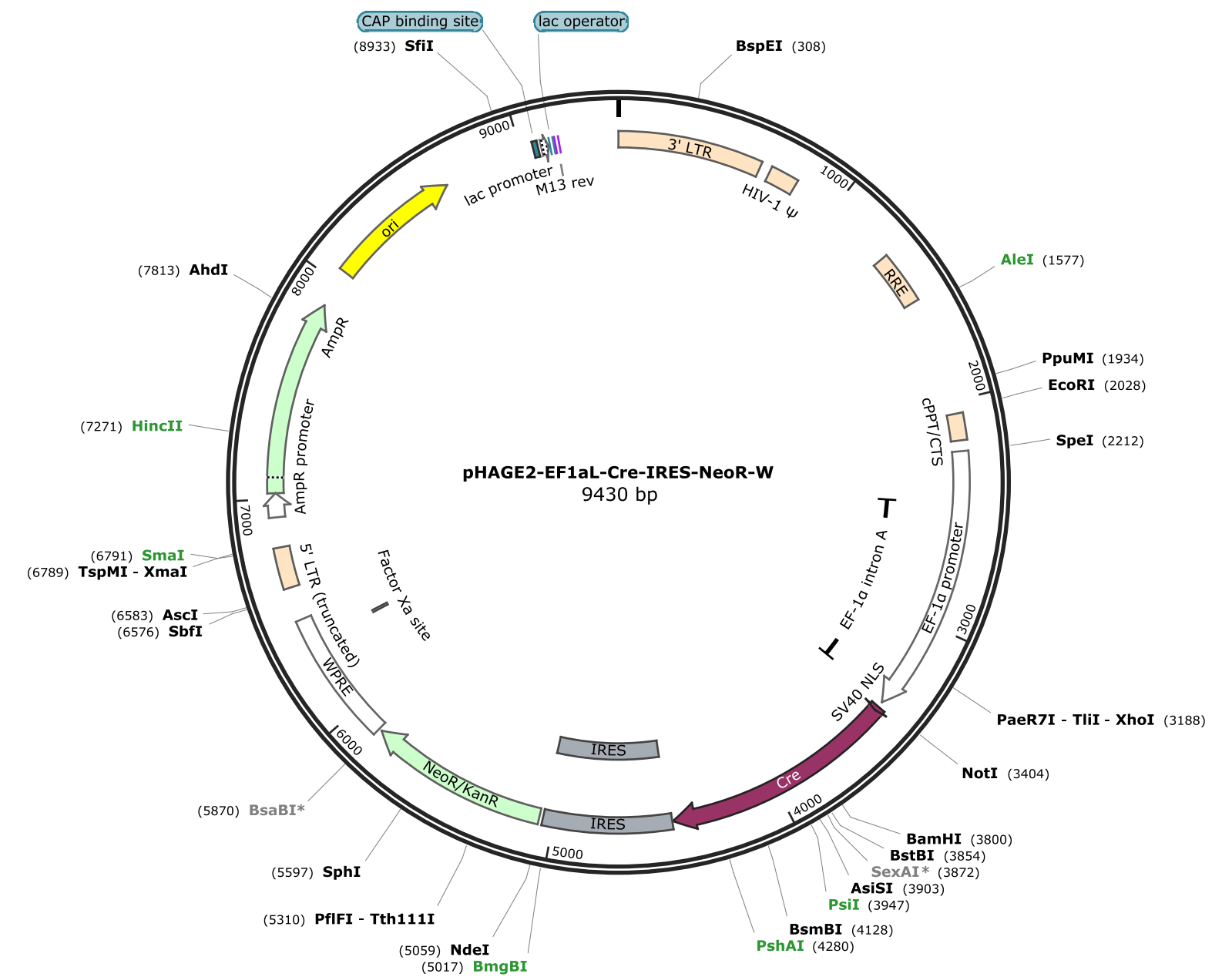




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1625

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PpuMI

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2015

EcoRI

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2080

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2145

cPPT/CTS

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2210

cPPT/CTS

SpeI

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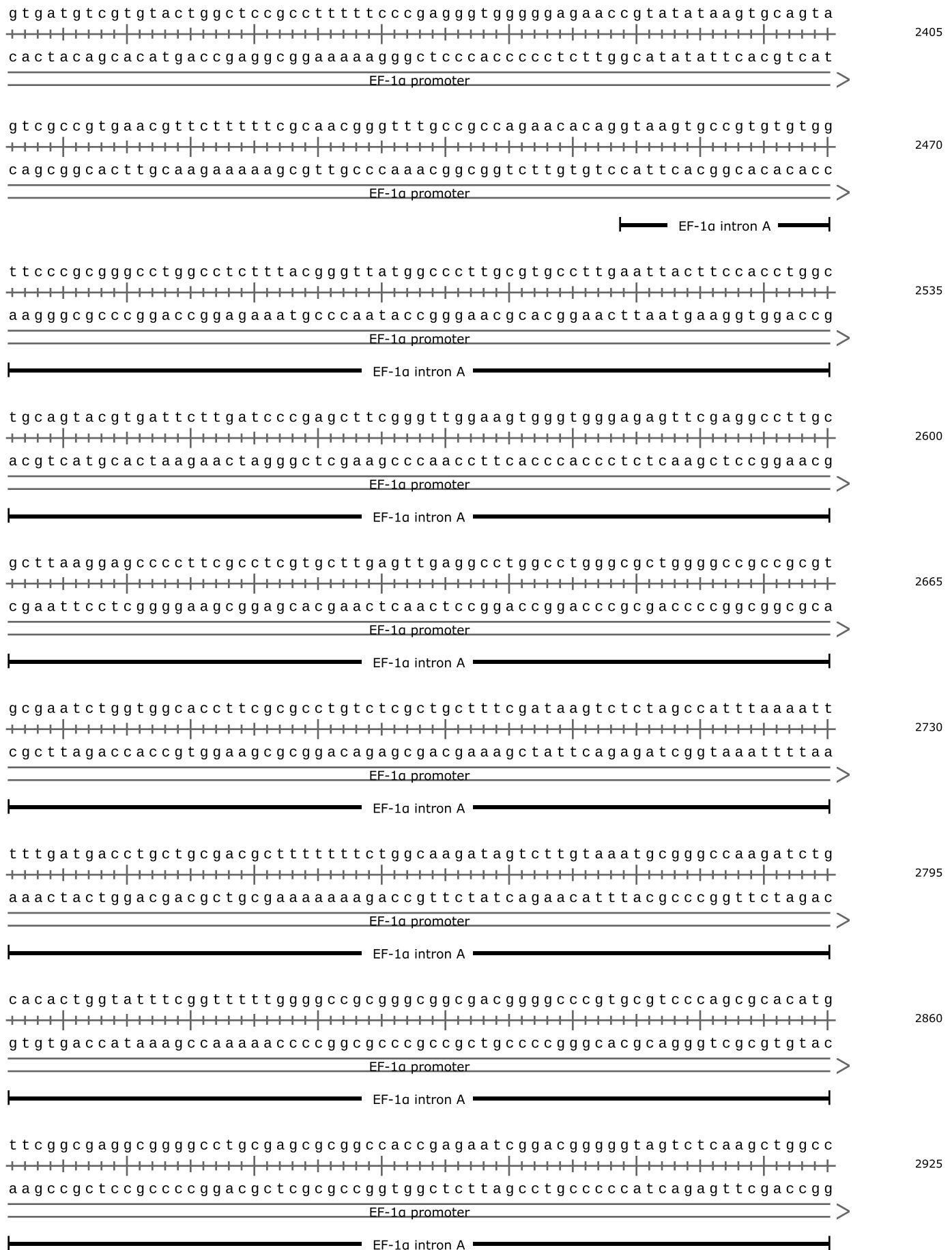
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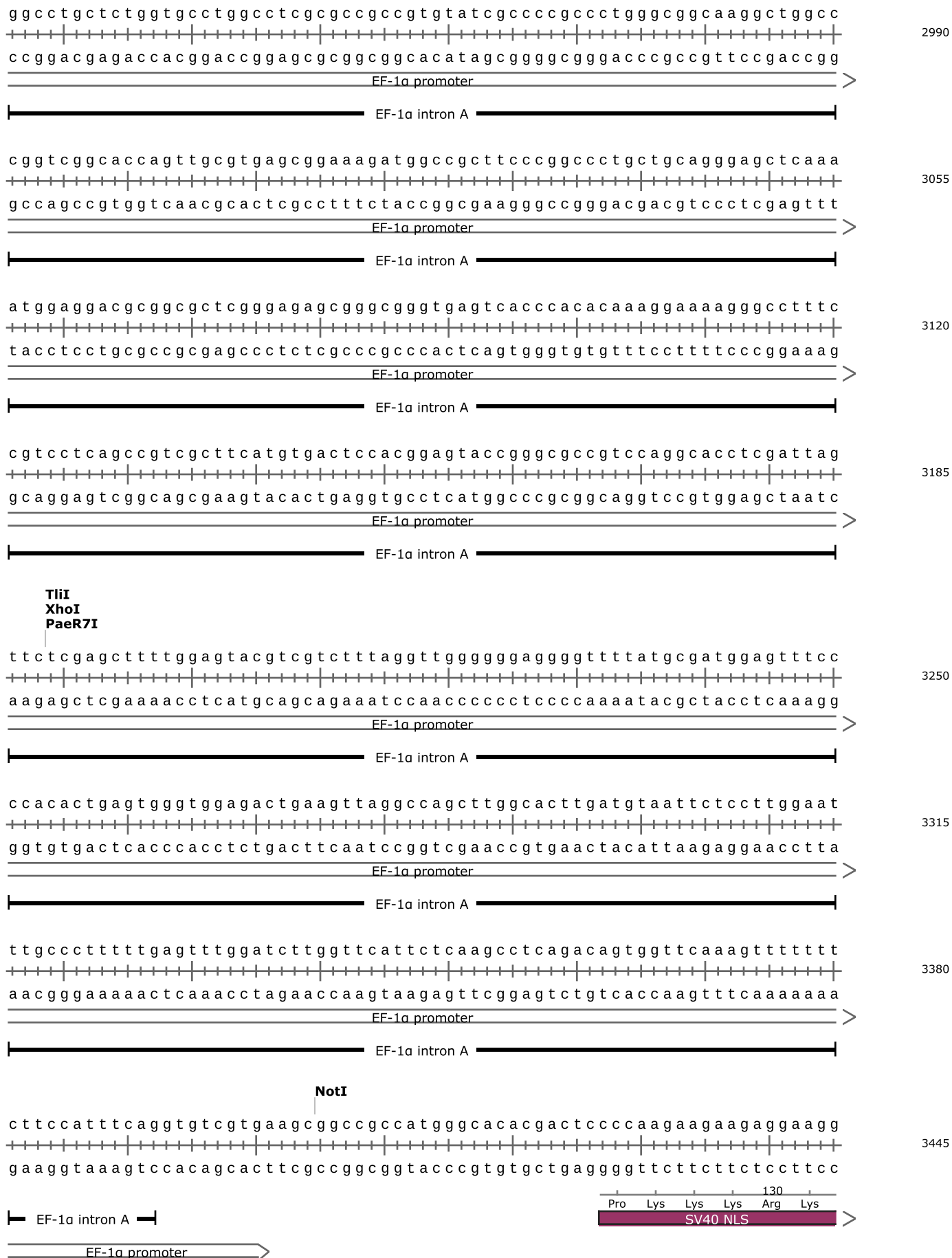
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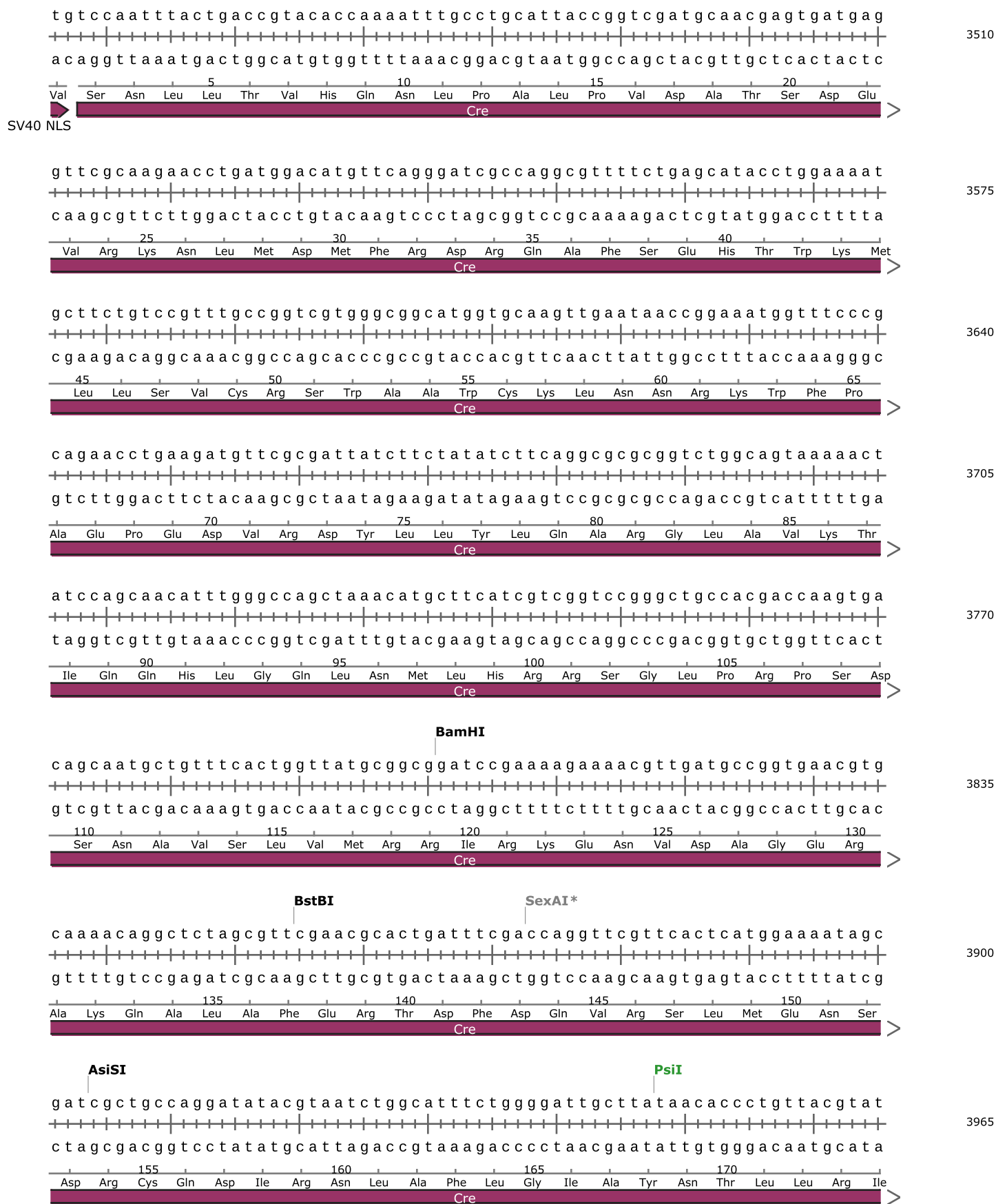
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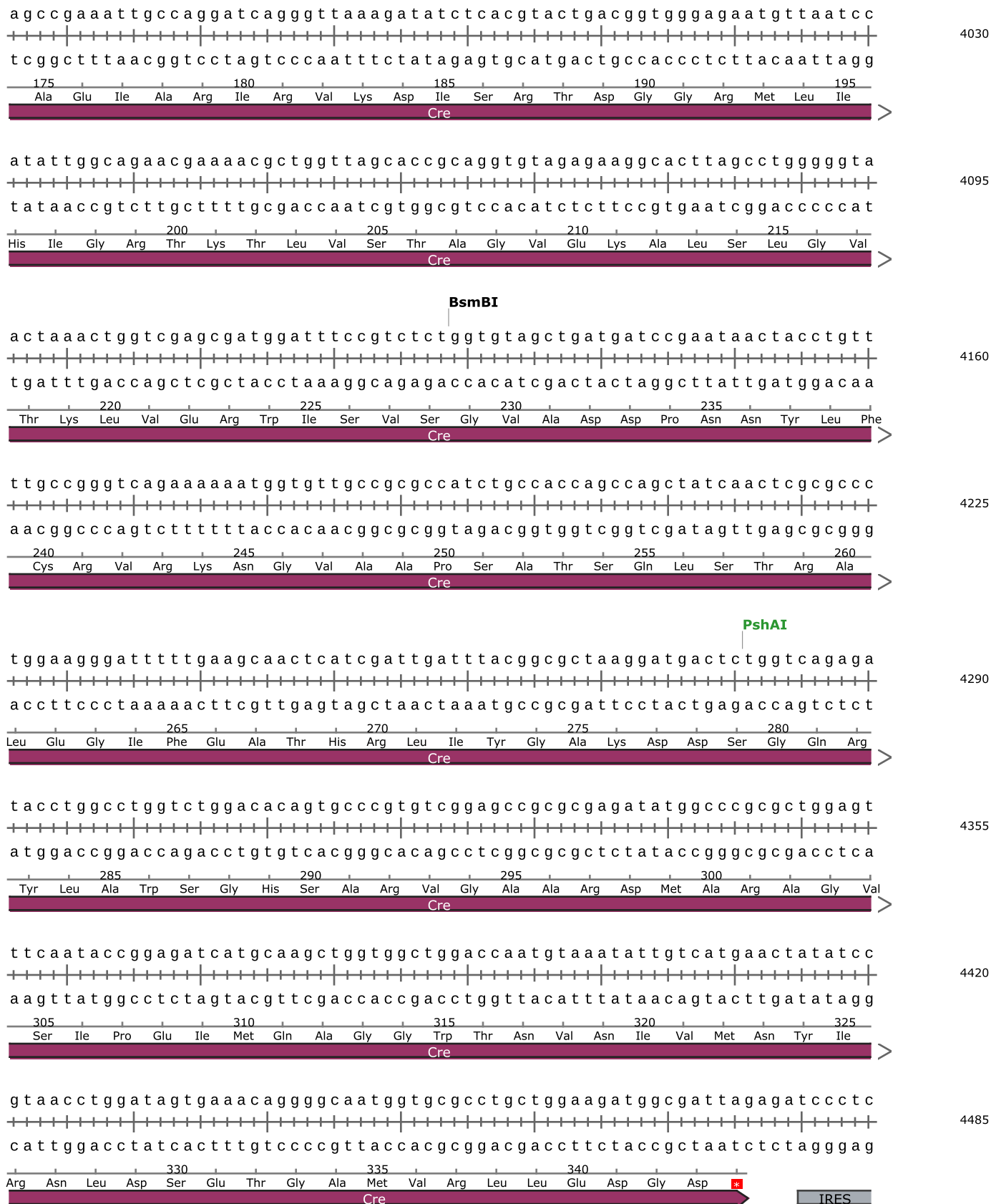
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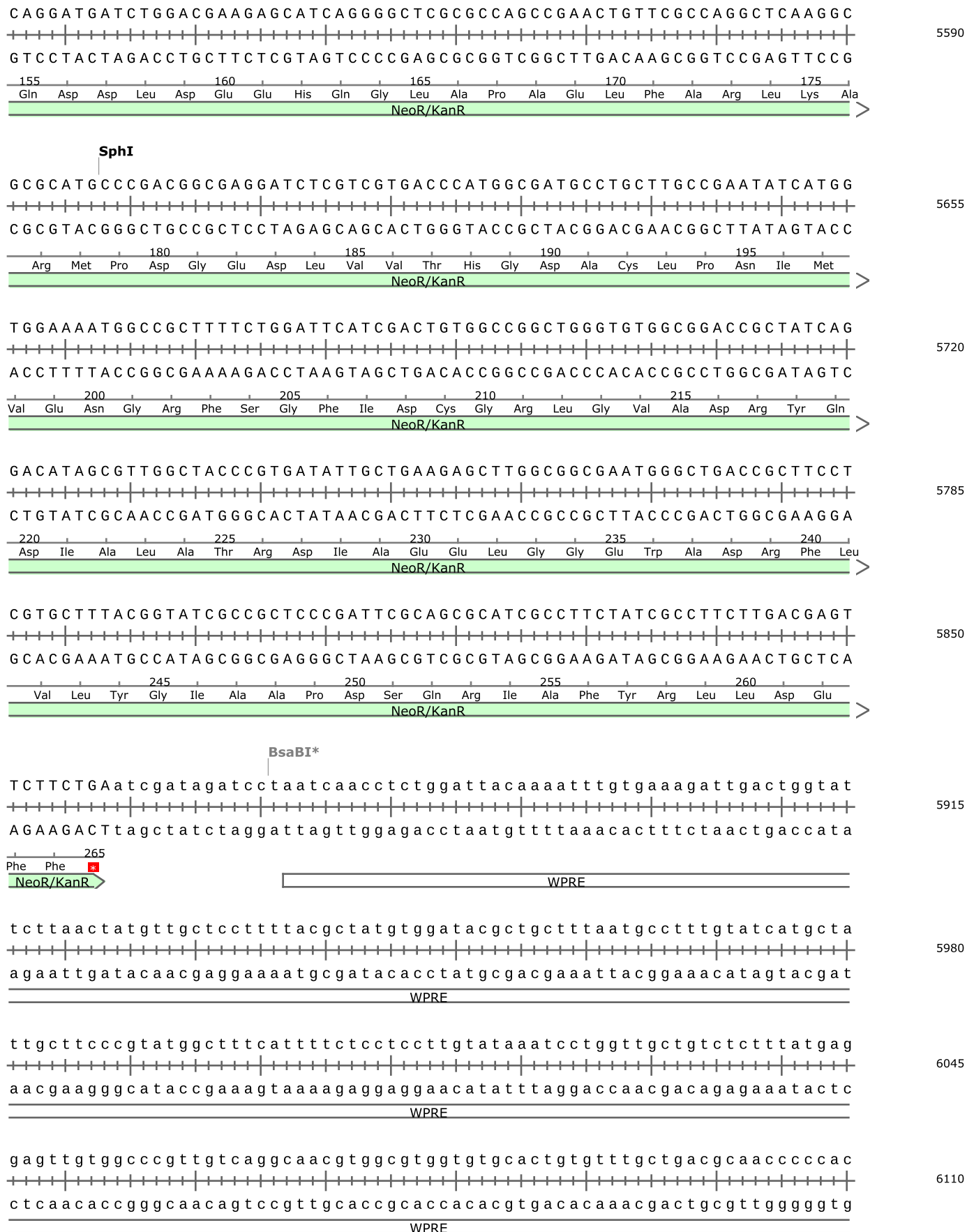
EF-1a promoter

