

**Gene:** *Cyba*

**Colony prefix:** MFAC

**ESC clone ID:** EPD0372\_5\_B08

**Allele:** *Cyba*<sup>tm1a(EUCOMM)Wtsi</sup>

**Allele type:** Knockout First, Reporter-tagged insertion with conditional potential

**Allele information:**

Further information about the allele can be found on the IKMC web site at <http://www.knockoutmouse.org/martsearch/project/74663>. Details on how to determine the floxed exon can be found at <http://www.knockoutmouse.org/kb/entry/21/>

**Mouse QC information**



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

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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/74663>

## 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.

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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	Cyba_83569_F2	Cyba_83569_R2	212
Standard PCR	Mutant	Cyba_83569_F	CAS_R1_Term	179
Standard PCR	Cassette	LacZ_2_small_F	LacZ_2_small_R	108

## Primer sequences

Primer Name	Primer Sequence (5' > 3')
CAS_R1_Term	TCGTGGTATCGTTATGCGCC
Cyba_83569_F	TGAAATGAAGGAGGTTGCC
Cyba_83569_F2	ACATCTGCCTGCCTCTGTCT
Cyba_83569_R2	AGCAAGCTGAACTCCACCAT
LacZ_2_small_F	ATCACGACGCGCTGTATC
LacZ_2_small_R	ACATCGGGCAAATAATATCG

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## Reaction setup

Reagent	$\mu$ l
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 $\mu$ M)	0.4
Primer 2 (10 $\mu$ M)	0.4
ddH <sub>2</sub> O	15.2
<b>Total</b>	<b>20</b>

## 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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### Genotyping using universal copy number qPCR assays designed to the selection cassette

The cassette qPCR assays use a hydrolysis probe assay (eg Applied Biosystems TaqMan technology) to determine genotype via the copy number of the selection cassette in a sample. Homozygotes will possess two copies, heterozygotes one copy and wild type mice will show no amplification when compared to known homozygote controls.

These FAM®-labeled assays are multiplexed with a VIC® labeled endogenous control assay (for example TaqMan® Copy Number Reference Assay, Mouse, Tfrc; Applied Biosystems part #4458366).

Please note that these assays are not gene-specific – other information should be used in conjunction with the universal cassette assays (for example the mutant-specific srPCR) when confirming the gene identity.

Primer type	Assay Name	Forward Primer Seq.	Reverse Primer Seq.	Probe Primer Seq.
Cassette	Neo	GGTGGAGAGGCTATTCGGC	GAACACGGCGGCATCAG	TGGGCACAACAGACAATCGGCT G

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
20x target assay	0.5
ddH2O	3
Tfrc endogenous 20x assay	0.5
DNA	1

### Amplification conditions

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

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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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