

# ***F2r Cas9-CKO Strategy***

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Reviewer: Longyun Hu

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# Project Overview

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**Project Name**

***F2r***

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**Project type**

**Cas9-CKO**

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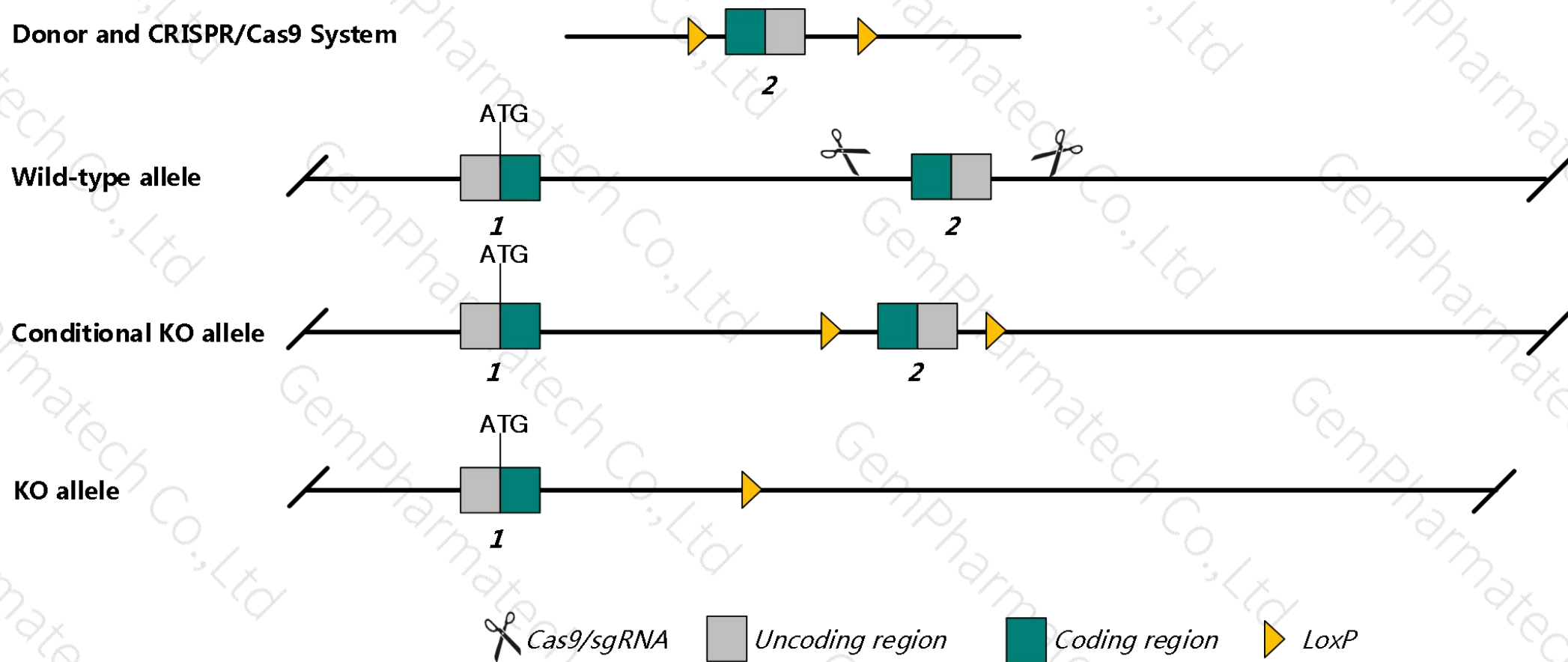
**Strain background**

**C57BL/6JGpt**

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# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *F2r* gene. The schematic diagram is as follows:



- The *F2r* gene has 1 transcript. According to the structure of *F2r* gene, exon2 of *F2r*-201 ( ENSMUST00000059193.6) transcript is recommended as the knockout region. The region contains 1205bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *F2r* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA 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 or cell types.

- According to the existing MGI data , Targeted mutations of this locus result in increased midgestational lethality, with up to ~50% of mutants surviving to adulthood. Gene deficiency does not affect thrombin signaling in mouse platelets but markedly attenuates thrombin signaling in mouse microvascular endothelial cells.
- The KO region deletes most of the coding sequence and result in frameshift.
- The *F2r* gene is located on the Chr13. 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.



# Gene information ( NCBI )

## F2r coagulation factor II (thrombin) receptor [ *Mus musculus* (house mouse) ]

Gene ID: 14062, updated on 6-Nov-2018

### Summary

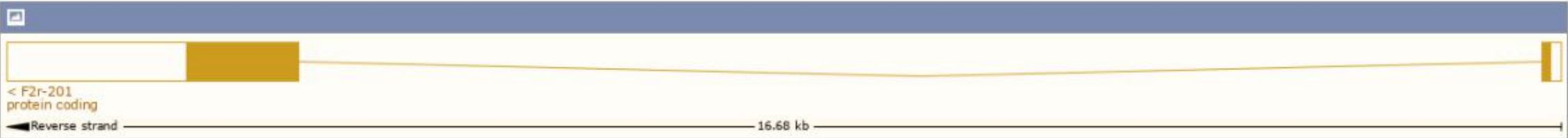
Official Symbol	F2r provided by <a href="#">MGI</a>
Official Full Name	coagulation factor II (thrombin) receptor provided by <a href="#">MGI</a>
Primary source	<a href="#">MGI:MGI:101802</a>
See related	<a href="#">Ensembl:ENSMUSG00000048376</a>
Gene type	protein coding
RefSeq status	VALIDATED
Organism	<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	Cf2r; Par1; ThrR; AI482343
Expression	Broad expression in lung adult (RPKM 53.2), adrenal adult (RPKM 42.2) and 24 other tissues <a href="#">See more</a>
Orthologs	<a href="#">human</a> <a href="#">all</a>

# Transcript information ( Ensembl )

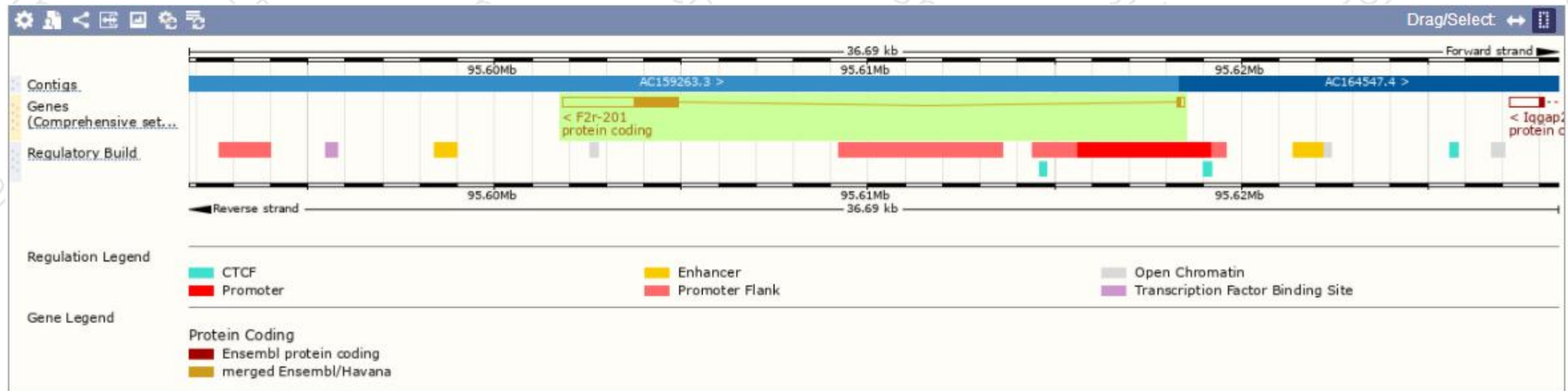
The gene has 1 transcript, and the transcript is shown below:

Show/hide columns (1 hidden)								Filter		
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags		
F2r-201	<a href="#">ENSMUST00000059193.6</a>	3336	<a href="#">430aa</a>	Protein coding	<a href="#">CCDS26701</a>	<a href="#">P30558</a>	<a href="#">NM_010169</a> <a href="#">NP_034299</a>	TSL:1	GENCODE basic	APPRIS P1

The strategy is based on the design of *F2r-201* transcript, The transcription is shown below

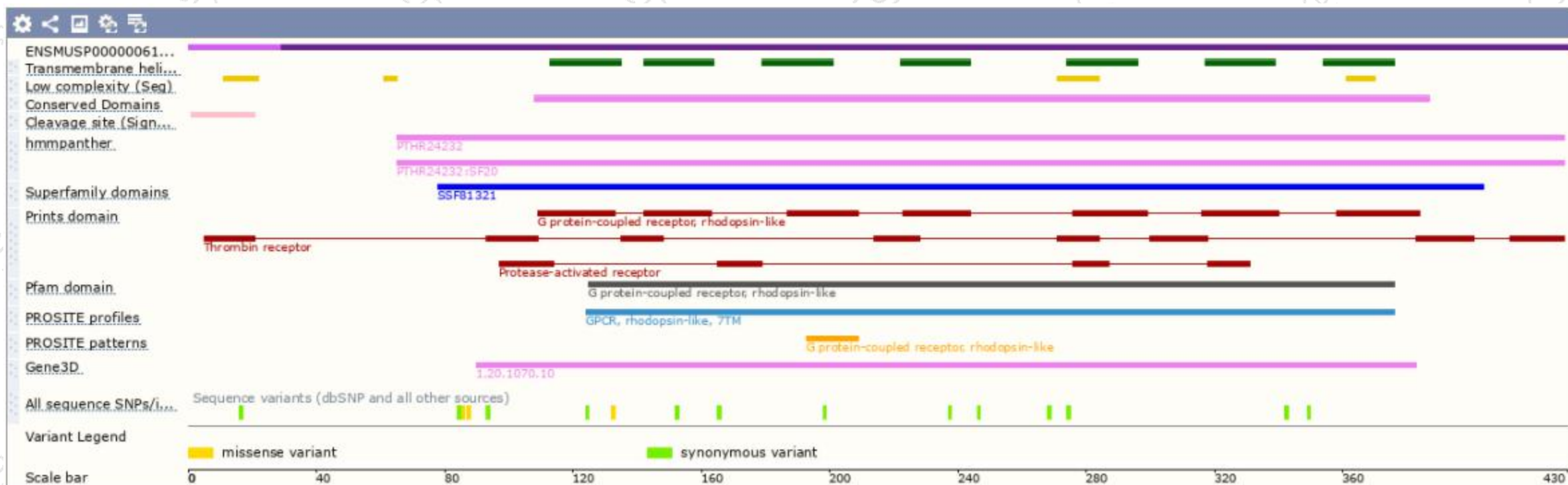


# Genomic location distribution

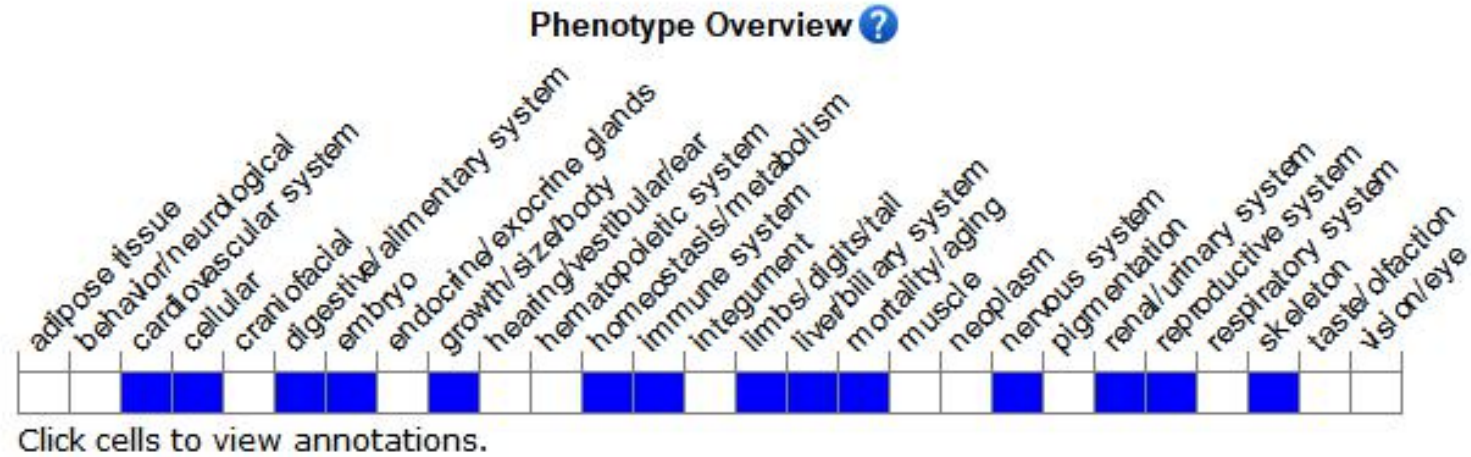




# Protein domain



# Mouse phenotype description(MGI)



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

Targeted mutations of this locus result in increased midgestational lethality, with up to ~50% of mutants surviving to adulthood. Gene deficiency does not affect thrombin signaling in mouse platelets but markedly attenuates thrombin signaling in mouse microvascular endothelial cells.

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