"HotSHOT" genomic DNA preperation

(hot sodium hydroxide and tris)

from *Biotechniques. 2000 Jul;29(1):52,54*

Alkaline Lysis Reagent

Reagent	[Final]	Add	Of
NaOH	25mM	125λ	10N NaOH
EDTA	0.2mM	20λ	0.5M EDTA
		50ml	ddH_2O

*pH will be **12***

EDTA = disodium EDTA

Neutralization Buffer

Reagent	[Final]	Add	Of
Tris-HCl	40mM	325mg	Tris-HCl
		50ml	ddH_2O

*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 75λ of Alkaline Lysis Reagent** 50ul for zfish
4. **Heat to 95°C for 10min to 1h (30min is optimal)**
5. **Cool to 4°C**
6. **Add 75λ Neutralization Buffer** 50ul
7. **Use 1 to 5 λ per PCR reaction** 3ul

Notes:

- DNA is suitable for PCR reactions but **NOT** for Southerns
- 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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