EMMA ID: *09104*
Gene: *Bach2*
Common name: *EPD0689_1_H02*
Allele: *Bach2tm1b(EUCOMM)Wtsi*

Allele Information

Further information about the allele can be found on IMPC website at (copy the link to web browser)
http://www.mousephenotype.org/data/alleles/MGI:894679/tm1b(EUCOMM)Wtsi

Links to the general information

About IKMC resource
https://www.infrafrontier.eu/knowledgebase/protocols/ikmc-products

IKMC allele types
http://www.i-dcc.org/kb/entry/89/

Allele conversion guide - genotyping tm1b, tm1c and tm1d mice (assays infos available when required)
http://www.mousephenotype.org/about-ikmc/targeting-strategies

IMPC mouse phenotype data, search by the gene name
http://www.mousephenotype.org/

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)
Mutant	Bach2_232916_F	CAS_R1_Term	377
Wildtype	Bach2_232916_F	Bach2_232916_R	555

Primer Name	Sequence 5' --> 3'
Bach2_232916_F	CTCACCCTACTCTCTTTGAACCAAC
Bach2_232916_R	ATATCCTGGCAAAAATGCGG
CAS_R1_Term	TCGTGGTATCGTTATGCGCC

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
Primer 3 (10 pmol/µl)	1	0,4
Taq Polymerase (5 U/µl)	0,3	0,06
H_2O*	12,7	
Final volume	25	

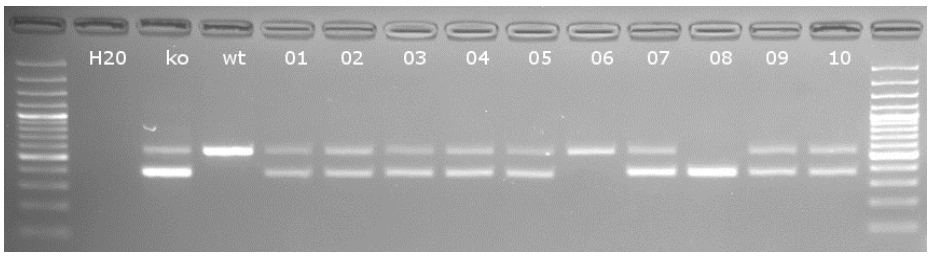
*** The amount of H_2O is adjusted with the number of primer.**

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 58°C 72°C	30 sec 45 sec 45 sec	39
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



ko = het control **work as triplex**

Separated by gel electrophoresis on a 2% agarose gel.