Ccm2 Cas9-CKO *Strategy*

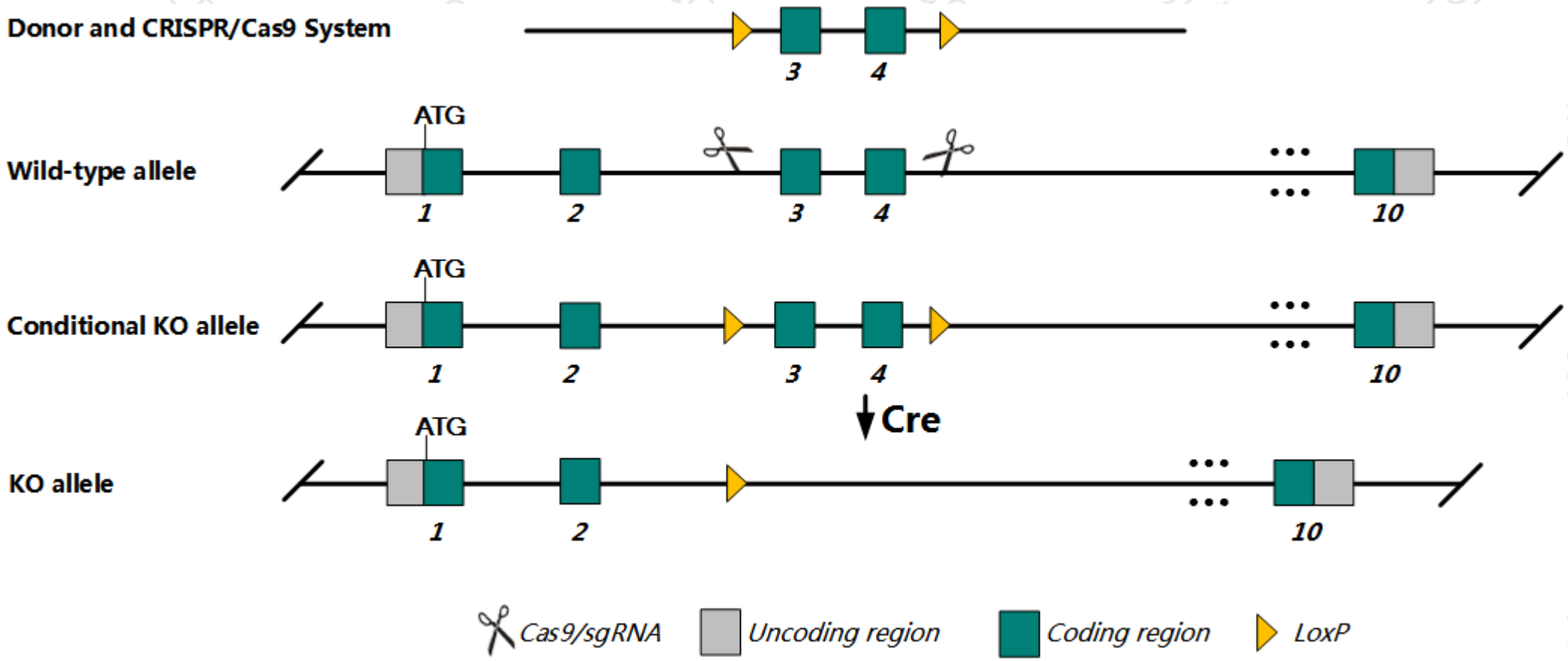
Designer: Xiaojing Li
Design Date: 2019-9-16
Reviewer: JiaYu

Project Overview

Project Name	***Ccm2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ccm2* gene. The schematic diagram is as follows:



Technical routes

- The *Ccm2* gene has 8 transcripts.According to the structure of *Ccm2* gene, exon3-4 of *Ccm2*-201 transcript （ ENSMUST00000000388.14 ） is recommended as the knockout region.The region contains 268bp coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Ccm2* 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 cell types.

Notice

- According to the existing MGI data，Homozygous null mice die during embryonic development with vasculature defects in the heart and placenta.
- The Ccm2 gene is located on the Chr11. 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

集萃药康 GemPharmatech

Ccm2 cerebral cavernous malformation 2 [*Mus musculus* (house mouse)]

Gene ID: 216527, updated on 31-Jan-2019

Summary

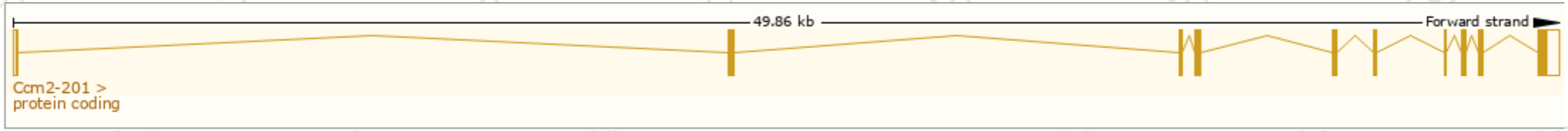
Official Symbol	Ccm2 provided by MGI
Official Full Name	cerebral cavernous malformation 2 provided by MGI
Primary source	MGI:MGI:2384924
See related	Ensembl:ENSMUSG00000000378
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	BC029157
Expression	Ubiquitous expression in thymus adult (RPKM 84.9), spleen adult (RPKM 58.8) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

