Yeast FACS Prep

DAY ONE

1) *Use hemocytometer to determine volume of original solution required to obtain ~1X10^7 . . . add dH$_2$O to final volume 1 mL*
   I just use 200 uL culture into 800 uL dH$_2$O
2) Pellet (2500 rpm, 3 minutes) and resuspend in 1 mL of cold 70% ethanol
3) Fix at least 60 minutes at room temperature or several days at 4°C

DAY TWO

4) Pellet (2500 rpm, 3 minutes)
5) Add 1mL sodium citrate, 50 mM pH 7
6) Pellet (2500 rpm, 3 minutes) and resuspend in sodium citrate.
7) Sonicate for 10-15 seconds at 30% -> this step isn't necessary
8) Pellet, resuspend in sodium citrate -> if didn't sonicate, unnecessary
9) Add RNAse A to 0.25 mg/mL (25 μL of 10mg/mL solution). Incubate overnight at 37°C.

DAY THREE

10) Pellet and resuspend twice (to wash off RNAse)
11) Resuspend in 970 μl sodium citrate and 30 μl of 50μm sytox green dye. Wrap the rack in aluminum foil and keep at room temperature overnight (on the bench)

DAY FOUR

13) Sonicate at lowest setting for 15 seconds immediately before FACS analysis -> also unnecessary, the analysis program Flow-Jo corrects for doublets

Italics : things that can be done if problems arise

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2007