

GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: STOCK *Myh10*^{tm3.1Rsad}/Mmcd MMRRC# 015983-UCD

DNA Extraction Method: NaOH _____ Proteinase K X Other _____

Protocol:

Reagent/ Constituent	Volume (uL)
DNA Sample (100 nM/uL)	2
10x Buffer (contains 15mM MgCl ₂)	2.5
dNTPs (stock concentration is 2mM)	2
Primer 1 (stock concentration is 100 pM) Name:	1
Primer 2 (stock concentration is 100 pM) Name:	1
Primer 3 (stock concentration is 100 pM) Name:	1
Water	14.5
Taq Polymerase	1
Other ?	
TOTAL VOLUME OF REACTION:	25 ul

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

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE []	95	2:00	1
2. Denaturation	95	0:45	
3. Annealing } steps 2-3-4 will cycle in sequence	60	1:00	32x
4. Elongation	72	0:45	
5. Amplification (i.e., 72°C, 10 min)	72	10:00	1
6. Finish (i.e., 4°C, indefinite)		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:	A	T	A	G	G	G	C	A	G	G	A	T	T	T	T	G	C	A	G	T	G													
2:	T	C	A	T	G	G	T	C	T	C	A	C	C	A	T	T	A	G	G	A	G													
3:																																		

Electrophoresis Protocol:

% Agarose: 1 mV : 100

Estimated Running Time (min): 2 hours

Number	Band (kB)	Genotype
1	525 bp	Wild-type
2	600 bp	Mutant