



Plasmid #855

Plasmid name pPBH-P-TREtight-EGFP-C

Plasmid description:

Name pPBH-P-TREtight-EGFP-C

Brief Description of the plasmid:

This is a PiggyBac vector for tet-inducible eukaryotic protein expression of N-terminal tagged EGFP fusion proteins. Between the inverted terminal repeat sequences, the plasmid contains the hygromycin resistant gene expression cassette for eukaryotic selection. 3' to the hygromycin resistant cassette, it contains a human phosphoglycerate kinase 1 (PGK) promoter driven TetON3G expression cassette. 5' to the hygromycin resistant cassette, it contains a TREtight promoter driven EGFP expression cassette. Immediately 3' to the EGFP, it contains a multiple cloning site to allow insertion of protein coding DNA sequences.

When transfected into eukaryotic cell lines, the TetON3G transactivator is constitutively expressed. In the presence of doxycycline, the TetON3G transactivator binds to the TREtight promoter within the plasmid to drive EGFP or EGFP-fusion protein expression in a dose dependent fashion. Such inducible expression can be achieved in both transiently or stably transfected cells.

The only difference between this vector and plasmid #734, pPBH-TREtight-EGFP-C is the usage of PGK promoter instead of EF-1 α promoter to drive TetON3G protein expression. Because the PGK promoter is generally much weaker than EF-1 α promoter, this leads to relatively low TetON3G expression in transfected cells. Use of this vector over #734 may limit cellular changes induced by TetON3G. Although this may also lead to reduced Tet-induced expression, PGK promoter activity is sufficient to support high levels of Tet-inducible protein expression in Caco-2 cells.

When co-transfected with pSPB-transposase (plasmid #709), the PiggyBac transposase expressed from pSPB-transposase will recognize the inverted terminal repeat and insert the DNA sequence between these terminal repeats into TTAA sites of the host cell chromosomes, allowing site-specific integration of plasmid DNA with high efficiency.

This vector has a spectinomycin resistant gene expression cassette for bacterial amplification.

Summary

1. Cloning Strategy

The 1.4 kb TRE-Tight-EGFP gene expression cassette was PCR amplified from the pPBH-TREtight-EGFP-C (plasmid #734). Purified PCR product was inserted into the SfiI site of pPBH-PGK-TetON3G (plasmid #851) by using a directional ligation independent cloning kit (InFusion HD cloning kit, Clontech 639645). Successful insertion was confirmed by restriction digestion with and sequencing.

2. *In silico* compilation of the plasmid sequence:

The plasmid sequence listed in this document was generated by combining the sequencing results and the preexisting sequence of pPBH-PGK-TetON3G (plasmid #851). The plasmid was not completely sequenced.

This plasmid contains the following unique restriction enzyme recognizing sites within the multiple cloning site for insertion of protein coding DNA sequences.

Acc65I, KpnI, NheI, BmtI, NotI, ClaI

Commonly used restriction enzyme recognizing sequences are highlighted with in the sequence of the multiple cloning site: **KpnI**, **NheI**, **NotI**, **ClaI**

TCCGGA^{CTCAGATCTCGAGCTCAAGCTTCGAACTCTGCAGTCGAC}**GGTACC**GCGGGC
CCGGGATCCTCTAGTCAGCTGACGCGT**GCTAGCGCGGCCGCATCGATA**AAGCTTGTCG
ACGATATCTCTAGA

Key Features:

5' core insulator	264..502
3' PiggyBac terminal repeat	25..257
P tight tet-responsive promoter	524..839
EGFP-MCS	864..1708
Multiple cloning site (MCS)	1581..1708
HSV TK promoter	2297..3334
hygromycin resistant gene	2297..3334
HSV TK polyadenylation sequence	2297..3334
PGK promoter	3879..4516
Tet-On 3G	4517..5263
SV40 polyadenylation sequence	5286..5728
3' core insulator	5993..6260
5' PiggyBac terminal repeat	6261..6295
Spectinomycin resistant gene	8638..9666

Primers:

x pMPB F81-104	5'-ACGCATGTGTTTTATCGGTCTGTAT-3' binds to 81..105 of pPBH-TREtight-EGFP-C
x EGFP beginning R	5'-GCCGGTGGTGCAGATGAACT-3' binds to complement(1000..1019)of pPBH-TREtight-EGFP-C
x EGFP 1/4F	5'-TTCAAGGACGACGGCAACTACAAG-3' binds to 1164..1187 of pPBH-TREtight-EGFP-C
x EGFP-C sequencing primer (Clontech #6478-1)	5'-CATGGTCCTGCTGGAGTTCGTG-3' binds to 1517..1538 of pPBH-TREtight-EGFP-C This primer can be used to sequence DNA sequences inserted into the multiple cloning site.

x TK prom 1/3 R	5'-TGGGCGGGGTTTGTGTCATCATAGA-3' binds to 2112..2136 of pPBH-TREtight-EGFP-C
x hygro 1/3R	5'-ACAGCGGGCAGTTCGGTTTCAG-3' binds to 2607..2628of pPBH-TREtight-EGFP-C
x hygro 1/3F	5'-CGCAAGGAATCGGTCAATACACT-3' binds to 2709..2731of pPBH-TREtight-EGFP-C
x hygro 2/3F	5'-CCGTGGTTGGCTTGTATGGA-3' binds to 3005..3024of pPBH-TREtight-EGFP-C
x TKpolyA 1/2F	5'ATGGTTTATGGTTCGTGGGGGTTATTCTTT-3' binds to 3631..3660of pPBH-TREtight-EGFP-C
x TET-ON 3G 1/2F	5'-CACTGGGCTGCGTATTGGAGGAACA-3' binds to 4938..4962 of pPBH-TREtight-EGFP-C
x SV40polyA 1/2F	5'-GGCCGGACCACGCTATCTGT-3' binds to 5533..5552of pPBH-TREtight-EGFP-C
x pMPB R 2549-2523	5'-CGCATGATTATCTTTTACGTGACTTTT-3' binds to 6077..6103of pPBH-TREtight-EGFP-C
x pMPB F 2563-2582	5'-CGCGGTCGTTATAGTTCAAA-3' binds to 6117..6136of pPBH-TREtight-EGFP-C
x pMPB F 6058-6083	5'-GAGATCACCAAGGTAGTCGGCAAATA-3' binds to 9622..9647of pPBH-TREtight-EGFP-C

