

# **Bbc3** Cas9-CKO Strategy

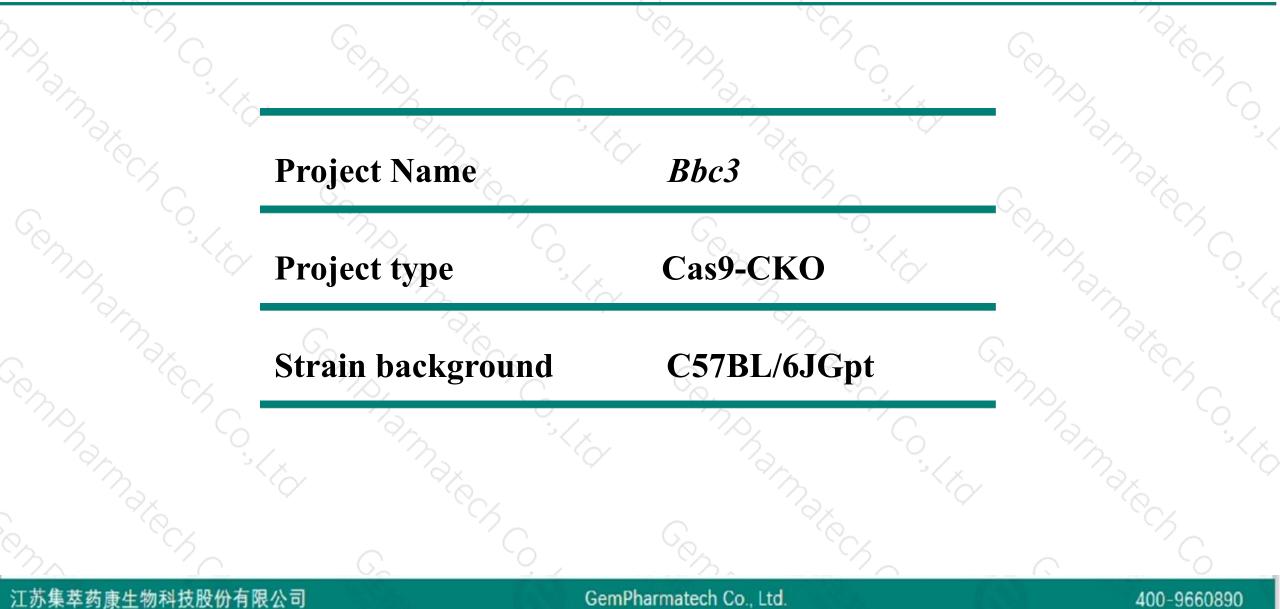
Designer: Reviewer:

**Design Date:** 

Daohua Xu Huimin Su 2019-9-28

## **Project Overview**



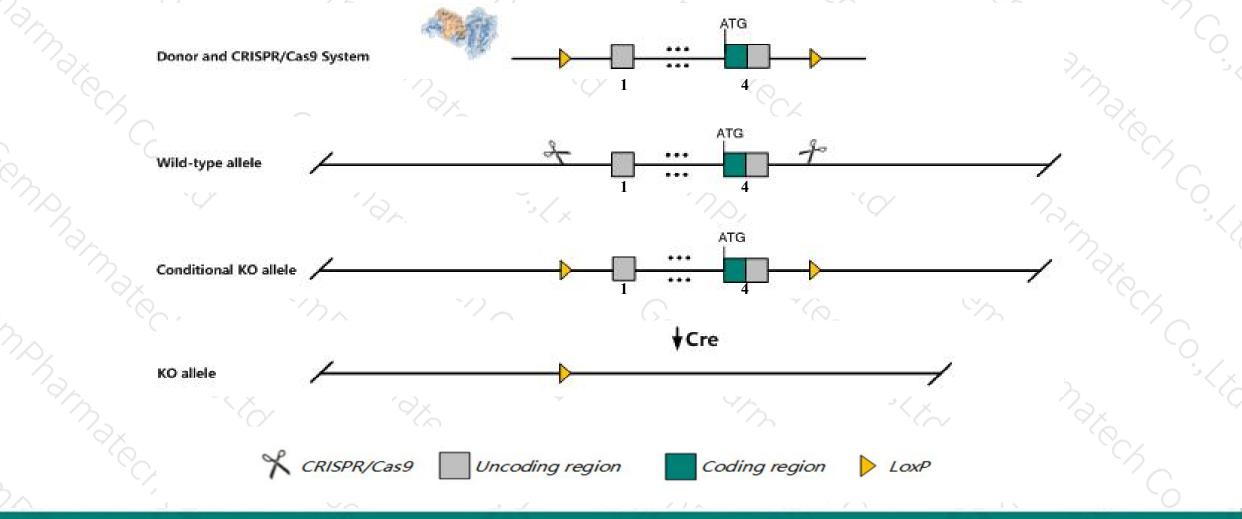


## **Conditional Knockout strategy**



400-9660890

This model will use CRISPR/Cas9 technology to edit the *Bbc3* gene. The schematic diagram is as follows:



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The Bbc3 gene has 4 transcripts. According to the structure of Bbc3 gene, exon1-exon4 of Bbc3-201 (ENSMUST0000002152.12) transcript is recommended as the knockout region. The region contains all 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 *Bbc3* 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 disruptions in this gene display abnormalities in apoptosis but otherwise are phenotypically normal.
- The *Bbc3* gene is located on the Chr7. 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)**



\$ ?

