

**Gene:** Arid1b

**Colony prefix:** PMAX

**ESC clone ID:**

**Allele:** Arid1b<sup>tm1b(EUCOMM)Hmgu</sup>

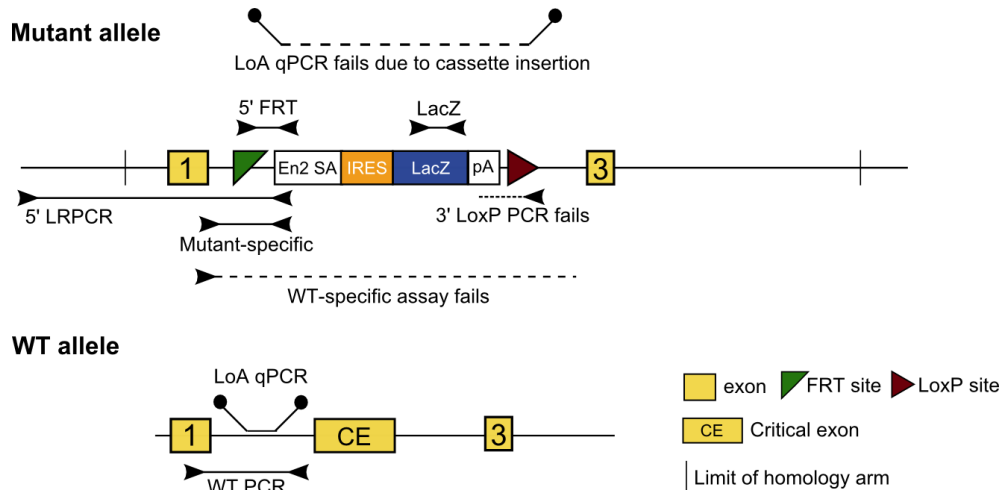
**Allele type:** Reporter-tagged deletion allele (post-cre)

### Allele information:

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

### Mouse QC information

**Promoter Driven:**



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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/78751>

## 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	Wild type	Arid1b_47092_F	Arid1b_47092_R	545
Standard PCR	Mutant	Arid1b_47092_F	CAS_R1_Term	200
Standard PCR	Cassette	LacZ_2_small_F	LacZ_2_small_R	108

## Primer sequences

Primer Name	Primer Sequence (5' > 3')
CAS_R1_Term	TCGTGGTATCGTTATGCGCC
LacZ_2_small_F	ATCACGACGCGCTGTATC
LacZ_2_small_R	ACATCGGGCAAATAATATCG
Arid1b_47092_F	GTGTCCTCTCCCATCTTGCC
Arid1b_47092_R	ACAAATGGCTTGACGGTTCC

## 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
<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	LacZ_reg	GGAGTGCGATCTTCCTGAGG	CGCATCGTAACCGTGCATC	CGATACTGTCGTCGTCGCCCTCAAACCTG

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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## Genotyping by loss of WT allele qPCR Assay (gene-specific assay)

The wild type loss of allele (LoA) qPCR assay uses a hydrolysis probe assay (for example Applied Biosystems TaqMan® technology) to determine the copy number of the wild type allele in a sample. Homozygotes will show no amplification, heterozygotes one copy and wild type mice will show two copies when compared to a wild type control.

The number of copies of the wild type allele can be detected using a FAM-labelled custom qPCR TaqMan® assay. These are multiplexed with a VIC® labelled endogenous control assay (for example TaqMan® Copy Number Reference Assay, Mouse, Tfr; Applied Biosystems part #4458366). Reference DNA controls of known genotypes should also be included to facilitate correct analysis.

## Primers for LoA qPCR assay

Gene	Forward Primer Seq.	Reverse Primer Seq.	Probe Primer Seq.
Arid1b	CCCCTGTAGTAAGGATCCTCCAT	CCCCAAGTTATACACATGGCTGATA	CAGTGCCTGTCCTGAAAAT

### Reaction setup

Reaction setup and amplification conditions are the same as those used for the LacZ<sub>reg</sub> cassette qPCR assay.

## Relevant publications

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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 flox 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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