Diagnostic Digests

AflII and KpnI (double digest) 305bp and 9384 bp

HindIII: 9689bp

Plasmid History

Plasmid generated by Le Shen

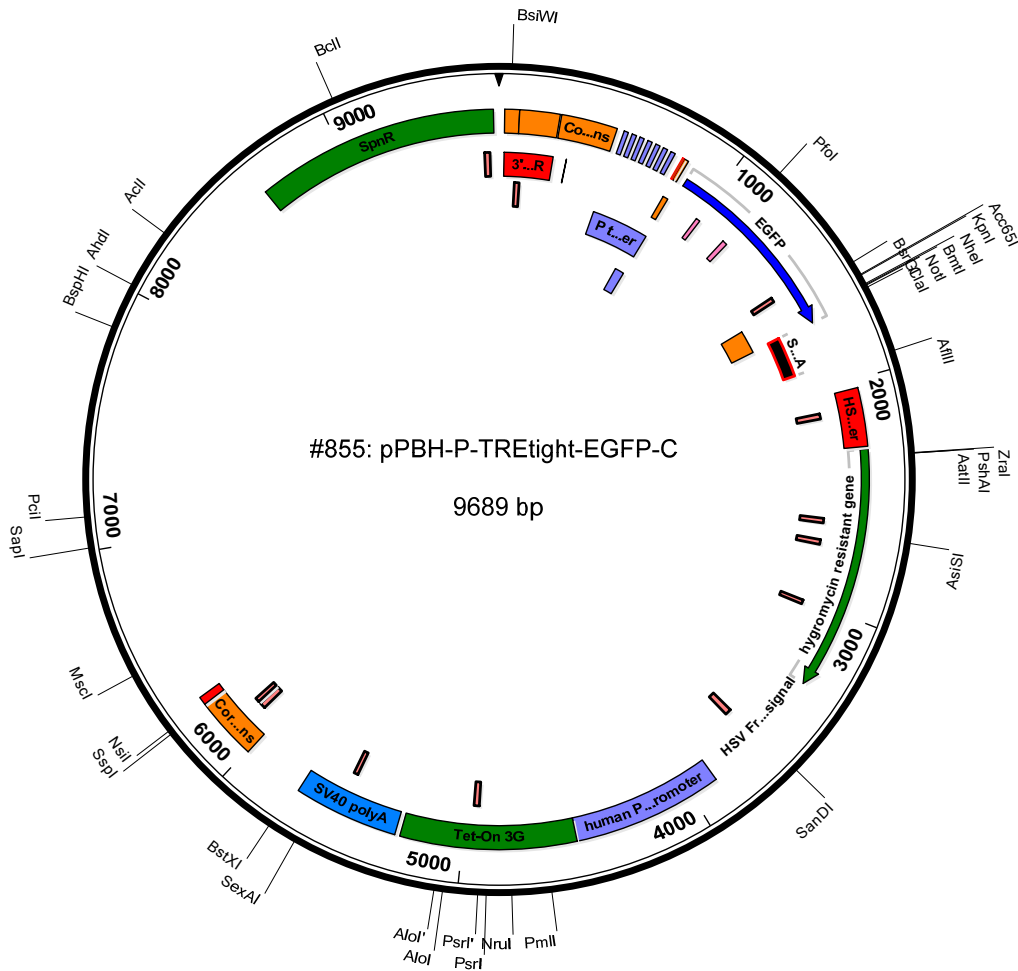
Date plasmid generated 12/2012

Data file generated by Amulya Lingaraju and Le Shen

Data file generated 01/2013,

Data file version history V1.0, 01/2013

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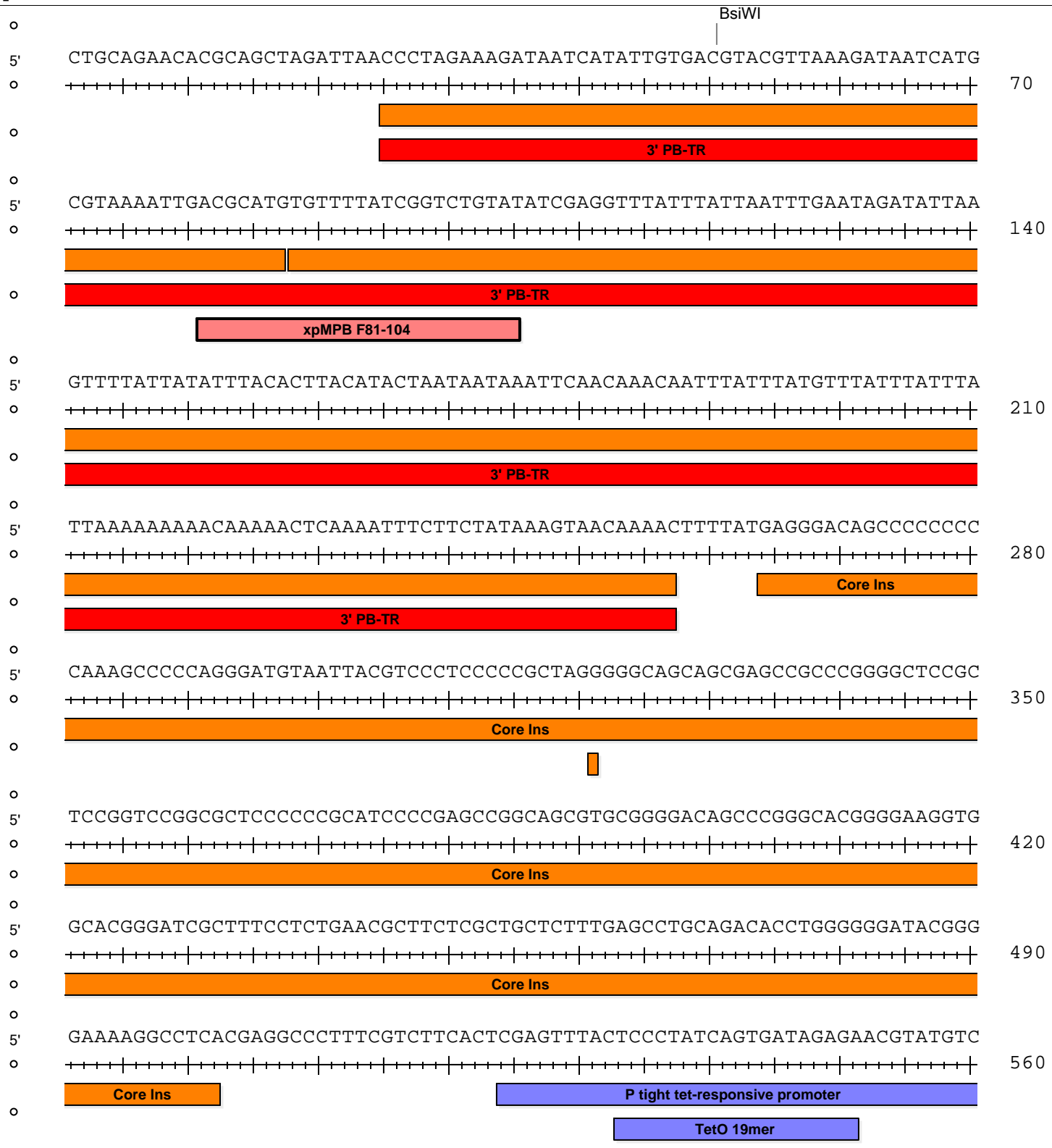


