2-5 MAT/ste4 KO of MA strains set 4

THURSDAY, 2018-12-27

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:
73–96

ste4 knock-out

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

FRIDAY, 2018-12-28

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

SUNDAY, 2019-01-13

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

MONDAY, 2019-01-14

- [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).

THURSDAY, 2019-01-17

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

MONDAY, 2019-01-21

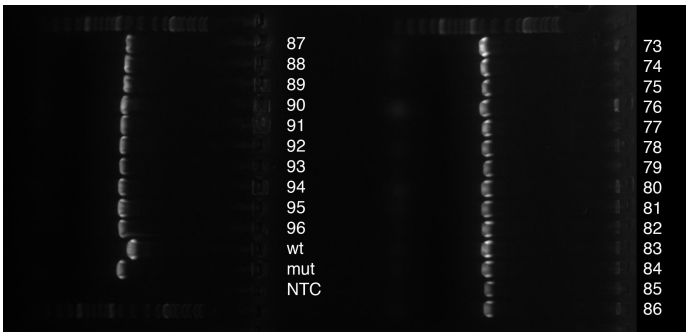
Put plates at 4°C.

THURSDAY, 2019-01-24

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

MONDAY, 2019-01-28

190128_2-5_STE4_Colony_PCR_Set_4.jpg



All strains are ste4Δ::TRP1.

- [x] If ste4Δ::TRP1 genotype is confirmed, proceed with MAT knockout; otherwise repeat streak-out and colony PCR with another transformant.

ste4Δ::TRP1

	A	B	C	D	E
1	Strain #	colony .1	colony .2	colony .3	colony .4
2	73	TRUE			
3	74	TRUE			
4	75	TRUE			
5	76	TRUE			
6	77	TRUE			
7	78	TRUE			
8	79	TRUE			
9	80	TRUE			
10	81	TRUE			
11	82	TRUE			
12	83	TRUE			
13	84	TRUE			
14	85	TRUE			
15	86	TRUE			
16	87	TRUE			
17	88	TRUE			
18	89	TRUE			
19	90	TRUE			
20	91	TRUE			
21	92	TRUE			
22	93	TRUE			
23	94	TRUE			
24	95	TRUE			
25	96	TRUE			

MONDAY, 2019-02-04

- [x] Make backup patch plate of all strains (73–96) on SC-Trp. Christina Hsu

SATURDAY, 2019-03-09

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).

SUNDAY, 2019-03-10

- [x] Prepare 1 mL competent cells (see MAT KO Transformation protocol day 2). Linnea Sandell . Line 80 had not grown over night, I cheated and diluted a bigger part of the back up patch into the 2.5 mL YPAD before taking 200µL of it.
- [x] Transform with 1 µg MAT KO PCR product and plate on SC-His-Trp (see MAT KO Transformation protocol day 2). Linnea Sandell

MONDAY, 2019-03-11

- [x] Put plates into fridge while waiting for new, proper SC-Trp-His pates to be made.

TUESDAY, 2019-03-12

- [x] Replica plated lines 81, 87, 93 onto new SC-Trp-His.

THURSDAY, 2019-03-14

Observe single colonies on all three of the replica plated lines.

- [x] Replica plated remaining lines onto the new SC-Trp-His plates.

FRIDAY, 2019-03-15

- [x] Move plates 81, 87, 93 into fridge while waiting for others.
- [x] Move remaining plates to RT.

MONDAY, 2019-03-18

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

Did not receive any colonies for strains #80 and 92.
Forgot 81, 87, and 93.

TUESDAY, 2019-03-19

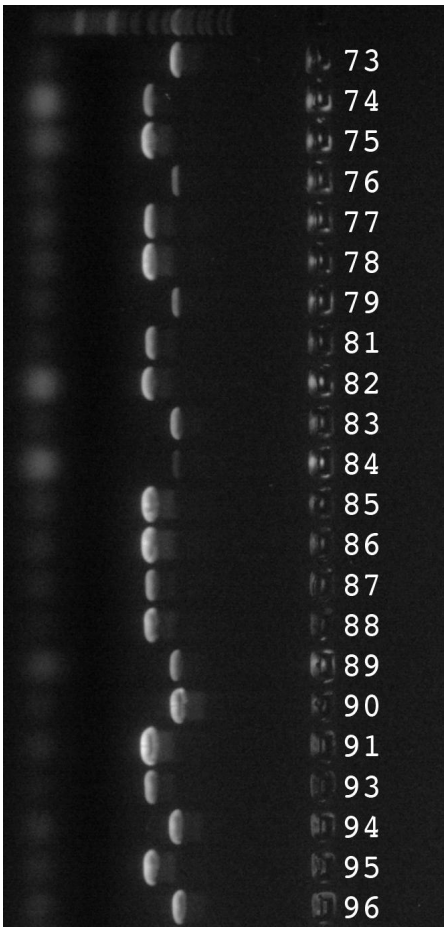
- [x] Streak-out 4 transformants/strain to single colonies on a single SC-His-Trp plate for 81, 87, and 93.

