Grm7-I154T Mouse Genotyping

1. Isolate DNA using NaOH method
    a. Add 300ul of 50mM NaOH to tail and heat at 95C with occasional vortexing until tail is dissolved
    b. Add 30ul Tris HCl
2. Set up PCR as below:
    a. Include water (blank) and HET (positive) controls

Reagent	1 Reaction (ul)	MasterMix
5X HF Phusion Buffer **Vortex well before use!**	4	
5mM dNTPs	1	
10uM Primer 12	1	
10uM Primer 15	1	
DNA	2	n/a
Water	10.8	
Phusion HF polymerase	0.2	
Total Volume	20	

3. Run PCR program:

Temp	Time	
98	0:30	
98	0:10	35 cycles
60	0:20	
72	0:30	
72	7:00	
4	Hold	

Primer sequences:

NF12: TCCTGGACACTTGTTCCAGGGACAC
NF15: CATGAAGTCCAAACCAGCTTTT

Product size: 285

4. Use Zymo kit to purify PCR product- elute in 15 ul of H20
5. Set up a BsrI digest:

Reagent	1 Reaction (ul)	MasterMix
PCR product	10	n/a
Bsr1	1	
Buffer 3.1	2	
Water	7	
Total Volume	20	

6. Incubate in PCR machine at 65C for 15 minutes.
7. Add DNA loading dye and run 15ul of product on a 3% DNA gel (small comb) for 15-20 min at 150V
8. Expected results:
    a. WT (+/+): 285 bp
    b. HET (+/I154T): 285, 150, 135 bp
    c. HOMO (I154T/I154T): 150, 135 bp