



## Welcome to The Genotyping Protocol System

## Master Protocol

**Strain Name:** C57BL/6NJ-Rab11b<sup>em1J</sup>/J  
**Stock Number:** 029635  
**Allele:** Rab11b<sup>em1J</sup>  
**Protocol Name:** Rab11b<sup>em1J</sup>  
**Method:** Probe  
**Created:** 13-September-2016 (LUCASM) **Updated:** 15-November -2016 (ESCHAAB)

## Notes

Notes: Taqman qPCR protocols are run on a real time PCR instrument. Use an appropriate instrument specific Fluorophore/Quencher combination. The transgene genotype is determined by comparing  $\Delta C_t$  values of each unknown sample against known homozygous and hemizygous controls, using appropriate endogenous references.

Expected Results:

- Mutant= 93 bp
- Wild Type = 108 bp
- Fam=Mut
- Hex=Wt

## Attachments



View Rab11b-29635 EP.jpg

- View Rab11b genomic.gcs

- View Rab11bem1jin progress 8-29-16.docx

## Protocol Primers

| Primer | 5' Label | Sequence 5' --> 3'             | 3' Label              | Description       | Reaction |
|--------|----------|--------------------------------|-----------------------|-------------------|----------|
| 29679  | -        | CAC CTG TGC TGT TTC TGA AG     | -                     | Common            | A        |
| 29680  | -        | CAT ATC CAC CCA GCA AAC CT     | -                     | Wild type Reverse | A        |
| 29681  | -        | GCT AAG ACT GGG AGA AGA TGC    | -                     | Mutant Reverse    | A        |
| 29682  | Hex      | TGG TGG CTC CCT GAT TTC T      | Black Hole Quencher 1 | WT Probe          | -        |
| 29683  | 6-FAM    | CAG GAA TAG AAA AGC AGG TAG GC | Black Hole Quencher 1 | MUT Probe         | -        |

Number Of Reactions 1

| Reaction A (Endpoint 3 primer) |                     | Cycling (EndPoint) |        |       |                                |
|--------------------------------|---------------------|--------------------|--------|-------|--------------------------------|
| Component                      | Final Concentration | Step #             | Temp°C | Time  | Note                           |
| Kapa Probe Fast QPCR           | 1 X                 | 1                  | 95     | 3min  | -                              |
| ddH2O                          |                     | 2                  | 95     | 5sec  | -                              |
| 29680                          | .4 uM               | 3                  | 60     | 30sec | -                              |
| 29681                          | .4 uM               | 4                  | -      | -     | repeat steps 2-3 for 40 cycles |

| Reaction A (Endpoint 3 primer) |        | Cycling (EndPoint) |   |   |         |
|--------------------------------|--------|--------------------|---|---|---------|
| 29679                          | .4 uM  | 5                  | 4 | - | Forever |
| Wt Probe                       | .15 uM |                    |   |   |         |
| Mutant Probe                   | .15 uM |                    |   |   |         |
| DNA                            |        |                    |   |   |         |

Version 3.2