Ploidy by PI stain and flow cytometry

Note: This requires fixing cells. Controls must be stained in the same PI working solution as strains of interest.

Materials

- Strains of interest
- Haploid control FY4 (DGY1)
- Diploid control FY4/5 (DGY3)
- Propidium iodide (PI stock, 20 mM, stored in -20°C)
- Sodium Citrate Buffer 50mM (pH 7.5)
  - 5.9g of tri-sodium citrate
  - 400mL of water
  - citric acid anhydrous to adjust the pH to 7.5
  - autoclave to sterilize
- RNaseA (100mg/ml)
- YPD or other media

Procedure

1. Start a 5 ml YPD overnight preculture for each strain and haploid and diploid controls (in 30°C incubator, rotating).
2. Prepare a 1 :10 (or 1:5) dilution of each preculture in YPD medium (1ml in 2ml tubes) - Incubate 2 hours at 30°C
   a. You want the cells to be actively dividing.
   b. You also want to make sure you have 2 mL tubes so you have enough room.
3. Centrifuge 5min at 3000 rpm ; Remove the supernatant ; Rinse with 1ml of water
4. Centrifuge 5min at 3000 rpm ; Remove the supernatant ; Add 1ml of 70% ethanol to fix the cells
   a. **OPTION: at this stage the cells can be saved some weeks at 4°C**
5. Centrifuge 5min at 3000 rpm ; Remove the supernatant ; Rinse the sample with 1ml of sodium citrate buffer 50mM (pH 7.5)
6. Centrifuge 5min at 3000 rpm ; Remove the supernatant gently with a pipette ; Resuspend cells in 1ml of sodium citrate buffer 50mM (pH 7.5) containing 5µl of RNaseA a 100mg/ml
7. Incubate 2 hours at 37°C
8. Sonicate samples
9. Add 1mL of citrate buffer + 10 µl 20 mM PI to each sample
10. Incubate 12-48 hours in the dark, 4°C
   a. The bleed through between channels should be sufficiently minimal that this will accurately measure ploidy in strains with GFP.
12. There is some code in the Gresham Lab flow cytometry pipeline to visualize these data, further work needs to be done for quantification.