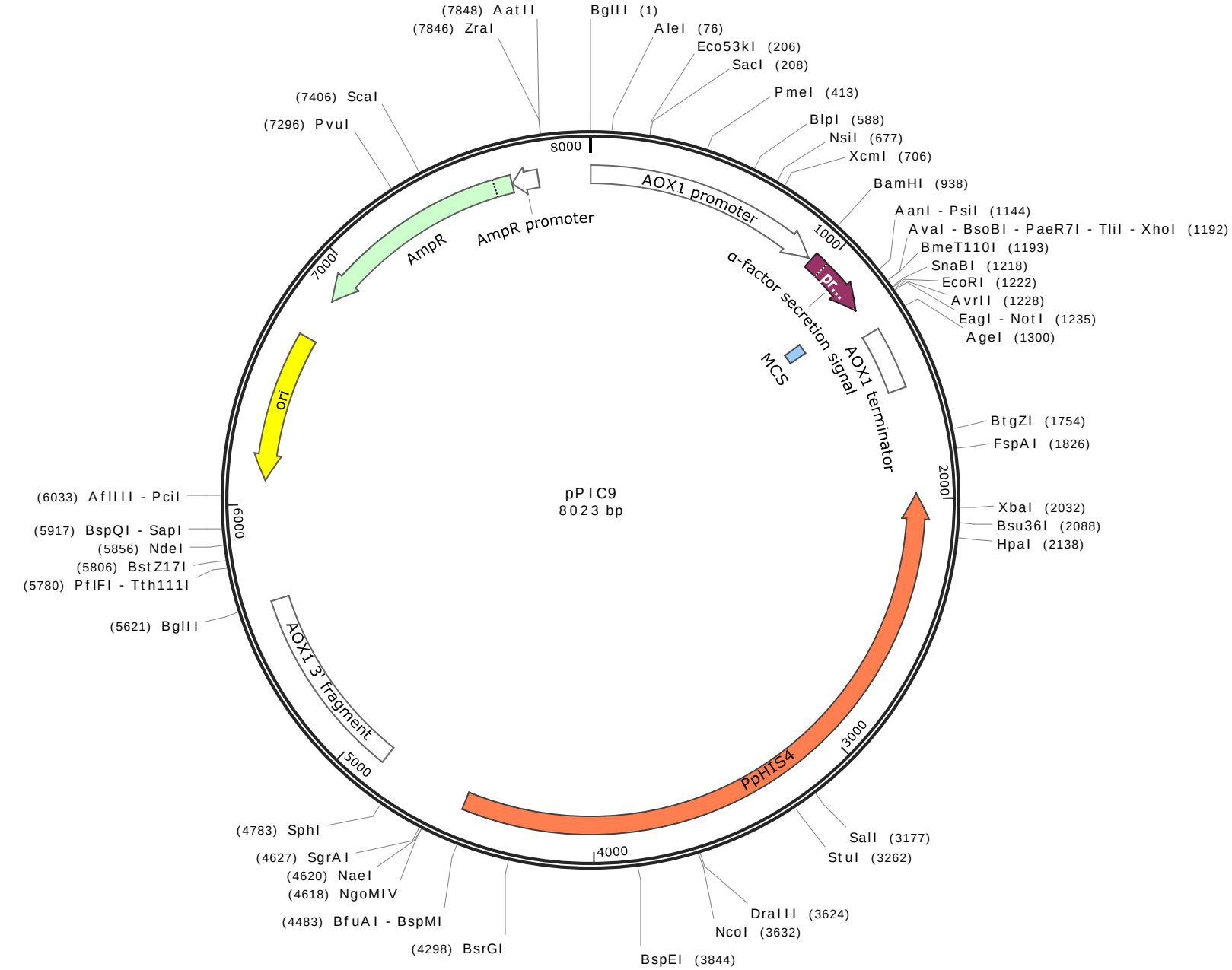
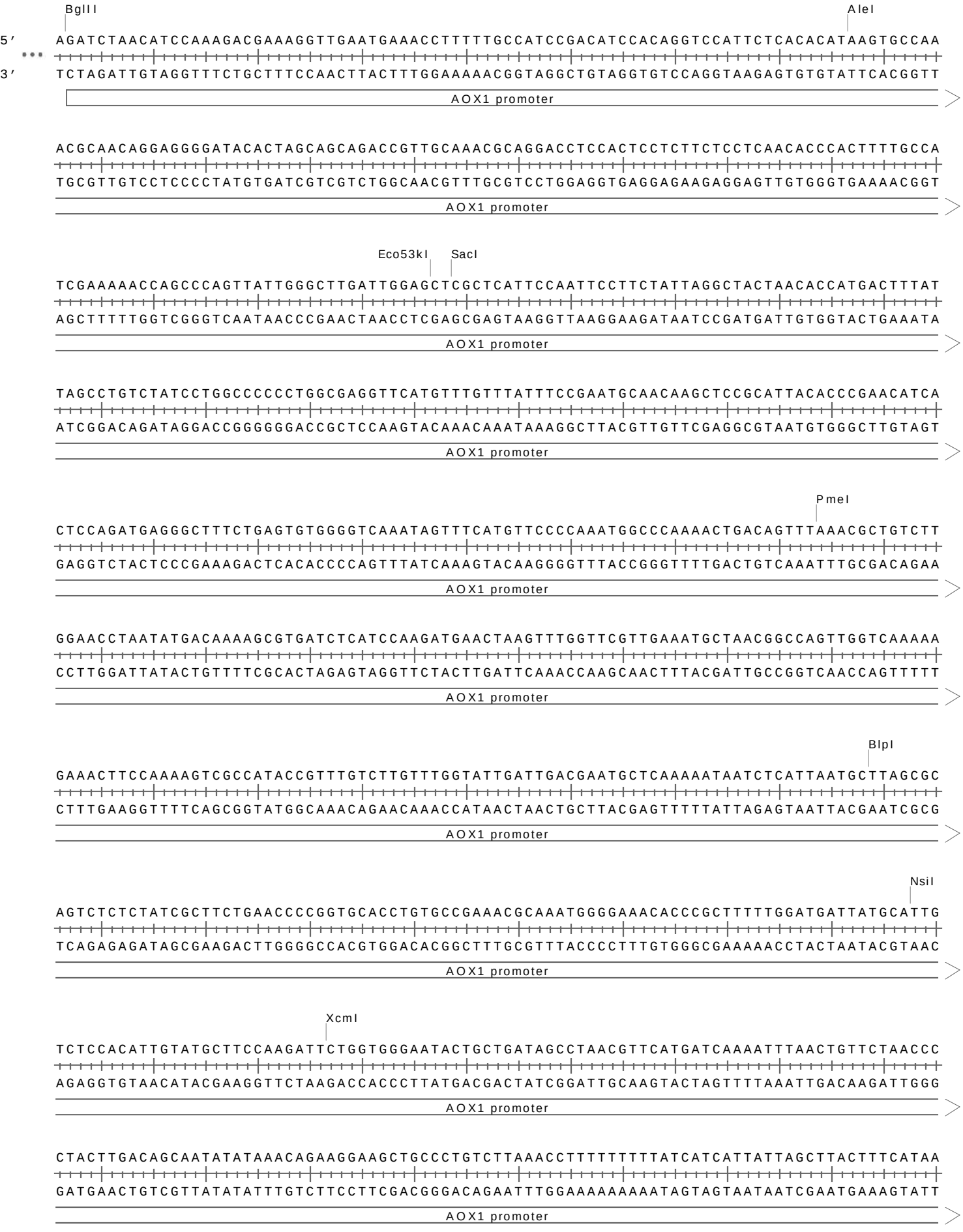
Pichia pastoris HIS4 vector for methanol-inducible expression of a secreted protein.

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pro region
(in frame with α-factor secretion signal)
α-factor secretion signal
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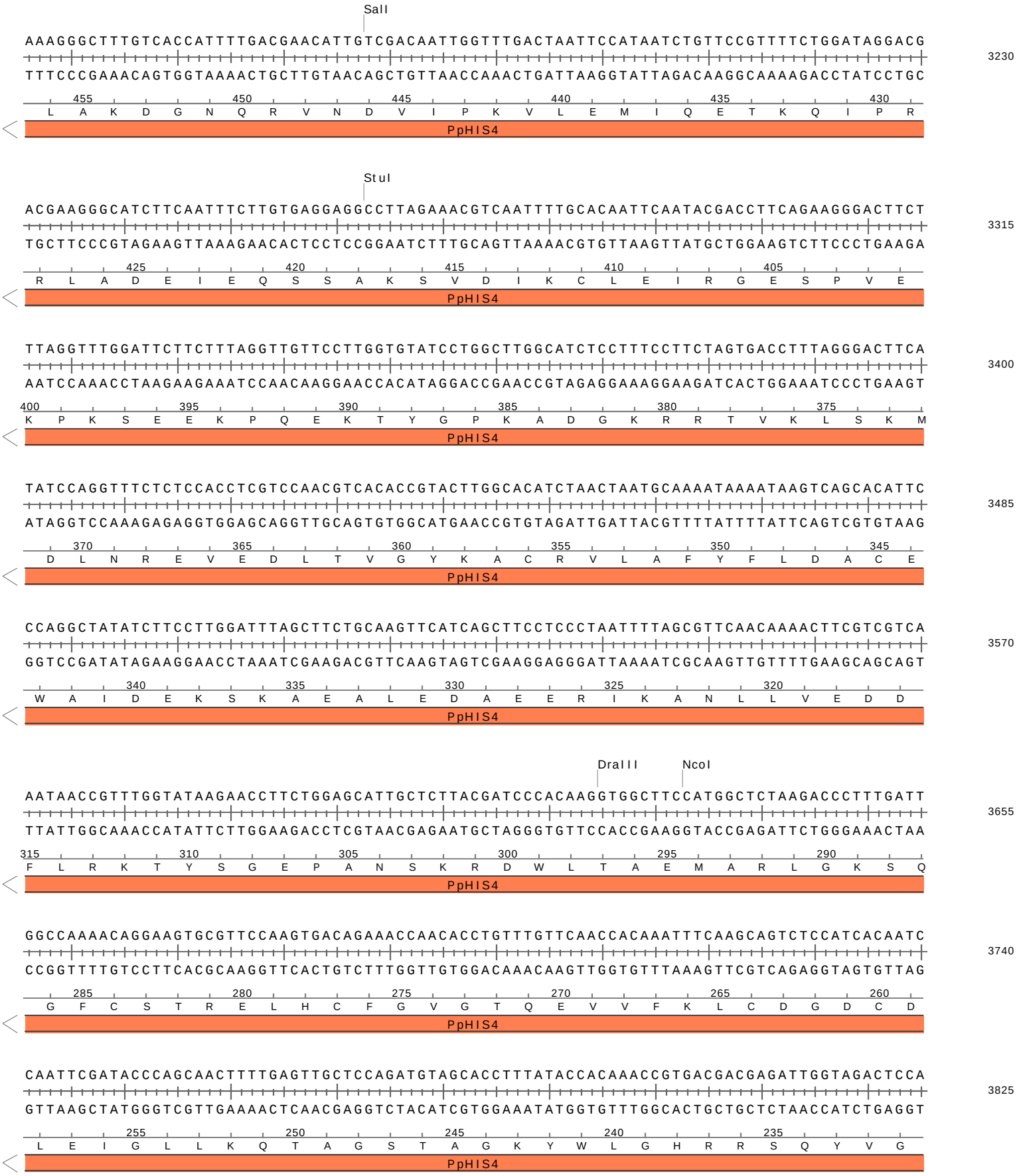
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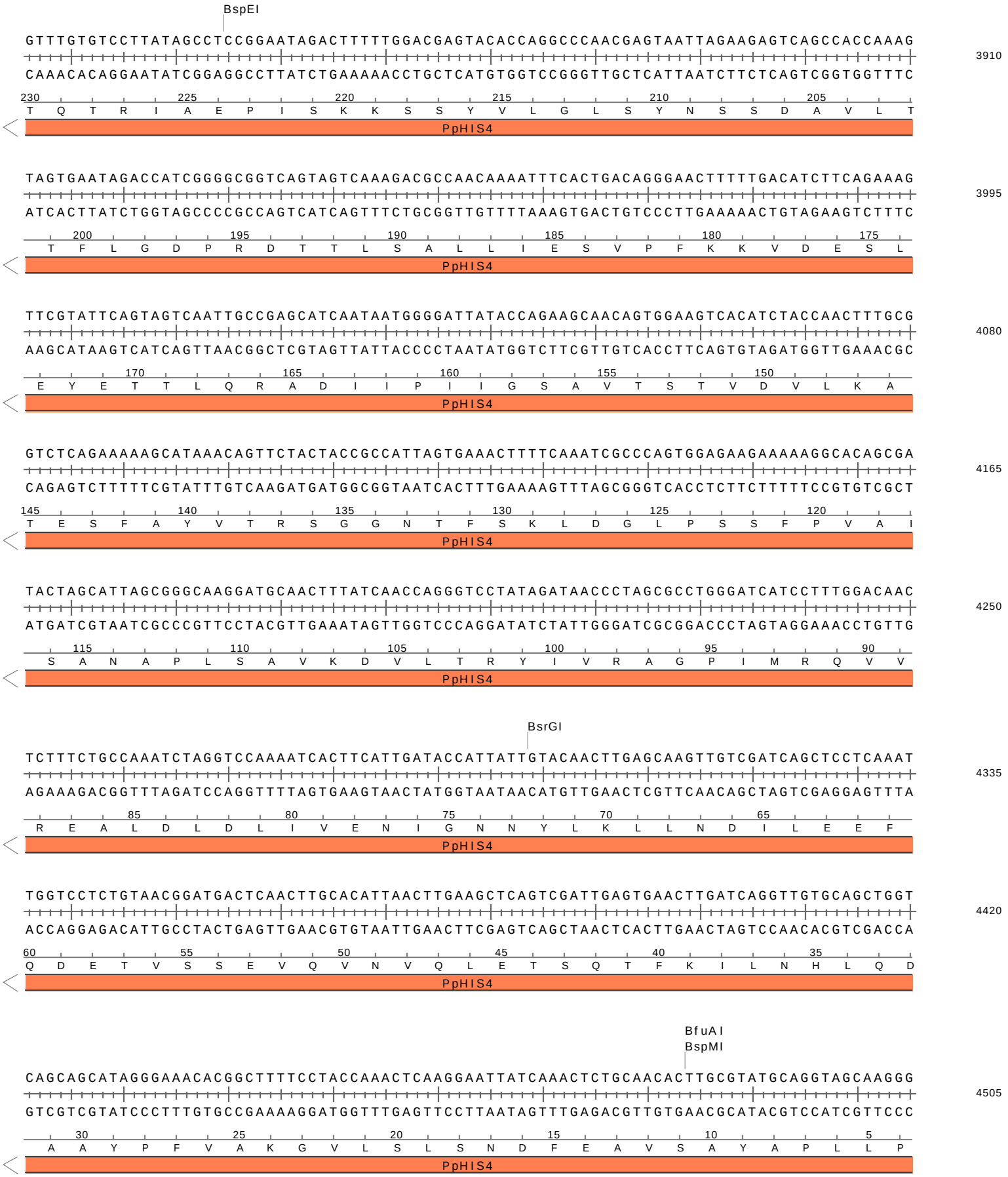
