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Genotyping of Tail DNA by PCR
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Hammer Lab

I. DNA Extraction

1. Cut ~0.5cm of tail from a 21 day old mouse. Place the segment of tail into a 14ml Falcon "2059" tube. Add 4mls SNET buffer + 60 µl Proteinase K stock solution (see next page).

2. Place tubes in 55°C shaking water bath overnight.

3. Pipette 600 µl phenol/chloroform / isoamyl alcohol (25/24/1) (Invitrogen # 15593-031) into a 1.5 ml Eppendorf tube clearly marked with animal/tail identification number.

4. Add 600 µl of the digested tail solution into each tube and vortex vigorously.

5. Centrifuge (14K) for 5 mins at room temp to separate the aqueous phase containing the DNA (top) and the organic (bottom) phase.

6. Clearly label a new set of 1.5 ml tubes and pipette 800 µl of cold 100% EtOH into each tube. Remove 400 µl from the top (aqueous) layer of the centrifuged digested tail preparation and transfer it into the appropriate 1.5 ml Eppendorf tube containing the EtOH. Vortex the tube briefly and place it into a -20°C freezer for at least 2 hours to permit precipitation of the genomic DNA. Note: Addition of salt is not necessary since the SNET contains 400 mM NaCl.

7. Pellet the precipitated DNA by centrifuging for 15 mins (14K, 4°C).

8. Carefully pour off EtOH – watch to make sure that the pellet doesn't slip out. To avoid extra work resuspending the pelleted DNA, do not allow the pellets to dry completely.

9. Resuspend the pellet in 50 µl of deionized, autoclaved PCR-dedicated water.

II. PCR amplification (using Qiagen reagents)

1. PCR core mix/per single 50µl rxn is shown below. To make a master mix for multiple samples, multiply each of the volumes below by the number of DNA samples to be genotyped. Always include a positive control (100pg plasmid or 100pg of injected transgene or 2ul of a previously identified positive tail sample) and negative controls that include: H_2O and a wild-type, non-transgenic tail DNA.

Reagents	1X PCR Core Mix
10 x PCR buffer	5.0 µl
5 x Q Solution	10.0 µl
dNTP Mix (10mM each dNTP)	1.0 µl
Forward Primer	5.0 µl (10 pmoles/µl)
Reverse Primer	5.0 µl (10 pmoles/µl)
PCR-dedicated H_20	21.75 µl
Taq Polymerase(5U/µl)	0.25 µl
	48 µl

2. Clearly label PCR tubes and pipette 48 µl of PCR core mix into each tube.

3. Add 2 µl of tail DNA from unknown samples and from known samples that include wild-type tail DNA, positive control DNA and H_2O, to each tube.

4. Vortex and spin for 2-5 seconds to make sure all reagents are mixed.

5. Run PCR Program

6. Run 20 µl of the reaction on an appropriate % agarose gel.

Solutions

1. SNET Buffer (1L)
   820 mls H_20
   100 mls 10 x SET Buffer
   80 mls 5M NaCl

2. 10 x SET Buffer (2L)
   100 mM Tris-HCl pH 8.0
   50 mM EDTA pH 8.0
   200 g SDS

3. Proteinase K (Fisher # M24568-2) 100 mg/vial
   Resuspend entire 100 mgs in 10 mls TE (10mM Tris-HCl ,pH8.0; 1mM EDTA).
   Final Proteinase K Conc. is 10mg/ml. The bottle should be stored at 4°C.

4. Qiagen Taq PCR Core Kit: # 20122.3