EMMA ID: *EM:16834*
Gene: *Il7r*
Common name: *B6.Il7rtm1.1(icre)Hrr*
Allele: *Il73* $^{tm1.1(icre)Hrr}$

Allele Information

Codon-improved Cre (iCre) was inserted by homologous recombination in ES cells into the first exon of the Il7r gene.The targeting construct consisted, from 5' to 3', of a homology arm with sequence upstream of the Il7r ATG start site, a splice donor site, an intron and a splice acceptor site from the rabbit b-globin gene, the coding sequence of codon-improved cre (icre), a poly-adenylation signal from the rabbit b-globin gene.
There is also a single FRT site in the genome (Schlenner SM et al., 2010).

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	Il7r_wtF	Il7r_wtR	584
Mutant	Il7r_wtF	Il7r_KI_R	677

Primer sequences

Primer Name	Sequence 5' --> 3'
Il7r_KI_R	CCT GAA AAC TTT GCC CCC TCC ATA
Il7r_wtF	CCA TAG AAT AGT GCA GCC TTG CCT C
Il7r_wtR	AGC GAA AGC TCT ACC CAG AGC

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	

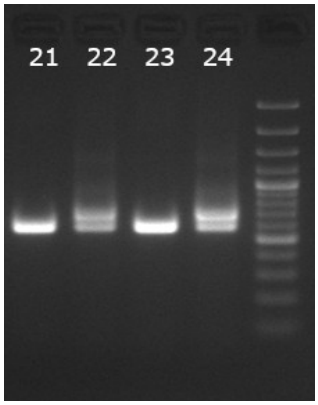
*** The amount of H_2O is adjusted with the number of primer.**

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 60°C 72°C	30 sec 45 sec 45 sec	39
3 Polymerisation	72°C	10 min	1
4 Cooling	4°C	hold	1

Touch-Down cycling protocol: first 10 cycles anneal at 65°C, decreasing 1°C per cycle, next 30 cycles aneal at 55°C.These PCR conditions have been optimized for our methods and preparation

Gel Image



21,23 wt
22,24 het

Separated by gel electrophoresis on a 2% agarose gel.