Appendix for

Engineering editopes through programmable RNA editing toward tumor neoantigen generation

Pecori *et al.*

Table of Contents:

a. Appendix Tables

#	Name	Sequence	Purpose	Figure
1	pENTR_plasm_F	TTTTTTCTAGACCCAGCTTTCTTGTA	To open via PCR pENTR-U6 plasmid	
2	pENTR_plasm_R	GGTGTTTCGTCCTTTCCACA	To open via PCR pENTR-U6 plasmid	
3	p2a-eGFP-NLS-6xHis_F	agaagaagaggaaagtctaaccggtGCTACTAACTTCAGCCTG	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	GTTTTAGAGCTAGAAATAGC	To open via PCR pFYF1320 plasmid	
6	pFYF1320_R	GGTGTTTCGTCCTTTCCACA	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	TGGACTTGGAGGGATTTCAG	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	acactctttccctacacgacgctcttccgatctAACTTCAGCCTGCTCAAACAAGCC	MiSeq amplicon sequencing on eGFP W58X.	1, S1
16	MiSeq eGFP R	gactggagttcagacgtgtgctcttccgatctCAGCCCTGGTCTTGTAGTTG	MiSeq amplicon sequencing on eGFP W58X.	1, S1
17	RT-PCR TP53 F	TTGATTCCACACCCCCGCCC	To amplify the Y220 site of the *TP53* mRNA.	2
18	RT-PCR TP53 R	TGTTGTTGGGCAGTGCTCGC	To amplify the Y220 site of the *TP53* mRNA.	2
19	TP53 sequencing	GTCCCAGTAGATTACCAC	Primer for Sanger sequencing and quantify editing the Y220 site of the *TP53* mRNA.	2
20	RT-PCR USP9X F	AGCGTATGTCATACAGGCGCCAGA	To amplify the Y2009 site of the *USP9X* mRNA.	2
21	RT-PCR USP9X R	CACTGGCAGAGCCACGGACTACT	To amplify the Y2009 site of the *USP9X* mRNA.	2
22	USP9X sequencing	CGAATGGACACAATAGACC	Primer for Sanger sequencing and quantify editing the Y2009 site of the *USP9X* mRNA.	2
23	RT-PCR C6orf89 F	TTTTGTGCCAGTACCCTGAGGCGA	To amplify the Y813 site of the *C6orf89* mRNA.	2
24	RT-PCR C6orf89 R	GCCTTGCAGCTCACAGCTGGTT	To amplify the Y813 site of the *C6orf89* mRNA.	2
25	C6orf89 sequencing	TCCTTTCTTCACCCAG	Primer for Sanger sequencing and quantify editing the Y813 site of the *C6orf89* 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

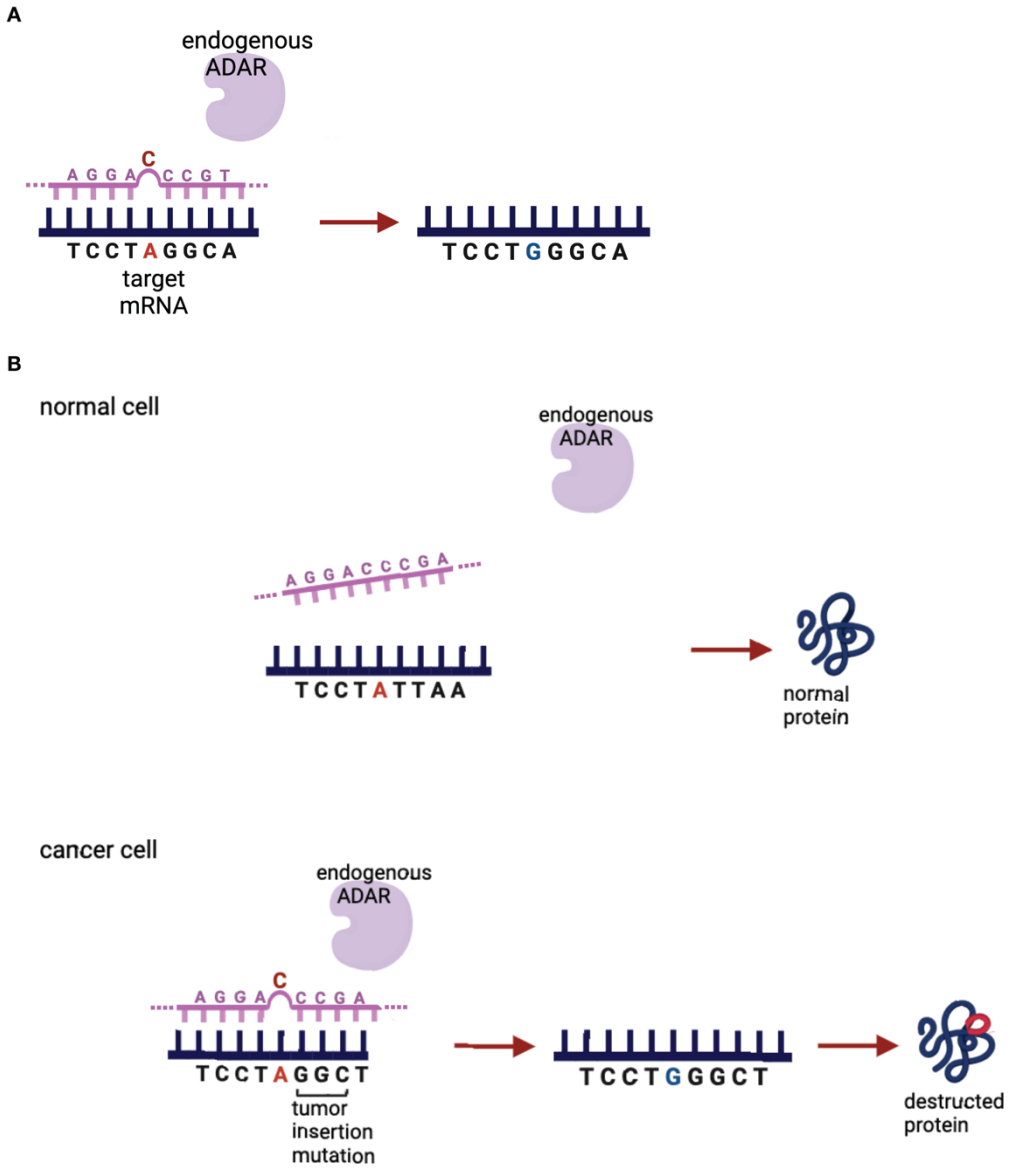
			MART1^{G31S} T and NT-SPEAR gRNA stable cell lines.	
28	RT-qPCR ADAR2 F	GACCCTGTCTGTCAACACGG	To quantify the ADAR2 expression in 624-mel MART1^{G31S} T and NT-SPEAR gRNA stable cell lines.	S3
29	RT-qPCR ADAR2 R	CCCACGTAAAAGGGAGGCTC	To quantify ADAR2 expression in 624-mel MART1^{G31S} T and NT-SPEAR gRNA 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.

