



LOCUS Exported 8782 bp DNA circular SYN 21-DEC-2021
 DEFINITION synthetic circular DNA
 ACCESSION .
 VERSION .
 KEYWORDS pZIP-hEF1a-mCherry-Blasticidin
 SOURCE synthetic DNA construct
 ORGANISM recombinant plasmid
 REFERENCE 1 (bases 1 to 8782)
 AUTHORS Transomic
 TITLE Direct Submission
 JOURNAL Exported Dec 21, 2021 from SnapGene 6.0.0
<https://www.snapgene.com>
 FEATURES Location/Qualifiers
 source 1..8782
 /mol_type="other DNA"
 /organism="recombinant plasmid"
 enhancer 27..404
 /label=CMV enhancer
 /note="human cytomegalovirus immediate early enhancer"

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                   /note="truncated 5' long terminal repeat (LTR) from
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                   /label=HIV-1 Psi
                   /note="packaging signal of human immunodeficiency
virus
  misc_feature      type 1"
                   1337..1570
                   /label=RRE
                   /note="The Rev response element (RRE) of HIV-1
allows for
  misc_feature      Rev-dependent mRNA export from the nucleus to the
                   2062..2178
                   /label=cPPT/CTS
                   /note="central polypurine tract and central
termination
  misc_feature      sequence of HIV-1 (lacking the first T)"
                   2175..2212
                   /label=cts
  misc_feature      2213..2214
                   /label=MCS
  misc_feature      2220..3402
                   /label=pEF1 alpha
  CDS               3420..4130
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protein
                   (Shaner et al., 2004)"
                   /label=mCherry
                   /note="mammalian codon-optimized"

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                   /label=BSD
                   /note="confers resistance to blasticidin"

/translation="MAKPLSQEESTLIERATATINSIPISEDYSVASAALSSDGRIFTG
VNVYHFTGGPCAELVVLGTAAAAAAGNLTCIVAIGNENRGILSPCGRCRQVLLDLHPGI

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 /label=shRNA
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 /label=3'-UltramiR
 misc_feature 5356..5377
 /label=For 5' for Pool qPCR
 /note="Twelve replicate reactions containing 825 ng
 gDNA
 were amplified and each carried out to a different
 cycle
 number from 15-27. Each replicate reaction vessel
 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?aatttataaccatttttaattcagctttg), generating a product
 of 127
 bp."
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 /label=UltramiR3'
 misc_feature 5487..6075
 /label=WPRE
 /note="woodchuck hepatitis virus posttranscriptional
 regulatory element"
 CDS complement(5958..5969)
 /codon_start=1
 /product="Factor Xa recognition and cleavage site"
 /label=Factor Xa site
 /translation="IEGR"
 LTR 6283..6516

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(LTR) from
        /label=3' LTR (Delta-U3)
        /note="self-inactivating 3' long terminal repeat
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        /label=SV40 promoter
        /note="SV40 enhancer and early promoter"
rep_origin 6792..6927
        /label=SV40 ori
        /note="SV40 origin of replication"
rep_origin complement(7030..7615)
        /direction=LEFT
        /label=ori
        /note="high-copy-number ColE1/pMB1/pBR322/pUC origin
of
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carbenicillin, and
        related antibiotics"

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