GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

Investigator/PI: __Barbara B. Kahn_____ Address: _____ Contact: Janice Zabolotny__
Telephone: _617-667-0258____ FAX: __617-667-2927____ email:__jzabolot@bidmc.harvard.edu

Protocol: **NAME OF PCR:__FVB – Tg(Mck-PTPRF)13Bbk MMRRC #:15507** _____

Reagent/ Constituent	Volume (uL)
DNA Sample	2
10x Buffer (contains 15mM MgCl2)	5
dNTPs (stock concentration is 2mM)	20
Primer **1** (stock concentration is 20 uM) Name: hLARpf	1
Primer **2** (stock concentration is 20 uM) Name: hLARpr	1
Primer **3** (stock concentration is 20 uM) Name: CTLA4S	1
Primer **4** (stock concentration is 20 uM) Name: CTLA4AS	1
Water	18
Taq Polymerase **from Invitrogen**	0.5
Other ? **$MgCl_2$ 50 mM**	0.5
TOTAL VOLUME OF REACTION:	**50 ul**

Comments on protocol *(e.g.,* different concentration of MgCl2, etc): __final concentration $MgCl_2$ should be 2 mM __

Strategy:

Steps	Temp (^oC)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [ ]	94^{o}C	1 min	**1**
2. Denaturation } steps 2-3-4 will cycle in sequence	94^{o}C	1 min	} 35x
3. Annealing	54^{o}C	1 min	
4. Elongation	72^{o}C	1 min	
5. Amplification (i.e., 72^{o}C, 10 min)	72^{o}C	10 min	**1**
6. Finish (i.e., 4^{o}C, indefinite)	4^{o}C	n/a	n/a

Primers:

Nucleotide sequence (5$^-$ - 3$^-$)

Primer Name	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: hLARpf	C	G	G	G	G	T	G	A	A	G	A	A	G	C	A	A	A	G	A	C													
2: hLARpr	C	C	C	T	G	C	C	C	C	A	T	C	A	T	C	A	A	A	C	T	C			} LAR									
3: CTLA4S	T	G	G	T	G	T	T	G	G	C	T	A	G	C	A	G	C	C	A	T	G												
4: CTLA4AS	T	G	G	G	A	T	G	G	T	G	A	G	G	T	T	C	A	C	T	G				} control									

Electrophoresis Protocol:

% Agarose: __2___ **mV : __50-100___**

Estimated Running Time (min): __60____

Number	Band (kB)	genotype
1	218	Control
2	332	Human LAR
3		

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