



NGS Prep Kit for Barcode Libraries in pRSI16/17 (CloneTracker™)

Product Name:	NGS Prep Kit for Barcode Libraries in pRSI16/17 (CloneTracker™)	
Catalog #:	LNGS-200	
Kit Lot #:	190111001	
Number of Samples:	6 - 48 samples (48 samples of 50 µg DNA each)	
Shipment Contents:	PCR Reagents Only (BOX 1 of 2) Lot# 181127005	— Store at -20°C
	PCR/Index/NGS Primers (BOX 2 of 2) Lot# 171002918	— Store at -20°C

Description:

Cellecta's NGS Prep Kit for Barcode Libraries in pRSI16/17 provides the protocol and reagents for PCR amplification and NGS sequencing of barcode inserts from genomic DNA isolated from cell populations transduced with pRSI16/pRSI17 vector-based Barcode Libraries, including the CloneTracker (formerly CellTracker) 50M Lentiviral Barcode Library and CloneTracker (formerly CellTracker) 13K Lentiviral 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 pRSI16/17 (CloneTracker™) is quality tested for functionality by following the protocols in the User Manual.



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-B Primer	blue	10 µM	150 µl
Reverse-B Primer	blue	10 µM	150 µl
NFwd-B Primer	green	20X (10 µM)	240 µl
NRev-B Index Primer A	white	20X (10 µM)	20 µl
NRev-B Index Primer B	white	20X (10 µM)	20 µl
NRev-B Index Primer C	white	20X (10 µM)	20 µl
NRev-B Index Primer D	white	20X (10 µM)	20 µl
NRev-B Index Primer E	white	20X (10 µM)	20 µl
NRev-B Index Primer F	white	20X (10 µM)	20 µl
NRev-B Index Primer G	white	20X (10 µM)	20 µl
NRev-B Index Primer H	white	20X (10 µM)	20 µl
NRev-B Index Primer I	white	20X (10 µM)	20 µl
NRev-B Index Primer J	white	20X (10 µM)	20 µl
NRev-B Index Primer K	white	20X (10 µM)	20 µl
NRev-B Index Primer L	white	20X (10 µM)	20 µl
Seq-B NGS Primer	dark blue	100 µM	40 µl
Index-B NGS Primer	dark 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
18ntBC+4nt+18ntBC: Read 1 (Reverse orientation)	Seq-B	#20	44
6-nt Index: Index 1 (Reverse orientation)	Index-B	#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
18ntBC+4nt+18ntBC: Read 1 (Reverse orientation)	Seq-B	See <i>HiSeq manual</i>	44
6-nt Index: Index 1 (Reverse orientation)	Index-B	See <i>HiSeq manual</i>	6

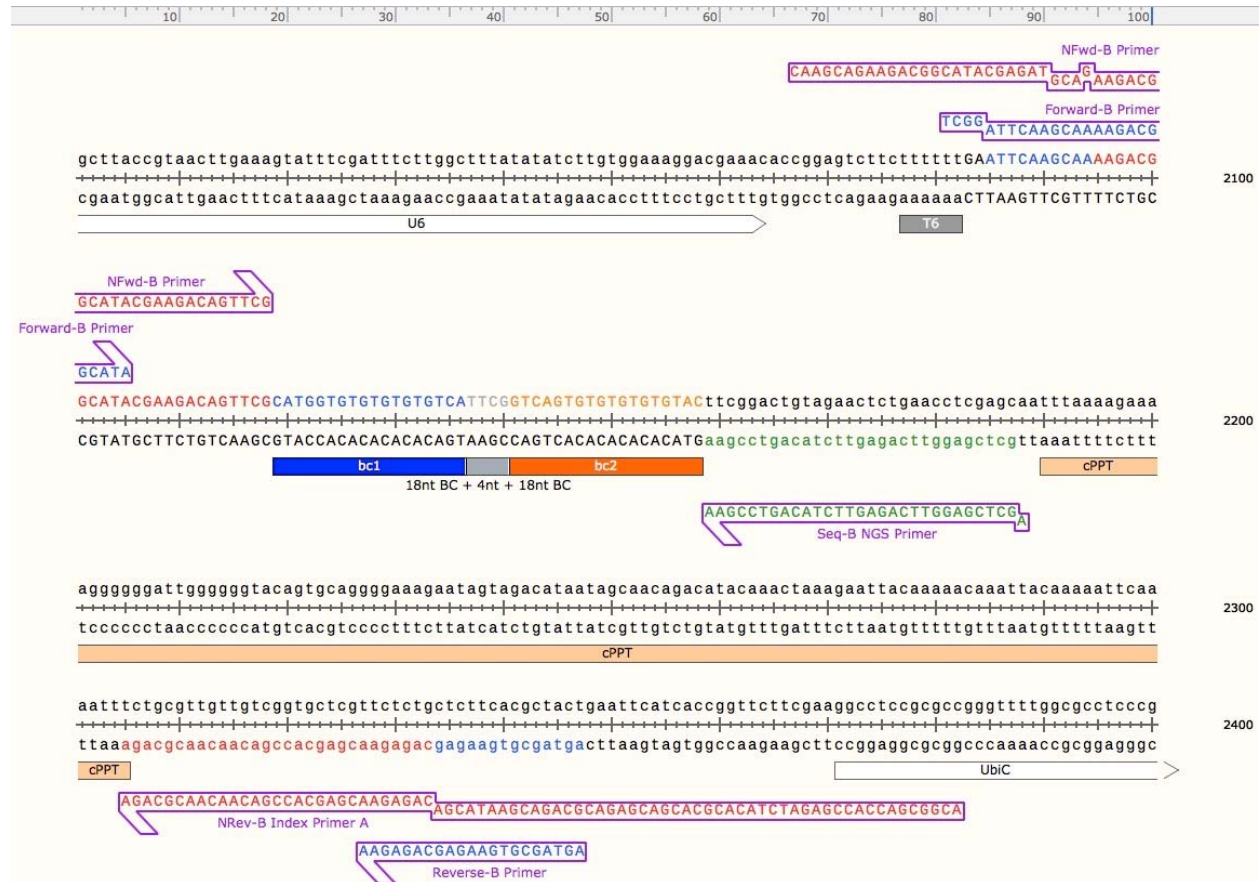


Indexes

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

NGS Cassette Diagram (13K x 13K Barcode in pRSI16/pRSI17)

Example NGS cassette diagram for an example 13K x 13K barcode library 18nt BC1 + 4nt + 18nt BC2 construct in pRSI16, from Cellecta's CloneTracker™ 50M Barcode Library.

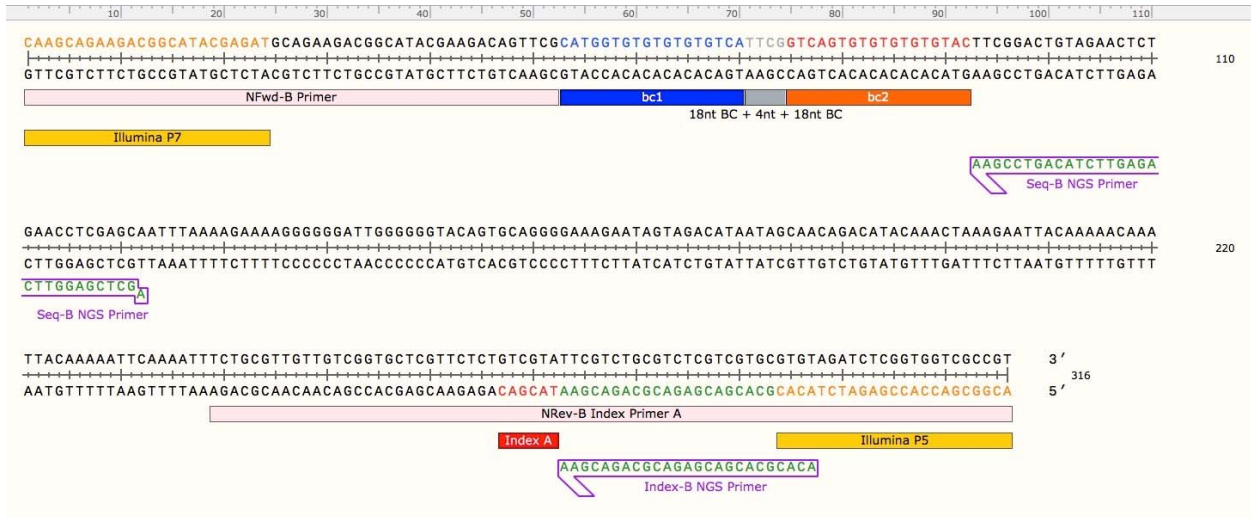




Second-Round PCR Amplicon Diagram (13K x 13K Barcode in pRSI16/pRSI17)

Example Second-Round (Nested) PCR amplicon diagram for a 13K x 13K barcode construct in pRSI16, from Cellecta's CloneTracker™ 50M Barcode Library. The **NFwd-B Primer** contains the minimal sequence of P7 (5' end, sequence in orange) and **NRev-B Index Primer A** contains the minimal sequence of P5 (3' end, sequence in orange) required for compatibility with all Illumina flow cells. The **Index-B NGS Primer** binding site is created by the **NRev-B Index Primer A**.

- Size of Second-Round (Nested) PCR amplicon: **316 bp**
- Starting library concentration (see NGS Sample Purification step): **10 nM**



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

Phone: +1 (650) 938-3910, option 2
 Toll-Free: (877) 938-3910, option 2

E-mail:
 Technical Support: tech@cellecta.com
 General Information: info@cellecta.com
 Sales: sales@cellecta.com
 Orders: orders@cellecta.com

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



NGS Prep Kit for Barcode Libraries in pRSI16/17 (CloneTracker™)

Terms and Conditions

Cellecta, Inc. Limited License

Cellecta grants the end user (the "Recipient") of the NGS Prep Kit for Barcode Libraries in pRSI16/17 (CloneTracker™) (the "Product") a non-transferable, non-exclusive license to use the reagents for internal research use only as described in the enclosed protocols; in particular, research use only excludes and without limitation, resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of Cellecta, Inc. -- separate licenses are available for non-research use or applications. The Product is not to be used for human diagnostics or included/used in any drug intended for human use. Care and attention should be exercised in handling the Product by following appropriate research laboratory practices.

Cellecta's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. Cellecta's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. Cellecta does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose. Use of the Product for any use other than described expressly herein may be covered by patents or subject to rights other than those mentioned. Cellecta disclaims any and all responsibility for injury or damage that may be caused by the failure of the Recipient or any other person to use the Product in accordance with the terms and conditions outlined herein.

The Recipient may refuse these licenses by returning the enclosed Product unused. By keeping or using the enclosed Product, you agree to be bound by the terms of these licenses. The laws of the State of California shall govern the interpretation and enforcement of the terms of these Licenses.

Terms and Conditions are also available online at <https://www.cellecta.com/company/legal-information/terms-and-conditions/>.

© 2018 Cellecta, Inc. All Rights Reserved.

Trademarks

CELLECTA is a registered trademark of Cellecta, Inc. CloneTracker is a trademark of Cellecta, Inc.