



11-20-12 R5 (9/7/16). Design of shRNA libraries in HTS6cb-13kCB18 vectors (e.g. Cat.# HGW*), WITH 18nt Clonal Barcodes:

The pRSI-HTS6cb-13kCB18 Library Cassette Design allows standard two-round amplification of the shRNA cassette from genomic DNA. In the first round of PCR, the cassette is amplified with F16 + R16 primers from genomic DNA. In the second round of PCR, the enriched cassette is amplified with P7-NF16 + P5-NR16 primers, PCR products are purified, and samples are sequenced on the NextSeq 500, HiSeq, or GAIIX.

Cassette design: 2M6-HTS6cb-13kCB18
Library Oligo size: 139nt
shRNA Design: GG214-GT-C7
HTS cassette: HTS6cb-13kCB18

Visit the Cellecta website for User Manuals and Vector information:

User Manuals: https://www.cellecta.com/resources/product-manuals-and-certificates/
Cellecta Vectors: https://www.cellecta.com/resources/vector-information/

Library Insert with shRNA Barcode (Inserts should not contain BpiI sites):

BpiI Sense (21)+GT-mm Stem4-LoopC7-Stem4 Antisense (21) EcoRI shRNA Barcode (22) BpiI
TCAGAAGACGCACCGNNNNNNNNNNNNNNNNNNNNNGTTAATATTCATAGCNNNNNNNNNNNNNNNNNNNNTTTTTGAATTCGCACCAGCAGCTACGCANNNNNNNNNNNNNNNNNNNTTCCGGTCTTCGT

Design of shRNA Library in HTS6cb-13kCB18 cloning vector with shRNA Library insert, WITH Clonal Barcodes:

ClaI-FwdU6-3>U6-shRNA-T6-F16>P7-NF16>FSeq16BC>shRNA BC22-13kCB18-cPPT-<P5-NR16<R16

F16
P7-NF16 5' -TCGGATTTCGCACCAGCAGCTA>
5' CAAGCAGAAGACGGCATAACGAGATCGCACCAGCAGCTACGCA>
FSeq16BC--> ACGAGATCGCACCAGCAGCTACGCA> 22n shRNA barcode 18n clonal barcode XhoI
U6-ACCGG-shRNA-TTTTTTGAATTCGCACCAGCAGCTACGCANNNNNNNNNNNNNNNNNNNTTTTCCGGACTGTAGAACTCTGAACCTCGAGCAA
U6-TGCC-shRNA-AAAAACTTAAGCGTGGTCTGCGATGCGTNNNNNNNNNNNNNNNNNNNAAGCCTGACATCTGAGACTTGAGACTCGTT

cPPT
TTTAAAAGAAAAGGGGGGATGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAACTAAAGAATTACAAAACAAATTACAAAAATTCAAAATTT
AAATTTTCTTTTCCCCCCTAACCCCATGTACGTCACGTCCTTTCTTATCATCTGTATTATCGTTGTCTGTATGTTGATTTCTTAATGTTTTTGTTAATGTTTTTAAGTTTTAA

EcoRI AgeI BstBI
TCTGCGTTGTTGTCGGTGTCTGTTCTCTGCTCTTCACGCTACTGAATTCATCACCGGTTCTTCGAAAGGCCTCCGCGCCGGGTTTGGCGCCTCCCGCGGGCGCCCCCTCCTCACGGCG
AGACGCAACAACAGCCACGAGCAAGAGACGAGAAGTGCATGACTTAAGTAGTGGCCAAGAAGCTTCCGGAGGCGCGGCCAAAACCGCGGAGGGCGCCCGGGGGAGGAGTGCCTCG
<AGACGCAACAACAGCCACGAGCAAGAGACAGCATAAGCAGACGCAGAGCAGCAGCAGCACATCTAGAGCCACCAGCGGCA-5' UbiC promoter-->
P5-NR16
<AAGAGACGAGAAGTGCGATGA-5'
R16



First Round PCR

F16 TCGGATTTCGCACCAGCACGCTA
R16 AGTAGCGTGAAGAGCAGAGAA

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

P7-NF16 CAAGCAGAAGACGGCATAACGAGATCGCACCAGCACGCTACGCA
P5-NR16 ACGGCGACCACCGAGATCTACACGCACGACGAGACGCAGACGAATACGACAGAGAACGAGCACCGACAACAACGCAGA

NGS Primer (44 cycles with clonal barcode, 22 cycles without)

FSeq16BC ACGAGATCGCACCAGCACGCTACGCA (HPLC Purified)

Standard shRNA Insert Screening PCR Primers

FwdU6-1 CAAGGCTGTTAGAGAGATAATTGGAA
Rev-cPPT10 TGTATGTCTGTTGCTATTATGTCTAC

Standard Sanger Sequencing Primer

FwdU6-3 ATTAGTACAAAATACGTGACGTAGAA