Quick & Easy Yeast Transformation Mix Protocol-At-A-Glance

The **Quick & Easy Yeast Transformation Mix** (Cat. No. 631851) allows you to perform non-library scale transformations in the budding yeast, *S. cerevisiae*, with an easy-to-use and time-saving protocol. The protocol can be completed in less than 1.5 hours.

Protocol: Yeast Transformation

The Quick & Easy Yeast Transformation Mix protocol allows for the transformation of yeast cells grown in liquid culture, or direct transformation of yeast colonies picked from culture plates. You will obtain higher transformation efficiencies with liquid culture than with colonies picked from plates.

1. Collect and resuspend your yeast cells:

From culture plates	From liquid culture
Resuspend a small, freshly plated colony or colony-sized amount of yeast scraped from a culture plate in 300–400 µl sterile water, in a microfuge tube. **NOTE:** You can use cells stored at 4°C for up to one month, although freshly plated cells provide higher transformation efficiencies.	a. Transfer ≥ 5 x 10^7 cells to a microfuge tube. For best results, use a saturated culture grown overnight. b. Centrifuge at 11,000 x g for 15 sec. c. Aspirate the supernatant and resuspend cells in 300–400 µl sterile water.

2. Pellet the resuspended cells by centrifugation.
    a. Centrifuge the cells at 3,000 x g for 3 min. (A slow spin allows for easier resuspension in subsequent steps.)
    b. During centrifugation, prepare the transformation mixture as described in Step 3.
    c. Carefully remove the supernatant by manual pipetting and proceed to Step 4.
3. Prepare a transformation mixture to be added to the cell pellet.
    a. Prepare individual reagents
        - **Denature the Yeastmaker Carrier DNA** at 95°C for 5 min. Place the tube on ice to prevent reannealing.
        - **Thoroughly vortex the Quick & Easy Yeast Transformation Mix tube** before use, as a precipitate may form. If frozen, allow it to thaw, then vortex.
        - **Make sure that the transforming DNA is sufficiently concentrated** so that it can be dispensed in a volume of 1.0–1.5 µl. Using > 1.5 µl of DNA may result in suboptimal transformation efficiency.

        NOTE: The denatured Yeastmaker Carrier DNA can be premixed with the vortexed Quick & Easy Yeast Transformation Mix in the proportions shown in Step 3.b without adding the transforming DNA, and stored at –20°C for up to 6 months. This premix should be thawed (**do not heat**) and vortexed before adding it to the transforming DNA (1.0–1.5 µl) for a total volume of 100 µl per transformation reaction.

    b. Prepare transformation mixture

Vol. Per Rxn	Reagent
93.5–94 µl	Quick & Easy Yeast Transformation Mix
5 µl	Denatured Yeastmaker Carrier DNA
1.0–1.5 µl	Transforming DNA (150–200 ng)
100 µl	**Total volume**

4. Add 100 µl of the transformation mixture from Step 3 to the cell pellet. Vortex for 3 x 1 sec to resuspend.

   NOTE: If the suspension is not homogenous, flick the tube with your finger until the cell pellet is fully resuspended.

5. **Incubate at 45°C** for 65–70 min.

   NOTE: This incubation temperature is critical.

6. Dilute the transformations 10-fold and 100-fold with sterile water.
7. Plate 100 µl of each dilution onto selective media.

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