2-22 Transformation of MATΔ ste4Δ strains with OLP003 or OLP004

MONDAY, 2019-06-17

First, a double knock-out of the strains of interest resulted in a MATΔ::His3 and ste4Δ::TRP1 genotype. 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:
Set.1 01–12, 14–20, 22-24
Set.2 25–48
Set.3 49–72
Set.4 73–96
Set.5 97–120
Set.6 121–131, 133–136, and repeat: 9, 39, 42, 98, 99, 100
Set.7 39, 58, 83, 84

Plasmid Transformation

- [x] Set.1 Inoculate overnight culture (see Plasmid transformation.1 day 1).

TUESDAY, 2019-06-18

- [x] Set.1 Prepare 2 x 1 mL competent cells for each strain (see Plasmid transformation.1 day 2). Linnea Sandell Note: 5 mL culture will produce 2 x 1 mL competent cells, i.e. just follow the protocol. *Line 4 was less dense than others (by visual comparison), added an additional 400 µL ONC of #4 (after 1 h). Line 9 did not seem to grow at all.*
  Prepare frozen aliquot by mixing 500 µL ONC with 500 µL 30% glycerol for all strains. *Did not freeze line 9, as it did not grow.*
  Prepare 2 additional frozen aliquots of strain #1 and #2.
- [x] Set.1 Transform either with 1 µg OPL003 and plate on SC-LEU or 1 µg OLP004 and plate on SC-Ura (see Plasmid transformation.1 day 2).
- [x] Set.2 Inoculate overnight culture (see Plasmid transformation.2 day 1).

WEDNESDAY, 2019-06-19

- [x] Set.2 Prepare 2 x 1 mL competent cells for each strain (see Plasmid transformation.2 day 2). Linnea Sandell
  Only line 34, 37 and 38 had visible growth. I needed to compare to YPAD media to confirm growth in the others. Therefore inoculated 800 µL ONC for the remaining lines, and also put the tubes back in the incubator to grow for longer before continuing to prepare the frozen aliquots.
  Prepare frozen aliquot by mixing 400 µL ONC with 500 µL 30% glycerol for all strains.
  Prepare 2 additional frozen aliquots of strain #34.
- [x] Set.2 Transform either with 1 µg OPL003 and plate on SC-LEU or 1 µg OLP004 and plate on SC-Ura (see Plasmid transformation.2 day 2). Matthew Stasiuk

SATURDAY, 2019-06-22

Set.2 No colonies for #39 and 42, Retransform with Set.6

SUNDAY, 2019-06-23

- [x] Set.3 Inoculate overnight culture (see Plasmid transformation.3 day 1). Linnea Sandell

MONDAY, 2019-06-24

- [x] Set.3 Prepare 2 x 1 mL competent cells for each strain (see Plasmid transformation.3 day 2). Linnea Sandell
  Prepare frozen aliquot by mixing 400 µL ONC with 500 µL 30% glycerol for all strains.
- [x] Set.3 Transform either with 1 µg OPL003 and plate on SC-LEU or 1 µg OLP004 and plate on SC-Ura (see Plasmid transformation.3 day 2). Matthew Stasiuk
- [x] Set.4 Inoculate overnight culture (see Plasmid transformation.4 day 1).

TUESDAY, 2019-06-25

- [x] Set.4 Prepare 2 x 1 mL competent cells for each strain (see Plasmid transformation.4 day 2). Linnea Sandell
  Prepare frozen aliquot by mixing 400 µL ONC with 500 µL 30% glycerol for all strains.
  Strain #84 did not grow.
- [x] Restreak strains #39, 42, 84, 98, 99, 100, 122, and 127 from frozen aliquots on YPAD.
- [x] Set.4 Transform either with 1 µg OPL003 and plate on SC-LEU or 1 µg OLP004 and plate on SC-Ura (see Plasmid transformation.4 day 2). Christina Hsu
- [x] Set.5 Inoculate overnight culture (see Plasmid transformation.5 day 1).
  Did use reference plates from 23 Apr 2019 for #98, 99, or 100 (Those strains did not grow on the new reference plates).

WEDNESDAY, 2019-06-26

- [x] Set.5 Prepare 2 x 1 mL competent cells for each strain (see Plasmid transformation.5 day 2). Linnea Sandell
  Prepare frozen aliquot by mixing 400 µL ONC with 500 µL 30% glycerol for all strains.
  Prepare 2 frozen aliquots of strain #73, 80, and 92.
  Prepare 2 additional frozen aliquots of strain #106, and 116.
- [x] Set.5 Transform either with 1 µg OPL003 and plate on SC-LEU or 1 µg OLP004 and plate on SC-Ura (see Plasmid transformation.5 day 2). Matthew Stasiuk
- [x] Set.6 Inoculate overnight culture (see Plasmid transformation.6 day 1). Make sure to include strains #9, and if ready also 39, 42, 84.
  #39, 42, 84 (also 98, 99, 100, 122 and 127) were streaked out from glycerol stocks onto new YPAD plates but will only have only grown for 1 day. They are in box labeled "Stephan" in incubator. We might have to transform those strains separately.

THURSDAY, 2019-06-27

- [x] Set.6 Prepare 2 x 1 mL competent cells for each strain (see Plasmid transformation.6 day 2).
  Prepare frozen aliquot by mixing 400 µL ONC with 500 µL 30% glycerol for all strains.
  Inoculated Line 122 and 127 with 800µL because of little overnight growth.
- [x] Move Set.3 to 4°C and note any strains without any transformants.
  Line 58 had no transformants (neither plasmid).
- [x] Set.6 Transform either with 1 µg OPL003 and plate on SC-LEU or 1 µg OLP004 and plate on SC-Ura (see Plasmid transformation.6 day 2). Christina Hsu

FRIDAY, 2019-06-28

- [x] Move Set.4 to 4°C and note any strains without any transformants.
  No transformant for #83/OLP003.

Colonies on plates with strains #78, 79, 80, 86, 90, 92 transformed with OLP004 had different morphology from regular yeast, look flatter and larger, maybe were contaminants. Christina and Stephan checked in microscope and cells looked like yeast. Maybe different morphology was caused by excess condensation on affected plates. Ask Linnea Sandell for her opinion on cell morphology.

- [x] Move Set.5 to RT.
- [x] Move Set.6 to RT.

TUESDAY, 2019-07-02

- [x] Move Set.5 to 4°C and note any strains without any transformants.
- [x] Move Set.6 to 4°C and note any strains without any transformants.

No transformants for #39/OLP004.

MONDAY, 2019-07-08

- [x] Transformation: Set.7 Inoculate overnight culture (see Plasmid transformation.7 day 1) for strains 39, 58, 83, 84.

TUESDAY, 2019-07-09

- [x] Set.7 Prepare 2 x 1 mL competent cells for each strain (see Plasmid transformation.7 day 2). Linnea Sandell
- [x] Set.7 Transform either with 1 µg OPL003 and plate on SC-LEU or 1 µg OLP004 and plate on SC-Ura (see Plasmid transformation.6 day 2). Matthew Stasiuk

Evaluating the success of transformation:
39 only successfully transformed with the OLP003 plasmid (same as in set 6).
58 transformed successfully with both.
83 only successfully transformed with the OLP004 plasmid (same as in set 4).
84 transformed successfully with both, but teeny tiny colonies with the OLY003 plasmid even after three days of growth. Colonies tiny with the OLP004 as well, but slightly bigger.

MONDAY, 2019-07-15

Line 39 has MA code 81. It has mutations in the following genes:
S000001900
S000003834
S000003949
S000005667
S000005898

Line 83 has MA code 157.It has mutations in the following genes:
S000002718
S000003118
S000004443: a.k.a LEU3, Leucine biosynthesis, make sense it doesn't grow (LEU2 alone on OLP003 cannot fully complement the LEU auxotroph phenotype).

Plasmid Transformation.1

Introduction

MATΔ::His3 STE4Δ::TRP1 yeast strain derived from SEYa or SEYα are transformed with OLP003 or OLP004. 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
- OLP003
- OLP004
- Sterile H20
- Water bath or heating block at 42°C
- Disposable 17x100 mm culture tubes with closures (VWR Catalog # 60818-725)
- SC-Leu plates
- SC-Ura 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 5 mL of YPAD in disposable 10 mL culture tubes with 400 µL of ONC and grow it on the shaker at 30°C/200 rpm/4 h

  This will make 2 x 1 mL competent cells.

- [x] 3. Set water bath or heating block to 42°C.

- [x] 4. Indicate number of transformations and plasmid concentration:

Settings

	# of Transformations	OLP003 [ng/µL]	OLP004 [ng/µL]
1	24	610	550

- [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	5.2
3	sterile H2O	46.8
4	**Total**	**52**

- [x] 7. Centrifuge 3,000 g/5 min; discard supernatant and resuspend cell pellet in 2 mL sterile H_2O.

- [x] 8. Centrifuge 3,000 g/5 min. Discard supernatant and resuspend cells in 2 mL of 0.1 M LiAc. Split into 2 x 1 mL in microcentrifuge tubes (transform one with OLP003 and one with OLP004 below).

- [x] 9. Centrifuge 3,000 g/5 min (use 7,500 rpm in microcentrifuge); discard supernatant.

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

Transformation master mix

	A	C	C
1		26 X OLP003 [µL]	26 X OLP004 [µL]
2	50% PEG	6240	6240
3	1 M LiAc	936	936
4	ssDNA (2 mg/ml)	1300	1300
5	Plasmid	42.6	47.3
6	ddH2O	841.4	836.7
7	**Total**	**9360**	**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 (use 7,500 rpm in microcentrifuge); discard supernatant and resuspend each cell pellet in 100 µL sterile H_2O, then plate cells on SC-Leu for OLP003 transformations or SC-Ura for OLP004 transformations.

Additional resources

Plasmid amount pe...

	Plasmid [ng]
1	1000

Transformation single reaction

	A	B	C
1		OLP003 [µL]	OLP004 [µL]
2	50% PEG	240	240
3	1 M LiAc	36	36
4	2mg/ml ssDNA	50	50
5	PCR product	1.6	1.8
6	dHd2O	32.4	32.2
7	**Total**	**360**	**360**

Plasmid Transformation.2

Introduction

MATΔ::His3 STE4Δ::TRP1 yeast strain derived from SEYa or SEYα are transformed with OLP003 or OLP004. 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
- OLP003
- OLP004
- Sterile H20
- Water bath or heating block at 42°C
- Disposable 17x100 mm culture tubes with closures (VWR Catalog # 60818-725)
- SC-Leu plates
- SC-Ura 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 5 mL of YPAD in disposable 10 mL culture tubes with 400 µL of ONC and grow it on the shaker at 30°C/200 rpm/4 h

  This will make 2 x 1 mL competent cells.
  Only line 34, 37 and 38 had visible growth. I needed to compare to YPAD media to confirm growth in the others. Therefore inoculated 800 µL ONC for the remaining lines, and also put the tubes back in the incubator to grow for longer before continuing to prepare the frozen aliquots.
  Because the lines did not grow well, we incubated them from 8.30 until 2pm (5.5 hours) to get more competent cells. Line 34, 37 and 38 might have gone beyond their exponential phase..?

- [x] 3. Set water bath or heating block to 42°C.

4. Indicate number of transformations and plasmid concentration:

Settings

	# of Transformations	OLP003 [ng/µL]	OLP004 [ng/µL]
1	24	610	550

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		26 X [mL]
2	1 M LiAc	5.2
3	sterile H2O	46.8
4	**Total**	**52**

7. Centrifuge 3,000 g/5 min; discard supernatant and resuspend cell pellet in 2 mL sterile H_2O.

8. Centrifuge 3,000 g/5 min. Discard supernatant and resuspend cells in 2 mL of 0.1 M LiAc. Split into 2 x 1 mL in microcentrifuge tubes (transform one with OLP003 and one with OLP004 below).

9. Centrifuge 3,000 g/5 min (use 7,500 rpm in microcentrifuge); discard supernatant.

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

Transformation master mix

	A	C	C
1		26 X OLP003 [µL]	26 X OLP004 [µL]
2	50% PEG	6240	6240
3	1 M LiAc	936	936
4	ssDNA (2 mg/ml)	1300	1300
5	Plasmid	42.6	47.3
6	ddH2O	841.4	836.7
7	**Total**	**9360**	**9360**

11. Add 360 µL of transformation master mix to each tube.

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 (use 7,500 rpm in microcentrifuge); discard supernatant and resuspend each cell pellet in 100 µL sterile H_2O, then plate cells on SC-Leu for OLP003 transformations or SC-Ura for OLP004 transformations.

Additional resources

Plasmid amount pe...

	Plasmid [ng]
1	1000

Transformation single reaction

	A	B	C
1		OLP003 [µL]	OLP004 [µL]
2	50% PEG	240	240
3	1 M LiAc	36	36
4	2mg/ml ssDNA	50	50
5	PCR product	1.6	1.8
6	dHd2O	32.4	32.2
7	**Total**	**360**	**360**

Plasmid Transformation.3

Introduction

MATΔ::His3 STE4Δ::TRP1 yeast strain derived from SEYa or SEYα are transformed with OLP003 or OLP004. 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
- OLP003
- OLP004
- Sterile H20
- Water bath or heating block at 42°C
- Disposable 17x100 mm culture tubes with closures (VWR Catalog # 60818-725)
- SC-Leu plates
- SC-Ura 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.

  Because line 58 had not gone on the new reference plates that Matt started growing on Thursday, I took yeast from the line 58 patch on the previous reference plate. Started lines 5.45 pm.

Day 2

- [x] 2. Inoculate 5 mL of YPAD in disposable 10 mL culture tubes with 400 µL of ONC and grow it on the shaker at 30°C/200 rpm/4 h

  This will make 2 x 1 mL competent cells.

  Took longer than usual, it seems that the 5 mL of YPAD varies between tubes and it got me thinking I had inoculated the same tube twice... Anyhow, put into incubator at 9.10am.

- [x] 3. Set water bath or heating block to 42°C.

4. Indicate number of transformations and plasmid concentration:

Settings

	# of Transformations	OLP003 [ng/µL]	OLP004 [ng/µL]
1	24	610	550

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		26 X [mL]
2	1 M LiAc	5.2
3	sterile H2O	46.8
4	**Total**	**52**

7. Centrifuge 3,000 g/5 min; discard supernatant and resuspend cell pellet in 2 mL sterile H_2O.

8. Centrifuge 3,000 g/5 min. Discard supernatant and resuspend cells in 2 mL of 0.1 M LiAc. Split into 2 x 1 mL in microcentrifuge tubes (transform one with OLP003 and one with OLP004 below).

9. Centrifuge 3,000 g/5 min (use 7,500 rpm in microcentrifuge); discard supernatant.

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

Transformation master mix

	A	C	C
1		26 X OLP003 [µL]	26 X OLP004 [µL]
2	50% PEG	6240	6240
3	1 M LiAc	936	936
4	ssDNA (2 mg/ml)	1300	1300
5	Plasmid	42.6	47.3
6	ddH2O	841.4	836.7
7	**Total**	**9360**	**9360**

11. Add 360 µL of transformation master mix to each tube.

12. Vortex tubes until the cells are smoothly suspended.

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

01:00:00

14. Centrifuge 3,000 g/5 min (use 7,500 rpm in microcentrifuge); discard supernatant and resuspend each cell pellet in 100 µL sterile H_2O, then plate cells on SC-Leu for OLP003 transformations or SC-Ura for OLP004 transformations.

Additional resources

Plasmid amount pe...

	Plasmid [ng]
1	1000

Transformation single reaction

	A	B	C
1		OLP003 [µL]	OLP004 [µL]
2	50% PEG	240	240
3	1 M LiAc	36	36
4	2mg/ml ssDNA	50	50
5	PCR product	1.6	1.8
6	dHd2O	32.4	32.2
7	**Total**	**360**	**360**

Plasmid Transformation.4

Introduction

MATΔ::His3 STE4Δ::TRP1 yeast strain derived from SEYa or SEYα are transformed with OLP003 or OLP004. 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
- OLP003
- OLP004
- Sterile H20
- Water bath or heating block at 42°C
- Disposable 17x100 mm culture tubes with closures (VWR Catalog # 60818-725)
- SC-Leu plates
- SC-Ura 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 5 mL of YPAD in disposable 10 mL culture tubes with 400 µL of ONC and grow it on the shaker at 30°C/200 rpm/4 h

  This will make 2 x 1 mL competent cells.

- [x] 3. Set water bath or heating block to 42°C.

- [x] 4. Indicate number of transformations and plasmid concentration:

Settings

	# of Transformations	OLP003 [ng/µL]	OLP004 [ng/µL]
1	24	610	550

- [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	5.2
3	sterile H2O	46.8
4	**Total**	**52**

- [x] 7. Centrifuge 3,000 g/5 min; discard supernatant and resuspend cell pellet in 2 mL steri le H_2O.

- [x] 8. Centrifuge 3,000 g/5 min. Discard supernatant and resuspend cells in 2 mL of 0.1 M LiAc. Split into 2 x 1 mL in microcentrifuge tubes (transform one with OLP003 and one with OLP004 below).

- [x] 9. Centrifuge 3,000 g/5 min (use 7,500 rpm in microcentrifuge); discard supernatant.

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

Transformation master mix

	A	C	C
1		26 X OLP003 [µL]	26 X OLP004 [µL]
2	50% PEG	6240	6240
3	1 M LiAc	936	936
4	ssDNA (2 mg/ml)	1300	1300
5	Plasmid	42.6	47.3
6	ddH2O	841.4	836.7
7	**Total**	**9360**	**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

14. Centrifuge 3,000 g/5 min (use 7,500 rpm in microcentrifuge); discard supernatant and resuspend each cell pellet in 100 µL sterile H_2O, then plate cells on -Leu for OLP003 transformations or SC-Ura for OLP004 transformations.

Additional resources

Plasmid amount pe...

	Plasmid [ng]
1	1000

Transformation single reaction

	A	B	C
1		OLP003 [µL]	OLP004 [µL]
2	50% PEG	240	240
3	1 M LiAc	36	36
4	2mg/ml ssDNA	50	50
5	PCR product	1.6	1.8
6	dHd2O	32.4	32.2
7	**Total**	**360**	**360**

Plasmid Transformation.5

Introduction

MATΔ::His3 STE4Δ::TRP1 yeast strain derived from SEYa or SEYα are transformed with OLP003 or OLP004. 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
- OLP003
- OLP004
- Sterile H20
- Water bath or heating block at 42°C
- Disposable 17x100 mm culture tubes with closures (VWR Catalog # 60818-725)
- SC-Leu plates
- SC-Ura plates

Procedure

Competent cells

Day 1

- [ ] 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

- [ ] 2. Inoculate 5 mL of YPAD in disposable 10 mL culture tubes with 400 µL of ONC and grow it on the shaker at 30°C/200 rpm/4 h

  This will make 2 x 1 mL competent cells.

- [ ] 3. Set water bath or heating block to 42°C.

- [ ] 4. Indicate number of transformations and plasmid concentration:

Settings

	# of Transformations	OLP003 [ng/µL]	OLP004 [ng/µL]
1	24	610	550

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		26 X [mL]
2	1 M LiAc	5.2
3	sterile H2O	46.8
4	**Total**	**52**

7. Centrifuge 3,000 g/5 min; discard supernatant and resuspend cell pellet in 2 mL sterile H_2O.

8. Centrifuge 3,000 g/5 min. Discard supernatant and resuspend cells in 2 mL of 0.1 M LiAc. Split into 2 x 1 mL in microcentrifuge tubes (transform one with OLP003 and one with OLP004 below).

9. Centrifuge 3,000 g/5 min (use 7,500 rpm in microcentrifuge); discard supernatant.

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

Transformation master mix

	A	C	C
1		26 X OLP003 [µL]	26 X OLP004 [µL]
2	50% PEG	6240	6240
3	1 M LiAc	936	936
4	ssDNA (2 mg/ml)	1300	1300
5	Plasmid	42.6	47.3
6	ddH2O	841.4	836.7
7	**Total**	**9360**	**9360**

11. Add 360 µL of transformation master mix to each tube.

12. Vortex tubes until the cells are smoothly suspended.

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

01:00:00

14. Centrifuge 3,000 g/5 min (use 7,500 rpm in microcentrifuge); discard supernatant and resuspend each cell pellet in 100 µL sterile H_2O, then plate cells on SC-Leu for OLP003 transformations or SC-Ura for OLP004 transformations.

