2-4 MAT/ste4 KO of MA strains set 3

THURSDAY, 2019-01-10

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:
49–72

ste4 knock-out

- [x] Patch each strain on grid on YPG plate [NOTE: used frozen strains from Aliquot 2 of MA lines].

SATURDAY, 2019-01-12

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

MONDAY, 2019-01-14

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

TUESDAY, 2019-01-15

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

FRIDAY, 2019-01-18

Strain #56 and 61 contaminated

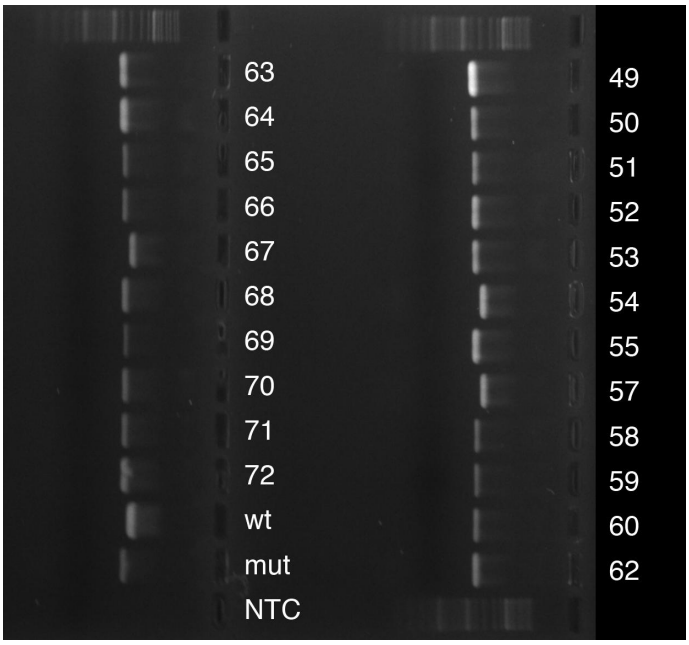
- [x] Streak-out a single transformant to single colonies on SC-Trp plate.

TUESDAY, 2019-01-22

- [x] Confirm genotype of single colony 49–55, 57–60, 62–72 (see ste4 colony PCR protocol). Christina Hsu

FRIDAY, 2019-01-25

190125_2-4_STE4_Colony_PCR_Set_3.jpg



19 strains were ste4Δ::TRP1 (see table below).

3 strains were ste4 wt: 54, 57, and 67.

FRIDAY, 2019-02-01

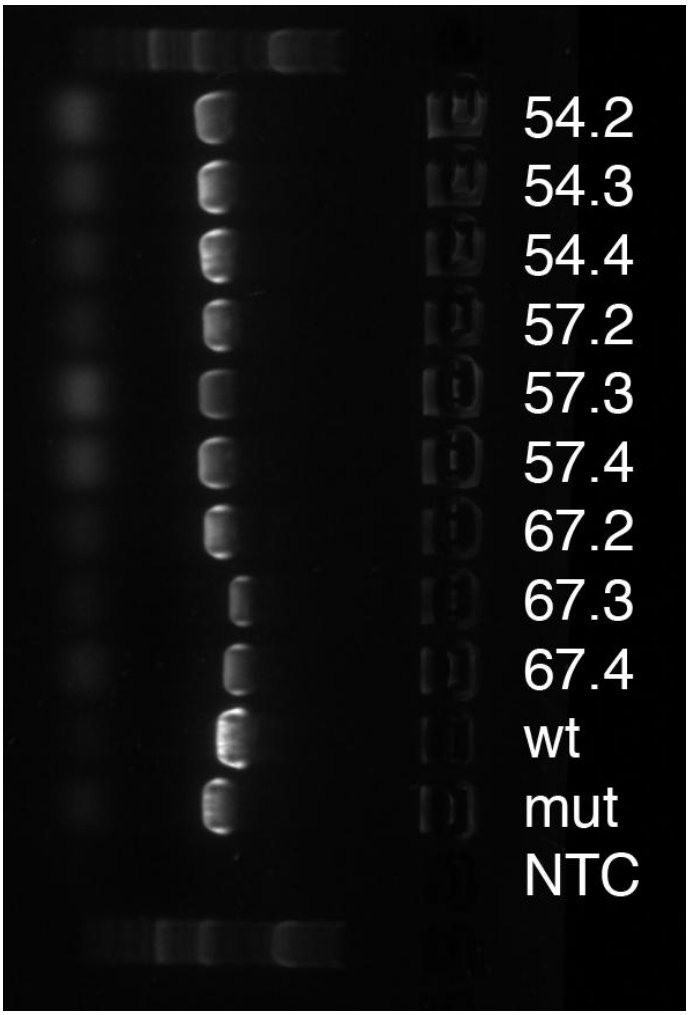
- [x] Confirm genotype of single colony of strains 54, 57, and 67 for colonies .2, .3, and .4 (see ste4 colony PCR.2 protocol).
  Christina Hsu

NTC showed a wt band, PCR master mix was likely contaminated, repeat.

MONDAY, 2019-02-04

- [x] Repeat: Confirm genotype of single colony of strains 54, 57, and 67 for colonies .2, .3, and .4 (see ste4 colony PCR.3 protocol).

190204_2-4_MAT_Colony_PCR.3_Set_3.jpg



7 strains were ste4Δ::TRP1 (see table below).
2 strains were ste4 wt: 67.3, 67.4.

TUESDAY, 2019-02-05

- [x] Make backup plate on SC-Trp.

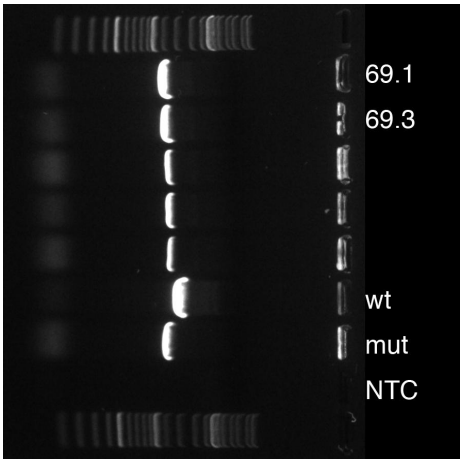
FRIDAY, 2019-02-08

Strain #69 did not grow on backup plate. Retest single colony from streak out plate in colony pcr.

TUESDAY, 2019-02-12

- [x] Confirm genotype of single colony of strain 69 for colonies .1, and .3 (see ste4 colony PCR.4 protocol, combined PCR with samples from 2-6).

190212_2-4_STE4_Colony_PCR.4_Set_3.jpg



All clones are ste4Δ::TRP1.

WEDNESDAY, 2019-02-27

- [x] Make backup plate and consolidate with ste4Δ::TRP1 strains of 61 from 2-9 MAT/ste4 KO of MA strains set 6 and of 56 from 2-11 MAT/ste4 KO of MA strains set 7 .

ste4Δ::TRP1

	A	B	C	D	E
1	Strain #	colony .1	colony .2	colony .3	colony .4
2	49	TRUE			
3	50	TRUE			
4	51	TRUE			
5	52	TRUE			
6	53	TRUE			
7	54	FALSE	TRUE	TRUE	TRUE
8	55	TRUE			
9	56	NA			
10	57	FALSE	TRUE	TRUE	TRUE
11	58	TRUE			
12	59	TRUE			
13	60	TRUE			
14	61	NA			
15	62	TRUE			
16	63	TRUE			
17	64	TRUE			
18	65	TRUE			
19	66	TRUE			
20	67	FALSE	TRUE	FALSE	FALSE
21	68	TRUE			
22	69	TRUE		TRUE	
23	70	TRUE			
24	71	TRUE			
25	72	TRUE			

THURSDAY, 2019-02-28

MAT knock-out

- [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).

FRIDAY, 2019-03-01

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

MONDAY, 2019-03-04

Only plate with strain #57 with distinct colonies, all others had a lawn. Cause for lawn was traced back to batch of SC-His-Trp plates: 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.

THURSDAY, 2019-03-07

- [x] Inoculate overnight culture for strains 49–72 (see MAT KO Transformation.2 day 1, included #57 as backup as it was just one additional transformation).

FRIDAY, 2019-03-08

- [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, 2019-03-11

- [x] Streak-out 4 transformants/strain to single colonies on a single SC-His-Trp plate.

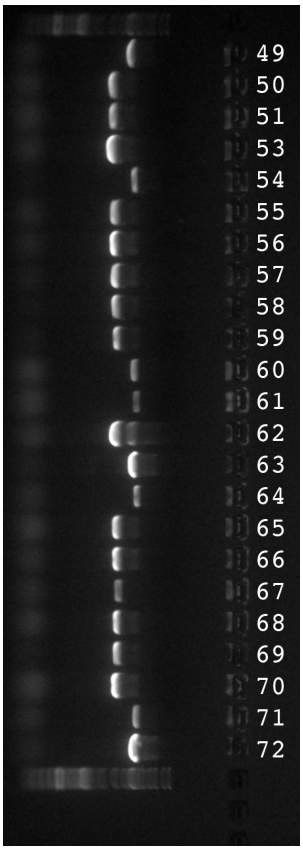
Did not receive any colonies for strain #52.

WEDNESDAY, 2019-03-13

- [x] Confirm genotype of single colony (combined with other samples, see 2-3 MAT/ste4 KO of MA strains set 2 MAT Colony PCR).

FRIDAY, 2019-03-15

190322_2-4_MAT_Colony_PCR.jpg



8 strains were MAT wt: 49, 54, 60, 61, 63, 64, 71, 72.

1 strain was apparently diploid MAT wt/MATΔ::His3: 62.

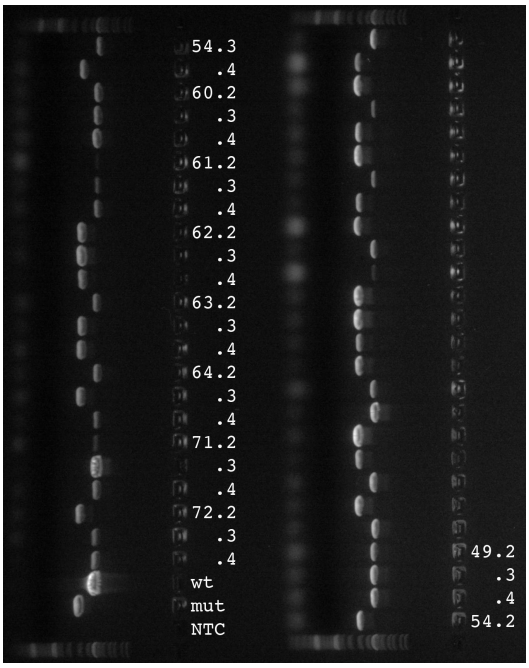
All other strains were MATΔ::His3.

TUESDAY, 2019-03-26

- [x] Confirm genotype of colonies .2, .3 and .4 for strains 49, 54, 60, 61, 62, 63, 64, 71, 72 combined with other samples (see 2-5 MAT/ste4 KO of MA strains set 4 MAT Colony PCR).

WEDNESDAY, 2019-03-27

190327_2-4_MAT_Colony_PCR.jpg



4 strains were MAT wt: 49, 60, 61, 71.

5 strain were MATΔ::His3: 54, 62, 63, 64, 72.

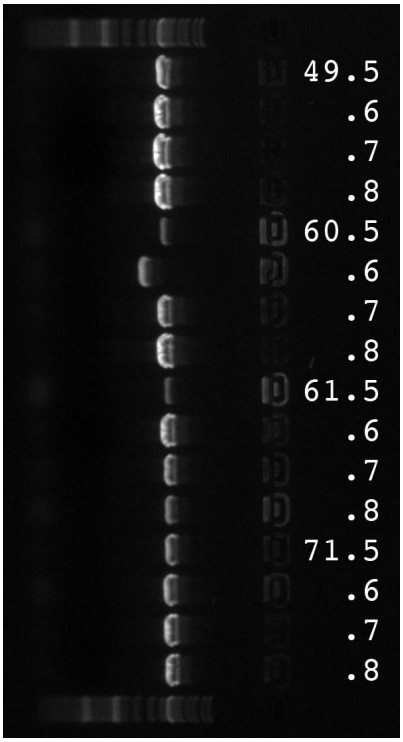
- [x] Restreak-out 4 transformants/strain to single colonies on a single SC-His-Trp plate for strains 49, 60, 61, 71.

FRIDAY, 2019-03-29

- [x] Confirm genotype of colonies .5, .6, .7 and .8 for strains 49, 60, 61, and 71 (combine samples with 2-5 MAT/ste4 KO of MA strains set 4 MAT Colony PCR.2). Matthew Stasiuk

TUESDAY, 2019-04-02

190402_2-4_MAT_Colony_PCR.2.jpg



3 strains were MAT wt: 49, 61, 71.

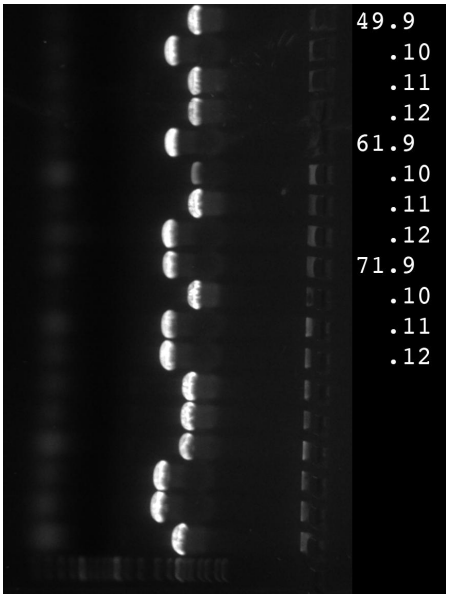
1 strain were MATΔ::His3: 60.

- [x] Restreak 4 transformants/strain to single colonies on a single SC-His-Trp plate for strains 49, 61, 71.

THURSDAY, 2019-04-11

- [x] Confirm genotype of colonies .9, .10, .11 and .12 for strains 49, 61, 71 (combine samples with 2-5 MAT/ste4 KO of MA strains set 4 MAT Colony PCR.3).

190411_2-4_MAT_Colony_PCR.3.jpg



All strain were MATΔ::His3: 49, 61, 71.

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	49	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE
3	50	TRUE											
4	51	TRUE											
5	52	NA	NA	NA	NA								
6	53	TRUE											
7	54	FALSE	TRUE	FALSE	TRUE								
8	55	TRUE											
9	56	TRUE											
10	57	TRUE											
11	58	TRUE											
12	59	TRUE											
13	60	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE				
14	61	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	TRUE
15	62	FALSE	TRUE	TRUE	TRUE								
16	63	FALSE	FALSE	TRUE	TRUE								
17	64	FALSE	FALSE	TRUE	FALSE								
18	65	TRUE											
19	66	TRUE											
20	67	TRUE											
21	68	TRUE											
22	69	TRUE											
23	70	TRUE											
24	71	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE	TRUE
25	72	FALSE	TRUE	FALSE	FALSE								

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/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.

- [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.

Results

	A	B
1	Strain #	# colonies
2	49	36
3	50	2
4	51	101
5	52	26
6	53	6
7	54	8
8	55	11
9	56	contaminated
10	57	4
11	58	6
12	59	9
13	60	9
14	61	contaminated
15	62	8
16	63	53
17	64	76
18	65	16
19	66	8
20	67	5
21	68	9
22	69	5
23	70	7
24	71	19
25	72	16

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
1	25

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		**25.5 X [µL]**
2	ddH2O	300.9
3	10X LA PCR Buffer	51
4	25 mM MgCl2	51
5	dNTP Mix (2.5 mM each)	81.6
6	10 µM OLPr022 STE4 -133 F	10.2
7	10 µM OLPr023 STE4 1402 R	10.2
8	LA Taq Polymerase (5 U/µL)	5.1
9	**Total**	**510**

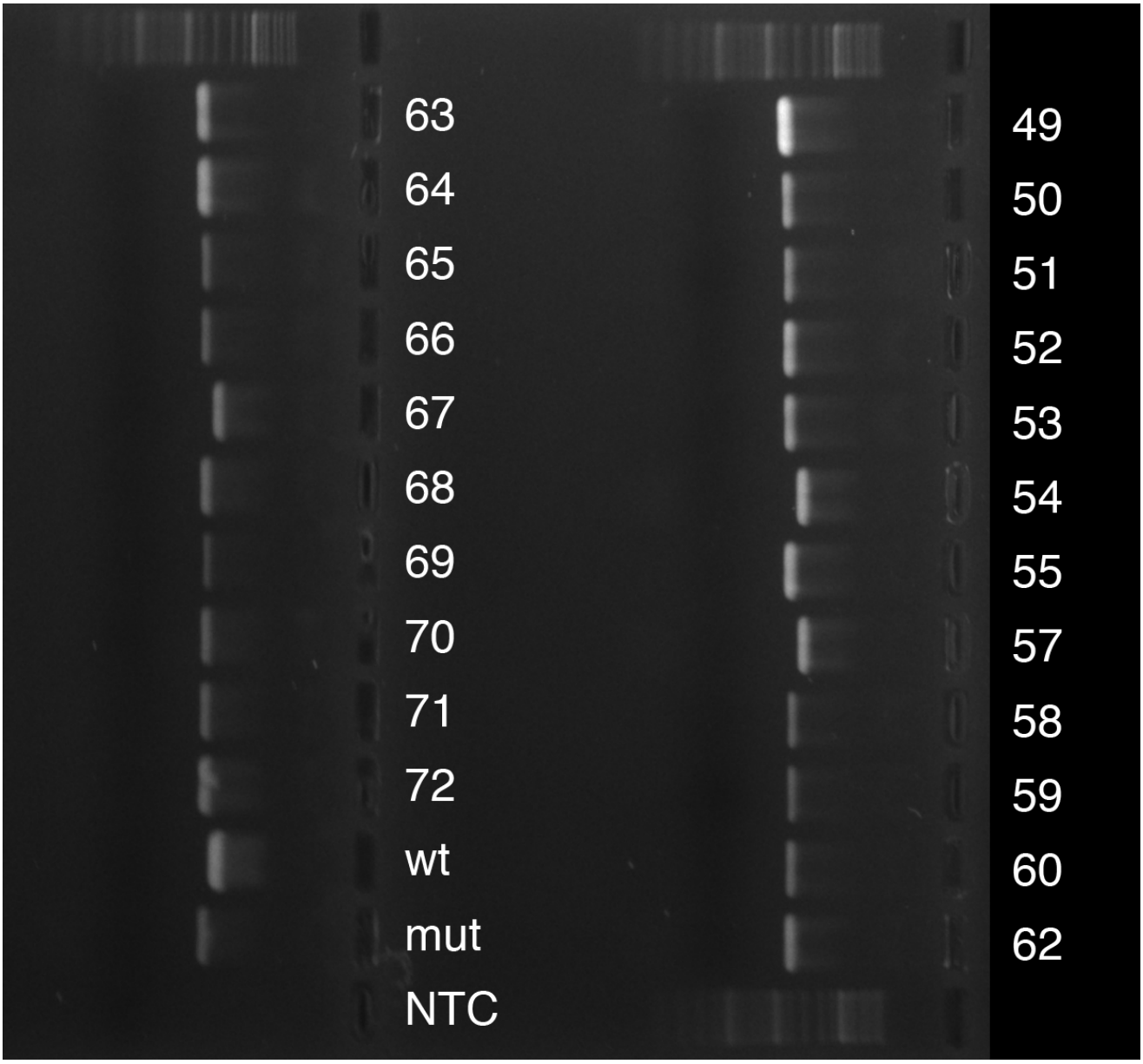
- 3. In sterile work environement (e.g. laminar flow), gently touch a yeast colony with a 10 µL pipette tip so a small number of cells stick to it and resuspend cells in PCR reaction.

  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/∞

- 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	12

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		**12.5 X [µL]**
2	ddH2O	147.5
3	10X LA PCR Buffer	25
4	25 mM MgCl2	25
5	dNTP Mix (2.5 mM each)	40
6	10 µM OLPr022 STE4 -133 F	5
7	10 µM OLPr023 STE4 1402 R	5
8	LA Taq Polymerase (5 U/µL)	2.5
9	**Total**	**250**

- ✓ 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.

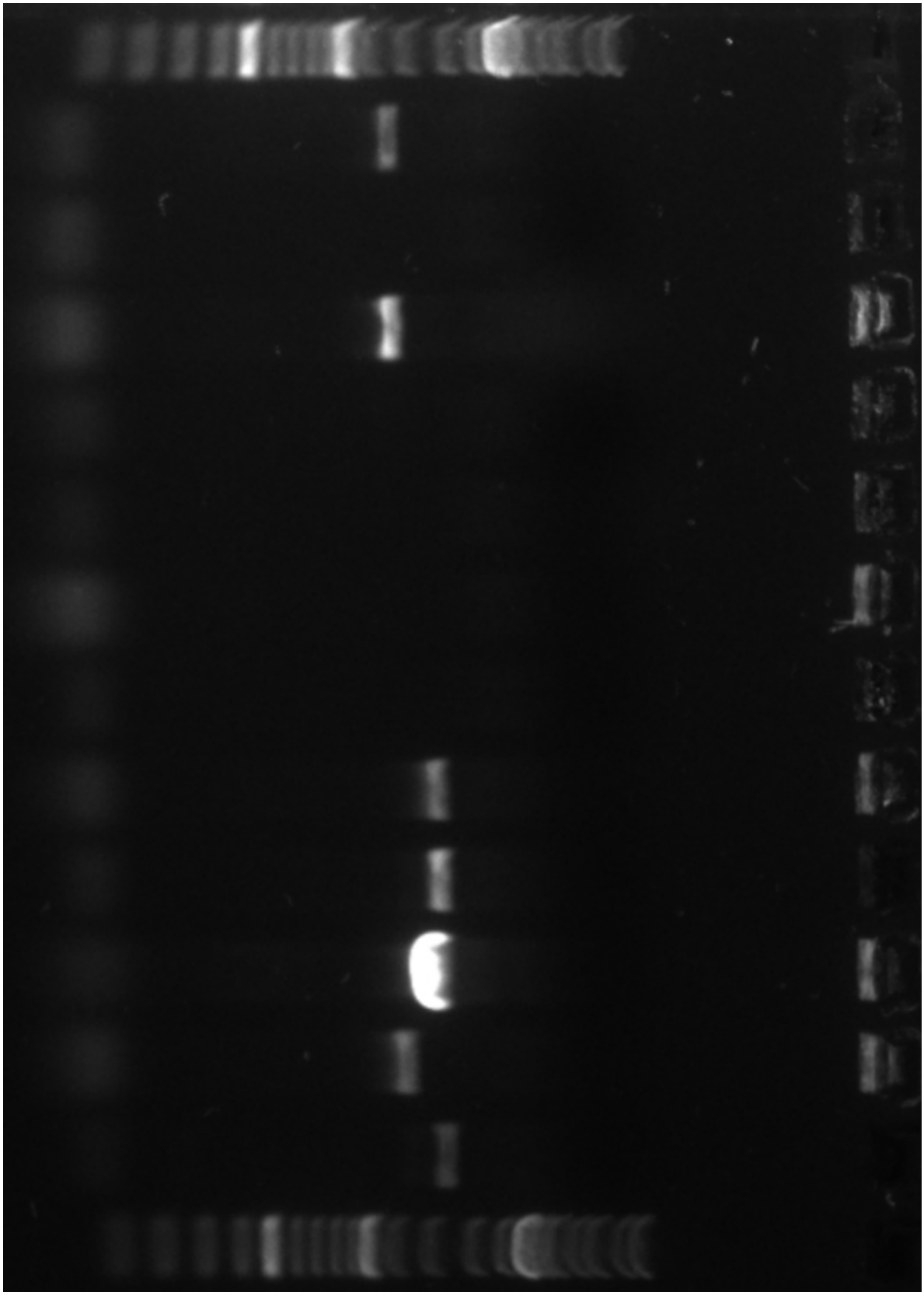
  Do not use too many cells as they might inhibit the PCR reaction. The PCR reaction should only look slightly opaque from the resuspended cells. If the reaction looks milky then you might have used too many cells.

- ✓ 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 (54, 57, 67)

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	12

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		**12.5 X [µL]**
2	ddH2O	147.5
3	10X LA PCR Buffer	25
4	25 mM MgCl2	25
5	dNTP Mix (2.5 mM each)	40
6	10 µM OLPr022 STE4 -133 F	5
7	10 µM OLPr023 STE4 1402 R	5
8	LA Taq Polymerase (5 U/µL)	2.5
9	**Total**	**250**

- 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.

