**Kurlytm304 aka cfap298tm304 Genotyping v2**

PCR reaction: 242bp amplicon

kurly\_geno\_fwd2 AATCCGCAAGCGAGAGATG

kurly\_geno\_rev2 CCTGACACAACTGTTAAACCAAAT

12.5ul GoTaq MM

4.5ul water

3ul primers (F+R mixed)

5ul Fin Clip DNA

55C annealing temperature

HinfI Digest:

1.5ul CutSmart Buffer

0.5ul HinfI

8ul water

5ul PCR product

WT allele will cut, mutant does not digest with HinfI

**Protocol from Burdine Lab (never worked for BRT when tried in DPRI)**

Primers : 225 F/R

PCR temperature : 55°C 800bp amplicon – needs 1min extension

Enzymatic digestion : HinfI overnight @ 37 °C

WT : 560bp/140bp/100bp

Mut : 560bp/240bp

Genotyping was performed from fin clip DNA isolated by treatment with Proteinase K overnight. The *kurtm304* mutation was genotyped by PCR using Forward (CCCCATGGGCAGATTATTTAGC) and Reverse (CCTGACACAACTGTTAAACC) primers to generate an 800 bp product. Wild-type sequence was cleaved by *HinfI* to produce 560, 140, and 100 bp bands whereas one *HinfI* site is eliminated by the *kurtm304* mutation such that mutant DNA is cleaved once to produce 560 and 240 bp bands.