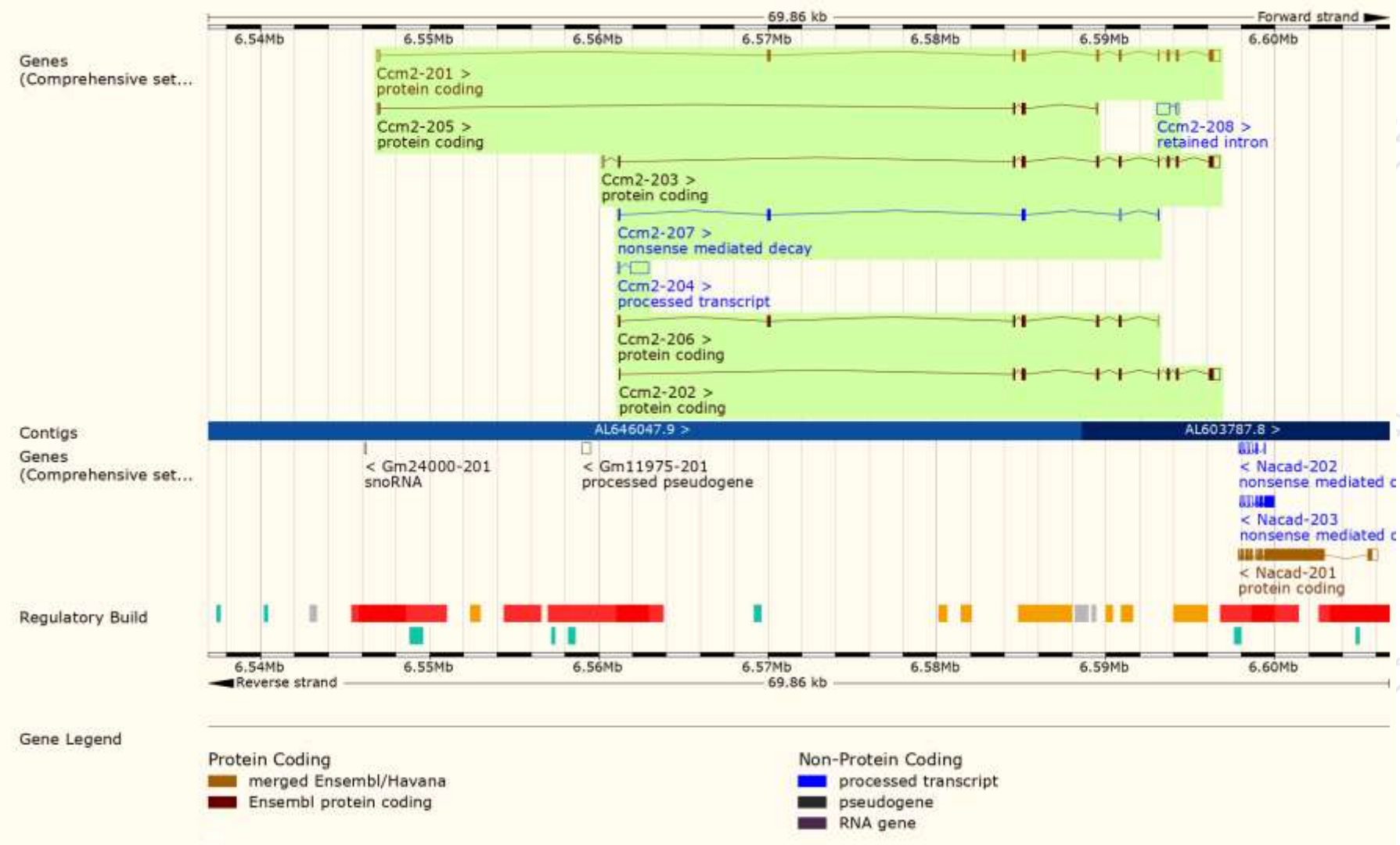
The gene has 8 transcripts, and all transcripts are shown below：

