DNA Extraction Protocol
Ryan Kerney, May 2010
Modified from Shrey and Coon[1]

Overview: This protocol describes the most commonly used method of purifying and concentrating DNA from samples. The DNA solution is first extracted with a phenol/chloroform/isoamyl alcohol mixture to remove protein contaminants, then precipitated with 100% ethanol. The DNA is pelleted after the precipitation step, washed with 70% ethanol to remove salts and small organic molecules, and resuspended in buffer at a concentration suitable for further experimentation. While this method is inexpensive, and efficient, it can be time consuming, taking up to three days to complete.

Materials:
- Extraction buffer
- Proteinase K (can find at www.fishersci.com)
- Phenol-chloroform isoamyl alcohol
- Chloroform isoamyl alcohol
- Ice cold 95-100% ethanol (keep aliquot in the freezer)
- 70% Ethanol
- Ultrapure (DNA- & DNase-free) water
- TE buffer

**Phenol-Chloroform tips, tubes, and liquid waste is a biohazard. Please contact your EH&S department for proper disposal.

Lab equipment needed:
-Incubator -Fume hood -Vortex
-Sterile 1.5mL microcentrifuge tubes - -20C Freezer
- Microcentrifuge - Rocking platform

Tissue Digestion:
This step lyses the tissue, cells, and organelles from which you want to extract DNA. This step, however, may not be sufficient if the organisms/cells you want to extract from have very thick cell walls (e.g. plants, coccidia).

1. Label enough autoclaved 1.5 ml tubes. Normally use:
    a. 100 ul sample
        i. Extract DNA from blood that is preserved in a lysis buffer (5 uL blood: 250 uL buffer), and we use 100uL of this mixture for digestion
    b. 400 ul extraction buffer per sample
    c. 5 ul proteinase K (10 mg/ml stock - 0.5-1mg/ml final)
        i. For 10 mL: add 200 mg (0.2 g) to 10 mL ultrapure water
        ii. Proteinase K should be kept in a -20C freezer

[1] http://ccoon.myweb.usf.edu/ecoimmunology.org/PCI_extraction.html

Incubate microorganisms and blood for a minimum of 2 hours, tissues should incubate overnight, both at 55C with gentle shaking, until the sample is clear.
**Always include a negative control (water) during extraction

Extraction and Ethanol Precipitation of the DNA:
1. Add an equal volume of PCI (phenol/chloroform/isoamyl alcohol) to the digested DNA solution to be purified in a 1.5-ml microcentrifuge tube.

a. Extracting volumes <100 ul is difficult; small volumes should be diluted to obtain a volume that is easy to work with.
b. High salt concentrations can cause the inversion of the aqueous and organic phases. If this happens, the organic phase can be identified by its yellow color.

2. Mix gently for 5 min (rocking platform) and microcentrifuge 10 min at 10,000 rpm at room temperature.

a. Phases should be well separated. If DNA solution is viscous or contains a large amount of protein, it should be spun longer.

3. Carefully remove the top (aqueous) phase containing the DNA using a 1000-ul pipetor and transfer to a new tube.

a. Repeat steps 1-3.
b. If after repeating a white precipitate is present at the aqueous/organic interface, repeat steps 1 to 3 once again.

4. Add an equal volume of CI (chloroform:isoamyl alcohol 24:1). Mix gently for 2 min and centrifuge for 1 min at 10,000.

5. Carefully remove the top (aqueous) phase containing the DNA using a 1000-ul pipettor and transfer to a new tube.

6. Add 2 to 2.5 vol of ice-cold 100% ethanol. Mix gently and place in -20 C overnight or in -70oC for 1 hr.

7. Spin 20 min in a fixed-angle microcentrifuge at maximum speed and remove the supernatant.

a. For large pellets the supernatant can simply be poured off.
b. For small pellets (<1 ug), aspirate off the ethanol supernatant with a pipetting device such as a Pasteur pipet or pipettor. This is best accomplished by drawing off liquid from the side of the tube opposite the DNA pellet. Start at the top and move downward as the liquid level drops.

8. Carefully add 1 ml of room-temperature 70% ethanol. Invert the tube gently and microcentrifuge as in step 7.

a. If the DNA molecules being precipitated are very small (<200 bases), use 95% ethanol at this step.

9. Remove the supernatant as in step 5. Dry the pellet at room temp.
    a. The DNA pellet will not stick well to the walls of the tube after the 70% ethanol wash and care must be taken to avoid aspirating the pellet out of the tube.

10. Dissolve in 20-50 ul TE buffer, pH 8.0.
    a. DNA pellets will not dissolve well in high-salt buffers. To facilitate resuspension, the DNA concentration of the final solution should be kept at <1 mg/ml. If DNA is resuspended in a volume of TE buffer or water to yield a DNA concentration of <1 mg/ml, small quantities (<25 ug) of precipitated plasmids or restriction fragments should dissolve quickly upon gentle vortexing or flicking of the tube. However, larger quantities of DNA may require vortexing and brief heating (5 min at 65oC) to resuspend.
    b. High-molecular-weight genomic DNA may require one to several days to dissolve and should be shaken gently (not vortexed) to avoid shearing, particularly if it is to be used for cosmid cloning or other applications requiring high-molecular-weight DNA. Gentle shaking on a rotating platform or a rocking apparatus is recommended.

Extraction Buffer	**Sigma #**	**For 500ml use:**
NaCl	S-9625	10ml of 5M stock
Tris Base (Trizma)	T-1503	10ml of 1M, pH 8.0 stock
EDTA, disodium	E-5134	25ml of 0.5M, pH8.0 stock
SDS	L-4390	2.5g

- Bring to 500ml with ddH2O

Filter with 0.45mm filter and autoclave before use