

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

Protocol Name: C57BL/6N-Myo1dem1(IMPC)J/Mmucd **MMRRC: 048705-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 65°C decreasing in temperature by 1.0°C; next 30 cycles anneal at 55°C.

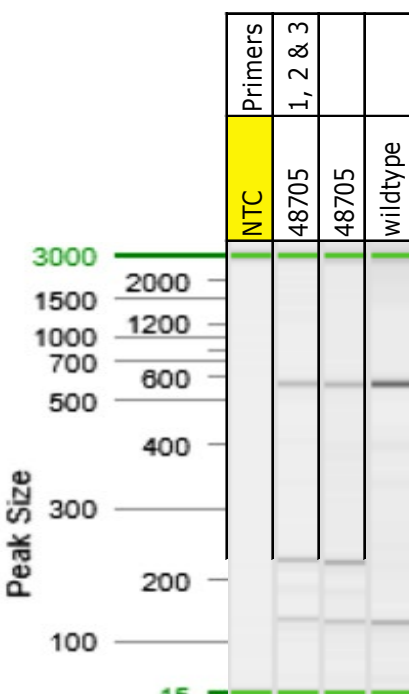
Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00 (↑20sec/cycle)	

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5%	V: 90
1. 29337-comF	TTTAAAAGTAAATTGAGGGCTGA	Estimated Running Time: 90 min.	
2. 29337-wtR	GCAAGAACAAAAGGTTGCTG	Primer Combination	Band (bp)
3. 29337-mutR	GCCTTCAGAACAAAGACTAAAGG	1 & 3	227, 561
		2 & 3	132
			Genotype
			Mutant, wildtype
			wildtype





Welcome to The Genotyping Protocol System

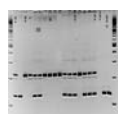
Master Protocol

Strain Name: C57BL/6NJ-Myo1d^{em1J/J}
Stock Number: 029337
Allele: Myo1d^{em1J}
Protocol Name: Myo1d^{em1J}
Method: High Resolution Melting
Created: 17-June -2016 (JKELMEN) **Updated:** 24-August -2016 (ESCHAAB)

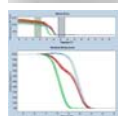
Notes

Expected Results: Mutant = 227 bp
 Heterozygote = 132 bp and 227 bp
 Wild type = 132 bp

Attachments



View 29337.jpg



View 29337 gs.JPG

- View Myo1dem1j in progress 6-13-16.docx
- View Myo1d genomic1.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
28553	-	TTT AAA AGT AAA TTG AGG GCT GA	-	Common	A
28554	-	GCA AGA ACA AAA GGT TGC TG	-	Wild type Reverse	A
28555	-	GCC TTC AGA ACA AAG ACT AAA GG	-	Mutant Reverse	A

Number Of Reactions 1

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
Component	Final Concentration	Step #	Temp°C	Time	Note
ddH2O		1	94	2 min	-
Kapa 2G HS buffer	1.3 X	2	94	20sec	-
MgCl2	2.6 mM	3	65	15sec	-0.5 C per cycle decrease
dNTP KAPA	.26 mM	4	68	10sec	-
28553	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
28554	.5 uM	6	94	15sec	-
28555	.5 uM	7	60	15sec	-
Glycerol	6.5 %	8	72	10sec	-
		9	-	-	repeat steps 6-8 for 28 cycles

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
Dye	1 X	10	72	2 min	-
Kapa 2G HS taq polymerase	.03 U/ul	11	10	-	hold
DNA					

Version 3.2