



**Plasmid #742**

**Plasmid name** pPBH-TREtight

Plasmid description:

Name: pPBH-TREtight

Brief Description of the plasmid:

This is a PiggyBac vector for tet-inducible eukaryotic protein expression. 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 EF1 $\alpha$  promoter driven TetOn3G expression cassette. 5' to the hygromycin resistant cassette, it contains a TREtight promoter driven expression cassette. Immediately 3' to the TREtight promoter, 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 insertion protein expression in a dose-dependent fashion. Such inducible expression can be achieved in both transiently or stably transfected 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 700bp TRE-Tight promoter driven expression cassette was PCR amplified from a mutated pTRE-tight-EGFP vector (plasmid #379) with deleted EGFP sequence between the EcoRI and BglII site. Purified PCR product was inserted into the SpeI site of pPBH-EF-TetON3G (plasmid #739) by using a directional ligation independent cloning kit (InFusion HD cloning kit, Clontech 639645). Successful insertion was confirmed by restriction digestion with SpeI and sequencing.

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

The plasmid sequence listed in this document was generated by combining the sequencing result and the preexisting sequence of pPBH-EF-TetON3G.

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, **Clal**

Commonly used restriction enzyme recognizing sequences are highlighted with in the sequence of the multiple cloning site:

GATCTCGAGCTCAAGCTTCGAACTCTGCAGTCGAC**GGTACC**GCGGGCCCCGGGATCCT  
CTAGTCAGCTGACGCGT**GCTAGC**GCGGCCGC**ATCGAT**AAGCTTGTCGACGATATCTC  
TAGA

## Key Features

5' core insulator	264..510
3' PiggyBac terminal repeat	25..257
P tight tet-responsive promoter	540..855
multiple cloning site	865-982
HSV TK promoter	1297-1545
hygromycin resistant gene	1558..2595
HSV TK polyadenylation sequence	2573..3085
EF1 $\alpha$ promoter	3130..4464
Tet-On 3G	4572..5318
SV40 polyadenylation sequence	5341..5783
3' core insulator	6048..6315
5' PiggyBac terminal repeat	6316..6350
spectinomycin resistant gene	8693..9721

## Primers

x pMPB F81-104	5'-ACGCATGTGTTTTATCGGTCTGTAT-3'	binds to 81..105 of pPBH-TREtight
x TK prom 1/3 R	5'-TGGGCGGGGTTTGTGTCATCATAGA-3'	binds to 1373..1397 of pPBH-TREtight
x hygro 1/3R	5'-ACAGCGGGCAGTTCGGTTTCAG-3'	binds to 1868..1889 of pPBH-TREtight
x hygro 1/3F	5'-CGCAAGGAATCGGTCAATACACT-3'	binds to 1970..1992 of pPBH-TREtight
x hygro 2/3F	5'-CCGTGGTTGGCTTGTATGGA-3'	binds to 2266..2285 of pPBH-TREtight
x TKpolyA 1/2F	5'-ATGGTTTATGGTTCGTGGGGGTTATTCTTT-3'	binds to 2892..2921 of pPBH-TREtight
x EFprom begin F	5'-GCCACAGTCCCCGAGAAGTTG-3'	binds to 3317..3338 of pPBH-TREtight
x EFprom 1/3 F	5'-CAAGATCTGCACACTGGTATTTC-3'	binds to 3850..3872 of pPBH-TREtight
x EFprom 3/4 F	5'-CTCAAGCCTCAGACAGTGGTTCAAAG-3'	binds to 4411..4436 of pPBH-TREtight
x TET-ON 3G 1/2F	5'-CACTGGGCTGCGTATTGGAGGAACA-3'	binds to 4993..5017 of pPBH-TREtight
x SV40polyA 1/2F	5'-GGCCGGACCACGCTATCTGT-3'	binds to 5588..5607 of pPBH-TREtight
x pMPB R 2549-2523	5'-CGCATGATTATCTTTTACGTGACTTTT-3'	binds to 6132..6158 of pPBH-TREtight
x pMPB F 2563-2582	5'-CGCGGTCGTTATAGTTCAAA-3'	binds to 6172..6191 of pPBH-TREtight
x pMPB F 6058-6083	5'-GAGATCACCAAGGTAGTCGGCAAATA-3'	

binds to 9677..9702 of pPBH-TREtight

Diagnostic Digests

SpeI 0.7 kb and 9.0 kb

KpnI 9.7 kb

Plasmid History

Plasmid generated by Juanmin Zha

Date plasmid generated 06/2012

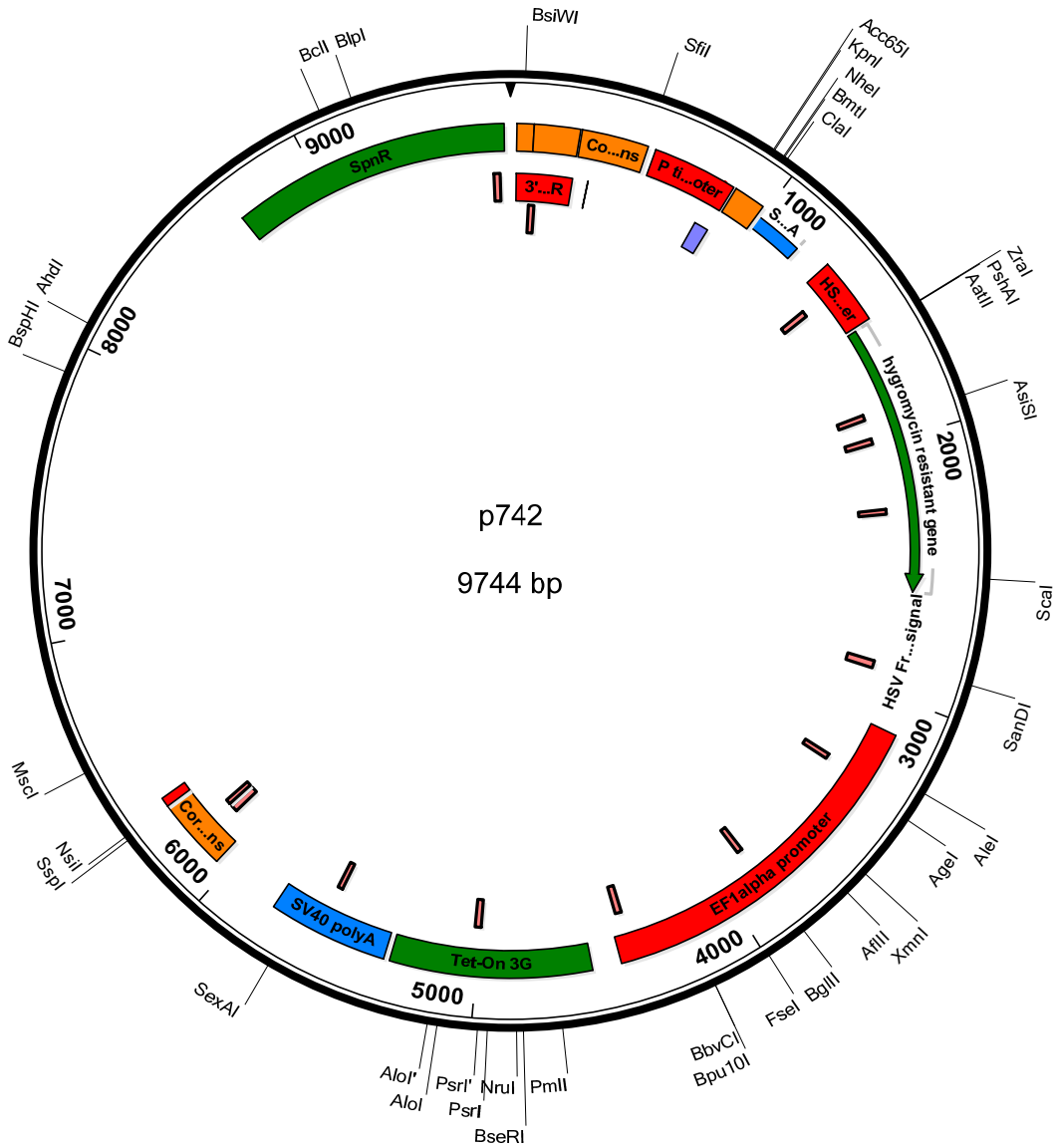
Data file generated by Juanmin Zha

Data file generated 06/2012

Data file version history V1.0

Attachments:

Plasmid map:



