Plasmid Transformation

Introduction

MATΔ::His3 STE4Δ::TRP1 yeast strain derived from SEYa or SEYα are transformed with OLP003 or OLP004. A heat shock time of 1 h increases transformation efficiency for SEY derived strains when compared to 20 min heat shock.

Materials

- YPAD
- 50% PEG
- 1 M LiAc
- 2mg/ml ssDNA
- OLP003
- OLP004
- Sterile H20
- Water bath or heating block at 42°C
- Disposable 17x100 mm culture tubes with closures (VWR Catalog 60818-725)
- SC-Leu plates
- SC-Ura plates

Procedure

Competent cells

Day 1

1. Overnight culture (ONC): Inoculate 2 mL of YPAD with a single yeast colony and incubate culture on the shaker at 30°C/200 rpm/overnight.

Day 2

2. Inoculate 5 mL of YPAD in disposable 10 mL culture tubes with 400 µL of ONC and grow it on the shaker at 30°C/200 rpm/4 h

   This will make 2 x 1 mL competent cells.

3. Set water bath or heating block to 42°C.

4. Indicate number of transformations and plasmid concentration:

Settings

	# of Transformations	OLP003 [ng/µL]	OLP004 [ng/µL]
1	24	610	550

5. If the carrier DNA has not been denatured yet, denature the carrier DNA in heating block at 95°C for 5 min and chill immediately on ice before use. If carrier DNA has already been denatured then thaw it on ice.

6. Prepare 0.1 M LiAc, freshly diluted from 1 M LiAc:

0.1 M Li Ac

	Materials	mL
1		26 X [mL]
2	1 M LiAc	5.2
3	sterile H2O	46.8
4	**Total**	**52**

7. Centrifuge 3,000 g/5 min; discard supernatant and resuspend cell pellet in 2 mL sterile H_2O.

8. Centrifuge 3,000 g/5 min. Discard supernatant and resuspend cells in 2 mL of 0.1 M LiAc. Split into 2 x 1 mL in microcentrifuge tubes (transform one with OLP003 and one with OLP004 below).

9. Centrifuge 3,000 g/5 min (use 7,500 rpm in microcentrifuge); discard supernatant.

10. Prepare one transformation master mix for each plasmid by adding reagents in order (pipetting 50% PEG slowly), two additional reactions are included to account for pipetting discrepancies:

Transformation master mix

	A	C	C
1		26 X OLP003 [µL]	26 X OLP004 [µL]
2	50% PEG	6240	6240
3	1 M LiAc	936	936
4	ssDNA (2 mg/ml)	1300	1300
5	Plasmid	42.6	47.3
6	ddH2O	841.4	836.7
7	**Total**	**9360**	**9360**

11. Add 360 µL of transformation master mix to each tube.

12. Vortex tubes until the cells are smoothly suspended.

13. Heat shock cells in water-bath or heat-block: 42°C/1 h.

01:00:00

14. Centrifuge 3,000 g/5 min (use 7,500 rpm in microcentrifuge); discard supernatant and resuspend each cell pellet in 100 µL sterile H_2O, then plate cells on SC-Leu for OLP003 transformations or SC-Ura for OLP004 transformations.

Additional resources

Plasmid amount pe...

	Plasmid [ng]
1	1000

Transformation single reaction

	A	B	C
1		OLP003 [µL]	OLP004 [µL]
2	50% PEG	240	240
3	1 M LiAc	36	36
4	2mg/ml ssDNA	50	50
5	PCR product	1.6	1.8
6	dHd2O	32.4	32.2
7	**Total**	**360**	**360**