EMMA ID: *EM:15605*
Gene: *Gt(ROSA)26Sor, Cdh23*
Common name: *Cdh23Ahl+-AOX-Rosa26*
Allele: *Gt(ROSA)26Sor $^{tm1.1(CAG-AOX)Htj}$, Cdh23Ahl+*

Allele Information

An expression cassette for alternative oxidase (AOX) from Ciona intestinalis targeting the mouse Gt(ROSA)26Sor locus was introduced into the genome of v6.5 mouse embryonic stem cells by homologous recombination. AOX expression is controlled by a CAG promoter (MGI:5824337allele ID). It is recommended to use B6.CAST-Cdh23Ahl+/Kjn (available from The Jackson Laboratory) as oocyte donors for rederivation and afterwards produce homozygous AOX knock-in mice by intercrossing. In case the rederivation is done in the C57BL/6J background, the recipient will need to produce homozygous mice (i.e. homzoygous for AOX knock-in and for B6.CAST-Cdh23Ahl+ background), by appropriate crossing within the F1

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	Rosa26-F	Aox-F	532 bp
Mutant	Rosa26-F	Rosa26-R	317 bp

Primer sequences

Primer Name	Sequence 5' --> 3'
Rosa26-F	GACCTCCATCGCGCACTCCG
Rosa26-R	CTCCGAGGCGGATCACAAGC
Aox-F	GCGATGCAAGATGGAGGGTA

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	

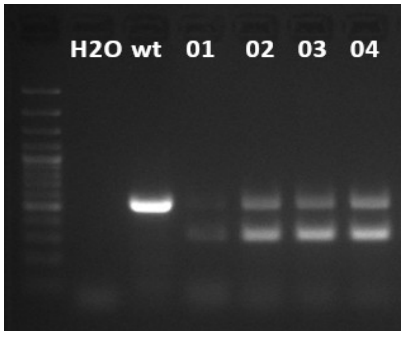
*** The amount of H_2O is adjusted with the number of primer.**

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

Gel Image



1/2/3/4 **het**

Separated by gel electrophoresis on a 2% agarose gel.