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

Donating Investigator/PI **John Rubenstein**		
Email **John.rubenstein@ucsf.edu**		
Institution **University of California at San Francisco**		
Address **Rock Hall, RH282 1550 4th st. University of California at San Francisco**		
City **San Francisco**	State **CA**	Zip **94158**
Lab Contact **Carol Kim**		
Email **carol.kim@ucsf.edu**		
Telephone **(415)476-7872**	FAX **(415)476-7884**	
Strain Name Gad2En1-CreERT2-GFP		44024

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NAME OF PCR: Gad2En1-CreERT2-GFP **MMRRC:** 44024

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

Reagent/Constituent	Volume (µL)
Water	17
10x Buffer	2.5
$MgCl_2$ (stock concentration is mM)	
Betaine (stock concentration is 5M) *Optional*	2.5
dNTPs (stock concentration is 10mM)	1
DMSO *Optional*	
Primer 1. (stock concentration is 20µM)	0.25
Primer 2. (stock concentration is 20µM)	0.25
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:**	**25µL**

Comments on protocol:

- For routine genotyping we use Proteinase K digested tail DNA without column purification, diluted with TE. We generally use GoTaq 2X Mastermix according to the manufacturer's instructions in place of separate buffer, dNTPs and Taq.

Strategy:

Steps	Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☒	95	5:00	**1**
2. Denaturation	95	0:30	
3. Annealing steps 2-3-4 cycle in sequence	60	0:30	32**x**
4. Elongation	72	1:00	
5. Amplification	72	10:00	**1**
6. Finish	12	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. Gad2-CT2IG F	CTC CAG AGG TCT CCA CGG CGC
2. Gad2-CT2IG R	TTA TTC AAC TTG CAC CAT GCC
3.	
4.	
5.	

Electrophoresis Protocol:

Argarose: 1% V:120

Estimated Running:Time: 20 min.

Primer Combination	Band	Genotype
	1127 bp	Tg
	bp	
	bp	

Please size gel images to fit in this space

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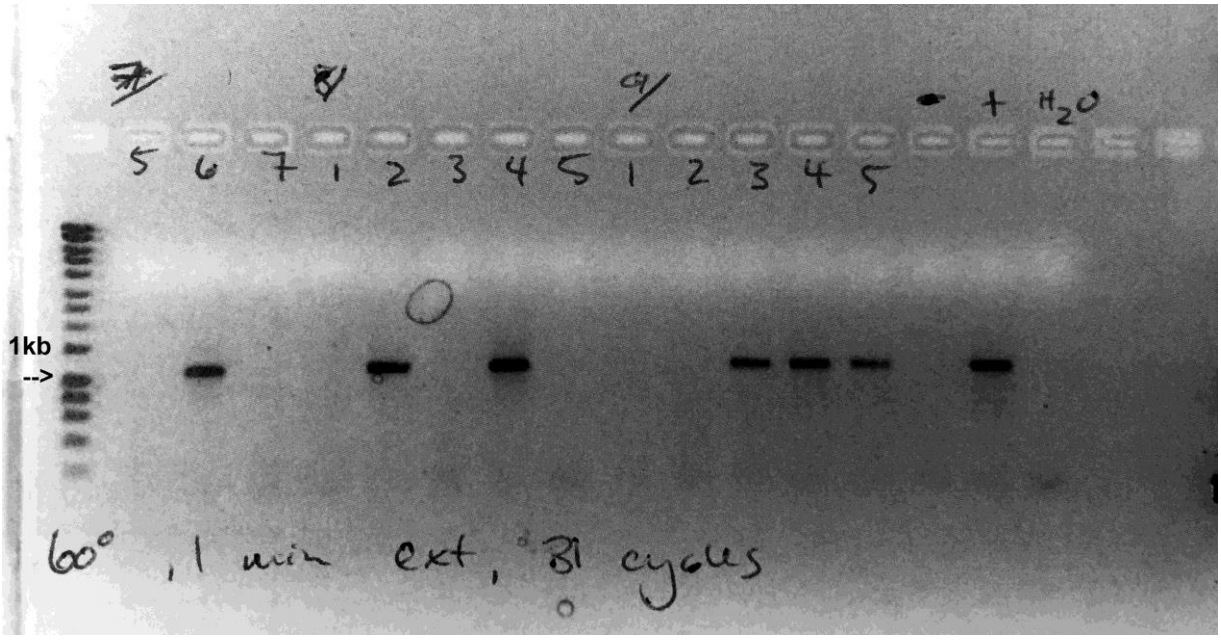
Protocol / Gel Comments:

Gel pictures:



PCR protocol provided by Donating Investigator