Isolation of genomic DNA from tail biopsies to check for transgenic founder animals

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Equipment and reagents

- Lysis buffer (0.3 M sodium acetate pH 5.5, 10 mM Tris pH 7.0, 1 mM EDTA, 1% SDS)
- Proteinase K (10 mg/ml), e.g. from Boehringer
- RNase A solution (4 mg/ml), e.g. from Sigma
- Phenol-chloroform-isoamylalcohol (25:24:1 by volume) equilibrated with Tris-HCl pH 8.0
- Isopropanol
- TE buffer (10 mM Tris pH 8.0, 1 mM EDTA)
- Saturated NaCl (6 M)

A. Preparation of genome DNA

1. Add 500 μl of lysis buffer and 10 μl of proteinase K (10 mg/ml) to each tail biopsy.[a]
2. Place the tubes on a rocking platform and incubate at 55 °C overnight.
3. Incubate the samples on ice for 5 min.
4. Add 200 μl of a saturated NaCl (6 M), mix well and incubate on ice for another 5 min.
5. Centrifuge the tube for 20 min at 15 000 r.p.m. to pellet the cell debris and transfer the supernatant to a fresh tube.
6. Add 2 μl of a 4 mg/ml RNase A solution and incubate at 37 C for 15 min.
7. Add 1 volume of phenol-choroform-isoamylalcohol (25:24:1 by volume) equilibrated with Tris-HCl pH 8.0 and mix the phases well.[b]
8. Centrifuge for 10 min at 12 000 r.p.m. and transfer the aqueous phase to a fresh tube.[b]
9. Precipitate the DNA with 1 vol. of isopropanol at room temperature. The DNA should be immediately visible as a white stringy precipitate. Either pull out the

DNA using a pipette tip and placing it in a new tube, or pellet it by centrifugation and discard the supernatant.

10 Wash the DNA once in 70% ethanol, shake gently and centrifuge again.

11 Dry the DNA pellet at room temperature.

12 Add 100 μl of TE buffer to the pellet and leave the sample at 37 °C for 30 min to dissolve. Vortexing of the genomic DNA should be avoided in all steps.

13 Quantify the DNA and use about 10 ng for PCR and 10 μg for Southern blot analysis.

14 Store the DNA at 4 °C.

B. Alternative method for very crude DNA preparations

1. Incubate the tail biopsy overnight at 55 °C in 500 μl lysis buffer (see above).
2. Freeze the tubes at –20 °C overnight.
3. Centrifuge the frozen sample at 14 000 r.p.m. at 4 °C until melted (this takes about 10 min). This pellets most of the SDS.
4. Transfer the supernatant containing the genomic DNA into a fresh tube.
5. Use 0.2 μl of the supernatant for a 20 μl PCR reaction.

Notes

a Tail tipping and ear punching is performed at weaning age (3 weeks). To anaesthetise the animals pipette a few ml of ether on paper towels in a 1 litre beaker. Put the mouse into the beaker and take it out as soon as it is anaesthetised. Cut off about 1 cm of the tail tip with a sterile razor blade. To stop the bleeding a fibrin containing ointment should be applied. Place the tail biopsy in a microfuge tube and store at -20°C until isolation of DNA. For identification of the mice the ears are quickly clipped according to an identification scheme usually used to number the animals.

b Steps 7 and 8 are optional. Alternatively, proceed directly with step 9 after the RNase treatment.