EMMA ID: *EM:16731*
Gene: *Tg(Tcra/Tcrb)1Vbo, Rag2*
Common name: C.Cg-Rag2^{tm1Fwa} Tg(Tcra/Tcrb)1Vbo/Ieg
Allele: Tg(Tcra/Tcrb)1Vbo, Rag2^{tm1Fwa}

Allele Information

Mice are homozygous for the Rag2 knock-out allele and for the TCR transgene, which has specificity for the hemagglutinin (HA) peptide in the context of I-E^{d} class II MHC molecules. Rag2^{tm1Fwa}: targeting in CCE ES-cells, thus Rag2 knock-out allele on 129S/SvEv-Gpi1^{c} background (Shinkai Y, Koyasu S, Nakayama K, Murphy KM, Loh DY, Reinherz EL, Alt FW, Science. 1993 259(5096):822-5). Tg(HA-TCRa,HA-TCRb)17Bii (MGI transgene: Tg(Tcra/Tcrb)1Vbo, MGI:2680176) Transgenesis by pronuclear injection into (B6 x DBA/2)F2 fertilized eggs (Kirberg, J., A. Baron, S. Jakob, A. Rolink, K. Karjalainen, and H. von Boehmer. Thymic selection of CD8+ single positive cells with a class II major histocompatibility complex-restricted receptor. 1994, J Exp Med 180:25-34).

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 Rag2	11377 (wt for)	11378 (wt rev)	606
Mutant Rag2	11377 (wt for)	11379 (rev)	425 (+496)
Wild type HA_TCR	5915 (HA_TCR wt for)	5918 (HA_TCR con rev)	741
Mutant HA_TCR	5915 (HA_TCR wt for)	5916 (HA_TCR rev)	480

Primer sequences

Gene	Primer Name	Sequence 5' --> 3'
Rag2	11377 (wt for)	GCA ACA TGT TAT CCA GTA GCC GGT
	11379 (rev)	GCT TCC TCT TGC AAA ACC ACA CTG
	11378 (wt rev)	TTG GGA GGA CAC TCA CTT GCC AGT
HA-TCR	5915 (HA_TCR wt for)	CCT GAA CTG GGG ATT CTA CTC TTC C
	5916 (HA_TCR rev)	AGT CAG CTT ATT ATT GCC TCC ACT C
	5918 (HA_TCR con rev)	AGC CTG GGC TAG ATA AAG TTC CAT C

PCR setup (AllTaq)

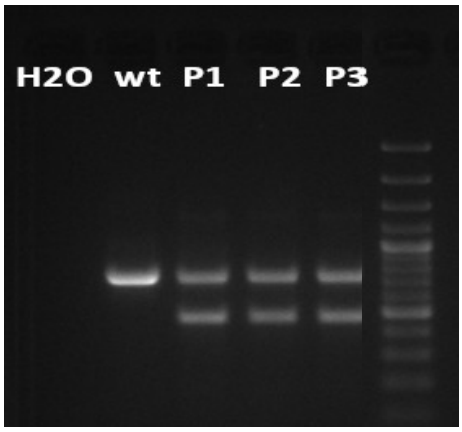
Component	Volume (µl) 1x	Final conc.
DNA (~ 50-100 ng)	2	
Q-Solution (5x)	2,5	0,5
PCR-Buffer (5x)	5	1
DNTP mix (10 mM)	0,5	0,2
$MgCl_2$ (25 mM)	1,5	1,5
Primer 1 (10 pmol/µl)	1	0,4
Primer 2 (10 pmol/µl)	1	0,4
Primer 3 (10 pmol/µl)	1	0,4
Taq Polymerase (5 U/µl)	0,5	2,5 U/rxn
H_2O*	10	
Final volume	25	

*** The amount of H_2O is adjusted with the number of primer.**

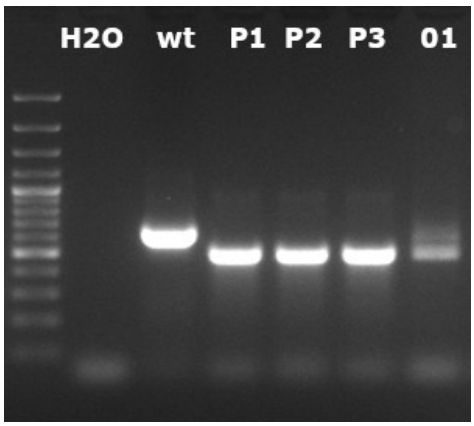
Amplification conditions

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

Gel Image



HA-TCR
55-65°C TD (HA-TCR)
P1-P3 het



Rag2
58°C (Rag2)
P1-P3 hom; 01 het

Separated by gel electrophoresis on a 2% agarose gel.