

Appendix for

Engineering editopes through programmable RNA editing toward tumor neoantigen generation

Pecori et al.

Table of Contents:

1. Appendix Table S1	pages 2-3
2. Appendix Table S2	page 3
3. Appendix Figure S1	page 4
4. Appendix Figure S2	pages 5-6
5. Appendix Figure S3	page 7
6. Appendix Methods	page 8
7. Plasmid maps (provided as text)	pages 9-93

a. Appendix Tables

#	Name	Sequence	Purpose	Figure
1	pENTR_plasm_F	TTTTTCTAGACCCAGCTTTCTTGTA	To open via PCR pENTR-U6 plasmid	
2	pENTR_plasm_R	GGTGTTCGTCCTTTCCACA	To open via PCR pENTR-U6 plasmid	
3	p2a-eGFP-NLS-6xHis_F	agaagaagaggaaagtctaaccggtGCTACTA ACTTCAGCCTG	To amplify p2a-GFP-SV40 NLS-6xHis from Addgene plasmid #140005	
4	p2a-eGFP-NLS-6xHis_R	ATGGTGATGGTGATGATG	To amplify p2a-GFP-SV40 NLS-6xHis from Addgene plasmid #140005	
5	pFYF1320_F	GTTTATAGAGCTAGAAATAGC	To open via PCR pFYF1320 plasmid	
6	pFYF1320_R	GGTGTTCGTCCTTTCCACA	To open via PCR pFYF1320 plasmid	
7	gDNA MART1_F	CTGCTCTGGGTCGTCAAAGT	To screen for MART1 ^{G31S} positive clones upon DNA base editing (624-mel).	
8	gDNA MART1_R	TGGAATTGGAGGGATTTCAG	To screen for MART1 ^{G31S} positive clones upon DNA base editing (624-mel).	
9	coMART1 F	ATGCCCAGGGAGGACGCCCA	To quantify editing at the G31S site of the codon optimized MART1 (HEK293T).	3
10	coMART1 R	CTCCACCATACAGCCCCGGT	To quantify editing at the G31S site of the codon optimized MART1 (HEK293T).	3
11	RT-PCR MART1 F	ACGGCCACTCTTACACCACG	To quantify editing at the G31S site of the endogenous MART1 (624-mel).	3, S3
12	RT-PCR MART1 R	TGGAGCATTGGGAACCACAGG	To quantify editing at the G31S site of the endogenous MART1 (624-mel).	3, S3
13	RT-PCR eGFP F	AACTTCAGCCTGCTCAAACAAGCC	To quantify editing at the W58X site of the mCherry-2A-eGFP ^{W58X} reporter.	1, S1
14	RT-PCR eGFP R	CAGCCCTGGTCTTGTAGTTG	To quantify editing at the W58X site of the mCherry-2A-eGFP ^{W58X} reporter.	1, S1
15	MiSeq eGFP F	acactcttccctacacgacgctctccgatctAACT TCAGCCTGCTCAAACAAGCC	MiSeq amplicon sequencing on eGFP W58X.	1, S1
16	MiSeq eGFP R	gactggagttcagacgtgtgctctccgatctCAGC CCTGGTCTTGTAGTTG	MiSeq amplicon sequencing on eGFP W58X.	1, S1
17	RT-PCR TP53 F	TTGATTCCACACCCCCGCC	To amplify the Y220 site of the <i>TP53</i> mRNA.	2
18	RT-PCR TP53 R	TGTTGTTGGGCAGTGCTCGC	To amplify the Y220 site of the <i>TP53</i> mRNA.	2
19	TP53 sequencing	GTCCCAGTAGATTACCAC	Primer for Sanger sequencing and quantify editing the Y220 site of the <i>TP53</i> mRNA.	2
20	RT-PCR USP9X F	AGCGTATGTCATACAGGCGCCAGA	To amplify the Y2009 site of the <i>USP9X</i> mRNA.	2
21	RT-PCR USP9X R	CACTGGCAGAGCCACGGACTACT	To amplify the Y2009 site of the <i>USP9X</i> mRNA.	2
22	USP9X sequencing	CGAATGGACACAATAGACC	Primer for Sanger sequencing and quantify editing the Y2009 site of the <i>USP9X</i> mRNA.	2
23	RT-PCR C6orf89 F	TTTTGTGCCAGTACCCTGAGGCGA	To amplify the Y813 site of the <i>C6orf89</i> mRNA.	2
24	RT-PCR C6orf89 R	GCCTTGCAGCTCACAGCTGGTT	To amplify the Y813 site of the <i>C6orf89</i> mRNA.	2
25	C6orf89 sequencing	TCCTTTCTTCACCCAG	Primer for Sanger sequencing and quantify editing the Y813 site of the <i>C6orf89</i> mRNA.	2
26	RT-qPCR ADAR1 F	ATCAGCGGGCTGTTAGAATATG	To quantify the ADAR1 (p110 and p150 isoforms) expression in 624-mel MART1 ^{G31S} T and NT-SPEAR gRNA stable cell lines.	S3
27	RT-qPCR ADAR1 R	AAACTCTCGGCCATTGATGAC	To quantify the ADAR1 (p110 and p150 isoforms) expression in 624-mel	S3

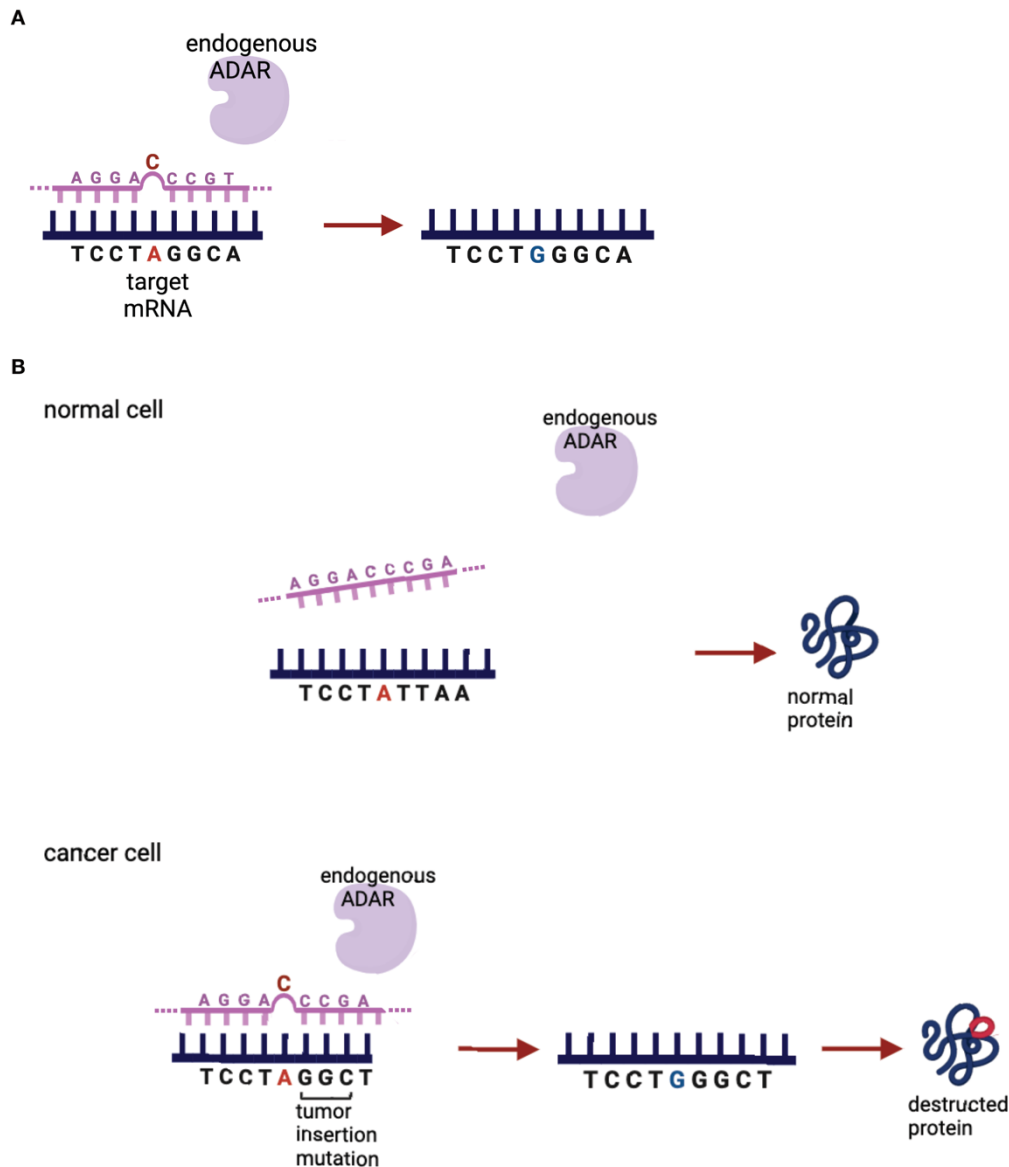
			MART1 ^{G31S} T and NT-SPEAR gRNA stable cell lines.	
28	RT-qPCR ADAR2 F	GACCCTGTCTGTCAACACGG	To quantify the ADAR2 expression in 624-mel MART1 ^{G31S} T and NT-SPEAR gRNA stable cell lines.	S3
29	RT-qPCR ADAR2 R	CCCACGTAAAAGGGAGGCTC	To quantify ADAR2 expression in 624-mel MART1 ^{G31S} T and NT-SPEAR gRNA stable cell lines.	S3
30	RT-qPCR MLANA F	ACAGTGATCCTGGGAGTCTTAC	To quantify MART-1 (MLANA) expression in 624-mel MART1 ^{G31S} T and NT-SPEAR gRNA stable cell lines.	S3
31	RT-qPCR MLANA R	TTGAAGAGACACTTTGCTGTCC	To quantify MART-1 (MLANA) expression in 624-mel MART1 ^{G31S} T and NT-SPEAR gRNA stable cell lines.	S3
32	RT-qPCR PGK1 F	ATCAGCGGGCTGTTAGAATATG	To quantify PGK1 (housekeeping gene) expression in 624-mel MART1 ^{G31S} T and NT-SPEAR gRNA stable cell lines.	S3, S6
33	RT-qPCR PGK1 R	AAACTCTCGGCCATTGATGAC	To quantify PGK1 (housekeeping gene) expression in 624-mel MART1 ^{G31S} T and NT-SPEAR gRNA stable cell lines.	S3, S6
34	RT-qPCR NT-gRNA F	TCGGGGTACCTGCTGAAGCA	To quantify NT-gRNA expression in 624-mel MART1 ^{G31S} NT-SPEAR gRNA stable cell lines.	S3, S6
35	RT-qPCR NT-gRNA R	CTACCTGTTCCATGGCCAACACTT	To quantify NT-gRNA expression in 624-mel MART1 ^{G31S} NT-SPEAR gRNA stable cell lines.	S3, S6
36	RT-qPCR T-gRNA F	TCCGGCAGTACCAGCAGC	To quantify T-gRNA expression in 624-mel MART1 ^{G31S} T-SPEAR gRNA stable cell lines.	S3, S6
37	RT-qPCR T-gRNA R	GGAGCTTGCTGGGATCGGC	To quantify T-gRNA expression in 624-mel MART1 ^{G31S} T-SPEAR gRNA stable cell lines.	S3, S6

Appendix Table S1 | Primers used in this study. Uppercase letters represent the specific sequence complementary to the specific target to amplify.

