

Isolation of DNA from Yeast Cells

1. For each of your 4 samples, start by pipetting 100 μ l of 200mM LiOAc, 1% SDS solution into a 1.5ml tube.
2. Using a sterile toothpick, collect a sesame seed-sized sample of yeast from each of your parent haploid strains into the LiOAc/SDS solution.
3. Incubate for 5 minutes at 70°C.
4. Add 300 μ l of 95% EtOH to each tube and vortex for 5-10 seconds.
5. Spin down DNA and cell debris at 15000xg for 3 minutes.
6. Decant and discard the liquid from the tubes into a waste container.
7. Add 400 μ l of 70% EtOH to each tube.
8. Spin down DNA and cell debris at 15000xg for 3 minutes.
9. Decant and discard the liquid from the tubes into a waste container.
10. Place your tubes upside down on a paper towel and air dry for up to ten minutes or until it seems the majority of EtOH has evaporated, being careful not to lose your pellet. You may use a KimWipe to manually wipe any accessible EtOH to accelerate the process, being careful not to disturb the pellet.
11. After the EtOH has evaporated, add 80 μ l TE to each tube and allow to sit at room temperature for two minutes.
12. Spin the samples for 15 seconds at 15000xg to pellet any debris remaining.
13. Collect only the supernatant (liquid) from each tube and transfer to a new 1.5ml tube, making sure not to disturb the waste pellet. Label your tubes appropriately.
14. Check on the quality and quantity of your DNAs by loading \sim 1 μ l of your samples onto the Nanodrop.
15. Take a picture of your data from the Nanodrop and record the information in your lab notebooks.