GENOTYPING by Real-Time PCR PROTOCOL
KOMP Repository: UC DAVIS

CR837 Nr1d2
2bp insertion Frameshift Indel

Protocol:

Reagent/Constituent	Volume (µL)
Cat No./ID 204541	
QuantiTect Multiplex PCR Master Mix	5.0
Water	3.6
Probe mix	0.4
-21 µM Forward primer	
-21 µM Reverse primer	
-7 µM Mutant probe	
-7 µM Wildtype probe	
Sample	1.0
TOTAL VOLUME OF REACTION:	**10.0 µL**

Comments on protocol:

- Protocol may work with other DNA extraction methods or reagent mixes.
- Real-Time probes were ordered with 6FAM (MGBF) and VIC (MGBV) dyes. Both probes use Life Technologies minor groove binder non-fluorescent quencher (MGB-NFQ)

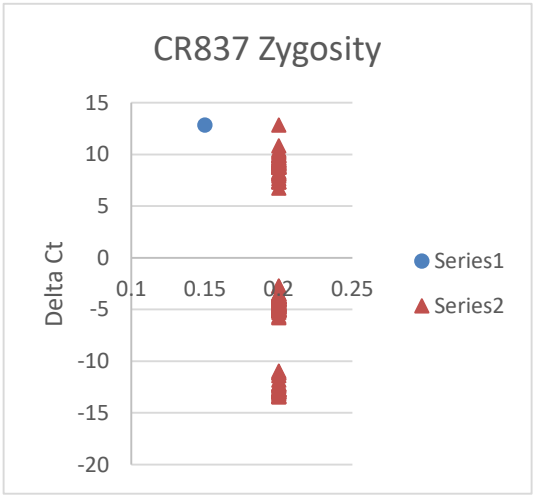
Strategy:

Steps	Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	95	15:00	**1**
2. Denaturation	95	0:30	**40x**
3. Annealing/Elongation	60	1:00	**40x**
4. To step 2 for 40 cycles			

Primers:

Name	Nucleotide Sequence (5' - 3')
1. TM_CR837-F	GCACCCTGTCCAGCGCCAT
2. TM_CR837-R	CCCGAGCCTCCCTCCTACATT
3. TM_CR837-MGBF	AGCTGAAAACGCAGG
4. TM_CR837-MGBV	GAGCTGAACGCAGGT

Allele Description: Exon 1 (ENSMUSE00000746927) received a 2bp insertion (**aa**)from the Nhlh2 gene (ENSMUST00000090543.5) 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.



most positive dCT cluster=WT

most negative dCT cluster=HOM

cluster between WT and HOM=HET