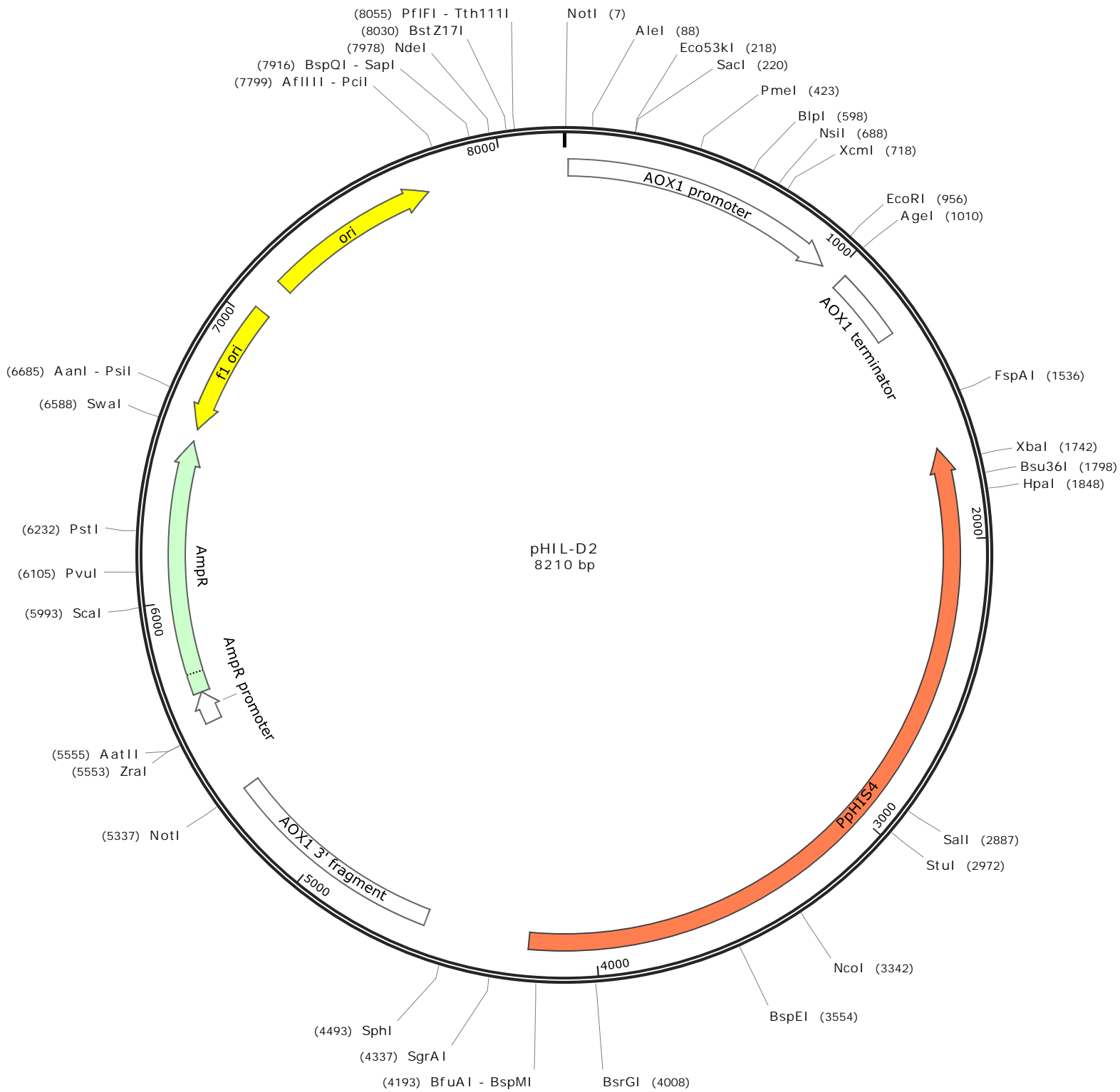


Pichia pastoris HIS4 vector for methanol-inducible intracellular expression of a protein.





