

EMMA ID: *EM:13665*
Gene: *Pax6*
Common name: *Aey11*
Allele: *Pax6*^{*Mhdaaey11*}

Allele Information

C/T mutation in exon 8 creating a stop codon in front of the homeodomain.
Single point mutation. Inheritance: dominant

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)
Mutant	pax6-Ex8 L1	pax6-Ex8 R1	242

Primer sequences

Primer Name	Sequence 5' --> 3'
pax6-Ex8 L1	GCCGTTCCGATTTCTTATTGTCC
pax6-Ex8 R1	AGGTTTCTCTGCTTTGGAAATTCC

HindIII digestion or sequencing of the PCR product

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 ₂ O*		11	
Final volume		25	

* The amount of H₂O is adjusted with the number of primer.

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cycles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C	30 sec	40
	62°C	45 sec	
	72°C	45 sec	
3 Polymerisation	72°C	10 min	1
4 Cooling	4°C	hold	1

Gel Image

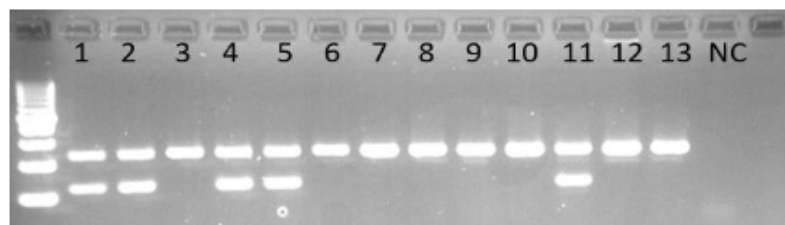
- **HindIII digestion:**

- 8ul PCR product
- 2ul Buffer from HindIII
- 1ul HindIII

Incubate samples at 37°C for at least 1 hour (longer time or ON incubation gives better results)

Run 10ul from each sample on a [3% agarose gel](#)

Example of genotyping results:



Samples 1, 2, 4, 5 and 11 show heterozygous typical bands; rest are wild type samples; NC is the negative control.

run on a 3% Agarose gel