Print Logout

The Jackson Laboratory
Leading the search for tomorrow's cures

**Welcome to The Genotyping Protocol System**

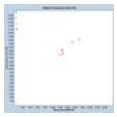
## Master Protocol

**Strain Name:** C57BL/6NJ-*Tusc1*$^{em1J}$/J
**Stock Number:** 029494
**Allele:** Tusc1$^{em1J}$
**Protocol Name:** Tusc1$^{em1J}$
**Method:** End Point Analysis
**Created:** 26-August -2016 (LUCASM) **Updated:** 23-November -2016 (JKELMEN)

## 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 ΔCt values of each unknown sample against known homozygous and hemizygous controls, using appropriate endogenous references. |
| Expected Results: | Mutant= 97 bp<br>Wild Type = 100 bp |

## Attachments



View Tusc1-29494 EP.jpg

- View Tusc1em1jMolDesJuly2016.docx
- View Tusc1 genomic.gcs
- View Tusc1 delete.gcs

## Sequence

Sequence

Wt Sequence:
GAGCCACCGCTCCAAGAAACTGCCACCGGCCTGTGCGCCCATGATCCGGACgttCCGAG
GCCCTGGCTGTAGTCCGAGGGCACGGAGGGAGGTGGGACCTGCGCGCG

Mutant
Sequence:GAGCCACCGCTCCAAGAAACTGCCACCGGCCTGTGCGCCCATGATCCGGACc
cgaggccctggctgtagtccgagggcacggagggaggtgggacctgcgcgcgc

## Protocol Primers

| Primer | 5' Label | Sequence 5' --> 3' | 3' Label | Description | Reaction |
|---|---|---|---|---|---|
| 29387 | - | CAC CGC TCC AAG AAA CTG C | - | Forward | A |
| 29388 | - | GCG CAG GTC CCA CCT C | - | Reverse | A |
| 29389 | Hex | ATC CGG ACG TTC CGA GG | Black Hole Quencher 1 | WT Probe | - |
| 29390 | 6-FAM | ATG ATC CGG ACC CGA GG | Black Hole Quencher 1 | MUT Probe | - |

Number Of Reactions 1

**Reaction A (Endpoint 2 primer)**

| Component | Final Concentration |
|---|---|
| Kapa Probe Fast QPCR | 1 X |
| ddH2O | |
| 29387 | .4 uM |
| 29388 | .4 uM |
| Wt Probe | .15 uM |
| Mutant Probe | .15 uM |
| DNA | |

**Cycling (EndPoint)**

| Step # | Temp°C | Time | Note |
|---|---|---|---|
| 1 | 95 | 3min | - |
| 2 | 95 | 5sec | - |
| 3 | 60 | 30sec | - |
| 4 | - | - | repeat steps 2-3 for 40 cycles |
| 5 | 4 | - | Forever |

Version 3.2