Yeast DNA 1C/2C analysis with Sytox Green

From the Gresham Lab / Bot Lab binder:

**50mM Na Citrate pH7.2:**

14.71g sodium citrate (FW=294.1g/mol) in 1L H2O, pH using "a few grains" of citric acid monohydrate to pH 7.2. Filter sterilize 0.45um to prevent fermentation.

1. Pellet 5e6 cells. Remove sup and resusp in ddH2O and pellet again. You can pellet more than this at this point, but don't use more than this for later analysis without first re-optimizing the dye concentration.
2. Remove sup and resusp in 400ul ddH2O. Should be in an eppendorf at this point.
3. Sonicate with small probe (~0.5cm), duty cycle 50%, output setting 3, 3 pulses (for the Branson sonicator model 450).
4. Add 950ul 100% etOH (to 70% final). Fix 1 hour at RT or O/N at 4C or -20C. Can store at -20C for a few weeks.
5. Pellet yeast. Remove sup and resusp in 800ul na citrate.
6. Pellet, remove sup and resusp in 500ul na citrate with 0.25mg/ml RNAse A. Incubate 50C at least 1-2 hours, O/N works better.
7. Add 50ul 20mg/ml Proteinase K and return to 50C for 1-2 hours.
8. Sonicate as above.
9. Add 500ul Na citrate with 2-4uM SYTOX Green, mix and transfer to 2054 falcon tubes. Incubate in dark for 1 hour, but no longer (without sonication, beware that SYTOX aerosolizes on sonication so wear a mask).
10. Run on flow cytometer.