2-31 FACS 2

WEDNESDAY, 2019-11-20

Preliminary test

Inoculate our samples: control SEY a and SEY alpha and SEY diploid.
Nathaniel: "Perhaps the best ancestor to compare with the mated diploids would be one of the RDH-delta control lines. The RDH+ control lines have an extra ChrXI, which won't be the case for the mated diploids."

We will use a-1, a-2, a-3, alpha-1, alpha-2, alpha-3, dip-1, dip-2, dip-3 three replicates each, 27 in total. Thaw freezer stocks. (Use MA line aliquot 2 controls).

Do 50 µg /mL of ampicillin. The stock is at 50 µg /µL. So basically take as many µL as media you want to use.

- [x] Inoculate with thawed freezer stock RDH-delta controls lines. Use MA lines aliquot 2. Incubate at 30C at 150rpm overnight.

Materials for FACS

	A	B	C	D
1	Reagent	Per sample	In total	Number of samples
2	Sodium citrate	3	126	40
3	RNAase A	25	1050	
4	Sytox Green	30	1260	
5	Ethanol	1	42	

THURSDAY, 2019-11-21

- [x] Continue following the protocol in Flow Cytometry test.

FRIDAY, 2019-11-22

- [x] Boil RNAase Matthew Stasiuk
- [x] Continue with day 3 in Flow Cytometry test. Incubating at 5.10pm

SATURDAY, 2019-11-23

- [x] Linnea Sandell Stain the cells according to day 4 in Flow Cytometry test

MONDAY, 2019-11-25

- [x] Linnea Sandell and Matthew Stasiuk run samples on LSC Attune at 9am.

Results of preliminary FACS are uncertain. The curves had unexpected shapes and plateaus. Running the samples slower may help, so we will run the samples again this week at a different speed. (Try 25 µL/min).

WEDNESDAY, 2019-11-27

- [x] Matthew Stasiuk Re-run preliminary samples on LSC Attune at 10am - 1230pm. Use a slower acquisition rate.

Preliminary FACS - RUN 2:

Samples run in same plate as previously - A1 is now A5, A2 is now A6, etc.

Using slower acquisition rate of 25 µL/min, 1 or 2 mixes. Stopping at 10,000 events. Peaks are much sharper on all the samples. We see no 'plateau' that appeared in curves last time (25 Nov). For future use, we estimate sample run time at 1 min. Considering running one sample at first before running a whole plate in the future. This can help us get an idea of quality before committing to reading all the samples.
Also, we will export statistics with the FACS results.
Potential to decrease total sample volume, which can alleviate reagent usage.

TUESDAY, 2019-12-03

- [ ] Inoculate lines from 15 µL of the fridge cultures of KOMA lines. Also use 15 µL of two control lines a-2 and dip-2, but made a mistake and took them from aliquot 3 rather than aliquot 2. Cultures grown at 6pm.

FACS_codes_december.csv

WEDNESDAY, 2019-12-04

- [x] Started Day 1 in FLOW CYTOMETRY at 9am. We use 96-well storage plates rather than tubes this time around.

THURSDAY, 2019-12-05

- [x] Day 2 in FLOW CYTOMETRY

FRIDAY, 2019-12-06

- [x] Day 3 in FLOW CYTOMETRY (stain)
- [x] Matthew Stasiuk did day 1-3 of the flow cytometry for the contaminant. We do this so that we can see if it would influence our results. However, it looks like the samples look good, so I'm not sure we need to worry about it...

SATURDAY, 2019-12-07

- [x] Run samples on LSC Attune (7-8 hour day). Leave time for the other user who contacted Linnea to use the machine.

SUNDAY, 2019-12-08

- [x] Continue running samples on LSC Attune. **Linnea spotted triploid yeast lines. Follow to** 2-32 Triploid Lines .

WEDNESDAY, 2019-12-11

- [x] Fast prep samples to run Thursday 12 Dec: 33x33, 35x35, 35x46, 48x48, 106x106, 111x111, 111x93, 134x134.

Ethanol step done at 9:50AM - 11:30AM
RNase at 11:50AM for overnight.

THURSDAY, 2019-12-12

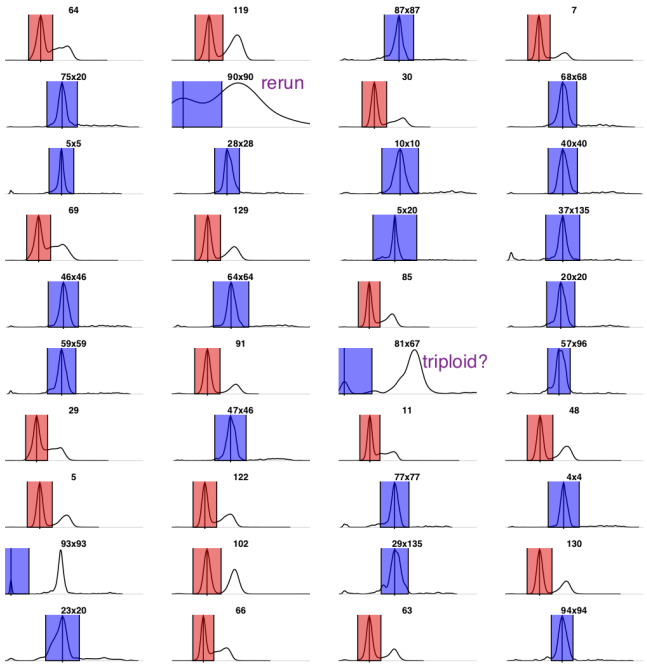
- [x] Run final plate of samples on LSC Attune.
- [x] Prep samples with sytox dye.

7.5 µL * 14 samples = 105 µL sytox stock
242.5*14 = 3395 µL sodium citrate.
😂 gosh I was tired, we only have 8 samples to run

FRIDAY, 2019-12-13

FACS went well. We have to discuss the results, specifically line 81 and 39. Matthew Stasiuk

facs_december.png



FACS_w:_events.pdf

FACS RESULTS:

Linnea and Matt think 81 and 39 may have been diploid before they were mated. This may be why they are triploid. We will grow up and perform FACS on the 39 and 81 KO lines to see if this is the case. At the same time repatch/remate 81Ax81B, 81Ax67B, and 39Ax67 in case they work a second time around.

Also lines 61x61 and 80x80 look haploid, while 61 and 80 look haploid themselves. We will remate these lines.

2-29 Mating.2 of MATΔ and ste4Δ strains

Lines 44 and 73 look diploid, but their diploid counterparts look diploid and normal. Linnea thinks the lines may have been able to mate while in the process of losing their plasmid. For line 73 this seems possible because some cells could act as mata once they lose the matalpha plasmid, though we aren't sure how they could if they no longer have the STE4 locus.

We will have to redo some lines that have poor curves. For some, low event number may have contributed to poor quality. Linnea thinks it is because we grew up these cultures for a shorter time, and not because of the plate prep method. We will grow them longer next time so we have more cells. What should we do for the samples that had a low count (<500?) but look acceptable? Standardizing the event amounts is ideal but may not be possible for the lines that grow worse...
REDO: 90x90, 29x135, 32x32, 56x56, 134x135, 128x135, 22x22, 95x95. Consider whether to redo specifically low count curves.

FRIDAY, 2020-01-03

Follow protocol named FACS: lagging lines 2.

Samples to run:

Rerun samples (poor curves/low counts:

90x90, 29x135, 32x32, 56x56, 134x135, 42x42, 128x135, 95x95

Lagging strain samples:

55x55, 61x61, 80x80, 81x81, 81x67, 44, 73, 81

(Very low counts ~500 or less but visually ok: 130x130, 51x51, 88, 104x104, 15x15, 22x22, 3x3)

Controls:

a-2, dip-2 (aliquot 3)

8+2+ 8 +7 = 25 samples

Flow cytometry: lagging lines 2 well ...

	A	B
1	61x61	A1
2	81x81	B1
3	15x15	C1
4	55x55	D1
5	51x51	E1
6	42x42	F1
7	95x95	G1
8	22x22	H1
9	130x130	A2
10	80x80	B2
11	81	C2
12	dip-2	D2
13	90x90	E2
14	73	F2
15	128x135	G2
16	88	H2
17	29x135	A3
18	56x56	B3
19	a-2	C3
20	3x3	D3
21	44	E3
22	104x104	F3
23	81x67	G3
24	32x32	H3
25	134x135	A4
26	Blank	B4
27	Blank	C4

Flow Cytometry: Lagging lines 2 reagents

	A	B	C	D
1	Reagent	Per sample (µL)	In total	Number of samples
2	Sodium citrate	3000	81000	25
3	RNAase A	25	675	
4	Sytox Green	30	810	
5	Ethanol	1	27	

(inventory: no sodium citrate stock, have 1.5mL RNaseA , ~1mL Sytox green solution, 100mL ethanol).

