GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

Protocol Name: STOCK Pdx1tm1Egs/Mmucd **MMRRC:** 017223-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:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65ºC decreasing in temperature by 1.0ºC; next 30 cycles anneal at 55ºC.

Strategy:

Steps		Temp (ºC)	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 (↓1ºC/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:

Name	Nucleotide Sequence (5' - 3')
1. 17223-GFP498F	CAAGATCCGCCACAACATCGAGGAC
2. 17223-GFP721R	CTTTACTTGTACAGCTCGTCCATGCC

Electrophoresis Protocol:

Argarose: **1.5%** V: **90**

Estimated Running:Time: **90** min.

Primer Combination	Band (bp)	Genotype
1 & 2	225	mutant

No gel image available

GFP PCR protocol

Primers

GFP498F: 5'-CAA GAT CCG CCA CAT CGA GGA C
GFP721R: 5'-CTT TAC TTG TAC AGC TCG TCC ATG CC

Reaction Mixture

DNA	1ul
GFP498F	1ul
GFP721R	1ul
10x redtaq buffer	5ul
dNTPmix(10mM)	4ul
Redtaq	1ul
DDW	37ul
TOTAL	50ul

Program Conditions

1. 94 °C for 5min
2. 94 °C for 30sec
3. 60 °C for 30sec
4. 72 °C for 30sec
5. 72 °C for 7min

2-4 x 35 cycles

1.5% Gel

~225bp band

Primer concentrations in the reaction mixture equal 100ng/ul.