2-24 FACS of diploids

WEDNESDAY, 2019-07-24

Materials for FACS

	A	B	C	D
1	Reagent	Per sample	In total	Number of samples
2	Sodium citrate	3	792	262
3	RNAase A	25	6600	
4	Sytox Green	30	7920	
5	Ethanol	1	264	

THURSDAY, 2019-07-25

- [x] Start Set 1, see Flow cytometry Day 1
- [x] Inoculate Set 2

Set 1

	Well	Mating	Well	Mating	Well	F	G	H	I	J	K	L	Well	N	O	P
1	A1	mata control (unstained)	B1	4x134	C1	12x12	D1	19x19	E1	27x27	F1	33x33	G1	39x92	H1	45x67
2	A2	matalpha control (unstained)	B2	5x5	C2	12x136	D2	19x92	E2	27x136	F2	33x136	G2	40x40	H2	46x46
3	A3	diploid control (unstained	B3	5x136	C3	14x14	D3	20x20	E3	28x28	F3	34x34	G3	40x48	H3	46x48
4	A4	mata control (stained)	B4	6x6	C4	14x48	D4	20x136	E4	28x67	F4	34x95	G4	41x41	H4	47x47
5	A5	matalpha control (stained)	B5	6x95	C5	15x15	D5	22x22	E5	29x29	F5	35x35	G5	41x134	H5	47x134
6	A6	diploid control (stained)	B6	7x48	C6	15x67	D6	22x134	E6	29x95	F6	35x48	G6	42x42	H6	48x48
7	A7	1x1	B7	8x8	C7	16x16	D7	23x23	E7	30x30	F7	36x36	G7	42x136	H7	48x95
8	A8	1x67	B8	8x67	C8	16x134	D8	23x67	E8	30x92	F8	36x67	G8	43x43	H8	49x95
9	A9	2x2	B9	9x9	C9	17x17	D9	24x24	E9	31x31	F9	37x37	G9	43x92	H9	50x50
10	A10	2x92	B10	10x10	C10	17x95	D10	24x95	E10	31x48	F10	37x134	G10	44x44	H10	50x48
11	A11	3x48	B11	10x95	C11	18x18	D11	25x25	E11	32x32	F11	38x38	G11	44x136	H11	51x51
12	A12	**4x4**	B12	**11x92**	C12	**18x92**	D12	**25x48**	E12	**32x134**	F12	**38x95**	G12	45x45	H12	**51x67**

FRIDAY, 2019-07-26

- [x] Start Set 2, see Flow Cytometry 2

Set 2

	Well	Mating	Well	Mating	Well	F	G	H	I	J	K	L	Well	N	O	P
1	A1	53x53	B1	59x136	C1	65x92	D1	71x134	E1	77x95	F1	85x92	G1	93x93	H1	100x100
2	A2	53x134	B2	60x60	C2	66x66	D2	72x72	E2	78x92	F2	86x86	G2	93x67	H2	102x102
3	A3	54x54	B3	60x134	C3	66x136	D3	72x95	E3	79x79	F3	86x134	G3	94x94	H3	102x67
4	A4	54x95	B4	61x61	C4	67x67	D4	73x73	E4	79x48	F4	88x88	G4	94x48	H4	103x103
5	A5	55x55	B5	61x95	C5	67x92	D5	73x95	E5	80x80	F5	88x136	G5	95x95	H5	103x48
6	A6	55x136	B6	62x62	C6	68x68	D6	74x74	E6	80x134	F6	89x89	G6	95x134	H6	104x104
7	A7	56x56	B7	62x67	C7	68x136	D7	74x48	E7	81x81	F7	89x48	G7	96x96	H7	105x105
8	A8	56x67	B8	63x63	C8	69x69	D8	75x75	E8	81x136	F8	90x90	G8	96x95	H8	105x136
9	A9	57x57	B9	63x48	C9	69x67	D9	75x67	E9	82x82	F9	90x48	G9	97x97	H9	106x106
10	A10	58x58	B10	64x64	C10	70x70	D10	76x76	E10	82x67	F10	91x91	G10	97x67	H10	106x134
11	A11	58x92	B11	64x134	C11	70x48	D11	76x134	E11	84x48	F11	92x92	G11	98x98	H11	107x107
12	A12	**59x59**	B12	65x65	C12	71x71	D12	77x77	E12	85x85	F12	92x136	G12	98x92	H12	**107x95**

SATURDAY, 2019-07-27

- [x] Inoculate ancestral SEYa+, SEYalpha+, SEYdip+

SUNDAY, 2019-07-28

- [x] Start control lines Linnea Sandell and incorporate into Set 2. Remember you want to have one sample for staining and one without staining. RNAase treatment overnight.

MONDAY, 2019-07-29

- [x] Last step of Set 2.