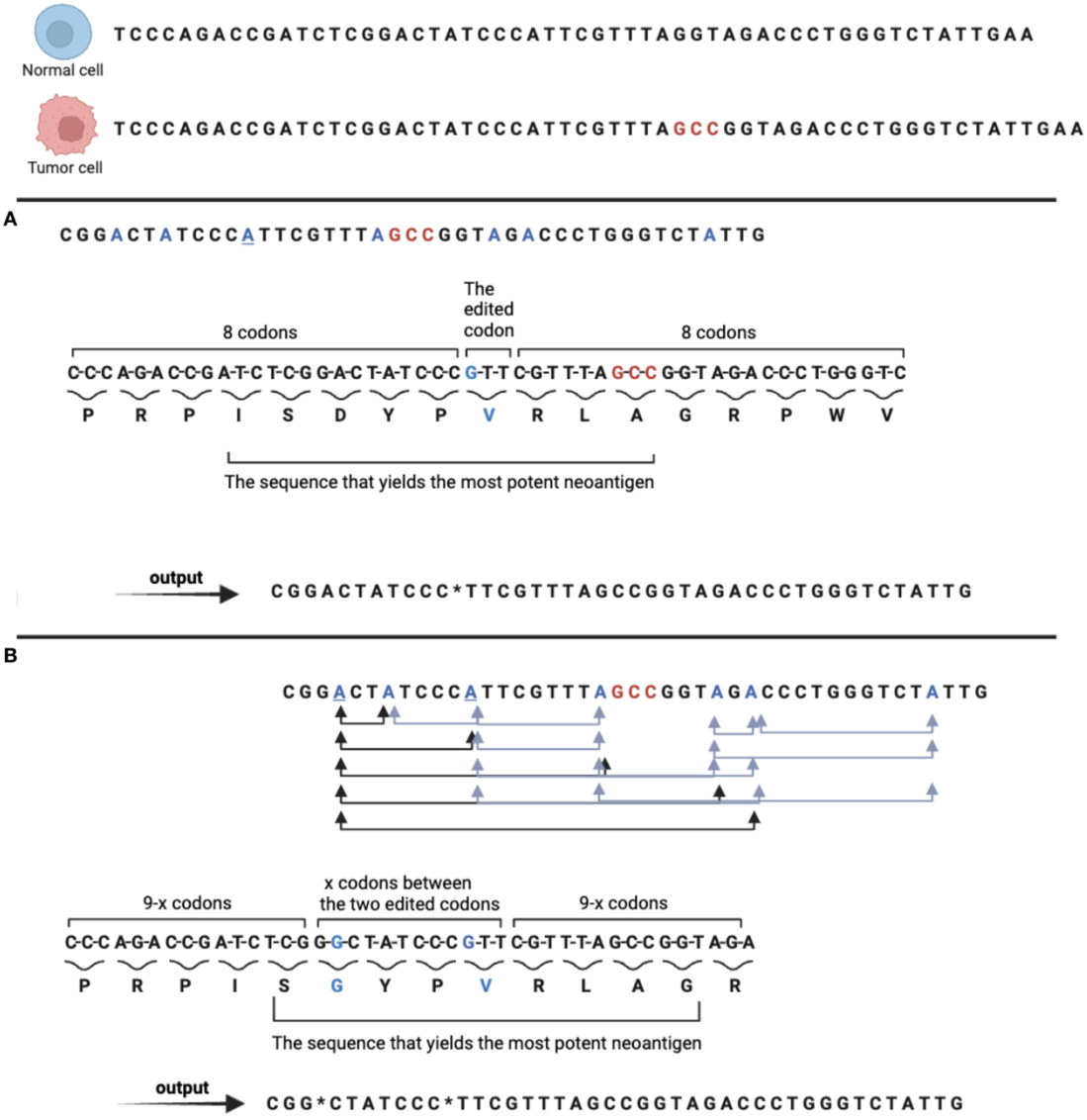
TCGA project	***Individuals in this project***	***Individuals included in the analysis***	***DNA files used***	***Whole genome files used***
BRCA	969	965	755	211
SKCM	470	467 (primary:102 metastatic:365)	467	-
OV	419	289	26	263
GBM	374	147	147	-
LIHC	369	362	362	-
LUAD	559	507	505	2
LUSC	490	487	481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

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

b. Appendix Figures

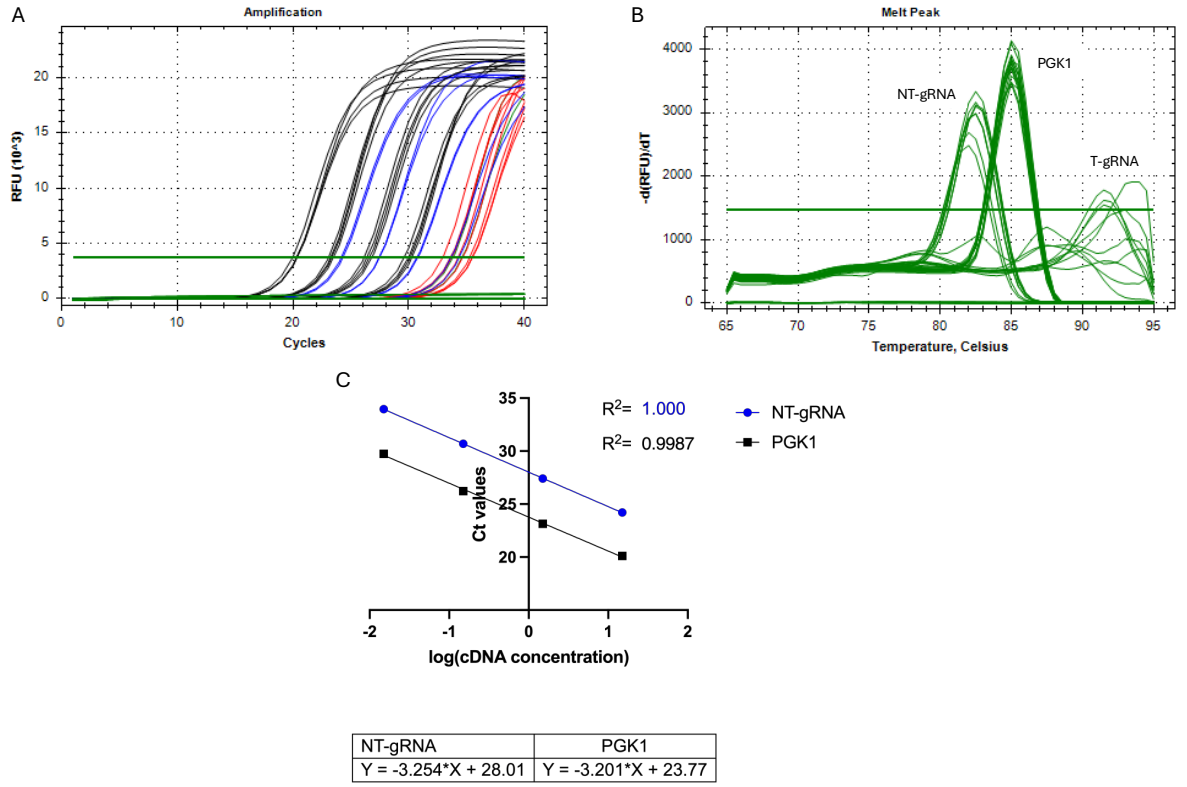


Appendix Figure S1. Schematic presentation of Endogenous-ADAR and of our method. (**A**) Illustration of the endogenous-ADAR editor that converts adenosine into inosine at the RNA level. The editing depends solely on a gRNA that recruits the endogenous cellular ADAR enzyme. The gRNA ensures target specificity 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 hypothetic 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.



NT-gRNA	PGK1
Y = -3.254*X + 28.01	Y = -3.201*X + 23.77

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

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     1501 gatgagatgg tcagactaaa ctggctgacg gaatttatgc ctcttccgac catcaagcat
     1561 tttatccgta ctcctgatga tgcatggtta ctcaccactg cgatccccgg aaaaacagca
     1621 ttccaggtat tagaagaata tcctgattca ggtgaaaata ttgttgatgc gctggcagtg
     1681 ttcctgcgcc ggttgcattc gattcctgtt tgtaattgtc cttttaacag cgatcgcgta
     1741 tttcgtctcg ctcaggcgca atcacgaatg aataacggtt tggttgatgc gagtgatttt
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     1861 ccattctcac cggattcagt cgtcactcat ggtgatttct cacttgataa ccttatttttt
     1921 gacgaggggga aattaatagg ttgtattgat gttggacgag tcggaatcgc agaccgatac
     1981 caggatcttg ccatcctatg gaactgcctc ggtgagtttt ctccttcatt acagaaacgg
     2041 ctttttcaaa aatatggtat tgataatcct gatatgaata aattgcagtt tcatttgatg
     2101 ctcgatgagt ttttctaatc agaattggtt aattggttgt aacactggca gagcattacg
     2161 ctgacttgac gggacggcgc aagctcatga ccaaaatccc ttaacgtgag ttacgcgtcg
     2221 ttccactgag cgtcagaccc cgtagaaaag atcaaaggat cttcttgaga tccttttttt
     2281 ctgcgcgtaa tctgctgctt gcaaacaaaa aaaccaccgc taccagcggt ggtttgtttg
     2341 ccggatcaag agctaccaac tctttttccg aaggtaactg gcttcagcag agcgcagata
     2401 ccaaatactg tTcttctagt gtagccgtag ttaggccacc acttcaagaa ctctgtagca
     2461 ccgcctacat acctcgctct gctaatcctg ttaccagtgg ctgctgccag tggcgataag
     2521 tcgtgtctta ccgggttgga ctcaagacga tagttaccgg ataaggcgca gcggtcgggc
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     2761 gcctggtatc tttatagtcc tgtcgggttt cgccacctct gacttgagcg tcgatttttg
     2821 tgatgctcgt caggggggcg gagcctatgg aaaaacgcca gcaacgcggc ctttttacgg
     2881 ttcctggcct tttgctggcc ttttgctcac atgtt
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Plasmid pENTR-U6 GluRv9.4-16 (eGFP^{W58X}) sequence (GenBank format, provided as text)

