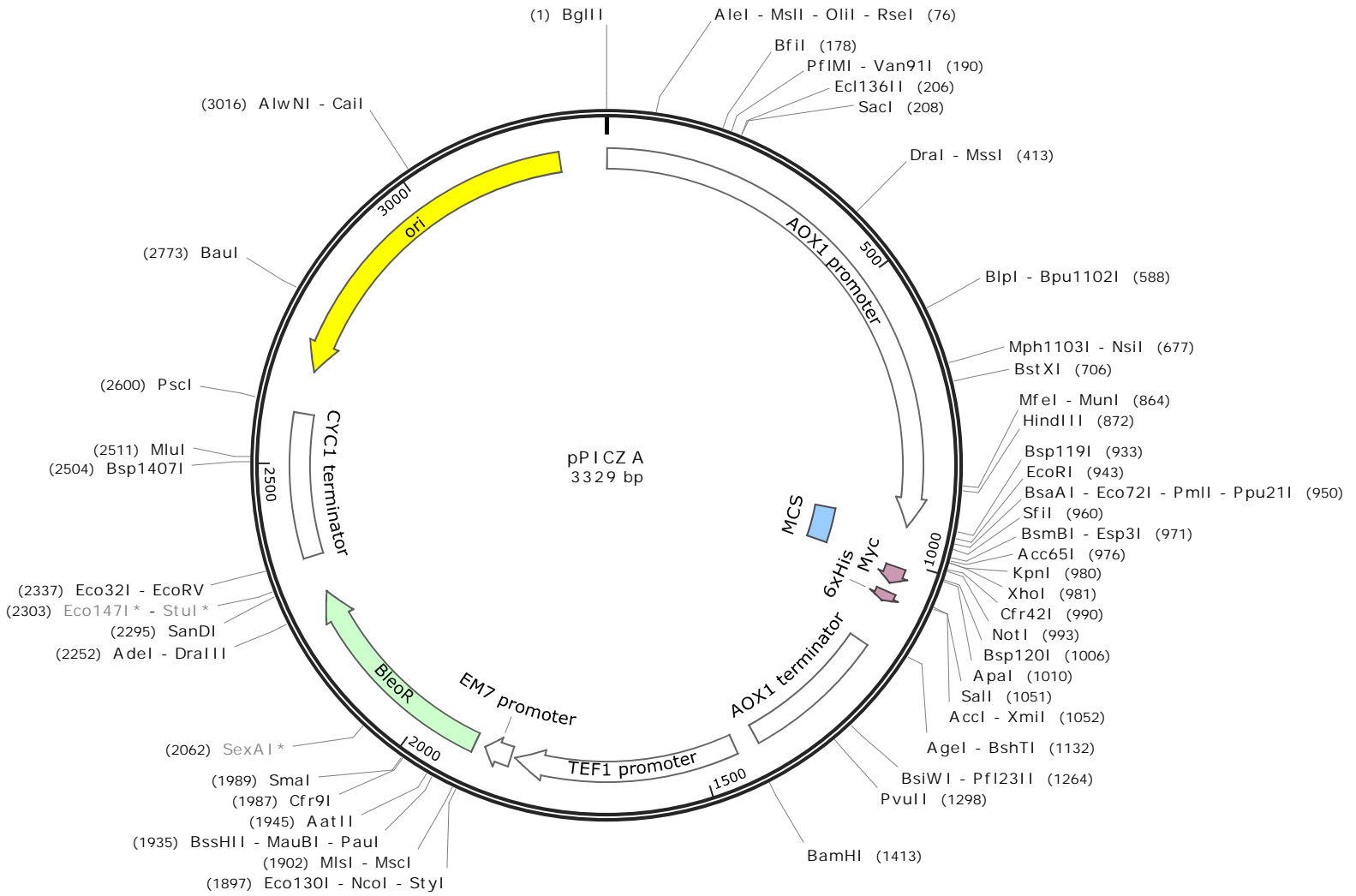


Pichia pastoris vector for methanol-inducible intracellular expression of a protein.





