Flow Cytometry of Yeast DNA

1. Remove 1mL of cells from liquid culture at 0.5 to 1.0 OD₆₀₀ 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 careful 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).

**Materials**

70% Ethanol (250 mL):
Mix 184.2 mL of 95% ethanol with 65.8 mL H₂O
Filter sterilize

1M Tris-Cl pH 7.5/8.0 (500 mL each):
Add 60.57g of Tris into 300 mL H₂O
Calibrate pH meter
Add HCl until solution reaches appropriate pH
Bring volume to 500 mL with H₂O
Autoclave

5M NaCl (500 mL):
Add 146.1g of NaCl to 300 mL H₂O
Bring solution to near 500 mL and add heat to dissolve NaCl
Bring volume to 500 mL with H₂O
Autoclave

1M MgCl₂ (100mL):
Add 20.33g MgCl₂ to H₂O
When dissolved, bring to 100 mL with H₂O
Autoclave

**RNAsel 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 H₂O

Filter sterilize

**FACS Buffer (100mL):**

- 200 mM Tris-Cl pH 7.5
- 200 mM NaCl
- 78 mM MgCl₂
- H₂O

- 20 mL of 1M stock
- 4 mL of 5M stock
- 7.8 mL of 1M stock
- 68.2 mL

Reference: