

Robyn's Gel Extraction Protocol

1. Run your product of interest on a 0.7-0.8% agarose gel. Make sure you use the low-melt agarose (I recommend casting the gel in the cold room...it will speed up the time of solidification).
2. Cut out a gel slice around your product of interest. Cut as close to the bands as possible to reduce the amount of agarose.
3. Melt each gel slice individually in 2.0mL eppendorf tubes at 65°C with mixing (easiest in Thermomixer, if available. Otherwise, use a water bath.). Make sure the slice is *completely* melted. I usually melt until it appears melted (about 10-15 min), then melt 10 more minutes to ensure it is melted.
4. Dilute gel slice volume four times in pre-warmed 1x TBE (so if the volume of the gel slice is 200uL, use 800uL TBE). Vortex thoroughly.
5. Check that the volume in each 2.0mL tube does not exceed 1mL. If over volume, split into multiple tubes as necessary.
6. Add an equal volume of phenol/chloroform to each tube. Vortex 30 seconds. Centrifuge at top speed 1 min. **See phenol precautions listed at end of protocol under notes.
7. Three layers will be visible. The bottom layer is agarose. The middle layer is cloudy-white. The top layer is clear. Remove the cloudy-white layer and clear layer and place in a new tube. It's ok if not all of the white layer is able to be pipetted out.
8. To the tube containing agarose and any leftover white layer, add an equal volume of phenol/chloroform. Vortex 30 seconds, and centrifuge 1 min at top speed.
9. Remove supernatant and cloudy-white layer and add to the tube containing the other cloudy-white and clear layer. Then add an equal volume of phenol, vortex 30 seconds, and centrifuge at top speed for 1 min.
10. Remove supernatant (clear layer only) and place in new tube. Discard old tube. Add to the supernatant an equal volume of phenol/chloroform. Vortex 30 seconds, then centrifuge 1 min. Remove supernatant (clear layer only) and discard old tube. Wash once more with phenol/chloroform if a middle layer is still highly visible.*

*Remember that with each wash you will get cleaner DNA, but you will lose some yield. I find that 2-3 washes gives the cleanest DNA for the best yield.

11. Proceed to EtOH precipitation after a total of 2-3 phenol/chloroform washes.

Ethanol Precipitation

1. Add to the tube containing the supernatant a 2x volume of 100% ethanol (sometimes I add up to 2.5x ethanol), 1/10 volume of 3.0M sodium acetate (pH 5.0), and 1uL of glycol blue (to help you see the pellet). Place tube in the -80°C freezer for at least 20 minutes
2. Remove from freezer. Be careful to not disturb or shake the tube. Place directly in centrifuge (room temperature or 4°C) and centrifuge at top speed for 30 minutes.

3. Pipette off the ethanol, being careful to not disturb the pellet. Wash pellet with 300uL of 70% EtOH. Mix tube by “flicking” and centrifuge 3-5 minutes at room temperature.
4. Pipette off the ethanol, being careful to not disturb the pellet. Let dry in chemical hood, or place on thermomixer at 40-50°C (do not go higher than 50°C!) until dry.
5. Resuspend in 30uL of H₂O.

Other Notes:

****Take extra precaution when handling phenol!** I wear 2 pairs of gloves and a lab coat. Be sure to clean up all spills, and clean the centrifuge rotor with ethanol when done. If you spill phenol on your gloves, remove them immediately, wash your hands, and put on new gloves. Read hazard label on bottle before use.

For a 50uL reaction, I typically end with a concentration of 60-100ng/uL if I had an average band “brightness” on the gel doc.

Approximate time of protocol from extraction to precipitation, but not including gel run time or gel slicing, is 2-2.5 hours. It is best to do the extraction at once, but if you must stop before the precipitation you can leave your product in the -80°C freezer overnight (after step 1 of precipitation).