

# *Ano7* Cas9-CKO Strategy

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# Project Overview

**Project Name**

*Ano7*

**Project type**

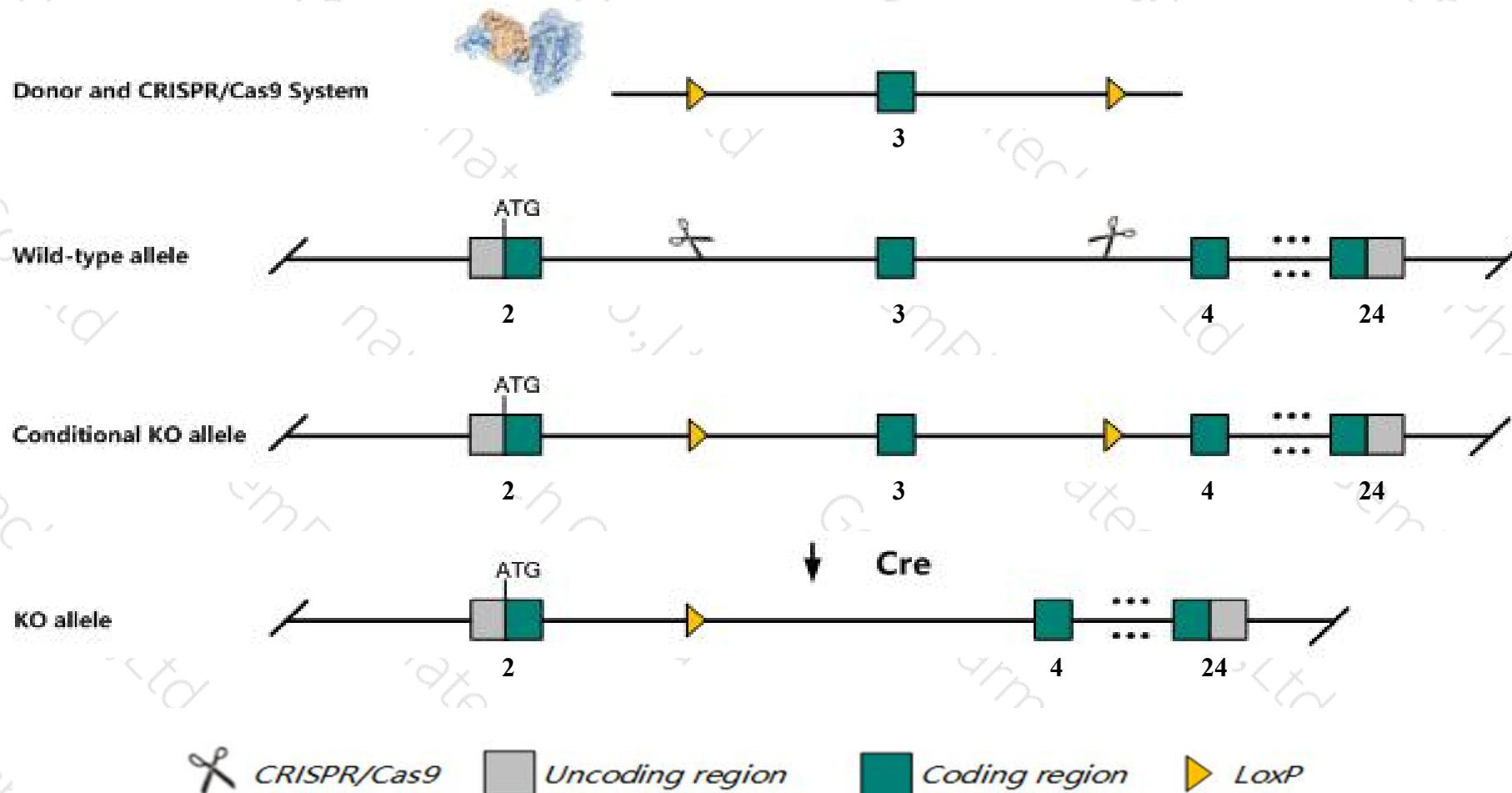
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ano7* gene. The schematic diagram is as follows:



# Technical routes

- The *Ano7* gene has 3 transcripts. According to the structure of *Ano7* gene, exon3 of *Ano7-202* (ENSMUST00000186641.6) transcript is recommended as the knockout region. The region contains 58bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ano7* 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.

- Transcript *Ano7-203* is incomplete, so the effect on it is unknown.
- The *Ano7* gene is located on the Chr1. 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)

## Ano7 anoctamin 7 [ *Mus musculus* (house mouse) ]

Gene ID: 404545, updated on 3-May-2020

### Summary

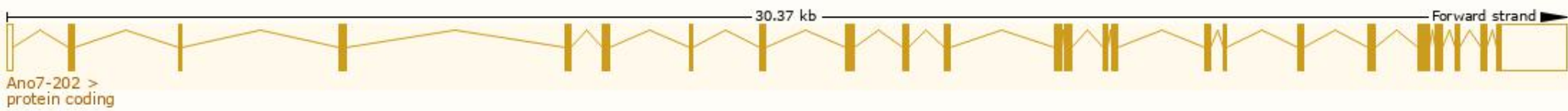
Official Symbol	Ano7 provided by MGI
Official Full Name	anoctamin 7 provided by MGI
Primary source	MGI:MGI:3052714
See related	<a href="#">Ensembl:ENSMUSG00000034107</a>
Gene type	protein coding
RefSeq status	REVIEWED
Organism	<a href="#">Mus musculus</a>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Ngep; Ngep-L; Pcanap5; Tmem16g
Summary	This gene encodes a member of the anoctamin family, which in mammals is comprised of 10 members. Anoctamin proteins are proposed to have eight transmembrane domains with both termini facing the cytoplasm and a C-terminal domain of unknown function. While some members have been characterized as calcium-activated chloride channels, this protein is reported to have little anion conductance activity. In humans, this protein is primarily found in prostate tissues and may serve as a target for prostate cancer immunotherapy. Alternative splicing results in multiple transcript variants that encode different isoforms. [provided by RefSeq, Dec 2012]
Expression	Biased expression in stomach adult (RPKM 32.3), colon adult (RPKM 26.4) and 3 other tissues <a href="#">See more</a>
Orthologs	<a href="#">human</a> <a href="#">all</a>

# Transcript information (Ensembl)

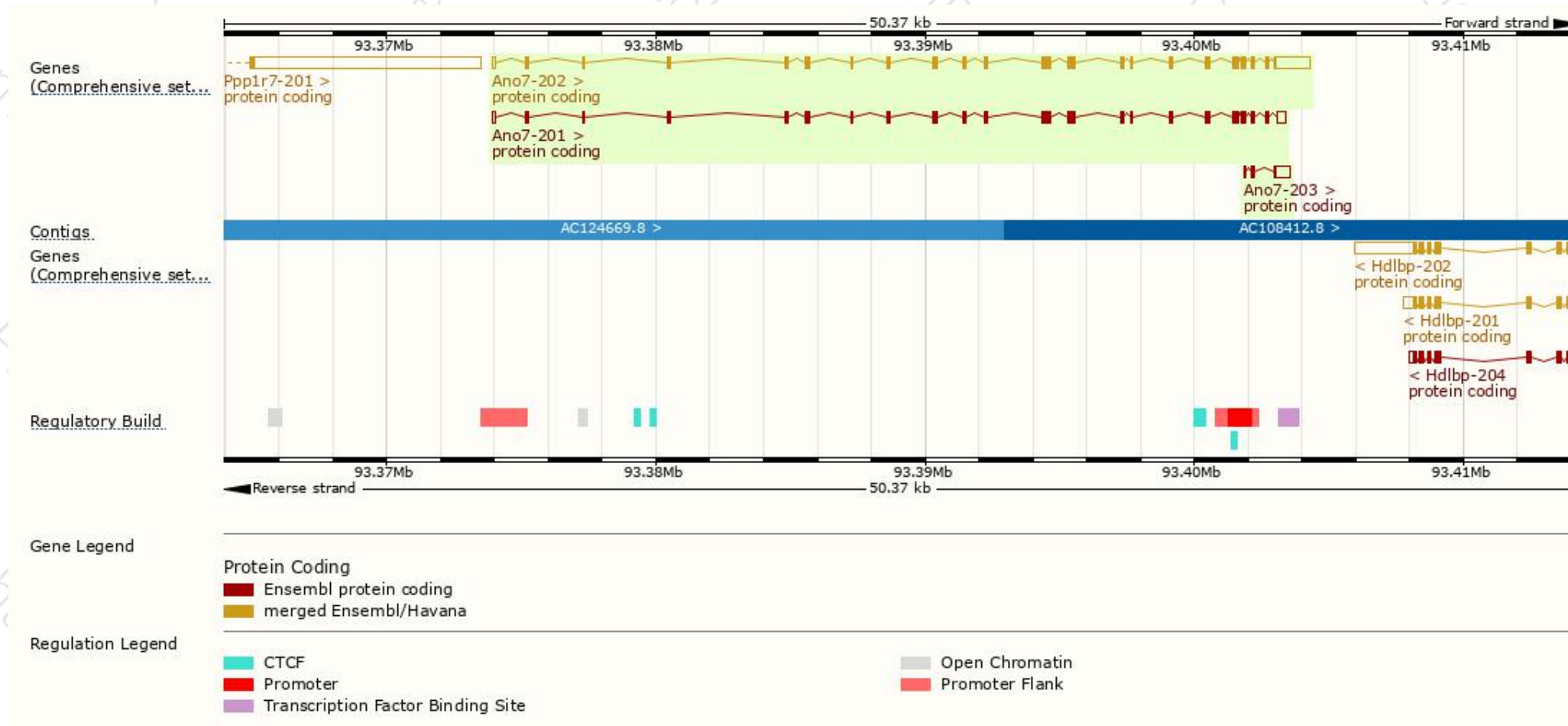
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ano7-202	<a href="#">ENSMUST00000186641.6</a>	3984	<a href="#">859aa</a>	Protein coding	<a href="#">CCDS15188</a>	<a href="#">Q14AT5</a>	TSL:5 GENCODE basic APPRIS P3
Ano7-201	<a href="#">ENSMUST00000058682.10</a>	2963	<a href="#">843aa</a>	Protein coding	<a href="#">CCDS78656</a>	<a href="#">Q14AT5</a>	TSL:1 GENCODE basic APPRIS ALT2
Ano7-203	<a href="#">ENSMUST00000190340.1</a>	755	<a href="#">69aa</a>	Protein coding	-	<a href="#">A0A087WPD8</a>	CDS 5' incomplete TSL:3

The strategy is based on the design of *Ano7-202* transcript, the transcription is shown below

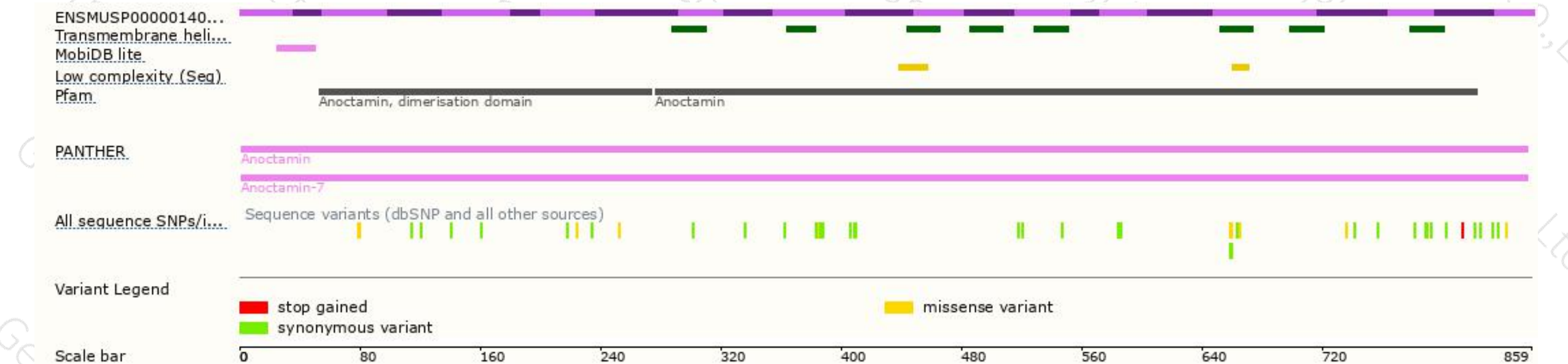


# Genomic location distribution



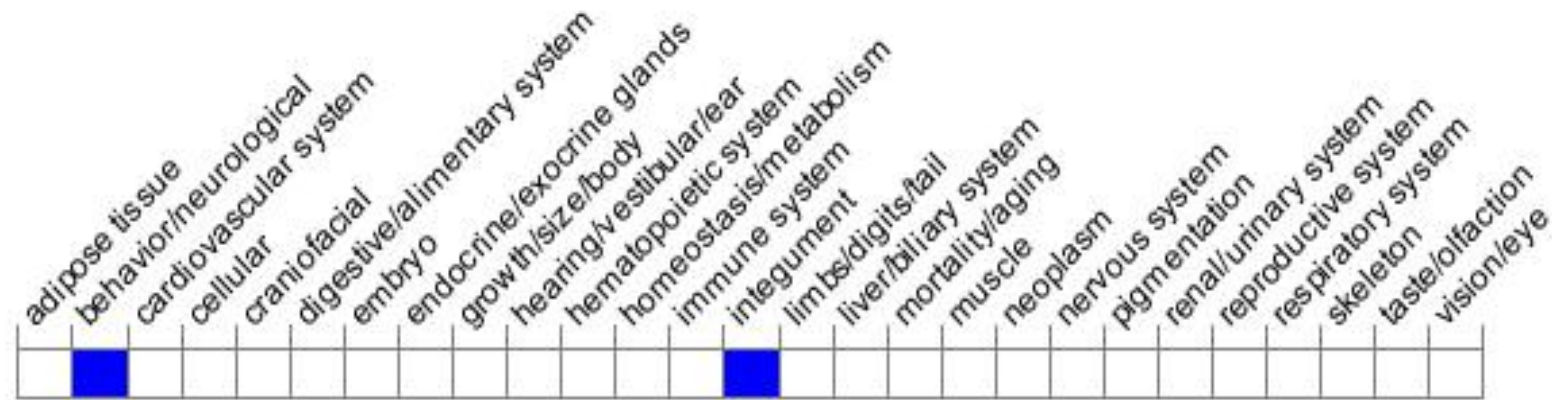


# Protein domain



# Mouse phenotype description(MGI)

Phenotype Overview



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

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

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