Tac1-P2A-iCre Cas9-KI Strategy

Designer:	**Yanhua Shen**
Reviewer:	**Xiaojing Li**
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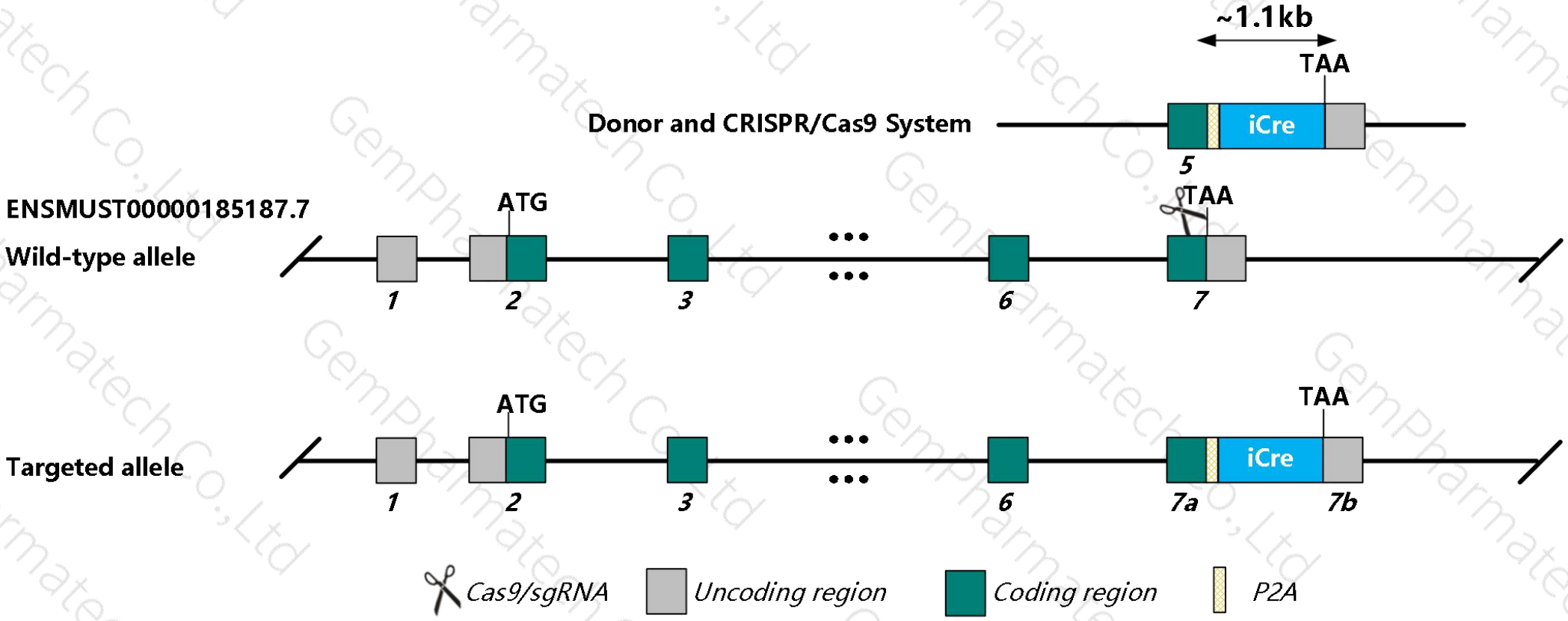
GemPharmatech Co.,Ltd

Project Overview

Project Name	***Tac1-P2A-iCre***
Project type	**Cas9-KI**
Strain background	**C57BL/6JGpt**

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Tac1* gene. The schematic diagram is as follows:



Technical routes

- The *Tac1* gene has 5 transcripts.According to the structure of *Tac1* gene, *Tac1-205*(ENSMUST00000185187.7) is selected for presentation of the recommended strategy.

- *Tac1-205* gene has 7 exons, with the ATG start codon in exon2 and TAA stop codon in exon7.

- We make *Tac1-P2A-iCre* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near stop coding(TAA) of *Tac1* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in *P2A-iCre* before stop coding(TAA) of *Tac1* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

Notice

- According to the existing MGI data, mice homozygous for a knock-out allele exhibit absence of agonist-induced hyperalgesia and an impaired response to chemical irritation of the urinary tract. Mice homozygous for a different knock-out allele display hypoalgesia, and reduced anxiety- and depression-related behaviors.
- According to the existing JAX data, Cre recombinase expression is observed in accessory olfactory bulb and anterior olfactory nucleus, thalamus, ventromedial hypothalamus and midbrain structures (such as superior colliculus), as well as in scattered cells of the caudate, septum, hypothalamus, midbrain, hindbrain and cerebellum.
- Insertion of iCre may affect the regulation of the 3' end of the *Tac1* gene.
- There may be 2 to 4 bases mutation in intron6-7 or 3'UTR of *Tac1* gene in this strategy.
- The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide.
- The *Tac1* gene is located on the Chr6. If the knockin mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- This strategy is designed based on genetic information in existing databases. Due to the complexity of gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information （NCBI）

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Tac1 tachykinin 1 [*Mus musculus* (house mouse)]

Gene ID: 21333, updated on 12-Aug-2019

Summary

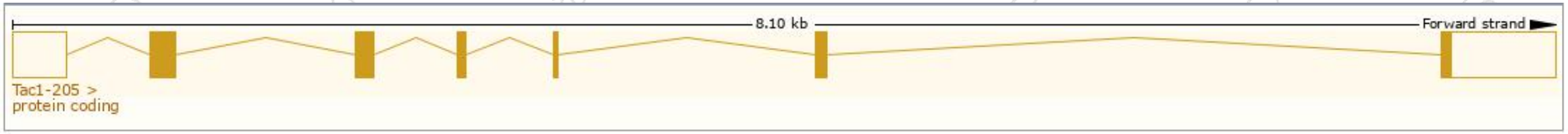
Official Symbol	Tac1 provided by MGI
Official Full Name	tachykinin 1 provided by MGI
Primary source	MGI:MGI:98474
See related	Ensembl:ENSMUSG00000061762
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	SP; NK1; NK-1; Nkna; PPTA; PPT-A; 4930528L02Rik
Expression	Biased expression in CNS E18 (RPKM 16.9), whole brain E14.5 (RPKM 7.7) and 8 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

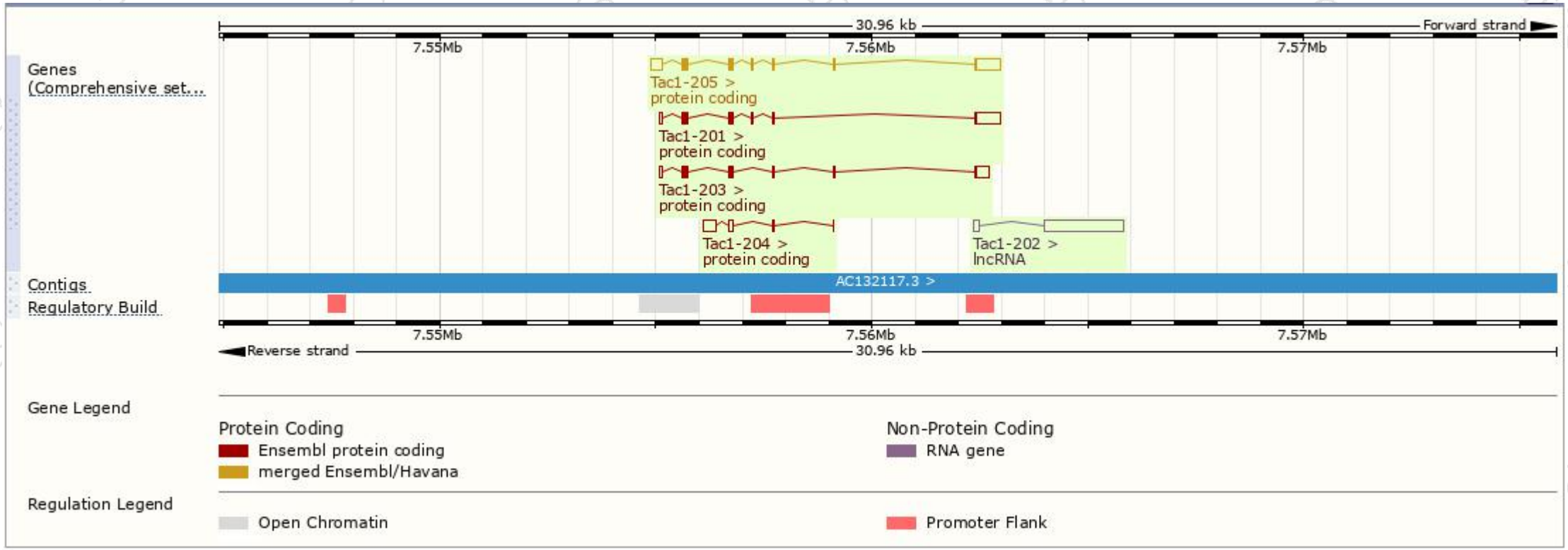
The gene has 5 transcripts, and the transcript is shown below：

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tac1-205	ENSMUST00000185187.7	1230	130aa	Protein coding	CCDS19906	P41539 Q149W7	TSL:1 GENCODE basic APPRIS P1
Tac1-203	ENSMUST00000184986.1	691	115aa	Protein coding	CCDS80486	B7ZMQ2 P41539	TSL:1 GENCODE basic
Tac1-201	ENSMUST00000090679.8	980	112aa	Protein coding	-	A0A0A0MQA8	TSL:5 GENCODE basic
Tac1-204	ENSMUST00000185130.1	434	15aa	Protein coding	-	V9GX83	CDS 3' incomplete TSL:5
Tac1-202	ENSMUST00000184481.1	1975	No protein	lncRNA	-	-	TSL:1

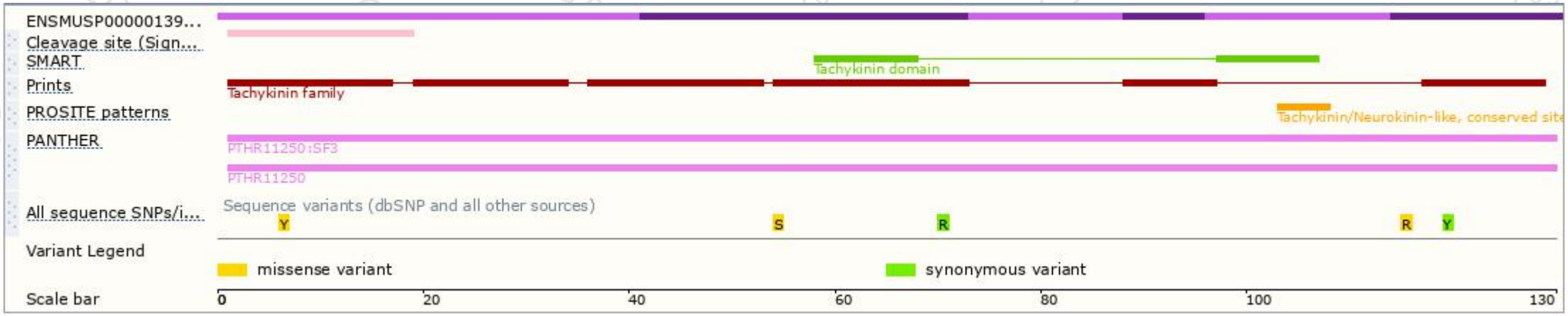
The strategy is based on the design of *Tac1-205* transcript,The transcription is shown below



Genomic location distribution



Protein domain



Targeted Progress (from JAX)

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Detailed Description

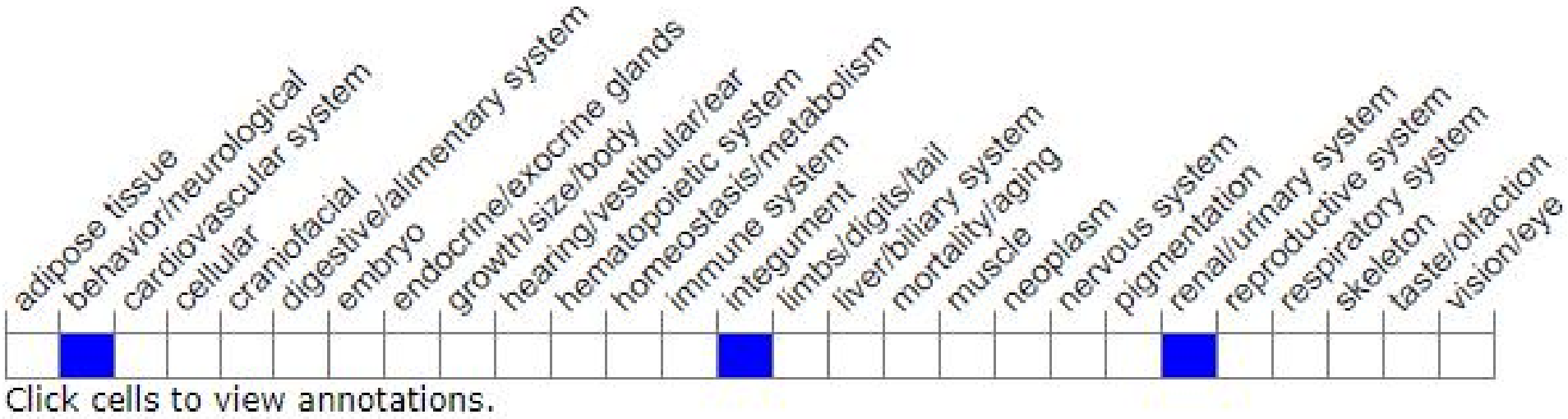
The Tac1-IRES2-Cre-D (also called Tac1-IRES2-Cre-Δ or Tac1-IRES2-Cre-Δneo/hygro) targeted mutation has an IRES2 sequence and a Cre recombinase gene inserted downstream of the translational STOP codon of the tachykinin 1 gene (*Tac1*). As such, Tac1-IRES2-Cre-D mice have both endogenous gene and Cre recombinase expression directed to *Tac1*-expressing cells by the endogenous promoter/enhancer elements of the tachykinin 1 locus. When Tac1-IRES2-Cre-D mice are bred with mice containing *loxP*-flanked sequences, Cre-mediated recombination will result in deletion of the floxed sequences in *Tac1*-expressing cells in the offspring. Cre recombinase expression is observed in a subset of Tac1+ cells, with a pattern very similar to that of the endogenous gene. Specifically, Cre recombinase expression is observed in accessory olfactory bulb and anterior olfactory nucleus, thalamus, VMH and midbrain structures (such as superior colliculus), as well as in scattered cells of the caudate, septum, hypothalamus, midbrain, hindbrain and cerebellum. The donating investigator did not examine *cre* expression in tissues other than brain. Heterozygous mice are viable and fertile with no reported gross physical or behavioral abnormalities. The donating investigator has not attempted to generate homozygous mice to date (April 2013).

For characterization information, see images at the Allen Institute for Brain Science website (Tac1-IRES2-Cre images).

Expressed Gene	*cre*, cre recombinase, bacteriophage P1
Site of Expression	Cre recombinase expression is observed in accessory olfactory bulb and anterior olfactory nucleus, thalamus, ventromedial hypothalamus and midbrain structures (such as superior colliculus), as well as in scattered cells of the caudate, septum, hypothalamus, midbrain, hindbrain and cerebellum.
Strain of Origin	(129S6/SvEvTac x C57BL/6NCrl)F1
Chromosome	6

https://www.jax.org/strain/021877

Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/) .

Mice homozygous for a knock-out allele exhibit absence of agonist-induced hyperalgesia and an impaired response to chemical irritation of the urinary tract. Mice homozygous for a different knock-out allele display hypoalgesia, and reduced anxiety- and depression-related behaviors.

If you have any questions, you are welcome to inquire.
Tel: 025-5864 1534

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GemPharmatech Co.,Ltd