



## Welcome to The Genotyping Protocol System

## Master Protocol

**Strain Name:** C57BL/6NJ-Ttll10<sup>em1J</sup>/<sub>J</sub>  
**Stock Number:** 026909  
**Allele:** Ttll10<sup>em1J</sup>  
**Protocol Name:** Ttll10<sup>em1J</sup>  
**Method:** Sanger sequencing  
**Created:** 30-December -2014 (JKELMEN) **Updated:** 29-January -2015 (JKELMEN)

## Sequence

Sequence SEQ

## Protocol Primers

| Primer | 5' Label | Sequence 5' --> 3'            | 3' Label | Description | Reaction |
|--------|----------|-------------------------------|----------|-------------|----------|
| 22348  | -        | CCA GCA ACA AGA CTG ATA GGC   | -        | Forward     | -        |
| 22349  | -        | ATG TAG TCC CTA TAC CCC TTC G | -        | Reverse     | -        |

## Reaction/Components A

| Reaction Components                | Volume Amt | Final Concentration | Total Volume Amt |
|------------------------------------|------------|---------------------|------------------|
| ddH2O                              | 42.55      | -                   | -                |
| 5 X Kapa 2G HS buffer              | 2.40       | .24                 | -                |
| 25 mM MgCl2                        | 0.96       | .48                 | -                |
| 10 mM dNTPS-kapa                   | 0.24       | .05                 | -                |
| 20 U Primer 1                      | 0.30       | .12                 | -                |
| 20 U Primer 2                      | 0.30       | .12                 | -                |
| 50 % Glycerol                      | 1.20       | 1.2                 | -                |
| 2.5 U/ul Kapa 2G HS taq polymerase | 0.05       | 0                   | -                |
| DNA                                | 2.00       | -                   | -                |

## Cycling

| Step # | Temp°C | Time  | Note                           |
|--------|--------|-------|--------------------------------|
| 1      | 94     | 2 min | -                              |
| 2      | 94     | 20sec | -                              |
| 3      | 65     | 15sec | -0.5 C per cycle decrease      |
| 4      | 68     | 10sec | -                              |
| 5      | -      | -     | repeat steps 2-4 for 10 cycles |
| 6      | 94     | 15sec | -                              |
| 7      | 60     | 15sec | -                              |
| 8      | 72     | 10sec | -                              |
| 9      | -      | -     | repeat steps 6-8 for 28 cycles |
| 10     | 72     | 2 min | -                              |
| 11     | 10     | -     | hold                           |

Number Of Reactions 1

Version 3.2