Trim28 Cas9-CKO Strategy

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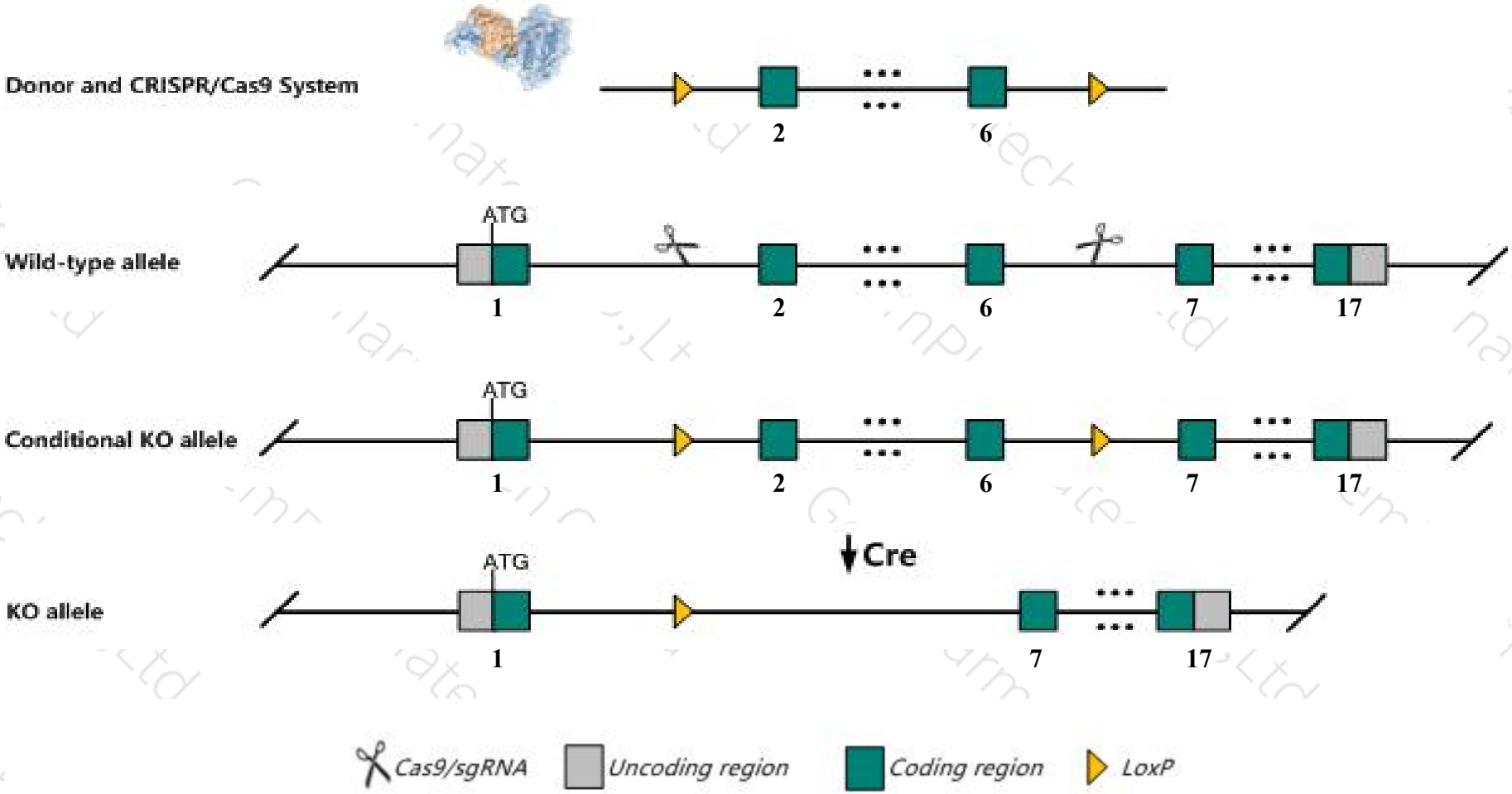
Design Date	**2018-6-21**

Project Overview

Project Name	***Trim28***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Trim28* gene. The schematic diagram is as follows:



Technical routes

- The *Trim28* gene has 10 transcripts. According to the structure of *Trim28* gene, exon2-exon6 of *Trim28-201* (ENSMUST00000005705.7) transcript is recommended as the knockout region.The region contains 614bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Trim28* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice.Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

- The flox mice was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Notice

➢ According to the existing MGI data,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.

