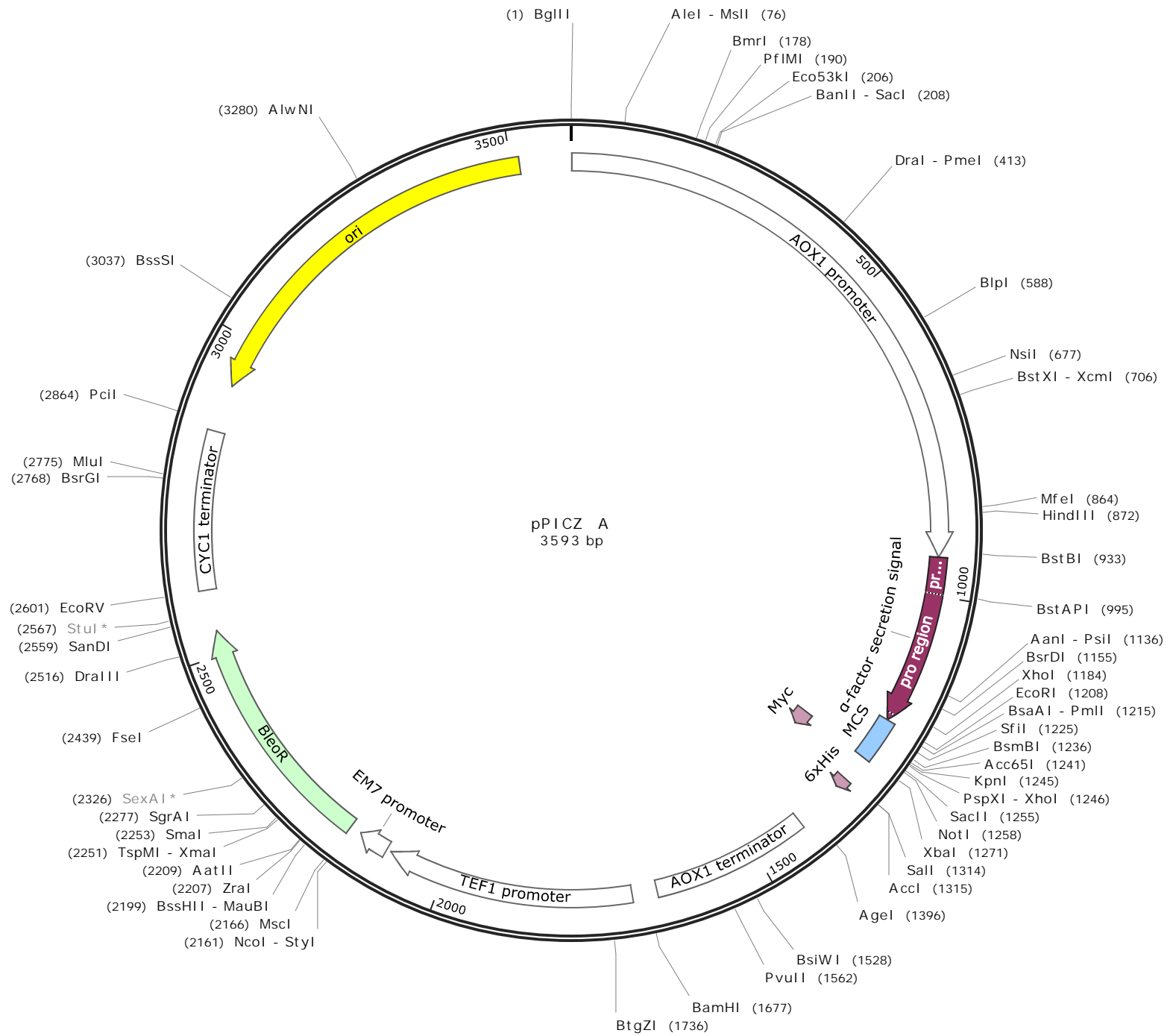
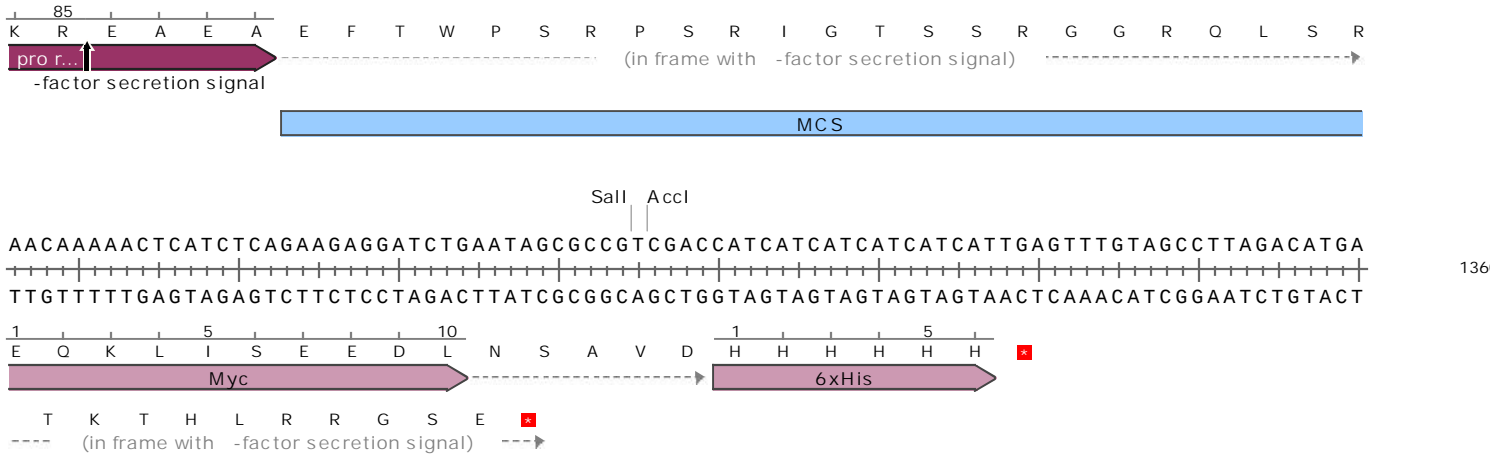
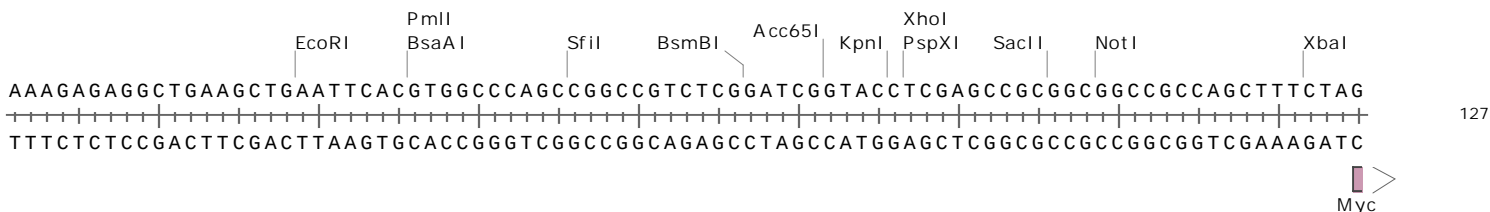
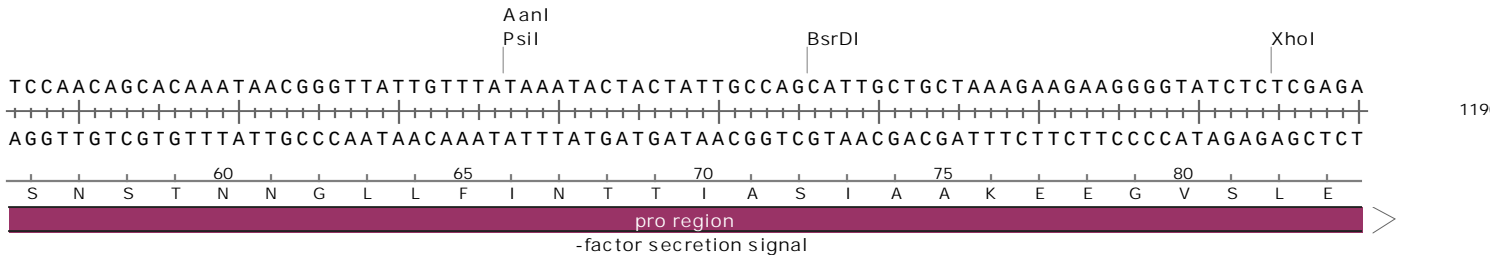
