

**EMMA ID:** 05697

**Gene:** *Trmt1*

**Common name:** *Trmt1 (TRM1 tRNA methyltransferase 1 homolog (S. cerevisiae))*

## 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	Trmt1 wt FV84 U184	Trmt1 wt FV85 L753	569
Mutant	Trmt1 wt FV84 U184	PT1betageo RB8 L81	350

### Primer sequences

Primer Name	Sequence 5' --> 3'
Trmt1 wt FV84 U184	TCCAGGAGGCATATCTTTTA
Trmt1 wt FV85 L753	TAAAAGGGCAGCAGTTGTA
PT1betageo RB8 L81	CAACCTCCGCAAACCTCCT

### 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 <sub>2</sub> (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 <sub>2</sub> O*	13,7	
Final volume	25	

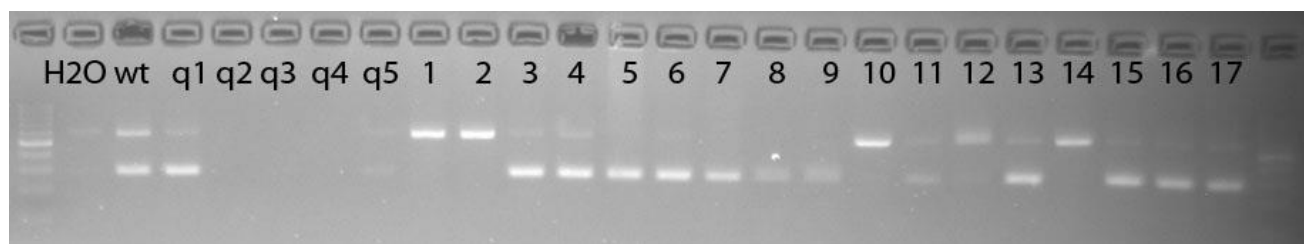
\* The amount of H<sub>2</sub>O is adjusted with the number of primer.

### Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cycles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C	30 sec	39
	59°C	45 sec	
	72°C	45 sec	
3 Polymerisation	72°C	10 min	1
4 Cooling	4°C	hold	1

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

### Gel Image



Separated by gel electrophoresis on a 2% agarose gel.