

# John Start Trim21 Cas9-CKO Strategy Ronald Color

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# **Project Overview**



Project Name Trim21

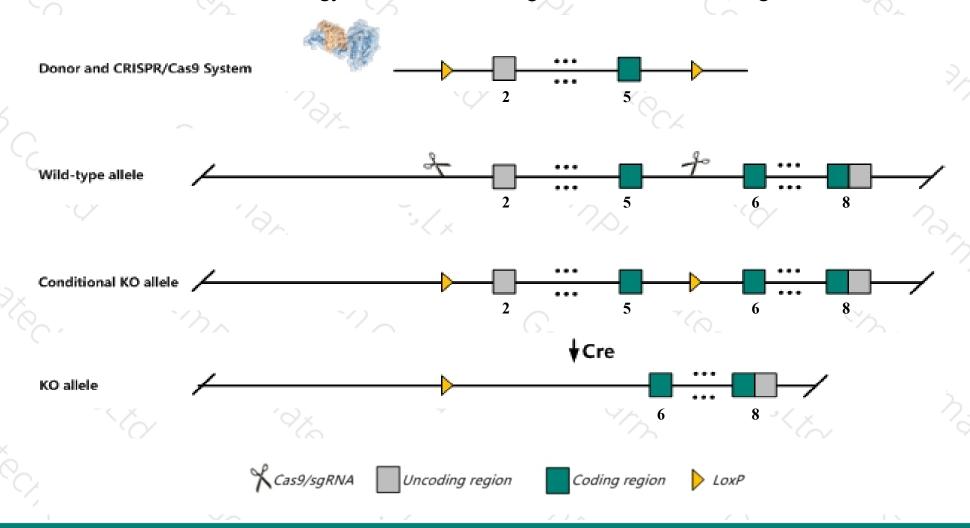
Project type Cas9-CKO

Strain background C57BL/6J

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Trim21* gene. The schematic diagram is as follows:



## **Technical routes**



- ➤ The *Trim21* gene has 5 transcripts. According to the structure of *Trim21* gene, exon2-exon5 of *Trim21-201* (ENSMUST00000033264.11) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Trim21* 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/6J 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/6J 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, Unmanipulated homozygous mice are normal, but leads to tissue inflammation and systemic autoimmunity in vivo and reduced number of CD11c+ dendritic cells from mutant bone marrow in vitro.
- > The *Trim21* 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)



#### Trim21 tripartite motif-containing 21 [Mus musculus (house mouse)]

Gene ID: 20821, updated on 7-Apr-2019

#### Summary

☆ ?

Official Symbol Trim21 provided by MGI

Official Full Name tripartite motif-containing 21 provided by MGI

Primary source MGI:MGI:106657

See related Ensembl: ENSMUSG00000030966

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 Ro52, Ssa1

Expression Ubiquitous expression in thymus adult (RPKM 5.4), bladder adult (RPKM 4.4) and 27 other tissuesSee more

Orthologs <u>human</u> <u>all</u>

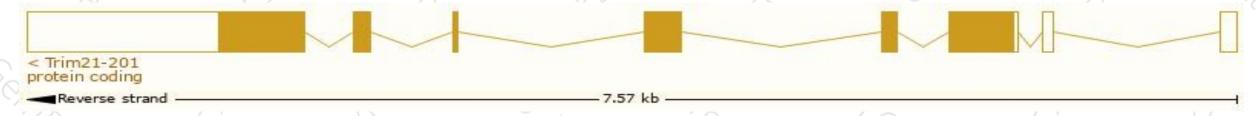
# Transcript information (Ensembl)



The gene has 5 transcripts, all transcripts are shown below:

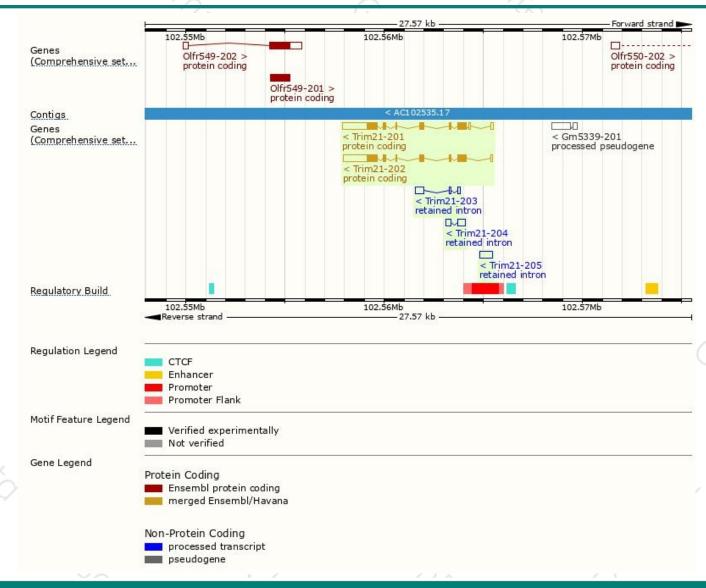
N	Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Trir	m21-201	ENSMUST00000033264.11	2793	<u>462aa</u>	Protein coding	CCDS40051	<u>Q3U7K7</u>	TSL:1 GENCODE basic APPRIS P1
Trin	m21-202	ENSMUST00000106913.2	2682	<u>462aa</u>	Protein coding	CCDS40051	<u>Q3U7K7</u>	TSL:1 GENCODE basic APPRIS P1
Trin	m21-203	ENSMUST00000209679.1	666	No protein	Retained intron	-	-	TSL:2
Trin	m21-204	ENSMUST00000209907.1	642	No protein	Retained intron	-	-	TSL:2
Trin	m21-205	ENSMUST00000210261.1	634	No protein	Retained intron	-	-	TSL:NA

The strategy is based on the design of *Trim21-201* transcript, The transcription is shown below



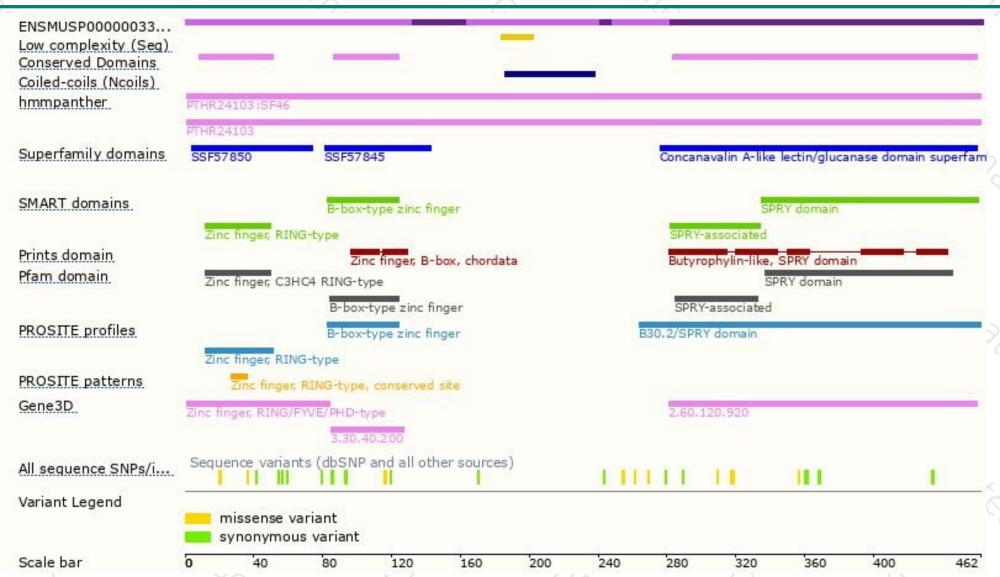
## Genomic location distribution





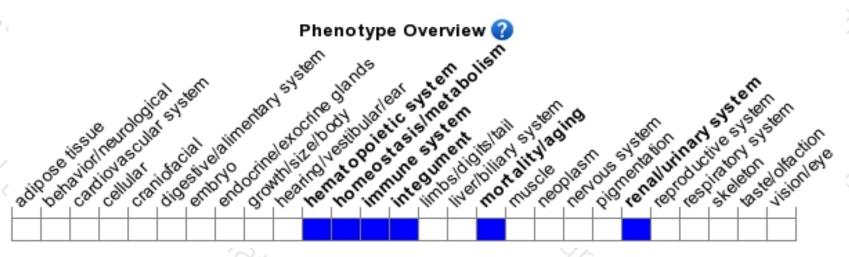
### Protein domain





## Mouse phenotype description(MGI)





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

According to the existing MGI data, Unmanipulated homozygous mice are normal, but leads to tissue inflammation and systemic autoimmunity in vivo and reduced number of CD11c+ dendritic cells from mutant bone marrow in vitro.



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

Tel: 025-5864 1534





