GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

2795 2nd Street, Suite 400, Davis, CA 95618
mmrrc@ucdavis.edu
530-754-MMRRC

NAME OF PCR: B6Crl.129-*Akap5*^{tm2Gsm}/Mmcd **MMRRC #** 034250-UCD

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

Protocol: Wild-type PCR

Reagent/ Constituent	Volume (µL)
Water	23.9
10X RP-PCR Buffer, 166mM $(NH_4)_2SO_4$, 670 Tris pH 8.8, 67 $MgCl_2$),	3.0
dNTPs (stock concentration is 2mM)	1.0
Primer 1 (stock concentration is 20µM) AKAP5 FOR	0.4
Primer 2 (stock concentration is 20µM) AKAP5 REV	0.4
Taq Polymerase	0.3
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	**30.0µL**

Comments on protocol:

If 700bp band does not appear, then run Akap exon2 reaction separately from Neo reaction (next page).The wild-type allele still has exon 2.

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	98	1:00	**1**
2. Denaturation		94	1:00	
3. Annealing	steps 2-3-4 will cycle in sequence	50	1:00	**30x**
4. Elongation		72	1:00	
5. Amplification		72	10:00	**1**
6. Finish		4	n/a	n/a

Primers:

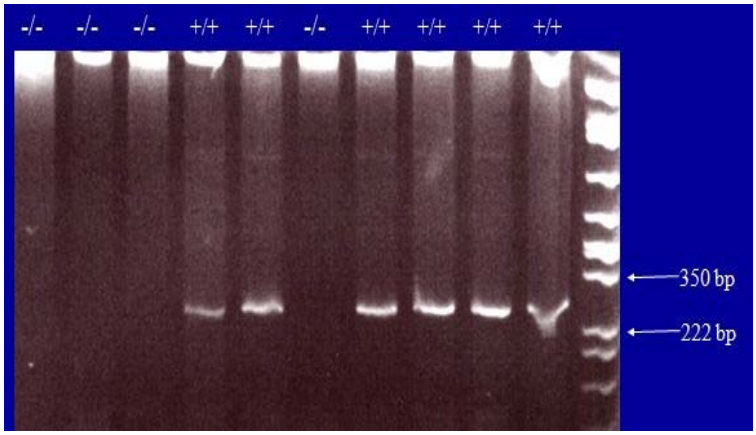
Primer Name	Nucleotide Sequence (5' - 3')
1: AKAP5 FOR	5'-AGA CCA GCG TTT CTG AG-3'
2: AKAP5 REV	5'-TTC CTG TGT GTC ACA AG-3'

Electrophoresis Protocol:

% Agarose: 1 **V:** 180

Estimated Running Time (min): 40

Expected Band	Genotype
258 bp	WT



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NAME OF PCR: B6Crl.129-*Akap5*^{tm2Gsm}/Mmcd **MMRRC #** 034250-UCD

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

Protocol: Akap5 KO PCR

Reagent/ Constituent	Volume (µL)
Water	11.1
10X RP-PCR Buffer, 166mM $(NH_4)_2SO_4$, 670 Tris pH 8.8, 67 $MgCl_2$),	3.0
dNTPs (stock concentration is 2mM)	2.0
Primer 1 (stock concentration is 20µM) Neo FOR	1.0
Primer 2 (stock concentration is 20µM) Neo REV	1.0
BSA, 0.1mg/ml	1.0
Taq Polymerase	0.3
DNA Sample	0.5
TOTAL VOLUME OF REACTION:	**20.0µL**

Comments on protocol:

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	2:00	**1**
2. Denaturation		94	0:30	
3. Annealing	steps 2-3-4 will cycle in sequence	53	0:30	**40x**
4. Elongation		72	1:30	
5. Amplification		72	10:00	**1**
6. Finish		4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: Neo FOR	5' TCG CAT GAT TGA ACA AG 3'
2: Neo REV	5' AAG CAC GAG GAA GCG GT 3'

Electrophoresis Protocol:

% Agarose: 1 **V:** 180

Estimated Running Time (min): 40 min

Primer Combination	Band	Genotype
1 and 2	700 bp	KO
	None	WT