

9009 GAATTGTGAGCGGATAACAAGCTCAACACTTGAATTTAGGAAAAGAGCAGAATTTGGCAA 9068
 HindIII
 9069 AAAAAATAAAAAAAAAAACAACACACTACTCATCGAGAAGCTTGAIAAAATGAAATTC 22
 M K F
 23 TCTACTATATTAGCCGATCTACTGCTTTAATTTCCGTTGTTATGGCTGCTCCAGTTTCT 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 ATTGACTTAACCGGGGATGAAGTTTCCCTGTTGCTGTTAATAACGGAACCCACACTGGT 202
 I D L T G D E V S L L P V N N G T H T G
 XhoI
 203 ATTCTATTCTTAAACACCACCTCGCTGAAGCTGCTTCGCTGACAAGGATGATCTCGAG 262
 I L F L N T T I A E A A F A D K D D L E
 NdeI NcoI NotI EcoRV
 263 AAAAAAGAGGCTGAAGCTAGAAGGCTCATATGTCATGGCGGCCGCGATATCGTCGAC 322
 K R E A E A R R A H M S M G G R D I V D
 EcoRI SbfI
 323 GGATCCGAATTCCTCGAGGTGACTCCTGGGCTGTTACAAGAGCTAAAGAAATTAACGAA 382
 G S E F P A G D S W A V T R A K E L N E
 383 CAATTTGTAAGGGTGAAGTAAATGGTAAGGACTTCTGCTGGATGGCGAAATCTCATGC 442
 Q F V K G E L N G K D S C S D G E I S C
 443 ACTGCTGATGGTAAGATTGCCATCTGTAACCTACGGAGCATGGGTTTATACAGAATGTGCT 502
 T A D G K I A I C N Y G A W V Y T E C A
 503 GCTGGTACAACATGTTTGGCTTATGACTCTGGTACTCCGTTTACACTTCTGTAACTTC 562
 A G T T C F A Y D S G D S V Y T S C N F
 SmaI
 563 ACTTATTGAAACCCGACGCTGCTTCAATTAATAAAGGCCTTGAATCGAGAATTTATA 622
 T Y L K P D V V F N *
 623 CTAGATAAGTATGACTACAGGTATATTTCTATGAGACTGATGTATACATGCATGA 682
 683 TAATATTTAAACGGTATTAGTGCCGATTGCTTGGCGATAATGACGTTCTTATCAAAAG 742
 CAATACACTACCACCTATTA

pKLCF-c 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-c:

- P_{LAC4-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-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.

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

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 G S E F P A G D S W A V T R A K E L N E
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