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VERSION
SOURCE      .
  ORGANISM  .
COMMENT
COMMENT
COMMENT     ApEinfo:methylated:1
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                     width 5 offset 0"
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                     width 5 offset 0"
     misc_feature    969..1023
                     /locus_tag="GluR loop"
                     /ApEinfo_fwdcolor="cyan"
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      121 gcgcccaata cgcaaaccgc ctctccccgc gcgttggccg attcattaat gcagctggca
      181 cgacaggttt cccgactgga aagcgggcag tgagcgcaac gcaattaata cgcgtaccgc
      241 tagccaggaa gagtttgtag aaacgcaaaa aggccatccg tcaggatggc cttctgctta
      301 gtttgatgcc tggcagttta tggcgggcgt cctgcccgcc accctccggg ccgttgcttc
      361 acaacgttca aatccgctcc cggcggattt gtcctactca ggagagcgtt caccgacaaa
      421 caacagataa aacgaaaggc ccagtcttcc gactgagcct ttcgttttat ttgatgcctg
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      541 aacgacggcc agtcttaagc tcgggcccca aataatgatt ttattttgac tgatagtgac
      601 ctgttcgttg caacaaattg atgagcaatg ctttttttata atgccaactt tgtacaaaaa
      661 agcaggcttt aaaggaacca attcagtcga ctggatccgg taccaaggtc gggcaggaag
      721 agggcctatt tcccatgatt ccttcatatt tgcatatacg atacaaggct gttagagaga
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      901 tgcttaccgt aacttgaaag tatttcgatt tcttggcttt atatatcttg tggaaaggac
      961 gaaacaccgg tgTCGAGaaG agGaGaacaa tatgctaaat gttgttCtCg tCtcCTCGAc
     1021 accgttggcC atggaacagt tttttctaga cccagctttc ttgtacaaag ttggcattat
     1081 aagaaagcat tgcttatcaa tttgttgcaa cgaacaggtc actatcagtc aaaataaaat
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     1321 gagccatatt caacgggaaa cgtcgaggcc gcgattaaat tccaacatgg atgctgattt
     1381 atatgggtat aaatgggctc gcgataatgt cgggcaatca ggtgcgacaa tctatcgctt
     1441 gtatgggaag cccgatgcgc cagagttgtt tctgaaacat ggcaaaggta gcgttgccaa
     1501 tgatgttaca gatgagatgg tcagactaaa ctggctgacg gaatttatgc ctcttccgac
     1561 catcaagcat tttatccgta ctcctgatga tgcatggtta ctcaccactg cgatccccgg
     1621 aaaaacagca ttccaggtat tagaagaata tcctgattca ggtgaaaata ttgttgatgc
     1681 gctggcagtg ttcctgcgcc ggttgcattc gattcctgtt tgtaattgtc cttttaacag
     1741 cgatcgcgta tttcgtctcg ctcaggcgca atcacgaatg aataacggtt tggttgatgc
     1801 gagtgatttt gatgacgagc gtaatggctg gcctgttgaa caagtctgga aagaaatgca
     1861 taaacttttg ccattctcac cggattcagt cgtcactcat ggtgatttct cacttgataa
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     1981 agaccgatac caggatcttg ccatcctatg gaactgcctc ggtgagtttt ctccttcatt
     2041 acagaaacgg ctttttcaaa aatatggtat tgataatcct gatatgaata aattgcagtt
     2101 tcatttgatg ctcgatgagt ttttctaatc agaattggtt aattggttgt aacactggca
     2161 gagcattacg ctgacttgac gggacggcgc aagctcatga ccaaaatccc ttaacgtgag
     2221 ttacgcgtcg ttccactgag cgtcagaccc cgtagaaaag atcaaaggat cttcttgaga
     2281 tccttttttt ctgcgcgtaa tctgctgctt gcaaacaaaa aaaccaccgc taccagcggt
     2341 ggtttgtttg ccggatcaag agctaccaac tctttttccg aaggtaactg gcttcagcag
     2401 agcgcagata ccaaatactg tTcttctagt gtagccgtag ttaggccacc acttcaagaa
     2461 ctctgtagca ccgcctacat acctcgctct gctaatcctg ttaccagtgg ctgctgccag
     2521 tggcgataag tcgtgtctta ccgggttgga ctcaagacga tagttaccgg ataaggcgca
     2581 gcggtcgggc tgaacggggg gttcgtgcac acagcccagc ttggagcgaa cgacctacac
     2641 cgaactgaga tacctacagc gtgagcattg agaaagcgcc acgcttcccg aagggagaaa
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     2761 agggggaaac gcctggtatc tttatagtcc tgtcgggttt cgccacctct gacttgagcg
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Plasmid pENTR-U6 GluRv9.4-38 (eGFP^{W58X}) sequence (GenBank format, provided as text)

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ACCESSION
VERSION
SOURCE      .
  ORGANISM  .
COMMENT
COMMENT
COMMENT     ApEinfo:methylated:1
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     terminator      1062..1067
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ORIGIN
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      121 gcgcccaata cgcaaaccgc ctctccccgc gcgttggccg attcattaat gcagctggca
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      421 caacagataa aacgaaaggc ccagtcttcc gactgagcct ttcgttttat ttgatgcctg
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      541 aacgacggcc agtcttaagc tcgggcccca aataatgatt ttattttgac tgatagtgac
      601 ctgttcgttg caacaaattg atgagcaatg ctttttttata atgccaactt tgtacaaaaa
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     1261 gttacattgc acaagataaa aatatatcat catgaacaat aaaactgtct gcttacataa
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     1501 atggcaaagg tagcgttgcc aatgatgtta cagatgagat ggtcagacta aactggctga
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     1861 aacaagtctg gaaagaaatg cataaacttt tgccattctc accggattca gtcgtcactc
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     2161 ttaattggtt gtaacactgg cagagcatta cgctgacttg acgggacggc gcaagctcat
     2221 gaccaaaatc ccttaacgtg agttacgcgt cgttccactg agcgtcagac cccgtagaaa
     2281 agatcaaagg atcttcttga gatccttttt ttctgcgcgt aatctgctgc ttgcaaacaa
     2341 aaaaaccacc gctaccagcg gtggtttgtt tgccggatca agagctacca actcttttc
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     2521 tgttaccagt ggctgctgcc agtggcgata agtcgtgtct taccgggttg gactcaagac
     2581 gatagttacc ggataaggcg cagcggtcgg gctgaacggg gggttcgtgc acacagccca
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Plasmid pENTR-U6 LEAPER-70 (eGFP^{W58X}) sequence (GenBank format, provided as text)