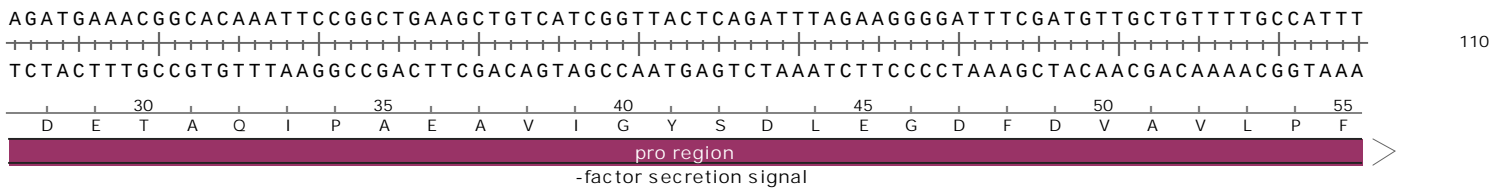
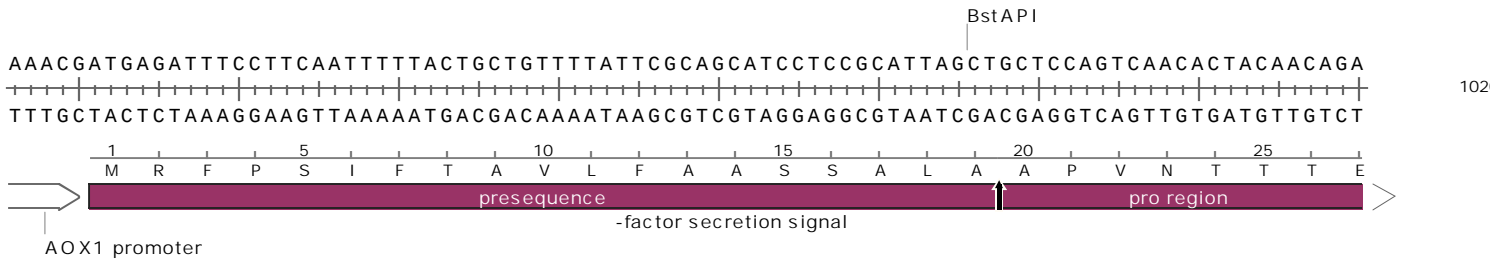
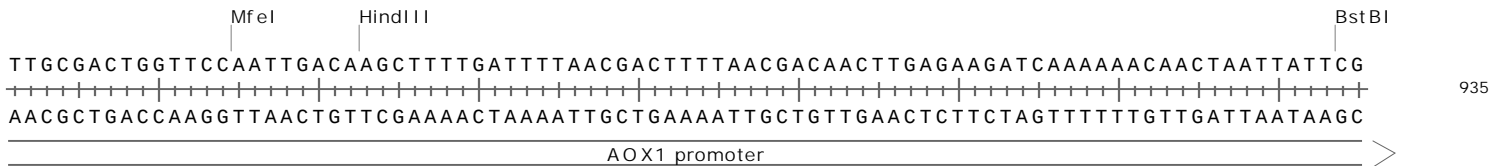


