Transformation Protocol (C1007)

Introduction

The following steps should be conducted using aseptic technique. Care should be taken to ensure that pipet tips, tubes, solutions and deionized water are sterilized prior to use.

Protocol

. Thaw a tube of *K. lactis* Competent Cells on ice. Add 620 µl NEB Yeast Transformation Reagent to the cells. Briefly shake or invert the tube until the solution is homogeneous. *Do not vortex.*
. Add 1 µg of linearized pKLAC2 DNA containing the gene of interest to the cell mixture. Briefly shake or invert the tube to mix.

Do not vortex. The total volume of transforming DNA should not exceed 15 µl.

. Incubate the mixture at 30°C for 30 minutes.
. Heat shock the cell mixture by incubation at 37°C for 1 hour in a water bath.
. Pellet cells by microcentrifugation at ~7000 r.p.m for 2 minutes and discard the supernatant.
. Resuspend the cell pellet in 1 ml sterile YPGlu medium (see Media & Solutions).
. Pellet cells by microcentrifugation at ~7000 r.p.m for 2 minutes and discard the supernatant.
. Resuspend the cell pellet in 1 ml YPGlu medium (see Media & Solutions) and transfer the cell mixture to a sterile culture tube. Incubate with shaking (250–300 r.p.m.) at 30°C for 3–4 hours.

Incubations shorter than 3 hours are not recommended due to a decline in transformation efficiency.

. Transfer the cell mixture to a sterile 1.5 ml microcentrifuge tube. Pellet the cells by microcentrifugation at ~7000 r.p.m for 2 minutes and discard the supernatant. Resuspend the cell pellet in 1 ml sterile 1X PBS.

0. Remove 10, 50 and 100 µl of the cell suspension to separate fresh sterile 1.5 ml microcentrifuge tubes each containing 50 µl of sterile deionized water. Mix briefly and spread the entire cell mixture from each tube onto separate YCB Agar Medium plates containing 5 mM acetamide (see Media & Solutions). Incubate plates inverted at 30°C for 3–4 days until colonies form.

1. Streak or patch 10–20 individual colonies onto fresh YCB Agar Medium plates containing 5 mM acetamide. Incubate at 30°C for 1–2 days.
   Patches of approximately 1.0 cm^2 are recommended. Plates containing patched cells may be stored at 4°C for up to 3 days prior to performing whole-cell PCR (optional steps 12, 13).
2. [OPTIONAL] Transformants can be tested to verify that they have correctly integrated the expression fragment.
3. [OPTIONAL] Correctly integrated transformants can be further screened to identify cells that have integrated multiple tandem copies of the expression fragment.