2-6 MAT/ste4 KO of MA strains set 5

MONDAY, 2019-01-21

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:
97-120

ste4 knock-out

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

TUESDAY, 2019-01-22

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

THURSDAY, 2019-01-24

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

FRIDAY, 2019-01-25

- [x] Prepare 1 mL competent cells (see ste4 KO Transformation protocol day 2).

Strain #99 liquid culture looked different from other strains, maybe is a contamination and did not prepare competent cells for it (but looked like yeast on backup plate).

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

MONDAY, 2019-01-28

Noticed contamination on plates #102 and 110, but possible to pick uncontaminated colonies.

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

FRIDAY, 2019-02-01

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

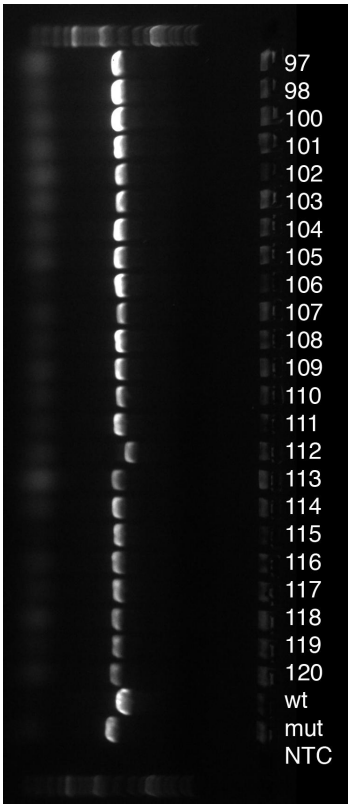
wt signal in NTC, likely due to contamination, unclear if PCR master mix was affected.

WEDNESDAY, 2019-02-06

- [x] Repeat: Confirm genotype of single clone, testing 10 µL reaction volume (see ste4 colony PCR.2 protocol).

THURSDAY, 2019-02-07

190207_2-6_STE4_Colony_PCR.2_set_5.jpg



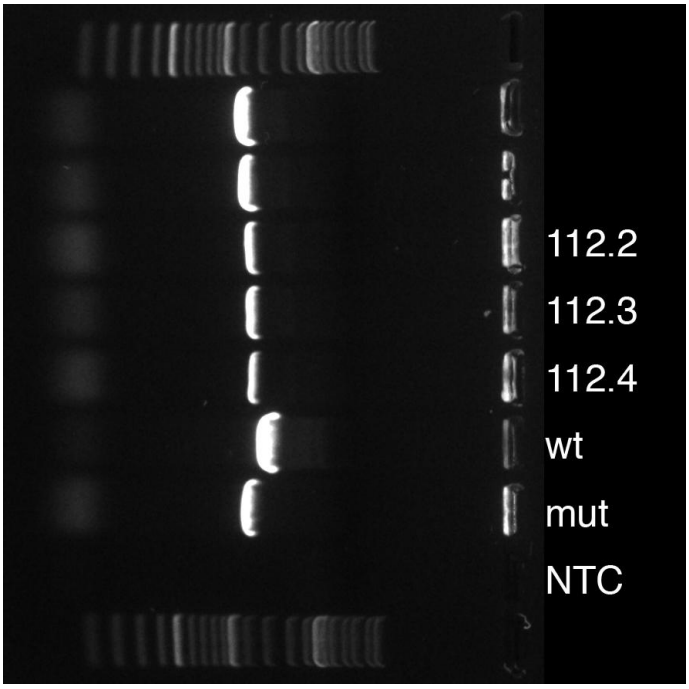
1 strain is ste4 wt: #112.

All other strains are ste4Δ::TRP1 (see table below).

TUESDAY, 2019-02-12

- [x] Confirm genotype of clone .2, .3, and .4 of strain #112 (see ste4 colony PCR.3 protocol, combined PCR with samples from 2-4).

190212_2-6_Colony_PCR.3_Set_5.jpg



All are ste4Δ::TRP1.

