DNA Genotyping Protocol

A. Zovein

Lysis Buffer
0.5M EDTA 50ml
5M NaCl 10ml
1M Tris pH7.4 5ml
10%SDS 50ml

Proteinase K (10mg/ml)
10mM Tris pH7.3
20mM CaCl2
50% glycerol

1. Place 1 cm tail sample in 1.5 ml eppendorf (may be stored at –20°C)
2. Add 600 ul lysis buffer and 20 ul proteinase K (10mg/ml) per tail: if a lot of tails: calculate out total amount to mix and aliquot.
3. Incubate at 55-60°C hot water bath overnight
4. Transfer solution to prespun (1500g x1-2min) PLG2 2ml heavy tube.
5. Add 0.5 ml Phenol: Chloroform: Isomyl Alcohol (PCI, 25:24:1 commercial) to sample in PLG 2ml tube and mix well by repeated inversion (DO NOT VORTEX).
6. Centrifuge at full speed (12,000 x g or greater) for 5 min and transfer resultant aqueous supernatant to fresh pre-spun PLG2 2ml eppendorf.
7. Add 0.5 ml Chloroform: Isoamyl alcohol(C:IA, 24:1 homemade) to sample and mix well by repeated inversion. (DO NOT VORTEX)
8. Centrifuge at full speed (12,000 x g or greater) for 5 min and transfer resultant aqueous supernatant to regular 1.5 ml eppendorf.
9. Fill sample tube with 1 ml of 100% Ethanol and mix by repeated inversion. DO NOT VORTEX. A visible DNA precipitate should form.
10. Place in –20°C for 1+ hr.
11. Then fast-cool centrifuge at 4°C 14,000 rpm x 20 min
12. Wash DNA with 70% Ethanol x 1:
   12a: decant 100% Ethanol leaving precipitate
   12b: add 1 ml 70% ethanol, mix with precipitate
   12c: centrifuge 14,000 rpm for 5 min 4°C
   12d: remove 70% ethanol leaving pellet
13. Allow DNA to partially dry, open tubes to air upside down on kim-wipes.
14. Resuspend DNA with 100 ul DNASE-free H₂O.
15. Resolubilize DNA by placing in water bath at 55°C for <= 1 hr
16. Spec plates: 98 ul ddH₂O and 2 ul sample, spec dilution = 50
17. Then PCR and Run on gel.
Gel

Check what % gel needed*:

<table>
<thead>
<tr>
<th>Agarose</th>
<th>effective Resol DNA (kb)</th>
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<tbody>
<tr>
<td>0.5%</td>
<td>30 to 1</td>
</tr>
<tr>
<td>0.7%</td>
<td>12 to 0.8</td>
</tr>
<tr>
<td>1%</td>
<td>10 to 0.5</td>
</tr>
<tr>
<td>1.2%</td>
<td>7 to 0.4</td>
</tr>
<tr>
<td>1.5%</td>
<td>3 to 0.2</td>
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</tbody>
</table>

*from current protocol in Neuroscience

1. Mix agarose (gms) in 1XTAE according to %
2. Microwave until clear
3. Add 3ul EtBr to gel as it cools (do not inhale or expose skin/eyes to vapor)
4. Pour gel

Large wells need 150ml total for both
Small wells need 25ml each

Load buffer (see prep reagents and buffers)
5ml of 6x of #III
1.5ml glycerol
0.0125g bromo blue
0.0125g xylene cyanol FF
3.475ml H2O

DNA Ladder (usually 100bp)
100ul stock
83ul 6xLB
317ul sterile H2O

Load: 3-5ul ladder
5ul LB to 25ul PCR rxn product
Run gel at 100V