

GENOTYPING BY PCR PROTOCOL FORM
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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Protocol:

NAME OF PCR: Fak Floxed

Reagent/ Constituent	Volume (uL)
Water	12.25
10x Buffer w/o MgCl ₂	2.5
25 mM MgCl ₂	2.5
Q-solution	5
10 mM dNTPs	0.5
Primer 1, 10 uM: P1	0.5
Primer 2, 10 uM: GenoRV	0.5
Taq Polymerase	0.25
DNA Sample	1
TOTAL VOLUME OF REACTION:	25 ul

Comments on protocol: See Beggs et al (2003) Neuron 40: 501-513 for other primers.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	95	5:00	1
2. Denaturation	94	0:30	35
3. Annealing	62	0:30	35
4. Elongation			
5. Finish	25	Hold	n/a

} steps 2-3-4 will cycle in sequence

Primers:

Primer Name	Nucleotide sequence (5' - 3')																																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33		
1:P1	g	A	g	A	A	T	C	C	A	g	C	T	T	T	g	g	C	T	g	T	T	g													
2:GenoRV	g	A	A	T	g	C	T	A	C	A	g	g	A	A	C	C	A	A	A	T	A	A	C												

Electrophoresis Protocol:

% Agarose: 2 mV : 110

Estimated Running Time (min): 60

Number	Band (bp)	genotype
1	290	Wild Type
2	400	Floxed
3	290/400	+F

