

# “HotSHOT” genomic DNA preparation

(hot sodium hydroxide and tris)

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## Alkaline Lysis Reagent

Reagent	[Final]	Add	Of
NaOH	25mM	125 $\lambda$	10N NaOH
EDTA	0.2mM	20 $\lambda$	0.5M EDTA
		50ml	ddH <sub>2</sub> O

*pH will be 12*

*EDTA = disodium EDTA*

## Neutralization Buffer

Reagent	[Final]	Add	Of
Tris-HCl	40mM	325mg	Tris-HCl
		50ml	ddH <sub>2</sub> O

*pH will be 5*

## Protocol:

1. Obtain tissue
  - a. 0.2cm tail snip
  - b. 2mm ear punch biopsy
2. Place tissue in 96 well plate
3. Add 75 $\lambda$  of Alkaline Lysis Reagent
4. Heat to 95°C for 10min to 1h (30min is optimal)
5. Cool to 4°C
6. Add 75 $\lambda$  Neutralization Buffer
7. Use 1 to 5  $\lambda$  per PCR reaction

## Notes:

- DNA is not suitable for PCR reactions but **NOT** for Southernns
- Heating for longer than 30 min does not increase [DNA]
- pH of Reagents does not need to be altered
- Don't worry about undigested floating tissue
- DNA yield is similar for tail snips and ear punches
- Too much tissue will destroy PCR attempts
- DNA must be stored at 4°C or -20°C

Mike Charles 10/15/03  
mikchar@umich.edu