Duox2 Cas9-CKO Strategy

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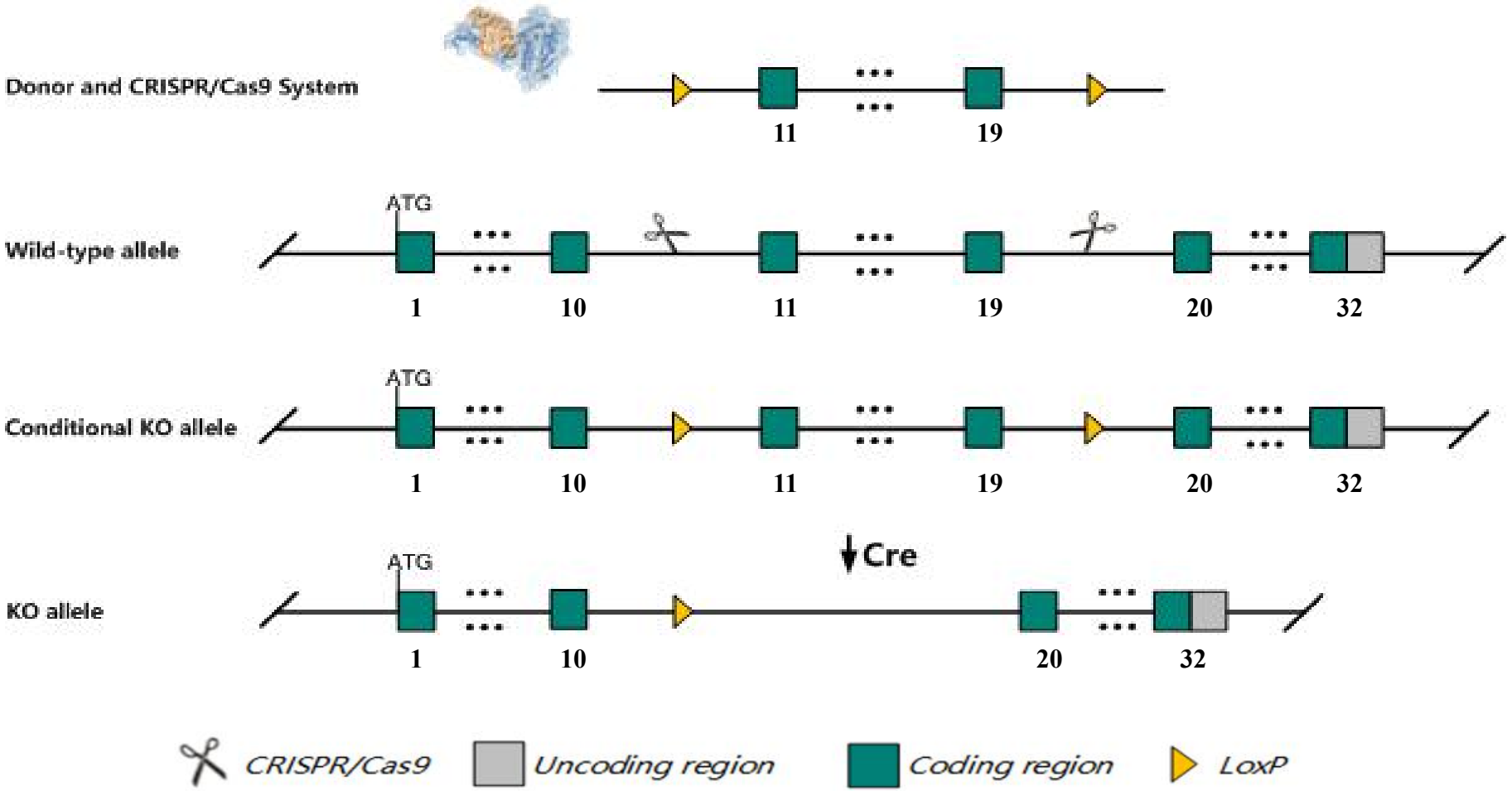
Project Overview

Project Name	***Duox2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Duox2* gene. The schematic diagram is as follows:



Technical routes

The *Duox2* gene has 3 transcripts. According to the structure of *Duox2* gene, exon11-exon19 of *Duox2-201*(ENSMUST00000053734.5) transcript is recommended as the knockout region.The region contains 1420bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Duox2* 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,mice homozygous for a spontaneous mutation fail to breed and are congenitally hypothyroid (low T4, high TSH), dwarf, and hearing impaired. Anterior pituitaries are dysplastic. Cochlear defects include delayed formation of the inner sulcus and tunnel of Corti and a thickened tectorial membrane.

The Intron10 and Intron19 are only 523bp and 2094bp,loxp insertion may affect mRNA splicing.

The *Duox2* gene is located on the Chr2. 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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Duox2 dual oxidase 2 [Mus musculus (house mouse)]

Gene ID: 214593, updated on 24-Feb-2019

Summary

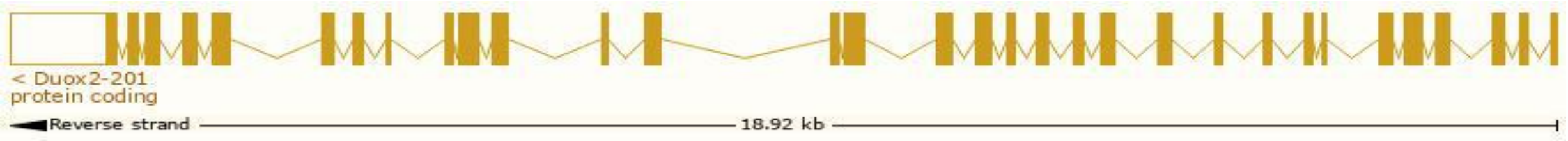
Official Symbol	Duox2 provided by MGI
Official Full Name	dual oxidase 2 provided by MGI
Primary source	MGI:MGI:3036280
See related	Ensembl:ENSMUSG00000068452
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	A430065P05Rik, LNOX2, NOXEF2, P138-TOX, THOX2
Expression	Biased expression in large intestine adult (RPKM 68.1), small intestine adult (RPKM 25.6) and 2 other tissues See more
Orthologs	human all

Transcript information Ensembl

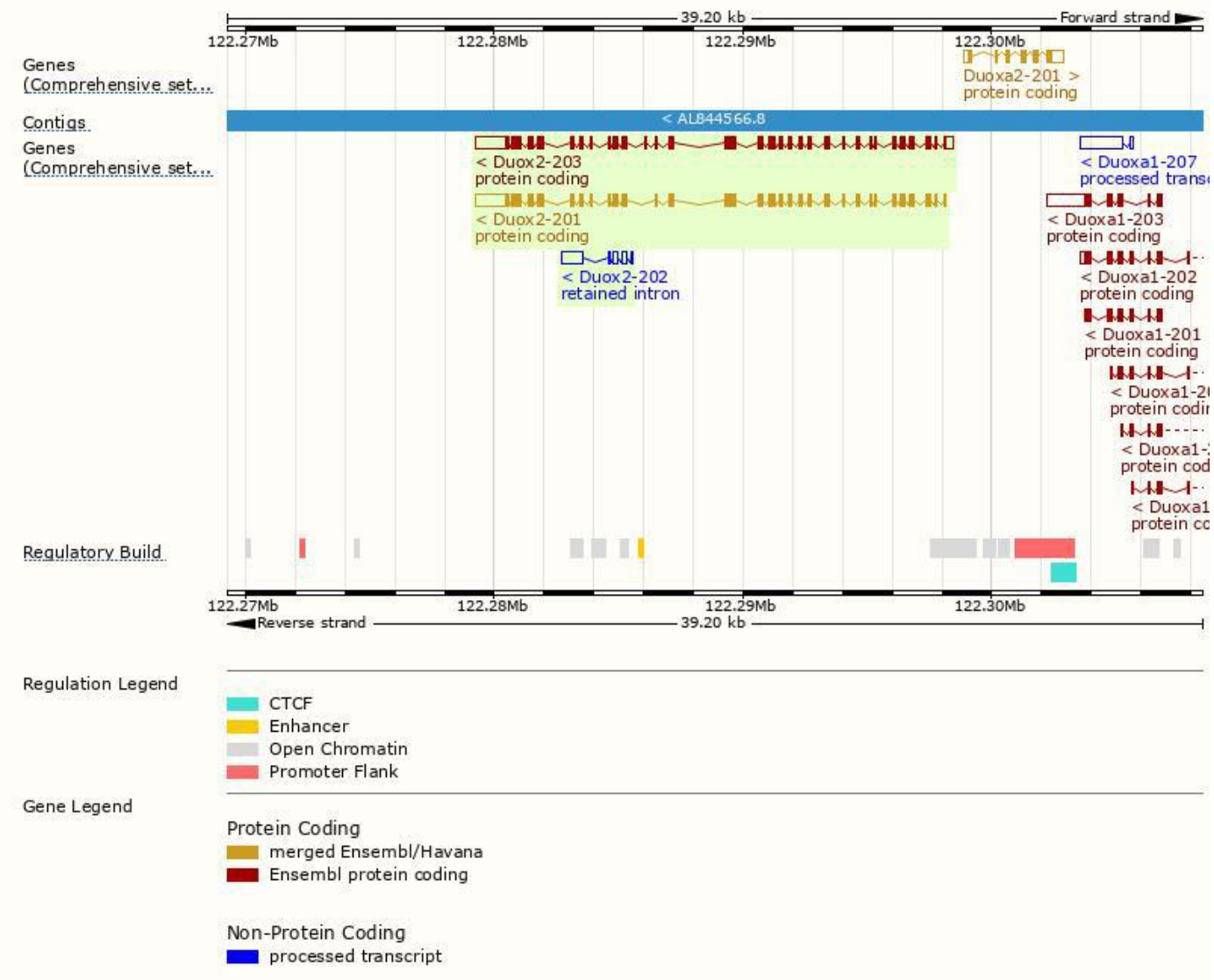
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Duox2-201	ENSMUST00000053734.5	5744	1517aa	Protein coding	CCDS38221	A2AQ99	TSL:2 GENCODE basic APPRIS P2
Duox2-203	ENSMUST00000237546.1	6112	1545aa	Protein coding	-	-	GENCODE basic APPRIS ALT2
Duox2-202	ENSMUST00000155820.1	1451	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Duox2-201* transcript,the transcription is shown below:

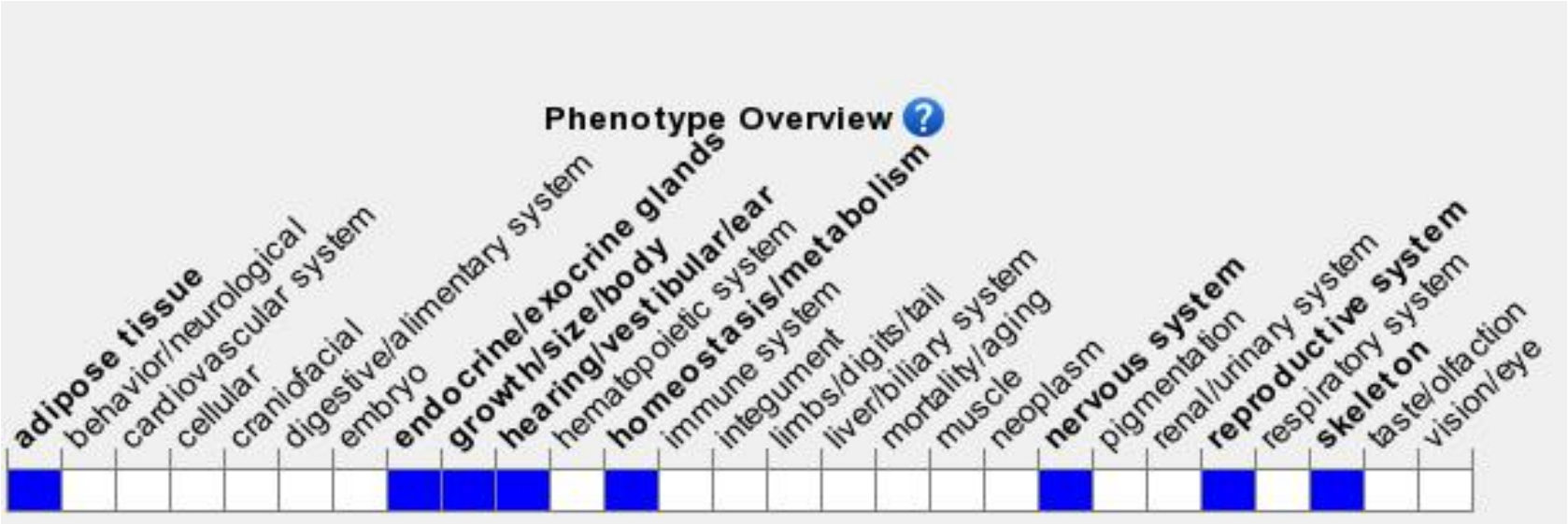


Genomic location distribution



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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