One-Step Transformation of Yeast

Alan Cone

Abstract

Quick and easy method to transform a free plasmid into either budding or fission yeast.

Citation: Alan Cone One-Step Transformation of Yeast. protocols.io

dx.doi.org/10.17504/protocols.io.ddv265

Published: 14 Jul 2015

Before start

Grow at least a 1 mL liquid yeast culture overnight, or plate yeast on minimal medium overnight for fresh yeast.

Materials

- Lithium acetate dihydrate by Contributed by users
- ✓ Polyethylene Glycol 3350 by Contributed by users

Protocol

Step 1.

Add 90 uL of 50% Polyethylene Glycol 3350 to a 1.5 mL microcentrifuge tube.

■ AMOUNT

90 µl Additional info:



✓ Polyethylene Glycol 3350 by Contributed by users.

Step 2.

Add 10 uL of 0.2 N Lithium Acetate to the microcentrifuge tube.

AMOUNT

10 µl Additional info:



Lithium acetate dihydrate by Contributed by users

₽ NOTES

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In this case, 0.2 N Lithium Acetate and 0.1 M Lithium Acetate are the same thing.

Step 3.

Pellet yeast from 1 mL liquid culture or take a toothpick full of freshly grown yeast on a plate and resuspend in the solution.

Step 4.

Add 1 ug of DNA and briefly vortex.

■ AMOUNT

1 μg Additional info:

Step 5.

Incubate at 45°C for 30 minutes to 1 hour, vortexing briefly every 10 minutes.

© DURATION

01:00:00

NOTES

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I have found that usually 30 minutes is just fine. Some people swear that longer is better though.

Step 6.

Add 1 mL water to the tube and pipette to mix, then centrifuge at 14,000 RPM for 15 seconds.

AMOUNT

1 ml Additional info:



REAGENTS

Nuclease-free Water by Contributed by users

O DURATION

00:00:15

Step 7.

Remove the supernatant and resuspend the pellet in 200 uL water, then spread on selective plates and incubate at 30°C for 3-6 days.

AMOUNT

200 µl Additional info:



✓ Nuclease-free Water by Contributed by users

NOTES

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Budding yeast should show colonies in 3-4 days while fission yeast may take 4-6. This difference has to do with the type of medium classified as minimal medium for each organism, along with fission yeast having a longer generation time.