Hsf1 genotyping protocol

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.

8. Set up the following reaction:

C;129-Hsf1tm1Ijb/J
Jackson Stock No: 010543

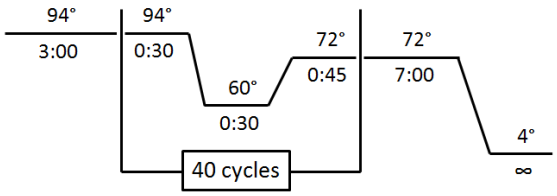
Conc.	Reagent	ul/rxn	Mastermix
2x	DreamTaq PCR Mastermix	12.5	
10uM	8975 WT Forward	1	
10uM	8976 Common	1	
10uM	9535 Mutant Forward	1	
	DNA	2	
	H20	2.5	

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

Date:

Mice:

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



10. Run products on a 1.5% gel for 30 min.

8975_WT_Forward: CCAGCAGCAAAAAGTTGTCA
8976_Common: TGCACACTTACTGGCAGTCC
9535_Mutant_Forward: GGGAGGATTGGGAAGACAAT

Product Sizes:
Mutant = **168bp**
Heterozygote = **168 bp and 418 bp**
Wild type = **418bp**