GRM7b C-ON Genotyping Protocol

Tail Prep:

1. Turn heat block to 95 degrees prior to going to the animal facility so that it is at temperature when you return.
2. Perform tail biopsy on pre-weaning pup, preferably prior to P15, when bones begin to ossify.
3. Place tail in 1.5mL microcentrifuge tube and chemically cauterize the wound with a silver stick
4. Add 300ul of 50mM NaOH to each tube and vortex
5. Place tubes at 95 degrees for 15 minutes, vortexing once every 5 minutes (the heat will blanch the skin and the NaOH will then lyse the cells. Vortexing assists in the blanching process.)
6. Remove tubes from heat block
7. Stop the lysis by addition of 30ul 1M Tris-HCl to each tube.

For the C-ON allele:

1. Set up the following reaction:

Conc.	Reagent	ul/rxn	Mastermix
2x	DreamTaq PCR Mastermix	12.5	
10uM	Primer 246	1	
10uM	Primer 247	1	
10uM	Primer 248	1	
	DNA	2	
	H20	7.5	

Date:

Mice:

** Make sure to do a control tube with water instead of DNA**

2. Run PCR in the thermal cycler under the following conditions:

Temp	Time
94	3:00
94	0:30
60	0:45
72	1:00
72	7:00
4	Hold

x 40 cycles

Primer 246: 5'-CAGACTTGTGGGATACAGAAGAC-3'
Primer 247: 5'-AGTCCACCTCACTCCTCATAAC-3'
Primer 248: 5'-CAATGGAAAGTCCCTATTGGCG-3'

Product Sizes:
WT: 758 bp
Flx/Flx or ON/ON: 535 bp
Flx/+ or ON/+: 758 + 535 bp

3. Run products on a 1.5% gel

For the Recombined allele:

1. Set up the following reaction:

Conc.	Reagent	ul/rxn	Mastermix
2x	DreamTaq PCR Mastermix	12.5	
10uM	Primer 264	1	
10uM	Primer 265	1	
	DNA	2	
	H20	8.5	

** Make sure to do a control tube with water instead of DNA**

2. Run PCR in the thermal cycler under same conditions as above:
3. Run products on a 1.5% gel

Primer 264: 5'-GCAGCAGGGGAACTTCATCAAAG-3'
Primer 265: 5'-GGCAACGTGCTGGTTATTGTG-3'

Product Sizes:

Flx/+ or Flx/Flx: 1099 bp

ON/+ or ON/ON (recombined): 222 bp