# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

**NAME OF PCR:** **C57BL/6J-*Ikbkg*$^{m1Btlr}$/Mmcd (*panr2*)** **MMRRC #** **030659-UCD**

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

| Reagent/Constituent | Volume (µL) |
|---|---|
| Water | 20.0 |
| 10x Buffer (contains 15mM $MgCl_2$) | 2.5 |
| dNTPs (stock concentration is 25mM) | 0.5 |
| Primer 1 (stock concentration is 20µM) | 0.5 |
| Primer 2 (stock concentration is 20µM) | 0.5 |
| Taq Polymerase 5Units/µL | 0.5 |
| DNA (50-100ng/ul) extracted w/ " ☒ NaOH ☐ Proteinase K ☐ Other: | 0.5 |
| **TOTAL VOLUME OF REACTION:** | **25.000 µL** |

***Comments on protocol:***

- *Panr2* genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide change. Use primers 1 & 2 for amplification and 3 & 4 for sequencing. The lab uses JumpStart®REDTaq®ReadyMix® (P1107- Sigma) 12.5 ul in a 25 ul reaction (includes Taq, buffer, dNTPs).

**Strategy:**

| Steps | Temp (°C ) | Time (m:ss) | # of Cycles |
|---|---|---|---|
| 1. Initiation/Melting HOT START? ☒ | 94 | 2:00 | **1** |
| 2. Denaturation | 94 | 0:50 | |
| 3. Annealing } steps 2-3-4 cycle in sequence | 56 | 0:50 | **30x** |
| 4. Elongation | 72 | 1:00 | |
| 5. Amplification | 72 | 7:00 | **1** |
| 6. Finish | 4 | ∞ | n/a |

**Primers:**

| Name | Nucleotide Sequence (5' - 3') |
|---|---|
| 1. Panr2(F) | TGATGAGCCTTCTTGATGGTTCAGC |
| 2. Panr2(R) | CCCCTTGGCAAAAGAGAGTGAGTG |
| 3. Panr2_seq(F) | CTACCACTTGAGAGTCAAGGTTG |
| 4. Panr2_seq(R) | TTCAGATGCTCAAGCTAGGC |

**Electrophoresis Protocol:**

**Agarose:** _____ **V:** _____

**Estimated Running Time:** _____ **min.**

| Primer Combination | Band | Genotype |
|---|---|---|
| 1 and 2 | 1.42 kB | *panr2* |
| **SNP found at position ~ 588 of sequencing** | | |

**Mutation site (red) and flanking sequence:**

**WT** agaac**T**ccag
***panr2*** agaac**C**ccag

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

The following sequence of 1424 nucleotides (from Genbank genomic region **NC_000086** for linear DNA sequence of *Ikbkg*) is amplified:

```
7079                                                                  tg
7081 atgagccttc ttgatggttc agctctcagt atttaaaaaa ttccagtctc ctctcttatt
7141 ctgaaacttc tcacctgttg tataatgtgg actaggtgtt aatttttgtt gctgtctttg
7201 agtctagcct ccttttacag agtctcaaaa gagactgaga aaggttcact accacttgag
7261 agtcaaggtt ggtgactgga ggaaggcaaa tagccattgg gctaatgagg cttcaaagta
7321 ccaaggttta taggtcttga gaggcaggag aagccctcca tatatcagct gcttgaaggc
7381 tgccttaggt cttagaagtg ccaaaagtgg gaagcttggg agaaagcagt actgataggg
7441 aatgaccttt tttcttgtag cagatggctg aggacaaggc ctctgtgaaa gctcaggtga
7501 catcattgct cggagaactc caggagagcc agagccgttt ggaggctgcc accaaggatc
7561 ggcaagcttt agagggaagg tgagtgggga ggggaagcta acccaggagg ccccttccag
7621 ggtttttaat gagacaaatg taggagttct aggaattcct ctctctgaaa ggaaggaccg
7681 agtcttttta gattccccag agccctcagg gcttccttgt gagaagcctg gtctggtatt
7741 cagggattta gttctagctg ttgctggaaa gactctgagt ctgtactggg tgcttagtta
7801 gggcttccgt tgctgtggaa agatagcacg acaatgacaa cttttataag gaaaacattc
7861 tatctggctt acattcagag gtttagtcca ttattgtcat ggtagcaagc atgatggtaa
7921 gcagacagac atgatgctgg acaggtgtct gaaagttctt catcgggatt ggcaggcagc
7981 aggaagagag agtaaaccag tgagcctagc ttgagcatct gaaacctcaa agtccatccc
8041 cagtgatccg cttcttttaa caaggccaca cctactctta acaaggccac ttatcctaat
8101 ccttttaaat aattccactc tctagaatcc tacgggggcc attttcactc aaaacatcat
8161 actgggatct agcttattct ttaggaagca ctgcagggcc cctgagacca cactcaagag
8221 ctcactatcc agactgatat gcagtgggat tctaccaagg cagaaagtcc taggtgaaca
8281 agactggata cattccacag caaccctgtg aatagcacag agccctcatg aactacttct
8341 cattaaagac tatggattcc atgtttctgt tgggagttgt tcacgttggt atcctctgcc
8401 aggcatgtac cagtatttct gaattctggc agaaaagtga gtttgactga ctttgtcaac
8461 taatggtctt gggactgtca ctcactctct tttgccaagg gg
```

PCR primer binding sites are underlined; sequencing primer binding sites are highlighted in gray; the mutated T is shown in red text.