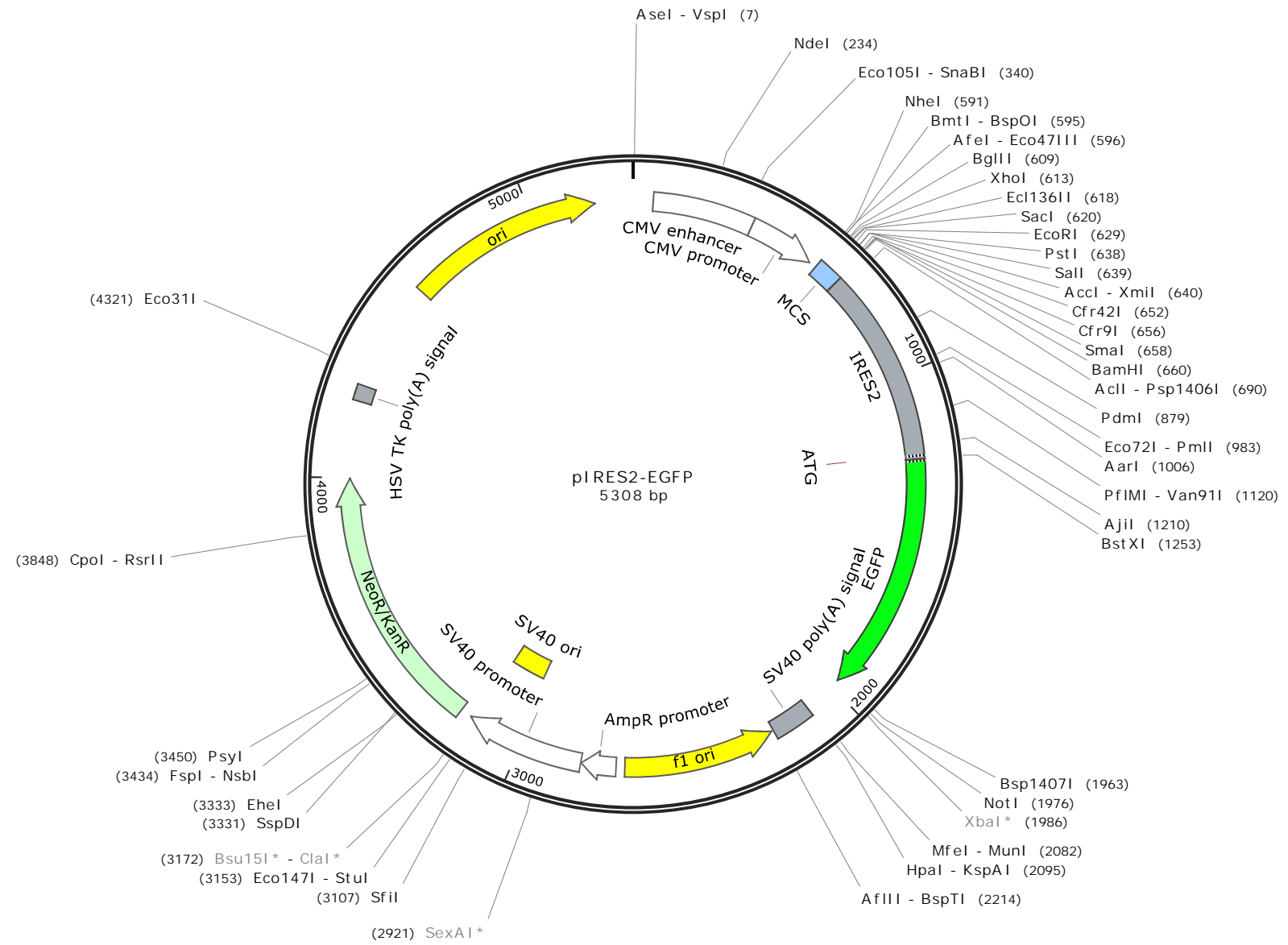


IRES-containing bicistronic vector for expressing a gene together with EGFP.





TTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATG
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850

IRES2

CAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCTTTGCAGGC
 GTTCCAGACAACCTTACAGCACTTCTTTCGTCAAGGAGACCTTCAAGAAGCTTCTGTTTGTTCAGACATCGCTGGGAAACGTCCG

935

IRES2

AGCGGAACCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGGGCACAACCCCA
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1020

IRES2

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1105

IRES2

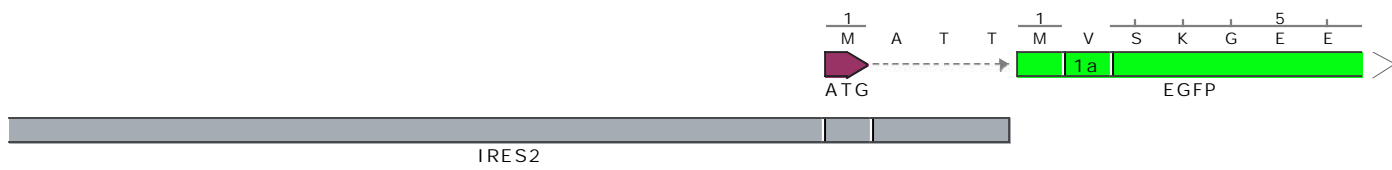
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1190

IRES2

GCCCCCGAACCACGGGGACGTGGTTTTCTTTGAAAAACACGATGATAATATGGCCACAACCATGGTGAGCAAGGGCGAGGAGC
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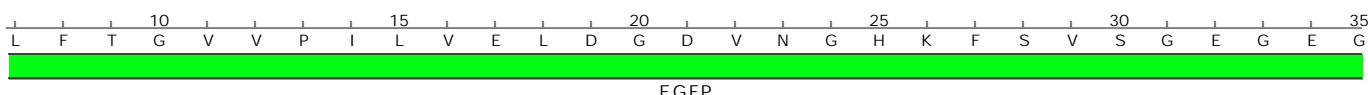
1275



IRES2

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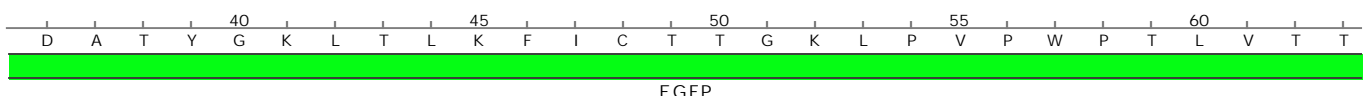
1360



EGFP

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1445



EGFP

CTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCAGCACTTCTTCAAGTCCGCCATGCCCGAAGGCT
 GACTGGATGCCGCACGTACCGAAGTCGGCGATGGGGCTGGTGTACTTCGTCGTGCTGAAGAAAGTTCAGGCGGTACGGGCTTCCGA
 65 70 75 80 85 90
 L T Y G V Q C F S R Y P D H M K Q H D F F K S A M P E G
 EGFP

ACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGT
 TGCAGGTCTCGCGTGGTAGAAGAAGTTCCTGCTGCCGTTGATGTTCTGGGCGCGGCTCCACTTCAAGCTCCCGCTGTGGGACCA
 95 100 105 110 115 120
 Y V Q E R T I F F K D D G N Y K T R A E V K F E G D T L V
 EGFP

GAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACAGCCAC
 CTTGGCGTAGCTCGACTTCCCGTAGCTGAAGTTCCTCCTGCCGTTGTAGGACCCCGTGTTCGACCTCATGTTGATGTTGTCGGTG
 125 130 135 140 145
 N R I E L K G I D F K E D G N I L G H K L E Y N Y N S H
 EGFP

AACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACCTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGC
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 150 155 160 165 170 175
 N V Y I M A D K Q K N G I K V N F K I R H N I E D G S V
 EGFP

AGCTCGCCGACCACTACCAGCAGAACACCCCATCGGGCAGCGGCCCGTGTGCTGCCCGACAACCACTACCTGAGCACCCAGTC
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 180 185 190 195 200 205
 Q L A D H Y Q Q N T P I G D G P V L L P D N H Y L S T Q S
 EGFP

CGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATG
 GCGGGACTCGTTTCTGGGGTGTCTTTCGCGCTAGTGTACCAGGACGACCTCAAGCACTGGCGGGCGCCCTAGTGAGAGCCGTAC
 210 215 220 225 230
 A L S K D P N E K R D H M V L L E F V T A A G I T L G M
 EGFP

Bsp14071 NotI XbaI*
 GACGAGCTGTACAAGTAAAGCGGCCGCGACTCTAGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTTAAAAAA
 CTGCTCGACATGTTTCATTTCCGCCGCGCTGAGATCTAGTATTAGTCGGTATGGTGTAAACATCTCCAAAATGAACGAAATTTTTT
 235
 D E L Y K
 EGFP

MfeI MunI HpaI KspAI
 CCTCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTATTGAGCTTATAATGGTTAC
 GGAGGGTGTGGAGGGGGACTTGGACTTTGTATTTTACTTACGTTAACAACAACAATTGAACAAATAACGTCGAATATTACCAATG
 2125
 SV40 poly(A) signal

AAATAAAGCAATAGCATCACAAATTTACAAATAAAGCATTTTTTCTACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATG
TTTATTTTCGTTATCGTAGTGTTTAAAGTGTTTATTTTCGTAATAAAGTACGTAAGATCAACACCAAACAGGTTTGAGTAGTTAC

2210

SV40 poly(A) signal

TATCTTAAGGCGTAAATTGTAAGCGTTAATATTTTGTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAAT
ATAGAATTCGCGATTAAACATTTCGCAATTATAAAACAATTTAAGCGCAATTTAAAAACAATTTAGTCGAGTAAAAAATTGGTTA

2295

SV40 poly(A) signal

f1 ori

AGGCCGAAATCGGC AAAATCCCTTATAAATCAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAAACAAGAGTCC
TCCGGCTTTAGCCGTTTTAGGGAATATTTAGTTTTCTTATCTGGCTCTATCCCAACTCACAACAAGGTCAAACCTTGTTCTCAGG

2380

f1 ori

ACTATTAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCTAA
TGATAATTTCTTGACCTGAGGTTGCAGTTTCCCGCTTTTTGGCAGATAGTCCCGCTACCGGGTGATGCACTTGGTAGTGGGATT

2465

f1 ori

TCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCTAAAGGGAGCCCCGATTTAGAGCTTGACGGGGAAAGC
AGTTCAAAAAACCCAGCTCCACGGCATTTCGTGATTTAGCCTTGGGATTTCCCTCGGGGGCTAAATCTCGAACTGCCCTTTTCG

2550

f1 ori

CGGCGAACGTGGCGAGAAAGGAAGGGAAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTG TAGCGGTACGCTGCGCGT
GCCGCTTGACCCGCTCTTTCTTCCCTTCTTTCGCTTTCTCGCCCGCGATCCCGCGACCGTTACATCGCCAGTGCGACGCGCA

2635

f1 ori

AACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATT
TTGGTGGTGTGGGCGGCGCAATTACGCGGCATGTCCC GCGCAGTCCACC GTGAAAAGCCCTTTACACGCGCCTTGGGGATAA

2720

f1 ori

AmpR promoter

TGTTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGA
ACAAATAAAAAGATTTATGTAAGTTTATACATAGGCGAGTACTCTGTTATTGGGACTATTTACGAAGTTATTATAACTTTTTCTT

2805

AmpR promoter

AGAGTCCTGAGGCGGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGT
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2890

