

**Gene:** Hsp90aa1

**Colony prefix:** MCPD

**ESC clone ID:** EPD0296\_2\_C08

**Allele:** *Hsp90aa1<sup>tm1(KOMP)Wtsi</sup>*

**Allele type:** Reporter-tagged deletion allele

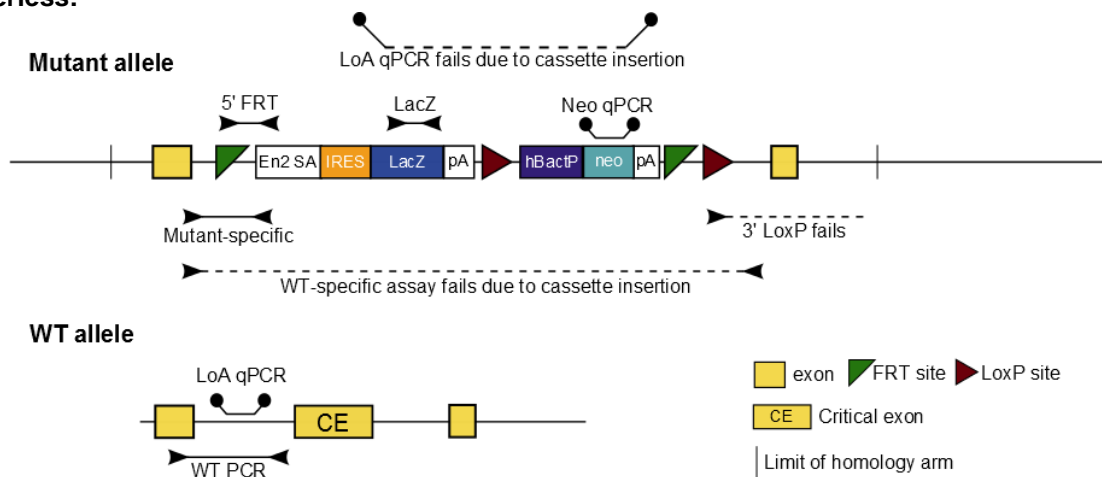
### Allele information:

Further information about the allele can be found on the IKMC web site at <https://www.mousephenotype.org/data/genes/MGI:96250>. Details on how to determine the deleted exon can be found at <http://www.i-dcc.org/kb/entry/21/>

### Mouse QC information

**Promoter Driven:**

**Promoterless:**



<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>	na
<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>	na	<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/69506>

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

General targeting strategies:

<http://www.mousephenotype.org/about-ikmc/targeting-strategies>

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.infrafrontier.eu/sites/infrafrontier.eu/files/upload/public/pdf/Resources%20and%20Services/eucomm\\_komp-csd\\_allele\\_conversion\\_guide\\_v3a\\_2016.pdf](http://www.infrafrontier.eu/sites/infrafrontier.eu/files/upload/public/pdf/Resources%20and%20Services/eucomm_komp-csd_allele_conversion_guide_v3a_2016.pdf)

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	Hsp90aa1_88547_F	Hsp90aa1_88547_R	420
Standard PCR	Mutant	Hsp90aa1_88547_F	CAS_R1_Term	328
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
Hsp90aa1_88547_F	TTAGCACGCCTGCAGCCATTCT
Hsp90aa1_88547_R	AGGGTTCCGGTCCTGTTTGCTGG

## 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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## 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. *Mamm. Genome*, 24, 286–294.

Ryder, E., Doe, B., Gleeson, D., Houghton, R., Dalvi, P., Grau, E., ... Ramirez-Solis, R. (2013). Rapid conversion of EUCOMM/KOMP-CSD alleles in mouse embryos using a cell-permeable Cre recombinase. *Transgenic research*. doi:10.1007/s11248-013-9764-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 floxer 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 FLP<sub>er</sub> (flipper) mice. *Genesis* 28, 106–110.

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