Ces5a Cas9-CKO Strategy

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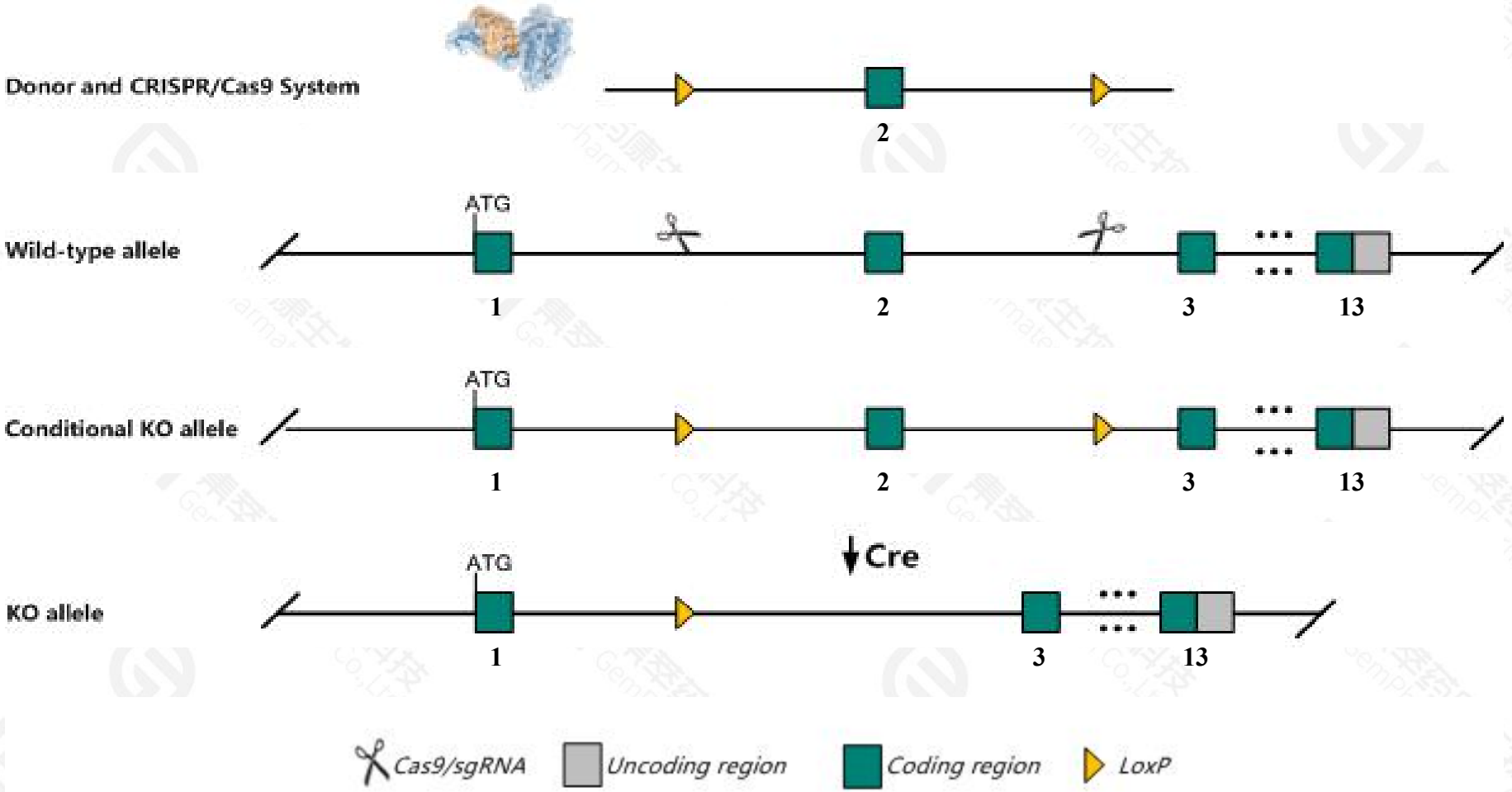
Project Overview

Project Name	***Ces5a***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ces5a* gene. The schematic diagram is as follows:



Technical routes

➢ The *Ces5a* gene has 6 transcripts. According to the structure of *Ces5a* gene, exon2 of *Ces5a-203*(ENSMUST00000212009.2) transcript is recommended as the knockout region.The region contains 205bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Ces5a* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor 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

➢ The *Ces5a* gene is located on the Chr8. 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.

➢Transcript *Ces5a*-202 may not be affected.

➢ 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）

集萃药康 GemPharmatech

Ces5a carboxylesterase 5A [Mus musculus (house mouse)]

Gene ID: 67935, updated on 29-Jan-2021

Summary

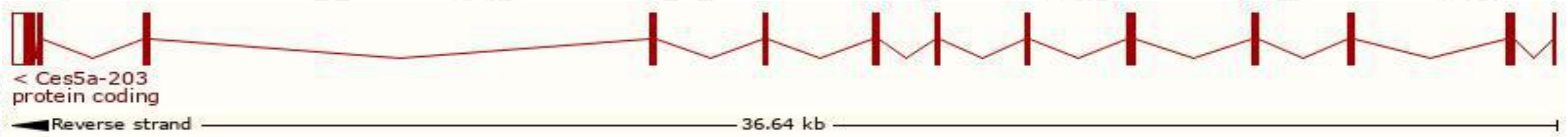
Official Symbol	Ces5a provided by MGI
Official Full Name	carboxylesterase 5A provided by MGI
Primary source	MGI:MGI:1915185
See related	Ensembl:ENSMUSG00000058019
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1700081L16Rik, 1700122C07Rik, BB081581, Ces, Ces7, Gm503, cau, cauxin
Expression	Restricted expression toward genital fat pad adult (RPKM 296.4) See more
Orthologs	human all

Transcript information（Ensembl）

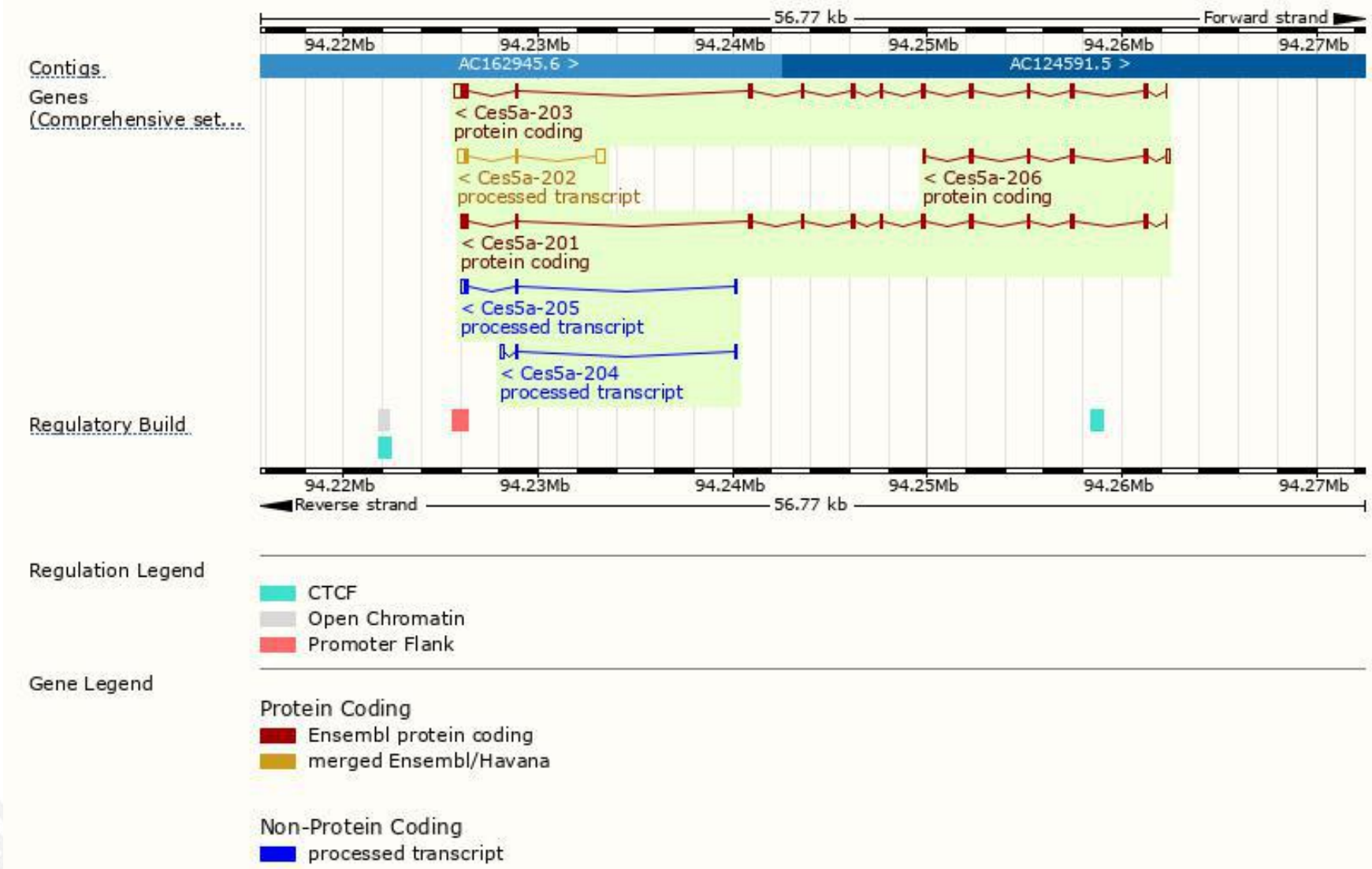
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ces5a-203	ENSMUST00000212009.2	2042	575aa	Protein coding	CCDS85584		TSL:1 , GENCODE basic , APPRIS P1 ,
Ces5a-201	ENSMUST00000077816.7	1728	575aa	Protein coding	-		TSL:5 , GENCODE basic , APPRIS P1 ,
Ces5a-206	ENSMUST00000212722.2	914	263aa	Protein coding	-		CDS 3' incomplete , TSL:3 ,
Ces5a-202	ENSMUST00000080391.14	987	No protein	Processed transcript	-		TSL:1 ,
Ces5a-205	ENSMUST00000212690.2	525	No protein	Processed transcript	-		TSL:3 ,
Ces5a-204	ENSMUST00000212407.2	456	No protein	Processed transcript	-		TSL:1 ,

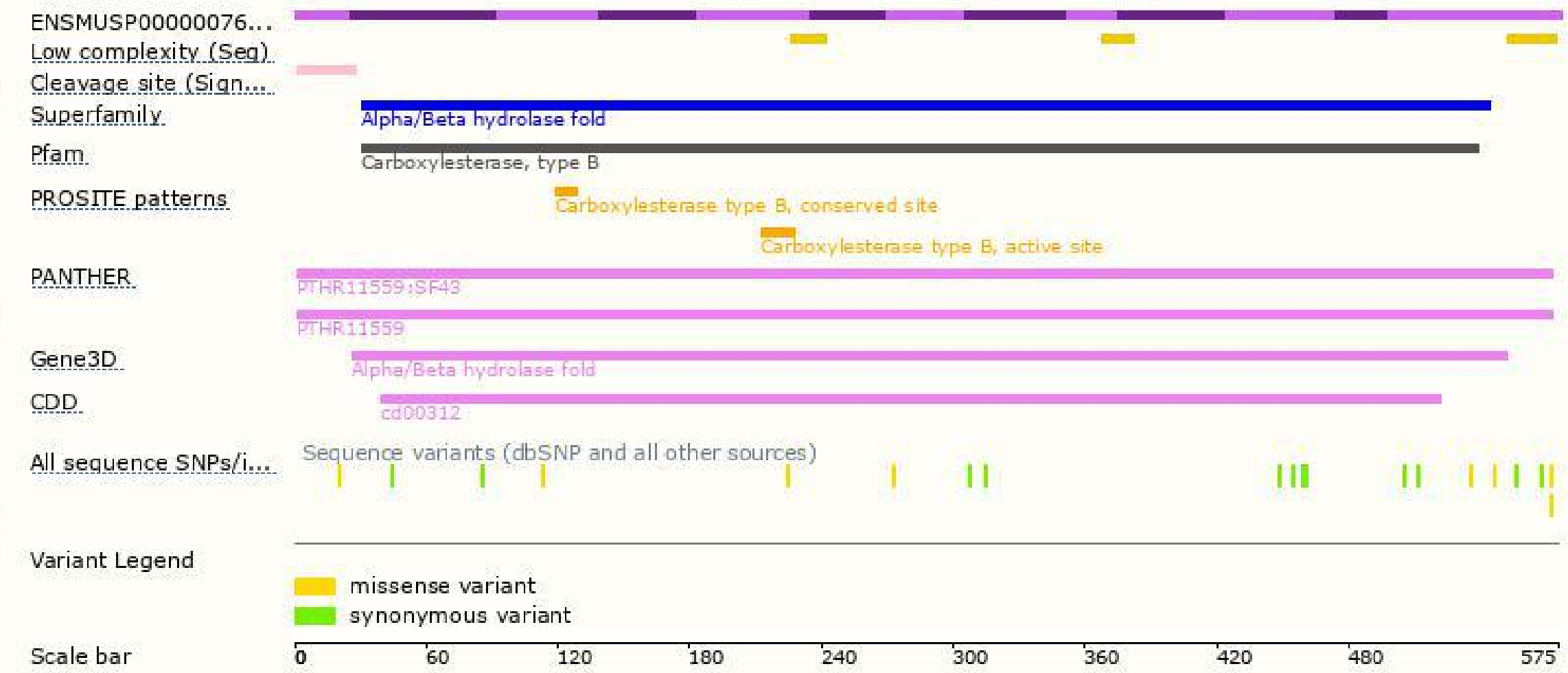
The strategy is based on the design of *Ces5a-203* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

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