CTACTTGACAGCAATATATAAACAGAAGGAAGCTGCCCTGTCTTAAACCTTTTTTTTTATCATCATTATTAGCTTACTTTTCATAA
GATGAACTGTCGTTATATATTTGTCTTCTTCGACGGGACAGAATTTGGAAAAAAAAAATAGTAGTAATAATCGAATGAAAGTATT

850

AOX1 promoter

TTGCGACTGGTTCCAATTGACAAGCTTTTTGATTTTAAACGACTTTTAAACGACAACCTTGAGAAGATCAAAAAACAACCTAATTATTCG
AACGCTGACCAAGGTTAACTGTTTCGAAAACTAAAATTGCTGAAAATTGCTGTTGAACTCTTCTAGTTTTTTGTTGATTAATAAGC

935

AOX1 promoter

MCS

AAACGAGGAATTCACGTGGCCAGCCGGCCGTCTCGGATCGGTACCTCGAGCCGCGGGCGGCCGAGCTTGGGCCCGAACAAAA
TTTGCTCCTTAAGTGCACCGGGTCGGCCGGCAGAGCCTAGCCATGGAGCTCGGCGCCGCCGGCGGTTCGAACCCGGGCTTGTTTTT

1020

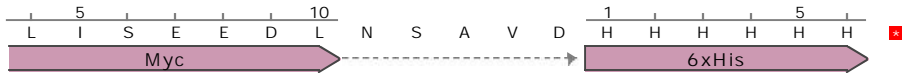
MCS

Myc

AOX1 promoter

CTCATCTCAGAAGAGGATCTGAATAGCGCCGTCGACCATCATCATCATCATTGAGTTTTAGCCTTAGACATGACTGTTCCCTC
GAGTAGAGTCTTCTCCTAGACTTATCGCGGCAGCTGGTAGTAGTAGTAGTAAGTCAAATCGGAATCTGTACTGACAAGGAG

1105



AGTTCAAGTTGGGCACCTTACGAGAAGACCGGTCTTGCTAGATTCTAATCAAGAGGATGTCAGAATGCCATTTGCCTGAGAGATGC
TCAAGTTCAACCCGTGAATGCTCTTCTGGCCAGAACGATCTAAGATTAGTTCTCCTACAGTCTTACGGTAAACGGACTCTCTACG

1190

AOX1 terminator

AGGCTTCATTTTTGATACTTTTTTATTTGTAACCTATATAGTATAGGATTTTTTTTGTCAATTTGTTTCTTCTCGTACGAGCTTG
TCCGAAGTAAAACTATGAAAAATAAACATTGGATATATCATATCCTAAAAAAAACAGTAAAAACAAAGAAGAGCATGCTCGAAC

1275

AOX1 terminator

CTCCTGATCAGCCTATCTCGCAGCTGATGAATATCTTGTGGTAGGGGTTTGGGAAAAATCATTTCGAGTTTGATGTTTTCTTGGTA
GAGGACTAGTCGGATAGAGCGTCGACTACTTATAGAACCACATCCCCAAACCCTTTTAGTAAGCTCAAACCTACAAAAAGAACCAT

1360

AOX1 terminator

TTTCCCACTCCTCTTCAGAGTACAGAAGATTAAGTGAGACCTTCGTTTGTGCGGATCCCCCACACACCATAGCTTCAAATGTTT
 AAAGGGTGAGGAGAAGTCTCATGTCTTCTAATTCACCTCTGGAAGCAAACACGCCTAGGGGGTGTGTGGTATCGAAGTTTTACAAA
 AOX1 terminator
 BamHI
 TEF1 promoter

CTACTCCTTTTTACTCTTCCAGATTTTCTCGGACTCCGCGCATCGCCGTACCCTTCAAACACCCAAGCACAGCATACTAAAT
 GATGAGGAAAAAATGAGAAGGTCTAAAAGAGCCTGAGGCGCGTAGCGGCATGGTGAAGTTTTGTGGGTTCTGTGTCGTATGATTTA
 TEF1 promoter

TTTCCCTCTTTCTTCTCTAGGGTGTCTGTTAATTACCCGTAATAAGGTTTGGAAAAGAAAAAGAGACCGCCTCGTTTCTTTTT
 AAAGGGAGAAAAGAGGAGATCCCACAGCAATTAATGGGCATGATTTCAAACCTTTTTCTTTTTTCTCTGGCGGAGCAAAGAAAA
 TEF1 promoter

CTTCGTCGAAAAAGGCAATAAAAAATTTTATCACGTTTCTTTTTCTTGAAATTTTTTTTTTTAGTTTTTTTCTCTTTCAGTGACC
 GAAGCAGCTTTTTCCGTTATTTTTAAAAATAGTGCAAAGAAAAAGAAGCTTTAAAAAATAAATCAAAAAAGAGAAAGTCACTGG
 TEF1 promoter

TCCATTGATATTTAAGTTAATAAACGGTCTTCAATTTCTCAAGTTTCAGTTTCATTTTTCTTGTCTATTACAACTTTTTTTACT
 AGGTAACATAAATTCATTTATTTGCCAGAAGTTAAAGAGTTCAAAGTCAAAGTAAAAAGAACAAGATAATGTTGAAAAAATGA
 TEF1 promoter

TCTTGTTCAATAGAAAAGAAAGCATAGCAATCTAATCTAAGGGGCGGTGTTGACAATTAATCATCGGCATAGTATATCGGCATAGT
 AGAACAAAGTAATCTTTCTTTCTGATCGTTAGATTAGATTCCCCGCCACAAGTGTAAATAGTAGCCGTATCATATAGCCGTATCA
 TEF1 promoter
 EM7 promoter

ATAATACGACAAGGTGAGGAACTAAACCATGGCCAAGTTGACCAGTGCCGTTCCGGTGCTCACCGCGCGCGACGTTCGCCGGAGCG
 TATTATGCTGTTCCACTCCTTGATTTGGTACCGGTTCAACTGGTACAGGCAAGGCCACGAGTGGCGCGCGCTGCAGCGGCTCGC
 Styl
 NcoI
 Eco130I
 MlsI
 MscI
 Paul
 MauBI
 BssHI I
 AatII
 1 5 10 15
 M A K L T S A V P V L T A R D V A G A
 EM7 promoter
 BleoR

GTCGAGTTCTGGACCGACCGGCTCGGGTTCTCCCGGGACTTCGTGGAGGACGACTTCGCCGGTGTGGTCCGGGACGACGTGACCC
 CAGCTCAAGACCTGGCTGGCCGAGCCCAAGAGGGCCCTGAAGCACCTCCTGCTGAAGCGGCCACACCAGGCCCTGCTGCACTGGG
 20 25 30 35 40 45
 V E F W T D R L G F S R D F V E D D F A G V V R D D V T
 Cfr9I
 SmaI
 BleoR

TGTTTCATCAGCGCGGTCCAGGACCAAGGTGGTGC CGGACAACACCCTGGCCTGGGTGTGGGTGCGCGGCCTGGACGAGCTGTACGC
 ACAAGTAGTCGCGCCAGGTCTGTTCCACCACGGCCTGTTGTGGGACCGGACCCACACCCACGCGCCGGACCTGCTCGACATGCG
 SexAI *
 50 55 60 65 70 75
 L F I S A V Q D Q V V P D N T L A W V W V R G L D E L Y A
 BleoR

CGAGTGGTCTGGAGGTCGTGTCCACGAACTTCCGGGACGCCTCCGGGCCGGCCATGACCGAGATCGGGCAGCAGCCGTGGGGGGCGG
 GCTCACCAGCCTCCAGCACAGGTGCTTGAAGGCCCTGCGGAGGCCCGGCCGGTACTGGCTCTAGCCGCTCGTCGGCACCCCCGCC

