

***Flot2* Cas9-CKO Strategy**

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Project Overview

Project Name

Flot2

Project type

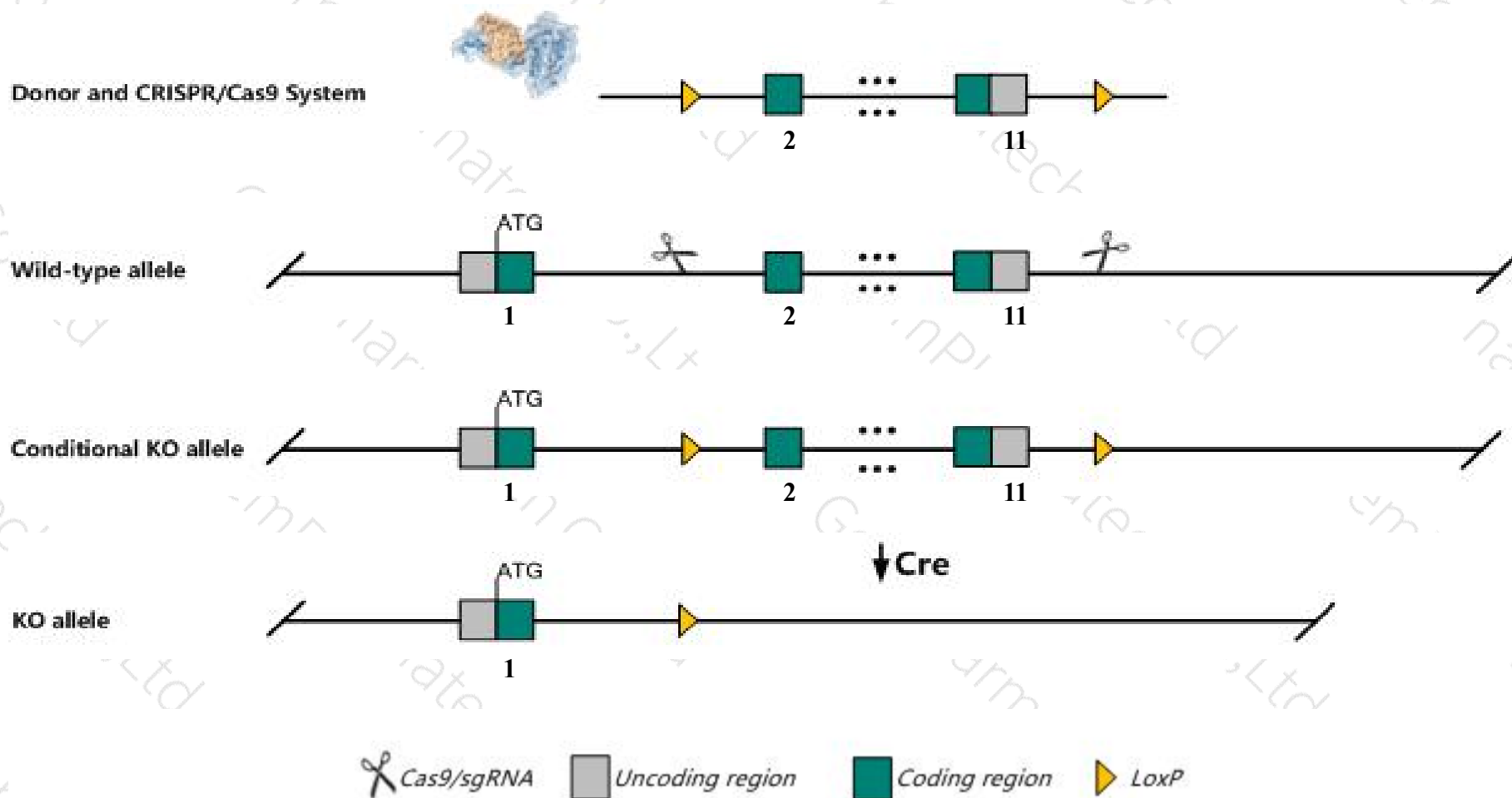
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Flot2* gene. The schematic diagram is as follows:



- The *Flot2* gene has 5 transcripts. According to the structure of *Flot2* gene, exon2-exon11 of *Flot2-201* (ENSMUST00000072289.11) 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 *Flot2* 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.

- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit reduced metastase into the lungs in a breast cancer model.
- The *Flot2* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)

Flot2 flotillin 2 [*Mus musculus* (house mouse)]

Gene ID: 14252, updated on 13-Jan-2020

Summary

Official Symbol	Flot2 provided by MGI
Official Full Name	flotillin 2 provided by MGI
Primary source	MGI:MGI:103309
See related	Ensembl:ENSMUSG00000061981
Gene type	protein coding
RefSeq status	VALIDATED
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	Esa; AI573412; reggie-2
Expression	Ubiquitous expression in adrenal adult (RPKM 67.2), mammary gland adult (RPKM 55.9) and 28 other tissues See more
Orthologs	human all

Genomic context

Location: 11 B5; 11 46.74 cM [See Flot2 in Genome Data Viewer](#)

Exon count: 14

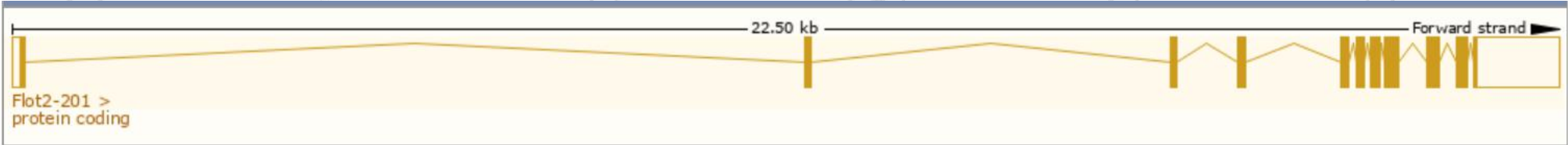
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	11	NC_000077.6 (78037931..78060434)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	11	NC_000077.5 (77851443..77873934)

Transcript information (Ensembl)

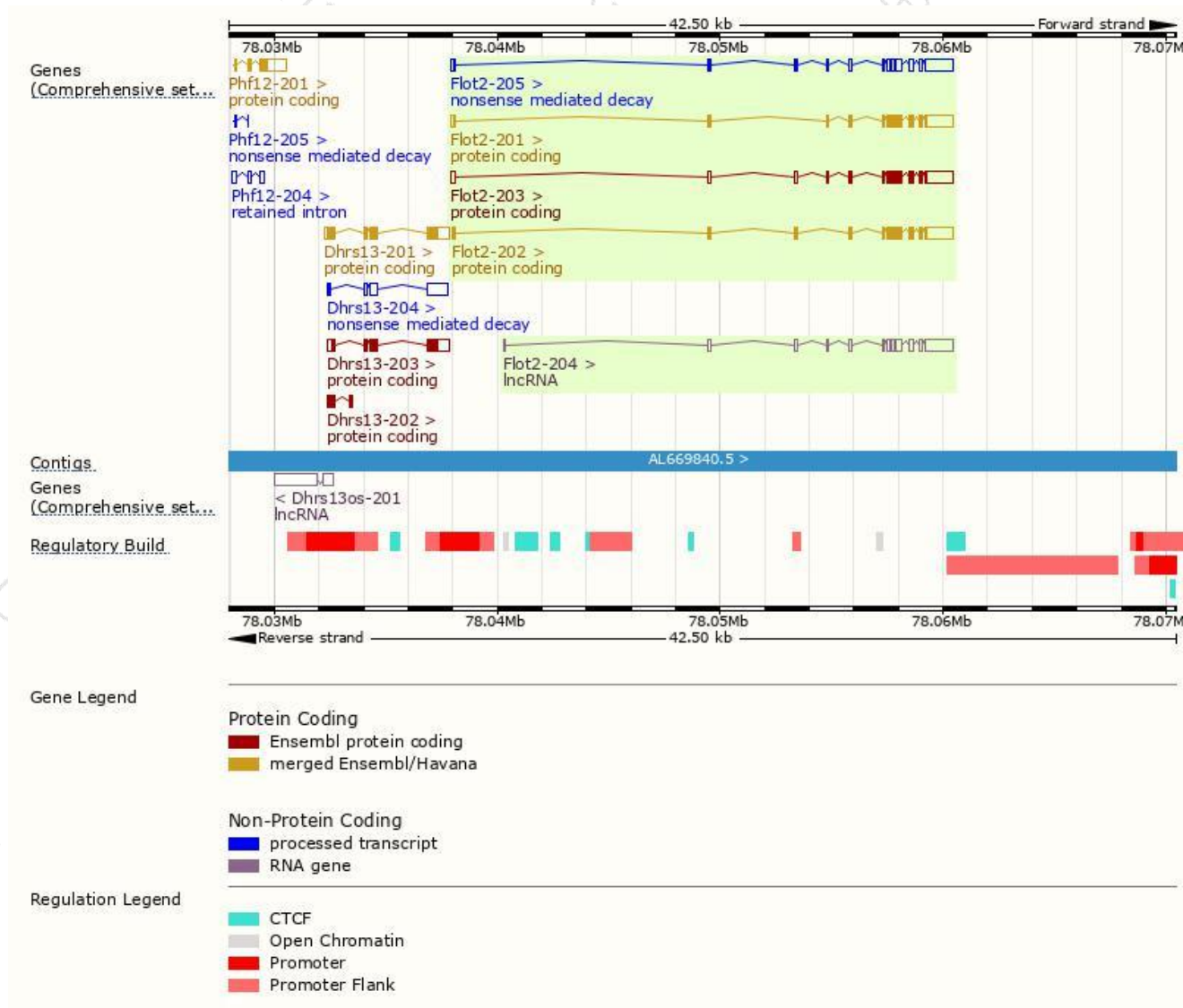
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Flot2-203	ENSMUST00000100784.8	2699	379aa	Protein coding	CCDS25088	Q60634	TSL:1 GENCODE basic APPRIS P3
Flot2-201	ENSMUST00000072289.11	2621	428aa	Protein coding	CCDS36237	Q60634	TSL:1 GENCODE basic APPRIS ALT 1
Flot2-202	ENSMUST00000073660.6	2568	428aa	Protein coding	-	Q5SS83	TSL:5 GENCODE basic APPRIS ALT 1
Flot2-205	ENSMUST00000148162.8	2712	86aa	Nonsense mediated decay	-	E9PV09	TSL:1
Flot2-204	ENSMUST00000136353.1	2558	No protein	lncRNA	-	-	TSL:1

