

# *Rfx6* Cas9-KO Strategy

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# Project Overview

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**Project Name**

***Rfx6***

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**Project type**

**Cas9-KO**

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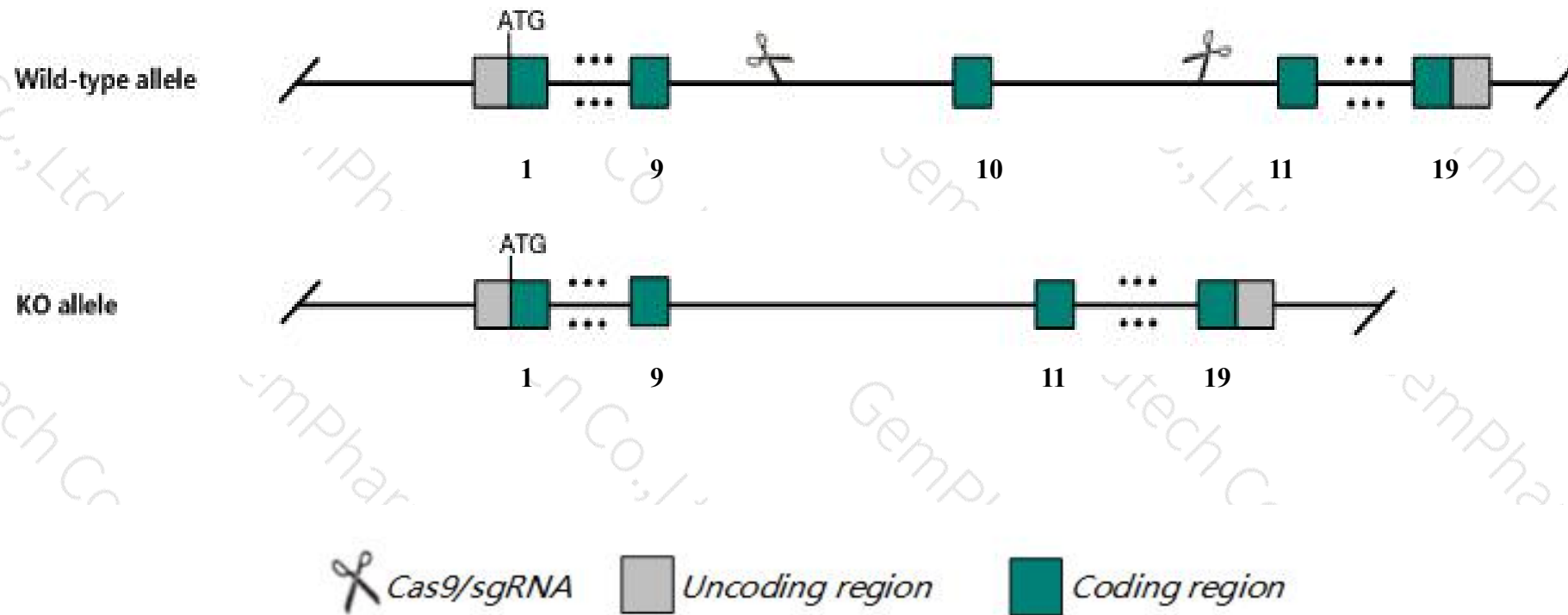
**Strain background**

**C57BL/6J**

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# Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rfx6* gene. The schematic diagram is as follows:



- The *Rfx6* gene has 5 transcripts. According to the structure of *Rfx6* gene, exon10 of *Rfx6-202* (ENSMUST00000122922.9) transcript is recommended as the knockout region. The region contains 50bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rfx6* gene. The brief process is as follows: sgRNA was transcribed in vitro. Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.

- According to the existing MGI data, Homozygotes fail to feed normally, show small bowel obstruction and die within 2 days of birth. Mutants fail to generate any of the normal islet cell types except for pancreatic-polypeptide-producing cells. Some display a reduced pancreas size; however, primary cilia formation in islets is normal.
- The *Rfx6* gene is located on the Chr10. 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 gene transcription and translation processes, all risks cannot be predicted under existing information.



# Gene information (NCBI)

## Rfx6 regulatory factor X, 6 [Mus musculus (house mouse)]

Gene ID: 320995, updated on 31-Jan-2019

### Summary



<b>Official Symbol</b>	Rfx6 provided by <a href="#">MGI</a>
<b>Official Full Name</b>	regulatory factor X, 6 provided by <a href="#">MGI</a>
<b>Primary source</b>	<a href="#">MGI:MGI:2445208</a>
<b>See related</b>	<a href="#">Ensembl:ENSMUSG00000019900</a>
<b>Gene type</b>	protein coding
<b>RefSeq status</b>	VALIDATED
<b>Organism</b>	<a href="#">Mus musculus</a>
<b>Lineage</b>	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
<b>Also known as</b>	4930572O07Rik, Rfxdc1
<b>Expression</b>	Low expression observed in reference dataset <a href="#">See more</a>
<b>Orthologs</b>	<a href="#">human</a> <a href="#">all</a>

# Transcript information (Ensembl)

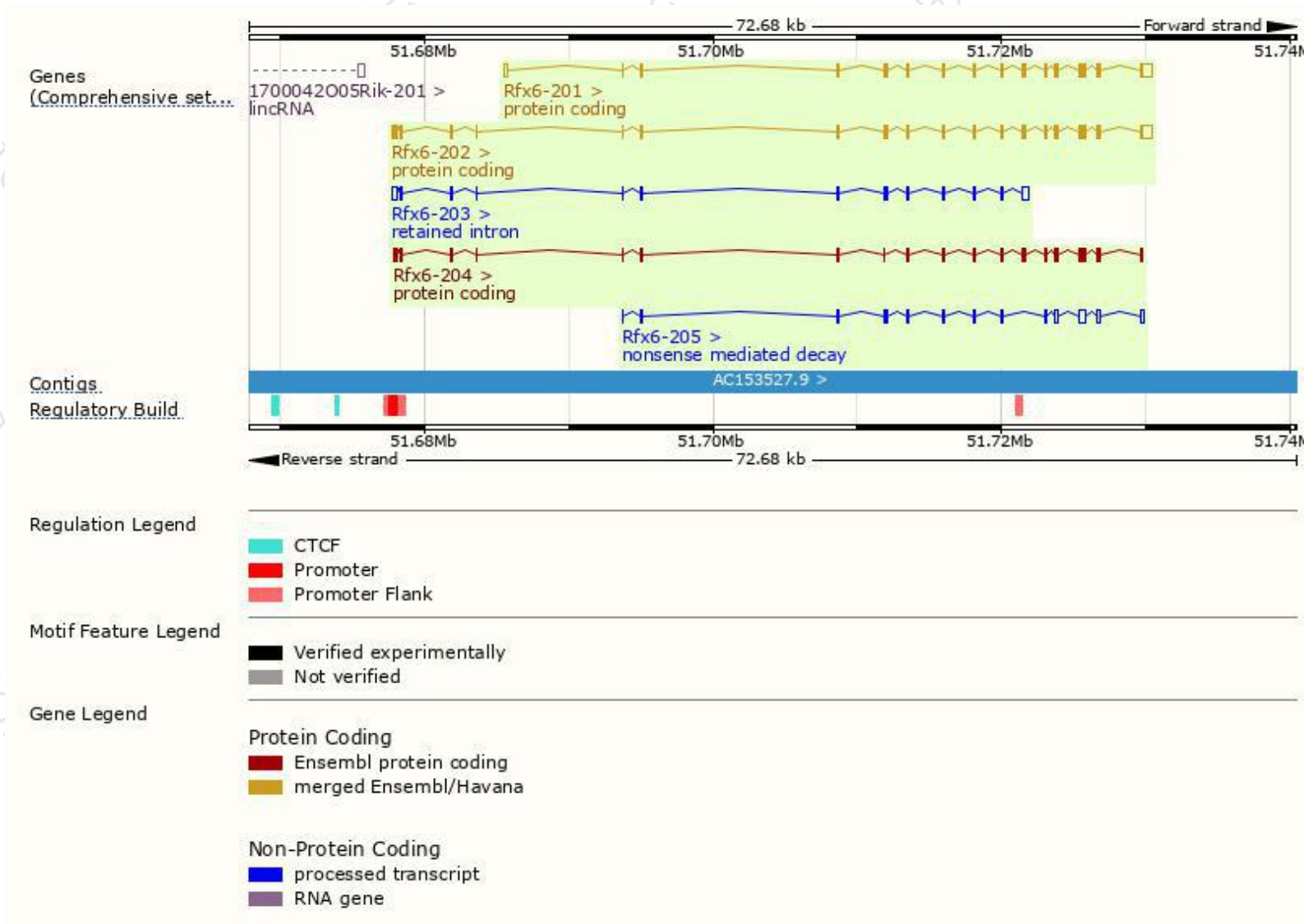
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rfx6-202	<a href="#">ENSMUST00000122922.9</a>	3464	<a href="#">927aa</a>	Protein coding	<a href="#">CCDS48558</a>	<a href="#">Q8C7R7</a>	TSL:5 GENCODE basic APPRIS P1
Rfx6-201	<a href="#">ENSMUST00000050455.4</a>	3088	<a href="#">663aa</a>	Protein coding	<a href="#">CCDS23835</a>	<a href="#">Q8C7R7</a>	TSL:1 GENCODE basic
Rfx6-204	<a href="#">ENSMUST00000219364.1</a>	2682	<a href="#">893aa</a>	Protein coding	-	<a href="#">D5FLC2</a>	TSL:1 GENCODE basic
Rfx6-205	<a href="#">ENSMUST00000219771.1</a>	2101	<a href="#">270aa</a>	Nonsense mediated decay	-	<a href="#">A0A1W2P7F2</a>	CDS 5' incomplete TSL:5
Rfx6-203	<a href="#">ENSMUST00000217662.1</a>	1896	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Rfx6-202* transcript,The transcription is shown below

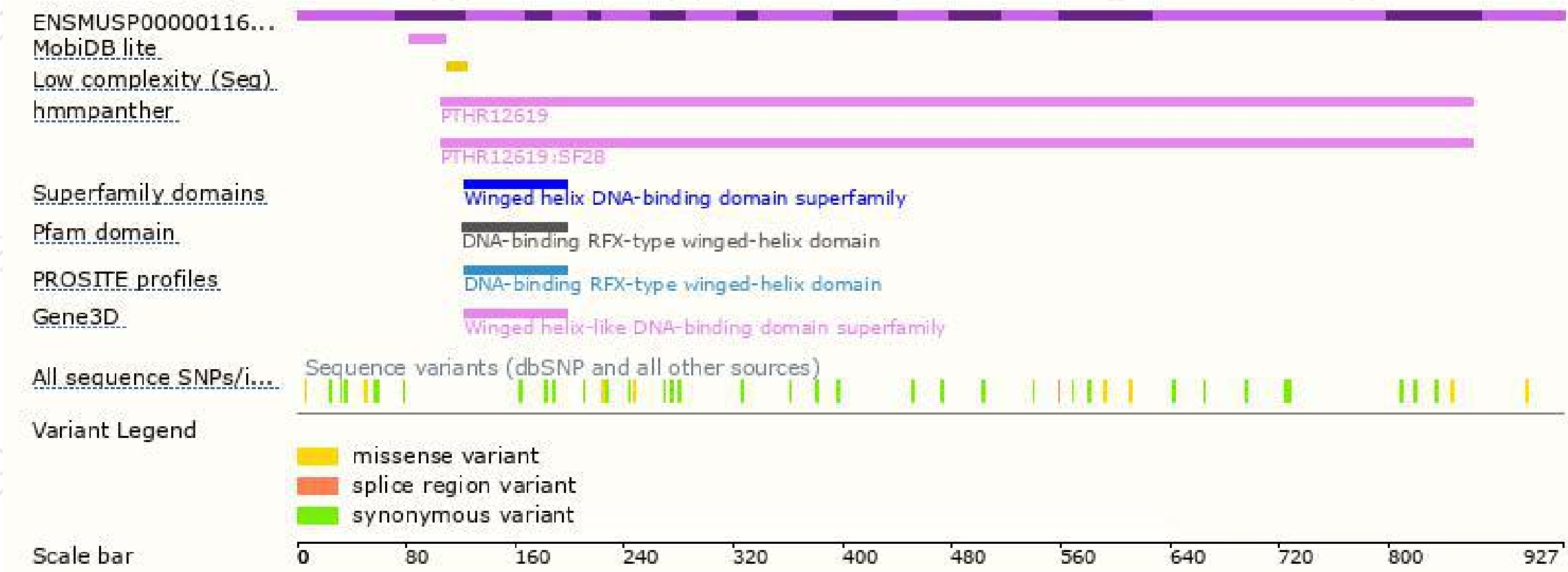


# Genomic location distribution





# 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, Homozygotes fail to feed normally, show small bowel obstruction and die within 2 days of birth. Mutants fail to generate any of the normal islet cell types except for pancreatic-polypeptide-producing cells. Some display a reduced pancreas size; however, primary cilia formation in islets is normal.

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

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