2-10 Mating of MATΔ ste4Δ strains

THURSDAY, 2019-07-04

We are going to first test MATΔ ste4Δ strains (without plasmids) for sterility. Then, we will make homozygous and heterozygous crosses of these strains that were transformed with either OLP003 or OLP004 (see 2-22 Transformation of MATΔ ste4Δ strains with OLP003 or OLP004).

Testing for sterility

We need to make sure that the double knockout lines are sterile. Therefore, we need to make new mating testers from the freezer. I would say to make new freezer stocks of them as well. To make freezer stocks inoculate some into ypad, and then spread them tomorrow, and inoculate 500 µL into 500 µL of 30% glycerol.

- [x] Inoculate small amount of freezer stock of OLY005 and OLY006 into 2 mL of liquid YPAD in the 10 mL glass tubes. Grow shaking overnight at 30°C.
- [x] Make new plates of all lines on YPAD from reference plates. Grow at 30°C overnight.

FRIDAY, 2019-07-05

- [x] Make two tubes of freezer stocks for each MAT tester: take 500 µL of ONC into 500 µL of 30% glycerol. into screw cap tubes. Label "OLY005" and "OLY006" and put into freezer box.
- [x] Spread 100 µL of ONC of each tester onto 5 YPAD plates (each), using glass beads.

The YPAD plates from yesterday have some lines that did not grow: 2, 9, 13, 21, 52, 58, 132, 99.

- [x] I will replica plate the plates and fill in the squares that did not grow with some from the reference plate.

The ones that grew overnight (which I replica plate from) will be labelled x a and the ones that I replica plate to are labelled x alpha.

SATURDAY, 2019-07-06

- [x] Replica plate the mat a testers onto all plates saying "x a". *Remember to switch velvet between each replicate.*
- [x] Replica plate the mat alpha testers onto all plates saying "x alpha". *Remember to switch velvet between each replicate.*
- [x] Incubate all plates at 30°C for four hours (10 am to 2 pm)
- [x] Replica plate all plates onto SC-8 plate labelled with the corresponding name. *Remember to switch velvet between each replicate.*
  After this step the YPAD plates can be put to waste.
- [x] Incubate the SC-8 plates at 30°C for two days.

Note if any crosses grew on SC-8:

Mating lines

MONDAY, 2019-07-08

- [x] Label YPAD plates into square pattern with 8 squares on each. Label each square according to mating scheme (Linnea prints scheme).
- [x] Label 130 SC-Ura-Leu plates according to mating scheme.
- [x] Follow Colony mating scheme for all plates, note start and end time:
  Started mating: 9.01 am **Finished mating: 10.55**

Started spreading on SC-Ura-Leu: 2.10 pm **Finished spreading on SC-Ura-Leu: 4.10pm**

- [x] Label 130 YPAD+5Foa plates according to mating scheme.

WEDNESDAY, 2019-07-10

- [x] Streak out from to single colony (the SC-Ura-Leu plate) onto YPAD+5foa. Me(Linnea), Christina, and Matt did this.

The following matings were unsuccessful:

7x7, The colonies on 7A are tiny and many, could be contamination or spread too dense, leading to weaker selection?
26x134, 26A looks perfectly normal. 134B maybe it's a little denser than the other control mates?
42x42, 42B looks like it could be a contamination. The colonies are not perfectly round but squiggly at edges and the colour is very pale yellow.
53x53, 53A potentially same phenotype as 42B. 53B on the other hand has small colonies.
49x49, looks normal
63x48, 63A slightly yellow in colour?
72x72, 72A looks a lot like 42B
89x48, 89A looks very "wet": the colonies are flatter than usual.
100x100, 100B also extremely "wet"
106x106, normal
106x134,
107x107, 107B severly contaminated, but we use colonies as far away from the contamination as possible. 107A seems quite yellow in colour.
117x117, 117B squiggly phenotype
133x92. 133A "wet" colonies.