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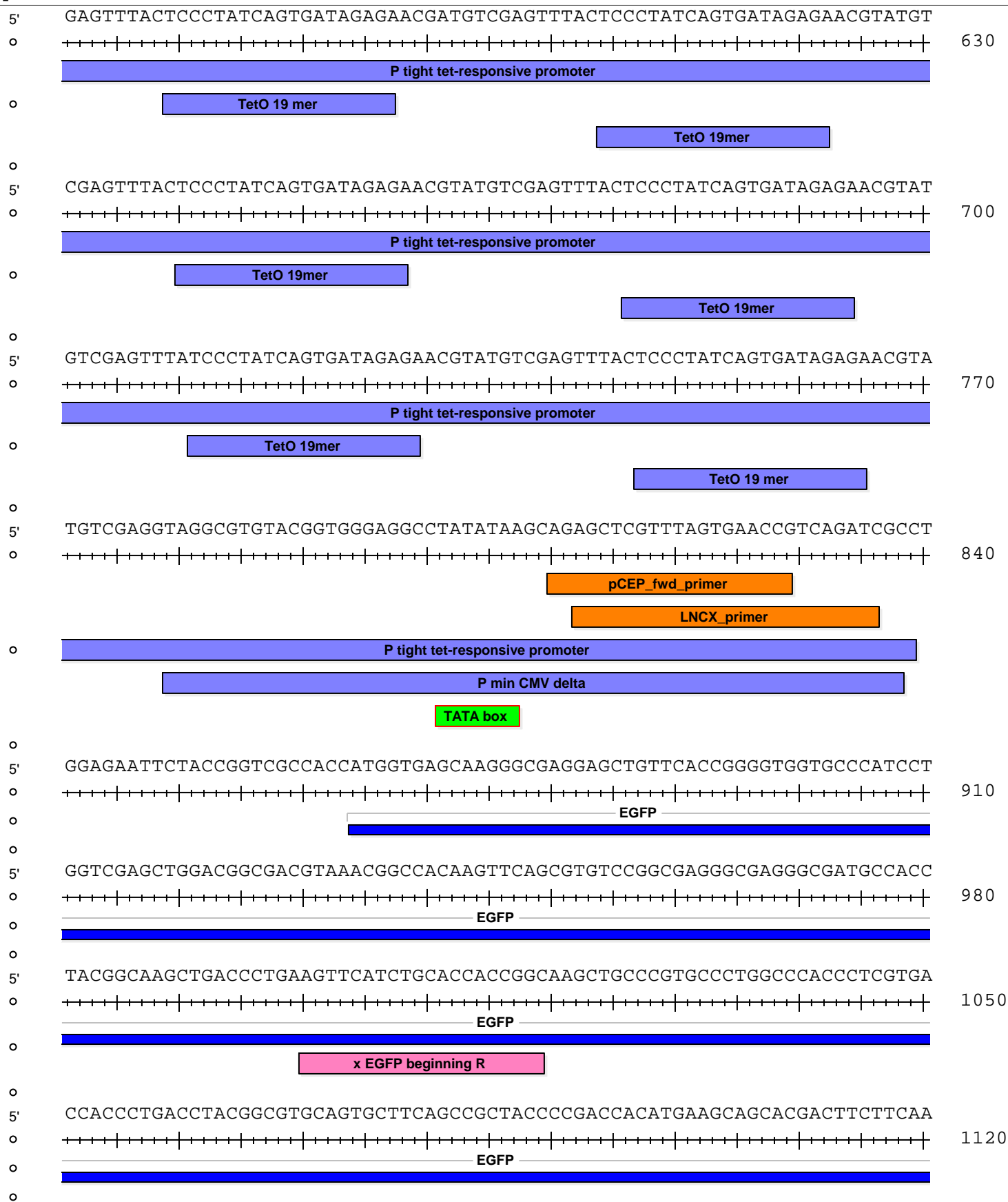
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