### Plasmid sequence

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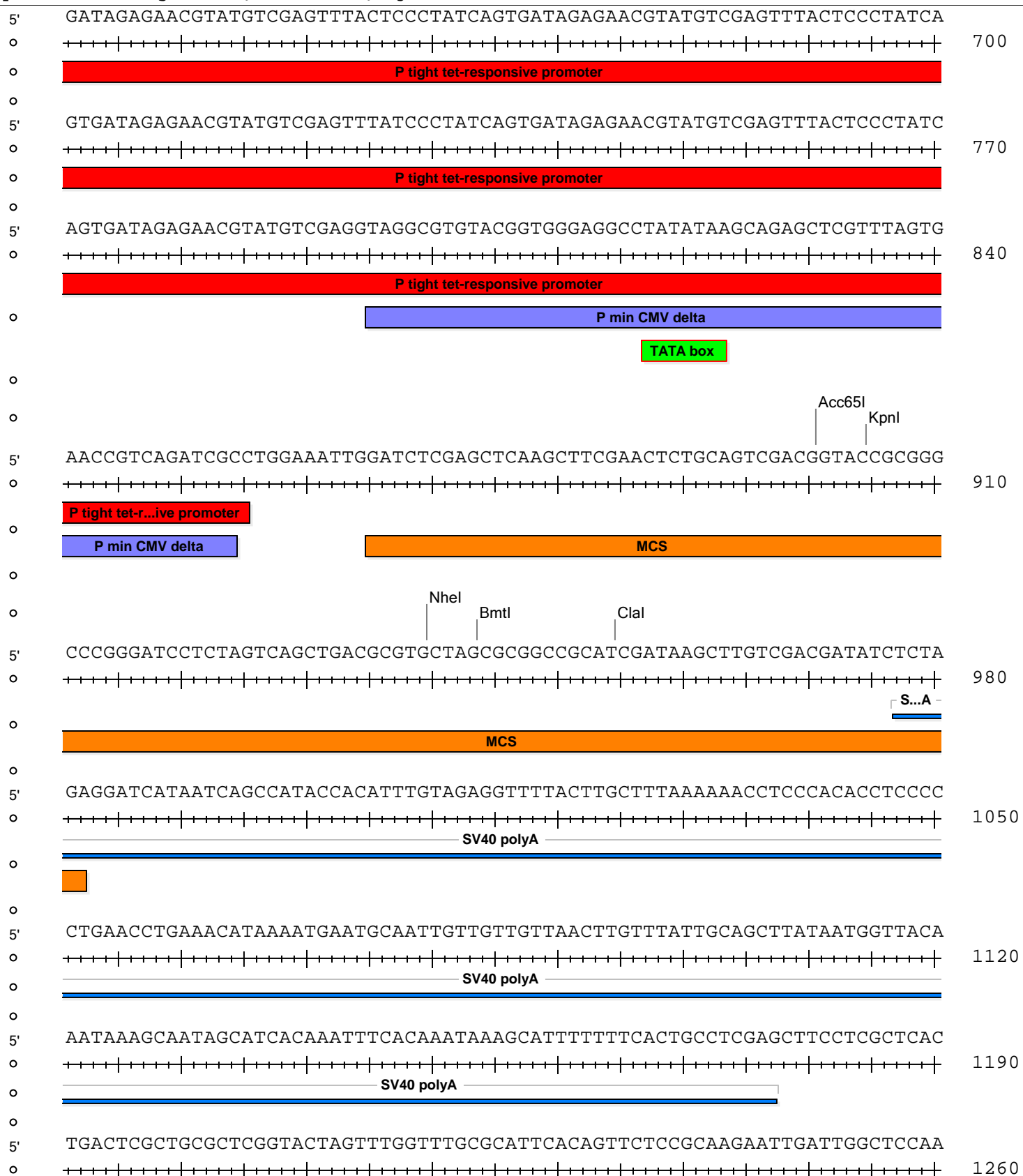