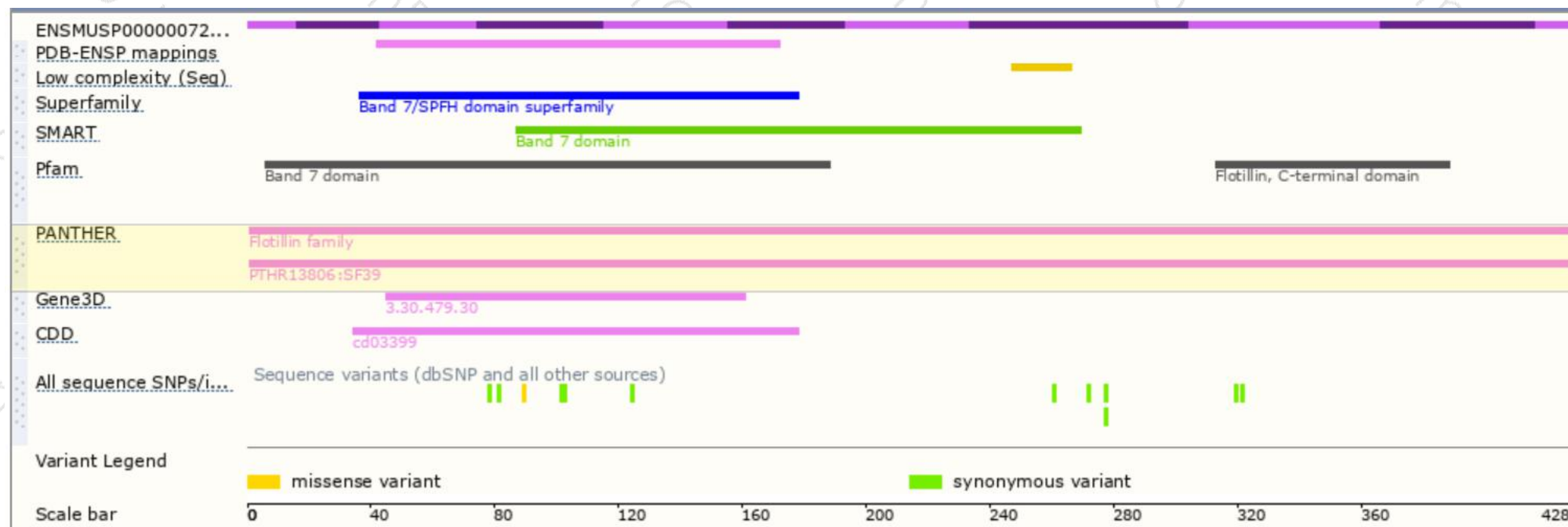
The strategy is based on the design of *Flot2-201* transcript,The transcription is shown below



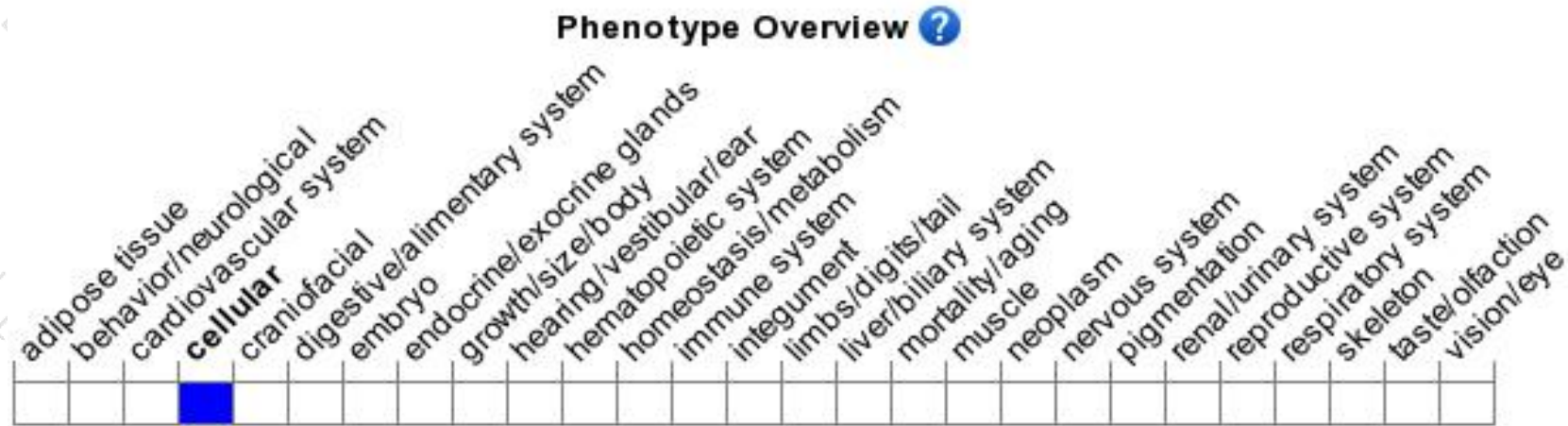
Genomic location distribution



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/>).

According to the existing MGI data, Mice homozygous for a knock-out allele exhibit reduced metastase into the lungs in a breast cancer model.

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

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