2-1 MAT/ste4 KO of MA strains set 1

MONDAY, 2018-11-26

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 transfomed with either OLP003 (carries ste4; transformant will mate like a MATa strain) or OLP004 (carries both ste4 and MATα; tranformant will mate like a MATα strain) and the two resulting transformants are mated to each other which result in entirely homozygous strains which are either diploid or polyploid. Finally, each candidate strain is tested for ploidy-level by flow cytometry.

- [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:
1–12, 14–20, 22-24

ste4 knock-out

- [x] Streak-out to single colonies on YPG plate and then patch on grid (provided by Nathaniel [NOTE: used frozen strains from Aliquot 2]).

MONDAY, 2018-12-03

- [x] Prepare ste4 KO PCR product (see ste4 KO PCR protocol).

Estimated PCR product concentration: 100 ng/µL

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

TUESDAY, 2018-12-04

- [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).

Not enough transformation mix for strain #24 (less than 200 µL, instead of 360 µL).

FRIDAY, 2018-12-07

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

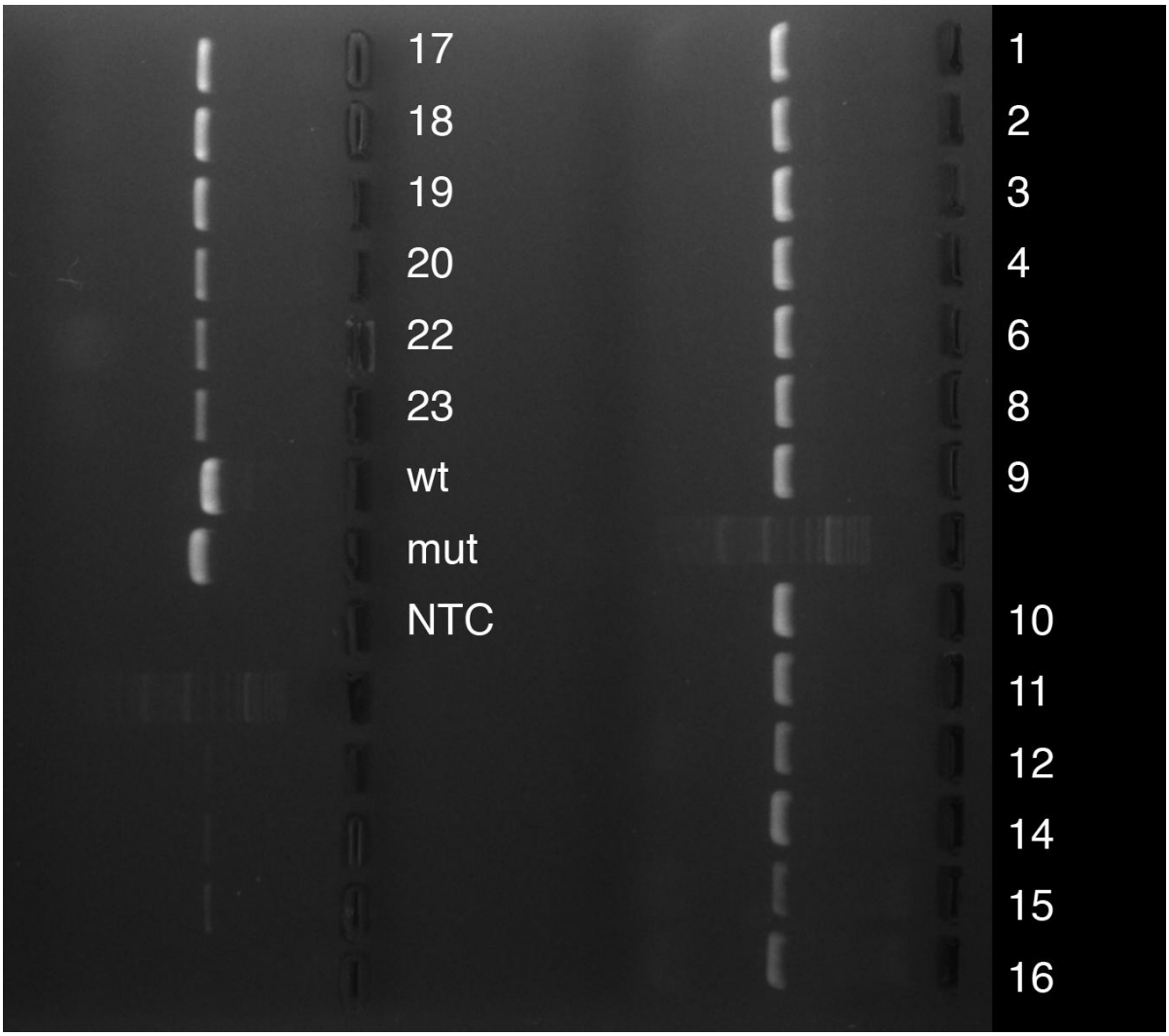
Did not receive transformants for strain #5, 7, and 24 (#24 likely due to reduced amount of transformation mix).

MONDAY, 2018-12-10

- [x] Confirm genotype of single colony of #1, 2, 3, 4, 6, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 22, 23 (see ste4 Colony PCR protocol).

TUESDAY, 2018-12-11

181211_2-1_STE4_Colony_PCR_MAT_KO_PCR_Set_1.jpg



- [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
1	Strain #	colony .1
2	1	TRUE
3	2	TRUE
4	3	TRUE
5	4	TRUE
6	5	Retransform
7	6	TRUE
8	7	Retransform
9	8	TRUE
10	9	TRUE
11	10	TRUE
12	11	TRUE
13	12	TRUE
14	14	TRUE
15	15	TRUE
16	16	TRUE
17	17	TRUE
18	18	TRUE
19	19	TRUE
20	20	TRUE
21	22	TRUE
22	23	TRUE
23	24	Retransform

MAT knock-out

- [x] Inoculate overnight culture (see STE4 Colony PCR or MAT KO Transformation protocol day 1).
- [x] Prepare MAT KO PCR product (see MAT KO PCR protocol, executed by Christina).

WEDNESDAY, 2018-12-12

- [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, 2018-12-14

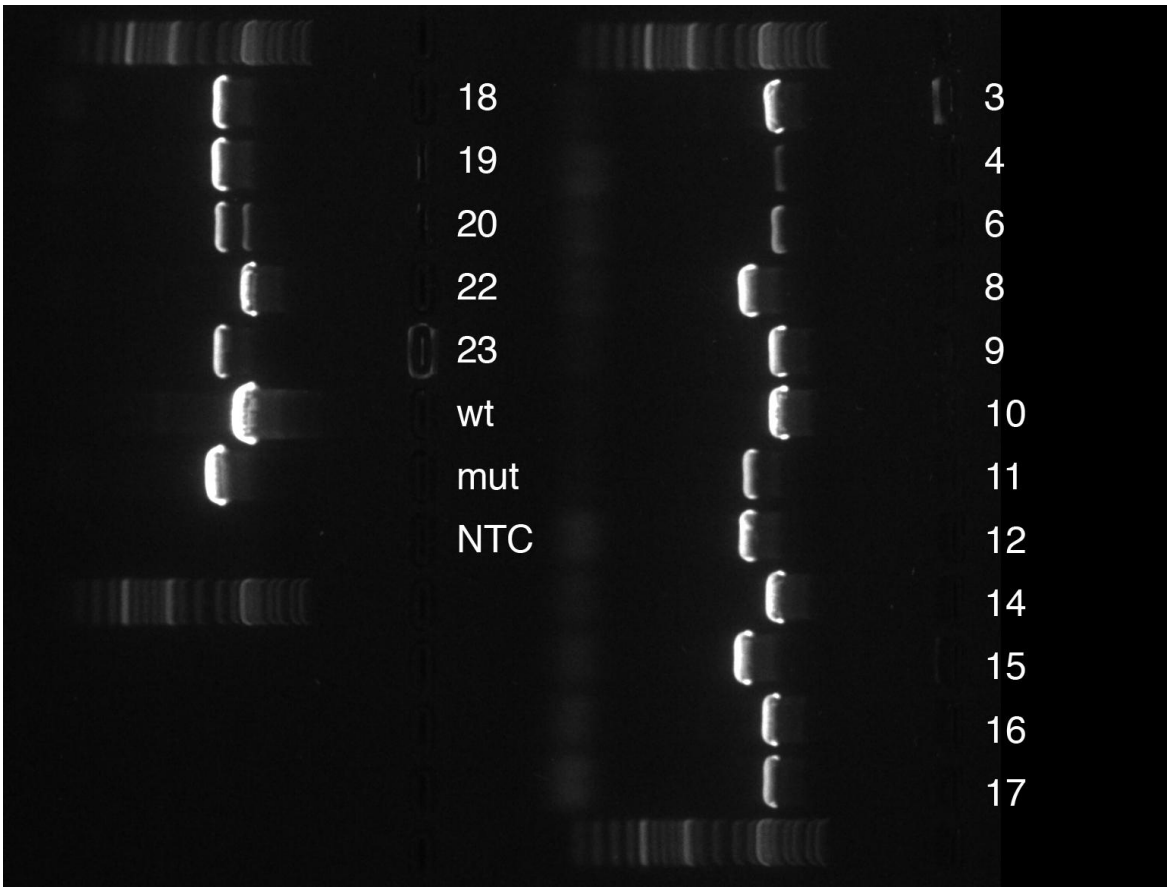
- [x] Streak-out a single transformant to single colonies on SC-His-Trp plate.

