Cul4b Cas9-CKO Strategy

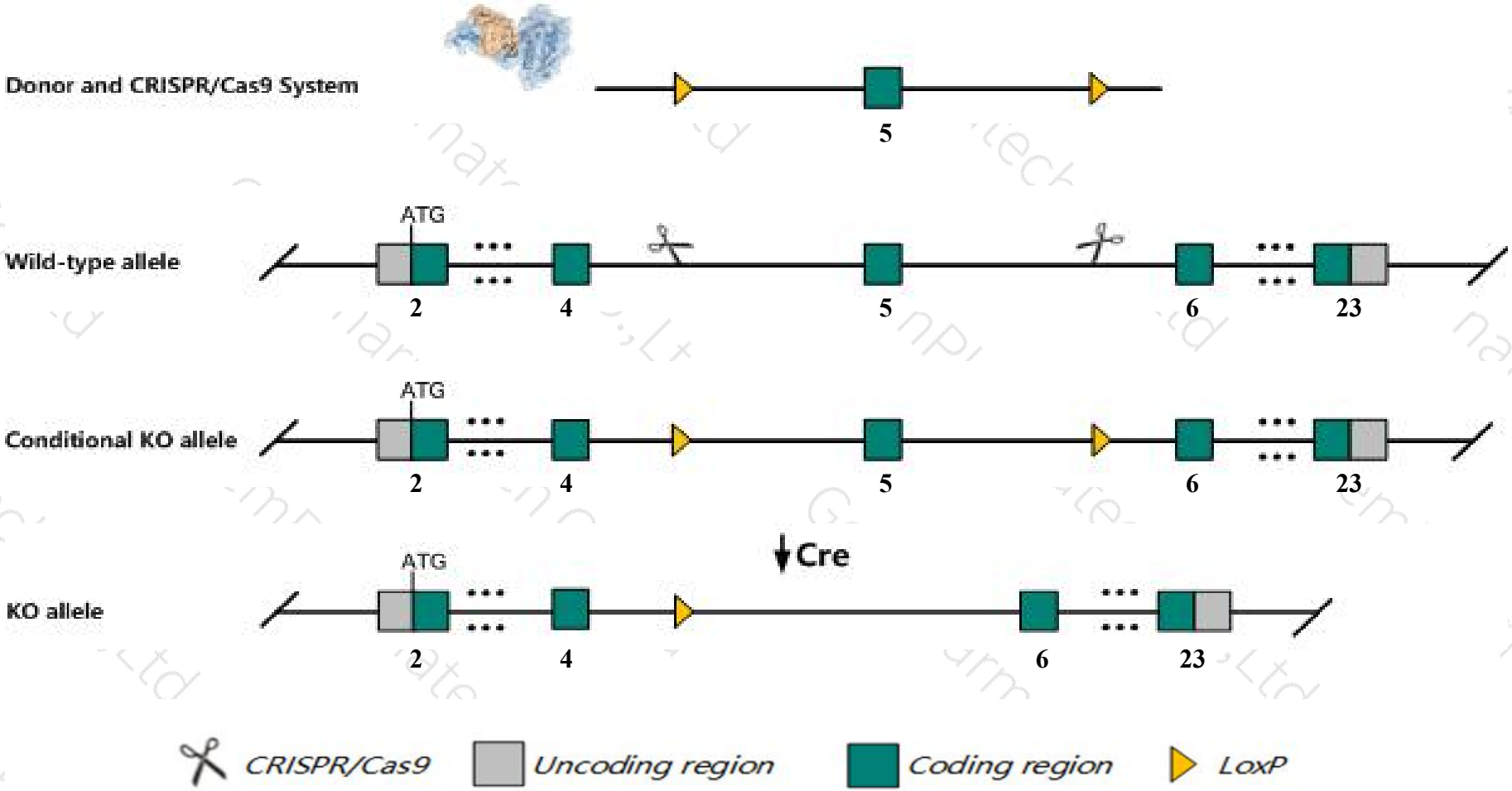
Designer:Lixin LYU
Design Date:2019-8-12

Project Overview

Project Name	***Cul4b***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cul4b* gene. The schematic diagram is as follows:



Technical routes

- The *Cul4b* gene has 4 transcripts. According to the structure of *Cul4b* gene, exon5 of *Cul4b-203* (ENSMUST00000115118.7) transcript is recommended as the knockout region.The region contains 116bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Cul4b* 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 conditional allele activated in the brain exhibit impaired spatial learning and memory, increased susceptibility to PTZ-induced seizures, abnormal dendrite morphology on hippocampal neurons. Mice homozygous for a null allele exhibitembryonic lethality and abnormal placenta.