CTTACTTTCATAATTGCGACTGGTTCCAATTGACAAGCTTTTGGATTTTAACGACTTTTAACGACAACCTTGAGAAGATCAAAAAAC
GAATGAAAGTATTAACGCTGACCAAGGTTAACTGTTTCGAAAACATAAAATTGCTGAAAATTGCTGTTGAACTCTTCTAGTTTTTTG

935

AOX1 promoter

AACTAATTATTCGAAACGAGGAATTCGCCTTAGACATGACTGTTTCTCAGTTCAAGTTGGGCACCTTACGAGAAGACCGGTCTTGC
TTGATTAATAAGCTTTGCTCCTTAAGCGGAATCTGTACTGACAAGGAGTCAAGTTCAACCCGTGAATGCTCTTCTGGCCAGAACG

1020

AOX1 promoter

TAGATTCTAATCAAGAGGATGTCAGAATGCCATTTGCCTGAGAGATGCAGGCTTCATTTTTGATACTTTTTTATTTGTAACCTAT
ATCTAAGATTAGTTCTCCTACAGTCTTACGGTAAACGGACTCTCTACGTCCGAAGTAAAAACTATGAAAAAATAAACATTGGATA

1105

AOX1 terminator

ATAGTATAGGATTTTTTTTGTCATTTTGTTTCTTCTCGTACGAGCTTGCTCCTGATCAGCCTATCTCGCAGCTGATGAATATCTT
TATCATATCCTAAAAAACAGTAAAAACAAAGAAGAGCATGCTCGAACGAGGACTAGTCGGATAGAGCGTCGACTACTTATAGAA

1190

AOX1 terminator

GTGGTAGGGGTTTTGGGAAAATCATTGAGTTTGGATTTTTTCTTGGTATTTCCCACTCCTCTTCAGAGTACAGAAGATTAAGTGA
CACCATCCCCAACCCCTTTAGTAAGCTCAAACCTACAAAAAGAACCATAAAGGGTGAGGAGAAGTCTCATGTCTTCTAATTCACT

1275

AOX1 terminator

GAAGTTCGTTTGTGCAAGCTTATCGATAAGCTTTAATGCGGTAGTTTATCACAGTTAAATTGCTAACGCAGTCAGGCACCGTGTA
CTTCAAGCAAACACGTTTGAATAGCTATTGAAATTACGCCATCAAATAGTGTCAATTTAACGATTGCGTCAGTCCGTGGCACAT

1360

AOX1 terminator

TGAAATCTAACAATGCGCTCATCGTCATCCTCGGCACCGTCACCCTGGATGCTGTAGGCATAGGCTTGGTTATGCCGGTACTGCC
ACTTTAGATTGTTACGCGAGTAGCAGTAGGAGCCGTGGCAGTGGGACCTACGACATCCGTATCCGAACCAATACGGCCATGACGG

1445

GGGCCTCTTGGCGGATATCGTCCATTCCGACAGCATCGCCAGTCACTATGGCGTGCTGCTAGCGCTATATGCGTTGATGCAATTT
CCCGGAGAACGCCCTATAGCAGGTAAGGCTGTCGTAGCGGTGAGTATACCGCACGACGATCGCGATATACGCAACTACGTTAAA

1530

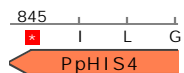
FspAI

CTATGCGCACCCGTTCTCGGAGCACTGTCCGACCGCTTTGGCCGCCGCCAGTCTCTGCTCGCTTCTGCTACTTGGAGCCACTATCG
GATACGCGTGGGCAAGAGCCTCGTGACAGGCTGGCGAAACCGGCGGGTTCAGGACGAGCGAAGCGATGAACCTCGGTGATAGC

1615

ACTACGCGATCATGGCGACCACACCCGTCCTGTGGATCTATCGAATCTAAATGTAAGTTAAAACTCTAAATAATTAATAAGTC
TGATGCGCTAGTACCGCTGGTGTGGGACAGGACACCTAGATAGCTTAGATTTACATTTTATAGAGATTTATTAATTTATTCAG

1700



CCAGTTTCTCCATACGAACCTTAACAGCATTGCGGTGAGCATCTAGACCTTCAACAGCAGCCAGATCCATCACTGCTTGGCCAAT
GGTCAAAGAGGTATGCTTGGAAATTGTCGTAACGCCACTCGTAGATCTGGAAGTTGTCGTGCGTCTAGGTAGTGACGAACCGGTTA

1785

XbaI

L K E M R V K V A N R H A D L G E V A A L D M V A Q G I

PpHIS4



GCAACCAGCAACTTTGGCAGGAACACCCAGCATCAGGGAAGTGGAAAGGCAGAATTGCGGTTCCACCAGGAATATAGAGGCCAACT
CGTTGGTCGTTGAAACCGTCTTTGTGGGTCGTAGTCCCTTACCTTCCGTCTTAACGCCAAGGTGGTCTTATATCTCCGGTTGA

2635

C G A V K A P V G L M L S T S P L I A T G G P I Y L G V
PpHIS4

TTCTCAATAGGTCTTGCAAAACGAGAGCAGACTACACCAGGGCAAGTCTCAACTTGCAACGTCTCCGTTAGTTGAGCTTCATGGA
AAGAGTTATCCAGAACGTTTTGCTCTCGTCTGATGTGGTCCCGTTCAGAGTTGAACGTTGCAGAGGCAATCAACTCGAAGTACCT

2720

K E I P R A F R S C V V G P C T E V Q L T E T L Q A E H F
PpHIS4

ATTTCTGACGTTATCTATAGAGAGATCAATGGCTCTCTTAACGTTATCTGGCAATTGCATAAGTTCTCTGGGAAAGGAGCTTC
TAAAGGACTGCAATAGATATCTCTCTAGTTACCGAGAGAAATTGCAATAGACCGTTAACGTATTCAAGGAGACCTTTCTCGAAG

2805

K R V N D I S L D I A R K V N D P L Q M L E E P F P A E
PpHIS4

TAAACACAGGTGCTTCAAAGCGACTCCATCAAACCTTGGCAGTTAGTTCTAAAAGGGCTTTGTCACCATTTTGACGAACATTGTCG
ATTGTGTCCACAGAAGTTTTGCTGAGGTAGTTTGAACCGTCAATCAAGATTTTTCCCGAAACAGTGGTAAACTGCTTGTAAACAGC

2890

L V P T K L A V G D F K A T L E L L A K D G N Q R V N D
PpHIS4

ACAATTGGTTTTGACTAATTCCATAATCTGTTCCGTTTTCTGGATAGGACGACGAAGGGCATCTTCAATTTCTTGTGAGGAGGCCT
TGTTAAACCAAACCTGATTAAGGTATTAGACAAGGCAAAAGACCTATCTGCTGCTTCCCGTAGAAGTTAAAGAACAACCTCTCCGGA

2975

V I P K V L E M I Q E T K Q I P R R L A D E I E Q S S A K
PpHIS4

TAGAAACGTCAATTTTGACAATTCAATACGACCTTCAGAAGGGACTTCTTTAGGTTTGGATTCTTCTTTAGGTTGTTCTTTGGT
ATCTTTGCAGTTAAAACGTGTTAAGTTATGCTGGAAGTCTTCCCTGAAGAAATCCAAACCTAAGAAGAAATCCAACAAGGAACCA

3060

S V D I K C L E I R G E S P V E K P K S E E K P Q E K T
PpHIS4

GTATCCTGGCTTGGCATCTCCTTTCTTTCTAGTGACCTTTAGGGACTTCATATCCAGGTTTCTCTCCACCTCGTCCAACGTCA
CATAGGACCGAACCGTAGAGGAAAGGAAGATCACTGGAATCCCTGAAGTATAGGTCCAAGAGAGGTGGAGCAGGTTGCAGTGT

3145

Y G P K A D G K R R T V K L S K M D L N R E V E D L T V
PpHIS4

CCGTACTIONGGCACATCTAACTAATGCAAAATAAAATAAGTCAGCACATTCCAGGCTATATCTTCCCTTGGATTAGCTTCTGCAA
GGCATGAACCGTGTAGATTGATTACGTTTTATTTTATTTCAGTCGTGTAAGGGTCCGATATAGAAGGAACCTAAATCGAAGACGTT

3230

G Y K A C R V L A F Y F L D A C E W A I D E K S K A E A L
PpHIS4

GTTTCATCAGCTTCTCCCTAATTTTAGCGTTCAACAAAACCTTCGTCTGTCAAATAACCGTTTGGTATAAGAACCTTCTGGAGCATT
CAAGTAGTCGAAGGAGGGATTAAAATCGCAAGTTGTTTTGAAGCAGCAGTTTATTGGCAAACCATATTCTTGAAGACCTCGTAA

3315

E D A E E R I K A N L V E D D F L R K T Y S G E P A N
PpHIS4



AACTTGAAGCTCAGTCGATTGAGTGAACCTTGATCAGGTTGTGCAGCTGGTCAGCAGCATAGGGAAACACGGCTTTTCTACCAAAA
TTGAACCTTCGAGTCAGCTAACTCACTTGAACCTAGTCCAACACGTCGACCAGTCGTCGTATCCCTTTGTGCCGAAAAGGATGGTTT

