

**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 | | |
| John C. Crabbe, PhD | | |
| Email | | |
| crabbe@ohsu.edu | | |
| Institution | | |
| VA Portland Health Care System | | |
| Address | | |
| 3710 SW US Veterans Hospital Road, R&D-12 | | |
| City | State | Zip |
| Portland | OR | 97239 |
| Lab Contact | | |
| Dr. Pamela Metten | | |
| Email | | |
| mettenp@ohsu.edu | | |
| Telephone | FAX | |
| 503-220-8262 x56680 | 503-721-1029 | |
| Strain Name | | MMRRC Stock Number |
| Withdrawal Seizure-Prone, Replicate 2 | | |

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NAME OF PCR: Not applicable, this is a selected line **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) | |
| Primer 2. (stock concentration is 20µM) | |
| Primer 3. (stock concentration is 20µM) | |
| Primer 4. (stock concentration is 20µM) | |
| Taq Polymerase 5Units/µL | |
| DNA (50-200ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits" | |
| <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: |
| | 0.000 µL |

Comments on protocol:

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Strategy:

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

Primers:

Electrophoresis Protocol:

| Name | Nucleotide Sequence (5' - 3') | Agarose: | V: |
|------|-------------------------------|------------------------------|------|
| 1. | | Estimated Running:Time: min. | |
| 2. | | Primer Combination | Band |
| 3. | | | bp |
| 4. | | | bp |
| 5. | | | bp |

Please size gel images to fit in this space

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Protocol / Gel Comments:

Gel pictures: