

Ddx41 Cas9-CKO Strategy

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Design Date: 2022-09-20

Overview

Target Gene Name

• Ddx41

Project Type

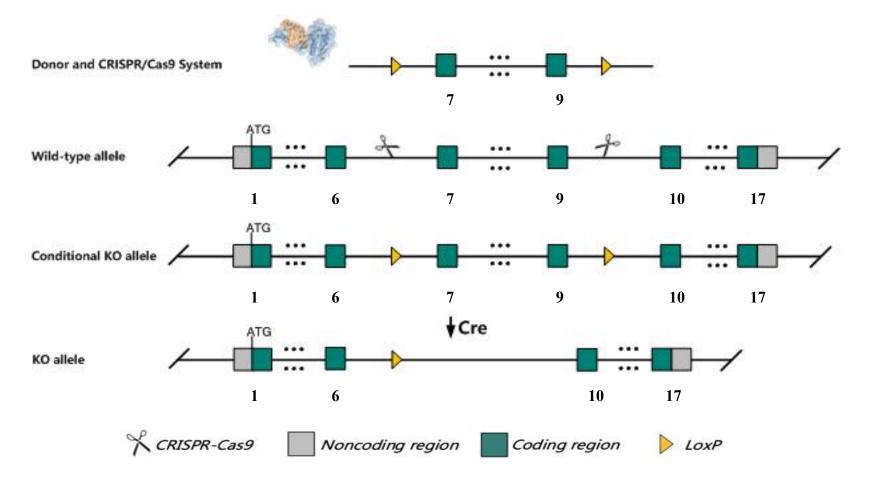
• Cas9-CKO

Genetic Background

• C57BL/6JGpt



Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Ddx41* gene.

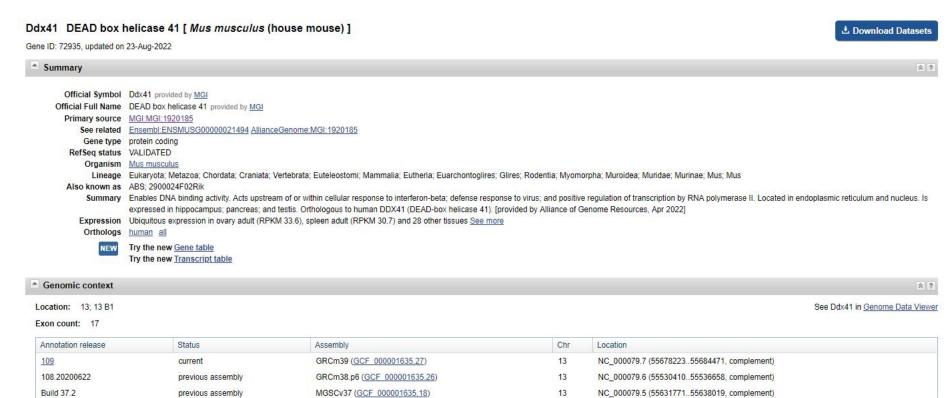


Technical Information

- The *Ddx41* gene has 5 transcripts. According to the structure of *Ddx41* gene, exon7-9 of *Ddx41*-201 (ENSMUST0000021956.9) transcript is recommended as the knockout region. The region contains 364 bp of coding sequences. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Ddx41* 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- 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.



Gene Information



Chromosome 13 - NC 000079.7

Gn46416-

Mir6945 -

[55722288 **b**

Gm31364

Source: https://www.ncbi.nlm.nih.gov/

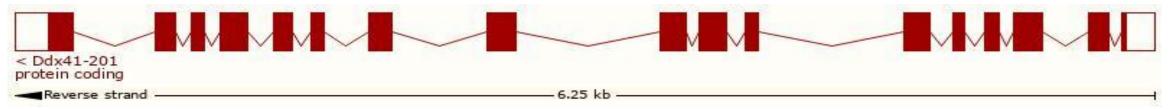


Transcript Information

The gene has 5 transcripts, all transcripts are shown below:

Transcript ID	Name	bp 🌲	Protein	Biotype	CCDS A	UniProt Match	Flags
ENSMUST00000224765.2	Ddx41-204	2199	633aa	Protein coding		A0A1S6GWJ4®	Ensembl Canonical GENCODE basic
ENSMUST00000224686.2	Ddx41-203	835	No protein	Retained intron		75	
ENSMUST00000225783.2	Ddx41-205	710	No protein	Retained intron		=	
ENSMUST00000224125.2	Ddx41-202	705	No protein	Retained intron		5	17
ENSMUST00000021956.9	Ddx41-201	2205	<u>622aa</u>	Protein coding	CCDS26549 €	Q91VN6 &	GENCODE basic APPRIS P1 TSL:1

