Yeast transformation

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1 Works for me

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ABSTRACT

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

MATERIALS

NAME	CATALOG #	VENDOR
Polyethylene Glycol 3350		
Salmon Sperm DNA Carrier	15632011	Thermofisher
100ml Lithium acetate [1M]	R039	G-Biosciences
YPD Broth	A1374501	Thermo Fisher

MATERIALS TEXT

Synthetic complete (SC) medium + agar

BEFORE STARTING

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

2 Centrifuge the 1.5 mL tubes with 100 µL of yeast competent cells for 2 minutes at 5000x g and remove the supernatant.

3 Add 360 µL of the transformation mix into the 1.5 mL with the cells and vortex trhoughly.

4 Heat-shock the solution at 42 ºC for 40 minutes.

5 Centrifige 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 ºC 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 ºC.