<i>TCGA project</i>	<i>Individuals in this project</i>	<i>Individuals included in the analysis</i>	<i>DNA files used</i>	<i>Whole genome files used</i>
<i>BRCA</i>	969	965	755	211
<i>SKCM</i>	470	467 (primary:102 metastatic:365)	467	-
<i>OV</i>	419	289	26	263
<i>GBM</i>	374	147	147	-
<i>LIHC</i>	369	362	362	-
<i>LUAD</i>	559	507	505	2
<i>LUSC</i>	490	487	481	6

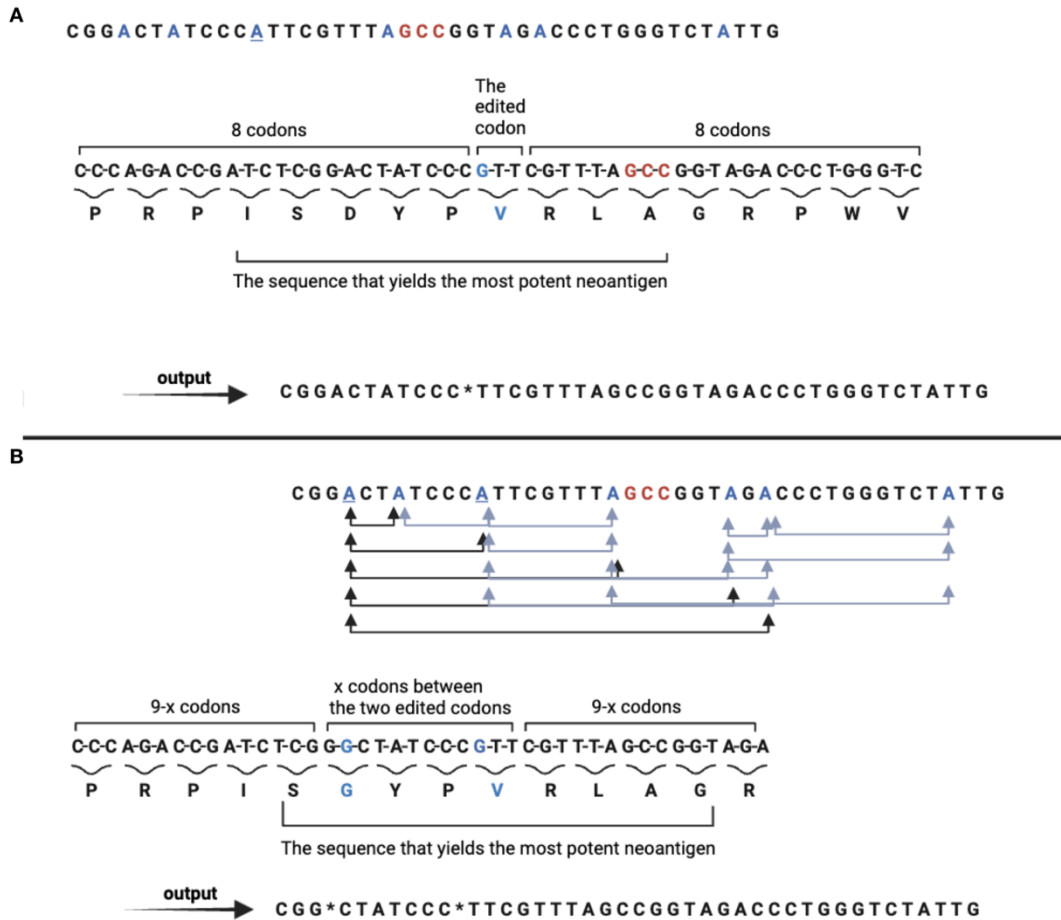
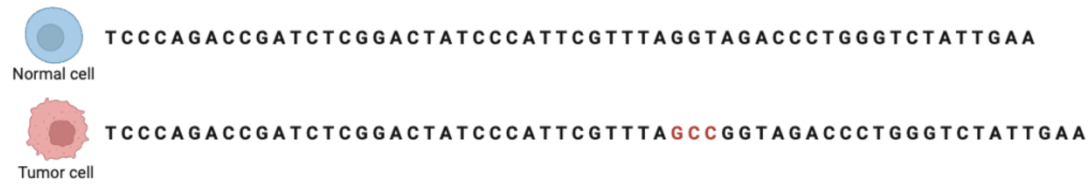
Appendix Table S2 | The seven TCGA projects utilized for the computational analysis in this study.

b. Appendix Figures



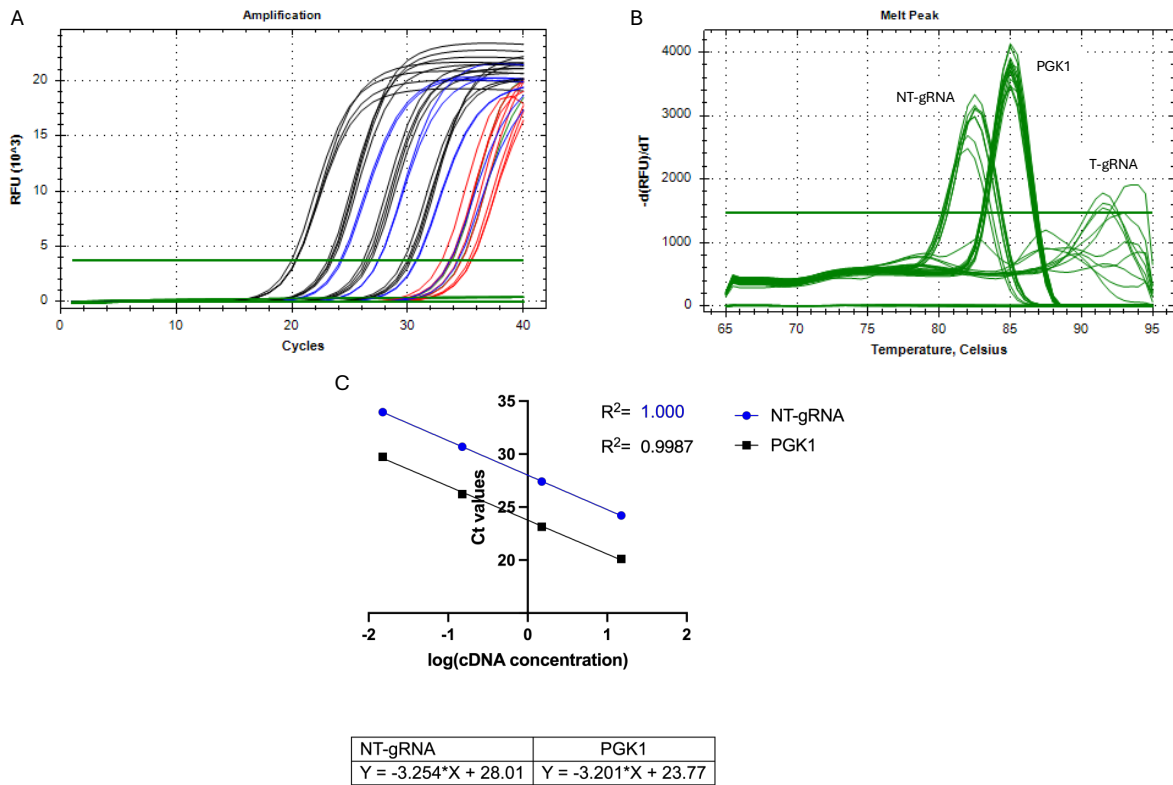
Appendix Figure S1. Schematic presentation of Endogenous-ADAR and of our method.

(A) Illustration of the endogenous-ADAR editor that converts adenosine into inosine at the RNA level. The editing depends solely on a gRNA that recruits the endogenous cellular ADAR enzyme. The gRNA ensures target specificity through Watson-Crick base pairing to the desired RNA region. (B) A carefully designed gRNA binds to a sequence of a somatic mutation that exists in the tumor but not in healthy cells, ensuring the binding specificity in tumor cells. Then, an adenosine in close proximity is edited to an inosine (read as guanosine from the ribosome), creating a peptide that can serve as a potent neoantigen in the tumor cell.



Appendix Figure S2. Constructing the codon sequence for submission to the external prediction tool. In this hypothetical example, an insertion mutation (GCC, marked in red) exists in the tumor cell and is absent in the normal one, and is considered for the next steps only if no potent neoantigen is currently predicted to be formed by this alteration. The algorithm retrieves the 20 bases upstream and downstream the mutation. This region serves as the locus to which the programmed gRNA binds. Within this span all adenosines are considered as possible targets for editing and the algorithm examines the potential of each to create a potent neoantigen upon conversion to guanosine. (A) Single-editing algorithm: For each candidate adenosine, the algorithm constructs a sequence spanning 17 codons—comprising the codon in which the adenosine is converted to guanosine, along with eight codons upstream and eight codons downstream. From this 17-mer sequence, the external prediction tool identifies the optimal 9-mer peptide according to the patient's HLA type. Constructing the codon sequence in this manner ensures that the edited adenosine is included within any

candidate 9-mer. As an example, we illustrate the analysis of the third adenosine within the region. The algorithm ultimately selects, among all analyzed adenosines, the optimal edit site that yields the strongest predicted neoepitope for the given somatic mutation. The output is the cDNA sequence with the edited adenosine represented by an asterisk (*). B) **Double-editing algorithm:** The same procedure is applied to every possible double-editing combination. For each candidate pair of adenosines, the algorithm constructs a codon sequence that includes all codons between the two edited sites (a total of x codons), supplemented by $(9 - x)$ codons upstream and $(9 - x)$ codons downstream. As a result, the generated sequence can range from a minimum of 9 codons - when the two adenosines are separated by exactly 9 codons - to a maximum of 17 codons when both adenosines reside within the same codon (pairs of adenosines located more than 9 codons apart are excluded from consideration). Constructing the codon sequence in this way ensures that both edited adenosines are encompassed within any 9-mer sequence selected by the prediction tool. As an example, we illustrate the analysis of the first and third adenosines within the region. Ultimately, the algorithm selects the optimal double-editing configuration that yields the highest predicted neoepitope score for the given somatic mutation.



Appendix Figure S3. RT-qPCR assay performance and primer efficiency for gRNA and PGK1. (A) Representative amplification curves for targeting gRNA (T-gRNA, red), non-targeting gRNA (NT-gRNA, blue), and the reference gene PGK1 (black) obtained using serial dilutions of cDNA and SYBR Green chemistry on a CFX96 Touch Real-Time PCR system (Bio-Rad). (B) Corresponding melt-curve analysis showing single, specific melt peaks for NT-gRNA and PGK1 assays, whereas the T-gRNA primer pair generated multiple low-temperature peaks consistent with nonspecific amplification and primer–dimer formation; T-gRNA was therefore excluded from further analyses. (C) Standard curves generated by plotting Ct values against log₁₀(cDNA concentration), yielding slopes of -3.25 (NT-gRNA) and -3.20 (PGK1), respectively, with $R^2 \geq 0.998$ for both assays. Simple linear regression analysis was done with GraphPad Prism.

c. Appendix Methods

RT-qPCR to determine gRNA expression and primer efficiencies

Quantification of the expression of our 74 nt long SPEAR gRNAs is challenging due to several aspects: limited amplicon size and therefore primer design, GC-content of the region, and sequence similarity to the target mRNA. Therefore, we performed a primer efficiency test to evaluate the primer pairs to quantify T- and NT-gRNA used in this study.

Serial ten-fold dilutions corresponding to 15, 1.5, 0.15, 0.015 ng of cDNA per reaction were used. T- and NT-gRNA primer pairs, alongside the PGK1 primer pair (see **Appendix Table S1**), were tested. The qPCR program used was adapted to the primer melting temperatures and manufacturer's instructions as follows:

Step	Temperature (°C)	Duration (s)	Cycles
Initial Denaturation	95	20	1
Denaturation	95	3	40
Annealing/Elongation	63	30	
Melt Curve	95	15	1
	60	60	
	95	15	
	60	15	

For the primer efficiency run, all reagents and cycling conditions were kept identical to those described in the RT-qPCR methods. A successful quantification was achieved with the NT-gRNA and PGK1 primer pairs, whereas the T-gRNA primer pair did not yield efficient amplicon quantification, as indicated by the melting curve analysis (**Appendix Figure S3A, B**). The T-gRNA primer pair exhibited amplification around 30-35 cycles, similar to their NTC controls, suggesting primer-driven amplification (**Appendix Figure S3A**). Additionally, this amplification resulted in non-uniform low peaks in melt curve analysis (**Appendix Figure S3B**). Since the T-gRNA and PGK1 primer pairs resulted in a single, sharp melt peak, with comparable Ct values for respective cDNA concentrations, primer efficiency was assessed only for them.

Amplification efficiency (E) was calculated from the slope (m) of the standard curve using the equation $E = (10^{-1/m} - 1) \times 100\%$, which assumes a theoretical doubling of the template in each cycle for an ideal efficiency of 100% (Bustin *et al*, 2009). NT-gRNA and PGK1 amplification resulted in amplification efficiencies of ~103% and ~105%, respectively.

With this experiment, we validated the primer pairs to later accurately quantify the gRNA expression in the mel624 cell lines (see **Figure EV3**).

d. Plasmid maps

To retrieve the GenBank-formatted plasmid sequence provided below, first open a plain text editor (e.g., TextEdit on macOS or Notepad on Windows). Ensure the editor is set to **Plain Text** mode (in TextEdit: Format → Make Plain Text). Then, copy the GenBank text for the plasmid of interest from below and paste it into the plain text editor. Save the file with a .gb extension to preserve formatting and enable compatibility with standard sequence analysis tools.

Plasmid pENTR-U6 GluRv1-16 (eGFP^{W58X}) sequence (GenBank format, provided as text)

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ACCESSION
VERSION
SOURCE
ORGANISM
COMMENT
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Plasmid pENTR-U6 GluRv4-16 (eGFP^{W58X}) sequence (GenBank format, provided as text)

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ACCESSION .
VERSION .
SOURCE .
  ORGANISM .
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Plasmid pENTR-U6 GluRv9.4-16 (eGFP^{w58x}) sequence (GenBank format, provided as text)

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VERSION
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ORGANISM     .
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COMMENT
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Plasmid pENTR-U6 GluRv9.4-38 (eGFP^{W58X}) sequence (GenBank format, provided as text)

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VERSION    .
SOURCE     .
  ORGANISM  .
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COMMENT    .
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Plasmid pENTR-U6 LEAPER-70 (eGFP^{W58X}) sequence (GenBank format, provided as text)

LOCUS RP325_pENTR_U6_L 2926 bp ds-DNA circular 14-SEP-2021

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ACCESSION

VERSION

SOURCE .

ORGANISM .

COMMENT

COMMENT

COMMENT ApEinfo:methylated:1

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Plasmid pENTR-U6 LEAPER-151 (Qu et al 2019) (eGFP^{W58X}) sequence (GenBank format, provided as text)

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ORGANISM   .
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Plasmid pENTR-U6 LEAPER-151 (38-4ntMM-33-C-75; Uzonyi et al 2021) (eGFP^{W58X})
sequence (GenBank format, provided as text)
LOCUS RP327_pENTR_U6_L 3007 bp ds-DNA circular 23-OCT-2021

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ORGANISM .
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Plasmid pENTR-U6 38-4ntMM-33-C-31 (sdl 107) (eGFP^{W58X}) sequence (GenBank format, provided as text)

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ORGANISM   .
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Plasmid pENTR-U6 38-4ntMM-33-C-16 (sdl 92) (eGFP^{W58X}) sequence (GenBank format, provided as text)

LOCUS RP382_pENTR_U6_L 2948 bp ds-DNA circular 14-MAR-2022

DEFINITION .

ACCESSION

VERSION

SOURCE .

ORGANISM .

COMMENT

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                   /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {{}} 0"
                   width 5 offset 0"

terminator        1063..1068
                   /locus_tag="PolIII terminator"
                   /ApEinfo_fwdcolor="#9d1b1c"
                   /ApEinfo_revcolor="#9d1b1c"
                   /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {{}} 0"
                   width 5 offset 0"

misc_binding      1009..1012
                   /locus_tag="4nt mismatch (as in Uzonyi et al 2021)"
                   /ApEinfo_fwdcolor="#ff4809"
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                   /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {{}} 0"
                   width 5 offset 0"

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ORIGIN

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1 ctttcctgcg ttatcccctg attctgtgga taaccgtatt accgcctttg agtgagctga
61 taccgctcgc cgcagccgaa cgaccgagcg cagcgagtca gtgagcgagg aagcggaaga
121 gcgccaata cgcaaaccgc ctctcccgc gcgttgccg attcattaat gcagctggca
181 cgacaggttt cccgactgga aagcgggcag tgagcgcaac gcaattaata cgcgtaccgc
241 tagccaggaa gagttttag aaacgcaaaa aggccatccg tcaggatggc cttctgctta
301 gtttgatgcc tggcagttta tggcgggcgt cctgcccgcc accctccggg ccgttgcttc
361 acaacgttca aatccgctcc cggcggatgt gtcctactca ggagagcgtt caccgacaaa
421 caacagataa aacgaaaggc ccagtcttcc gactgagcct ttcgttttat ttgatgcctg

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481 gcagttccct actctcgcgt taacgctagc atggatgttt tcccagtcac gacgttgtaa
541 aacgacggcc agtcttaagc tcggggccca aataatgatt ttatitttgac tgatagtgc
601 ctgttcgttg caacaaattg atgagcaatg cttttttata atgccaaactt tgtacaaaaa
661 agcaggcttt aaaggaacca attcagtcga ctggatccgg taccaaggctc gggcaggaag
721 agggcctatt tcccatgatt ccttcattatt tgcataacg atacaaggct gttagagaga
781 taattagaat taatttgact gtaaacacaa agatattagt acaaaatacg tgacgtagaa
841 agtaataatt tcttggttag tttgcagttt taaaattatg ttttaaaatg gactatcata
901 tgcttaccgt aacttgaaag tatttcgatt tcttggtttt atatatcttg tggaaaggac
961 gaaacaccGG tcgtgctgtt tcatgtggtc ggggtacctg ctgaagcaaa cgacgccgta
1021 tgtcagggta gtgacaagtg ttggcCatgg aacaggtagt ttttttttct agaccagct
1081 ttcttgtaca agttggcat tataagaaag cattgcttat caatttggtg caacgaacag
1141 gtcactatca gtcaaaataa aatcattatt tgccatccag ctgatatccc ctatagttag
1201 tcgtattaca tgggtcatagc tgtttcctgg cagctctggc ccgtgtctca aaatctctga
1261 tgttacattg cacaagataa aaatatatca tcatgaacaa taaaactgtc tgcttacata
1321 aacagtaata caaggggtgt tatgagccat attcaacggg aaacgtcgag gccgcgatta
1381 aattccaaca tggatgctga tttatatggg tataaatggg ctcgcgataa tgtcgggcaa
1441 tcaggtgcga caatctatcg cttgtatggg aagcccgatg cgccagagtt gtttctgaaa
1501 catggcaaaag gtacggtgac caatgatggt acagatgaga tggtcagact aaactggctg
1561 acggaattta tgcctcttcc gaccatcaag cattttatcc gtactcctga tgatgcattg
1621 ttactacca ctgcgatccc cggaaaaaca gcattccagg tattagaaga atatcctgat
1681 tcaggtgaaa atattgttga tgcgctggca gtgttcctgc gccggttgca ttcgattcct
1741 gtttgtaatt gtccttttaa cagcgatcgc gtatttcgtc tcgctcaggc gcaatcacga
1801 atgaataacg gtttggttga tgcgagtgat tttgatgacg agcgtaatgg ctggcctgtt
1861 gaacaagtct ggaaagaaat gcataaactt ttgccattct caccggattc agtcgtcact
1921 catggtgatt tctcacttga taaccttatt tttgacgagg ggaaattaat aggttgattt
1981 gatgttggtgac gagtcggaat cgcagaccga taccaggatc ttgccatcct atggaactgc
2041 ctcggtgagt tttctccttc attacagaaa cggctttttc aaaaatatgg tattgataat
2101 cctgatatga ataaattgca gtttcatttg atgctcgatg agtttttcta atcagaattg
2161 gttaattggt tgtaaacactg gcagagcatt acgctgactt gacgggacgg cgcaagctca
2221 tgacaaaaat cccttaacgt gagttacgcg tcgttccact gacgctcaga ccccgtagaa
2281 aagatcaaag gatcttcttg agatcctttt tttctgcgcg taatctgctg cttgcaaaaca
2341 aaaaaaccac cgctaccagc ggtggtttgt ttgccggatc aagagctacc aactctttt
2401 ccgaaggtaa ctggcttcag cagagcgagc ataccaaata ctgtTcttct agtgtagccg
2461 tagttaggcc accacttcaa gaactctgta gcaccgccta catacctcgc tctgctaatac
2521 ctgttaccag tggctgctgc cagtggcgat aagtcgtgtc ttaccgggtt ggactcaaga
2581 cgatagttac cggataaagg gcagcggtcg ggctgaacgg ggggttcgtg cacacagccc
2641 agcttggagc gaacgaccta caccgaactg agatacctac agcgtgagca ttgagaaagc
2701 gccacgcttc ccgaaggagc aaaggcgagc aggtatccgg taagcggcag ggtcggaaca
2761 ggagagcgca cgagggagct tccaggggga aacgcctggt atctttatag tcctgtcggg
2821 tttcgccacc tctgacttga gcgtcgattt ttgtgatgct cgtcaggggg gcggagccta
2881 tggaaaaacg ccagcaacgc ggccttttta cggttccttg ccttttgctg gccttttgct
2941 cacatggt

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//

Plasmid pENTR-U6 20-4ntMM-33-C-75 (sdl 133) (eGFP^{W58X}) sequence (GenBank format, provided as text)

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LOCUS      RP383_pENTR_U6_L          2989 bp ds-DNA      circular      14-MAR-2022
DEFINITION .
ACCESSION
VERSION
SOURCE     .
ORGANISM   .
COMMENT
COMMENT
COMMENT    ApEinfo:methylated:1
FEATURES             Location/Qualifiers
     misc_feature     1383..2192
                        /vntifkey="21"
                        /locus_tag="KanR"
                        /ApEinfo_fwdcolor="#7eff74"
                        /ApEinfo_revcolor="#7eff74"
                        /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {{}} 0"
                        width 5 offset 0"
     promoter         962..968
                        /vntifkey="29"
                        /locus_tag="hU6"
                        /ApEinfo_fwdcolor="#346ee0"

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promoter
    /ApEinfo_revcolor="#346ee0"
    /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {} 0"
    width 5 offset 0"
    705..961
    /vntifkey="29"
    /locus_tag="hU6(1)"
    /ApEinfo_label="hU6"
    /ApEinfo_fwdcolor="#346ee0"
    /ApEinfo_revcolor="#346ee0"
    /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {} 0"
    width 5 offset 0"
rep_origin
    2323..2942
    /vntifkey="33"
    /locus_tag="pBR322ori"
    /ApEinfo_fwdcolor="#999999"
    /ApEinfo_revcolor="#999999"
    /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {} 0"
    width 5 offset 0"
terminator
    1104..1109
    /locus_tag="PolIII terminator"
    /ApEinfo_fwdcolor="#9d1b1c"
    /ApEinfo_revcolor="#9d1b1c"
    /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {} 0"
    width 5 offset 0"
misc_binding
    991..994
    /locus_tag="4nt mismatch (as in Uzonyi et al 2021)"
    /ApEinfo_fwdcolor="#ff4809"
    /ApEinfo_revcolor="#ff4809"
    /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {} 0"
    width 5 offset 0"
ORIGIN
1  ttttctctgcg ttatcccctg attctgtgga taaccgtatt accgcctttg agtgagctga
61  taccgctcgc cgcagccgaa cgaccgagcg cagcgagtca gtgagcgagg aagcgggaaga
121 ggcgcccaata cgcaaaccgc ctctcccgc gcgttggccg attcattaat gcagctggca
181 cgacaggttt cccgactgga aagcggcgag tgagcgcaac gcaattaata cgcgtaccgc
241 tagccaggaa gagttttag aaacgcaaaa aggccatccg tcaggatggc cttctgctta
301 gtttgatgcc tggcagttta tggcgggctt cctgcccggc accctccggg ccgttgcttc
361 acaacgttca aatccgctcc cggcggattt gtcctactca ggagagcggt caccgacaaa
421 caacagataa aacgaaaggc ccagtcctcc gactgagcct ttcgttttat ttgatgcctg
481 gcagttccct actctcgcgt taacgctagc atggatgttt tcccagtcac gacgttgtaa
541 aacgacggcc agtcttaagc tcgggcccc aataatgatt ttattttgac tgatagtgc
601 ctgttcgttg caacaaattg atgagcaatg cttttttata atgccaaact tgtacaaaaa
661 agcaggcttt aaaggaacca attcagtcga ctggatccgg taccaagggtc gggcaggaag
721 agggcctatt tcccatgatt ccttcataat tgcataatag atacaaggct gttagagaga
781 taattagaat taatttgact gtaaacacaa agatattagt acaaaatagc tgacgtagaa
841 agtaataatt tcttggttag tttgcagttt taaaattatg ttttaaaatg gactatcata
901 tgcttaccgt aacttgaaag tatttcgatt tcttggtttt atatatcttg tggaaaggac
961 gaaacaccGG tcggggtacc tgctgaagca aacgacgccg tatgtcaggg tagtgacaag
1021 tgttggcCat ggaacaggta gttttccagt agtgcaata aacttcaggg tcagcttgcc
1081 ataggtggca tcgccctctc cttttttttc tagaccagc tttcttgtag aaagttggca
1141 ttataagaaa gcattgctta tcaattgttt gcaacgaaca ggtcactatc agtcaaaata
1201 aaatcattat ttgccatcca gctgatatcc cctatagtga gtcgtattac atggtcatag
1261 ctgtttcctg gcagctctgg cccgtgtctc aaaatctctg atgttacatt gcacaagata
1321 aaaatatatc atcatgaaca ataaaactgt ctgcttacat aaacagtaat acaaggggtg
1381 ttatgagcca tattcaacgg gaaacgtcga ggccgcgatt aaattccaac atggatgctg
1441 atttatatgg gtataaatgg gctcgcgata atgtcgggca atcagggtcg acaatctatc
1501 gcttgatgg gaagccgat gcgccagat tgtttctgaa acatggcaaa ggtagcgttg
1561 ccaatgatgt tacagatgag atggtcagac taaactggct gacggaattt atgcctcttc
1621 cgaccatcaa gcattttatc cgtactcctg atgatgcatg gttactcacc actgcgatcc
1681 ccgaaaaaac agcattccag gtattagaag aatatcctga ttcaggtgaa aatattgttg
1741 atgcgctggc agtggtcctg cgccggttgc attcgattcc tgtttgtaat tgtcctttta
1801 acagcgatcg cgtatttcgt ctgcgtcagg cgcaatcacg aatgaataac ggtttggttg
1861 atgcgagtga ttttgatgac gagcgtaatg gctggcctgt tgaacaagtc tggaaaagaa
1921 tgcataaact tttgccattc tcaccggatt cagtcgtcac tcatggtgat tttcactttg
1981 ataactttat ttttgacgag gggaaattaa taggttgatg tgatgttgga cgagtcggaa
2041 tcgcagaccg ataccaggat cttgccatcc tatggaactg cctcgggtgag ttttctcctt
2101 cattacagaa acggcttttt caaaaatag gtattgataa tcctgatatg aataaattgc
2161 agtttcatatt gatgctcgat gagtttttct aatcagaatt ggttaattgg ttgtaacact