FRIDAY, 2019-03-01

- [x] Make backup plate and consolidate with ste4Δ::TRP1 strain of 99 from 2-9 MAT/ste4 KO of MA strains set 6.

ste4Δ::TRP1

	A	B	C	D	E
1	Strain #	colony .1	colony .2	colony .3	colony .4
2	97	TRUE			
3	98	TRUE			
4	99	NA			
5	100	TRUE			
6	101	TRUE			
7	102	TRUE			
8	103	TRUE			
9	104	TRUE			
10	105	TRUE			
11	106	TRUE			
12	107	TRUE			
13	108	TRUE			
14	109	TRUE			
15	110	TRUE			
16	111	TRUE			
17	112	FALSE	TRUE	TRUE	TRUE
18	113	TRUE			
19	114	TRUE			
20	115	TRUE			
21	116	TRUE			
22	117	TRUE			
23	118	TRUE			
24	119	TRUE			
25	120	TRUE			

SUNDAY, 2019-03-03

MAT knock-out

- [x] Inoculate overnight culture (see ste4 Colony PCR or MAT KO Transformation protocol day 1). Linnea Sandell
- [x] Prepare MAT KO PCR product (see 2-13 MAT KO PCR product for double KO).

MONDAY, 2019-03-11

Delayed transformation because of problems with SC-His-Trp plates. Christina prepared new plates. Will start new ONC.

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

TUESDAY, 2019-03-12

- [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).

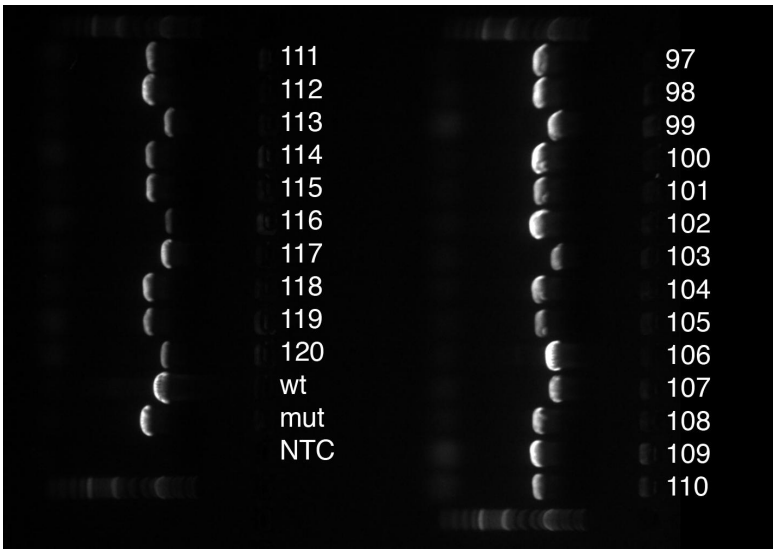
FRIDAY, 2019-03-15

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

TUESDAY, 2019-03-19

- [x] Confirm genotype of single colony (see MAT Colony PCR).

190319_2-6_MAT_Colony_PCR.jpg



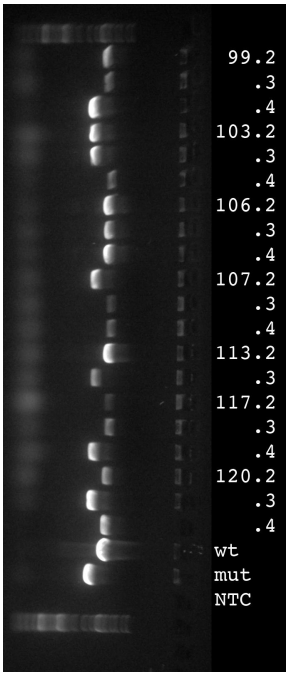
8 strains are MAT wt: #99, 103, 106, 107, 113, 116, 117, 120.
All other strains are MATΔ::His3 (see table below).
Retransform strain #116 with 2-9 MAT/ste4 KO of MA strains set 6.

WEDNESDAY, 2019-03-20

- [ ] Repeat: Confirm genotype of single colony for strains #99, 103, 106, 107, 113, 117, 120 of colonies .2, .3 and .4 (combined with other samples, see 2-3 MAT/ste4 KO of MA strains set 2 MAT Colony PCR).