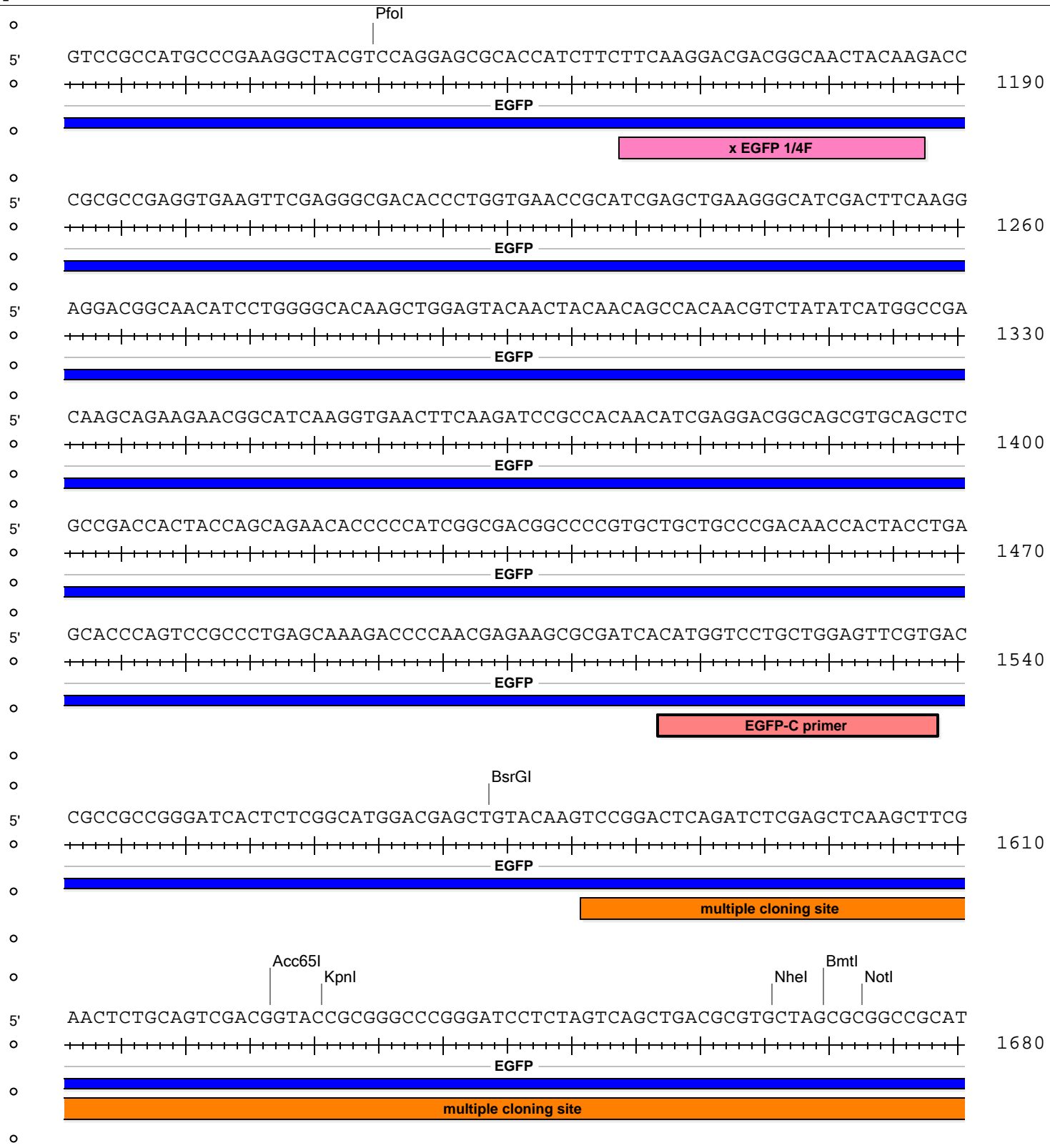
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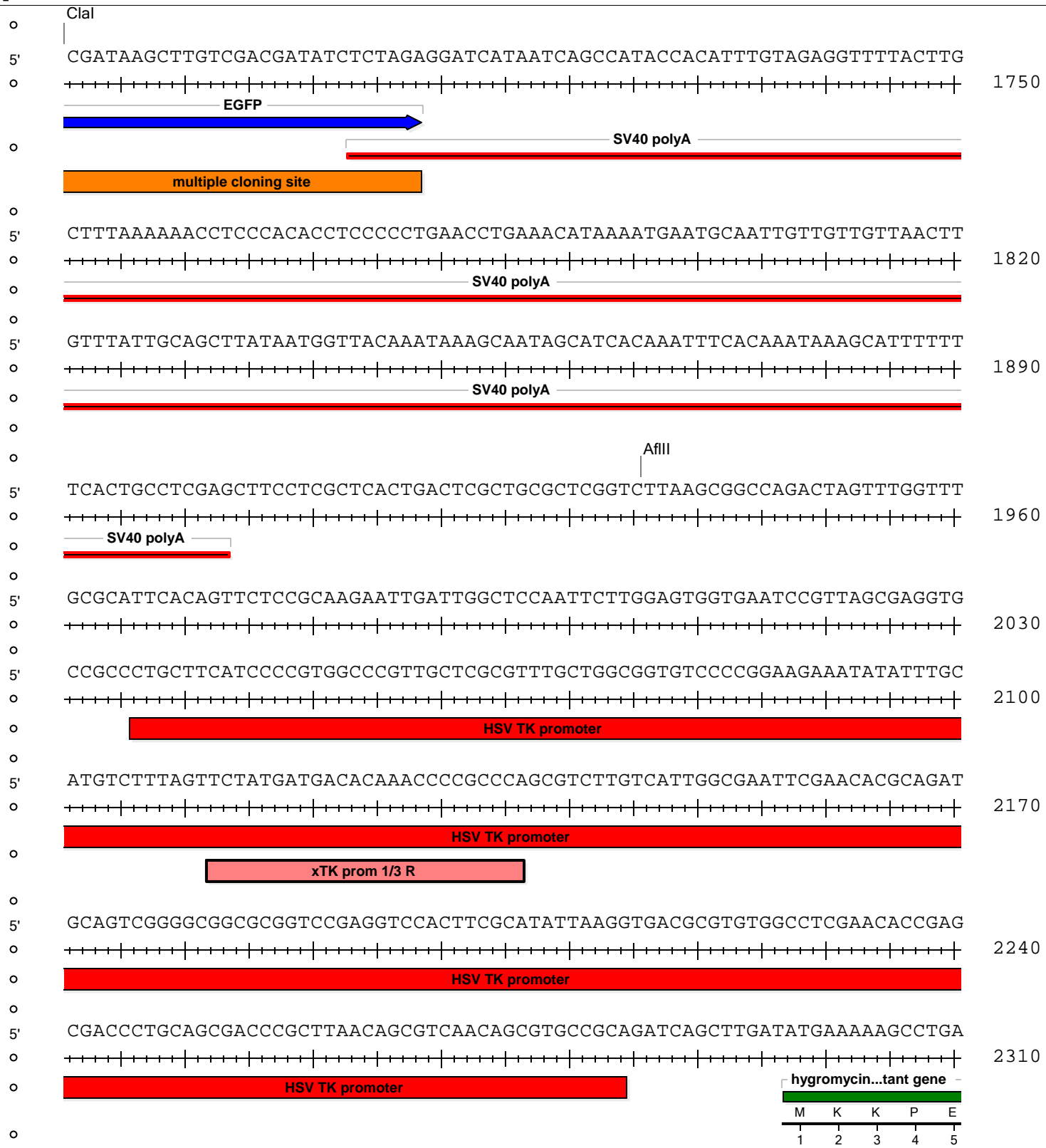


