Flow cytometry (Plate Variant)

Introduction

Version: 09 December 2019 MS

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.

This Flow Cytometry protocol is optimised for using plates. In particular, it uses a quarter of the volume of reagents that the normal procedure uses, and it allows greater amounts of samples to be prepared faster.

Materials

- YPAD + Ampicillin (2.5 µL/mL)
- Round-bottom storage plates
- Plate centrifuge (ask Irwin lab)
- Sterile water
- 70% Ethanol (kept cold in fridge)
- Sodium Citrate (50 mM, ph 7)
- RNase A (10 mg/mL) *Can be boiled before using to denature any DNAase*
- SYTOX Green Stock (50 mg/mL)
- Aluminium Foil

Procedure

Day 1

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.
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 980 µL of YPAD + Ampicillin into all wells using multichannel pipettor.
5. Grow shaking at 30°C overnight.

Day 2

6. From the overnight cultures, sample 5 µL from each well into round-bottom storage plates.

7. Add 250 µL of sterile water.
8. Centrifuge plates at 2500 rpm for 5 minutes.
9. Pipette off supernatant with a multichannel pipette.
10. Add 250 µL of cold 70% Ethanol with a multichannel pipette.
11. Pipette up and down to gently mix the samples.
12. Incubate plates overnight at 4°C, or at room temperature for > 1 hour.

Day 3

13. Centrifuge plates at 2500 rpm for 5 minutes.
14. Pipette off supernatant.
15. Add 250 µL of sodium citrate into all samples.
16. Pipette up and down to gently mix the samples.
17. Centrifuge plates at 2500 rpm for 5 minutes.
18. Pipette off supernatant.
19. Add 250 µL of Sodium Citrate + RNAase A mixture.

    Sodium citrate + RNAase mixture for 250 µL volume
    (25 ÷ 4) µg of RNAse A (10 mg/mL) times number of samples
    (975 ÷ 4) µL of sodium citrate per sample

RNAase mixture

	Number of samples	RNAase needed [µL]	Sodium citrate needed [mL]
1	96	612.5	23.8875
2			

20. Pipette up and down to gently mix the samples.
21. Incubate at 37 °C overnight without shaking.

Day 4

22. Centrifuge plates at 2500 rpm for 5 minutes.
23. Pipette off supernatant.
24. Add 250 µL of Sodum Citrate + Sytox Green dye mixture

Sodium citrate + Sytox Green dye mixture for 250 µL volume

(30 ÷ 4) µg of Sytox Green dye (50 mg/mL) times number of samples

(970 ÷ 4) µL of sodium citrate per sample

Sytox green mixture

	Number of samples	Sytox Green needed [µL]	Sodium citrate needed [mL]
1	96	735	23.765
2			

25. Pipette up and down to gently mix the samples.

26. Cover plates with lids, then with aluminum foil, and place under a cardboard box to avoid light exposure. Leave overnight at room temperature.

Day 5

27. Cover plates with aluminum foil and set up on a FACSAttune machine.