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  ORGANISM  .
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COMMENT
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     2581 agcggtcggg ctgaacgggg ggttcgtgca cacagcccag cttggagcga acgacctaca
     2641 ccgaactgag atacctacag cgtgagcatt gagaaagcgc cacgcttccc gaagggagaa
     2701 aggcggacag gtatccggta agcggcaggg tcggaacagg agagcgcacg agggagcttc
     2761 cagggggaaa cgcctggtat ctttatagtc ctgtcgggtt tcgccacctc tgacttgagc
     2821 gtcgattttt gtgatgctcg tcaggggggc ggagcctatg gaaaaacgcc agcaacgcgg
     2881 cctttttacg gttcctggcc ttttgctggc cttttgctca catgtt
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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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ACCESSION
VERSION
SOURCE      .
  ORGANISM  .
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                     /ApEinfo_revcolor="#7eff74"
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                     width 5 offset 0"
     promoter        962..968
                     /vntifkey="29"
                     /locus_tag="hU6"
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                     /ApEinfo_revcolor="#346ee0"
                     /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1} {} 0}
                     width 5 offset 0"
     promoter        705..961
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                     /locus_tag="hU6(1)"
                     /ApEinfo_label="hU6"
                     /ApEinfo_fwdcolor="#346ee0"
                     /ApEinfo_revcolor="#346ee0"
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      121 gcgcccaata cgcaaaccgc ctctccccgc gcgttggccg attcattaat gcagctggca
      181 cgacaggttt cccgactgga aagcgggcag tgagcgcaac gcaattaata cgcgtaccgc
      241 tagccaggaa gagtttgtag aaacgcaaaa aggccatccg tcaggatggc cttctgctta
      301 gtttgatgcc tggcagttta tggcgggcgt cctgcccgcc accctccggg ccgttgcttc
      361 acaacgttca aatccgctcc cggcggattt gtcctactca ggagagcgtt caccgacaaa
      421 caacagataa aacgaaaggc ccagtcttcc gactgagcct ttcgttttat ttgatgcctg
      481 gcagttccct actctcgcgt taacgctagc atggatgttt tcccagtcac gacgttgtaa
      541 aacgacggcc agtcttaagc tcgggcccca aataatgatt ttattttgac tgatagtgac
      601 ctgttcgttg caacaaattg atgagcaatg ctttttttata atgccaactt tgtacaaaaa
      661 agcaggcttt aaaggaacca attcagtcga ctggatccgg taccaaggtc gggcaggaag
      721 agggcctatt tcccatgatt ccttcatatt tgcatatacg atacaaggct gttagagaga
      781 taattagaat taatttgact gtaaacacaa agatattagt acaaaatacg tgacgtagaa
      841 agtaataatt tcttgggtag tttgcagttt taaaattatg ttttaaaatg gactatcata
      901 tgcttaccgt aacttgaaag tatttcgatt tcttggcttt atatatcttg tggaaaggac
      961 gaaacaccGG tcgtgctgtt tcatgtggtc ggggtacctg ctgaagcatt gcacgccgta
     1021 tgtcagggta gtgacaagtg ttggcCatgg aacaggtagt tttccagtag tgcaaataaa
     1081 cttcagggtc agcttgccat aggtggcatc gccctctcct ttttttttcta gacccagctt
     1141 tcttgtacaa agttggcatt ataagaaagc attgcttatc aatttgttgc aacgaacagg
     1201 tcactatcag tcaaaataaa atcattattt gccatccagc tgatatcccc tatagtgagt
     1261 cgtattacat ggtcatagct gtttcctggc agctctggcc cgtgtctcaa aatctctgat
     1321 gttacattgc acaagataaa aatatatcat catgaacaat aaaactgtct gcttacataa
     1381 acagtaatac aaggggtgtt atgagccata ttcaacggga aacgtcgagg ccgcgattaa
     1441 attccaacat ggatgctgat ttatatgggt ataaatgggc tcgcgataat gtcgggcaat
     1501 caggtgcgac aatctatcgc ttgtatggga agcccgatgc gccagagttg tttctgaaac
     1561 atggcaaagg tagcgttgcc aatgatgtta cagatgagat ggtcagacta aactggctga
     1621 cggaatttat gcctcttccg accatcaagc attttatccg tactcctgat gatgcatggt
     1681 tactcaccac tgcgatcccc ggaaaaacag cattccaggt attagaagaa tatcctgatt
     1741 caggtgaaaa tattgttgat gcgctggcag tgttcctgcg ccggttgcat tcgattcctg
     1801 tttgtaattg tccttttaac agcgatcgcg tatttcgtct cgctcaggcg caatcacgaa
     1861 tgaataacgg tttggttgat gcgagtgatt ttgatgacga gcgtaatggc tggcctgttg
     1921 aacaagtctg gaaagaaatg cataaacttt tgccattctc accggattca gtcgtcactc
     1981 atggtgattt ctcacttgat aaccttattt ttgacgaggg gaaattaata ggttgtattg
     2041 atgttggacg agtcggaatc gcagaccgat accaggatct tgccatccta tggaactgcc
     2101 tcggtgagtt ttctccttca ttacagaaac ggctttttca aaaatatggt attgataatc
     2161 ctgatatgaa taaattgcag tttcatttga tgctcgatga gtttttctaa tcagaattgg
     2221 ttaattggtt gtaacactgg cagagcatta cgctgacttg acgggacggc gcaagctcat
     2281 gaccaaaatc ccttaacgtg agttacgcgt cgttccactg agcgtcagac cccgtagaaa
     2341 agatcaaagg atcttcttga gatccttttt ttctgcgcgt aatctgctgc ttgcaaacaa
     2401 aaaaaccacc gctaccagcg gtggtttgtt tgccggatca agagctacca actcttttc
     2461 cgaaggtaac tggcttcagc agagcgcaga taccaaatac tgtTcttcta gtgtagccgt
     2521 agttaggcca ccacttcaag aactctgtag caccgcctac atacctcgct ctgctaatcc
     2581 tgttaccagt ggctgctgcc agtggcgata agtcgtgtct taccgggttg gactcaagac
     2641 gatagttacc ggataaggcg cagcggtcgg gctgaacggg gggttcgtgc acacagccca
     2701 gcttggagcg aacgacctac accgaactga gatacctaca gcgtgagcat tgagaaagcg
     2761 ccacgcttcc cgaagggaga aaggcggaca ggtatccggt aagcggcagg gtcggaacag
     2821 gagagcgcac gagggagctt ccagggggaa acgcctggta tctttatagt cctgtcgggt
     2881 ttcgccacct ctgacttgag cgtcgatttt tgtgatgctc gtcaggggggg cggagcctat
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     3001 acatgtt
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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)

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ACCESSION
VERSION
SOURCE      .
  ORGANISM  .
COMMENT
COMMENT     ApEinfo:methylated:1
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                     /ApEinfo_revcolor="#7eff74"
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     promoter        962..968
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                     width 5 offset 0"
     promoter        705..961
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      121 gcgcccaata cgcaaaccgc ctctccccgc gcgttggccg attcattaat gcagctggca
      181 cgacaggttt cccgactgga aagcgggcag tgagcgcaac gcaattaata cgcgtaccgc
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      301 gtttgatgcc tggcagttta tggcgggcgt cctgcccgcc accctccggg ccgttgcttc
      361 acaacgttca aatccgctcc cggcggattt gtcctactca ggagagcgtt caccgacaaa
      421 caacagataa aacgaaaggc ccagtcttcc gactgagcct ttcgttttat ttgatgcctg
      481 gcagttccct actctcgcgt taacgctagc atggatgttt tcccagtcac gacgttgtaa
      541 aacgacggcc agtcttaagc tcgggcccca aataatgatt ttattttgac tgatagtgac
      601 ctgttcgttg caacaaattg atgagcaatg ctttttttata atgccaactt tgtacaaaaa
      661 agcaggcttt aaaggaacca attcagtcga ctggatccgg taccaaggtc gggcaggaag
      721 agggcctatt tcccatgatt ccttcatatt tgcatatacg atacaaggct gttagagaga
      781 taattagaat taatttgact gtaaacacaa agatattagt acaaaatacg tgacgtagaa
      841 agtaataatt tcttgggtag tttgcagttt taaaattatg ttttaaaatg gactatcata
      901 tgcttaccgt aacttgaaag tatttcgatt tcttggcttt atatatcttg tggaaaggac
      961 gaaacaccGG tcgtgctgtt tcatgtggtc ggggtacctg ctgaagcaaa cgacgccgta
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     1321 gttacattgc acaagataaa aatatatcat catgaacaat aaaactgtct gcttacataa
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     1441 attccaacat ggatgctgat ttatatgggt ataaatgggc tcgcgataat gtcgggcaat
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     1921 aacaagtctg gaaagaaatg cataaacttt tgccattctc accggattca gtcgtcactc
     1981 atggtgattt ctcacttgat aaccttattt ttgacgaggg gaaattaata ggttgtattg
     2041 atgttggacg agtcggaatc gcagaccgat accaggatct tgccatccta tggaactgcc
     2101 tcggtgagtt ttctccttca ttacagaaac ggctttttca aaaatatggt attgataatc
     2161 ctgatatgaa taaattgcag tttcatttga tgctcgatga gtttttctaa tcagaattgg
     2221 ttaattggtt gtaacactgg cagagcatta cgctgacttg acgggacggc gcaagctcat
     2281 gaccaaaatc ccttaacgtg agttacgcgt cgttccactg agcgtcagac cccgtagaaa
     2341 agatcaaagg atcttcttga gatccttttt ttctgcgcgt aatctgctgc ttgcaaacaa
     2401 aaaaaccacc gctaccagcg gtggtttgtt tgccggatca agagctacca actcttttc
     2461 cgaaggtaac tggcttcagc agagcgcaga taccaaatac tgtTcttcta gtgtagccgt
     2521 agttaggcca ccacttcaag aactctgtag caccgcctac atacctcgct ctgctaatcc
     2581 tgttaccagt ggctgctgcc agtggcgata agtcgtgtct taccgggttg gactcaagac
     2641 gatagttacc ggataaggcg cagcggtcgg gctgaacggg gggttcgtgc acacagccca
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     2821 gagagcgcac gagggagctt ccagggggaa acgcctggta tctttatagt cctgtcgggt
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     2941 ggaaaaacgc cagcaacgcg gccttttac ggttcctggc cttttgctgg ccttttgctc
     3001 acatgtt
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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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VERSION
SOURCE      .
  ORGANISM  .
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COMMENT
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                     width 5 offset 0"
     promoter        962..968
                     /vntifkey="29"
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                     /ApEinfo_fwdcolor="#346ee0"
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                     width 5 offset 0"
     promoter        705..961
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     misc_binding    1009..1012
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ORIGIN
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      121 gcgcccaata cgcaaaccgc ctctccccgc gcgttggccg attcattaat gcagctggca
      181 cgacaggttt cccgactgga aagcgggcag tgagcgcaac gcaattaata cgcgtaccgc
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      301 gtttgatgcc tggcagttta tggcgggcgt cctgcccgcc accctccggg ccgttgcttc
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     1681 gaagaatatc ctgattcagg tgaaaatatt gttgatgcgc tggcagtgtt cctgcgccgg
     1741 ttgcattcga ttcctgtttg taattgtcct tttaacagcg atcgcgtatt tcgtctcgct
     1801 caggcgcaat cacgaatgaa taacggtttg gttgatgcga gtgattttga tgacgagcgt
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     2101 tatggtattg ataatcctga tatgaataaa ttgcagtttc atttgatgct cgatgagttt
     2161 ttctaatcag aattggttaa ttggttgtaa cactggcaga gcattacgct gacttgacgg
     2221 gacggcgcaa gctcatgacc aaaatccctt aacgtgagtt acgcgtcgtt ccactgagcg
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     2581 gggttggact caagacgata gttaccggat aaggcgcagc ggtcgggctg aacggggggt
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     2881 gggggggcgga gcctatggaa aaacgccagc aacgcggcct ttttacggtt cctggccttt
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Plasmid pENTR-U6 38-4ntMM-33-C-16 (sdl 92) (eGFP^{W58X}) sequence (GenBank format, provided as text)

