

Cellecta HTS6C (pRSG16/17) sgRNA Expression NGS Cassette

Cellecta's HTS6C NGS (Next-Generation Sequencing) cassette for sgRNA expression from the U6 promoter is the standard cassette for pRSG16/pRSG17 vector-based Cellecta CRISPR sgRNA libraries, including the CRISPR Human Genome Knockout (KOHGW) Single-Module 80K, Three-Module 150K libraries, and Human Genome-Wide CRISPRa/CRISPRi libraries in pRSG16.

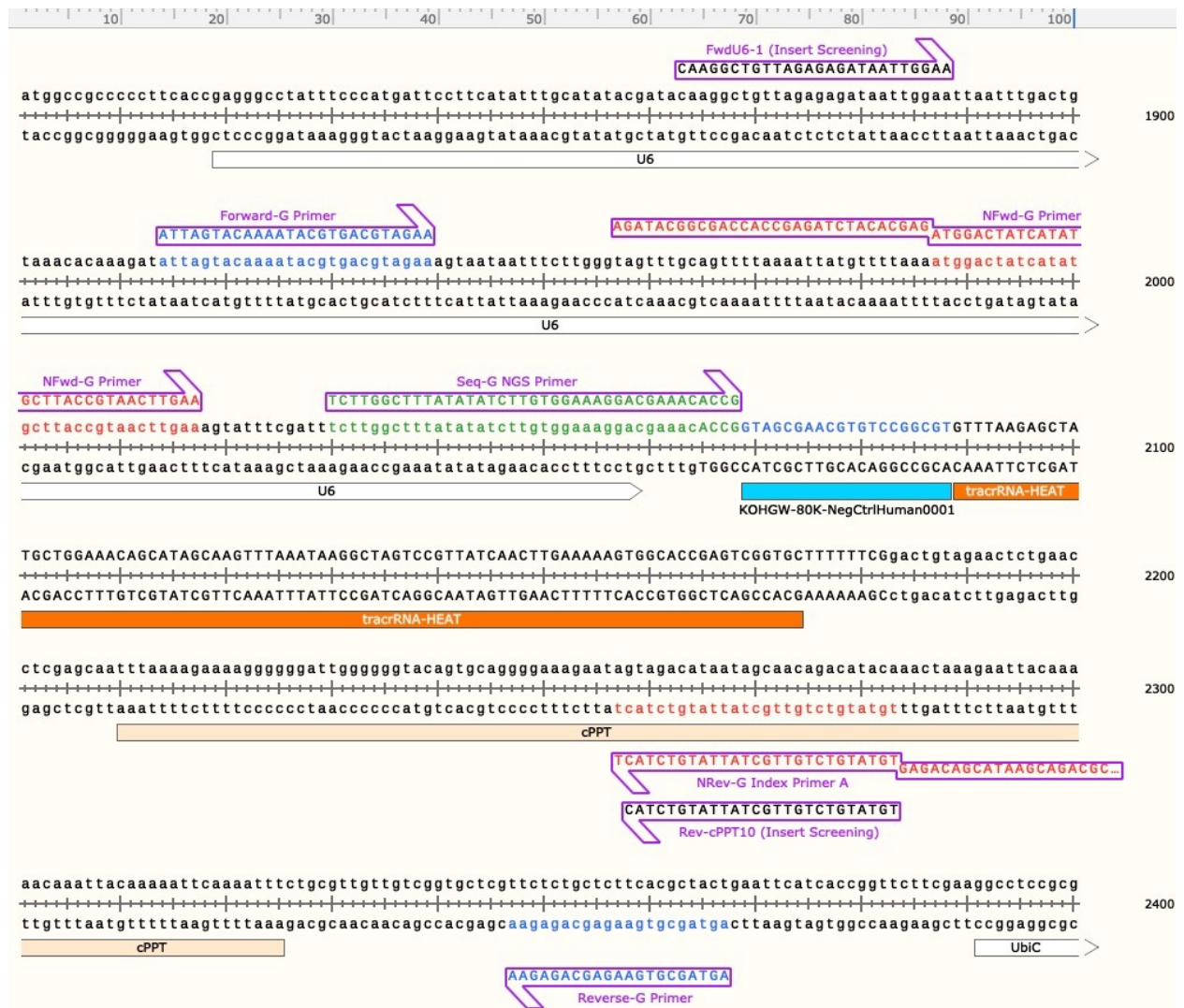
The HTS6C cassette is also present in most custom sgRNA libraries in pRSG16, pRSG17, or other pRSG16-based vectors. If you are not sure which NGS cassette your library contains, please contact us at tech@cellecta.com.

