EMMA ID: *EM:16832*
Gene: *Cpa3*
Common name: *B6-Cpa3tm3(icre)Hrr*
Allele: *Cpa3* $^{tm3(icre)Hrr}$

Allele Information

In the first exon of the Cpa3 gene, the 28 nucleotides coding for the first 9 amino acids were replaced with a codon-improved cre recombinase expression cassette and a Frt-flanked neomycin selection cassette. The cre recombinase expression cassette consists of a splice donor, intron, splice acceptor from rabbit beta-globin and the codon-improved cre (Shimshek et al. 2002) followed by the rabbit beta-globin polyadenylation site. Transient expression of flp recombinase in a clone of correctly targeted ES cells removed the selection cassette.

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	Cpa3_wtF	Cpa3_wtR	328
Mutant	Cpa3_wtF	Cpa3_KI_R	503

Primer sequences

Primer Name	Sequence 5' --> 3'
Cpa3_KI_R	GTC CGG ACA CGC TGA ACT TG
Cpa3_wtF	GGA CTG TTC ATC CCC AGG AAC C
Cpa3_wtR	CTG GCG TGC TTT TCA TTC 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	

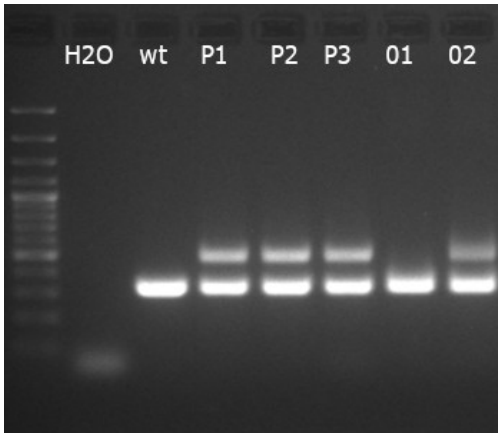
*** The amount of H_2O is adjusted with the number of primer.**

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 62°C 72°C	30 sec 45 sec 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



P1-P3 het positive control
1 wt
2 het

Separated by gel electrophoresis on a 2% agarose gel.