Pichia pastoris vector for methanol-inducible expression of a secreted protein.







MCS

AgeI

CTGTTCTCAGTTCAAGTTGGGCACTTACGAGAAGACCGGTCCTTGCTAGATTCTAATCAAGAGGATGTCAGAATGCCATTTGCCT
GACAAGGAGTCAAGTTCAACCCGTGAATGCTCTTCTGGCCAGAACGATCTAAGATTAGTTCTCCTACAGTCTTACGGTAAACGGA

1445

AOX1 terminator

BsiW I

GAGAGATGCAGGCTTCATTTTTGATACTTTTTTATTTGTAACCTATATAGTATAGGATTTTTTTTTGTCATTTTGTTCCTTCTCGT
CTCTCTACGTCCGAAGTAAAAACTATGAAAAAATAAACATTGGATATATCATATCCTAAAAAAAACAGTAAAAACAAAGAAGAGCA

1530

AOX1 terminator

PvuII

ACGAGCTTGCTCCTGATCAGCCTATCTCGCAGCTGATGAATATCTTGTGGTAGGGGTTTTGGGAAAATCATTGAGTTTTGATGTTT
TGCTCGAACGAGGACTAGTCGGATAGAGCGTCGACTACTTATAGAACCACCATCCCCAAACCCTTTTAGTAAGCTCAAACCTACAAA

1615

AOX1 terminator

BamHI

TTCTTGGTATTTCCCACTCCTCTTCAGAGTACAGAAGATTAAGTGAGACCTTCGTTTGTGCGGATCCCCACACACCATAGCTTC
AAGAACCATAAAGGGTGAGGAGAAGTCTCATGTCTTCTAATCACTCTGGAAGCAAACACGCCTAGGGGGTGTGTGGTATCGAAG

1700

AOX1 terminator

BtgZI

AAAATGTTTCTACTCCTTTTTTACTCTTCCAGATTTTCTCGGACTCCGCGCATCGCCGTACCACTTCAAACACCCAAAGCACAGC
TTTTACAAAGATGAGGAAAAAATGAGAAGGTCTAAAAGAGCCTGAGGCGCGTAGCGGCATGGTGAAGTTTTGTGGGTTTCGTGTCCG

1785

TEF1 promoter

ATACTAAATTTTCCCTCTTTCTTCTCTAGGGTGTCGTTAATTACCCGTACTAAAGGTTTGGAAAAGAAAAAGAGACCGCCTCG
TATGATTTAAAAGGGAGAAAGAAGGAGATCCACAGCAATTAATGGGCATGATTTCCAAACCTTTTCTTTTTTCTCTGGCGGAGC

1870

TEF1 promoter

TTTCTTTTTCTTCGTCGAAAAAGGCAATAAAAAATTTTATCACGTTTCTTTTTCTTGAAATTTTTTTTTTAGTTTTTTTCTCTT
AAAGAAAAAGAAGCAGCTTTTTCCGTTATTTTTAAAAATAGTGCAAAGAAAAAGAAGCTTTAAAAAAAATAAATAAAGAGAA

1955

TEF1 promoter

TCAGTGACCTCCATTGATATTTAAGTTAATAAACGGTCTTCAATTTCTCAAGTTTCAGTTTCATTTTTCTTGTTCATTACAAC
AGTCACTGGAGGTAACATAAATTCAATTATTTGCCAGAAGTTAAGAGTTCAAAGTCAAAGTAAAAAGAACAAGATAATGTTGA

2040

TEF1 promoter

TTTTTACTTCTTGTTCATTAGAAAGAAAGCATAGCAATCTAATCTAAGGGGCGGTGTTGACAATTAATCATCGGCATAGTATAT
AAAAAATGAAGAACAAGTAATCTTTCTTTCGTATCGTTAGATTAGATTCCCCGCCACAACCTGTTAATTAGTAGCCGTATCATATA