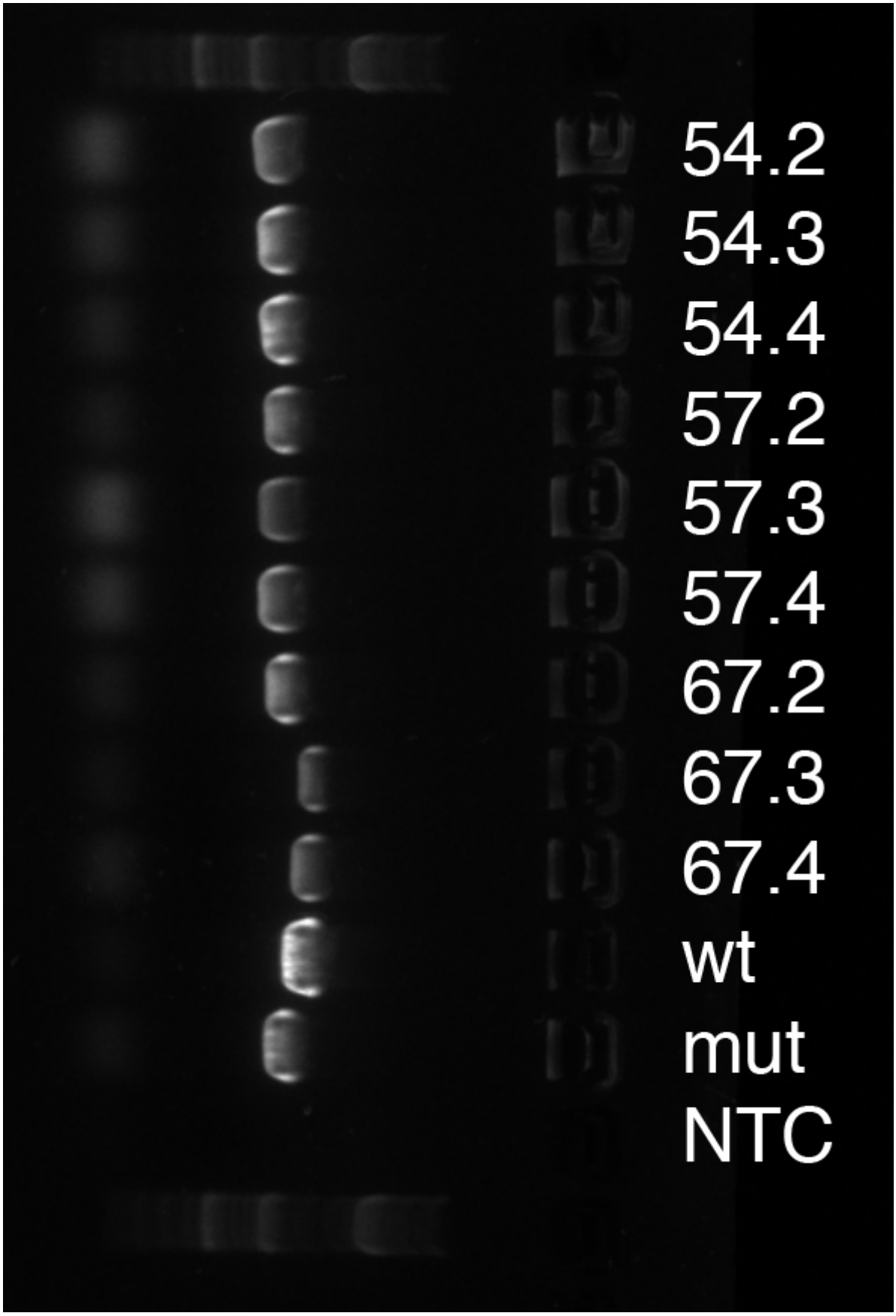
  Do not use too many cells as they might inhibit the PCR reaction. The PCR reaction should only look slightly opaque from the resuspended cells. If the reaction looks milky then you might have used too many cells.

