

EMMA ID: 02112

Gene: *Rosa26Sor*

Common name: *Rosa-tdRFP*

Allele: *Gt(ROSA)26Sor^{tm1Hjf}*

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.

PCR primer pairs and expected size bands

Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Wildtype	HL152	HL54	209
Mutant	HL152	HL15	301

Primer sequences

Primer Name	Sequence 5' --> 3'
HL152	5' AAG GGA GCT GCA GTG GAG TA 3'
HL54	5' TAA GCC TGC CCA GAA GAC TCC 3'
HL15	5'AAGACCGCGAAGAGTTGTCC3'

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 ₂ O*	13,7	
Final volume	25	

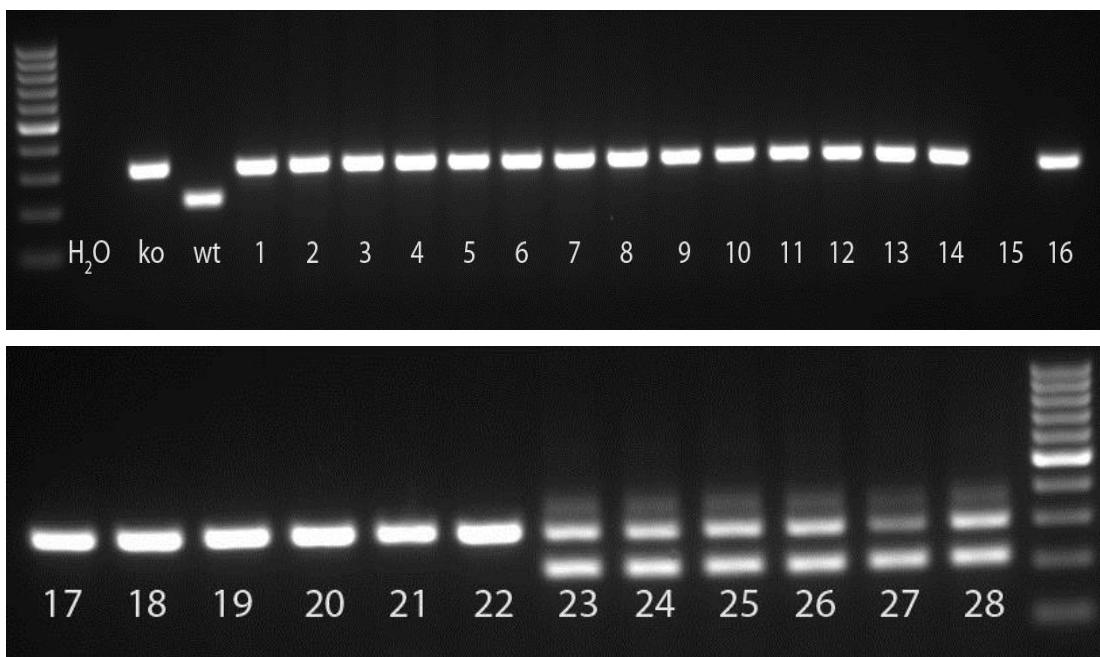
* The amount of H₂O is adjusted with the number of primer.

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cycles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 62°C 72°C	30 sec 45 sec 45 sec	39
3 Polymerisation	72°C	10 min	1
4 Cooling	12°C	hold	1

These PCR conditions have been optimized for our methods and preparation kits. Adoptions may be required.

Gel Image



Separated by gel electrophoresis on a 2% agarose gel.