

EMMA ID: *EM 07443*

Gene: *Paox*

Common name: *EPD0321_3_E09*

Allele: *Paox*^{tm1(KOMP)Wtsi}

Allele Information

Further information about the allele can be found on IMPC website at (copy the link to web browser)
<https://www.mousephenotype.org/data/alleles/MGI:1916983/tm1.1%2528KOMP%2529Wtsi?>

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 gene-specific 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	Paox 5'arm	LAR3	344
Wildtype	Paox 5'arm	Paox 3'arm	-

Primer sequences

Primer Name	Sequence 5' --> 3'
Paox 5'arm	gaaatctggtctgaccaagc
Paox 3'arm	aggccttaattagcagcagga
LAR3	CAACGGTTCTTCTGTTAGTCC

Genotyping by end-point PCR based on gel is composed of a gene-specific short range PCR using primers on wild type all

PCR setup (Qiagen, Hot Start Plus)

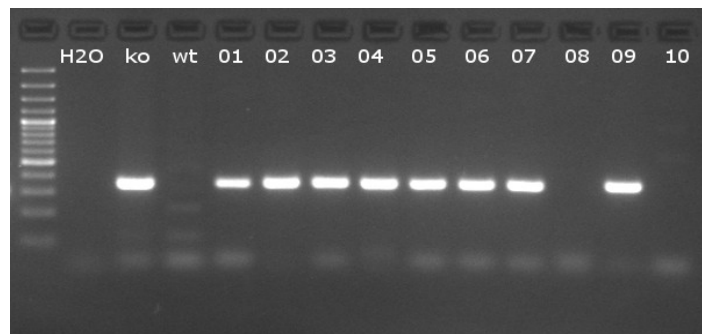
Component	Volume (µl) 1x	Final conc.
DNA (~ 50-100 ng)	2	
Q-Solution (5x)	2,5	0,5
PCR-Buffer (10x)	2,5	1
DNTP mix (10 mM)	0,5	0,2
MgCl ₂ (25 mM)	1,5	1,5
Primer 1 (10 pmol/µl)	1	0,4
Primer 2 (10 pmol/µl)	1	0,4
Taq Polymerase (5 U/µl)	0,3	0,06
H ₂ O *	13,7	
Final volume	25	

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

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cycles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C	30 sec	39
	65°C	45 sec	
	72°C	45 sec	
3 Polymerisation	72°C	10 min	1
4 Cooling	12°C	hold	1

Gel Image



Separated by gel electrophoresis on a 2% agarose gel.

deletion line

ele and a mutant allele-specific short range PCR. The combined results show the genotype of the mice. For example: |

Genotyping using PCR-assays for cassette detection

LacZ reporter, Neo selection cassettes are inserted into the Knockout-first mutant allele.

Cassette changes by allele conversion can be found on:

<http://www.mousephenotype.org/about-ikmc/targeting-strategies>.

For example, tm1b allele contains still lacZ reporter cassette, Neo selection cassette is deleted (promotor-driven only). Please note that these assays are with universal cassette primers other than gene-specific. The confirmation on gene identity performed by e.g. sr genespecific PCR as provided is

PCR primer pairs and expected size bands

Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
lacZ	LacZ_multi_Deen_2F	LacZ_multi_Deen_2R	mut 81 bp,wt without ban
Neo	Neo_long_Deen_F1	Neo_long_Deen_R1	mut 186 bp,wt without ba

Primer sequences

PCR setup (Qiagen, Hot Start Plus)

Primer Name	Sequence 5' --> 3'
LacZ_multi_Deen_2F	TACTGGAGGCTGAAGTTCAGAT
LacZ_multi_Deen_2R	GCGTTTCACCTGCCATAA
Neo_long_Deen_F1	TTGAACAAGATGGATTGCACGC
Neo_long_Deen_R1	CCTCGTCTGCAGTTCATT

Component	Volume (µl)	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 (25mM)	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 ₂ O	13,7	
Final volume	25	

PCR Settings	Temperature (°C)	Time	# of cycles
Denaturation (Melting)	95°C	5 min	1
Amplification (Melting, An- nealing, Polym.)	94°C 58°C 72°C	30 sec 45 sec 45 sec	39
Polymerisation	72°C	10 min	1
Cooling	12°C	hold	1

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

mutant positive, wild type positive = Heterozygous.

Tm1.1 Allele Conversion PCR-assays

Allele conversion guide - genotyping tm1b, tm1c and tm1d mice

<http://www.mousephenotype.org/about-ikmc/targeting-strategies>

Tm1.1 allele is reporter-tagged deletion allele (post-Cre). Neo selection cassette is removed together in promoter-driven strains only. LacZ reporter cassette is kept for visualising gene expression.

Assay	Forward Prime	Reverse P	Size Band (bp)	Allele
Tm1b Promotor-driven	tm1b_forw	Floxed LR	380 bp others	tm1b, Promotor-driven tm1a or partially conversion
Flox Promotorless	Floxed PNF	Floxed LR	128 bp ~ 1 kb	tm1b, Promotorless tm1a

Primer sequences

Primer Name	Sequence 5' --> 3'
tm1b_forw	CGGTCGCTACCATTACCACT
Floxed LR	ACTGATGGCGAGCTCAGACC
Floxed PNF	ATCCGGGGGTACCGCGTCGAG

PCR setup (Phire Hot Start II)

Amplification conditions

Component	Volume (µl)	PCR Settings	Temperature (°C)	Time
DNA (~ 50-100 ng)	2,0	1	98°C	30 sec
H ₂ O	12,7	2	98°C	5 sec
PCR-Buffer (5x)	4,0	3	58°C	10 sec
DNTP mix (10 mM)	0,4	4	72°C	10 sec
Primer mixed (10	0,5	5	to 2 + 34 cycles	
Phire Tag (1 U/µl)	0,4	6	72°C	1 min
Final volume	20	7	12°C	hold

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