

EMMA ID: 00196

Gene: *Ceacam10*

Common name: *Ceacam 10 Ko*

Allele: *Ceacam10*^{tm1Wzm}

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.

PCR primer pairs and expected size bands

| Assay | Forward Primer | Reverse Primer | Expected Size Band (bp) |
|----------|----------------|----------------|-------------------------|
| Wildtype | Ceacam10-f | Ceacam10-r | 350 |
| Mutant | Ceacam10-Neo | Ceacam10-N1 | 197 |

Primer sequences

| Primer Name | Sequence 5' --> 3' |
|--------------|------------------------|
| Ceacam10-f | TTAGCCTCACTTTTAACTTAC |
| Ceacam10-r | GCACAGAAATCGGAGTAATT |
| Ceacam10-N1 | TTGTAACATCCATTAATAGGAG |
| Ceacam10-Neo | GAATGTGTGCGAGGCCAG |

PCR setup (Qiagen, Hot Start Plus)

| Component | Volume (µl) 1x | Final conc. |
|---------------------------|----------------|-------------|
| DNA (~ 50-100 ng) | 2 | |
| Q-Solution (5x) | 2,5 | 0,5 |
| PCR-Buffer (10x) | 2,5 | 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 |
| Taq Polymerase (5 U/µl) | 0,3 | 0,06 |
| H ₂ O* | 13,7 | |
| Final volume | 25 | |

* The amount of H₂O is adjusted with the number of primer.

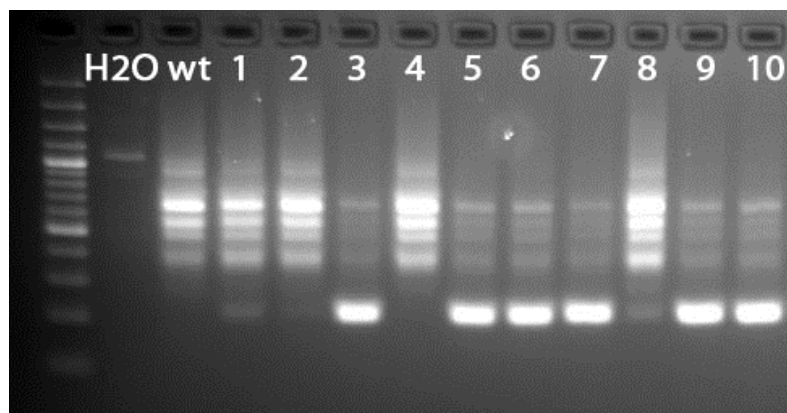
Amplification conditions

| PCR Settings | Temperature (°C) | Time | # of cycles |
|--|------------------|--------|-------------|
| 1 Denaturation (Melting) | 95°C | 5 min | 1 |
| 2 Amplification (Melting, Annealing, Polym.) | 94°C | 30 sec | 39 |
| | 62°C | 45 sec | |
| | 72°C | 45 sec | |
| 3 Polymerisation | 72°C | 10 min | 1 |
| 4 Cooling | 12°C | hold | 1 |

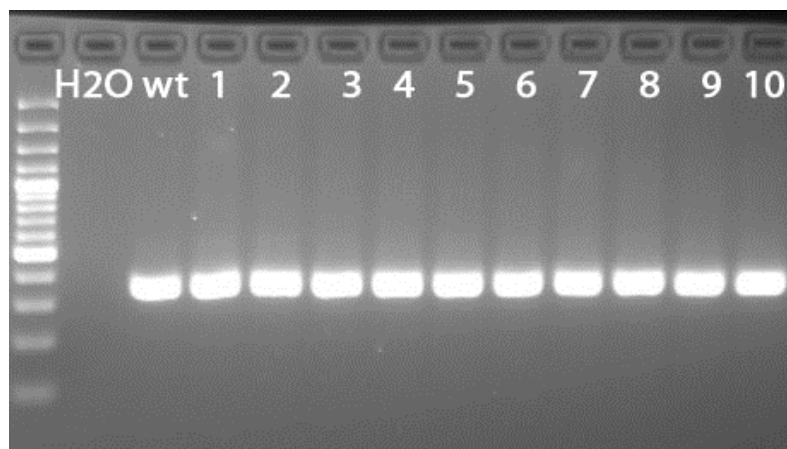
These PCR conditions have been optimized for our methods and preparation kits. Adaptions may be required.

Gel Image

Mutant-PCR



WT-PCR



Separated by gel electrophoresis on a 2% agarose gel.
 ko/wt separate