HIGH EFFICIENCY TRANSFORMATION

Gietz, R.D. & R.A. Woods. (2002) TRANSFORMATION OF YEAST BY THE Liac/SS CARRIER DNA/PEG METHOD. Methods in Enzymology 350: 87-96**.**

Matériel et Solution :

- Liquid and plate media
- 0.1M LiOAc
- ssDNA
- PEG 3500 50% w/v
- H_2O nano sterile

Méthode :

1. From a culture 10 ml of cells, dilute at 5.10^6 cell/ml in 50ml for PCR fragment or 25mL for plasmid of YPD, stay 4h at 30˚C.

2. When the cell titer is at least 2 x 10^7 cells/ml, harvest 50 ml of cells by centrifugation at 3000 *g* for 5 min (Beckman Coulter Avanti J-20 XP centrifuge, rotor JS-5.3, 3513 rpm).

3. Wash the cells in 25 ml of sterile water + 10 ml of 0.1M LiOAc, centrifuge and resuspend the cell in 1 ml of 0.1M LiOAc.

4. Boil a XX ml sample of ssDNA for 10 min and chill in an ice/water bath while harvesting the cells.

5. Transfer the cell suspension to a 1.5 ml microcentrifuge tube, centrifuge at 10,000 rpm for 30 sec and discard the supernatant.

6. Add 0.1M LiOAc to a final volume of 1.0 ml for PCR fragment or 500 µl for plasmid (Eppendorf mark of 1 ml) and mix to resuspend cells.

7. Pipette 100 µl samples for PCR fragment or 50 µl for plasmid (ie. 1 x 10^8 cells) into 1.5 ml microfuge tubes, one for each transformation, centrifuge at 10,000 rpm for 30 sec and remove the supernatant.

8. Make up sufficient *Transformation Mix* for the planned number of transformations.

TRANSFORMATION MIX	1x	10x
PEG 3500 50% w/v	240 µl	2400 µl
LiOAc 1.0 M	36 µl	360 µl
Boiled ssDNA	50 µl	500 µl
DNA & Water (DNA: 3µg min; Plasmid: 500-100ng)	34 µl	---
TOTAL	360 µl	3600 µl
		326 µl/tube

9. Add 326 µl of *Transformation Mix* to each transformation tube containing 34 µl of DNA. Use this mixture to resuspend the cells up and down with pipette (not forget H_2O as negative control). Use 10 µl of PCR + 24 µl of water.

10. Incubate the tubes in a 30°C water bath for 45 min.

11. Incubate the tubes in a 42°C water bath for 30 min.

12. Microcentrifuge at 10,000 rpm for 30 sec and remove the Transformation Mix with a micropipettor.

13. Add 600 µl of H_2O into each tube; stir the pellet with a blue tip (We like to be a gentle as possible at this step if high efficiency is important. Excessive washing washes away transformants).

14. Plate 100, 200 and what's left µl (PCR fragment) or 50, 350 and what's left (plasmid) of the cell suspension onto plate with appropriate media.

15. Incubate the plates at 30°C for 3 days.

Conseil:

Avec 50 ml de culture pour un fragment de PCR ou bien 25ml de culture pour un plasmide on peut faire 10 transformations.