TUESDAY, 2019-03-26

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

WEDNESDAY, 2019-03-27

190327_2-5_MAT_Colony_PCR.jpg

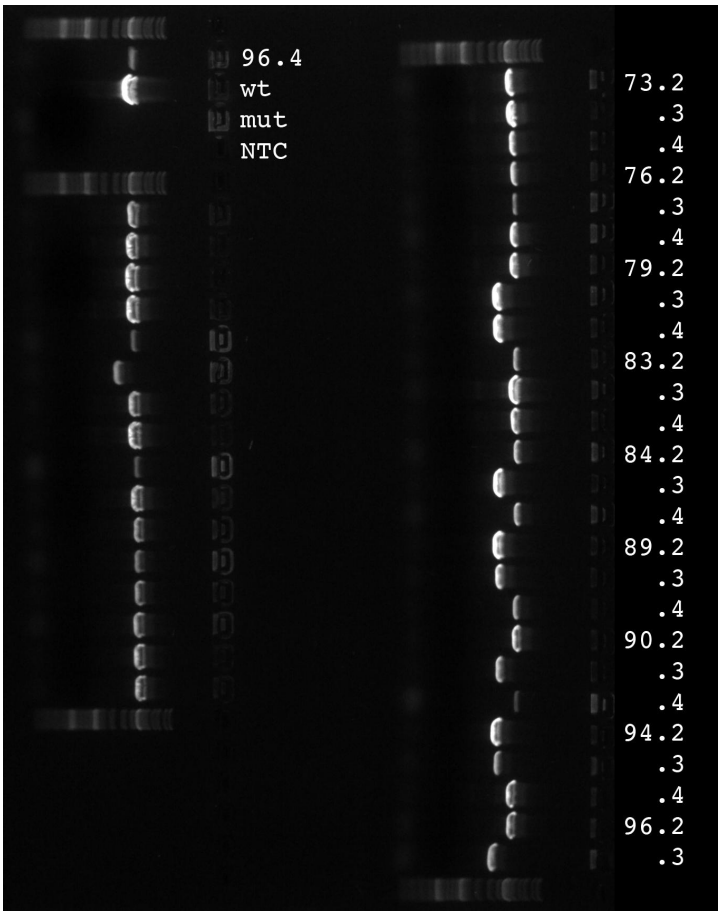


9 strains were MAT wt: 73, 76, 79, 83, 84, 89, 90, 94, 96.
All other strains were MATΔ::His3.

- [x] Repeat: Confirm genotype of colonies .2, .3, .4 for strains 73, 76, 79, 83, 84, 89, 90, 94, 96 (see MAT Colony PCR.2).
  Matthew Stasiuk

TUESDAY, 2019-04-02

190402_2-5_MAT_Colony_PCR.2.jpg



9 strains were MAT wt: 73, 76, 83.

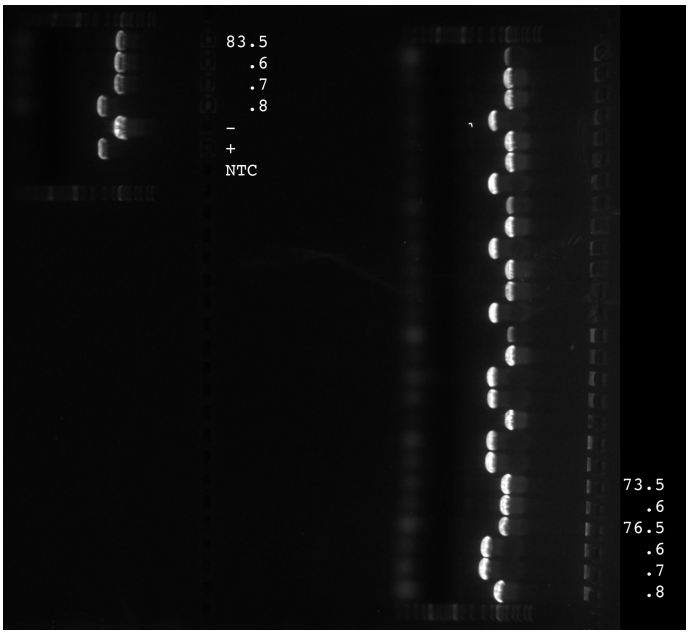
All other strains were MATΔ::His3: 79, 84, 89, 90, 94, 96.

