

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

STOCK Gt(ROSA)26Sortm4(ACTB-tdTomato,-EGFP)Luo

Protocol Name: Rb1tm3Tyj Rbl2tm2Tyj Trp53tm1Brn/Mmucd **MMRRC: 043692-UCD**

Protocol:

Reagent/Constituent	Volume (μL)
Water	5.6
GoTaq® G2 Colorless Master Mix, 2X	7.5
Primer 1. (stock concentration is 20μM)	0.45
Primer 2. (stock concentration is 20μM)	0.45
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME	
15	

Comments on protocol:

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5:00	1x
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓ 1°C/cycle)	0:30	10x
4. Elongation	72	0:40	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	30X
7. Elongation	72	0:40	

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. 43692-Rb L	CTCTAGATCCTCTCATTCTTCCC	Estimated Running Time: 90 min.		
2. 43692-Rb R	CCTTGACCATAGCCCAGCAC	Primer Combination	Band (bp)	Genotype
3. 43692-P53 L	GAAGACAGAAAAGGGGAGGG	1 & 2	310/260	Lox/wt
4. 43692-P53 R	AAGGGGTATGAGGGACAAGG	3 & 4	584/431	Lox/wt
5. 43692-P130 L	GTGTTGTAACATTCTCGTGGG	5 & 6	320/280	Lox/wt
6. 43692-P130 R	GACTGCTGGTATTAGAACCC			

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