

**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.

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Strain Name		MMRRC Stock Number
Zcchc11		RRR277

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NAME OF PCR: Zcchc11 mutant

MMRRC: 0-CTR

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

Reagent/Constituent	Volume (µL)
Water	34
5x Buffer (GoTaq buffer, already has MgCl ₂)	10
dNTPs (stock concentration is 10mM)	1
Primer 1. (stock concentration is 25µM) Beta-Gal forward	0.25
Primer 2. (stock concentration is 25µM) Beta-Gal reverse	0.25
Primer 3. (stock concentration is 50µM) Zcchc11 forward	0.25
Primer 4. (stock concentration is 20µM) Zcchc11 reverse	0.25
Taq Polymerase 5Units/µL	0.7
DNA (50-200ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	3
<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: 50 µL

Comments on protocol:

- This PCR will detect two products. The first is Beta-gal which is present only in the Hets and Knockouts. The second is Zcchc11 but only detected in Hets and WTs.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	94	2:00	1
2. Denaturation	94	0:30	
3. Annealing steps 2-3-4 cycle in sequence	57	0:30	34x
4. Elongation	72	0:30	
5. Amplification	72	7:00	1
6. Finish	4	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 2%	V:105
1. Beta-Gal	caaatggcgattaccgttga	Estimated Running:Time: 60 min.	
2. Beta-Gal	tgcccagtcataagccgaata	Primer Combination	Band
3. Zcchc11 forward	tctgccactttgttacctcttctg		317 bp
4. Zcchc11 reverse	ctacagcctcaataaatgatatccttaataag		bp
5.			bp

Please size gel images to fit in this space

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Protocol / Gel Comments:

Gel pictures:

