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-*Akap1*^{tm1Gsm}/Mmcd **MMRRC #** 034251-UCD

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

Protocol:

Reagent/ Constituent	Volume (µL)
Water	24.1
10X RP-PCR Buffer, 166mM $(NH_4)_2SO_4$, 670 Tris pH 8.8, 67 $MgCl_2$),	3.0
dNTPs (stock concentration is 25mM)	0.6
Primer 1 (stock concentration is 1mg/ml) AKAP1 FOR	0.25
Primer 2 (stock concentration is 1mg/ml) AKAP1 REV	0.25
Primer 3 (stock concentration is 1mg/ml) NEO FOR	0.25
Primer 4 (stock concentration is 1mg/ml) NEO REV	0.25
Taq Polymerase	0.30
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? ☐	94	2:00	**1**
2. Denaturation		94	0:30	
3. Annealing	steps 2-3-4 will cycle in sequence	61	0:30	**25x**
4. Elongation		72	1:00	
5. Amplification		72	10:00	**1**
6. Finish		4	n/a	n/a

Primers:

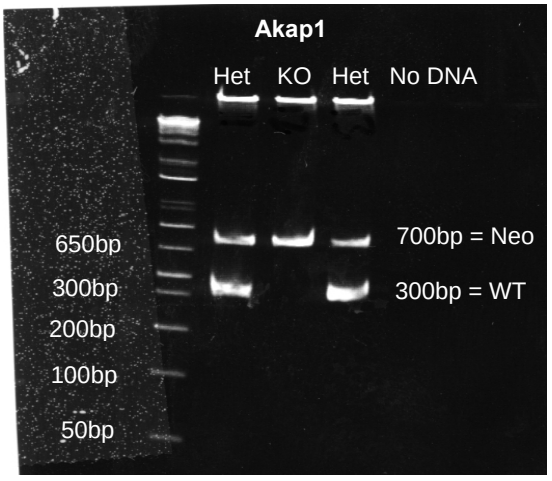
Primer Name	Nucleotide Sequence (5' - 3')
1: AKAP1 FOR	5' –ATG GCA ATC CAG TTG CGT TCG-3'
2: AKAP1 REV	5' –GCG GAC ATC CAA GAG GAA TA-3'
3: NEO FOR	5' –GGA TGA TCT GGA CGA AGA GC-3'
4: NEO REV	5' –AAT ATC ACG GGT AGC CAA CG-3'

Electrophoresis Protocol:

% Agarose: 1 **V:** 180

Estimated Running Time (min): 40 min

Primer Combination	Band	Genotype
1 and 2	300 bp	WT
1, 2, 3 and 4	300 / 700 bp	HET
3 and 4	700 bp	Akap1 KO



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-*Akap1*^{tm1Gsm}/Mmcd **MMRRC #** 034251-UCD

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

Protocol: Neo Reaction

Reagent/ Constituent	Volume (µL)
Water	15.1
10X RP-PCR Buffer, 166mM $(NH_4)_2SO_4$, 670 Tris pH 8.8, 67 $MgCl_2$),	3.0
dNTPs (stock concentration is 2mM)	0.6
Primer 1 (stock concentration is 1mg/ml) SM33	0.25
Primer 2 (stock concentration is 1mg/ml) SM34	0.25
BSA, 0.1mg/ml	0.25
Taq Polymerase	0.3
DNA Sample	0.5
TOTAL VOLUME OF REACTION:	**20.0µL**

Comments on protocol:

The neomycin cassette replaces Akap1 exon 2 in the knockout allele.

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	2:00	**1**
2. Denaturation	} steps 2-3-4 will cycle in sequence	94	0:30	} **40x**
3. Annealing		53	0:30	
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: SM33	5' TCG CAT GAT TGA ACA AG 3'
2: SM34	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