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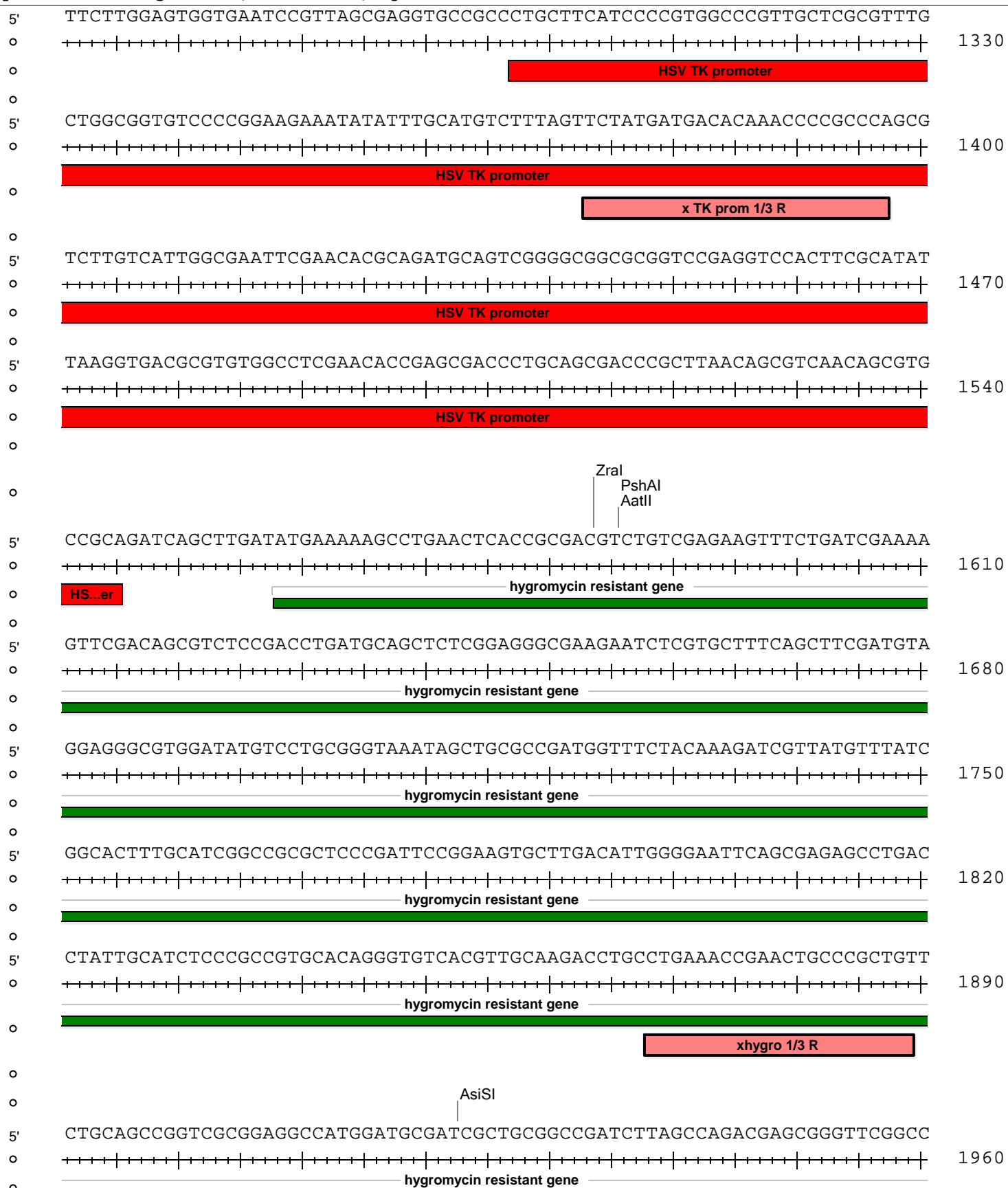




## pMPH2-EF1-TREtight-EGFP(MPH+611+376) Zjm



pMPH2-EF1-TREtight-EGFP(MPH+611+376) Zjm



pMPH2-EF1-TREtight-EGFP(MPH+611+376) Zjm

5' CATTTCGGACCGCAAGGAATCGGTCAATACACTACATGGCGTGATTTCATATGCGCGATTGCTGATCCCCA

2030

hygromycin resistant gene

xhygro 1/3F

5' TGTGTATCACTGGCAAACCTGTGATGGACGACACCGTCAGTGGTCCGTCGCGCAGGCTCTCGATGAGCTG

2100

hygromycin resistant gene

5' ATGCTTTGGGCCGAGGACTGCCCCGAAGTCCGGCACCTCGTGCACGCGGATTTTCGGCTCCAACAATGTCC

2170

hygromycin resistant gene

5' TGACGGACAATGGCCGCATAACAGCGGTCATTGACTGGAGCGAGGCGATGTTTCGGGGATTCCCAATACGA

2240

hygromycin resistant gene

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2310

hygromycin resistant gene

xhygro 2/3F

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2380

hygromycin resistant gene

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2450

hygromycin resistant gene

5' ATCCGGAGCCGGGACTGTTCGGGCGTACACAAATCGCCCGCAGAAGCGCGGCCGTCTGGACCGATGGCTGT

2520

hygromycin resistant gene

Scal

5' GTAGAAGTACTCGCCGATAGTGGAAACCGACGCCCCAGCACTCGTCCGGATCGGGAGATGGGGGAGGCTA

2590

hygromycin resistant gene

HSV Fragment...ly A signal

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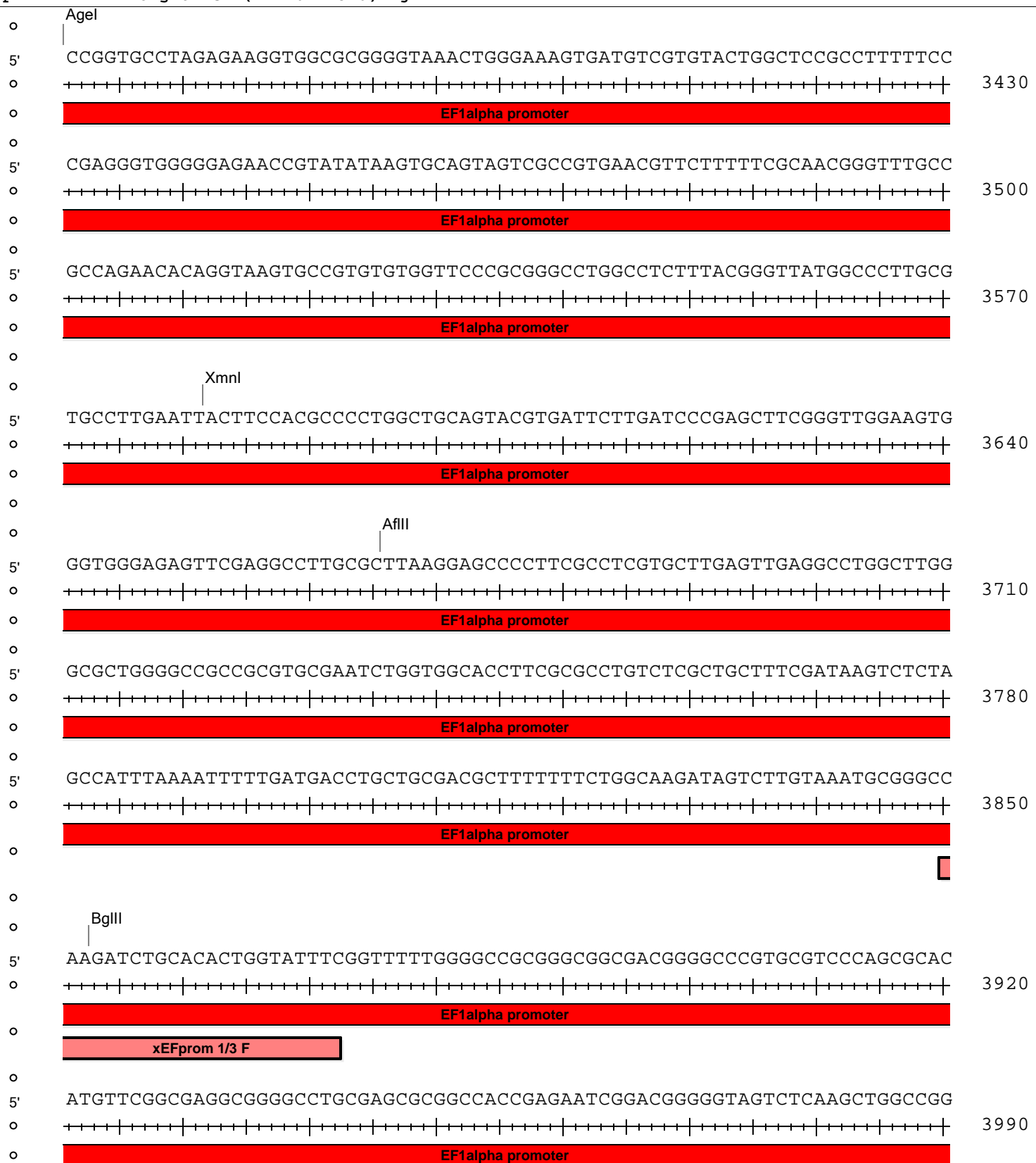
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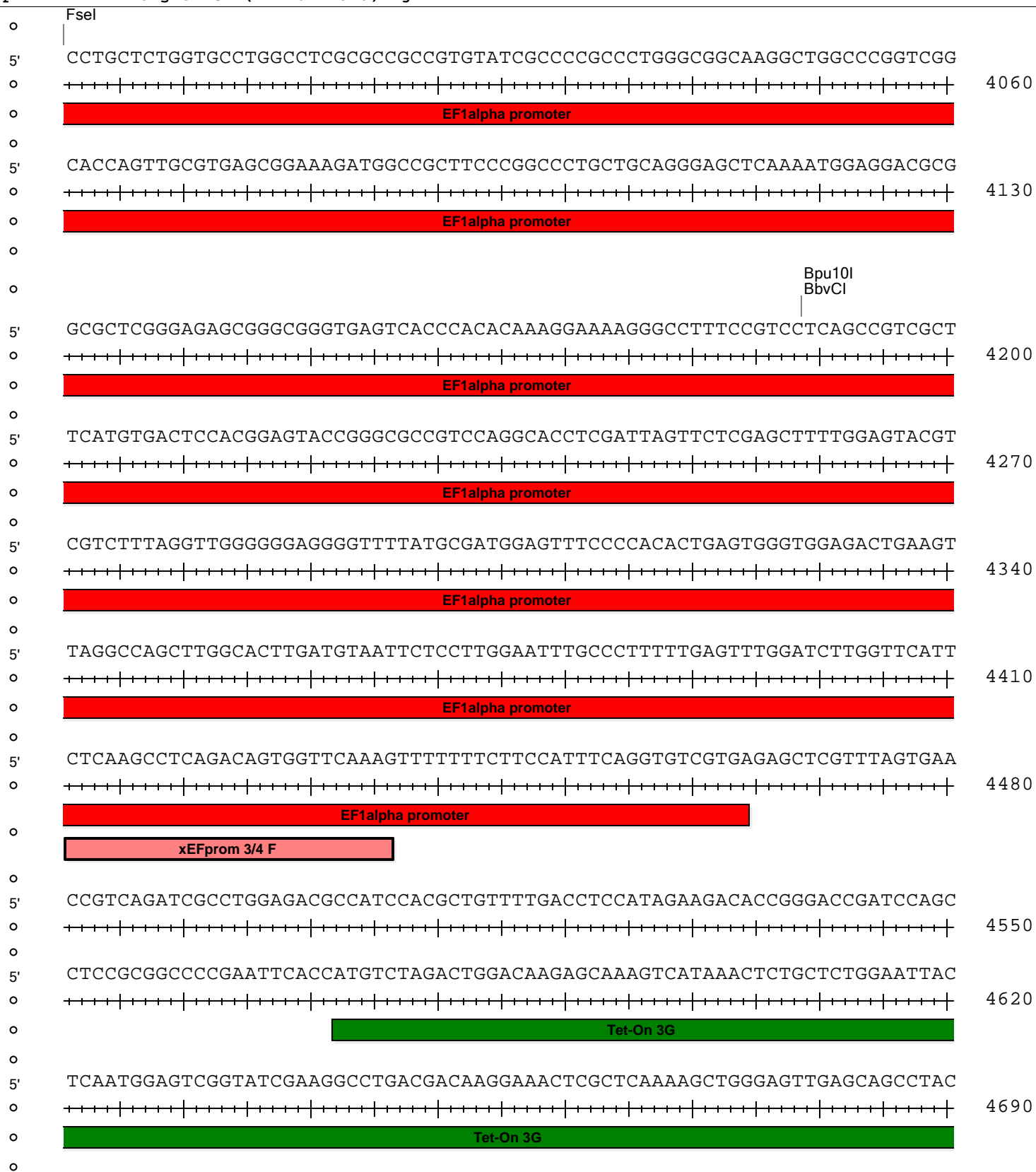
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pMPH2-EF1-TREtight-EGFP(MPH+611+376) Zjm



pMPH2-EF1-TREtight-EGFP(MPH+611+376) Zjm









## pMPH2-EF1-TREtight-EGFP(MPH+611+376) Zjm

