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Yeast transformation

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 We use this protocol and it's working

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ABSTRACT

This is a protocol for the transformation of yeast cells with linear or circular DNA.

MATERIALS

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⊗ Polyethylene Glycol 3350 **Contributed by users**

⊗ Salmon Sperm DNA Carrier **Thermofisher Catalog #15632011**

⊗ 100ml Lithium acetate [1M] **G-Biosciences Catalog #R039**

⊗ YPD Broth **Thermo Fisher Catalog #A1374501**

Synthetic complete (SC) medium + agar

BEFORE START INSTRUCTIONS

Have all the DNA fragments that will be transformed in high enough concentrations to reach from 0.5-4 µg DNA per transformation.

Have SC plates without the right amino acid that will be used as a selective marker.

1 Make a transformation mix consisting of:

DNA	34 μ L (0.5-4 μ g per fragment)
Salmon sperm DNA 2 mg/mL	50 μ L
PEG-3350	240 μ L
1M LiOAc	36 μ L
MQ water	up to a final volume of 360 μ L

- 2 Centrifuge the 1.5 mL tubes with 100 μ L of yeast competent cells for 2 minutes at 5000x g and remove the supernatant.
- 3 Add 360 μ L of the transformation mix into the 1.5 mL with the cells and vortex thoroughly.
- 4 Heat-shock the solution at 42 °C for 40 minutes.
- 5 Centrifuge the cells for 2 minutes at 5000x g and remove the supernatant.
- 6 Resuspend the cells in 1 mL of YPD medium. Divide the volume into two different 1.5 mL tubes.

- 7** Leave cells to recover for 1.5 hours at 30 °C and 300 rpm.
- 8** Centrifuge cells for 2 minutes at 5000x g and decant supernatant, leaving a bit of the medium inside the tubes.
- 9** Resuspend cells in the resting medium and plate 50 µL onto a SC plate without the amino acid used as a selection marker.
- 10** Incubate the cells for 3 days at 30 °C.