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2221 ggcagagcat tacgctgact tgacgggacg ggcgaagctc atgacacaaa tcccttaacg
2281 tgagttacgc gtcgttcac tgagcgtag accccgtaga aaagatcaaa ggatcttctt
2341 gagatccttt ttttctgcgc gtaatctgct gcttgcaaac aaaaaaacca ccgctaccag
2401 cgggtggttg tttgccgat caagagctac caactctttt tccgaaggta actggcttca
2461 gcagagcgca gataccaaat actgtTcttc tagtgtagcc gtagttaggc caccacttca
2521 agaactctgt agcaccgcct acatacctcg ctctgctaata cctgttacca gtggctgctg
2581 ccagtggcga taagtcgtgt cttaccgggt tggactcaag acgatatgta ccggataagg
2641 cgcagcggtc gggctgaacg ggggggttcgt gcacacagcc cagcttgag cgaacgacct
2701 acaccgaact gagataccta cagcgtagac attgagaaag cgccacgctt cccgaaggga
2761 gaaaggcgga caggtatccg gtaagcgga gggtcggaac aggagagcgc acgagggagc
2821 ttccaggggg aaacgcctgg tatctttata gtcctgtcgg gtttcgccac ctctgacttg
2881 agcgtcgatt tttgtgatgc tcgtcagggg ggcggagcct atggaaaaac gccagcaacg
2941 cggccttttt acggttcctg gccttttctg ggccttttgc tcacatggt
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```

Plasmid pENTR-U6 10-4ntMM-33-C-75 (sdl 133) (eGFP^{W58X}) sequence (GenBank format, provided as text)

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LOCUS      RP384_pENTR_U6_L          2979 bp ds-DNA      circular      14-MAR-2022
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VERSION
SOURCE
ORGANISM   .
COMMENT
COMMENT    ApEinfo:methylated:1
FEATURES   Location/Qualifiers
    misc_feature   1373..2182
                    /vntifkey="21"
                    /locus_tag="KanR"
                    /ApEinfo_fwdcolor="#7eff74"
                    /ApEinfo_revcolor="#7eff74"
                    /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                    width 5 offset 0"
    promoter       962..968
                    /vntifkey="29"
                    /locus_tag="hU6"
                    /ApEinfo_fwdcolor="#346ee0"
                    /ApEinfo_revcolor="#346ee0"
                    /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                    width 5 offset 0"
    promoter       705..961
                    /vntifkey="29"
                    /locus_tag="hU6(1)"
                    /ApEinfo_label="hU6"
                    /ApEinfo_fwdcolor="#346ee0"
                    /ApEinfo_revcolor="#346ee0"
                    /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                    width 5 offset 0"
    rep_origin     2313..2932
                    /vntifkey="33"
                    /locus_tag="pBR322ori"
                    /ApEinfo_fwdcolor="#999999"
                    /ApEinfo_revcolor="#999999"
                    /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                    width 5 offset 0"
    terminator     1094..1099
                    /locus_tag="PolIII terminator"
                    /ApEinfo_fwdcolor="#9d1b1c"
                    /ApEinfo_revcolor="#9d1b1c"
                    /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                    width 5 offset 0"
    misc_binding   981..984
                    /locus_tag="4nt mismatch (as in Uzonyi et al 2021)"
                    /ApEinfo_fwdcolor="#ff4809"
                    /ApEinfo_revcolor="#ff4809"
                    /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {{}} 0}
                    width 5 offset 0"

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ORIGIN

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1 ctttctcgcg ttatcccctg attctgtgga taaccgtatt accgcctttg agtgagctga
61 taccgctcgc cgcagccgaa cgaccgagcg cagcgagtca gtgagcgagg aagcgggaaga
121 gcgccaataa cgcaaaccgc ctctccccgc gcgttgcccg attcattaat gcagctggca
181 cgacaggttt cccgactgga aagcgggcag tgagcgcaac gcaattaata cgcgtaccgc
241 tagccaggaa gagttttagg aaacgcacaa aggccatccg tcaggatggc cttctgctta
301 gttttagtgc tggcagttta tggcgggcgt cctgcccgcc accctccggg ccgttgcttc
361 acaacgttca aatccgctcc cggcggatgt gtcctactca ggagagcggt caccgacaaa
421 caacagataa aacgaaaggc ccagtcttcc gactgagcct ttcgttttat ttgatgcctg
481 gcagttccct actctcgcgt taacgctagc atggatgttt tcccagtcac gacgttgtaa
541 aacgacggcg agtcttaagc tcgggcccc aataatgatt ttattttgac tgatagtac
601 ctgttcgttg caacaaattg atgagcaatg cttttttata atgccaaact tgtacaaaaa
661 agcaggcttt aaaggaacca attcagtcga ctggatccgg taccaaggtc gggcaggaag
721 agggcctatt tcccatgatt cttcatatt tgcatatacg atacaaggct gttagagaga
781 taattagaat taatttgact gtaaacacaa agatattagt acaaaatagc tgacgtagaa
841 agtaataatt tcttggttag tttgcagttt taaaattatg ttttaaaatg gactatcata
901 tgcttaccgt aacttgaaag tatttcgatt tcttggtttt atatatcttg tggaaaggac
961 gaaacaccGG tgctgaagca aacgacgcg tatgtcaggg tagtgacaag tgttgccCat
1021 ggaacaggta gttttccagt agtgcaata aacttcaggg tcagcttgcc ataggtggca
1081 tcgccctctc cttttttttc tagaccagc tttcttgtag aaagttggca ttataagaaa
1141 gcattgctta tcaatttggt gcaacgaaca ggctactatc agtcaaaata aaatcattat
1201 ttgccatcca gctgatatcc cctatagtga gtcgtattac atgggtcatag ctgtttcctg
1261 gcagctctgg cccgtgtctc aaaatctctg atgttacatt gcacaagata aaaatatatc
1321 atcatgaaca ataaaactgt ctgcttaaat aaacagtaat acaagggtg ttatgagcca
1381 tattcaacgg gaaacgtcga ggccgcgatt aaattccaac atggatgctg atttatatgg
1441 gtataaatgg gctcgcgata atgtcgggca atcagggtgc acaatctatc gcttgtaggg
1501 gaagcccgat gcgccagagt tgtttctgaa acatggcaaa ggtagcgttg ccaatgatgt
1561 tacagatgag atggtcagac taaactggct gacggaattt atgcctcttc cgaccatcaa
1621 gcattttatc cgtactcctg atgatgcagt gttactcacc actgcgatcc ccgaaaaaac
1681 agcattccag gtattagaag aatatcctga ttcaggtgaa aatattgttg atgcgtggc
1741 agtgttcctg cgccggttgc attcgattcc tgtttgtaat tgcctttta acagcgatcg
1801 cgtatttcgt ctgcgtcagg cgcaatcacg aatgaataac ggtttggttg atgcgagtga
1861 tttttagtgc gagcgtaatg gctggcctgt tgaacaagtc tggaaagaaa tgcataaact
1921 tttgccattc tcaccggatt cagtcgtcac tcatggtgat ttctcacttg ataacttat
1981 ttttgacgag gggaaattaa taggttgtag tgatgttggg cgagtcggaa tcgcagaccg
2041 ataccaggat cttgccatcc tatggaactg cctcggtgag ttttctcctt cattacagaa
2101 acggcttttt caaaaatatg gtattgataa tcctgatatg aataaattgc agtttcattt
2161 gatgttcgat gagtttttct aatcagaatt ggttaattgg ttgtaacact ggcagagcat
2221 tacgctgact tgacgggacg gcgcaagctc atgacaaaaa tcccttaacg tgagttacgc
2281 gtcgtttccac tgagcgtcag acccgtaga aaagatcaaa ggatcttctt gagatccttt
2341 ttttctgcgc gtaatctgct gcttgcaaac aaaaaaacca ccgctaccag cgggtggttg
2401 tttgccgat caagagctac caactctttt tccgaaggta actggcttca gcagagcgca
2461 gataccaaat actgtTctt tagtgtagcc gtagttaggc caccacttca agaactctgt
2521 agcacgcct acatacctcg ctctgctaatt cctgttaccg gtggctgctg ccagtgccga
2581 taagtctgtg cttaccgggt tggaactcaag acgatagtta ccgataaagg cgcagcggtc
2641 gggctgaacg gggggttcgt gcacacagcc cagcttgtag cgaacgacct acaccgaact
2701 gagataccta cagcgtgagc attgagaaag cgccacgctt cccgaaggga gaaaggcgga
2761 caggtatccg gtaagcgga gggtcggaac aggagagcgc acgagggagc ttccaggggg
2821 aaacgcctgg tatctttata gtctgtcgg gtttcgccac ctctgacttg agcgtcgatt
2881 tttgtgatgc tcgtcagggg ggccgagcct atggaaaaac gccagcaacg cggccttttt
2941 acggttcctg gccttttgct ggccttttgc tcacatgtt

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//

Plasmid pENTR-U6 20-4ntMM-33-C-16 (sdl 74, SPEAR) (eGFP^{W58X}) sequence (GenBank format, provided as text)

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LOCUS      408_RP_pENTR_U6_          2930 bp ds-DNA      circular      28-APR-2022
DEFINITION .
ACCESSION
VERSION
SOURCE     .
ORGANISM   .
COMMENT
COMMENT    ApEinfo:methylated:1
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            misc_feature      1324..2133
                               /vntifkey="21"
                               /locus_tag="KanR"

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promoter
    /ApEinfo_fwdcolor="#7eff74"
    /ApEinfo_revcolor="#7eff74"
    /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {} 0"
    width 5 offset 0"
    705..968
    /vntifkey="29"
    /locus_tag="hU6"
    /ApEinfo_fwdcolor="#346ee0"
    /ApEinfo_revcolor="#346ee0"
    /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {} 0"
    width 5 offset 0"

rep_origin
    2264..2883
    /vntifkey="33"
    /locus_tag="pBR322ori"
    /ApEinfo_fwdcolor="#999999"
    /ApEinfo_revcolor="#999999"
    /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {} 0"
    width 5 offset 0"

terminator
    1045..1050
    /locus_tag="PolIII terminator"
    /ApEinfo_fwdcolor="#9d1b1c"
    /ApEinfo_revcolor="#9d1b1c"
    /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {} 0"
    width 5 offset 0"

misc_binding
    991..994
    /locus_tag="4nt mismatch (as in Uzonyi et al 2021)"
    /ApEinfo_fwdcolor="#ff4809"
    /ApEinfo_revcolor="#ff4809"
    /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {} 0"
    width 5 offset 0"

ORIGIN
1  ttttctgtcg  ttatcccctg  attctgtgga  taaccgtatt  accgcctttg  agtgagctga
61  taccgctcgc  cgcagccgaa  cgaccgagcg  cagcgagtca  gtgagcgagg  aagcgggaaga
121 ggcaccaata  cgcaaaccgc  ctctcccgcg  gcgttggccg  attcattaat  gcagctggca
181 cgacaggttt  cccgactgga  aagcgggcag  tgagcgcaac  gcaattaata  cgcgtaaccgc
241 tagccaggaa  gagttttag  aaacgcaaaa  aggccatccg  tcaggatggc  cttctgctta
301 gtttgatgcc  tggcagttta  tggcgggctg  cctgcccggc  accctccggg  ccgttgcttc
361 acaacgttca  aatccgctcc  cggcggtttt  gtcctactca  ggagagcggt  caccgacaaa
421 caacagataa  aacgaaaggc  ccagtccttc  gactgagcct  ttcgttttat  ttgatgcctg
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601 ctgttcgttg  caacaaattg  atgagcaatg  cttttttata  atgccaaact  tgtacaaaaa
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841 agtaataatt  tcttggttag  tttgcagttt  taaaattatg  ttttaaaatg  gactatcata
901 tgcttaccgt  aacttgaaag  tatttcgatt  tcttggtttt  atatatcttg  tggaaaggac
961 gaaacaccGG  tcgggggtacc  tgctgaagca  aacgacgccg  tatgtcaggg  tagtgacaag
1021 tgttggcCat  ggaacaggta  gttttttttt  ctagaccagc  ctttcttgta  caaagttggc
1081 attataagaa  agcattgctt  atcaatttgt  tgcaacgaac  aggtcactat  cagtcaaaat
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2041 tcattacaga  aacggctttt  tcaaaaatat  ggtattgata  atcctgatat  gaataaattg
2101 cagtttcatt  tgatgctcga  tgagtttttc  taatcagaat  tgggttaatt  gttgtaaac
2161 tggcagagca  ttacgctgac  ttgacgggac  ggcgcaagct  catgaccaa  atccctaac

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2341 gcggtggttt gtttgccgga tcaagagcta ccaactctt ttccgaaggt aactggcttc
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2881 gcggcctttt tacggttcct ggccttttg tgcccttttg ctcacatggt
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```

Plasmid hU6 NT-SPEAR (control)_hPGK coMART1^{WT}-T2A-BFP-P2A-Bsd sequence (GenBank format, provided as text)

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LOCUS      RP468_pLV_hU6_AS              8620 bp ds-DNA      circular      28-JAN-2023
DEFINITION
ACCESSION  VB210525-1302qyy
VERSION    VB210525-1302qyy
KEYWORDS   .
SOURCE
  ORGANISM  .
COMMENT
COMMENT
COMMENT    ApEinfo:methylated:1
FEATURES   Location/Qualifiers
    Promoter      1..229
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                  /__level__="0"
                  /application_notes="Strong promoter; drives transcription
                  of viral RNA in packaging cells."
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                  /full_name="Rous sarcoma virus (RSV) enhancer/promoter"
                  /name="RSV promoter"
                  /note="color: #946a00; direction: RIGHT"
                  /official_designation="RSV"
                  /uid="3f2ddca7f0cb4015b6abcec8a25c834e"
                  /vntifkey="21"
                  /locus_tag="RSV promoter"
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    LTR           230..410
                  /application_notes="Allows transcription of viral RNA and
                  its packaging into virus."
                  /description="[Truncated HIV-1 5' long terminal repeat]"
                  /full_name="HIV-1 truncated 5' LTR"
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    Miscellaneous 521..565
                  /application_notes="Allows packaging of viral RNA into
                  virus."

