

**Gene:** Son

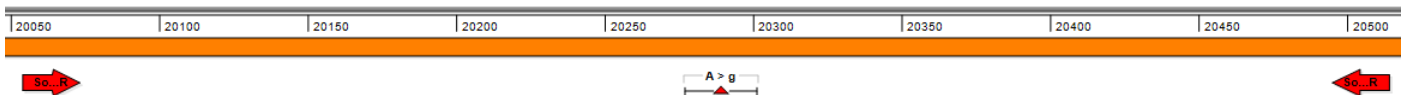
**Colony prefix:** DANR

**Allele:** *Son*<sup>em1(IMPC)Wtsi</sup>

**Allele type:** Crispr/Cas9 mediated Point mutation

**Allele information:** E1249G

Further information about the allele can be found on the 'International Mouse Phenotyping Consortium' (IMPC) web site at <http://www.mousephenotype.org/>



## Mouse QC information

Loss of WT Allele (LOA) qPCR	na	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
CCGAGTCTTCAGTTACATCAGCA	16	91658109	91658131
GGAACCATTTTCATGTTCTGCTACTACAGCAGACTCAACT GGTGCTGATGTAAGTGAAGACCCGGGCTCTCGTGACAG GTTCTGGAACAGTCATAACAGCCTCTGATGCTAACATT GAAGTT	16	91658053	91658172

This technical data sheet and information ("Datasheet") is supplied by Genome Research Limited ("GRL").

Although reasonable care is taken in the preparation of this Datasheet, GRL gives no warranties express or implied for any use of the Datasheet or for the accuracy of the Datasheet. GRL assumes no responsibility or liability for any decisions based upon the Datasheet. Without limiting the foregoing the Datasheet was prepared for mice supplied directly from GRL and where copies of this Datasheet are available from third party repositories or distribution centres ("Third Parties") GRL shall not be liable for any inconsistency between the mouse strain supplied by the Third Party and the Datasheet howsoever arising.  
Report Generated on: 29-September-2020

## Mutant allele sequence:

GAGCCTATGGCAGTACCTGCTAATTATTCAATGTCAGAATCAGAACTTCAATGTTAGCATCAGAGGCTGTTATGACTGTTCCAG  
AACCTGCACGAGAGCCCG[A/G]GTCTTCAGTTACATCAGCACCCAGTTGAGTCTGCTGTAGTAGCAGAACATGAAATGGTCCAGA  
GAGACCGATGACTTACATGGTTTCTGAGACTACCATG

## 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*	Son_PM_WT_F	Son_PM_WT_R	461

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

**We recommend that mice are sequence-verified with the screening primers to confirm the genotyping qPCR results when establishing the colony, in case of any cross-talk between the assays.**

### Primer sequences

Primer Name	Primer Sequence (5' > 3')
Son_PM_WT_F	CCAGAGGGTCCAGCATTATC
Son_PM_WT_R	GCTGGCTCCAAGAGTGACAT

### Reaction setup

Reagent	µl
DNA (~50-100 ng)	1
10x Buffer	2
MgCl <sub>2</sub> (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
ddH <sub>2</sub> O	15.2
Total	20

This technical data sheet and information ("Datasheet") is supplied by Genome Research Limited ("GRL").

Although reasonable care is taken in the preparation of this Datasheet, GRL gives no warranties express or implied for any use of the Datasheet or for the accuracy of the Datasheet. GRL assumes no responsibility or liability for any decisions based upon the Datasheet. Without limiting the foregoing the Datasheet was prepared for mice supplied directly from GRL and where copies of this Datasheet are available from third party repositories or distribution centres ("Third Parties") GRL shall not be liable for any inconsistency between the mouse strain supplied by the Third Party and the Datasheet howsoever arising.  
Report Generated on: 29-September-2020

### 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

### Genotyping by SNP qPCR

#### Primers for LoA qPCR assay

Gene	Source	Forward Primer Seq.	Reverse Primer Seq.	Probe Primer Seq.
Son	Life Technologies	GCTGTTATGACTGTTCCA GAACCT	CAGACTCAACTGGTGCTGATGT AA	[VIC]AGCCCGAGTCTTCAG [FAM]CCCGGGTCTTCAG

Reactions are performed in a 10µl volume using an Applied Biosystems 7900HT Fast Real-Time PCR System or Applied Biosystems Vii7 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 GTXpress™ 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-read	60°C	30 sec

This technical data sheet and information ("Datasheet") is supplied by Genome Research Limited ("GRL").

Although reasonable care is taken in the preparation of this Datasheet, GRL gives no warranties express or implied for any use of the Datasheet or for the accuracy of the Datasheet. GRL assumes no responsibility or liability for any decisions based upon the Datasheet. Without limiting the foregoing the Datasheet was prepared for mice supplied directly from GRL and where copies of this Datasheet are available from third party repositories or distribution centres ("Third Parties") GRL shall not be liable for any inconsistency between the mouse strain supplied by the Third Party and the Datasheet howsoever arising.  
Report Generated on: 29-September-2020

## 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/>

## 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.

Mali P, Yang L, Esvelt KM, et al (2013) RNA-guided human genome engineering via Cas9. *Science* 339:823–6. doi: 10.1126/science.1232033

Jinek M, Chylinski K, Fonfara I, et al (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 337:816–21. doi: 10.1126/science.1225829

Cong L, Ran FA, Cox D, et al (2013) Multiplex genome engineering using CRISPR/Cas systems. *Science* 339:819–23. doi: 10.1126/science.1231143

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

Brandl C, Ortiz O, Röttig B, et al (2015) Creation of targeted genomic deletions using TALEN or CRISPR/Cas nuclease pairs in one-cell mouse embryos. *FEBS Open Bio* 5:26–35. doi: 10.1016/j.fob.2014.11.009

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

Kraft K, Geuer S, Will AJ, et al (2015) Deletions, Inversions, Duplications: Engineering of Structural Variants using CRISPR/Cas in Mice. *Cell Rep*. doi: 10.1016/j.celrep.2015.01.016

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

Wang H, Yang H, Shivalila CS, et al (2013) One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. *Cell* 153:910–8. doi: 10.1016/j.cell.2013.04.025

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

This technical data sheet and information ("Datasheet") is supplied by Genome Research Limited ("GRL").

Although reasonable care is taken in the preparation of this Datasheet, GRL gives no warranties express or implied for any use of the Datasheet or for the accuracy of the Datasheet. GRL assumes no responsibility or liability for any decisions based upon the Datasheet. Without limiting the foregoing the Datasheet was prepared for mice supplied directly from GRL and where copies of this Datasheet are available from third party repositories or distribution centres ("Third Parties") GRL shall not be liable for any inconsistency between the mouse strain supplied by the Third Party and the Datasheet howsoever arising.  
Report Generated on: 29-September-2020