**EMMA ID:** *00050*
**Gene:** *Ceacam9*
**Common name:** *Ceacam9 KO*
**Allele:** *Ceacam9* $^{tm1Wzm}$

## 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 | WT up | WT down | 349 |
| Mutant | ceacam9KO up | ceacam9KO down | 560 |

**Primer sequences**

| Primer Name | Sequence 5' --> 3' |
|---|---|
| ceacam9KO up | ATGCGGCGGCTGCATACGCTTGATCC |
| ceacam9KO down | CGTCAAGAAGGCGATAGAAGGCGATGC |
| WT up | CTTAACCTGCTGGAATGCACCCGCCG |
| WT down | GCACTTCCAGATGCACATGTGTTAATTCG |

**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 |
| $H_2O$* | 13,7 | |
| Final volume | 25 | |

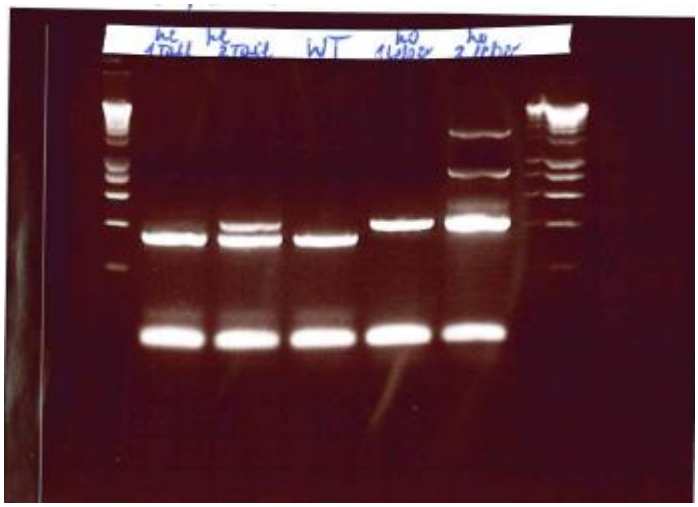
*** The amount of $H_2O$ is adjusted with the number of primer.**

**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 | 12°C | hold | 1 |

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

**Gel Image**




Separated by gel electrophoresis on a 2% agarose gel.