EMMA ID: *EM:15088*
Gene: *Vti1b, Vti1a*
Common name: *Vti1a-/- Vti1b-/-*
Allele: *Vti1b* ^{tm1Gfvm} *,Vti1b* ^{tm1Gfvm}

Allele Information

A neomycine resistant cassette has been inserted into exon 4 of Vti1b encoding amino acid residues 123-180 in ES cells by homologous recombination.
A neomycine resistant cassette has been inserted into exon 6 of Vti1a encoding amino acid residues 115-142 in ES cells by homologous recombination.

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 Vti1b	Vti1b seq6	Vti1 AK32 (Vit1b AK32)	510
Mutant Vti1b	Vti1b seq6	Vti1b seq7	410
Wild type Vti1a	Vti1a SalC	Vti1 AK32 (Vit1b AK32)	370
Mutant Vti1a	Vti1a SalC	Vti1a AXhoA	530

Primer sequences

Primer Name	Sequence 5' --> 3'
Vti1b seq6	ATACCTTCTCCTGGCATCCATG
Vti1b seq7	TACCAATTTGATCAGTTTCTGTGG
Vti1 AK32 (Vit1b AK32)	CCTGCGTGCAATCCATCTTG
Vti1a SalC	GCAAATAAGCTCCCAATTGTCTC
Vti1a AXhoA	ATG AAC TGT CTG CTA AAA TTT AAC

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.**

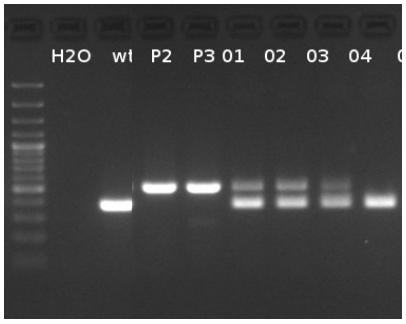
Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 52°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

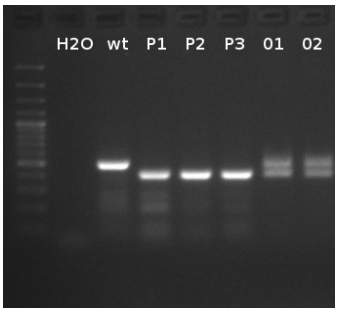
For each mutant line (Vti1b or Vti1a) use a separate PCR.

Vti1a



P2,P3 positiv control hom
01,02,03 het
4 wt

Vti1b



P1,P2,P3 positiv control hom
01,02 het