Rapid Yeast Transformation

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Abstract

This protocol is our lab's version of the standard lithium acetate-based transformation protocol originally developed by the Gietz lab (see eg Gietz and Schiestl 2007).

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Before start

Before you start, you will need:

- A suitable yeast strain to be transformed
- A suitable plasmid, with a yeast origin of replication (centromeric or 2µ) and a selectable marker that matches the genome of the yeast strain you are transforming (eg a *URA3* marker if this gene is non-functioal in your strain's genome). Typical minipreps, which give yields of 50 ng/µl or higher, are suitable.
- Suitable selective plates that match the selectable marker on the plasmid (eg standard dropout plates lacking uracil for a *URA3* marker plasmid)
- Suitable medium for growing the non-transformed yeast strain (normally YPD medium, ie 2% glucose, 1% Yeast extract, 2% peptone in water).

Materials

PEG-4000 81240 by Sigma Aldrich

Lithium Acetate Dihydrate L4158 by Sigma Aldrich

2-mercaptoethanol M6250 by Sigma Aldrich

Protocol

Step 1.

Inocculate an over night culture with the yeast strain to be transformed. The medium for this culture can be standard YPD, or if the strain already contains plasmids that need to be selected for, a defined medium. Per planned transformation reaction, 0.7 to 1 ml of culture are required. Place the culture in a 30°C incubator over night (other temperatures are fine for ts strains).

Step 2.

Spin down the cells from 0.7 of over night culture in a microcentrifuge (1 minute, max speed). This volume is fine for vigorously gowing strains like BY4741 in YPD, but higher volumes may be better for slow growing strains. After centrifugation, remove the medium as completely as possible using a pipette.

Step 3.

Pipet the following reagents on top of the yeast cell pellet, in this order:

1. 240 µl of 50% PEG 4000 in water
2. 36 µl of 1 M lithium acetate
3. 10 µl of 1 mg/ml single stranded DNA (eg fish sperm DNA)
4. 2.5 µl of 2-mercaptoethanol
5. 2 µl of the plasmid to be transformed
6. 69.5 µl water to give a total volume of 360 µl

Step 4.

Vortex the mixture for 1 minute. The yeast cell pellet should be well dispersed and no cells should remain at the bottom of the tube.

Step 5.

Incubate the transformation mixture at room temperaure for 20 minutes, then at 42°C for 20 minutes.

NOTES
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For temperature sensitive strains, a single incubation at room temperature over night can give improved yields.

Step 6.

Spin down the cells by centifugation in a microcentrifuge, at 2000 rpm, for 2 minutes. Remove the supernatant using a micropipette.

Step 7.

Resuspend the cells in 200 µl sterile water, by slowly pipetting up and down with a blue pipette tip (the narrower opening of yellow tips can shear the yeast cells which can be/ fragile after the LiAc

treatment). Pipet the resuspended cells into the center of a suitable selective plate, and spread the cells evenly over the plate using a sterile plastic or glass spreader. Incubate the plates for 2 to 4 days at 30°C.

Warnings

2-mercaptoethanol must be handled in a fume hood.