4165

V Q L E T S Q T F K I L N H L Q D A A Y P F V A K G V L
PpHIS4

BfuAI
BspMI

CTCAAGGAATTATCAAACCTCTGCAACACTTGCATATGCAGGTAGCAAGGGAAATGTCATACTTGAAGTCGGACAGTGAAGTGTAGT
GAGTTCCTTAATAGTTTGTGACGTTGTGAACGCATACGTCCATCGTTCCCTTTACAGTATGAACCTTCAGCCTGTCACCTCACATCA

4250

S L S N D F E A V S A Y A P L L P F T M
PpHIS4

CTTGAGAAATTCTGAAGCCGTATTTTTATTATCAGTGAGTCAGTCATCAGGAGATCCTCTACGCCGGACGCATCGTGGCCGGCAT
GAACTCTTTAAGACTTCGGCATAAAAATAATAGTCACTCAGTCAGTAGTCCTCTAGGAGATGCGGCCCTGCGTAGCACCGGCCGTA

4335

SgrAI

CACCGGCGCCACAGGTGCGGTTGCTGGCGCCTATATCGCCGACATCACCGATGGGGAAGATCGGGCTCGCCACTTCGGGCTCATG
GTGGCCGCGGTGTCCACGCCAACGACCGCGGATATAGCGGCTGTAGTGGCTACCCCTTCTAGCCCCGAGCGGTGAAGCCCCGAGTAC

4420

SphI

AGCGCTTGTTCGGCGTGGGTATGGTGGCAGGCCCGTGGCCGGGGGACTGTTGGGCGCCATCTCCTTGCATGCACCATTCTTTG
TCGCGAACAAGCCGACCCATACCACCGTCCGGGGCACCAGGCCCGCTGACAACCCGCGGTAGAGGAACGTACGTGGTAAGGAAC

4505

CGGCGGCGGTGCTCAACGGCCTCAACCTACTACTGGGCTGCTTCTAATGCAGGAGTCGCATAAGGGAGAGCGTTCGAGTATCTAT
GCCGCGCCACGAGTTGCCGGAGTTGGATGATGACCCGACGAAGGATTACGTCTCAGCGTATTCCCTCTCGCAGCTCATAGATA

4590

AOX1 3' fragment

GATTGGAAGTATGGGAATGGTGATACCCGCATTCTTCAGTGTCTTGAGGTCTCCTATCAGATTATGCCCAACTAAAGCAACCGGA
CTAACCTTCATACCCTTACCCTATGGGCGTAAGAAGTCACAGAAGTCCAGAGGATAGTCTAATACGGGTTGATTTGTTGGCCT

4675

AOX1 3' fragment

GGAGGAGATTTTCATGGTAAATTTCTCTGACTTTTGGTCATCAGTAGACTCGAACTGTGAGACTATCTCGGTTATGACAGCAGAAA
CCTCTCTAAAGTACCATTTAAAGAGACTGAAAACCAGTAGTCATCTGAGCTTGACACTCTGATAGAGCCAATACTGTCGTCTTT

4760

AOX1 3' fragment

TGTCCTTCTTGAGACAGTAAATGAAGTCCCACCAATAAAGAAATCCTTGTTATCAGGAACAACTTCTTGTTTGAAGTCTTTTC
ACAGGAAGAACCTCTGTCACTTACTTCAGGGTGGTTATTTCTTTAGGAACAATAGTCCTTGTTTGAAGAACAAGCTTGAAAAAG

4845

AOX1 3' fragment

GGTGCCTTGAACATAAAAATGTAGAGTGGATATGTCGGGTAGGAATGGAGCGGGCAAATGCTTACCTTCTGGACCTTCAAGAGGT
CCACGGAACCTTGATATTTTACATCTCACCTATACAGCCCATCCTTACCTCGCCCGTTTACGAATGGAAGACCTGGAAGTTCTCCA

4930

AOX1 3' fragment

ATGTAGGGTTTGTAGATACTGATGCCAACTTCAGTGACAACGTTGCTATTTTCGTTCAAACCATTCCGAATCCAGAGAAATCAAAG
TACATCCCAAACATCTATGACTACGGTTGAAGTCACTGTTGCAACGATAAAGCAAGTTTGGTAAGGCTTAGGTCTCTTTAGTTTC

5015

AOX1 3' fragment

TTGTTTGTCTACTATTGATCCAAGCCAGTGCGGTCTTGAAACTGACAATAGTGTGCTCGTGTTTTGAGGTCATCTTTGTATGAAT
 AACAAACAGATGATAACTAGGTTTCGGTCACGCCAGAACTTTGACTGTTATCACACGAGCACAAAACCTCCAGTAGAAACATACTTA

5100

AOX1 3' fragment

AAATCTAGTCTTTGATCTAAATAATCTTGACGAGCCAAGGCGATAAATACCCAAATCTAAAACCTTTTTAAAACGTTAAAAGGAC
 TTTAGATCAGAAACTAGATTTATTAGAAGCTGCTCGGTTCCGCTATTTATGGGTTTAGATTTTGAGAAAATTTTGCAATTTTCTG

5185

AOX1 3' fragment

AAGTATGTCTGCCTGTATTAACCCCAAATCAGCTCGTAGTCTGATCCTCATCAACTTGAGGGGCACTATCTTGTTTTAGAGAAA
 TTCATACAGACGGACATAATTTGGGGTTTAGTCGAGCATCAGACTAGGAGTAGTTGAACTCCCCGTGATAGAACAAAATCTCTTT

5270

AOX1 3' fragment

TTTGCGGAGATGCGATATCGAGAAAAAGGTACGCTGATTTTAAACGTGAAATTTATCTCAAGATCGCGGCCGCGATCTCGAATAA
 AAACGCCTCTACGCTATAGCTCTTTTTCCATGCGACTAAAATTTGCACTTTAAATAGAGTTCTAGCGCCGGCGCTAGAGCTTATT

5355

AOX1 3' fragment

NotI

TAACTGTTATTTTTAGTGTTCCTGATCTGCGTCTATTTTACAATACCAACATGAGTCAGCTTATCGATGATAAGCTGTCAAACA
 ATTGACAATAAAAAGTCACAAGGGCTAGACGCAGATAAAGTGTTATGGTTGTA CT CAGTCGAATAGCTACTATTCGACAGTTTGT

5440

TGAGAATTAATTCGATGATAAGCTGTCAAACATGAGAAATCTTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTA
 ACTCTTAATTAAGCTACTATTCGACAGTTTGTACTCTTTAGA ACTTCTGCTTTCCCGGAGCACTATGCGGATAAAAATATCCAAT

5525

ATGTCATGATAATAATGGTTTTCTTAGACGTCAGGTGGCACTTTTCCGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTA
 TACAGTACTATTATTACCAAAGAATCTGCAGTCCACCGTGAAAAGCCCCCTTACACGCGCCTTGGGGATAAACAAATAAAAAGAT

5610

ZraI AatII

AmpR promoter

AATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATT
 TTATGTAAGTTTATACATAGGCGAGTACTCTGTTATTGGGACTATTTACGAAGTTATTATAACTTTTTCTTCTCATACTCATAA

5695

AmpR promoter

1
M S I
signal seq...
AmpR

CAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTGCTTCCCTGTTTTTGTCTACCCAGAAACGCTGGTGAAAGTAA
 GTTGTAAGGCACAGCGGGAATAAGGGAAAAACGCGTAAAACGGAAGGACAAAAACGAGTGGGTCTTTGCGACCACTTTTCATT

5780

5 10 15 20 25 30

Q H F R V A L I P F F A A F C L P V F A H P E T L V K V

signal sequence

AmpR

AAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCC
 TTCTACGACTTCTAGTCAACCCACGTGCTCACCCAATGTAGCTTGACCTAGAGTTGTCGCCATTCTAGGA ACTCTCAAAAAGCGGG

5865

35 40 45 50 55 60

K D A E D Q L G A R V G Y I E L D L N S G K I L E S F R P

AmpR

CGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAA
 GCTTCTTGCAAAAAGGTTACTACTCGTGAAAATTTCAAGACGATACACCGCGCCATAATAGGGCACAACCTGCGGCCGTTCTCGTT
 E E R F P M M S T F K V L L C G A V L S R V D A G Q E Q
 AmpR

CTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAG
 GAGCCAGCGGCGTATGTGATAAGAGTCTTACTGAACCAACTCATGAGTGGTCAGTGTCTTTTCGTAGAATGCCTACCGTACTGTC
 L G R R I H Y S Q N D L V E Y S P V T E K H L T D G M T
 AmpR

TAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAAACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGA
 ATTCTCTTAATACGTACGACGGTATTGGTACTCACTATTGTGACGCCGGTTGAATGAAGACTGTTGCTAGCCTCCTGGCTTCTCT
 V R E L C S A A I T M S D N T A A N L L L T T I G G P K E
 AmpR

GCTAACCGCTTTTTTGCAACATGGGGGATCATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAAC
 CGATTGGCGAAAAAACGTGTTGTACCCCTAGTACATTGAGCGGAACCTAGCAACCCTTGGCCTCGACTTACTTCGGTATGGTTTG
 L T A F L H N M G D H V T R L D R W E P E L N E A I P N
 AmpR

GACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACCTATTAACCTGGCGAACTACTTACTCTAGCTTCCC
 CTGCTCGCACTGTGGTGCTACGGACGTCGTTACCGTTGTTGCAACGCGTTTGATAATTGACCGCTTGATGAATGAGATCGAAGGG
 D E R D T T M P A A M A T T L R K L L T G E L L T L A S
 AmpR

GGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGC
 CCGTTGTTAATTATCTGACCTACCTCCGCCTATTTCAACGTCTCTGGTGAAGACGCGAGCCGGGAAGGCCGACCGACCAAATAACG
 R Q Q L I D W M E A D K V A G P L L R S A L P A G W F I A
 AmpR

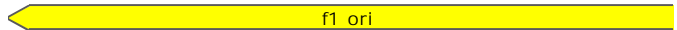
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 D K S G A G E R G S R G I I A A L G P D G K P S R I V V
 AmpR

ATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGT
 TAGATGTGCTGCCCTCAGTCCGTTGATACCTACTTGTCTTATCTGTCTAGCGACTCTATCCACGGAGTGACTAATTCGTAACCA
 I Y T T G S Q A T M D E R N R Q I A E I G A S L I K H W
 AmpR

Swal

AACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAATTGTAACGTTAATATTTTGTAAAATTCGCGTTAAATT
TTGACAGTCTGGTTCAAATGAGTATATATGAAATCTAACTAAATTTAACATTTGCAATTATAAAACAATTTTAAGCGCAATTTAA

6630



AmpR

AanI
PstI

TTTGTAAATCAGCTCATTTTTAAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAGAATAGACCGAGATAGGGTT
AAACAATTTAGTCGAGTAAAAAATTGGTTATCCGGCTTTAGCCGTTTTAGGGAATATTTAGTTTTCTTATCTGGCTCTATCCCAA

6715



GAGTGTGGTTCCAGTTTGAACAAGAGTCCACTATTAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGC
CTCACAAACAAGGTCAAACCTTGTCTCAGGTGATAATTTCTTGACCTGAGGTTGCAGTTTTCCCGCTTTTTGGCAGATAGTCCCG

6800



GATGGCCCACTACGTGAACCATCACCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCTAAAGGGA
CTACCGGGTGATGCACTTGGTAGTGGGATTAGTTCAAAAAACCCAGCTCCACGGCATTTCGTGATTTAGCCTTGGGATTTCCCT

6885



GCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGC
CGGGGGCTAAATCTCGAACTGCCCTTTGGCCGCTTGCACCGCTCTTTCCTTCCCTTCTTTCGCTTTCCTCGCCCGGATCCCG

6970



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CGACCGTTACATCGCCAGTGCACGCGCATTGGTGGTGTGGGGCGCGCGAATTACGCGGCGATGTCCCAGCGCATTTCCTAGAT

7055



GGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAG
CCACTTCTAGGAAAAACTATTAGAGTACTGGTTTTAGGGAATTGCACTCAAAGCAAGGTGACTCGCAGTCTGGGGCATCTTTTTC

7140

ATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTT
TAGTTTTCTAGAAGAACTCTAGGAAAAAAAGACGCGCATTAGACGACGAACGTTTTGTTTTTTGGTGGCGATGGTCCGCCACAAA

7225



GTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACGTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCTTCTAGT
CAAACGGCCTAGTTCTCGATGGTTGAGAAAAAGGCTTCCATTGACCGAAGTCGTCTCGCGTCTATGGTTTATGACAGGAAGATCA

