

# ***Tmbim4 Cas9-CKO Strategy***

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**Reviewer:**

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# Project Overview

**Project Name**

***Tmbim4***

**Project type**

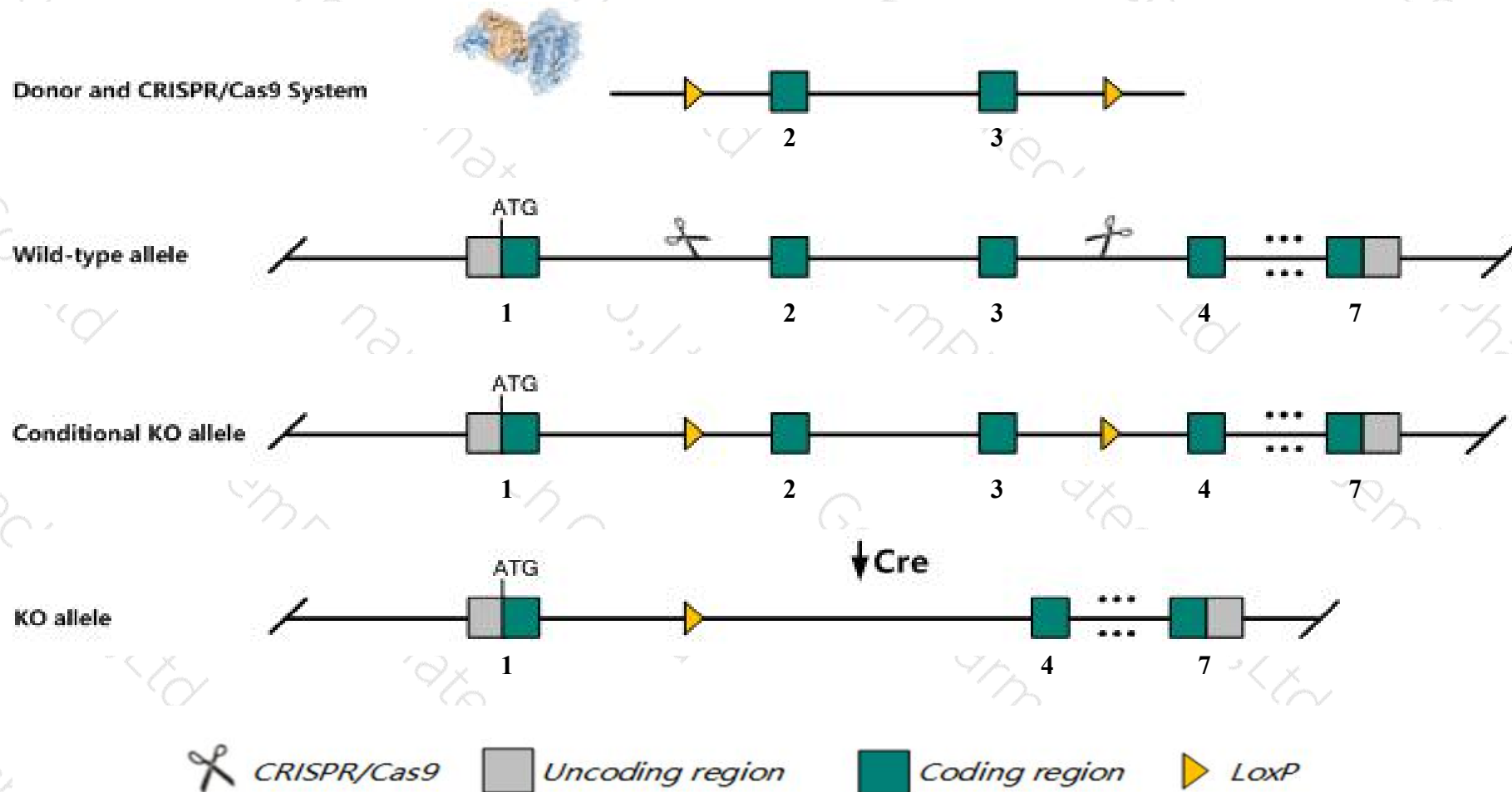
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Tmbim4* gene. The schematic diagram is as follows:



# Technical routes

- The *Tmbim4* gene has 10 transcripts. According to the structure of *Tmbim4* gene, exon2-exon3 of *Tmbim4-201*(ENSMUST00000020446.10) transcript is recommended as the knockout region. The region contains 215bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Tmbim4* gene. The brief process is as follows: CRISPR/Cas9 system 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 will be 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

- The *Tmbim4* gene is located on the Chr10. 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)

## Tmbim4 transmembrane BAX inhibitor motif containing 4 [ *Mus musculus* (house mouse) ]

Gene ID: 68212, updated on 26-Jun-2020

### Summary

<b>Official Symbol</b>	Tmbim4 provided by <a href="#">MGI</a>
<b>Official Full Name</b>	transmembrane BAX inhibitor motif containing 4 provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:1915462</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000020225</a>
<b>Gene type</b>	protein coding
<b>RefSeq status</b>	PROVISIONAL
<b>Organism</b>	<a href="#">Mus musculus</a>
<b>Lineage</b>	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
<b>Also known as</b>	AU022431; 0610007H07Rik
<b>Expression</b>	Ubiquitous expression in kidney adult (RPKM 273.2), genital fat pad adult (RPKM 142.9) and 28 other tissues <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>

# Transcript information (Ensembl)

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

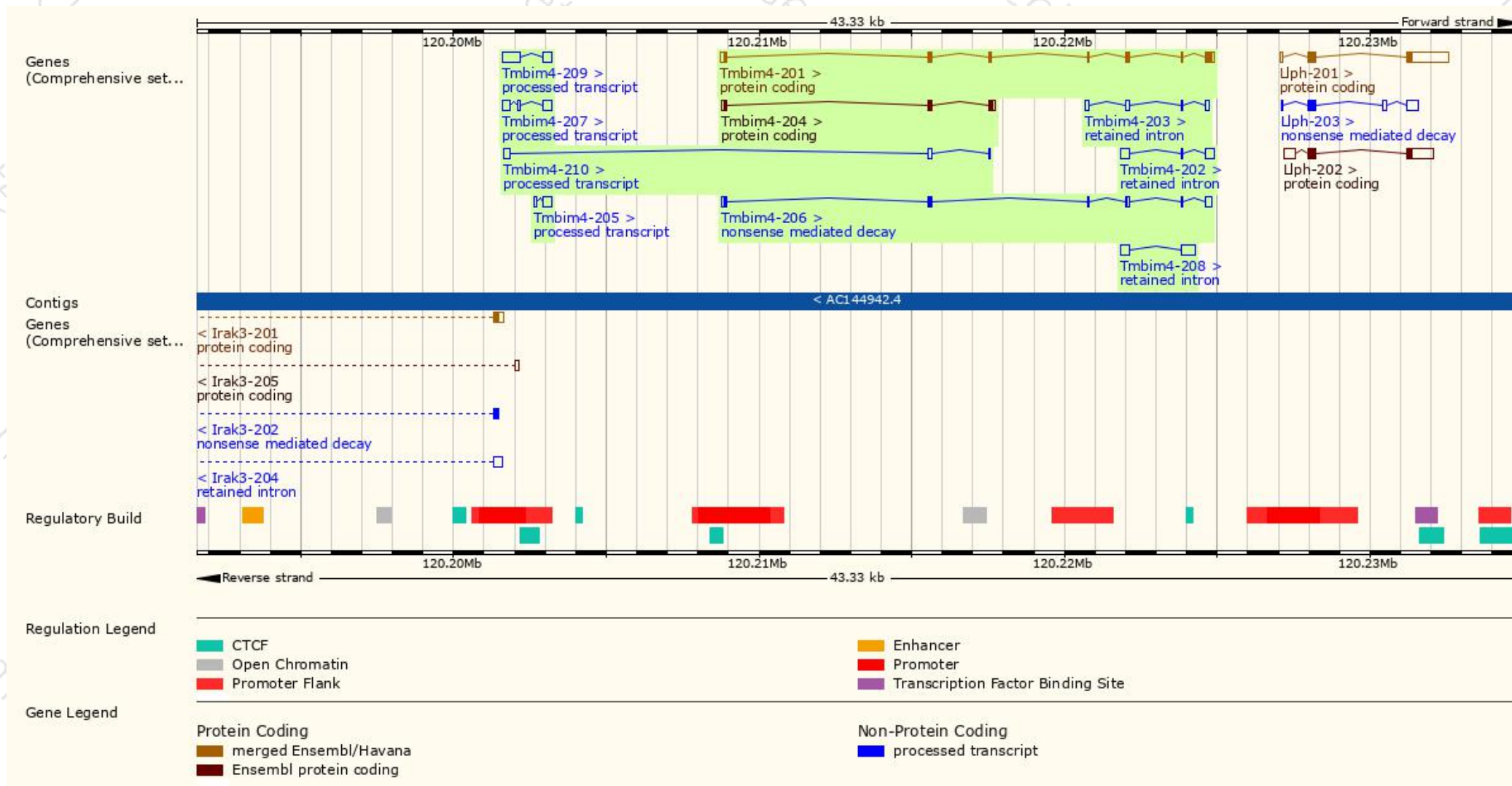
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tmbim4-208	<a href="#">ENSMUST00000148678.2</a>	739	No protein	Retained intron	-	-	TSL:2
Tmbim4-202	<a href="#">ENSMUST00000123802.6</a>	647	No protein	Retained intron	-	-	TSL:2
Tmbim4-203	<a href="#">ENSMUST00000134203.7</a>	381	No protein	Retained intron	-	-	TSL:2
Tmbim4-201	<a href="#">ENSMUST00000020446.10</a>	904	238aa	Protein coding	<a href="#">CCDS24205</a>	<a href="#">Q9DA39</a>	TSL:1 GENCODE basic APPRIS P1
Tmbim4-204	<a href="#">ENSMUST00000134797.7</a>	486	120aa	Protein coding	-	<a href="#">Q8K191</a>	TSL:1 GENCODE basic
Tmbim4-209	<a href="#">ENSMUST00000156247.1</a>	874	No protein	Processed transcript	-	-	TSL:1
Tmbim4-207	<a href="#">ENSMUST00000145015.7</a>	593	No protein	Processed transcript	-	-	TSL:1
Tmbim4-210	<a href="#">ENSMUST00000156877.7</a>	360	No protein	Processed transcript	-	-	TSL:2
Tmbim4-205	<a href="#">ENSMUST00000135794.1</a>	318	No protein	Processed transcript	-	-	TSL:5
Tmbim4-206	<a href="#">ENSMUST00000141206.7</a>	677	93aa	Nonsense mediated decay	-	<a href="#">D6RIR2</a>	TSL:1

The strategy is based on the design of *Tmbim4-201* transcript,the transcription is shown below:





# Genomic location distribution





# Protein domain



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

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