

# PRODUCT CERTIFICATE



**Product Name:** NGS Prep Kit for Dual-sgRNA Libraries in pRSL10 (KADHGW/KIDHGW)  
**Catalog #:** LNGS-400  
**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 Dual-sgRNA Libraries in pRSL10 provides the protocol and reagents for PCR amplification and NGS sequencing of sgRNA inserts from genomic DNA isolated from cell populations screened with pRSL10 vector-based dual-sgRNA libraries, including the CRISPRa and CRISPRi Genome-Wide Dual-sgRNA Libraries. A total of 18 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-400-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-DG Primer	blue	10 µM	150 µl
Reverse-DG Primer	blue	10 µM	150 µl
NFwd-DG Primer	green	20X (10 µM)	240 µl
NRev-DG Index Primer A	white	20X (10 µM)	20 µl
NRev-DG Index Primer B	white	20X (10 µM)	20 µl
NRev-DG Index Primer C	white	20X (10 µM)	20 µl
NRev-DG Index Primer D	white	20X (10 µM)	20 µl
NRev-DG Index Primer E	white	20X (10 µM)	20 µl
NRev-DG Index Primer F	white	20X (10 µM)	20 µl
NRev-DG Index Primer G	white	20X (10 µM)	20 µl
NRev-DG Index Primer H	white	20X (10 µM)	20 µl
NRev-DG Index Primer I	white	20X (10 µM)	20 µl
NRev-DG Index Primer J	white	20X (10 µM)	20 µl
NRev-DG Index Primer K	white	20X (10 µM)	20 µl
NRev-DG Index Primer L	white	20X (10 µM)	20 µl
Seq-DG1 NGS Primer	purple	100 µM	40 µl
Seq-DG2 NGS Primer	purple	100 µM	40 µl
Index-DG NGS Primer	purple	100 µM	40 µl

**6-nt Collecta Indexes**

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

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

**Illumina NextSeq 500/550\*\* (Paired-End)**

What to Sequence	Program (orientation)	NGS Primer	Cartridge Well	Number of Cycles
sgRNA 1 (19 or 20-nt)	<b>READ 1</b> (Forward)	Seq-DG1	#20	26
sgRNA 2 (19 or 20-nt)	<b>INDEX 1</b> (Forward)	Seq-DG2	#22	20
6-nt Collecta Index	<b>READ 2</b> (Reverse)	Index-DG	#21	7

**Illumina HiSeq 2000/2500\*\* (Paired-End)**

What to Sequence	Program (orientation)	NGS Primer	Cartridge Well	Number of Cycles
sgRNA 1 (19 or 20-nt)	<b>READ 1</b> (Forward)	Seq-DG1	See HiSeq manual	26
sgRNA 2 (19 or 20-nt)	<b>INDEX 1</b> (Forward)	Seq-DG2	See HiSeq manual	20
6-nt Collecta Index	<b>READ 2</b> (Reverse)	Index-DG	See HiSeq manual	7

\*\* Newer Illumina models may require different parameters.

**Quality Control**

Each lot of the NGS Prep Kit for Dual-sgRNA Libraries in pRSL10 (KADHGW/KIDHGW) is quality tested for functionality by following the protocols in the User Manual and the information in this Product Insert.

**Lot #s:** 191010006 (Box 1: 190524001, Box 2: 191010005)  
 200218007 (Box 1: 200218003, Box 2: 191010005)  
 201221040 (Box 1: 201201003, Box 2: 201221039)  
 210803002 (Box 1: 210803003, Box 2: 210803001)  
 230102020 (Box 1: 210803003, Box 2: 230102019)

