# GENOTYPING PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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**Protocol Name:** **CR1782 Pcnp EXDEL**

**Protocol:** ***GoTaq® G2 Colorless Master Mix(Promega)***

| Reagent/Constituent | Volume (µL) |
|---|---|
| Water | 4.5 |
| GoTaq® G2 Colorless Master Mix,2X | 7.5 |
| Primer 1. (stock concentration is 20µM) comF | 0.5 |
| Primer 2. (stock concentration is 20µM) wtR | 0.5 |
| Primer 3. (stock concentration is 20µM) mutR | 0.5 |
| DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits" | 1.5 |
| **TOTAL VOLUME OF REACTION:** | **15.00 µL** |

***Comments on protocol:***

- Protocol may work with other DNA extraction methods.

**Strategy:**

| Steps | | Temp (ºC ) | Time (m:ss) | # of Cycles |
|---|---|---|---|---|
| 1. Initiation/Melting | HOT START? ☐ | 94 | 2:00 | **1x** |
| 2. Denaturation | | 94 | 0:10 | |
| 3. Annealing | steps 2-3-4 cycle in sequence | 65 (↓1ºC/cycle) | 0:30 | **10x** |
| 4. Elongation | | 68 | 2:00 | |
| 5. Denaturation | | 94 | 0:15 | |
| 6. Annealing | steps 5-6-7 cycle in sequence | 55 | 0:30 | **25x** |
| 7. Elongation | | 68 | 2:00 (↑20sec/cycle) | |
| 8. Finish | | 4 | ∞ | n/a |

**Primers:**

| Name | Nucleotide Sequence (5' - 3') |
|---|---|
| 1. CR_Pcnp_comF | TGCTAGACCAGCTTTCAGAGTAGGG |
| 2. CR_Pcnp_wtR | GCAGTACAAGAGGCTCCCATGAATT |
| 3. CR_Pcnp_mutR | GCATATACTGTGCAGCAGAGCTACAAC |
| | |

**Electrophoresis Protocol:**

Agarose: **1.5%** **V:** **90**

Estimated Running Time: **90** min.

| Primer Combination | **Band** (bp) | **Genotype** |
|---|---|---|
| 1 & 2,1 & 3 | 532,1306* | wildtype |
| 1 & 3 | 401 | mutant |

**Allele Description:** Exon 1-2 (ENSMUSE00000699358 ,ENSMUSE00000618093)and flanking splicing regions were constitutively deleted from the Pcnp gene ENSMUSG00000071533 using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

***May not see larger wildtype band