"HotSHOT" genomic DNA preperation
(hot sodium hydroxide and tris)
from Biotechniques. 2000 Jul;29(1):52,54

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

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

<table>
<thead>
<tr>
<th>Alkaline Lysis Reagent</th>
<th>Neutralization Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reagent</strong></td>
<td><strong>Reagent</strong></td>
</tr>
<tr>
<td>[Final]</td>
<td>[Final]</td>
</tr>
<tr>
<td>NaOH 25mM</td>
<td>Tris-HCl 40mM</td>
</tr>
<tr>
<td>125λ</td>
<td>325mg</td>
</tr>
<tr>
<td>10N NaOH</td>
<td>Tris-HCl</td>
</tr>
<tr>
<td>EDTA 0.2mM</td>
<td>50ml</td>
</tr>
<tr>
<td>20λ</td>
<td>ddH₂O</td>
</tr>
<tr>
<td>0.5M EDTA</td>
<td></td>
</tr>
<tr>
<td>50ml</td>
<td></td>
</tr>
</tbody>
</table>

*pH will be 12
EDTA = disodium EDTA

*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
4. Heat to 95°C for 10min to 1h (30min is optimal)
5. Cool to 4°C
6. Add 75λ Neutralization Buffer
7. Use 1 to 5 λ per PCR reaction