Nested PCR from Genomic DNA

The HTS6C Cassette Design allows standard two-round amplification (Nested PCR) of the sgRNA cassette from genomic DNA. In the First Round of PCR, the cassette is amplified with **Forward-G + Reverse-G** primers from genomic DNA (see diagram below). In the Second Round of PCR, the enriched cassette is amplified with **NFwd-G + NRev-G** primers, PCR products are purified, and samples are sequenced on the NextSeq 500 or HiSeq (see *sgRNA Library User Manual for details*).

Example HTS6C NGS Cassette Diagram, from KOHW-80K Library Construct (pRSG16/pRSG17)

SnapGene format: <https://www.cellecta.com/Cellecta-pRSG16-HTS6C-sgRNA-Cassette-Diagram.dna>



Second Round PCR Amplicon Diagram (pRSG16/pRSG17)

Example Second Round (Nested) PCR amplicon diagram for a 20-nt sgRNA construct in pRSG16, from Collecta's KOHW-80K sgRNA Library. The **NFwd-G** Primer contains the minimal sequence of P5 (5' end, sequence in orange) and **NRev-G** Index Primer contains the minimal sequence of P7 (3' end, sequence in orange) required for compatibility with all Illumina flow cells.

The sgRNA is sequenced using the **Seq-G** NGS Primer in Read 1.

- Size of Second-Round (Nested) PCR amplicon: **382 bp**
- Starting library concentration (see [NGS Sample Purification](#) step in User Manual): **10 nM**

SnapGene format: <https://www.collecta.com/Collecta-pRSG16-HTS6C-sgRNA-PCR-Amplicon-Standard.dna>



Primers for Nested PCR and NGS (all 5' → 3')*

Forward-G Primer	ATTAGTACAAAATACGTGACGTAGAA
Reverse-G Primer	AGTAGCGTGAAGAGCAGAGAA
NFwd-G Primer	AGATACGGCGACCACCGAGATCTACACGAGATGGACTATCATATGCTTACCGTAACTTGAA
NRev-G Index Primer	CAAGCAGAAGACGGCATAACGATGCACGACGAGACGCAGACGAATACGACAGAGTGTATGCTGTGCTATTATGTCTACT
Seq-G NGS Primer	TCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG (<i>HPLC-purified</i>)

* These primers as well as additional multiplexing primers are available in the NGS Prep Kit for sgRNA Libraries (see page 3)

Primers for sgRNA Insert Screening (all 5' → 3')

FwdU6-1	CAAGGCTGTTAGAGAGATAATTGGAA
Rev-cPPT10	TGTATGCTGTGCTATTATGCTACT
Forward-G Primer	ATTAGTACAAAATACGTGACGTAGAA

NGS Cassette in Formats other than SnapGene

The HTS6C NGS Cassette and Nested PCR Amplicon diagrams are available in other formats. Contact us at tech@collecta.com.

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

Illumina NextSeq (Single Read or Paired-End – **If multiplexing, Paired-End only*)

What to Sequence	Program (orientation)	NGS Primer	Cartridge Well	Number of Cycles
19-nt or 20-nt sgRNA	Read 1 (Forward)	Seq-G	#20	26
Index*	<i>For multiplexing up to 24 samples, we offer the NGS Prep Kits listed below.</i>			

Illumina HiSeq 2000/2500** (Single Read or Paired-End – **If multiplexing, Paired-End only*)

What to Sequence	Program (orientation)	NGS Primer	Cartridge Well	Number of Cycles
19-nt or 20-nt sgRNA	Read 1 (Forward)	Seq-G	<i>See HiSeq manual</i>	26
Index*	<i>For multiplexing up to 24 samples, we offer the NGS Prep Kits listed below.</i>			

**** Newer HiSeq models may require different parameters.**

Cellecta NGS Prep Kits (<https://www.cellecta.com/ngs-analysis-kits-for-pooled-libraries/>)

Cat.#	Description	Quantity
LNGS-120	NGS Prep Kit for sgRNA Libraries in pRSG16 (KOHGW)	Kit for 6-48 samples (48 preps of 50ug each, 12 multiplex)
LNGS-120-SP	Supplementary Primer Set for LNGS-120 (12 Additional Index Primers)	48 Reactions (12 Index Primers x 4 reactions each)

Contact Us

Phone: +1 (650) 938-3910 (option 2 for Technical Support)
Toll-Free: (877) 938-3910 (option 2 for Technical Support)

E-mail:

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

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