

Gene: Crb2

Colony prefix: MMBE

ESC clone ID: EPD0603_1_B06

Allele: *Crb2*^{tm1c(KOMP)Wtsi}

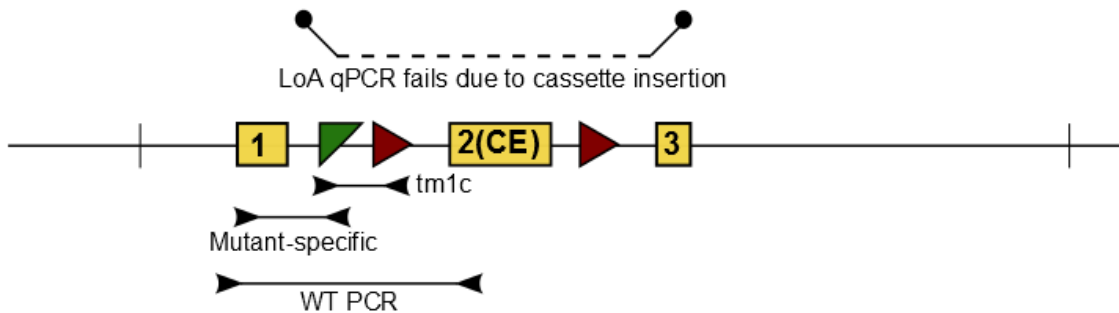
Allele type: Conditional allele (post-Flp)

Allele information:

Further information about the allele can be found on the IKMC web site at <http://www.mousephenotype.org/data/genes/MGI:2679260> Details on how to determine the floxed exon can be found at <http://www.knockoutmouse.org/kb/entry/21/>

Mouse QC information

Mutant allele



WT allele



Southern Blot	na	TV Backbone Assay	N/A	5' LR-PCR	N/A
Loss of WT Allele (LOA) qPCR	pass	Homozygous Loss of WT Allele (LOA) SR-	pass	Neo Count (qPCR)	N/A
LacZ SR-PCR	N/A	5' Cassette Integrity	N/A	Neo SR-PCR	N/A
Mutant Specific SR-PCR	pass	LoxP Confirmation	N/A	3' LR-PCR	N/A
Genotyping Comment					

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Southern blot confirmation:

Southern blots are not routinely performed at the Sanger Institute due to throughput constraints. A southern blot experiment design tool can be found on the IKMC web site at <http://www.knockoutmouse.org/martsearch/project/69506>

Links to information and frequently asked questions about the EUCOMM/KOMP alleles and MGP projects

General targeting strategies:
<http://www.knockoutmouse.org/about/targeting-strategies>

MGP mouse phenotype data:
<http://www.sanger.ac.uk/mouseportal/>

IKMC allele types:
<http://www.knockoutmouse.org/kb/entry/89/>

MGP mouse quality control tests :
<http://www.knockoutmouse.org/kb/25/>

Allele conversion guide - genotyping tm1b, tm1c and tm1d mice:
<http://www.knockoutmouse.org/kb/entry/105/>

How the "critical" exon is decided:
<http://www.knockoutmouse.org/kb/entry/102/>

Genotyping Information

Genotyping by end-point PCR

These mice may be genotyped through a combination of separate PCR reactions that detect the cassette, 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.

For example: cassette positive, mutant positive, wild type positive = heterozygous.

Please note that for tm1c mice the mutant allele will still amplify a band in the WT assay, but the size will be different to the native WT product (exact size difference is allele-specific).

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PCRs primer pairs and expected size bands

Assay Type	Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Standard PCR	Wildtype	Crb2_102679_F2	Crb2_102679_R2	. tm1c converted WT = 198
Standard PCR	Mutant	Crb2_102679_F	CAS_R1_Term	204
Standard PCR	Cassette	Tm1c_F	Tm1c_R	218

Primer sequences

Primer Name	Primer Sequence (5' > 3')
CAS_R1_Term	TCGTGGTATCGTTATGCGCC
Tm1c_F	AAGGCGCATAACGATACCAC
Tm1c_R	CCGCCTACTGCGACTATAGAGA
Crb2_102679_F2	ACCTTCTCTGTGAGGGCAAG
Crb2_102679_F	CAAGGTGTCATTGGGCTGTG
Crb2_102679_R2	CCAGCCACTCACCTTGGTAT

Reaction setup

Reagent	µl
DNA (~50-100 ng)	1
10x Buffer	2
MgCl ₂ (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 ₂ O	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	45 sec
5	Go to '2' + 34 cycles	-
6	72°C	5 min
7	12°C	forever

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Relevant publications

Ryder, E., Gleeson, D., Sethi, D., Vyas, S., Miklejewska, E., Dalvi, P., Habib, B., Cook, R., Hardy, M., Jhaveri, K., et al. (2013). Molecular Characterization of Mutant Mouse Strains Generated from the EUCOMM/KOMP-CSD ES Cell Resource. *Mammalian Genome*. Doi: 10.1007/s00335-013-9467-x

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.

Ryder, E., Wong, K., Gleeson, D., Keane, T.M., Sethi, D., Vyas, S., Wardle-Jones, H., Bussell, J.N., Houghton, R., Salisbury, J., et al. (2013). Genomic analysis of a novel spontaneous albino C57BL/6N mouse strain. *Genesis* 51, 523–528.

Bradley, A., Anastassiadis, K., Ayadi, A., Battey, J.F., Bell, C., Birling, M.-C., Bottomley, J., Brown, S.D., Bürger, A., Bult, C.J., et al. (2012). The mammalian gene function resource: the international knockout mouse consortium. *Mamm Genome* 23, 580–586.

Birling, M.-C., Dierich, A., Jacquot, S., Héroult, Y., and Pavlovic, G. (2011). Highly-efficient, fluorescent, locus directed Cre and flopo deleter mice on a pure C57BL/6N genetic background. *Genesis*.

Skarnes, W.C., Rosen, B., West, A.P., Koutsourakis, M., Bushell, W., Iyer, V., Mujica, A.O., Thomas, M., Harrow, J., Cox, T., et al. (2011). A conditional knockout resource for the genome-wide study of mouse gene function. *Nature* 474, 337–342.

Pettitt, S.J., Liang, Q., Rairdan, X.Y., Moran, J.L., Prosser, H.M., Beier, D.R., Lloyd, K.C., Bradley, A., and Skarnes, W.C. (2009). Agouti C57BL/6N embryonic stem cells for mouse genetic resources. *Nat Methods* 6, 493–495.

Liang, Q., Conte, N., Skarnes, W.C., and Bradley, A. (2008). Extensive genomic copy number variation in embryonic stem cells. *Proc Natl Acad Sci U S A* 105, 17453–17456.

Farley, F.W., Soriano, P., Steffen, L.S., and Dymecki, S.M. (2000). Widespread recombinase expression using FLPeR (flipper) mice. *Genesis* 28, 106–110.

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