

Dio2 Cas9-CKO Strategy

Designer:

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Project Overview

Project Name

Dio2

Project type

Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

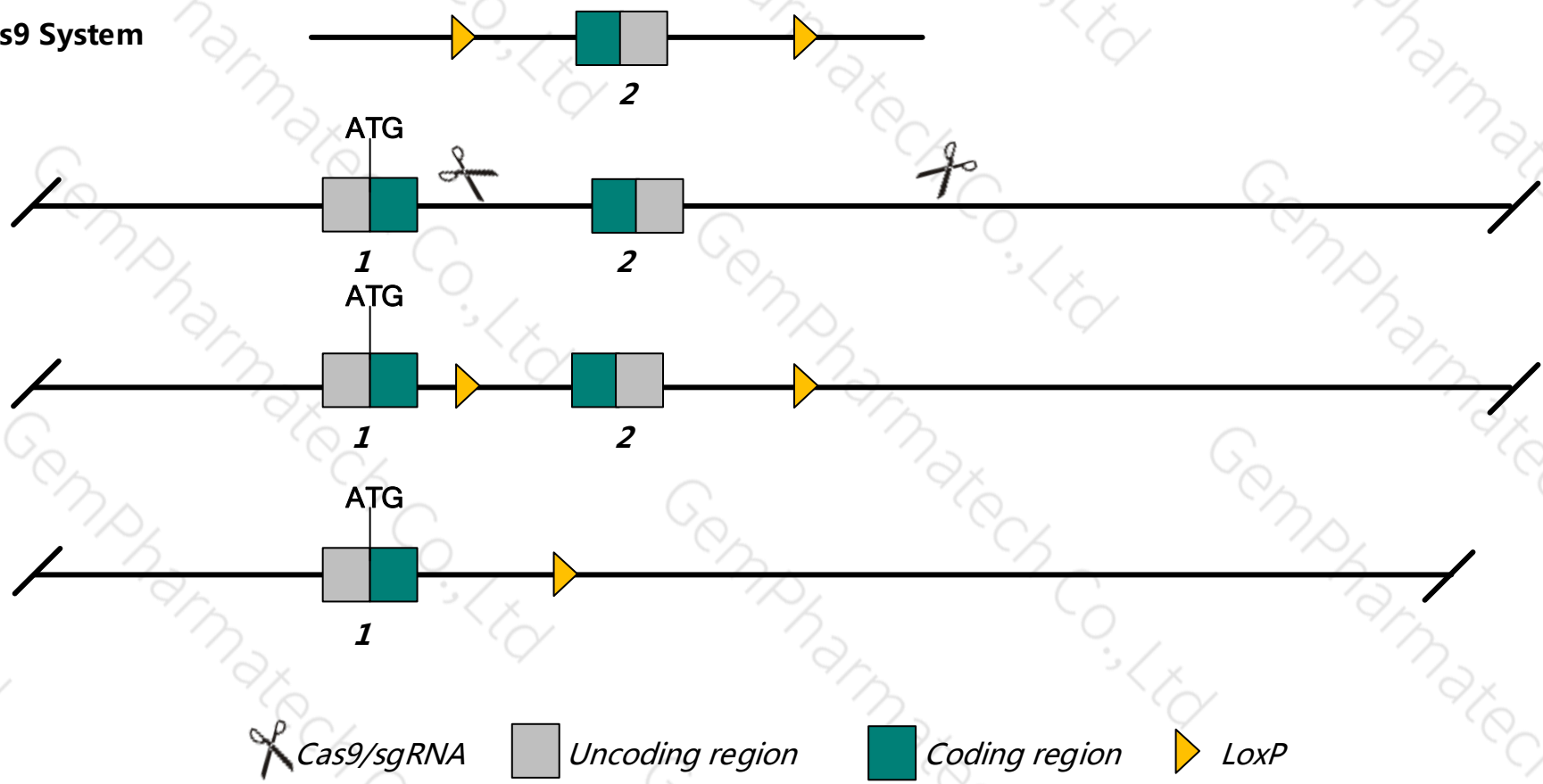
This model will use CRISPR/Cas9 technology to edit the *Dio2* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System

Wild-type allele

Conditional KO allele

KO allele



- The *Dio2* gene has 1 transcript. According to the structure of *Dio2* gene, exon2 of *Dio2*-201 transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Dio2* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed. Cas9, sgRNA 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 was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues or *Dio2* types.

- The *Dio2* gene is located on the Chr12. 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.

Gene information (NCBI)

Dio2 deiodinase, iodothyronine, type II [*Mus musculus* (house mouse)]

Gene ID: 13371, updated on 7-May-2019

Summary

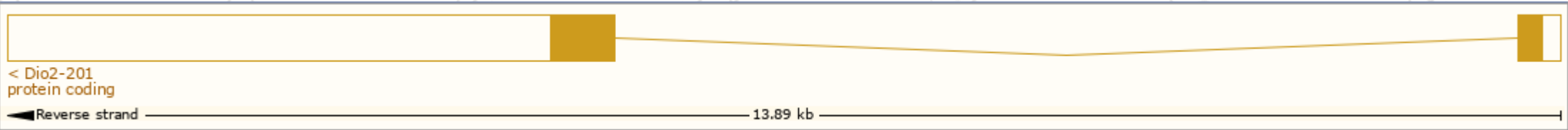
Official Symbol	Dio2 provided by MGI
Official Full Name	deiodinase, iodothyronine, type II provided by MGI
Primary source	MGI:MGI:1338833
See related	Ensembl:ENSMUSG00000007682
Gene type	protein coding
RefSeq status	REVIEWED
Organism	<i>Mus musculus</i>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	5DII; DIOII; AI324267
Summary	<p>The protein encoded by this gene belongs to the iodothyronine deiodinase family. It catalyzes the conversion of prohormone thyroxine (3,5,3',5'-tetraiodothyronine, T4) to the bioactive thyroid hormone (3,5,3'-triiodothyronine, T3) by outer ring 5'-deiodination. This gene is highly expressed in brain, placenta and mammary gland. It is thought to be responsible for the 'local' production of T3, and thus important in influencing thyroid hormone action in these tissues. Knockout studies in mice suggest that this gene may play an important role in brown adipose tissue lipogenesis, auditory function, and bone formation. This protein is a selenoprotein containing the non-standard amino acid, selenocysteine (Sec), which is encoded by the UGA codon that normally signals translation termination. The 3' UTRs of selenoprotein mRNAs contain a conserved stem-loop structure, designated the Sec insertion sequence (SECIS) element, that is necessary for the recognition of UGA as a Sec codon, rather than as a stop signal. Unlike the other two members (DIO1 and DIO3) of this enzyme family, the mRNA for this gene contains an additional in-frame UGA codon that has been reported (in human) to function either as a Sec or a stop codon, resulting in two potential isoforms with one or two Sec residues; however, only the upstream Sec (conserved with the single Sec residue found at the active site in DIO1 and DIO3) was shown to be essential for enzyme activity (PMID:10403186). In addition, the lack of conservation of the protein extension past the second TGA codon suggests that the one-Sec containing isoform represents the canonical form. [provided by RefSeq, Oct 2018]</p>
Expression	Biased expression in subcutaneous fat pad adult (RPKM 14.7), frontal lobe adult (RPKM 8.9) and 4 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

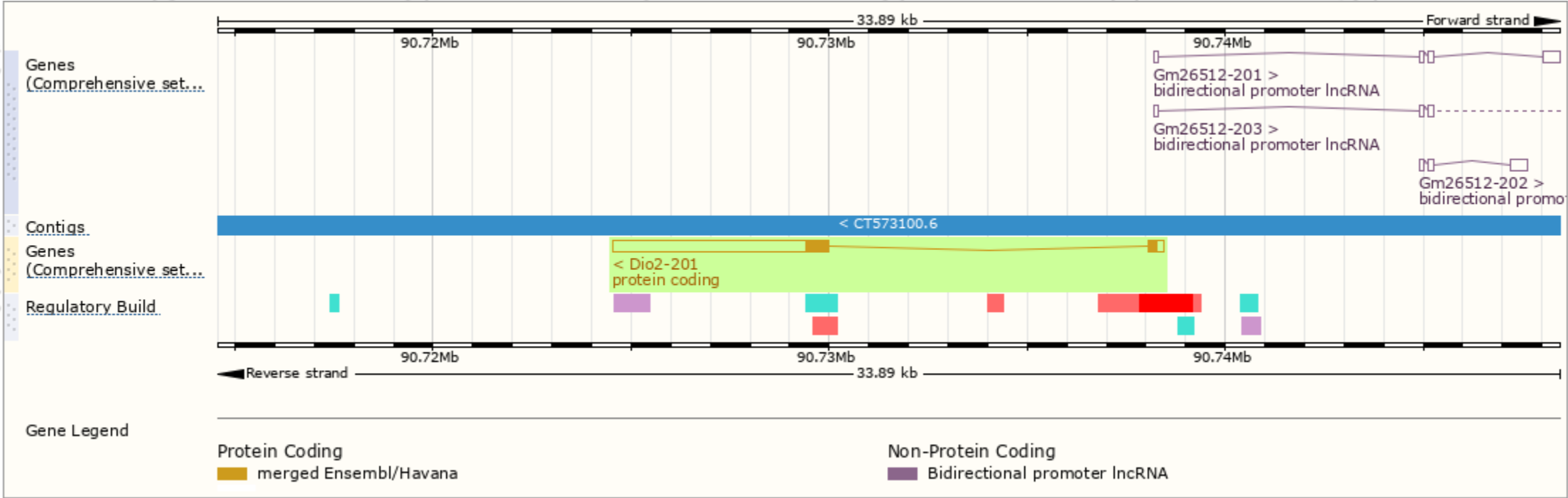
The gene has 1 transcript, and all transcripts are shown below :

Show/hide columns (1 hidden)							Filter	
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Dio2-201	ENSMUST00000082432.4	5815	266aa	Protein coding	CCDS36514	Q9Z1Y9	TSL:1	GENCODE basic APPRIS P1

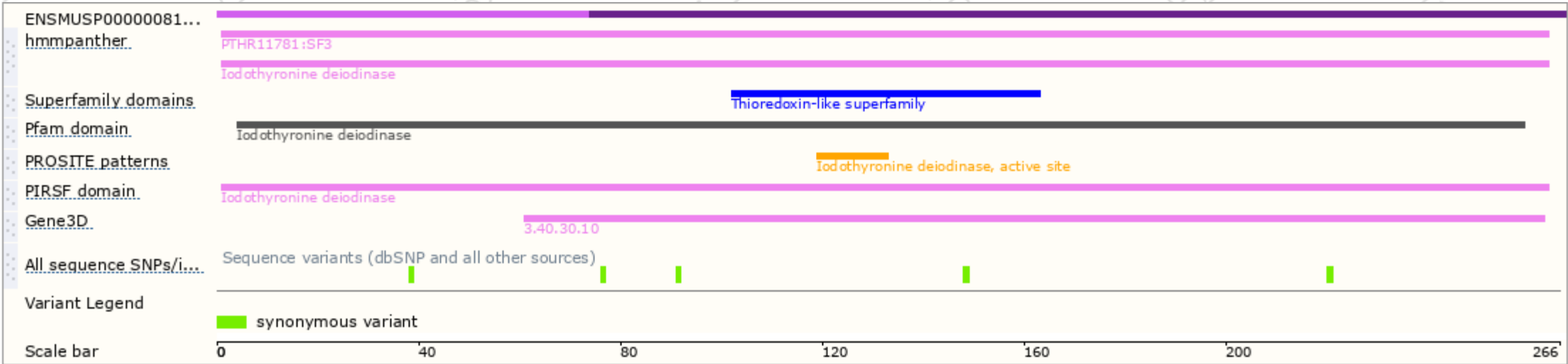
The strategy is based on the design of *Dio2-201* transcript, The transcription is shown below



Genomic location distribution

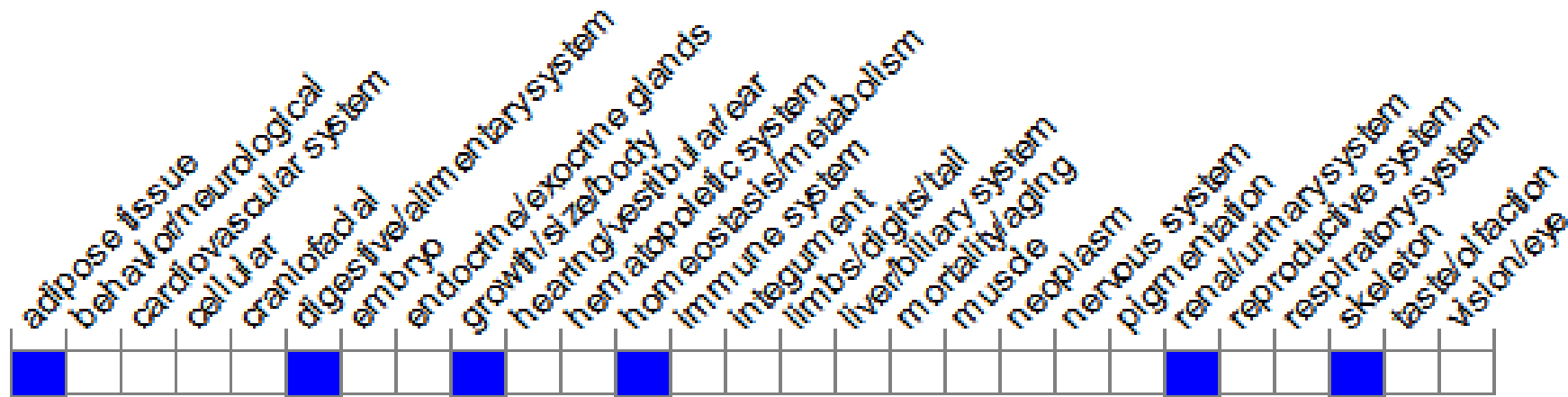


Protein domain



Mouse phenotype description(MGI)

Phenotype Overview ?



Click cells to view annotations.

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 display elevated thyroxine (T4) and thyroid-stimulating hormone levels, changes in the metabolism and excretion of iodothyronines, and impaired adaptive thermogenesis.

If you have any questions, you are welcome to inquire.
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