Mouse Genotyping by PCR / Tail DNA Extraction

I. Protocol for Processing Blood for Flow Cytometry

1. Place mouse in isoflurane chamber in order to briefly anesthetize.
2. Snip end of tail, and tag each mouse appropriately.
3. Gently squeeze blood from tail, and collect 2-3 drops of blood.
4. Place blood into 50 µL of heparin (100 USP Heparin units/mL).
5. After collection, stop bleeding by applying Kwik Stop styptic powder using a moistened cotton applicator to end of tail.
6. After collection of blood samples is completed, wash heparin by adding 150 µL of 1X PBS, and spin at 1500 rpm for 5 min.
7. Discard supernatant.
8. Resuspend in 200 µL of Red Blood Cell Lysis buffer (NH_4Cl (155 mM) and $NaHCO_3$ (10 mM), pH 7.2).
9. Spin and discard supernatant.
10. Resuspend in 200 µL of 1X PBS.
11. Spin and discard supernatant
12. Resuspend in 50 µL of a 1:50 dilution of Fc block in FACS buffer (0.1% sodium azide in 1X PBS).
13. Incubate on ice for 15-20 min.
14. Wash by adding 150 µL of FACS buffer.
15. Spin and discard supernatant.
16. Resuspend in 50 µL of the appropriate antibody mix for each strain.
17. Incubate on ice for 45- 60 min.
18. Wash by adding 150 µL of FACS buffer.
19. Spin and discard supernatant.
20. Resuspend in 200 µL of FACS buffer.

Samples are now ready for flow cytometry.

II. Protocol for Tail Extraction

1. Snip 0.5 cm of tail and place in 1.5 mL clean labeled eppendorf tube.
    - (Store in -20°C if necessary)
2. Add 500 µL of tail cocktail (2% SDS, 50 mM TRIS pH 8.0, 20 mM EDTA pH 8.0, Water) and 50 µL of Proteinase K to each tube.
3. Incubate tubes in 60°C circulating waterbath overnight.
4. Place tubes at 4°C until ready to extract DNA.
5. Add 200 µL of saturated NaCl to each tube and invert to mix.
6. Spin at 4°C for 20 min at 13,000 rpm.
7. Transfer supernatants into clean, labeled eppendorf tubes.
8. Add cold 100% ethanol to the top of each tube and invert to mix until DNA strands precipitate out.
9. Spin at 4°C for 10 min at 13,000 rpm.
10. Discard supernatant and resuspend pellets in 300 µL ddH_20 and briefly vortex.
11. Incubate tubes in 60°C water bath for 1 hour to resolubilize DNA.
12. Store at -20°C.

III. Protocol for PCR Amplification

1. Mix PCR reaction components together as described in protocol for given gene.
2. Visualize DNA bands with agarose gel electrophoresis
    a. Add solid agarose to 1X TBE buffer to desired concentration.
    b. Microwave to melt the agarose in the buffer, and add 10 µL of 1% ethidium bromide solution per 100 mL and pour into gel box.
    c. Let the gel solidify and load samples with appropriate base pair markers and loading dye.
    d. Run at approximately 100V for 60 min.
    e. Image and record results.

PCR Protocol for STAT^{fl} PCR

Master Mix

Reagent	uL/ sample
5X PCR Buffer	5.0
25 mM $MgCl_2$	2.0
10 mM DNTP	0.5
10 µM Forward Primer 5'-GCA TGA CAT GAT CAG CAT TGC- 3'	1.0
10 µM Reverse Primer 5'- ACT GAC GTC AAC CAA GCC TG- 3'	1.0
Platinum Taq DNA polymerase (Invitrogen)	0.2
Water	38.3
DNA	2.0

Cycling Parameters:

Step 1: 94°C 2 min

Step 2: 94°C 20 sec
65°C 15 sec For 10 cycles
68°C 10 sec

Step 3: 94°C 20 sec
60°C 15 sec For 28 cycles
72°C 10 sec

Step 4: 72°C 2 min

Step 5: 4°C hold

PCR Protocol for Lambda Receptor^{fl} PCR

Master Mix

Reagent	uL/ sample
5X PCR Buffer	5.0
25 mM $MgCl_2$	2.0
10 mM DNTP	0.5
20 µM Forward Primer 5'- GGC CGC CCA AAT ATA AAC C - 3'	1.0
20 µM Reverse Primer 5'- TCT GAC ATC CGC TCA GCA CCA A - 3'	1.0
Platinum Taq DNA polymerase (Invitrogen)	0.2
Water	38.3
DNA	2.0

Cycling Parameters:

Step 1: 94°C 5 min

Step 2: 94°C 30 sec

48°C 30 sec For 35 cycles

72°C 30 sec

Step 3: 72°C 10 min

Step 4: 4°C hold