

# pKLMF-FX Vector



## N3744S

**20 µg**      **Lot: 0011309**      **Exp: 9/15**  
**1,000 µg/ml**      **Store at -20°C**

**Description:** The vector pKLMF-FX directs high-level intracellular expression of a recombinant protein fused to the maltose binding protein (MBP) in the yeast *Kluyveromyces lactis*. It is compatible with the *K. lactis* Protein Expression Kit (NEB #E1000). MBP fusion proteins expressed from pKLMF-FX can be affinity purified from cell lysates using amylose resin (NEB #E8021). MBP can be removed from purified fusion proteins by digestion with Factor Xa protease (NEB #P8010).

Vector pKLMF-FX contains the strong *K. lactis* P<sub>LAC4-PBI</sub> promoter (1), DNA encoding *E. coli* maltose binding protein (MBP) with a C-terminal Factor Xa site, a universal multiple cloning site (MCS), the *K. lactis* LAC4 transcription terminator (TT), and a fungal acetamidase selectable marker gene (*amdS*) expressed from the yeast ADH1 promoter (P<sub>ADH1</sub>). An *E. coli* replication origin (*ori*) and ampicillin resistance gene (*Ap<sup>R</sup>*) is present for propagation of pKLMF-FX in *E. coli*. SacII linearized pKLMF-FX integrates into the LAC4 locus of the *K. lactis* genome upon transformation of *K. lactis* competent cells.

The sequence of the pKLMF-FX vector (GenBank # FJ010197) and additional pKLMF-FX information are available at [www.neb.com](http://www.neb.com).

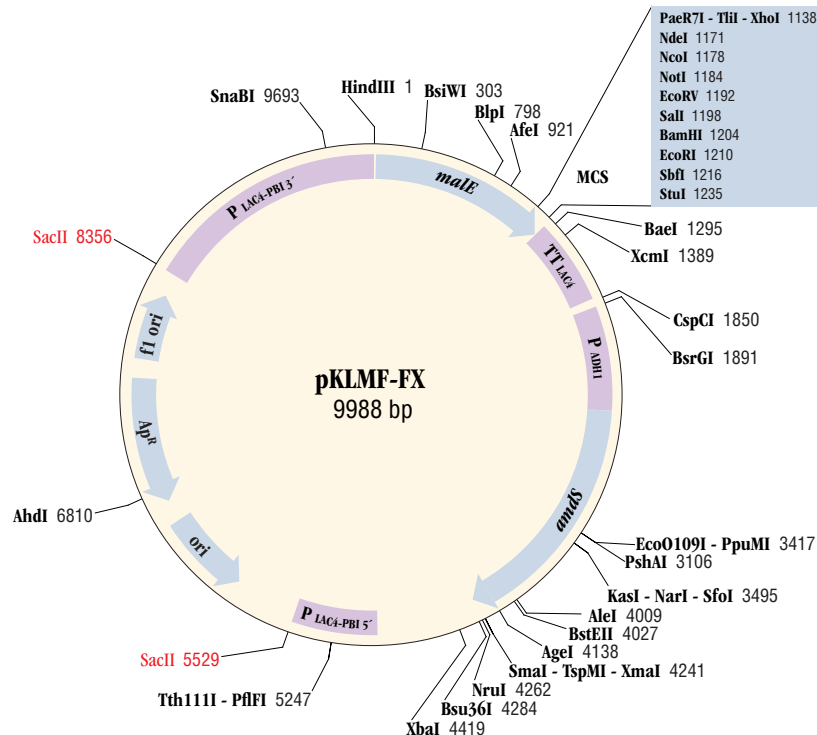
**Source:** pKLMF-FX is isolated from *E. coli* strain ER2268 by a standard DNA purification procedure.

Supplied in: 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA.

### Features of pKLMF-FX

- P<sub>LAC4-PBI</sub> promoter does not express in *E. coli* allowing toxic genes to be cloned prior to their expression in yeast.
- Universal MCS lies downstream of DNA encoding MBP and P<sub>LAC4-PBI</sub> promoter.
- Acetamidase expression for non-antibiotic selection in *K. lactis*.
- Ampicillin resistance for propagation in *E. coli*.

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GAATTGTGAGCGGATAACAAGCTCAACACTTGAATTTAGGAAAGAGCAGAATTTGGCAA
                                     HindIII
AAAAAATAAAAAAAAAATAACACACATACTCATCGAGAAGCTTGCCACCATGAAAAGTGMKT
AAGAAGGTAAGTGGTAATCTGGATTAACGGCGATAAAGGCTATAACGGTCTCGCTGAAG
E E G K L V I W I N G D K G Y N G L A E
TCGGTAAGAAATTCGAGAAAGATACCGGAATTAAGTCACCGTTGAGCATCCGGATAAAC
V G K K F E K D T G I K V T V E H P D K
TGGAAGAGAAATCCCACAGGTTGCGGCAACTGGCGATGGCCCTGACATTATCTTCTGGG
L E E K F P Q V A A T G D G P D I I F W
CACACGACCGCTTTGGTGGCTACGCTCAATCTGGCCTGTTGGCTGAAATCACCCCGGACA
A H D R F G G Y A Q S G L L A E I T P D
AAGCGTTCCAGGACAAGCTGTATCCGTTTACTGGGATGCGGTACGTTACAACGGCAAGC
K A F Q D K L Y P F T W D A V R Y N G K
TGATTGCTTACCCGATCGCTGTTGAAGCGTTATCGCTGATTATAACAAGATCTGCTGC
L I A Y P I A V E A L S L I Y N K D L L
CGAACCCGCCAAAAACCTGGGAAGAGATCCCGGCGCTGGATAAAGAAGCTGAAAGCGAAAG
P N P P K T W E E I P A L D K E L K A K
GTAAGAGCGCGCTGATGTTCAACCTGCAAGAACCCTACTTACCTGCGCGCTGATTGCTG
G K S A L M F N L Q E P Y F T W P L I A
CTGACGGGGTTATGCGTTCAAGTATGAAAACGGCAAGTACGACATTAAGACGTGGGCG
A D G G Y A F K Y E N G K Y D I K D V G
TGATAACGCTGGCGGAAAGCGGGTCTGACCTTCTGGTGGCTGATTAAAAACAAC
V D N A G A K A G L T F L V D L I K N K
ACATGAATGCAGACACCGATTACTCCATCGCAGAAGCTGCCTTTAATAAAGCGAAAACAG
H M N A D T D Y S I A E A A F N K G E T
CGATGACCATCAACGGCCGCTGGGATGTTCCAAACATCGACACCAAGTGAATTATG
A M T I N G P W A W S N I D T S K V N Y
GTGTAACGGTACTGCCGACCTTCAAGGGTCAACCATCCAACCGTTTCGTTGGCTGCTGA
G V T V L P T F K G Q P S K P F V G V L
GCGCAGGTATTAACGCCGCGAGTCCGAACAAAGAGCTGGCAAAGAGTTCCTCGAAAAC
S A G I N A A S P N K E L A K E F L E N
ATCTGCTGACTGATGAAGGTCTGGAAGCGGTTAATAAAGACAACCGCTGGGTGCCGTAG
Y L L T D E G L E A V N K D K P L G A V
CGCTGAAGTCTTACGAGGAAGAGTTGGCGAAAGATCCACGTATTGCCGCCACTATGGAAA
A L K S Y E E E L A K D P R I A A T M E
ACGCCGAGAAAGGTGAAATCATGCCGAACATCCCGCAGATGTCGCTTCTGGTATGCCG
N A Q K G E I M P N I P Q M S A F W Y A
TGCCTACTGCGGTGATCAACGCCGCCAGCGGTGCTGACTGTCGATGAAGCCCTGAAAG
V R T A V I N A A S G R Q T V D E A L K
                                     Factor Xa site XhoI
ACGCGCAGACTAATTCGAGCTCGATCGAGGGAAGGCTCGAGAAAAGAGAGGCTGAAGCTA
D A Q T N S S S I E G R L E K R E A E A
                                     NdeI NcoI NotI EcoRV Sall BamHI EcoRI SbfI
GAAGAGCTCATATGTCATGGGCGCGCGATATCGTCGACGGATCCGAATTCCTCGAC
R R A H M S M G G R D I V D G S E F P A
                                     StuI
GTAATTAATAAAGGCCTTGAATCGAGAATTTACTTAGATAAATTTAAACGGTTATTAGTGC
G N *
ATATTTCTATGAGATACTGATGTATACATGCATGATAAATTTAAACGGTTATTAGTGC
GATTGCTTGTGCGATAATGACGTTCTATCAAAGCAATACACTACCACCTATTAC
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pKLMF-FX plasmid map. Unique restriction sites are shown in bold. SacII sites are shown in red.

**Usage Notes:** For proper integration into the LAC4 promoter region of the *K. lactis* chromosome, pKLMF-series vectors containing a gene of interest must be linearized with SacII prior to their introduction into *K. lactis* cells. The cloned gene of interest must have no internal SacII sites.

After transformation of *K. lactis* cells by a pKLMF-series vector, its targeted integration into the LAC4 promoter locus can be confirmed by whole-cell PCR using an adaptation of Optional Methods I and II of the *K. lactis* Protein Expression Kit Instruction Manual (#E1000). In these methods, Integration Primer 2 is a reverse primer that anneals to a specific region of DNA encoding the  $\alpha$ -mating factor secretion leader sequence in pKLAC-series vectors, a region that is absent from pKLMF-series vectors. For assessment of the integration patterns of pKLMF-series vectors, a primer with the sequence 5' d(GTTTACCTTCTCAGTTTTCAT) 3' should be used in place of the kit's Integration Primer 2 in these protocols (2). Whole cell PCR using this primer in conjunction with either Integration Primer 1 or Integration Primer 3 will each yield a 2.3 kb diagnostic amplicon. (see other side)

pKLMF-FX multiple cloning site (MCS). The *K. lactis*  $\alpha$ -mating factor secretion domain is shown with a blue background. Only unique restriction sites are shown.

NEB 5-alpha Competent *E. coli* (High Efficiency) (NEB #C2987), NEB 5-alpha Electrocompetent *E. coli* (NEB #C2989) and NEB 5-alpha Competent *E. coli* (Subcloning Efficiency) (NEB #C2988) are all recommended for propagation and subcloning this vector.

**References:**

1. Colussi, P.A. and Taron, C.H. (2005) *Appl. Environ. Microbiol.*, 71, 7092–7098.
2. Foster, J. et al. (2008) *Parasitol. Res.* In press.

**NOTICE TO BUYER/USER:** The vector pKLMF-FX is a component of an expression system that was developed from basic research at New England Biolabs, Inc. and DSM Biologics Company B.V. The buyer/user has a non-exclusive sublicense to use this system or any component thereof, including vector pKLMF-FX, for **RESEARCH PURPOSES**

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U.S. Patent No. 5,643,758