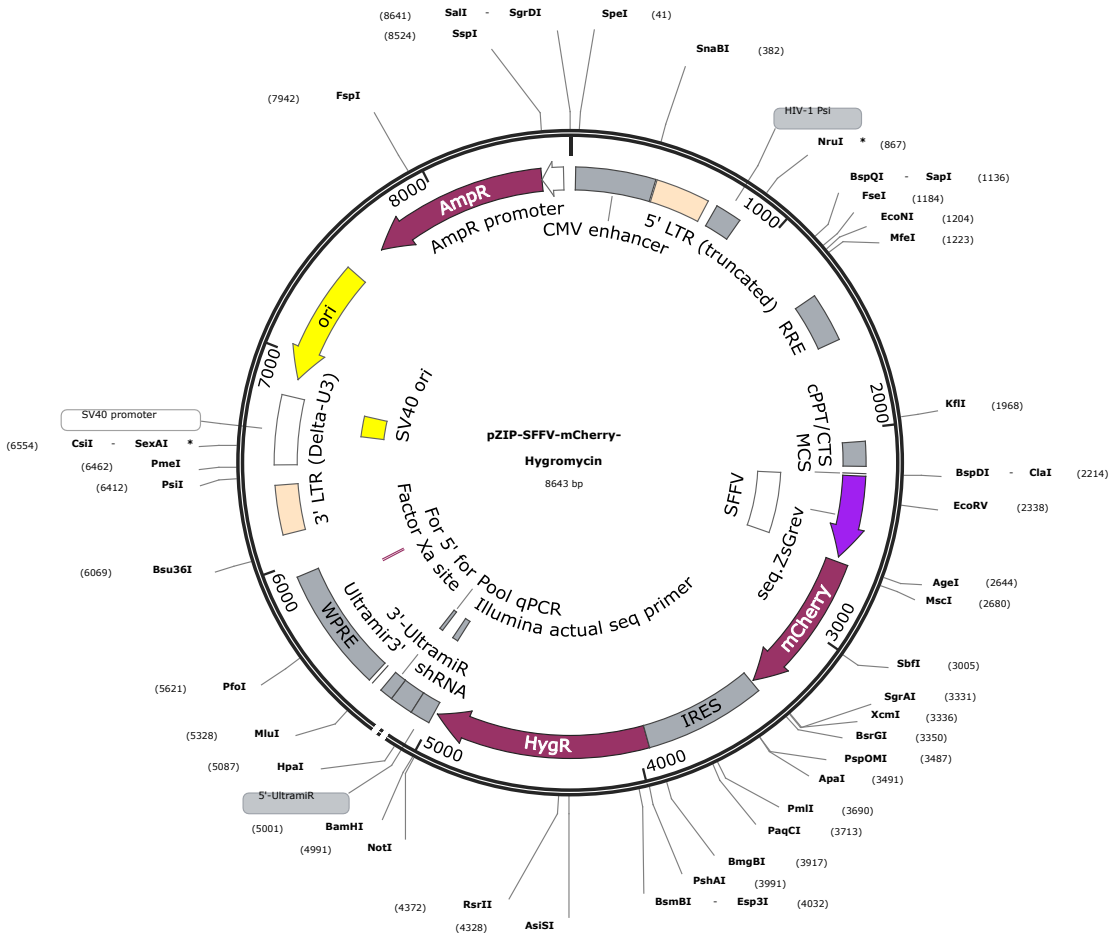


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2021
DEFINITION  synthetic circular DNA
ACCESSION   .
VERSION     .
KEYWORDS    pZIP-SFFV-mCherry-Hygromycin
SOURCE      synthetic DNA construct
  ORGANISM  recombinant plasmid
REFERENCE   1  (bases 1 to 8643)
  AUTHORS   Transomic
  TITLE     Direct Submission
  JOURNAL   Exported Dec 21, 2021 from SnapGene 6.0.0
            https://www.snapgene.com
FEATURES             Location/Qualifiers
     source          1..8643
                     /mol_type="other DNA"
                     /organism="recombinant plasmid"
     enhancer        27..404
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                     /note="human cytomegalovirus immediate early
enhancer"
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                     /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..2178
                     /label=cPPT/CTS
                     /note="central polypurine tract and central
termination
                     sequence of HIV-1 (lacking the first T)"
     misc_feature    2213..2214
                     /label=MCS
     primer_bind     2225..2625
                     /label=seq.ZsGrev
     promoter        2225..2625
                     /gene="SFFV"
                     /label=SFFV
     primer_bind     2488..2625
                     /label=seq.SFFVfw
     CDS             2650..3360
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                     /product="monomeric derivative of DsRed fluorescent
protein
                     (Shaner et al., 2004)"
                     /label=mCherry
                     /note="mammalian codon-optimized"

/translation="MVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEG

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EDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALK

GEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERA
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coli"
                     /label=HygR
                     /note="confers resistance to hygromycin"
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LQDLPETELPAVLQPVAEAMDAIAAADLSQTSGFGPFGPQGIGQYTTWRDFICAIADPH
VYHWQTVMDDTVSASVAQALDELMLWAEDCPEVRHLVHADFGSNNVLTDNGRITAVIDW
SEAMFGDSQYEVANIFFWRPWLACMEQQTRYFERRHPELAGSPRLRAYMLRIGLDQLYQ
SLVDGNFDDAAWAQGRCDAIVRSGAGTVGRTQIARRSAAVWTDGCVEVLADSGNRRPST
                     RPRAKE"
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                     /label=5'-UltramiR
     misc_feature    5097..5134
                     /label=Illumina actual seq primer
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                     /label=shRNA
     gap             5135..5156
                     /estimated_length=22
     gap             5176..5197
                     /estimated_length=22
     misc_feature    5214..5276
                     /label=3'-UltramiR
     misc_feature    5217..5238
                     /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?caaggggctactttaggagcaa, Rev-
                     5?aatttataccatttaattcagctttg), generating a product
of 127
                     bp."
```

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     misc_feature    5348..5936
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                     /label=Factor Xa site
                     /translation="IEGR"
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                     /note="self-inactivating 3' long terminal repeat
(LTR) from
                     HIV-1"
     promoter        6473..6802
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                     /note="SV40 enhancer and early promoter"
     rep_origin      6653..6788
                     /label=SV40 ori
                     /note="SV40 origin of replication"
     rep_origin      complement(6891..7476)
                     /direction=LEFT
                     /label=ori
                     /note="high-copy-number ColE1/pMB1/pBR322/pUC origin
of
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     CDS             complement(7647..8507)
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                     /label=AmpR
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carbenicillin, and
                     related antibiotics"

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EPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPLLRSA

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                     LIKHW"
     promoter        complement(8508..8612)
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                     /label=AmpR promoter
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Stratech

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aacttacggt
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taatgacgta
      181 tgttcccata gtaacgccaa tagggacttt ccattgacgt caatgggtgg
agtatttacg
      241 gtaaactgcc cacttggcag tacatcaagt gtatcatatg ccaagtacgc
cccctattga
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gctaactagg
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tgtggaaaat
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ggagctctct
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Stratech

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cccgacaggc
     1981 ccgaaggaat agaagaagaa ggtggagaga gagacagaga cagatccatt
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Stratech

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Stratech

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