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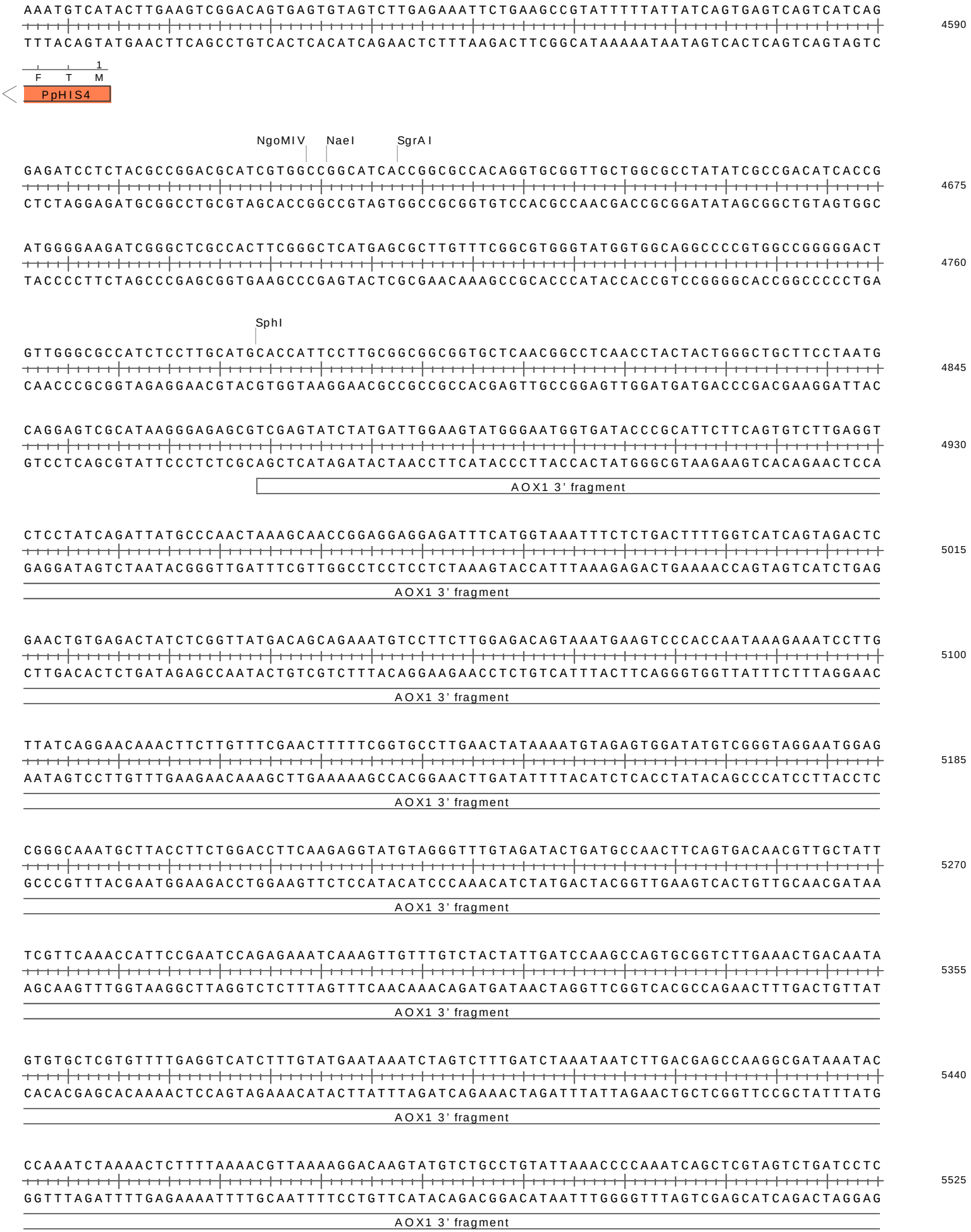
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AOX1 3' fragment
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5780
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PflFI
Tth111I
BstZ17I
NdeI
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5865
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6290
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6375
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6460
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ori


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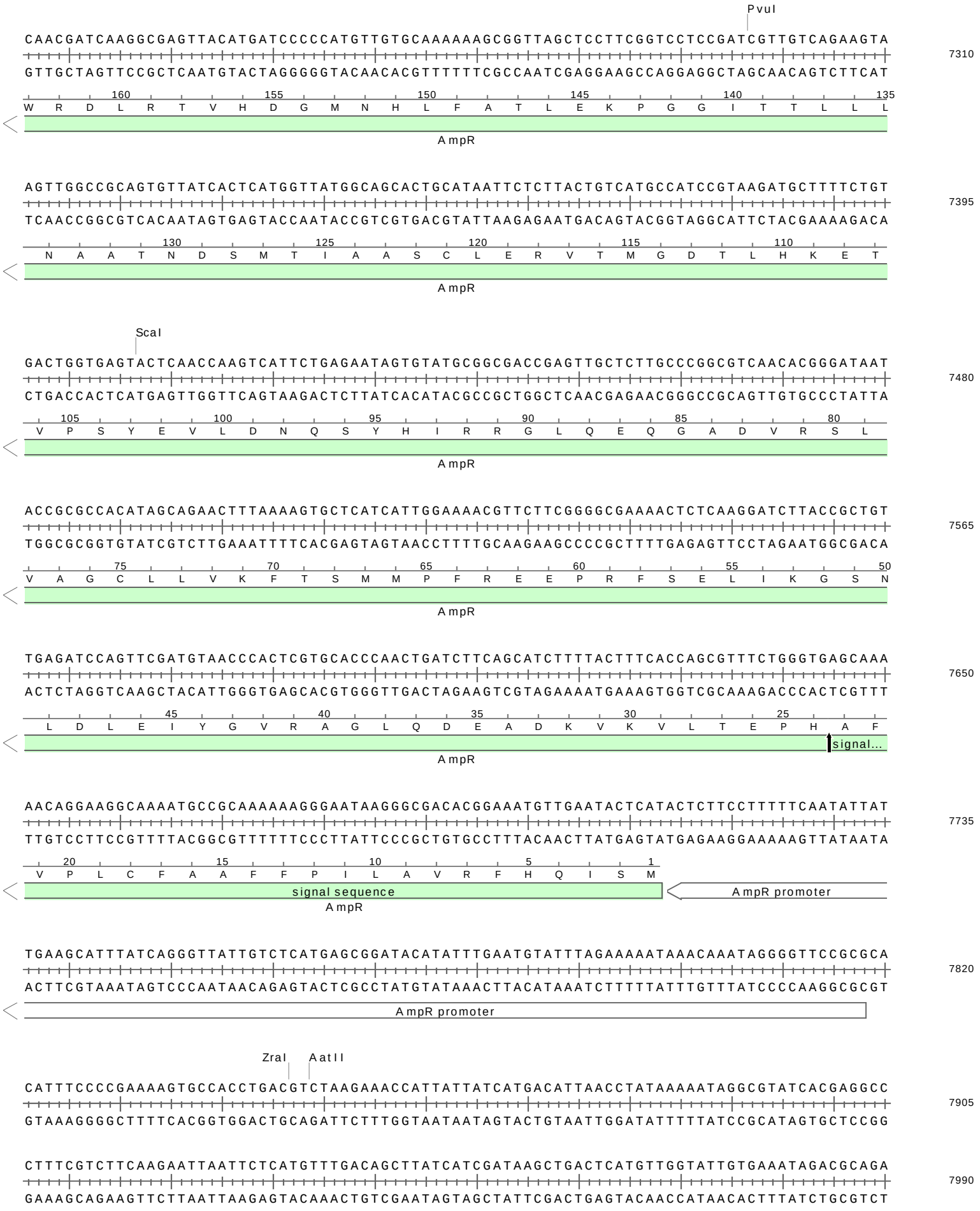
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AmpR
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7735
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V P L C F A A F F P I L A V R F H Q I S M
signal sequence
AmpR promoter
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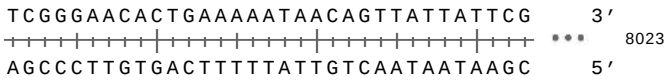
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Enzymes	Sites	
AanI	1	1144
AatII	1	7848
AflIII	1	6033
AgeI	1	1300
AleI	1	76
AvaI	1	1192
AvrII	1	1228
BamHI	1	938
BfuAI	1	4483
BglII	2	1 5621
BlpI	1	588
BmeT110I	1	1193
BsoBI	1	1192
BspEI	1	3844
BspMI	1	4483
BspQI	1	5917
BsrGI	1	4298
BstZ17I	1	5806
Bsu36I	1	2088
BtgZI	1	1754
DraIII	1	3624
EagI	1	1235
Eco53kI	1	206
EcoRI	1	1222
FspAI	1	1826
HpaI	1	2138
NaeI	1	4620
NcoI	1	3632
NdeI	1	5856
NgoMIV	1	4618
NotI	1	1235
NsiI	1	677
PaeR7I	1	1192
PciI	1	6033
PflFI	1	5780
PmeI	1	413
PsiI	1	1144
PvuI	1	7296
SacI	1	208
SalI	1	3177
SapI	1	5917
ScaI	1	7406
SgrAI	1	4627
SnaBI	1	1218
SphI	1	4783
StuI	1	3262
TliI	1	1192
Tth111I	1	5780
XbaI	1	2032
XcmI	1	706
XhoI	1	1192
ZraI	1	7846

