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LOCUS       Exported                               7020 bp ds-DNA   circular SYN 03-
NOV-2020
DEFINITION synthetic circular DNA
ACCESSION  .
VERSION    .
KEYWORDS   pZIP-mCMV-Puromycin
SOURCE     synthetic DNA construct
  ORGANISM synthetic DNA construct
REFERENCE  1 (bases 1 to 7020)
  AUTHORS  Transomic
  TITLE    Direct Submission
  JOURNAL  Exported Tuesday, Nov 3, 2020 from SnapGene 5.2.1
           https://www.snapgene.com
FEATURES   Location/Qualifiers
     source          1..7020
                   /organism="synthetic DNA construct"
                   /mol_type="other DNA"
     enhancer        27..404
                   /label=CMV enhancer
  
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                                /note="human cytomegalovirus immediate early
enhancer"
  LTR                          411..668
                                /label=5' LTR (truncated)
                                /note="truncated 5' long terminal repeat (LTR) from
HIV-1"
  misc_feature                 715..840
                                /label=HIV-1 Psi
                                /note="packaging signal of human immunodeficiency
virus
                                type 1"
  misc_feature                 1337..1570
                                /label=RRE
                                /note="The Rev response element (RRE) of HIV-1
allows for
                                Rev-dependent mRNA export from the nucleus to the
                                cytoplasm."
  misc_feature                 2062..2177
                                /label=cPPT/CTS
                                /note="central polypurine tract and central
termination
  promoter                     sequence of HIV-1 (lacking the first T)"
                                2219..2755
                                /label=mCMV promoter
                                /note="mouse cytomegalovirus (CMV) immediate early
                                promoter"
  CDS                          2764..3366
                                /codon_start=1
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                                /product="puromycin N-acetyltransferase"
                                /label=PuroR
                                /note="confers resistance to puromycin"

/translation="MATEYKPTVRLATRDDVPRAVRTLAAAFADYPATRHTVDPDRHIE
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  misc_feature                 3474..3511
                                /label=Illumina actual seq primer
  gap                          3512..3533
                                /estimated_length=22
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                                /estimated_length=22
  misc_feature                 3594..3615
                                /label=For 5' for Pool qPCR
                                /note="Twelve replicate reactions containing 825 ng
gDNA
                                were amplified and each carried out to a different
cycle

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was placed on ice immediately after the designated number of cycles completed to arrest the reaction. 10 µl of product from each reaction was analyzed using agarose gel electrophoresis. An aliquot of each product was serially diluted 25 000-, 100 000- and 400 000-fold in water. An aliquot from each dilution of each PCR replicate served as template for SYBR qPCR reactions that were prepared using Absolute Blue qPCR SYBR Green master mix (Thermo Scientific, Epsom, UK) and primers that amplify common sequence of the shRNA barcode PCR products (For-5?caaggggctacttttaggagcaa, Rev-5?aatttataaccattttaattcagctttg), generating a product of 127 bp."

misc_feature 3632..3705
 /label=Ultramir3'

misc_feature 3725..4313
 /label=WPRE
 /note="woodchuck hepatitis virus posttranscriptional regulatory element"

LTR 4521..4754
 /label=3' LTR (Delta-U3)
 /note="self-inactivating 3' long terminal repeat (LTR) from HIV-1"

promoter 4850..5179
 /label=SV40 promoter
 /note="SV40 enhancer and early promoter"

rep_origin 5030..5165
 /label=SV40 ori
 /note="SV40 origin of replication"

rep_origin complement(5268..5853)
 /direction=LEFT
 /label=ori
 /note="high-copy-number ColE1/pMB1/pBR322/pUC origin of replication"

CDS complement(6024..6884)
 /codon_start=1
 /gene="bla"
 /product="beta-lactamase"
 /label=AmpR
 /note="confers resistance to ampicillin, carbenicillin, and related antibiotics"

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promoter        complement(6885..6989)  
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ORIGIN

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