Brca2 Cas9-CKO Strategy

Designer: Jinling Wang

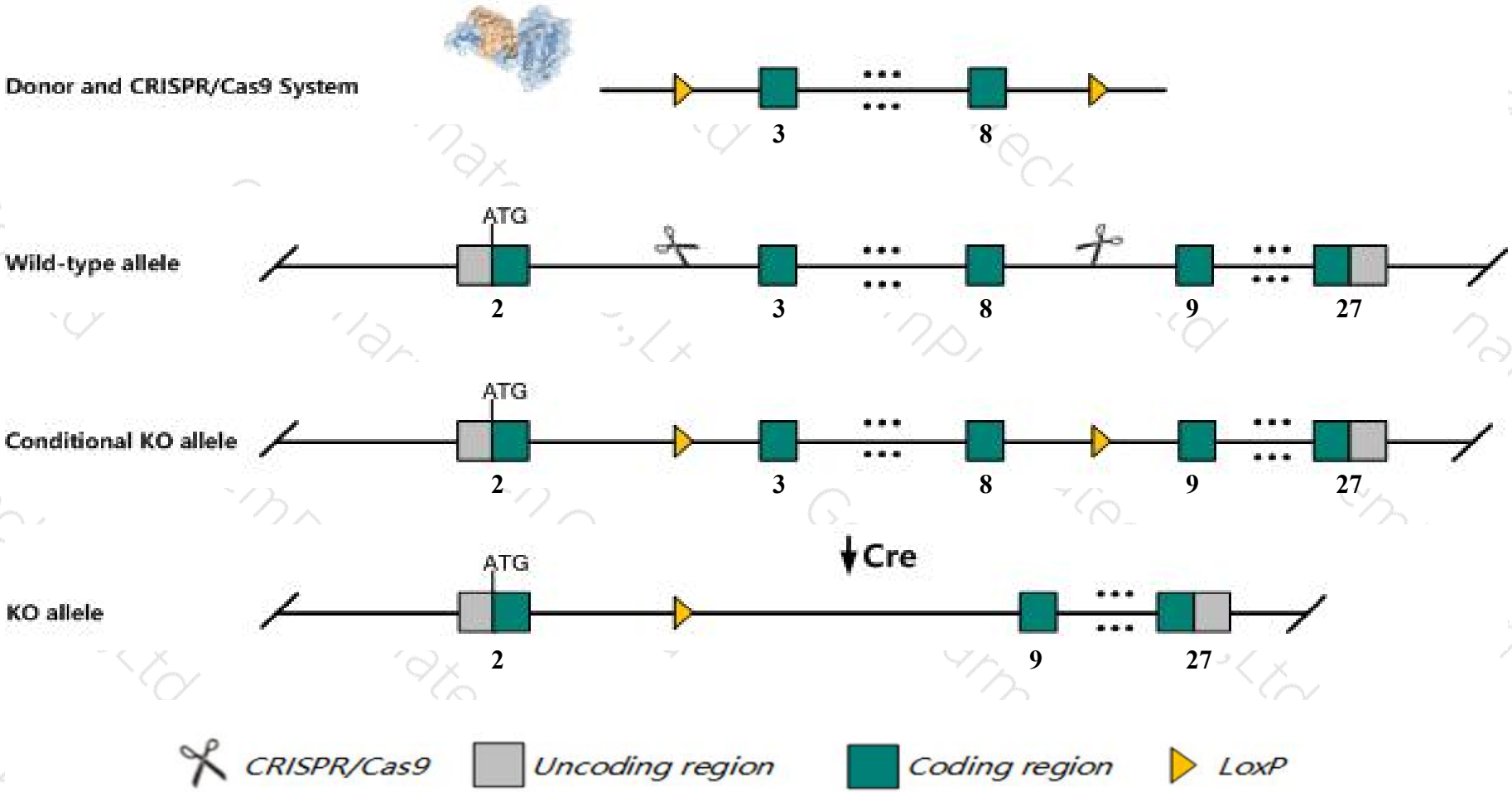
Design Date: 2019-7-19

Project Overview

Project Name	***Brca2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Brca2* gene. The schematic diagram is as follows:



Technical routes

- The *Brca2* gene has 14 transcripts. According to the structure of *Brca2* gene, exon3-exon8 of *Brca2-201* (ENSMUST00000044620.10) transcript is recommended as the knockout region.The region contains 590bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Brca2* 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,Homozygous null mutants are embryonic lethal with abnormalities including growth retardation, neural tube defects, and mesoderm abnormalities; conditional mutations cause genetic instability and enhanced tumor formation; mutants with truncated BRCA2 protein survive, are small, infertile, show improper tissue differentiation and develop lymphomas and carcinomas.

- The *Brca2* gene is located on the Chr5. 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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Brca2 breast cancer 2, early onset [Mus musculus (house mouse)]

Gene ID: 12190, updated on 9-Apr-2019

Summary

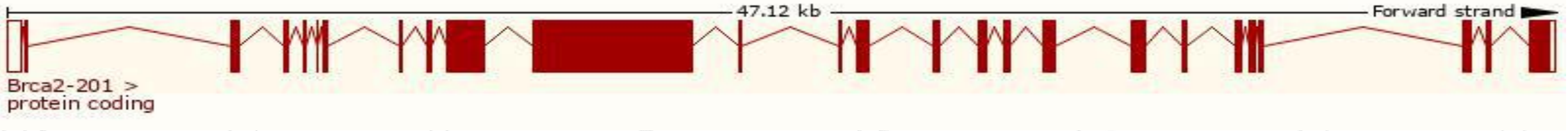
Official Symbol	Brca2 provided by MGI
Official Full Name	breast cancer 2, early onset provided by MGI
Primary source	MGI:MGI:109337
See related	Ensembl:ENSMUSG00000041147
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Fancd1, RAB163
Expression	Broad expression in CNS E11.5 (RPKM 2.0), liver E14 (RPKM 1.6) and 24 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

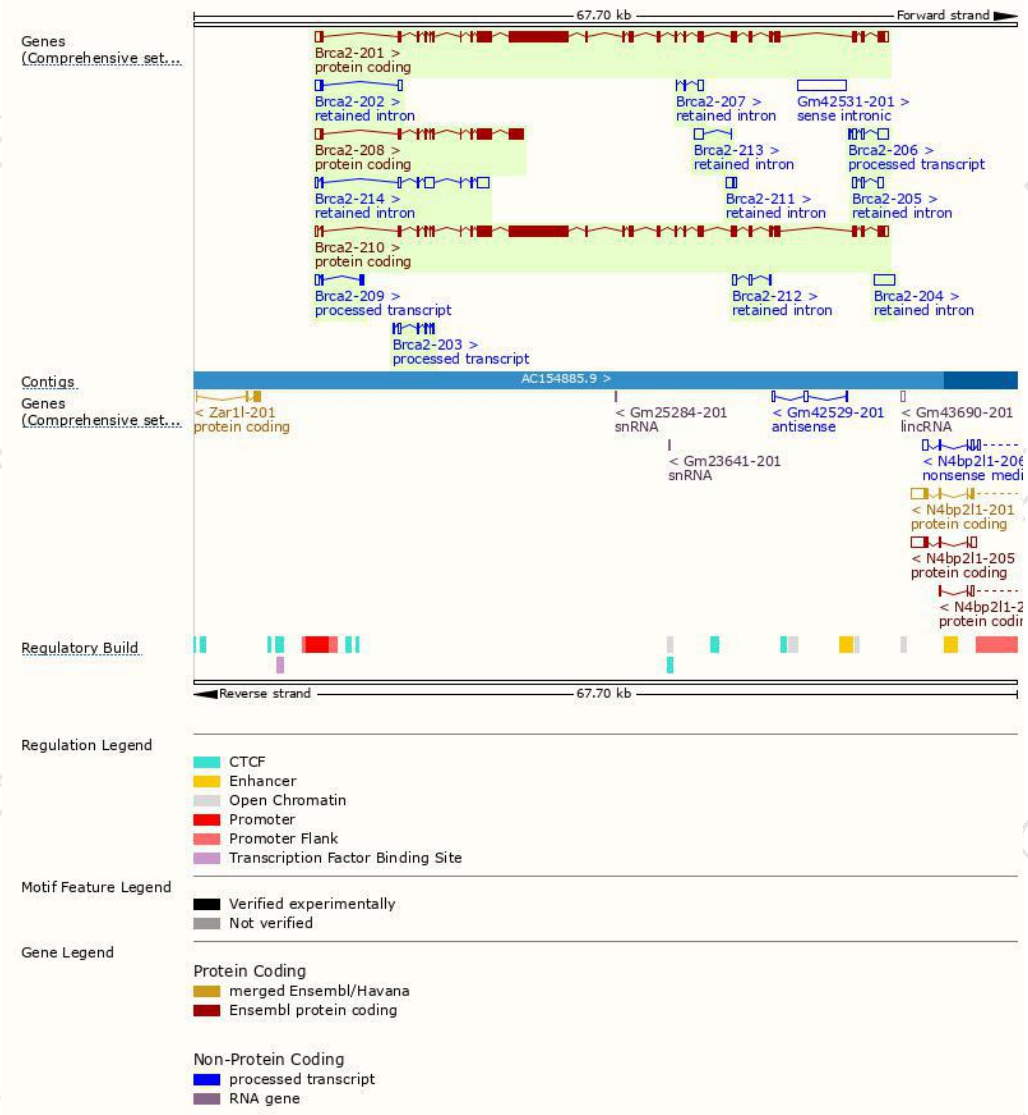
The gene has 14 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Brca2-201	ENSMUST00000044620.10	10724	3329aa	Protein coding	CCDS39411	P97929	TSL:5 GENCODE basic APPRIS P1
Brca2-210	ENSMUST00000202313.1	10517	3329aa	Protein coding	CCDS39411	P97929	TSL:1 GENCODE basic APPRIS P1
Brca2-208	ENSMUST00000202003.3	3473	992aa	Protein coding	-	A0A0J9YVI7	CDS 3' incomplete TSL:1
Brca2-206	ENSMUST00000201309.3	1340	No protein	Processed transcript	-	-	TSL:1
Brca2-203	ENSMUST00000201149.1	634	No protein	Processed transcript	-	-	TSL:5
Brca2-209	ENSMUST00000202192.1	588	No protein	Processed transcript	-	-	TSL:2
Brca2-214	ENSMUST00000202975.3	2534	No protein	Retained intron	-	-	TSL:2
Brca2-204	ENSMUST00000201165.1	1742	No protein	Retained intron	-	-	TSL:NA
Brca2-202	ENSMUST00000200686.3	937	No protein	Retained intron	-	-	TSL:2
Brca2-205	ENSMUST00000201226.1	864	No protein	Retained intron	-	-	TSL:2
Brca2-211	ENSMUST00000202693.1	688	No protein	Retained intron	-	-	TSL:3
Brca2-213	ENSMUST00000202837.1	669	No protein	Retained intron	-	-	TSL:3
Brca2-207	ENSMUST00000201678.1	646	No protein	Retained intron	-	-	TSL:3
Brca2-212	ENSMUST00000202727.1	479	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Brca2-201* transcript,The transcription is shown below

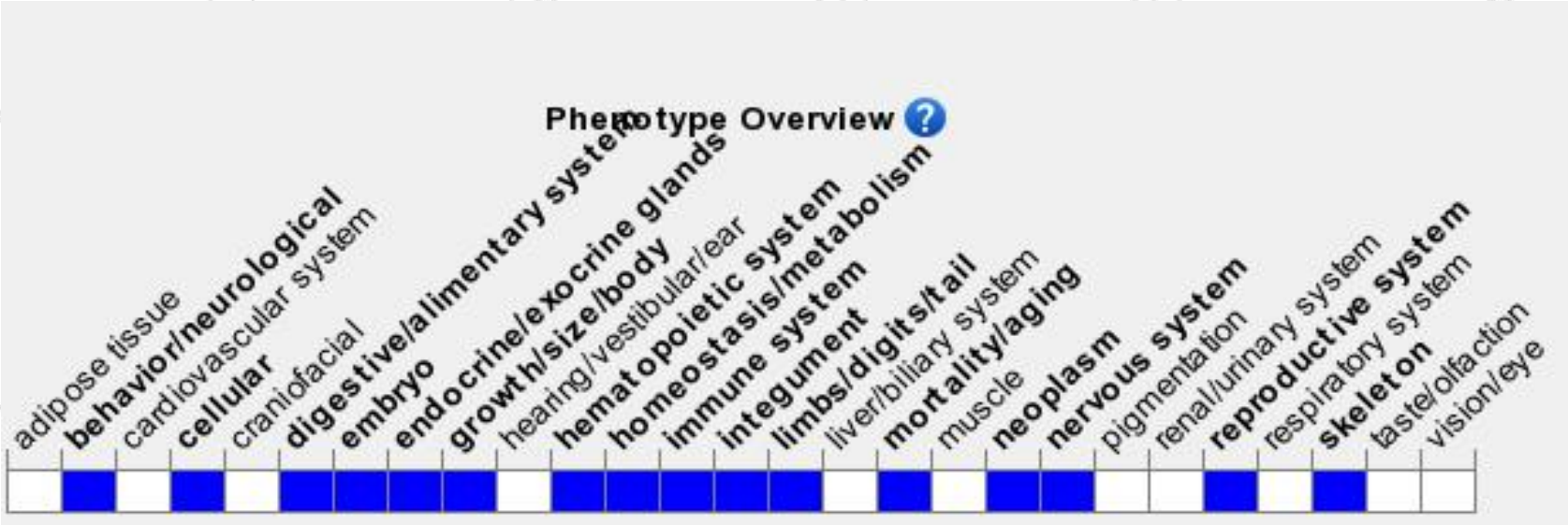


Genomic location distribution



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/).

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集萃药康
GemPharmatech

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

Tel: 400-9660890

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GemPharmatech Co.,Ltd