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/ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0"
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/ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0"
terminator 2267..2272
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/ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {{}} 0}
width 5 offset 0"
Miscellaneous 1075..1308
/___SeqFeature___="True"
/___level___="0"
/application_notes="Rev protein binding site that allows
Rev-dependent nuclear export of viral RNA during viral
packaging."
/description="['HIV-1 Rev response element']"
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width 5 offset 0"
ORF 3267..3980
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mutagenesis"
/full_name="Enhanced blue fluorescent protein(ns)"
/note="color: #6ddaae; direction: RIGHT"
/official_designation="EBFP(ns)"
/uuid="c22d3886879b4745b895a6f25c150792"
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Miscellaneous 1803..1920
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HIV-1 cDNA through a central DNA flap."
/description="['Central polypurine tract']"
/full_name="Central polypurine tract"
/name="cPPT"
/note="color: #db6901; direction: RIGHT"
/official_designation="cPPT"
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Linker
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    the encoded polypeptide. Multiple proteins can be made
    from a polycistronic transcript containing multiple ORFs
    separated by 2A. P2A and T2A have higher cleavage
    efficiency compared to other 2As."
    /description="Self-cleaving 2A peptide from Porcine
    teschovirus-1"
    /full_name="Porcine teschovirus-1 2A peptide"
    /note="color: #1f36a9; direction: RIGHT"
    /official_designation="P2A"
    /uuid="26656daa74294a0f8b331614d72d5f5a"
    /locus_tag="P2A"
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    2300..2804
    promoter
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        /description="['Human phosphoglycerate kinase 1
        promoter']"
        /name="hPGK promoter"
        /note="color: #5566f5; direction: RIGHT"
        /official_designation="hPGK promoter"
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        4047..4445
        CDS
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            /__level__="0"
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            blasticidin."
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            /name="Bsd"
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            /locus_tag="Bsd"
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                /application_notes="Enhances virus stability in packaging
                cells, leading to higher titer of packaged virus; enhances
                higher expression of transgenes."
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                posttranscriptional regulatory element']"
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                regulatory element"
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5140..5374
/application_notes="Allows packaging of viral RNA into
virus; self-inactivates the 5' LTR by a copying mechanism
during viral genome integration; contains polyadenylation
signal for transcription termination."
/description="[Truncated HIV-1 3' long terminal repeat ]"
/full_name="HIV-1 truncated 3' LTR"
/note="color: #c54b7c; direction: RIGHT"
/official_designation="DeltaU3/3' LTR"
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/locus_tag="U3/3' LTR"
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polyA_signal
5447..5581
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/___level___="0"
/application_notes="Allows transcription termination and
polyadenylation of mRNA transcribed by Pol II RNA
polymerase."
/description="['Simian virus 40 early polyadenylation
signal']"
/full_name="SV40 early polyadenation signal"
/name="SV40 early pA"
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ORF
6535..7395
/___SeqFeature___="True"
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ampicillin."
/description="['Ampicillin resistance gene']"
/full_name="Ampicillin resistance gene"
/name="Ampicillin"
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Rep_origin
7566..8154
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/application_notes="Facilitates plasmid replication in E.
coli; regulates high-copy plasmid number (500-700)."
/description="['pUC origin of replication']"
/full_name="pUC origin of replication"
/name="pUC ori"
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/ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0"
primer_bind complement(5791..5808)
/locus_tag="M13-fwd"
/ApEinfo_fwdcolor="cyan"
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primer_bind 8540..8560
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/ApEinfo_revcolor="#e9d024"
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width 5 offset 0"
sig_peptide 3213..3266
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2701 ggccctgttc ctgcccgcgc ggtgttcgcg attctgcaag cctccggagc gcacgtcggc
2761 agtcgggtcc ctcgttgacc gaatcaccga cctctctccc caggcaagtt tgtacaaaaa

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2821 agcaggctgc caccatgccc agggaggacg cccacttcat ctacggctac cccaagaagg
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//

Plasmid hU6 NT-SPEAR (control)_hPGK coMART1^{G31S}-T2A-BFP-P2A-Bsd sequence (GenBank format, provided as text)

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LOCUS      RP469_pLV_hU6_AS              8620 bp ds-DNA      circular      04-JAN-2023
DEFINITION
ACCESSION  VB210525-1302qyy
VERSION    VB210525-1302qyy
KEYWORDS   .
SOURCE
  ORGANISM  .
COMMENT
COMMENT
COMMENT    ApEinfo:methylated:1
FEATURES   Location/Qualifiers
    Promoter      1..229
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                  /__level__="0"
                  /application_notes="Strong promoter; drives transcription
                  of viral RNA in packaging cells."
                  /description="['Rous sarcoma virus enhancer/promoter']"
                  /full_name="Rous sarcoma virus (RSV) enhancer/promoter"
                  /name="RSV promoter"
                  /note="color: #946a00; direction: RIGHT"
                  /official_designation="RSV"
                  /uid="3f2ddca7f0cb4015b6abcec8a25c834e"
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/locus_tag="5' LTR"
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Miscellaneous 521..565
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virus."
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/note="color: #d84e4e; direction: RIGHT"
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/ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0"
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terminator 2267..2272
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width 5 offset 0"
Miscellaneous 1075..1308
/___SeqFeature___="True"
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/application_notes="Rev protein binding site that allows
Rev-dependent nuclear export of viral RNA during viral
packaging."
/description="['HIV-1 Rev response element']"
/full_name="HIV-1 Rev response element"
/name="RRE"
/note="color: #1f36a9; direction: RIGHT"
/official_designation="RRE"
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mutagenesis"
/full_name="Enhanced blue fluorescent protein(ns)"
/note="color: #6ddaae; direction: RIGHT"
/official_designation="EBFP(ns) "
/uuid="c22d3886879b4745b895a6f25c150792"
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    /full_name="Central polypurine tract"
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    /note="color: #db6901; direction: RIGHT"
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Linker
    3981..4046
    /application_notes="Causes co-translational cleavage of
    the encoded polypeptide. Multiple proteins can be made
    from a polycistronic transcript containing multiple ORFs
    separated by 2A. P2A and T2A have higher cleavage
    efficiency compared to other 2As."
    /description="Self-cleaving 2A peptide from Porcine
    teschovirus-1"
    /full_name="Porcine teschovirus-1 2A peptide"
    /note="color: #1f36a9; direction: RIGHT"
    /official_designation="P2A"
    /uuid="26656daa74294a0f8b331614d72d5f5a"
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promoter
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    promoter']"
    /name="hPGK promoter"
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CDS
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/___level___="0"
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cells, leading to higher titer of packaged virus; enhances
higher expression of transgenes."
/description="['Woodchuck hepatitis virus
posttranscriptional regulatory element']"
/full_name="Woodchuck hepatitis virus posttranscriptional
regulatory element"
/name="WPRE"
/note="color: #ef6cdf; direction: RIGHT"
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virus; self-inactivates the 5' LTR by a copying mechanism
during viral genome integration; contains polyadenylation
signal for transcription termination."
/description="['Truncated HIV-1 3' long terminal repeat ]"
/full_name="HIV-1 truncated 3' LTR"
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/official_designation="DeltaU3/3' LTR"
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polyadenylation of mRNA transcribed by Pol II RNA
polymerase."
/description="['Simian virus 40 early polyadenylation
signal']"
/full_name="SV40 early polyadenation signal"
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coli; regulates high-copy plasmid number (500-700).\"
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Plasmid hU6 T-SPEAR (coMART-1^{G31S})_hPGK coMART1^{G31S}-T2A-BFP-P2A-Bsd sequence (GenBank format, provided as text)

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VERSION    VB210525-1302qyy
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SOURCE
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COMMENT
COMMENT    ApEinfo:methylated:1
FEATURES
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the encoded polypeptide. Multiple proteins can be made
from a polycistronic transcript containing multiple ORFs
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efficiency compared to other 2As."
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8221 cctgcgttat cccctgattc tgtggataac cgtattaccg cctttgagtg agctgatacc
8281 gctcgccgca gccgaacgac cgagcgcagc gaggcagtg ggcaggaagc ggaagagcgc
8341 ccaatacgca aaccgcctct ccccgcgctg tggccgattc attaatgcag ctggcacgac
8401 aggtttcccg actggaaagc gggcagtgag cgcaacgcaa ttaatgtgag ttagctcact
8461 cattaggcac cccaggcttt acactttatg cttccggctc gtatgttggtg tggaattgtg
8521 agcggataac aatttcacac aggaacacgc tatgaccatg attacgcaa gcgcgcaatt
8581 aaccctcact aaaggaaca aaagctggag ctgcaagctt
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Plasmid pT_hU6 NT-SPEAR (control)_hPGK BFP-P2A-Bsd sequence (GenBank format, provided as text)

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LOCUS      RP641_pT_U6_20_4          5929 bp      DNA            circular      29-JAN-2025
DEFINITION  synthetic circular DNA
ACCESSION   .
VERSION     .
KEYWORDS    .
SOURCE      synthetic DNA construct
  ORGANISM   synthetic DNA construct
REFERENCE   1  (bases 1 to 5504)
  AUTHORS    .
  TITLE      Direct Submission
  JOURNAL     Exported Jul 23, 2024 from SnapGene Viewer 7.2.1
             https://www.snapgene.com
COMMENT     [DEFINITION]: Sequence
COMMENT     ApEinfo:methylated:1
FEATURES             Location/Qualifiers
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/full_name="Enhanced blue fluorescent protein(ns)"
/note="color: #6ddaae; direction: RIGHT"
/official_designation="EBFP(ns)"
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-
VKDSEDVPMVLVGNKCDLPSRTVDTKQAQELARSYGIPFIETSAKTRQGVDADFYTLLV
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the encoded polypeptide. Multiple proteins can be made
from a polycistronic transcript containing multiple ORFs
separated by 2A. P2A and T2A have higher cleavage
efficiency compared to other 2As."
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teschovirus-1"
    /full_name="Porcine teschovirus-1 2A peptide"
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promoter']"
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width 5 offset 0"
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width 5 offset 0"
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-
VIDGETCLLDILDITAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKR
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VKDSEDVPMVLVGNKCDLPSRTVDTKQAQELARSYGIPFIETSAKTRQGVDDAFYTLV
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primer_bind complement(3313..3329)
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variants"
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misc_feature complement(3326..3348)
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-
VKDSEDVPMVLVGNKCDLPSRTVDTKQAQELARSYGIPFIETSAKTRQGVDDAFYTLV
- REIRKHKEKMSKDGKKKKKSRTRCTVM*" - complement("
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protein_bind 3337..3353
/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).\"
/locus_tag="lac operator"
/label="lac operator"
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               /locus_tag="lac promoter"
               /label="lac promoter"
               /ApEinfo_label="lac promoter"
               /ApEinfo_fwdcolor="#346ee0"
               /ApEinfo_revcolor="#346ee0"
               /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
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               -
               VKDSEDVPMVLVGNKCDLPSRTVDTKQAQELARSYGIPFIETSAKTRQGVDDAFYTLV
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protein_bind   3406..3427
               /bound_moiety="E. coli catabolite activator protein"
               /note="CAP binding activates transcription in the presence
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               /locus_tag="CAP binding site"
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source        complement(2600..2824)
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               -
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-
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- DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQQLIDWMEA"
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-
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-
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
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-
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
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Plasmid pT_hU6 T-SPEAR (TP53^{Y220C})_hPGK BFP-P2A-Bsd sequence (GenBank format, provided as text)

