Flow Cytometry of Yeast DNA

1. Remove 1mL of cells from liquid culture at 0.5 to 1.0 OD_{600} and put into an eppendorf tube (be sure to always include an asynchronous wild-type haploid culture to calibrate the flow cytometer).

2. Spin down the cells in a microfuge (14,000 rpm for 1 minute).

3. Aspirate off the supernatant (be carful not to suck up pellet!).

4. Add 1 mL of 70% EtOH and resuspend the pellet by vortexing.

5. Let the cells sit at room temperature for at least 1 hour, then store at 4°C or continue with the protocol.

6. Spin down 0.5 mL of cells and carefully aspirate off the supernatant. Keep the other 0.5 mL of ethanol fixed cells at 4°C.

7. Resuspend the cells in 0.5 mL ddH2O.

8. Spin down the cells and aspirate off the supernatant (the pellet may not be visible, so be careful when aspirating).

9. Resuspend in 200 µL of RNase A solution (4 µL of 10mg/mL RNase A + 196 µL 50 mM Tris-Cl pH 8.0; make up a mix for all your samples).

10. Incubate the cells at 37°C for 2-4 hours.

11. Spin down the cells and aspirate off the supernatant.

12. Resuspend the pellet in 200 µL proteinase K solution (2 mg/mL proteinase K in 50 mM Tris-Cl pH7.5; make up a mix for all your samples).

13. Incubate at 50°C for 30-60 minutes.

14. Spin down the cells and aspirate off the supernatant.

15. Resuspend in 200-400 µL FACS buffer (can leave in FACS buffer at 4°C, but no longer than 1 week).

16. Transfer 10 µL of cells into 96-well plate. Add 200 µL SybrGreen (diluted 5,000X from stock in 50 mM Tris-Cl pH7.5) to each well. Samples are light sensitive, keep in dark place where possible.

27. Sonicate each sample for 3 seconds at 10% power (5-10 watt output).

28. Run samples immediately on the Flow Cytometer.

Materials

70% Ethanol (250 mL):

Mix 184.2 mL of 95% ethanol with 65.8 mL H_2O

Filter sterilize

1M Tris-Cl pH 7.5/8.0 (500 mL each):

Add 60.57g of Tris into 300 mL H_2O

Calibrate pH meter

Add HCl until solution reaches appropriate pH

Bring volume to 500 mL with H_2O

Autoclave

5M NaCl (500 mL):

Add 146.1g of NaCl to 300 mL H_2O

Bring solution to near 500 mL and add heat to dissolve NaCl

Bring volume to 500 mL with H_2O

Autoclave

1M $MgCl_2$ (100mL):

Add 20.33g $MgCl_2$ to H_2O

When dissolved, bring to 100 mL with H_2O

Autoclave

RNAse A Solution:

10 mg/mL of RNase A stored at -20°C freezer

Working Solutions:

50mM Tris-Cl pH 7.5/8.0 (250mL):

Add 12.5 mL of 1M stock to 237.5 mL of H_2O

Filter sterilize

FACS Buffer (100mL):

200 mM Tris-Cl pH 7.5	20 mL of 1M stock
200 mM NaCl	4 mL of 5M stock
78 mM $MgCl_2$	7.8 mL of 1M stock
H_2O	68.2 mL

Reference:

Dunham, M.J., Gartenberg, M.R., and Brown, G.W. (2015). *Methods in Yeast Genetics and Genomics, A Cold Spring Harbor Laboratory Course Manual, 2015 Edition.* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.