

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

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:**

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