

**EMMA ID:** 05138

**Gene:** *Cryba2*

**Common name:** *Aca30*

**Allele:** *Cryba2*<sup>*Aca30*</sup>

## 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.

**Cut PCR-products with BseNI enzyme: Take 10µl of PCR-Product and incubate at 37°C over night**

### PCR primer pairs and expected size bands

Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Wildtype	Aca30-L1	Aca30-R1	320
Mutant	same as wt	same as wt	242 + 147

### Primer sequences

Primer Name	Sequence 5' --> 3'
Aca30-L1	CAGGCTTAGGCTAAGTAGAGTGTCC
Aca30-R1	GCACTGGAGTGAAGTTGAAGTCC

### PCR setup (Qiagen, Hot Start Plus)

Component	Volume ( $\mu$ l) 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 <sub>2</sub> (25 mM)	1,5	1,5
Primer 1 (10 pmol/ $\mu$ l)	1	0,4
Primer 2 (10 pmol/ $\mu$ l)	1	0,4
Taq Polymerase (5 U/ $\mu$ l)	0,3	0,06
H <sub>2</sub> O*	13,7	
Final volume	25	

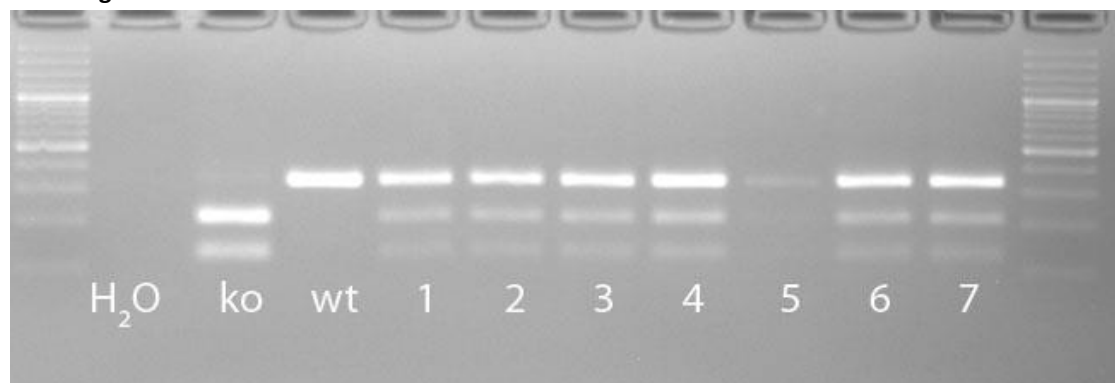
\* The amount of H<sub>2</sub>O is adjusted with the number of primer.

### Amplification conditions

PCR Settings	Temperature ( $^{\circ}$ C)	Time	# of cycles
1 Denaturation (Melting)	95 $^{\circ}$ C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94 $^{\circ}$ C	30 sec	39
	65 $^{\circ}$ C	45 sec	
	72 $^{\circ}$ C	45 sec	
3 Polymerisation	72 $^{\circ}$ C	10 min	1
4 Cooling	12 $^{\circ}$ 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.