

## Espn (MCRP; EPD0409\_1\_A08)

Allele: *Espn*<sup>tm1e(EUCOMM)Wtsi</sup>

### 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    | Espn_40631_F   | CAS_R1_Term x  | 105                     |
| Wild type PCR | Espn_40631_F   | Espn_40631_R   | 306                     |
| LacZ PCR      | LacZ_2_small_F | LacZ_2_small_R | 108                     |

#### ➤ Primer sequences

| Primer name    | Primer sequence (5' > 3') |
|----------------|---------------------------|
| CAS_R1_Term    | TCGTGGTATCGTTATGCGCC      |
| Espn_40631_F   | ACCGGAACAGTCGCAGGTAG      |
| Espn_40631_R   | GTTAGGTCCAAGGGCACAGG      |
| 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 <sub>2</sub> (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 <sub>2</sub> O                  | <u>15.2</u> |
| <b>Total</b>                      | <b>20.0</b> |

➤ **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 |

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