# 2-3 MAT/ste4 KO of MA strains set 2

**MONDAY, 2019-01-07**

The workflow assumes that the strains in question were derived from SEYa or SEYα, are haploid and do not carry HIS3 or TRP1 selection markers. The protocol can be modified to use other selection markers for knock-out if necessary. The protocol can also be adapted for other parental strains than SEYa or SEYα by optimizing the transformation protocol parameters.

First, a double knock-out of the strains of interest results in a MATΔ::His3 and ste4Δ::TRP1 genotype (ste4 is first knocked-out to render the strain sterile and prevent uncontrolled selfing of a MATα strain in the presence of a MATΔ::His3 strain during MAT knockout). Each knock-out strain is then selfed.

- [x] Enter batch of yeast strains being processed below [NOTE: these are new codes for this procedure, not MA line codes].

List of yeast strains:
25–48

## ste4 knock-out

- [x] Patch each strain on grid on YPG plate [NOTE: used frozen strains from Aliquot 2 of MA lines].
- [x] Prepare STE4 KO PCR product (see 2-2 ste4 KO PCR product for double KO ).

**TUESDAY, 2019-01-08**

- [x] Streak-out to single colonies on YPAD plate.

**THURSDAY, 2019-01-10**

- [x] Inoculate overnight culture (see ste4 KO Transformation protocol day 1).

**FRIDAY, 2019-01-11**

- [x] Prepare 1 mL competent cells (see ste4 KO Transformation protocol day 2).
- [x] Transform with 1 µg ste4 KO PCR product and plate on SC-Trp (see ste4 KO Transformation protocol day 2).

**MONDAY, 2019-01-14**

Received lawn on all plates and no single colonies. Checked the YPAD bottle and it showed some growth, i.e. was contaminated. Repeat transformation.

**MONDAY, 2019-01-21**

- [x] Inoculate overnight culture (see ste4 KO Transformation.2 protocol day 1).

**TUESDAY, 2019-01-22**

- [x] Prepare 1 mL competent cells (see ste4 KO Transformation protocol day 2).
- [x] Transform with 1 µg ste4 KO PCR product and plate on SC-Trp (see ste4 KO Transformation.2 protocol day 2).

**FRIDAY, 2019-01-25**

- [x] Streak-out a single transformant to single colonies on SC-Trp plate.

**MONDAY, 2019-01-28**

- [x] Confirm genotype of single colony (see ste4 colony PCR protocol).

**TUESDAY, 2019-01-29**

- [x] Run gel (see ste4 colony PCR protocol). Christina Hsu

wt signal in NTC, likely due to contamination, unclear if PCR master mix was affected. Christina is going to discard all reagent she has been using (except for Taq) and repeat colony PCR.

**FRIDAY, 2019-02-01**

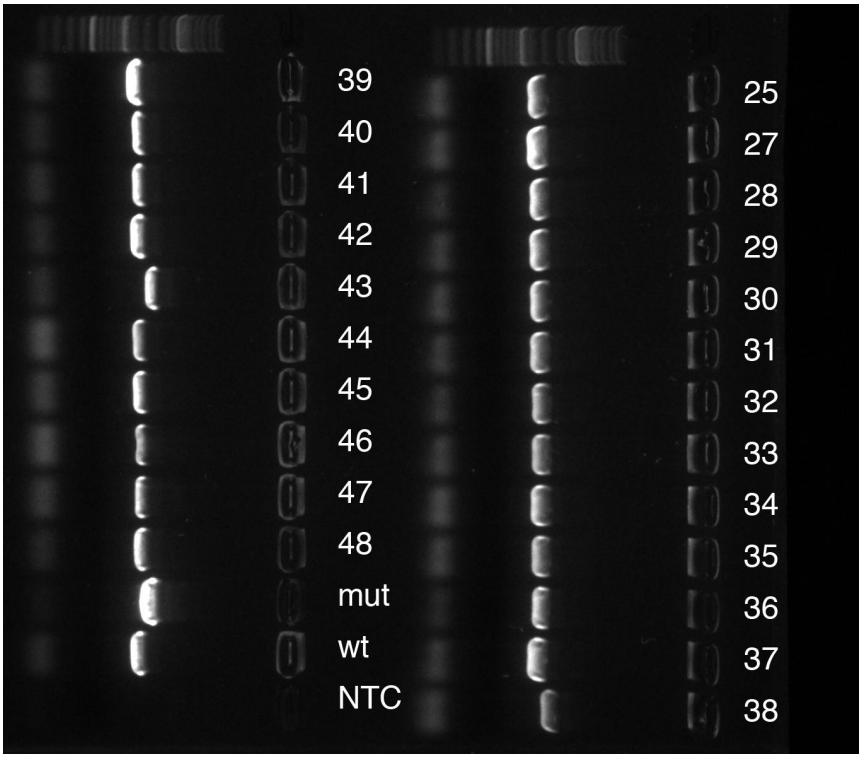
- [x] Confirm genotype of single colony from the same quadrant (quadrant 1) (see ste4 colony PCR.2 protocol). Christina Hsu

Again wt signal in NTC, likely PCR master mix contamination.

**TUESDAY, 2019-02-05**

- [x] Repeat: Confirm genotype of single colony.1 (see ste4 colony PCR.3 protocol).

190205_2-3_STE4_Colony_PCR.3_Set_2.jpg



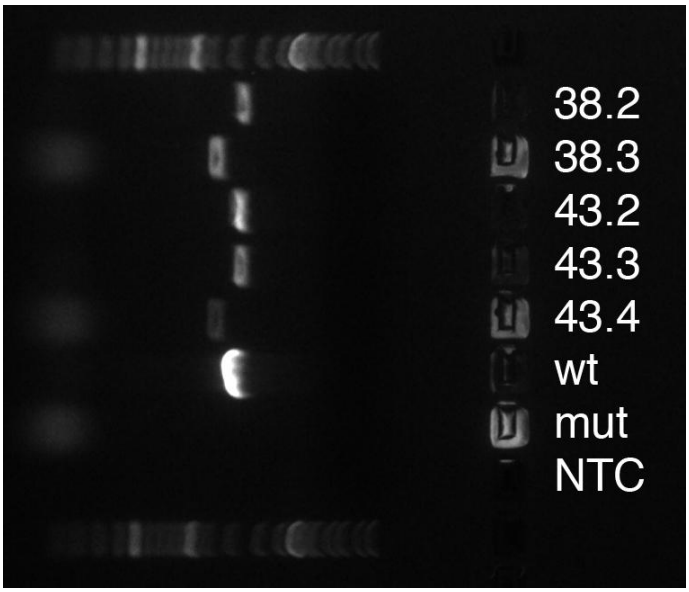

2 strains are ste4 wt: #38 and 43.
All other strains are ste4Δ::TRP1 (see table below).

**WEDNESDAY, 2019-02-06**

- [x] Confirm genotype of single colony .2, .3 of strain #38 and .2, .3, and .4 of #43 using 10 µL reaction volume (see ste4 colony PCR.4 protocol). First PCR done by Matt!

**THURSDAY, 2019-02-07**

190207_2-3_STE4_Colony_PCR.4_Set_2.jpg




3 strains are ste4 wt: #38.2, 43.2, and 43.3.

2 strains are ste4Δ::TRP1: #38.3 and 43.4.

**THURSDAY, 2019-02-28**

- [x] Make backup plate and consolidate with ste4Δ::TRP1 strain of 26 from 2-9 MAT/ste4 KO of MA strains set 6.
- [x] If ste4Δ::TRP1 genotype is confirmed, proceed with MAT knockout; otherwise repeat streak-out and colony PCR with another transformant.

ste4Δ::TRP1

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Strain # | colony .1 | colony .2 | colony .3 | colony .4 |
| 2 | 25 | TRUE | | | |
| 3 | 26 | NA | | | |
| 4 | 27 | TRUE | | | |
| 5 | 28 | TRUE | | | |
| 6 | 29 | TRUE | | | |
| 7 | 30 | TRUE | | | |
| 8 | 31 | TRUE | | | |
| 9 | 32 | TRUE | | | |
| 10 | 33 | TRUE | | | |
| 11 | 34 | TRUE | | | |
| 12 | 35 | TRUE | | | |
| 13 | 36 | TRUE | | | |
| 14 | 37 | TRUE | | | |
| 15 | 38 | FALSE | FALSE | TRUE | |
| 16 | 39 | TRUE | | | |
| 17 | 40 | TRUE | | | |
| 18 | 41 | TRUE | | | |
| 19 | 42 | TRUE | | | |
| 20 | 43 | FALSE | FALSE | FALSE | TRUE |
| 21 | 44 | TRUE | | | |
| 22 | 45 | TRUE | | | |
| 23 | 46 | TRUE | | | |
| 24 | 47 | TRUE | | | |
| 25 | 48 | TRUE | | | |

# MAT knock-out

**WEDNESDAY, 2019-03-06**

- [x] Inoculate overnight culture (see MAT KO Transformation day 1).
- [x] Prepare MAT KO PCR product (see 2-13 MAT KO PCR product for double KO ).