Extra matings

FRIDAY, 2019-07-12

At 9.17 I started the mating of (Colony Mating.2): 7x7, 26x134, 39x39, 42x42, 53x53, 49x49, 63x48, 72x72, 89x48, 100x100, 106x106, 106x134, 107x107, 117x117, 133x92

39x39 Could not be done because failed to be transformed with the OLP004 plasmid twice. (but has succesfully mated into a heterozygous state with line 92).
83x83 Could not be done because failed to be transformed with the OLP003 plasmid twice. (look for heterozygote Linnea Sandell)
Did not succeed to mate with heterozygote, because mated to wrong control (83A missing, can't mate to 95B. Must mate 83B with 95A.)

Spread out on SC-Ura-Leu on 2.17pm.

Plan was to, for each YPAD + 5FoA plate sample a colony with a toothpick and transfer it into the appropriate labelled square on a YPAD plate and subsequently (using the same toothpick) onto SC-Leu plate. HOWEVER, the lines did not grow on the YPAD + 5FoA plates. We are keeping those in the incubator, seeing if anything will have grown by Monday. Meanwhile, we will:

- [x] Streak out the mated lines to single colony on YPAD. We do two matings per plate. I ask Christina to help with this.

Select against OLP003

MONDAY, 2019-07-15

- [x] Streak out all the lines from YPAD to 5FoA (Colony Mating). Christina Hsu

- [x] Streak out combination of colonies from a single mating plate onto a single YPAD plate (Colony Mating.2). Stephan Koenig

THURSDAY, 2019-07-18

Colony Mating: Colonies still small on YPAD+5FoA, check again tomorrow.

- [x] Streak out all the lines from YPAD to 5FoA (Colony Mating.2). Christina Hsu Matthew Stasiuk

SATURDAY, 2019-07-20

- [x] For matings 1-8, from YPAD + 5FoA sample a colony with a toothpick and transfer it into the appropriate labelled square on a YPAD plate and subsequently (using the same toothpick) onto SC-Leu plate (Colony Mating). Repeat for 3 colonies per plate.

Select against OLP004

MONDAY, 2019-07-22

We have success with lines 1-8, and feel encouraged to proceed with the remaining matings.

- [x] Mating 3x3 had been unsuccessful but not caught earlier. I started the mating at 11.30 (streak on Sc-ura-leu at 4.30 pm) Linnea Sandell
- [x] Mating 7x7 grew too dense. We stroke out from YPAD onto 5Foa.
- [x] For each YPAD + 5FoA plate sample a colony with a toothpick and transfer it into the appropriate labelled square on a YPAD plate and subsequently (using the same toothpick) onto SC-Leu plate (Colony Mating). Repeat for 3 colonies per plate. Did all up to #56 except:

Set 1: did not have enough single colonies on 5FoA

	A	B	C
1	3x3	remated 22 July	
2	7x7	streaked on 5FoA 22 July	
3	26x26	streaked on 5FoA 23 July	
4	26x134	streaked on 5FoA 23 July	
5	35x35	streaked on 5FoA 23 July	
6	41x41	streaked on 5FoA 23 July	worked for one colony
7	48x95	streaked on 5FoA 23 July	
8	49x49	streaked on 5FoA 23 July	
9	53x53	streaked on 5FoA 23 July	
10	54x54	streaked on 5FoA 23 July	

TUESDAY, 2019-07-23

- [x] Streak out the matings that were not used yesterday (26-54) from their YPAD plate onto YPAD + 5FoA.

- [x] For each YPAD + 5FoA plate sample a colony with a toothpick and transfer it into the appropriate labelled square on a YPAD plate and subsequently (using the same toothpick) onto SC-Leu plate (Colony Mating). Starting with #57. Repeat for 3 colonies per plate.

