

***Trim28-p.K290R* Mouse Model Strategy**

-CRISPR/Cas9 technology

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Project Overview

Project Name

Trim28-p.K290R

Project type

cas9-ki(PM)

Strain background

C57BL/6JGpt

Technical Description

- The mouse *Trim28* gene has 10 transcripts.
- This project produced *Trim28*-p.K290R point mutation on exon 6 of the transcript of *Trim28*-201(ENSMUST00000005705.7). The 869th nucleotide of *Trim28* CDS is mutated from A to G, The 290th amino acid will be mutated from K(Lys) to R(Arg).
- In this project, *Trim28* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: In vitro, sgRNA and donor vectors were constructed. Cas9, sgRNA and donor were injected into the fertilized eggs of C57BL/6JGpt mice for homologous recombination, and obtained positive F0 mice identified by PCR and sequencing analysis. The stable inheritable positive F1 mice model was obtained by mating F0 mice with C57BL/6JGpt mice.

Analysis of Homology

	1		100
mTrim28-P	(1)	MAASAAATAAASAATAASAASGSPGSGEGSAGGEKR---PAASSAAAASAAASSPAGGGGEAQELLEHCVCRCRRLRPERDPRLLPCLHSACSACLGPAT	
hTRIM28-P	(1)	---MAASAAAASAAAASAASGSPGPEGGSAGGEKRSTAPSAASASASAAASSPAGGGAEALELLEHCVCRCRRLRPEREPRLLPCLHSACSACLGPAA	
Consensus	(1)	AASAAAAA AASAASGSPG GEGSAGGEKR PAAAAAAAASAAASSPAGGGAEA ELLEHCVCRCRRLRPERDPRLLPCLHSACSACLGPA	
	101		200
mTrim28-P	(98)	PAAANNSSGDGGSAGDGMVDCPVCKQCCYSKDIVENYFMRDSGSKASSDSQDANQCCSTCEDNAPATSYCVCSEPLCETCVEAHQRVKYTKDHTVRSSTG	
hTRIM28-P	(97)	PAAANSSGDGGAAGDGTVVDCPVCKQCCFSKDIVENYFMRDSGSKAATDAQDANQCCSTCEDNAPATSYCVCSEPLCETCVEAHQRVKYTKDHTVRSSTG	
Consensus	(101)	PAAAN SDDGGAAGDG MVDCPVCKQCCFSKDIVENYFMRDSGSKAASDAQDANQCCSTCEDNAPATSYCVCSEPLCETCVEAHQRVKYTKDHTVRSSTG	
	201		300
mTrim28-P	(198)	PAKTRDGERIVYCNVHKHEPLVLFCECSDTLTCRDCQLNAHKDHQYQFLEDAVRNQRKLLASLVKRLGDKHATLQKNTKEVRSSIRQVSDVCKRVQVDVK	
hTRIM28-P	(197)	PAKSRDGERIVYCNVHKHEPLVLFCECSDTLTCRDCQLNAHKDHQYQFLEDAVRNQRKLLASLVKRLGDKHATLQKSTKEVRSSIRQVSDVCKRVQVDVK	
Consensus	(201)	PAKSRDGERIVYCNVHKHEPLVLFCECSDTLTCRDCQLNAHKDHQYQFLEDAVRNQRKLLASLVKRLGDKHATLQK TKEVRSSIRQVSDVCKRVQVDVK	
