EMMA ID: *EM:13608*
Gene: *Arsg*
Common name: *Arsgtm1Tdi*
Allele: *Arsg* ^{tm1Tdi}

Allele Information

Exon 2 was disrupted by insertion of a neo cassette.
RT-PCR confirmed the absence of mRNA expression in homozygous mice.

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	Arsg_F	Arsg_R	200
Mutant	Arsg_F	Arsg_R	1300

Primer sequences

Primer Name	Sequence 5' --> 3'	0
Arsg_F	GTTTGTAGACTTCCACGCAGC	
Arsg_R	AGGGCACGTTACCTATCATG	

PCR setup (Biotaq)

Component	Volume (µl) 1x
DNA (~ 50-100 ng)	1
PCR-Buffer (10x)	5
DNTP mix (10 mM)	1
Primer 1 (10 pmol/µl)	1
Primer 2 (10 pmol/µl)	1
Biotaq DNA Polymerase	0,5
H_2O*	40,5
Final volume	50

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

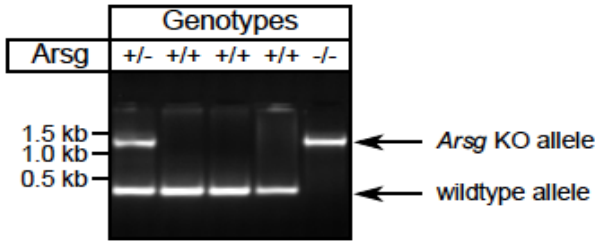
Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	94°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 58°C 72°C	30 sec 30 sec 3 min	40
3 Polymerisation	72°C	3 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

Gel Image

In *Arsg* KO mice, the amplicon size is increased to 1.3 kB due to the presence of the neomycin cassette, which disrupts *Arsg* exon 2. PCR reaction batch and program are displayed in table 4.5.



Separated by gel electrophoresis on a 2% agarose gel.