Set 2: did not have enough colonies on 5FoA

	A	B	C	D
1	57x48			24 July
2	58x92	only one colony	Our one sampled colony had correct genotype!	
3	69x69	only one colony	Our one sampled colony had correct genotype!	
4	78x78			24 July
5	83x95			24 July
6	84x84			24 July
7	87x87			24 July
8	87x95			24 July
9	88x136	only 2 tiny colonie	Had correct genotype!	
10	91x92			24 July
11	99x99			24 July
12	99x134			24 July
13	100x95			24 July
14	101x101			24 July
15	101x136			24 July
16	104x92			24 July

Linnea asked Matt to reorder the YPAD plates and we found a number were missing (must have nver been streaked out from SC-Ura-Leu onto YPAD). Linnea restreaked from SC-Ura-Leu (diploid selection plate) to YPAD:

Missing YPAD plates

	Mating	Restreaked on YPAD	Streaked on 5FoA
1	99x99	23 July	
2	99x134	23 July	
3	100x95	23 July	
4	101x101	23 July	
5	101x136	23 July	
6	123x123	23 July	
7	123x92	23 July	
8	124x124	23 July	
9	124x136	23 July	
10			

WEDNESDAY, 2019-07-24

Set 1

- [x] Select and circle one colony for each mating that did not grow on SC-Leu from set 1.
  Lines where all three sampled colonies grew on SC-Leu (need to sample a different colony or restreak on YPAD): 11x11
- [x] Inoculate into 2 mL of YPAD. Grow in incubator over night.

Set 2

- [x] Restreak the lines missing from Set 2 onto 5 FoA

Lagging

- [x] Streak the 3x3 cross from SC-Ura-Leu onto YPAD
- [x] Realized that the 83x95 mating does not exist because the 83A did not exist and I made the heterozygotes by mating to the controls transformed with the OLP004 plasmid. I need to mate 83B with 95A. Started 10.47, goes on SC-Ura-Leu at 15.47

Set 3

- [x] For each YPAD + 5FoA plate sample a colony with a toothpick and transfer it into the appropriate labelled square on a YPAD plate and subsequently (using the same toothpick) onto SC-Leu plate (Colony Mating). Starting with #. Repeat for 3 colonies per plate.
- [x] Three new colonies of mating 11x11 are sampled and put on YPAD and SC-Leu, sharing with lines from Set 3.

Set 3: Did not have enough colonies ...

	A	B
1	123x123	
2	123x92	
3	124x124	
4	124x136	
5	130x95	
6	134x134	
7	134x48	
8	135x135	
9	135x134	
10		

THURSDAY, 2019-07-25

Set 1

- [x] Freeze set 1 lines with 500 µL of culture and 500 µL of 30% glycerol.

Set 2

- [x] Select and circle one colony for each mating that did not grow on SC-Leu.
  Lines where all three sampled colonies grew on SC-Leu (need to sample a different colony or restreak on YPAD): none
- [x] Inoculate into 2 mL of YPAD. Grow in incubator over night.

Set 3

- [x] Restreak the lines missing from Set 3 onto 5FoA
- [x] Label new YPAD and SC-Leu plates for the missing lines of set 1, 2, and 3 (square pattern.

FRIDAY, 2019-07-26

Set 2

- [x] Freeze set 2 lines with 500 µL of culture and 500 µL of 30% glycerol.

Set 3

- [x] Select and circle one colony for each mating that did not grow on SC-Leu.
  Lines where all three sampled colonies grew on SC-Leu (need to sample a different colony or restreak on YPAD): 129x129

Lagging

- [x] Streak out 83x95 mating onto YPAD (the YPAD of the 3x3 mating is put in the fridge)
- [x] Put the 7x7 mating (5FoA) into fridge to wait

Set 1 and 2

We want to verify the identity of our samples, and will do so by checking the RDH status of the lines, by G418 resistance. This should tell us whether the lines are in the expected order or not.

- [x] Dip a cottontip into the culture and streak out into labelled square on G418 plate. Stephan did this.