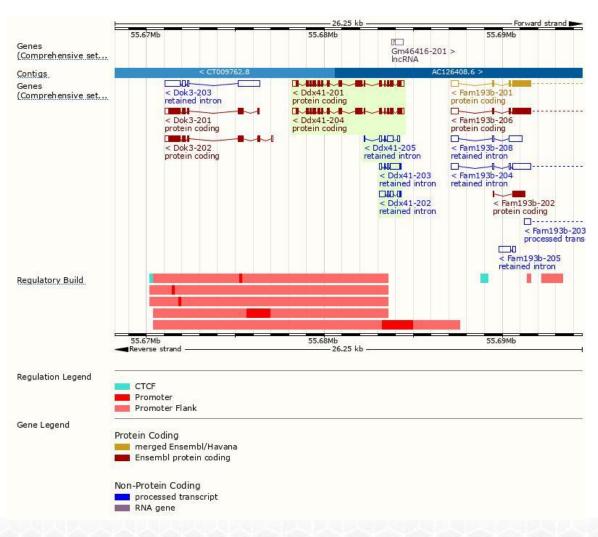
The strategy is based on the design of Ddx41-201 transcript, the transcription is shown below:

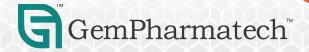


Source: https://www.ensembl.org



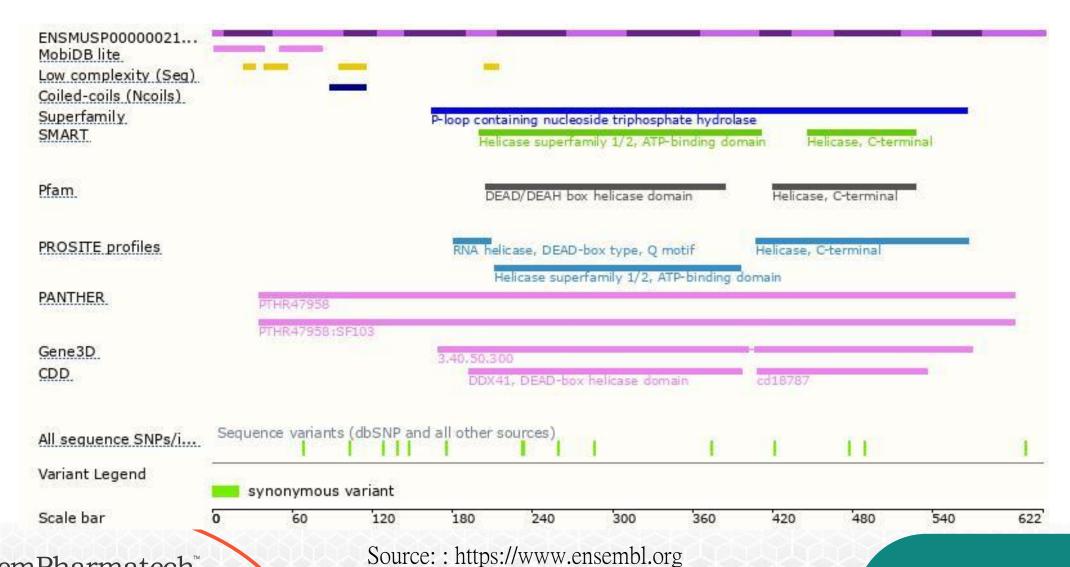
Genomic Information



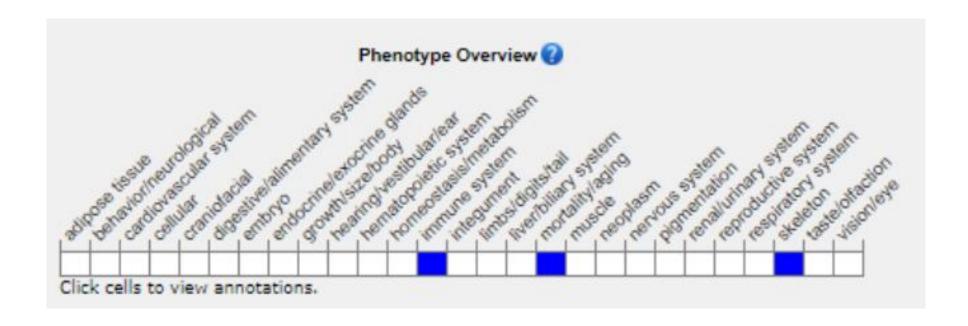


Source: : https://www.ensembl.org

Protein Information



Mouse Phenotype Information (MGI)



• Constitutive homozygous knockout is embryonic lethal. Conditional homozygous KO in macrophages and DCs (dendritic cells) leads to a depressed immune response to viral infection.



Important Information

- According to the existing MGI data, constitutive homozygous knockout is embryonic lethal. Conditional homozygous KO in macrophages and DCs (dendritic cells) leads to a depressed immune response to viral infection.
- This strategy may affect the 5-terminal regulation of *Dok3* gene and *Gm46416* gene.
- The Ddx41 gene is located on the Chr13. 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.