**THURSDAY, 2019-03-07**

- [x] Prepare 1 mL competent cells (see MAT KO Transformation day 2).
- [x] Transform with 1 µg MAT KO PCR product and plate on SC-His-Trp (see MAT KO Transformation day 2).

**SUNDAY, 2019-03-10**

28, 30, 33, 34, 40, 42 had single colonies, all other strains grew as a lawn.

- [x] Streak-out 4 transformants/strain to single colonies on a single SC-His-Trp plate for strains 28, 30, 33, 34, 40, 42. Linnea Sandell

**MONDAY, 2019-03-11**

Traced back cause of lawn: Plates with single colonies were from one batch (prepared by Jocelyn), plates with lawn were from another batch (prepared by Christina). Repeat transformation for missing strains using newly prepared plates.

**WEDNESDAY, 2019-03-13**

- [x] Inoculate overnight culture for strains 25, 26, 27, 28, 29, 31, 32, 35, 36, 37, 38, 39, 41, 43, 44, 45, 46, 47, 48 (see MAT KO Transformation.2 day 1, also include #28 because so far only a single colony was received). Linnea Sandell

**THURSDAY, 2019-03-14**

- [x] Prepare 1 mL competent cells (see MAT KO Transformation.2 day 2).
- [x] Transform with 1 µg MAT KO PCR product and plate on SC-His-Trp (see MAT KO Transformation.2 day 2).

**MONDAY, 2019-03-18**

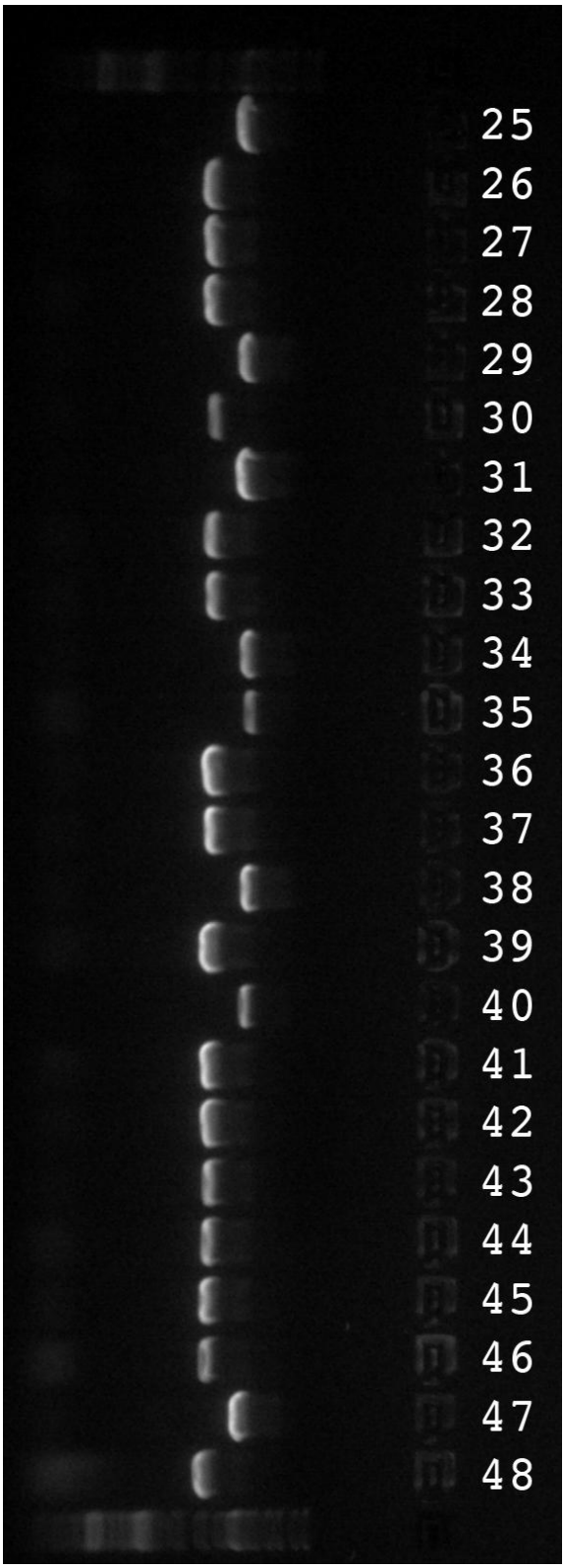
- [x] Streak-out 4 transformants/strain to single colonies on a single SC-His-Trp plate for strains 28, 30, 33, 34, 40, 42.

**WEDNESDAY, 2019-03-20**

- [x] Confirm genotype of single colony (see MAT Colony PCR), together with samples from 2-4 MAT/ste4 KO of MA strains set 3 and 2-6 MAT/ste4 KO of MA strains set 5 (2-6 has controls).

**FRIDAY, 2019-03-22**

190322_2-3_MAT_Colony_PCR.jpg



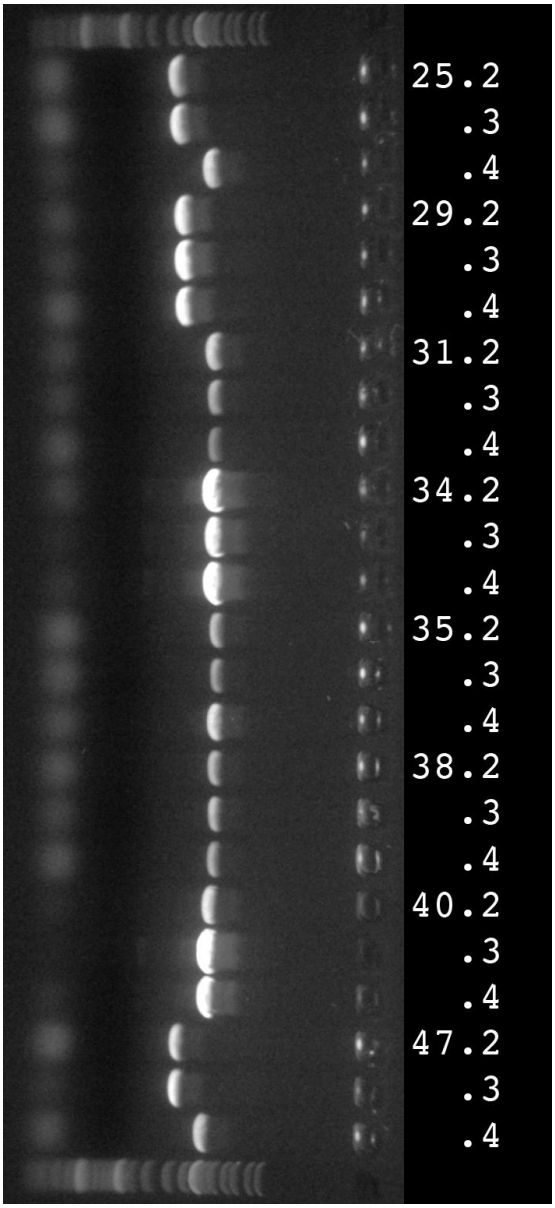

8 strains were MAT wt: 25, 29, 31, 34, 35, 38, 40, 47.
All others MATΔ::His3.

**TUESDAY, 2019-03-26**

- [x] Confirm genotype of colonies .2, .3, and .4 for 25, 29, 31, 34, 35, 38, 40, 47 combined with other samples (see 2-5 MAT/ste4 KO of MA strains set 4 MAT Colony PCR).

## WEDNESDAY, 2019-03-27

190327_2-3_MAT_Colony_PCR.jpg




5 strains were MAT wt: 31, 34, 35, 38, 40.

3 strains were MATΔ::His3: 25, 29, 47.

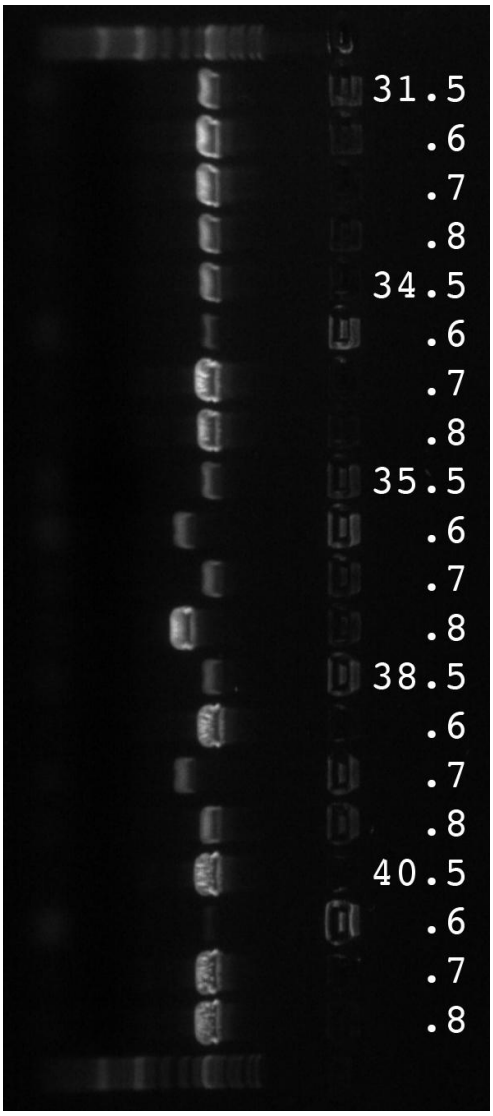
- [x] Restreak 4 transformants/strain to single colonies on a single SC-His-Trp plate for strains 31, 34, 35, 38, 40.

## FRIDAY, 2019-03-29

- [x] Confirm genotype of colonies .5, .6, .7 and .8 for strains 31, 34, 35, 38, 40 (combine samples with 2-5 MAT/ste4 KO of MA strains set 4 MAT Colony PCR.2). Matthew Stasiuk

## TUESDAY, 2019-04-02

190402_2-3_MAT_Colony_PCR.2.jpg




3 strains were MAT wt: 31, 34, 40.

2 strains were MATΔ::His3: 35, 38.

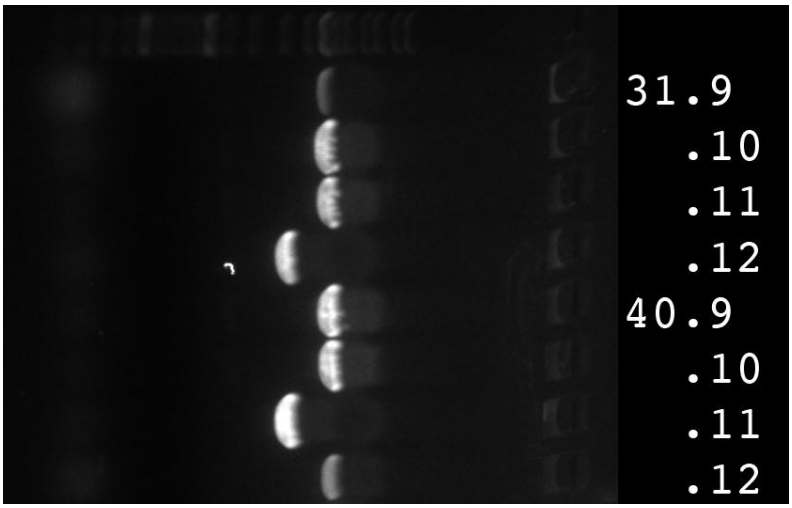
Retransform #34 with 2-9 MAT/ste4 KO of MA strains set 6.

- [x] Restreak 4 transformants/strain to single colonies on a single SC-His-Trp plate for strains 31, 40.

**THURSDAY, 2019-04-11**

- [x] Confirm genotype of colonies .9, .10, .11 and .12 for strains 31, 40 (combine samples with 2-5 MAT/ste4 KO of MA strains set 4 MAT Colony PCR.3).

190411_2-3_MAT_Colony_PCR.3.jpg




2 strains were MATΔ::His3: 31, 40.

**THURSDAY, 2019-04-25**

- [x] Inoculate overnight culture in 2 mL YPAD for all isolated ste4Δ::TRP1 MATΔ::His3 strains.

**FRIDAY, 2019-04-26**

- [x] Prepare glycerol stock and store in -80°C freezer.

MATΔ::His3

| | A | B | C | D | E | F | G | H | I | J | K | L | M |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Strain # | colony .1 | colony .2 | colony .3 | colony .4 | colony .5 | colony .6 | colony .7 | colony .8 | colony .9 | colony .10 | colony .11 | colony .12 |
| 2 | 25 | FALSE | TRUE | TRUE | FALSE | | | | | | | | |
| 3 | 26 | TRUE | | | | | | | | | | | |
| 4 | 27 | TRUE | | | | | | | | | | | |
| 5 | 28 | TRUE | | | | | | | | | | | |
| 6 | 29 | FALSE | TRUE | TRUE | TRUE | | | | | | | | |
| 7 | 30 | TRUE | | | | | | | | | | | |
| 8 | 31 | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | TRUE |
| 9 | 32 | TRUE | | | | | | | | | | | |
| 10 | 33 | TRUE | | | | | | | | | | | |
| 11 | 34 | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | | | | |
| 12 | 35 | FALSE | FALSE | FALSE | FALSE | FALSE | TRUE | FALSE | | | | | |
| 13 | 36 | TRUE | | | | | | | | | | | |
| 14 | 37 | TRUE | | | | | | | | | | | |
| 15 | 38 | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | TRUE | FALSE | | | | |
| 16 | 39 | TRUE | | | | | | | | | | | |
| 17 | 40 | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | FALSE | TRUE | FALSE |
| 18 | 41 | TRUE | | | | | | | | | | | |
| 19 | 42 | TRUE | | | | | | | | | | | |
| 20 | 43 | TRUE | | | | | | | | | | | |
| 21 | 44 | TRUE | | | | | | | | | | | |
| 22 | 45 | TRUE | | | | | | | | | | | |
| 23 | 46 | TRUE | | | | | | | | | | | |
| 24 | 47 | FALSE | TRUE | TRUE | FALSE | | | | | | | | |
| 25 | 48 | TRUE | | | | | | | | | | | |
| 26 | | | | | | | | | | | | | |
| 27 | | | | | | | | | | | | | |
| 28 | | | | | | | | | | | | | |
| 29 | | | | | | | | | | | | | |
| 30 | | | | | | | | | | | | | |
| 31 | | | | | | | | | | | | | |
| 32 | | | | | | | | | | | | | |
| 33 | | | | | | | | | | | | | |
| 34 | | | | | | | | | | | | | |
| 35 | | | | | | | | | | | | | |
| 36 | | | | | | | | | | | | | |
| 37 | | | | | | | | | | 73.5 | | | |
| 38 | | | | | | | | | | 0.6 | | | |
| 39 | | | | | | | | | | 76.5 | | | |
| 40 | | | | | | | | | | 0.6 | | | |
| 41 | | | | | | | | | | 0.7 | | | |
| 42 | | | | | | | | | | 0.8 | | | |

# 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 H20
- 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

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.
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 | 24 | 100 |

- [x] 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.

- [x] 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 | sterile H2O | 23.4 |
| 4 | **Total** | **26** |

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

- [x] 8. Centrifuge 3,000 g/5 min. Discard supernatant and resuspend cells in 1 mL of 0.1 M LiAc.

- [x] 9. Centrifuge 3,000 g/1 min; discard supernatant.

- [x] 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 | C |
|---|---|---|
| 1 | | 26 X [µL] |
| 2 | 50% PEG | 6240 |
| 3 | 1 M LiAc | 936 |
| 4 | ssDNA (2 mg/ml) | 1300 |
| 5 | ste4 KO PCR product | 260 |
| 6 | ddH2O | 624 |
| 7 | **Total** | **9360** |

- [x] 11. Add 360 µL of transformation master mix to each tube.

- [x] 12. Vortex tubes until the cells are smoothly suspended.

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

01:00:00

- [x] 14. Centrifuge 3,000 g/5 min; discard supernatant and resuspend each cell pellet in 100 µL sterile $H_2O$, then plate cells on the SC-Trp.

## Additional resources

**PCR product amou...**

| | STE4 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 | dHd2O | 24 |
| 7 | **Total** | **360** |

# ste4 KO Transformation.2

## 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 H20
- 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

- [x] 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.
- [x] 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

- [x] 3. Set water bath or heating block to 42°C.
- [x] 4. Indicate number of transformations and PCR product concentration:

**Settings**

| | # of Transformations | ste4 KO PCR product [ng/µL] |
|---|---|---|
| 1 | 24 | 100 |

- [x] 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.

- [x] 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 | sterile H2O | 23.4 |
| 4 | **Total** | **26** |

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

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

- [x] 9. Centrifuge 3,000 g/5 min; discard supernatant.

- [x] 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 | C |
|---|---|---|
| 1 | | 26 X [µL] |
| 2 | 50% PEG | 6240 |
| 3 | 1 M LiAc | 936 |
| 4 | ssDNA (2 mg/ml) | 1300 |
| 5 | ste4 KO PCR product | 260 |
| 6 | ddH2O | 624 |
| 7 | **Total** | **9360** |

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

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

01:00:00

- [x] 13. Centrifuge 3,000 g/5 min; discard supernatant.

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

Results

| | A | B |
|---|---|---|
| 1 | Strain # | # colonies |
| 2 | 25 | 7 |
| 3 | 26 | contaminated |
| 4 | 27 | 5 |
| 5 | 28 | 4 |
| 6 | 29 | 4 |
| 7 | 30 | 5 |
| 8 | 31 | 5 |
| 9 | 32 | 5 |
| 10 | 33 | 12 |
| 11 | 34 | 9 |
| 12 | 35 | 16 |
| 13 | 36 | 12 |
| 14 | 37 | 1 |
| 15 | 38 | 3 |
| 16 | 39 | 28 |
| 17 | 40 | 4 |
| 18 | 41 | 6 |
| 19 | 42 | 5 |
| 20 | 43 | 5 |
| 21 | 44 | 8 |
| 22 | 45 | 4 |
| 23 | 46 | 5 |
| 24 | 47 | 7 |
| 25 | 48 | 4 |

## 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 | dHd2O | 24 |
| 7 | **Total** | **360** |

# ste4 Colony PCR

## Introduction

For genotyping of ste4 locus: the primers bind upstream (F) and downstream (R) of ste4, and PCR is used to determine if TRP1 has replaced ste4 by homologous recombination.

## Materials

- TaKaRa LA Taq® DNA Polymerase (Mg2+ free buffer) RR002A
- 10 µM Primer F: OLPr022 STE4 -133 F
- 10 µM Primer R: OLPr023 STE4 1402 R
- SC-Trp plates
- Template: yeast colonies transformed with ste4 KO PCR product

## Procedure

### PCR

1. Chose the number of 20 µL reactions (include 3 additional reactions to account for wild-type, mutant, and no-template control):

**Settings**

| | # of reactions | B |
|---|---|---|
| 1 | 26 | plate 35 is contaminated by fungus |

2. Combine the following reagents to prepare the master mix (contains 0.5 additional reaction to account for pipetting discrepancies):

**Master mix**

| | A | B |
|---|---|---|
| 1 | | **26.5 X [µL]** |
| 2 | ddH2O | 312.7 |
| 3 | 10X LA PCR Buffer | 53 |
| 4 | 25 mM MgCl2 | 53 |
| 5 | dNTP Mix (2.5 mM each) | 84.8 |
| 6 | 10 µM OLPr022 STE4 -133 F | 10.6 |
| 7 | 10 µM OLPr023 STE4 1402 R | 10.6 |
| 8 | LA Taq Polymerase (5 U/µL) | 5.3 |
| 9 | **Total** | **530** |

- 3. In sterile work environement (e.g. laminar flow), gently touch a yeast colony with a 10 µL pipette tip so a small number of cells stick to it and resuspend cells in PCR reaction.

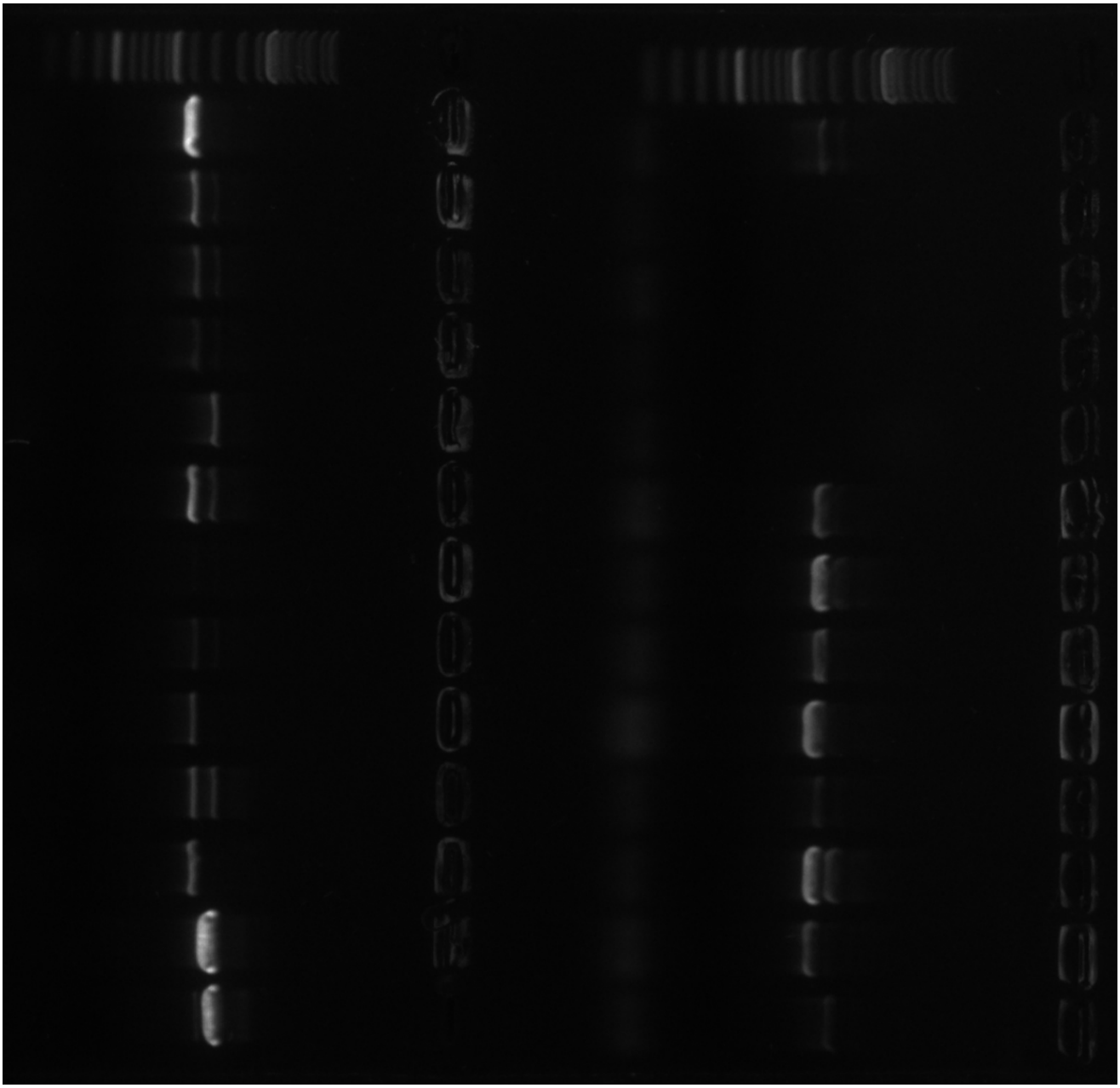
  Do not use too many cells as they might inhibit the PCR reaction. The PCR reaction should only look slightly opaque from the resuspended cells. If the reaction looks milky then you might have used too many cells.

- 4. PCR program: 94°C/5 min—[94°C/0.5 min—61°C/0.5 min—68°C/1.5 min]x35—68°C/5 min—10°C/∞

## Gel

- 5. Gel: 10 µL/0.5 µg O'GeneRuler DNA Ladder Mix/1% TAE/120 V/35 min

  Expected product sizes:
  ste4 1535 bp
  ste4Δ::TRP1 1232 bp
  Stop processing any yeast strains with the wrong genotype (i.e. discard any corresponding overnight cultures and patches on reference plate).



## Additional resources

**Primers and template**

| | A | B |
|---|---|---|
| 1 | Forward Primer | OLPr022 STE4 -133 F |
| 2 | Reverse Primer | OLPr023 STE4 1402 R |
| 3 | Template | yeast colonies |

**PCR Volume and reagent concentrations**

| | A | B |
|---|---|---|
| 1 | PCR Volume [µL] | 20 |
| 2 | Starting buffer conc [X] | 10 |
| 3 | Final buffer conc [X] | 1 |
| 4 | Starting  Mg2+ conc [mM] | 25 |
| 5 | Final Mg2+ conc [mM] | 2.5 |
| 6 | Starting dNTP conc each [mM] | 2.5 |
| 7 | Final dNTP conc each [mM] | 0.4 |
| 8 | Starting Primer F conc [µM] | 10 |
| 9 | Final Primer F conc [µM] | 0.2 |
| 10 | Starting Primer R conc [µM] | 10 |
| 11 | Final Primer R conc [µM] | 0.2 |
| 12 | Starting Polymerase conc [U/µL] | 5 |
| 13 | Final Polymerase conc [U/µL] | 0.05 |
| 14 | Starting Template conc [ng/µL] | 5 |
| 15 | Final Template conc [ng/µL] | 0 |

**Single PCR reaction**

| | A | B |
|---|---|---|
| 1 | | [µL] |
| 2 | ddH2O | 11.8 |
| 3 | 10X PCR Buffer | 2 |
| 4 | 25 mM MgCl2 | 2 |
| 5 | dNTP Mix (2.5 mM each) | 3.2 |
| 6 | 10 µM Primer F | 0.4 |
| 7 | 10 µM Primer R | 0.4 |
| 8 | LA Taq Polymerase (5 U/µL) | 0.2 |
| 9 | Template (5 ng/µL) | 0 |
| 10 | Total | 20 |

# ste4 Colony PCR.2

## Introduction

For genotyping of ste4 locus: the primers bind upstream (F) and downstream (R) of ste4, and PCR is used to determine if TRP1 has replaced ste4 by homologous recombination.

## Materials

- TaKaRa LA Taq® DNA Polymerase (Mg2+ free buffer) RR002A
- 10 µM Primer F: OLPr022 STE4 -133 F
- 10 µM Primer R: OLPr023 STE4 1402 R
- SC-Trp plates
- Template: yeast colonies transformed with ste4 KO PCR product

## Procedure

### PCR

1. Chose the number of 20 µL reactions (include 3 additional reactions to account for wild-type, mutant, and no-template control):

**Settings**

| | # of reactions |
|---|---|
| 1 | 26 |

2. Combine the following reagents to prepare the master mix (contains 0.5 additional reaction to account for pipetting discrepancies):

**Master mix**

| | A | B |
|---|---|---|
| 1 | | **26.5 X [µL]** |
| 2 | ddH2O | 312.7 |
| 3 | 10X LA PCR Buffer | 53 |
| 4 | 25 mM MgCl2 | 53 |
| 5 | dNTP Mix (2.5 mM each) | 84.8 |
| 6 | 10 µM OLPr022 STE4 -133 F | 10.6 |
| 7 | 10 µM OLPr023 STE4 1402 R | 10.6 |
| 8 | LA Taq Polymerase (5 U/µL) | 5.3 |
| 9 | **Total** | **530** |

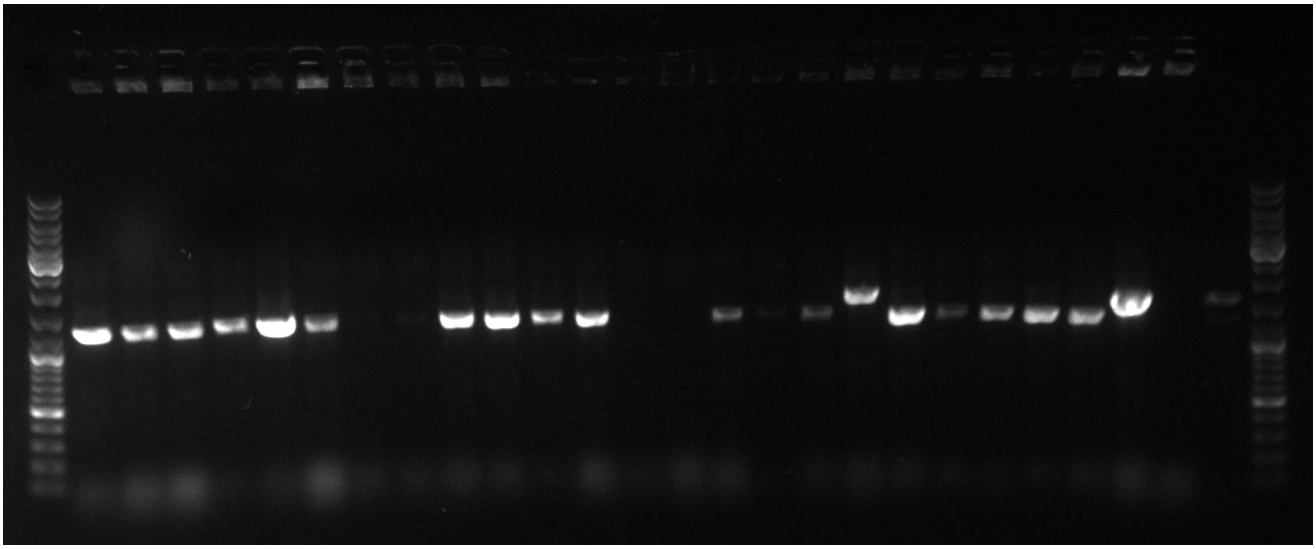
- 3. In sterile work environement (e.g. laminar flow), gently touch a yeast colony with a 10 µL pipette tip so a small number of cells stick to it and resuspend cells in PCR reaction.

  Do not use too many cells as they might inhibit the PCR reaction. The PCR reaction should only look slightly opaque from the resuspended cells. If the reaction looks milky then you might have used too many cells.
- 4. PCR program: 94°C/5 min—[94°C/0.5 min—61°C/0.5 min—68°C/1.5 min]x35—68°C/5 min—10°C/∞

## Gel

- 5. Gel: 10 µL/0.5 µg O'GeneRuler DNA Ladder Mix/1% TAE/120 V/35 min

  Expected product sizes:
  ste4 1535 bp
  ste4Δ::TRP1 1232 bp
  Stop processing any yeast strains with the wrong genotype (i.e. discard any corresponding overnight cultures and patches on reference plate).



# Additional resources

**Primers and template**

| | A | B |
|---|---|---|
| 1 | Forward Primer | OLPr022 STE4 -133 F |
| 2 | Reverse Primer | OLPr023 STE4 1402 R |
| 3 | Template | yeast colonies |

**PCR Volume and reagent concentrations**

| | A | B |
|---|---|---|
| 1 | PCR Volume [µL] | 20 |
| 2 | Starting buffer conc [X] | 10 |
| 3 | Final buffer conc [X] | 1 |
| 4 | Starting  Mg2+ conc [mM] | 25 |
| 5 | Final Mg2+ conc [mM] | 2.5 |
| 6 | Starting dNTP conc each [mM] | 2.5 |
| 7 | Final dNTP conc each [mM] | 0.4 |
| 8 | Starting Primer F conc [µM] | 10 |
| 9 | Final Primer F conc [µM] | 0.2 |
| 10 | Starting Primer R conc [µM] | 10 |
| 11 | Final Primer R conc [µM] | 0.2 |
| 12 | Starting Polymerase conc [U/µL] | 5 |
| 13 | Final Polymerase conc [U/µL] | 0.05 |
| 14 | Starting Template conc [ng/µL] | 5 |
| 15 | Final Template conc [ng/µL] | 0 |

**Single PCR reaction**

| | A | B |
|---|---|---|
| 1 | | [µL] |
| 2 | ddH2O | 11.8 |
| 3 | 10X PCR Buffer | 2 |
| 4 | 25 mM MgCl2 | 2 |
| 5 | dNTP Mix (2.5 mM each) | 3.2 |
| 6 | 10 µM Primer F | 0.4 |
| 7 | 10 µM Primer R | 0.4 |
| 8 | LA Taq Polymerase (5 U/µL) | 0.2 |
| 9 | Template (5 ng/µL) | 0 |
| 10 | Total | 20 |

# ste4 Colony PCR.3

## Introduction

For genotyping of ste4 locus: the primers bind upstream (F) and downstream (R) of ste4, and PCR is used to determine if TRP1 has replaced ste4 by homologous recombination.