SATURDAY, 2019-07-27

- [x] Put the **Set 1 Restreak on 5FoA** into the fridge to wait

SUNDAY, 2019-07-28

- [x] Streak out the 3x3 and the 83x95 mating on 5FoA Linnea Sandell
- [x] Put the **Set 2 Restreak on 5FoA** into the fridge to wait. The cross 84x84 does not have any colonies on 5FoA. I'm doubtful that incubating it longer will change that. Need to streak out on 5FoA but now we are out of 5FoA plates :(I will streak out the 84x84 mating from YPAD onto YPAD instead. Then, when we patch them, for the 84x84, we will also patch on SC-Ura to test for the loss of the OLP003 plasmid. Also want to note that the 84x84 mating even on YPAD grew very poorly, so it may just be the growth rate. It's the 88x136 mating that's on that same 5FoA plate, and we already have that crossing, so I can just leave it in the incubator for longer, wiho!

MONDAY, 2019-07-29

- [x] Put the **Set 3 Restreak on 5FoA** into the fridge to wait
- [x] Check growth on G418 Stephan Koenig (I made a new sheet in the Excel file on Dropbox)
- [x] Compare expected and predicted phenotype of diploid strains

```
# Load dependencies
library(tidyverse)

# Path to excel file
excel_filename <- paste0("~/Dropbox/Otto Lab General/experiments/",
                         "mating_of_ma_lines/knockout_records.xlsx")

# Load strain data, determine kanR genotype and keep only it and KO_code
kanR_genotype <- read_excel(excel_filename, sheet = "KO records") %>%
    mutate(kanR = RDH == "del") %>%
    select(KO_code, kanR)

# Read table with all homozygous and heterozygous matings
mating <- read_excel(excel_filename, sheet = "Mating scheme") %>%
    select(mateA, mateB, mating)

# Join once for mate A and once for mate B to determine their kanR genotype,
# then predict G418 phenotype of diploids
diploid_kanR_genotype <- inner_join(mating, kanR_genotype, c("mateA" = "KO_code")) %>%
    rename(mateA_kanR = kanR) %>%
    inner_join(kanR_genotype, c("mateB" = "KO_code")) %>%
    select(mating,
           mateA,
           mateA_kanR,
           mateB,
           mateB_kanR = kanR) %>%
    mutate(growth_on_g418_predicted = mateA_kanR & mateB_kanR)

# Load observed g418 phenotype for each diploid strain
g418_phenotype <- read_excel(excel_filename, sheet = "G418 resistance test",
                      col_types = c("text", "logical"))

# Join genoptype with phenotype it see if they match
final <- inner_join(diploid_kanR_genotype, g418_phenotype) %>%
    mutate(expected_outcome = if_else(growth_on_g418_predicted == growth_on_g418_observed,
                                      TRUE, FALSE))

# List of all strains with unexpected phenotype
unexpected_phenotype <- filter(final, expected_outcome == FALSE)
```

Test phenotype of 81A and B, 93A and B by patching on G418.
The following matings show growth on G418 although they should not grow:
81x81: 81x136 is correct, possible 81B (i.e w/ OLP004) is the incorrect strain, since 136B shows the correct phenotype in all other matings.
93x93
93x67: 67B shows the correct phenotype in all other matings, possibly 93A is incorrect strain.

- [x] Test phenotype of 81A and B, 93A and B by patching on G418.

TUESDAY, 2019-07-30

81A, 81B, and 93B show the correct phenotype on G418 (did not grow), but 93A grew (wrong phenotype).
Remate 81Ax81B.
Retransform 93 with OLP003? No, Sally suggested to drop 93 homozygote.
Hypothesis: 93A was switched with 94A during plating of transformation mix. Test 94A for G418 phenotype. If 94A does not grow (94A should be kanR), then it is likely 93A.
84A, 84B and 84x84 generally have very small colonies on YPAD or drop-out media, and do not seem to grow on 5FoA. 84 is possibly petite? Test on YPG.
Patch the lagging lines of Set 1, Set 2, Set 3:

- [x] For each YPAD + 5FoA plate sample a colony with a toothpick and transfer it into the appropriate labelled square on a YPAD plate and subsequently (using the same toothpick) onto SC-Leu plate (Colony Mating). Repeat for 3 colonies per plate.

