Isolation of Genomic DNA

From Sambrook et al., Molecular Cloning Manual Vol 2, p.9.16-9.19.

1. Grind tissue in liquid Nitrogen and then place in approximately 10 volumes of **extraction buffer** . Shake and submerge material and then incubate for 1 hr at 37 C.

2. Add **Protease K** to a final concentration of 100µg/mL. Gently mix the enzyme into the solution.

3. Place at 50 C for 3 hours. Swirl solution periodically.

4. Cool solution to room temperature and add an equal volume of **Tris-equilibrated Phenol (pH 8.0)** and gently mix the two phases by turning tube end over end for 10 minutes. Separate the two phases by centrifugation at 5,000x**g** for 15 minutes at room temperature.

5. With a wide bore pipette transfer the aqueous phase to a new tube and extract the aqueous phase with **Tris-equilibrated Phenol (pH 8.0)** two more times.

   (note: Do not disturb the interface when transferring the aqueous phase)

6. Precipitate the genomic DNA by adding 0.2 vol **10M Ammonium Acetate** and 2 volumes of 100% ethanol. Swirl to mix and the DNA will precipitate out. Centrifuge at 5000x**g** for 5 minutes at room temperature.

7. Wash the DNA twice with 70% ethanol and store the pellet in an open tube at room temperature until the last traces of ethanol have evaporated. **Do not allow the pellet to completely dry because it will be difficult to resuspend.**

8. Add 1 mL of 10mM TE (pH 8.0) and gently resuspend the DNA.

Store genomic DNA at 4 C. Repeated freeze thaws will shear genomic DNA.

Extraction Buffer:

10 mM Tris-Cl (pH 8.0)
0.1 M EDTA (pH 8.0)
20 µg/mL Rnase A
0.5 % SDS