## Materials

- TaKaRa LA Taq® DNA Polymerase (Mg2+ free buffer) RR002A
- 10 µM Primer F: OLPr022 STE4 -133 F
- 10 µM Primer R: OLPr023 STE4 1402 R
- SC-Trp plates
- Template: yeast colonies transformed with ste4 KO PCR product

## Procedure

### PCR

1. Chose the number of 20 µL reactions (include 3 additional reactions to account for wild-type, mutant, and no-template control):

**Settings**

| | # of reactions |
|---|---|
| 1 | 26 |

2. Combine the following reagents to prepare the master mix (contains 0.5 additional reaction to account for pipetting discrepancies):

**Master mix**

| | A | B |
|---|---|---|
| 1 | | **26.5 X [µL]** |
| 2 | ddH2O | 312.7 |
| 3 | 10X LA PCR Buffer | 53 |
| 4 | 25 mM MgCl2 | 53 |
| 5 | dNTP Mix (2.5 mM each) | 84.8 |
| 6 | 10 µM OLPr022 STE4 -133 F | 10.6 |
| 7 | 10 µM OLPr023 STE4 1402 R | 10.6 |
| 8 | LA Taq Polymerase (5 U/µL) | 5.3 |
| 9 | **Total** | **530** |

- 3. In sterile work environement (e.g. laminar flow), gently touch a yeast colony with a 10 µL pipette tip so a small number of cells stick to it and resuspend cells in PCR reaction.

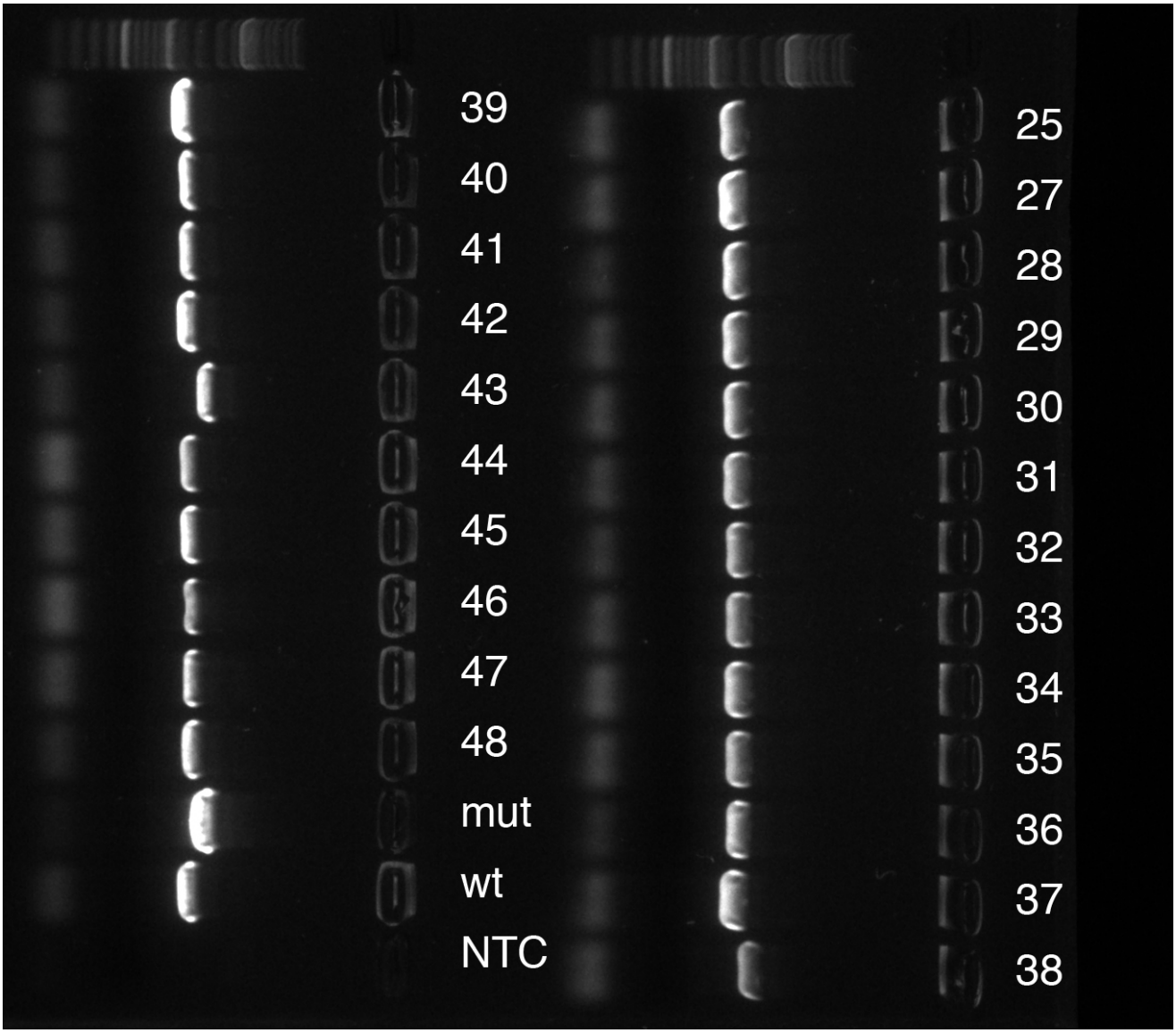
  Do not use too many cells as they might inhibit the PCR reaction. The PCR reaction should only look slightly opaque from the resuspended cells. If the reaction looks milky then you might have used too many cells.

- 4. PCR program: 94°C/5 min—[94°C/0.5 min—61°C/0.5 min—68°C/1.5 min]x35—68°C/5 min—10°C/∞

## Gel

- 5. Gel: 10 µL/0.5 µg O'GeneRuler DNA Ladder Mix/1% TAE/120 V/35 min

  Expected product sizes:
  ste4 1535 bp
  ste4Δ::TRP1 1232 bp
  Stop processing any yeast strains with the wrong genotype (i.e. discard any corresponding overnight cultures and patches on reference plate).




## Additional resources

**Primers and template**

| | A | B |
|---|---|---|
| 1 | Forward Primer | OLPr022 STE4 -133 F |
| 2 | Reverse Primer | OLPr023 STE4 1402 R |
| 3 | Template | yeast colonies |

**PCR Volume and reagent concentrations**

| | A | B |
|---|---|---|
| 1 | PCR Volume [µL] | 20 |
| 2 | Starting buffer conc [X] | 10 |
| 3 | Final buffer conc [X] | 1 |
| 4 | Starting  Mg2+ conc [mM] | 25 |
| 5 | Final Mg2+ conc [mM] | 2.5 |
| 6 | Starting dNTP conc each [mM] | 2.5 |
| 7 | Final dNTP conc each [mM] | 0.4 |
| 8 | Starting Primer F conc [µM] | 10 |
| 9 | Final Primer F conc [µM] | 0.2 |
| 10 | Starting Primer R conc [µM] | 10 |
| 11 | Final Primer R conc [µM] | 0.2 |
| 12 | Starting Polymerase conc [U/µL] | 5 |
| 13 | Final Polymerase conc [U/µL] | 0.05 |
| 14 | Starting Template conc [ng/µL] | 5 |
| 15 | Final Template conc [ng/µL] | 0 |

**Single PCR reaction**

| | A | B |
|---|---|---|
| 1 | | [µL] |
| 2 | ddH2O | 11.8 |
| 3 | 10X PCR Buffer | 2 |
| 4 | 25 mM MgCl2 | 2 |
| 5 | dNTP Mix (2.5 mM each) | 3.2 |
| 6 | 10 µM Primer F | 0.4 |
| 7 | 10 µM Primer R | 0.4 |
| 8 | LA Taq Polymerase (5 U/µL) | 0.2 |
| 9 | Template (5 ng/µL) | 0 |
| 10 | Total | 20 |

# ste4 Colony PCR.4

## Introduction

For genotyping of ste4 locus: the primers bind upstream (F) and downstream (R) of ste4, and PCR is used to determine if TRP1 has replaced ste4 by homologous recombination.

