**Genotyping Tailsnips**

David Hoey

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**Materials:**

* Tweezers
* Scissors
* PCR tubes
* H2O (RNAse and DNase free)
* 10% Triton X-100
* Proteinase K (Roche 20mg/ml)
* 2M Tris pH 8.8
* 1M (NH4)2SO4
* 0.5M MgCl2
* Agarose
* PCR Master Mix
* Custom Primers
* Blue Juice
* DNA Ladder
* Agarose
* EtBR
* TBE

**Snipping of mice tails**

1. Place large kimwipe under and surrounding area of cage.
2. Prepare PCR tubes with the mouse number and ear tag ID written on the side.
3. Wipe down scissors and tweezers using 70% ethanol.
4. Remove mouse from cage by grabbing its tail. Place the mouse on top of cage and hold the tip of the tail so that it is hanging over the edge of the cage.
5. Using a scissors cut off approximately 1mm from the tip of the tail.
6. Collect the tip using the tweezers and place in the correctly marked PCR tube and place on ice.
7. Repeat steps 2-6 for each mouse.

**Tissue Lysis**

1. Prepare lysis buffer (1ml =22 samples)
   1. 0.85ml H2O
   2. 0.10ml 10x Gitschiers Buffer
      1. 3.35ml 2M Tris pH 8.8 (Final concentration – 670mM Tris pH8.8)
      2. 1.66ml 1M (NH4)2SO4 (Final concentration – 1.66mM Ammonium Sulphate)
      3. 1.34ml 0.5M MgCl2 (Final concentration – 67mM Magnesium Chloride)
      4. 3.65ml nanopure H2O
   3. 0.05ml 10% Triton-X 100
   4. 0.002ml Proteinase K (Roche 20mg/ml)
2. Add 40ul of lysis buffer per sample PCR tube containing tail snip.
3. Lyse in PCR machine
   1. 55C – 60min
   2. 95C – 5min
4. Aliquot and store at -20C

**PCR**

1. Prepare complete PCR mix

Volume per 25ul reaction

* 1. 22.5ul 2X Master Mix (Promega)
  2. 1ul per 10uM custom primer (x3 for Kif3A reaction)
  3. 8.5ul H2O

1. Add 24ul per PCR tube
2. Add 1ul DNA from tissue lysis (or water for negative control)
3. Run PCR
   1. Initial denature 95C 5min
   2. Denature 94C 45secs
   3. Anneal 60C 45secs
   4. Extension 72C 1min
   5. Final extension 72C 10min
   6. Cycles 35

**Running the DNA gel**

1. Prepare 1L 0.5x TBE with nanopure water
2. Prepare 2% agarose gel
   1. 1g agarose
   2. 50ml of 0.5x TBE
3. Microwave for 1min at power level 5
4. Add 10ul EtBr per 1L in fume hood
5. Allow to cool for a few minutes and pour into gel mould using large tooth combs
6. Allow to set for at least an hour
7. Add 5ul Blue Juice to 25ul of PCR sample and pipette into gel
8. Add 6ul of DNA ladder
9. Run gel at 100V
10. Once DNA has reached the bottom of the gel image immediately