80 85 90 95 100
 E W S E V V S T N F R D A S G P A M T E I G E Q P W G R

BleoR

2210

GAGTTCGCCCTGCGCGACCCGGCCGGCAACTGCGTGCACCTTCGTGGCCGAGGAGCAGGACTGACACGTCCGACGGCGGCCACGG
 CTCAAGCGGGACGCGCTGGGCCGGCCGTTGACGCACGTGAAGCACCGGCTCCTCGTCTGACTGTGCAGGCTGCCGCCGGGTGCC

105 110 115 120 125
 E F A L R D P A G N C V H F V A E E Q D

BleoR

2295

GTCCCAGGCCTCGGAGATCCGTCCCCCTTTTCTTTTGTGCGATATCATGTAATTAGTTATGTCACGCTTACATTACAGCCCTCCCC
 CAGGGTCCGGAGCCTCTAGGCAGGGGAAAAGGAAACAGCTATAGTACATTAATCAATACAGTGCGAATGTAAGTGCGGGAGGGG

SanDI Stul* EcoRV
 Eco1471* Eco321

CYC1 terminator

2380

CCACATCCGCTCTAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTATTTTTTATAGTTATGTTAGTATT
 GGTGTAGGCGAGATTGGCTTTTCTTCTCAATCTGTTGGACTTCAGATCCAGGGATAAATAAAAAAATATCAATACAATCATAA

CYC1 terminator

2465

AAGAACGTTATTTATATTTCAAATTTTTCTTTTTTTTCTGTACAGACGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTT
 TTCTTGCAATAAATATAAAGTTTTAAAAAGAAAAAAGACATGTCTGCGCACATGCGTACATTGTAATATGACTTTTGGAACGAA

Bsp14071 MluI

CYC1 terminator

2550

GAGAAGGTTTTGGGACGCTCGAAGGCTTTAATTTGCAAGCTGGAGACCAACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACC
 CTCTTCCAAAACCTGCGAGCTTCCGAAATTAACGTTTCGACCTCTGGTTGTACACTCGTTTTCCGGTCTTTTTCCGGTCTTTGG

PscI

CYC1 terminator

2635

GTAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTG
 CATTTTTCCGGCGCAACGACCGCAAAAAGGTATCCGAGGCGGGGGACTGCTCGTAGTGTTTTAGCTGCGAGTTCAGTCTCCAC

ori

2720

GCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTT
 CGCTTTGGGCTGTCTGATATTTCTATGGTCCGCAAAGGGGGACCTTCGAGGGAGCACGCGAGAGGACAAGGCTGGGACGGCGAA

BauI

ori

2805

ACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCCGGTGTAGG
 TGGCCTATGGACAGGCGGAAAGAGGGAAGCCCTTCGCACCGCGAAAGAGTTACGAGTGCGACATCCATAGAGTCAAGCCACATCC

ori

2890

TCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTTCAGCCCAGCCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTC
 AGCAAAGCGAGGTTCCGACCCGACACACGTGCTTGGGGGGCAAGTCGGGCTGGCGACGCGGAATAGGCCATTGATAGCAGAAGTCAAG

ori

2975

CaiI
AlwNI

CAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACA
 GTTGGGCCATTCTGTGCTGAATAGCGGTGACCGTCGTCGGTGACCATTGTCCTAATCGTCTCGCTCCATACATCCGCCACGATGT

3060

ori

GAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCG
 CTC AAGA AACTTCA CCA CCGG ATTGATGCCGATGTGATCTTCTGT CATAA ACCATAGACGCGGAGACGACTTCGGTCAATGGAAGC

3145

ori

GAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCG
 CTTTTTCTCAACCATCGAGA AACTAGGCCGTTTGGTTGGTGGCGACCATCGCCACCAAAAAAACA AACGTTTCGTCTAATGCGC

3230

ori























CAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAA AACTCACGTTAAGGGATT
 GTCTTTTTTTCTTAGAGTTCTTCTAGGAAACTAGAAAAGATGCCCCAGACTGCGAGTCACCTTGCTTTTGGAGTGCAATTCCTAA