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VERSION
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COMMENT
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    3337..3353
    /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).\"
    /locus_tag="lac operator"
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Plasmid pT_hU6 T-SPEAR (USP9X^{Y2009c})_hPGK BFP-P2A-Bsd sequence (GenBank format, provided as text)

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ACCESSION
VERSION
SOURCE
ORGANISM
COMMENT
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                        -0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
     promoter          710..716
                        /vntifkey="29"
                        /locus_tag="hU6"
                        /label="hU6"
                        /ApEinfo_label="hU6"
                        /ApEinfo_fwdcolor="#346ee0"
                        /ApEinfo_revcolor="#346ee0"

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misc_feature      /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {} 0}
                  width 5 offset 0"
                  219..250
                  /locus_tag="LIR"
                  /label="LIR"
                  /ApEinfo_label="LIR"
                  /ApEinfo_fwdcolor="#7eff74"
                  /ApEinfo_revcolor="#7eff74"
                  /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
misc_feature      -0.5}} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
                  complement(382..402)
                  /note="label="pBABE_3_primer" - complement(382..402)"
                  /locus_tag="pBABE_3_primer"
                  /label="pBABE_3_primer"
                  /ApEinfo_label="pBABE_3_primer"
                  /ApEinfo_fwdcolor="#7eff74"
                  /ApEinfo_revcolor="#7eff74"
                  /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
promoter          -0.5}} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
                  453..709
                  /vntifkey="29"
                  /locus_tag="hU6(1)"
                  /label="hU6(1)"
                  /ApEinfo_label="hU6"
                  /ApEinfo_fwdcolor="#346ee0"
                  /ApEinfo_revcolor="#346ee0"
                  /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {} 0}
ORF               width 5 offset 0"
                  1418..2134
                  /application_notes="Low fluorescence and low
                  photostability."
                  /description="Blue variant of EGFP generated by
                  mutagenesis"
                  /full_name="Enhanced blue fluorescent protein(ns)"
                  /note="color: #6ddaae; direction: RIGHT"
                  /official_designation="EBFP(ns)"
                  /uuid="c22d3886879b4745b895a6f25c150792"
                  /locus_tag="EBFP(ns)"
                  /label="EBFP(ns)"
                  /ApEinfo_label="EBFP(ns)"
                  /ApEinfo_fwdcolor="#0f7ffe"
                  /ApEinfo_revcolor="pink"
                  /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {} 0}
CDS               width 5 offset 0"
                  complement(join(719..738,743..792))
                  /locus_tag="CDS"
                  /label="CDS"
                  /ApEinfo_label="CDS"
                  /ApEinfo_fwdcolor="#e9d024"
                  /ApEinfo_revcolor="#e9d024"
                  /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
primer_bind       -0.5}} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
                  complement(2835..2856)
                  /locus_tag="pT3_rev"
                  /label="pT3_rev"
                  /ApEinfo_label="pT3_rev"
                  /ApEinfo_fwdcolor="#14c0bd"
                  /ApEinfo_revcolor="#4ec02b"
                  /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
variation         -0.5}} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
                  complement(775..777)
                  /locus_tag="Y2009C"
                  /label="Y2009C"
                  /ApEinfo_label="Y2009C"
                  /ApEinfo_fwdcolor="#ff9e39"
                  /ApEinfo_revcolor="#ff9e39"
                  /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
                  -0.5}} {} 0} width 5 offset 0"

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misc_feature 2845..2864
 /note="label="EBV_rev_primer" -
 translation="MTEYKLVVVGAVGVGKSALTIQLIQNHVFDEYDPTIEDSYRKQV
 -
 VIDGETCLLDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKR
 -
 VKDSEDVPMVLVGNKCDLPSRTVDTKQAQELARSYGIPFIETSAKTRQGVDDAFYTLV
 - REIRKHKEKMSKDGKKKKKSRTCTVM*" - 2420..2439"
 /locus_tag="EBV_rev_primer"
 /label="EBV_rev_primer"
 /ApEinfo_label="EBV_rev_primer"
 /ApEinfo_fwdcolor="#7eff74"
 /ApEinfo_revcolor="#7eff74"
 /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
 -0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"

misc_binding complement(739..742)
 /bound_moiety=""
 /locus_tag="4 nt mismatch (as in Uzonyi et al 2021)"
 /label="4 nt mismatch (as in Uzonyi et al 2021)"
 /ApEinfo_label="4 nt mismatch (as in Uzonyi et al 2021)"
 /ApEinfo_fwdcolor="#ff4809"
 /ApEinfo_revcolor="#ff4809"
 /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
 -0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"

Linker 2135..2200
 /application_notes="Causes co-translational cleavage of
 the encoded polypeptide. Multiple proteins can be made
 from a polycistronic transcript containing multiple ORFs
 separated by 2A. P2A and T2A have higher cleavage
 efficiency compared to other 2As."
 /description="Self-cleaving 2A peptide from Porcine
 teschovirus-1"
 /full_name="Porcine teschovirus-1 2A peptide"
 /note="color: #1f36a9; direction: RIGHT"
 /official_designation="P2A"
 /uid="26656daa74294a0f8b331614d72d5f5a"
 /locus_tag="P2A"
 /label="P2A"
 /ApEinfo_label="P2A"
 /ApEinfo_fwdcolor="#20fefe"
 /ApEinfo_revcolor="pink"
 /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1} {} 0}
 width 5 offset 0"

primer_bind 3004..3025
 /locus_tag="RIR Primer"
 /label="RIR Primer"
 /ApEinfo_label="RIR Primer"
 /ApEinfo_fwdcolor="#14c0bd"
 /ApEinfo_revcolor="#4ec02b"
 /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
 -0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"

promoter 883..1387
 /__SeqFeature__="True"
 /__level__="0"
 /application_notes="Medium-strength promoter."
 /description="['Human phosphoglycerate kinase 1
 promoter']"
 /name="hPGK promoter"
 /note="color: #5566f5; direction: RIGHT"
 /official_designation="hPGK promoter"
 /uid="a105ee8f48814e769c9a41c38cfff41e"
 /vntifkey="21"
 /locus_tag="hPGK promoter"
 /label="hPGK promoter"
 /ApEinfo_label="hPGK promoter"
 /ApEinfo_fwdcolor="#346ee0"
 /ApEinfo_revcolor="#346ee0"
 /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1} {} 0}

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misc_feature      width 5 offset 0"
                  3032..3062
                  /locus_tag="RIR"
                  /label="RIR"
                  /ApEinfo_label="RIR"
                  /ApEinfo_fwdcolor="#7eff74"
                  /ApEinfo_revcolor="#7eff74"
                  /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
CDS               -0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
                  2201..2599
                  /__SeqFeature__="True"
                  /__level__="0"
                  /application_notes="Allows cells to be resistant to
                  blasticidin."
                  /description="['Blasticidin resistance gene']"
                  /full_name="Blasticidin resistance gene"
                  /name="Bsd"
                  /note="color: #68e66d; direction: RIGHT"
                  /official_designation="Bsd"
                  /uuid="70e7f83c244f451dae86de92afa03717"
                  /vntifkey="21"
                  /locus_tag="Bsd"
                  /label="Bsd"
                  /ApEinfo_label="Bsd"
                  /ApEinfo_fwdcolor="#e9d024"
                  /ApEinfo_revcolor="#e9d024"
                  /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1} {} 0}
misc_feature      width 5 offset 0"
                  3229..3260
                  /locus_tag="ROR"
                  /label="ROR"
                  /ApEinfo_label="ROR"
                  /ApEinfo_fwdcolor="#7eff74"
                  /ApEinfo_revcolor="#7eff74"
                  /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
promoter          -0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
                  complement(3309..3327)
                  /note="label="M13_reverse_primer" -
                  translation="MTEYKLVVVGAVGVGKSALTIQLIQNHVFVEYDPTIEDSYRKQV
                  -
                  VIDGETCLLDILDITAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKR
                  -
                  VKDSEDVPMVLVGNKCDLPSRTVDTKQAQELARSYGIPFIETSAKTRQGVDDAFYTLV
                  - REIRKHKEKMSKDGKKKKKKSRTCTVM*" - complement("
                  /locus_tag="M13_reverse_primer"
                  /label="M13_reverse_primer"
                  /ApEinfo_label="M13_reverse_primer"
                  /ApEinfo_fwdcolor="#346ee0"
                  /ApEinfo_revcolor="#346ee0"
                  /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
primer_bind       -0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
                  complement(3313..3329)
                  /note="common sequencing primer, one of multiple similar
                  variants"
                  /locus_tag="M13 rev"
                  /label="M13 rev"
                  /ApEinfo_label="M13 rev"
                  /ApEinfo_fwdcolor="#14c0bd"
                  /ApEinfo_revcolor="#4ec02b"
                  /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
misc_feature      -0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
                  complement(3326..3348)
                  /note="label="M13_pUC_rev_primer" -
                  translation="MTEYKLVVVGAVGVGKSALTIQLIQNHVFVEYDPTIEDSYRKQV
                  -
                  VIDGETCLLDILDITAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKR
                  -
                  VKDSEDVPMVLVGNKCDLPSRTVDTKQAQELARSYGIPFIETSAKTRQGVDDAFYTLV

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- REIRKHKEKMSKDGKKKKKSRTRCTVM*" - complement("
/locus_tag="M13_pUC_rev_primer"
/label="M13_pUC_rev_primer"
/ApEinfo_label="M13_pUC_rev_primer"
/ApEinfo_fwdcolor="#7eff74"
/ApEinfo_revcolor="#7eff74"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
protein_bind 3337..3353
/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)."
```

```

/locus_tag="lac operator"
/label="lac operator"
/ApEinfo_label="lac operator"
/ApEinfo_fwdcolor="#00D95A"
/ApEinfo_revcolor="#00D95A"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
promoter complement(3361..3391)
/note="promoter for the E. coli lac operon"
/locus_tag="lac promoter"
/label="lac promoter"
/ApEinfo_label="lac promoter"
/ApEinfo_fwdcolor="#346ee0"
/ApEinfo_revcolor="#346ee0"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
promoter complement(3362..3391)
/note="label="lac promoter" -
translation="MTEYKLVVVGAVGVGKSALTIQLIQNHVFVEYDPTIEDSYRKQV
-
VIDGETCLLDILDTAGQEEYSAMRDQYMRGTGEGFLCVFAINNNTKSFEDIHHYREQIKR
-
VKDSEDVPMVLVGNKCDLPSRTVDTKQAQELARSYGIPFIETSAKTRQGVDADFYTTLV
- REIRKHKEKMSKDGKKKKKSRTRCTVM*" - complement(2937.."
/locus_tag="lac promoter"
/label="lac promoter"
/ApEinfo_label="lac promoter"
/ApEinfo_fwdcolor="#346ee0"
/ApEinfo_revcolor="#346ee0"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
protein_bind 3406..3427
/bound_moiety="E. coli catabolite activator protein"
/note="CAP binding activates transcription in the presence
of cAMP."
/locus_tag="CAP binding site"
/label="CAP binding site"
/ApEinfo_label="CAP binding site"
/ApEinfo_fwdcolor="#008065"
/ApEinfo_revcolor="#008065"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
source complement(2600..2824)
/locus_tag="source"
/label="source"
/ApEinfo_label="source"
/ApEinfo_fwdcolor="#00DF87"
/ApEinfo_revcolor="#00DF87"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
/ApEinfo_hidden
rep_origin complement(3715..4303)
/direction=LEFT
/note="high-copy-number ColE1/pMB1/pBR322/pUC origin of
```