- The *Cul4b* gene is located on the ChrX. 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）

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Cul4b cullin 4B [Mus musculus (house mouse)]

Gene ID: 72584, updated on 12-Mar-2019

Summary

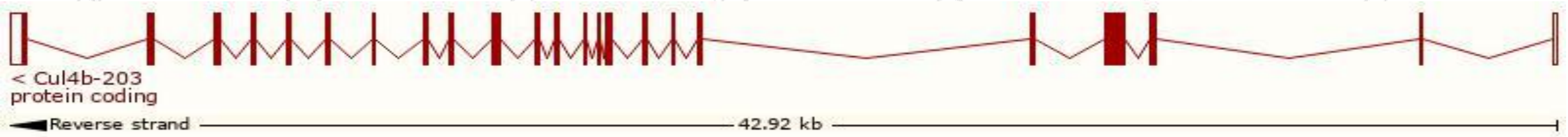
Official Symbol	Cul4b provided by MGI
Official Full Name	cullin 4B provided by MGI
Primary source	MGI:MGI:1919834
See related	Ensembl:ENSMUSG00000031095
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2700050M05Rik, AA409770, CUL-4B, mKIAA0695
Expression	Broad expression in placenta adult (RPKM 24.3), CNS E11.5 (RPKM 9.0) and 20 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

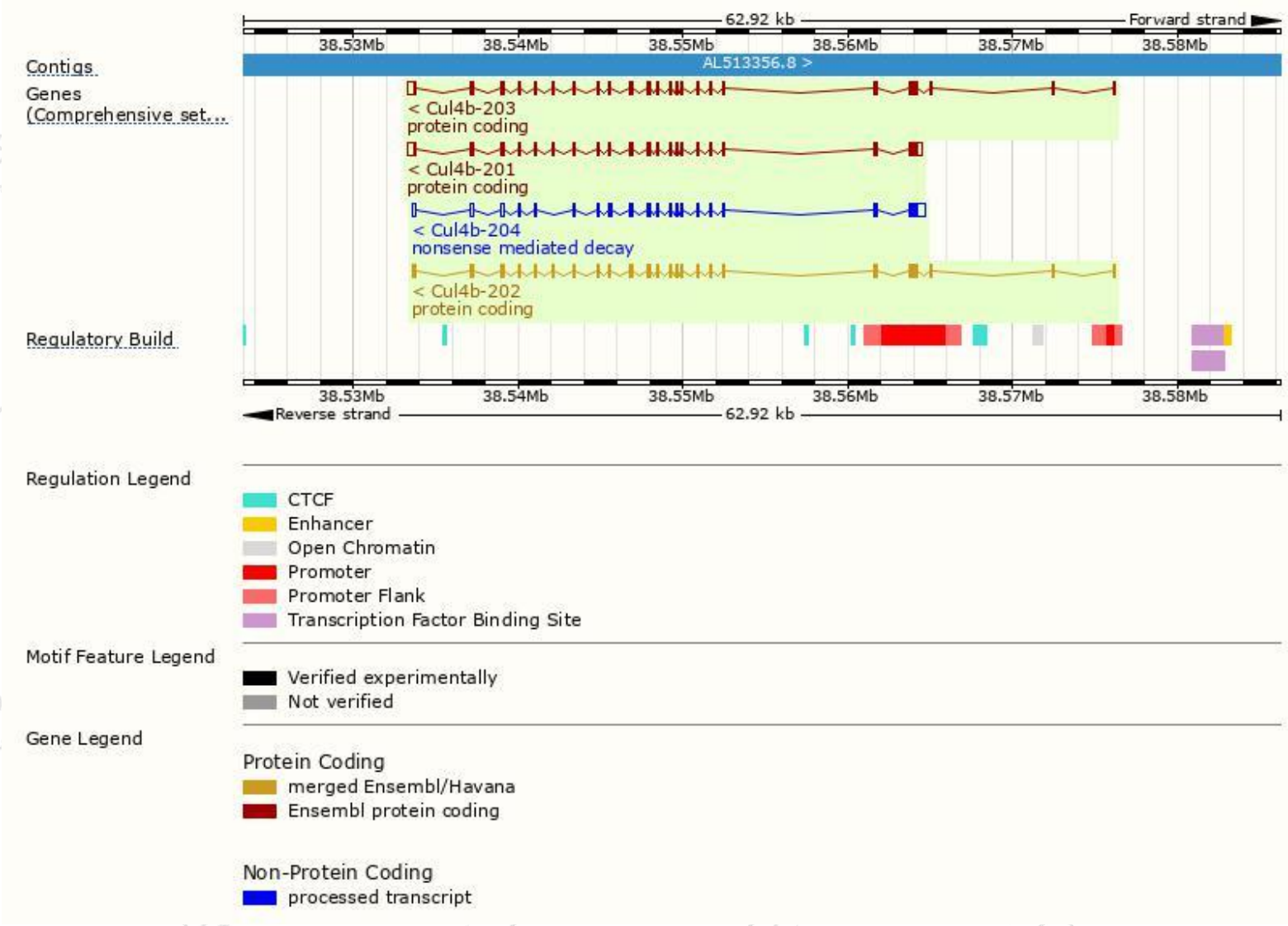
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cul4b-203	ENSMUST00000115118.7	3344	970aa	Protein coding	CCDS40948	A2A432	TSL:1 GENCODE basic APPRIS P2
Cul4b-202	ENSMUST00000050083.5	3090	970aa	Protein coding	CCDS40948	A2A432	TSL:1 GENCODE basic APPRIS P2
Cul4b-201	ENSMUST00000016681.14	3354	896aa	Protein coding	-	E9PXY1	TSL:5 GENCODE basic APPRIS ALT2
Cul4b-204	ENSMUST00000147129.7	3103	552aa	Nonsense mediated decay	-	J3QJX0	TSL:2

