

Finishing Maxipreps without Finalizers: Blaise's Protocol

1. **ELUTE DNA FROM COLUMN INTO ORANGE-CAPPED TUBES:** Do the regular maxiprep up through the WASH step. Elute the DNA using 20 mls of ELU, collecting into an orange-capped 50 ml conical Falcon type tube. (The orange-capped tubes can be spun harder than the blue-capped tubes.)
2. **PRECIPITATE DNA WITH ISOPROPANOL:** Add 14 mls isopropanol to the ELU/DNA solution. Invert tube 6-10 times to mix. Do not vortex, as this can break the DNA. Let solution sit 3-5 minutes.
3. **SPIN:** at 4000 x g for one hour.
4. **LOOK FOR PELLETT ON BOTTOM/SIDE OF TUBE:** Carefully pour off supernatant without discarding the DNA pellet.
5. **WASH WITH 70% ETHANOL:** Carefully add 5 ml 70% EtOH (filtered) to wash the pellet. Let sit for 2-5 minutes, long enough to wash but not long enough to dissolve the DNA.
6. **SPIN:** at 4000 x g for 30-60 minutes.
7. **FIND DNA PELLETT** and carefully pour off ethanol, leaving pellet behind. Circle the location of the pellet so that once it dries and is barely visible you will know where the DNA is.
8. **DRY PELLETT:** Allow pellet to air dry by placing the conical tube so that it is inverted and supported at an angle, draining onto a clean, large kimwipe in a clean place. Once it is dry, you will probably not be able to see the pellet anymore, so be sure that you have marked its location.
9. **REDISSOLVE DNA WITH TRIS BUFFER:** For maxipreps we use 1 ml. Let the TRIS sit over the DNA spot for 5-20 minutes at room temperature (or overnight at 4 degrees if you want). Resuspend a little with a pipette – the solution becomes slightly syrupy.

Hint: If a higher concentration is desired, dissolve DNA in 250-500 ul of TRIS and check OD. If you have what you need, great! If not, or to collect every plasmid possible from the prep, rinse the conical tube with another 500-750 ul of Tris and pipette gently to redissolve any remaining DNA. Measure OD, and, if desired, combine this DNA with the first portion collected.

10. **TRANSFER TO CLEAN EPPENDORF TUBE:** OD and label as usual.