# HOTSHOT Method of DNA Preparation

1. Cut 1 to 2 mm tail or ear notch and place in a 0.5 ml microfuge tube. Caution - larger pieces of tail can inhibit the PCR.

2. Add 50 µl Alkaline Lysis Reagent. Assure that the tail/ear fragment is completely submerged.

3. Incubate at 95°C for at least one hour (longer may be better – see below) and then store at 4°C until you proceed to the next step. A thermocycler is convenient for this step.

4. Add 50 µl Neutralization Reagent using a new aerosol-barrier tip for each sample. Mix well, using tip to break up tissue. Some people like to centrifuge the tubes after this step and transfer the neutralized supernatant to a new tube, but this is not necessary.

5. When genotyping animals that are six weeks and older, we find that increasing the 95°C incubation time to two-hour yields better results. Preps made from tail pieces longer than 2 mm may inhibit the PCR. Use 1 µl of neutralized supernatant per 20 µl PCR reaction.

## Alkaline Lysis Reagent

To 25 ml water, add:

- 62.5 µl of 10 N NaOH (final concentration is 25 mM.)
- 10.0 µl of 0.5 M disodium EDTA (final concentration is 0.2 mM, pH should be about 12 but should not have to be adjusted.)

Make fresh every one to two months. Keep solution at room temperature.

## Neutralization Reagent

To 24 ml water add:

- 1 ml of 1 M Tris-HCl (final concentration is 40 mM, pH should be about 5 but should not have to be adjusted.)

Keep solution at room temperature.

Make 1 M Tris-HCl with Tris hydrochloride salt.

**Note:** Because of the presence of EDTA in the alkaline lysis reagent, you may need to increase the amount of magnesium chloride in your PCR master mix. Your PCR conditions may need to be re-optimized when switching to this method of DNA preparation.

(protocol obtained from http://gttf.uchc.edu/protocols/hotshot.html)