pKLAC2

GenBank Accession #: EU196354
See page 231 for ordering information.

There are no restriction sites for the following enzymes: AatII, AbsI(x), Acc65I, AfeI, AflII, ApaI, AscI, AsiSI, AvrII, BbvCI, BlpI, Bpu10I, BsiWI, FseI, FspAI(x), KflI(x), KpnI, MauBI(x), MluI, MreI(x), MscI, MteI(x), PacI, PaqCI, PasI(x), PmeI, PmlI, PspOMI, PspXI, PsrI(x), RsrII, SfiI, SgrAI, SpeI, SrfI, SwaI, ZraI

(x) = enzyme not available from NEB

We recommend NEBcutter at NEBcutter.com to identify the restriction sites within your DNA sequence. NEBcutter indicates cut frequency and methylation-state sensitivity.

pKLAC2 is an expression vector capable both of replication in *E. coli* and stable integration into the genome of the yeast *Kluyveromyces lactis* (1). It is designed for high-level expression of recombinant protein in *K. lactis* using the *K. lactis* Protein Expression Kit (NEB #E1000). pKLAC2 contains a universal multiple cloning site (MCS) that is compatible with all NEB expression systems.

In *E. coli*, it replicates using the pMB1 origin of replication from pBR322 (although the *rop* gene is missing) and carries the *bla* (Ap^R) marker for selection with ampicillin. Upon transformation of *K. lactis* GG799 competent cells (NEB #C1001), SacII- or BstXI-linearized pKLAC2 integrates into the *K. lactis* chromosome at the *LAC4* locus. Yeast transformants can be selected using the acetamidase selectable marker (*amdS*), which is expressed from the yeast *ADH1* promoter. Acetamidase expressed from pKLAC2 permits transformed cells to utilize acetamide as a sole nitrogen source on defined medium (2).

The multiple cloning site (MCS) is positioned to allow translational fusion of the *K. lactis* α-mating factor secretion domain (α-MF) to the N-terminus of the recombinant target protein. This directs the fusion protein to the general secretory pathway, but the α-MF domain is cleaved off in the Golgi apparatus by the Kex protease, resulting in secretion of the recombinant protein alone.

Expression of the recombinant fusion protein is driven by the *K. lactis LAC4* promoter, which has been modified to be transcriptionally silent in *E. coli* (1). This facilitates the cloning of proteins that are toxic to *E. coli*. This promoter is split such that when pKLAC2 is cleaved with SacII or BstXI, the recombinant protein and selectable marker are flanked by the two halves of the promoter. When these ends recombine with the *LAC4* promoter in the *K. lactis* chromosome, the result is integration of the recombinant fusion protein (driven by the *LAC4* promoter) and *amdS* upstream of the *LAC4* gene (driven by a duplicate copy of the *LAC4* promoter) (2).

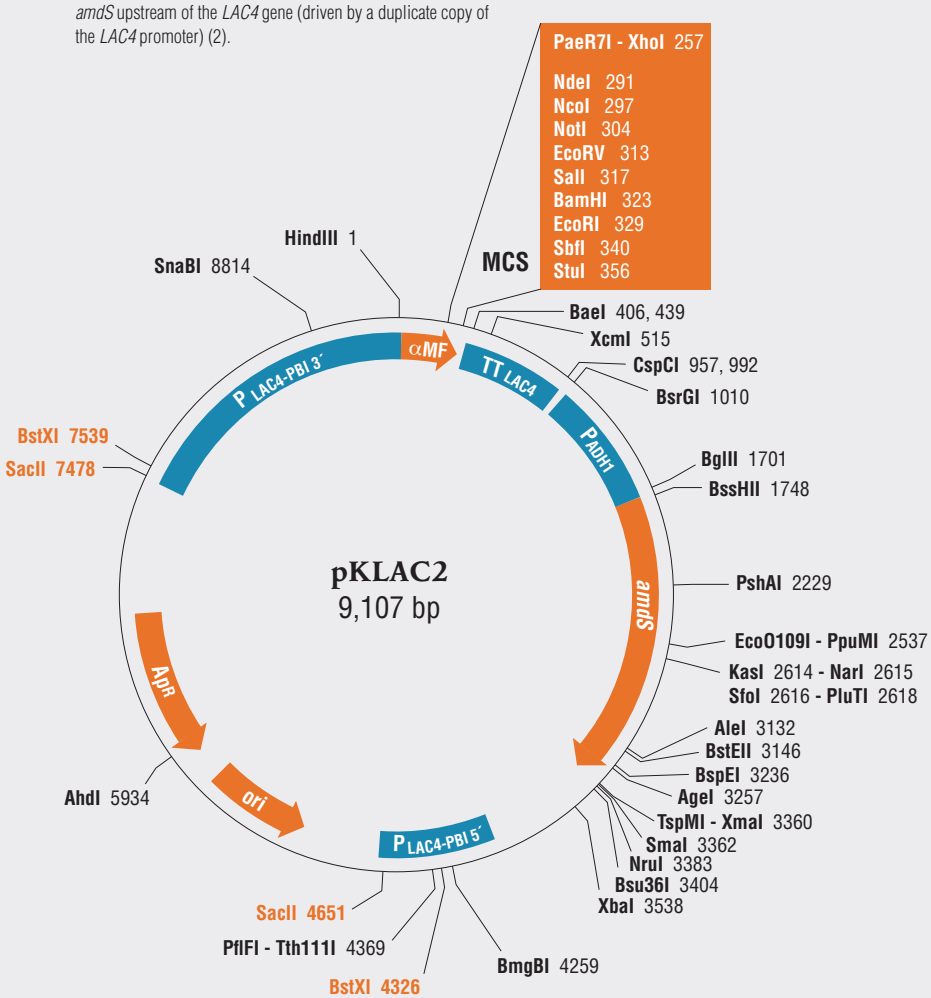
Enzymes with unique restriction sites are shown in **bold** type and selected enzymes with two restriction sites are shown in regular type. **Coordinates indicate position of cutsite on the top strand. In previous catalogs, coordinates referred to the position of the 5´ most base on the top strand, please make note of new numbering system.**

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons. Components of coordinated regions are indented below the region itself.

pMB1 origin of replication coordinates include the region from the –35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. Promoter and transcription terminator coordinates represent cloned regions and not necessarily the precise functional elements.

Feature	Coordinates	Source
expression region:		
α-mating factor		
leader sequence	14-349	*K. lactis*
MCS	257-354	–
LAC4 TT region	371-953	*K. lactis*
AdH1 promoter region	1010-1712	*S. cerevisiae*
amdS	1713-3359	*A. nidulans*
LAC4 promoter		
region (5´ end)	4068-4648	*K. lactis*
origin	5102-5690	pMB1
bla (Ap^R)	6721-5861	Tn3
LAC4 promoter		
region (3´ end)	7475-9107	*K. lactis* (modified)

ori = origin of replication
Ap = ampicillin
TT = transcription terminator



References
(1) Colussi, P.A. and Taron, C.H. (2005) *Appl. Environ. Microbiol.*, 71, 7092–7098.
(2) van Ooyen, A.J. et al. (2006) *FEMS Yeast Res.*, 6, 381–392.