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

661 gtgagcaaaa acaggaaggc aaaatgccgc aaaaaaggga ataagggcga cacggaaatg

721 ttgaatactc atactcttcc tttttcaata ttattgaagc atttatcagg gttattgtct

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901 aatttttgtt aaatcagctc attttttaac caataggccg aaatcggcaa aatcccttat

961 aaatcaaaag aatagaccga gatagggttg agtgttgttc cagtttggaa caagagtcca

1021 ctattaaaga acgtggactc caacgtcaaa gggcgaaaaa ccgtctatca gggcgatggc

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1861 cataatactg taaaacacaa catatccagt cactatggtc gacctgcaga ctggctgtgt

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1981 ttttcgcggt ggctgagatc agccacttct tccccgataa cggagaccgg cacactggcc

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3481 attcactggc cgtcgtttta cggtacgcta gcggtctctc ctgggtacca ttaatagtgt

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