This protocol describes how to obtain size estimates for MATα haploid yeast strains using a Coulter counter.

NB, tested on *S. cerevisiae* and *S. paradoxus*

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**Day 1: Inoculate**

- Inoculate each sample strain into 10 mL of the desired medium, as well as any supplemental nutrients.  
- Incubate cultures at 30° C, 225 rpm.

**Day 2: Transfer**

- Dilute an appropriate volume (e.g. 20 µL) of each culture into 10 mL of fresh medium, as well as any supplemental nutrients.  
- Incubate cultures at 30° C, 225 rpm.

**Day 3: Transfer**

- Dilute an appropriate volume (e.g. 20 µL) of each culture into 10 mL of fresh medium, as well as any supplemental nutrients.  
- Incubate cultures at 30° C, 225 rpm.

**Day 4: Arrest and measure**

1. Dilute culture to an OD of approximately 0.3, making sure that the volume of the dilution is equal to or greater than 10 mL.  
2. Return the cultures to the incubator (30° C, 225 rpm) and allow to grow for 120 minutes (to readjust to fresh media).  
3. Synchronize/arrest the strains:  
   - Pellet cells by transferring each culture to a 15 mL Falcon tube.  
   - Centrifuge each tube for 2 minutes at 5,000 rpm.  
   - Discard the supernatant (media).  
   - Wash the contents of each falcon tube twice with 4° C minimal medium (SD-glucose).  
   - Add 10 mL of 4° C minimal medium to each pellet (NB also add supplemental nutrients, for consistency).  
   - Add alpha factor to each tube, to final 4 µM concentration.  
   - Vortex each tube and pour contents into a 50 mL flask.  
   - Incubate the flasks at desired temperature for size measurements (e.g. 18°, 30° C), 225 rpm, for 120 min. NB, incubation time may be strain-specific, and as low as 90 min. Want to synchronize for minimal time until culture has < 10% buds and shmoo.  
4. Remove cultures from incubator, vortex well, and pipette an appropriate amount (e.g. 80 µL) into cuvette of 10 mL of cold (4° C) Isoton solution (filtered NaCL).  
5. Sonicate samples for 1 min, power 4.5—5.0.  
6. Measure sample on the Coulter counter.