



Design of shRNA libraries in HTS3 vectors.

The 26M6-HTS3 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 FwdHTS2 + RevcPPT-5 primers from genomic DNA. In the second round of PCR, the enriched cassette is amplified with Gex1MS + Gex2M primers, PCR products are mixed, and the sample is sequenced on the HiSeq 2500 (or 2000). Amplified PCR products (approx. 150bp) are compatible only with single read (SR) Illumina flow cells.

Cassette design: 26M6-HTS3, wherein NN are CA, GT, AC, TG
Library Oligo size: 138nt
shRNA Design: GG214-GT-C7
HTS cassette: Version 3

Diagram showing the HTS3 cassette sequence with various regions labeled: Sense (21)-GT, Stem4-LoopC7-Stem4, Antisense (21), FwdHTS2, Gex1MS, Bar-code (18), and RevcPPT-5.

HTS3 cassette in pRSI Cloning Vector (without clonal barcodes)

Diagram showing the HTS3 cassette in a cloning vector with restriction sites BpiI, EcoRI, and cPPT. It includes sequences for U6, GexSeqN, Gex2M, and RevcPPT-5.

shRNA Construct:
Fwd640SA>ClaI-U6-FwdU6-1>FwdU6-2>shRNA-T6-FwdHTS2>Gex1MS>NNBarCode18<GexSeqN<Gex2M<RevcPPT5<RevcPPT4<RevcPPT3<RevcPPT2<RevcPPT1

Diagram showing the shRNA construct sequence with various regions labeled: FwdHTS2, Gex1MS, BarCode (18), GexSeqN, Gex2M, and RevcPPT-5.

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

First Round PCR

FwdHTS2 (FwdHTS) TTCTCTGGCAAGCAAAGACGGCATA
RevcPPT-5 TGCCATTTGTCTCGAGGTCGAGAA

Second Round PCR (primers contain Illumina Gex1 and Gex2 sequences required for HT sequencing)

Gex1MS (FwdGex) CAAGCAGAAGACGGCATAACGAGA
Gex2M (RevGex) AATGATACGGCGACCACCGAGA

HT Sequencing Primer (20 cycles)

GexSeqN ACAGTCGGAAACCCCAACGCACGAA (HPLC Purified)



CELLECTA

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Standard Sanger Sequencing Primers

FwdU6-1 CAAGGCTGTTAGAGAGATAATTGGAA
FwdU6-2 CCTAGTACAAAATACGTGACGTAGAA

Visit the Cellecta website for User Manuals and Vector information:

User Manuals, PACs: <http://www.cellecta.com/resources/product-manuals-and-certificates/>

Cellecta Vectors: <http://www.cellecta.com/resources/vector-information/>