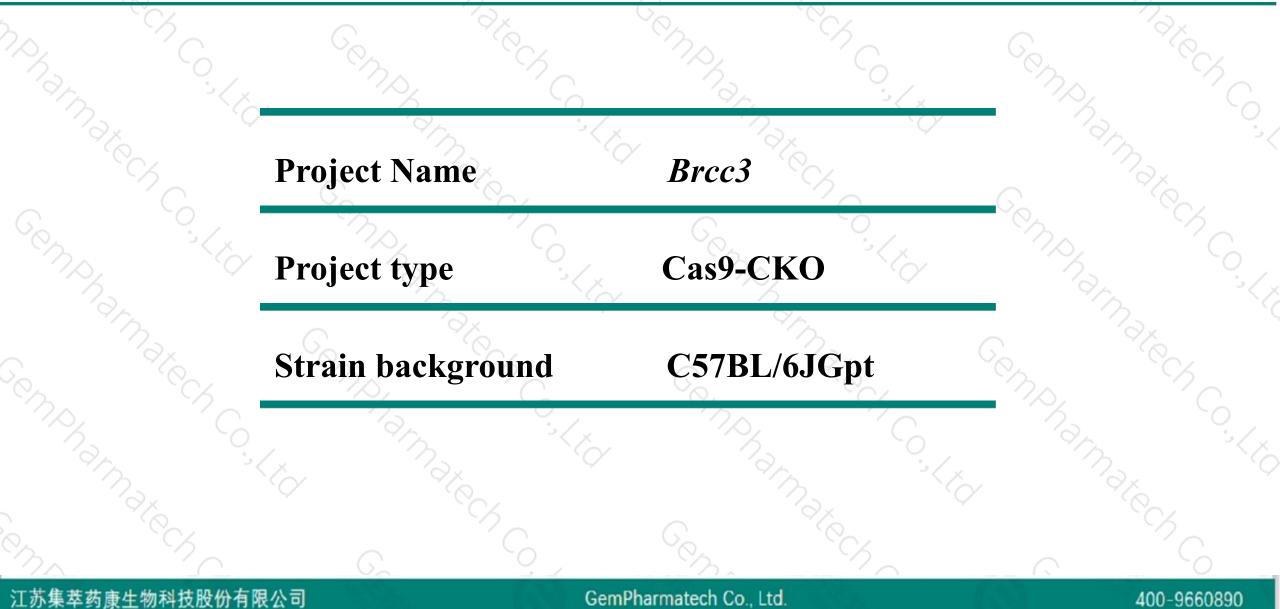


Mohamak G. G. S. L. J. Cemphamatech **Brcc3** Cas9-CKO Strategy Annak Cherry

Cemphamatech, Designer: Yanhua Shen Design Date: 2019-08-07

Project Overview

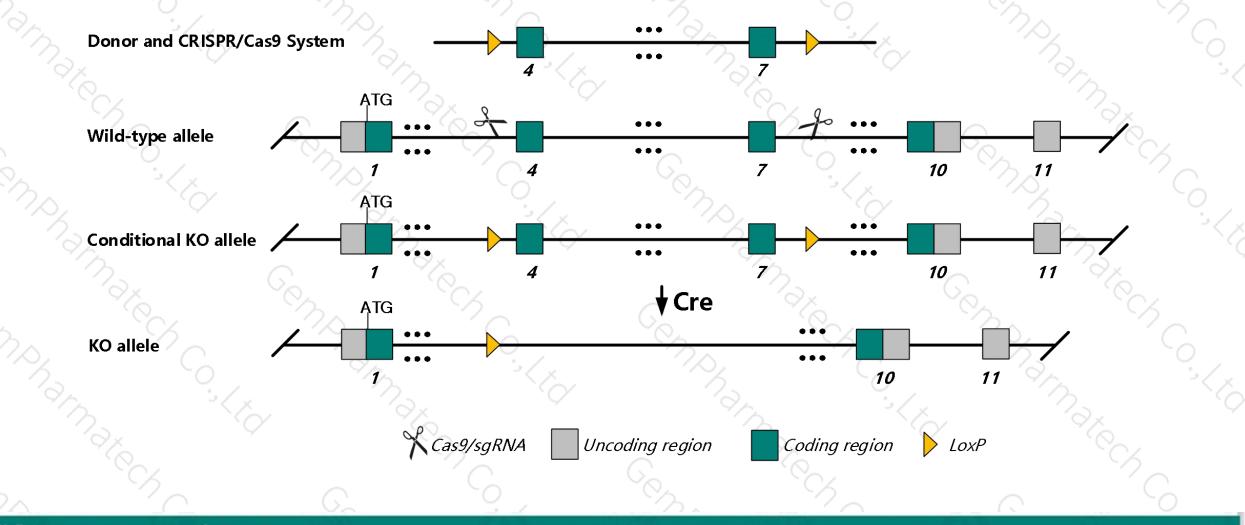




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Brcc3 gene. The schematic diagram is as follows:



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The Brcc3 gene has 7 transcripts. According to the structure of Brcc3 gene, exon4-exon7 of Brcc3-201 (ENSMUST00000033544.13) transcript is recommended as the knockout region. The region contains 353bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Brcc3* 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.