Did not receive any transformants for strain #1, and 2.

MONDAY, 2018-12-17

- [x] Confirm genotype of single colony of #3, 4, 6, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 22, 23 (see MAT Colony PCR protocol).

181217_2-1_MAT_Colony_PCR_Set_1.jpg



7 strains are MATΔ::His3 (#8, 11, 12 15, 18, 19, 23), i.e. 41%.
9 strains are MATwt (#3, 4, 6, 9, 10, 14, 16, 17, 22).
Apparently 1 is diploid MATΔ::His3/MATwt (#20).

MONDAY, 2019-01-07

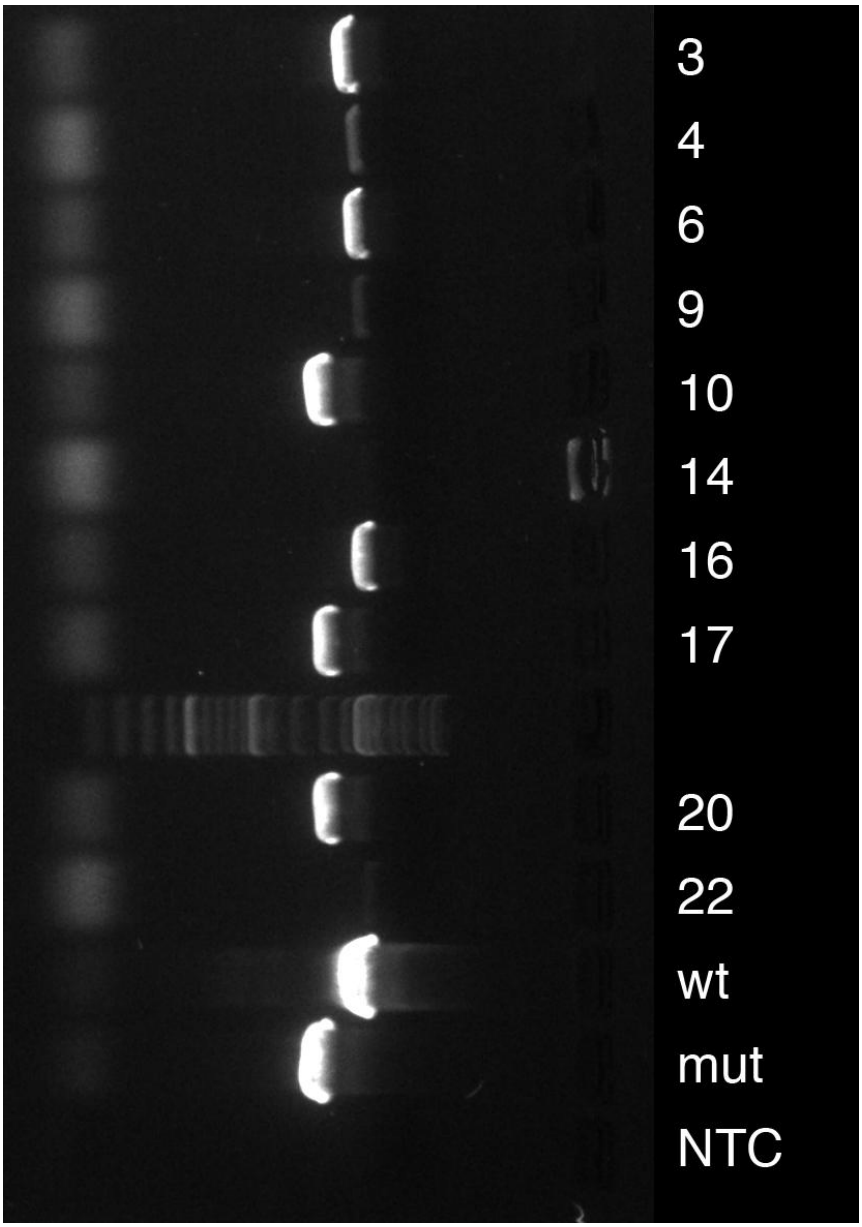
- [x] If MATΔ::His3 genotype is confirmed, proceed with plasmid transformation; otherwise repeat streak-out and colony PCR with another transformant.

Restreaked #3, 4, 6, 9, 10, 14, 16, 17, 20, 22.

WEDNESDAY, 2019-01-09

- [x] Confirm genotype of single colony of #3, 4, 6, 9, 10, 14, 16, 17, 20, 22 (see MAT Colony PCR.2).

190109_2-1_MAT_Colony_PCR_Set_1_restreaks.jpg

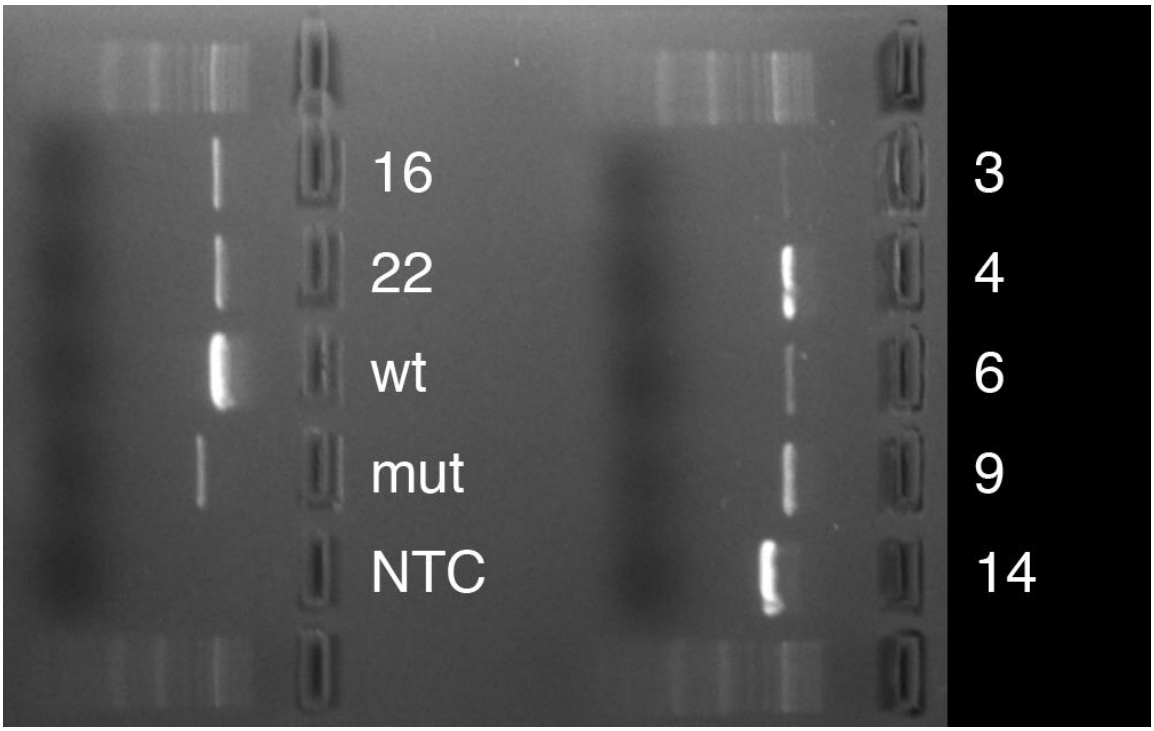


3 strains are MATΔ::His3: #10, 17, and 20.
7 strains are MATwt: #3, 4, 6, 9, 14, 16, 22.
Restreaked #3, 4, 6, 9, 14, 16, 22.

