电转法酵母转化*

Materials

1 M dithiothreitol (DTT; filter sterilize and store at −20°C)
1 M sorbitol
Sorbitol selection plates
Gene Pulser with Pulse Controller (Bio-Rad) *or* Cell-Porator (GIBCO/BRL)
0.2-cm-gap disposable electroporation cuvettes (Bio-Rad) *or* 0.15-cm-gap microelectroporation chambers (GIBCO/BRL); ice-cold

Grow and harvest the yeast cells

1. Two days before the experiment, inoculate 5 ml of YPD medium with a single yeast colony of the strain to be transformed. Grow overnight to saturation at 30°C.

The saturated overnight culture may be prepared up to several weeks in advance of the transformation and stored at 4°C.

2. The night before transformation, inoculate a 2-liter sterile flask containing 500 ml YPD with an appropriate amount of the saturated culture and grow overnight with vigorous shaking at 30°C to 1 × 10^8 cells/ml (OD_{600} ~ 1.3 to 1.5, depending on strain).

This cell density is achieved in mid- to late-log phase. It is often difficult to know how much inoculum from the overnight culture will produce a cell density of 1 × 10^8 cells/ml at a reasonable time the next day. If the exact inoculum for a given strain is not known, inoculate three independent flasks with varying amounts of the saturated culture as described in step 2 of the basic protocol.

3. Harvest culture by centrifuging at 4000 × *g* (5000 rpm) 4°C, and resuspend vigorously in 80 ml sterile H_2O. To increase electrocompetence of the cells, proceed to step 4. If this treatment is not required, proceed to step 7.

The rotor, the exact speed, and the duration of centrifuge spins are not critical; the principal consideration is to centrifuge sufficiently hard (usually ~4000 × g) to pellet all of the yeast, but not so hard as to make resuspension difficult. For large volumes, 5000 rpm in a Sorvall GSA rotor will suffice; for smaller volumes, 7000 rpm in a Sorvall SS-34 rotor is recommended. This and subsequent centrifugation steps should be conducted at 4°C.

Treat with lithium acetate and DTT to increase electrocompetence

This treatment is optional. It increases the handling time in preparing the yeast and should not be performed if the cells are to be frozen for subsequent use.

4. Add 10 ml of 10× TE buffer, pH 7.5. Swirl to mix.

5. Add 10 ml of 10× lithium acetate stock solution. Swirl to mix. Shake gently 45 min at 30°C.

6. Add 2.5 ml of 1 M DTT while swirling. Shake gently 15 min at 30°C.

This treatment increases the transformation efficiency >5-fold. Both lithium acetate and DTT, if used individually, will increase transformation efficiency. Used together, the improvement is approximately additive.

Concentrate and wash the yeast cells

7. Dilute yeast suspension to 500 ml with water.

8. Wash and concentrate the cells three times by centrifuging at 4000 to 6000 × *g*, resuspending the successive pellets as follows:

First pellet—250 ml ice-cold water

Second pellet—20 to 30 ml ice-cold 1 M sorbitol

Third pellet—0.5 ml ice-cold 1 M sorbitol.

Resuspension should be vigorous enough to completely dissociate each pellet. The final volume of resuspended yeast should be 1.0 to 1.5 ml and the final OD_{600} should be ~200. Yeast can be stored at −70°C for subsequent use by addition of glycerol to 15% (v/v) followed by freezing in a dry ice/ethanol bath. Frozen aliquots should be thawed slowly, pelleted in a sterile microcentrifuge tube, and resuspended to the same volume in 1 M sorbitol. The wash is necessary to remove ions liberated by lysis of some yeast cells. Transformation efficiency will drop ≥10-fold with freezing.

Electroporate the yeast cells

Using the Bio-Rad Gene Pulser:

9a. In a sterile, ice-cold 1.5-ml microcentrifuge tube, mix 40 µl concentrated yeast cells with ≤100 ng transforming DNA contained in ≤5 µl.

Transforming DNA should be in a low-ionic-strength buffer such as TE or in sterile high-quality water (see reagents and solutions). There is no required length of incubation; this time can be varied to convenience. Do not include carrier DNA in this procedure; it drastically reduces transformation efficiency. Maximal efficiency (transformants/g) will be obtained with <10 ng of transforming DNA, while the largest number of transformants will be obtained with 100 ng. Using >100 ng will cause a precipitous drop in both transformation efficiency and the total number of transformants, with few colonies evident in transformation with 1 g DNA.

10a. Transfer to an ice-cold 0.2-cm-gap disposable electroporation cuvette.

11a. Pulse at 1.5 kV, 25 µF, 200 Ω. It is crucial that the Bio-Rad Pulse Controller be included in the circuit; failure to do so will result in damage to the Gene Pulser.

The time constant reported by the Gene Pulser will vary from 4.2 to 4.9 msec. Times <4 msec or the presence of a current arc (evidenced by a spark and smoke) indicate that the conductance of the yeast/DNA mixture is too high.

12a. Add 1 ml ice-cold 1 M sorbitol to the cuvette and recover the yeast, with gentle mixing, using a sterile 9-inch Pasteur pipet.

13a. Spread aliquots of the yeast suspension directly on sorbitol selection plates. Incubate at 30°C until colonies appear.

Although there is a conflicting report (Lorow-Murray and Jessee, 1991), we find that the addition of sorbitol as an osmotic protectant to the agar increases survival of transformed yeast, yielding 10-fold higher transformation efficiency (Becker and Guarente, 1991). Transformants will be visible on the agar surface 3 to 6 days after transformation, depending on the strain, the plasmid, and the selection. This is usually 1 day longer than with the lithium acetate procedure.

Using the BRL Cell-Porator:

9b. In a sterile, ice-cold 1.5-ml microcentrifuge tube, mix 20 µl concentrated yeast with ≤100 ng transforming DNA contained in ≤5 µl.

Incubation time can be varied for convenience (step 9a).

10b. Transfer to an ice-cold, 0.15-cm-gap micro-electroporation chamber.

11b. Pulse at 400V, 10 µF, low resistance.

12b. Remove 10 µl of electroporated mixture to a sterile 1.5-ml microcentrifuge tube containing 0.5 ml ice-cold 1 M sorbitol.

13b. Spread aliquots of the yeast suspension directly on sorbitol selection plates. Incubate at 30°C until colonies appear.

REAGENTS AND SOLUTIONS

10× lithium acetate stock solution:

1 M lithium acetate, pH 7.5 (adjust PH with dilute acetic acid), filter sterilized

*:出自 *Current Protocols in Molecular Biology* [B], *Wiley Online Library*,2007