**Campbell Lab Genotyping (R403Q Mice)**

**Agarose Gel PCR**

**Tail snips**

1.  Cut a 2-4 mm section from tip of tail and place in 0.2 uL microcentrifuge tube

2.  Add **100 µL** of 50 mM NaOH (make sure tail snip is completely submerged)

3.  Cook tails at 95°C for 1 hour or 98C for 30 min. (I’ve had success with both) and hold at 15-22 C.

**\*Note**: Can place tails in the 4°C following cooking until ready for use.

4.  Vortex (to break up tail pieces)

5.  Add **100 µL** of DNAse/RNAse free water

6.  Add **10 µL** of 1 M Tris-HCl (pH 8.0)

**\*Note**: I do this to neutralize the NaOH

7.  Centrifuge at 3,000-4,000 rpm for 5 min.

**\*Note**: I like to remove the supernatant and place the supernatant in a new microcentrifuge tube. Discard the hair pellet after the centrifugation because I vortex samples prior to adding them to the PCR reaction tube but this is not necessary.

Again, you can either place in the 4°C or go straight into making your PCR reactions from here.

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**PCR Master Mix** (per reaction/tail snip) – the online protocol says to make a 25 µL reaction.  I make a 12.5 µL reaction to save on reagent and have had good results in doing so.  I have also compared the 25 µL to the 12.5 µL reaction and the results are the same.

**6.25 µL** of Green GoTaq® Master Mix

**5.15** **µL** of RNAse free H2O

**0.05** **µL** of F. Primer (stock: 100 uM)

**0.05** **µL** of R. Primer (stock: 100 uM)

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**1.00** **µL** of tail DNA sample

Total Reaction: **12.5** **µL** (11.5 µL of Master Mix + 1 µL of tail DNA sample)

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**PCR Reaction Protocol**

**TnI-G203S** (Undigested/Unspliced product: bp)

1. 95°C for 2 min

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1. 95°C for 30 sec
2. 58°C for 30 sec
3. 72°C for 1 min

---------------------35 cycles (steps 2-4)

1. 72°C for 5 min
2. 4°C Hold

Proceed to endonuclease digestion step

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**AvaI Restriction Enzyme Digestion**

To the PCR reaction (post-thermocycler) add the following to each reaction:

**1 µL** (10 Units) of AvaI Restriction Enzyme (comes as 10,000 units per mL)

**5 µL** of CutSmart 1X Buffer (comes as 10X)

**1.5 µL** of DNAse/RNAse free water

Total Reaction: **12.5 µL** (original PCR reaction) + **7.5 µL** (restriction enzyme cocktail)

Place the **20 µL** reaction in the thermocycler and incubate at 37°C for 5 hours followed by an 80°C inactivation step for 20 minutes, hold at 12°C.

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**Make and Load Agarose Gel**

Make a 2% agarose gel with 5% EtBr.

-Large gel: 2.2 g of agarose into 110 mL of 1x TAE buffer.

-Small gel: 1.1 g of agarose into 55 mL of 1x TAE buffer.

Cook in an Erlenmeyer flask in the microwave for 2:45 min covered with a paper towel. Be sure to stop the microwave every 30 sec or so to swirl the mixture so that all the agarose melts and is in solution.

You will want to let the agarose cool for about 5-10 min by placing it on the work bench OR run cool water from the faucet over the base of the flask for ~45 seconds.

**Under a fume hood:**

Add **6 µL** of EtBr for a large gel or **3 µL** of EtBr for a small gel into the agarose mixture and swirl and mix well.

**\*Note**: I then let sit for 15-30 sec and then add it to the gel casting unit.

**\*Note**: If you allow the gel to be poured and formed under the fume hood, it reduces the time that you have to wait on it to set and harden (~15-20 min rather than +1 hr).

**\*Note:** Alternatives to EtBr that I would recommend are SYBR Safe DNA gel stain (10,000x) from Invitrogen Thermo Fisher or GelRed Nucleic Acid Gel Stain from Biotium. We use EtBr and I do all my gel pouring and EtBR mixing under a fume hood.

Once gel has set, place gel in running apparatus filled with 1X TAE Buffer, load **5** **µL** of DNA loading ladder (500 bp), then load **18-19 µL** into the gel for genotyping because you normally lose **0.5-1** **µL** of volume while making the PCR reaction.

Run gel at 90V for 60 minutes.