Dars Cas9-CKO Strategy

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Project Overview

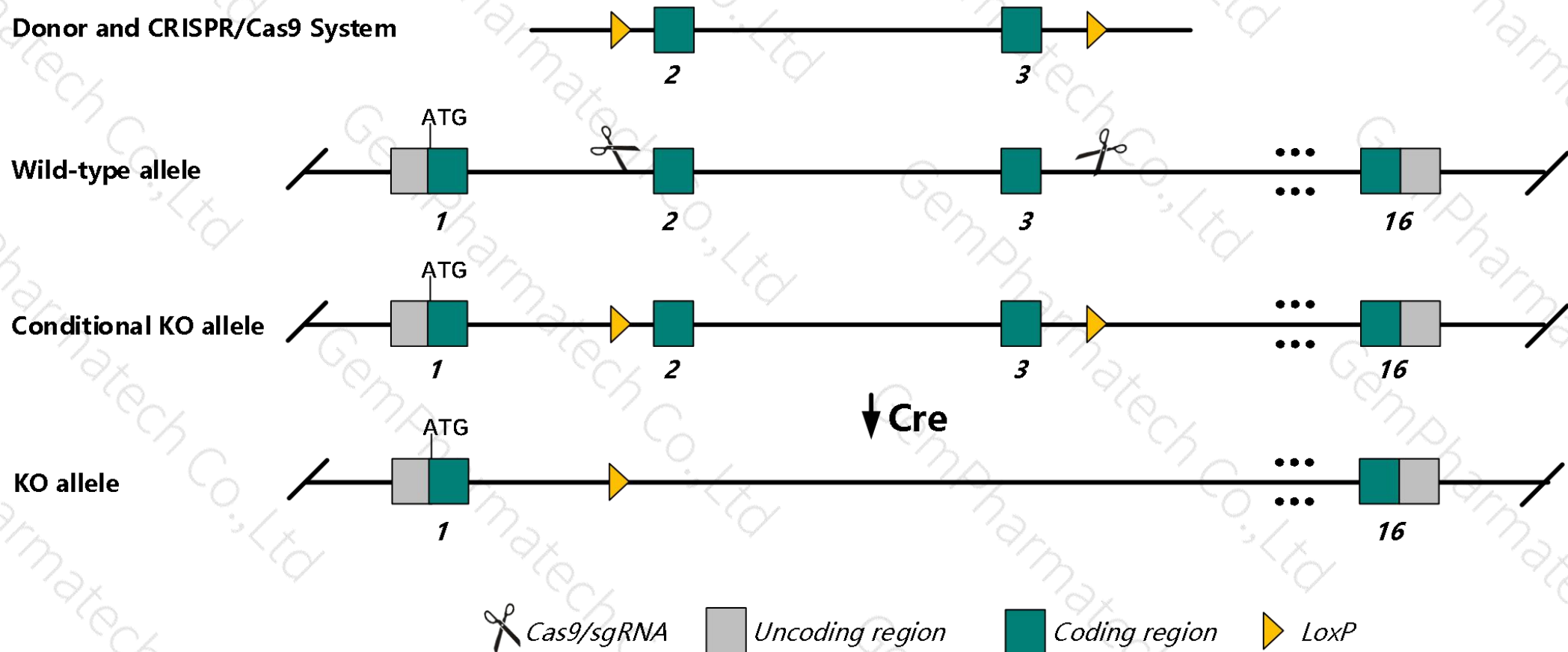| Project Name	*Dars*
Project type	Cas9-CKO
Strain background	C57BL/6JGpt

Conditional Knockout strategy

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

Donor and CRISPR/Cas9 System

Wild-type allele

Conditional KO allele

↓ **Cre**

KO allele

Cas9/sgRNA   Uncoding region   Coding region   LoxP

Technical routes

➤ The *Dars* gene has 3 transcripts. According to the structure of *Dars* gene, exon2-exon3 of *Dars-201* (ENSMUST00000027602.14) transcript is recommended as the knockout region.The region contains 151bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Dars* 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 die between e11 and e14. Mice heterozygous for the allele exhibit decreased ppi.

➢ Transcript 203 may not be affected.

➢ The *Dars* gene is located on the Chr1. 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）

Dars aspartyl-tRNA synthetase [Mus musculus (house mouse)]

Gene ID: 226414, updated on 13-Mar-2020

Summary

|	
Official Symbol	Dars provided by MGI
Official Full Name	aspartyl-tRNA synthetase provided byMGI
Primary source	MGI:MGI:2442544
See related	Ensembl:ENSMUSG00000026356
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	5730439G15Rik
Expression	Ubiquitous expression in CNS E11.5 (RPKM 27.3), placenta adult (RPKM 26.4) and 25 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dars-201	ENSMUST00000027602.14	2175	501aa	Protein coding	CCDS15253	Q922B2	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Dars-202	ENSMUST00000064309.8	645	87aa	Protein coding	CCDS48348	Q8BJY7	TSL:1 GENCODE basic
Dars-203	ENSMUST00000186398.1	2144	No protein	Retained intron	-	-	TSL:2

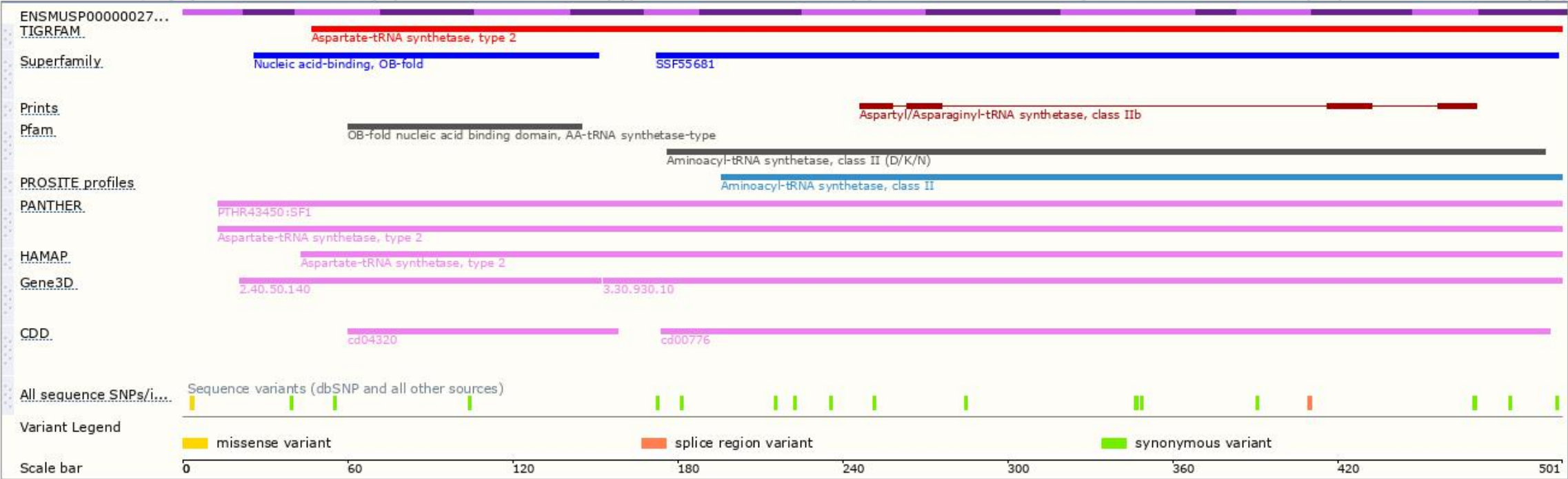The strategy is based on the design of *Dars-201* transcript,the transcription is shown below:
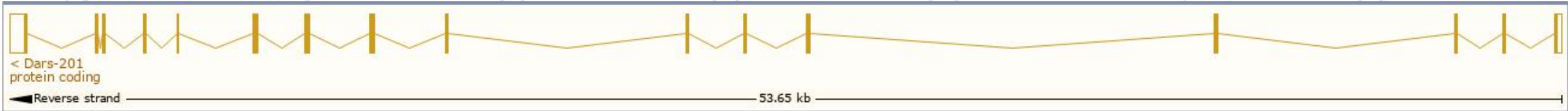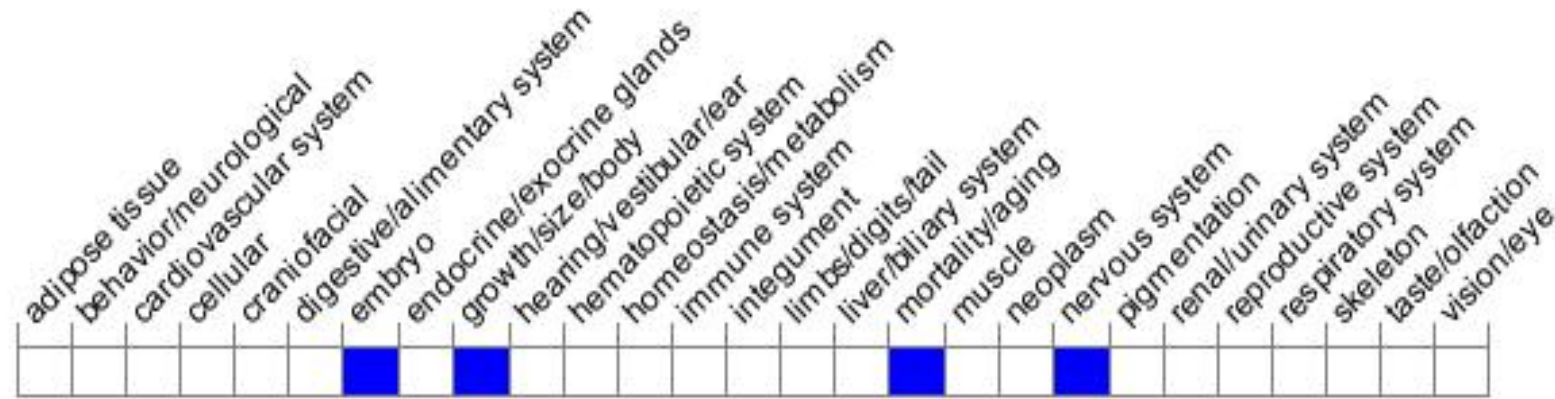
Genomic location distribution
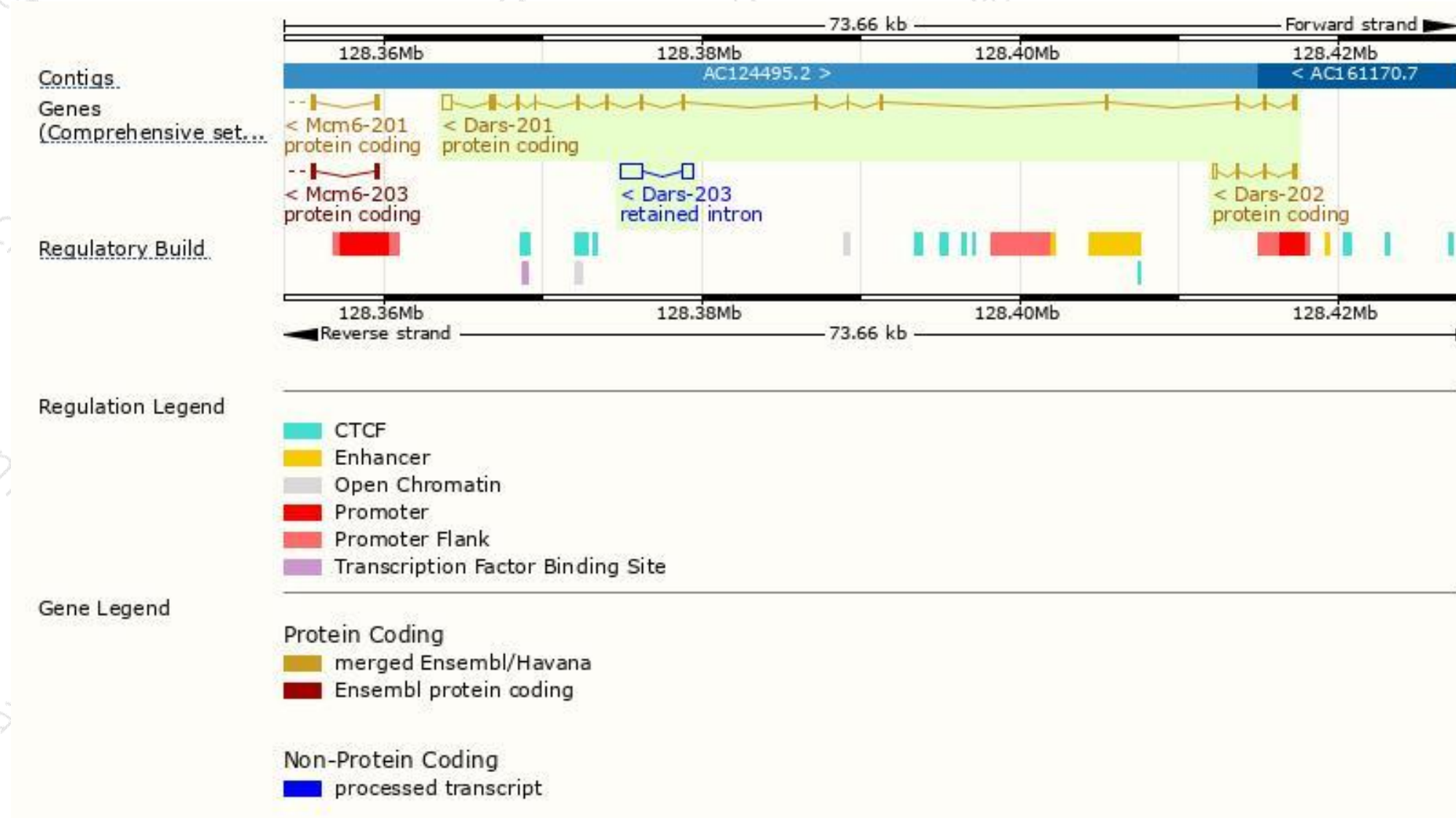
Protein domain

Mouse phenotype description(MGI)

Phenotype Overview

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 die between E11 and E14. Mice heterozygous for the allele exhibit decreased PPI.

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