WEDNESDAY, 2019-07-31

- [x] Run set 1 and set 2 in FACS (up to 107x95)

Everything is so saaaad

FACS_snafu.pdf

FRIDAY, 2019-08-02

I used aliquot 1 of the Double Knockout MA lines for inoculating cultures for FACS. Line 84 did not grow, I put 1 mL of the culture thinking there might still be cells in there, and washed it one extra time with water before proceeding to the ethanol step. It looked like 134x48 might be contaminated (if it turns out weird you know why).
I did three dilutions for the controls, to test the hypothesis that cell to stain ratio affects the height of the fluorescence peak.

Controls, dilution test

	Sample name	µL used
1	a+1.1	20
2	a+1.2	40
3	a+1.3	200
4		

SUNDAY, 2019-08-04

Did FACS on Set 3.

facs.pdf

MONDAY, 2019-08-05

The following lines need to be diluted, vortexed, re-run:

Table1

	Rerun with final set	B
1		
2	9x9	
3	24x24	
4	46x46	
5	47x47	
6	51x67	
7	53x53	
8	53x134	
9	54x54	
10	59x59	
11	59x136	
12	60x60	
13	61x61	
14	63x63	
15	65x65	
16	65x92	
17	70x70	
18	71x134	
19	72x72	
20	72x95	
21	73x73	
22	75x75	
23	76x134	
24	77x77	
25	78x92	
26	79x48	
27	88x88	
28	90x48	
29	94x94	
30	94x48	
31	95x95	
32	103x103	
33	103x48	
34	104x104	
35	108x67	
36	109x136	
37	110x67	

38	111x111	
39	111x92	
40	113x67	
41	116x116	
42	117x117	
43	118x136	
44	119x119	
45	121x136	
46	122x122	
47	122x67	
48	123x123	
49	126x126	
50	126x92	
51	128x95	
52	129x129	
53	130x95	
54	135x134	
55	136x136	
56	11x11	
57	26x26	
58	49x49	
59	57x48	
60	87x87	
61	99x99	
62	99x134	
63	101x136	
64	104x92	

Plus all controls and the haploid DO KO.

WEDNESDAY, 2019-08-07

Starting cultures of a new set of lines, to run replicates to see if there is consistent variation between lines in the fluorescence of their lower peak (G1
cells).

Lines included in the fourth set, FLOW CYTOMETRY 5

	set	well	sample
1	4	A1	48x95
2	4	A2	29x95
3	4	A3	39x92
4	4	A4	8x67
5	4	A5	32x134
6	4	A6	18x92
7	4	A7	25x48
8	4	A8	a-1
9	4	A9	45x67
10	4	A10	dip-2
11	4	A11	dip-2
12	4	A12	a-2
13	4	B1	2x92
14	4	B2	29x95
15	4	B3	25x48
16	4	B4	25x48
17	4	B5	23x23
18	4	B6	135x135
19	4	B7	45x67
20	4	B8	4x4
21	4	B9	117x134
22	4	B10	35x35
23	4	B11	8x67
24	4	B12	35x35
25	4	C1	4x134
26	4	C2	8x67
27	4	C3	4x134
28	4	C4	12x12
29	4	C5	48x95
30	4	C6	95x134
31	4	C7	35x35
32	4	C8	130x130
33	4	C9	dip-1
34	4	C10	95x134
35	4	C11	22x134
36	4	C12	32x134
37	4	D1	29x29

38	4	D2	135x135
39	4	D3	29x29
40	4	D4	24x95
41	4	D5	2x2
42	4	D6	4x4
43	4	D7	2x92
44	4	D8	117x134
45	4	D9	dip-2
46	4	D10	115x115
47	4	D11	22x134
48	4	D12	dip-1
49	4	E1	130x130
50	4	E2	15x15
51	4	E3	2x2
52	4	E4	18x92
53	4	E5	10x10
54	4	E6	4x4
55	4	E7	131x131
56	4	E8	12x136
57	4	E9	12x12
58	4	E10	34x95
59	4	E11	131x131
60	4	E12	115x115
61	4	F1	a-2
62	4	F2	34x95
63	4	F3	48x95
64	4	F4	45x67
65	4	F5	a-2
66	4	F6	18x92
67	4	F7	131x131
68	4	F8	32x134
69	4	F9	12x136
70	4	F10	10x10
71	4	F11	23x23
72	4	F12	117x134
73	4	G1	a-1
74	4	G2	29x95
75	4	G3	39x92
76	4	G4	dip-1
77	4	G5	29x29

78	4	G6	34x95
79	4	G7	115x115
80	4	G8	2x92
81	4	G9	24x95
82	4	G10	24x95
83	4	G11	10x10
84	4	G12	12x136
85	4	H1	23x23
86	4	H2	22x134
87	4	H3	15x15
88	4	H4	15x15
89	4	H5	95x134
90	4	H6	39x92
91	4	H7	12x12
92	4	H8	130x130
93	4	H9	4x134
94	4	H10	135x135
95	4	H11	2x2
96	4	H12	a-1

MONDAY, 2019-08-12

- [x] Inoculated lines for the fifth set, **FLOW CYTOMETRY 6 @ 5.50 pm**

Flow cytometry 1

Introduction

This protocol tells you how to prepare samples for use in flow cytometry. It was adapted from a previous protocol designed by Aleeza Gerstein. To use the UBC flow cytometry, you need to go through training. See ubcflow.ca.
The username of our lab is otto, and our password is test123.

Schedule the time for FACS here: ubcflow.ca
Lines in set 1: matings 1x1 until 55x136

Materials

- YPAD + Ampicillin (2.5 µL mL^{-1})
- 1.5 mL centrifuge tubes
- Sterile water
- 70% Ethanol (kept cold in fridge)
- Sodium citrate (50 mM, ph 7)
- RNAase A (10 mg mL^{-1})
- Sytox green (50 mg mL^{-1})
- Aluminium foil

Procedure

Day 1 Thursday 25 July

- [x] 1. Make a sheet of what sample goes into what well. If you are planning to do replicates, randomization is key. If you are only interested in ploidy, a single run is probably enough and randomization is optional.
- [x] 2. From the overnight cultures, sample 20 µL from each well into 1.5 mL centrifuge tubes.
- [x] 3. Add 1 mL of sterile water.
- [x] 4. Centrifuge tubes at 2500 rpm for 5 minutes (bc it takes that long for me to process the other samples).
- [x] 5. Remove supernatant.
- [x] 6. Add 1 mL of cold 70 % Ethanol (use the Finntip Stepper for ease).
- [x] 7. Invert the tubes to gently mix the samples.
- [x] 8. Incubate tubes one hour at room temp. Except samples 56x56 and 56x57 that are put at 4 °C, and incorporated with Set 2 after two days.

01:00:00

- [x] 9. Centrifuge tubes at 2500 rpm for 5 minutes.
- [x] 10. Remove supernatant.
- [x] 11. Add 1 mL of sodium citrate to all samples.
- [x] 12. Invert tubes to gently mix samples.
- [x] 13. Centrifuge tubes at 2500 rpm for 5 minutes.
- [x] 14. Remove supernatant.
- [x] 15. Add 1 mL of Sodium Citrate + RNAase A mixture.

    Sodium citrate + RNAase mixture

    25 µg of RNAse A (10 mg mL^{-1}) times number of samples

    975 µL of sodium citrate per sample

RNAase mixture

	Number of samples	RNAase needed [µL]	Sodium citrate needed [mL]
1	96	2450	95.55
2			

- [x] 16. Invert tubes to gently mix samples.
- [x] 17. Incubate at 37 °C overnight without shaking.

Day 2, Friday 26th July

- [x] 18. Centrigfuge tubes at 2500 rpm for 5 minutes.
- [x] 19. Pour off supernatant.
- [x] 20. Add 1 mL of Sodum Citrate + Sytox Green dye mixture

    Sodium citrate + Sytox Green dye mixture

    30 µg of Sytox Green dye (50 mg mL^{-1}) times number of samples

    1 mL of sodium citrate per sample

Sytox green mixture

	Number of samples	Sytox Green needed [µL]	Sodium citrate needed [mL]
1	96	2940	95.06
2			

- [x] 21. Invert tubes to gently mix samples.
- [x] 22. Cover tube racks with aluminum foil, and place racks under cardboard box to avoid light exposure. Leave overnight at room temperature.

Wednesday 31st July

- [x] 23. Put plates into 4ºC fridge until ready to read on FACS.
- [x] 24. On the day of reading, 200 µL of each sample put into assay plate. Be aware to keep the planned order of samples, and pipette up and down before sampling.
- [x] 25. Cover plates with aluminum foil and set up on a FACSAttune machine.

Flow cytometry 2

Introduction

This protocol tells you how to prepare samples for use in flow cytometry. It was adapted from a previous protocol designed by Aleeza Gerstein. To use the UBC flow cytometry, you need to go through training. See ubcflow.ca.
The username of our lab is otto, and our password is test123.

Schedule the time for FACS here:
Lines in set 2: 56x56 to 107x95

Materials

- YPAD + Ampicillin (2.5 µL mL^{-1})
- 1.5 mL centrifuge tubes
- Sterile water
- 70% Ethanol (kept cold in fridge)
- Sodium citrate (50 mM, ph 7)
- RNAase A (10 mg mL^{-1})
- Sytox green (50 mg mL^{-1})
- Aluminium foil

Procedure

Day 0

- [x] 1. Make a sheet of what sample goes into what well. If you are planning to do replicates, randomization is key. If you are only interested in ploidy, a single run is probably enough and randomization is optional.

Day 1, Friday 26 July

- [x] 2. From the overnight cultures, sample 20 µL from each well into 1.5 mL centrifuge tubes.
- [x] 3. Add 1 mL of sterile water.
- [x] 4. Centrifuge tubes at 2500 rpm for 2 minutes
- [x] 5. Remove supernatant.
- [x] 6. Add 1 mL of cold 70 % Ethanol (use the Finntip Stepper for ease).
- [x] 7. Invert the tubes to gently mix the samples.

- [x] 8. Incubate tubes two nights at 4 °C (crossings 108-114 are left in fridge to be incorporated into Flow Cytometry 3).

Day 3, Sunday 28th July

- [x] 9. Centrifuge tubes at 2500 rpm for 5 minutes.
- [x] 10. Remove supernatant.
- [x] 11. Add 1 mL of sodium citrate into all samples.
- [x] 12. Invert tubes to gently mix samples.
- [x] 13. Centrifuge tubes at 2500 rpm for 5 minutes.
- [x] 14. Remove supernatant.
- [x] 15. Add 1 mL of Sodium Citrate + RNAase A mixture.

    Sodium citrate + RNAase mixture
    25 µL of RNAse A (10 mg mL^{-1}) times number of samples
    975 µL of sodium citrate per sample

RNAase mixture

	Number of samples	RNAase needed [µL]	Sodium citrate needed [mL]
1	96	2450	95.55
2			

- [x] 16. Invert tubes to gently mix samples.
- [x] 17. Incubate at 37 °C overnight without shaking.

Day 4, Monday 29 July

- [x] 18. Centrigfuge tubes at 2500 rpm for 5 minutes.
- [x] 19. Pour off supernatant.
- [x] 20. Add 1 mL of Sodum Citrate + Sytox Green dye mixture

    Sodium citrate + Sytox Green dye mixture
    30 µg of Sytox Green dye (50 mg mL^{-1}) times number of samples
    970 µL of sodium citrate per sample

Sytox green mixture

	Number of samples	Sytox Green needed [µL]	Sodium citrate needed [mL]
1	93	2850	92.15
2			

- [x] 21. Invert tubes to gently mix samples.
- [x] 22. Cover tube racks with aluminum foil, and place racks under cardboard box to avoid light exposure. Leave overnight at room temperature.

Day 5, Wednesday 31st July

- [x] 23. 200 µL of each sample put into assay plate (be aware to keep the planned order of samples).
- [x] 24. Cover plates with aluminum foil and set up on a FACSAttune machine.

Sytox Green

Introduction

Sytox green is needed for the preparation of cells for flow cytometry

Version: 23 July 2019 CH

Materials

› SYTOX Green (Invitrogen S7020)

Procedure

✓ 1. Enter desired volume

Sytox Green Volume

	Volume [uL]
1	7000

✓ 2. Add indicated volume of sytox green the indicated amount of DMSO:

Recipe

	A	B	C
1		amount	
2	5mM Sytox Green	70	uL
3	combine with	6930	uL of volume with DMSO

✓ 3. If desired, alliquiot.

Additional Resources

1000 uL recipe

	A	B	C
1	5mM Sytox Green	10	uL
2	combine with	990	uL of volume of DMSO

✓ 4.

Flow cytometry 3

Introduction

This protocol tells you how to prepare samples for use in flow cytometry. It was adapted from a previous protocol designed by Aleeza Gerstein. To use the UBC flow cytometry, you need to go through training. See ubcflow.ca. Go to the WebCalendar. The username of our lab is Otto, and our password is test123.
To sign up for use of the FACSAttune machine, click on Views (top left corner) and choose "View another User's calendar". Choose FACSLSC Attune.

Materials

- YPAD + Ampicillin (2.5 µL mL^{-1})
- 1.5 mL centrifuge tubes
- Sterile water
- 70% Ethanol (kept cold in fridge)
- Sodium citrate (50 mM, ph 7)
- RNAase A (10 mg mL^{-1})
- Sytox green (50 mg mL^{-1})
- Aluminium foil

Procedure

Day 1

- [x] 1. Thaw frozen tubes of yeast stocks
- [x] 2. Add 20 µL of each sample into individual wells of a 96-well plate according to predetermined scheme.
- [x] 3. Add 980 µL of YPAD + Ampicillin into all wells using multichannel pipettor.
- [x] 4. Grow shaking at 30 °C overnight.

Day 2

- [x] 5. From the overnight cultures, sample 20 µL from each well into 1.5 mL centrifuge tubes.
- [x] 6. Add 1 mL of sterile water.
- [x] 7. Centrifuge tubes at 2500 rpm for 2 minutes
- [x] 8. Remove supernatant.

- [x] 9. Add 1 mL of cold 70 % Ethanol (use the Finntip Stepper for ease).
- [x] 10. Invert the tubes to gently mix the samples.
- [x] 11. Incubate tubes at room temperature for 60 minutes

01:00:00

- [x] 12. Incorporate into the FLOW CYTOMETRY 2 set.

Flow cytometry 4

Introduction

Version 2: 31 July 2019 CH (Materials)
This protocol tells you how to prepare samples for use in flow cytometry. It was adapted from a previous protocol designed by Aleeza Gerstein. To use the UBC flow cytometry, you need to go through training. See ubcflow.ca. Go to the WebCalendar. The username of our lab is Otto, and our password is test123.
To sign up for use of the FACSAttune machine, click on Views (top left corner) and choose "View another User's calendar". Choose FACSLSC Attune.

Lines: haploid 1-136
I used aliquot 1 of the Double Knockout MA lines for inoculating cultures for FACS.
Line 84 did not grow, I put 1 mL of the culture thinking there might still be cells in there, and washed it one extra time with water before proceeding to the ethanol step.
It looked like 134x48 might be contaminated (if it turns out weird you know why).

Materials

- YPAD + Ampicillin (2.5 µL mL^{-1})
- 1.5 mL centrifuge tubes
- Sterile water
- 70% Ethanol (kept cold in fridge)
- Sodium Citrate (50 mM, ph 7)
- RNAase A (10 mg mL^{-1})
- SYTOX Green Stock (50 mg mL^{-1})
- Aluminium Foil

Procedure

Day 1, 1st august

- [x] 1. Make a sheet of what sample goes into what well. If you are planning to do replicates, randomization is key. If you are only interested in ploidy, a single run is probably enough and randomization is optional.
- [x] 2. Thaw frozen tubes of yeast stocks
- [x] 3. Add 20 µL of each sample into individual 5 mL tubes with 2 mL of YPAD.
- [x] 4. Grow shaking at 30 °C overnight.

Day 2, 2nd august

- [x] 5. From the overnight cultures, sample 20 µL from each well into 1.5 mL centrifuge tubes.
- [x] 6. I did three dilutions for the controls, to test the hypothesis that cell to stain ratio affects the height of the fluorescence peak.

Table1

	A	B
1	Sample name	µL used
2	a+1.1	20
3	a+1.2	40
4	a+1.3	200
5		

- [x] 7. Add 1 mL of sterile water.
- [x] 8. Centrifuge tubes at 2500 rpm for 5 minutes
- [x] 9. Remove supernatant.

    For Control lines that had 200 µL in them, resuspend in 1 mL ddH20 and centrifuge, remove supernatant before proceeding.

- [x] 10. Add 1 mL of cold 70 % Ethanol (use the Finntip Stepper for ease).
- [x] 11. Invert the tubes to gently mix the samples.
- [x] 12. Leave for >1 hour at room temp
- [x] 13. Centrifuge tubes at 2500 rpm for 5 minutes.
- [x] 14. Remove supernatant.
- [x] 15. Add 700 µL of sodium citrate into all samples.
- [x] 16. Invert tubes to gently mix samples.
- [x] 17. Centrifuge tubes at 2500 rpm for 5 minutes.
- [x] 18. Remove supernatant.
- [x] 19. Add 1 mL of Sodium Citrate + RNAase A mixture.

    Sodium citrate + RNAase mixture

    25 µg of RNAse A (10 mg mL^{-1}) times number of samples

    975 µL of sodium citrate per sample

RNAase mixture

	Number of samples	RNAase needed [µL]	Sodium citrate needed [mL]
1	258	6500	253.5
2			

- [x] 20. Invert tubes to gently mix samples.
- [x] 21. Incubate at 37 °C overnight without shaking.

Day 4, Saturday 3rd august

- [x] 22. Centrigfuge tubes at 2500 rpm for 5 minutes.
- [x] 23. Pour off supernatant.
- [x] 24. Add 1 mL of Sodum Citrate + Sytox Green dye mixture

    Sodium citrate + Sytox Green dye mixture

    30 µg of Sytox Green dye (50 mg mL^{-1}) times number of samples

    970 µL of sodium citrate per sample

Sytox green mixture

	Number of samples	Sytox Green needed [µL]	Sodium citrate needed [mL]
1	258	7800	252.2
2			

- [x] 25. Invert tubes to gently mix samples.
- [x] 26. Cover tube racks with aluminum foil, and place racks under cardboard box to avoid light exposure. Leave overnight at room temperature.

Day 5

- [x] 27. 200 µL of each sample put into assay plate (be aware to keep the planned order of samples).
- [x] 28. Cover plates with aluminum foil and set up on a FACSAttune machine.

Sytox Green Stock 2

Introduction

Version: 23 July 2019 CH, 30 July 2019 MS, 31 July 2019 CH

Sytox green is a green-fluorescent nucleic acid stain needed for the preparation of cells for flow cytometry.

Materials

› SYTOX Green (Invitrogen S7020)

Procedure

✓ 1. Enter desired volume:

Sytox Green Stock Volume

	Volume [uL]
1	7800

✓ 2. Add indicated volume of sytox green in the indicated amount of DMSO:

Recipe

	Materials	Amount	Units
1	5mM Sytox Green	78	uL
2	DMSO	7722	uL

✓ 3. If desired, aliquot.

Additional Resources

1000 uL recipe

	A	B	C
1	5mM Sytox Green	10	uL
2	DMSO	990	uL

Flow cytometry 5

Introduction

Version 3: 7 August 2019 LS

Version 2: 31 July 2019 CH (Materials)

This protocol tells you how to prepare samples for use in flow cytometry. It was adapted from a previous protocol designed by Aleeza Gerstein. To use the UBC flow cytometry, you need to go through training. See ubcflow.ca. Go to the WebCalendar. The username of our lab is Otto, and our password is test123.

