ste4 KO Transformation

Introduction

Yeast strain derived from SEYa or SEYα are transformed with ste4 KO PCR product to replace ste4 with TRP1 and render the strain sterile. 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
- ste4 KO PCR product (at least 30 ng/µL)
- Sterile H_2O
- Water bath or heating block at 42°C
- Disposable 17x100 mm culture tubes with closures (VWR Catalog # 60818-725)
- SC-Trp 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 2.5 mL of YPAD in disposable 10 mL culture tubes with 200 µL of ONC and grow it on the shaker at 30°C/200 rpm/4 h.

04:00:00

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

4. Indicate number of transformations and PCR product concentration:

Settings

	# of Transformations	ste4 KO PCR product [ng/µL]
1	22	100

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		24 X [mL]
2	1 M LiAc	2.4
3	ddH2O	21.6
4	**Total**	**24**

7. Centrifuge 3,000 g/5 min; discard supernatant and resuspend cell pellet in 1 mL sterile H_2O.

8. Centrifuge 3,000 g/5 min; discard supernatant and resuspend cells in 1 mL of 0.1 M LiAc.

9. Centrifuge 3,000 g/5 min; discard supernatant.

Transformation

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

Transformation master mix

	A	B
1		24 X [µL]
2	50% PEG	5760
3	1 M LiAc	864
4	ssDNA (2 mg/ml)	1200
5	ste4 KO PCR product	240
6	ddH2O	576
7	**Total**	**8640**

11. Add 360 µL of transformation master mix to each tube and immediately vortex until the cells are smoothly suspended.

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

01:00:00

13. Centrifuge 3,000 g/5 min; discard supernatant.

14. Resuspend each cell pellet in 100 µL sterile H_2O, then plate cells on the SC-Trp.

Additional resources

PCR product amou...

	KO PCR product [ng]
1	1000

Transformation single reaction

	A	B
1		[µL]
2	50% PEG	240
3	1 M LiAc	36
4	2mg/ml ssDNA	50
5	PCR product	10
6	ddH2O	24
7	**Total**	**360**