7310



GTAGCCGTAGTTAGGCCACCCTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCT
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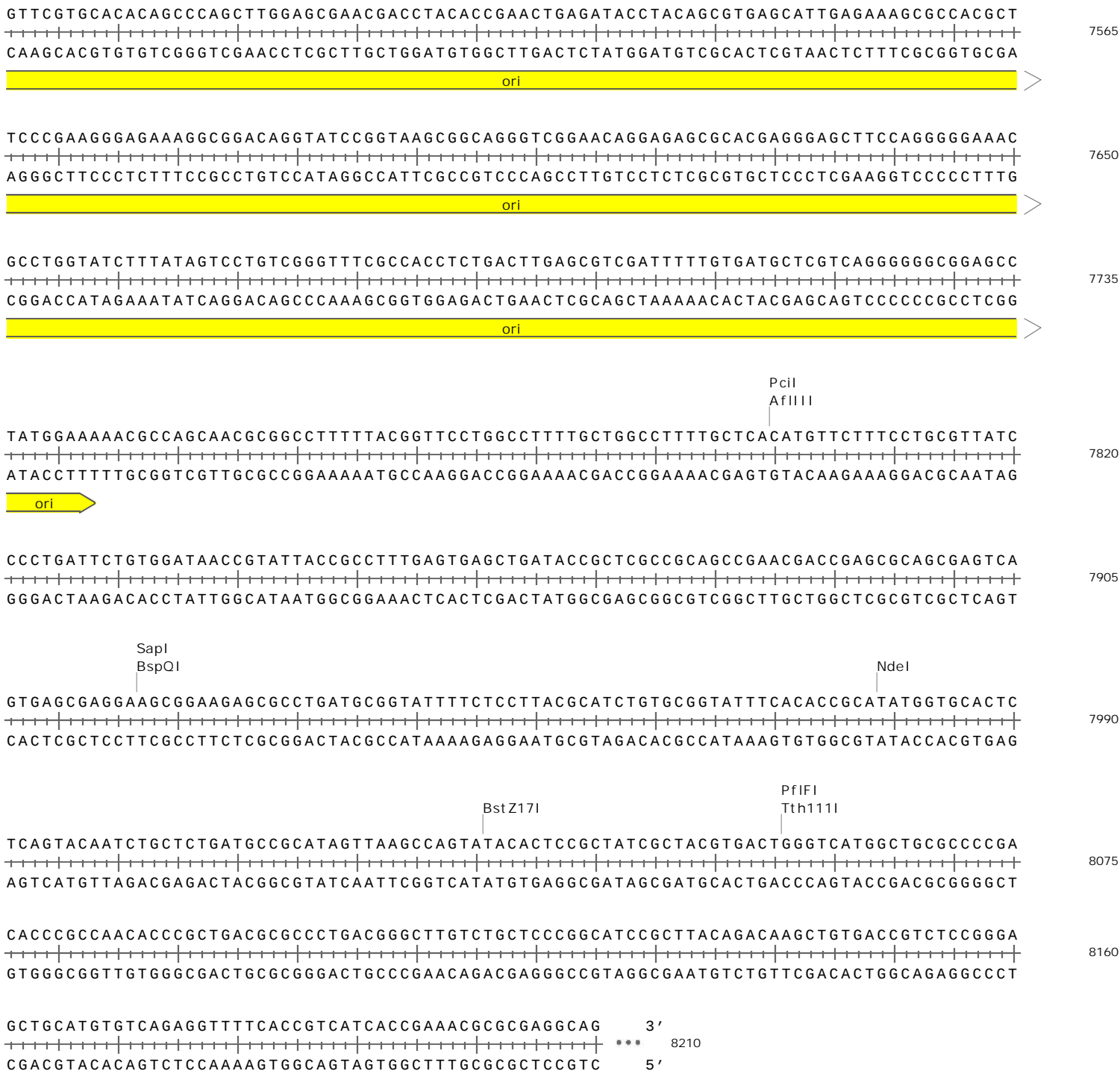
7395





















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CGGTCAACCGCTATTGACGACAGAATGGCCCAACCTGAGTTCTGCTATCAATGGCCTATTCCGCGCTCGCCAGCCCGACTTGCCCCC

7480





Enzymes	Sites	
AanI	1	6685
AatII	1	5555
AflIII	1	7799
AgeI	1	1010
AleI	1	88
BfuAI	1	4193
BipI	1	598
BspEI	1	3554
BspMI	1	4193
BspQI	1	7916
BsrGI	1	4008
BstZ17I	1	8030
Bsu36I	1	1798
Eco53kI	1	218
EcoRI	1	956
FspAI	1	1536
HpaI	1	1848
NcoI	1	3342
NdeI	1	7978
NotI	2	7 5337
NsiI	1	688
PciI	1	7799
PfIF1	1	8055
PmeI	1	423
PsiI	1	6685
PstI	1	6232
PvuI	1	6105
SacI	1	220
Sall	1	2887
SapI	1	7916
ScaI	1	5993
SgrAI	1	4337
SphI	1	4493
StuI	1	2972
Swal	1	6588
Tth111I	1	8055
XbaI	1	1742
XcmI	1	718
ZraI	1	5553