## Materials

- TaKaRa LA Taq® DNA Polymerase (Mg2+ free buffer) RR002A
- 10 µM Primer F: OLPr022 STE4 -133 F
- 10 µM Primer R: OLPr023 STE4 1402 R
- SC-Trp plates
- Template: yeast colonies transformed with ste4 KO PCR product

## Procedure

### PCR

1. Chose the number of 10 µL reactions (include 3 additional reactions to account for wild-type, mutant, and no-template control):

**Settings**

| | # of reactions |
|---|---|
| 1 | 8 |

2. Combine the following reagents to prepare the master mix (contains 0.5 additional reaction to account for pipetting discrepancies):

**Master mix**

| | A | B |
|---|---|---|
| 1 | | **8.5 X [µL]** |
| 2 | ddH2O | 50.15 |
| 3 | 10X LA PCR Buffer | 8.5 |
| 4 | 25 mM MgCl2 | 8.5 |
| 5 | dNTP Mix (2.5 mM each) | 13.6 |
| 6 | 10 µM OLPr022 STE4 -133 F | 1.7 |
| 7 | 10 µM OLPr023 STE4 1402 R | 1.7 |
| 8 | LA Taq Polymerase (5 U/µL) | 0.85 |
| 9 | **Total** | **85** |

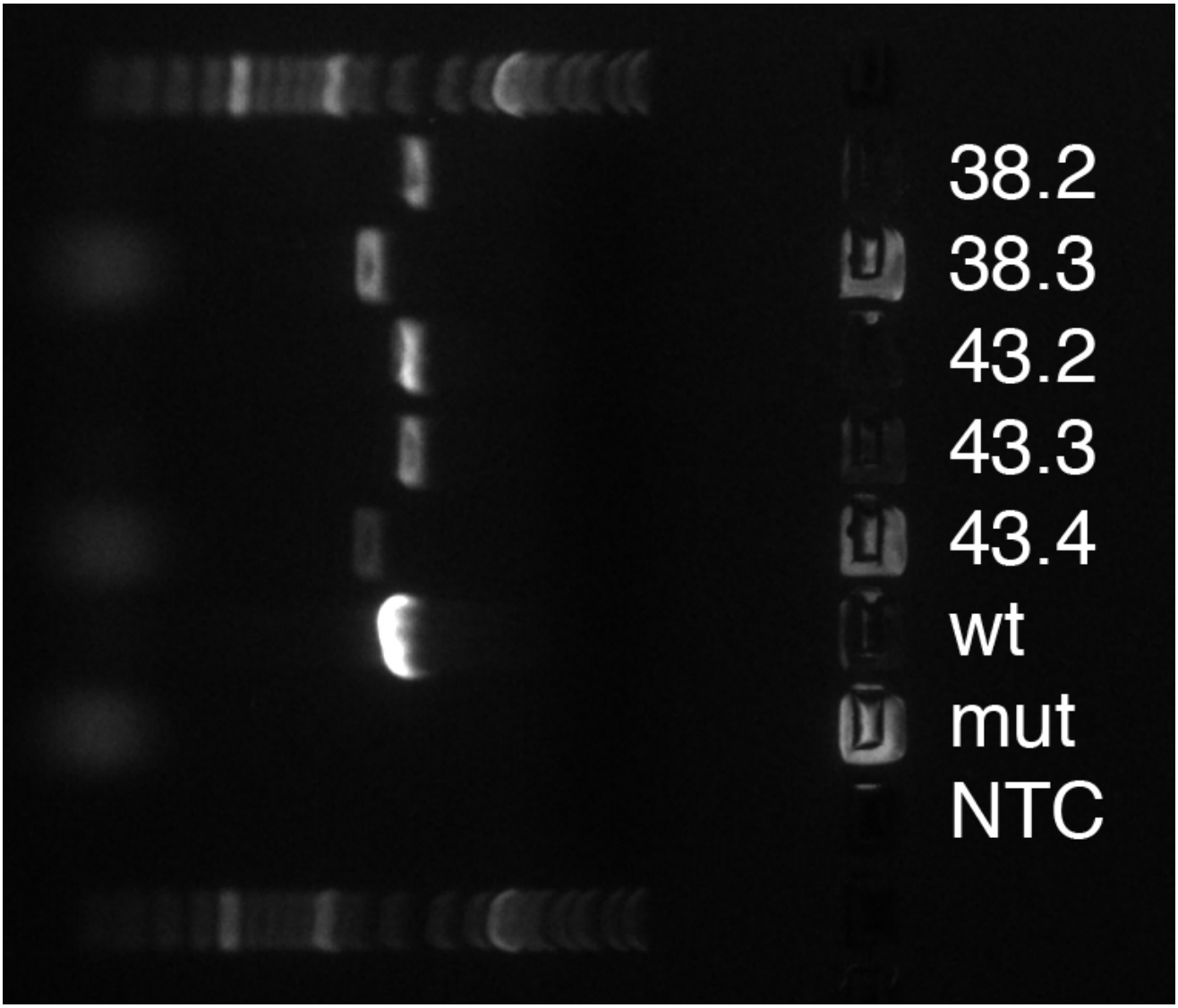
- 3. In sterile work environement (e.g. laminar flow), gently touch a yeast colony with a 10 µL pipette tip so a small number of cells stick to it and resuspend cells in PCR reaction.

  Do not use too many cells as they might inhibit the PCR reaction. The PCR reaction should only look slightly opaque from the resuspended cells. If the reaction looks milky then you might have used too many cells.
- 4. PCR program: 94°C/5 min—[94°C/0.5 min—61°C/0.5 min—68°C/1.5 min]x35—68°C/5 min—10°C/∞

## Gel

- 5. Gel: 10 µL/0.5 µg O'GeneRuler DNA Ladder Mix/1% TAE/120 V/35 min

  Expected product sizes:
  ste4 1535 bp
  ste4Δ::TRP1 1232 bp
  Stop processing any yeast strains with the wrong genotype (i.e. discard any corresponding overnight cultures and patches on reference plate).




## Additional resources

**Primers and template**

| | A | B |
|---|---|---|
| 1 | Forward Primer | OLPr022 STE4 -133 F |
| 2 | Reverse Primer | OLPr023 STE4 1402 R |
| 3 | Template | yeast colonies |

**PCR Volume and reagent concentrations**

| | A | B |
|---|---|---|
| 1 | PCR Volume [µL] | 10 |
| 2 | Starting buffer conc [X] | 10 |
| 3 | Final buffer conc [X] | 1 |
| 4 | Starting  Mg2+ conc [mM] | 25 |
| 5 | Final Mg2+ conc [mM] | 2.5 |
| 6 | Starting dNTP conc each [mM] | 2.5 |
| 7 | Final dNTP conc each [mM] | 0.4 |
| 8 | Starting Primer F conc [µM] | 10 |
| 9 | Final Primer F conc [µM] | 0.2 |
| 10 | Starting Primer R conc [µM] | 10 |
| 11 | Final Primer R conc [µM] | 0.2 |
| 12 | Starting Polymerase conc [U/µL] | 5 |
| 13 | Final Polymerase conc [U/µL] | 0.05 |
| 14 | Starting Template conc [ng/µL] | 5 |
| 15 | Final Template conc [ng/µL] | 0 |

**Single PCR reaction**

| | A | B |
|---|---|---|
| 1 | | [µL] |
| 2 | ddH2O | 5.9 |
| 3 | 10X PCR Buffer | 1 |
| 4 | 25 mM MgCl2 | 1 |
| 5 | dNTP Mix (2.5 mM each) | 1.6 |
| 6 | 10 µM Primer F | 0.2 |
| 7 | 10 µM Primer R | 0.2 |
| 8 | LA Taq Polymerase (5 U/µL) | 0.1 |
| 9 | Template (5 ng/µL) | 0 |
| 10 | Total | 10 |

# MAT KO Transformation

## Introduction

ste4Δ::TRP1 yeast strain derived from SEYa or SEYα are transformed with MAT KO PCR product to replace MATa or MATα 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

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.
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 | MAT KO PCR product [ng/µL] |
|---|---|---|
| 1 | 24 | 100 |

- [x] 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.

