LOCUS pGGdestISceIT2EG 7408 bp ds-DNA circular SYN 11-AUG-2021

SOURCE synthetic DNA construct

 ORGANISM synthetic DNA construct

REFERENCE 1 (bases 1 to 7408)

 AUTHORS .

 TITLE Direct Submission

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 NTI(R) format

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COMMENT VNTDATE|956188800|

COMMENT VNTDBDATE|957571200|

COMMENT VNTNAME|pGGdestISceIT2EG-4sgRNA|

FEATURES Location/Qualifiers

 promoter complement(733..837)

 /gene="bla"

 /label=AmpR promoter

 misc\_feature 1478..1627

 /label=Right end of Tol2

 misc\_feature 1651..1668

 /label=I-SceI forward

 primer\_bind 1738..1754

 /label=M13 rev

 /note="common sequencing primer, one of multiple similar

 variants"

 protein\_bind 1773..1897

 /gene="mutant version of attR"

 /label=attR4

 /bound\_moiety="LR Clonase(TM)"

 /note="recombination site for the Gateway(R) LR reaction"

 CDS complement(1938..2243)

 /codon\_start=1

 /gene="ccdB"

 /product="CcdB, a bacterial toxin that poisons DNA gyrase"

 /label=ccdB

 /note="Plasmids containing the ccdB gene cannot be

 propagated in standard E. coli strains."

 CDS complement(2585..3244)

 /codon\_start=1

 /gene="cat"

 /product="chloramphenicol acetyltransferase"

 /label=CmR

 /note="confers resistance to chloramphenicol"

 promoter complement(3298..3328)

 /label=lac UV5 promoter

 /note="E. coli lac promoter with an ""up"" mutation"

 protein\_bind complement(3354..3477)

 /gene="mutant version of attR"

 /label=attR3

 /bound\_moiety="LR Clonase(TM)"

 /note="recombination site for the Gateway(R) LR reaction"

 primer\_bind complement(3485..3501)

 /label=M13 fwd

 /note="common sequencing primer, one of multiple similar

 variants"

 misc\_feature 3520..3523

 /label=4sgRNA feature sequence

 promoter 3557..4034

 /label=EF1-alpha promoter

 intron 4073..4645

 /label=beta-globin intron

 /note="intron from rabbit beta-globin gene"

 primer complement(4696..4718)

 /label=pdestdGFP2-

 /note="sequence: GACCGGTGGATCCGTCGAGGAAT"

 misc\_feature 4719..4728

 /label=Kozak sequence

 CDS 4725..5444

 /codon\_start=1

 /product="enhanced GFP"

 /label=EGFP

 /note="mammalian codon-optimized"

 primer 5480..5518

 /label=pdestdGFP2+

 /note="sequence: ATCGATGATGATCCAGACATGATAAGATACATTGATGAG"

 polyA\_signal 5489..5623

 /label=SV40 poly(A) signal

 /note="SV40 polyadenylation signal"

 misc\_feature complement(5743..5760)

 /label=I-SceI reverse

 misc\_feature 5794..5993

 /label=Left end of Tol2

 primer\_bind complement(6027..6043)

 /label=SK primer

 /note="common sequencing primer, one of multiple similar

 variants"

 promoter complement(6080..6098)

 /label=T3 promoter

 /note="promoter for bacteriophage T3 RNA polymerase"

 primer\_bind complement(6119..6135)

 /label=M13 rev

 /note="common sequencing primer, one of multiple similar

 variants"

 protein\_bind 6143..6159

 /label=lac operator

 /bound\_moiety="lac repressor encoded by lacI"

 /note="The lac repressor binds to the lac operator to

 inhibit transcription in E. coli. This inhibition can be

 relieved by adding lactose or

 isopropyl-beta-D-thiogalactopyranoside (IPTG)."

 promoter complement(6167..6197)

 /label=lac promoter

 /note="promoter for the E. coli lac operon"

 protein\_bind 6212..6233

 /label=CAP binding site

 /bound\_moiety="E. coli catabolite activator protein"

 /note="CAP binding activates transcription in the presence

 of cAMP."

 rep\_origin complement(6521..7109)

 /direction=LEFT

 /label=ori

 /note="high-copy-number ColE1/pMB1/pBR322/pUC origin of

 replication"

 CDS complement(join(7280..7408,1..732))

 /codon\_start=1

 /gene="bla"

 /product="beta-lactamase"

 /label=AmpR

 /note="confers resistance to ampicillin, carbenicillin, and

 related antibiotics"

ORIGIN

 1 aatgataccg cgggacccac gctcaccggc tccagattta tcagcaataa accagccagc

 61 cggaagggcc gagcgcagaa gtggtcctgc aactttatcc gcctccatcc agtctattaa

 121 ttgttgccgg gaagctagag taagtagttc gccagttaat agtttgcgca acgttgttgc

 181 cattgctaca ggcatcgtgg tgtcacgctc gtcgtttggt atggcttcat tcagctccgg

 241 ttcccaacga tcaaggcgag ttacatgatc ccccatgttg tgcaaaaaag cggttagctc

 301 cttcggtcct ccgatcgttg tcagaagtaa gttggccgca gtgttatcac tcatggttat

 361 ggcagcactg cataattctc ttactgtcat gccatccgta agatgctttt ctgtgactgg

 421 tgagtactca accaagtcat tctgagaata gtgtatgcgg cgaccgagtt gctcttgccc

 481 ggcgtcaata cgggataata ccgcgccaca tagcagaact ttaaaagtgc tcatcattgg

 541 aaaacgttct tcggggcgaa aactctcaag gatcttaccg ctgttgagat ccagttcgat

 601 gtaacccact cgtgcaccca actgatcttc agcatctttt actttcacca gcgtttctgg

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 1261 gacggctact acatggtgcc attccttcct ctttatagga atggagacta cctcctgtcc

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 1801 aaacgtaaaa tgatataaat atcaatatat taaattagat tttgcataaa aaacagacta

 1861 cataatactg taaaacacaa catatccagt cactatggtc gacctgcaga ctggctgtgt

 1921 ataagggagc ctgacattta tattccccag aacatcaggt taatggcgtt tttgatgtca

 1981 ttttcgcggt ggctgagatc agccacttct tccccgataa cggagaccgg cacactggcc

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 3421 atatattgat atttatatca ttttacgttt ctcgttcaac tttattatac atagttgata

 3481 attcactggc cgtcgtttta cggtacgcta gcggtctctc ctgggtacca ttaatagtgt

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