Additional resources

Plasmid amount pe...

	Plasmid [ng]
1	1000

Transformation single reaction

	A	B	C
1		OLP003 [µL]	OLP004 [µL]
2	50% PEG	240	240
3	1 M LiAc	36	36
4	2mg/ml ssDNA	50	50
5	PCR product	1.6	1.8
6	dHd2O	32.4	32.2
7	**Total**	**360**	**360**

Plasmid Transformation.6

Introduction

MATΔ::His3 STE4Δ::TRP1 yeast strain derived from SEYa or SEYα are transformed with OLP003 or OLP004. 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
- OLP003
- OLP004
- Sterile H20
- Water bath or heating block at 42°C
- Disposable 17x100 mm culture tubes with closures (VWR Catalog # 60818-725)
- SC-Leu plates
- SC-Ura plates

Procedure

Competent cells

Day 1

- [ ] 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

- [ ] 2. Inoculate 5 mL of YPAD in disposable 10 mL culture tubes with 400 µL of ONC and grow it on the shaker at 30°C/200 rpm/4 h

  This will make 2 x 1 mL competent cells.

- [ ] 3. Set water bath or heating block to 42°C.

- [ ] 4. Indicate number of transformations and plasmid concentration:

Settings

	# of Transformations	OLP003 [ng/µL]	OLP004 [ng/µL]
1	24	610	550

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		26 X [mL]
2	1 M LiAc	5.2
3	sterile H2O	46.8
4	**Total**	**52**

7. Centrifuge 3,000 g/5 min; discard supernatant and resuspend cell pellet in 2 mL sterile H_2O.

8. Centrifuge 3,000 g/5 min. Discard supernatant and resuspend cells in 2 mL of 0.1 M LiAc. Split into 2 x 1 mL in microcentrifuge tubes (transform one with OLP003 and one with OLP004 below).

9. Centrifuge 3,000 g/5 min (use 7,500 rpm in microcentrifuge); discard supernatant.

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

Transformation master mix

	A	C	C
1		26 X OLP003 [µL]	26 X OLP004 [µL]
2	50% PEG	6240	6240
3	1 M LiAc	936	936
4	ssDNA (2 mg/ml)	1300	1300
5	Plasmid	42.6	47.3
6	ddH2O	841.4	836.7
7	**Total**	**9360**	**9360**

11. Add 360 µL of transformation master mix to each tube.

12. Vortex tubes until the cells are smoothly suspended.

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

01:00:00

14. Centrifuge 3,000 g/5 min (use 7,500 rpm in microcentrifuge); discard supernatant and resuspend each cell pellet in 100 µL sterile H_2O, then plate cells on SC-Leu for OLP003 transformations or SC-Ura for OLP004 transformations.

Additional resources

Plasmid amount pe...

	Plasmid [ng]
1	1000

Transformation single reaction

	A	B	C
1		OLP003 [µL]	OLP004 [µL]
2	50% PEG	240	240
3	1 M LiAc	36	36
4	2mg/ml ssDNA	50	50
5	PCR product	1.6	1.8
6	dHd2O	32.4	32.2
7	**Total**	**360**	**360**

Plasmid Transformation.7

Introduction

MATΔ::His3 STE4Δ::TRP1 yeast strain derived from SEYa or SEYα are transformed with OLP003 or OLP004. 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
- OLP003
- OLP004
- Sterile H20
- Water bath or heating block at 42°C
- Disposable 17x100 mm culture tubes with closures (VWR Catalog # 60818-725)
- SC-Leu plates
- SC-Ura 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 5 mL of YPAD in disposable 10 mL culture tubes with 400 µL of ONC and grow it on the shaker at 30°C/200 rpm/4 h

  This will make 2 x 1 mL competent cells.

04:00:00

- [x] 3. Set water bath or heating block to 42°C.