WEDNESDAY, 2019-07-31

94A and 94B both grew on G418 (correct phenotype), i.e. identity of 93A is unclear but does not show correct phenotype fot 93A.
Patch 3x3, 83x95, 84x84 :

- [x] For each YPAD + 5FoA plate sample a colony with a toothpick and transfer it into the appropriate labelled square on a YPAD plate and subsequently (using the same toothpick) onto SC-Leu plate (Colony Mating). Repeat for 3 colonies per plate. For the 84x84 cross, also streak out colonies onto SC-Ura. Can also be replica plated from the YPAD.
- [x] Mate 81Ax81B and 93Bx67A. Linnea Sandell Started 2.20pm, stroke out at 7.50 pm.

THURSDAY, 2019-08-01

- [x] Select and circle one colony for each mating that did not grow on SC-Leu.
  Lines where all three sampled colonies grew on SC-Leu (need to sample a different colony or restreak on YPAD): 124x136, 100x95, 101x101.
- [x] Inoculate lines: 108x108 -136x92, plus extra (lagging) lines

Because the FACS results are odd, and we will need to run the haploids and remate some lines, we decide to retransform line 93.

FRIDAY, 2019-08-02

- [x] Select and circle one colony for each mating (the 3x3 and 84x84). that did not grow on SC-Leu.
- [x] Streak out 9x9, 9x134, 100x95, 101x101, 124x136 onto 5FoA from YPAD plates (lagging)
- [x] Streak out 81Ax81B and 93Bx67A from SC-Ura-Leu to YPAD.
- [x] Freeze set 3 lines with 500 µL of culture and 500 µL of 30% glycerol. Stephan did.
- [x] Use cottontip to streak out Set 3 cultures on G418 to verify order. Stephan did.
- [x] Christina retransformed Line 93.

Set 3 Linnea Sandell
Matings 108x108 to 136x92

Extras in set 3

	A
1	7x7
2	11x11
3	26x26
4	26x134
5	49x49
6	57x48
7	78x78
8	83x95
9	84x84
10	87x87
11	87x95
12	91x92
13	99x99
14	99x134
15	101x136
16	104x92
17	93x67

SUNDAY, 2019-08-04

- [x] Streak out 81Ax81B and 93Bx67A from YPAD to 5FoA

MONDAY, 2019-08-05

- [x] Because cross 29x29 looks haploid from the FACS, need to be remated Linnea Sandell
- [x] Remate 93Ax93B and 93Ax67B
- [x] Update the MA knockout excel file

TUESDAY, 2019-08-06

- [x] Score G418 phenotype for haploid strains.
- [x] Compare expected and predicted phenotype of haploid strains.

```
# Load dependencies
library(tidyverse)
library(readxl)

# Path to excel file
excel_filename <- paste0("~/Dropbox/Otto Lab General/experiments/",
                         "mating_of_ma_lines/knockout_records.xlsx")

# Load strain data, determine kanR genotype and predicted phenotype on G418 (both the same)
# and keep only them and KO_code
kanR_genotype <- read_excel(excel_filename, sheet = "KO records") %>%
  mutate(kanR = RDH == "del",
         growth_on_g418_predicted = kanR) %>%
  select(KO_code, kanR, growth_on_g418_predicted)

# Load observed g418 phenotype for each haploid strain
g418_phenotype <- read_excel(excel_filename, sheet = "G418 resistance test haploid",
                             col_types = c("numeric", "logical"))

# Join genoptype with phenotype it see if they match
final <- inner_join(kanR_genotype, g418_phenotype) %>%
  mutate(expected_outcome = if_else(growth_on_g418_predicted == growth_on_g418_observed,
                                    TRUE, FALSE))

# List of all strains with unexpected phenotype
unexpected_phenotype <- filter(final, expected_outcome == FALSE)
```

All haploid strains have the expected G418 phenotype!

