

Genotyping Protocol Received 21 February 2019

1. Following tail digestion with the previously described protocol, create a mixture of Taq Master Mix (2x), the primers, and the ultrapure water that comes with the Taq. These are the ratios per sample.

Green Taq – Remember this is 2X!!	10 uL	
Primer Forward	1 uL	150 ng of primer is either 1.0 or 1.5 uL of each. Trial and error will result in procedure changes.
Primer Reverse	1 uL	
Primer Internal Control Forward	1 uL	
Primer Internal Control Reverse	1 uL	
Ultrapure H ₂ O	6 uL	Total before DNA should be 20 uL, H ₂ O total depends on primer volumes used
DNA Mixture from Tail Digestion Plate	2 uL	
Total	22 uL	

2. Put new DNA + Taq mixture into a new COVERED 96-well plate and run the “JAX 023598 PCR” program on a thermocycler. “JAX 023598 PCR” is just the temperature cycle from below. We use a different program for our other sequence of interest.
3. Following the thermocycler, mix samples 1:1 with the sample buffer, no more than 15 uL total.
 - a. Our PCR products are ~750 and ~150 so we use a 1000 bp DNA ladder mixed with a gel-loading buffer to disperse in wells among the samples
4. Run in a 1.5% Agarose gel at 140 V for roughly an hour. We use EtBr to stain the gel
5. Image the gel on a UV light system and take note of which individuals have the proper band lighting up.
6. Repeat this process for other genes to check for a hybrid offspring as needed.

Annealing Temperature Calculation:

$$T_a \text{ Opt} = 0.3 \times (T_m \text{ of primer}) + 0.7 \times (T_m \text{ of product}) - 14.9$$

Where T_m of primer is the melting temperature of the less stable primer-template pair, and T_m of product is the melting temperature of the PCR product.

JAX 023598 PCR:

Step #	Temp. (°C)	Time	Note
1	94	2 mins	
2	94	20 s	
3	65	15 s	-0.5° C per cycle DECREASE
4	68	10 s	
5			Repeat steps 2-4 10 times (touchdown)
6	94	15 s	
7	60	15 s	
8	72	10 s	
9			Repeat steps 6-8 for 28 cycles
10	72	2 mins	
11	10	∞	