GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

NAME OF PCR: **B6.129-*Kif3a*^{tm2Gsn}/Mmucd** **MMRRC #** **000135-UCD**

DNA Extraction Method: ☐ NaOH ☐ Proteinase K ☒ Other: Qiagen

Protocol:

Reagent/ Constituent	Volume (µL)
Water	13.35
10x Buffer (contains 15mM $MgCl_2$)	2.5
$MgCl_2$ (stock concentration is 25mM)	1.7
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 20µM) 3A wild-type	0.4
Primer 2 (stock concentration is 20µM) 3A common	0.4
Primer 3 (stock concentration is 20µM) 3A deletion (optional; see notes)	
Taq Polymerase	0.15
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

Use the deletion primer only if mice have been crossed to Cre and detection of recombination event is necessary. Use 0.25uL of Deletion primer and adjust water accordingly. For lox site detection, best results come when using the Wild-type and Common primers only.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☒	95	5:00	**1**
2. Denaturation	94	0:45	
3. Annealing } steps 2-3-4 will cycle in sequence	65	0:45	} **35x**
4. Elongation	72	1:00	
5. Amplification	72	10:00	**1**
6. Finish	25	n/a	n/a

Primers:

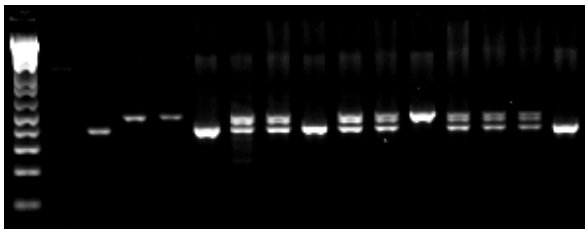
Primer Name	Nucleotide Sequence (5' - 3')
1: 3A Wild-type	TCT GTG AGT TTG TGA CCA GCC
2: 3A Common	AGG GCA GAC GGA AGG GTG G
3: 3A Deletion	TGG CAG GTC AAT GGA CGC AG

Electrophoresis Protocol:

% Agarose: 2 **mV:** 80

Estimated Running Time (min): 90

Expected Bands	Genotype
~360 bp	WT +/+
~490 bp	Floxed
~200 bp	Deletion



From Left to Right:
1 Kb+ Ladder (Invitrogen, Cat. No. 10787-018)
Controls: Water, B6, female F/F, male F/F
Samples 34 through 44