Genotyping Protocol:

Materials:

Digestion Buffer:

50 mM NaCl

10 mM Tris pH 9.0

5 mM EDTA

0.2% SDS

Immediately before digestion, add 20 l/ 1ml of a 20 mg/ml stock Proteinase K solution (stored at -20C).

1. Cut P19-P21 mouse tails (1mm)
2. Add 250 l tail lysis buffer supplemented with Proteinase K
3. Digest 6hr-O/N at 55 C
4. Before starting DNA isolation process, label 1.5 ml Eppendorf tubes with mouse genotype and animal number.
5. After complete digestion – shouldn’t see clumps, only hair, spin solution down at maximum speed (14,000 rpm) in a desktop centrifuge.
6. Transfer supernatant to labeled, new tube (made in step 4).
7. Add 250 l Isopropanol (bottle labeled 2-propanol) – mix tube by inverting 4-6 times.
8. Spin for 15 minutes at 14,000 rpm in a desktop centrifuge.
9. After spin, carefully remove supernatant, leaving clear pellet (DNA).
10. Add 1 ml 70% Ethanol (EtOH) to each tube to remove salts and other impurities from DNA – DO NOT VORTEX OR MIX.
11. Spin tubes for 5-10 minutes at 14,000 rpm.
12. Carefully remove supernatant, leaving DNA pellet. Try to remove as much liquid as possible.
13. Place tubes, with the lid open, in the safety hood for 15-30 minutes to dry DNA and remove Ethanol.
14. Add 300-350 l Tris pH 9.0 to DNA to resuspend. Put tube on a heatblock set to 55 C for 15-30 min (while you make PCR mix) to allow for complete resuspension.

PCR

10x PCR Buffer:

100 mM Tris pH ~8.0

500 mM KCl

1% Triton X-100

25 mM MgCl2

20 mM Forward and Reverse Primer (stocks are at 200 mM)

2.5 mM dNTPs

5 M Betaine

DNA Taq Polymerase

1. Follow generic PCR recipe and add primers specific to each genotype (Master Mix) – make enough for each DNA sample.
2. Label PCR tubes with genotype/primer set and animal number.
3. Add 1 l purified genomic DNA per tube.
4. Add Taq to Master Mix.
5. Add 24 l Master Mix to each PCR tube.
6. Cap tubes and run PCR.

**Critical PCR steps:**

Determine annealing temperature for primer set

ALWAYS include a positive and negative control

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 rxn | 10 rxns | 20 | 30 | 40 | 50 | 75 | 100 |
| **Water** | 12.575 | 125.75 | 251.5 | 377.25 | 503 | 628.75 | 943.125 | 1257.5 |
| **10x PCR Buffer** | 2.5 | 25 | 50 | 75 | 100 | 125 | 187.5 | 250 |
| **25mM MgCl** | 1.5 | 15 | 30 | 45 | 60 | 75 | 112.5 | 150 |
| **5M Betaine** | 5 | 50 | 100 | 150 | 200 | 250 | 375 | 500 |
| **25mM dNTPs** | 0.2 | 2 | 4 | 6 | 8 | 10 | 15 | 20 |
| **DMSO** | 1.25 | 12.5 | 25 | 37.5 | 50 | 62.5 | 93.75 | 125 |
| **Primer 1 (20uM)** | 0.5 | 5 | 10 | 15 | 20 | 25 | 37.5 | 50 |
| **Primer 2 (20uM)** | 0.5 | 5 | 10 | 15 | 20 | 25 | 37.5 | 50 |
| **Taq** | 0.4 | 4 | 8 | 12 | 16 | 20 | 30 | 40 |