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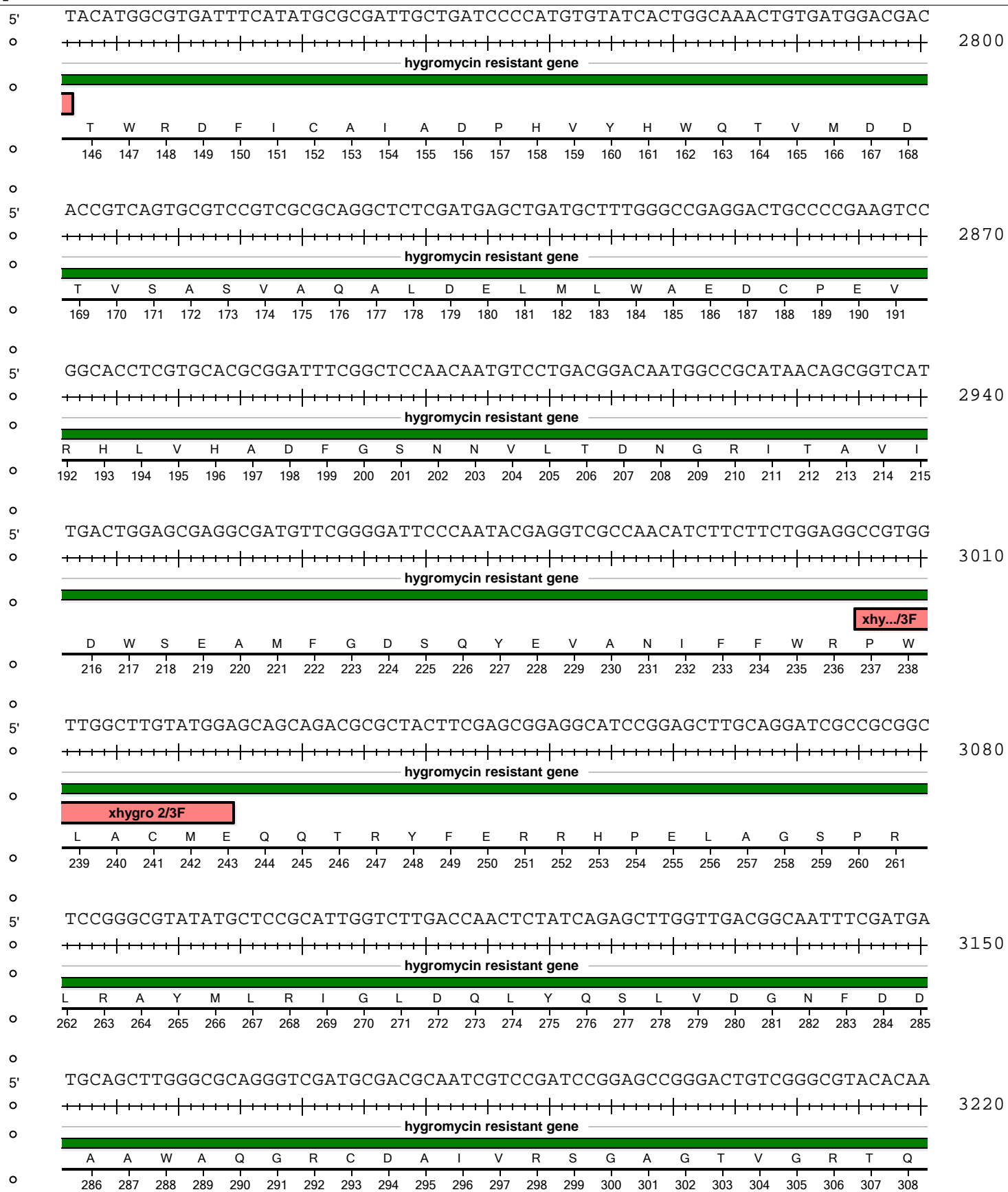




