GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: C57BL/6N-Rab12em1(IMPC)J/Mmucd **MMRRC:** 049312-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. 49312-F	GGTCCACCTGTAAGGCTCAG
2. 49312-R	CAGTGCCCCATCCGTAGC

Electrophoresis Protocol:

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

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

Primer Combination	**Band** (bp)	Genotype
1 & 2	150	mutant
1 & 2	414	wildtype

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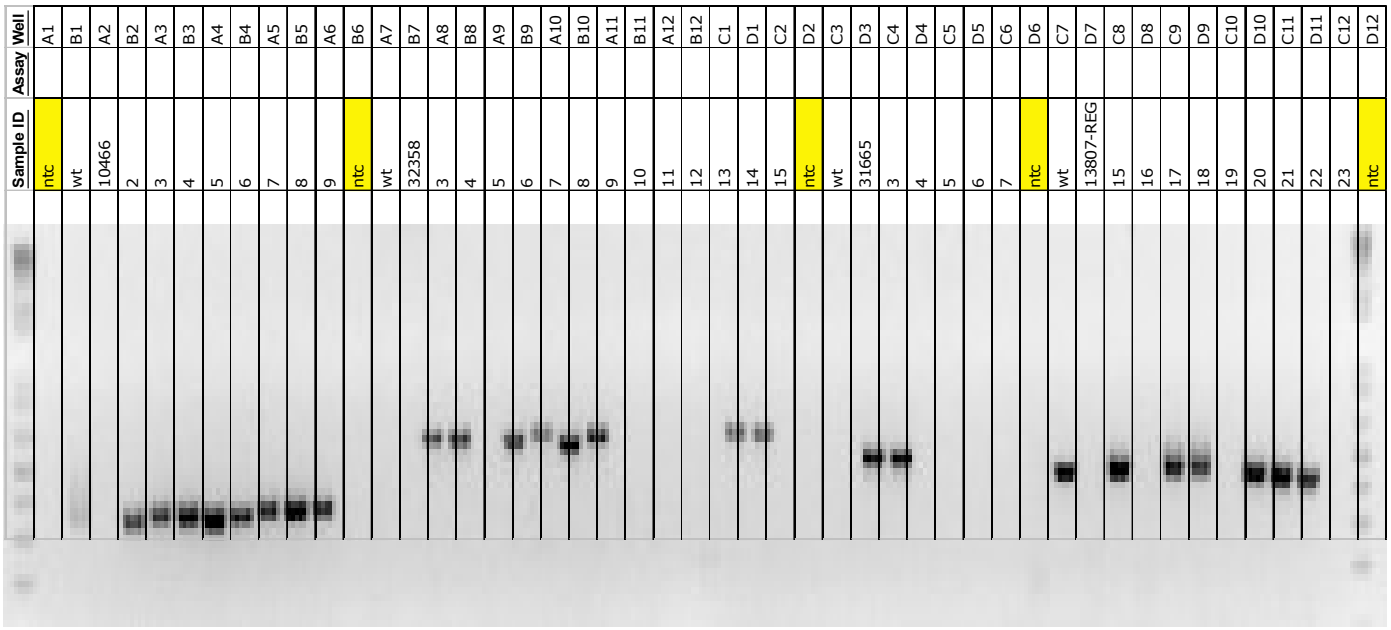

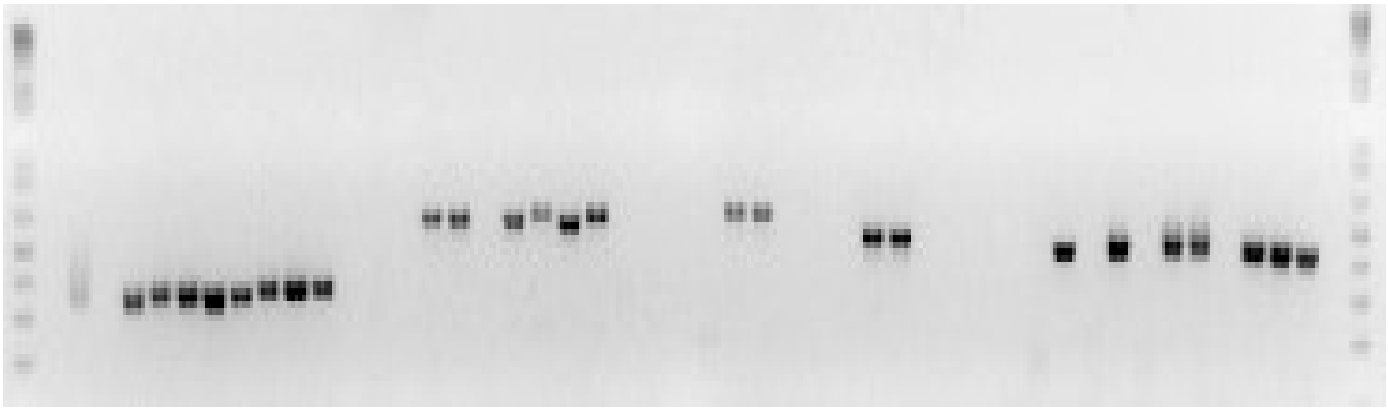

PCR protocol developed by MMRRC at University of California, Davis

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Welcome to The Genotyping Protocol System

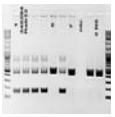
Master Protocol

Strain Name: C57BL/6NJ-*Rab12*^{em1J}/J
Stock Number: 028989
Allele: Rab12^{em1J}
Protocol Name: Rab12^{em1J}
Method: Standard PCR
Created: 05-April -2016 (JKELMEN) **Updated:** 12-September-2016 (LUCASM)

Notes

Expected Results:	Mutant = 150 bp Heterozygote = 150 bp and 414 bp Wild type = 414 bp

Attachments



View 28989 Rab12 2 primer gel.jpg

\- View Rab12em1jin progress 3-24-16.docx

\- View Rab12 genomic1.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27521	-	GGT CCA CCT GTA AGG CTC AG	-	Common	A
27523	-	CAG TGC CCC ATC CGT AGC	-	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
27521	.5 uM
27523	.5 uM
Glycerol	6.5 %
Dye	1 X
Kapa 2G HS taq polymerase	.03 U/ul
DNA	

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	-
11	10	-	hold

Version 3.2