

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: C57BL/6N-Wscd2em1(IMPC)J/Mmucd **MMRRC: 050393-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.
- The mutant PCR is a general LacZ PCR. The wild type is specific for this strain.

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. 50393-comF	CTCCTGTGCCCTACTGTCTGT	Estimated Running Time: 90 min.		
2. 50393-mutR	TCCTCGGCACACTTCATACA	Primer Combination	Band (bp)	Genotype
3. 50393-wtR	GTACTTGGCTGTGGGGAAAG	1 & 2	434	mutant
		1 & 3	209	wildtype



Welcome to The Genotyping Protocol System

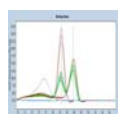
Master Protocol

Strain Name: C57BL/6NJ-Wscd2^{em1J}/J
Stock Number: 029160
Allele: Wscd2^{em1J}
Protocol Name: Wscd2^{em1J}
Method: MELT
Created: 09-May -2016 (LUCASM) **Updated:** 17-August -2016 (ESCHAAB)

Notes

Expected Results: Mutant = 434 bp
Heterozygote = 209 bp and 434 bp
Wild type = 209 bp

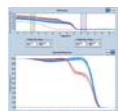
Attachments



View Wscd2-29160 MCA.JPG



View Wscd2-29160 Gel.jpg



View Wscd Mplex HRM.jpg

- View Wscd2 Genomic.gcs

- View Wscd2em1jMolDesApr2016.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27966	-	CTC CTG TGC CCT ACT GTC TGT	-	Common	A
27967	-	GTA CTT GGC TGT GGG GAA AG	-	Wild type Reverse	A
27968	-	TCC TCG GCA CAC TTC ATA CA	-	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	-
27968	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
		6	94	15sec	-

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
27967	.5 uM	7	60	15sec	-
27966	.5 uM	8	72	10sec	-
Glycerol	6.5 %	9	-	-	repeat steps 6-8 for 28 cycles
Dye	1 X	10	72	2 min	-
Kapa 2G HS taq polymerase	.03 U/ul	11	10	-	hold
DNA					

Version 3.2