- [x] Compare expected and predicted phenotype of lagging diploid strains.
  All strains have the expected phenotype!

Several lines looked haploid from the flow cytometry. This requires remating of lines: 115Ax115B, 95Ax134B, 135Ax135B, 131Ax131B, 117Ax134B. Linnea does.

WEDNESDAY, 2019-08-07

- [x] Streak out 29x29 and the retransformed 93Ax93B and retransformed 93Ax67B matings from SC-Ura-Leu to YPAD
- [x] Patch colonies from 9x134, 81x81, 93Bx67A, 100x95, 101x101, 124x136 onto SC-Leu and YPAD.

THURSDAY, 2019-08-08

- [x] Matt restreak 115Ax115B, 95Ax134B, 135Ax135B, 131Ax131B, 117Ax134B from SC-Ura-Leu onto YPAD.

FRIDAY, 2019-08-09

- [x] Streak out 29x29 and 93Ax93B and 93Ax67B matings from YPAD to 5FoA
- [x] Select and circle one patch from 9x134, 81x81, 93Bx67A, 100x95, 101x101, 124x136.
- [x] Inoculate set 4: **3x3, 83x95, 84x84, 9x134, 100x95, 101x101, 124x136, 81x81**

SATURDAY, 2019-08-10

- [x] Linnea Sandell restreak 115Ax115B, 95Ax134B, 135Ax135B, 131Ax131B, 117Ax134B from YPAD onto 5FoA (at this point, you should have found out whether they are true haploids or actual diploids from the FACS).
- [x] Freeze set 4 lines with 500 µL of culture and 500 µL of 30% glycerol.

MONDAY, 2019-08-12

- [x] 29x29 streak out on 5FoA

TUESDAY, 2019-08-13

- [x] Patch colonies from 29x29 and 93Ax93B and 93Ax67B, 115Ax115B, 95Ax134B, 135Ax135B, 131Ax131B, 117Ax134B matings onto SC-Leu and YPAD

WEDNESDAY, 2019-08-14

- [x] Select and circle one patch from 29x29 and 93Ax93B and 93Ax67B, 115Ax115B, 95Ax134B, 135Ax135B, 131Ax131B, 117Ax134B matings
- [x] Inoculate set 29x29, 93x93, 93x67, 115x115, 95x134, 135x135, 131x131, 117x134 growing shaking in incubator overnight at 30°

FRIDAY, 2019-08-16

- [x] Freeze set 5 lines with 500 µL of culture and 500 µL of 30% glycerol.
- [x] Use cottontip to streak out Set 5 cultures on G418 to verify RDH status.

Colony mating

Introduction

This protocol mates lines transformed with OLP003 and OLP004. Successful mating is evaluated by plating on SC-Ura-Leu. Loss of the plasmid is confirmed by streaking on 5FoA.

Materials

- Line transformed with OLP003 (growing on SC-Leu)
- Line transformed with OLP004 (growing on SC-Ura)
- YPAD plate
- SC-Ura-Leu plate
- YPAD+5FoA plate

Procedure

Mating

- [ ] 1. Use a toothpick to transfer a small amount of OLP003 transformed line to a labelled square on YPAD plate.
- [ ] 2. Use a toothpick to transfer a small amount of OLP004 transformed line to the same labelled square on YPAD plate.
- [ ] 3. Incubate 5 hours @ 30°C.
- [ ] 4. Use cottontip to transfer the spot to a large plate of SC–Ura–Leu, streaking the cottontip over the whole surface of the plate. Incubate at 30°C for 2 d.

Selection to lose plasmids

- [ ] 5. Streak full colonies growing on SC-Ura-Leu plate to single colonies onto YPAD+5FoA plate. Incubate at 30°C for 3 d.