

Capzb (MBLU; EPD0105_5_A09)

Allele: *Capzb*^{tm1a(EUCOMM)Wtsi}

Genotyping Information

These mice may be genotyped through a combination of separate PCR reactions that detect *LacZ*, the gene-specific wild type allele, and a mutant allele-specific short range PCR. Interpretation of the consolidated results produces the genotype of the mice.

For example: *LacZ* positive, mutant positive, wild type positive = heterozygous.

➤ PCRs primer pairs and expected size bands

| PCR type | Forward primer | Reverse primer | Expected size band (bp) |
|---------------|----------------|----------------|-------------------------|
| Mutant PCR | Capzb_47322_F | CAS_R1_Term x | 111 |
| Wild type PCR | Capzb_47322_F | Capzb_47322_R | 419, 461, 503* |
| LacZ PCR | LacZ_2_small_F | LacZ_2_small_R | 108 |

➤ Primer sequences

| Primer name | Primer sequence (5' > 3') |
|----------------|---------------------------|
| CAS_R1_Term | TCGTGGTATCGTTATGCGCC |
| Capzb_47322_F | GACTCGAACAGAAAGGCATCC |
| Capzb_47322_R | GATCCAGGCTGTCCATAGGTG |
| LacZ_2_small_F | ATCACGACGCGCTGTATC |
| LacZ_2_small_R | ACATCGGGCAAATAATATCG |

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➤ **Reaction**

| Reagent | μl |
|-----------------------------------|-------------|
| DNA (~50-100 ng) | 1.0 |
| 10x Buffer | 2.0 |
| MgCl ₂ (50 mM) | 0.6 |
| PtTaq (Platinum Taq (Invitrogen)) | 0.2 |
| dNTPs (100 mM) | 0.2 |
| Primer 1 (10 μM) | 0.4 |
| Primer 2 (10 μM) | 0.4 |
| H ₂ O | <u>15.2</u> |
| Total | 20.0 |

➤ **Cycling conditions**

Wild type and mutant PCRs

| Cycle | Temp | Time |
|-------|-----------------------|---------|
| 1 | 94 °C | 5 min |
| 2 | 94 °C | 30 sec |
| 3 | 58 °C | 30 sec |
| 4 | 72 °C | 45 sec |
| 5 | Go to '2' + 34 cycles | |
| 6 | 72 °C | 5 min |
| 7 | 12 °C | forever |

LacZ PCR

| Cycle | Temp | Time |
|-------|-----------------------|---------|
| 1 | 94 °C | 5 min |
| 2 | 94 °C | 30 sec |
| 3 | 60 °C | 30 sec |
| 4 | 72 °C | 30 sec |
| 5 | Go to '2' + 34 cycles | |
| 6 | 72 °C | 5 min |
| 7 | 12 °C | forever |

*Multiple primer binding sites

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