SV40 promoter

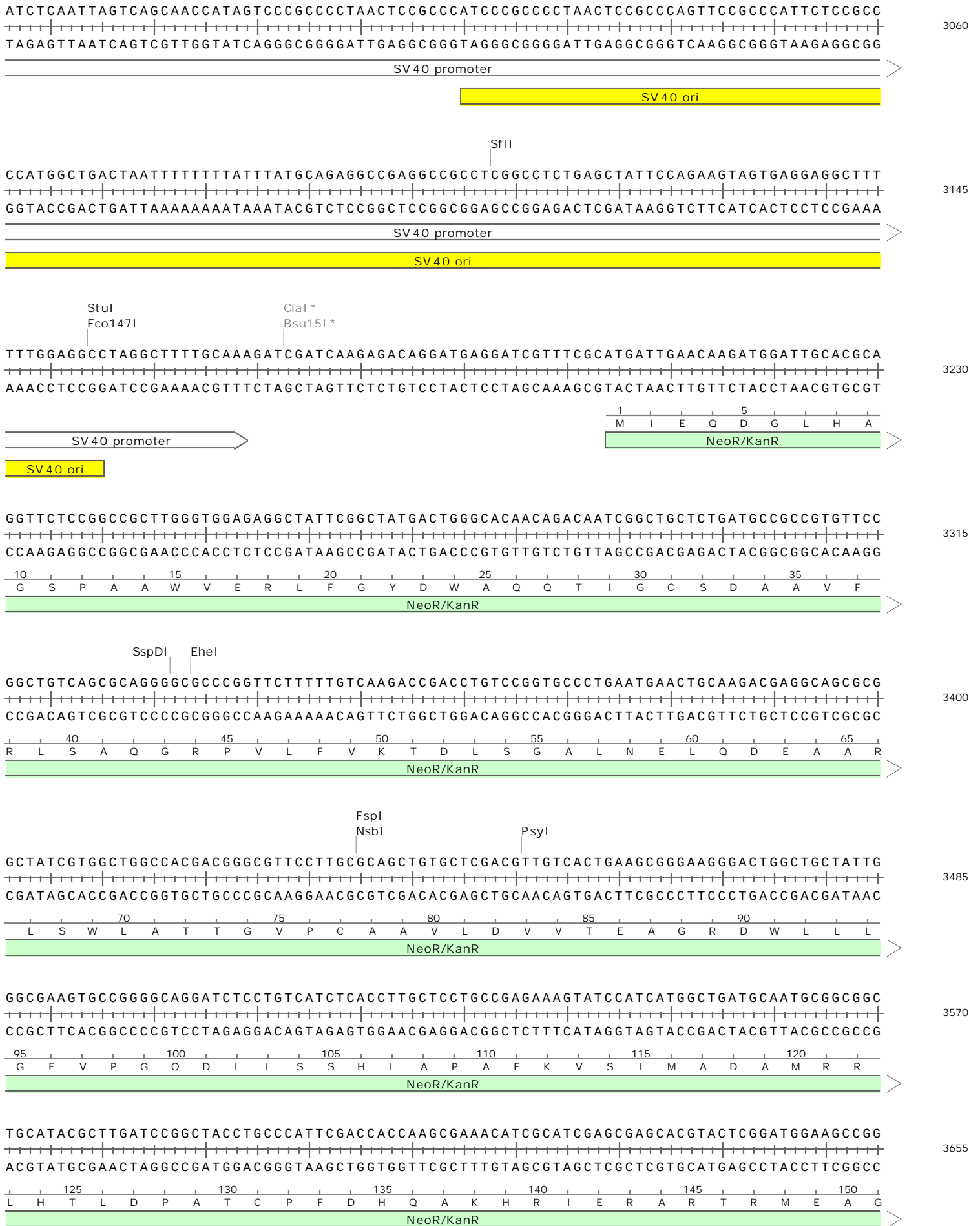
AmpR promoter

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TACGTTTCGTACGTAGAGTTAATCAGTCGTTGGTCCACACCTTT CAGGGGTCCGAGGGGTCTGTCCTTTCATACGTTTCGTACG