- [x] Restreak 4 transformants/strain to single colonies on a single SC-His-Trp plate for strains 73, 76, 83.

THURSDAY, 2019-04-11

- [x] Confirm genotype of colonies .5, .6, .7, .8 for strains 73, 76, 83 (see MAT Colony PCR.3 together with samples from 2-3 MAT/ste4 KO of MA strains set 2 and 2-4 MAT/ste4 KO of MA strains set 3).

190411_2-5_MAT_Colony_PCR.3.jpg



1 strain was MAT wt: 73.

All other strains were MATΔ::His3: 76, 83.

Include strain 73 with 2-11 MAT/ste4 KO of MA strains set 7 MAT KO Transformation.

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
1	Strain #	colony .1	colony .2	colony .3	colony .4	colony .5	colony .6	colony .7	colony .8
2	73	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE		
3	74	TRUE							
4	75	TRUE							
5	76	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	FALSE
6	77	TRUE							
7	78	TRUE							
8	79	FALSE	FALSE	TRUE	TRUE				
9	80	NA	NA	NA	NA				
10	81	TRUE							
11	82	TRUE							
12	83	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE
13	84	FALSE	FALSE	TRUE	FALSE				
14	85	TRUE							
15	86	TRUE							
16	87	TRUE							
17	88	TRUE							
18	89	FALSE	TRUE	TRUE	FALSE				
19	90	FALSE	FALSE	TRUE	FALSE				
20	91	TRUE							
21	92	NA	NA	NA	NA				
22	93	TRUE							
23	94	FALSE	TRUE	TRUE	FALSE				
24	95	TRUE							
25	96	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

- [x] 1. Overnight culture (ONC): Inoculate 2 mL of YPAD with a single yeast colony and incubate culture on the shaker at 30°C/200 rpm/overnight.
- [x] 2. Inoculate 2.5 mL of YPAD in disposable 10 mL culture tubes with 200 µL of ONC and grow it on the shaker at 30°C/200 rpm/4 h.

04:00:00

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

Settings

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

- [x] 5. If the carrier DNA has not been denatured yet, denature the carrier DNA in heating block at 95°C for 5 min and chill immediately on ice before use. If carrier DNA has already been denatured then thaw it on ice.

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

0.1 M Li Ac

	Materials	mL
1		26 X [mL]
2	1 M LiAc	2.6
3	sterile H2O	23.4
4	**Total**	**26**

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

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

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

- [x] 10. Prepare transformation master mix by adding reagents in order (pipetting 50% PEG slowly), two additional reactions are included to account for pipetting discrepancies:

Transformation master mix

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

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

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

01:00:00

- [x] 13. Centrifuge 3,000 g/5 min; discard supernatant and resuspend each cell pellet in 100 µL sterile H_2O, then plate cells on the SC-Trp

Table1

	A	B
1	Strain #	# colonies
2	73	11
3	74	12
4	75	9
5	76	9
6	77	14
7	78	10
8	79	12
9	80	4
10	81	12
11	82	6
12	83	14
13	84	6
14	85	7
15	86	17
16	87	13
17	88	14
18	89	11
19	90	19
20	91	16
21	92	9
22	93	12
23	94	20
24	95	10
25	96	19

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	28

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		**28.5 X [µL]**
2	ddH2O	336.3
3	10X LA PCR Buffer	57
4	25 mM MgCl2	57
5	dNTP Mix (2.5 mM each)	91.2
6	10 µM OLPr022 STE4 -133 F	11.4
7	10 µM OLPr023 STE4 1402 R	11.4
8	LA Taq Polymerase (5 U/µL)	5.7
9	**Total**	**570**

