



Welcome to The Genotyping Protocol System

Master Protocol

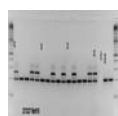
Strain Name: C57BL/6NJ-*Rnf10*^{em1J/J}
Stock Number: 027327
Allele: *Rnf10*^{em1J}
Protocol Name: *Rnf10*^{em1J}
Method: MELT
Created: 24-July -2015 (JKELMEN) **Updated:** 21-October -2015 (ESCHAAB)

Notes

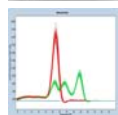
Notes: This allele from project *Rnf10*-6732J-M6624 was generated at The Jackson Laboratory by injecting Cas9 RNA and 3 guide sequences: GTGAATTGGAGCGACGGGAC, AGACACGGCCAATTTCTATC and GCACGCACTCACACTTTGAC, which resulted in a 302 bp deletion beginning in intron 2 at Chromosome 5 negative strand position 115,260,367 bp at GCCGTGTCTGGGAAAACATTTAA and ending after CCTGTCAAAGTGTGAGTGCGTG in intron 3 at 115,260,066 bp (GRCm38/mm10). This mutation deletes all of exon 2 and is predicted to cause amino acid sequence changes after 52 residues and early truncation 9 amino acid residues later.

Expected Results: Mutant = 261 bp
 Heterozygote = 201 bp and 261 bp
 Wild type = 201 bp

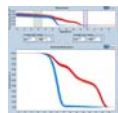
Attachments



View [Rnf-27327 Gel.jpg](#)



View [Rnf Mplex Melt.jpg](#)



View [Rnf HRM.JPG](#)

- View [Rnf10em1jMolDescrJuly2015.docx](#)

- View [Rnf10 genomic.gcc](#)

Protocol Primers

| Primer | 5' Label | Sequence 5' --> 3' | 3' Label | Description | Reaction |
|--------|----------|----------------------------|----------|-------------------|----------|
| 24387 | - | AGA GCA CCA GTG CTA TGG AC | - | Common | - |
| 24388 | - | TTC ACG CTT GCG ATT GTA AC | - | Wild type Reverse | - |
| 24389 | - | CTC CAG AGG GCA CAT GAA GT | - | Mutant Reverse | - |

Reaction/Components A

Cycling

| Reaction/Components A | | Cycling | | | |
|-----------------------|-------|---------|--------|------|------|
| Final | Total | Step | Temp°C | Time | Note |

| Reaction/Components A | | | | Cycling | | | |
|------------------------------------|------------|---------------|------------|---------|----|-------|--------------------------------|
| Reaction Components | Volume Amt | Concentration | Volume Amt | # | | | |
| ddH2O | 4.85 | - | - | 1 | 94 | 2 min | - |
| 5 X Kapa 2G HS buffer | 2.40 | 1 | - | 2 | 94 | 20sec | - |
| 25 mM MgCl2 | 0.96 | 2 | - | 3 | 65 | 15sec | -0.5 C per cycle decrease |
| 10 mM dNTP KAPA | 0.24 | .2 | - | 4 | 68 | 10sec | - |
| 20 uM 24387 | 0.30 | .5 | - | 5 | - | - | repeat steps 2-4 for 10 cycles |
| 20 uM 24388 | 0.30 | .5 | - | 6 | 94 | 15sec | - |
| 20 uM 24389 | 0.30 | .5 | - | 7 | 60 | 15sec | - |
| 2.5 U/ul Kapa 2G HS taq polymerase | 0.05 | .01 | - | 8 | 72 | 10sec | - |
| 20 X EvaGreen | 0.60 | 1 | - | 9 | - | - | repeat steps 6-8 for 28 cycles |
| DNA | 2.00 | - | - | 10 | 72 | 2 min | - |
| | | | | 11 | 10 | - | hold |

Number Of Reactions 1

Version 3.2