EMMA ID: *02273*
Gene: *Cnr2, Cnr1*
Common name: *Cnr1 tm1zim/ Cnr2 tm2zim*
Allele: *Cnr1* ^{tm1Zim} *Cnr2* ^{tm1Zim}

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.

Combination of Cnr1 tm1zim and Cnr2 tm2zim protocols. 4 different aproaches/PCRs are needed (Cnr1 WT / KO separate, Cnr2 WT / KO separate)

PCR primer pairs and expected size bands

Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Cnr1 Wildtype	CB1C	CB1WT	284
Cnr1 Mutant	CB1C	CB1KO	334
Cnr2 Wildtype	mCB2	CB2wt2	1100
Cnr2 Mutant	mCB2	pPNT	850

Primer sequences

Primer Name	Sequence 5' --> 3'
CB1C	CTCCTGGCACCTCTTTCTCAGTCACG
CB1WT	TGTGTCTCCTGCTGGAACCAACGG
CB1KO	TCTCTCGTGGGATCATTGTTTTTCTCTTGA
mCB2	AAATGCTTGATTGGTGTCAGCCTCTC
pPNT	TAAAGCGCATGCTCCAGACTGCCTT
CB2wt2	GGCTCCTAGGTGGTTTTCACATCAGCCTCT

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	

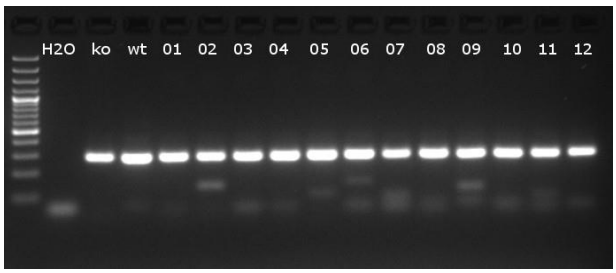
*** The amount of H_2O is adjusted with the number of primer.**

Amplification conditions Cnr1

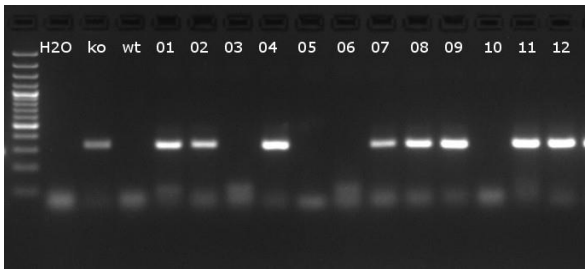
PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 65°C 72°C	30 sec 45 sec 45 sec	39
3 Polymerisation	72°C	10 min	1
4 Cooling	4°C	hold	1

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

Gel Image



WT-PCR



Mutant-PCR

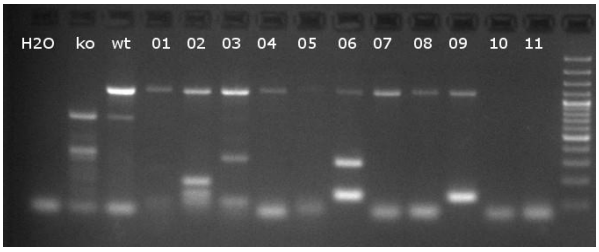
Separated by gel electrophoresis on a 2% agarose gel.

Amplification conditions Cnr2

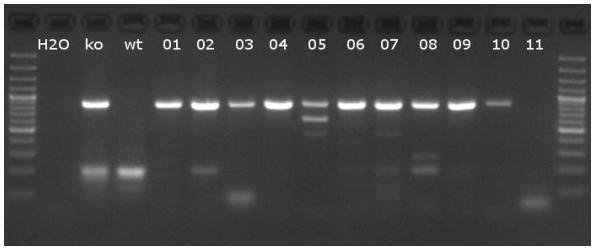
PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 60°C 72°C	30 sec 45 sec 45 sec	39
3 Polymerisation	72°C	10 min	1
4 Cooling	4°C	hold	1

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

Gel Image



WT-PCR



Mutant-PCR

Separated by gel electrophoresis on a 2% agarose gel.