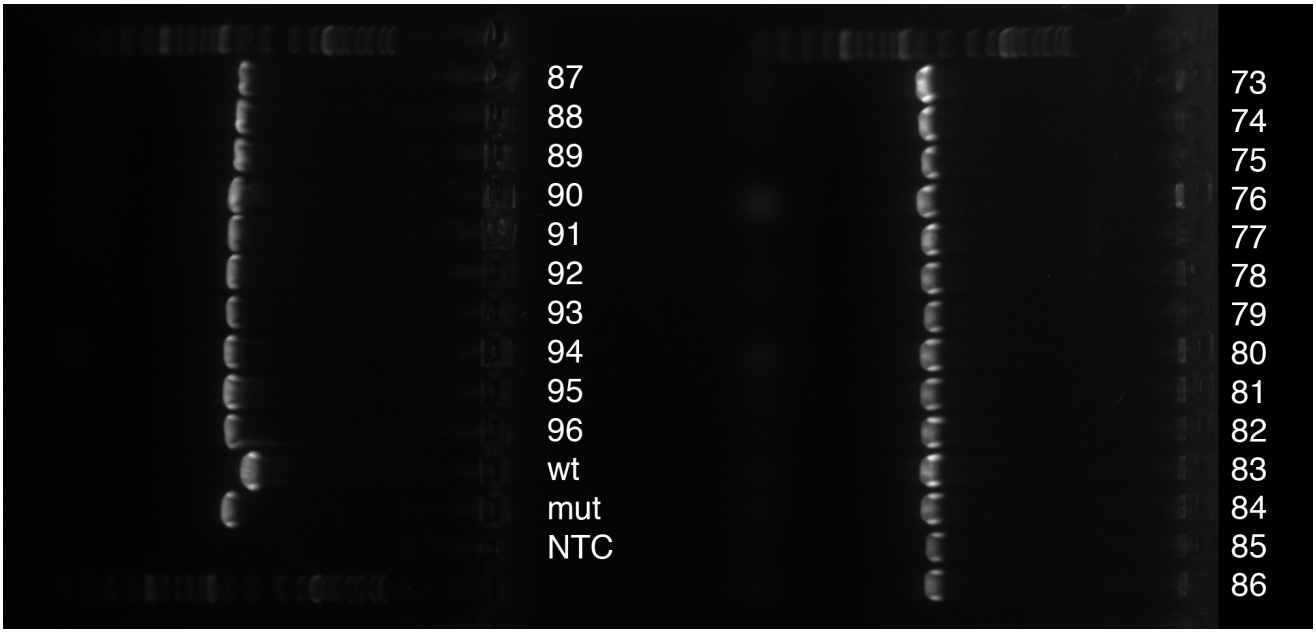
- 3. In sterile work environement (e.g. laminar flow), gently touch a yeast colony with a 10 µL pipette tip so a small number of cells stick to it and resuspend cells in PCR reaction.

  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/60 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

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 small amount of the patched colony from the backup plate, 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. Line 80 had not grown, so I cheated and took a big part of the patch on the backup plate in the empty media and then took 200µL of that.

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	73	8
3	74	6
4	75	1
5	76	21
6	77	12
7	78	3
8	79	22
9	80	0
10	81	2
11	82	1
12	83	13
13	84	30
14	85	19
15	86	121
16	87	5
17	88	6
18	89	56
19	90	31
20	91	1
21	92	0
22	93	1
23	94	5
24	95	17
25	96	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	10.0
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	78

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		**78.5 X [µL]**
2	ddH2O	463.15
3	10X LA PCR Buffer	78.5
4	25 mM MgCl2	78.5
5	dNTP Mix (2.5 mM each)	125.6
6	10 µM OLPr014 MAT -283 F	15.7
7	10 µM OLPr015 MAT +295 R	15.7
8	LA Taq Polymerase (5 U/µL)	7.85
9	**Total**	**785**

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

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.

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::TRP1 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	68.5

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		**69 X [µL]**
2	ddH2O	407.1
3	10X LA PCR Buffer	69
4	25 mM MgCl2	69
5	dNTP Mix (2.5 mM each)	110.4
6	10 µM OLPr014 MAT -283 F	13.8
7	10 µM OLPr015 MAT +295 R	13.8
8	LA Taq Polymerase (5 U/µL)	6.9
9	**Total**	**690**

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, touch the tip to a new SC-His-Trp plate and thenr esuspend 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

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::TRP1 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	34.5

2. Combine the following reagents to prepare the master mix (contains 0.5 additional reaction to account for pipetting discrepancies), and aliquot 10 µL master mix/pcr tube:

Master mix

	A	B
1		**35 X [µL]**
2	ddH2O	206.5
3	10X LA PCR Buffer	35
4	25 mM MgCl2	35
5	dNTP Mix (2.5 mM each)	56
6	10 µM OLPr014 MAT -283 F	7
7	10 µM OLPr015 MAT +295 R	7
8	LA Taq Polymerase (5 U/µL)	3.5
9	**Total**	**350**

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, touch the tip to a fresh reference SC-His-Trp plate, 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.
   The reference plate is incubated 30°C/2–3 days.

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