



LOCUS Exported 6806 bp ds-DNA circular SYN 06-
 NOV-2020
 DEFINITION synthetic circular DNA.
 ACCESSION .
 VERSION .
 KEYWORDS pINT-gRNA-EFS-Puromycin
 SOURCE synthetic DNA construct
 ORGANISM synthetic DNA construct
 REFERENCE 1 (bases 1 to 6806)
 AUTHORS Transomic
 TITLE Direct Submission
 JOURNAL Exported Friday, Nov 6, 2020 from SnapGene 5.2.1
<https://www.snapgene.com>
 FEATURES Location/Qualifiers
 source 1..6806
 /organism="synthetic DNA construct"
 /mol_type="other DNA"
 misc_feature 23..667
 /label=CMV-LTR

```

misc_feature      720..745
                  /label=psi
misc_feature      746..840
                  /label=HIV-1 Psi
                  /note="packaging signal of human immunodeficiency
virustype
                  1"
misc_feature      1333..1566
                  /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      2093..2210
                  /label=cPPT/CTS
                  /note="central polypurine tract and central
                  terminationsequence of HIV-1"
promoter          2261..2501
                  /label=U6 Promoter
                  /note="RNA polymerase III promoter for human U6
snRNA"
gap              2511..2530
                  /estimated_length=20
misc_RNA          2531..2606
                  /label=gRNA scaffold
                  /note="guide RNA scaffold for the CRISPR/Cas9
system"
misc_feature      2648..2880
                  /label=EFS promoter
CDS              2899..3498
                  /codon_start=1
                  /gene="pac from Streptomyces alboniger"
                  /product="puromycin N-acetyltransferase"
                  /label=PuroR
                  /note="confers resistance to puromycin"

/translation="MTEYKPTVRLATRDDVPRAVRTLAAAFADYPATRHTVDPDRHIER
VTELQELFLTRVGLDIGKVVVADDGAAVAVWTTTPESVEAGAVFAEIGPRMAELSGSRLA
AQQQMEGLLAPHRPKEPAWFLATVGVSPDHQKGLGSAVVLPGVAAAERAGVPAFLETS
APRNLPFYERLGFTVTADVEVPEGPRTWCMTRKPGA"
misc_feature      3500..3505
                  /label=MCS
misc_feature      3512..4103
                  /label=WPRE
misc_feature      4308..4543
                  /label=3'LTR
promoter          4637..4966
                  /label=SV40
                  /note="SV40 enhancer and early promoter"
rep_origin        complement(5055..5640)
                  /direction=LEFT
                  /label=ori

```

```

of                                     /note="high-copy-number ColE1/pMB1/pBR322/pUC origin
                                     replication"
CDS                                     complement (5811..6671)
                                     /codon_start=1
                                     /gene="bla"
                                     /product="beta-lactamase"
                                     /label=AmpR
                                     /note="confers resistance to ampicillin,
carbenicillin, and                    related antibiotics"

/translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGYI
ELDLSNGKILESFRPEERFPMMSSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVEYS
PVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAF LHNMGDHSVTRLDRW
EPELNEAIPNDERDITMPVAMATTLRKLTLGELLTLASRQQLIDWMEADKVAGPLLRSA
LPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGAS
                                     LIKHW"
promoter                               complement (6672..6776)
                                     /gene="bla"
                                     /label=AmpR promoter

ORIGIN
      1 gacggatcgg gagatcctcg cgcggtgaca ttgattattg actagttatt
aatagtaatc
      61 aattacgggg tcattagttc atagcccata tatggagttc cgcggtacat
aacttacggt
     121 aaatggcccc cctggctgac cgcccaacga cccccgcca ttgacgtcaa
taatgacgta
     181 tgttcccata gtaacgcaa tagggacttt ccattgacgt caatgggtgg
agtatttacg
     241 gtaaactgcc cacttggcag tacatcaagt gtatcatatg ccaagtacgc
ccctattga
     301 cgtcaatgac ggtaaatggc cgcctggca ttatgccag tacatgacct
tatgggactt
     361 tcctacttgg cagtacatct acgtattagt catcgctatt accagctagc
gagcggtggc
     421 ctggggcgga ctggggagtg gcgagccctc agatcctgca tataagcagc
tgctttttgc
     481 ctgtactggg tctctctggt tagaccagat ctgagcctgg gagctctctg
gctaactagg
     541 gaaccactg cttaagcctc aataaagctt gccttgagtg cttcaagtag
tgtgtgcccc
     601 tctgttgtgt gactctggta actagagatc cctcagacct ttttagtcag
tgtggaaaat
     661 ctctagcagt ggcgccccgaa cagggacttg aaagcgaaag ggaaaccaga
ggagctctct
     721 cgacgcagga ctcggcttgc tgaagcgcgc acggcaagag gcgaggggcg
gcgactggtg
     781 agtacgcaa aaattttgac tagcggaggc tagaaggaga gagatgggtg
cgagagcgtc

```

841 agtattaagc gggggagaat tagatcgcg tgggaaaaaa ttcggttaag
gccaggggga
901 aagaaaaaat ataaattaa acatatagta tgggcaagca gggagctaga
acgattcgca
961 gttaatcctg gcctgttaga aacatcagaa ggctgtagac aaatactggg
acagctacaa
1021 ccatcccttc agacaggatc agaagaactt agatcattat ataatacagt
agcaaccctc
1081 tattgtgtgc atcaaaggat agagataaaa gacaccaagg aagctttaga
caagatagag
1141 gaagagcaaa acaaaagtaa gaccaccgca cagcaagcgg ccgctgatct
tcagacctgg
1201 aggaggagat atgagggaca attggagaag tgaattatat aaatataaag
tagtaaaaat
1261 tgaaccatta ggagtagcac ccaccaaggc aaagagaaga gtggtgcaga
gagaaaaaag
1321 agcagtggga ataggagctt tgttccttgg gttcttggga gcagcaggaa
gcactatggg
1381 cgcagcgtca atgacgctga cggtagcagg cagacaatta ttgtctggta
tagtgcagca
1441 gcagaacaat ttgctgaggg ctattgaggc gcaacagcat ctgttgcaac
tcacagtctg
1501 gggcatcaag cagctccagg caagaatcct ggctgtggaa agatacctaa
aggatcaaca
1561 gtcctcgggg atttgggggt gctctggaaa actcatttgc accactgctg
tgccttggaa
1621 tgctagttagt agtaataaat ctctggaaca gatttggaat cacacgacct
ggatggagtg
1681 ggacagagaa attaacaatt acacaagctt aatacactcc ttaattgaag
aatcgcaaaa
1741 ccagcaagaa aagaatgaac aagaattatt ggaattagat aaatgggcaa
gtttgtggaa
1801 ttggtttaac ataacaaatt ggctgtggta tataaaatta ttcataatga
tagtaggagg
1861 cttggttagt ttaagaatag tttttgctgt actttctata gtgaatagag
ttaggcaggg
1921 atattcacca ttatcgtttc agaccacct cccaaccccg aggggacccg
