

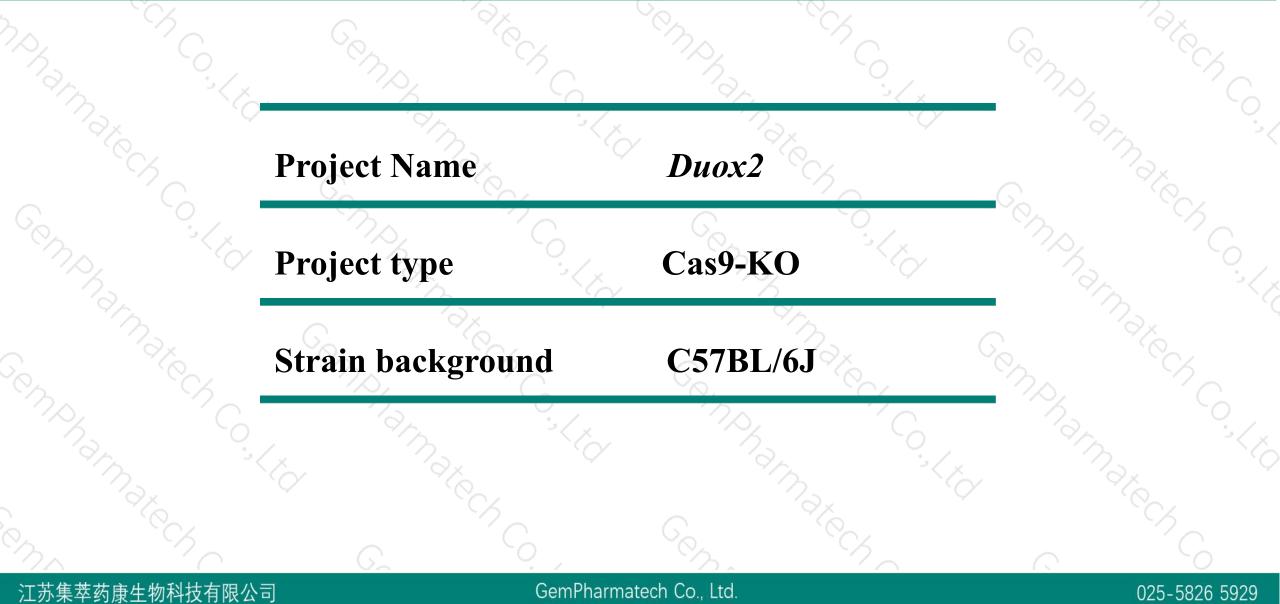
Comparing to Compare of Compare o **Duox2** Cas9-KO Strategy Cempharmateco Romphamater Control

Cemphamatech, Comphannated Co Designer: Yanhua Shen

empharmatech,

Project Overview

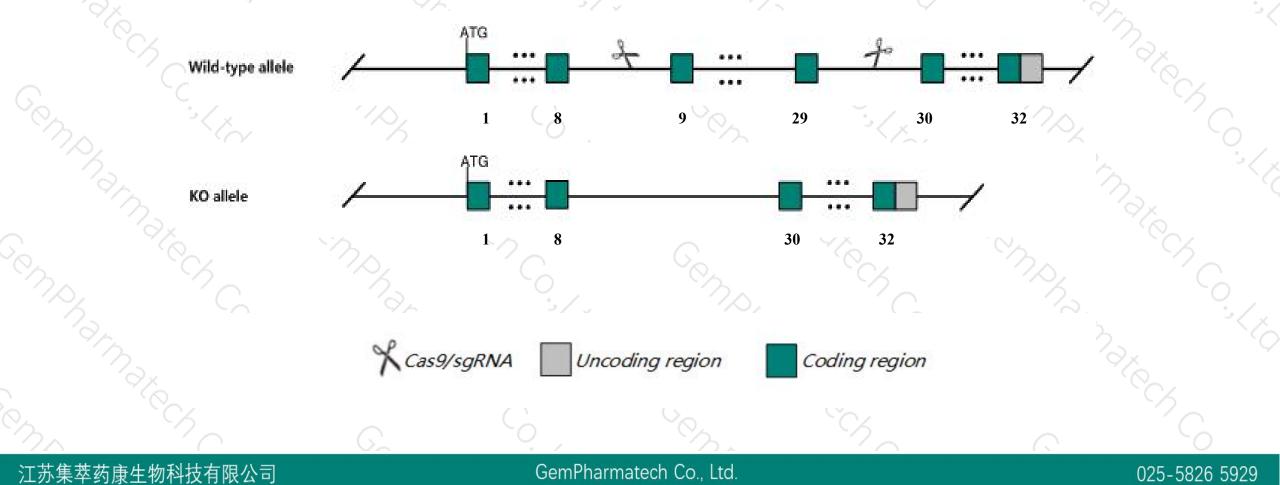




Knockout strategy



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





- The Duox2 gene has 3 transcripts. According to the structure of Duox2 gene, exon9-exon29 of Duox2-201 (ENSMUST00000053734.5) transcript is recommended as the knockout region. The region contains 3106bp 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: 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.



025-5826 5929

- 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 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 gene transcription and translation processes, all risks cannot be predicted under existing information.

江苏集萃药康生物科技有限公司

Gene information (NCBI)



< ?

025-5826 5929

Duox2 dual oxidase 2 [Mus musculus (house mouse)]

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

Summary

Official SymbolDuxx2 provided by MGIOfficial Full Namedual oxidase 2 provided by MGIPrimary soureMGI:MGI:3036280See relateEnsembl:ENSMUSG0000068452Gene typeprotein codingprotein codingVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
Muroidea; Murinae; Mus; MusAlso knownasA430065P05Rik, LNOX2, NOXEF2, P138-TOX, THOX2ExpressionBiased expression in large intestine adult (RPKM 68.1), small intestine adult (RPKM 25.6) and 2 other tissuesSee more
Muma all

江苏集萃药康生物科技有限公司

GemPharmatech Co., Ltd

Transcript information (Ensembl)

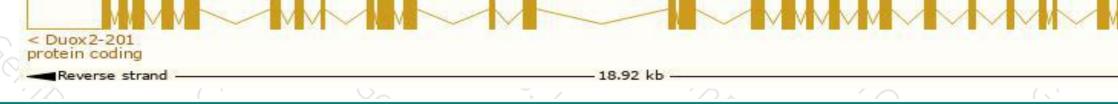


025-5826 5929

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

1 m							d kees C 1
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Duox2-201	ENSMUST00000053734.5	5744	<u>1517aa</u>	Protein coding	CCDS38221	A2AQ99	TSL:2 GENCODE basic APPRIS P2
Duox2-203	ENSMUST00000237546.1	6112	<u>1545aa</u>	Protein coding	-	-	GENCODE basic APPRIS ALT2
Duox2-202	ENSMUST00000155820.1	1451	No protein	Retained intron	2	22	TSL:1

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



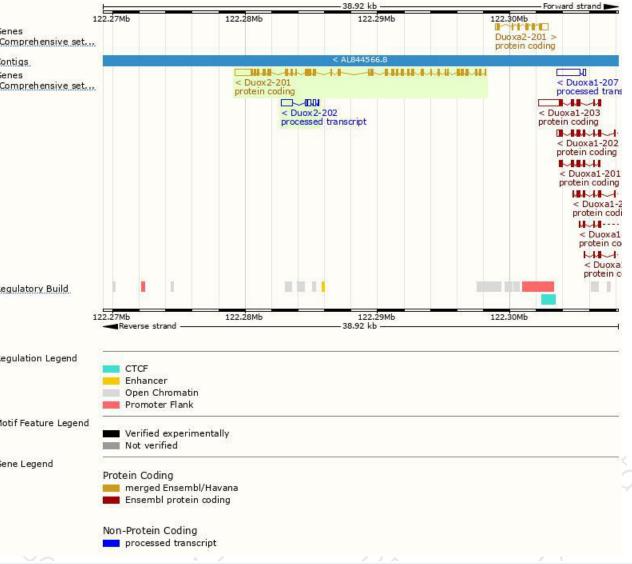
江苏集萃药康生物科技有限公司

GemPharmatech Co., Ltd.

Genomic location distribution







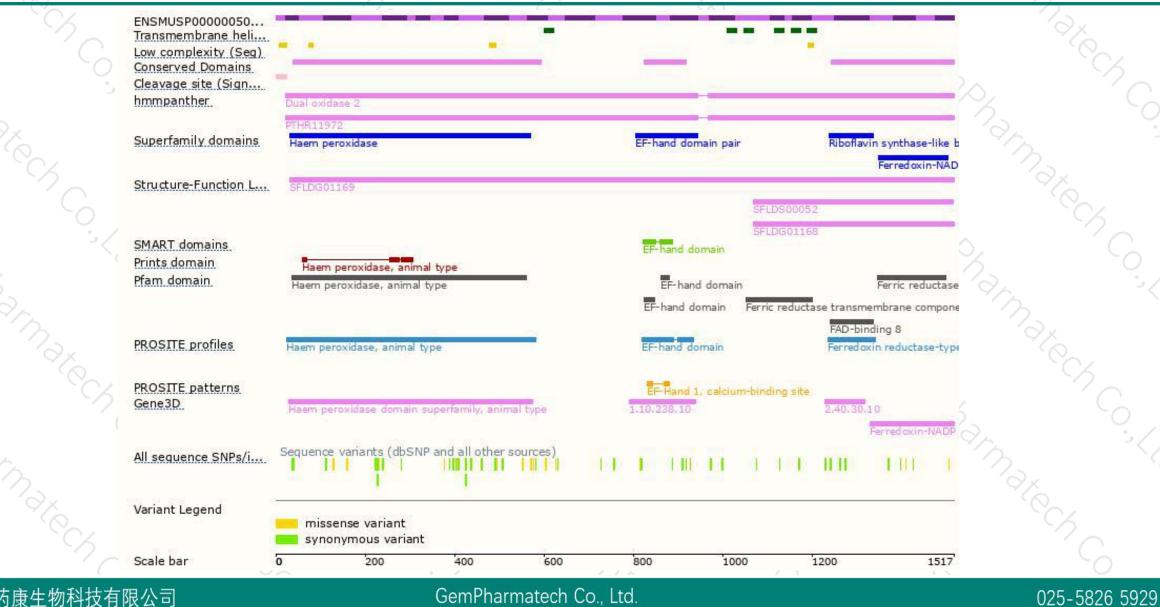
江苏集萃药康生物科技有限公司

GemPharmatech Co., Ltd.

025-5826 5929

Protein domain



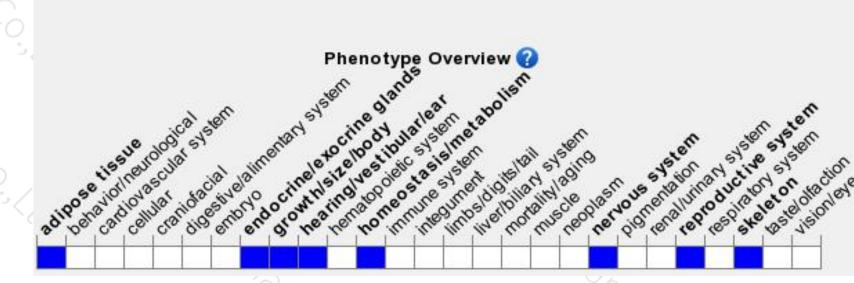


江苏集萃药康生物科技有限公司

GemPharmatech Co., Ltd.

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 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.

江苏集萃药康生物科技有限公司

GemPharmatech Co., Ltd,

025-5826 5929



If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