3315

ori

TTGGTCATGAGATC 3'
 3329
 AACCAGTACTCTAG 5'

Enzymes	Sites	
Aat II	1	1945
AccI	1	1052
Acc65I	1	976
Adel	1	2252
AgeI	1	1132
AleI	1	76
AlwNI	1	3016
ApaI	1	1010
BamHI	1	1413
BauI	1	2773
BfiI	1	178
BglI I	1	1
BlpI	1	588
Bpu1102I	1	588
BsaAI	1	950
BshTI	1	1132
BsiWI	1	1264
BsmBI	1	971
Bsp119I	1	933
Bsp120I	1	1006
Bsp1407I	1	2504
BssHI I	1	1935
BstXI	1	706
CaiI	1	3016
Cfr9I	1	1987
Cfr42I	1	990
DraI	1	413
DraI I I	1	2252
Ecl136I I	1	206
Eco32I	1	2337
Eco72I	1	950
Eco130I	1	1897
Eco147I	1*	2303*
EcoRI	1	943
EcoRV	1	2337
Esp3I	1	971
HindI I I	1	872
KpnI	1	980
MauBI	1	1935
MfeI	1	864
MisI	1	1902
MluI	1	2511
Mph1103I	1	677
MscI	1	1902
MslI	1	76
MssI	1	413
MunI	1	864
NcoI	1	1897
Not I	1	993
NsiI	1	677
OliI	1	76
Paul	1	1935
PfI23I I	1	1264
PfIMI	1	190
PmlI	1	950
Ppu21I	1	950
PscI	1	2600
PvuII	1	1298
RseI	1	76
SacI	1	208
Sall	1	1051
SanDI	1	2295
SexAI	1*	2062*
SfiI	1	960
SmaI	1	1989
StuI	1*	2303*
StyI	1	1897
Van91I	1	190
XhoI	1	981
XmiI	1	1052

Feature	Location	Size (bp)			Type
✓ AOX1 promoter /gene = Pichia pastoris AOX1 /note = inducible promoter, regulated by methanol	2 .. 940	939			promoter
✓ MCS /note = multiple cloning site	932 .. 1011	80			misc_feature
✓ Myc /product = Myc (human c-Myc oncogene) epitope tag /translation = EQKLISEEDL 10 amino acids = 1.2 kDa	1012 .. 1041	30			CDS
✓ 6xHis /product = 6xHis affinity tag /translation = HHHHHH 6 amino acids = 840.9 Da	1057 .. 1074	18			CDS
✓ AOX1 terminator /gene = Pichia pastoris AOX1 /note = transcription terminator for AOX1	1153 .. 1399	247			terminator
✓ TEF1 promoter /gene = S. cerevisiae TEF1 /note = promoter for EF-1	1439 .. 1825	387			promoter
✓ EM7 promoter /note = synthetic bacterial promoter	1833 .. 1880	48			promoter
✓ BleoR /gene = Sh ble from Streptoalloteichus hindustanus /product = antibiotic-binding protein /note = confers resistance to bleomycin, phleomycin, and Zeocin™ /translation = MAKLTSAVPVLTARDVAGAVEFWTDRDLGFSRDFVEDDFAGVVRDDVTLFISAVQDQVVPDNTLAWVWVRGLDELYAEWSEVVST NFRDASGPAMTEIGEQPWGREFALRDPAGNCVHFVAEEQD* 124 amino acids = 13.8 kDa	1899 .. 2273	375			CDS
✓ CYC1 terminator /gene = S. cerevisiae CYC1 /note = transcription terminator for CYC1	2339 .. 2586	248			terminator
✓ ori /direction = LEFT /note = high-copy-number CoIE1/pMB1/pBR322/pUC origin of replication	2661 .. 3249	589			rep_origin

Description: Pichia pastoris vector for methanol-inducible intracellular expression of a protein.

Created: Monday, Feb 4, 2013

Last Modified: Monday, Feb 4, 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: