

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER
mmrrc@ucdavis.edu
530-754-MMRRC

NAME OF PCR: STOCK *Myh9*^{tm10.1(GFP/Myh9*)Rsad}/Mmucd **MMRRC: 037506-UCD**

Protocol: *(PCR protocol provided by Donating Investigator)*

Reagent/Constituent	Volume (μL)
Water	
10x Buffer	
MgCl ₂ (stock concentration is mM) 1.5mM final	
Betaine (stock concentration is 5M) <i>Optional</i>	
dNTPs (stock concentration is 10mM) 250uM each final	
DMSO <i>Optional</i>	
Primer 1. (stock concentration is 20μM) 20pmole total	
Primer 2. (stock concentration is 20μM) 20pmole total	
Primer 3. (stock concentration is 20μM) 20pmol total	
Primer 4. (stock concentration is 20μM)	
Taq Polymerase 5Units/μL 1 unit HotStart polymerase	
DNA (50-200ng/ μL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	
<i>The total volume is auto-calculated based on volumes entered, right click the total and update field to show/recalculate the total volume.</i>	TOTAL VOLUME OF REACTION:
	20.0 μL

Comments on protocol:

- Protocol is used with Bioneer Accupower HotStart PCR beads

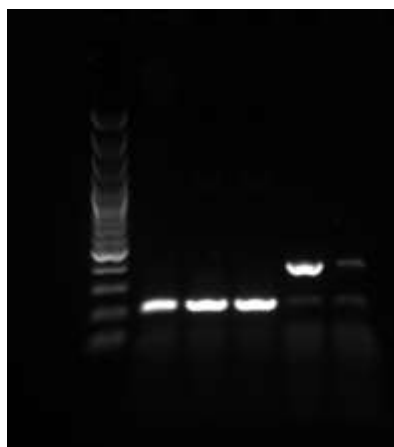
Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? X	95	2:00	1
2. Denaturation	95	00:20	
3. Annealing steps 2-3-4 cycle in sequence	58	00:30	40x
4. Elongation	72	00:30	
5. Amplification	72	10:00	1
6. Finish	4	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.4% V:100		
1. R1	CTG TCA CAT GGC TCA TGT TC	Estimated Running:Time: 60 min.		
2. R2	GCC GGA CAC GCT GAA CTT GT	Primer Combination	Band	Genotype
3. R3	GCC CTG AGT AGT ATC GCT CC	R1 R2 R3	400 bp	WT
		R1 R2 R3	200 bp	targeted



Lane 1 Fermentas GeneRuler 100bp DNA Ladder #SM0321
Lanes 2-4 Homozygous GFP-IIA mouse tail DNA PCR product
Lane 5 Wild-type mouse tail DNA PCR product
Lane 6 Water blank