ste4 KO Transformation

Introduction

Yeast strain derived from SEYa or SEY α 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 H₂O
- > 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 μ L 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:

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

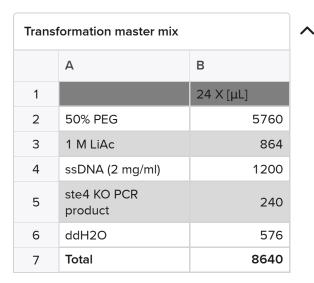
- 5. If the carrier DNA has not been denatured yet, denature the carrier DNA in heating block at 95°C for 5 min and chill immediately on ice before use. If carrier DNA has already been denatured then thaw it on ice.
- 6. Prepare 0.1 M LiAc, freshly diluted from 1 M LiAc:

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:



- 11. Add 360 μ L 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.

- 13. Centrifuge 3,000 g/5 min; discard supernatant.
- 14. Resuspend each cell pellet in 100 μL sterile $\text{H}_2\text{O},$ then plate cells on the SC-Trp.

Additional resources



Transformation single reaction				
	Α	В		
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		