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ACCESSION
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  ORGANISM  .
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                     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
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                     /locus_tag="hU6(1)"
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                     /ApEinfo_fwdcolor="#346ee0"
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                     /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1} {} 0}
                     width 5 offset 0"
     rep_origin      2282..2901
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                     /locus_tag="pBR322ori"
                     /ApEinfo_fwdcolor="#999999"
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                     width 5 offset 0"
     terminator      1063..1068
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                     /locus_tag="4nt mismatch (as in Uzonyi et al 2021)"
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       61 taccgctcgc cgcagccgaa cgaccgagcg cagcgagtca gtgagcgagg aagcggaaga
      121 gcgcccaata cgcaaaccgc ctctccccgc gcgttggccg attcattaat gcagctggca
      181 cgacaggttt cccgactgga aagcgggcag tgagcgcaac gcaattaata cgcgtaccgc
      241 tagccaggaa gagtttgtag aaacgcaaaa aggccatccg tcaggatggc cttctgctta
      301 gtttgatgcc tggcagttta tggcgggcgt cctgcccgcc accctccggg ccgttgcttc
      361 acaacgttca aatccgctcc cggcggattt gtcctactca ggagagcgtt caccgacaaa
      421 caacagataa aacgaaaggc ccagtcttcc gactgagcct ttcgttttat ttgatgcctg
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      481 gcagttccct actctcgcgt taacgctagc atggatgttt tcccagtcac gacgttgtaa
      541 aacgacggcc agtcttaagc tcgggcccca aataatgatt ttattttgac tgatagtgac
      601 ctgttcgttg caacaaattg atgagcaatg ctttttttata atgccaactt tgtacaaaaa
      661 agcaggcttt aaaggaacca attcagtcga ctggatccgg taccaaggtc gggcaggaag
      721 agggcctatt tcccatgatt ccttcatatt tgcatatacg atacaaggct gttagagaga
      781 taattagaat taatttgact gtaaacacaa agatattagt acaaaatacg tgacgtagaa
      841 agtaataatt tcttgggtag tttgcagttt taaaattatg ttttaaaatg gactatcata
      901 tgcttaccgt aacttgaaag tatttcgatt tcttggcttt atatatcttg tggaaaggac
      961 gaaacaccGG tcgtgctgtt tcatgtggtc ggggtacctg ctgaagcaaa cgacgccgta
     1021 tgtcagggta gtgacaagtg ttggcCatgg aacaggtagt tttttttttct agacccagct
     1081 ttcttgtaca aagttggcat tataagaaag cattgcttat caatttgttg caacgaacag
     1141 gtcactatca gtcaaaataa aatcattatt tgccatccag ctgatatccc ctatagtgag
     1201 tcgtattaca tggtcatagc 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 catggcaaag gtagcgttgc caatgatgtt acagatgaga tggtcagact aaactggctg
     1561 acggaattta tgcctcttcc gaccatcaag cattttatcc gtactcctga tgatgcatgg
     1621 ttactcacca 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 aggttgtatt
     1981 gatgttggac gagtcggaat cgcagaccga taccaggatc ttgccatcct atggaactgc
     2041 ctcggtgagt tttctccttc attacagaaa cggctttttc aaaaatatgg tattgataat
     2101 cctgatatga ataaattgca gtttcatttg atgctcgatg agtttttcta atcagaattg
     2161 gttaattggt tgtaacactg gcagagcatt acgctgactt gacgggacgg cgcaagctca
     2221 tgaccaaaat cccttaacgt gagttacgcg tcgttccact gagcgtcaga ccccgtagaa
     2281 aagatcaaag gatcttcttg agatcctttt tttctgcgcg taatctgctg cttgcaaaca
     2341 aaaaaaccac cgctaccagc ggtggtttgt ttgccggatc aagagctacc aactcttttt
     2401 ccgaaggtaa ctggcttcag cagagcgcag ataccaaata ctgtTcttct agtgtagccg
     2461 tagttaggcc accacttcaa gaactctgta gcaccgccta catacctcgc tctgctaatc
     2521 ctgttaccag tggctgctgc cagtggcgat aagtcgtgtc ttaccgggtt ggactcaaga
     2581 cgatagttac cggataaggc gcagcggtcg ggctgaacgg ggggttcgtg cacacagccc
     2641 agcttggagc gaacgaccta caccgaactg agatacctac agcgtgagca ttgagaaagc
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     2761 ggagagcgca cgagggagct tccagggggа aacgcctggt atctttatag tcctgtcggg
     2821 tttcgccacc tctgacttga gcgtcgattt ttgtgatgct cgtcaggggg gcggagccta
     2881 tggaaaaacg ccagcaacgc ggccttttta cggttcctgg ccttttgctg gccttttgct
     2941 cacatgtt
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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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ACCESSION
VERSION
SOURCE      .
  ORGANISM  .
COMMENT
COMMENT
COMMENT     ApEinfo:methylated:1
FEATURES             Location/Qualifiers
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                     /ApEinfo_revcolor="#7eff74"
                     /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1} {} 0}
                     width 5 offset 0"
     promoter        962..968
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                     /locus_tag="hU6"
                     /ApEinfo_fwdcolor="#346ee0"
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     misc_binding    991..994
                     /locus_tag="4nt mismatch (as in Uzonyi et al 2021)"
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                     width 5 offset 0"
ORIGIN
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       61 taccgctcgc cgcagccgaa cgaccgagcg cagcgagtca gtgagcgagg aagcggaaga
      121 gcgcccaata cgcaaaccgc ctctccccgc gcgttggccg attcattaat gcagctggca
      181 cgacaggttt cccgactgga aagcgggcag tgagcgcaac gcaattaata cgcgtaccgc
      241 tagccaggaa gagtttgtag aaacgcaaaa aggccatccg tcaggatggc cttctgctta
      301 gtttgatgcc tggcagttta tggcgggcgt cctgcccgcc accctccggg ccgttgcttc
      361 acaacgttca aatccgctcc cggcggattt gtcctactca ggagagcgtt caccgacaaa
      421 caacagataa aacgaaaggc ccagtcttcc gactgagcct ttcgttttat ttgatgcctg
      481 gcagttccct actctcgcgt taacgctagc atggatgttt tcccagtcac gacgttgtaa
      541 aacgacggcc agtcttaagc tcgggcccca aataatgatt ttattttgac tgatagtgac
      601 ctgttcgttg caacaaattg atgagcaatg ctttttttata atgccaactt tgtacaaaaa
      661 agcaggcttt aaaggaacca attcagtcga ctggatccgg taccaaggtc gggcaggaag
      721 agggcctatt tcccatgatt ccttcatatt tgcatatacg atacaaggct gttagagaga
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      841 agtaataatt tcttgggtag tttgcagttt taaaattatg ttttaaaatg gactatcata
      901 tgcttaccgt aacttgaaag tatttcgatt tcttggcttt atatatcttg tggaaaggac
      961 gaaacaccGG tcggggtacc tgctgaagca aacgacgccg tatgtcaggg tagtgacaag
     1021 tgttggcCat ggaacaggta gttttccagt agtgcaaata aacttcaggg tcagcttgcc
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     1261 ctgtttcctg gcagctctgg cccgtgtctc aaaatctctg atgttacatt gcacaagata
     1321 aaaatatatc atcatgaaca ataaaactgt ctgcttacat aaacagtaat acaaggggtg
     1381 ttatgagcca tattcaacgg gaaacgtcga ggccgcgatt aaattccaac atggatgctg
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     1501 gcttgtatgg gaagcccgat gcgccagagt tgtttctgaa acatggcaaa ggtagcgttg
     1561 ccaatgatgt tacagatgag atggtcagac taaactggct gacggaattt atgcctcttc
     1621 cgaccatcaa gcattttatc cgtactcctg atgatgcatg gttactcacc actgcgatcc
     1681 ccggaaaaac agcattccag gtattagaag aatatcctga ttcaggtgaa aatattgttg
     1741 atgcgctggc agtgttcctg cgccggttgc attcgattcc tgtttgtaat tgtcctttta
     1801 acagcgatcg cgtatttcgt ctcgctcagg cgcaatcacg aatgaataac ggtttggttg
     1861 atgcgagtga ttttgatgac gagcgtaatg gctggcctgt tgaacaagtc tggaaagaaa
     1921 tgcataaact tttgccattc tcaccggatt cagtcgtcac tcatggtgat ttctcacttg
     1981 ataaccttat ttttgacgag gggaaattaa taggttgtat tgatgttgga cgagtcggaa
     2041 tcgcagaccg ataccaggat cttgccatcc tatggaactg cctcggtgag ttttctcctt
     2101 cattacagaa acggcttttt caaaaatatg gtattgataa tcctgatatg aataaattgc
     2161 agtttcattt gatgctcgat gagtttttct aatcagaatt ggttaattgg ttgtaacact
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     2281 tgagttacgc gtcgttccac tgagcgtcag accccgtaga aaagatcaaa ggatcttctt
     2341 gagatccttt ttttctgcgc gtaatctgct gcttgcaaac aaaaaaacca ccgctaccag
     2401 cggtggtttg tttgccggat caagagctac caactctttt tccgaaggta actggcttca
     2461 gcagagcgca gataccaaat actgtTcttc tagtgtagcc gtagttaggc caccacttca
     2521 agaactctgt agcaccgcct acatacctcg ctctgctaat cctgttacca gtggctgctg
     2581 ccagtggcga taagtcgtgt cttaccgggt tggactcaag acgatagtta ccggataagg
     2641 cgcagcggtc gggctgaacg gggggttcgt gcacacagcc cagcttggag cgaacgacct
     2701 acaccgaact gagataccta cagcgtgagc attgagaaag cgccacgctt cccgaaggga
     2761 gaaaggcgga caggtatccg gtaagcggca gggtcggaac aggagagcgc acgagggagc
     2821 ttccaggggg aaacgcctgg tatctttata gtcctgtcgg gtttcgccac ctctgacttg
     2881 agcgtcgatt tttgtgatgc tcgtcagggg ggcggagcct atggaaaaac gccagcaacg
     2941 cggccttttt acggttcctg gccttttgct ggccttttgc tcacatgtt
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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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  ORGANISM  .
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                     width 5 offset 0"
     promoter        962..968
                     /vntifkey="29"
                     /locus_tag="hU6"
                     /ApEinfo_fwdcolor="#346ee0"
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                     width 5 offset 0"
     promoter        705..961
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                     /locus_tag="pBR322ori"