THURSDAY, 2019-03-21

190322_2-6_MAT_Colony_PCR.2.jpg



Only MAT wt colonies for strain 116. Include in MAT KO transformation of 2-9 MAT/ste4 KO of MA strains set 6.

All other strains at least one colony that is MATΔ::His3 (see table below).

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
1	Strain #	colony .1	colony .2	colony .3	colony .4
2	97	TRUE			
3	98	TRUE			
4	99	FALSE	FALSE	FALSE	TRUE
5	100	TRUE			
6	101	TRUE			
7	102	TRUE			
8	103	FALSE	TRUE	TRUE	FALSE
9	104	TRUE			
10	105	TRUE			
11	106	FALSE	FALSE	FALSE	FALSE
12	107	FALSE	TRUE	FALSE	FALSE
13	108	TRUE			
14	109	TRUE			
15	110	TRUE			
16	111	TRUE			
17	112	TRUE			
18	113	FALSE	FALSE	TRUE	
19	114	TRUE			
20	115	TRUE			
21	116	FALSE			
22	117	FALSE	FALSE	FALSE	TRUE
23	118	TRUE			
24	119	TRUE			
25	120	FALSE	FALSE	TRUE	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.

01:20:00

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	23	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		25 X [mL]
2	1 M LiAc	2.5
3	sterile H2O	22.5
4	**Total**	**25**

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.

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		25 X [µL]
2	50% PEG	6000
3	1 M LiAc	900
4	ssDNA (2 mg/ml)	1250
5	ste4 KO PCR product	250
6	ddH2O	600
7	**Total**	**9000**

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.

Table1

	A	B	C
1	Strain #	# colonies	comments
2	97	28	
3	98	15	
4	100	15	
5	101	24	
6	102	21	contaminated by fungus
7	103	34	
8	104	14	
9	105	18	
10	106	17	
11	170	24	This is supposed to be 107, right?
12	108	14	
13	109	37	
14	110	15	contaminated by fungus
15	111	9	
16	112	19	
17	113	23	
18	114	7	
19	115	22	
20	116	10	
21	117	11	
22	118	19	
23	119	11	
24	120	13	

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 KO PCR

Introduction

The primers contain homologous sequences upstream (F) and downstream (R) of STE4 and bind to the Universal F1 or Universal R1 region flanking TRP1 on OLP44. The PCR product is used to delete STE4 with TRP1.

Materials

- TaKaRa LA Taq® DNA Polymerase (Mg2+ free buffer) RR002A
- 10 µM Primer F: OLPr010 STE4 -37 UKO F
- 10 µM Primer R: OLPr011 STE4 1309 UKO R
- Template: OLP044

Procedure

PCR

1. Chose the number of 50 µL reactions (include one additional reaction for no-template control):

Settings

	# of reactions
1	20

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		**20.5 X [µL]**
2	ddH2O	584.25
3	10X LA PCR Buffer	102.5
4	25 mM MgCl2	102.5
5	dNTP Mix (2.5 mM each)	164
6	10 µM OLPr010 STE4 -37 UKO F	20.5
7	10 µM OLPr011 STE4 1309 UKO R	20.5
8	LA Taq Polymerase (5 U/µL)	10.25
9	OLP044 (pFA6a-TRP1) (5 ng/µL)	20.5
10	**Total**	**1025**

- 3. PCR program: 94°C/2 min—[94°C/0.5 min—61°C/0.5 min—68°C/1 min]x30—68°C/5 min—10°C/∞

Gel

- 4. Pool all PCR reactions except for controls.
- 5. Gel: sample volume see below/0.5 µg O'GeneRuler DNA Ladder Mix/1% TAE/120 V/35 min

  Load 0.1, 0.2, 0.4 and 0.8 µL of the PCR product (i.e. by diluting PCR product 1:10 by mixing 2 µL product with 18 µL H_2O, then load 1, 2, 4, and 8 µL of the diluted product).
  Load 10 µL of no-template control.
  Expected product size: 1043 bp

Additional Resources

Primers and template

	A	B
1	Forward Primer	OLPr010 STE4 -37 UKO F
2	Reverse Primer	OLPr011 STE4 1309 UKO R
3	Template	OLP044 (pFA6a-TRP1)

