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 **Frank Furnari**		
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Telephone **858 534 7809**	FAX **858 534 7830**	
Strain Name **K125R-FVB**	MMRRC Stock Number **43571**	

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NAME OF PCR: K125 **MMRRC:** 0-CTR

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

Reagent/Constituent	Volume (µL)
Water	42.1
10x Buffer	5
$MgCl_2$ (stock concentration is mM)	
Betaine (stock concentration is 5M) *Optional*	
dNTPs (stock concentration is 10mM)	1
DMSO *Optional*	
Primer 1. (stock concentration is 20µM)	0.2
Primer 2. (stock concentration is 20µM)	0.2
Primer 3. (stock concentration is 20µM)	
Primer 4. (stock concentration is 20µM)	
Taq Polymerase 5Units/µL	0.5
DNA (50-200ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1
The total volume is auto-calculated based on volumes entered, right click the total and update field to show/recalculate the total volume. **TOTAL VOLUME OF REACTION:**	**50.000 µL**

Comments on protocol:

•

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	3.0	1
2. Denaturation	94	0.30	
3. Annealing steps 2-3-4 cycle in sequence	50	0.45	30x
4. Elongation	72	1.0	
5. Amplification	72	5.0	1
6. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. RTL1	CCTGAAAGTTAG GCTTCTTTTAAG
2. RTL2	AACAGTTCTCAAAGCATCACACTG
3.	
4.	
5.	

Electrophoresis Protocol:

Argarose: 1% V:110

Estimated Running:Time: min.

Primer Combination	Band	Genotype
Rtl1 + Rtl2	520 bp	Sequence
	bp	
	bp	

Please size gel images to fit in this space

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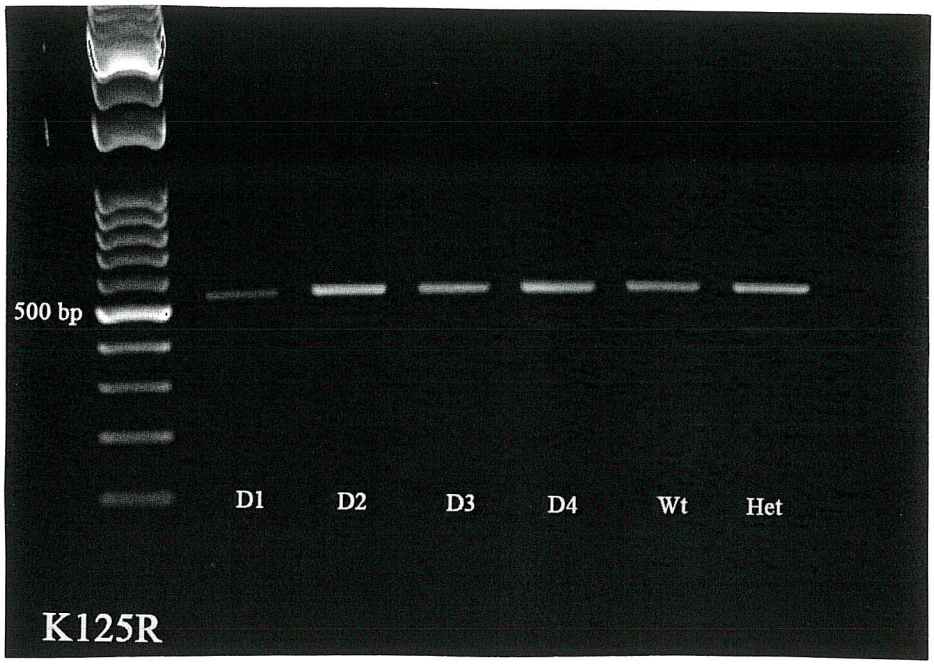
Protocol / Gel Comments:

After running the PCR, purify, and send to sequencing with RLT1 and see if wildtype, homo, or het.

Gel pictures:



Trace: D1_Rtl.ab1