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                     width 5 offset 0"
     terminator      1094..1099
                     /locus_tag="PolIII terminator"
                     /ApEinfo_fwdcolor="#9d1b1c"
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     misc_binding    981..984
                     /locus_tag="4nt mismatch (as in Uzonyi et al 2021)"
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      121 gcgcccaata cgcaaaccgc ctctccccgc gcgttggccg attcattaat gcagctggca
      181 cgacaggttt cccgactgga aagcgggcag tgagcgcaac gcaattaata cgcgtaccgc
      241 tagccaggaa gagtttgtag aaacgcaaaa aggccatccg tcaggatggc cttctgctta
      301 gtttgatgcc tggcagttta tggcgggcgt cctgcccgcc accctccggg ccgttgcttc
      361 acaacgttca aatccgctcc cggcggattt gtcctactca ggagagcgtt caccgacaaa
      421 caacagataa aacgaaaggc ccagtcttcc gactgagcct ttcgttttat ttgatgcctg
      481 gcagttccct actctcgcgt taacgctagc atggatgttt tcccagtcac gacgttgtaa
      541 aacgacggcc agtcttaagc tcgggcccca aataatgatt ttattttgac tgatagtgac
      601 ctgttcgttg caacaaattg atgagcaatg ctttttttata atgccaactt tgtacaaaaa
      661 agcaggcttt aaaggaacca attcagtcga ctggatccgg taccaaggtc gggcaggaag
      721 agggcctatt tcccatgatt ccttcatatt tgcatatacg atacaaggct gttagagaga
      781 taattagaat taatttgact gtaaacacaa agatattagt acaaaatacg tgacgtagaa
      841 agtaataatt tcttgggtag tttgcagttt taaaattatg ttttaaaatg gactatcata
      901 tgcttaccgt aacttgaaag tatttcgatt tcttggcttt atatatcttg tggaaaggac
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     1021 ggaacaggta gttttccagt agtgcaaata aacttcaggg tcagcttgcc ataggtggca
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     1201 ttgccatcca gctgatatcc cctatagtga gtcgtattac atggtcatag ctgtttcctg
     1261 gcagctctgg cccgtgtctc aaaatctctg atgttacatt gcacaagata aaaatatatc
     1321 atcatgaaca ataaaactgt ctgcttacat aaacagtaat acaaggggtg ttatgagcca
     1381 tattcaacgg gaaacgtcga ggccgcgatt aaattccaac atggatgctg atttatatgg
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     1561 tacagatgag atggtcagac taaactggct gacggaattt atgcctcttc cgaccatcaa
     1621 gcattttatc cgtactcctg atgatgcatg gttactcacc actgcgatcc ccggaaaaac
     1681 agcattccag gtattagaag aatatcctga ttcaggtgaa aatattgttg atgcgctggc
     1741 agtgttcctg cgccggttgc attcgattcc tgtttgtaat tgtcctttta acagcgatcg
     1801 cgtatttcgt ctcgctcagg cgcaatcacg aatgaataac ggtttggttg atgcgagtga
     1861 ttttgatgac gagcgtaatg gctggcctgt tgaacaagtc tggaaagaaa tgcataaact
     1921 tttgccattc tcaccggatt cagtcgtcac tcatggtgat ttctcacttg ataaccttat
     1981 ttttgacgag gggaaattaa taggttgtat tgatgttgga cgagtcggaa tcgcagaccg
     2041 ataccaggat cttgccatcc tatggaactg cctcggtgag ttttctcctt cattacagaa
     2101 acggcttttt caaaaatatg gtattgataa tcctgatatg aataaattgc agtttcattt
     2161 gatgctcgat gagtttttct aatcagaatt ggttaattgg ttgtaacact ggcagagcat
     2221 tacgctgact tgacgggacg gcgcaagctc atgaccaaaa tcccttaacg tgagttacgc
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     2401 tttgccggat caagagctac caactctttt tccgaaggta actggcttca gcagagcgca
     2461 gataccaaat actgtTcttc tagtgtagcc gtagttaggc caccacttca agaactctgt
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     2581 taagtcgtgt cttaccgggt tggactcaag acgatagtta ccggataagg cgcagcggtc
     2641 gggctgaacg gggggttcgt gcacacagcc cagcttggag cgaacgacct acaccgaact
     2701 gagataccta cagcgtgagc attgagaaag cgccacgctt cccgaaggga gaaaggcgga
     2761 caggtatccg gtaagcggca gggtcggaac aggagagcgc acgagggagc ttccaggggg
     2821 aaacgcctgg tatctttata gtcctgtcgg gtttcgccac ctctgacttg agcgtcgatt
     2881 tttgtgatgc tcgtcagggg ggcggagcct atggaaaaac gccagcaacg cggccttttt
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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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ACCESSION
VERSION
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  ORGANISM  .
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COMMENT     ApEinfo:methylated:1
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     promoter        705..968
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     1021 tgttggcCat ggaacaggta gttttttttt ctagacccag ctttcttgta caaagttggc
     1081 attataagaa agcattgctt atcaatttgt tgcaacgaac aggtcactat cagtcaaaat
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     1981 atcgcagacc gataccagga tcttgccatc ctatggaact gcctcggtga gttttctcct
     2041 tcattacaga aacggctttt tcaaaaatat ggtattgata atcctgatat gaataaattg
     2101 cagtttcatt tgatgctcga tgagtttttc taatcagaat tggttaattg gttgtaacac
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     2341 gcggtggttt gtttgccgga tcaagagcta ccaactcttt ttccgaaggt aactggcttc
     2401 agcagagcgc agataccaaa tactgttctt ctagtgtagc cgtagttagg ccaccacttc
     2461 aagaactctg tagcaccgcc tacatacctc gctctgctaa tcctgttacc agtggctgct
     2521 gccagtggcg ataagtcgtg tcttaccggg ttggactcaa gacgatagtt accggataag
     2581 gcgcagcggt cgggctgaac gggggttcg tgcacacagc ccagcttgga gcgaacgacc
     2641 tacaccgaac tgagatacct acagcgtgag cattgagaaa gcgccacgct tcccgaaggg
     2701 agaaaggcgg acaggtatcc ggtaagcggc agggtcggaa caggagagcg cacgagggag
     2761 cttccagggg gaaacgcctg gtatctttat agtcctgtcg ggtttcgcca cctctgactt
     2821 gagcgtcgat ttttgtgatg ctcgtcaggg gggcggagcc tatggaaaaa cgccagcaac
     2881 gcggcctttt tacggttcct ggccttttgc tggccttttg ctcacatgtt
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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
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                     /__level__="0"
                     /application_notes="Strong promoter; drives transcription
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                     /name="RSV  promoter"
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     LTR             230..410
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                     its packaging into virus."
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                     /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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                /application_notes="Rev protein binding site that allows
                Rev-dependent nuclear export of viral RNA during viral
                packaging."
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ORF             3267..3980
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                /description="Blue variant of EGFP generated by
                mutagenesis"
                /full_name="Enhanced blue fluorescent protein(ns)"
                /note="color: #6ddaae; direction: RIGHT"
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                /uuid="c22d3886879b4745b895a6f25c150792"
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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"
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                     cells, leading to higher titer of packaged virus; enhances
                     higher expression of transgenes."
                     /description="['Woodchuck hepatitis virus
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                     /full_name="Woodchuck hepatitis virus posttranscriptional
                     regulatory element"
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                     virus; self-inactivates the 5' LTR by a copying mechanism
                     during viral genome integration; contains polyadenylation
                     signal for transcription termination."
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                     polyadenylation of mRNA transcribed by Pol II RNA
                     polymerase."
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                     signal']"
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                     /name="SV40 early pA"
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                     /application_notes="Facilitates plasmid replication in E.
                     coli; regulates high-copy plasmid number (500-700)."
                     /description="['pUC origin of replication']"
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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
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                Rev-dependent nuclear export of viral RNA during viral
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ORF             3267..3980
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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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LTR             5140..5374
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                virus; self-inactivates the 5' LTR by a copying mechanism
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                signal for transcription termination."
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                polyadenylation of mRNA transcribed by Pol II RNA
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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
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ORIGIN

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Plasmid pT_hU6 NT-SPEAR (control)_hPGK BFP-P2A-Bsd sequence (GenBank format, provided as text)

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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
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ORF             1418..2134
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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
COMMENT
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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
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primer_bind     complement(3313..3329)
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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"
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promoter        complement(3361..3391)
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                -
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protein_bind    3406..3427
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gene            complement(4474..5334)
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                -
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promoter             complement(5376..5404)
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3601 ctcaaaggcg gtaatacggt tatccacaga atcaggggat aacgcaggaa agaacatgtg
3661 agcaaaaggc cagcaaaagg ccaggaaccg taaaaaggcc gcgttgctgg cgtttttcca
3721 taggctccgc ccccctgacg agcatcacaa aaatcgacgc tcaagtcaga ggtggcgaaa
3781 cccgacagga ctataaagat accaggcgtt tccccctgga agctccctcg tgcgctctcc
3841 tgttccgacc ctgccgctta ccggatacct gtccgccttt ctcccttcgg gaagcgtggc
3901 gctttctcat agctcacgct gtaggtatct cagttcggtg taggtcgttc gctccaagct
3961 gggctgtgtg cacgaacccc ccgttcagcc cgaccgctgc gccttatccg gtaactatcg
4021 tcttgagtcc aacccggtaa gacacgactt atcgccactg gcagcagcca ctggtaacag
4081 gattagcaga gcgaggtatg taggcggtgc tacagagttc ttgaagtggt ggcctaacta
4141 cggctacact agaagaacag tatttggtat ctgcgctctg ctgaagccag ttaccttcgg
4201 aaaaagagtt ggtagctctt gatccggcaa acaaaccacc gctggtagcg gtggtttttt
4261 tgtttgcaag cagcagatta cgcgcagaaa aaaaggatct caagaagatc ctttgatctt
4321 ttctacgggg tctgacgctc agtggaacga aaactcacgt taagggattt tggtcatgag
4381 attatcaaaa aggatcttca cctagatcct tttaaattaa aaatgaagtt ttaaatcaat
4441 ctaaagtata tatgagtaaa cttggtctga cagttaccaa tgcttaatca gtgaggcacc
4501 tatctcagcg atctgtctat ttcgttcatc catagttgcc tgactccccg tcgtgtagat
4561 aactacgata cgggagggct taccatctgg ccccagtgct gcaatgatac cgcgagaccc
4621 acgctcaccg gctccagatt tatcagcaat aaaccagcca gccggaaggg ccgagcgcag
4681 aagtggtcct gcaactttat ccgcctccat ccagtctatt aattgttgcc gggaagctag
4741 agtaagtagt tcgccagtta atagtttgcg caacgttgtt gccattgcta caggcatcgt
4801 ggtgtcacgc tcgtcgtttg gtatggcttc attcagctcc ggttcccaac gatcaaggcg
4861 agttacatga tcccccatgt tgtgcaaaaa agcggttagc tccttcggtc ctccgatcgt
4921 tgtcagaagt aagttggccg cagtgttatc actcatggtt atggcagcac tgcataattc
4981 tcttactgtc atgccatccg taagatgctt ttctgtgact ggtgagtact caaccaagtc
5041 attctgagaa tagtgtatgc ggcgaccgag ttgctcttgc ccggcgtcaa tacgggataa
5101 taccgcgcca catagcagaa ctttaaaagt gctcatcatt ggaaaacgtt cttcggggcg
5161 aaaactctca aggatcttac cgctgttgag atccagttcg atgtaaccca ctcgtgcacc
5221 caactgatct tcagcatctt ttactttcac cagcgtttct gggtgagcaa aaacaggaag
5281 gcaaaatgcc gcaaaaaagg gaataagggc gacacggaaa tgttgaatac tcatactctt
5341 cctttttcaa tattattgaa gcatttatca gggttattgt ctcatgagcg gatacatatt