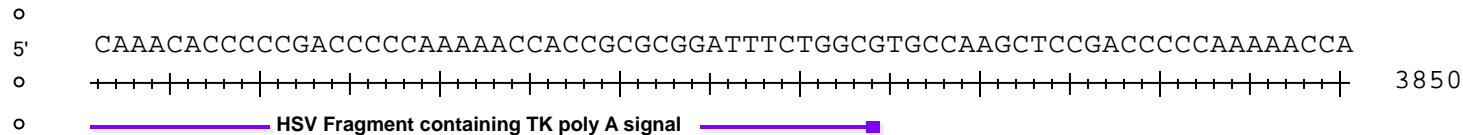
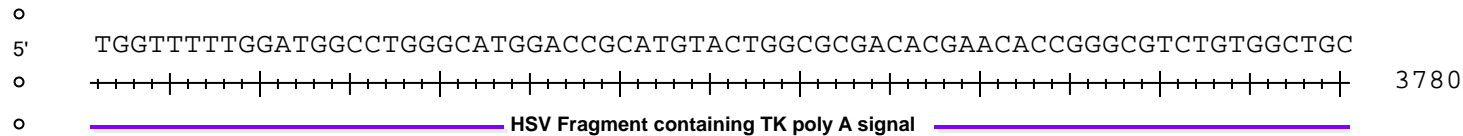
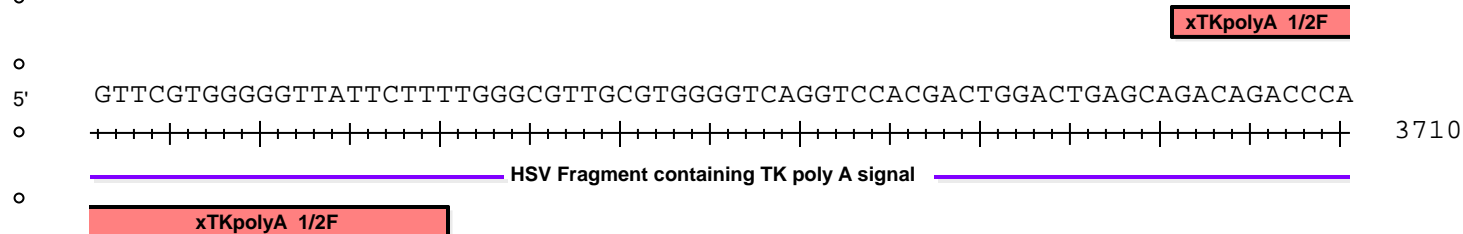
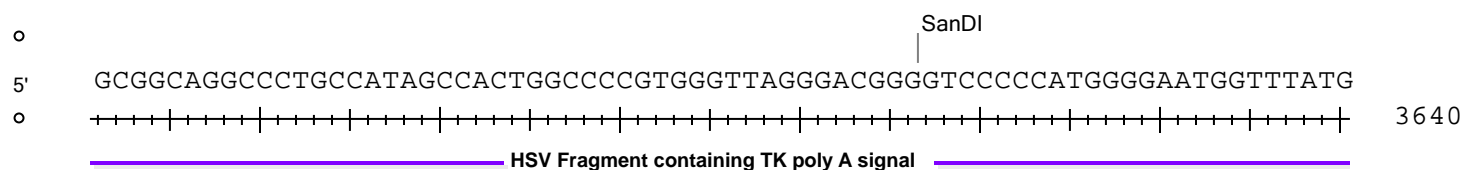
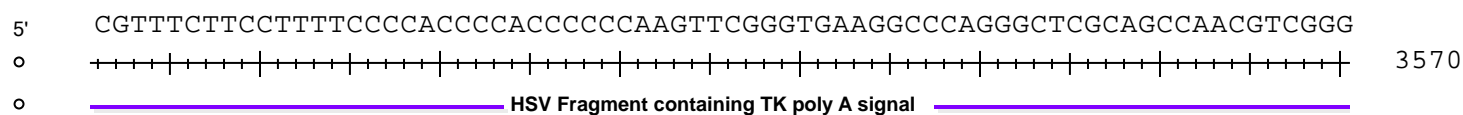
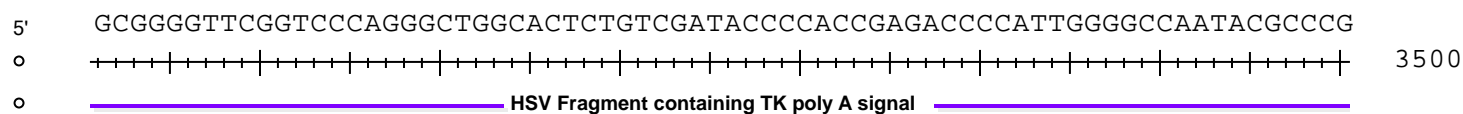
