Co-expression of Multiple Proteins in *Kluyveromyces lactis*

Introduction

Most expression systems utilize a vector that permits expression of a single heterologous gene. However, it is sometimes desirable to over-produce more than one polypeptide in the same cell. For example, co-expression of individual subunits of a multi-subunit protein, or expression of multiple proteins that form a protein complex, are important yet difficult protein expression challenges.

In yeast expression systems that use integrative transformation to insert an expression cassette into the host chromosome, co-expression of multiple genes typically requires either construction of a complex vector containing multiple promoters each driving a different gene of interest, or the longer process of successively introducing different expression vectors into a host cell through iterative rounds of transformation and selection.

Recently, Read *et al.* observed that selection of *K. lactis* cells transformed with pKLAC-series vectors by growth on YCB Agar Medium containing 5 mM acetamide nearly completely enriched transformant populations for cells that had inserted multiple tandem copies of the linear vector into the genome. This phenomenon was exploited to rapidly create *K. lactis* strains that expressed multiple heterologous proteins using a single round of transformation and selection (1). This was accomplished by co-transforming *K. lactis* cells with two or more pKLAC-series vectors, each containing a different heterologous gene (Fig. 1 & 2). Successful expression of up to four secreted proteins was reported using this method (1), although co-expression of more proteins may be possible. This application note describes an easy protocol for constructing *K. lactis* strains that co-express multiple heterologous proteins.

General Protocol

1. Separately digest 2 µg of each pKLAC2 vector containing a gene of interest with 20 units of SacII in 50 µl of 1X NEBuffer 4 (supplied as a 10X stock) at 37°C for 2 hours. For example, to produce two proteins (protein A and protein B), the two constructs pKLAC2-gene-A and pKLAC2-gene-B would each be digested. The procedure can be performed with the pKLAC-, pKLMF- or the pKLCF-series of vectors.

2. Pool the restriction digests and desalt the digested vectors using a commercially available DNA fragment purification kit (e.g., Qiagen's QIAquick™ PCR Purification Kit). Elute in 30 µl deionized water. 1 µg of each linearized vector in a total 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 transformation.

3. Transform *K. lactis* GG799 cells with 15 µl of linearized vector DNA (containing 1 µg of each vector) using the method for "Transformation of *K. lactis* GG799 cells" as described in the *K. Lactis* manual (NEB #E1000). It is important to note that not all transformants will express both proteins. Acetamide selection almost completely enriches transformant populations for multicopy integrants, however, it does not select for each vector individually. Therefore, it is important to screen multiple transformants for co-expression of proteins. In one study, 70–93% of strains transformed with two expression vectors produced both heterologous proteins, and 63% of cells transformed with three vectors produced all three proteins (1).

Materials

- 2 µg each of two or more pKLAC-series vectors (e.g., NEB #N3742) each containing a different gene of interest
- SacII (NEB #R0157)
- NEBuffer 4
- A DNA fragment purification column (e.g., Qiagen QIAquick #28104)
- *K. lactis* GG799 Competent Cells (NEB #C1001) or *K. lactis* Protease Deficient Competent Cells (NEB #C1007)

