

**GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER**

sacoord@mmrrc.org

800-910-2291 North America, +1-530-757-5710 International

Please provide the following information required for genetic analysis of your mutant mice.

Note to MAC users: to ensure your graphic can be viewed on a PC please follow the steps below when inserting the graphic into this document. DO NOT drag and drop or copy/paste the graphic into this document.

- Open the original graphic in the program that created it
- Choose File, Save As
- Select No Compression in the save options.
- Save as JPG or PNG or similar format that's compatible with both PC and Mac Word versions.
- Switch to Word, choose Insert, Picture, From File and choose the newly saved picture.

These instructions are very generic. The menu options for your graphics program may be different.

Donating Investigator/PI		
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734-975-9315	734-975-9329	
Strain Name		MMRRC Stock Number
C57BL/6JFam38h<tm#Jpsi>/Mmucd		037476UCD

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NAME OF PCR: Fam83h Dune Genotyping **MMRRC:** 0-CTR

Protocol: *(PCR protocol provided by Donating Investigator)*

Reagent/Constituent	Volume (µL)
Water	-
10x Buffer	-
MgCl ₂ (stock concentration is mM)	-
Betaine (stock concentration is 5M) <i>Optional</i>	-
dNTPs (stock concentration is 10mM)	-
DMSO <i>Optional</i>	-
Primer 1. (stock concentration is 20µM)	1
Primer 2. (stock concentration is 20µM)	1
Primer 3. (stock concentration is 20µM)	-
Primer 4. (stock concentration is 20µM)	-
Invitrogen SuperMix	17
DNA (50-200ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1
<i>The total volume is auto-calculated based on volumes entered, right click the total and update field to show/recalculate the total volume.</i>	TOTAL VOLUME OF REACTION: 20.000 µL

Comments on protocol:

- We use Invitrogen SuperMix for PCR reaction. The components of the SuperMix include: 22 U/mL complexed recombinant Taq DNA polymerase with Platinum® Taq Antibody, 22 mM Tris-HCl (pH 8.4), 55 mM KCl, 1.65 mM MgCl₂, 220 µM dGTP, 220 µM dATP, 220 µM dTTP, 220 µM dCTP, and stabilizers.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input checked="" type="checkbox"/>	94	5m	1
2. Denaturation	92	30s	
3. Annealing steps 2-3-4 cycle in sequence	58	45s	35x
4. Elongation	72	90s	
5. Amplification	72	7m	1
6. Finish	4	∞	n/a

Primers:

Electrophoresis Protocol:

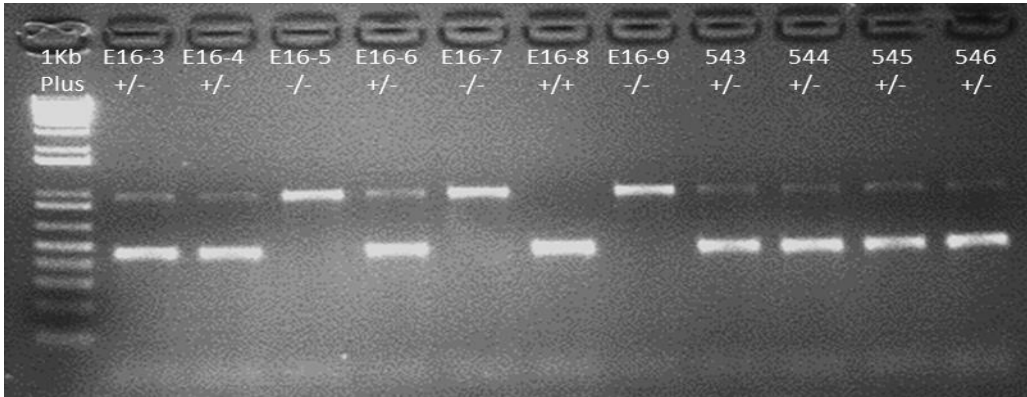
Name	Nucleotide Sequence (5' - 3')	Argarose:	V:
1. WTDUNELacZF	GTCTGTCTTCACTGGGGCTC	Estimated Running:Time: min.	
2. WTR	CCTGGTCTGAGTTCACCTGGC	Primer Combination	Band
3. DUNELacZR	TGCGTTTCACCCTGCCATAA	1&2	467 bp
4.		1&3	972 bp
5.			bp
			Genotype
			WT
			Null

Please size gel images to fit in this space

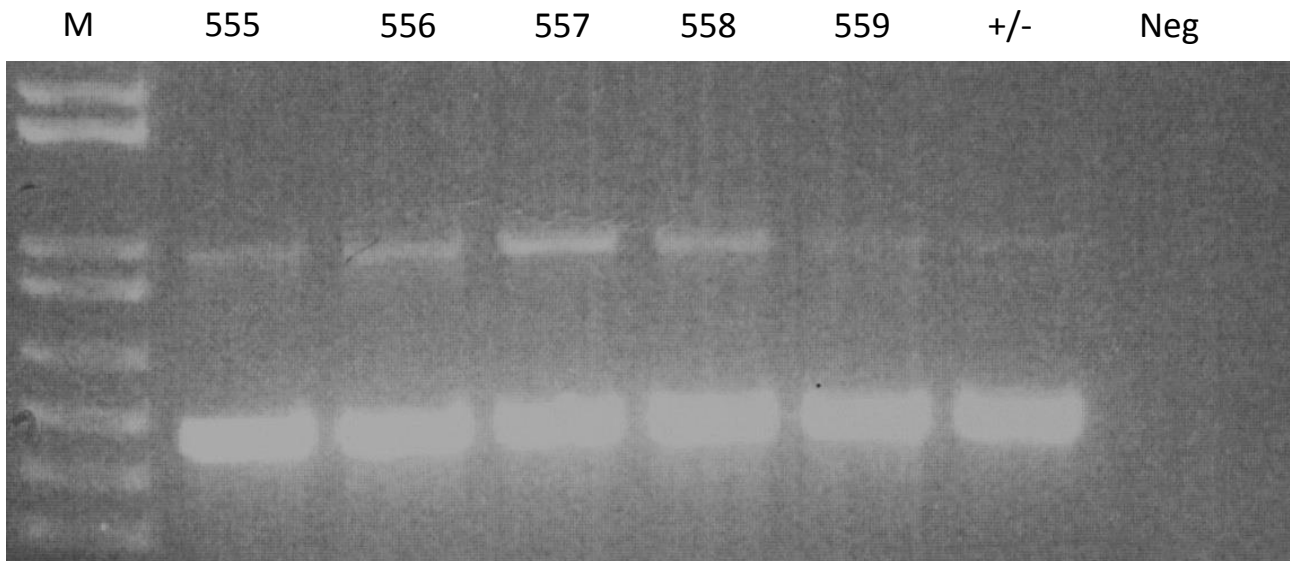
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Protocol / Gel Comments:



Gel pictures:



10/8/2014