



Ret Cas9-CKO Strategy

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Design Date: 2019-7-31

Project Overview

Project Name

Ret

Project type

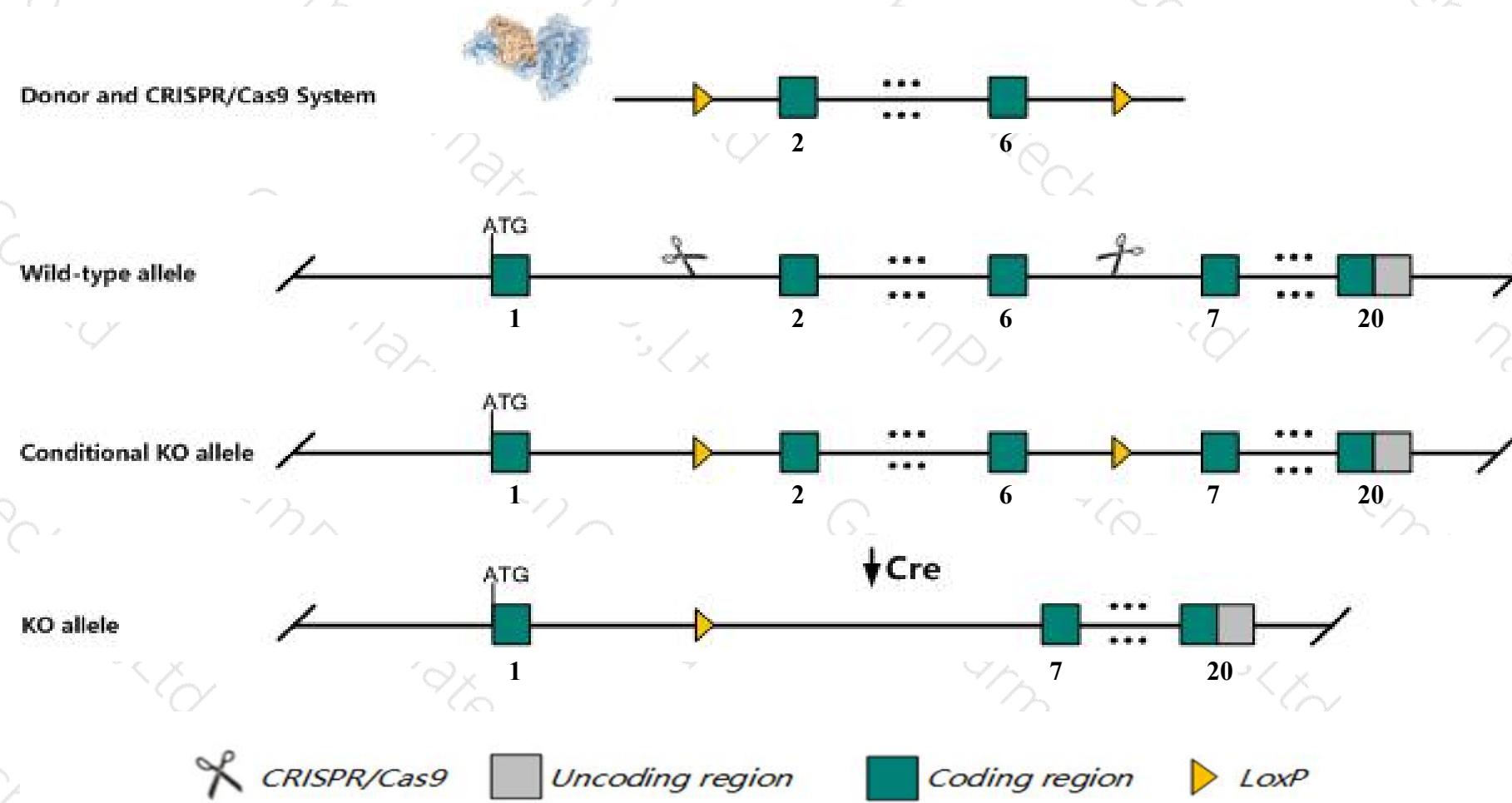
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



Technical routes

- The *Ret* gene has 2 transcripts. According to the structure of *Ret* gene, exon2-exon6 of *Ret-201* (ENSMUST00000032201.7) transcript is recommended as the knockout region. The region contains 1196bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ret* 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.



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Notice

- According to the existing MGI data, Mice homozygous for some point mutations or knock-out alleles exhibit premature lethality, defects in neurogenesis, and abnormal kidney, ureter, ovary, muscle, and intestine morphology.
- The *Ret* gene is located on the Chr6. 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)

Ret ret proto-oncogene [Mus musculus (house mouse)]

Gene ID: 19713, updated on 26-Mar-2019

Summary



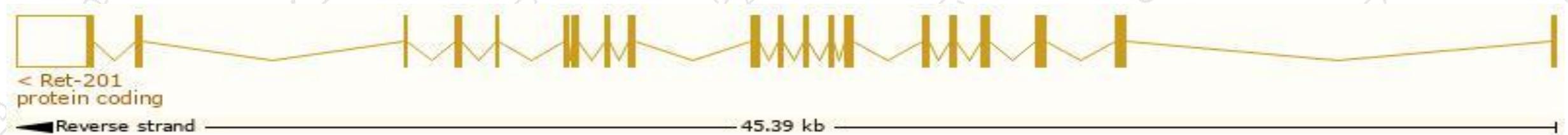
| | |
|---------------------------|---|
| Official Symbol | Ret provided by MGI |
| Official Full Name | ret proto-oncogene provided by MGI |
| Primary source | MGI:MGI:97902 |
| See related | Ensembl:ENSMUSG00000030110 |
| Gene type | protein coding |
| RefSeq status | VALIDATED |
| Organism | Mus musculus |
| Lineage | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| Also known as | PTC, RET51, RET9, c-Ret |
| Expression | Broad expression in cerebellum adult (RPKM 5.3), subcutaneous fat pad adult (RPKM 4.5) and 18 other tissues See more |
| Orthologs | human all |

Transcript information (Ensembl)

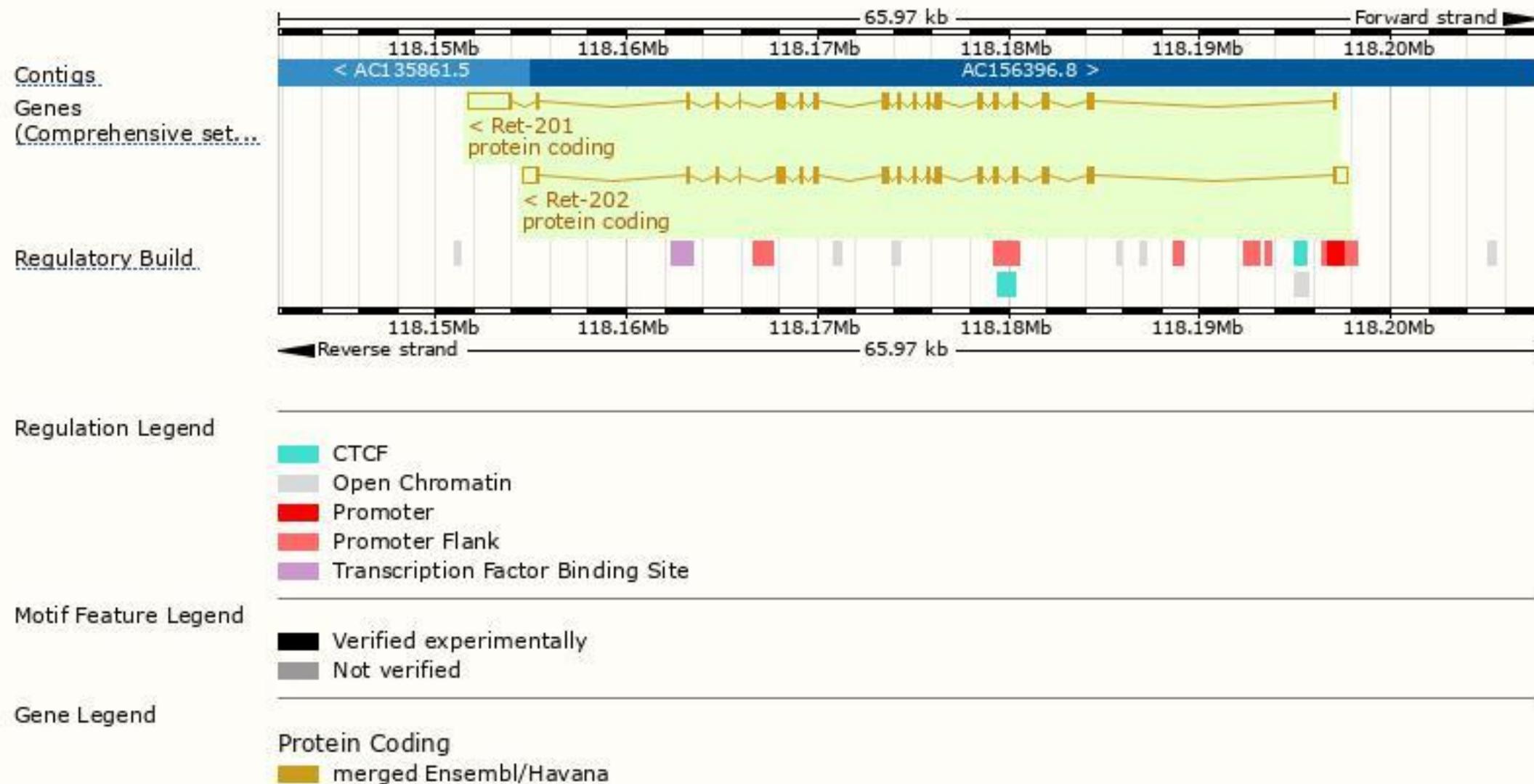
The gene has 2 transcripts, all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|---------|--------------------------------------|------|------------------------|----------------|---------------------------|------------------------|---------------------------------|
| Ret-201 | ENSMUST00000032201.7 | 5456 | 1115aa | Protein coding | CCDS20470 | P35546 | TSL:1 GENCODE basic APPRIS P3 |
| Ret-202 | ENSMUST00000088790.8 | 4497 | 1073aa | Protein coding | CCDS39608 | P35546 | TSL:1 GENCODE basic APPRIS ALT2 |

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



Genomic location distribution



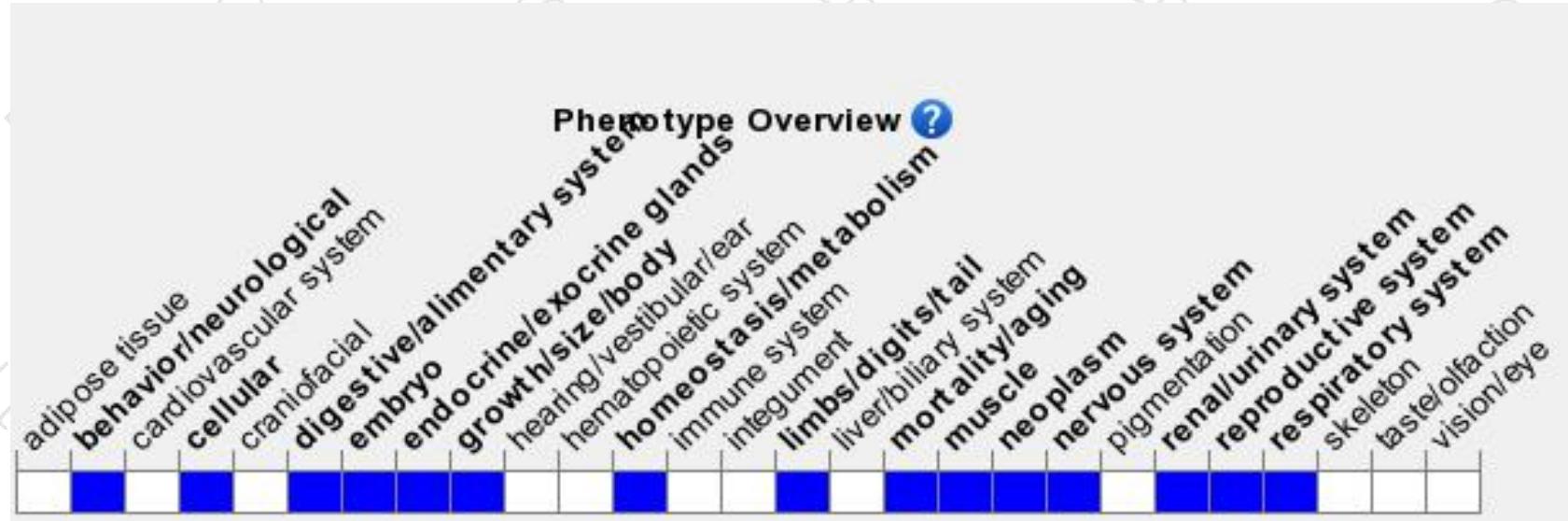
Protein domain





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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, Mice homozygous for some point mutations or knock-out alleles exhibit premature lethality, defects in neurogenesis, and abnormal kidney, ureter, ovary, muscle, and intestine morphology.



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

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