PCR Volume and reagent concentrations

	A	B
1	PCR Volume [µL]	50
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]	0.1

Single PCR reaction

	A	B
1		[µL]
2	ddH2O	28.5
3	10X PCR Buffer	5
4	25 mM MgCl2	5
5	dNTP Mix (2.5 mM each)	8
6	10 µM Primer F	1
7	10 µM Primer R	1
8	LA Taq Polymerase (5 U/µL)	0.5
9	Template (5 ng/µL)	1
10	Total	50

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	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**

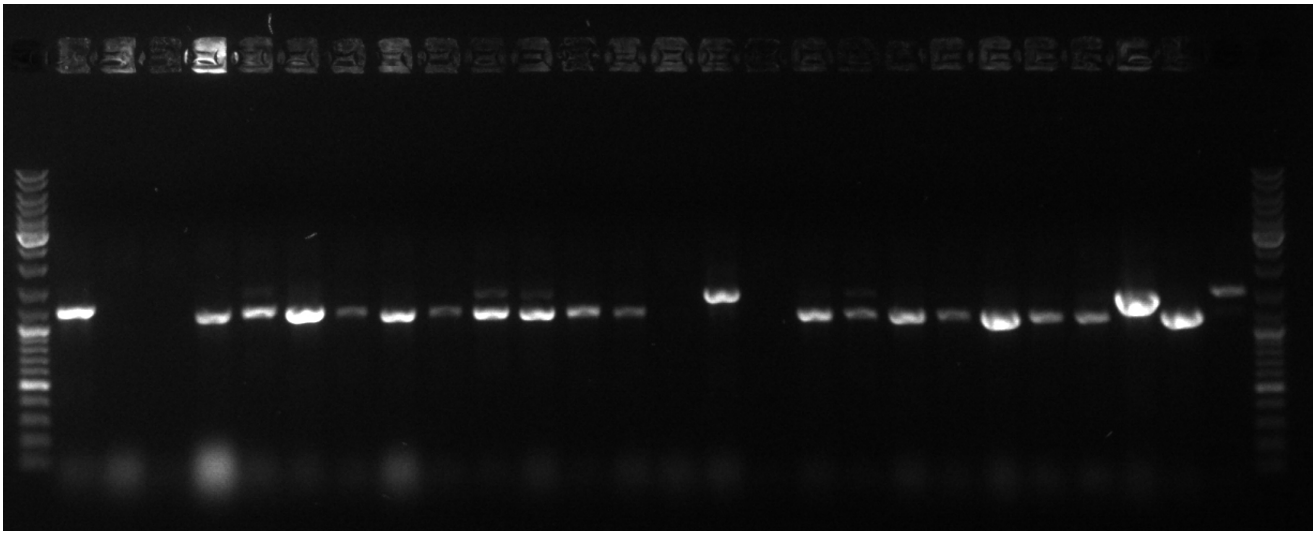
- 3. In sterile work environement (e.g. laminar flow), gently touch a yeast colony with a 10 µL pipette tip so a small number of cells stick to it and resuspend cells in PCR reaction.

  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.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	156.35
3	10X LA PCR Buffer	26.5
4	25 mM MgCl2	26.5
5	dNTP Mix (2.5 mM each)	42.4
6	10 µM OLPr022 STE4 -133 F	5.3
7	10 µM OLPr023 STE4 1402 R	5.3
8	LA Taq Polymerase (5 U/µL)	2.65
9	**Total**	**265**

- 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.

