



## Welcome to The Genotyping Protocol System

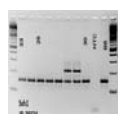
## Master Protocol

**Strain Name:** C57BL/6NJ-Ssh1<sup>em1J</sup>/<sub>J</sub>  
**Stock Number:** 029324  
**Allele:** Ssh1<sup>em1J</sup>  
**Protocol Name:** Ssh1<sup>em1J</sup>  
**Method:** MELT  
**Created:** 13-June -2016 (JKELMEN) **Updated:** 06-October -2016 (ESCHAAB)

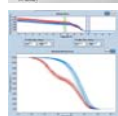
## Notes

Expected Results: Mutant = 215 bp  
Heterozygote = 145 bp and 215 bp  
Wild type = 145 bp

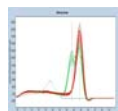
## Attachments



View 29324 ssh1.jpg



View ssh1 HRM JW.JPG



View ssh1 MCA JW.jpg

- View Ssh1em1jMolDesJun2016.docx
- View Ssh1 genomic.gcs

## Protocol Primers

| Primer | 5' Label | Sequence 5' --> 3'          | 3' Label | Description       | Reaction |
|--------|----------|-----------------------------|----------|-------------------|----------|
| 28483  | -        | GAG CGA GAG TGA CTC AGA GGA | -        | Common            | A        |
| 28484  | -        | AGC CGC CAC TAT TAG CTC TG  | -        | Wild type Reverse | A        |
| 28485  | -        | CCG GAA CGT GCT ATG TAG AG  | -        | 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 | -                                          |
| 28483                 | .5 uM               | 5                                  | -      | -     | repeat steps 2-4 for 10 cycles (Touchdown) |
|                       |                     | 6                                  | 94     | 15sec | -                                          |

| Reaction A (3 primer)     |          | Cycling (TouchDown 65-60_12-12-11) |    |       |                                |
|---------------------------|----------|------------------------------------|----|-------|--------------------------------|
| 28484                     | .5 uM    | 7                                  | 60 | 15sec | -                              |
| 28485                     | .5 uM    | 8                                  | 72 | 10sec | -                              |
| Glycerol                  | 6.5 %    | 9                                  | -  | -     | repeat steps 6-8 for 28 cycles |
| Dye                       | 1 X      | 10                                 | 72 | 2 min | -                              |
| Kapa 2G HS taq polymerase | .03 U/ul | 11                                 | 10 | -     | hold                           |
| DNA                       |          |                                    |    |       |                                |

Version 3.2