EMMA ID: *05691*
Gene: *Diaph2*
Common name: *Diap2 (diaphanous homolog 2 (Drosophila))*
Allele: *Diaph2* $^{Gt(A011B06)Wrst}$

Allele Information

The Diaph2 gene is X-chromosome-linked. Depending on the parental genotype the male offspring can only be wildtype or hemizygous and the female offspring can be wildtype, hetero-, or homozygous.

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	Diap2 Diap10 U1338	Diap2 Diap13 L1635	297
Mutant	Diap2 Diap10 U1338	Diap2 RB1 L1533	457

Primer sequences

Primer Name	Sequence 5' --> 3'
Diap2 Diap10 U1338	TCCCTTCCCTTGCATCACTG
Diap2 Diap13 L1635	TGCCCCTTTCCCTGAGTTC
Diap2 RB1 L1533	GTGCCCACTGACCAGAAG

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	

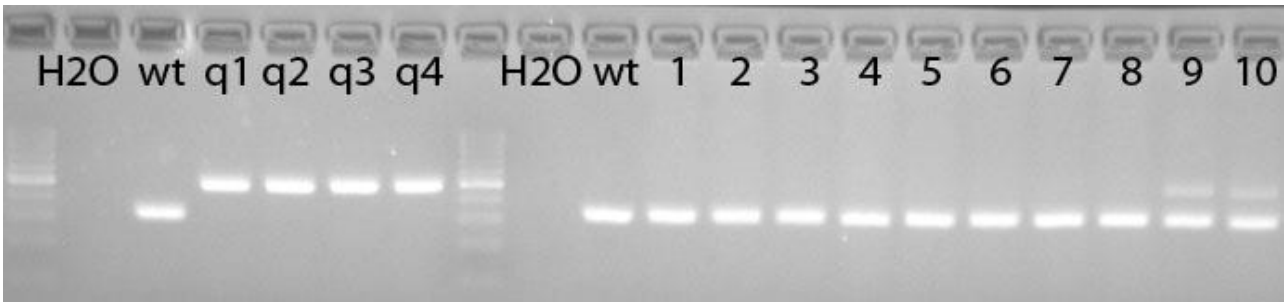
*** The amount of H_2O is adjusted with the number of primer.**

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 59°C 72°C	30 sec 45 sec 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.

> q1 - q4 = fathers (hemizygous males)
> 1 - 10 = offspring (males are wildtype, females are heterozygous)