2125

TEF1 promoter

EM7 promoter

StyI

NcoI

MscI

MauBI

BssHII

ZraI

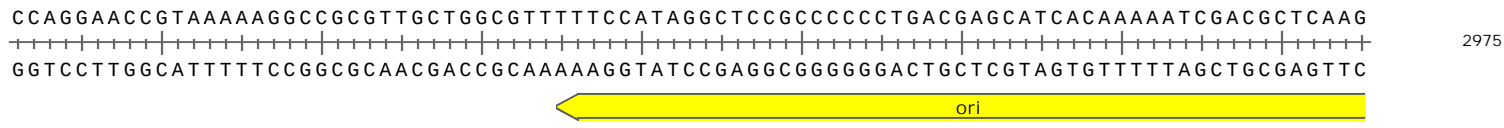
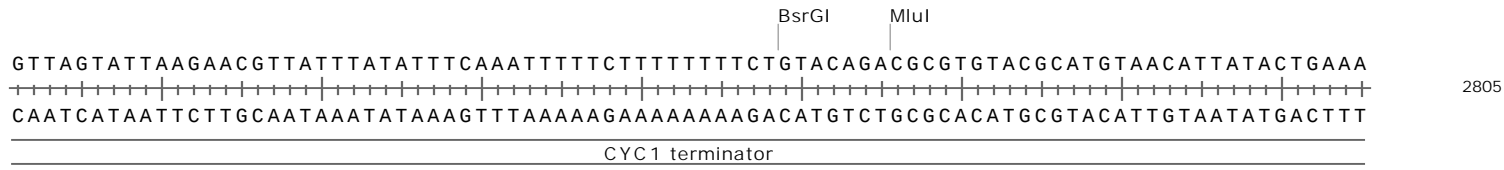
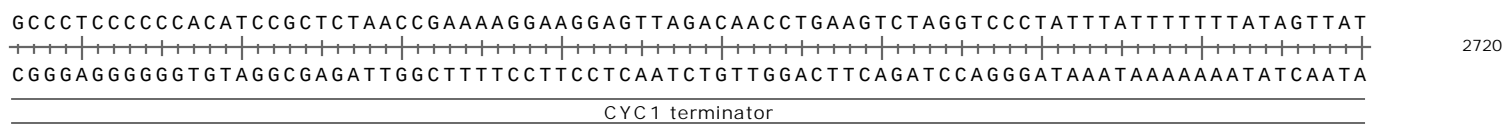
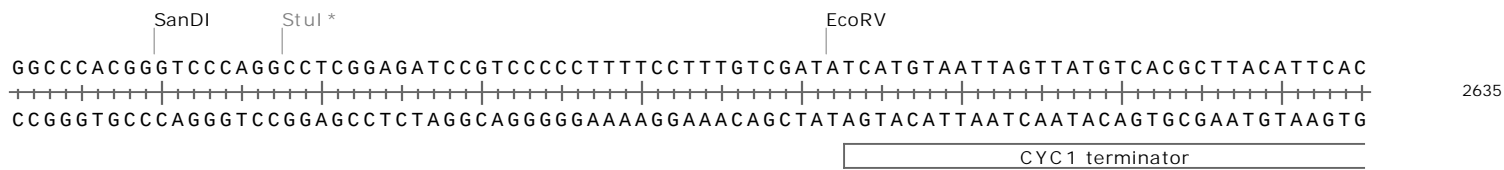
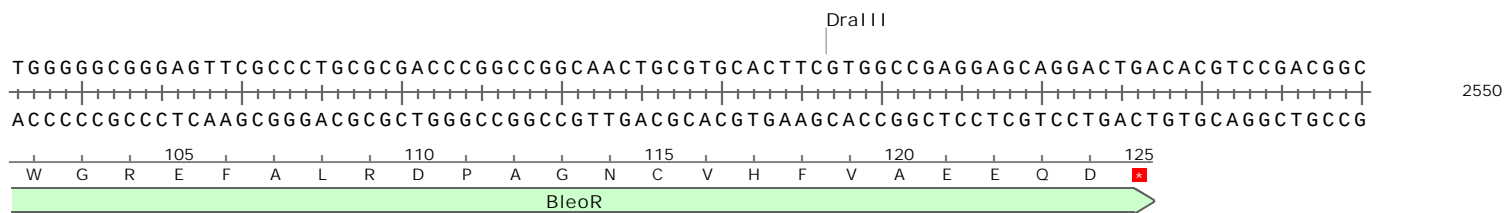
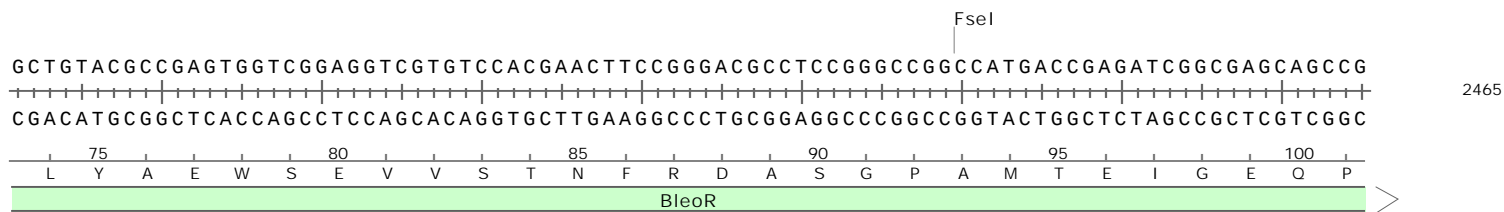
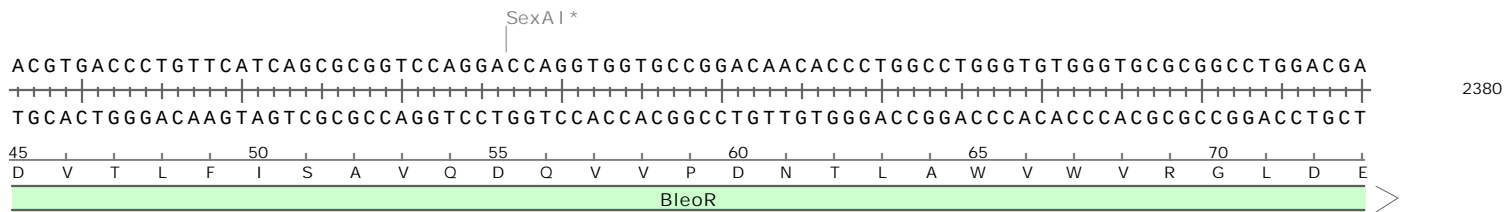
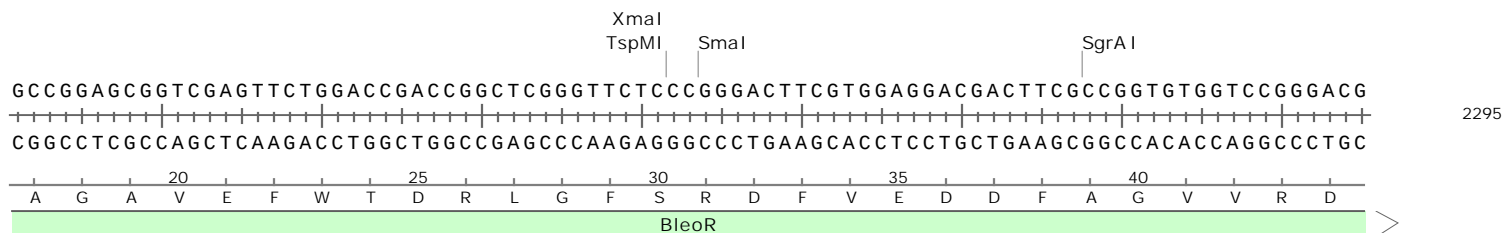
AatII