acaggcccga
1981 aggaatagaa gaagaagggt gagagagaga cagagacaga tccattcgat
tagtgaacgg
2041 atcggcactg cgtgcgcaa ttctgcagac aaatggcagt attcatccac
aattttaaaa
2101 gaaaaggggg gattgggggg tacagtgcag gggaaagaat agtagacata
atagcaacag
2161 acatacaaac taaagaatta caaaaacaaa ttacaaaaat tcaaaatfff
cgggtttatt
2221 acagggacag cagagatcca gtttggttaa ttaaggtacc gagggcctat
ttccatgat
2281 tccttcatat ttgcatatac gatacaaggc tgtagagag ataattagaa
ttaatttgac
2341 tgtaaacaca aagatattag taaaaatac gtgacgtaga aagtaataat
ttcttgggta
2401 gtttgcagtt ttaaaattat gttttaaaaat ggactatcat atgcttaccg
taacttgaaa

2461 gtatttcgat ttcttggctt tatatatcctt gtggaaagga cgaaacaccg
nnnnnnnnnn
2521 nnnnnnnnnn gttttagagc tagaaatagc aagttaaaat aaggctagtc
cgttatcaac
2581 ttgaaaaagt ggcaccgagt cggtgctttt ttgaattcgc tagctaggtc
ttgaaaggag
2641 tgggaattgg ctccggtgcc cgtcagtggg cagagcgcac atcgcccaca
gtccccgaga
2701 agttgggggg aggggtcggc aattgatccg gtgcctagag aagggtggcg
ggggtaaact
2761 gggaaagtga tgtcgtgtac tggctccgcc tttttcccga ggggtggggga
gaaccgtata
2821 taagtgcagt agtcgccgtg aacgttcttt ttcgcaacgg gtttgccgcc
agaacacagg
2881 accggttcta gagccacat gaccgagtac aagcccacgg tgcgcctcgc
caccgcgac
2941 gacgtcccca gggccgtacg caccctcgcc gccgcgttcg ccgactacc
cgccacgcgc
3001 cacaccgtcg atccggaccg ccacatcgag cgggtcaccg agctgcaaga
actcttctc
3061 acgcgcgtcg ggctcgacat cggcaagggtg tgggtcgcgg acgacggcg
cgccgtggcg
3121 gtctggacca cgccggagag cgtcgaagcg ggggcggtgt tcgccgagat
cggcccgcgc
3181 atggccgagt tgagcggttc ccggctggcc gcgcagcaac agatggaag
cctcctggcg
3241 ccgcaccggc ccaaggagcc cgcgtggttc ctggccaccg tcggagtctc
gcccgaccac
3301 cagggcaagg gtctgggcag cgccgtcgtg ctccccggag tggaggcggc
cgagcgcgc
3361 ggggtgcccg ctttctgga gacctccgcg ccccgaacc tccccttcta
cgagcggctc
3421 ggcttcaccg tcaccgccga cgtcgaggtg cccgaaggac cgcgcacctg
gtgcatgacc
3481 cgcaagcccg gtgcctgaac gcgtctggaa caatcaacct ctggattaca
aaatttgtga
3541 aagattgact ggtattctta actatgttgc tccttttacg ctatgtggat
acgtgcttt
3601 aatgcctttg tatcatgcta ttgcttcccg tatggctttc attttctct
ccttgataa
3661 atcctggttg ctgtctcttt atgaggagtt gtggcccgtt gtcaggcaac
gtggcgtggt
3721 gtgcaactgtg tttgctgacg caacccccac tggttggggc attgccacca
cctgtcagct
3781 cttttccggg actttcgctt tccccctccc tattgccacg gcggaactca
tcgccgctg
3841 cottgcccgc tgctggacag gggctcggct gttgggcaact gacaattccg
tggtgttgtc
3901 ggggaagctg acgtccttcc catggctgct cgcctgtggt gccacctgga
ttctgcgcgg
3961 gacgtccttc tgctacgtcc cttcggccct caatccagcg gaccttctt
cccgcggcct
4021 gctgccggct ctgcggcctc ttccgcgtct tcgccttcgc cctcagacga
gtcggatctc

4081 cctttgggcc gcctccccgc ctggaattaa ttctgcagtc gagacctaga
aaaacatgga
4141 gcaatcacia gtagcaatac agcagctacc aatgctgatt gtgcctggct
agaagcacia
4201 gaggaggagg aggtggggtt ttccagtcac acctcaggta cctttaagac
caatgactta
4261 caaggcagct gtagatctta gccacttttt aaaagaaaag aggggactgg
aagggcta
4321 tcaactccaa cgaagacaag atctgctttt tgcttgact gggctctctt
ggttagacca
4381 gatctgagcc tgggagctct ctggctaact agggaacca ctgcttaagc
ctcaataaag
4441 cttgccttga gtgcttcaag tagtgtgtgc ccgtctgttg tgtgactctg
gtaactagag
4501 atccctcaga cccttttagt cagtgtggaa aatctctagc agtagtagtt
catgtcatct
4561 tattattcag tattataaac ttgcaaagaa atgaatatca gagagtgaga
ggccttgaca
4621 ttgtttaaac tggaatgtgt gtcagttagg gtgtggaaag tccccaggct
ccccagcagg
4681 cagaagtatg caaagcatgc atctcaatta gtcagcaacc aggtgtggaa
agtccccagg
4741 ctccccagca ggcagaagta tgcaaagcat gcatctcaat tagtcagcaa
ccatagtccc
4801 gccccctaact cgcgccatcc cgccccaac tccgcccagt tccgccatt
ctccgcccc
4861 tggctgacta atttttttta tttatgcaga ggccgaggcc gcctctgcct
ctgagctatt
4921 ccagaagtag tgaggaggct tttttggagg cctaggcttt tgcaaaaagc
tcccgggagc
4981 ttgtatatcc attacatgtg agcaaaaggc cagcaaaagg ccaggaaccg
taaaaaggcc
5041 gcgttgctgg cgtttttcca taggctccgc ccccctgacg agcatcacia
aaatcgacgc
5101 tcaagtcaga ggtggcgaaa cccgacagga ctataaagat accaggcgtt
tccccctgga
5161 agctccctcg tgcgctctcc tgttccgacc ctgccgctta ccggatacct
gtccgccttt
5221 ctcccttcgg gaagcgtggc gctttctcat agctcacgct gtaggtatct
cagttcgggtg
5281 taggtcgttc gtcceaagct gggctgtgtg cacgaacccc ccgttcagcc
cgaccgctgc
5341 gccttatccg gtaactatcg tcttgagtcc aaccggtaa gacacgactt
atcgccactg
5401 gcagcagcca ctggtaacag gattagcaga gcgaggtatg taggcggtgc
tacagagttc
5461 ttgaagtggg ggcttaacta cggctacact agaagaacag tatttggtat
ctgcgctctg
5521 ctgaagccag ttaccttcgg aaaaagagtt ggtagctctt gatccggcaa
acaaccacc
5581 gctggtagcg gtttttttgt ttgcaagcag cagattacgc gcagaaaaaa
aggatctcaa
5641 gaagatcctt tgatcttttc tacgggggtct gacgctcagt ggaacgaaaa
ctcacgttaa

```
5701 gggatthtttg tcatgagatt atcaaaaagg atcttcacct agatcctttt
aaattaaaaaa
5761 tgaagthttta aatcaatcta aagtatatat gagtaaactt ggtctgacag
ttaccaatgc
5821 ttaatcagtg aggcacctat ctcagcgate tgtctatttc gttcatccat
agttgcctga
5881 ctccccgtcg tgtagataac tacgatacgg gagggcttac catctggccc
cagtgctgca
5941 atgataccgc gagaccacg ctcaccggct ccagatttat cagcaataaa
ccagccagcc
6001 ggaagggccg agcgcagaag tggctctgca actttatccg cctccatcca
gtctattaat
6061 tgthgcccgg aagctagagt aagtagttcg ccagthtaata gththgcccga
cgtthgthgccc
6121 attgctacag gcatcgtggg gtcacgctcg tcgththggta tggcttcatt
cagctcccgg
6181 tcccaacgat caagggcagat tacatgatcc cccatgthtgt gcaaaaaagc
gththagctcc
6241 thcggctctc cgatecgttht cagaagthaa thggcccgcag ththtatcact
catgththtath
6301 gcagcactgc ataattctct tactgtcatg ccatccgthaa gatgctththc
ththgactggg
6361 gagthactcaa ccaagthcatt ctgagaathag thththgcccgc gaccgagtht
ctctthgcccg
6421 gcgthcaatac gggataatac cgcgccacat agcagaactt thaaaagthgt
catcaththga
6481 aaacgthtctt cggggcgaaa actctcaagg atctthaccgc thththgagatc
cagththcgate
6541 thaaaccactc gthgcacccaa ctgatectthca gatectththta cththcaccag
cgtththctggg
6601 thgagcaaaaa caggaaggca aathgcccgc aaaaagggaa thaaagggcgc
acggaathgt
6661 thgaathactca tactctthct thththcaathat thththgagca thththcaggg
thththgtctc
6721 atgagcggat acathththga atgthththtag aaaaathaaac aaththgggg
thcgcgcaca
6781 ththccccgaa aagthgccacc thgacgt
//
```