

GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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Protocol:

NAME OF PCR: CYP7A1-tg (MMRRC #12048 and 12049)

Reagent/ Constituent	Volume (uL)
DNA Sample	2
10x Buffer (contains 15mM MgCl ₂)	5
dNTPs (stock concentration is 2mM)	3.125
Primer 1 (stock concentration is 20 uM) Name: DM5	2
Primer 2 (stock concentration is 20 uM) Name: 630R	2
Primer 3 (stock concentration is 20 uM) Name: Actin-F	2
Primer 4 (stock concentration is 20 uM) Name: Actin-R	2
Water	31.375
Taq Polymerase	0.5
Other ?	
TOTAL VOLUME OF REACTION:	50 ul

Comments on protocol (e.g., different concentration of MgCl₂, etc): _____

Strategy:

Steps	Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE []			1
2. Denaturation	95	1	40
3. Annealing	60	1	40
4. Elongation	72	1	40
5. Amplification (i.e., 72°C, 10 min)	72	5	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: DM5	T	T	G	A	T	T	C	C	G	T	A	C	C	T	G	G	G	C	T	G															
2: 630R	A	G	T	G	C	T	T	T	T	G	T	G	T	G	T	C	T	G	T	C															
3: Actin-F	C	G	A	G	C	A	C	A	G	C	T	T	C	T	T	T	G	C	A	G															
4: Actin-R	A	T	G	A	G	G	T	A	G	T	C	T	G	T	C	A	G	G	T	C															

Electrophoresis Protocol:

% Agarose: 1.5 mV : 100V

Estimated Running Time (min): 60

Number	Band (kB)	genotype
1	0.5	CYP7A1
2	~0.62	Actin
3		