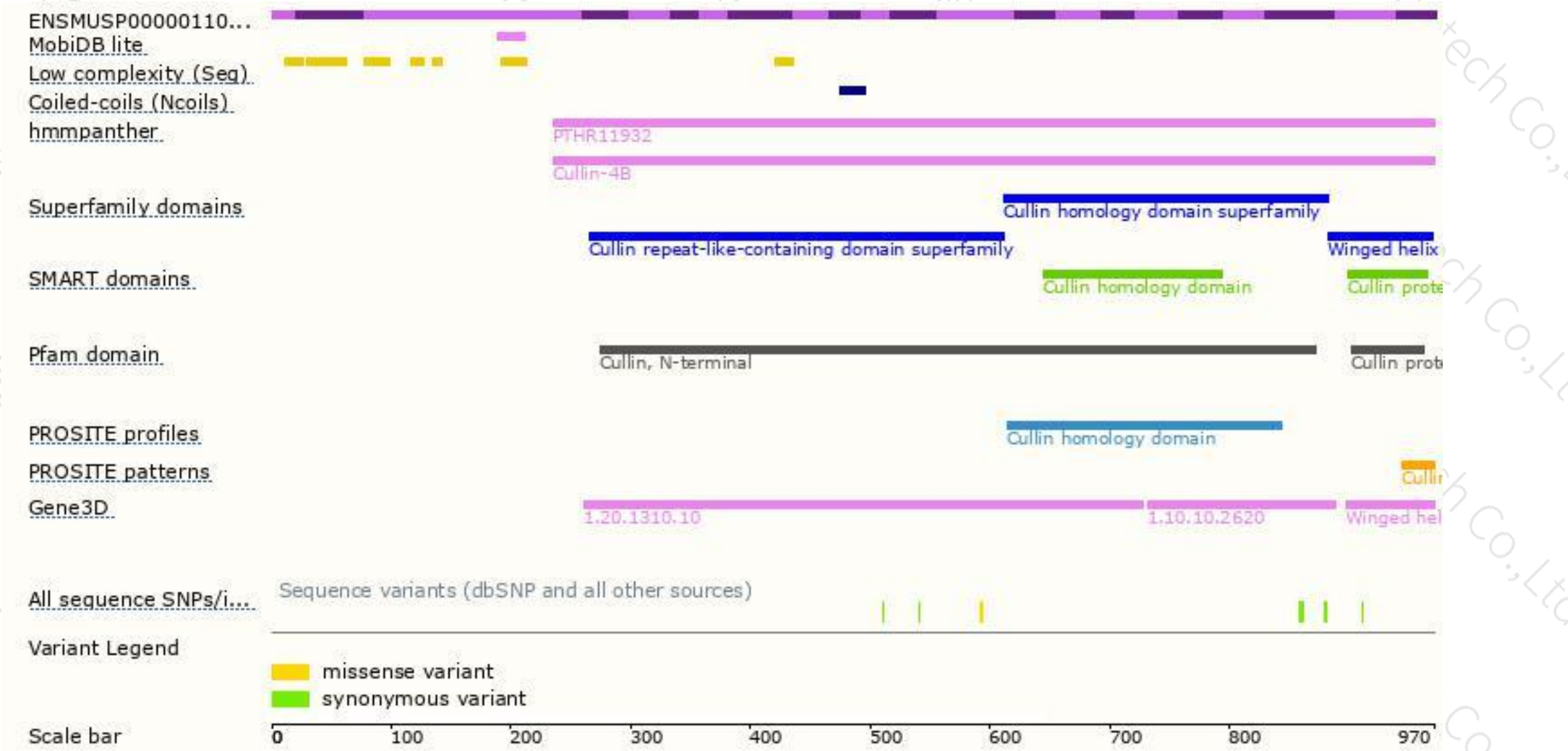
The strategy is based on the design of *Cul4b-203* transcript,The transcription is shown below



