# GENOTYPING BY PCR PROTOCOL
## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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**NAME OF PCR:** **C57BL/6- *Rab27a*$^{cct}$/Mmcd, (*concrete*)** **MMRRC #** **016836-UCD**

### Protocol:

| Reagent/ Constituent | Volume (µL) |
|---|---|
| Water | 12.675 |
| 10x Buffer (contains 15mM $MgCl_2$) | 2.5 |
| Betaine (stock concentration is 5M) *Optional* | 6.5 |
| dNTPs (stock concentration is 25mM) | 0.5 |
| DMSO *Optional* | 0.325 |
| Primer 1 (stock concentration is 20µM) Con PCR F | 0.5 |
| Primer 2 (stock concentration is 20µM) Con PCR R | 0.5 |
| Taq Polymerase | 0.5 |
| DNA sample extracted with ☐ NaOH ☐ Proteinase K ☒ Other: Any | 1.0 |
| **TOTAL VOLUME OF REACTION:** | **25µL** |

### *Comments on protocol:*

- PCR products are verified to contain the correct amplicon size by running ~10µl of the reaction on a gel and the remaining 15µl purified via column based PCR purification method for sequencing.
- 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 and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non specific amplifications.

### Strategy:

| Steps | | Temp (°C ) | Time (m:ss) | # of Cycles |
|---|---|---|---|---|
| 1. Initiation/Melting HOT START? ☐ | | 94 | 5:00 | **1** |
| 2. Denaturation | | 94 | 0:15 | |
| 3. Annealing | steps 2-3-4 will cycle in sequence | 65 to 55 (↓1°C/cycle) | 0:30 | **10x** |
| 4. Elongation | | 72 | 0:40 | |
| 5. Denaturation | | 94 | 0:15 | |
| 6. Annealing | steps 5-6-7 will cycle in sequence | 55 | 0:30 | **30x** |
| 7. Elongation | | 72 | 0:40 | |
| 8. Amplification | | 72 | 5:00 | **1** |
| 9. Finish | | 15 | ∞ | n/a |

### Primers:

| Name | Nucleotide Sequence (5' - 3') |
|---|---|
| 1: Con PCR F | TTC GGG ACT CAA TTC TCT TGA |
| 2: Con PCR R | TCT GGC AGC TCA TCT TAC CA |
| 3: Concrete Sequencing | Use PCR primers for sequencing |

### Electrophoresis Protocol:

**Agarose:** 2% **mV:** 80 **Estimated Running Time:** 90 **min**

| Primer Combination | Band | Genotype |
|---|---|---|
| 1 and 2 | 170 bp | *concrete* |
| **SNP found at position ~ 137 of sequencing** | | |

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

**WT** aggtgtac**A**gagcca

***concrete*** aggtgtac**T**gagcca