



## Welcome to The Genotyping Protocol System

## Master Protocol

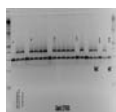
**Strain Name:** C57BL/6NJ-*Galnt12<sup>em1J/J</sup>*  
**Stock Number:** 027693  
**Allele:** Galnt12-6964-KOMP CRISPR  
**Protocol Name:** Galnt12-6964-KOMP CRISPR  
**Method:** Standard PCR  
**Created:** 23-November -2015 (DLINDNER) **Updated:** 23-November -2015 (ESCHAAB)

## Notes

Notes: This allele from project Galnt12-6964J-M4370 was generated at The Jackson Laboratory by injecting Cas9 RNA and 4 guide sequences: GCTTGCTCTGCCAAAGACGT, TGCTTGCTCTGCCAAAGACG, AAGCAAGGACAGATTCACCT, GGA ACTCTGTGATGGTACAA, which resulted in a 347 bp deletion beginning in intron 3 at Chromosome 4 positive strand position 47, 108,346 (GCCAAAGACGTGGGACAATGACC) and ending after (ACTCTGTGATGGTACAATG) at position 47,108,692 bp (GRCm38/mm10). This mutation deletes exon 3 and is predicted to cause an amino acid change after 175 amino acids and early truncation 15 residues later.

Expected Results: Mutant = 393 bp  
 Heterozygote = 300 bp and 393 bp  
 Wild type = 300 bp

## Attachments



View Galnt-27693 Gel.jpg

- View Galnt12 genomic1.gcs

- View Galnt12em1j\_molecular description\_9-19-15 in progress..docx

## Protocol Primers

| Primer | 5' Label | Sequence 5' --> 3'         | 3' Label | Description       | Reaction |
|--------|----------|----------------------------|----------|-------------------|----------|
| 25085  | -        | ATG TAT TTG GTG CCC TTG GA | -        | Common            | A        |
| 25086  | -        | CTC GTT GGC CAA GCG TTC    | -        | Wild type Reverse | A        |
| 25087  | -        | AAG TGA GCC GTG CTT GAA 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 | -  |
| 25087                 | .5 uM               | 5                                  | -      | -     | repeat steps 2-4 for 10 cycles (Touchdown) |
| 25085                 | .5 uM               | 6                                  | 94     | 15sec | -  |
| 25086                 | .5 uM               | 7                                  | 60     | 15sec | -  |

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

Version 3.2