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 1cm tail sample in 1.5ml eppendorf (may be stored at –20°C)
2. Add 600ul lysis buffer and 20ul 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 0.5ml Phenol: Chloroform:Isoamyl 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 5min and transfer resultant aqueous supernatant to fresh pre-spun PLG2 2ml eppendorf.
7. Add 0.5ml 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 5min and transfer resultant aqueous supernatant to regular 1.5ml eppendorf.
9. Fill sample tube with 1ml 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 14,000rpm x 20min
12. Wash DNA with 70% Ethanol x 1:
    12a: decant 100% Ethanol leaving precipitate
    12b: add 1ml 70% ethanol, mix with precipitate
    12c: centrifuge 14,000 rpm for 5min 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 100ul DNASE-free H_2O.
15. Resolubilize DNA by placing in waterbatch at 55°C for <= 1hr
16. Spec plates: 98ul ddH_2O and 2ul sample, spec dilution = 50
17. Then PCR and Run on gel.

Gel
Check what % gel needed*:

Agarose	effective Resol DNA (kb)
0.5%	30 to 1

0.7%	12 to 0.8
1%	10 to 0.5
1.2%	7 to 0.4
1.5%	3 to 0.2

*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 H_2O

DNA Ladder (usually 100bp)
100ul stock
83ul 6xLB
317ul sterile H_2O

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