4. Indicate number of transformations and plasmid concentration:

Settings

	# of Transformations	OLP003 [ng/µL]	OLP004 [ng/µL]
1	4	610	550

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		6 X [mL]
2	1 M LiAc	1.2
3	sterile H2O	10.8
4	**Total**	**12**

7. Centrifuge 3,000 g/5 min; discard supernatant and resuspend cell pellet in 2 mL sterile H_2O.

8. Centrifuge 3,000 g/5 min. Discard supernatant and resuspend cells in 2 mL of 0.1 M LiAc. Split into 2 x 1 mL in microcentrifuge tubes (transform one with OLP003 and one with OLP004 below).

9. Centrifuge 3,000 g/5 min (use 7,500 rpm in microcentrifuge); discard supernatant.

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

Transformation master mix

	A	C	C
1		6 X OLP003 [µL]	6 X OLP004 [µL]
2	50% PEG	1440	1440
3	1 M LiAc	216	216
4	ssDNA (2 mg/ml)	300	300
5	Plasmid	9.8	10.9
6	ddH2O	194.2	193.1
7	**Total**	**2160**	**2160**

11. Add 360 µL of transformation master mix to each tube.

12. Vortex tubes until the cells are smoothly suspended.

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

01:00:00

14. Centrifuge 3,000 g/5 min (use 7,500 rpm in microcentrifuge); discard supernatant and resuspend each cell pellet in 100 µL sterile H_2O, then plate cells on SC-Leu for OLP003 transformations or SC-Ura for OLP004 transformations.

Additional resources

Plasmid amount pe...

	Plasmid [ng]
1	1000

Transformation single reaction

	A	B	C
1		OLP003 [µL]	OLP004 [µL]
2	50% PEG	240	240
3	1 M LiAc	36	36
4	2mg/ml ssDNA	50	50
5	PCR product	1.6	1.8
6	dHd2O	32.4	32.2
7	**Total**	**360**	**360**

15.

Plasmid Transformation Line 93

Introduction

MATΔ::His3 STE4Δ::TRP1 yeast strain derived from SEYa or SEYα are transformed with OLP003 or OLP004. 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
- OLP003
- OLP004
- Sterile H20
- Water bath or heating block at 42°C
- Disposable 17x100 mm culture tubes with closures (VWR Catalog # 60818-725)
- SC-Leu plates
- SC-Ura 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 5 mL of YPAD in disposable 10 mL culture tubes with 400 µL of ONC and grow it on the shaker at 30°C/200 rpm/4 h

  This will make 2 x 1 mL competent cells.

04:00:00

- [x] 3. Set water bath or heating block to 42°C.

- [x] 4. Indicate number of transformations and plasmid concentration:

Settings

	# of Transformations	OLP003 [ng/µL]	OLP004 [ng/µL]
1	3	610	550

- [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		5 X [mL]
2	1 M LiAc	1
3	sterile H2O	9
4	**Total**	**10**

- [x] 7. Centrifuge 3,000 g/5 min; discard supernatant and resuspend cell pellet in 2 mL sterile H_2O.

- [x] 8. Centrifuge 3,000 g/5 min. Discard supernatant and resuspend cells in 2 mL of 0.1 M LiAc. Split into 2 x 1 mL in microcentrifuge tubes (transform one with OLP003 and one with OLP004 below).

- [x] 9. Centrifuge 3,000 g/5 min (use 7,500 rpm in microcentrifuge); discard supernatant.

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

Transformation master mix

	A	C	C
1		5 X OLP003 [µL]	5 X OLP004 [µL]
2	50% PEG	1200	1200
3	1 M LiAc	180	180
4	ssDNA (2 mg/ml)	250	250
5	Plasmid	8.2	9.1
6	ddH2O	161.8	160.9
7	**Total**	**1800**	**1800**

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

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

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

01:00:00

14. Centrifuge 3,000 g/5 min (use 7,500 rpm in microcentrifuge); discard supernatant and resuspend each cell pellet in 100 µL sterile H_2O, then plate cells on SC-Leu for OLP003 transformations or SC-Ura for OLP004 transformations.

Additional resources

Plasmid amount pe...

	Plasmid [ng]
1	1000

Transformation single reaction

	A	B	C
1		OLP003 [µL]	OLP004 [µL]
2	50% PEG	240	240
3	1 M LiAc	36	36
4	2mg/ml ssDNA	50	50
5	PCR product	1.6	1.8
6	dHd2O	32.4	32.2
7	**Total**	**360**	**360**