**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-mal-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

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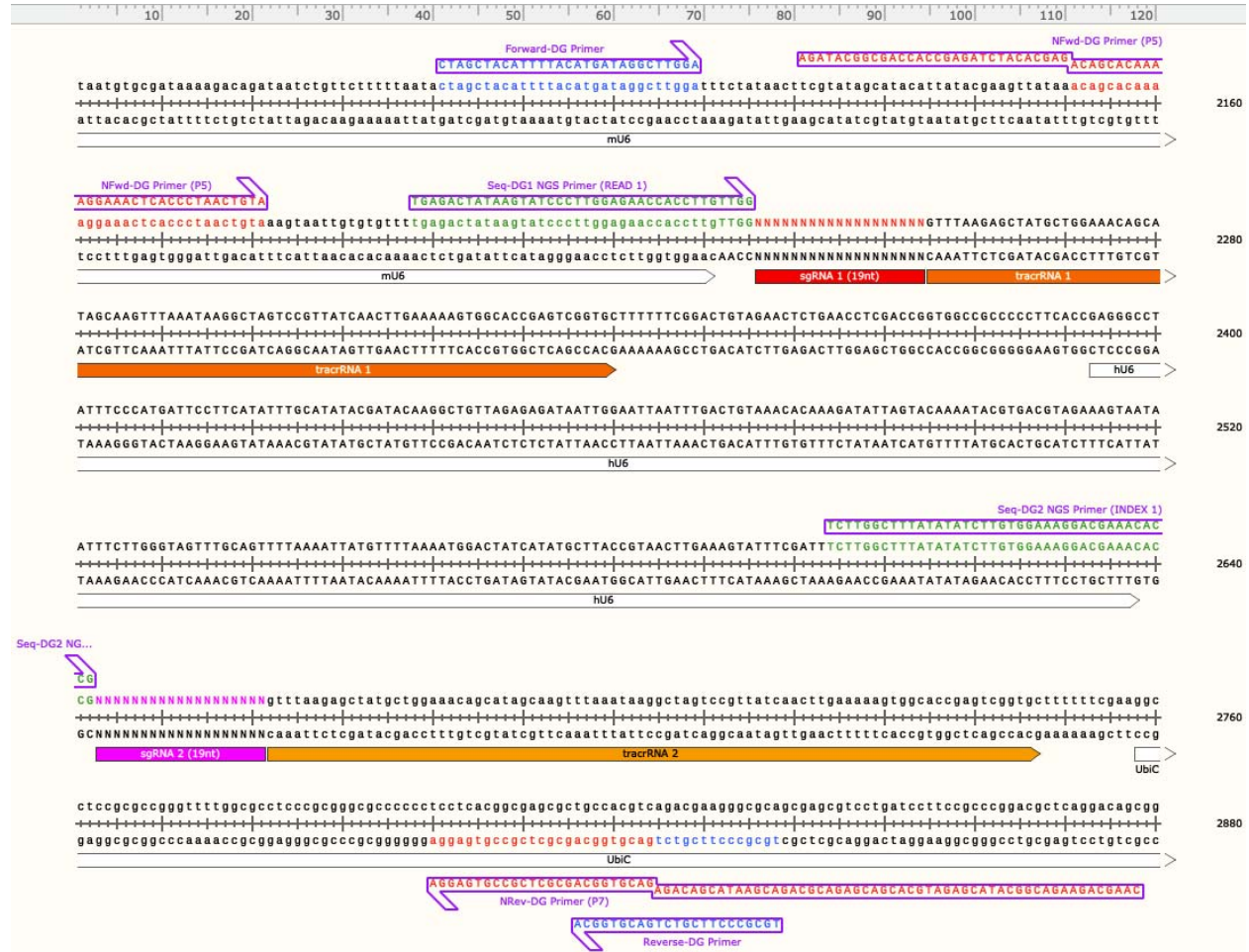


CANA-100SQD	NGS of DNA from Genetic Screen, >100M Reads	per DNA sample
CANA-DNA	DNA Isolation from Cell Pellets for Sequencing	per sample
CANA-DNAT	DNA Isolation from Tissues for Sequencing	per sample

## Example NGS Cassette Diagram, from the CRISPRa Genome-Wide Dual-sgRNA Library

Example NGS cassette diagram for an example Dual-sgRNA construct in pRSL10, from the CRISPRa Genome-Wide Dual-sgRNA Library.

Vector Sequence in SnapGene or GenBank format: email [tech@cellecta.com](mailto:tech@cellecta.com).



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## Second Round PCR Amplicon Diagram (pRSL10)

Example Second Round (Nested) PCR amplicon diagram for a dual 19-nt sgRNA construct in pRSL10, from the CRISPRa Genome-Wide Dual-sgRNA Library. The **NFwd-DG** Primer contains the minimal sequence of P5 (5' end, sequence in orange) and **NRev-DG Index Primer A** contains the minimal sequence of P7 (3' end, sequence in orange) required for compatibility with Illumina flow cells.

The sgRNA expressed from mU6 (sgRNA 1) is sequenced using the **Seq-DG1** NGS Primer in Read 1 using 26 cycles in the forward orientation, and the sgRNA expressed from hU6 (sgRNA 2) is sequenced using the **Seq-DG2** NGS Primer in Index 1, using 20 cycles also in the forward orientation. The **Index-DG** NGS Primer binding site is created by NRev-DG Index Primer A after Second Round PCR for reading the 6-nt Collecta Index in Index 1, in the reverse orientation.

- Size of Second-Round (Nested) PCR amplicon: **758 bp**
- Starting library concentration (see [NGS Sample Purification](#) step in User Manual): **10 nM (5.3 ng/μl)**

Amplicon Sequence in SnapGene or GenBank format: email [tech@cellecta.com](mailto:tech@cellecta.com).



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## Contact Us

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