- [x] DAY 1: Inoculate YPAD+amp with KOMA fridge stocks, lagging line colonies, and a-2/dip-2 controls. See scheme above. 20µL samples.

SATURDAY, 2020-01-04

Followed Day 2 and Day 3 protocol. Made sodium citrate solution.

SUNDAY, 2020-01-05

Followed Day 4 and 5 protocol. Ran on FACS. Everything looks pretty ugly.

facs_lagging.pdf

Especially worrying is 90x90, that looks haploid, and 81x67, that looks triploid.

MONDAY, 2020-01-06

Turns out there was a contaminant in the media, whole bottle is white of mold. Probably the reason why the samples all look suspicious...

WEDNESDAY, 2020-01-08

Re-inoculate diploid lines of interest from single colony. (take dip-2 from aliquot 2 of MA lines).
Start new FACS protocol for diploid lagging lines.

THURSDAY, 2020-01-09

DAY 2 Diploids lagging lines

Start new FACS protocol for haploid lagging lines. (take a-2 from aliquot 2 of MA lines)/

FRIDAY, 2020-01-10

DAY 3 Diploids lagging
Day 2-3 Haploids lagging

SATURDAY, 2020-01-11

Day 4 and 5 of lagging (run in same order as previously). Linnea Sandell

MONDAY, 2020-01-20

- [x] Follow DAY 1 for Flow Cytometry: Lagging 3. Matthew Stasiuk . We are re-running the FACS for these samples because of the contamination found previously. How should we make the YPAD this time around: we can just use the tubes that we have left from the growh of all the lines.

Inoculate lines from bioscreen stocks:

Lagging strain samples:
55x55, 61x61, 80x80, 81x81, 81x67, 44, 73, 81
(poor curves)
90x90, 29x135, 32x32, 56x56, 134x135, 42x42, 128x135, 95x95

(Very low counts ~500 or less but visually ok:
130x130, 51x51, 88, 104x104, 15x15, 22x22, 3x3)
Controls:
a-2, dip-2 (aliquot 3)

Table2

	A	B
1	A1	22x22
2	B1	81x81
3	C1	81
4	D1	128x135
5	E1	Blank
6	F1	15x15
7	G1	56x56
8	H1	80x80
9	A2	95x95
10	B2	104x104
11	C2	dip-2
12	D2	55x55
13	E2	130x130
14	F2	Blank
15	G2	81x67
16	H2	61x61
17	A3	32x32
18	B3	73
19	C3	51x51
20	D3	3x3
21	E3	44
22	F3	42x42
23	G3	134x135
24	H3	29x135
25	A4	a-2
26	B4	90x90
27	C4	88

Table1

	A	B	C	D
1	Reagent	Per sample (µL)	In total	Number of samples
2	Sodium citrate	3000	81000	25
3	RNAase A	25	675	
4	Sytox Green	30	810	
5	Ethanol	1	27	

TUESDAY, 2020-01-21

- [x] Follow DAY 2 and DAY 3 for Flow Cytometry: Lagging 3. Linnea Sandell

WEDNESDAY, 2020-01-22

- [x] Follow DAY 4 for Flow Cytometry: Lagging 3. Matthew Stasiuk .

FRIDAY, 2020-01-24

- [x] Run samples in FACSattune machine @ Friday Jan 24, 10AM-12PM. Matt saw that many looked good. A few had odder shapes or looked shifted, but that has to be checked over.

MONDAY, 2020-02-03

facs_lagging3.pdf

Flow Cytometry Lagging 4

Day 1: Kismet + Matt: See Flow Cytometry

- [x] Inoculate 33x33, 35x35, 35x46, 48x48, 106x106, 111x111, 111x93, 134x134, 35, 111.

TUESDAY, 2020-02-04

Need to do facs on lagging lines

Day 2:

- [x] Day 2 of facs prep Bryn Wiley

WEDNESDAY, 2020-02-05

FACS: Lagging 4

- [x] Day 3 of FACS prep Bryn Wiley

Table3

	A	B	C	D
1	Reagent	Per sample	In total	Number of samples
2	Sodium citrate	3	306	100
3	RNAase A	25	2550	
4	Sytox Green	30	3060	
5	Ethanol	1	102	

THURSDAY, 2020-02-06

FACS: Lagging 4

- [x] Day 4 of FACS prep Bryn Wiley

FRIDAY, 2020-02-07

FACS: Lagging 4

- [x] Transfer and run samples on LSCAttune at 2:00 pm - 3:15 pm.

  FCS samples are labelled A6=A1, B6=B1, and so on.

  48x48 looks very weird in FACS.