Feature	Location	Size (bp)			Type
✓ AOX1 promoter	14 .. 953	940			promoter
/gene	= Pichia pastoris AOX1				
/note	= inducible promoter, regulated by methanol				
✓ AOX1 terminator	1031 .. 1277	247			terminator
/gene	= Pichia pastoris AOX1				
/note	= transcription terminator for AOX1				
✓ PpHIS4	1690 .. 4224	2535			CDS
/gene	= Pichia pastoris HIS4				
/product	= multifunctional enzyme, required for histidine biosynthesis				
/note	= auxotrophic marker for Pichia pastoris				
/translation	= MTFPLLPAYASVAEFDNSLSLVGKAVFPYAADQLHNLKFTQSTELQVNVQVESSVTEdqFEELIDNLLKLYNNGINEVILDLDLAE RVVQRMIPGARVIYRTLVDKVASLPANASIAVPFSSPLGDLKSFNNGSRTVYAFSETAKLVDVTSTVASGIIPIIDARQLTTEYELS EDVKKFPVSEILLASLTDRPDGLFTTLVADSSNYSLGLVYSSKKSIPeAIRtQTGVYQSRRHGLWYKGATSGATQKLLGIELDCDGD CLKFVVEQTGVGFCHLERTSCFGOSKGLRAMEATLWDRKSNAPeGSYTKRLFDDEVLLNAKIREEADELAeAKSKEDIAWECADLF YFALVRCaKYGVTLDEVERNLDmKSLKVTRRKGDAKPGYTKEQPKeESKPKVEPSEGRIELCKIDVSKASSQeIeDALRRPIQKTEQ IMELVKPIVDNVRONGDKALLELTAKFDGVALKTPVLEAPFPEELMQLPDNVKRAIDLSIDNVRKFHEAQLTETLQVETCPGVVCSR FARPIEKVGLYIPGGTAiLPSTSLMLGVPaKvAGCKeIVFASPPKDGTLTPEVIYVAHKVgAKCIVLAGGAQAVAAAMAYGTETVP KCDKIFGPGNQFVTAaKMMVQNDTSALCSIDMPAGPSEVLVIADKYADPDFVAsDLLSOAEHGIDSOVILLAVDMDTKELARIED AVHNQAVQLPRVEIVRKCIAHSTTLsVATYEQALEMSNQYAPeHLILQIENASSYVDQVQHAGSVFVgAYSPeSCGDYSSGtNH TIPtYGYARoYSGVNTATFOkFITsODVTPFGIKHIGoAVMDIAAVFGIDAhRNaVKVRMFkIGI I * 844 amino acids = 92.2 kDa				
✓ AOX1 3' fragment	4579 .. 5335	757			misc_feature
/note	= region downstream of Pichia pastoris AOX1 gene				
✓ AmpR promoter	5582 .. 5686	105			promoter
/gene	= bla				
✓ AmpR	5687 .. 6547	861			CDS
▶ 2 segments					
/gene	= bla				
/product	= -lactamase				
/note	= confers resistance to ampicillin, carbenicillin, and related antibiotics				
/translation	= MSIQHFRVALIPFFAAFLPVFA,HPETLVKVKDAEDQLGARVGYIELDLSNGKILESFRPEERFPMSTFKVLLCGAVLSRVDAGQE QLGRRIHYSQNDLVEYSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHVTRLDRWEPELNEAIPN DERDRTMPAAMATTLRKLTLGELLTLASRQQLIDWMEADKVAGPLLSALPAWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIY TTGSQATMDFRNRIAFIGASIKHW* 286 amino acids = 31.5 kDa				
✓ f1 ori	6589 .. 7044	456			rep_origin
/direction	= LEFT				
/note	= f1 bacteriophage origin of replication; arrow indicates direction of (+) strand synthesis				
✓ ori	7155 .. 7743	589			rep_origin
/direction	= RIGHT				
/note	= high-copy-number ColE1/pMB1/pBR322/pUC origin of replication				

Description: Pichia pastoris HIS4 vector for methanol-inducible intracellular expression of a 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: The gene of interest can be inserted at the unique EcoRI site.

References: