

**Gene:** Tnp01

**Colony prefix:** DASX

**Allele:** *Tnp01<sup>em1(IMPC)Wtsi</sup>*

**Allele type:** Crispr/Cas9 mediated deletion

**Anticipated mutation:** Exon deleted and out of frame

**Allele information:**

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

<b>Loss of WT Allele (LOA) qPCR</b>	Pass	<b>Mutation Sequence confirmed</b>	Pass
<b>Mutant Specific SR-PCR</b>	Pass	<b>Off-target analysis complete</b>	na

### Mutant Allele sequence:

ACAGACTGTGCAACCCATTTCTCATTTTGC AAACAGTGAGGGA ACTGACATCAGCATCCAGTTAGCAAGAGAACG  
GTACTTACCCATTTAAATACCACAGTTGCAGGAAATGATCACGATGGCTCTTCTTGGCTCATCTGTTCTTTAGAAT  
GTCACAGCAGAGACTGCCATTCATGCACGTCTCCACACCAACTCTTCCCAG

**Deletion size (bp):** 460

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## Guide RNAs used in initial experiment

Sequence	Chr	Chr Start	Chr End
ACAGTGGTAGTAACTCTAGGCGG	13	98888335	98888357
CCACAGTGGTAGTAACTCTAGGC	13	98888333	98888355
CCACACGTTTCGCTCAAGAGCTTT	13	98888778	98888800
CGTTCGCTCAAGAGCTTTGCAGG	13	98888783	98888805

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## Genotyping by end-point PCR

These mice may be genotyped through a combination of separate PCR reactions that detect the gene-specific wild type allele and a mutant allele-specific short range PCR. Interpretation of the consolidated results produces the genotype of the mice. In addition to the expected product, the mutant assay may also amplify the endogenous wild type sequence which will appear as a larger band on an agarose gel. The presence of this extra band will depend on the size of the original deletion.

## PCRs primer pairs and expected size bands

Assay Type	Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Standard PCR	Wild type	Tnp01_DF1	Tnp01_ER1	496
Standard PCR	Wild type	Tnp01_EF1	Tnp01_DR1	470
Standard PCR	Mutant	Tnp01_DF1	Tnp01_DR1	313

## Primer sequences

Primer Name	Primer Sequence (5' > 3')
Tnp01_DF1	CACCAAGACAGACTGTGCAA
Tnp01_DR1	TGAGAGTCAGAGCTTGAAAGGTT
Tnp01_EF1	CCAGTTTCTAGAACAAACAGTGGT
Tnp01_ER1	GTCAGCCAGAACACAAATGGT

## Reaction setup

Reagent	µl
DNA (~50-100 ng)	1
10x Buffer	1.5
MgCl <sub>2</sub> (50 mM)	0.45
Platinum Taq (Invitrogen)	0.15
dNTPs (100 mM)	0.15
Primer 1 (10 µM)	0.3
Primer 2 (10 µM)	0.3
ddH <sub>2</sub> O	11.15
Total	15

## 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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## Links to information and frequently asked questions

MGP mouse phenotype data:

<http://www.mousephenotype.org>

## Useful 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* 194:169771–. 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

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