

Metap2 Cas9-CKO Strategy

Designer: Yun Li

Reviewer: Longyun Hu

Design Date: 2023-01-29

Overview

Target Gene Name

- Metap2

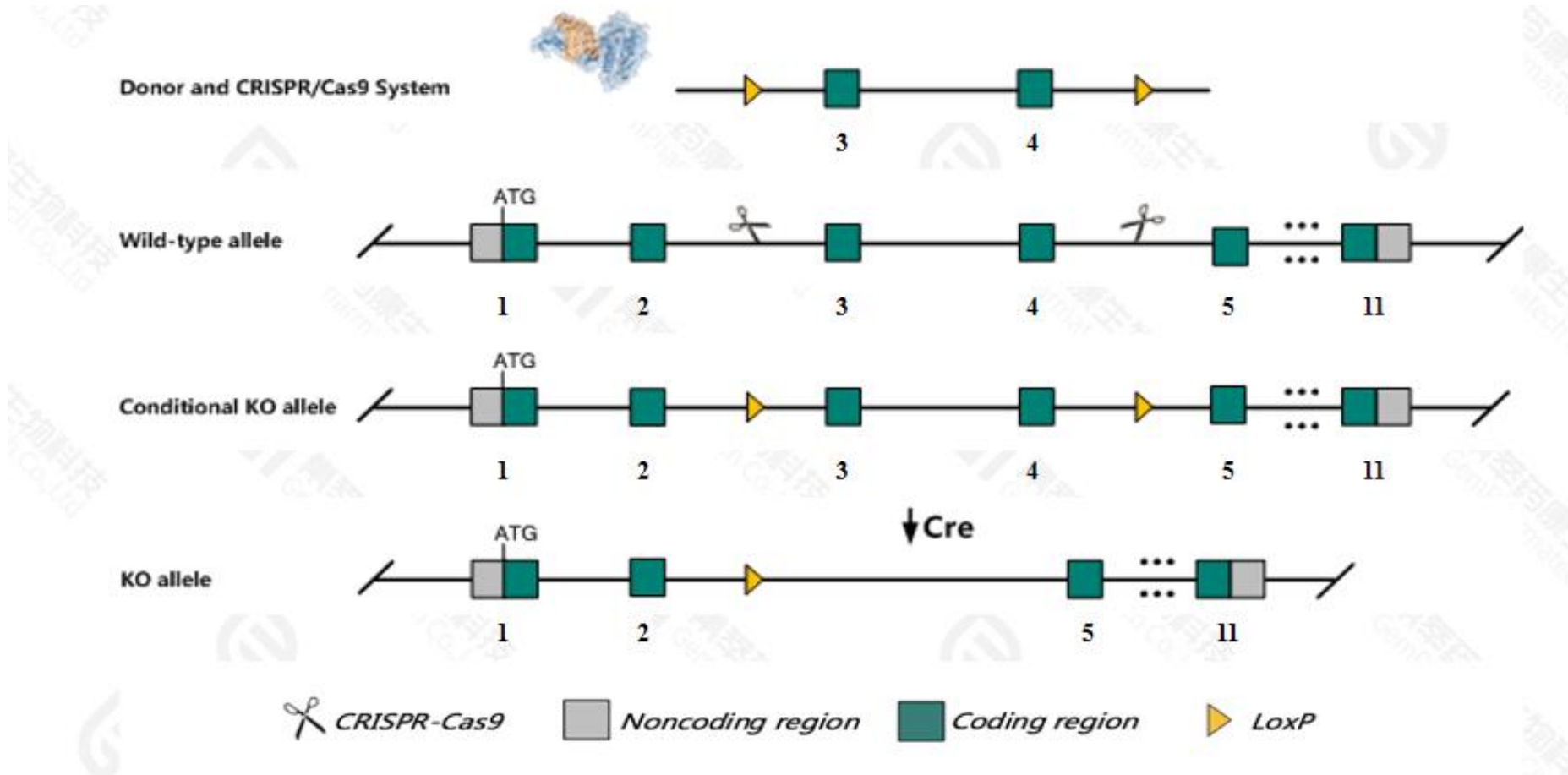
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Metap2* gene.

Technical Information

- The *Metap2* gene has 18 transcripts. According to the structure of *Metap2* gene, exon3-exon4 of *Metap2-201*(ENSMUST00000047910.15) transcript is recommended as the knockout region. The region contains 169bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Metap2* 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

Metap2 methionine aminopeptidase 2 [Mus musculus (house mouse)]

Gene ID: 56307, updated on 22-May-2022

Summary	
Official Symbol	Metap2 provided by MGI
Official Full Name	methionine aminopeptidase 2 provided by MGI
Primary source	MGI:MGI:1929701
See related	Ensembl:ENSMUSG00000036112
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	4930584B20Rik, A930035J23Rik, AI047573, AL024412, AU014659, Amp2, Mnpep, p67, p67eIF2
Expression	Broad expression in liver E14 (RPKM 47.5), liver E14.5 (RPKM 37.1) and 20 other tissues See more
Orthologs	human all

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

Transcript Information

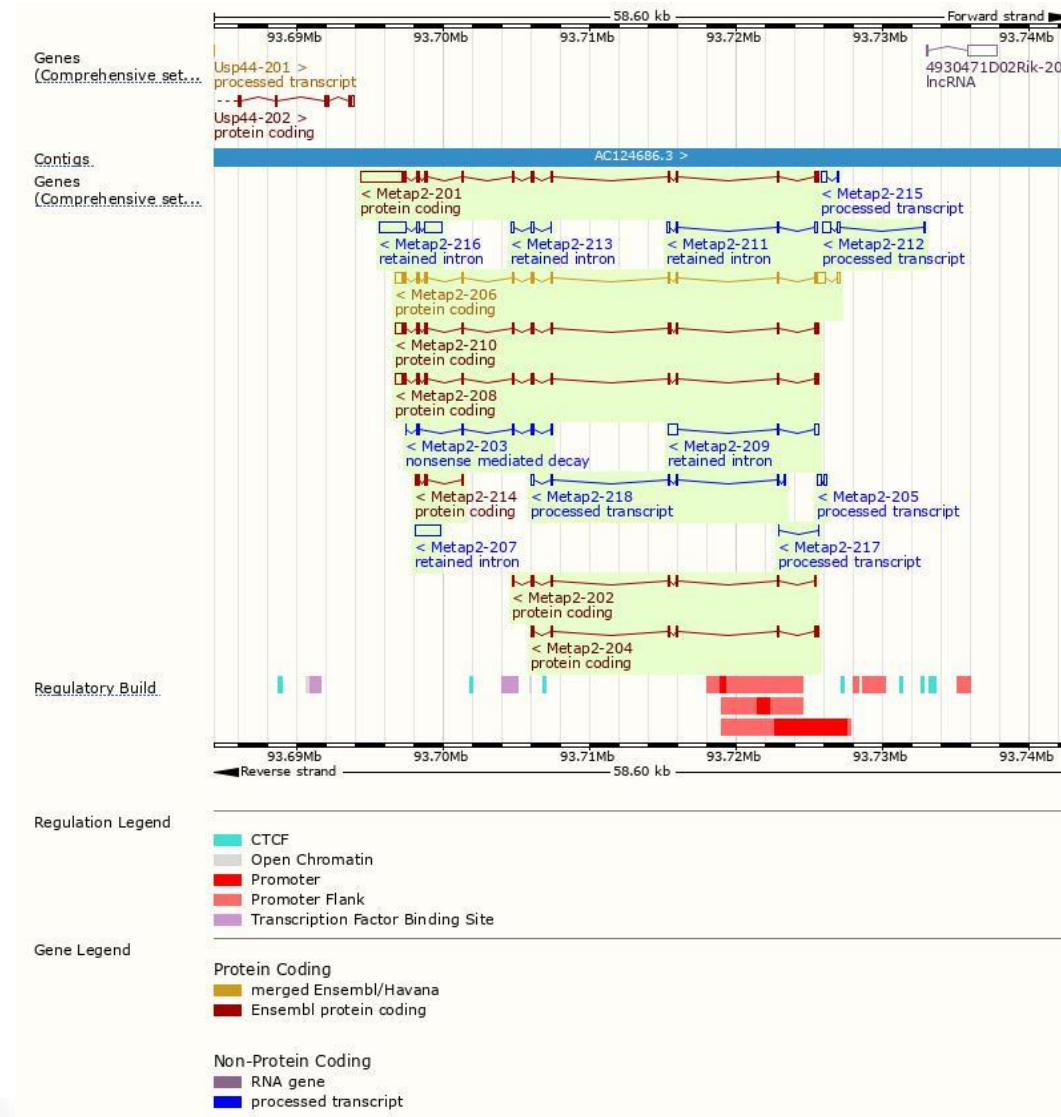
The gene has 18 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Metap2-201	ENSMUST00000047910.15	4424	478aa	Protein coding	CCDS48673		TSL:1 , GENCODE basic , APPRIS P1 ,
Metap2-206	ENSMUST00000180840.8	2551	478aa	Protein coding	CCDS48673		TSL:1 , GENCODE basic , APPRIS P1 ,
Metap2-210	ENSMUST00000181217.8	1984	488aa	Protein coding	-		TSL:1 , GENCODE basic ,
Metap2-208	ENSMUST00000181091.8	1876	455aa	Protein coding	-		TSL:1 , GENCODE basic ,
Metap2-204	ENSMUST00000180688.2	825	233aa	Protein coding	-		CDS 3' incomplete , TSL:3 ,
Metap2-202	ENSMUST00000180375.8	724	242aa	Protein coding	-		CDS 5' and 3' incomplete , TSL:5 ,
Metap2-214	ENSMUST00000181470.2	445	106aa	Protein coding	-		CDS 5' incomplete , TSL:2 ,
Metap2-203	ENSMUST00000180392.8	674	186aa	Nonsense mediated decay	-		CDS 5' incomplete , TSL:5 ,
Metap2-212	ENSMUST00000181434.2	793	No protein	Processed transcript	-		TSL:1 ,
Metap2-218	ENSMUST00000216232.2	643	No protein	Processed transcript	-		TSL:3 ,
Metap2-205	ENSMUST00000180739.2	505	No protein	Processed transcript	-		TSL:3 ,
Metap2-215	ENSMUST00000181740.2	404	No protein	Processed transcript	-		TSL:2 ,
Metap2-217	ENSMUST00000215642.2	116	No protein	Processed transcript	-		TSL:3 ,
Metap2-216	ENSMUST00000181894.2	3034	No protein	Retained intron	-		TSL:1 ,
Metap2-207	ENSMUST00000181009.2	1753	No protein	Retained intron	-		TSL:NA ,
Metap2-209	ENSMUST00000181104.2	931	No protein	Retained intron	-		TSL:2 ,
Metap2-211	ENSMUST00000181236.8	547	No protein	Retained intron	-		TSL:2 ,
Metap2-213	ENSMUST00000181442.2	331	No protein	Retained intron	-		TSL:3 ,

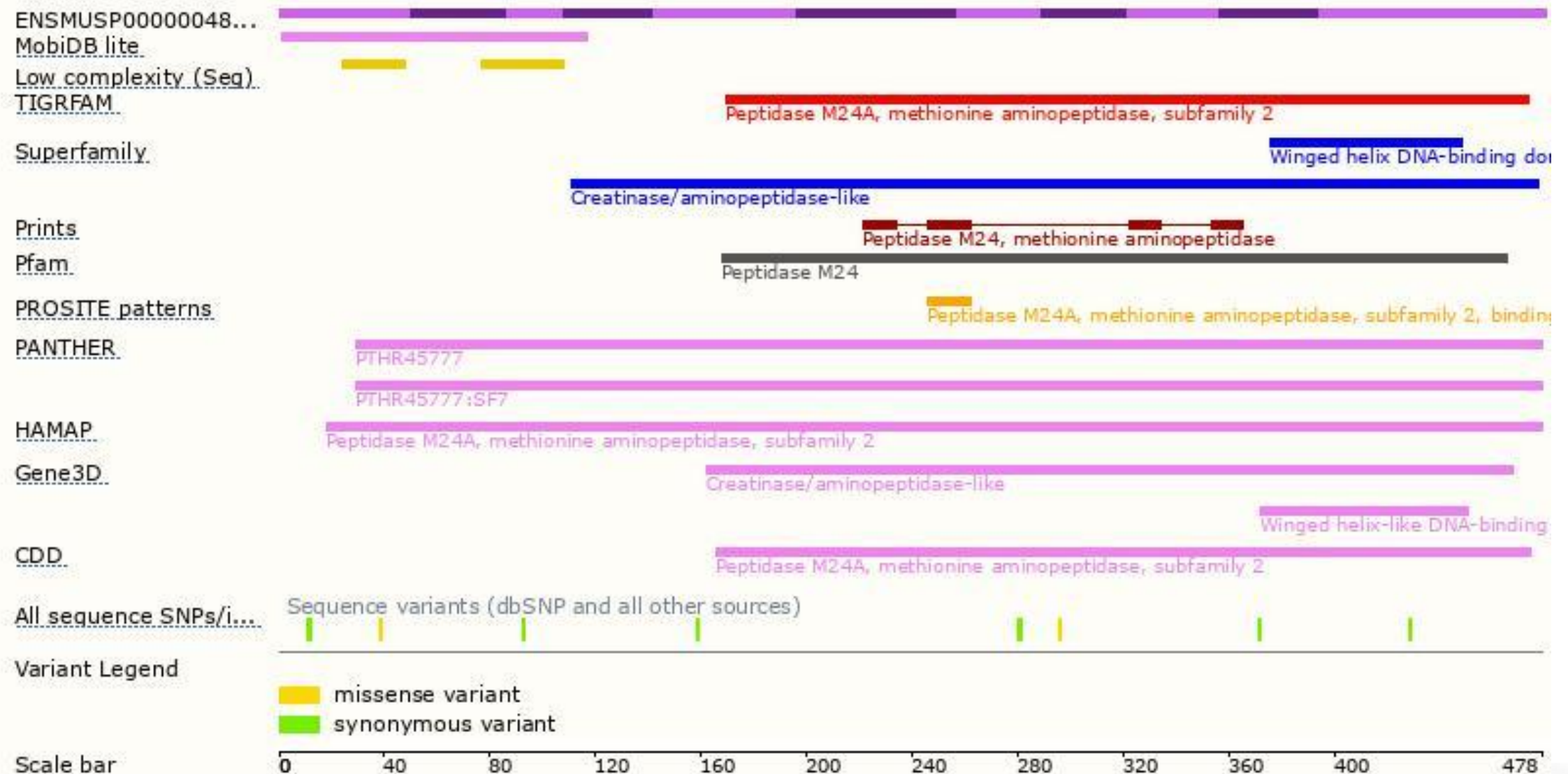
The strategy is based on the design of *Metap2-201* transcript, the transcription is shown below:



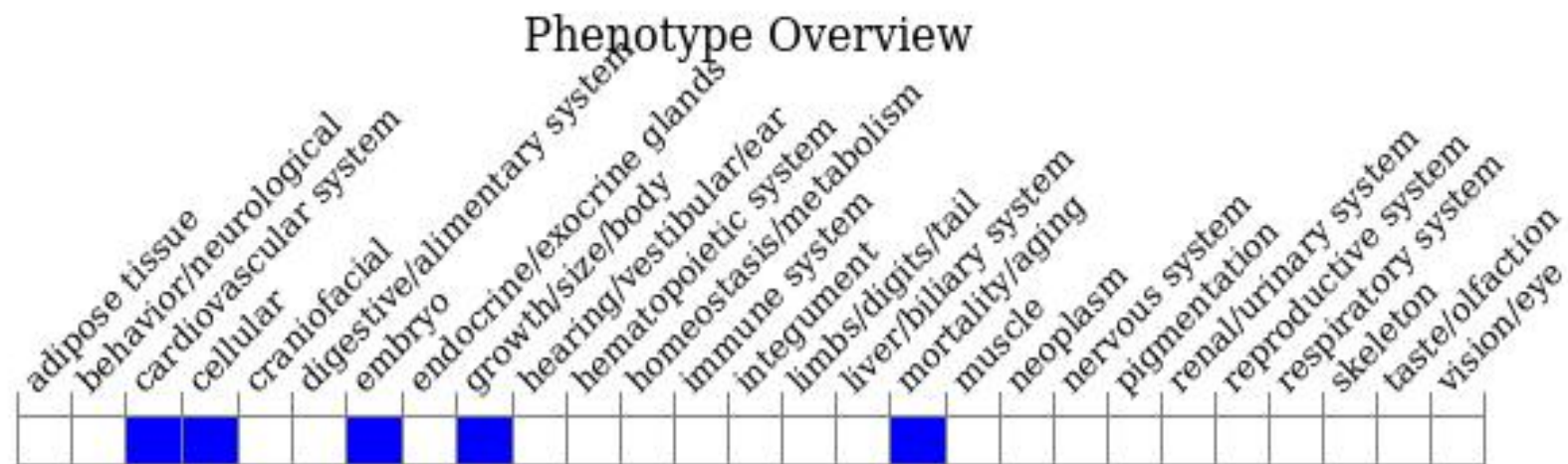
Genomic Information



Protein Information



Mouse Phenotype Information (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, homozygous mutation of this gene results in embryonic lethality around E8.5, smaller size, failure to gastrulate, reduced cell proliferation and absence of a distinct mesoderm.

Important Information

- According to the existing MGI data, homozygous mutation of this gene results in embryonic lethality around E8.5, smaller size, failure to gastrulate, reduced cell proliferation and absence of a distinct mesoderm..
- *Metap2* is located on Chr10. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is 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 the existing technology level.