- 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**

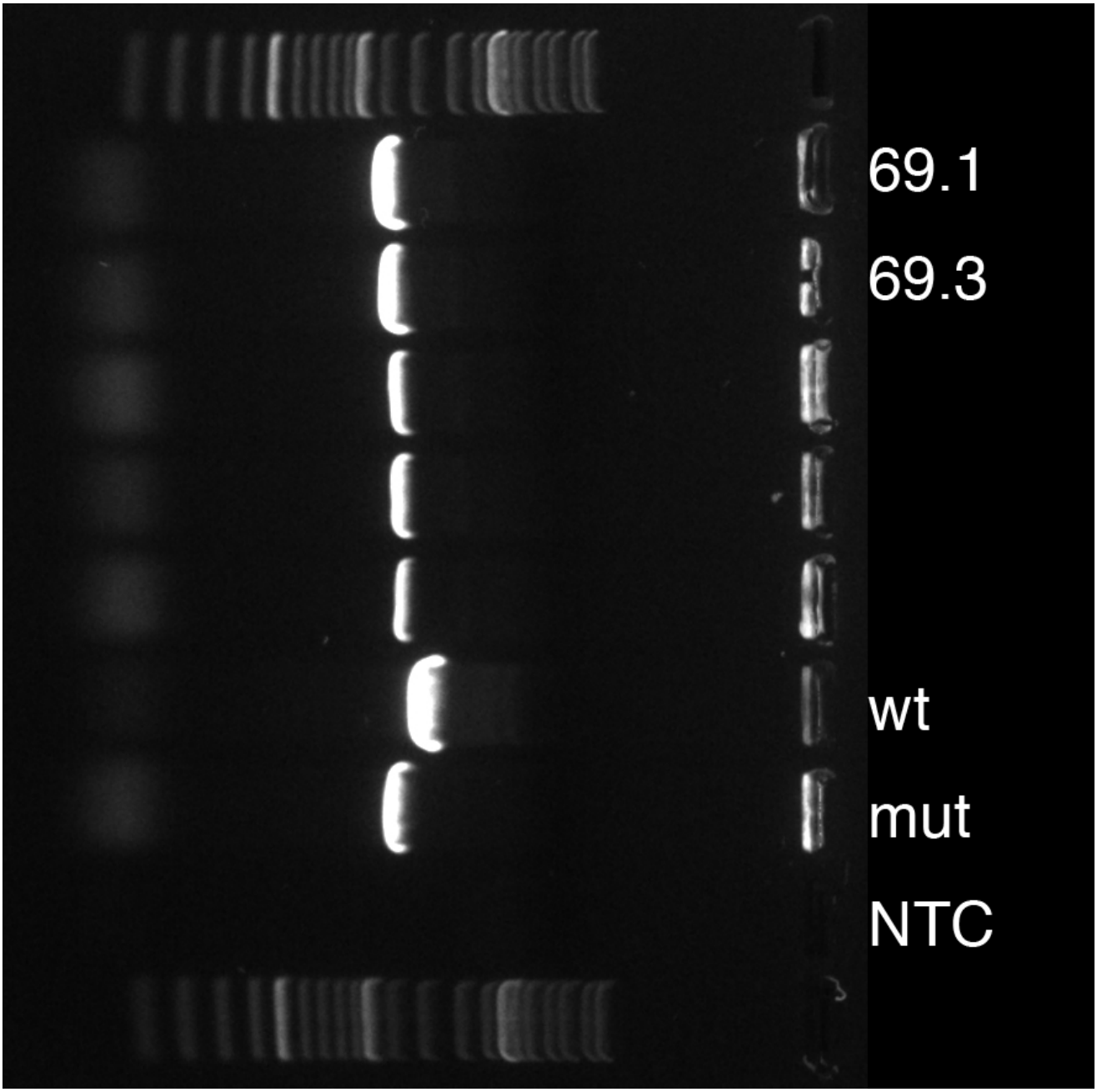
- 3. In sterile work environement (e.g. laminar flow), gently touch a yeast colony with a 10 µL pipette tip so a small number of cells stick to it and resuspend cells in PCR reaction.

  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

- [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	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 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 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	49	lawn
3	50	lawn
4	51	lawn
5	52	lawn
6	53	lawn
7	54	lawn
8	55	lawn
9	56	lawn
10	57	11
11	58	lawn
12	59	lawn
13	60	lawn
14	61	lawn
15	62	lawn
16	63	lawn
17	64	lawn
18	65	lawn
19	66	lawn
20	67	lawn
21	68	lawn
22	69	lawn
23	70	lawn
24	71	lawn
25	72	lawn

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	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.

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		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 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	49	27
3	50	37
4	51	7
5	52	0
6	53	32
7	54	42
8	55	32
9	56	8
10	57	38
11	58	4
12	59	5
13	60	42
14	61	30
15	62	10
16	63	33
17	64	20
18	65	10
19	66	6
20	67	18
21	68	15
22	69	24
23	70	35
24	71	36
25	72	34

Additional resources

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**