Vector pKLCF-c contains the strong K. lactis (PLACA-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 (ApR) is present for propagation of pKLCF-c in E. coli. Sacl or BstXI linearized pKLCF-c integrates into the LAC4 locus of the K. lactis genome upon transformation of K. lactis competent cells.

The sequence of the pKLCF-c vector (GenBank HQ236722) and additional pKLCF-c information are available at www.neb.com.

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

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Features of pKLCF-c:
- $P_{LAC4}$ 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 $P_{LAC4}$ 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:

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.