GENOTYPING BY PCR PROTOCOL MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

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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- 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.

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	ΓΔΧ					
(415)476-7872	(415)476-7884	·				
Strain Name		MN	MMRRC Stock Number			
1172 Croed IDES CED		0.2	7165			
11/2-VICEN-INED-OFI		0.5	/103			

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NAME OF PCR: <u>1172-CreER-IRES-GFP</u>

MMRRC: 037165-UCD

Protocol:

(PCR protocol provided by Donating Investigator)

Reagent/Constituent	Volume (µL)
Water	17.75
10x Buffer (contains 15mM MgCl2)	2.5
MgCl ₂ (stock concentration is mM)	0
Betaine (stock concentration is 5M) Optional	0
dNTPs (stock concentration is 10mM)	1.5
Qiagen Q Buffer	0
Primer 1. (stock concentration is 20µM) Name: 1172.for	0.5
Primer 2. (stock concentration is 20µM) Name: Cre.rev	0.5
Primer 3. (stock concentration is 20µM)	0
Primer 4. (stock concentration is 20µM)	0
Taq Polymerase 5Units/µL	0.25
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	2
The total volume is auto-calculated based on volumes entered, right click the total and update field to show/recalculate the total volume.	25 μL

Comments on protocol:

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START?	94	5min	1
2. Denaturation		94	30sec	
3. Annealing	steps 2-3-4 cycle in sequence	51	30sec	39 x
4. Elongation		72	30sec	
5. Amplification		72	10min	1
6. Finish		4	œ	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1	V:120	
1.		Estimated Running:Time: 45 min.		
2. 1172.for	GGTTTAATTAATGACTCACT	Primer Combination	Band	Genotype
3. Cre.rev	AGTGCTGCCTCTGACCTCAT	1172 F/R	500 bp	KO or MT/-
4.		1172 F/R	N/A bp	WT +/+
5.			bp	

Please size gel images to fit in this space

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Protocol / Gel Comments:

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