

# ***Tpo Cas9-CKO Strategy***

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**Reviewer:**

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# Project Overview

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**Project Name**

*Tpo*

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**Project type**

**Cas9-CKO**

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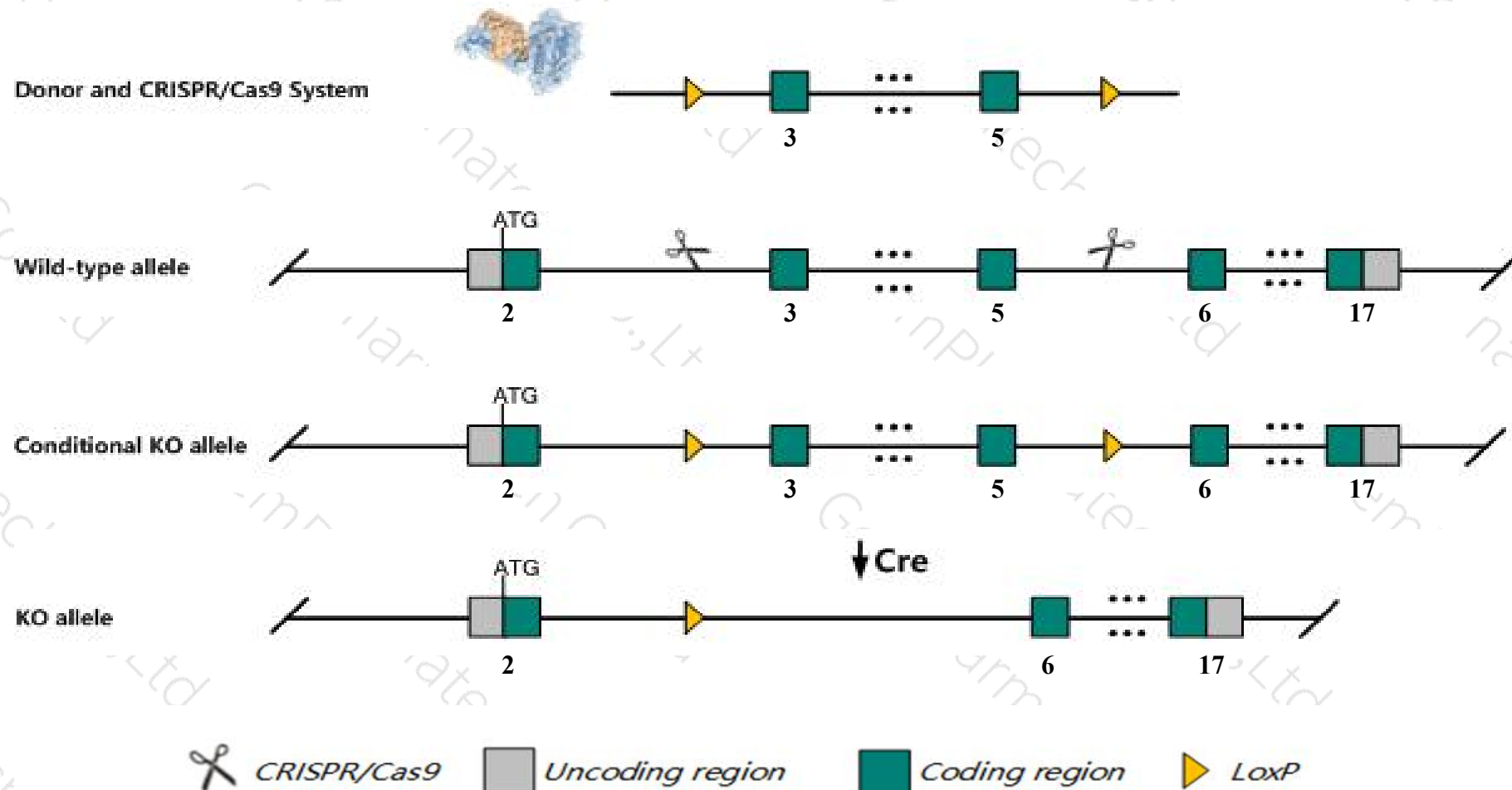
**Strain background**

**C57BL/6JGpt**

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# Conditional Knockout strategy

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



- The *Tpo* gene has 3 transcripts. According to the structure of *Tpo* gene, exon3-exon5 of *Tpo-201* (ENSMUST00000021005.14) transcript is recommended as the knockout region. The region contains 370bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Tpo* 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.

- According to the existing MGI data, Homozygous mice with a missense mutation exhibit hypothyroid dwarfism, including a goiter with colloid deficiency and abnormal follicle epithelium, reduced hematocrit and red blood cells and a lifespan of about 3 months.
- The *Tpo* gene is located on the Chr12. 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)

## Tpo thyroid peroxidase [ *Mus musculus* (house mouse) ]

Gene ID: 22018, updated on 12-Aug-2019

### Summary

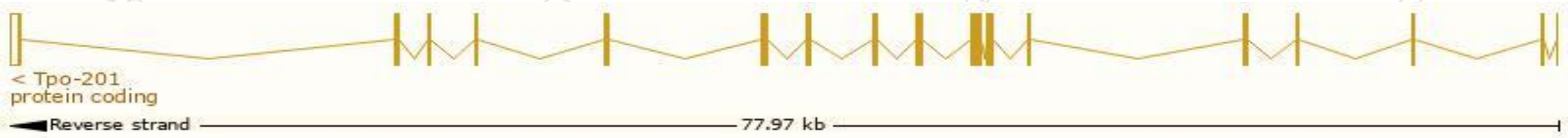
Official Symbol	Tpo provided by <a href="#">MGI</a>
Official Full Name	thyroid peroxidase provided by <a href="#">MGI</a>
Primary source	<a href="#">MGI:MGI:98813</a>
See related	<a href="#">Ensembl:ENSMUSG00000020673</a>
Gene type	protein coding
RefSeq status	REVIEWED
Organism	<a href="#">Mus musculus</a>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Summary	This gene encodes a membrane-bound glycoprotein. The encoded enzyme plays a central role in thyroid gland function. The enzyme functions in the iodination of tyrosine residues in thyroglobulin and phenoxy-ester formation between pairs of iodinated tyrosines to generate the thyroid hormones, thyroxine and triiodothyronine. Mice with homozygous missense mutations in this gene exhibit hypothyroid dwarfism and hearing impairment. [provided by RefSeq, Sep 2015]
Expression	Low expression observed in reference dataset <a href="#">See more</a>
Orthologs	<a href="#">human</a> <a href="#">all</a>

# Transcript information (Ensembl)

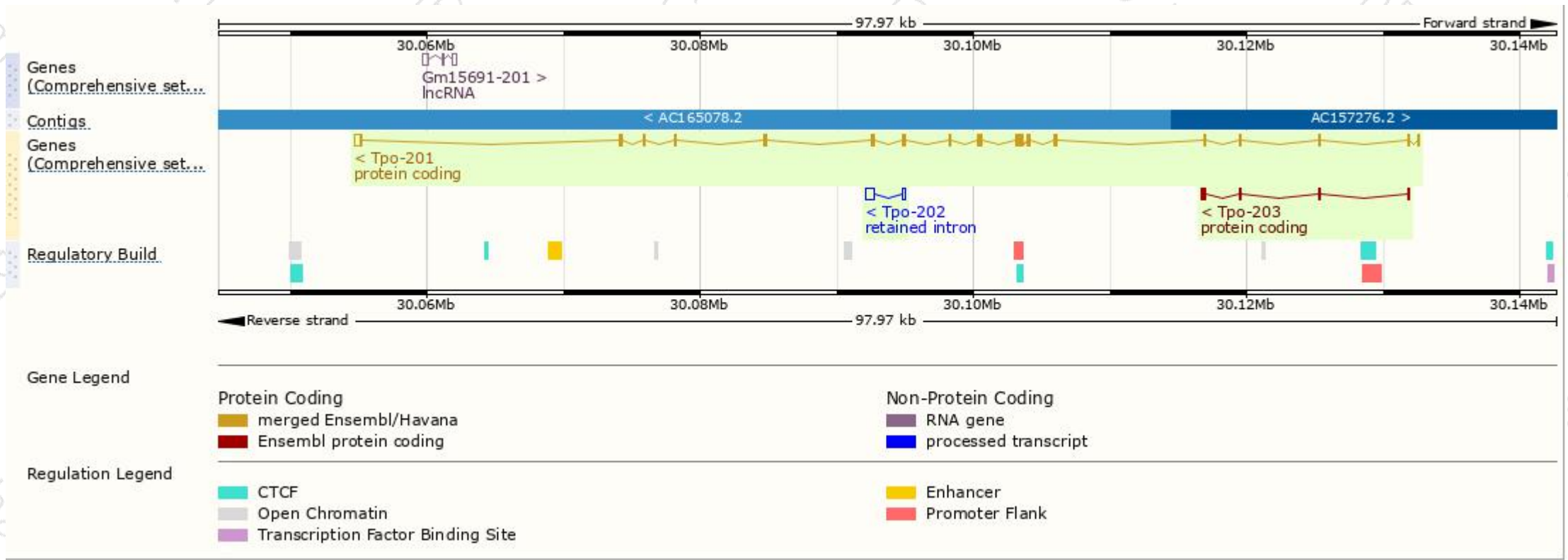
The gene has 3 transcripts,all transcripts are shown below:

Name ▲	Transcript ID ▲	bp ▲	Protein ▲	Biotype ▲	CCDS ▲	UniProt ▲	Flags ▲
Tpo-201	<a href="#">ENSMUST00000021005.14</a>	3299	<a href="#">914aa</a>	Protein coding	<a href="#">CCDS25857</a>	<a href="#">P35419</a>	TSL:1 GENCODE basic APPRIS P1
Tpo-202	<a href="#">ENSMUST00000140875.1</a>	851	No protein	Retained intron	-	-	TSL:3
Tpo-203	<a href="#">ENSMUST00000155263.1</a>	664	<a href="#">164aa</a>	Protein coding	-	<a href="#">G3UXW8</a>	TSL:1 GENCODE basic

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



# Genomic location distribution

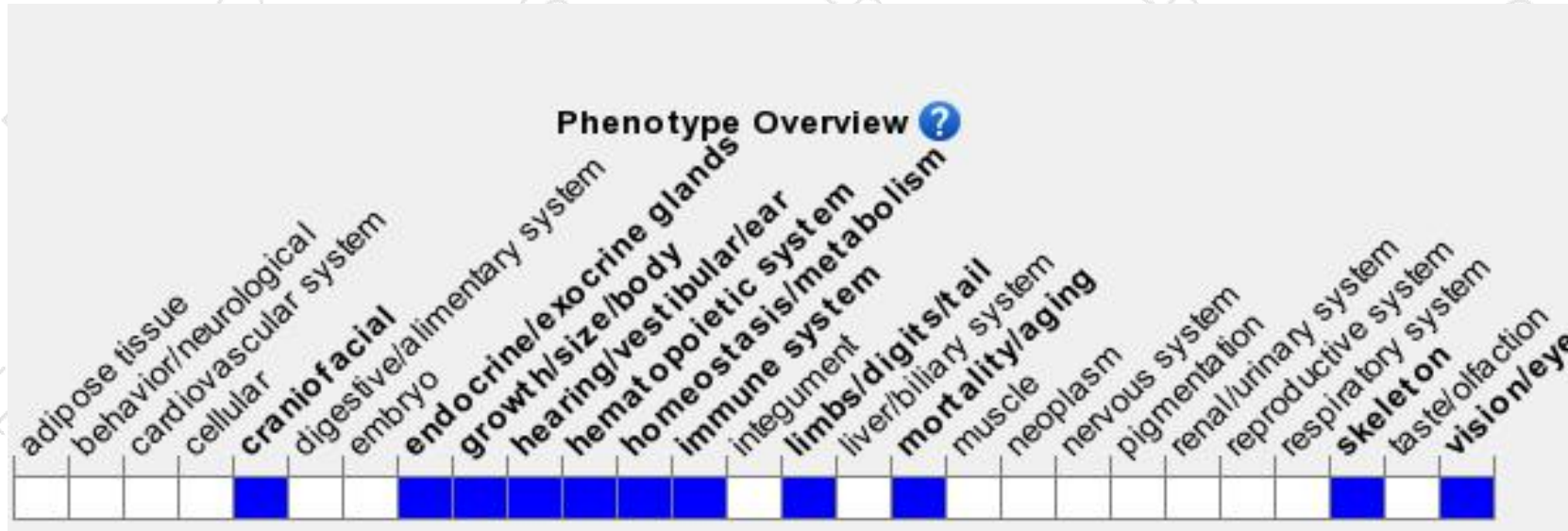




# Protein domain



# 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, Homozygous mice with a missense mutation exhibit hypothyroid dwarfism, including a goiter with colloid deficiency and abnormal follicle epithelium, reduced hematocrit and red blood cells and a lifespan of about 3 months.

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

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