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     5401 tgaatgtatt tagaaaaata aacaaatagg ggttccgcgc acatttcccc gaaaagtgcc
     5461 acctgacgtc taagaaacca ttattatcat gacattaacc tataaaaata ggcgtatcac
     5521 gaggcccttt cgtctcgcgc gtttcggtga tgacggtgaa aacctctgac acatgcagct
     5581 cccggagacg gtcacagctt gtctgtaagc ggatgccggg agcagacaag cccgtcaggg
     5641 cgcgtcagcg ggtgttggcg ggtgtcgggg ctggcttaac tatgcggcat cagagcagat
     5701 tgtactgaga gtgcaccata tgcggtgtga aataccgcac agatgcgtaa ggagaaaata
     5761 ccgcatcagg cgccattcgc cattcaggct gcgcaactgt tgggaagggc gatcggtgcg
     5821 ggcctcttcg ctattacgcc agctggcgaa aggggatgt gctgcaaggc gattaagttg
     5881 ggtaacgcca gggttttccc agtcacgacg ttgtaaaacg acggccagt
//
```

Plasmid pT_hU6 T-SPEAR (USP9X^{Y2009C})_hPGK BFP-P2A-Bsd sequence (GenBank format, provided as text)

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LOCUS       RP829_pT_U6_20_4        5929 bp    DNA        linear       28-JAN-2026
DEFINITION  .
ACCESSION
VERSION
SOURCE      .
  ORGANISM  .
COMMENT
COMMENT     ApEinfo:methylated:1
FEATURES             Location/Qualifiers
     source          join(1..452,823..877,2825..5929)
                     /note="organism="Sequence" - mol_type="other DNA" -
                     1..5504"
                     /organism="synthetic DNA construct"
                     /locus_tag="organism=Sequence - mol_type=other DNA -
                     1..5504"
                     /label="organism=Sequence - mol_type=other DNA - 1..5504"
                     /ApEinfo_label="organism=Sequence - mol_type=other DNA -
                     1..5504"
                     /ApEinfo_fwdcolor="#00DF87"
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                     /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
     misc_feature    complement(join(3..452,823..877,2825..5784))
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                     translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGY
                     -
                     IELDLNSGKILESFRPEERFPMMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQNDLVE
                     -
                     YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTRL
                     -
                     DRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLASRQQLIDWMEADKVAG"
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                     /label="lacZ_a"
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                     /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
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     misc_feature    22..53
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                     /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
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                     /locus_tag="hU6"
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                     /ApEinfo_label="hU6"
                     /ApEinfo_fwdcolor="#346ee0"
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                -0.5} {0 .5 .1 .5 .1 -.5 0 -.5} 0} width 5 offset 0"
misc_feature    complement(382..402)
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                /label="pBABE_3_primer"
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                /ApEinfo_fwdcolor="#7eff74"
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                /ApEinfo_label="hU6"
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                /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1} {} 0}
                width 5 offset 0"
ORF             1418..2134
                /application_notes="Low fluorescence and low
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                /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}
                width 5 offset 0"
CDS             complement(join(719..738,743..792))
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                /ApEinfo_label="CDS"
                /ApEinfo_fwdcolor="#e9d024"
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                /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(2835..2856)
                /locus_tag="pT3_rev"
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                /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"
variation       complement(775..777)
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                /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" -
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                -
                VIDGETCLLDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKR
                -
                VKDSEDVPMVLVGNKCDLPSRTVDTKQAQELARSYGIPFIETSAKTRQGVDDAFYTLV
                - REIRKHKEKMSKDGKKKKKKSRTRCTVM*" - 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)"
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                /ApEinfo_label="4 nt mismatch (as in Uzonyi et al 2021)"
                /ApEinfo_fwdcolor="#ff4809"
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                /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"
                /uuid="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
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promoter        883..1387
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                /name="hPGK promoter"
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                /official_designation="hPGK promoter"
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                /vntifkey="21"
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                /label="hPGK promoter"
                /ApEinfo_label="hPGK promoter"
                /ApEinfo_fwdcolor="#346ee0"
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                /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1} {} 0}
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                -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']"
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                /note="color: #68e66d; direction: RIGHT"
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                /vntifkey="21"
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                /ApEinfo_fwdcolor="#e9d024"
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                width 5 offset 0"
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                /ApEinfo_fwdcolor="#7eff74"
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promoter        complement(3309..3327)
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                -
                VIDGETCLLDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKR
                -
                VKDSEDVPMVLVGNKCDLPSRTVDTKQAQELARSYGIPFIETSAKTRQGVDDAFYTLV
                - REIRKHKEKMSKDGKKKKKKSRTRCTVM*" - complement("
                /locus_tag="M13_reverse_primer"
                /label="M13_reverse_primer"
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                /ApEinfo_fwdcolor="#346ee0"
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primer_bind     complement(3313..3329)
                /note="common sequencing primer, one of multiple similar
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                /ApEinfo_label="M13 rev"
                /ApEinfo_fwdcolor="#14c0bd"
                /ApEinfo_revcolor="#4ec02b"
                /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
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misc_feature    complement(3326..3348)
                /note="label="M13_pUC_rev_primer" -
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                -
                VIDGETCLLDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKR
                -
                VKDSEDVPMVLVGNKCDLPSRTVDTKQAQELARSYGIPFIETSAKTRQGVDDAFYTLV
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                - REIRKHKEKMSKDGKKKKKKSRTRCTVM*" - complement("
                /locus_tag="M13_pUC_rev_primer"
                /label="M13_pUC_rev_primer"
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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"
                /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"
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promoter        complement(3362..3391)
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                -
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                -
                VKDSEDVPMVLVGNKCDLPSRTVDTKQAQELARSYGIPFIETSAKTRQGVDDAFYTLV
                - REIRKHKEKMSKDGKKKKKKSRTRCTVM*" - complement(2937.."
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                -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"
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                /ApEinfo_graphicformat="arrow_data {{0 0.5 0 1 2 0 0 -1 0
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source          complement(2600..2824)
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                /ApEinfo_label="source"
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                /ApEinfo_revcolor="#00DF87"
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                /ApEinfo_hidden
rep_origin      complement(3715..4303)
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                /note="high-copy-number ColE1/pMB1/pBR322/pUC origin of
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                replication"
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gene            complement(4474..5334)
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                -
                VIDGETCLLDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHHYREQIKR
                -
                VKDSEDVPMVLVGNKCDLPSRTVDTKQAQELARSYGIPFIETSAKTRQGVDDAFYTLV
                - REIRKHKEKMSKDGKKKKKKSRTRCTVM*" -"
                /locus_tag="AmpR"
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CDS             complement(4474..5334)
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                -
                YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTRL
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promoter        complement(5376..5404)
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                -
                YSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTRL
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       61 cattaaaact cgtttttcaa ctactccaca aatttcttgt taacaaacaa tagttttggc
      121 aagtcagtta ggacatctac tttgtgcatg acacaagtca tttttccaac aattgtttac
      181 agacagatta tttcacttat aattcactgt atcacaattc cagtgggtca gaagtttaca
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      781 catactgtac tgtttttttct agacccagct ttcttgtaca aactcgagaa taaaggtacc
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     1561 ctgcaccacc ggcaagctgc ccgtgccctg gcccaccctc gtgaccaccc tgacccacgg
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//
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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
COMMENT     ApEinfo:methylated:1
FEATURES             Location/Qualifiers
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                     separated by 2A. P2A and T2A have higher cleavage
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                /note="The lac repressor binds to the lac operator to
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                isopropyl-beta-D-thiogalactopyranoside (IPTG)."
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3781 cccgacagga ctataaagat accaggcgtt tccccctgga agctccctcg tgcgctctcc
3841 tgttccgacc ctgccgctta ccggatacct gtccgccttt ctcccttcgg gaagcgtggc
3901 gctttctcat agctcacgct gtaggtatct cagttcggtg taggtcgttc gctccaagct
3961 gggctgtgtg cacgaacccc ccgttcagcc cgaccgctgc gccttatccg gtaactatcg
4021 tcttgagtcc aacccggtaa gacacgactt atcgccactg gcagcagcca ctggtaacag
4081 gattagcaga gcgaggtatg taggcggtgc tacagagttc ttgaagtggt ggcctaacta
4141 cggctacact agaagaacag tatttggtat ctgcgctctg ctgaagccag ttaccttcgg
4201 aaaaagagtt ggtagctctt gatccggcaa acaaaccacc gctggtagcg gtggtttttt
4261 tgtttgcaag cagcagatta cgcgcagaaa aaaaggatct caagaagatc ctttgatctt
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4381 attatcaaaa aggatcttca cctagatcct tttaaattaa aaatgaagtt ttaaatcaat
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4501 tatctcagcg atctgtctat ttcgttcatc catagttgcc tgactccccg tcgtgtagat
4561 aactacgata cgggagggct taccatctgg ccccagtgct gcaatgatac cgcgagaccc
4621 acgctcaccg gctccagatt tatcagcaat aaaccagcca gccggaaggg ccgagcgcag
4681 aagtggtcct gcaactttat ccgcctccat ccagtctatt aattgttgcc gggaagctag
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     5161 aaaactctca aggatcttac cgctgttgag atccagttcg atgtaaccca ctcgtgcacc
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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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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
COMMENT     ApEinfo:methylated:1
FEATURES             Location/Qualifiers
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ORF             1418..2134
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                /full_name="Enhanced blue fluorescent protein(ns)"
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                -
                VKDSEDVPMVLVGNKCDLPSRTVDTKQAQELARSYGIPFIETSAKTRQGVDDAFYTLV
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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
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                /description="Self-cleaving 2A peptide from Porcine
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                /full_name="Porcine teschovirus-1 2A peptide"
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                /ApEinfo_fwdcolor="#20fefe"
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primer_bind     3004..3025
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promoter        883..1387
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CDS             2201..2599
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protein_bind    3337..3353
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                /note="The lac repressor binds to the lac operator to
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                relieved by adding lactose or
                isopropyl-beta-D-thiogalactopyranoside (IPTG)."
                /locus_tag="lac operator"
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promoter        complement(3361..3391)
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                     -
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