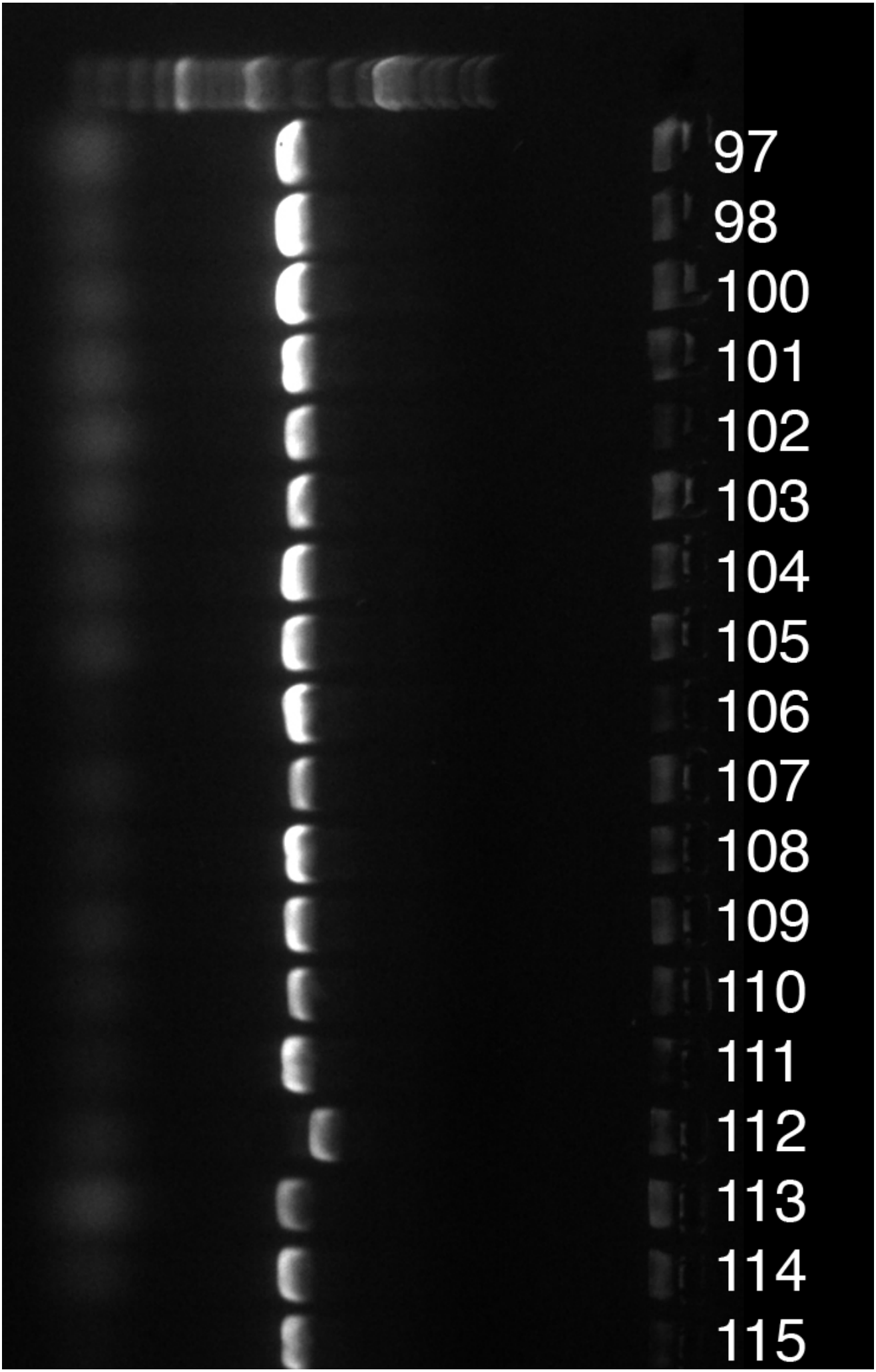
  Do not use too many cells as they might inhibit the PCR reaction. The PCR reaction should only look slightly opaque from the resuspended cells. If the reaction looks milky then you might have used too many cells.

