

Rfx5 Cas9-CKO Strategy

Designer: Xueting Zhang

Reviewer: Yanhua Shen

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Project Overview

Project Name

Rfx5

Project type

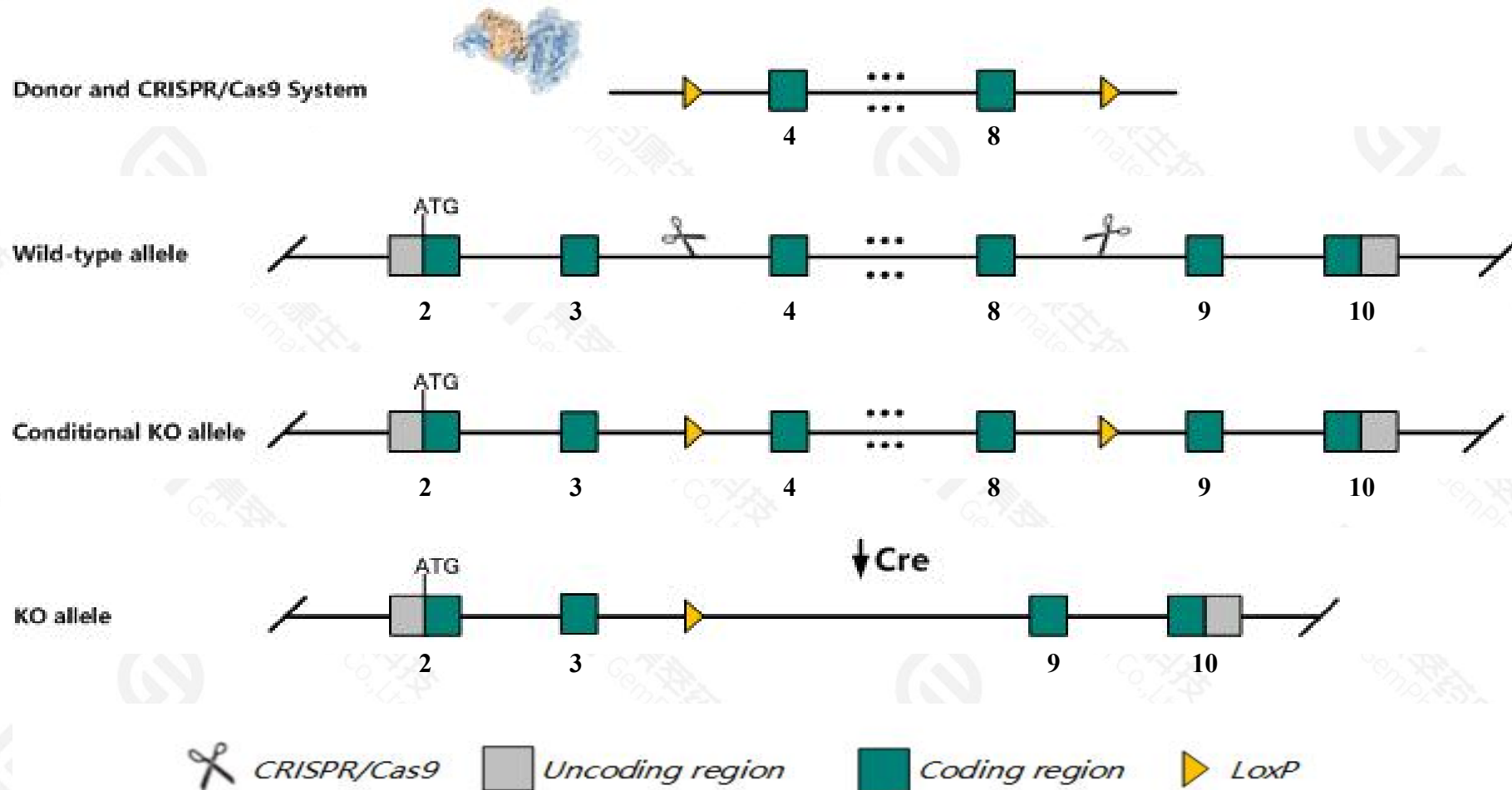
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rfx5* gene. The schematic diagram is as follows:



Technical routes

- The *Rfx5* gene has 17 transcripts. According to the structure of *Rfx5* gene, exon4-exon8 of *Rfx5*-207(ENSMUST00000137088.8) transcript is recommended as the knockout region. The region contains 607bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rfx5* 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.

- According to the existing MGI data, homozygous null mice have absent or decreased expression of MHC-II complexes on antigen presenting cells, which leads to reduced numbers of CD4.
- The Intron3 and Intron8 are only 360bp and 483bp, loxp insertion may affect mRNA splicing.
- The *Rfx5* gene is located on the Chr3. 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)

Rfx5 regulatory factor X, 5 (influences HLA class II expression) [Mus musculus (house mouse)]

Gene ID: 53970, updated on 17-Feb-2021

Summary



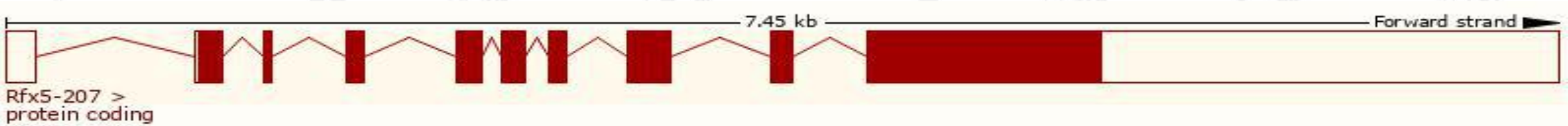
Official Symbol	Rfx5 provided by MGI
Official Full Name	regulatory factor X, 5 (influences HLA class II expression) provided by MGI
Primary source	MGI:MGI:1858421
See related	Ensembl:ENSMUSG00000005774
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Ubiquitous expression in limb E14.5 (RPKM 5.9), spleen adult (RPKM 5.5) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

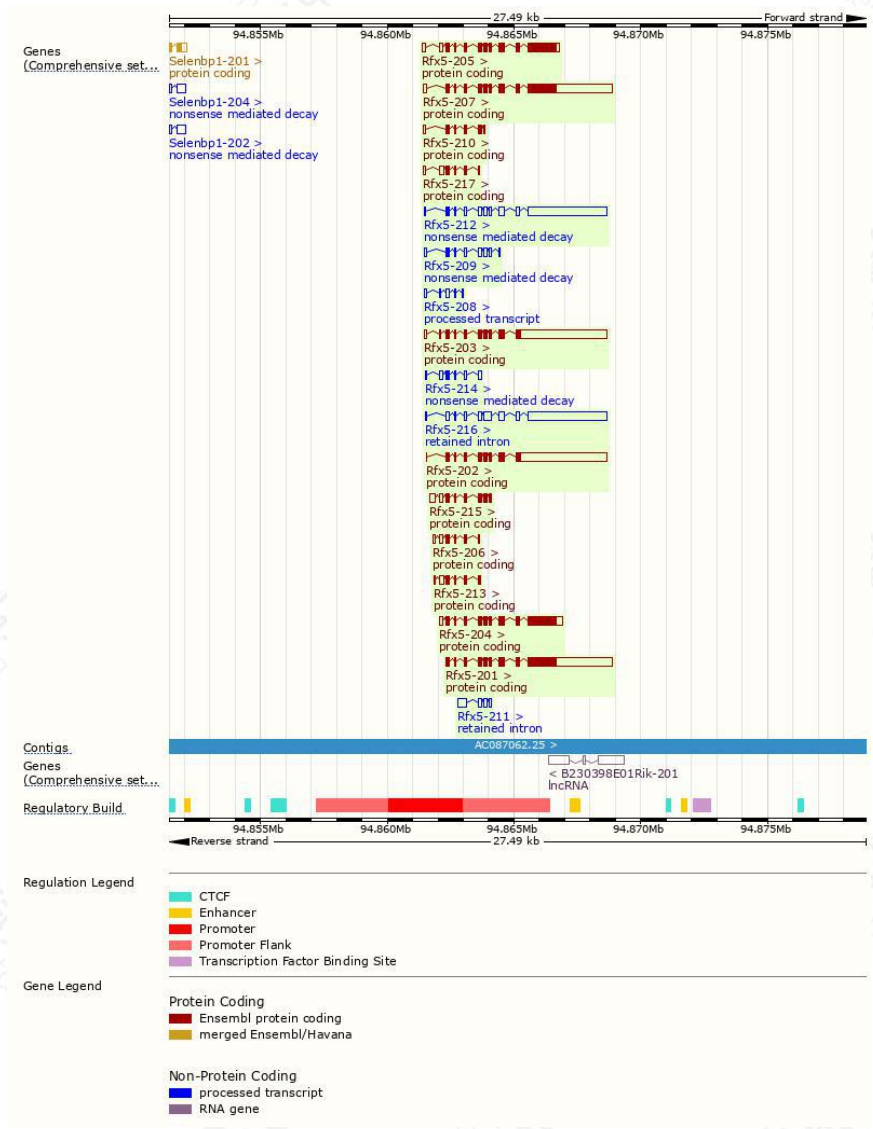
The gene has 17 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rfx5-207	ENSMUST00000137088.8	4333	658aa	Protein coding	CCDS17598		TSL:5 , GENCODE basic , APPRIS P1 ,
Rfx5-201	ENSMUST00000029772.3	4189	658aa	Protein coding	CCDS17598		TSL:1 , GENCODE basic , APPRIS P1 ,
Rfx5-205	ENSMUST00000107260.9	2355	658aa	Protein coding	CCDS17598		TSL:5 , GENCODE basic , APPRIS P1 ,
Rfx5-204	ENSMUST00000107255.8	2350	658aa	Protein coding	CCDS17598		TSL:5 , GENCODE basic , APPRIS P1 ,
Rfx5-203	ENSMUST00000107254.8	4450	303aa	Protein coding	-		TSL:1 , GENCODE basic ,
Rfx5-202	ENSMUST00000107253.8	4367	303aa	Protein coding	-		TSL:1 , GENCODE basic ,
Rfx5-215	ENSMUST00000147237.8	882	181aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Rfx5-213	ENSMUST00000145031.8	565	105aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Rfx5-217	ENSMUST00000152869.8	538	90aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Rfx5-206	ENSMUST00000132393.8	536	95aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Rfx5-210	ENSMUST00000142311.8	486	131aa	Protein coding	-		CDS 3' incomplete , TSL:3 ,
Rfx5-212	ENSMUST00000144132.8	4014	41aa	Nonsense mediated decay	-		TSL:1 ,
Rfx5-209	ENSMUST00000140331.8	705	41aa	Nonsense mediated decay	-		TSL:3 ,
Rfx5-214	ENSMUST00000145472.8	525	41aa	Nonsense mediated decay	-		TSL:5 ,
Rfx5-208	ENSMUST00000140294.8	346	No protein	Processed transcript	-		TSL:3 ,
Rfx5-216	ENSMUST00000148803.8	4139	No protein	Retained intron	-		TSL:2 ,
Rfx5-211	ENSMUST00000142708.2	680	No protein	Retained intron	-		TSL:5 ,

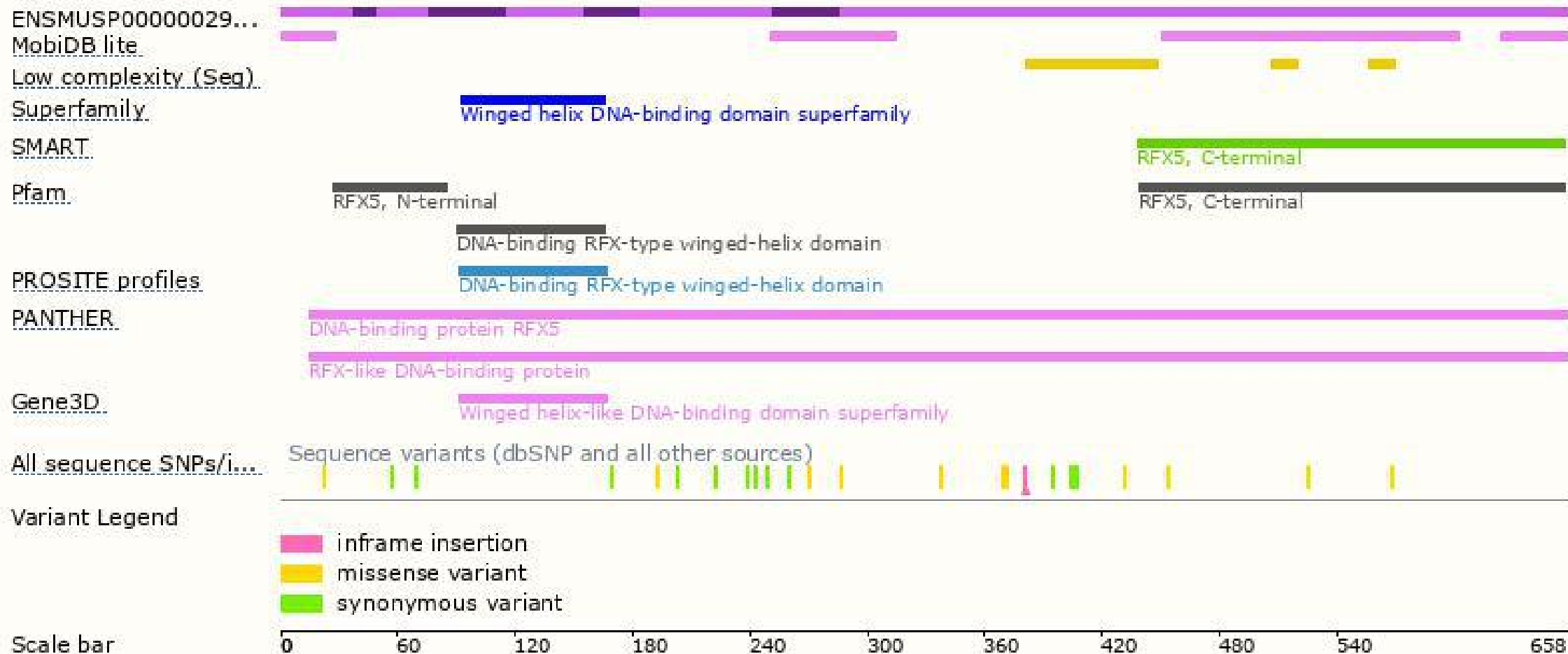
The strategy is based on the design of *Rfx5-207* transcript,the transcription is shown below:



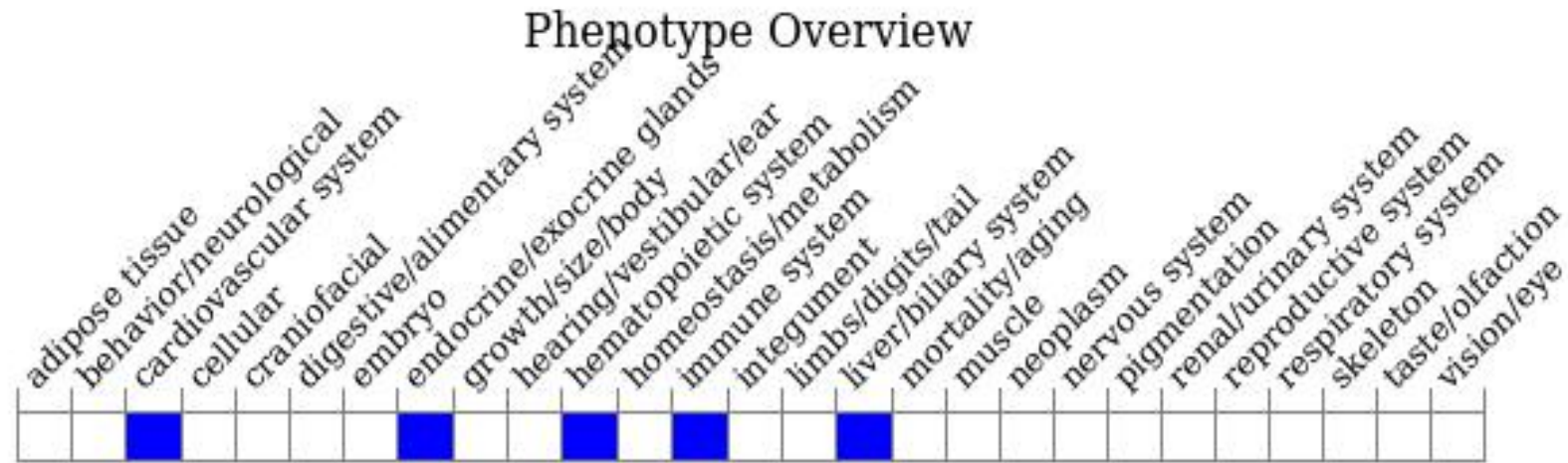
Genomic location distribution



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, homozygous null mice have absent or decreased expression of MHC-II complexes on antigen presenting cells, which leads to reduced numbers of CD4

If you have any questions, you are welcome to inquire.
Tel: 400-9660890

