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Gene: Ppm1d

Colony prefix: TBCQ

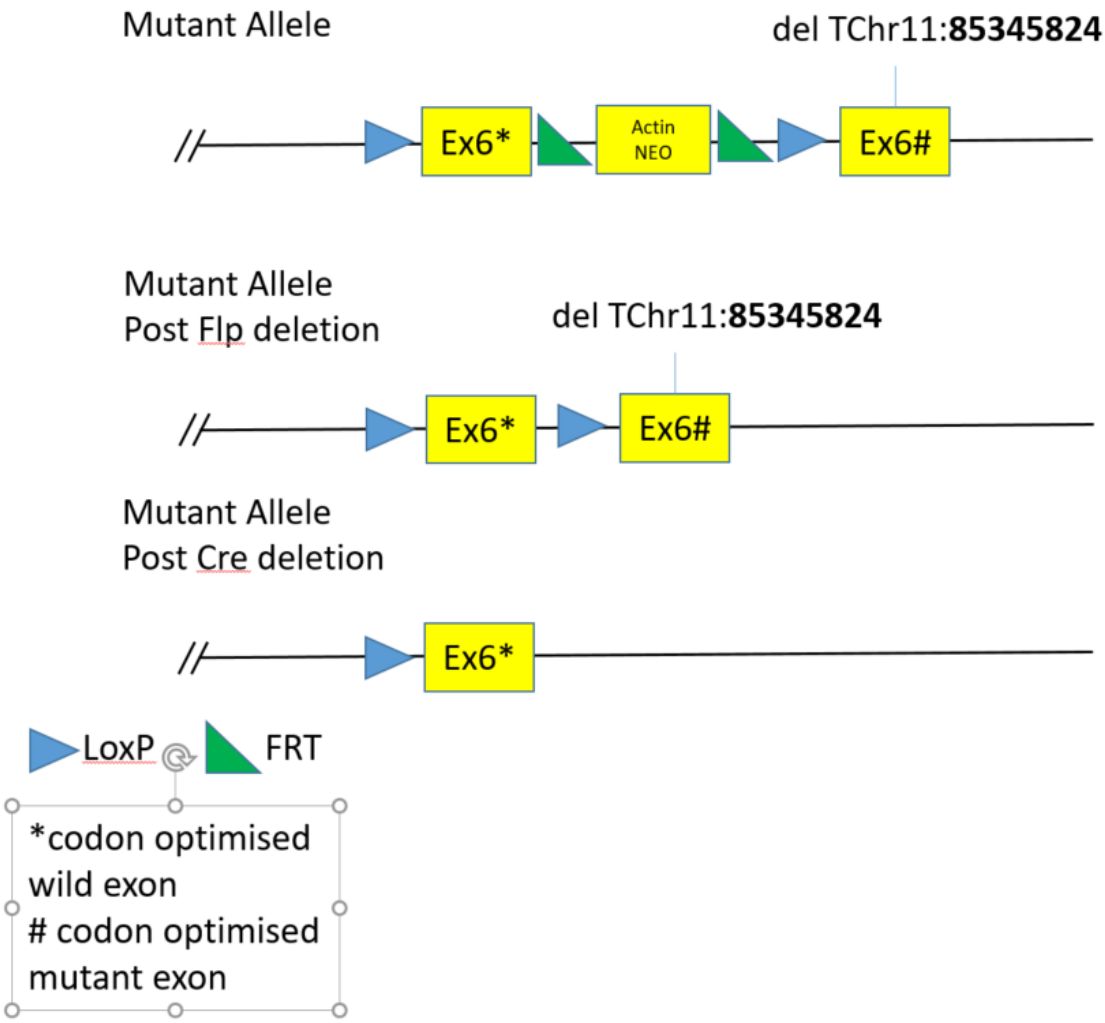
Allele: *Ppm1d*$^{tm1.2(IMPC)Wtsi}$

Allele type: Conditional Point mutation

Allele information:

A single bp deletion is introduced once the wild type exon has been deleted.

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



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Mouse QC information

Loss of WT Allele (LOA) qPCR	pass	**Mutation Sequence confirmed**	pass
Mutant Specific SR-PCR	pass		

Mutant allele sequence:

TCACCGGAAGAAGATGCATGGCCAAGGCTGAGCTCTAAGGACCATATACCTGCCCTTGTTCGCAGTAATGCCTTC
TCAGAGAAGTTTTTAGAGGTCCCAGCTGAGATAGCTAGAGGGAATATCCAGACTGTAGTGATGACCTCAAAAGAC
TCAGAGACACTTGAAGAAAATTGCCCCAAAGCCCTGACTTAAGGATTCATGATTCTTTGAATAATACTCTGTCAGT
TGGCCTCATTCCAACCAATTCAACAAATACTATCATGGACCAAAAAAACTTAAAGATGTCAACTCCAGGTCAAATG
AAAGCTCAAGAAGTTGAAAGAACCCCTCCAGCCAATTTTAAAAGGACATTAGAAGAATCCAACTCTGGCCCCCTTA
TGAAGAAGCACCGACGAAATGGCTTAAGTCGAAGTAGCGGGGCCCAGGCTTCCAGTCTCCCTACAGCATCCCAG
CGCAGGCACTCTGTCAAACTGACCCTGAGGCGCAGACTCAGGGGCCAGAGGAAGATGGGAAATCCTCTTCTCCA
CCAGCACCGGAAAACAGTGTGTGTGTGCTGAGATGGGCCTGGGAAGTGGGGGTCTCCCTACCTACGACTGAGG
GCTTTTTAAACTTGGTGCGAAGTTGAACTTTTTTAAGGGGATAAAATAAAGAGAATACAGTTTGACTTTTTGGAATT
TAACAGTTTTATTTTGGCCTTGTACTTGCCTGTATTATAATGTGAATTTTGTAGATGTAGGGAATAAGTTGCTGTAA
AATGTGTGTAAATTTGTATCCTTTACACAAGTTTAGTCTCTTACTCTGACACATAGTAATTGTGACAGCAGGGCTAA
TGTTGAAGAAAAGTCAGAAGAATCTTTAAGATTTTAAAAATGTCTTTAAAGTTTTTAAAATGCTTACTACATACTTAT
ATACACCCCTTGTGAAGAACACATGACTTTTTAAAGAAAATTACTAAGCAAACTGGAAAAGTGAAGTATTTTCATAG
TGATCTGTGCTCCACTTAATGTTTCCCAGGGACCATTAGTGTCTTTTTAAAATTACATTTTATTTCACATTTCATAAT
TCAGAAGTAAACCTTTCATAGGAAAAATACTGAGCTGTGCTAATGTAGCTGATTTTAGTCTCCTTGTCCCACTTACA
CTATGCAGTATCTCCTAACTTCAGTGCACTCTGCTAGAACAGTACATTTGCTGTATTTACTGAAATCTCTGGCACA
GAAGGAAGTGTGTTTGCCTCACACACCATTTGTCCCAGACCAGTGGCATTAGGCCATATATTCTCTTCTAGTGTTT
GCTTAAAATATGTGAAGTTTTTCTTGCTATTTCAATAACAAATGGTGCTGCTAACACCCAACATTTCCTAAATTATTT
TCTATCATACAGTTTTCATTGGTTATATGAGTATGTCTACCCAATAAATCACTGAATTTA

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Genotyping by end-point PCR

PCRs primer pairs and expected size bands

Assay Type	Forward Primer	Reverse Primer	Expected Size Band (bp)	Notes
Cassette	Ppm1d_5LOXP_F	LR	263	5' LoxP + Mutant
Cassette	SG_FRT_F	Ppm1d_3LOXP_R	283	3' LoxP
Wildtype	Ppm1d_5LOXP_F	Ppm1d_WT_R	378	
Mutant	Ppm1d_5LOXP_F	LR	263	

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')
Ppm1d_5LOXP_F	TGGGCACAGAGTGAATGTCT
SG_FRT_F	AAAGCAATAGCATCACAAATTTCA
LR	ACTGATGGCGAGCTCAGACC
Ppm1d_3LOXP_R	GGGCTCCAGAGAAAAACCTT
Ppm1d_WT_R	AGGAGACAGAGGCAAGTGGA

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

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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 LoA qPCR assay

Gene	Assay type	Assay Name	Forward Primer Seq.	Reverse Primer Seq.	Probe Primer Seq.
Ppm1d	LoA	Ppm1d_CPM_WT	AGAAACAAACTAAATACCTAACAACACGAT	GAGCCGGAGCTTTACTGAACA	CAGGTACAGCTTCTG
Neo	Cassette	Neo	GGTGGAGAGGCTATTCGGC	GAACACGGCGGCATCAG	TGGGCACAACAGACAATCGGCTG

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

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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

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