

Il12b Cas9-CKO Strategy

Designer: Xueting Zhang

Design Date: 2019-8-3

Project Overview



Project Name

Il12b

Project type

Cas9-CKO

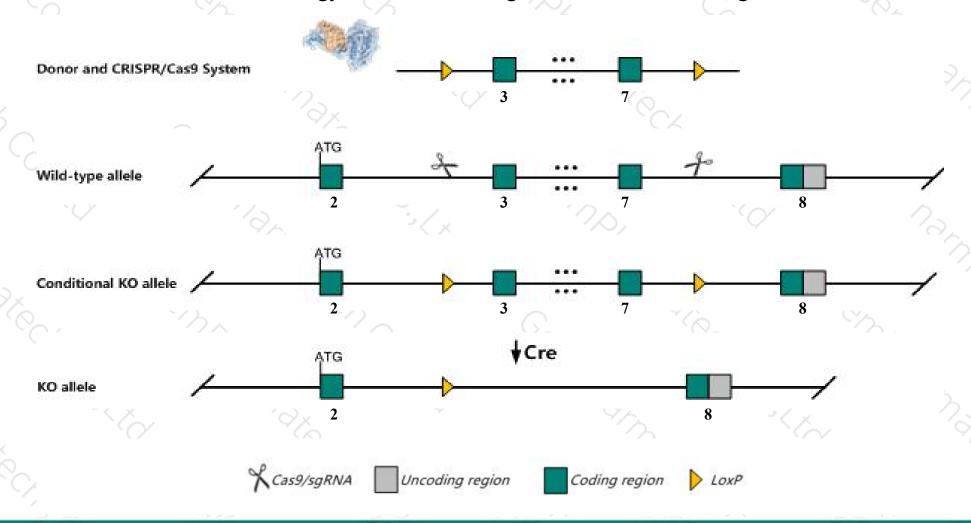
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Il12b* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Il12b* gene has 2 transcripts. According to the structure of *Il12b* gene, exon3-exon7 of *Il12b-201*(ENSMUST00000102796.9) transcript is recommended as the knockout region. The region contains 907bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Il12b* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector 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



- ➤ According to the existing MGI data, Mice homozygous for a null allele display impaired Th1 responses, defects in IFN gamma secretion and NK cell activity, increased susceptibility to bacterial and parasitic infection, alveolar bone loss, and resistance to chemically induced tumors and to delayed type hypersensitivity.
- > The *Il12b* gene is located on the Chr11. 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)



Il12b interleukin 12b [Mus musculus (house mouse)]

Gene ID: 16160, updated on 12-Feb-2019

Summary

↑ ?

Official Symbol II12b provided by MGI

Official Full Name interleukin 12b provided by MGI

Primary source MGI:MGI:96540

See related Ensembl:ENSMUSG00000004296

Gene type protein coding
RefSeq status REVIEWED
Organism Mus musculus

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;

Muroidea; Muridae; Murinae; Mus; Mus

Also known as II-12b, II-12p40, II12p40, p40

Summary This gene encodes the beta subunit p40 of the Interleukin 12 (IL-12) family of cytokines. Members of the IL-12 family form heterodimers

consisting of heavy and light subunits linked by disulfide bonds. The product of this gene, p40, is a subunit of interleukins IL-12 and IL-23.

[provided by RefSeq, Dec 2014]

Expression Biased expression in thymus adult (RPKM 1.0), spleen adult (RPKM 0.3) and 4 other tissuesSee more

Orthologs <u>human all</u>

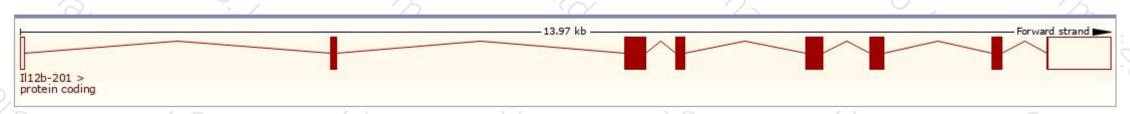
Transcript information (Ensembl)



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

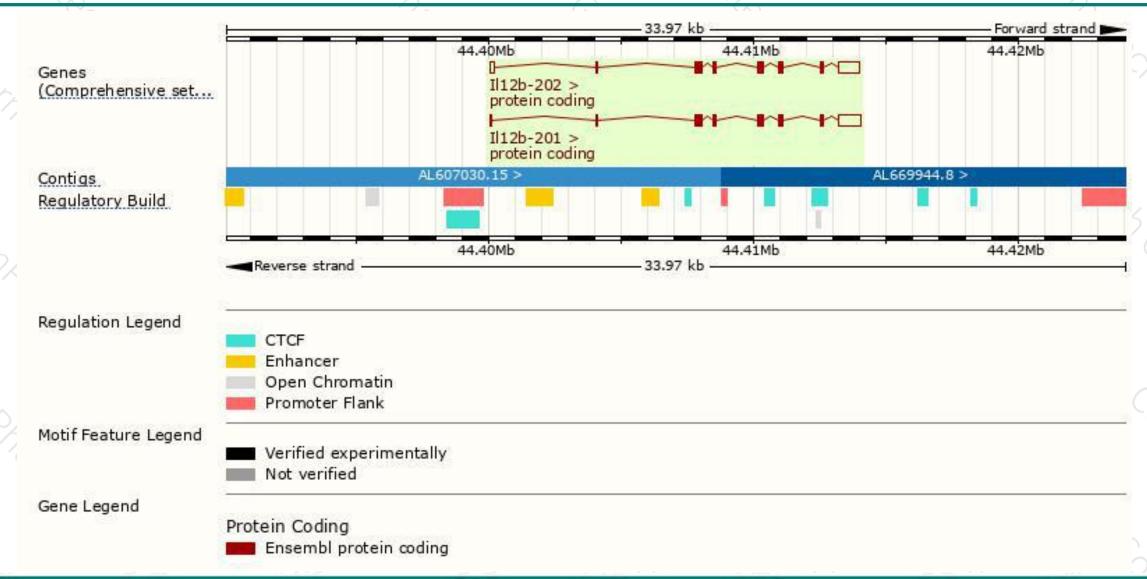
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
II12b-202	ENSMUST00000170513.2	1944	335aa	Protein coding	CCDS24563	P43432 Q3ZAX5	TSL:5 GENCODE basic APPRIS P1
II12b-201	ENSMUST00000102796.9	1861	335aa	Protein coding	CCDS24563	P43432 Q3ZAX5	TSL:1 GENCODE basic APPRIS P1

The strategy is based on the design of Il12b-201 transcript, The transcription is shown below



Genomic location distribution





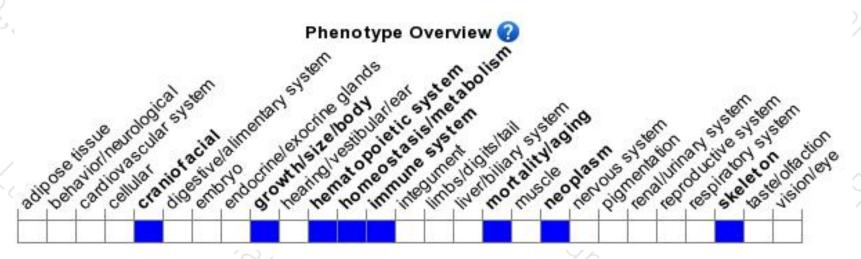
Protein domain





Mouse phenotype description(MGI)





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

According to the existing MGI data, Mice homozygous for a null allele display impaired Th1 responses, defects in IFN gamma secretion and NK cell activity, increased susceptibility to bacterial and parasitic infection, alveolar bone loss, and resistance to chemically induced tumors and to delayed type hypersensitivity.



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

Tel: 025-5864 1534





