

GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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Protocol:

NAME OF PCR: _____ Kr<ENU> _____

Reagent/ Constituent	Volume (uL)
DNA Sample	4
10x Buffer (contains 15mM MgCl ₂)	5
dNTPs (stock concentration is 2mM)	0.4
Primer 1 (stock concentration is 20 uM) Name: KrENU Upper	4
Primer 2 (stock concentration is 20 uM) Name: KrENU Lower	4
Primer 3 (stock concentration is 20 uM) Name:	
Water	31.4
Taq Polymerase	1.2
Other ?	
TOTAL VOLUME OF REACTION:	50 ul

Comments on protocol (e.g., different concentration of MgCl₂, etc): 25mM dNTPs, 5uM Primers, SEE ADDITIONAL ATTACHMENT

Strategy:

Steps	Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [x]	94	2	1
2. Denaturation	94	.75	40
3. Annealing	56	.75	40
4. Elongation	72	1	40
5. Amplification (i.e., 72°C, 10 min)	72	4	1
6. Finish (i.e., 4°C, indefinite)	4	n/a	n/a

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:KrENU UP	C	C	G	C	C	G	T	C	C	A	G	C	G	C	T	G	C	C	A	G	T	C													
2:KrENU LOW	G	C	C	G	G	A	C	C	C	G	C	C	A	G	G	A	C	T																	
3:																																			

Electrophoresis Protocol:

% Agarose: _____ 1.2 _____ mV : _____

Estimated Running Time (min): _____

Number	Band (kB)	genotype
1		
2		
3		

PASTE GEL PICTURE HERE, CLEARLY INDICATING LADDER, WATER CONTROL, DNA CONTROL, & DIAGNOSTIC SAMPLES

Additional genotyping notes for Kr<ENU>:

The genotyping protocol is a 2 step process, PCR reaction followed by a restriction digest with EarI. The PCR reaction will generate a 513 bp product in both WT and +/- . The mutation introduces a EarI site within the PCR product, so any product digested with EarI to generate a 260 and 253 bp fragments are positive for the mutation.