EMMA ID: *15641*
Gene: *Nos1*
Common name: *AGT1*
Allele: *Nos1* *tm1.1(cre/ERT2)Geno/RjIeg*

Allele Information

A targeting vector was designed to insert IRES.Cre-ERT2 in frame into exon 33 of the neuronal allele. nitric oxide (Nos1) gene, followed by a frt- flanked neomycin resistance cassette. Offspring was bred with C57BL/6 deleter mice to excise the neo cassette and to generate heterozygous mice carrying the knock-in

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	175965cof-AGT1	175969cof-AGT1	816 bp
Mutant	175965cof-AGT1	175967hom-AGT1	441 bp

Primer sequences

Primer Name	Sequence 5' --> 3'
175965cof-AGT1	CATCGCAGCCCAAGAGTCTAGGAGA
175967hom-AGT1	CCCCTAATCCAAAAGTCACAGGGGA
175969cof-AGT1	CCACATCAGGCACATGAGTAACAAAGG

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 60°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