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ccm2-201	ENSMUST00000000388.14	1858	453aa	Protein coding	CCDS24422	Q8K2Y9	TSL:1 GENCODE basic APPRIS P3
Ccm2-202	ENSMUST00000109721.2	1551	389aa	Protein coding	CCDS48751	F7AVU1	TSL:5 GENCODE basic APPRIS ALT2
Ccm2-203	ENSMUST00000109722.8	1760	389aa	Protein coding	CCDS48751	F7AVU1	TSL:1 GENCODE basic APPRIS ALT2
Ccm2-204	ENSMUST00000144293.1	1182	No protein	Processed transcript	-	-	TSL:1
Ccm2-205	ENSMUST00000159007.7	431	113aa	Protein coding	-	E0CZ84	CDS 3' incomplete TSL:3
Ccm2-206	ENSMUST00000160633.7	827	243aa	Protein coding	-	E0CXR5	CDS 3' incomplete TSL:5
Ccm2-207	ENSMUST00000161501.7	691	126aa	Nonsense mediated decay	-	E0CYY2	TSL:5
Ccm2-208	ENSMUST00000161667.1	815	No protein	Retained intron	-	-	TSL:2

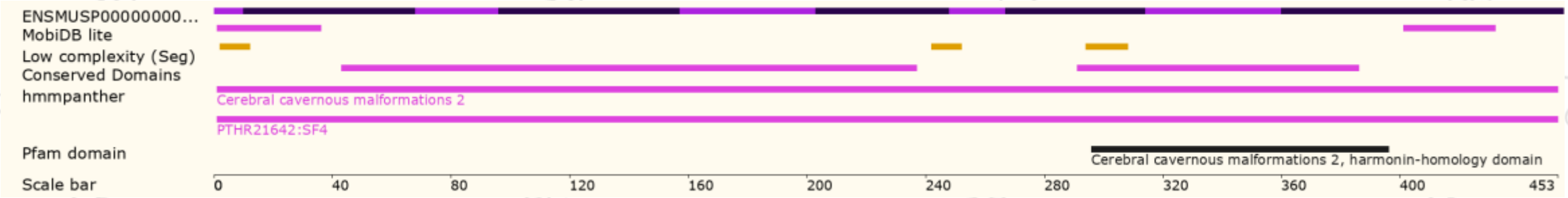
The strategy is based on the design of *Ccm2*-201 transcript,The transcription is shown below



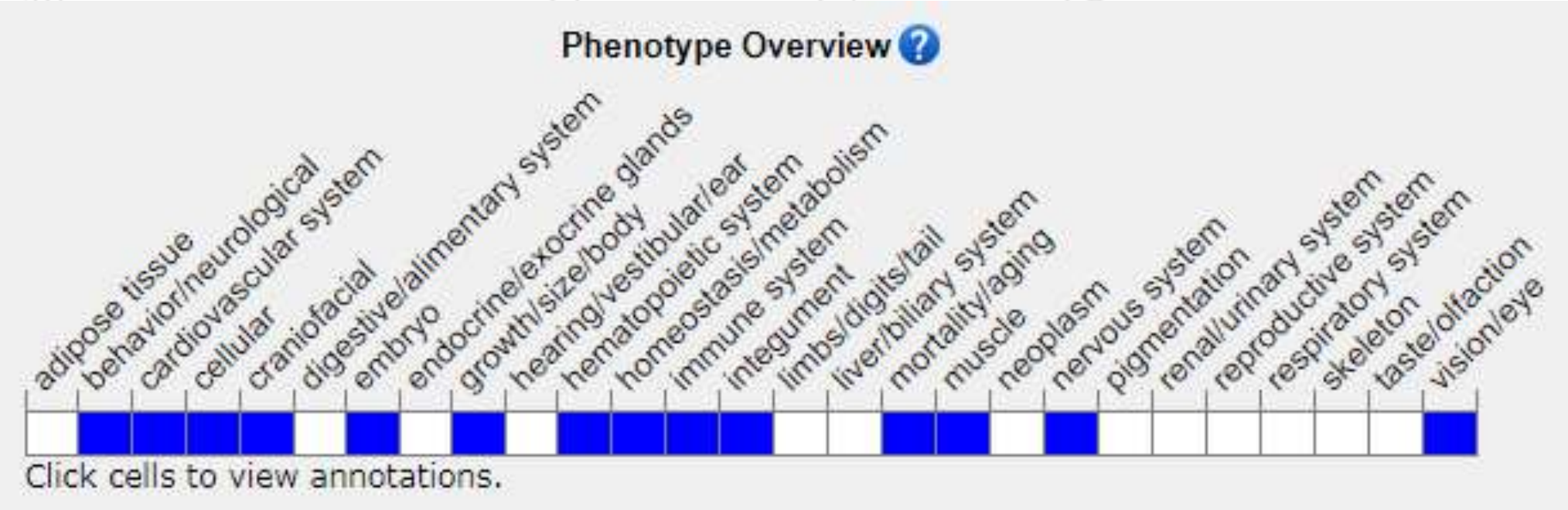
Genomic location distribution



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/) .

Homozygous null mice die during embryonic development with vasculature defects in the heart and placenta.

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

Tel: 025-5864 1534

集萃药康生物科技

GemPharmatech Co.,Ltd