Wellcome Trust Sanger Institute
Wellcome Trust Genome Campus
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Gene: Stat2

Colony prefix: DAMX

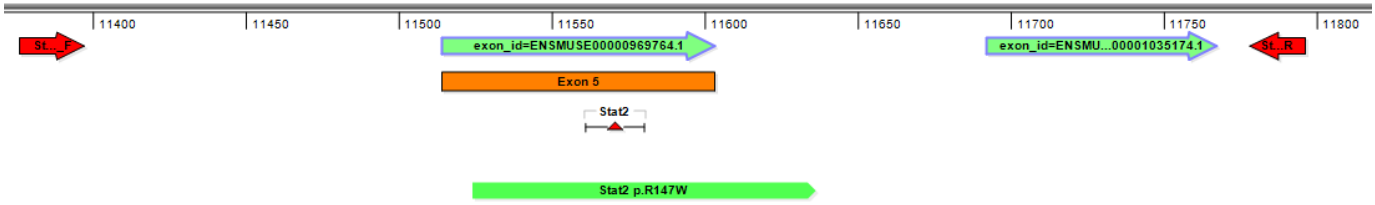
Allele: Stat2$^{em1(IMPC)Wtsi}$

Allele type: Crispr/Cas9 mediated Point Mutation Point mutation

Intended allele description: R147W

Allele information:

Further information about the allele can be found on the 'International Mouse Phenotyping Consortium' (IMPC) web site at
http://www.mousephenotype.org/data/alleles/MGI:103039/em1%2528IMPC%2529Wtsi?



Mouse QC information

SNP qPCR	pass	**Mutation Sequence confirmed**	pass
Mutant Specific SR-PCR	na	**Off-target analysis complete**	na

Guide RNAs and mutant oligos used in initial experiment

Sequence	Chr	Chr Start	Chr End	Strand
TGAGATTGAAAATCGAATCC(AGG)	10	128277691	128277713	plus
CCTCCCCACCCAGCACTTGCCACACCCGCCAACCTCAATGTCCA CATGTAAACCCTGGATCCAATTTTCAATCTCAAGCTGCTGGCTC TCCACAACTGCTTCGGGGGCTGGTG	10	128277766	128277654	minus

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Mutant allele sequence:

CACCAGCCCCCGAAGCAGTTGTGGAGAGCCAGCAGCTTGAGATTGAAAAT[CGA/tGg]ATCCAGGGTTTACATGTGGA
CATTGAGGTTGGCGGGTGTGGCAAGTGCTGGGTGGGGAGG

Genotyping by end-point PCR

PCRs primer pairs and expected size bands

Assay Type	Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Standard PCR	Screening*	Stat2_PM_WT_F	Stat2_PM_WT_R	421

*The screening PCR flanks the SNP region and can be used for sequence verification of the allele. The PCR will not distinguish wildtype from mutant mice, however, as a product will be amplified in all cases.

Primer sequences

Primer Name	Primer Sequence (5' > 3')
Stat2_PM_WT_F	CCCTGTCTTGAAAACAAACAAA
Stat2_PM_WT_R	TGTCCTCCTCCCACATTGA

Reaction setup

Reagent	µl
DNA (~50-100 ng)	1
10x Buffer	2
MgCl2 (50 mM)	0.6
Platinum Taq (Invitrogen)	0.2
dNTPs (100 mM)	0.2
Primer 1 (10 µM)	0.4
Primer 2 (10 µM)	0.4
ddH20	15.2
Total	20

Amplification conditions

Step	Conditions	Time
1	94°C	5 min
2	94°C	30 sec
3	58°C	30 sec
4	72°C	1:30 sec
5	Go to '2' + 34 cycles	-
6	72°C	5 min
7	12°C	forever

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Genotyping by SNP qPCR

Primers for qPCR assay

Gene	Source	Forward Primer Seq.	Reverse Primer Seq.	Probe Primer Seq.
Stat2	Life Technologies	GAGAGCCAGCAGCTTGAGAT	CAACCTCAATGTCCACATGTAAACC	[VIC]CTGGATTCGATTTTCA [FAM]TGGATCCAATTTTCA

Reactions are performed in a 10µl volume using an Applied Biosystems 7900HT Fast Real-Time PCR System or Applied Biosystems Viia7 with DNA prepared using the Sample-to-SNP™ kit (Applied Biosystems) from mouse ear biopsies. GTXpress™ buffer is also used (Applied Biosystems).

Reagent	µl
2x GTXpressTM buffer	5
40x target assay	0.25
ddH2O	3.75
DNA	1

Amplification conditions

Step	Conditions	Time
Pre-read	60°C	30 sec
1	95°C	20 sec
2	95°C	10 sec
3	60°C	30 sec
4	Go to '2' + 34	-
Post-red	60°C	30 sec

Links to information and frequently asked questions

MGP mouse phenotype data:
http://www.mousephenotype.org

How the "critical" exon is decided:
http://www.i-dcc.org/kb/entry/102/

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Relevant publications

White, J.K., Gerdin, A.-K., Karp, N.A., Ryder, E., Buljan, M., Bussell, J.N., Salisbury, J., Clare, S., Ingham, N.J., Podrini, C., et al. (2013). Genome-wide Generation and Systematic Phenotyping of Knockout Mice Reveals New Roles for Many Genes. Cell 154, 452–464.

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Singh P, Schimenti JC, Bolcun-Filas E (2014) A Mouse Geneticist's Practical Guide to CRISPR Applications. Genetics genetics.114.169771–. doi: 10.1534/genetics.114.169771

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Zhou J, Wang J, Shen B, et al (2014) Dual sgRNAs facilitate CRISPR/Cas9 mediated mouse genome targeting. FEBS J. doi: 10.1111/febs.12735

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Shen B, Zhang J, Wu H, et al (2013) Generation of gene-modified mice via Cas9/RNA-mediated gene targeting. Cell Res 23:720–3. doi: 10.1038/cr.2013.46

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Yang H, Wang H, Shivalila CS, et al (2013) One-Step Generation of Mice Carrying Reporter and Conditional Alleles by CRISPR/Cas-Mediated Genome Engineering. Cell 154:1370–1379. doi: 10.1016/j.cell.2013.08.022

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