

## STOCK *Insm1*<sup>tm1.1Mgn</sup>/Mmjax

Stock No: 023899

Protocol 11255: Standard PCR Assay - *Insm1*<tm1.1Mgn>

Version 1.0

### Notes

The genotyping protocol(s) presented here have been optimized for reagents and conditions used by The Jackson Laboratory (JAX). To genotype animals, JAX recommends researchers validate the assay independently upon receipt of animals into their facility. Reaction cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

### Expected Results

Mutant = ~550 bp

Heterozygote = 447 bp and ~550 bp

Wild type = 447 bp

### JAX Protocol

#### Protocol Primers

| PRIMER | 5' LABEL | SEQUENCE 5' → 3'            | 3' LABEL | PRIMER TYPE | REACTION | NOTE |
|--------|----------|-----------------------------|----------|-------------|----------|------|
| 18544  |          | CCT TGT ACA ACC GAC AGC TCT |          | Forward     |          |      |
| 18545  |          | GTG CCC TGT ATC TGC TGT GC  |          | Reverse     |          |      |

#### Reaction A

| COMPONENT            | FINAL CONCENTRATION |
|----------------------|---------------------|
| Kapa Probe Fast QPCR | 0.42                |
| ddH <sub>2</sub> O   |                     |
| Primer 1             | 0.03                |
| Primer 2             | 0.03                |
| Primer 3             | 0.02                |
| Primer 4             | 0.02                |
| DNA                  |                     |

#### Cycling

| STEP | TEMP °C | TIME | NOTE                           |
|------|---------|------|--------------------------------|
| 1    | 94.0    | --   |                                |
| 2    | 94.0    | --   |                                |
| 3    | 65.0    | --   | -0.5 C per cycle decrease      |
| 4    | 68.0    | --   |                                |
| 5    |         | --   | repeat steps 2-4 for 10 cycles |
| 6    | 94.0    | --   |                                |
| 7    | 60.0    | --   |                                |
| 8    | 72.0    | --   |                                |
| 9    |         | --   | repeat steps 6-8 for 28 cycles |
| 10   | 72.0    | --   |                                |
| 11   | 10.0    | --   | hold                           |

JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.

