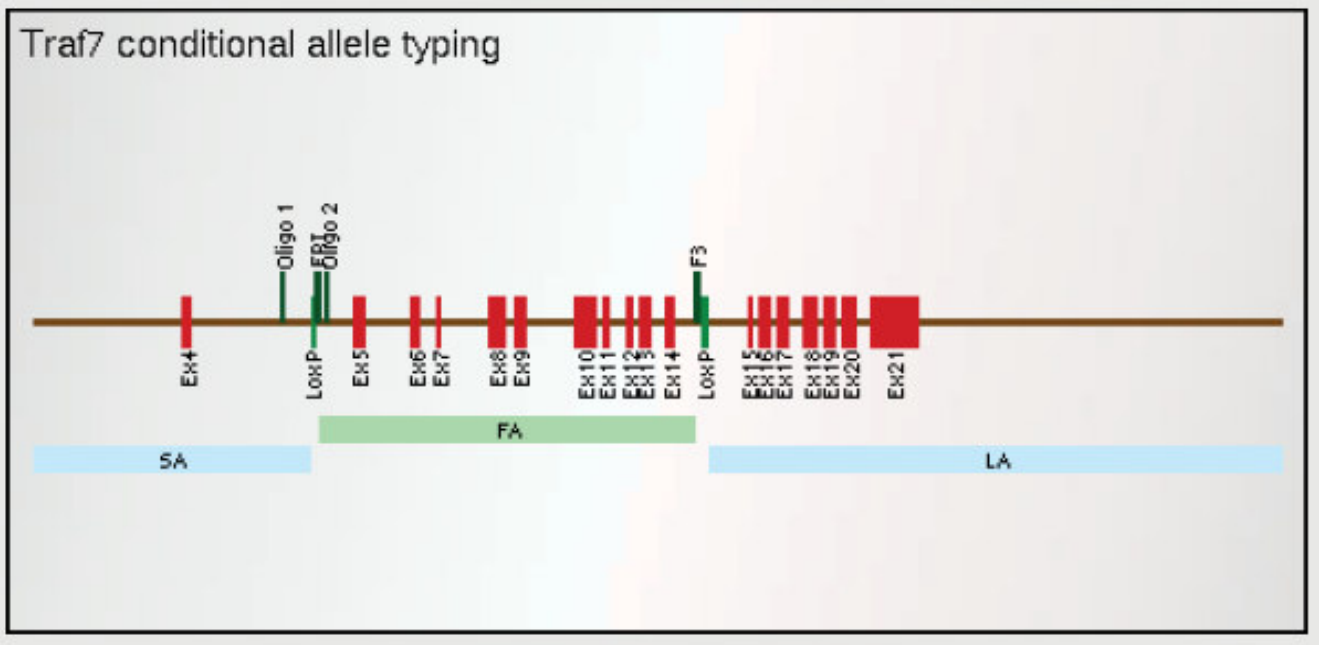
EMMA ID: *EM:05503*
Gene: *Traf7*
Common name: *TRAF7 KO b cond*

Allele Information



Genotyping Information

The fragment amplified with oligo 1 (1533_27) + oligo 2 (1533_28) detects heterozygous / homozygous wildtype and conditional alleles. Due to highly palindromic repeats structures (FRT, multiple cloning site, loxP) in the conditional allele, an additional shorter artifact fragment might be visible in case of long electrophoretic separation.

PCR primer pairs and expected size bands

Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Wildtype	*1533_27*	*1533_28*	*383*
Mutant	*same as wt*	*same as wt*	*502*

Primer sequences

Primer Name	Sequence 5' --> 3'
1533_27	*TGGGTCTCACACGGCATTCC*
1533_28	*CATCCAGCAGCCAATCATGG*

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
MgCl2 (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
H2O*	13,7	
Final volume	25	

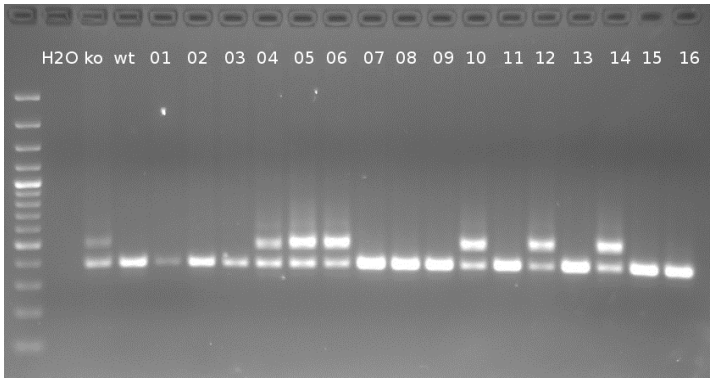
*** The amount of H2O is adjusted with the number of primer.**

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C	30 sec	39
	60°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



Separated by gel electrophoresis on a 2% agarose gel.