	Feature	Location	Size (bp)	Type
✓	AOX1 promoter	2 .. 937	936	promoter

/gene = Pichia pastoris AOX1

/note = inducible promoter, regulated by methanol

✓	α-factor secretion signal	949 .. 1215	267	CDS

3 segments

/gene = MFα1

/product = N-terminal secretion signal from S. cerevisiae alpha-factor

/note = Cleavage by the Kex2 protease occurs after the dibasic KR sequence. The EA dipeptides are then removed by dipeptidyl aminopeptidase A.

/translation = MRFPSIFTAVLFAASSALA,APVNTTTEDETAQIPAEAVIGYSDLEGDFDVAVLPFSNSTNNGLLFINTTIASIAAKEEGVSLEKR,EAEA
89 amino acids = 9.3 kDa

✓	MCS	1192 .. 1241	50	misc_feature

/note = multiple cloning site

✓	AOX1 terminator	1321 .. 1567	247	terminator

/gene = Pichia pastoris AOX1

/note = transcription terminator for AOX1

✓	PpHIS4	1980 .. 4514	2535	CDS

/gene = Pichia pastoris HIS4

/product = multifunctional enzyme, required for histidine biosynthesis

/note = auxotrophic marker for Pichia pastoris

/translation = MTFPLLPAYASVAEFDNSLSLVGKAVFPYAADQLHNLIKFTQSTELQVNVQVESSVTEDQFEELIDNLLKLYNNGINEVILDLDLAE
RVVQRMIPGARVIYRTLVDKVASLPANASIAVPFSSPLGDLKSFTNGGSRTVYAFSETAKLVDVTSTVASGIIPIIDARQLTTEYELS
EDVKKFPVSEILLASLTTDRPDGLFTTLVADSSNYSLGLVYSSKKSIPEAIRTQTGVYQSRRHGLWYKGATSGATQKLLGIELDCDGD
CLKFVVEQTGVGFCHLERTSCFGQSKGLRAMEATLWDRKSNAPEGSYTKRLFDDEVLLNAKIREEADELAEAKSKEDIAWECADLF
YFALVRCAKYGVTLDEVERNLDMKSLKVTRRKGDAKPGYTKEQPKEESKPKEVPSEGRIELCKIDVSKASSQEIEDALRRPIQKTEQ
IMELVKPIVDNVRQNGDKALLELTAKFDGVALKTPVLEAPFPEELMQLPDNVKRAIDLSIDNVRKFHEAQLTETLQVETCPGVVCSR
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TLPTYGYAROYSGVNTATFQKFITSQDVTPEGLKHIGQAVMDLAAVEGLDAHRNAVKVRMEKLGLL*
844 amino acids = 92.2 kDa

✓	AOX1 3' fragment	4869 .. 5625	757	misc_feature

/note = region downstream of Pichia pastoris AOX1 gene

✓	ori	6094 .. 6682	589	rep_origin

/direction = LEFT

/note = high-copy-number ColE1/pMB1/pBR322/pUC origin of replication

✓	AmpR	6853 .. 7713	861	CDS

2 segments

/gene = bla

/product = β-lactamase

/note = confers resistance to ampicillin, carbenicillin, and related antibiotics

/translation = MSIQHFRVALIPFFAAFCLPVFA,HPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRPEERFPMMSTFKVLLCGAVLSRVDAGQE
QLGRRIHYSQNDLVEYSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTRLDRWEPELNEAIPN
DERDTTMPAAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPLLRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIY
TTGSQATMDERNRQIAEIGASLIKHW*
286 amino acids = 31.5 kDa

	Feature	Location	Size (bp)			Type
✓	AmpR promoter	7714 .. 7818	105	☐	←	promoter
	/gene = bla					

Description:	Pichia pastoris HIS4 vector for methanol-inducible expression of a secreted protein.
Created:	Tuesday, Feb 5, 2013
Last Modified:	Tuesday, Feb 5, 2013
Accession Number:	
Code Number:	
Sequence Author:	Invitrogen (Life Technologies)
DNA Type:	Synthetic DNA
Laboratory Host Organism:	Pichia pastoris
Bacterial Transformation Strain:	Unspecified Dam$^+$ Dcm$^+$ EcoKI$^+$
Comments:	
References:	