EMMA ID: *04623*
Gene: *Cbx5*
Common name: *W205C07*
Allele: *Cbx5* $^{Gt(W205C07)Wrst}$

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	W205 hp1a2	W205 hp1a5	530
Mutant	W205 hp1a2	W205 splirev2	500

Primer sequences

Primer Name	Sequence 5' --> 3'
W205 hp1a2	CCACTTGCCCCCTTAACCATGC
W205 splirev2	GCCAAACCTACAGGTGGGGTCTTT
W205 hp1a5	GATGAAAGGTGTGTGCTACCACG

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
$MgCl_2$ (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_2O*	13,7	
Final volume	25	

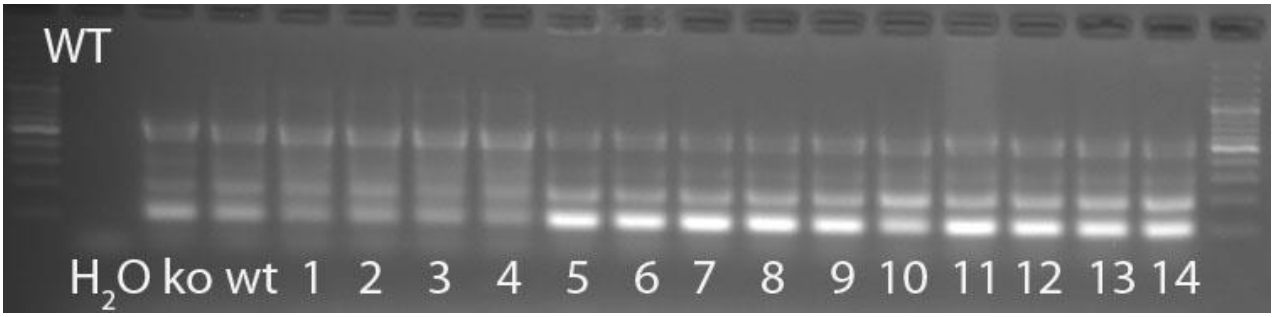
*** The amount of H_2O is adjusted with the number of primer.**

Amplification conditions

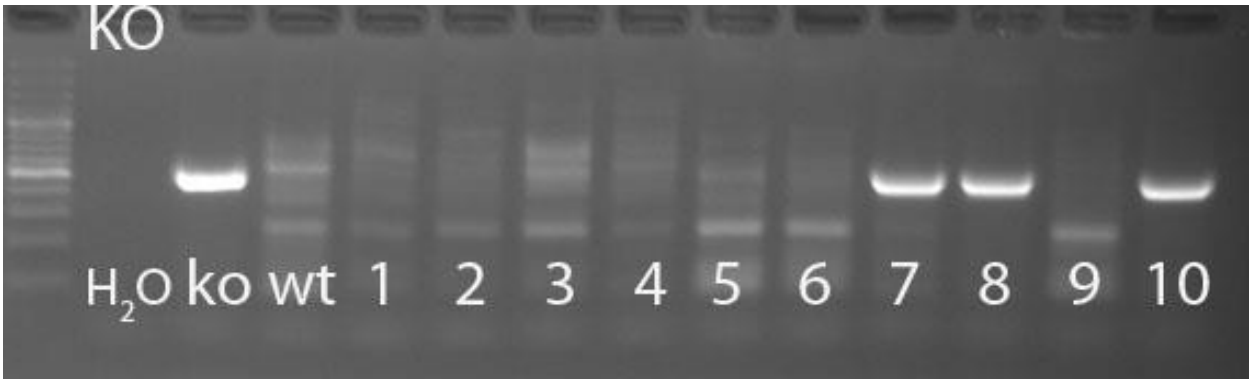
PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 60°C 72°C	30 sec 45 sec 45 sec	39
3 Polymerisation	72°C	10 min	1
4 Cooling	4°C	hold	1

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

Gel Image



WT-PCR



Mutant-PCR

Separated by gel electrophoresis on a 2% agarose gel.
ko/wt separate