DNA content flow cytometry with Sytox Green

1 - Darach’s protocol from December 2015

This protocol is designed/re-optimized for staining 5e6 cells. However, sytoxgreen is supposed to be fairly robust to cell number changes, so variation from this should be tolerated (see Haase and Reed 2002). Remember, PBS messes with Sytoxgreen staining.

All spins are at 6000 rcf 1min RT. You will need 50mM sodium citrate buffer (~7.2 pH), a 5mM solution of sytoxgreen in DMSO, RNAseA (100mg/ml), and ProteinaseK (20mg/ml) solutions.

- Perform an Ethanol fix in whatever condition you want. The following assumes that cells are be in 70% etOH and at -20C. You of course need to count the density of these fixed cells to proceed.
- Spin down 5e6 cells in an eppendorf.
- Aspirate ALL of the etOH. R/S in 1ml of citrate buffer. Spin.
- Put at 50C for one hour.
- Add 20ul of 20mg/ml ProteinaseK. Continue incubation for one hour (do not cut short without re-optimizing!).
- Spin.
- Aspirate and R/S in 1ml citrate buffer. Spin.
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- Sonicate standard settings (5s on/off, power 10), put on ice.
- Put 200ul of this cell suspension onto 200ul of 2uM Sytoxgreen in citrate buffer. Mix.
- Flow in the Accuri flow cytometer.

Expected results, for cells fixed during exponential growth in YPD:

Want quantification? Consider using the MLE scripts here (working on it).

2 - Older protocol, very very similar, yeast DNA content cytometry with Sytox Green
1. Pellet $5 \times 10^6$ cells. Remove sup and resuspend in ddH$_2$O and pellet again. (can pellet more than this, but don't USE more than this for the analysis.) Remove sup and resuspend pellet in 400 uL ddH$_2$O, and if not already in a 1.5 mL Eppendorf tube, transfer to one.
2. Sonicate w/ small probe (diameter at tip is ~ 0.5 cm), duty cycle 50%, output setting 3, 3 pulses (specs for Branson sonifier model 450).
3. Add 950 uL 100% EtOH (to 70%). Fix at 1 h at room temp or overnight at 4 °C or -20 °C (this is a good stopping point if you can't flow cytometry samples anytime soon, can store at -20 °C for a few weeks).
4. Pellet yeast. Remove sup and resuspend pellet in 800 uL Na citrate. Pellet again, remove sup, and resuspend pellet in 0.5 mL Na citrate containing 0.25 mg/mL RNase A. Incubate at 50 °C for at least 1-2 h, but overnight works even better.
5. Add 50 uL 20 mg/mL proteinase K and return to 50 °C for 1-2 h.
6. Sonicate as above. Add 0.5 mL Na citrate containing 2-4 uM SYTOX Green (stock is 5 mM in DMSO from Molecular Probes (now w/ Invitrogen)), mix, and transfer to Falcon 2054 tubes. Incubate in the dark for 1 h (no longer; if sample has to sit longer and flow cytometry doesn't look good, may need to sonicate the cells again, but be aware that the SYTOX green will aerosolize, so wear a mask).
7. Proceed w/ flow cytometry analysis.

Sample Dilutions:

For 14 samples

1. need at least 7 mL RNase solution: 7.125 mL Na citrate + 0.375 mL 5 mg/mL RNase A for 0.25 mg/mL final in 7.5 mL
2. need at least 7 mL SYTOX Green solution: 7.5 mL Na citrate + 6 uL 5 mM SYTOX Green for 4 uM final.

50 mM Na citrate pH 7.2:

- 14.71 g sodium citrate dehydrate (FW = 294.1 g/mol)
- In 1 L H$_2$O. Measure pH and pH w/ a few grains of citric acid monohydrate to pH 7.2. Sterile filter 0.45 um (will ferment if left unfiltered)

Notes

- You can also add 100% etOH directly to media to a final 70%. Seems to work okay for FY4 in SC, exponential.
- 10uL proteinase K also works okay with 1 hour incubation.
- 5uM final SYTOX Green works well, so 10uM input. Maybe more robust to overloading sample (too many cells per dye).
- SYTOX Green shows up in multiple channels because its so bright.
- Blue is the 488 laser, green filter is FL1, orange filter is FL2, green emission plot is Sytox Green, orange emission plot is PI.