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

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**NAME OF PCR:** **C57BL/6J-*Fam83h*$^{tm1Jcch}$/Mmucd** **MMRRC:** **037504-UCD**

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

| Reagent/Constituent | Volume (µL) |
|---|---|
| Water | |
| 10x Buffer | |
| $MgCl_2$ (stock concentration is mM) | |
| dNTPs (stock concentration is mM) | |
| Primer 1. (stock concentration is 20 µM) | 1 |
| Primer 2. (stock concentration is 20 µM) | 1 |
| Invitrogen SuperMix | 17 |
| DNA (50-200ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits | 1 |
| DNA extracted with ☐ NaOH ☐ Proteinase K ☐ Other: | |
| **TOTAL VOLUME OF REACTION:** | **20.000 µL** |

***Comments on protocol:***

- We use Invitrogen SuperMix for PCR reaction. The components of the SuperMix include: 22 U/mL complexed recombinant Taq DNA polymerase with Platinum® Taq Antibody, 22 mM Tris-HCl (pH 8.4), 55 mM KCl, 1.65 mM $MgCl_2$, 220 µM dGTP, 220 µM dATP, 220 µM dTTP, 220 µM dCTP, and stabilizers.

**Strategy:**

| Steps | Temp (ºC ) | Time (m:ss) | # of Cycles |
|---|---|---|---|
| 1. Initiation/Melting HOT START? ☒ | 94 | 5:00 | **1** |
| 2. Denaturation | 92 | 0:30 | |
| 3. Annealing steps 2-3-4 cycle in sequence | 58 | 0:45 | **35x** |
| 4. Elongation | 72 | 1:30 | |
| 5. Amplification | 72 | 7:00 | **1** |
| 6. Finish | 4 | ∞ | n/a |

**Primers:**

| Name | Nucleotide Sequence (5' - 3') |
|---|---|
| 1. Fang Ex4F DUNELacZF | TTCTTGCTTGTGGACTGTGC |
| 2. Fang Ex5R | GTAGCTATGCCGCTTGAAGG |

Argarose: V:

Estimated Running:Time: min.

| Primer Combination | **Band** (bp) | Genotype |
|---|---|---|
| 1 and 2 | 728 | WT |
| 1 and 2 | 1127 | Null |
| 1 and 2 | 728 and 1127 | +/- |