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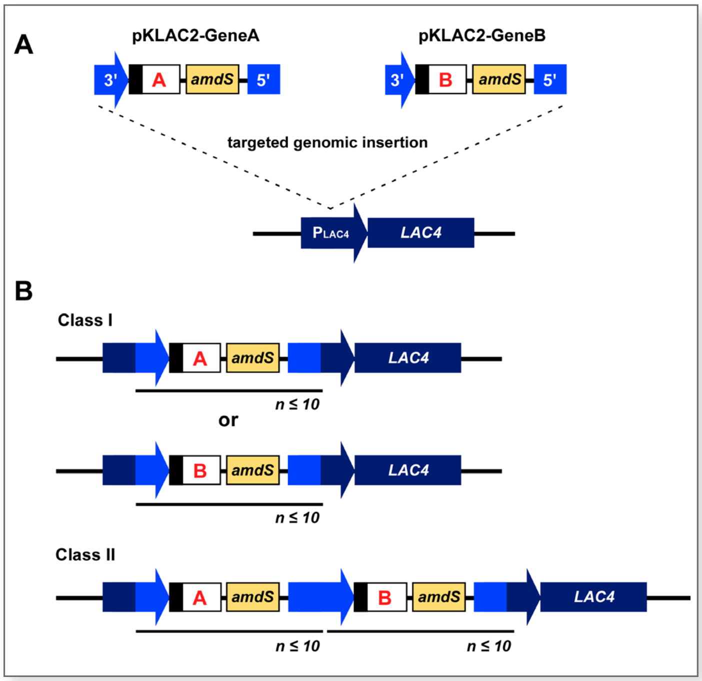
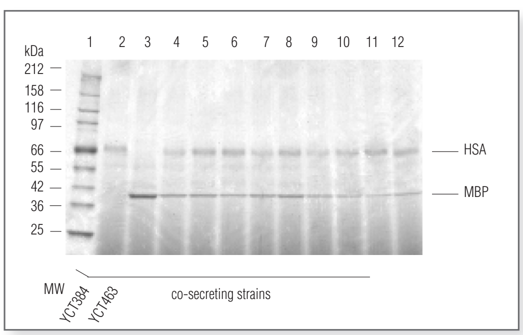
Figure 1: The principle of protein co-expression in *K. lactis*



A. Schematic representation of co-intergration of two expression vectors containing either gene A or gene B (pKLAC2-GeneA and pKLAC2-GeneB). The vectors are each linearized by digestion with SacII, pooled and simultaneously introduced into K. lactis GG799 cells in a single transformation reaction. Internalized fragments are targeted for insertion into the LAC4 promoter locus (dark blue arrow) by sequences homologous to the 5' and 3' ends of the LAC4 promoter region (light blue boxes). The amdS gene permits selection of transformants by enabling their growth on nitrogen-free agar medium (YCB Agar) containing 5 mM acetamide.

B. Example genotypes of transformed strains. Each vector is cable of tandemly integrating into the LAC4 locus up to ~10 times. Class I transformants have 1-10 integrated copies of only pKLAC2-GeneA or pKLAC2-GeneB. Class II transformants have 1-10 integrated copies of both pKLAC2-GeneA and pKLAC2-GeneB, and will be capable of producing both proteins. In one study, Class II isolates represented 70-93% of a transformant population for cells transformed by two pKLAC-series vectors (1).

Figure 2: Co-secretion of Human Serum Albumin (HSA) and Maltose Binding Protein (MBP).



Shown is a Coomassie-stained SDS-polyacrylamide gel with resolution of 13 µL of spent culture medium from growth of nine randomly selected Class II transformants obtained from co-transformation of pKLAC1-HSA and pKLAC1-MBP. Spend medium (13 µl) from cultures of reference strains that produce only HSA (YCT384) and MBP (YCT463) are shown in lanes 2 and 3, respectively. Lane 1 is the Protein Marker, Broad Range (NEB #P7702).

Summary

K. lactis is an efficient system for the simultaneous expression of two or more heterologous proteins. This application note describes a simple technique for constructing strains that co-express multiple proteins using our standard *K. lactis* integrative expression vectors (i.e. pKLAC-, pKLMF- and pKLCF-series vectors) and a single round of strain transformation. The power of this strain construction approach is its simplicity. Co-expressing strains can be created with a single round of cell transformation using the same vectors that would be utilized for expression of either protein alone. However, it is important to remember that this approach exploits the high multicopy insertion frequency of NEB's integrative *K. lactis* expression vectors. As such, co-expressing strains are not specifically selected for, but instead, randomly form in a high percentage of a transformant population. Thus, it is imperative to test several transformants for their ability to co-express the desired proteins to find an optimal strain. Even with this screening step, this method represents a significant time savings over existing techniques for co-expression. More information can be found in reference 1.

References

1. Read, J.D. et al. (2007) *Appl. Environ. Microbiol.*, 73, 5088–5096.

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