Old Yeast Cell Sorting

1. Grow 3-5 ml overnight culture in YPD. Next morning, measure $OD_{600}$. Dilute into 50 ml YPD to yield $OD_{600} \sim 0.05$. Grow at 25 - 30°C to yield $OD_{600} < 0.5$.

   - Place EZ-link biotin at room temperature.
   - Place avidin magnetic beads on rotator in cold room to disperse.

2. Collect $1 \times 10^8$ cells. Spin in IEC centrifuge 10 minutes at 2000 rpm. If convenient, collect 2 $\times 10^8$ cells and keep one tube on ice as a back up for step 5. For yeast strains we use, $1 \times 10^7$ cells/ml = $OD_{600} \sim 0.25$ (haploid), or $OD_{600} \sim 0.3$ (diploid). Use hemocytometer to be sure.

3. Wash twice with sterile PBS, pH 7.5. Resuspend $1 \times 10^8$ washed cells in 0.5 ml PBS.

4. Dissolve 8 mg of EZ-link biotin in 0.5 ml PBS. Repipet to dissolve. Add 0.5 ml of EZ-link biotin to 0.5 ml cells (8 mg EZ-link per $1 \times 10^8$ cells). Place on rotator at room temperature for 15 minutes.

   EZ-link biotin is sulfo-NHS-LC-biotin (Pierce #21335).

5. Microfuge at top speed for 15 seconds to collect cells. Wash with PBS 7 times to remove biotin.

6. Resuspend cells in 1 ml YPD. Transfer to 1 L YPD at room temperature. Shake at 25 – 30°C overnight. Aim for $OD_{600} < 0.5$ in the morning.

7. Place cells on ice. Once chilled (<15°C), spin in GSA rotor 5 minutes at 5000 rpm at 4°C. Pour off supernatant immediately after spin to ensure that cell pellet is tight.

8. Resuspend and pool cells with 20 ml ice cold PBS. Transfer to 2 $\times$ 15 ml centrifuge tubes.

9. Spin 10 minutes at 2000 rpm at 4°C. Wash each cell pellet with 10 ml ice cold PBS.

   - At this point, wash avidin-magnetic beads using magnetic sorter. Plan for 200 µl of magnetic bead stock solution per $1 \times 10^8$ cells. Dilute magnetic beads into 10 ml cold PBS in 15 ml tube. Mix gently. Do magnetic sort for 15 minutes. Remove supernatant. Leave some (~0.5 ml) PBS behind to avoid loss of beads. Repeat wash. Resuspend magnetic beads in residual PBS.

10. Resuspend each washed cell pellet in 10 ml ice cold PBS. Add washed magnetic beads. Place on rotator in cold room for 2 hours (3 hours for second sort).

11. Place 2 $\times$ 15 ml tubes in magnetic sorter in cold room. Sort for 20 minutes. Remove supernatant with 10 ml pipet. Submerge tip of pipet and slowly lower it as liquid is removed. Keep pipet against outer wall of tube. Do not disturb magnet beads. Leave 0.5 - 1 ml of supernatant behind to avoid loss of beads. Do one tube at a time so that beads do not dry out.

12. Add 10 ml cold YPD. Sort for 20 minutes. Remove supernatant. Repeat 6 more times.

13. For 2 sorts, resuspend in 1 ml YPD. Transfer to 1 L YPD. Grow at 25 - 30°C. Repeat steps 7 - 12. It is necessary to add more magnetic beads for 2nd sort.

14. Collect beads in PBS. Remove small aliquot, fix by addition of 1/10 volume 37% formaldehyde, store at 4°C, and count bud scars later. To store cells, microfuge and place dry pellet at -80°C.