

EMMA ID: 05694

Gene: *Vdac1*

Common name: *Vdac1 (voltage-dependent anion channel 1)*

Allele: *Vdac1*^{Gt(A023F03)Wrst}

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	Vdac1 CD26	Vdac1 CD31	595
Mutant	Vdac1 FV104	Vdac1 FV105	460

Primer sequences

Primer Name	Sequence 5' --> 3'
Vdac1 CD26	GGGTCCAGGCTCTATGAG
Vdac1 CD31	GATGGGCATTTACTCTAACAC
Vdac1 FV104	CGCGTTCGGTTGCACTAC
Vdac1 FV105	TGGCGGTTTCGCTAAATACT

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 ₂ O*	13,7	
Final volume	25	

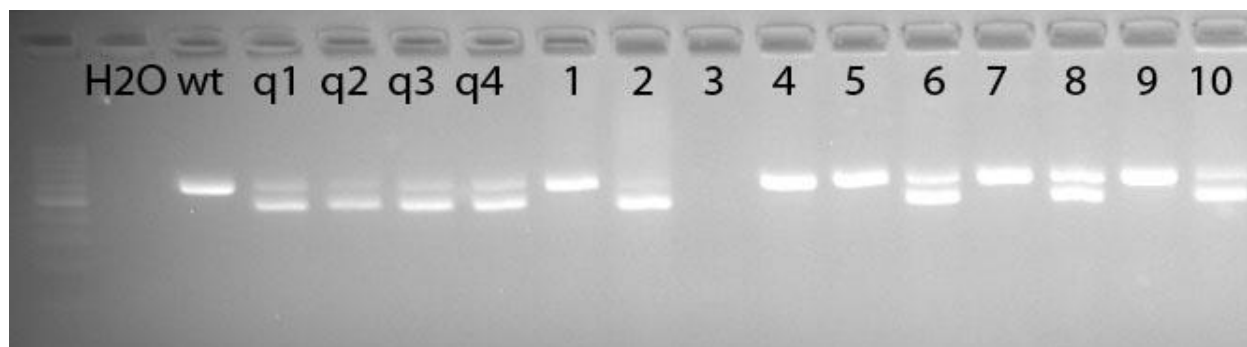
* The amount of H₂O is adjusted with the number of primer.

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	39
	59°C	45 sec	
	72°C	45 sec	
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



Separated by gel electrophoresis on a 2% agarose gel.