Yeast DNA Content Analysis Using Sytox Green
From Tina DeCoste (Princeton FACS facility manager)

1. Pellet 5 x 10^6 cells. Wash in 1 mL ddH_2O, pellet, and aspirate supernatant.
   NOTE - Don't use more cells than this for the analysis.

2. Resuspend cell pellet in 400 μL, and transfer to a microcentrifuge tube (if not there already).

3. Sonicate to break up cell clumps (Misonix Sonicator 3000; microtip; Output = 5.0, 10 - half-second pulses).

4. Add 950 μL of 100% ethanol (final concentration = 70%) to fix the cells. Fix 1 hour at room temperature or overnight at 4°C or -20°C.
   NOTE - Can store samples at -20°C for a few weeks before FACS analysis, if needed.

5. Pellet yeast. Remove supernatant and wash pellet in 800 μL of 50 mM sodium citrate (pH = 7.2). Pellet again and resuspend in 500 μL of RNase A Solution. Incubate at 37°C for at least 1-2 hours (overnight works even better).

6. Add 50 μL of 20 mg/mL proteinase K and incubate at 50°C for 1-2 hours.

7. Sonicate again to break up clumps (as in step #3).

8. Add 500 μL of Sytox Green Solution, mix, and transfer to Falcon 2054 tubes. Incubate in the dark for 1 hour (but no longer; if sample sits longer and FACS analysis is low quality, sonicate again but be aware that Sytox Green will aerosolize - so wear a mask).

9. Proceed with FACS analysis (If cell density is correct, each sample should take less than 1 minute to run using the BD LSRII Multi-Laser Analyzer).
   • When counting microbes (yeast or bacteria), don't count any faster than 5,000 events per second (faster counting results in more acquisition of multiple cells in the laser at one time)
   • For DNA content, Tina draws a gate to encompass 1N and 2N cells, then counts 100,000 events in that gate
   • Before "recording" data, allow data to "acquire" for at least 10 seconds (the first 10 seconds of acquisition are very noisy and not worth collecting)

<u>50 mM Sodium Citrate</u> (pH = 7.2)
14.71 g Sodium Citrate dihydrate (MW = 294.1) for 1L
(adjust pH using a few grains of citric acid monohydrate)
(sterilize using a 0.45 μm filter… will ferment if not sterilized)

<u>RNAse A Solution</u> (for 15 samples; 500 μL used per sample)
7.125 mL of 50 mM Sodium Citrate (pH = 7.2)
0.375 mL of 5 mg/mL RNAse A (or 112.5 μL of 100 mg/mL)(final concentration = 0.25 mg/mL)

<u>Sytox Green Solution</u> (for 15 samples; 500 μL used per sample)
7.5 mL of 50 mM Sodium Citrate (pH = 7.2)
6 μL of 5 mM Sytox Green (final concentration = 4 μM)

NOTE: Sytox Green is supplied from Invitrogen as a 5 mM stock in DMSO (previously from Molecular Probes). Sytox Green is a high affinity nucleic acid stain that penetrates cells with a compromised membrane (not live cells, and can therefore be used to screen live/dead cells based on membrane integrity). Sytox Green has a molecular weight of ~600, an excitation maximum at 504 nm and an emission maxima at 523 nm. Upon DNA binding, Sytox Green increases its fluorescence over 500-fold.