Protein expression using the *K. lactis* Protein Expression Kit - Linearization of pKLAC2 for integrative transformation of *K. lactis*

Overview

pKLAC2 containing any desired gene must be linearized to allow it to insert into the *K. lactis* genome at the *LAC4* locus. This is accomplished by digesting the construct with either SacII (supplied with kit) or BstXI to generate an "expression cassette" consisting of > 6.2 kb of DNA containing $P_{LAC4\text{-}PBI}$, the cloned gene and the *amdS* cassette, and a 2.8 kb fragment containing the remaining pKLAC2 vector DNA. The cloned gene must be free of SacII sites (or BstXI sites if digesting with BstXI) to allow for generation of the proper expression fragment. It is not necessary to purify the expression fragment from the remaining vector DNA following digestion as only the expression fragment will integrate into the *K. lactis* genome upon transformation.

Protocol

1. Digest 2 µg of pKLAC2 DNA containing the gene of interest with 20 units of SacII in 50 µl of 1X rCutSmart™ Buffer (NEB #B6004) (supplied as a 10X stock) at 37°C for 2 hours.*The pKLAC1-malE control vector can be linearized only with SacII due to the presence of a BstXI site in the malE gene.*

2. Desalt digested DNA using a commercially available DNA fragment purification kit (e.g., Monarch® Spin PCR & DNA Cleanup Kit (5 µg) (NEB #T1130). *A total of 1 µg of linearized DNA in a volume less than 15 µl will be needed to transform K. lactis cells. DNA may be stored frozen at -20 °C for up to one month prior to transforming K. lactis cells.*