pKLCF-n Vector

1-800-632-7799
info@neb.com
www.neb.com

N3746S

20 µg **1,000 µg/ml** **Lot: 0011111**

Store at –20°C **Exp: 11/13**

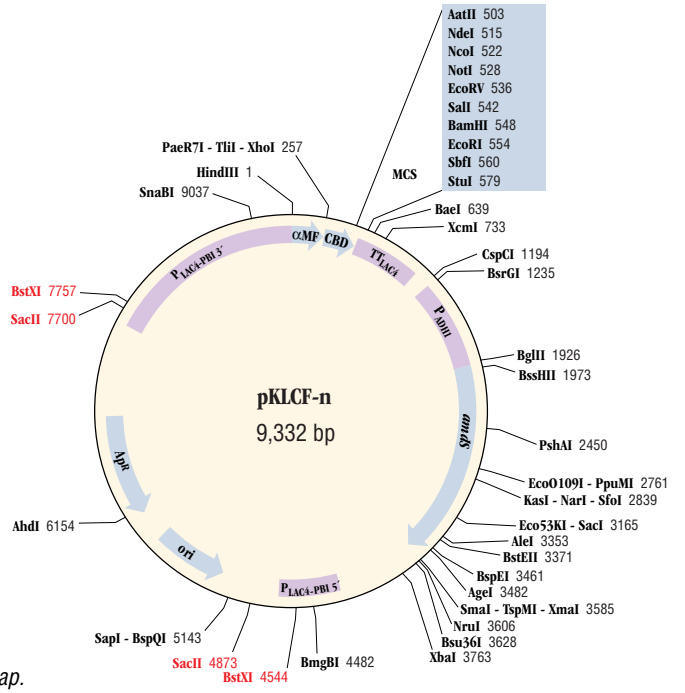
Description: The vector pKLCF-n permits secreted expression of a recombinant protein having a chitin-binding domain (CBD) affinity tag fused to its amino-terminus in the yeast *Kluyveromyces lactis*. It is compatible with the *K. lactis* Protein Expression Kit (NEB #E1000). CBD fusion proteins expressed from pKLCF-n can be affinity purified directly from untreated culture medium using Chitin Beads (NEB #S6651) or Chitin Magnetic Beads (NEB #E8036).

Vector pKLCF-n contains the strong *K. lactis* $P_{LAC4\text{-}PBI}$ promoter (1), DNA encoding the *K. lactis* **Cts1p** chitin-binding domain (2), 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_{ADH1}). An *E. coli* replication origin (ori) and ampicillin resistance gene (Ap^R) is present for propagation of pKLCF-n in *E. coli*. SacII or BstXI linearized pKLCF-n integrates into the *LAC4* locus of the *K. lactis* genome upon transformation of *K. lactis* competent cells.

The sequence of the pKLCF-n vector (GenBank HQ214066) and additional pKLCF-n information are available at www.neb.com.

Source: pKLCF-n 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.



pKLCF-n plasmid map.
Unique restriction sites are shown in bold.
SacII and BstXI sites are shown in red.

(see other side)

CERTIFICATE OF ANALYSIS

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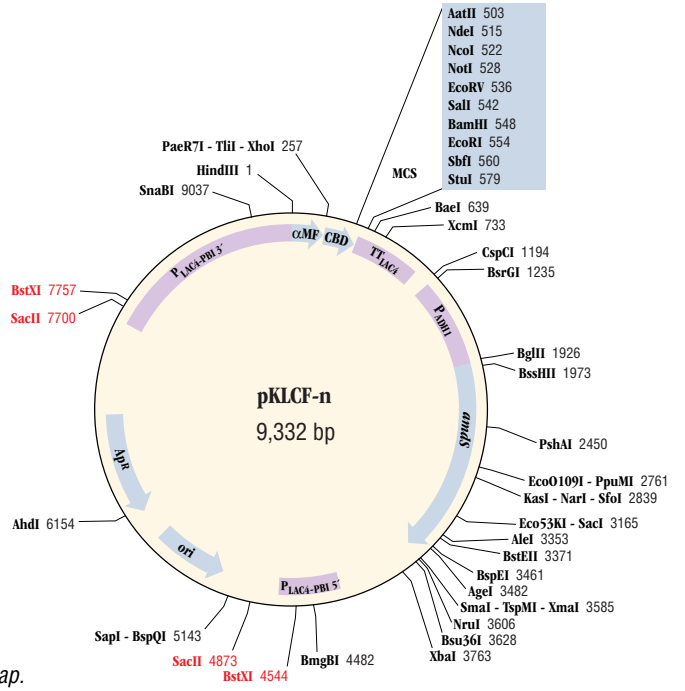

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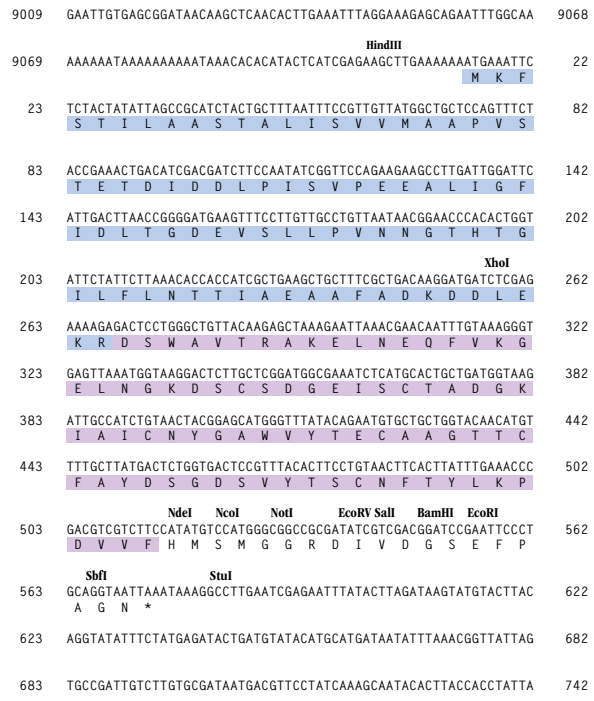

pKLCF-n multiple cloning site (MCS). The K. lactis α-mating factor is shown with a blue background and the chitin-binding domain is shown with a purple background. Only unique restriction sites are shown.

Features of pKLCF-n:

- $P_{LAC4\text{-}PBI}$ 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 CBD and $P_{LAC4\text{-}PBI}$ promoter.
- Acetamidase expression for non-antibiotic selection in *K. lactis*.
- Ampicillin resistance for propagation in *E. coli*.
- Permits expression of CBD-tagged fusion proteins and their one-step purification directly from growth medium.

Usage Notes: In applications where protease removal of the tag from a purified CBD-fusion protein is ultimately desired, DNA encoding a site-specific protease site should be included in-frame at the extreme 5´ end of the target gene's coding sequence. For example, including the sequence 5´-GAT GAC GAT GAC AAG-3´ (encoding an enterokinase cleavage site: DDDDK↓) immediately upstream of the target gene's start codon will place an enterokinase site between the CBD and the target protein. After purification of the CBD-fusion protein, digestion with enterokinase (NEB #P8070) will remove CBD from the protein leaving no non-native amino acids on the protein's amino-terminus. In this expression strategy, it is important to place the enterokinase site in the same translational reading frame as both the CBD and the target gene to ensure a full-length fusion protein is produced.

For proper integration into the *LAC4* promoter region of the *K. lactis* chromosome, pKLCF-series vectors containing a gene of interest must be linearized with either SacII or BstXI prior to their introduction into *K. lactis* cells. Therefore, the cloned gene of interest must lack either internal SacII or BstXI sites, depending upon which enzyme is used for linearization.

After transformation of *K. lactis* cells by a pKLCF-series vector, its targeted integration into the LAC4 promoter locus can be confirmed by whole-cell PCR using Optional Methods I and II of the *K. lactis* Protein Expression Kit Instruction Manual (NEB #E1000).

References:

1. Colussi, P.A. and Taron, C.H. (2005) *Appl. Environ. Microbiol.*, 71, 7092–7098.
2. Colussi, P.A., Specht, C.A. and Taron, C.H. (2005) *Appl. Environ. Microbiol.*, 71, 2862–2869.

9009 GAATTGTGAGCGGATAACAAGCTCAACACTTGAAATTTAGGAAAGAGCAGAATTTGGCAA 9068
HindIII
9069 AAAAAATAAAAAAAAAAATAAACACACATACTCATCGAGAAGCTTGAAAAAAATGAAATTC 22
M K F
23 TCTACTATATTAGCCGCATCTACTGCTTTAATTTCCGTTGTTATGGCTGCTCCAGTTTCT 82
S T I L A A S T A L I S V V M A A P V S
83 ACCGAAACTGACATCGACGATCTTCCAATATCGGTTCCAGAAGAAGCCTTGATTGGATTC 142
T E T D I D D L P I S V P E E A L I G F
143 ATTGACTTAACCGGGGATGAAGTTTCCTTGTTGCCTGTTAATAACGGAACCCACACTGGT 202
I D L T G D E V S L L P V N N G T H T G
XhoI
203 ATTCTATTCTTAAACACCACCATCGCTGAAGCTGCTTTCGCTGACAAGGATGATCTCGAG 262
I L F L N T T I A E A A F A D K D D L E
263 AAAAGAGACTCCTGGGCTGTTACAAGAGCTAAAGAATTAAACGAACAATTTGTAAAGGGT 322
K R D S W A V T R A K E L N E Q F V K G
323 GAGTTAAATGGTAAGGACTCTTGCTCGGATGGCGAAATCTCATGCACTGCTGATGGTAAG 382
E L N G K D S C S D G E I S C T A D G K
383 ATTGCCATCTGTAACTACGGAGCATGGGTTTATACAGAATGTGCTGCTGGTACAACATGT 442
I A I C N Y G A W V Y T E C A A G T T C
443 TTTGCTTATGACTCTGGTGACTCCGTTTACACTTCCTGTAACTTCACTTATTTGAAACCC 502
F A Y D S G D S V Y T S C N F T Y L K P
NdeI NcoI NotI EcoRV SalI BamHI EcoRI
503 GACGTCGTCTTCCATATGTCCATGGGCGGCCGCGATATCGTCGACGGATCCGAATTCCCT 562
D V V F H M S M G G R D I V D G S E F P
SbfI StuI
563 GCAGGTAATTAAATAAAGGCCTTGAATCGAGAATTTATACTTAGATAAGTATGTACTTAC 622
A G N *
623 AGGTATATTTCTATGAGATACTGATGTATACATGCATGATAATATTTAAACGGTTATTAG 682
683 TGCCGATTGTCTTGTGCGATAATGACGTTCCTATCAAAGCAATACACTTACCACCTATTA 742

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NOTICE TO BUYER/USER: The vector pKLCF-n 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 pKLCF-n, for **RESEARCH PURPOSES ONLY.** A license to use this system for manufacture of clinical grade material or commercial purposes is available from New England Biolabs, Inc. or DSM Biologics Company B.V.