ 1M Barcode-3' Library Pool 1 with RFP-Puro. RNA-Seq primer **FSeqRNA-BC14-XP** is also shown.

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



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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 Pool 1. The **P7-NFwd-XP Primer** contains the minimal sequence of P7 (5' end, sequence in orange) and **P5-NRev-XP Primer** contains the minimal sequence of P5 (3' end, sequence in orange) required for compatibility with all Illumina flow cells.

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



NGS Prep of Screening Samples

Collecta provides the following options for preparing and sequencing your screening samples. 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 50ug each, 12 multiplex)
CANA-SQD	NGS of DNA from Genetic Screen	Service (per DNA sample)

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Terms and Conditions

Cellecta, Inc. Limited License

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