GENOTYPING BY PCR PROTOCOL
KOMP Repository: UC DAVIS

Protocol:CR824 Ap4e1

Reagent/Constituent	Volume (µL)
Water	10.275
10x Buffer	2.5
$MgCl_2$ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) *Optional*	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO *Optional*	0.325
Primer 1. (stock concentration is 20µM)	0.5
Primer 2. (stock concentration is 20µM)	0.5
Primer 3. (stock concentration is 20µM)	0.5
Primer 4. (stock concentration is 20µM)	0.5
Taq Polymerase 5Units/µL	0.2
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	**25.000 µL**

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65ºC decreasing in temperature by 1.0ºC; next 30 cycles anneal at 55ºC.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non-specific amplifications.

Strategy:

Steps		Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	5:00	**1**
2. Denaturation		94	0:15	
3. Annealing	steps 2-3-4 cycle in sequence	65 to 55 (↓1ºC/cycle)	0:30	**40x**
4. Elongation		72	0:40	
5. Amplification		72	5:00	**1**
6. Finish		15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. Ap4e1_CR824-F	CTGTGAGATGCTTGGATACGATGC
2. Ap4e1_CR824-R	CCTTAAAAACGACAGTCAGGACAG

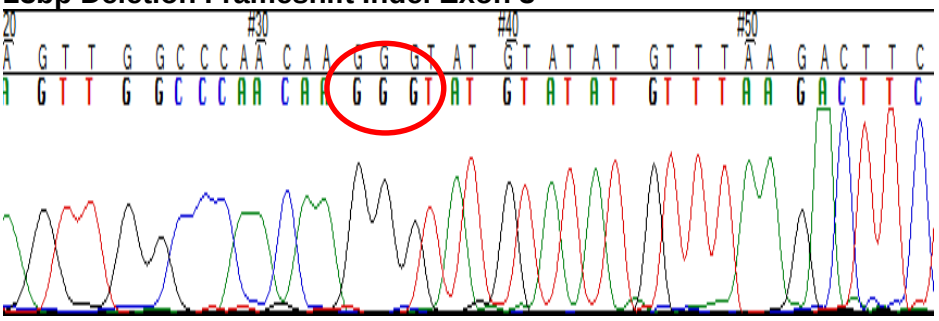
Electrophoresis Protocol:

Agarose: 1.5% V: 90

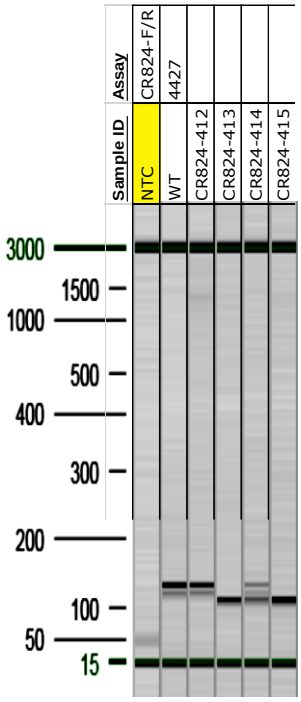
Estimated Running Time: 90 min

Primer Combination	Band (bp)	Genotype
1 & 2	134	wildtype
1 & 2	111	mutant

23bp Deletion Frameshift Indel Exon 3



Contig agttggcccaacaaggaaacctcttagaaaaaagagtgg**gtatgtatatgttta**



Allele Description: Exon 3 (ENSMUSE00001225189) received a 23bp deletion (**aaacctcttagaaaaaagagtgg**) from the Ap4e1 gene (ENSMUST00000177372.7) using CRISPR Cas9 gene editing technology in mouse zygotes. This causes a frameshifted transcript followed by early termination signal. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.