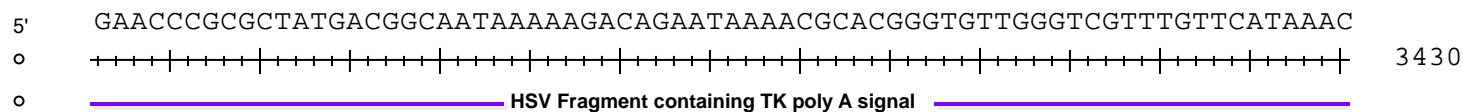
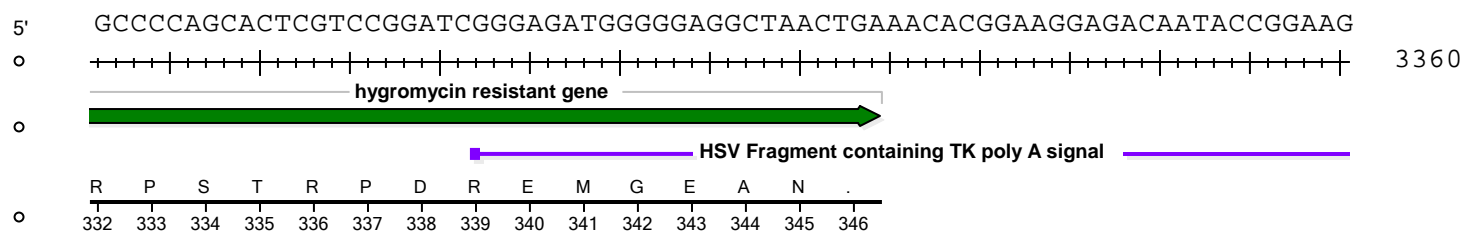
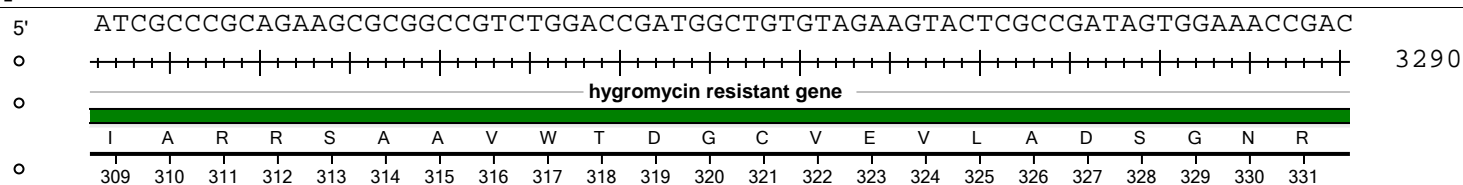
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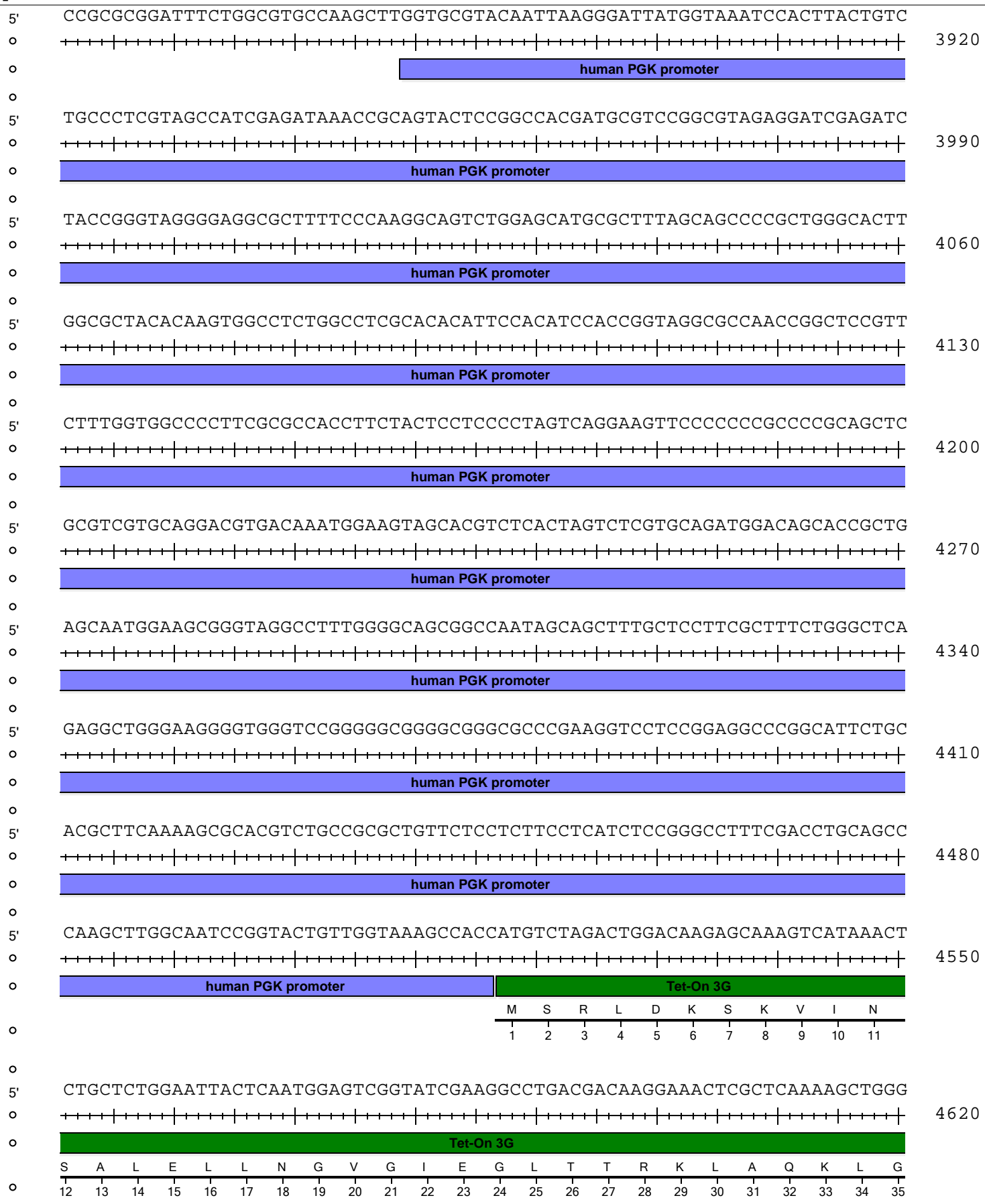
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Alol Alol'

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Tet-On 3G

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5110

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Tet-On 3G

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o

Tet-On 3G

A D A L D D F D L D M L P A D A L D D F D L D M

o

222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245

5250

5' GCTCCCCGGGTAACATAAGTAAGGATCCAGACATGATAAGATAACATTGATGAGTTTGGACAAACCACAAC

o

Tet-On 3G

L P G .

o

246 247 248 249

SV40 polyA

5320

5' AGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAA

o

SV40 polyA

5390

5' GCTGCAATAAACAAAGTTAACAAACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGGAGGTGTGGGA

o

SV40 polyA

5460

5' GGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGCTGATTATGATCCTGCAAGCCTCGTCGTC

o

SV40 polyA

5530

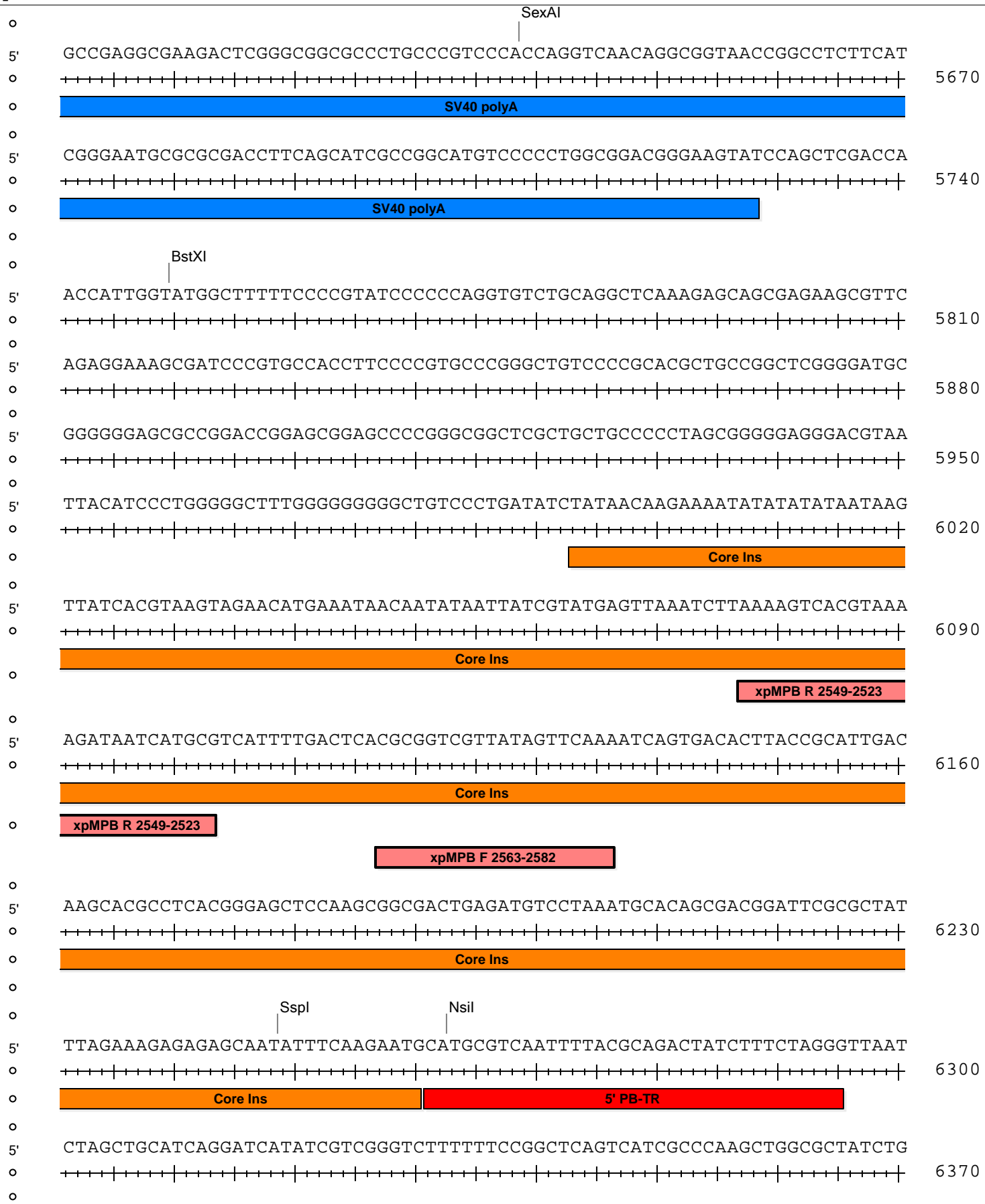
5' CTGGCCGGACCACGCTATCTGTGCAAGGTCCCCGGCCCCGGACGCGCTCCATGAGCAGAGCGCCCCGCC

o

SV40 polyA

xSV40polyA 1/2F

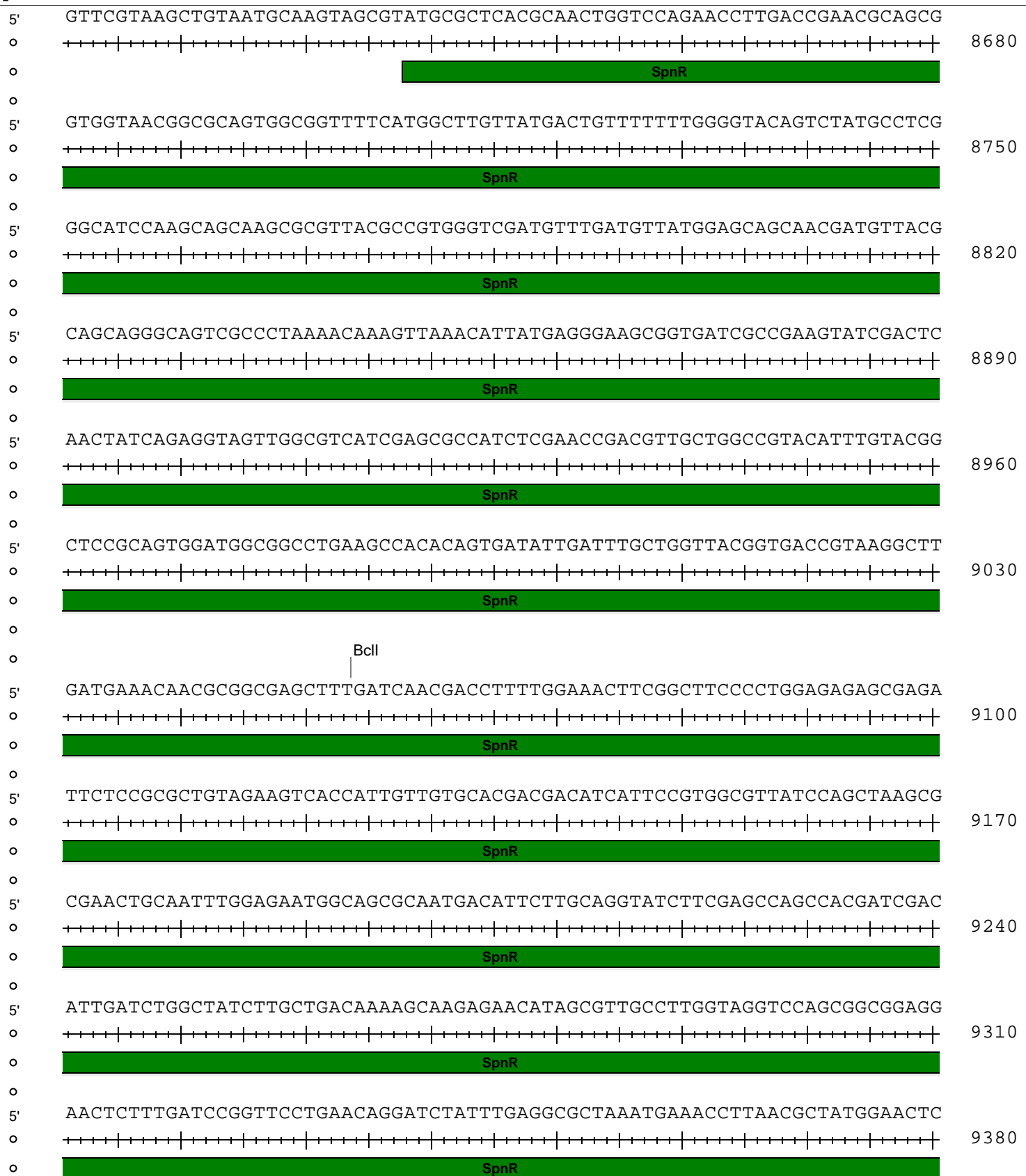
5600



p855data

5'	TCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGC	
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	7560
o		
5'	AGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGA	
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	7630
o		
5'	CAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGG	
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	7700
o		
5'	CAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGA	
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	7770
o		
5'	TCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAAACGAAAACACGTTAAGGGA	
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	7840
o		
o		
	BspHI	
o		
5'	TTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATC	
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	7910
o		
5'	AATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCA	
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	7980
o		
o		
	AhdI	
o		
5'	GCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGG	
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	8050
o		
5'	GCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGC	
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	8120
o		
5'	AATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCT	
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	8190
o		
o		
	AccI	
o		
5'	ATTAATTGTTGCCGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTG	
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	8260
o		
5'	CTACAGGCATCGTGGTGTACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCTAACGATCAAG	
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	8330
o		
5'	GCGAGTTACATGATCCCCATGTTGTGCAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGTCAGA	
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	8400
o		
5'	AGTAAGTTGGCCGAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCAT	
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	8470
o		
5'	CCGTAAGATGCTTTTCTGTGACTGGTGAGTGTACCAGCCAGGACAGAAATGCCTCGACTTCGCTGCTACC	
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	8540
o		
5'	CAAGGTTGCCGGGTGACGCACACCGTGGAAACGGATGAAGGCACGAACCCAGTGGACATAAGCCTGTTTCG	
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	8610
o		
o		

p855data



p855data