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GCCGTATCATATTATGCTGTTCCACTCCTTGATTTGGTACCGGTTCAACTGGTCACGGCAAGGCCACGAGTGGCGCGCGCTGCAG

2210

1 5 10 15
M A K L T S A V P V L T A R D V
BleoR

EM7 promoter



BssSI

TCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACC 3060
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 AGTCTCCACCGCTTTGGGCTGTCCTGATATTTCTATGGTCCGCAAAGGGGGACCTTCGAGGGAGCACGCGAGAGGACAAGGCTGG
 <-----ori----->

CTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTT 3145
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 GACGGCGAATGGCCTATGGACAGGCGGAAAGAGGGGAAGCCCTTCGCACCGCGAAAGAGTTACGAGTGCGACATCCATAGAGTCAA
 <-----ori----->

CGGTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCG 3230
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 GCCACATCCAGCAAGCGAGGTTTCGACCCGACACACGTGCTTGGGGGGCAAGTCGGGCTGGCGACGCGGAATAGGCCATTGATAGC
 <-----ori----->

AlwNI

TCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGC 3315
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
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





















GGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAG 3400
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
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

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 <-----ori----->

GATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGT 3570
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 CTAATGCGCGTCTTTTTTCTAGAGTTCTTCTAGGAACTAGAAAAGATGCCCCAGACTGCGAGTCACCTTGCTTTTGAGTGCA
 <-----ori----->

