



Design of CRISPR sgRNA Library in pRSg-U6-HTS6C cloning vector with CRISPR Library insert, WITHOUT barcodes:

ClaI-FwdU6-1>-FwdU6-3>P5-NFG16>-U6-FSeq-gRNA>-sgRNA-tracrRNA-T6-cPPT-<P7-NRG16-<Rev-1-UbiC

ClaI U6 FwdU6-1
ATCGATGGCCGCCCTTCACCGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGG
TAGCTACCGCGGGGGAAGTGGCTCCCGATAAAGGGTACTAAGGAAGTATAAACGTATATGCTATGTTCCGACAATCTCTCTATTAACC

FwdU6-1 (cont'd)

AA> U6 P5-NFG16
AATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAG
TTAATTAAACTGACATTTGTGTTTCTATAATCATGTTTTATGCACATGCTTTTATTAAAGAACCCATCAAACGTCAAATTTTAATACAAAATTTTACCTGATAGTATACGAATGGCATTGAACTTTC

FSeq-gRNA sgRNA dCas9-binding hairpin tracrRNA-HEAT
GGCTTTATATATCTTGTGGAAAGGACGAAACACCG>
TATTTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGNNNNNNNNNNNNNNNNNNNNGTTTAAGAGCTATGCTGGAAACAGCATAGCAAGTTAAATAAGGCTAGTCCGTTATCAACTTGA
ATAAAGCTAAAGAACCGAAATATATAGAACACCTTTCCTGCTTTGTGGCNNNNNNNNNNNNNNNNNNNNCAAATTCGATACGACCTTTGTCGTATCGTTCAAATTTATTCCGATCAGGCAATAGTTGAACT

T6
AAAAGTGGCACCAGTCGGTGCTTTTTTCGGACTGTAGAACTCTGAACCTCGAGCAATTTAAAGAAAAGGGGGATTGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAAA
TTTTACCGTGGCTCAGCCACGAAAAAGCCTGACATCTTGAGACTTGGAGCTCGTTAAATTTTCTTTTCCCCCTAACCCCCATGTCACGTCCCTTTTCTTATCATCTGTATTATCGTTGTCTGTATGTTT
XhoI cPPT <TCATCTGTATTATCGTTGTCTGTATGTGA
P7-NRG16

CTAAAGAATTACAAAACAAATTACAAAAATTCAAAATTTCTGCGTTGTTGTGCGGTGCTCGTTCTCTGCTCTTCACGCTACTGAATTCATCACCGGTTCTTCGAAGGCCCTCCGCGCC
GATTTCTTAATGTTTTTGTAAATGTTTTTAAGTTTTAAAGACGCAACAACAGCCACGAGCAAGAGACGAGAAGTGCATGACTTAAGTAGTGGCCAAGAAGCTTCCGGAGGCGCGG
GACAGCATAAGCAGACGACAGCAGCAGCTAGAGCATACGGCAGAGACGAAC UbiC Promoter-->

P7-NRG16 (cont'd)

<TAAGTTTTAAAGACGCAACAACAGC
Rev-1



First Round PCR

FwdU6-1 CAAGGCTGTTAGAGAGATAATTGGAA
Rev-1 CGACAACAACGCAGAAATTTGAAT

Second Round PCR (primers contain Illumina P5 and P7 sequences required for NGS on the NextSeq 500, HiSeq, or GAIIX)

P5-NFG16 AGATACGGCGACCACCGAGATCTACACGAGATGGACTATCATATGCTTACCGTAACTTGAA
P7-NRG16* CAAGCAGAAGACGGCATACGAGATGCACGACGAGACGCAGACGAATACGACAGAGTGTATGCTGTTGCTATTATGTCTACT

NGS Primer (20 cycles)

FSeq-gRNA GGCTTTATATATCTTGTGGAAAGGACGAAACACCG (HPLC Purified)

Standard sgRNA Insert Screening PCR Primers:

FwdU6-1 CAAGGCTGTTAGAGAGATAATTGGAA
Rev-cPPT10 TGTATGCTCTGTGCTATTATGTCTAC

Standard Sanger Sequencing Primer

FwdU6-3 ATTAGTACAAAATACGTGACGTAGAA

* The purpose of the black region in the Second Round **P7-NRG16** primer is to allow for multiplexing of samples. It contains an index primer binding site as well as 6-base index region. If you require multiplexing of your samples, Cellecta offers an NGS Prep Kit for sgRNA Libraries:

<u>Catalog#</u>	<u>Description</u>	<u>Base Unit for Price/ Quantity Provided</u>
LNGS-120	NGS Prep Kit for sgRNA Libraries in pRSG16/17 (KOHGW)	Kit for 6-48 samples (48 preps of 50ug each, 12 multiplex)

For more information or to place an order, please visit the following web page:

<https://www.cellecta.com/ngs-analysis-kits-for-pooled-libraries/>