



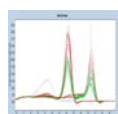
Welcome to The Genotyping Protocol System

**Master Protocol**

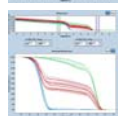
**Strain Name:** C57BL/6NJ-*Tgfr3<sup>em1J</sup>*J  
**Stock Number:** 029112  
**Allele:** *Tgfr3<sup>em1J</sup>*  
**Protocol Name:** *Tgfr3<sup>em1J</sup>*  
**Method:** MELT  
**Created:** 09-May -2016 (DTG)      **Updated:** 17-August -2016 (ESCHAAB)

**Notes**

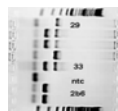
Expected Results: Mutant = 250 bp  
 Heterozygote = 250bp and 150 bp  
 Wild type = 150 bp

**Attachments**

View 29112 Tgfr3I MCA.jpg



View 29112 Tgfr3I HRM.JPG



View 373386 12-19-16.JPG

- View 29112 Tgfr3lem1JMolDesApr2016.docx

- View 29112-Tgfr3lem1J.gcs

**Protocol Primers**

| Primer | 5' Label | Sequence 5' --> 3'         | 3' Label | Description       | Reaction |
|--------|----------|----------------------------|----------|-------------------|----------|
| 27969  | -        | CTC TGC AAA TGG CAC AAA AG | -        | Common            | A        |
| 27970  | -        | GGT GGT CTC TTT ACC CAT GC | -        | Wild type Reverse | A        |
| 27971  | -        | GGA GGT GGT GAG AAG CTG AC | -        | Mutant Reverse    | A        |

Number Of Reactions 1

| Reaction A (3 primer) |                     | Cycling (TouchDown 65-60_12-12-11) |         |       |  |
|-----------------------|---------------------|------------------------------------|---------|-------|--|
| Component             | Final Concentration | Step #                             | Temp °C | Time  | Note                                       |
| ddH2O                 |                     | 1                                  | 94      | 2 min | -  |
| Kapa 2G HS buffer     | 1.3 X               | 2                                  | 94      | 20sec | -  |
| MgCl2                 | 2.6 mM              | 3                                  | 65      | 15sec | -0.5 C per cycle decrease                  |
| dNTP KAPA             | .26 mM              | 4                                  | 68      | 10sec | -  |
| 27969                 | .5 uM               | 5                                  | -       | -     | repeat steps 2-4 for 10 cycles (Touchdown) |
|                       |                     | 6                                  | 94      | 15sec | -  |

| <b>Reaction A (3 primer)</b> |          | <b>Cycling (TouchDown 65-60_12-12-11)</b> |    |       |                                |
|------------------------------|----------|---|----|-------|--------------------------------|
| 27970                        | .5 uM    | 7   | 60 | 15sec | -                              |
| 27971                        | .5 uM    | 8   | 72 | 10sec | -                              |
| Glycerol                     | 6.5 %    | 9   | -  | -     | repeat steps 6-8 for 28 cycles |
| Dye                          | 1 X      | 10  | 72 | 2 min | -                              |
| Kapa 2G HS taq polymerase    | .03 U/ul | 11  | 10 | -     | hold                           |
| DNA                          |          |   |    |       |                                |

Version 3.2