

# GENOTYPING BY PCR PROTOCOL FORM

## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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### PCR Protocol: B6.Cg-Ush1gjs-bkup/Mmucd Stock Number: 036536-UCD

| Reagent/ Constituent             | Volume (μL)  |
|----------------------------------|--------------|
| Water                            | 11.275       |
| 10x Buffer                       | 2.5          |
| 25 mM MgCl <sub>2</sub>          | 1.7          |
| 5 M Betaine                      | 6.5          |
| 10 mM dNTPs                      | 0.5          |
| DMSO                             | 0.325        |
| Primer 1: (20uM)                 | 0.5          |
| Primer 2: (20uM)                 | 0.5          |
| Taq Polymerase-5 Units/μl        | 0.2          |
| DNA Sample                       | 1.0          |
| <b>TOTAL VOLUME OF REACTION:</b> |              |
|                                  | <b>25 μl</b> |

#### Comments on protocol:

- Use Touch-Down cycling protocol-first 10 cycles anneal at 65° C decreasing in temperature by 1.0° C; next 30 cycles anneal at 55° C.
- Betaine/DMSO is standardized due to high GC content in promoter regions and protocol may be tested without. Also, may adjust MgCl<sub>2</sub> to increase reaction or decrease non specific amplifications.
- Use primer 3 (36536 F) to sequence the PCR product. Locate the SNP to determine genotype.

#### Strategy:

| Steps                                         | Temp (°C )            | Time (m:ss) | # of Cycles   |
|-----------------------------------------------|-----------------------|-------------|---------------|
| 1. Initiation/Melting                         | 94                    | 5:00        | <b>1</b>      |
| 2. Denaturation                               | 94                    | 0:15        | \             |
| 3. Annealing steps 2-4 will cycle in sequence | 65 to 55 (↓1°C/cycle) | 0:30        | > <b>x 40</b> |
| 4. Extension                                  | 72                    | 0:40        | /             |
| 5. Final Extension                            | 72                    | 5:00        | <b>1</b>      |
| 6. Finish                                     | 4                     | Hold        | --            |

#### Primers:

| Primer Name        | Nucleotide sequence (5'– 3') |
|--------------------|------------------------------|
| <b>1:</b> 36536 F2 | CGTTTAGGACCCAGGGGAAG         |
| <b>2:</b> 36536 R2 | CCCCACCTTAGGGTCTGTCC         |
| <b>3:</b> 36536 F  | CTCAGATGTCCCGGTAGTCT         |

#### Electrophoresis Protocol: (optional)

% Agarose: 1.5 Volts : 90

Estimated Running Time (min): 90

| Primer Combinations | Band size (bp) | genotype |
|---------------------|----------------|----------|
| 1 & 2               | 576            | mutant   |
|                     |                |          |

WT SNP ID = ccatgaatgac**C**agtatcac  
mutant SNP ID = ccatgaatgac**T**agtatcac

Found at position ~ 165 of sequencing

