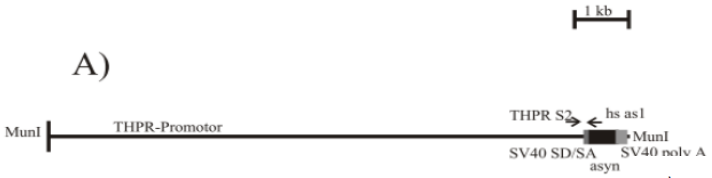
EMMA ID: *EM:14609*
Gene: *Th-Snca*
Common name: *Th-Snca15Hlu*
Allele: *Tg(Prnp-SNCA*)11Ccs*

Allele Information



A) Transgenic construct,
THPR: mouse tyrosine hydroxylase promoter
asyn: human α-Synuclein

Th-Snca transgenic mice express the human α-synuclein gene with two missense mutations (A53T and A30P) under the direction of the mouse tyrosine hydroxylase promoter

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)
Mutant	hsas1	THPR S2	450

Primer sequences

Primer Name	Sequence 5' --> 3'
hsas1	GAATTCCTTCCTGTGGGGCTCC
THPR S2	AGAACTGCTCCTCAGTGGATGT

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_2O*	13,7	
Final volume	25	

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

Amplification conditions

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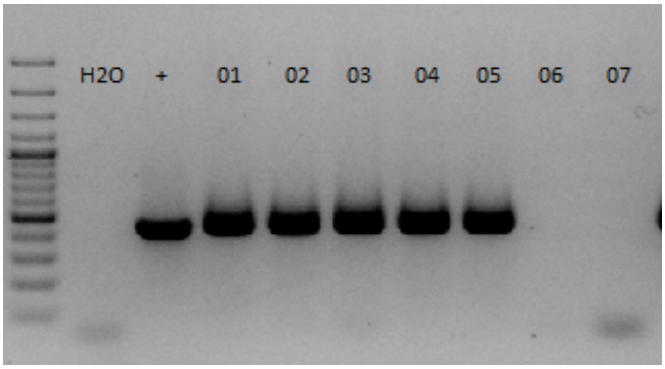
PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 55°C 72°C	30 sec 45 sec 45 sec	39
3 Polymerisation	72°C	10 min	1
4 Cooling	4°C	hold	1

Gel Image



PCR product of 450bp shows the positive transgene

Separated by gel electrophoresis on a 2% agarose gel.