To sign up for use of the FACSAttune machine, click on Views (top left corner) and choose "View another User's calendar". Choose FACSLSC Attune.

Materials

- YPAD + Ampicillin (2.5 µL mL^{-1})
- 1.5 mL centrifuge tubes
- Sterile water
- 70% Ethanol (kept cold in fridge)
- Sodium Citrate (50 mM, ph 7)
- RNAase A (10 mg mL^{-1})
- SYTOX Green Stock (50 mg mL^{-1})
- Aluminium Foil

Procedure

Day 1, Wednesday Aug 7th

- [x] 1. Make a sheet of what sample goes into what well. If you are planning to do replicates, randomization is key. If you are only interested in ploidy, a single run is probably enough and randomization is optional.

Samples in set 4, flow cytometry 5

	A	B	C
1	set	well	sample
2	4	A1	48x95
3	4	A2	29x95
4	4	A3	39x92
5	4	A4	8x67
6	4	A5	32x134
7	4	A6	18x92
8	4	A7	25x48
9	4	A8	a-1
10	4	A9	45x67
11	4	A10	dip-2
12	4	A11	dip-2
13	4	A12	a-2
14	4	B1	2x92
15	4	B2	29x95
16	4	B3	25x48
17	4	B4	25x48
18	4	B5	23x23
19	4	B6	135x135
20	4	B7	45x67
21	4	B8	4x4
22	4	B9	117x134
23	4	B10	35x35
24	4	B11	8x67
25	4	B12	35x35
26	4	C1	4x134
27	4	C2	8x67
28	4	C3	4x134
29	4	C4	12x12
30	4	C5	48x95
31	4	C6	95x134
32	4	C7	35x35
33	4	C8	130x130
34	4	C9	dip-1
35	4	C10	95x134
36	4	C11	22x134
37	4	C12	32x134

38	4	D1	29x29
39	4	D2	135x135
40	4	D3	29x29
41	4	D4	24x95
42	4	D5	2x2
43	4	D6	4x4
44	4	D7	2x92
45	4	D8	117x134
46	4	D9	dip-2
47	4	D10	115x115
48	4	D11	22x134
49	4	D12	dip-1
50	4	E1	130x130
51	4	E2	15x15
52	4	E3	2x2
53	4	E4	18x92
54	4	E5	10x10
55	4	E6	4x4
56	4	E7	131x131
57	4	E8	12x136
58	4	E9	12x12
59	4	E10	34x95
60	4	E11	131x131
61	4	E12	115x115
62	4	F1	a-2
63	4	F2	34x95
64	4	F3	48x95
65	4	F4	45x67
66	4	F5	a-2
67	4	F6	18x92
68	4	F7	131x131
69	4	F8	32x134
70	4	F9	12x136
71	4	F10	10x10
72	4	F11	23x23
73	4	F12	117x134
74	4	G1	a-1
75	4	G2	29x95
76	4	G3	39x92
77	4	G4	dip-1

78	4	G5	29x29
79	4	G6	34x95
80	4	G7	115x115
81	4	G8	2x92
82	4	G9	24x95
83	4	G10	24x95
84	4	G11	10x10
85	4	G12	12x136
86	4	H1	23x23
87	4	H2	22x134
88	4	H3	15x15
89	4	H4	15x15
90	4	H5	95x134
91	4	H6	39x92
92	4	H7	12x12
93	4	H8	130x130
94	4	H9	4x134
95	4	H10	135x135
96	4	H11	2x2
97	4	H12	a-1

- ✓ 2. Thaw frozen tubes of yeast stocks
- ✓ 3. Add 20 µL of each sample into individual wells of a 96-well plate according to predetermined scheme.
- ✓ 4. Add 1 mL of YPAD into all wells using multichannel pipettor.
- ✓ 5. Grow shaking at 30 °C for six hours (to an OD of 0.2-0.5), from 9am to 3pm.

  Because the culture didn't reach the 0.2 OD within 8 hours (and I wanted to go home), I let the culture continue growing overnight.

OD of culture from frozen (20 µL froz...

	Time	OD
1	4h15min	0.04
2	5h40min	0.04
3	7h	0.09
4	8h	0.1
5	22h	1.1

At 7.20h I diluted the cultures down with 80 µL into 1 mL YPAD (start OD at around 0.16).

After four hours of growth (7.20 to 11.20, then measured, then sat at RT for ~1h before I transferred them to tubes).

- [x] 6. Sample 200 µL from cultures and measure their OD in the biotek machine.

OD at sampling

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.548	0.477	0.441	0.245	0.615	0.586	0.261	0.547	0.449	0.389	0.453	0.516
B	0.510	0.530	0.271	0.303	0.524	0.492	0.571	0.515	0.491	0.506	0.275	0.483
C	0.568	0.235	0.491	0.504	0.493	0.493	0.487	0.388	0.453	0.452	0.522	0.495
D	0.442	0.553	0.420	0.516	0.520	0.577	0.553	0.525	0.517	0.487	0.541	0.468
E	0.446	0.507	0.453	0.512	0.486	0.527	0.403	0.518	0.446	0.422	0.411	0.438
F	0.564	0.371	0.498	0.486	0.546	0.512	0.385	0.536	0.522	0.520	0.507	0.453
G	0.520	0.715	0.419	0.460	0.429	0.339	0.430	0.456	0.483	0.494	0.460	0.460
H	0.530	0.605	0.543	0.552	0.591	0.480	0.556	0.474	0.556	0.584	0.503	0.547

Real OD (blank removed)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.388	0.317	0.281	0.085	0.455	0.426	0.101	0.387	0.289	0.229	0.293	0.356
B	0.35	0.37	0.111	0.143	0.364	0.332	0.411	0.355	0.331	0.346	0.115	0.323
C	0.408	0.075	0.331	0.344	0.333	0.333	0.327	0.228	0.293	0.292	0.362	0.335
D	0.282	0.393	0.26	0.356	0.36	0.417	0.393	0.365	0.357	0.327	0.381	0.308
E	0.286	0.347	0.293	0.352	0.326	0.367	0.243	0.358	0.286	0.262	0.251	0.278
F	0.404	0.211	0.338	0.326	0.386	0.352	0.225	0.376	0.362	0.36	0.347	0.293
G	0.36	0.555	0.259	0.3	0.269	0.179	0.27	0.296	0.323	0.334	0.3	0.3
H	0.37	0.445	0.383	0.392	0.431	0.32	0.396	0.314	0.396	0.424	0.343	0.387

- [x] 7. Transfer 100 µL of the cultures into 1.5 mL centrifuge tubes.

  Given that a 1:13.5 (80 µL in 1080 µL) dilution of the cultures gave us an OD of 0.16, the "actual" OD of an overnight culture should be around 0.16*13.5 = 2.16. 20 µL of 2.16 OD culture would mean (20 µL * 2.16 OD / 0.4 OD = 108 µL). 10^7 cells in 1 mL of 1 OD * 0.4 OD * 0.1 mL = 400 000 cells about.

- [x] 8. Centrifuge tubes at 2500 rpm for 5 minutes
- [x] 9. Remove supernatant.
- [x] 10. Add 1 mL of ddH20 (use the Finntip Stepper for ease).
- [x] 11. Centrifuge tubes at 2500 rpm for 5 minutes
- [x] 12. Add 1 mL of cold 70 % Ethanol (use the Finntip Stepper for ease).

- [x] 13. Invert the tubes to gently mix the samples.
- [x] 14. Incubate tubes at room temperature for > 1 hour.

01:00:00

- [x] 15. Centrifuge tubes at 2500 rpm for 5 minutes.
- [x] 16. Remove supernatant.
- [x] 17. Add 1 mL of sodium citrate into all samples.
- [x] 18. Invert tubes to gently mix samples.
- [x] 19. Centrifuge tubes at 2500 rpm for 5 minutes.
- [x] 20. Remove supernatant.
- [x] 21. Add 1 mL of Sodium Citrate + RNAase A mixture.

    Sodium citrate + RNAase mixture

    25 µg of RNAse A (10 mg mL^{-1}) times number of samples

    975 µL of sodium citrate per sample

RNAase mixture

	Number of samples	RNAase needed [µL]	Sodium citrate needed [mL]
1	96	2450	95.55
2			

- [x] 22. Invert tubes to gently mix samples.
- [x] 23. Incubate at 37 °C overnight without shaking.

Day 2, Friday Aug 9th

- [x] 24. Centrifuge tubes at 2500 rpm for 5 minutes.
- [x] 25. Pour off supernatant.
- [x] 26. Add 1 mL of Sodum Citrate + Sytox Green dye mixture

    Sodium citrate + Sytox Green dye mixture

    30 µg of Sytox Green dye (50 mg mL^{-1}) times number of samples

    970 µL of sodium citrate per sample

Sytox green mixture

	Number of samples	Sytox Green needed [µL]	Sodium citrate needed [mL]
1	96	2940	95.06
2			

- [x] 27. Invert tubes to gently mix samples.
- [x] 28. Cover tube racks with aluminum foil, and place racks under cardboard box to avoid light exposure. Leave overnight at room temperature.

Day 3

- [x] 29. 200 µL of each sample put into assay plate (be aware to keep the planned order of samples).
- [x] 30. Cover plates with aluminum foil and set up on a FACSAttune machine.

Flow cytometry 6

Introduction

Version: 08 August 2019 LS

This protocol tells you how to prepare samples for use in flow cytometry. It was adapted from a previous protocol designed by Aleeza Gerstein. To use the UBC flow cytometry, you need to go through training. See ubcflow.ca. Go to the WebCalendar. The username of our lab is Otto, and our password is test123.

To sign up for use of the FACSAttune machine, click on Views (top left corner) and choose "View another User's calendar". Choose FACSLSC Attune.

Materials

- YPAD + Ampicillin (2.5 µL/mL)
- 1.5 mL centrifuge tubes
- Sterile water
- 70% Ethanol (kept cold in fridge)
- Sodium Citrate (50 mM, ph 7)
- RNase A (10 mg/mL)
- SYTOX Green Stock (50 mg/mL)
- Aluminium Foil

Procedure

Day 1

- [x] 1. Make a sheet of what sample goes into what well. If you are planning to do replicates, randomization is key. If you are only interested in ploidy, a single run is probably enough and randomization is optional.
- [x] 2. Thaw frozen tubes of yeast stocks
- [x] 3. Add 20 µL of each sample into individual wells of a 96-well plate according to predetermined scheme.
- [x] 4. Add 1 mL of YPAD into all wells using multichannel pipettor.
- [x] 5. Grow shaking at 30^{o}C overnight.

Day 2

- [x] 6. From the overnight cultures, sample 20 µL from each well into 1.5 mL centrifuge tubes. Sample C3 in plate 2 may have been sampled twice.
- [x] 7. Add 1 mL of sterile water.

- [x] 8. Centrifuge tubes at 2500 rpm for 2 minutes, except for samples Ex and Fx in plate 2, which always have 5 minutes of centrifuge time.
- [x] 9. Remove supernatant.
- [x] 10. Add 1 mL of cold 70% Ethanol (use the Finntip Stepper for ease).
- [x] 11. Invert the tubes to gently mix the samples.
- [x] 12. Incubate at room temperature for > 1 hour.
- [x] 13. Centrifuge tubes at 2500 rpm for 2 minutes, except for samples Ex and Fx in plate 2, which always have 5 minutes of centrifuge time.
- [x] 14. Remove supernatant.
- [x] 15. Add 1 mL of sodium citrate into all samples. Use old sodium citrate for Ax and Bx of plate 2.
- [x] 16. Invert tubes to gently mix samples.
- [x] 17. Centrifuge tubes at 2500 rpm for 2 minutes, except for samples Ex and Fx in plate 2, which always have 5 minutes of centrifuge time.
- [x] 18. Remove supernatant.
- [x] 19. Add 1 mL of Sodium Citrate + RNAase A mixture. Use old sodium citrate for Ax and Bx of plate 2. Use old RNAase A for samples Cx and Dx.

    Sodium citrate + RNAase mixture
    25 µg of RNAse A (10 mg/mL) times number of samples
    975 µL of sodium citrate per sample

Ax and Bx plate 2, and similarly for Cx and Dx of plate 2

	Number of samples	RNAase needed [µL]	Sodium citrate needed [mL]
1	11	325	12.675
2			

RNAase mixture

	Number of samples	RNAase needed [µL]	Sodium citrate needed [mL]
1	120	3050	118.95
2			

- [x] 20. Invert tubes to gently mix samples.

- [x] 21. Incubate at 37 °C overnight without shaking.

Day 4

- [x] 22. Centrifuge tubes at 2500 rpm for 2 minutes, except for samples Ex and Fx in plate 2, which always have 5 minutes of centrifuge time.
- [x] 23. Pour off supernatant.
- [x] 24. Make new sytox green mixture. See SYTOX GREEN STOCK 3
- [x] 25. Add 1 mL of Sodum Citrate + Sytox Green dye mixture. Sample B12 in plate 1 got old sodium citrate and sytox green (both prepped last week).

  Sodium citrate + Sytox Green dye mixture
  30 µg of Sytox Green dye (50 mg/mL) times number of samples
  970 µL of sodium citrate per sample

For A&B (old sodium citrate)

	Number of samples	Sytox Green needed [µL]	Sodium citrate needed [mL]
1	12	390	13.58
2			

Sytox green mixture

	Number of samples	Sytox Green needed [µL]	Sodium citrate needed [mL]
1	132	3990	129.01
2			

- [x] 26. Invert tubes to gently mix samples.
- [x] 27. Place tubes in cardboard box to avoid light exposure. Leave overnight at room temperature.

Day 5

- [ ] 28. 200 µL of each sample put into assay plate (be aware to keep the planned order of samples).
- [ ] 29. Cover plates with aluminum foil and set up on a FACSAttune machine.

Sytox Green Stock 3

Introduction

Version: 23 July 2019 CH, 30 July 2019 MS, 31 July 2019 CH

Sytox green is a green-fluorescent nucleic acid stain needed for the preparation of cells for flow cytometry.

Materials

› SYTOX Green (Invitrogen S7020)

Procedure

✓ 1. Enter desired volume:

Sytox Green Stock Volume

	Volume [uL]
1	4000

✓ 2. Add indicated volume of sytox green in the indicated amount of DMSO:

Recipe

	Materials	Amount	Units
1	5mM Sytox Green	40	uL
2	DMSO	3960	uL

✓ 3. If desired, aliquot.

Additional Resources

1000 uL recipe

	A	B	C
1	5mM Sytox Green	10	uL
2	DMSO	990	uL