DNA Extraction Protocol

This is a low cost procedure for extracting DNA from tissue or blood samples. It does not produce the cleanest DNA, and therefore may not be the best option for sensitive or finicky reactions that require very clean DNA. However, I have successfully used DNA extracted using this protocol for a variety of applications including sequencing mitochondrial and nuclear genes, microsatellites, and SNP genotyping using TaqMan and Kasp chemistries.

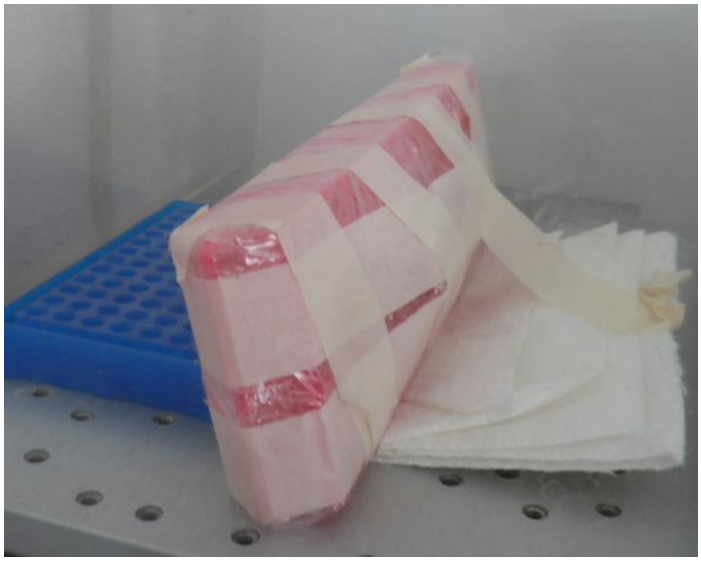
You will need two 1.7 tubes for each sample, one for dissolving the sample and a second to pour DNA into later in the protocol. This second tube will be the final tube the DNA is stored in. Label both sets of tubes before starting the extraction.

1. Add 600 uL of cell lysis solution (0.1m Tris, 0.1m EDTA, 1% SDS, pH 7.1) to a sterile 1.7 mL microcentrifuge tube. Be sure to keep the tube and solution cold during steps 1-3 if extracting from frozen samples. Add 20 uL of 1M DTT to each sample. This step could be omitted for blood samples.

2. Add 10-20 mg of tissue in the tube with the cell lysis solution. The tissue does not have to be weighed, but overly large tissues will not fully digest. However, very small samples will also result in DNA pellets that are more difficult to see in later steps.

3. Add 8 uL of Proteinase K (20 mg/mL) to the tube containing the tissue and cell lysis solution. Note: Proteinase K needs to be mixed in Pro K storage buffer if starting from powder.

4. Incubate the tubes at 55C for at least 3 hours (or until the tissue is completely dissolved) with low agitation (e.g., ~160-180 speed on an incubating mini shaker). Note: Make sure the tubes are closed tightly, wrap the 96 well tray with plastic wrap and seal well with masking tape. This will help prevent evaporation if any tubes are not tightly sealed.



This photo shows a 96-well tray wrapped in plastic wrap and taped closed. Here, the tray has also been propped up inside the mini shaker during incubation. The reason for propping up the tray, if you can, is that when placed horizontally the agitation results in only minimal movement of the liquid in the tubes. When the tubes are at more of an angle as in this photo, the motion results in more of a sloshing which agitates the tissue more and helps aid in quicker and more complete digestion.

5. Remove the tubes from the incubator and allow them to cool for ~15 minutes (closer to room temperature). Add 250 uL of protein precipitation solution (7.5M NH_4A_0C) to the tubes once they have cooled.

6. Vortex the tubes vigorously for 20 seconds and then centrifuge at 13,000-16,000g (= 12-13,000 rpm) for 5 minutes. Running this centrifuge spin at 4 C tends to result in cleaner DNA. Note: The DNA is in the supernatant after this step.

7. Label new 1.7mL tubes, add 600 uL of 100% ice cold isopropanol (store in the freezer) and pour the supernatant with the DNA into the new tube. Leave behind and discard the tubes with the precipitated protein pellet.

NOTE: Vigorous agitation of the tubes beyond this point can shear the DNA when it is in solution. **DO NOT VORTEX.**

8. Immediately mix by inverting the tubes 50 times. The most efficient way to do this is to complete step 7 for all the samples, then place an empty 96 well tray on top to hold the tubes in place and invert all of the samples 50 times at once, rather than just doing a couple at a time. Note: The DNA may be visible as a wad of "snot-looking" material in the solution, but not always. Don't worry if you can't see it.
After inverting 50 times, centrifuge the tubes for 10 minutes at 13-16,000g (12-13,000 rpm). This spin can be done at room temperature. The DNA should be visible as a small white pellet at the bottom of the tube after this spin.

9. Pour the supernatant into a waste container and drain the tube briefly on a paper towel or other absorbent material. NOTE: The pellet is often stuck to the tube, but may come lose. It is important to watch the tubes to avoid pouring out the pellet.
Add 600 uL of 70% ethanol and invert the tubes gently several times to wash the pellet. 70% ethanol can be prepared by mixing 48 mL ethanol with 18 mL ultra pure water. We tend to make this fresh every time.

10. Centrifuge 13-16,000g (12-13,000 rpm) for 10 minutes, then carefully pour off the ethanol. NOTE: The pellet may be loose. Pour carefully and watch to make sure the pellet is not poured out.

11. Invert and drain the tubes on a paper towel. Allow the samples to dry at least 15 minutes. The amount of time needed to dry depends upon how much isopropanol is left after draining. The tubes can be placed in an incubator at a low temperature (~37 C) to speed the drying process.

12. Add 52 uL of TE (1mM EDTA, 10mM Tris, pH 8.0) or other DNA storage solution or purified water. NOTE: The storage solution used will affect how stable the DNA is and whether it is best to ultimately store at 4C or lower temperature. If using TE solution, it is best to store samples for long-term at -20 or -80. Over shorter periods, DNA can be left in the fridge (2-8C).

13. Allow the DNA to rehydrate in the TE solution in the fridge (4C) overnight or at 37 C for 1 hour. Occasionally agitating the tube will aid in rehydrating the DNA pellet.

14. Store frozen or in the refrigerator.