MONDAY, 2019-01-14

- [x] Confirm genotype of single colony of #3, 4, 6, 9, 14, 16, 22 (see MAT Colony PCR.3).

190115_2-1_MAT_Colony_PCR.3.jpg



1 strains is MATΔ::His3: #14.

6 strains are MATwt: #3, 4, 6, 9, 16, 22.

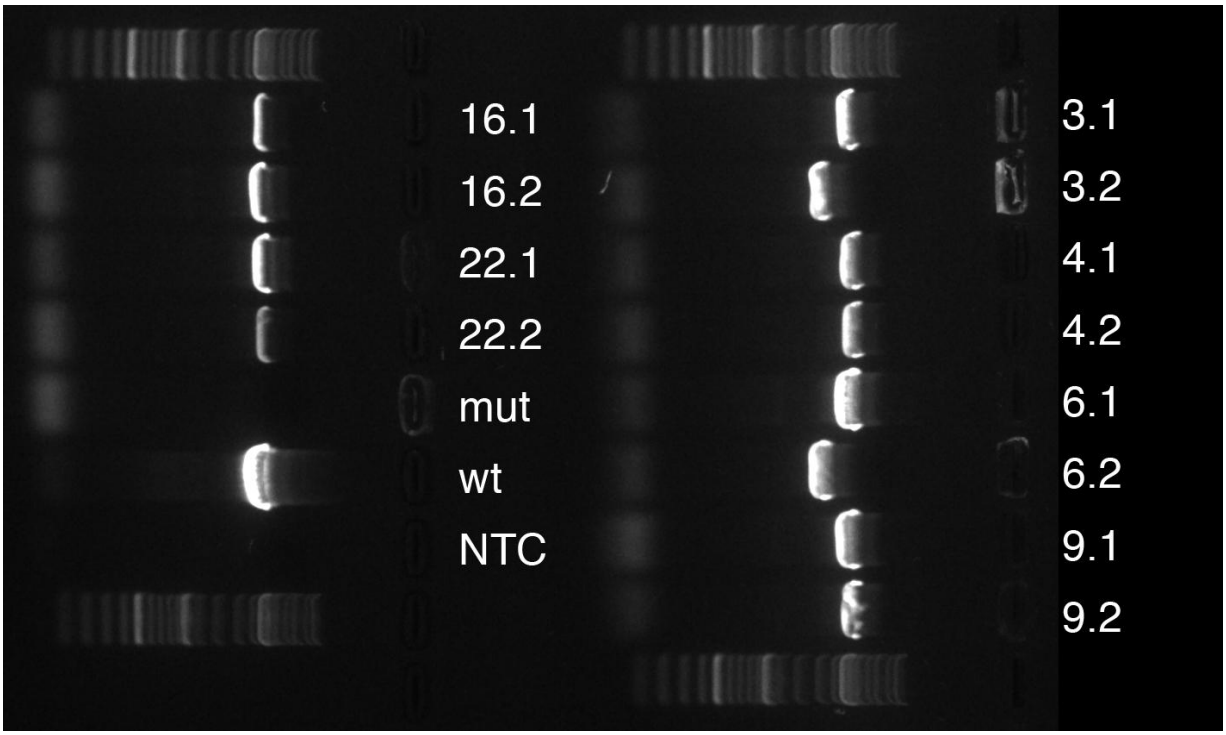
TUESDAY, 2019-01-15

- [x] Restreaked #3, 4, 6, 9, 16, 22 four colonies each (colonies .1 to .4).

FRIDAY, 2019-01-18

- [x] Confirm genotype of single colony of #3, 4, 6, 9, 16, 22 for colonies .1 and .2 (see MAT Colony PCR.4).

190118_2-1_MAT_Colony_PCR.4.jpg



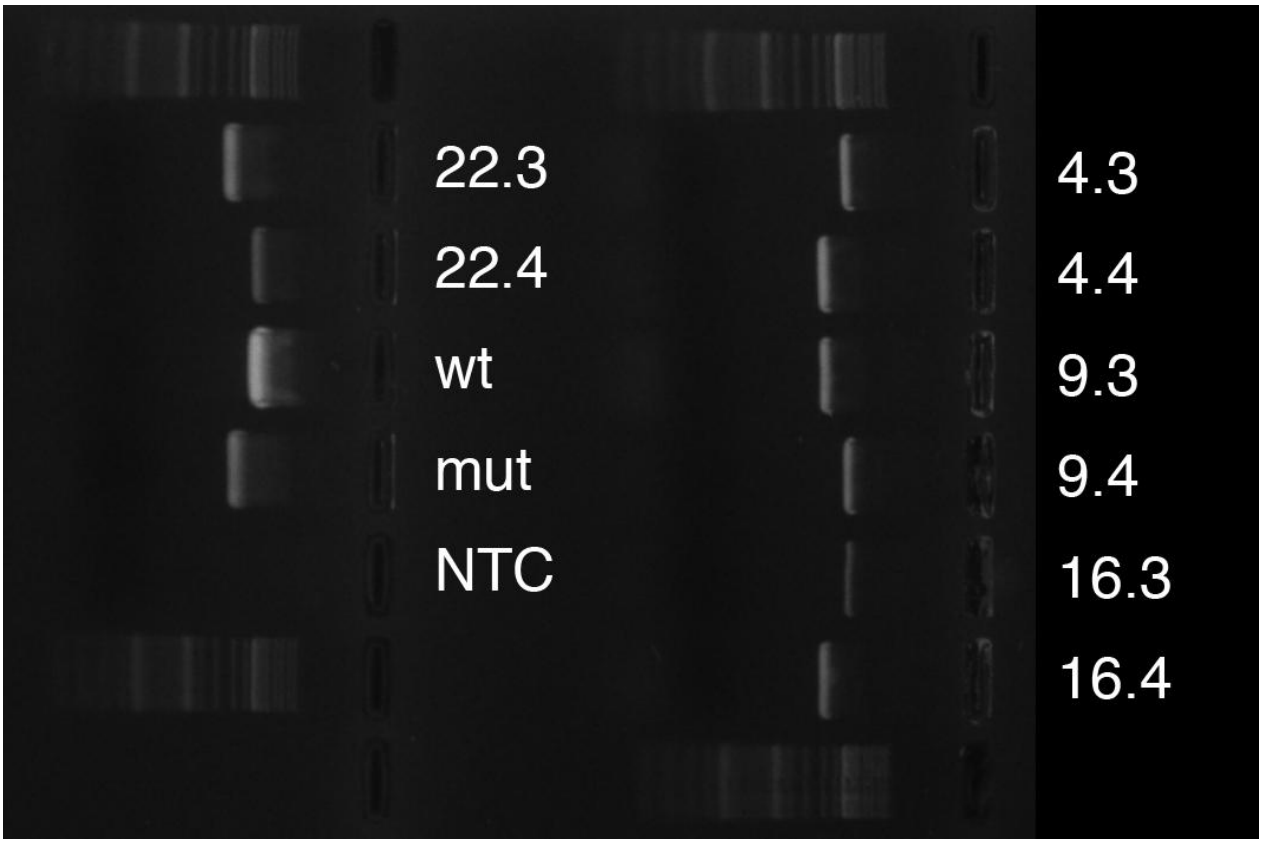
2 strains are MATΔ::His3: #3.2 and 6.2.

TUESDAY, 2019-01-22

- [x] Confirm genotype of single colony of #4, 9, 16, 22 for colonies .3 and .4 (see MAT Colony PCR.5). Christina Hsu

FRIDAY, 2019-01-25

190125_2-1_MAT_Colony_PCR.5.jpg



4 strains are MATΔ::His3: #4.4, 9.3, 16.4, 22.3.

FRIDAY, 2019-02-01

- [x] Prepare new patch plate of all successful double knock outs #3, 4, 6, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 22, 23 on SC-His-Trp.
  Christina Hsu

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.

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	B
1	6	

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

Master mix

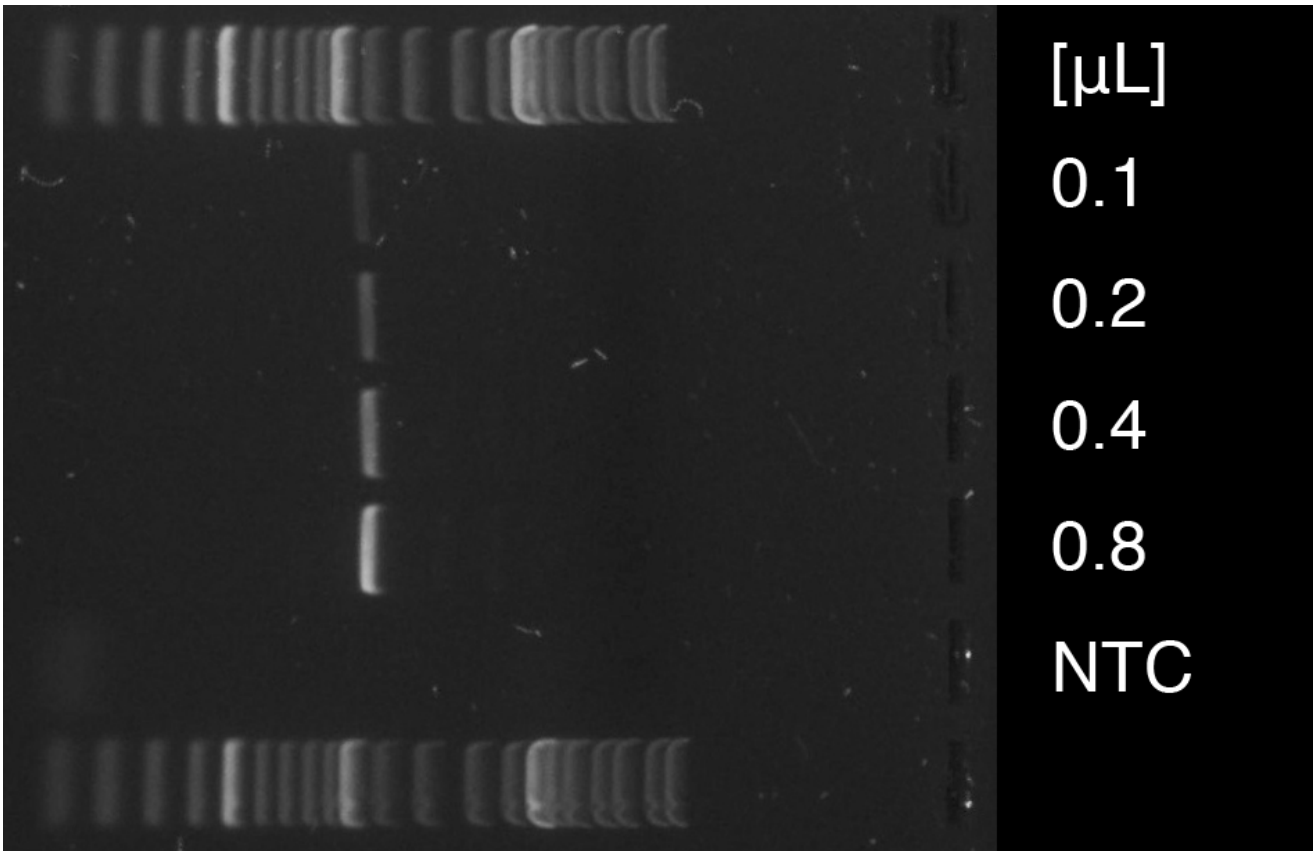
	A	B
1		**6.5 X [µL]**
2	ddH2O	185.25
3	10X LA PCR Buffer	32.5
4	25 mM MgCl2	32.5
5	dNTP Mix (2.5 mM each)	52
6	10 µM OLPr010 STE4 -37 UKO F	6.5
7	10 µM OLPr011 STE4 1309 UKO R	6.5
8	LA Taq Polymerase (5 U/µL)	3.25
9	OLP044 (pFA6a-TRP1) (5 ng/µL)	6.5
10	**Total**	**325**

- ✓ 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
  Product size seems correct. Estimated 60 ng PCR product in 0.6 µL = 100 ng/µL



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 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

- [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
- [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	20	100

- [x] 5. Prepare 0.1 M LiAc, freshly diluted from 1 M LiAc:

0.1 M Li Ac

	Materials	mL
1		22 X [mL]
2	1 M LiAc	2.2
3	sterile H2O	19.8
4	**Total**	**22**

- [x] 6. Centrifuge 3,000 g/5 min; discard supernatant and resuspend cell pellet in 1 mL sterile H_2O.
- [x] 7. Centrifuge 3,000 g/5 min. Discard supernatant and resuspend cells in 1 ml of 0.1 M LiAc.
- [x] 8. Centrifuge 13,000 g/1 min; discard supernatant
- [x] 9. 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		22 X [µL]
2	50% PEG	5280
3	1 M LiAc	792
4	2mg/ml ssDNA	1100
5	STE4 KO PCR product	220
6	ddH2O	528
7	**Total**	**7920**

- [x] 10. Add 360 µL of transformation master mix to each tube.
- [x] 11. Vortex tubes until the cells are smoothly suspended.
- [x] 12. Heat shock cells in water-bath or heat-block: 42°C/1 h.
- [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-Trp.

    There was not enough transformation mix for strain 24.

Received colonies

	A	B	C
1	Strain	Total colonies	Large colonies
2	1	9	2
3	2	4	1
4	3	5	2
5	4	3	1
6	5	1	0
7	6	7	3
8	7	0	0
9	8	7	1
10	9	45	8
11	10	9	5
12	11	5	2
13	12	9	5
14	14	3	1
15	15	14	4
16	16	8	3
17	17	6	2
18	18	16	5
19	19	12	1
20	20	17	6
21	22	10	2
22	23	14	1
23	24	0	0

Additional resources

PCR product amou...

	STE4 KO PCR product [µg]
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

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	22

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		**22.5 X [µL]**
2	ddH2O	265.5
3	10X LA PCR Buffer	45
4	25 mM MgCl2	45
5	dNTP Mix (2.5 mM each)	72
6	10 µM OLPr022 STE4 -133 F	9
7	10 µM OLPr023 STE4 1402 R	9
8	LA Taq Polymerase (5 U/µL)	4.5
9	**Total**	**450**

- 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/∞

Reference plate and overnight culture

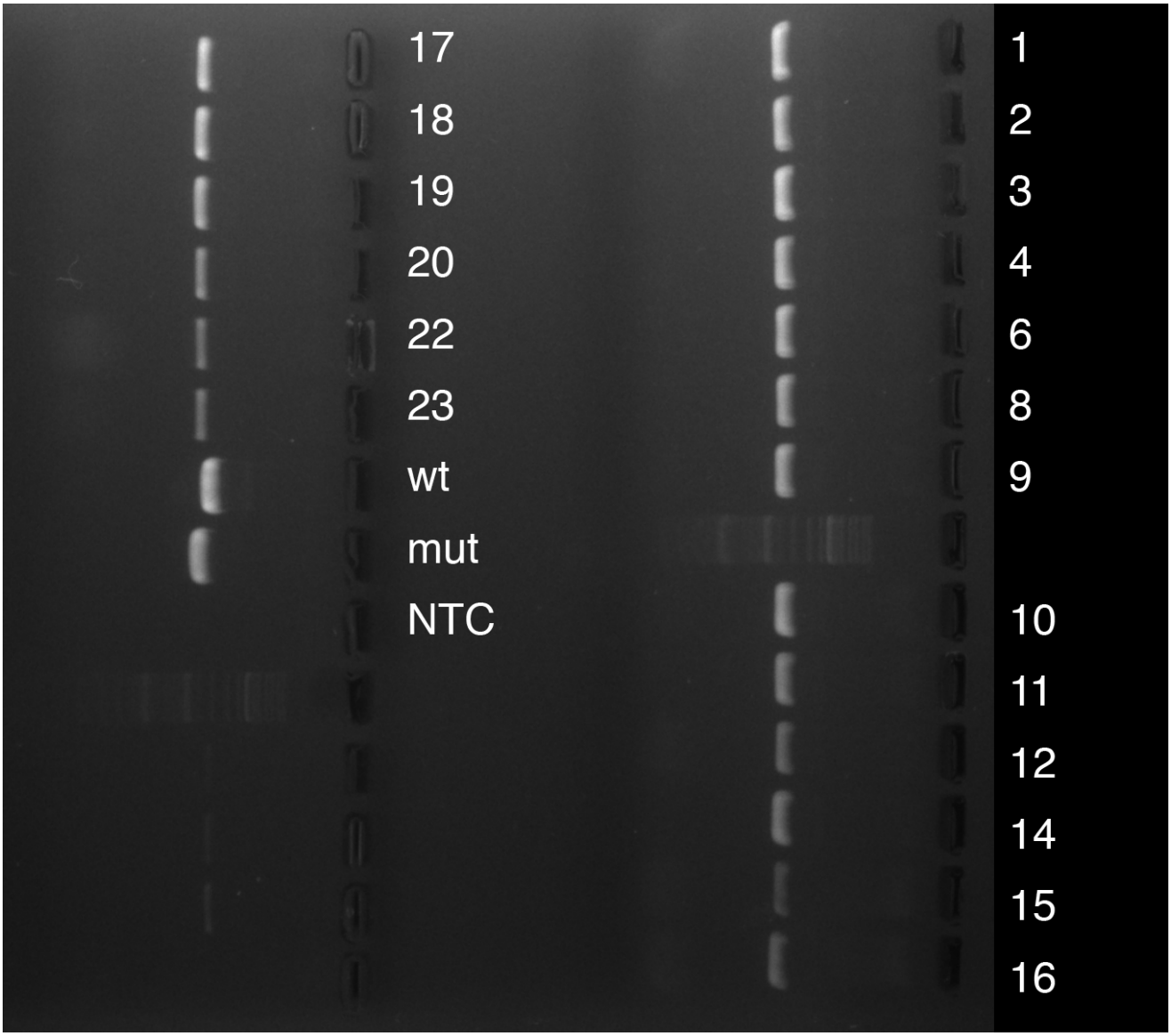
- 5. Scrape off the same colony used in the PCR with a cotton swab, inoculate a square patch on a SC-Trp reference plate and incubate plate at 30°C/overnight (or until yeast lawn is clearly visible. Optional: resuspend the remaining cells on the swab in 2 mL YPAD for use as an overnight culture for the next transformation and grow it on the shaker at 30°C/200 rpm/overnight.

  A reference plate contains multiple patches of different yeast strains on a grid.

Gel

- 6. 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).
  All tested strains show the ste4Δ::TRP1 genotype.



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

MAT KO PCR

Introduction

The primers contain homologous sequences upstream (F) and downstream (R) of MAT and bind to the Universal F1 or Universal R1 region flanking His3 on OLP45. The PCR product is used to delete MAT locus with His3.

Materials

- TaKaRa LA Taq® DNA Polymerase (Mg2+ free buffer) RR002A
- 10 µM OLPr008 MAT -33 UKO F
- 10 µM OLPr012 MAT +34 SEY UKO R
- Template: OLP045

Procedure

PCR

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

Settings

	# of reactions
1	6

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

Master mix

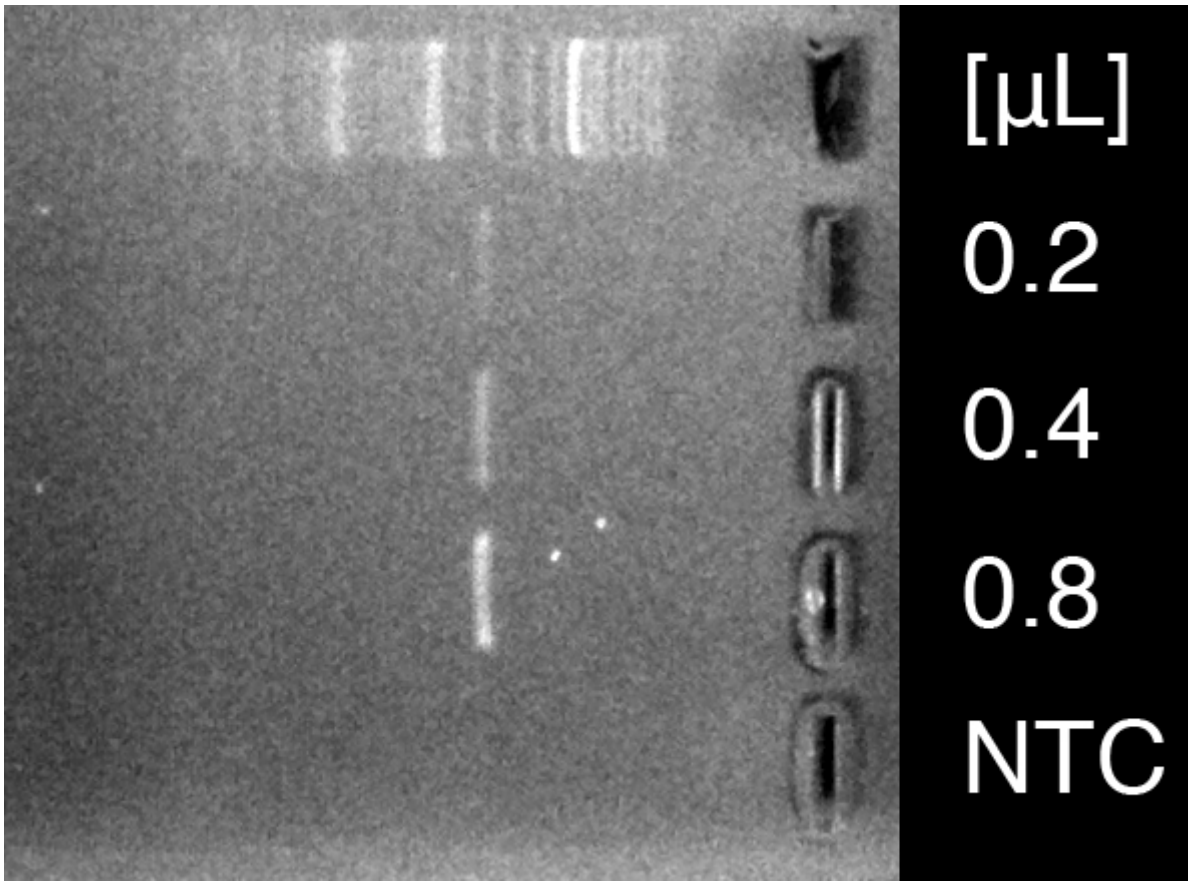
	A	B
1		**6.5 X [µL]**
2	ddH2O	185.25
3	10X LA PCR Buffer	32.5
4	25 mM MgCl2	32.5
5	dNTP Mix (2.5 mM each)	52
6	10 µM OLPr008 MAT -33 UKO F	6.5
7	10 µM OLPr012 MAT +34 SEY UKO R	6.5
8	LA Taq Polymerase (5 U/µL)	3.25
9	OLP045 (pFA6a-His3MX6) (5 ng/µL)	6.5
10	**Total**	**325**

✓ 3. PCR program: 94°C/2 min—[94°C/0.5 min—61°C/0.5 min—68°C/1.5 min]x30—68°C/5 min—10°C/∞

Gel

✓ 4. Gel: sample volume see below/0.5 µg O'GeneRuler DNA Ladder Mix/1% TAE/120 V/35 min

Load 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 2, 4, and 8 µL of the diluted product).
Load 10 µL of no-template control.
Expected product size: 1447 bp
Estimated 60 ng/0.8 µL = 75 ng/µL



Additional Resources

Primers and template

	A	B
1	Forward Primer	OLPr008 MAT -33 UKO F
2	Reverse Primer	OLPr012 MAT +34 SEY UKO R
3	Template	OLP045 (pFA6a-His3MX6)

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

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
- [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	19	75

- [x] 5. 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] 6. Centrifuge 3,000 g/5 min; discard supernatant and resuspend cell pellet in 1 mL sterile H_2O.
- [x] 7. Centrifuge 3,000 g/5 min. Discard supernatant and resuspend cells in 1 ml of 0.1 M LiAc.
- [x] 8. Centrifuge 13,000 g/1 min; discard supernatant
- [x] 9. 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		21 X [µL]
2	50% PEG	5040
3	1 M LiAc	756
4	2mg/ml ssDNA	1050
5	MAT KO PCR product	280
6	ddH2O	434
7	**Total**	**7560**

- [x] 10. Add 360 µL of transformation master mix to each tube.
- [x] 11. Vortex tubes until the cells are smoothly suspended.
- [x] 12. Heat shock cells in water-bath or heat-block: 42°C/1 h.
- [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.

Received colonies

	A	B	C
1	Strain	Total colonies	Large colonies
2	1	0	0
3	2	0	0
4	3	14	14
5	4	21	21
6	6	26	23
7	8	3	2
8	9	70	63
9	10	22	19
10	11	1	1
11	12	5	4
12	14	19	15
13	15	8	2
14	16	22	19
15	17	6	6
16	18	2	2
17	19	9	5
18	20	2	2
19	22	27	21
20	23	4	3

Additional resources

PCR product amou...

	MAT KO PCR product [µg]
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	13.3
6	dHd2O	20.7
7	**Total**	**360**

MAT Colony PCR

Introduction

For genotyping of the 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 20 µL reactions (include 3 additional reactions to account for wild-type, mutant, and no-template control):

Settings

	# of reactions
1	22

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		**22.5 X [µL]**
2	ddH2O	265.5
3	10X LA PCR Buffer	45
4	25 mM MgCl2	45
5	dNTP Mix (2.5 mM each)	72
6	10 µM OLPr014 MAT -283 F	9
7	10 µM OLPr015 MAT +295 R	9
8	LA Taq Polymerase (5 U/µL)	4.5
9	**Total**	**450**

- 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/3 min]x35—68°C/5 min—10°C/∞

Reference plate and overnight culture

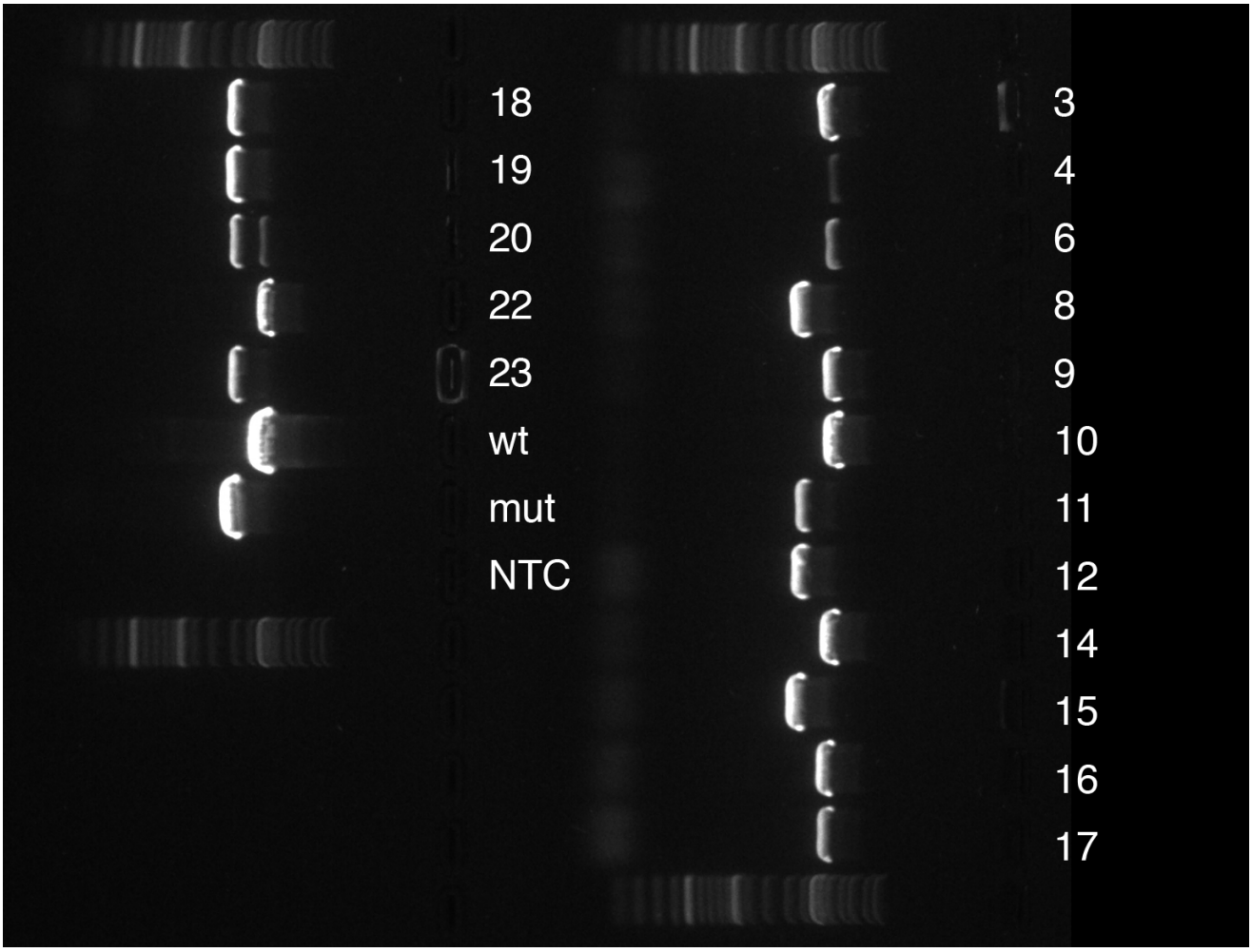
- 5. Scrape off the same colony used in the PCR with a cotton swab, inoculate a square patch on a SC-His-Trp reference plate and incubate plate at 30°C/overnight (or until yeast lawn is clearly visible. Optional: resuspend the remaining cells on the swab in 2 mL YPAD for use as an overnight culture for the next transformation and grow it on the shaker at 30°C/200 rpm/overnight.

  A reference plate contains multiple patches of different yeast strains on a grid.

Gel

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

  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).
  7 strains are MATΔ::His3 (#8, 11, 12 15, 18, 19, 23), i.e. 41%
  9 strains are MATwt (#3, 4, 6, 9, 10, 14, 16, 17, 22)
  Apparently 1 is diploid MATΔ::His3/MATwt (#20)



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]	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

MAT Colony PCR.2

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. Restreak of #3, 4, 6, 9, 10, 14, 16, 17, 20, 22

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, restreak of #3, 4, 6, 9, 10, 14, 16, 17, 20, 22.

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	13

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		**13.5 X [µL]**
2	ddH2O	159.3
3	10X LA PCR Buffer	27
4	25 mM MgCl2	27
5	dNTP Mix (2.5 mM each)	43.2
6	10 µM OLPr014 MAT -283 F	5.4
7	10 µM OLPr015 MAT +295 R	5.4
8	LA Taq Polymerase (5 U/µL)	2.7
9	**Total**	**270**

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/3 min]x35—68°C/5 min—10°C/∞

Reference plate and overnight culture

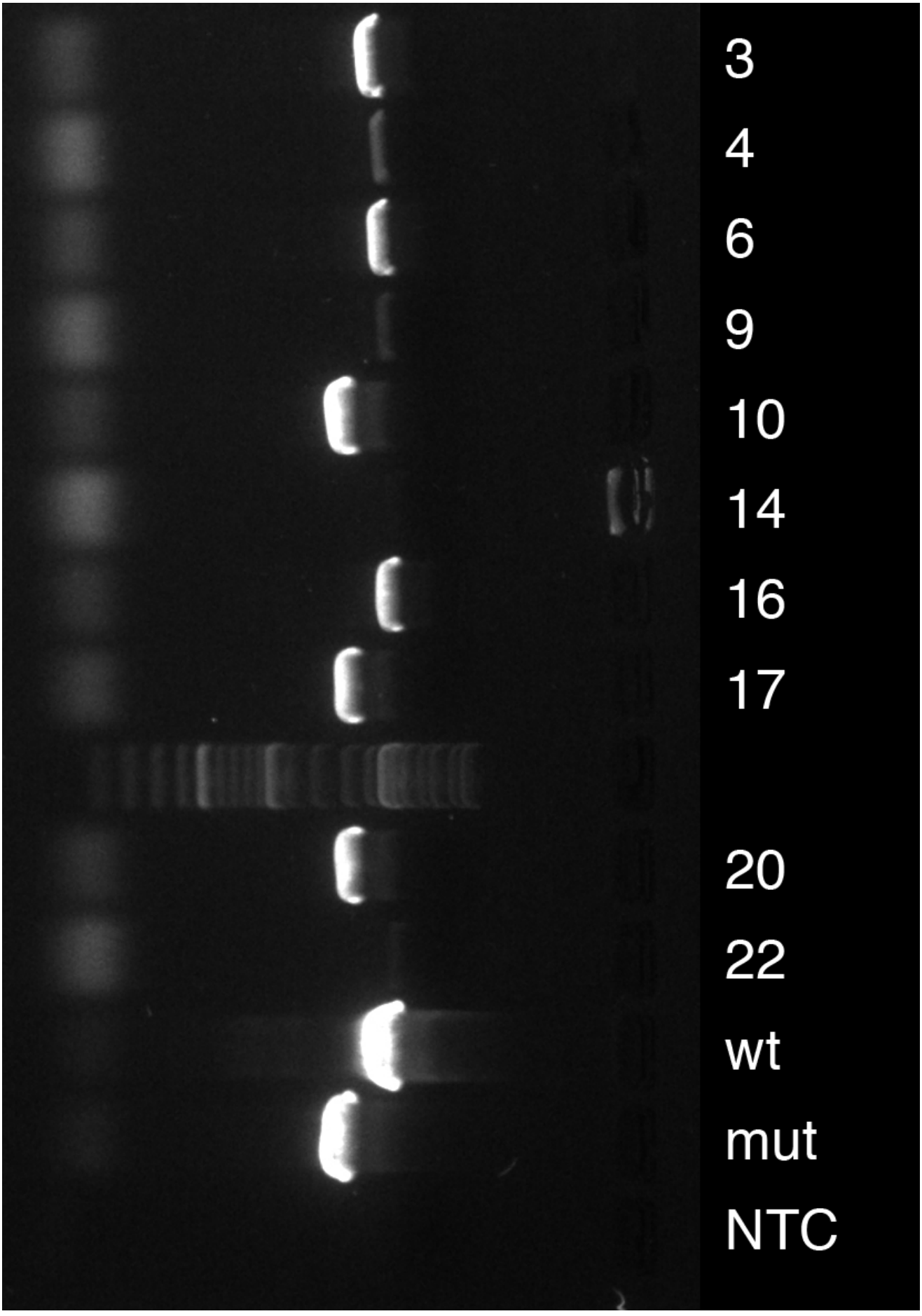
5. Scrape off the same colony used in the PCR with a cotton swab, inoculate a square patch on a SC-His-Trp reference plate and incubate plate at 30°C/overnight (or until yeast lawn is clearly visible). Optional: resuspend the remaining cells on the swab in 2 mL YPAD for use as an overnight culture for the next transformation and grow it on the shaker at 30°C/200 rpm/overnight.

   A reference plate contains multiple patches of different yeast strains on a grid.

Gel

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

   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).
   3 strains are MATΔ::His3: #10, 17, and 20
   7 strains are MATwt: #3, 4, 6, 9, 14, 16, 22


3
4
6
9
10
14
16
17
20
22
wt
mut
NTC

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]	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

MAT Colony PCR.3

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 20 µL reactions (include 3 additional reactions to account for wild-type, mutant, and no-template control):

Settings

	# of reactions
1	10

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		**10.5 X [µL]**
2	ddH2O	123.9
3	10X LA PCR Buffer	21
4	25 mM MgCl2	21
5	dNTP Mix (2.5 mM each)	33.6
6	10 µM OLPr014 MAT -283 F	4.2
7	10 µM OLPr015 MAT +295 R	4.2
8	LA Taq Polymerase (5 U/µL)	2.1
9	**Total**	**210**

- 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.

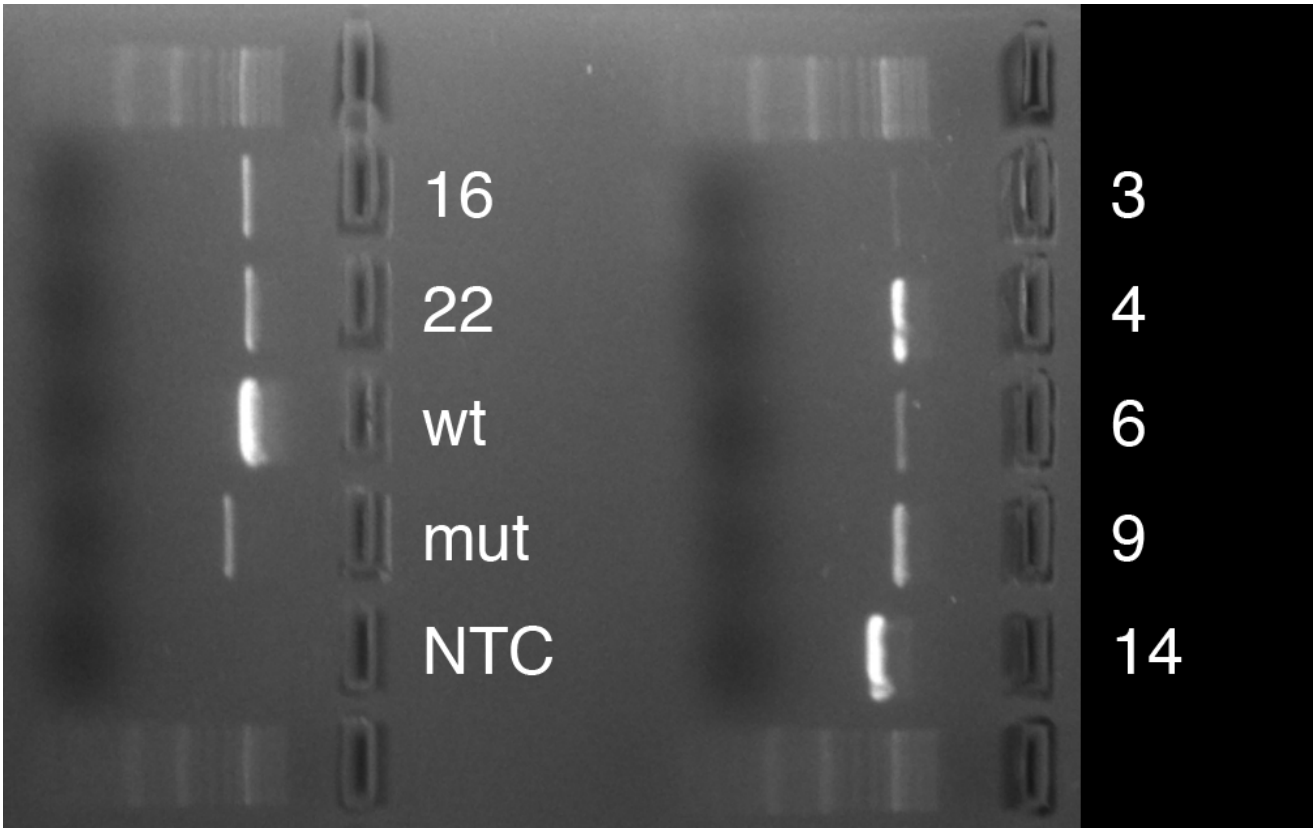
  Do not use too many cells as they might inhibit the PCR reaction. The PCR reaction should only look slightly opaque from the resuspended cells. If the reaction looks milky then you might have used too many cells.

- 4. PCR program: 94°C/5 min—[94°C/0.5 min—61°C/0.5 min—68°C/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

  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).
  1 strains is MATΔ::His3: #14.
  6 strains are MATwt: #3, 4, 6, 9, 16, 22.



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]	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

MAT Colony PCR.4

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 20 µL reactions (include 3 additional reactions to account for wild-type, mutant, and no-template control):

Settings

	# of reactions
1	15

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		**15.5 X [µL]**
2	ddH2O	182.9
3	10X LA PCR Buffer	31
4	25 mM MgCl2	31
5	dNTP Mix (2.5 mM each)	49.6
6	10 µM OLPr014 MAT -283 F	6.2
7	10 µM OLPr015 MAT +295 R	6.2
8	LA Taq Polymerase (5 U/µL)	3.1
9	**Total**	**310**

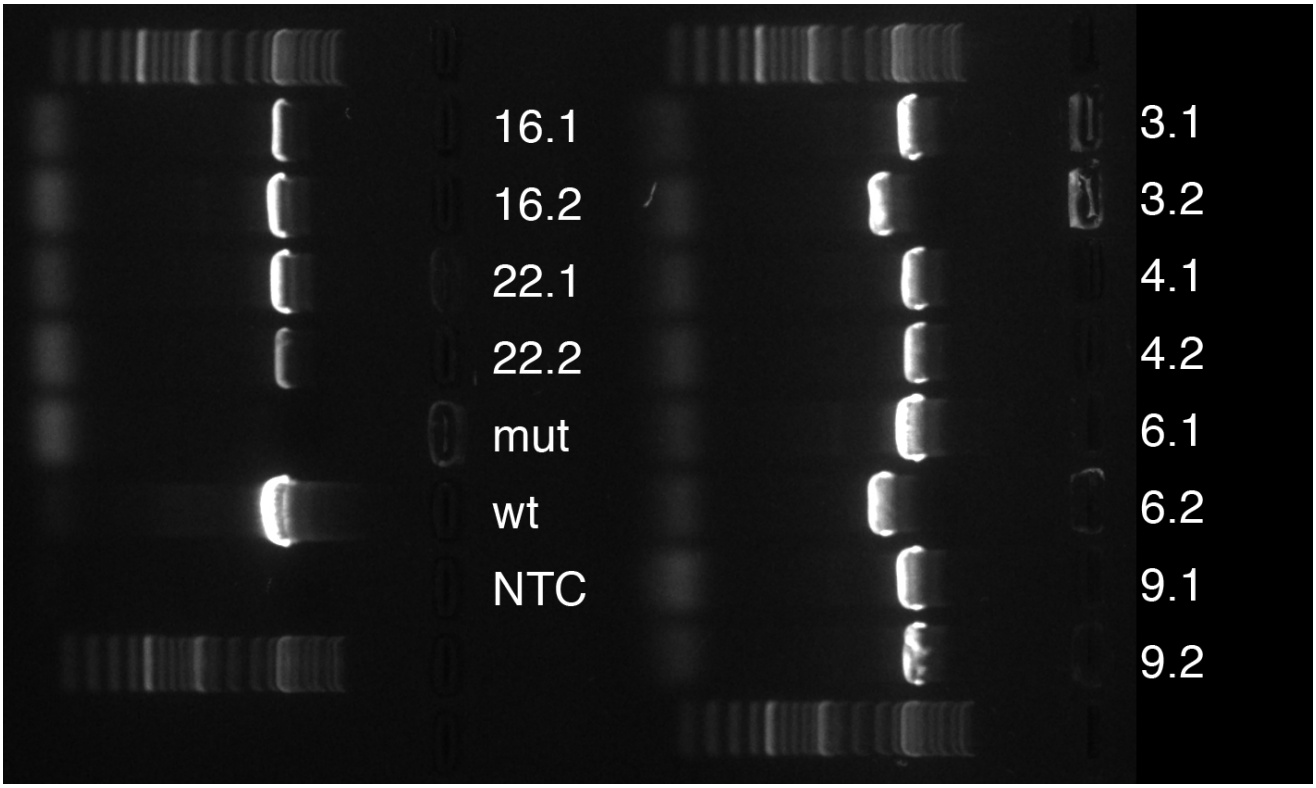
- 3. In sterile work environement (e.g. laminar flow), gently touch a yeast colony with a 10 µL pipette tip so a small number of cells stick to it and resuspend cells in PCR reaction.

  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/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

  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]	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

MAT Colony PCR.5

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 20 µL reactions (include 3 additional reactions to account for wild-type, mutant, and no-template control):

Settings

	# of reactions
1	11

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		**11.5 X [µL]**
2	ddH2O	135.7
3	10X LA PCR Buffer	23
4	25 mM MgCl2	23
5	dNTP Mix (2.5 mM each)	36.8
6	10 µM OLPr014 MAT -283 F	4.6
7	10 µM OLPr015 MAT +295 R	4.6
8	LA Taq Polymerase (5 U/µL)	2.3
9	**Total**	**230**

- 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.

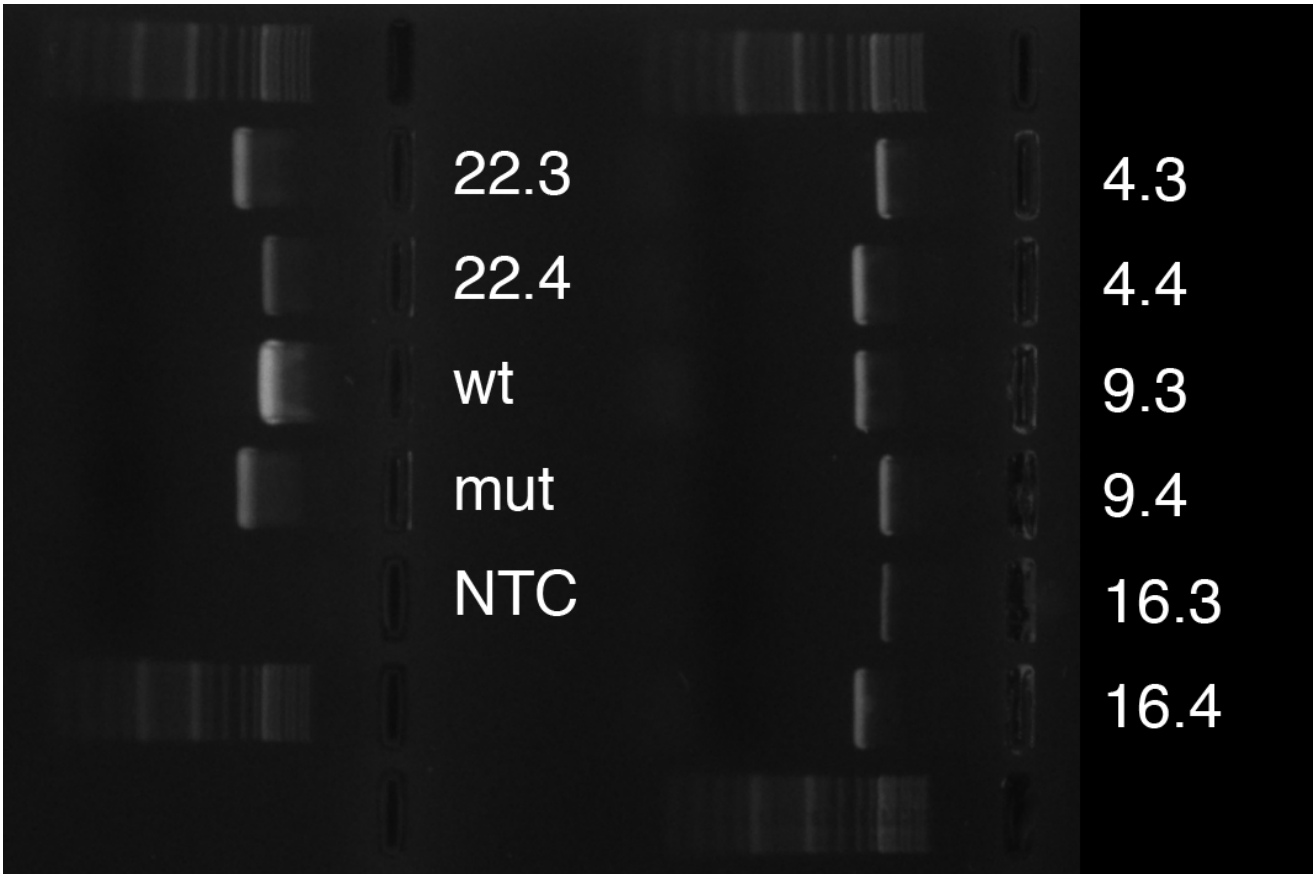
- 4. PCR program "MATCOLON" in folder "STEPHAN":

  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

  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]	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