

EMMA ID: *EM:15254*

Gene: *Grn*

Common name: *Gt(ROSA)26Sortm1.1(Ubc-Grn)lfe*

Allele: *Gt(ROSA)26Sor^{tm1.1(Ubc-Grn)lfe}*

Allele Information

A ubiquitin C promoter, a floxed stop cassette, mouse progranulin cDNA, and FRT flanked pgk-neo cassette were inserted via homologous recombination into the *Gt(ROSA)26Sor* locus; flp-mediated recombination removed the neo cassette.

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	Grn_wtF (Zug1)	Grn_wtR (Zug2)	747
Mutant	Grn_F (Zug4)	Grn_R (Zug5)	425

Primer sequences

Primer Name	Sequence 5' --> 3'
Grn_wtF (Zug1)	CGTGTTTCGTGCAAGTTGAGT
Grn_wtR (Zug2)	GGCGGATCACAAGCAATAAT
Grn_F (Zug4)	GGATCTGACATGGTAAGTAAGC
Grn_R (Zug5)	GCCATCACCACAAGACACAC

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 ₂ O*	10	
Final volume	25	

* The amount of H₂O is adjusted with the number of primer.

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cycles
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 anneal at 55°C. These PCR conditions have been optimized for our methods and preparation