```

replication"
/locus_tag="ori"
/label="ori"
/ApEinfo_label="ori"
/ApEinfo_fwdcolor="#999999"
/ApEinfo_revcolor="#999999"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
gene complement(4474..5334)
/note="label="Ampicillin" - gene="Ampicillin" -
translation="MTEYKLVVVGAVGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQV
-
VIDGETCLLDILDITAGQEEYSAMRDQYMRGTGEGFLCVFAINNTKSFEDIHHYREQIKR
-
VKDSEDEVPMVLVGNKCDLPSRTVDTKQAQELARSYGIPFIETSAKTRQGVDDAFYTLV
- REIRKHKEKMSKDGKKKKKSRTCTVM*" -"
/locus_tag="AmpR"
/label="AmpR"
/ApEinfo_label="AmpR"
/ApEinfo_fwdcolor="#ff797d"
/ApEinfo_revcolor="#ff102f"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
CDS complement(4474..5334)
/note="label="ORF frame 2" -
translation="MSIQHFRVALIPFFAAFCPLPVFAHPETLVKVKDAEDQLGARVG
-
IELDLNSGKILESFRPEERFPMMSSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
-
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHSVTRL
- DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEA"
/locus_tag="ORF frame 2"
/label="ORF frame 2"
/ApEinfo_label="ORF frame 2"
/ApEinfo_fwdcolor="#e9d024"
/ApEinfo_revcolor="#e9d024"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
promoter complement(5335..5439)
/gene="bla"
/locus_tag="AmpR promoter"
/label="AmpR promoter"
/ApEinfo_label="AmpR promoter"
/ApEinfo_fwdcolor="#346ee0"
/ApEinfo_revcolor="#346ee0"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
promoter complement(5376..5404)
/note="label="AmpR promoter" -
translation="MSIQHFRVALIPFFAAFCPLPVFAHPETLVKVKDAEDQLGARVG
-
IELDLNSGKILESFRPEERFPMMSSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
-
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHSVTRL
- DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWM"
/locus_tag="AmpR promoter"
/label="AmpR promoter"
/ApEinfo_label="AmpR promoter"
/ApEinfo_fwdcolor="#346ee0"
/ApEinfo_revcolor="#346ee0"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
misc_feature complement(5563..5585)
/note="label="pGEX_3_primer" -
translation="MSIQHFRVALIPFFAAFCPLPVFAHPETLVKVKDAEDQLGARVG
-
IELDLNSGKILESFRPEERFPMMSSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
-

```



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YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHVTRL
- DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQQLIDWM"
/locus_tag="pGEX_3_primer"
/label="pGEX_3_primer"
/ApEinfo_label="pGEX_3_primer"
/ApEinfo_fwdcolor="#7eff74"
/ApEinfo_revcolor="#7eff74"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
misc_feature 2600..2824
/___SeqFeature___="True"
/___level___="0"
/application_notes="Allows transcription termination and
polyadenylation of mRNA transcribed by Pol II RNA
polymerase."
/full_name="Bovine growth hormone polyadenylation"
/name="BGH pA"
/note="color: #46c6ef; direction: LEFT"
/official_designation="BGH pA"
/uuid="439e92d8cb8e406dbfacd87ce28d14dc"
/vntifkey="21"
/locus_tag="BGH pA"
/label="BGH pA"
/ApEinfo_label="BGH pA"
/ApEinfo_fwdcolor="#7eff74"
/ApEinfo_revcolor="#7eff74"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
misc_feature 5898..5920
/note="label="M13_pUC_fwd_primer" -
translation="MSIQHFRVALIPFFAAFCPLPVFAHPETLVKVKDAEDQLGARVGY
-
IELDLNSGKILESFRPEERFPMMSSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
-
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHVTRL
- DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQ"
/locus_tag="M13_pUC_fwd_primer"
/label="M13_pUC_fwd_primer"
/ApEinfo_label="M13_pUC_fwd_primer"
/ApEinfo_fwdcolor="#7eff74"
/ApEinfo_revcolor="#7eff74"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
promoter 5913..5929
/note="label="M13_forward20_primer" -
translation="MSIQHFRVALIPFFAAFCPLPVFAHPETLVKVKDAEDQLGARVGY
-
IELDLNSGKILESFRPEERFPMMSSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
-
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLTTIGGPKELTAFLHNMGDHVTRL
- DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASR"
/locus_tag="M13_forward20_primer"
/label="M13_forward20_primer"
/ApEinfo_label="M13_forward20_primer"
/ApEinfo_fwdcolor="#346ee0"
/ApEinfo_revcolor="#346ee0"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
primer_bind 5913..5929
/note="common sequencing primer, one of multiple similar
variants"
/locus_tag="M13 fwd"
/label="M13 fwd"
/ApEinfo_label="M13 fwd"
/ApEinfo_fwdcolor="#14c0bd"
/ApEinfo_revcolor="#4ec02b"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"

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terminator      793..798
                 /locus_tag="PolIII terminator"
                 /label="PolIII terminator"
                 /ApEinfo_label="PolIII terminator"
                 /ApEinfo_fwdcolor="#9d1b1c"
                 /ApEinfo_revcolor="#9d1b1c"
                 /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {} 0}
                 width 5 offset 0"
variation       complement(776..776)
                 /locus_tag="A:C mismatch"
                 /label="A:C mismatch"
                 /ApEinfo_label="A:C mismatch"
                 /ApEinfo_fwdcolor="#ff2600"
                 /ApEinfo_revcolor="#ff2600"
                 /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
                 -0.5}} {} 0} width 5 offset 0"

ORIGIN
1  gaattcgagc  tcggtaccct  acagttgaag  tcggaagttt  acatacactt  aagttggagt
61  cattaaaact  cgtttttcaa  ctactccaca  aatttcttgt  taacaaacaa  tagttttggc
121 aagtcagtta  ggacatctac  tttgtgcatg  acacaagtca  tttttccaac  aattgtttac
181 agacagatta  ttccacttat  aattcactgt  atcacaattc  cagtgggtca  gaagtttaca
241 tacactaagt  tgactgtgcc  tttaaacagc  ttggaaaatt  ccagaaaatg  atgtcatggc
301 tttagaagct  tctgatagac  taattgacat  catttgagtc  aattggaggt  gtacctgtgg
361 atgtatttca  aggaattctg  tggaatgtgt  gtcagttagg  gtgtggaaag  tccccaggct
421 ccccagcagg  cagaagtatg  caaagcatgc  ataaggtcgg  gcaggaagag  ggcctatttc
481 ccatgattcc  ttcataattg  catatacgat  acaaggctgt  tagagagata  attagaatta
541 atttgactgt  aaacacaaag  atattagtac  aaaatacgtg  acgtagaaag  taataatttc
601 ttgggtagtt  tgcagtttta  aaattatgtt  ttaaaatgga  ctatcatatg  cttaccgtaa
661 cttgaaagta  tttcgatttc  ttggctttat  atatcttgtg  gaaaggacga  aacaccGGgg
721 aggagggttt  aagtaaacCG  GTttacatgt  aagcagtttt  ttcataaact  gaaaaCactc
781 catactgtac  tgttttttct  agaccagctt  ttcttgtaca  aactcgagaa  taaaggtacc
841 ttattttcat  tagatttgtg  tgtttgtttt  ttgtgtgCTA  GCgggttgcg  ctttttcaa
901 ggcagccctg  ggtttgcgca  gggacgcggc  tgctctgggc  gtggttccgg  gaaacgcagc
961 ggcgcgcgac  ctgggtctcg  cacattcttc  acgtccgttc  gcagcgtcac  ccgatcttc
1021 gccgctaccc  ttgtgggccc  cccggcgacg  cttcctgctc  cgccccaaag  tcgggaaggt
1081 tccttgcggt  tcgcggcgtg  ccggacgtga  caaacggaag  ccgcacgtct  cactagtacc
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5761 ccgcatcagg cgccattcgc cattcaggct gcgcaactgt tgggaagggc gatcgggtcg
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//

Plasmid pT_hU6 T-SPEAR (C6orf89^{Y813C})_hPGK BFP-P2A-Bsd sequence (GenBank format, provided as text)

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DEFINITION .
ACCESSION
VERSION
SOURCE
ORGANISM   .
COMMENT
COMMENT
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FEATURES
            Location/Qualifiers
            source
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-
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Plasmid pT_hU6 T-SPEAR (MART-1^{G31S})_hPGK BFP-P2A-Bsd sequence (GenBank format, provided as text)

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VERSION     .
KEYWORDS    .
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REFERENCE   1  (bases 1 to 5504)
  AUTHORS   .
  TITLE     Direct Submission
  JOURNAL   Exported Jul 23, 2024 from SnapGene Viewer 7.2.1
            https://www.snapgene.com
COMMENT     [DEFINITION]: Sequence
COMMENT
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/locus_tag="hU6(1)"
/label="hU6(1)"
/ApEinfo_label="hU6"
/ApEinfo_fwdcolor="#346ee0"
/ApEinfo_revcolor="#346ee0"
/ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1} {} 0}
width 5 offset 0"
ORF 1418..2134
/application_notes="Low fluorescence and low
photostability."
/description="Blue variant of EGFP generated by
mutagenesis"
/full_name="Enhanced blue fluorescent protein(ns)"

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/feature
  /note="color: #6ddaae; direction: RIGHT"
  /official_designation="EBFP(ns) "
  /uid="c22d3886879b4745b895a6f25c150792"
  /locus_tag="EBFP(ns) "
  /label="EBFP(ns) "
  /ApEinfo_label="EBFP(ns) "
  /ApEinfo_fwdcolor="#0f7ffe"
  /ApEinfo_revcolor="pink"
  /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {{}} 0"
  width 5 offset 0"
primer_bind
  complement(2835..2856)
  /locus_tag="pT3_rev"
  /label="pT3_rev"
  /ApEinfo_label="pT3_rev"
  /ApEinfo_fwdcolor="#14c0bd"
  /ApEinfo_revcolor="#4ec02b"
  /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5}} {{0 .5 .1 .5 .1 -.5 0 -.5}} 0" width 5 offset 0"
misc_feature
  2845..2864
  /note="label="EBV_rev_primer" -
  translation="MTEYKLVVVGAVGVGKSALTIQLIQNHVFDEYDPTIEDSYRKQV
-
VIDGETCLLDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKR
-
VKDSEDVPMVLVGNKCDLPSRTVDTKQAQELARSYGIPFIETSAKTRQGVDDAFYTLV
- REIRKHKEKMSKDGKKKKKSRTRCTVM*" - 2420..2439"
  /locus_tag="EBV_rev_primer"
  /label="EBV_rev_primer"
  /ApEinfo_label="EBV_rev_primer"
  /ApEinfo_fwdcolor="#7eff74"
  /ApEinfo_revcolor="#7eff74"
  /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5}} {{0 .5 .1 .5 .1 -.5 0 -.5}} 0" width 5 offset 0"
Linker
  2135..2200
  /application_notes="Causes co-translational cleavage of
the encoded polypeptide. Multiple proteins can be made
from a polycistronic transcript containing multiple ORFs
separated by 2A. P2A and T2A have higher cleavage
efficiency compared to other 2As."
  /description="Self-cleaving 2A peptide from Porcine
teschovirus-1"
  /full_name="Porcine teschovirus-1 2A peptide"
  /note="color: #1f36a9; direction: RIGHT"
  /official_designation="P2A"
  /uid="26656daa74294a0f8b331614d72d5f5a"
  /locus_tag="P2A"
  /label="P2A"
  /ApEinfo_label="P2A"
  /ApEinfo_fwdcolor="#20fefe"
  /ApEinfo_revcolor="pink"
  /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {{}} 0"
  width 5 offset 0"
primer_bind
  3004..3025
  /locus_tag="RIR Primer"
  /label="RIR Primer"
  /ApEinfo_label="RIR Primer"
  /ApEinfo_fwdcolor="#14c0bd"
  /ApEinfo_revcolor="#4ec02b"
  /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5}} {{0 .5 .1 .5 .1 -.5 0 -.5}} 0" width 5 offset 0"
promoter
  883..1387
  /__SeqFeature__="True"
  /__level__="0"
  /application_notes="Medium-strength promoter."
  /description="['Human phosphoglycerate kinase 1
promoter']"
  /name="hPGK promoter"
  /note="color: #5566f5; direction: RIGHT"

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/official_designation="hPGK promoter"
/uid="a105ee8f48814e769c9a41c38cfff41e"
/vntifkey="21"
/locus_tag="hPGK promoter"
/label="hPGK promoter"
/ApEinfo_label="hPGK promoter"
/ApEinfo_fwdcolor="#346ee0"
/ApEinfo_revcolor="#346ee0"
/ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0"
misc_feature
3032..3062
/locus_tag="RIR"
/label="RIR"
/ApEinfo_label="RIR"
/ApEinfo_fwdcolor="#7eff74"
/ApEinfo_revcolor="#7eff74"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5}} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
CDS
2201..2599
/___SeqFeature___="True"
/___level___="0"
/application_notes="Allows cells to be resistant to
blasticidin."
/description="['Blasticidin resistance gene']"
/full_name="Blasticidin resistance gene"
/name="Bsd"
/note="color: #68e66d; direction:RIGHT"
/official_designation="Bsd"
/uid="70e7f83c244f451dae86de92afa03717"
/vntifkey="21"
/locus_tag="Bsd"
/label="Bsd"
/ApEinfo_label="Bsd"
/ApEinfo_fwdcolor="#e9d024"
/ApEinfo_revcolor="#e9d024"
/ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1}} {} 0}
width 5 offset 0"
misc_feature
3229..3260
/locus_tag="ROR"
/label="ROR"
/ApEinfo_label="ROR"
/ApEinfo_fwdcolor="#7eff74"
/ApEinfo_revcolor="#7eff74"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5}} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
promoter
complement(3309..3327)
/note="label="M13_reverse_primer" -
translation="MTEYKLVVVGAVGVGKSALTIQLIQNHVFDEYDPTIEDSYRKQV
-
VIDGETCLLDILDITAGQEEYSAMRDQYMRTGEGFLCVFAINNNTKSFEDIHHYREQIKR
-
VKDSEDVPMVLVGNKCDLPSRTVDTKQAQELARSYGIPFIETSAKTRQGVDDAFYTLV
- REIRKHKEKMSKDGKKKKKSRTRCTVM*" - complement("
/locus_tag="M13_reverse_primer"
/label="M13_reverse_primer"
/ApEinfo_label="M13_reverse_primer"
/ApEinfo_fwdcolor="#346ee0"
/ApEinfo_revcolor="#346ee0"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5}} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
primer_bind
complement(3313..3329)
/note="common sequencing primer, one of multiple similar
variants"
/locus_tag="M13 rev"
/label="M13 rev"
/ApEinfo_label="M13 rev"
/ApEinfo_fwdcolor="#14c0bd"
/ApEinfo_revcolor="#4ec02b"

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misc_feature      /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
complement(3326..3348)
/note="label="M13_pUC_rev_primer" -
translation="MTEYKLVVVGAVGVGKSALTIQLIQNHVFDEYDPTIEDSYRKQV
-
VIDGETCLLDILDITAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKR
-
VKDSEDVPMVLVGNKCDLPSRTVDTKQAQELARSYGIPFIETSAKTRQGVDDAFYTLV
- REIRKHKEKMSKDGKKKKKKSRTCTVM*" - complement("
/locus_tag="M13_pUC_rev_primer"
/label="M13_pUC_rev_primer"
/ApEinfo_label="M13_pUC_rev_primer"
/ApEinfo_fwdcolor="#7eff74"
/ApEinfo_revcolor="#7eff74"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
protein_bind     3337..3353
/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)."
```

