

“HotSHOT” genomic DNA preparation

(hot sodium hydroxide and tris)
from *Biotechniques*. 2000 Jul;29(1):52,54

Alkaline Lysis Reagent			
Reagent	[Final]	Add	Of
NaOH	25mM	125 λ	10N NaOH
EDTA	0.2mM	20 λ	0.5M EDTA
		50ml	ddH ₂ O

pH will be 12
EDTA = disodium EDTA

Neutralization Buffer			
Reagent	[Final]	Add	Of
Tris-HCl	40mM	325mg	Tris-HCl
		50ml	ddH ₂ 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 λ of Alkaline Lysis Reagent
4. Heat to 95°C for 10min to 1h (30min is optimal)
5. Cool to 4°C
6. Add 75 λ Neutralization Buffer
7. Use 1 to 5 λ per PCR reaction

Notes:

- DNA is 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

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