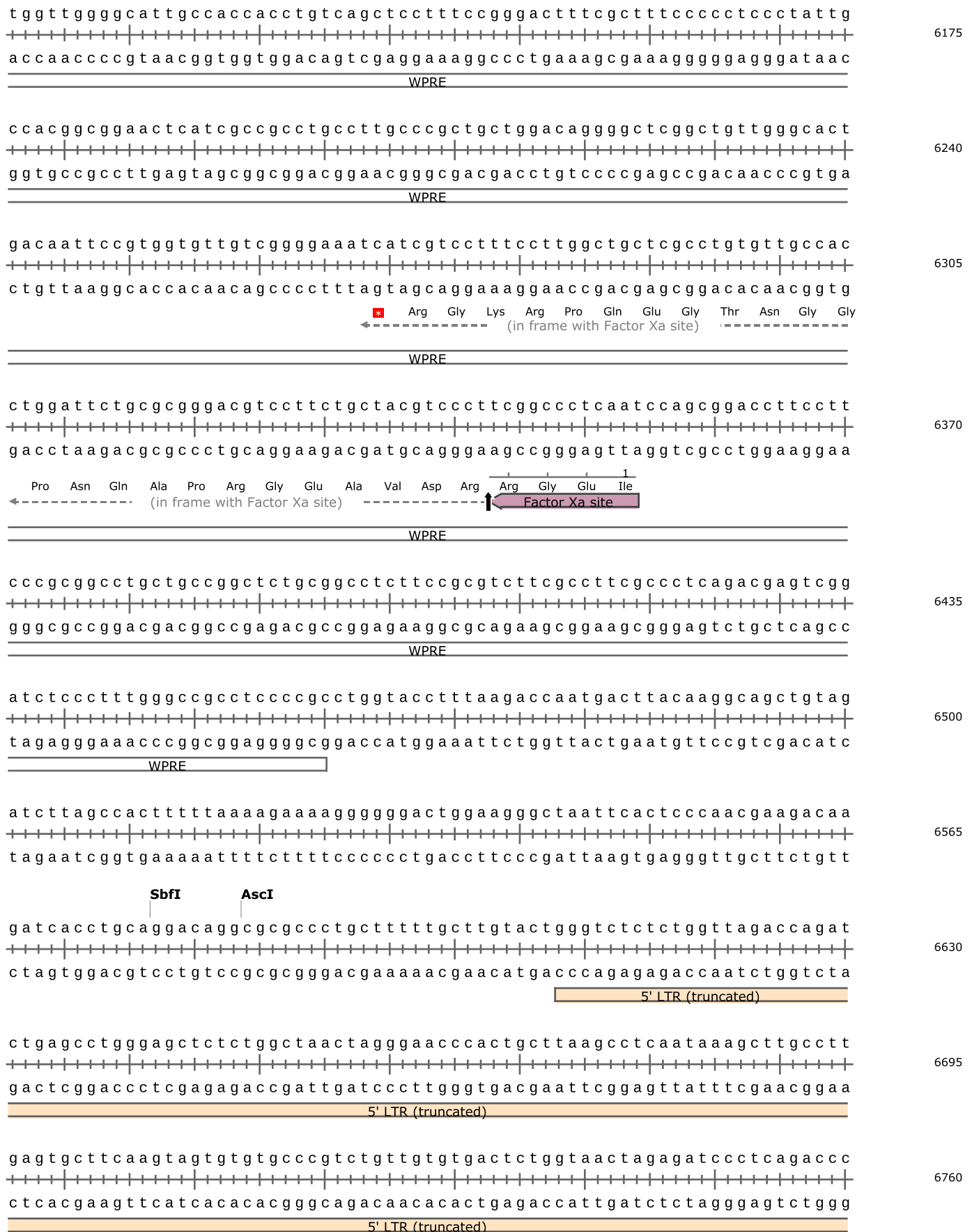


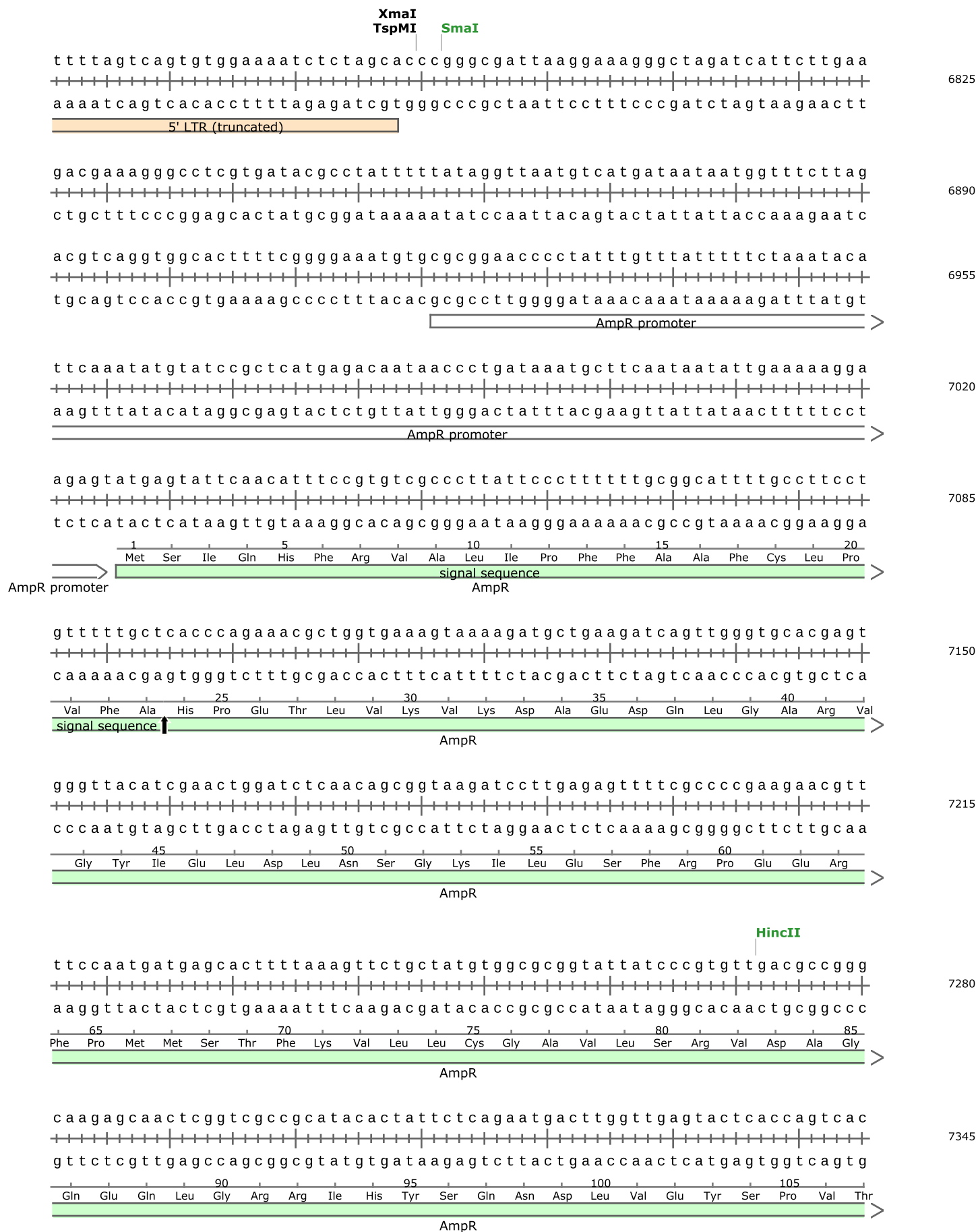


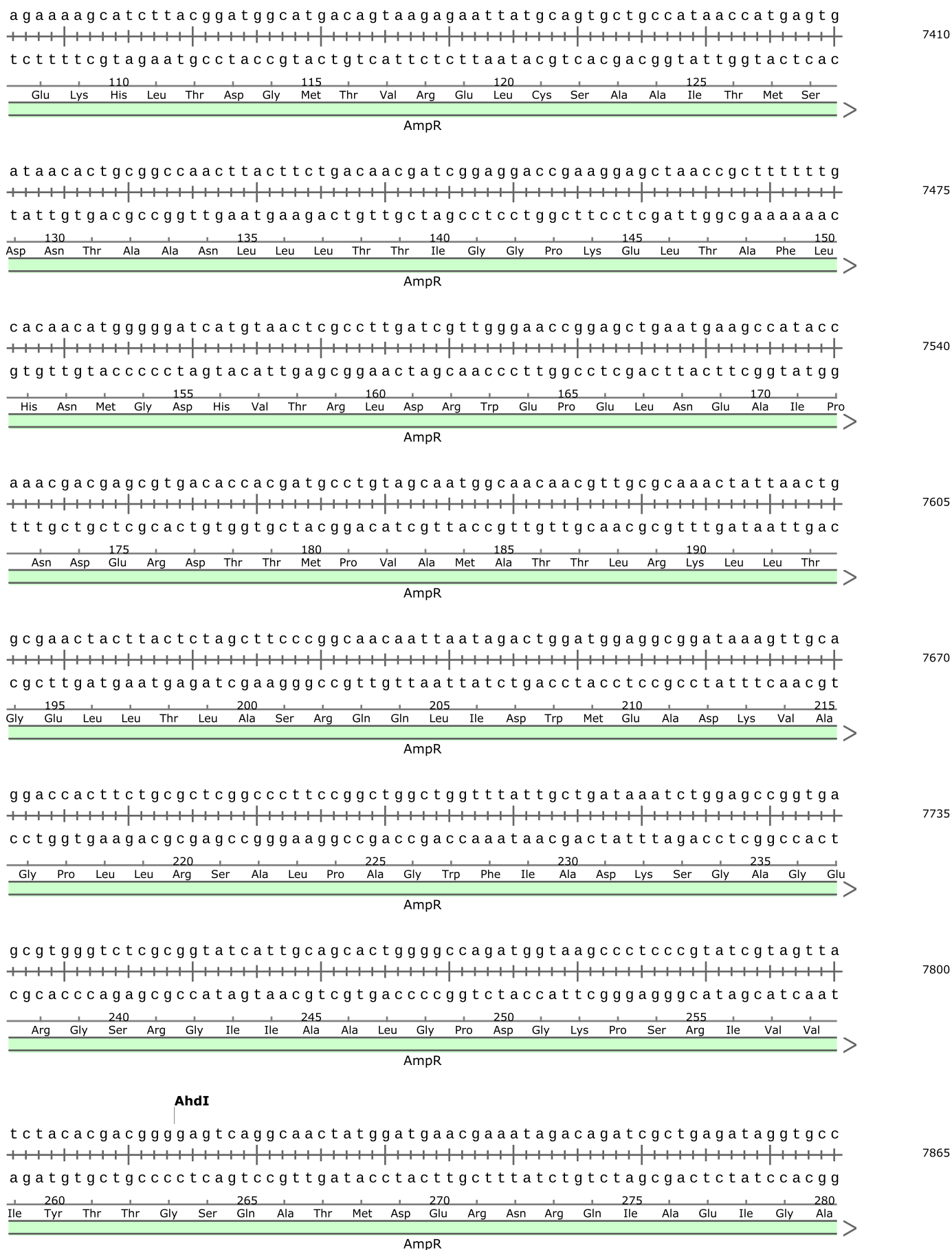


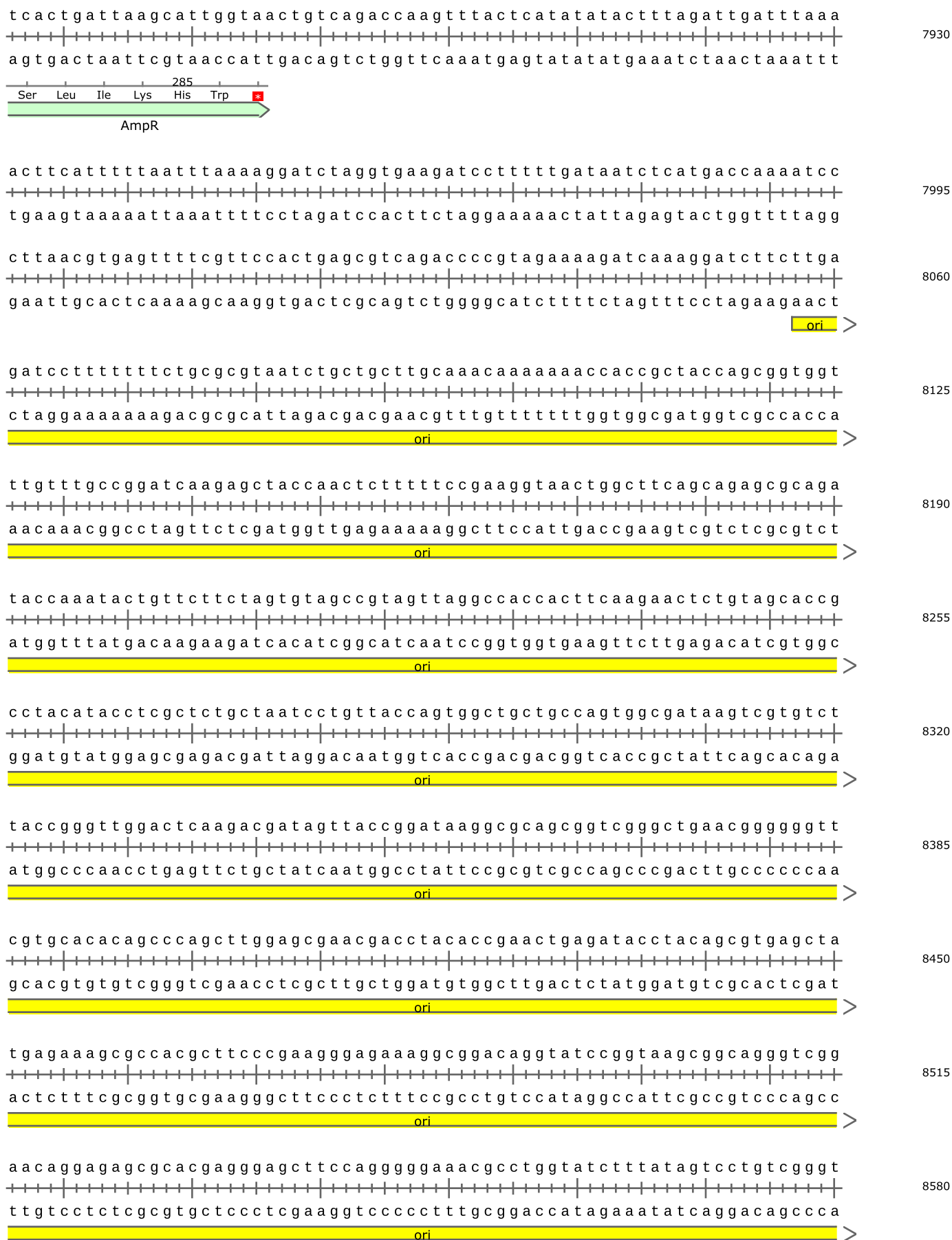


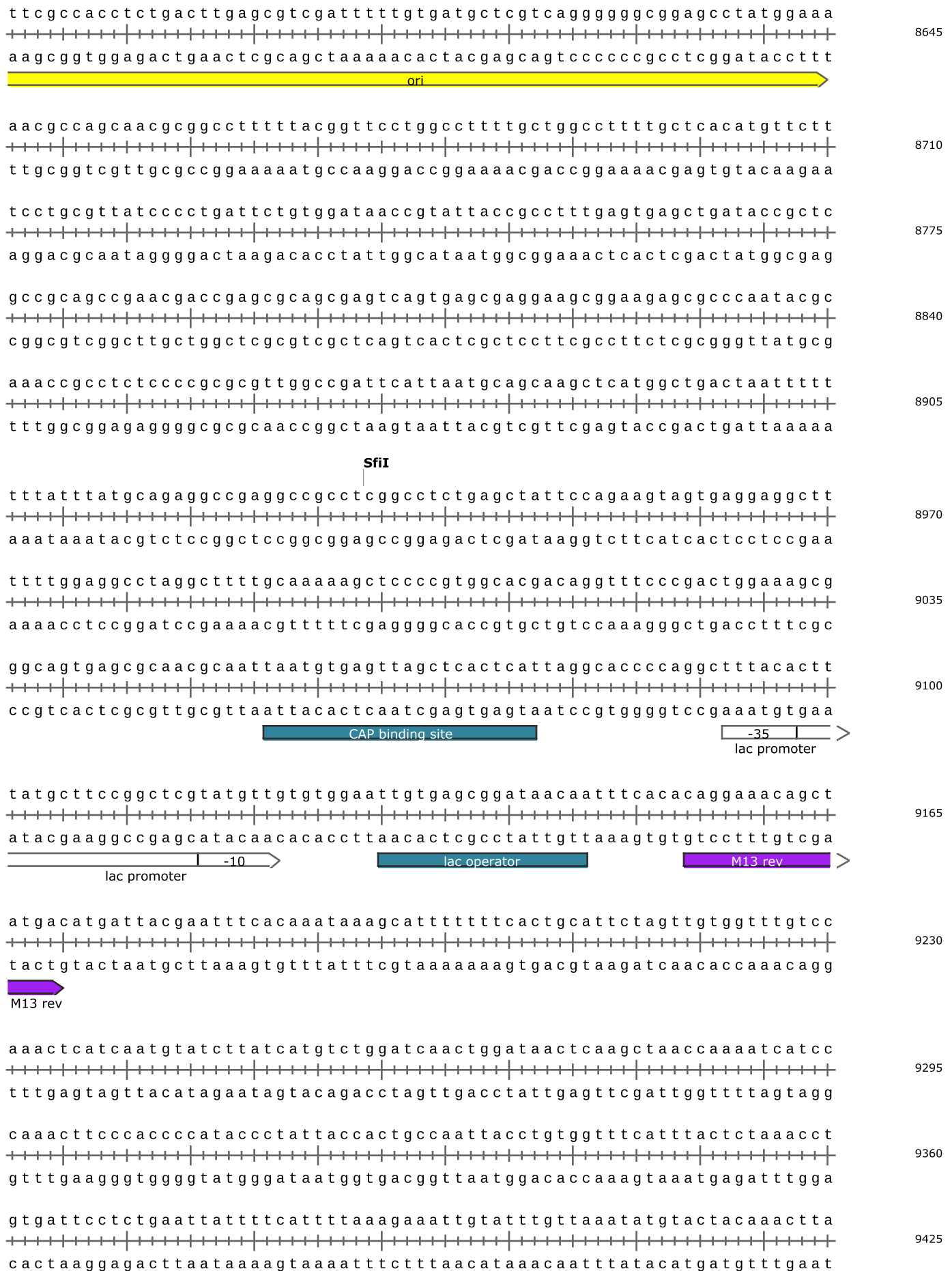












g t a g t 3 '
+ + + + + . . . 9430
c a t c a 5 '

Enzymes	Sites	
AhdI	1	7813
AleI	1	1577
AscI	1	6583
AsiSI	1	3903
BamHI	1	3800
BmgBI	1	5017
BsaBI	1*	5870*
BsmBI	1	4128
BspEI	1	308
BstBI	1	3854
EcoRI	1	2028
HincII	1	7271
NdeI	1	5059
NotI	1	3404
PaeR7I	1	3188
PfiFI	1	5310
PpuMI	1	1934
PshAI	1	4280
PsiI	1	3947
SbfI	1	6576
SexAI	1*	3872*
SfiI	1	8933
SmaI	1	6791
SpeI	1	2212
SphI	1	5597
TliI	1	3188
TspMI	1	6789
Tth111I	1	5310
XhoI	1	3188
XmaI	1	6789

Feature	Location	Size (bp)			Type
✓ 3' LTR	1 .. 634	634			LTR
/note	= 3' long terminal repeat (LTR) from HIV-1				
✓ HIV-1 Ψ	681 .. 806	126			misc_feature
/note	= packaging signal of human immunodeficiency virus type 1				
✓ RRE	1303 .. 1536	234			misc_feature
/note	= The Rev response element (RRE) of HIV-1 allows for Rev-dependent mRNA export from the nucleus to the cytoplasm.				
✓ cPPT/CTS	2058 .. 2175	118			misc_feature
/note	= central polypurine tract and central termination sequence of HIV-1				
✓ EF-1α promoter	2223 .. 3401	1179			promoter
/note	= strong constitutive promoter for human elongation factor EF-1α				
✓ EF-1α intron A	2454 .. 3392	939			intron
/note	= intron upstream of the start codon of human EF-1α				
✓ SV40 NLS	3427 .. 3447	21			CDS
/product	= nuclear localization signal of SV40 large T antigen				
/translation	= PKKKRKV 7 amino acids = 883.1 Da				
✓ Cre	3448 .. 4476	1029			CDS
/product	= site-specific recombinase				
/note	= Cre is a site-specific recombinase from bacteriophage P1. Recombination occurs at <i>loxP</i> sequences.				
/translation	= SNLLTVHQNLPALPVDATSDEVRKNLMDMFRDRQAFSEHTWKMLLSVCRSWAAWCKLNNRKWFPAEPEDVRDYLLYLQARGLAVKTIQQHLGQIRSGLPSPSDSNAVSLVMRRIRKENVDAGERAKQALAFERTDFDQVRSLMENS DRCQDIRNLAFLGIA YNTLLRIAETARIRVKDISRTDGG RMLIHI VSTAGVEKALSLGVTKLVERWISVSGVADDPNNYLF CRVRKNGVAAPSATSQ LSTRALEGIFEATHRLIYGAKDDSGQRYLAWSGHSARVGAARCSIPEIMQAGGWTNVNIVMNYIRNLDSETGAMVRLLLEDGD*				
	342 amino acids = 38.4 kDa				
✓ IRES	4480 .. 5047	568			misc_feature
/note	= internal ribosome entry site (IRES) of the encephalomyocarditis virus (EMCV)				
✓ IRES	4497 .. 5049	553			misc_feature
/note	= internal ribosome entry site (IRES) of the encephalomyocarditis virus (EMCV)				

Feature	Location	Size (bp)			Type
✓ NeoR/KanR	5064 .. 5858	795			CDS
/gene	= <i>aph(3')-II</i> (or <i>nptII</i>)				
/product	= aminoglycoside phosphotransferase from Tn5				
/note	= confers resistance to neomycin, kanamycin, and G418 (Geneticin®)				
/translation	= MIEQDGLHAGSPAAWVERLFGYDWAQQTIGCSDAAVFRLSAQGRPVLFVKTDLSGALNELQDEAARLSWLATTGVPCAAVLDDVVTEAGRDWLLL DLLSSHLAPAEKVSIMADAMRRLHTLDPATCPFDHQAKHRIERARTRMEAGLVDQDDLDEEHQGLAPAELEFARLKARMPDGEDLVVTHGDACLPN RFSGFIDCGRLGVADRYQDIALATRDIAEELGGEWADRFLVLYGIAAPDSQRIAFYRLLDEFF*				
	264 amino acids = 29.0 kDa				
✓ WPRE	5872 .. 6460	589			misc_feature
/note	= woodchuck hepatitis virus posttranscriptional regulatory element				
✓ Factor Xa site	6343 .. 6354	12			CDS
/product	= Factor Xa recognition and cleavage site				
/translation	= IEGR				
	4 amino acids = 473.5 Da				
✓ 5' LTR (truncated)	6608 .. 6788	181			LTR
/note	= truncated 5' long terminal repeat (LTR) from HIV-1				
✓ AmpR promoter	6921 .. 7025	105			promoter
/gene	= <i>bla</i>				
✓ AmpR	7026 .. 7886	861			CDS
▶ 2 segments					
/gene	= <i>bla</i>				
/product	= β-lactamase				
/note	= confers resistance to ampicillin, carbenicillin, and related antibiotics				
/translation	= MSIQHFRVALIPFFAAFCPLPVFA,HPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRPEERFPMMSSTFKVLLCGAVLSRVDAGQEQLGRRIHYSQ SPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTSLDRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTLA DWMEADKVAGPLLRSLAPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW*				
	286 amino acids = 31.5 kDa				
✓ ori	8057 .. 8645	589			rep_origin
/direction	= RIGHT				
/note	= high-copy-number ColE1/pMB1/pBR322/pUC origin of replication				
✓ CAP binding site	9056 .. 9077	22			protein_bind
/bound_moiety	= <i>E. coli</i> catabolite activator protein				
/note	= CAP binding activates transcription in the presence of cAMP.				

Feature	Location	Size (bp)			Type
✓ lac promoter ▶ 3 segments /note = promoter for the <i>E. coli lac</i> operon	9092 .. 9122	31			promoter
✓ lac operator /bound_moiety = <i>lac</i> repressor encoded by <i>lacI</i> /note = The <i>lac</i> repressor binds to the <i>lac</i> operator to inhibit transcription in <i>E. coli</i> . This inhibition can be relieved by adding lactose or isopropyl-β-D-thiogalactopyranoside (IPTG).	9130 .. 9146	17			protein_bind
✓ M13 rev /note = common sequencing primer, one of multiple similar variants	9154 .. 9170	17			primer_bind

Description: We generated this vector (pHAGE2-EF1a-Cre-IRES-NeoR-W) as follows: JCJ modified the CReM's lentiviral vector pHAGE2-EF1aL-Cre-IRES-PuroR-W by removing the PuromycinR sequence by digestion using Restriction sites NdeI and BsaBI and replacing this cassette with a NeomycinR insert generated by PCR with oligos attaching NdeI and BsaBI to generate compatible cohesive ends for ligating this new insert into the lentiviral backbone, resulting in pHAGE2-EF1a-Cre-IRES-NeoR-W.

Created: Mar 26, 2015

Last Modified: Sep 27, 2017

Accession Number:

Code Number:

Sequence Author:

DNA Type: Synthetic DNA

Laboratory Host Organism:

Bacterial Transformation Strain: DH5a™
Dam⁺ Dcm⁺ EcoKI⁺

Comments:

References: