# **MAT KO Transformation**

## Introduction

ste $4\Delta$ ::TRP1 yeast strain derived from SEYa or SEYa are transformed with MAT KO PCR product to replace MATa or MATa with His3. 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
- > MAT KO PCR product (at least 30 ng/µL)
- > Sterile H20
- > Water bath or heating block at 42°C
- > Disposable 17x100 mm culture tubes with closures (VWR Catalog # 60818-725)
- > SC-His-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  $\mu$ 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:

Settings				
	# of Transformations	MAT KO PCR product [ng/µL]		
1	24	100		

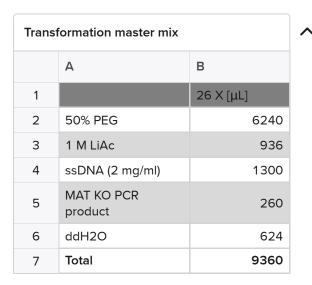
- 5. If the carrier DNA has not been denatured yet, denature the carrier DNA in heating block at 95°C for 5 min and chill immediately on ice before use. If carrier DNA has already been denatured then thaw it on ice.
- 6. Prepare 0.1 M LiAc, freshly diluted from 1 M LiAc:

0.1 M Li Ac			
	Materials	mL	
1		26 X [mL]	
2	1 M LiAc	2.6	
3	ddH2O	23.4	
4	Total	26	

- 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  $\mu$ 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 and resuspend each cell pellet in 100  $\mu$ L sterile H<sub>2</sub>O, then plate cells on the SC-His-Trp.

# Additional resources

PCR p	^	
	KO PCR prod- uct [ng]	
1	1000	

Transformation single reaction					
	А	В			
1		[μL]			
2	50% PEG	240			
3	1 M LiAc	36			
4	2mg/ml ssDNA	50			
5	PCR product	10.0			
6	dHd2O	24.0			
7	Total	360			