

Genomic DNA Isolation

Procedures

1. **IMPORTANT:** The key to success for PCR-based genotyping is not to get contamination from phenol. The goal is the get correct genotyping, not to recover DNA. Therefore, keep in mind that you can afford to lose DNA to trade for high quality DNA.
2. **Cut a small (~ 2 mm) piece of tail** by using a pair of scissors and place the tail sample in a 1.5-ml tube. This can be stored at 4°C or -20°C before the next step.
3. **Add 700 µl of Lysis buffer** with proteinase K (see below for recipe) to the tube.
4. **Shake at 55°C overnight.**
5. **Add 700 µl of phenol**, mix by shaking 30 times and spin in a microfuge at maximal speed for 5 min.
6. **Transfer 600 µl of top layer** to a clean tube. This step is critical. Transferring smaller volume (600 µl) helps to avoid contamination from the phenol.
7. **Add 600 µl of chloroform** to the tube, mix well and spin for 5 min.
8. **Transfer 500 µl of top layer** to a clean tube.
9. **Add 1 ml of 100% Ethanol**, Mix and spin for 3 min.
10. **Pour off the supernatant** gently. You should be able to see a small white pellet of DNA. Use your imagination.
11. **Add 500 µl of 70% Ethanol** and spin for 3 min.
12. **Remove all the liquid** and air-dry the pellet. Do not over-dry the sample. The DNA will be difficult to dissolve.
13. **Resuspend the pellet** in 100 µl of TE.
14. **Incubate at 55°C** for 30-60 min.
15. **Use only 0.5 µl** of DNA for PCR.

Reagents

Lysis Buffer (500 ml)

Stock	Volume	Final Conc.
Tris (1 M), pH 8	50 ml	100 mM
EDTA (0.5 M)	5 ml	5 mM
NaCl (5 M)	20 ml	200 mM
SDS (10%)	10 ml	0.2%
dH2O	415 ml	

Store the Lysis buffer at room temperature. Right before use, transfer enough Lysis buffer and add proteinase K (1 µl/100 µl lysis buffer from 20 mg/ml stock stored at -20°C) into tube. Mix well.