- 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).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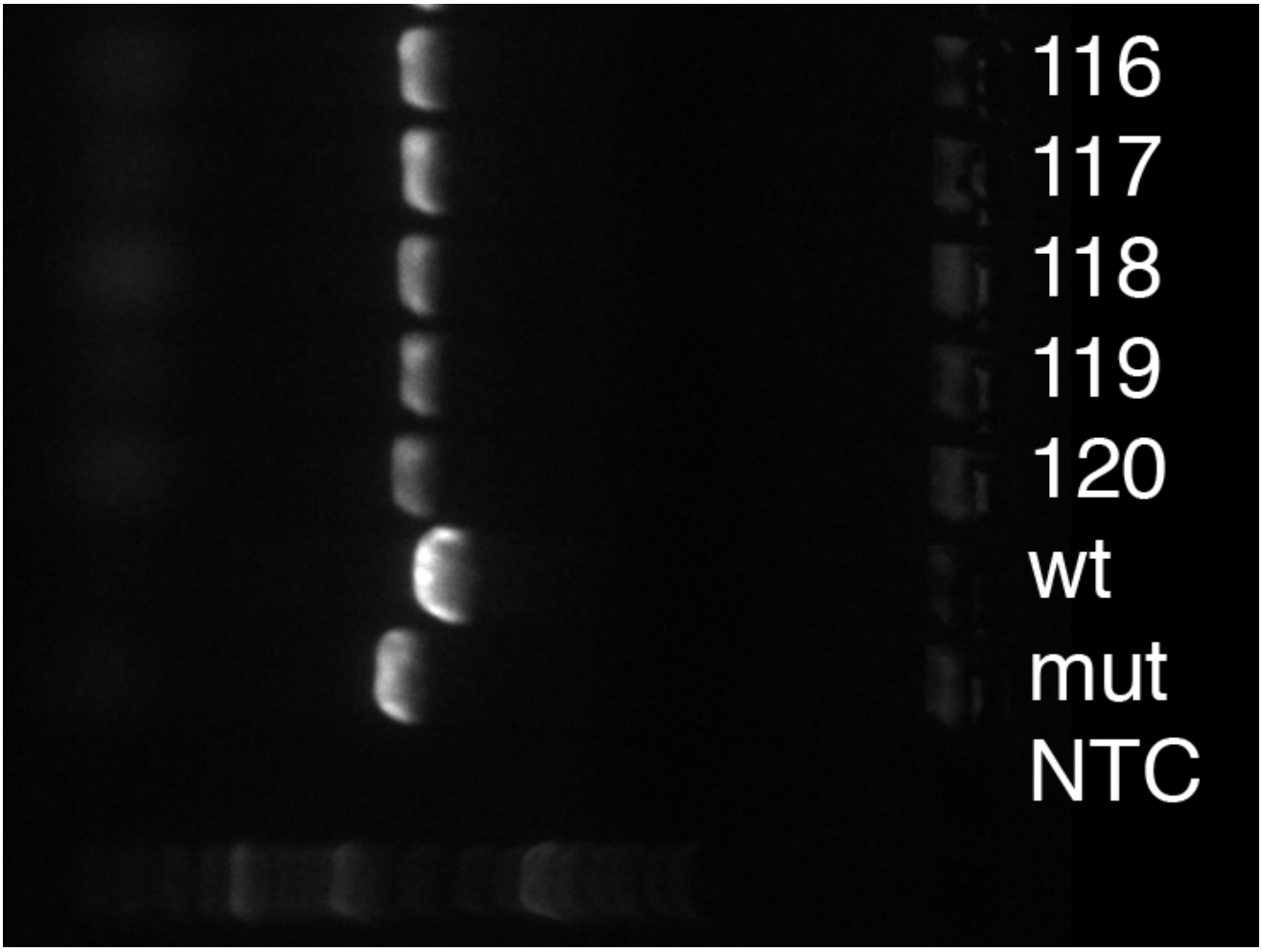


Additional resources

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

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 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

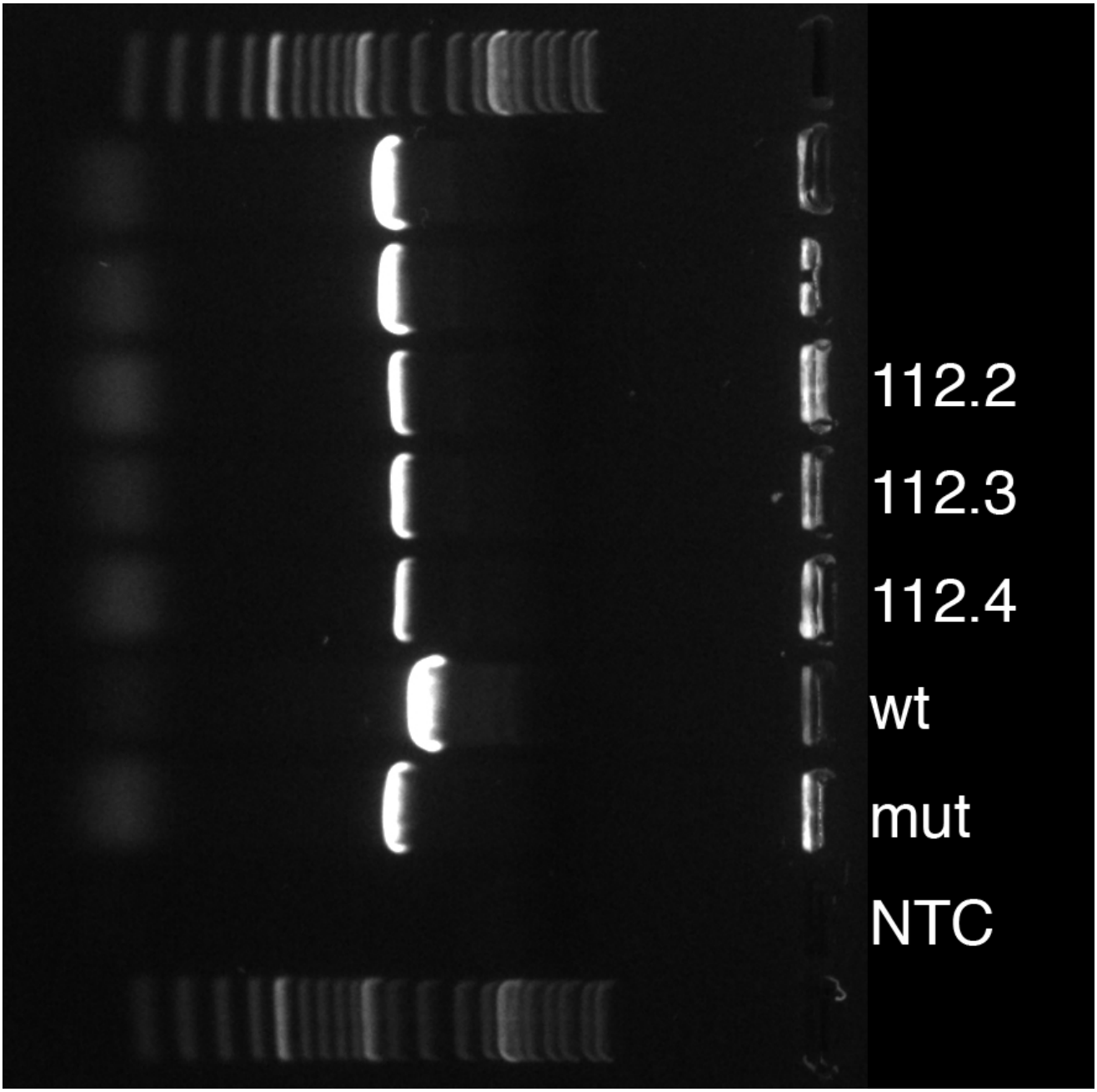
00:35:00

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.
Lines used for this transformation: 97, 98, 100-120

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	C
1	Strain #	# colonies	comments
2	97	2	
3	98	3	
4	99	98	
5	100	62	
6	101	3	
7	102	3	
8	103	10	
9	104	4	
10	105	2	
11	106	59	
12	107	6	
13	108	3	
14	109	5	
15	110	6	
16	111	7	
17	112	3	
18	113	3	
19	114	5	
20	115	1	
21	116	1	
22	117	10	
23	118	3	
24	119	1	
25	120	23	

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	27

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		**27.5 X [µL]**
2	ddH2O	162.25
3	10X LA PCR Buffer	27.5
4	25 mM MgCl2	27.5
5	dNTP Mix (2.5 mM each)	44
6	10 µM OLPr014 MAT -283 F	5.5
7	10 µM OLPr015 MAT +295 R	5.5
8	LA Taq Polymerase (5 U/µL)	2.75
9	**Total**	**275**