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/locus_tag="lac operator"
/label="lac operator"
/ApEinfo_label="lac operator"
/ApEinfo_fwdcolor="#00D95A"
/ApEinfo_revcolor="#00D95A"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
promoter         complement(3361..3391)
/note="promoter for the E. coli lac operon"
/locus_tag="lac promoter"
/label="lac promoter"
/ApEinfo_label="lac promoter"
/ApEinfo_fwdcolor="#346ee0"
/ApEinfo_revcolor="#346ee0"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
promoter         complement(3362..3391)
/note="label="lac_promoter" -
translation="MTEYKLVVVGAVGVGKSALTIQLIQNHVFDEYDPTIEDSYRKQV
-
VIDGETCLLDILDITAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKR
-
VKDSEDVPMVLVGNKCDLPSRTVDTKQAQELARSYGIPFIETSAKTRQGVDDAFYTLV
- REIRKHKEKMSKDGKKKKKKSRTCTVM*" - complement(2937.."
/locus_tag="lac_promoter"
/label="lac_promoter"
/ApEinfo_label="lac_promoter"
/ApEinfo_fwdcolor="#346ee0"
/ApEinfo_revcolor="#346ee0"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
protein_bind     3406..3427
/bound moiety="E. coli catabolite activator protein"
/note="CAP binding activates transcription in the presence
of cAMP."
/locus_tag="CAP binding site"
/label="CAP binding site"
/ApEinfo_label="CAP binding site"
/ApEinfo_fwdcolor="#008065"
/ApEinfo_revcolor="#008065"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
source           complement(2600..2824)
/locus_tag="source"
/label="source"

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/ApEinfo_label="source"
/ApEinfo_fwdcolor="#00DF87"
/ApEinfo_revcolor="#00DF87"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
/ApEinfo_hidden
rep_origin complement(3715..4303)
/direction=LEFT
/note="high-copy-number ColE1/pMB1/pBR322/pUC origin of
replication"
/locus_tag="ori"
/label="ori"
/ApEinfo_label="ori"
/ApEinfo_fwdcolor="#999999"
/ApEinfo_revcolor="#999999"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
gene complement(4474..5334)
/note="label="Ampicillin" - gene="Ampicillin" -
translation="MTEYKLVVVGAVGVGKSALTIQLIQNHVFDEYDPTIEDSYRKQV
-
VIDGETCLLDILDTAGQEEYSAMRDQYMRGTGEGFLCVFAINNTKSFEDIHHYREQIKR
-
VKDSEDVPMVLVGNKCDLPSRTVDTKQAQELARSYGIPFIETSAKTRQGVDDAFYTLV
- REIRKHKEKMSKDGKKKKKSRTCTVM*" -"
/locus_tag="AmpR"
/label="AmpR"
/ApEinfo_label="AmpR"
/ApEinfo_fwdcolor="#ff797d"
/ApEinfo_revcolor="#ff102f"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
CDS complement(4474..5334)
/note="label="ORF frame 2" -
translation="MSIQHFRVALIPFFAAFCPLPVFAHPETLVKVKDAEDQLGARVGY
-
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
-
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
- DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEA"
/locus_tag="ORF frame 2"
/label="ORF frame 2"
/ApEinfo_label="ORF frame 2"
/ApEinfo_fwdcolor="#e9d024"
/ApEinfo_revcolor="#e9d024"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
promoter complement(5335..5439)
/gene="bla"
/locus_tag="AmpR promoter"
/label="AmpR promoter"
/ApEinfo_label="AmpR promoter"
/ApEinfo_fwdcolor="#346ee0"
/ApEinfo_revcolor="#346ee0"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
promoter complement(5376..5404)
/note="label="AmpR promoter" -
translation="MSIQHFRVALIPFFAAFCPLPVFAHPETLVKVKDAEDQLGARVGY
-
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
-
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
- DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWM"
/locus_tag="AmpR_promoter"
/label="AmpR_promoter"
/ApEinfo_label="AmpR_promoter"
/ApEinfo_fwdcolor="#346ee0"

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/ApEinfo_revcolor="#346ee0"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
misc_feature complement(5563..5585)
/note="label="pGEX_3_primer" -
translation="MSIQHFRVALIPFFAAFCPLPVFAHPETLVKVKDAEDQLGARVGY
-
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
-
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
- DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQQLIDWM"
/locus_tag="pGEX_3_primer"
/label="pGEX_3_primer"
/ApEinfo_label="pGEX_3_primer"
/ApEinfo_fwdcolor="#7eff74"
/ApEinfo_revcolor="#7eff74"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
misc_feature 2600..2824
/___SeqFeature___="True"
/___level___="0"
/application_notes="Allows transcription termination and
polyadenylation of mRNA transcribed by Pol II RNA
polymerase."
/full_name="Bovine growth hormone polyadenylation"
/name="BGH pA"
/note="color: #46c6ef; direction: LEFT"
/official_designation="BGH pA"
/uuid="439e92d8cb8e406dbfacd87ce28d14dc"
/vntifkey="21"
/locus_tag="BGH pA"
/label="BGH pA"
/ApEinfo_label="BGH pA"
/ApEinfo_fwdcolor="#7eff74"
/ApEinfo_revcolor="#7eff74"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
misc_feature 5898..5920
/note="label="M13_pUC_fwd_primer" -
translation="MSIQHFRVALIPFFAAFCPLPVFAHPETLVKVKDAEDQLGARVGY
-
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
-
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
- DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQ"
/locus_tag="M13_pUC_fwd_primer"
/label="M13_pUC_fwd_primer"
/ApEinfo_label="M13_pUC_fwd_primer"
/ApEinfo_fwdcolor="#7eff74"
/ApEinfo_revcolor="#7eff74"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
promoter 5913..5929
/note="label="M13_forward20_primer" -
translation="MSIQHFRVALIPFFAAFCPLPVFAHPETLVKVKDAEDQLGARVGY
-
IELDLNSGKILESFRPEERFPMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
-
YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRL
- DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASR"
/locus_tag="M13_forward20_primer"
/label="M13_forward20_primer"
/ApEinfo_label="M13_forward20_primer"
/ApEinfo_fwdcolor="#346ee0"
/ApEinfo_revcolor="#346ee0"
/ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
-0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
primer_bind 5913..5929

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        /note="common sequencing primer, one of multiple similar
        variants"
        /locus_tag="M13 fwd"
        /label="M13 fwd"
        /ApEinfo_label="M13 fwd"
        /ApEinfo_fwdcolor="#14c0bd"
        /ApEinfo_revcolor="#4ec02b"
        /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
        -0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
misc_feature complement(776..776)
        /locus_tag="G31(1)"
        /label="G31(1)"
        /ApEinfo_label="G31"
        /ApEinfo_fwdcolor="#ff7d78"
        /ApEinfo_revcolor="green"
        /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1} {} 0}
        width 5 offset 0"
variation complement(776..776)
        /locus_tag="G-to-A mutation (target A)"
        /label="G-to-A mutation (target A)"
        /ApEinfo_label="G-to-A mutation (target A)"
        /ApEinfo_fwdcolor="#fe0300"
        /ApEinfo_revcolor="#ff9e39"
        /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
        -0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
terminator 793..798
        /locus_tag="PolIII terminator"
        /label="PolIII terminator"
        /ApEinfo_label="PolIII terminator"
        /ApEinfo_fwdcolor="#9d1b1c"
        /ApEinfo_revcolor="#9d1b1c"
        /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1} {} 0}
        width 5 offset 0"
misc_binding 739..742
        /bound_moiety=""
        /locus_tag="4nt mismatch (as in Uzonyi et al 2021)"
        /label="4nt mismatch (as in Uzonyi et al 2021)"
        /ApEinfo_label="4nt mismatch (as in Uzonyi et al 2021)"
        /ApEinfo_fwdcolor="#ff4809"
        /ApEinfo_revcolor="#ff4809"
        /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
        -0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
misc_feature complement(762..775)
        /locus_tag="EAAGIGILTV(1)"
        /label="EAAGIGILTV(1)"
        /ApEinfo_label="EAAGIGILTV"
        /ApEinfo_fwdcolor="#009192"
        /ApEinfo_revcolor="#ff7d78"
        /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1} {} 0}
        width 5 offset 0"
ORIGIN
1 gaattcgcagc tcggtaccct acagttgaag tcggaagttt acatacactt aagttggagt
61 cattaaaact cgtttttcaa ctactccaca aatttcttgt taacaaacaa tagttttggc
121 aagtcagtta ggacatctac tttgtgcatg acacaagtca tttttccaac aattgtttac
181 agacagatta tttcacttat aattcactgt atcacaaatc cagtgggtca gaagtttaca
241 tacactaagt tgactgtgcc tttaaacagc ttggaaaatt ccagaaaatg atgtcatggc
301 tttagaagct tctgatagac taattgacat catttgagtc aattggagggt gtacctgtgg
361 atgtatttca aggaattctg tggaatgtgt gtcagttagg gtgtggaaag tccccaggct
421 ccccgagcagg cagaagtatg caaagcatgc ataaggtcgg gcaggaagag ggcctatttc
481 ccatgattcc ttcataattg catatacgat acaaggctgt tagagagata attagaatta
541 atttgactgt aaacacaaag atattagtac aaaatacgtg acgtagaaag taataatttc
601 ttgggtagtt tgcagtttta aaattatggt ttaaaatgga ctatcatatg cttaccgtaa
661 cttgaaagta tttcgatttc ttggctttat atatcttgtg gaaaggacga aacaccGGtt
721 ctacaatacc aacagccgTA CTgcagtaag actcccagga tcactgtcag gatgcCgatc
781 ccagcggcct ctttttttct agaccagct ttcttgtaca aactcgagaa taaaggtacc
841 ttattttcat tagatttgtg tgttggtttt ttgtgtgCTA GCgggttgcg ctttttccaa
901 ggcagccctg ggtttgcgca gggacgcggc tgctctgggc gtggttccgg gaaacgcagc
961 ggcgccgacc ctgggtctcg cacattcttc acgtccgttc gcagcgtcac ccgatcttc

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1021 gccgctaccc ttgtgggccc cccggcgacg cttcctgctc cgcccctaag tcgggaaggt
1081 tccttgcggt tcgcgcggtg ccggacgtga caaacggaag ccgcacgtct cactagtacc
1141 ctgcgacagc gacagcgcca gggagcaatg gcagcgcgcc gaccgcgatg ggctgtggcc
1201 aatagcggtc gctcagcagg gcgcgccagg agcagcgggc gggaaggggc ggtgcgggag
1261 gcgggggtgtg gggcggtagt gtgggcctcg ttcctgcccg cgcggtgttc cgcattctgc
1321 aagcctccgg agcgcacgtc ggcagtcggc tccctcggtg accgaatcac cgacctctct
1381 cccagggcaa gtttgtacaa aaaagcaggc tgccaccatg gtgagcaagg gcgaggagct
1441 gttcacccgg gtggtgcca tcctggtcga gctggacggc gacgtaaacg gccacaagtt
1501 cagcgtgtcc ggcgagggcg agggcgatgc cacctacggc aagctgaccc tgaagttcat
1561 ctgcaccacc ggcaagctgc ccgtgccctg gccaccctc gtgaccaccc tgaccacagg
1621 cgtgacgctc ttcagccgct accccgacca catgaagcag cagcacttct tcaagtcggc
1681 catgcccga ggcctacgtc aggagcgcac catcttcttc aaggacgacg gcaactacaa
1741 gacccgcgcc gaggtgaagt tcgagggcga caccctggtg aaccgcatcg agctgaaggg
1801 catcgacttc aaggaggacg gcaacatcct ggggcacaag ctggagtaca acttcaacag
1861 ccacaacgtc tatatcatgg ccgacaagca gaagaacggc atcaaggtga acttcaagat
1921 ccgccacaac atcgaggacg gcagcgtgca gctcgccgac cactaccagg agaacacccc
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