➢ The *Trim28* gene is located on the Chr7. If the knockout 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 biological processes, all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information（NCBI）

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

Gene ID: 21849, updated on 21-Aug-2019

Summary

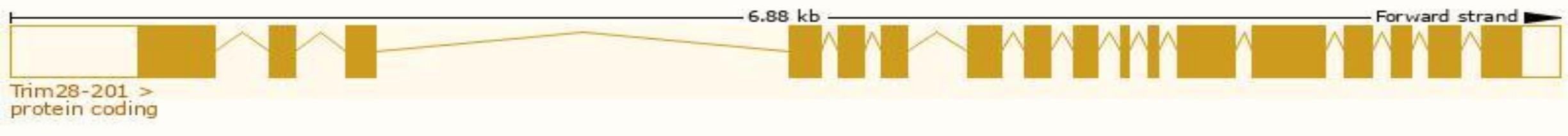
Official Symbol	Trim28 provided by MGI
Official Full Name	tripartite motif-containing 28 provided by MGI
Primary source	MGI:MGI:109274
See related	Ensembl:ENSMUSG00000005566
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	KAP-1; Tif1b; KRIP-1; 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）

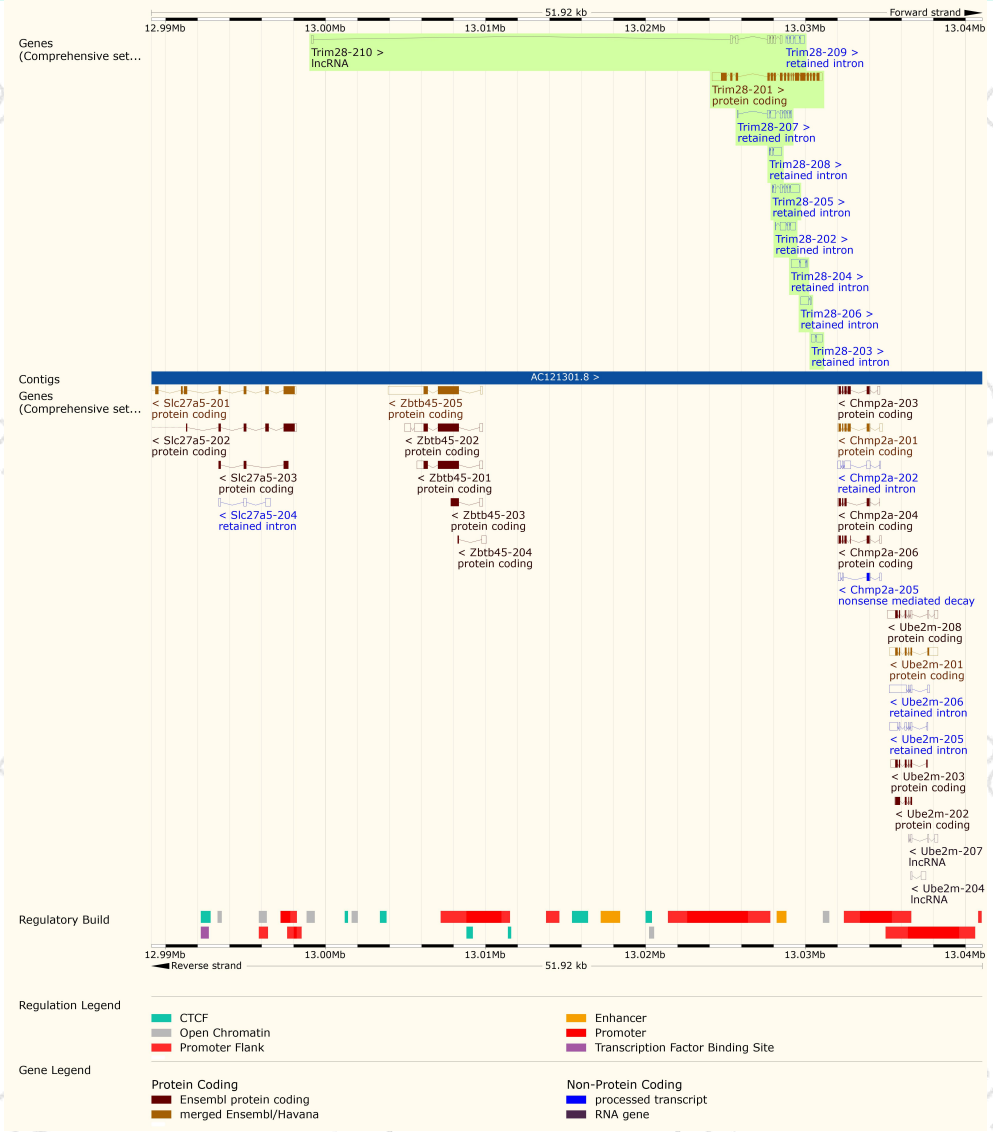
The gene has 10 transcripts,all transcripts are shown below:

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

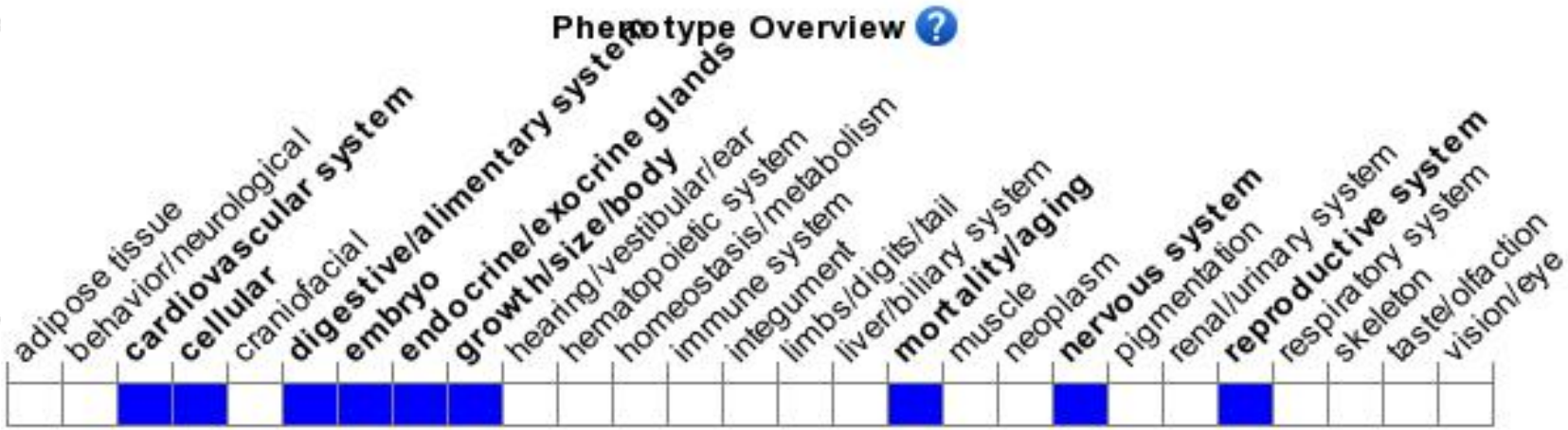


Genomic location distribution



Protein domain

Mouse phenotype description(MGI)



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

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If you have any questions, you are welcome to inquire.

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