

GENOTYPING by Real-Time PCR PROTOCOL

KOMP Repository: UC DAVIS

CR1012 Nhlh2

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:

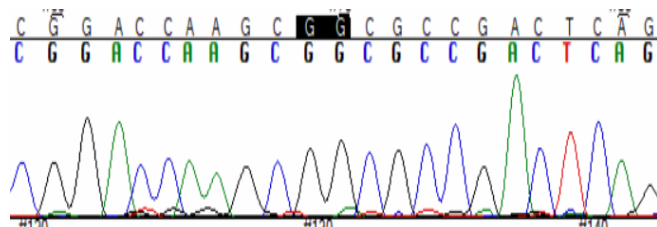
- Protocol may work with other DNA extraction methods or reagent mixes.
- Real-Time probes were ordered with 6FAM (MGBF) and VIC (MGBV) dyes. Both probes use Life Technologies minor groove binder non-fluorescent quencher (MGB-NFQ)

Strategy:

Steps	Temp (°C)	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 CR1012-F	GCCTCCAAAATGATGCTGAGTCC
2. TM CR1012-MGBF	gaccaagcggcg
3. TM CR1012-MGBV	CCAAGCCGCCG
4. TM CR1012-R	GTCGAGCTGGGGTGATCTGAG



most positive dCT cluster = WT

cluster between WT and HOM = HET

most negative dCT cluster = HOM

Allele Description: Exon 2 UTR

(ENSMUSE00001355406) received a 2bp insertion (gg) from the Nhlh2 gene (ENSMUST00000198675.1) 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.