	301		400
mTrim28-P	(298)	MATLQIMKELNKRGRVLVNDAQKVTGEGQERLERQHWMTTKIQKHQEHILRFASWALESDNNTALLSKKLIYFQLHRLAKMIVDPVEPHGEMKFQWDLN	
hTRIM28-P	(297)	MATLQIMKELNKRGRVLVNDAQKVTGEGQERLERQHWMTTKIQKHQEHILRFASWALESDNNTALLSKKLIYFQLHRLAKMIVDPVEPHGEMKFQWDLN	
Consensus	(301)	MATLQIMKELNKRGRVLVNDAQKVTGEGQERLERQHWMTTKIQKHQEHILRFASWALESDNNTALLSKKLIYFQLHRLAKMIVDPVEPHGEMKFQWDLN	
	401		500
mTrim28-P	(398)	AWTKSAEAFGKIVAERPNTSTGPGMAPPRAPGPLSKQGGSSQPMVEVQEGYGFSGS-DDPYSSAEPHVSVMKRSRSGEVEVSGLLRKYPRVSLERLDD	
hTRIM28-P	(397)	AWTKSAEAFGKIVAERPNTSTGPGMAPPRAPGPLSKQGGSSQPMVEVQEGYGFSGSDDPYSSAEPHVSVMKRSRSGEVEVSGLLRKYPRVSLERLDD	
Consensus	(401)	AWTKSAEAFGKIVAERPNTSTGPGMAPPRAPGPLSKQGGSSQPMVEVQEGYGFSGS DDPYSSAEPHVSVMKRSRSGEVEVSGLLRKYPRVSLERLDD	
	501		600
mTrim28-P	(497)	LTSDSQPPVFKVFPSTTEDYNLIVIERGAAAAAGQAGTVPPGAPGAPPLPGMAIVKEEETEAAIGAPPAEPGPKVLMPLTEGPGAEGPRLASPS	
hTRIM28-P	(497)	LTADSQPPVFKVFPSTTEDYNLIVIERGAAAAATGQPGTAPAGTAPGAPPLAGMAIVKEEETEAAIGAPPTATEGPKVLMALAEPGAEGPRLASPS	
Consensus	(501)	LTADSQPPVFKVFPSTTEDYNLIVIERGAAAAA GQ GT P G PGAPPL GMAIVKEEETEAAIGAPP A EGPETKPVLM L EPGAEGPRLASPS	
	601		700
mTrim28-P	(597)	GSTSSGLEVVAVEVTSAPVSGPGLDSDATICRVCQKPGDLVMCNQCEFCFHLDCPLALQDVPGEWVSCSLCHVLPDLKEEDGSLSLDGADSTGVVAKL	
hTRIM28-P	(597)	GSTSSGLEVVAVEVTSAPVSGPGLDSDATICRVCQKPGDLVMCNQCEFCFHLDCPLALQDVPGEWVSCSLCHVLPDLKEEDGSLSLDGADSTGVVAKL	
Consensus	(601)	GSTSSGLEVVAVEV TSAP GPG LDDSDATICRVCQKPGDLVMCNQCEFCFHLDCPLALQDVPGEWVSCSLCHVLPDLKEEDGSLSLDGADSTGVVAKL	
	701		800
mTrim28-P	(697)	SPANQRKRCERVLALFCHPCPRPLHQLATDSTFSLDQPGGTLDLTLIRARLQEKLSPPYSSPQEFADVGRMFKQFNKLTEDKADVQSIIGLQRFFETRM	
hTRIM28-P	(697)	SPANQRKRCERVLALFCHPCPRPLHQLATDSTFSLDQPGGTLDLTLIRARLQEKLSPPYSSPQEFADVGRMFKQFNKLTEDKADVQSIIGLQRFFETRM	
Consensus	(701)	SPANQRKRCERVLALFCHPCPRPLHQLATDSTFSLDQPGGTLDLTLIRARLQEKLSPPYSSPQEFADVGRMFKQFNKLTEDKADVQSIIGLQRFFETRM	
	801		839
mTrim28-P	(797)	NDAFGDTKFSAVLVEPPPLNLPAGLSSQELSG-PGDGP	
hTRIM28-P	(797)	NEAFGDTKFSAVLVEPPPLNLPAGLSSQELSG-PGDGP	
Consensus	(801)	NDAFGDTKFSAVLVEPPPL LP AGLSSQELSG PGDGP	



hTRIM28-P-289K-mTrim28-P-290K

Identity positions: 93.1%

Mutation Site

Before mutation

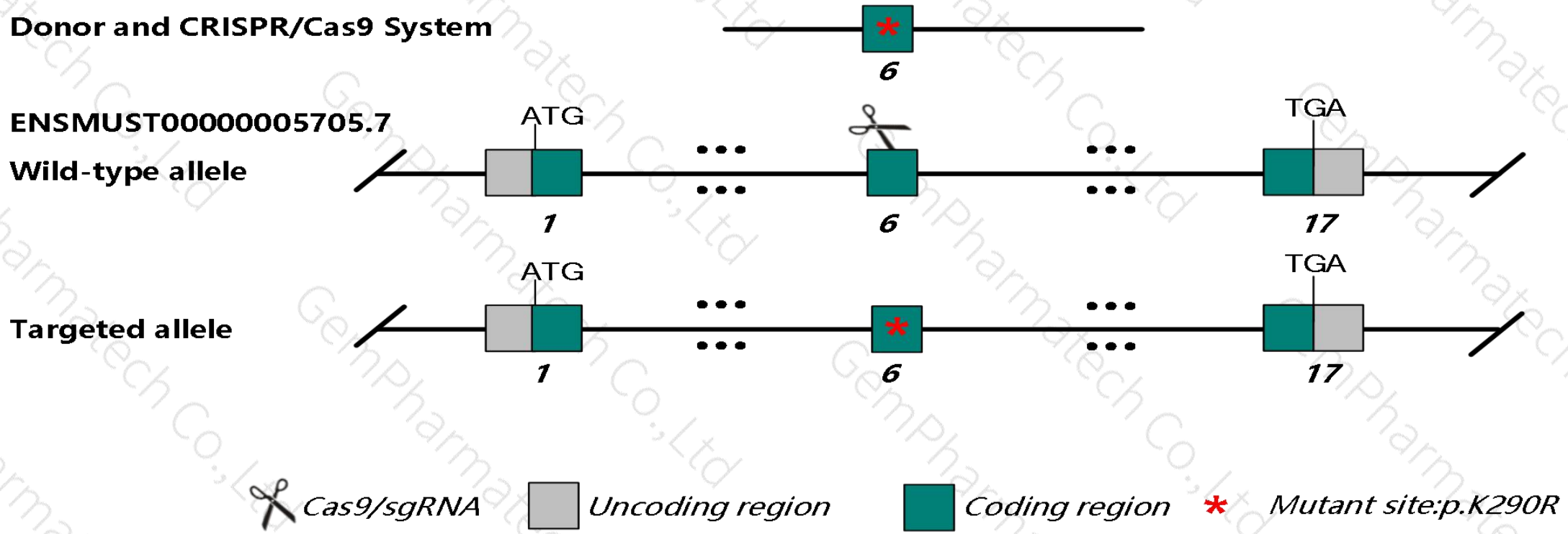
```
+2      I R Q V S D V Q K R V Q V D V K M A I L Q I M K E L N K R G R?  
38901  CCATTAGGAT CCGCCAGGTG TCTGATGTGC AGAAGCGAGT GCAGGTTGAT GTCAAGATGG CCATTCTGCA GATCATGAAG GAGCTGAATA AGCGGGGTCG  
      GGTAATCCTA GGCGGTCCAC AGACTACACG TCTCCGCTCA CGTCCAACCTA CAGTTCTACC GGTAAGACGT CTAGTACTTC CTCGACTTAT TCGCCCCAGC  
+2 ?R V L V N D A Q  
39001  AGTTCGGTC AATGATGCC AGGTAAACT TAGGGCTGCA AGGGTATTAA CCAACCTCTC CTTTCTTGTT TTTTGGTTTT TTTTGTGTTT TTGTTTTTTT  
      TCAAGACCAG TTACTIONG TCCATTTTGA ATCCCGACGT TCCATAAATT GGTTGGAGAG GAAAGAACAA AAAACCAAAA AAAAACAAAA AACAAAAAAA
```

After mutation

```
+2      I R Q V S D V Q R R V Q V D V K M A I L Q I M K E L N K R G R?  
38901  CCATTAGGAT CCGCCAGGTG TCTGATGTGC AGAGGCGAGT GCAGGTTGAT GTCAAGATGG CCATTCTGCA GATCATGAAG GAGCTGAATA AGCGGGGTCG  
      GGTAATCCTA GGCGGTCCAC AGACTACACG TCTCCGCTCA CGTCCAACCTA CAGTTCTACC GGTAAGACGT CTAGTACTTC CTCGACTTAT TCGCCCCAGC  
+2 ?R V L V N D A Q  
39001  AGTTCGGTC AATGATGCC AGGTAAACT TAGGGCTGCA AGGGTATTAA CCAACCTCTC CTTTCTTGTT TTTTGGTTTT TTTTGTGTTT TTGTTTTTTT  
      TCAAGACCAG TTACTIONG TCCATTTTGA ATCCCGACGT TCCATAAATT GGTTGGAGAG GAAAGAACAA AAAACCAAAA AAAAACAAAA AACAAAAAAA
```

The yellow region is exon6 of *Trim28-201*, and the red region represents the p.K290R mutation site.

This model uses CRISPR/Cas9 technology to edit the *Trim28* gene and the schematic diagram is as follow:



- According to the data of MGI, homozygotes for a targeted disruption of this gene develop normally until the blastocyst stage and undergo uterine implantation, but become arrested at the early egg-cylinder stage, fail to gastrulate, and are completely resorbed by E8.5.
- One or two synonymous mutations of amino acids will be introduced on exon6 of *Trim28*.
- Mouse *Trim28* gene is located on Chr7. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr7, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Gene name and location (NCBI)

Trim28 tripartite motif-containing 28 [*Mus musculus* (house mouse)]

Gene ID: 21849, updated on 4-Oct-2020

Summary



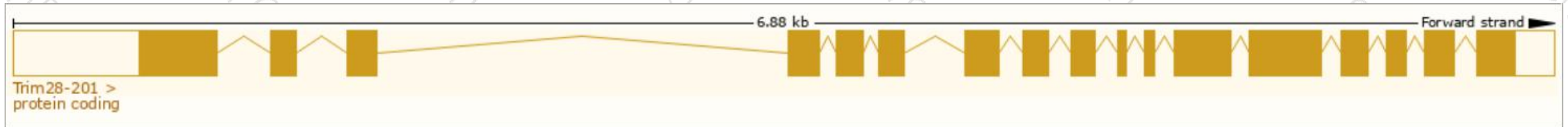
Official Symbol	Trim28 provided by MGI
Official Full Name	tripartite motif-containing 28 provided by MGI
Primary source	MGI:MGI:109274
See related	Ensembl:ENSMUSG00000005566
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	KAP; KRI; Tif1; KAP-1; Tif1b; KRIP-1; MommeD; MommeD9; AA408787; Tif1beta
Expression	Ubiquitous expression in ovary adult (RPKM 143.7), CNS E11.5 (RPKM 133.2) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

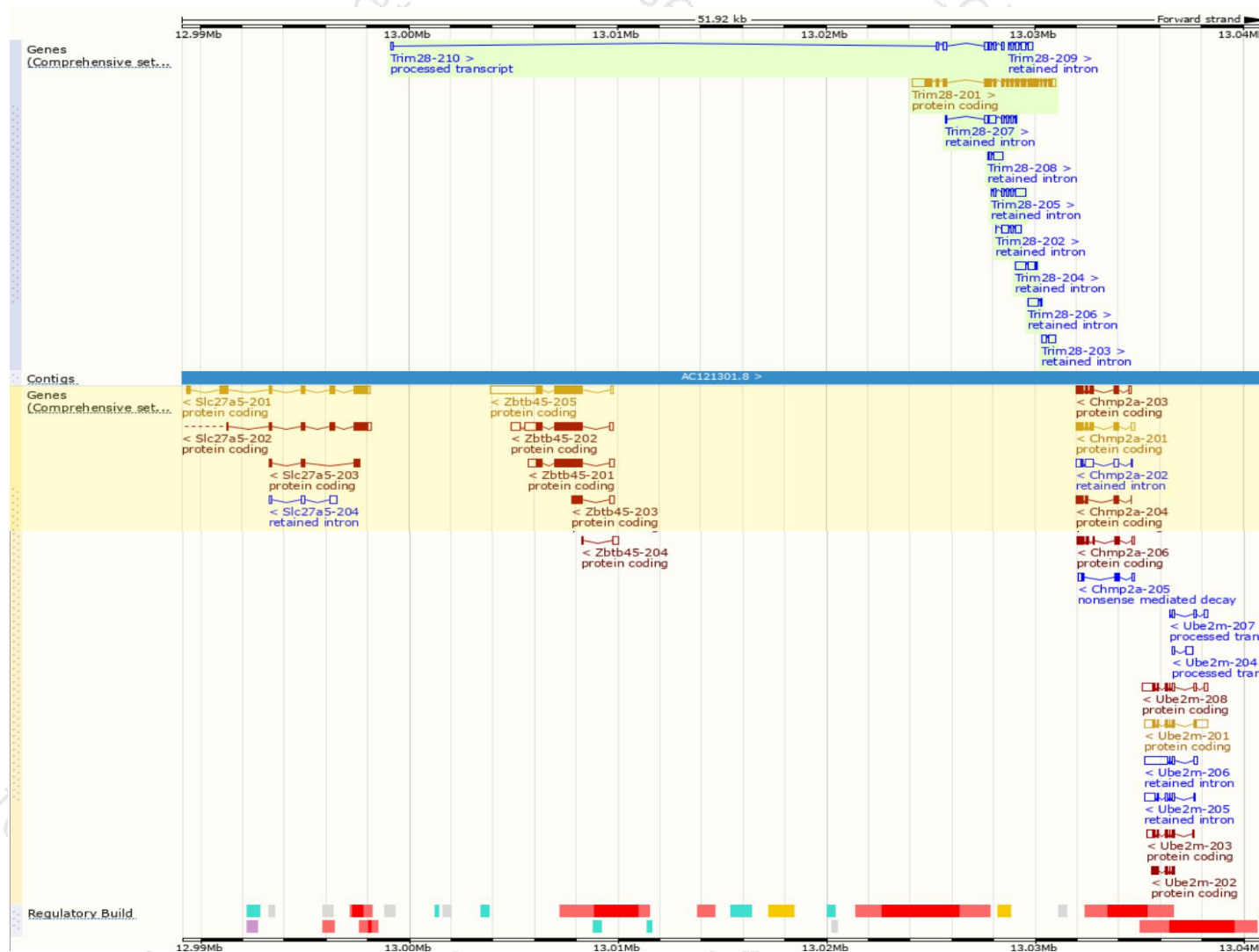
The gene has 10 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Trim28-201	ENSMUST00000005705.7	3245	834aa	Protein coding	CCDS20823	Q5EBP9 Q62318	TSL:1 GENCODE basic APPRIS P1
Trim28-210	ENSMUST00000209577.1	851	No protein	Processed transcript	-	-	TSL:3
Trim28-205	ENSMUST00000128951.8	1004	No protein	Retained intron	-	-	TSL:3
Trim28-207	ENSMUST00000142421.8	864	No protein	Retained intron	-	-	TSL:5
Trim28-204	ENSMUST00000125136.2	846	No protein	Retained intron	-	-	TSL:2
Trim28-209	ENSMUST00000149061.2	783	No protein	Retained intron	-	-	TSL:3
Trim28-202	ENSMUST00000123603.2	767	No protein	Retained intron	-	-	TSL:3
Trim28-208	ENSMUST00000148354.2	608	No protein	Retained intron	-	-	TSL:2
Trim28-203	ENSMUST00000123778.1	565	No protein	Retained intron	-	-	TSL:1
Trim28-206	ENSMUST00000129122.1	562	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Trim28-201* transcript, the transcription is shown below:



Genomic location distribution



Protein domain

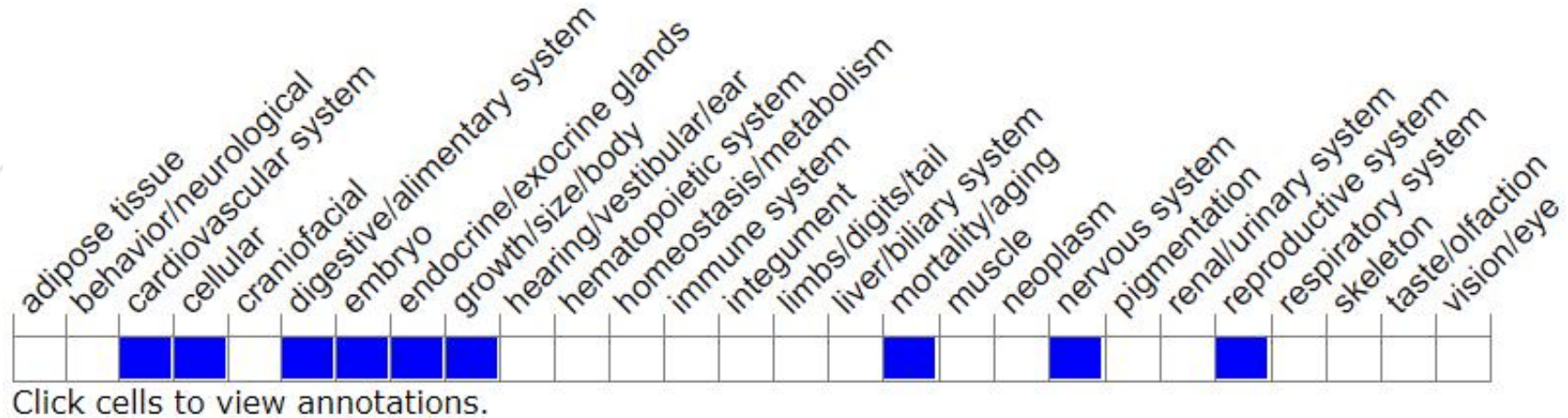


Mouse phenotype description(MGI)

URL link is as follows:

<http://www.informatics.jax.org/marker/MGI:109274>

Phenotype Overview ?



Homozygotes for a targeted disruption of this gene develop normally until the blastocyst stage and undergo uterine implantation, but become arrested at the early egg-cylinder stage, fail to gastrulate, and are completely resorbed by E8.5.

If you have any questions, please feel free to contact us.

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