TAAGGGATTTTGGTCATGAGATC 3'
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 ATTCCCTAAAACCAGTACTCTAG 5' *** 3593

Enzymes	Sites	
AanI	1	1136
AatII	1	2209
AccI	1	1315
Acc65I	1	1241
AgeI	1	1396
AleI	1	76
AlwNI	1	3280
BamHI	1	1677
BanII	1	208
BglII	1	1
BipI	1	588
BmrI	1	178
BsaAI	1	1215
BsiWI	1	1528
BsmBI	1	1236
BsrDI	1	1155
BsrGI	1	2768
BssHII	1	2199
BssSI	1	3037
BstAPI	1	995
BstBI	1	933
BstXI	1	706
BtgZI	1	1736
DraI	1	413
DraIII	1	2516
Eco53kI	1	206
EcoRI	1	1208
EcoRV	1	2601
FseI	1	2439
HindIII	1	872
KpnI	1	1245
MauBI	1	2199
MfeI	1	864
MluI	1	2775
MscI	1	2166
MslI	1	76
NcoI	1	2161
NotI	1	1258
NsiI	1	677
PciI	1	2864
PfIMI	1	190
PmeI	1	413
PmlI	1	1215
PsiI	1	1136
PspXI	1	1246
PvuII	1	1562
SacI	1	208
SacII	1	1255
SalI	1	1314
SanDI	1	2559
SexAI	1*	2326*
SfiI	1	1225
SgrAI	1	2277
SmaI	1	2253
StuI	1*	2567*
StyI	1	2161
TspMI	1	2251
XbaI	1	1271
XcmI	1	706
XhoI	2	1184 1246
XmaI	1	2251
ZraI	1	2207

Feature	Location	Size (bp)			Type
✓ AOX1 promoter	2 .. 940	939			promoter
/gene	= <i>Pichia pastoris</i> AOX1				
/note	= inducible promoter, regulated by methanol				
✓ -factor secretion signal	941 .. 1207	267			CDS
▶ 3 segments					
/gene	= MF 1				
/product	= N-terminal secretion signal from <i>S. cerevisiae</i> 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,APVNTTTEDETAQIPAEAVIGYSDLEGGDFDVAVLPFSNSTNNGLLFINTTIIASIAAKEEGVSLEKR,EA FA 89 amino acids = 9.3 kDa				
✓ MCS	1208 .. 1276	69			misc_feature
/note	= multiple cloning site				
✓ Myc	1275 .. 1304	30			CDS
/product	= Myc (human c-Myc oncogene) epitope tag				
/translation	= EQKLISEEDL 10 amino acids = 1.2 kDa				
✓ 6xHis	1320 .. 1337	18			CDS
/product	= 6xHis affinity tag				
/translation	= HHHHHH 6 amino acids = 840.9 Da				
✓ AOX1 terminator	1417 .. 1663	247			terminator
/gene	= <i>Pichia pastoris</i> AOX1				
/note	= transcription terminator for AOX1				
✓ TEF1 promoter	1703 .. 2089	387			promoter
/gene	= <i>S. cerevisiae</i> TEF1				
/note	= promoter for EF-1				
✓ EM7 promoter	2097 .. 2144	48			promoter
/note	= synthetic bacterial promoter				
✓ BleoR	2163 .. 2537	375			CDS
/gene	= <i>Sh ble</i> from <i>Streptoalloteichus hindustanus</i>				
/product	= antibiotic-binding protein				
/note	= confers resistance to bleomycin, phleomycin, and Zeocin™				
/translation	= MAKLTSAVPVLTARDVAGAVEFWTDRLGFSRDFVEDDFAGVVRDDVTLFISAVQDQVVPDNTLAWVWVRGLDELYAEWSEVVST NFRDASGPAMTEIGEOPWGREFALRDPAGNCVHFVAEEQD*				
	124 amino acids = 13.8 kDa				
✓ CYC1 terminator	2603 .. 2850	248			terminator
/gene	= <i>S. cerevisiae</i> CYC1				
/note	= transcription terminator for CYC1				

Feature	Location	Size (bp)			Type
✓ ori	2925 .. 3513	589			rep_origin
/direction	= LEFT				
/note	= high-copy-number ColE1/pMB1/pBR322/pUC origin of replication				

Description: Pichia pastoris 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: Linearize with SacI, PmeI, or BstXI for integration at the AOX1 locus.

References: