Brca1 Cas9-CKO Strategy

Designer: Jinling Wang

Design Date: 2019-7-17

Project Overview

Project Name	***Brca1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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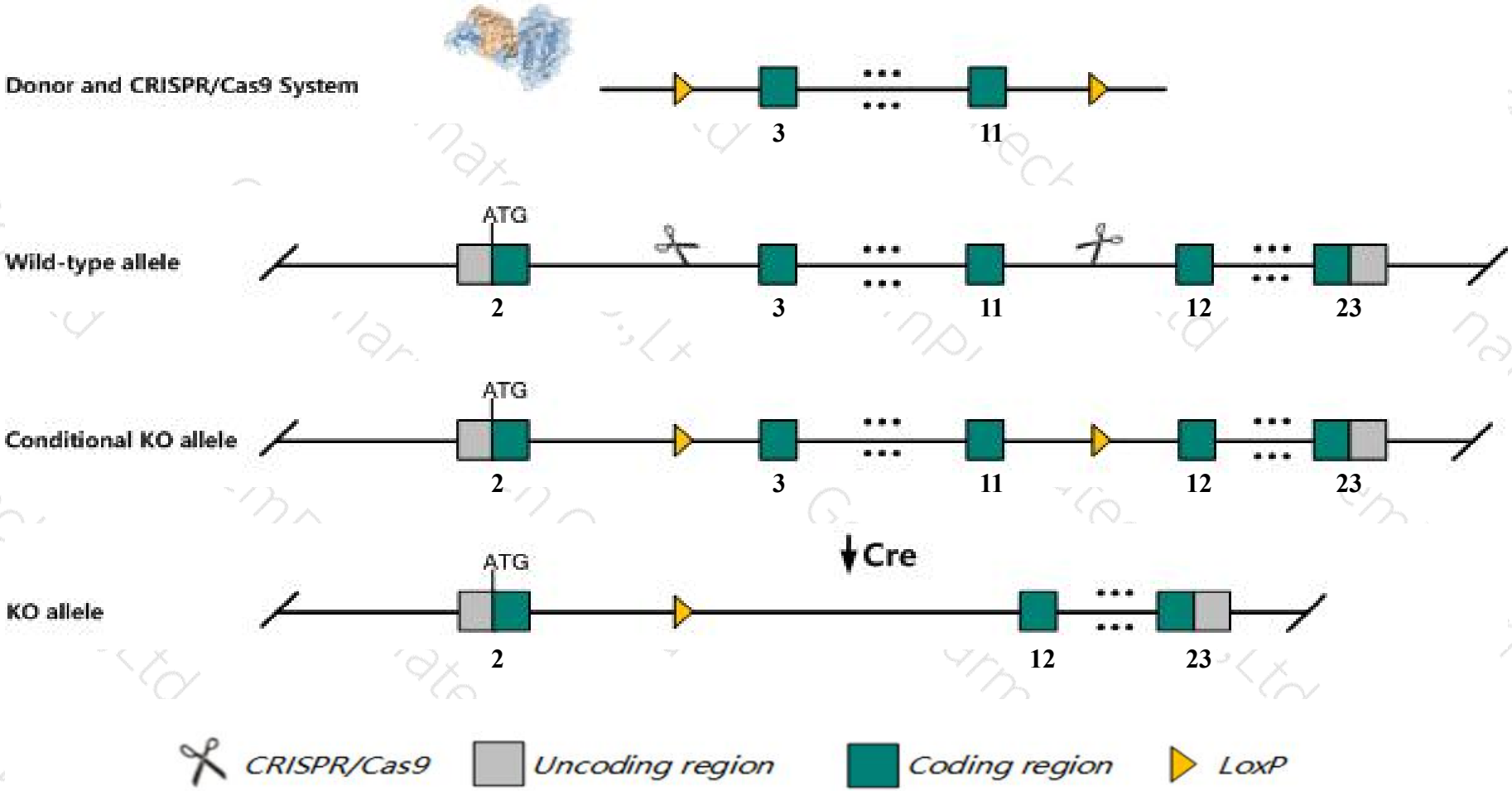
This model will use CRISPR/Cas9 technology to edit the *Brca1* gene. The schematic diagram is as follows:



Technical routes

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- The *Brca1* gene has 7 transcripts. According to the structure of *Brca1* gene, exon3-exon11 of *Brca1-201* (ENSMUST00000017290.10) transcript is recommended as the knockout region.The region contains 3973bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Brca1* 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 BRCA1 protein survive, have a kinky tail, pigmentation anomalies, male infertility and increased tumor incidence.

- The *Brca1* 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）

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Brca1 breast cancer 1, early onset [Mus musculus (house mouse)]

Gene ID: 12189, updated on 9-Apr-2019

Summary

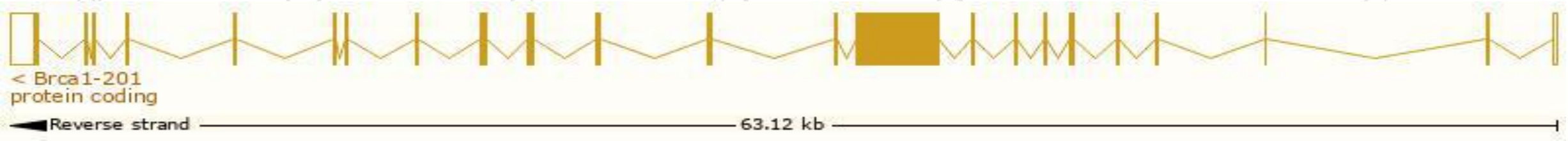
Official Symbol	Brca1 provided by MGI
Official Full Name	breast cancer 1, early onset provided byMGI
Primary source	MGI:MGI:104537
See related	Ensembl:ENSMUSG00000017146
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Biased expression in liver E14 (RPKM 5.9), CNS E11.5 (RPKM 5.7) and 12 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

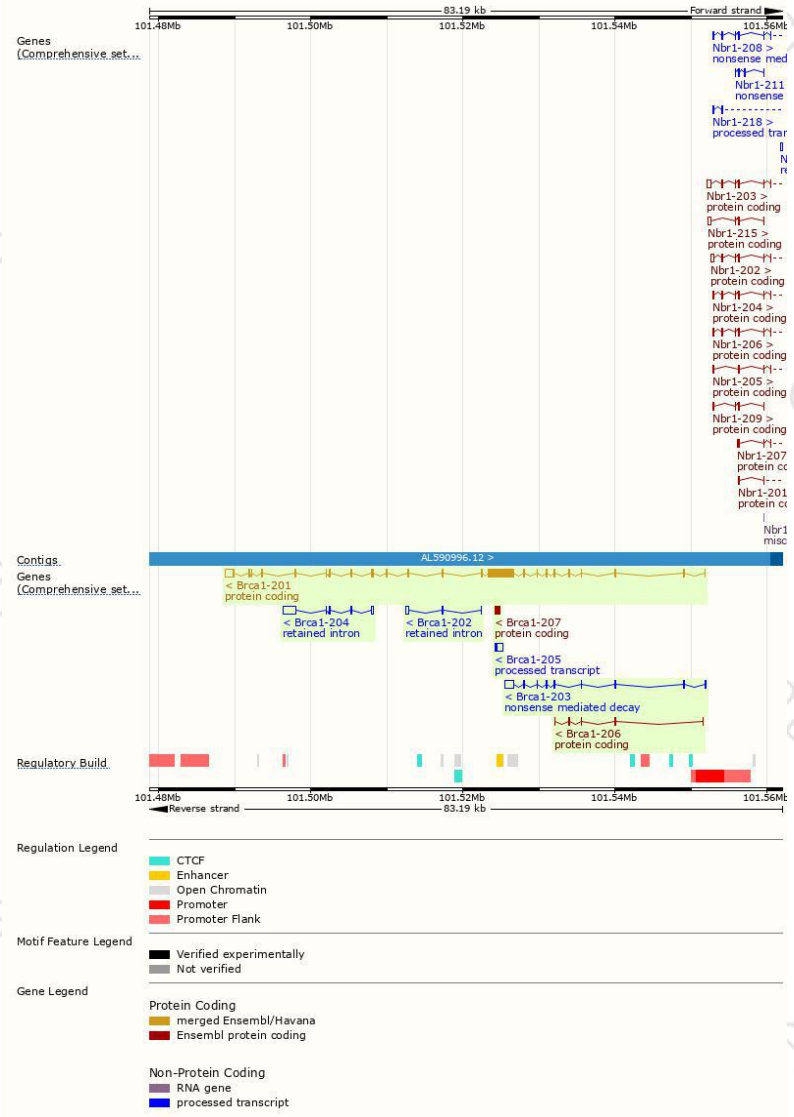
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Brca1-201	ENSMUST00000017290.10	6572	1812aa	Protein coding	CCDS25474	P48754	TSL:1 GENCODE basic APPRIS P1
Brca1-207	ENSMUST00000191198.1	531	177aa	Protein coding	-	A0A087WPE1	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Brca1-206	ENSMUST00000190862.1	355	79aa	Protein coding	-	A0A087WP26	CDS 3' incomplete TSL:5
Brca1-203	ENSMUST00000142086.2	1917	75aa	Nonsense mediated decay	-	A0A087WPK5	TSL:1
Brca1-205	ENSMUST00000188168.1	852	No protein	Processed transcript	-	-	TSL:3
Brca1-204	ENSMUST00000156843.1	2136	No protein	Retained intron	-	-	TSL:1
Brca1-202	ENSMUST00000131460.1	539	No protein	Retained intron	-	-	TSL:3

