

# Genomic DNA Isolation Methods:

## Genomic DNA extraction with "HotSHOT" Lysis

This is a "quick and dirty" (crude lysis) DNA extraction method. DNA extracted in this manner may or may not be suitable for downstream applications or PCR, depending on which locus you are amplifying and the length of the amplicon. Test the compatibility of this method with your experimental design before extracting "precious" samples.

Truett, G.E., et al. 2000. Preparation of PCR-Quality Mouse Genomic DNA with Hot Sodium Hydroxide and Tris (HotSHOT) *Biotechniques* 29:52-54 (July 2000)

### Materials:

- HotSHOT Lysis Solution- Store at Room Temperature
  - 25mM NaOH (0.5g)
  - 0.2mM Na<sub>2</sub>EDTA x 2H<sub>2</sub>O (0.037g)
  - H<sub>2</sub>O to 500mL
- HotSHOT Neutralization Solution
  - 40mM Tris Acid (3.15g)
    - Tris Hydroxymethyl Aminomethane Hydrochloride, NOT TRIS BASE
  - H<sub>2</sub>O to 500mL
- Heat block

### Protocol:

1. Ensure heat block is at  $\geq 95^{\circ}\text{C}$
2. Microfuge tubes should contain the following quantity of tissue
  - a. 2mm weanling mouse tail tip
  - b. Ear punches
  - c. 25mg spleen (or other tissue/need to test other tissues)
  - d. small zebrafish fin clip
  - e. a single zebrafish embryo 1 dpf
  - f. \*Too much tissue will cause PCR failure, if necessary transfer smaller pieces of tissue to a fresh tube.
3. Add 150 $\mu\text{L}$  for larger tissue amounts (i.e. tail tips, ear punches) or 30 $\mu\text{L}$  for smaller tissue amounts (i.e. fin clips or zebrafish embryos) HotSHOT lysis buffer to each sample. Ensure tissue sample is submerged in buffer. \*Note this amount can be adjusted depending on source tissue and amount of tissue.
4. Incubate at  $95^{\circ}\text{C}$  for 30 minutes
5. Place rack/samples on ice to chill to  $4^{\circ}\text{C}$
6. Vortex vigorously to mix
7. Centrifuge briefly to collect buffer
8. Add an equal volume HotSHOT neutralizing solution to each sample (i.e. 150 $\mu\text{L}$  to ear punches)
9. Vortex vigorously and spin samples down
10. Store  $4^{\circ}\text{C}$  until ready for PCR
11. PCR using 1-2 $\mu\text{L}$  of solution per 20 $\mu\text{L}$  reaction

## **DNA Isolation Using Proteinase K Method**

This is another crude genomic DNA isolation method, but unlike the HotSHOT lysis method it preserves longer DNA fragments and is suitable for long range PCR and Southern blot analyses. If purer genomic DNA is desired, it is best to use a column purification kit. Qiagen has many good options for high yield and high DNA molecular weight isolation from many cell and tissue types.

### **Materials:**

- Lysis Buffer (Store at room temp for up to 1 month)
  - 50mM Tris-HCL (pH 8.0)
  - 100 mM EDTA
  - 1% SDS
  - 100mM NaCl
- Molecular biology grade proteinase K (ProK) (20mg/ml)
- Heat block

### **Protocol:**

1. Ensure heat block is at 55°C
2. Microfuge tubes should contain the following quantity of tissue
  - a. 2mM weanling mouse tail tip
  - b. Ear punches
  - c. 25mg spleen (or other tissue/need to test other tissues)
  - d. \*Too much tissue will cause PCR failure, if necessary transfer smaller pieces of tissue to a fresh tube.
3. Add 500µL lysis buffer to tissue
4. Add 2µL ProK solution
5. Invert several times to mix well and briefly centrifuge to collect liquid
6. Incubate at 55°C overnight
7. Centrifuge at 20,000 g for 10 min.
8. Transfer the supernatant into a new tube, add 1 ml ice cold 100% ethanol
9. Gentle rock to precipitate DNA (can stow tubes at -20 °C for 30 minutes to overnight to increase yield)
10. Centrifuge at 20,000 g for 10 min
11. Decant ethanol
12. Gently wash with 0.5-1ml 70% ethanol
13. Decant ethanol, briefly dry and dissolve DNA in 100ul TE or nuclease free water (do not dry for more than a few minutes)
14. Store -20°C until ready for PCR
15. PCR using 1µL per 20µL reaction

## **Isolating genomic DNA for cell pellets**

This is by far the most consistent, rapid and easy method we have used for isolating genomic DNA from cell pellets. It is also suitable for other tissue samples such as zebrafish embryos, mouse ear clips and even 5-day old mouse embryos.

### **Materials:**

- Quick Extract (QE) Buffer (<https://www.lucigen.com/QuickExtract-DNA-Extraction-Solution/>)
- Heat block or thermocycler

### **Protocol:**

1. Spin down ~500,000 cells
2. Wash at least 2-3X with cold PBS
3. Resuspend pellet in appropriate volume of QE buffer, 200  $\mu$ L per 500,000 cells.
4. Vortex samples thoroughly for 15 seconds each
5. Heat samples at 65°C for 6 minutes
6. Briefly vortex samples again
7. Heat samples at 98°C for 6 minutes.
8. Chill on ice, and vortex again briefly
9. Extracts can be stored indefinitely at -20 °C.
10. PCR using 2-3 $\mu$ L per 20 $\mu$ L reaction