

**EMMA ID:** 09617

**Gene:** *Tg(CMV-rtTA)4Bjd, Tg(TRE-ORFeus,-lacZ)*

**Common name:** *ORFeus lacZ\_CMVrtTA; OFC*

## Genotyping Information

Genotyping by end-point PCR based on gel is composed of a genespecific short range PCR using primers on CMV-rt TA and a ORFeus lacZ specific short range PCR. The combined results show the genotype of the mice.

### PCR primer pairs and expected size bands

Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
ORFeus lacZ	SW29	SW30	1370
CMVrtTA	SW61	SW62	450

### Primer sequences

Primer Name	Sequence 5' --> 3'
SW29	ACCCAACACCCGTGCGTTTTATT
SW30	TATATCACCGCCGACAAGCAGAAGA
SW61	CGCTGTGGGGCATTCTTTACTTT
SW62	CATGTCCAGATCGAAATCGTC

### 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	

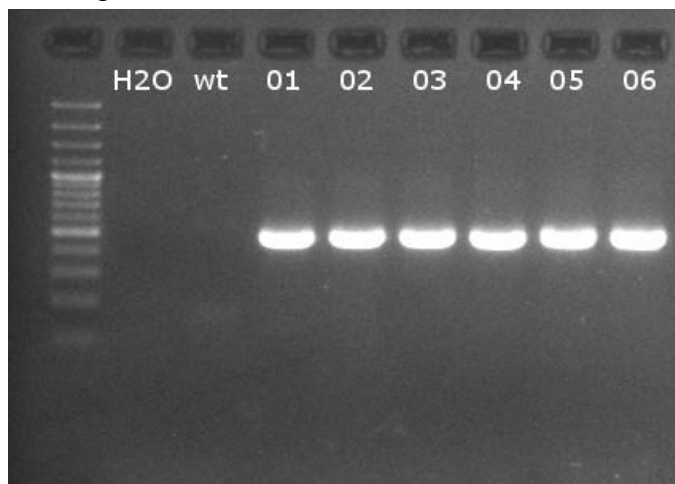
\* The amount of H<sub>2</sub>O is adjusted with the number of primer.

### Amplification conditions

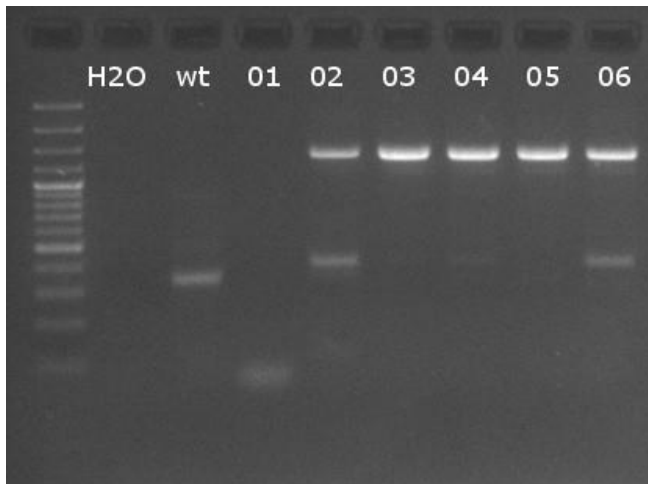
PCR Settings	Temperature (°C)	Time	# of cycles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C	30 sec	39
	55°C	45 sec	
	72°C	45 sec	
3 Polymerisation	72°C	10 min	1
4 Cooling	12°C	hold	1

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

### Gel Image



**CMV-rt TA PCR**



**ORFeus lacZ PCR**

Separated by gel electrophoresis on a 2% agarose gel.