GENOTYPING PROTOCOL

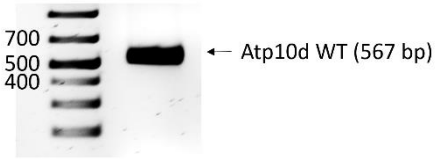
Investigator: Todd Graham and John Stafford
Genome Edit: Atp10d X817Q
Allele name: *Atp10d*$^{em1(ILAR\ lab\ code)}$

Forward Primer (Atp10dSTOP-Fwd1)**:** GACTCCCTGTGCCTTTGTGAGC
Reverse Primer (Atp10dSTOP-Rev1)**:** CACCATGGCAACGTTGTAAACATAC

Expected PCR Product: 567 bp

A high fidelity polymerase should be used to confirm the germline transmission of your desired gene edit in the N1 generation by Sanger sequencing. VAPRase polymerase is prepared by the Vanderbilt Antibody and Protein Resource. It can be purchased through the Molecular Cell Biology Resource Core: http://thecore.vanderbilt.edu/.

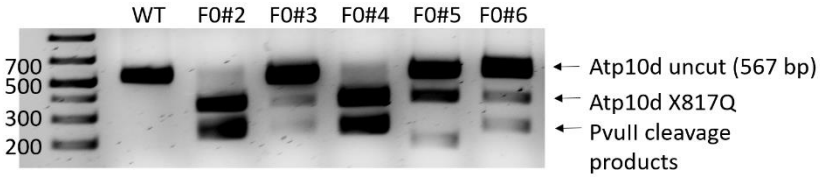
PCR reaction (50 ul)		PCR program
10x VAPRase buffer	5 ul	1. 98ºC, 30 sec
10 mM dTNPS	1 ul	2. 98ºC, 10 sec
10 µM Atp10dSTOP-Fwd1	2 ul	3. 64ºC, 20 sec
10 µM Atp10dSTOP-Rev1	2 ul	4. 72ºC, 25 sec
VAPRase HF polymerase	0.5 ul	5. Go to 2, 38X
Nuclease free water	37.5 ul	6. 72ºC, 2 min
DNA tail lysate	2 ul	7. 12ºC, forever



PvuII Enzyme Restriction Digest Assay:

Example Digest (25 µl volume)

0.2 to 0.5 µg purified PCR product
0.5 µl PvuII-HF (NEB)
2.5 µl CutSmart Buffer
To 25 µl volume with nuclease-free water



Incubate at 37°C for 30 minutes.
Run the full digest volume on a 2% agarose gel

Additional Notes:

- Atp10d X817Q animals are identified by the presence of PvuII-HF 343 and 224 base pair cleavage products. NEB PvuII-HF is available for purchase onsite: http://thecore.vanderbilt.edu/

- If conditions are fully optimized, Atp10d X817Q homozygous animals can be routinely identified by complete digestion of the PCR product and heterozygous animals by 50% product digest. Alternatively, a new genotyping assay may be developed using a primer that spans the point mutation.