Lithium acetate transformation of yeast

Maitreya Dunham August 2004
Original protocol from Katja Schwartz

Digest plasmid DNA so you cut in a region of homology, leaving at least a couple hundred basepairs of homology on both sides.
Gel purify it if desired.
Check concentration.

For a difficult transformation, you will need ~10 µg DNA. For an easier transformation, you can use less.

Maintain sterility throughout. Whenever shaking is called for, use the lowest setting that allows for complete mixing.
Inoculate 2 ml YPD with a fresh colony.
Grow overnight 30°C.
Inoculate 50 ml YPD with 200 µl of the overnight.
Monitor the growth until the OD600 is around 0.7-0.8 (~7 hours).
Spin down the cells.
Resuspend in 5 ml lithium acetate mix. Spin.
Resuspend in 0.5 ml lithium acetate mix. Transfer to an eppendorf tube.
Incubate 60 minutes at room temperature on the orbital shaker.

Mix:

10 µl	10 mg/ml boiled sheared salmon sperm DNA
~10 µl	~5-10 µg DNA (also set up a –DNA control)
200 µl	cells

Incubate 30 min at room temperature on the orbital shaker.
Add 1 ml PEG mix.
Incubate 30 min at room temperature on the orbital shaker.
Incubate 10 min 42°C.
Spin down.
Remove ALL of the supernatant with a pipet.
Resuspend the cells in 200 µl TE and plate to selective media.
For drug selections, you may want to outgrow first.
Once transformants appear, colony purify them.
Check the integration by PCR with one flanking region primer and one internal primer, or with flanking primers that give different size products.

Lithium acetate mix

10 ml	10X TE
10 ml	1 M lithium acetate
80 ml	water

Filter sterilize.

PEG mix

8 ml	50% PEG 3500
1 ml	10X TE
1 ml	1 M lithium acetate

Filter sterilize.

10X TE

10 ml	1 M Tris pH 7.5
2 ml	0.5 M EDTA pH 8
88 ml	water

Filter sterilize.