GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

Protocol Name: C57BL/6N-Sh3d19em1(IMPC)J/Mmucd **MMRRC:** 049641-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.
- The mutant PCR is a general LacZ PCR. The wild type is specific for this strain.

Strategy:

Steps		Temp (ºC)	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 (↓1ºC/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:

Name	Nucleotide Sequence (5' - 3')
1. 49641-F	TCCACTTTTCACAGGTCTGCT
2. 49641-R	TTAAGTAAGCACATGAGGTTTAACA

Electrophoresis Protocol:

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

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

Primer Combination	**Band** (bp)	Genotype
1 & 2	193	mutant
1 & 2	401	wildtype

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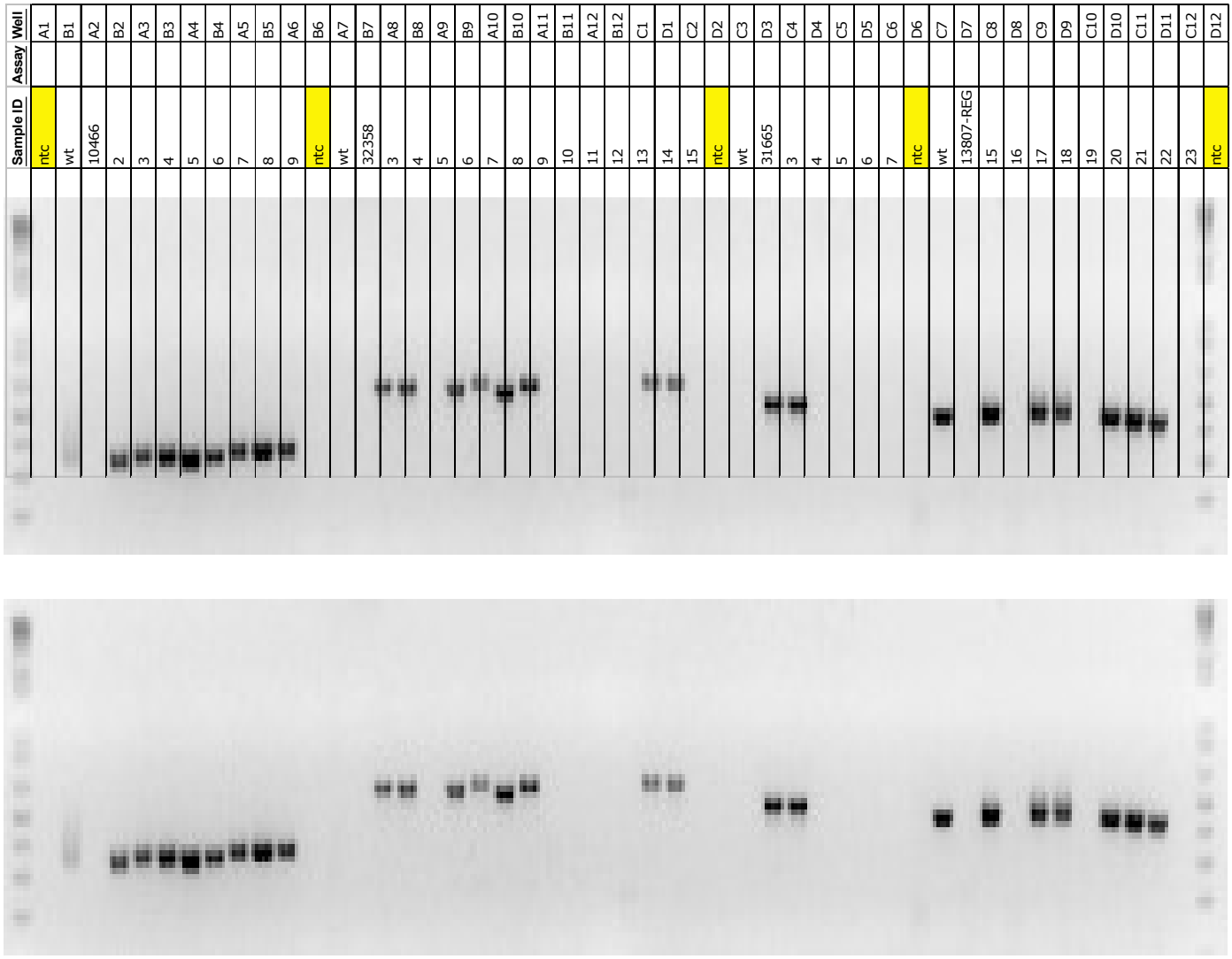

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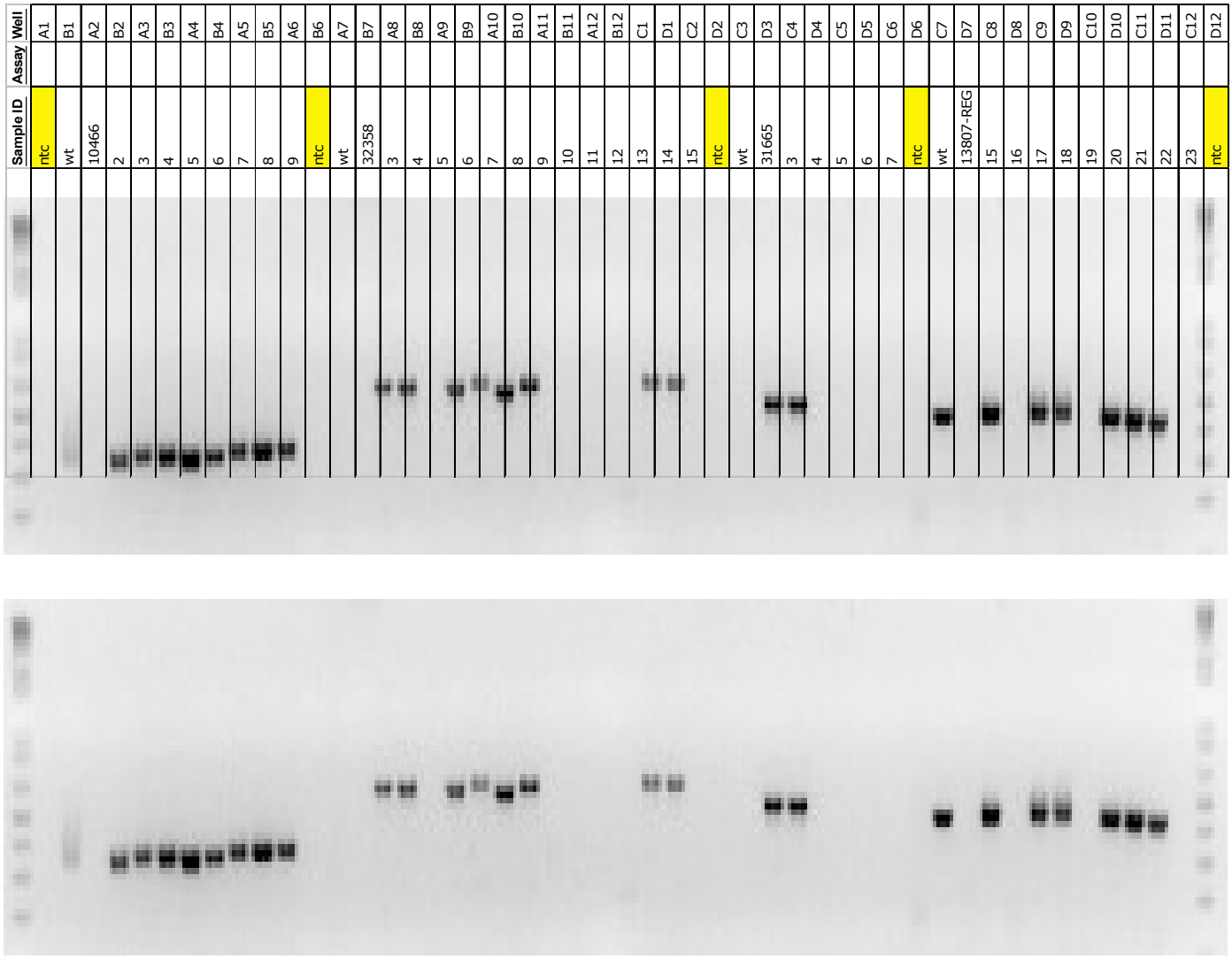

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Leading the search for tomorrow's cures

Welcome to The Genotyping Protocol System

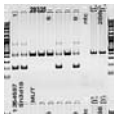
Master Protocol

Strain Name: C57BL/6NJ-*Sh3d19*^{em1J}/J
Stock Number: 029335
Allele: Sh3d19^{em1J}
Protocol Name: Sh3d19^{em1J}
Method: High Resolution Melting
Created: 17-June -2016 (JKELMEN) **Updated:** 09-September-2016 (ESCHAAB)

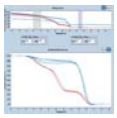
Notes

Expected Results:	Mutant = 193 bp Heterozygote = 401 bp and 193 bp Wild type = 401 bp

Attachments



View Sh3d19-29335 Gel.jpg



View Sh3d19 2 Primer HRM.JPG

\- View Sh3d19em1JMolDesJun2016.docx

\- View Sh3d19 genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
28561	-	TCC ACT TTT CAC AGG TCT GCT	-	Common	A
28563	-	TTA AGT AAG CAC ATG AGG TTT AAC A	-	Mutant Reverse	A

Number Of Reactions 1

Reaction A (2 Primer)

Component	Final Concentration
ddH2O	
Kapa 2G HS buffer	1.3 X
MgCl2	2.6 mM
dNTPS-kapa	.26 mM
28561	.5 uM
28563	.5 uM
Glycerol	6.5 %
Dye	1 X
Kapa 2G HS taq polymerase	.03 U/ul

Cycling (TouchDown 65-60_12-12-11)

Step #	Temp°C	Time	Note
1	94	2 min	-
2	94	20sec	-
3	65	15sec	-0.5 C per cycle decrease
4	68	10sec	-
5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
6	94	15sec	-
7	60	15sec	-
8	72	10sec	-
9	-	-	repeat steps 6-8 for 28 cycles
10	72	2 min	-

Reaction A (2 Primer)	Cycling (TouchDown 65-60_12-12-11)
DNA	11 10 - hold

Version 3.2