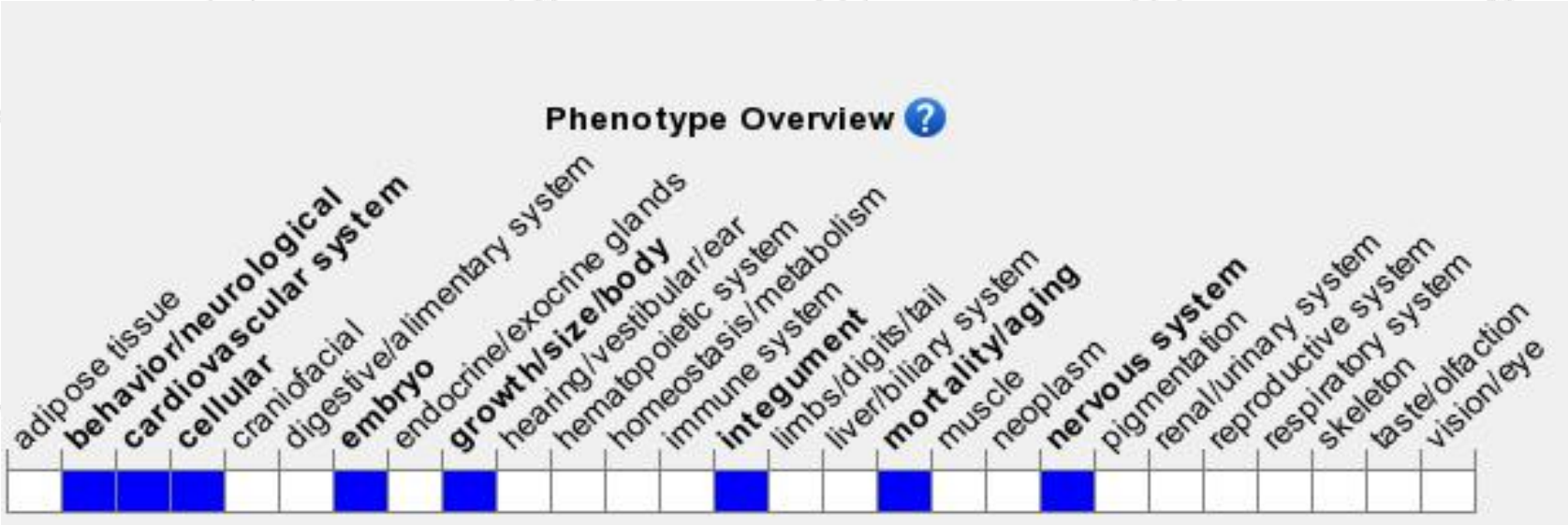
Genomic location distribution



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/).

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

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