Cellecta, Inc.
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Design of shRNA libraries in pMSCV vectors with the HTS6M cassette.

The GG214-GT-C7 HTS6M Library Cassette Design allows standard two-round amplification of the shRNA cassette from genomic DNA. In the first step, the cassette is amplified with F2 + R2 primers from genomic DNA. In the second step, the enriched cassette is amplified with Gex1-NF2 + Gex2-NR2 primers (Standard Next-Gen Sequencing) and PCR products are sequenced on the HiSeq 2000 or 2500. Amplified PCR products (approx. 200bp) are compatible only with single read (SR) Illumina flow cells.

Cassette design: 26M6-HTS6M
Oligo size: 139n
shRNA Design: GG214-GT-C7
NGS cassette: Version 6M

Related Vectors: pMSCVURP
NGS Platforms: HiSeq 2000/2500, GAIIx
(Please contact Cellecta at tech@cellecta.com for NextSeq primers)

Visit the Cellecta website for User Manuals and Vector information:
User Manuals, PACs: https://www.cellecta.com/resources/product-manuals-and-certificates/
Cellecta Vectors: https://www.cellecta.com/resources/vector-information/

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

```
TCAGAAGACGCACCGGNNNNNNNNNNNNNNNNNNNNNNGTTAATATTCATAGCNNNNNNNNNNNNNNNNNNNNNNTTTTTTGAATTCGCACCAGCACGCTACGCANNNNNNNNNNNNNNNNNNNNNNTTCGCCGTCTTCGT
                                                                                                                                                  BpiI
```

shRNA Construct/Library in pMSCV vector:

ClaI-FwdU6-1>FwdU6-3>U6-shRNA-T6-F2>Gex1-NF2>shRNA BC22<GexSeqS-cPPT<Gex2-NR2<R2<RevUbiC1

F2
Gex1-NF2 5′-TCGGATTCGCACCAGCACGCTA
5′-TCAAGCAGAAGACGGCATACGATCGCACCAGCACGCTACGCA XhoI Stuffer

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U6-ACCGG-shRNA-TTTTTTGAATTCGCACCAGCACGCTACGCA-BC22-TTCGGACTGTAGAACTCTGAACCTCGAGCAATAAAAGAATGGTACAGTGCAGAAACAATAGTAGACATAATAGCAACAGA
U6-TGGCC-shRNA-AAAAAACTTAAGCGTGGTCGTGCGATGCGT-BC22-AAGCCTGACATCTTGAGACTTGGAGCTCGTTATTTTCTTACCATGTCACGTCTTTGTTATCATCTGTATTATCGTTGTCT
                                                  AAGCCTGACATCTTGAGACTTGGAGA-5′
                                                  GexSeqS
```

EcoRI AgeI BstBI UbiC promoter

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CATACAAACTAAAGAATCTGCGTTGTTGTCGGTGCTCGTTCTCTGCTCTTCACGCTACTGAATTCATCACCGGTTCTTCGAAGGCCTCCGCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCCCCTCCTCAC
GTATGTTTGATTTCTTAGACGCAACAACAGCCACGAGCAAGAGACGAGAAGTGCGATGACTTAAGTAGTGGCCAAGAAGCTTCCGGAGGCGCGGCCCAAAACCGCGGAGGGCGCCCGCGGGGGGAGGAGTG
          AGACGCAACAACAGCCACGAGAGCCACCAGCGGCATAGTAA-5′                                                                    AGGAGTGCCGCTCGCGACGGA
                Gex2-NR2      AAGAGACGAGAAGTGCGATGA-5′                                                                    RevUbiC1
                              R2
```

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Amplification of shRNA-specific Barcodes using two rounds of amplification (starting from 100-200ug of genomic DNA with Clontech's Titanium Taq DNA polymerase)

First Round

F2 TCGGATTCGCACCAGCACGCTA
R2 AGTAGCGTGAAGAGCAGAGAA

Second Round – amplicon size ~200bp

Gex1-NF2 TCAAGCAGAAGACGGCATACGATCGCACCAGCACGCTACGCA
Gex2-NR2 AATGATACGGCGACCACCGAGAGCACCGACAACAACGCAGA

Sequencing Primer (22 cycles)

GexSeqS AGAGGTTCAGAGTTCTACAGTCCGAA

pMSCV Standard NGS Amplification/Sequencing Primers:

Primer Name	Used for	Sequence (IDT preferred)
F2	1st Round	5′-TCGGATTCGCACCAGCACGCTA-3′
R2	1st Round	5′-AGTAGCGTGAAGAGCAGAGAA-3′
Gex1-NF2	2nd Round	5′-TCAAGCAGAAGACGGCATACGATCGCACCAGCACGCTACGCA-3′
Gex2-NR2	2nd Round	5′-AATGATACGGCGACCACCGAGAGCACCGACAACAACGCAGA-3′
GexSeqS	NGS	5′-AGAGGTTCAGAGTTCTACAGTCCGAA-3′ (HPLC Purified)

Standard Insert Screening PCR Primers:

FwdU6-1 CAAGGCTGTTAGAGAGATAATTGGAA
Rev-cPPT10 TGTATGTCTGTTGCTATTATGTCTAC

Standard Sanger Sequencing Primer

FwdU6-3 ATTAGTACAAAATACGTGACGTAGAA