

# Transformation Protocol (C1007)

## Introduction

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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

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1. Thaw a tube of *K. lactis* Competent Cells on ice. Add 620  $\mu$ l NEB Yeast Transformation Reagent to the cells. Briefly shake or invert the tube until the solution is homogeneous. *Do not vortex.*
2. Add 1  $\mu$ g 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  $\mu$ l.*

3. Incubate the mixture at 30°C for 30 minutes.
4. Heat shock the cell mixture by incubation at 37°C for 1 hour in a water bath.
5. Pellet cells by microcentrifugation at  $\sim$ 7000 r.p.m for 2 minutes and discard the supernatant.
6. Resuspend the cell pellet in 1 ml sterile YPGlu medium (see Media & Solutions).
7. Pellet cells by microcentrifugation at  $\sim$ 7000 r.p.m for 2 minutes and discard the supernatant.
8. 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.*

9. Transfer the cell mixture to a sterile 1.5 ml microcentrifuge tube. Pellet the cells by microcentrifugation at  $\sim$ 7000 r.p.m for 2 minutes and discard the supernatant. Resuspend the cell pellet in 1 ml sterile 1X PBS.
10. Remove 10, 50 and 100  $\mu$ l of the cell suspension to separate fresh sterile 1.5 ml microcentrifuge tubes each containing 50  $\mu$ l 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.
11. 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<sup>2</sup> 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).*

12. [OPTIONAL] Transformants can be tested to verify that they have correctly integrated the expression fragment.
13. [OPTIONAL] Correctly integrated transformants can be further screened to identify cells that have integrated multiple tandem copies of the expression fragment.