Def6 Cas9-CKO Strategy

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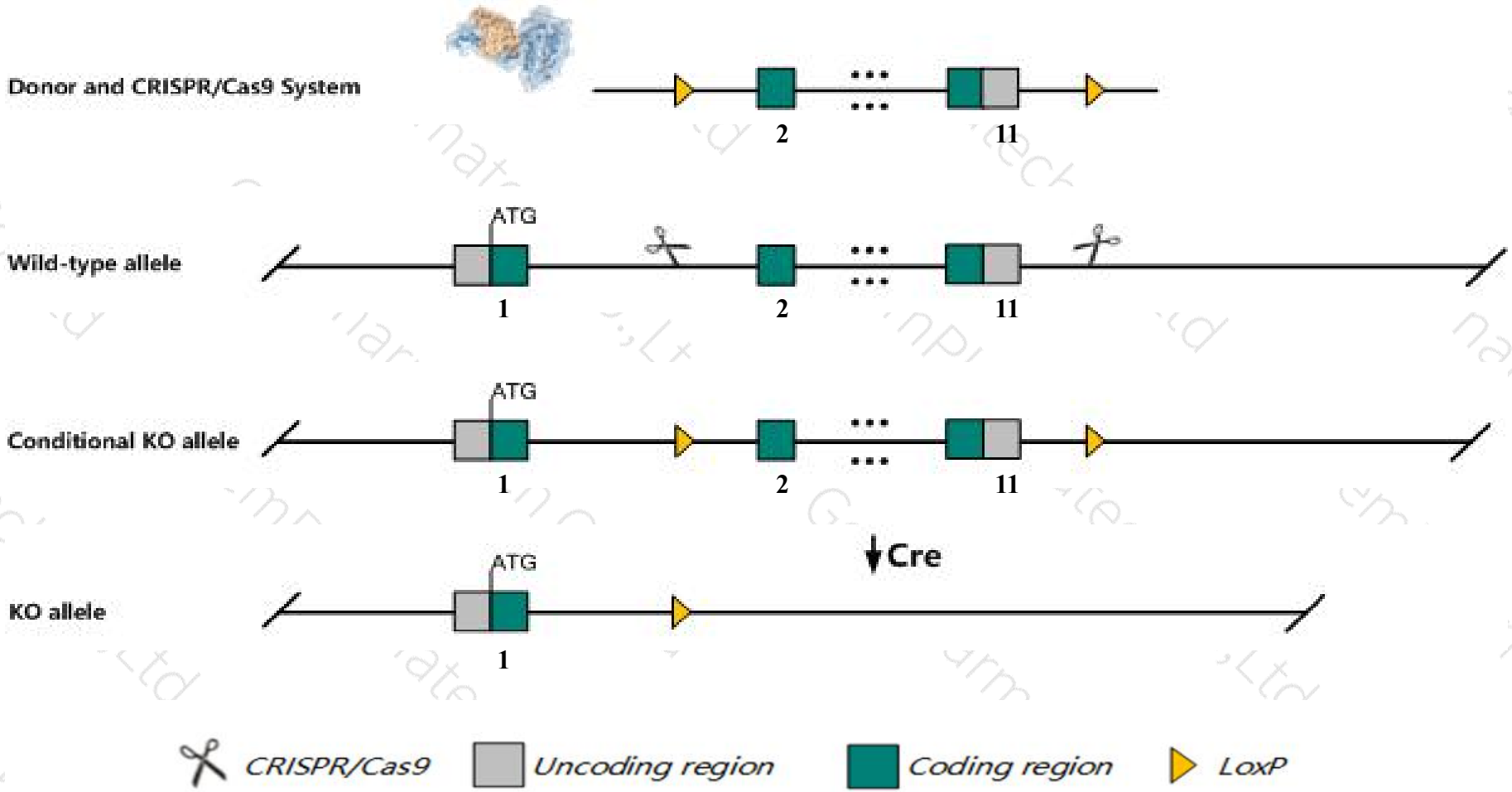
Project Overview

Project Name	***Def6***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Def6* gene. The schematic diagram is as follows:



Technical routes

- The *Def6* gene has 8 transcripts. According to the structure of *Def6* gene, exon2-exon11 of *Def6-201* (ENSMUST00000002327.5) transcript is recommended as the knockout region.The region contains most of the coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Def6* 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.

Notice

- According to the existing MGI data,Homozygous mutants spontaneously develop systemic autoimmunity. Females primarily are affected, displaying hypergammaglobulinemia, accumulation of effector/memory T cells and IgG+ B cells, and production of autoantibodies.
- The KO region contains the *Gm49874* gene.Knockout the region will affect the function of *Gm49874* gene.
- This strategy may affect the 5-terminal regulation of the Ppard gene.
- The *Def6* gene is located on the Chr17. 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）

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Def6 differentially expressed in FDCP 6 [Mus musculus (house mouse)]

Gene ID: 23853, updated on 31-Jan-2019

Summary

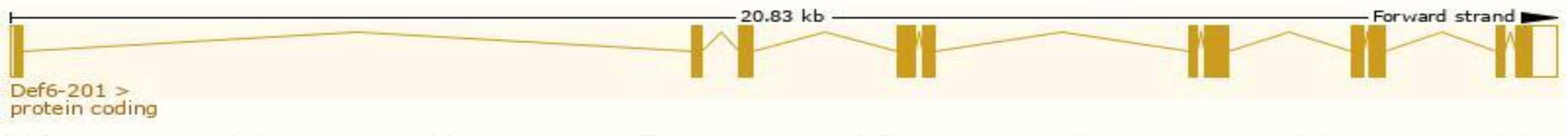
Official Symbol	Def6 provided by MGI
Official Full Name	differentially expressed in FDCP 6 provided byMGI
Primary source	MGI:MGI:1346328
See related	Ensembl:ENSMUSG00000002257
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2410003F05Rik, 6430538D02Rik, AV094905, Ibp, Slat, Slat2, Slat6
Expression	Biased expression in thymus adult (RPKM 67.0), spleen adult (RPKM 23.0) and 8 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

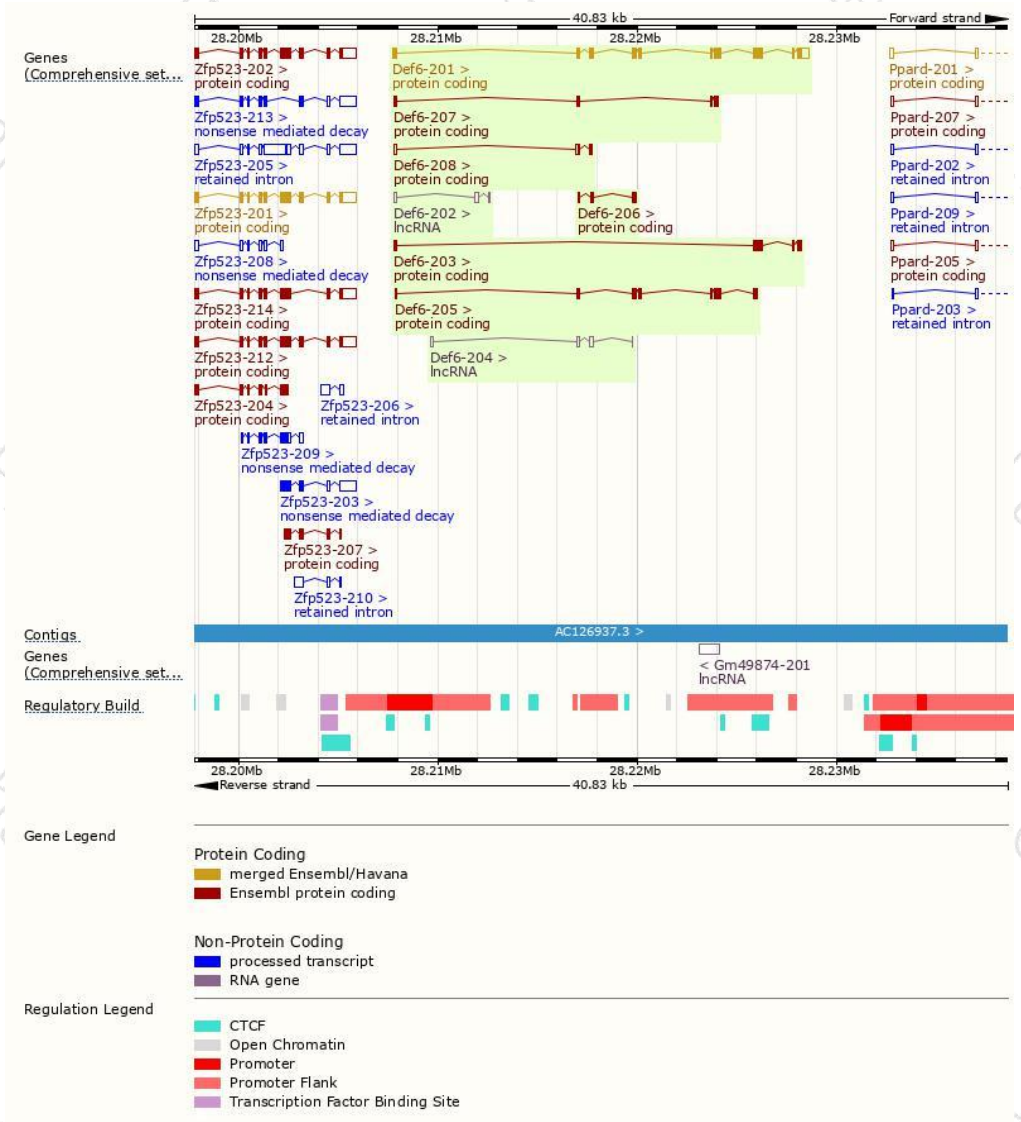
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Def6-201	ENSMUST00000002327.5	2277	630aa	Protein coding	CCDS28574	A0A0R4IZX1	TSL:1 GENCODE basic APPRIS P1
Def6-205	ENSMUST00000233264.1	1155	385aa	Protein coding	-	A0A3B2W417	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete
Def6-203	ENSMUST00000233170.1	774	257aa	Protein coding	-	Q8C2K1	GENCODE basic
Def6-207	ENSMUST00000233560.1	581	180aa	Protein coding	-	A0A3B2W860	CDS 3' incomplete
Def6-208	ENSMUST00000233958.1	432	56aa	Protein coding	-	A0A3B2WCX4	CDS 3' incomplete
Def6-206	ENSMUST00000233534.1	385	129aa	Protein coding	-	A0A3B2W486	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete
Def6-204	ENSMUST00000233205.1	445	No protein	lncRNA	-	-	
Def6-202	ENSMUST00000146724.1	349	No protein	lncRNA	-	-	TSL:3

The strategy is based on the design of *Def6-201* transcript,The transcription is shown below

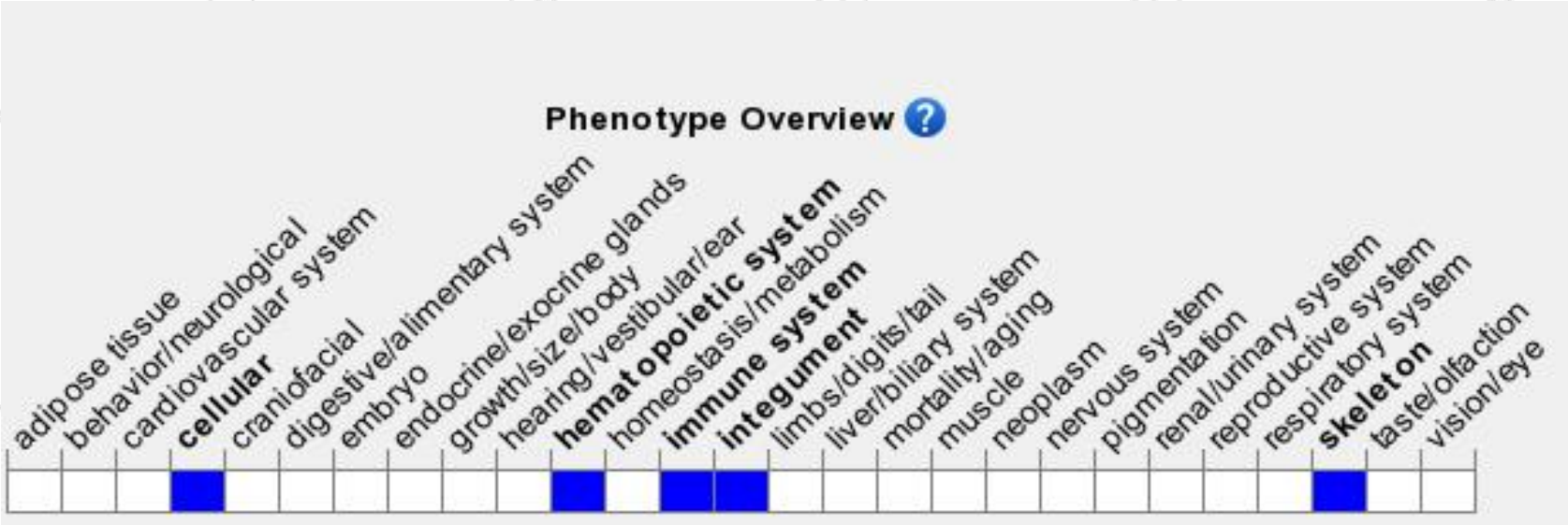


Genomic location distribution



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/).

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

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