- [x] 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 | sterile H2O | 23.4 |
| 4 | **Total** | **26** |

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

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

- [x] 9. Centrifuge 3,000 g/5 min; discard supernatant.

- [x] 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 | | 26 X [µL] |
| 2 | 50% PEG | 6240 |
| 3 | 1 M LiAc | 936 |
| 4 | ssDNA (2 mg/ml) | 1300 |
| 5 | MAT KO PCR product | 260 |
| 6 | ddH2O | 624 |
| 7 | **Total** | **9360** |

- [x] 11. Add 360 µL of transformation master mix to each tube.

- [x] 12. Vortex tubes until the cells are smoothly suspended.

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

01:00:00

- [x] 14. Centrifuge 3,000 g/5 min; discard supernatant and resuspend each cell pellet in 100 µL sterile $H_2O$, then plate cells on the SC-His-Trp.

**Results**

| | A | B |
|---|---|---|
| 1 | Strain # | # colonies |
| 2 | 25 | |
| 3 | 26 | |
| 4 | 27 | |
| 5 | 28 | 1 |
| 6 | 29 | |
| 7 | 30 | 3 |
| 8 | 31 | |
| 9 | 32 | |
| 10 | 33 | 9 |
| 11 | 34 | 7 |
| 12 | 35 | |
| 13 | 36 | |
| 14 | 37 | |
| 15 | 38 | |
| 16 | 39 | |
| 17 | 40 | 25 |
| 18 | 41 | |
| 19 | 42 | 21 |
| 20 | 43 | |
| 21 | 44 | |
| 22 | 45 | |
| 23 | 46 | |
| 24 | 47 | |
| 25 | 48 | |

## 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 | 10.0 |
| 6 | dHd2O | 24.0 |
| 7 | **Total** | **360** |

# MAT KO Transformation.2

## Introduction

ste4Δ::TRP1 yeast strain derived from SEYa or SEYα are transformed with MAT KO PCR product to replace MATa or MATα 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

- [x] 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

- [x] 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

- [x] 3. Set water bath or heating block to 42°C.
- [x] 4. Indicate number of transformations and PCR product concentration:

**Settings**

| | # of Transformations | MAT KO PCR product [ng/µL] |
|---|---|---|
| 1 | 19 | 100 |

- [x] 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.

- [x] 6. Prepare 0.1 M LiAc, freshly diluted from 1 M LiAc:

**0.1 M Li Ac**

| | Materials | mL |
|---|---|---|
| 1 | | 21 X [mL] |
| 2 | 1 M LiAc | 2.1 |
| 3 | sterile H2O | 18.9 |
| 4 | **Total** | **21** |

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

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

- [x] 9. Centrifuge 3,000 g/5 min; discard supernatant.

## Transformation

- [x] 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 | | 21 X [µL] |
| 2 | 50% PEG | 5040 |
| 3 | 1 M LiAc | 756 |
| 4 | ssDNA (2 mg/ml) | 1050 |
| 5 | MAT KO PCR product | 210 |
| 6 | ddH2O | 504 |
| 7 | **Total** | **7560** |

- [x] 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 and resuspend each cell pellet in 100 µL sterile $H_2O$, then plate cells on the SC-His-Trp.

**Results**

| | A | B |
|---|---|---|
| 1 | Strain # | # colonies |
| 2 | 25 | 41 |
| 3 | 26 | 21 |
| 4 | 27 | 9 |
| 5 | 28 | 8 |
| 6 | 29 | 36 |
| 7 | 31 | 34 |
| 8 | 32 | 36 |
| 9 | 33 | 35 |
| 10 | 36 | 14 |
| 11 | 37 | 44 |
| 12 | 38 | 35 |
| 13 | 39 | 1 |
| 14 | 41 | 54 |
| 15 | 43 | 18 |
| 16 | 44 | 8 |
| 17 | 45 | 8 |
| 18 | 46 | 40 |
| 19 | 47 | 25 |
| 20 | 48 | 42 |

## 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 | 10.0 |
| 6 | dHd2O | 24.0 |
| 7 | **Total** | **360** |

# MAT Colony PCR

## Introduction

For genotyping of MAT locus: the primers bind upstream (F) and downstream (R) of MAT locus, and PCR is used to determine if His3 has replaced MAT by homologous recombination.

## Materials

- TaKaRa LA Taq® DNA Polymerase (Mg2+ free buffer) RR002A
- 10 µM Primer F: OLPr014 MAT -283 F
- 10 µM Primer R: OLPr015 MAT +295 R
- SC-His-Trp plates
- Template: yeast colonies from transformation of STE4Δ::strains with MAT KO PCR product

## Procedure

### PCR

1. Chose the number of 10 µL reactions (include 3 additional reactions to account for wild-type, mutant, and no-template control):

**Settings**

| | # of reactions |
|---|---|
| 1 | 72 |

2. Combine the following reagents to prepare the master mix (contains 0.5 additional reaction to account for pipetting discrepancies):

**Master mix**

| | A | B |
|---|---|---|
| 1 | | **72.5 X [µL]** |
| 2 | ddH2O | 427.75 |
| 3 | 10X LA PCR Buffer | 72.5 |
| 4 | 25 mM MgCl2 | 72.5 |
| 5 | dNTP Mix (2.5 mM each) | 116 |
| 6 | 10 µM OLPr014 MAT -283 F | 14.5 |
| 7 | 10 µM OLPr015 MAT +295 R | 14.5 |
| 8 | LA Taq Polymerase (5 U/µL) | 7.25 |
| 9 | **Total** | **725** |

3. In sterile work environement (e.g. laminar flow), gently touch a yeast colony with a 10 µL pipette tip so a small number of cells stick to it and resuspend cells in PCR reaction.

   Do not use too many cells as they might inhibit the PCR reaction. The PCR reaction should only look slightly opaque from the resuspended cells. If the reaction looks milky then you might have used too many cells.

4. PCR program "MATCOLON" in folder "STEPHAN":

   94°C/5 min—[94°C/0.5 min—61°C/0.5 min—68°C/3 min]x35—68°C/5 min—10°C/∞

## Gel

5. Gel: 10 µL/0.5 µg O'GeneRuler DNA Ladder Mix/1% TAE/120 V/35 min

00:35:00

Expected product sizes:
MATa 2974 bp
MATα 3085 bp
MATΔ::His3 1958 bp
Stop processing any yeast strains with the wrong genotype (i.e. discard any corresponding overnight cultures and patches on reference plate).

## Additional resources

**Primers and template**

| | A | B |
|---|---|---|
| 1 | Forward Primer | OLPr014 MAT -283 F |
| 2 | Reverse Primer | OLPr015 MAT +295 R |
| 3 | Template | yeast colonies |

**PCR Volume and reagent concentrations**

| | A | B |
|---|---|---|
| 1 | PCR Volume [µL] | 10 |
| 2 | Starting buffer conc [X] | 10 |
| 3 | Final buffer conc [X] | 1 |
| 4 | Starting  Mg2+ conc [mM] | 25 |
| 5 | Final Mg2+ conc [mM] | 2.5 |
| 6 | Starting dNTP conc each [mM] | 2.5 |
| 7 | Final dNTP conc each [mM] | 0.4 |
| 8 | Starting Primer F conc [µM] | 10 |
| 9 | Final Primer F conc [µM] | 0.2 |
| 10 | Starting Primer R conc [µM] | 10 |
| 11 | Final Primer R conc [µM] | 0.2 |
| 12 | Starting Polymerase conc [U/µL] | 5 |
| 13 | Final Polymerase conc [U/µL] | 0.05 |
| 14 | Starting Template conc [ng/µL] | 5 |
| 15 | Final Template conc [ng/µL] | 0 |

**Single PCR reaction**

| | A | B |
|---|---|---|
| 1 | | [µL] |
| 2 | ddH2O | 5.9 |
| 3 | 10X PCR Buffer | 1 |
| 4 | 25 mM MgCl2 | 1 |
| 5 | dNTP Mix (2.5 mM each) | 1.6 |
| 6 | 10 µM Primer F | 0.2 |
| 7 | 10 µM Primer R | 0.2 |
| 8 | LA Taq Polymerase (5 U/µL) | 0.1 |
| 9 | Template (5 ng/µL) | 0 |
| 10 | Total | 10 |