- The Brcc3 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)



Brcc3 BRCA1/BRCA2-containing complex, subunit 3 [Mus musculus (house mouse)]

Gene ID: 210766, updated on 31-Jan-2019

- Summary

2

Official Symbol	Brcc3 provided by MGI
	BRCA1/BRCA2-containing complex, subunit 3 provided by MGI
Primary source	MGI:MGI:2389572
See related	Ensembl:ENSMUSG0000031201
Gene type	protein coding
RefSeq status	VALIDATED
Organism	
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;
	Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	C6.1A
Expression	Broad expression in CNS E11.5 (RPKM 5.2), liver E14 (RPKM 5.1) and 24 other tissues See more
Orthologs	

Transcript information (Ensembl)



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

Name 🖕	Transcript ID 💧	bp 🖕	Protein 🖕	Biotype 💧	CCDS 🍦	UniProt 🖕	Flags
Brcc3-201	ENSMUST0000033544.13	4310	<u>291aa</u>	Protein coding	<u>CCDS41031</u> &	<u>P46737</u> &	TSL:1 GENCODE basic APPRIS P2
Brcc3-202	ENSMUST00000114074.7	2473	<u>291aa</u>	Protein coding	<u>CCDS41031</u> &	<u>P46737</u> &	TSL:1 GENCODE basic APPRIS P2
Brcc3-203	ENSMUST00000118428.7	2093	<u>247aa</u>	Protein coding	170	P46737 &	TSL:5 GENCODE basic APPRIS ALT2
Brcc3-205	ENSMUST00000133781.7	479	<u>158aa</u>	Protein coding	170	A3KGA8函	CDS 3' incomplete TSL:5
Brcc3-207	ENSMUST00000152228.2	473	<u>50aa</u>	Protein coding	170	E9Q0P6&	CDS 3' incomplete TSL:5
Brcc3-204	ENSMUST00000124321.1	2614	No protein	Retained intron	170		TSL:1
Brcc3-206	ENSMUST00000151938.7	2521	No protein	Retained intron	172	-	TSL:2

The strategy is based on the design of *Brcc3-201* transcript, The transcription is shown below

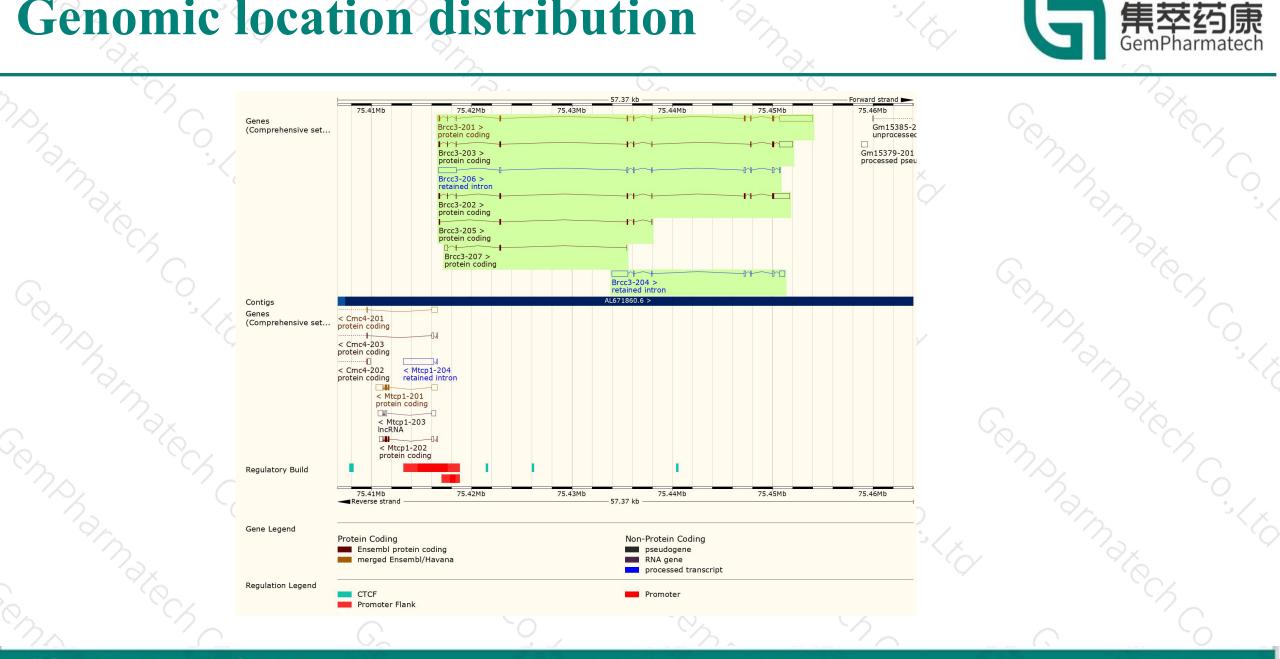


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Genomic location distribution



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Protein domain



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w complexity (Seg) piled-coils (Ncoils)							-	
uperfamily MART	SSF102712				_			
am	JAB1/MPN/MOV34 JAB1/MPN/MOV34 m	metalloenzyme do etalloenzyme doma			•	BRCC36, C-termi	nal helical domain	-
OSITE profiles	MPN domain							
ANTHER	PTHR10410:SF20							
ene3D	PTHR10410 3.40.140.10							
D		ase						
DD	Brcc36 isopeptid Sequence variants (db		sources)					
sequence SNPs/i	Brcc36 isopeptid	SNP and all other	sources)					
	Brcc36 isopeptid Sequence variants (db	SNP and all other	sources)	120	160	200	240	291
sequence SNPs/i riant Legend	Brcc36 isopeptid Sequence variants (db	SNP and all other		120	160	200	240	291

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If you have any questions, you are welcome to inquire. Tel: 400-9660890