- 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":

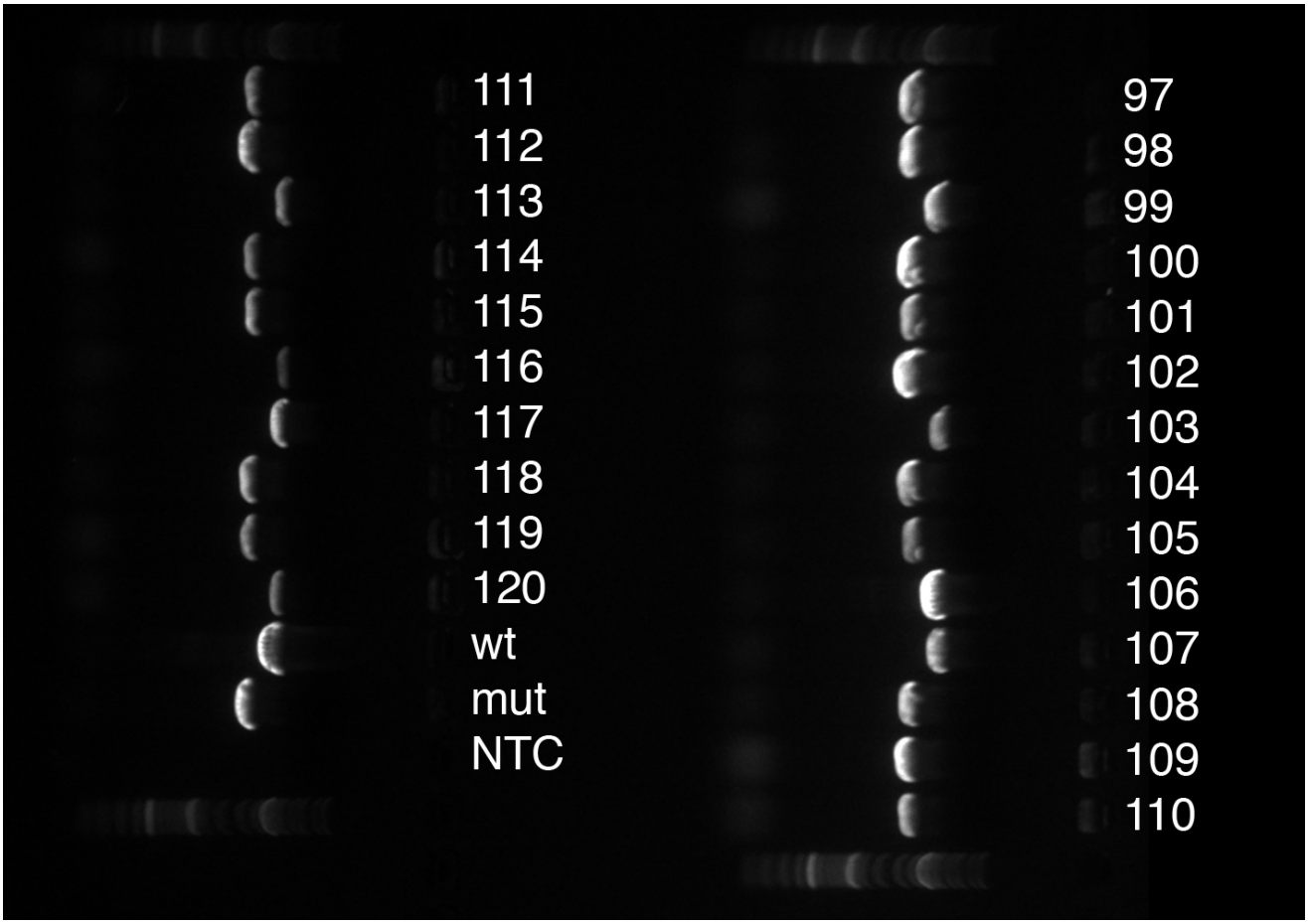
  94°C/5 min—[94°C/0.5 min—61°C/0.5 min—68°C/3 min]x35—68°C/5 min—10°C/∞

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