EMMA ID: *EM:14978*
Gene: Crybb1
Common name: *Crybb1_Asp167_ENU*
Allele: Crybb1^{m1Mhda}

Allele Information

ENU-induced mutation in Crybb1 gene: c500A>T; pAsp167Val.

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	Crybb1-Ex5-R1	Crybb1-Ex5-L1	400
Mutant	Crybb1-Ex5-R1	Crybb1-Ex5-L1	400

Sequencing of the PCR band

Primer sequences

Primer Name	Sequence 5' --> 3'
Crybb1-Ex5-R1	TGATGTGACCGTCCACC
Crybb1-Ex5-L1	TGTTACTGCAGGGTGATACC

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
Taq Polymerase (5 U/µl)	0,5	2,5 U/rxn
H_2O*	11	
Final volume	25	

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