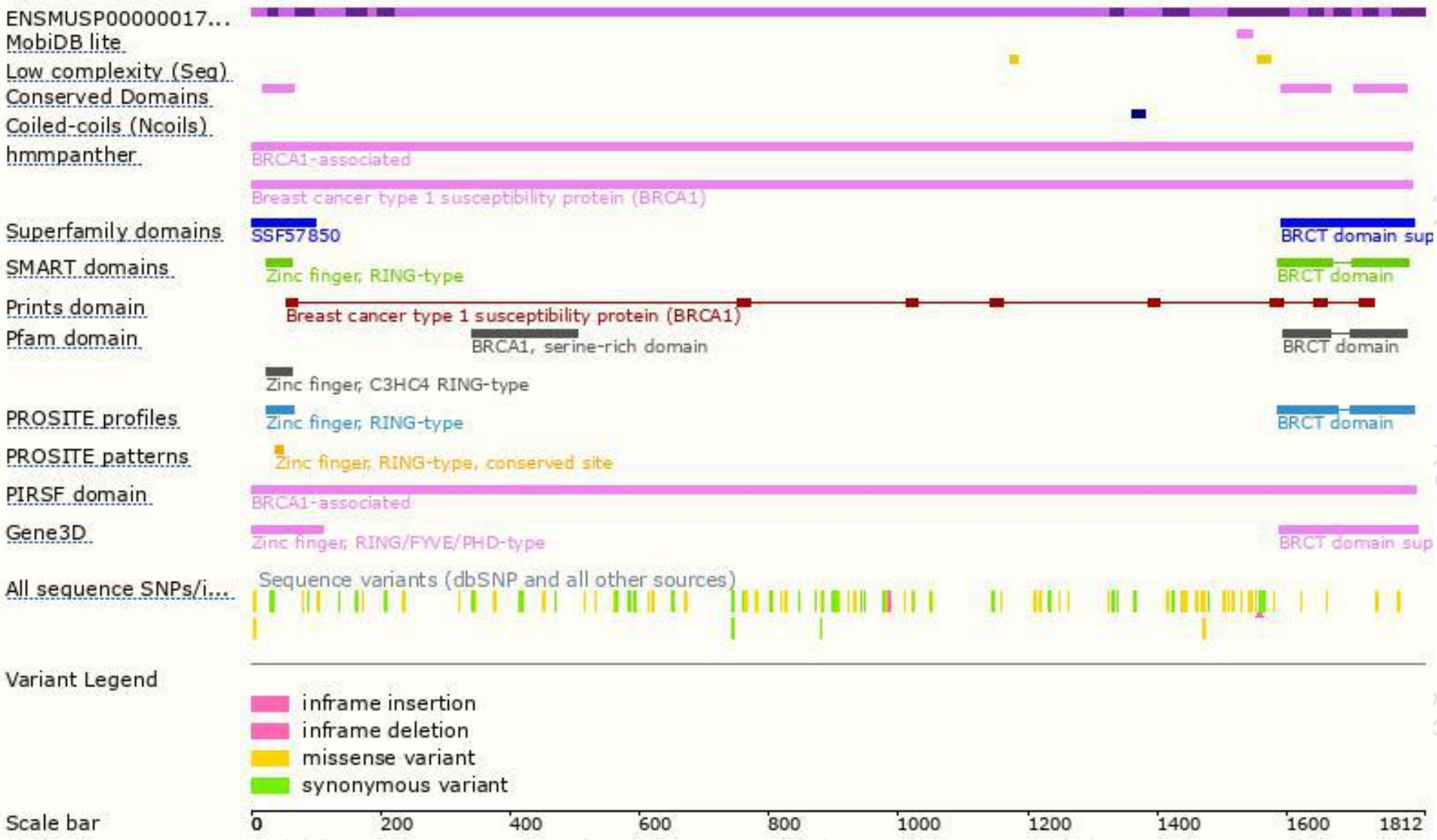
The strategy is based on the design of *Brca1-201* transcript,The transcription is shown below



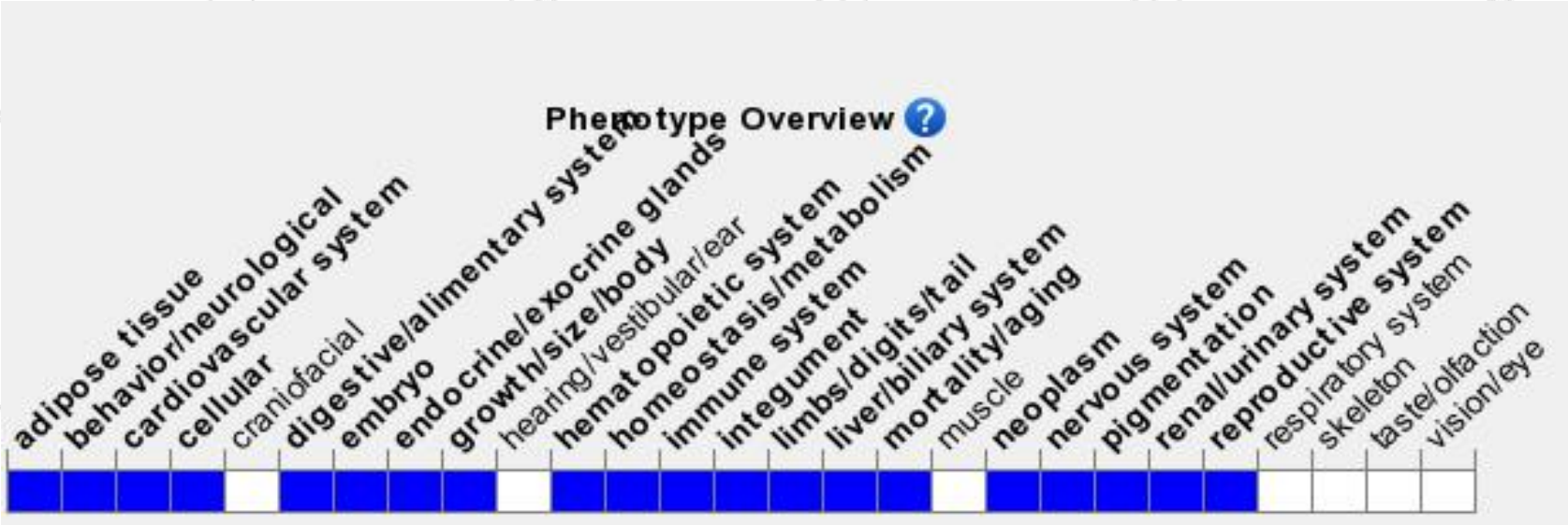
Genomic location distribution



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

Tel: 400-9660890

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GemPharmatech Co.,Ltd