**EMMA ID:** *EM:16839*
**Gene:** *Tek*
**Common name:** *Tektm1.1(icre/Esr1*)Hrr (Tie2-MicM)*
**Allele:** *Tek* $^{tm1.1(icre/Esr1*)Hrr}$

## Allele Information

The Tamoxifen-inducible Mer-iCre-Mer was introduced by homologous recombination in ES cells into the first exon of the Tie2 gene.
The targeting cassette inserted into exon 1 upstream of the translation start site comprises a rabbit HBB2 splice donor, intron and splice acceptor; an inducible improved cre fused to two modified Esr1 binding domains (icre/Esr1*); and an FRT-flanked neomycin resistance cassette. Flp-mediated recombination removed the selection cassette. (J:219867)

## Genotyping Information

Genotyping by end-point PCR based on gel is composed of a genespecific short range PCR using primers on wild type allele and a mutant allele-specific short range PCR. The combined results show the genotype of the mice. For example: mutant positive, wild type positive = Heterozygous. In addition to the expected product, the mutant assay may also amplify the endogenous wild type sequence, which will appear as a larger band on an agarose gel. The presence of this extra band will depend on the size of the original deletion.

**PCR primer pairs and expected size bands**

| Assay | Forward Primer | Reverse Primer | Expected Size Band (bp) |
|---|---|---|---|
| Wild type | Tie2_wtF | Tie2_wtR | 746 |
| Mutant | Tie2_wtF | Tie2_KI_R | 527 |

**Primer sequences**

| Primer Name | Sequence 5' --> 3' |
|---|---|
| Tie2_KI_R | AAT CAA GGG TCC CCA AAC TCA C |
| Tie2_wtF | CAT CGC ATA CCA TAC ATA GGT GGA GG |
| Tie2_wtR | GAG GCA GCA TCT GTC TAC AAG AGA TGG |

**PCR setup (AllTaq)**

| Component | Volume (µl) 1x | Final conc. |
|---|---|---|
| DNA (~ 50-100 ng) | 2 | |
| Q-Solution (5x) | 2,5 | 0,5 |
| PCR-Buffer (5x) | 5 | 1 |
| DNTP mix (10 mM) | 0,5 | 0,2 |
| $MgCl_2$ (25 mM) | 1,5 | 1,5 |
| Primer 1 (10 pmol/µl) | 1 | 0,4 |
| Primer 2 (10 pmol/µl) | 1 | 0,4 |
| Primer 3 (10 pmol/µl) | 1 | 0,4 |
| Taq Polymerase (5 U/µl) | 0,5 | 2,5 U/rxn |
| $H_2O$* | 10 | |
| Final volume | 25 | |

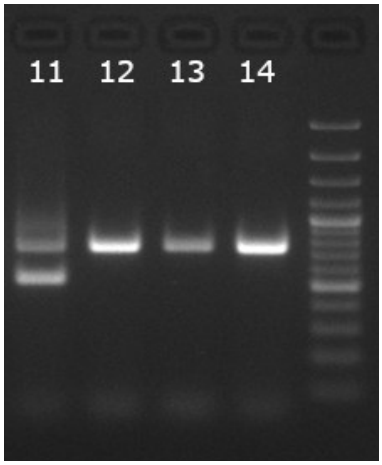
*** The amount of $H_2O$ is adjusted with the number of primer.**

**Amplification conditions**

| PCR Settings | Temperature (°C) | Time | # of cyles |
|---|---|---|---|
| 1 Denaturation (Melting) | 95°C | 5 min | 1 |
| 2 Amplification (Melting, Annealing, Polym.) | 94°C<br>60°C<br>72°C | 30 sec<br>45 sec<br>45 sec | 39 |
| 3 Polymerisation | 72°C | 10 min | 1 |
| 4 Cooling | 4°C | hold | 1 |

Touch-Down cycling protocol: first 10 cycles anneal at 65°C, decreasing 1°C per cycle, next 30 cycles aneal at 55°C.These PCR conditions have been optimized for our methods and preparation

**Gel Image**




**11 het**
**12,13,14 wt**

Separated by gel electrophoresis on a 2% agarose gel.