2975

SV40 promoter

SexAI *



TCTTGTGGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACCTGTTTCGCCAGGCTCAAGGGCAGCATGCC
 AGAACAGCTAGTCTACTAGACCTGCTTCTCGTAGTCCCCGAGCGCGGTTCGGCTTGACAAGCGGTCCGAGTTCCGCTCGTACGGG
 155 160 165 170 175
 L V D Q D D L D E E H Q G L A P A E L F A R L K A S M P
 NeoR/KanR


GACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTTCATCG
 CTGCCGCTCCTAGAGCAGCACTGGGTACCGCTACGGACGAACGGCTTATAGTACCACCTTTTACCAGGGCGAAAAGACCTAAGTAGC
 180 185 190 195 200 205
 D G E D L V V T H G D A C L P N I M V E N G R F S G F I
 NeoR/KanR

ACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGGCAATG
 TGACACCGGGCCGACCCACACCGCCTGGCGATAGTCTGTATCGCAACCGATGGGCACTATAACGACTTCTCGAACCAGCGCTTAC
 210 215 220 225 230 235
 D C G R L G V A D R Y Q D I A L A T R D I A E L G G E W
 NeoR/KanR

CpoI
RsrII

GGCTGACCGCTTCTCGTGCTTTACGGTATCGCCGCTCCCGATTTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTC
 CCGACTGGCGAAGGAGCAGCAAATGCCATAGCGGCGAGGGCTAAGCGTTCGCGTAGCGGAAGATAGCGGAAGAAGTCTCAAGAAG
 240 245 250 255 260
 A D R F L V L Y G I A A P D S Q R I A F Y R L L D E F F
 NeoR/KanR

TGAGCGGGACTCTGGGGTTCGAAATGACCGACCAAGCGACGCCAACCTGCCATCACGAGATTTTCGATTCCACCGCCGCTTCTA
 ACTCGCCCTGAGACCCCAAGCTTTACTGGCTGGTTTCGCTGCGGGTTGGACGGTAGTGCTCTAAAGCTAAGGTGGCGGGCGGAAGAT

265

 NeoR/KanR

TGAAAAGTTGGGCTTCGGAATCGTTTTCCGGGACGCCGGCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCC
 ACTTTCCAACCCGAAGCCTTAGCAAAAGGCCCTGCGGCCGACCTACTAGGAGGTTCGCGCCCCTAGAGTACGACCTCAAGAAGCGG
 CACCCTAGGGGAGGCTAACTGAAACACGGAAGGAGACAATACCGGAAGGAACCCGCGCTATGACGGCAATAAAAAGACAGAATA
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 HSV TK poly(A) signal

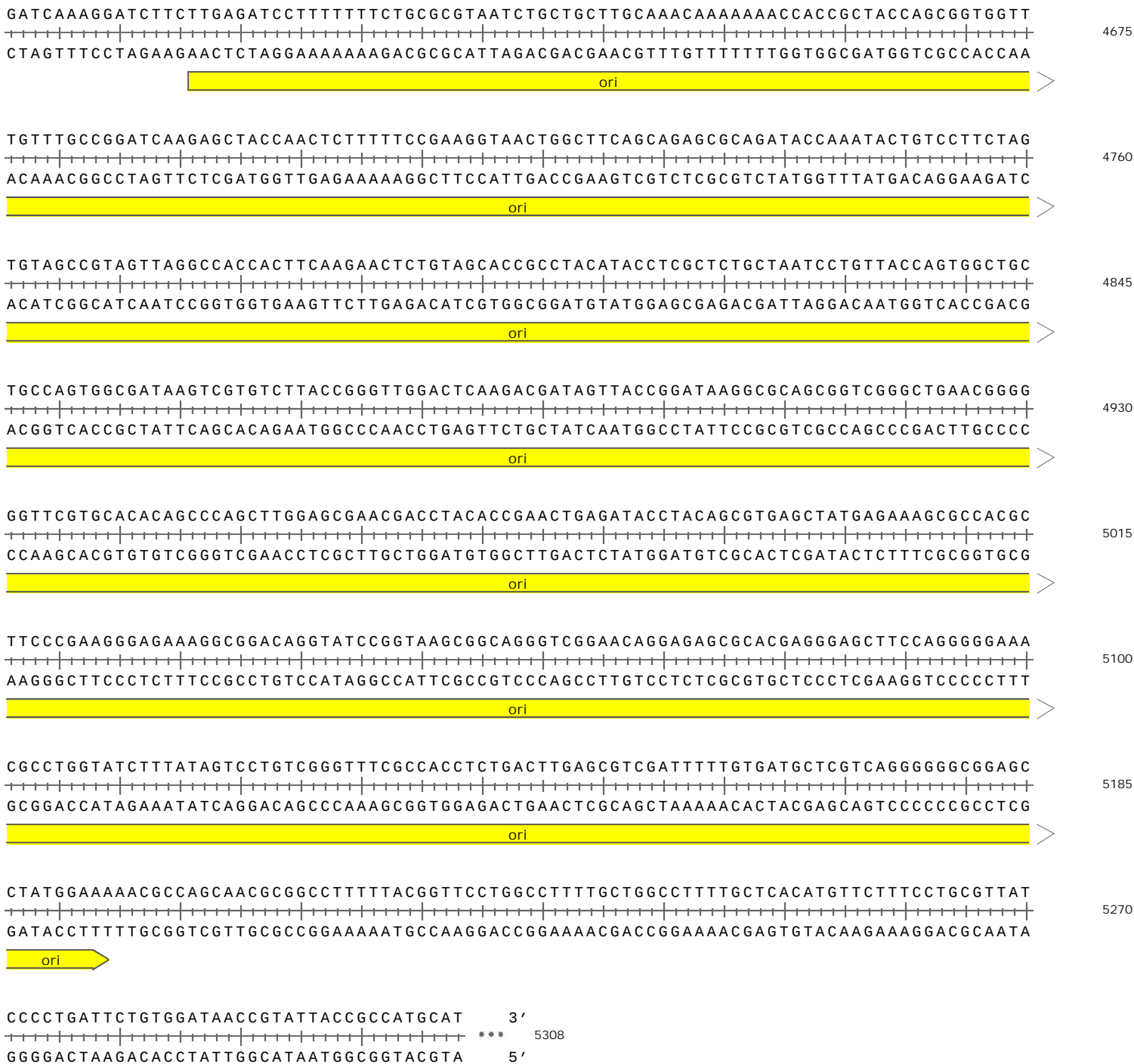
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 HSV TK poly(A) signal

Eco311



























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 TCCACTTCTAGGAAAACTATTAGAGTACTGGTTTTAGGGAATTGCACTCAAAGCAAGGTGACTCGCAGTCTGGGGCATCTTTT



Enzymes	Sites	
AarI	1	1006
AccI	1	640
AccII	1	690
AfeI	1	596
AfIII	1	2214
AjiI	1	1210
AseI	1	7
BamHI	1	660
BglII	1	609
BmtI	1	595
Bsp1407I	1	1963
BspOI	1	595
BspTI	1	2214
BstXI	1	1253
Bsu15I	1*	3172*
Cfr9I	1	656
Cfr42I	1	652
ClaI	1*	3172*
CpoI	1	3848
Ecl136I	1	618
Eco31I	1	4321
Eco47III	1	596
Eco72I	1	983
Eco105I	1	340
Eco147I	1	3153
EcoRI	1	629
EheI	1	3333
FspI	1	3434
HpaI	1	2095
KspAI	1	2095
MfeI	1	2082
MunI	1	2082
NdeI	1	234
NheI	1	591
NotI	1	1976
NsbI	1	3434
Pdml	1	879
PfIMI	1	1120
PmlI	1	983
Psp1406I	1	690
PstI	1	638
PsyI	1	3450
RsrII	1	3848
SacI	1	620
Sall	1	639
SexAI	1*	2921*
Sfil	1	3107
SmaI	1	658
SnaBI	1	340
SspDI	1	3331
StuI	1	3153
Van91I	1	1120
VspI	1	7
XbaI	1*	1986*
XhoI	1	613
XmiI	1	640

Feature	Location	Size (bp)			Type
✓ CMV enhancer /note = human cytomegalovirus immediate early enhancer	61 .. 364	304			enhancer
✓ CMV promoter /note = human cytomegalovirus (CMV) immediate early promoter	365 .. 568	204			promoter
✓ MCS /note = multiple cloning site of fluorescent protein plasmids	591 .. 665	75			misc_feature
✓ IRES2 ▶ 3 segments /note = internal ribosome entry site (IRES) of the encephalomyocarditis virus (EMCV)	667 .. 1253	587			misc_feature
✓ ATG /product = start codon for translation from IRES2 /translation = M 1 amino acid = 149.2 Da	1242 .. 1244	3			CDS
✓ EGFP ▶ 3 segments /product = enhanced GFP /note = mammalian codon-optimized /translation = M,V,SKGEELFTGVVPILVELDGDVNGHKFVS GEGEGDATY GKLTKFKICTTGKLPVPWPTLVTTLT YGVQCFSRYPDHMKQHDFFK SAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGI DFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIE DGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSA LSKDPNEKRDHMLLEFVTAAGITLGMDELYK* 239 amino acids = 26.9 kDa	1254 .. 1973	720			CDS
✓ SV40 poly(A) signal /note = SV40 polyadenylation signal	2096 .. 2217	122			polyA_signal
✓ f1 ori /direction = LEFT /note = f1 bacteriophage origin of replication; arrow indicates direction of (+) strand synthesis	2224 .. 2679	456			rep_origin
✓ AmpR promoter /gene = bla	2706 .. 2810	105			promoter
✓ SV40 promoter /note = SV40 enhancer and early promoter	2812 .. 3169	358			promoter
✓ SV40 ori /note = SV40 origin of replication	3020 .. 3155	136			rep_origin
✓ NeoR/ KanR /gene = aph(3')-II (or nptII) /product = aminoglycoside phosphotransferase from Tn5 /note = confers resistance to neomycin, kanamycin, and G418 (Geneticin) /translation = MIEQDGLHAGSPAAWVERLFGYDWAQQTIGCSDAAVFRLSAQGRPVLFVKTDLSGALNELODEAARLSWLATTGVPAAVLDVV TEAGRDWLLLGEVPGDLLSSHLAPAEKVSIMADAMRRLHTLDPATCPFDDHQA KHRIERARTRMEAGLVDQDDLDEEHQGLAPAE L FARLKA S MPDGEDLVVTHGDACLPNIMVENGRFSGFIDCGRLGVADRYQDIALATRDIAEELGGEWADRFLVLYGIAAPDSORIA FYRIIDFFF* 264 amino acids = 29.0 kDa	3204 .. 3998	795			CDS

Feature	Location	Size (bp)			Type
✓ HSV TK poly(A) signal	4230 .. 4277	48			misc_feature
/note	= herpesvirus thymidine kinase polyadenylation signal				
✓ ori	4606 .. 5194	589			rep_origin
/direction	= RIGHT				
/note	= high-copy-number CoIE 1/pMB1/pBR322/pUC origin of replication				

Description: IRES-containing bicistronic vector for expressing a gene together with EGFP.

Created: Thursday, Sep 13, 2012

Last Modified: Sunday, Jan 26, 2014

Accession Number:

Code Number:

Sequence Author: Clontech

DNA Type: Synthetic DNA

Laboratory Host Organism: Mammalian Cells

Bacterial Transformation Strain: Unspecified

Dam⁺ Dcm⁺ EcoKI⁺

Comments: The gene inserted into the MCS should contain start and stop codons.

References: