

# Donald Color Idh2 Cas9-CKO Strategy Rohalmakech Co.

Constant areas Consolarna de Co Designer:Lixin LYU

# **Project Overview**



**Project Name** 

Idh2

**Project type** 

Cas9-CKO

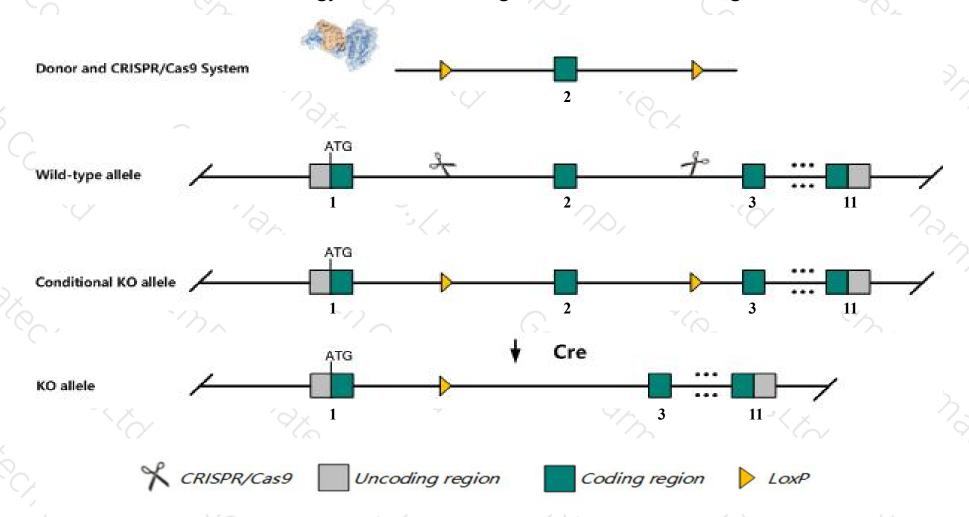
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Idh2* gene. The schematic diagram is as follows:



## Technical routes



- ➤ The *Idh2* gene has 7 transcripts. According to the structure of *Idh2* gene, exon2 of *Idh2-201*(ENSMUST00000107384.9) transcript is recommended as the knockout region. The region contains 92bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Idh2* 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 knock-out allele exhibit suppression of tumorigenesis from B16F10 melanoma cells.
- The *Idh2* gene is located on the Chr7. 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)



#### Idh2 isocitrate dehydrogenase 2 (NADP+), mitochondrial [Mus musculus (house mouse)]

Gene ID: 269951, updated on 19-Mar-2019

#### Summary

☆ ?

Official Symbol Idh2 provided by MGI

Official Full Name isocitrate dehydrogenase 2 (NADP+), mitochondrial provided by MGI

Primary source MGI:MGI:96414

See related Ensembl: ENSMUSG00000030541

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 E430004F23, IDPm, Idh-2

Expression Broad expression in heart adult (RPKM 329.1), kidney adult (RPKM 134.6) and 22 other tissuesSee more

Orthologs <u>human</u> all

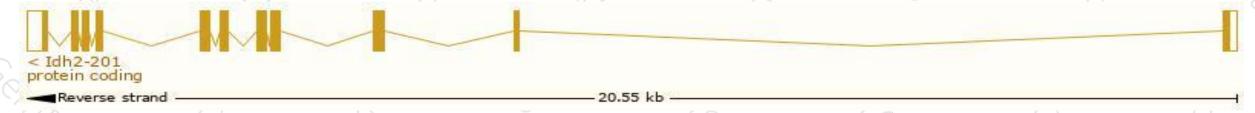
# Transcript information (Ensembl)



The gene has 7 transcripts, all transcripts are shown below:

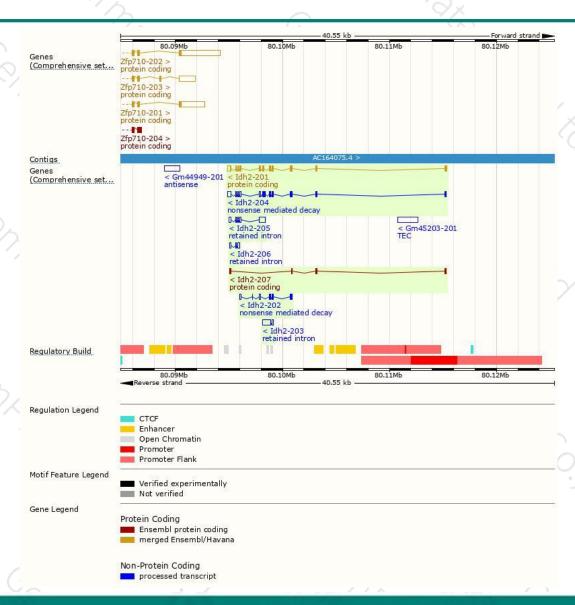
			1 100			
Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
ENSMUST00000107384.9	1745	452aa	Protein coding	CCDS39994	P54071	TSL:1 GENCODE basic APPRIS P1
ENSMUST00000206714.1	392	90aa	Protein coding		A0A0U1RPR1	TSL:3 GENCODE basic
ENSMUST00000134328.7	1790	294aa	Nonsense mediated decay	26	D6RIL6	TSL:1
ENSMUST00000125542.2	743	<u>150aa</u>	Nonsense mediated decay	0:	A0A0U1RP68	CDS 5' incomplete TSL:3
ENSMUST00000139178.7	1085	No protein	Retained intron	-	150	TSL:2
ENSMUST00000134070.1	891	No protein	Retained intron	8	6-8	TSL:1
ENSMUST00000156761.1	485	No protein	Retained intron	2	(2)	TSL:2
	ENSMUST00000107384.9 ENSMUST00000206714.1 ENSMUST00000134328.7 ENSMUST00000125542.2 ENSMUST00000139178.7 ENSMUST00000134070.1	ENSMUST00000107384.9 1745 ENSMUST00000206714.1 392 ENSMUST00000134328.7 1790 ENSMUST00000125542.2 743 ENSMUST00000139178.7 1085 ENSMUST00000134070.1 891	ENSMUST00000107384.9 1745 452aa  ENSMUST00000206714.1 392 90aa  ENSMUST00000134328.7 1790 294aa  ENSMUST00000125542.2 743 150aa  ENSMUST00000139178.7 1085 No protein  ENSMUST00000134070.1 891 No protein	ENSMUST00000107384.9         1745         452aa         Protein coding           ENSMUST00000206714.1         392         90aa         Protein coding           ENSMUST00000134328.7         1790         294aa         Nonsense mediated decay           ENSMUST00000125542.2         743         150aa         Nonsense mediated decay           ENSMUST00000139178.7         1085         No protein         Retained intron           ENSMUST00000134070.1         891         No protein         Retained intron	ENSMUST00000107384.9         1745         452aa         Protein coding         CCDS39994           ENSMUST00000206714.1         392         90aa         Protein coding         -           ENSMUST00000134328.7         1790         294aa         Nonsense mediated decay         -           ENSMUST00000125542.2         743         150aa         Nonsense mediated decay         -           ENSMUST00000139178.7         1085         No protein         Retained intron         -           ENSMUST00000134070.1         891         No protein         Retained intron         -	ENSMUST00000107384.9         1745         452aa         Protein coding         CCDS39994         P54071           ENSMUST00000206714.1         392         90aa         Protein coding         -         A0A0U1RPR1           ENSMUST00000134328.7         1790         294aa         Nonsense mediated decay         -         D6RIL6           ENSMUST00000125542.2         743         150aa         Nonsense mediated decay         -         A0A0U1RP68           ENSMUST00000139178.7         1085         No protein         Retained intron         -         -           ENSMUST00000134070.1         891         No protein         Retained intron         -         -

The strategy is based on the design of *Idh2-201* transcript, The transcription is shown below



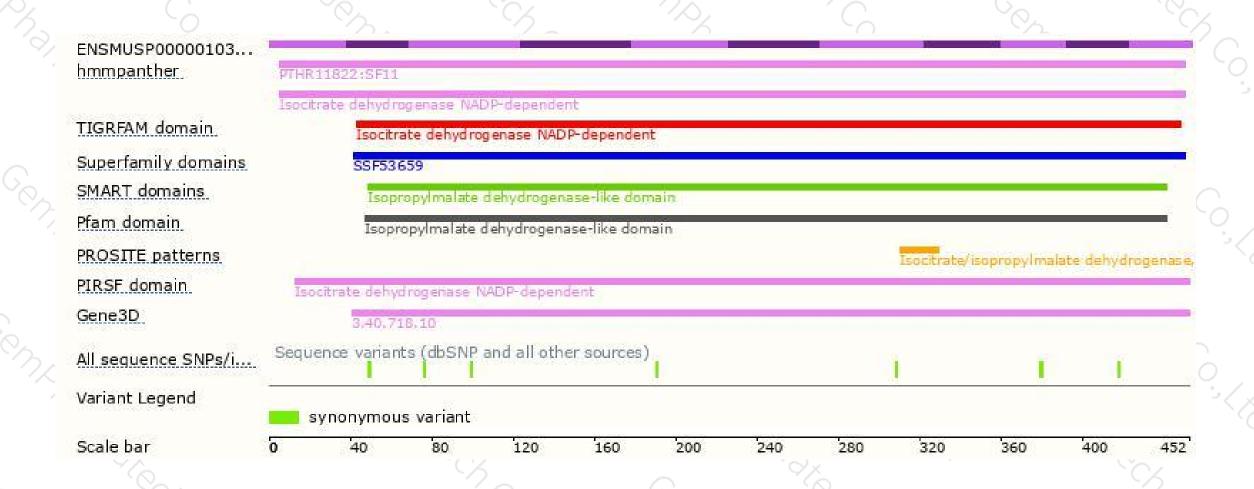
## Genomic location distribution





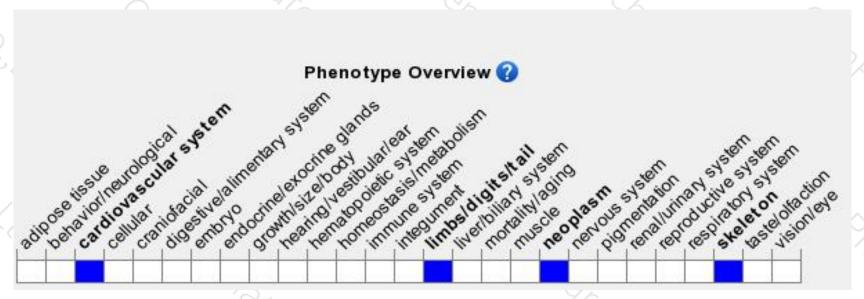
### Protein domain





# 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 knock-out allele exhibit suppression of tumorigenesis from B16F10 melanoma cells.



If you have any questions, you are welcome to inquire. Tel: 400-9660890