#### Bbc3 BCL2 binding component 3 [Mus musculus (house mouse)]

Gene ID: 170770, updated on 12-Mar-2019

#### Summary

Official Symbol	Bbc3 provided by MGI
Official Full Name	BCL2 binding component 3 provided by MGI
Primary source	MGI:MGI:2181667
See related	Ensembl:ENSMUSG0000002083
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	PUMA, PUMA/JFY1
Expression	Ubiquitous expression in adrenal adult (RPKM 7.3), mammary gland adult (RPKM 6.3) and 28 other tissues See more
Orthologs	human all

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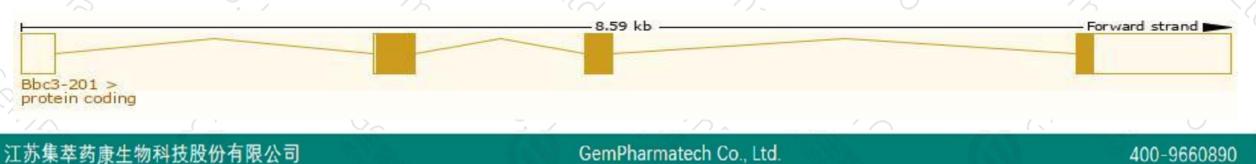
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#### The gene has 4 transcripts, all transcripts are shown below:

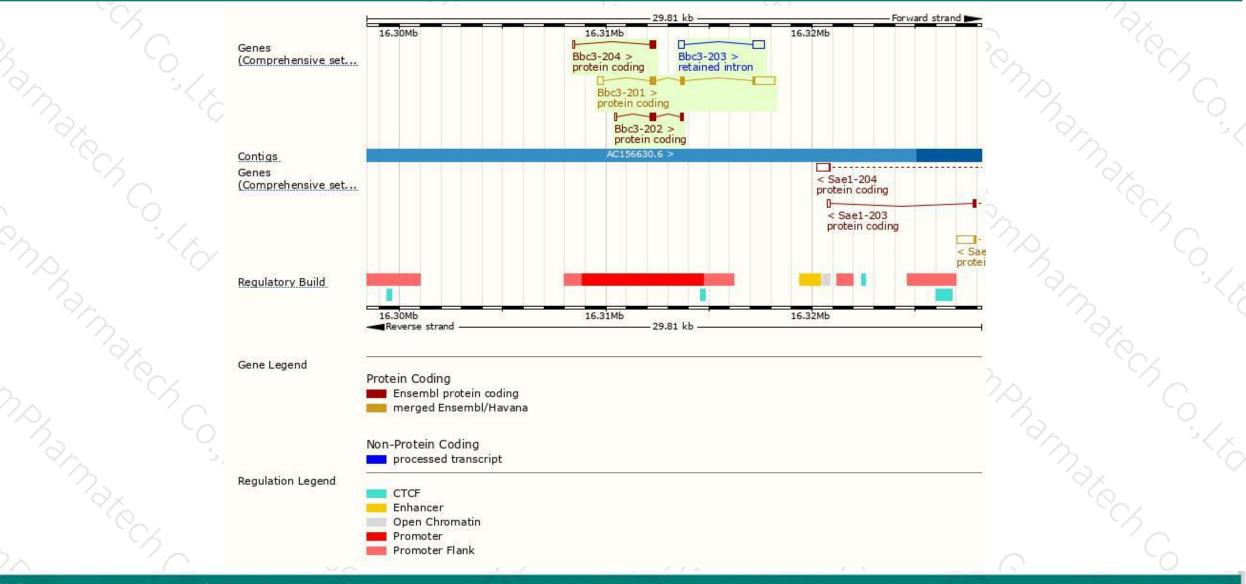
	Here II aller		2 1000 L			K Dira. 4
Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
ENSMUST0000002152.12	1814	<u>193aa</u>	Protein coding	CCDS20847	B2RVL4 Q99ML1	TSL:1 GENCODE basic APPRIS P1
ENSMUST00000136781.1	485	<u>128aa</u>	Protein coding	( <del>1</del> 91	D3Z2M5	CDS 3' incomplete TSL:3
ENSMUST00000209688.1	405	<u>91aa</u>	Protein coding	(2)	A0A1B0GR88	CDS 3' incomplete TSL:3
ENSMUST00000147267.1	881	No protein	Retained intron	100	12	TSL:2
	ENSMUST0000002152.12 ENSMUST00000136781.1 ENSMUST00000209688.1	ENSMUST0000002152.12 1814   ENSMUST00000136781.1 485   ENSMUST00000209688.1 405	ENSMUST0000002152.12 1814 193aa   ENSMUST00000136781.1 485 128aa   ENSMUST00000209688.1 405 91aa	ENSMUST0000002152.121814193aaProtein codingENSMUST00000136781.1485128aaProtein codingENSMUST00000209688.140591aaProtein coding	ENSMUST0000002152.121814193aaProtein codingCCDS20847ENSMUST00000136781.1485128aaProtein coding	ENSMUST000002152.121814193aaProtein codingCCDS20847B2RVL4 Q99ML1ENSMUST0000136781.1485128aaProtein coding-D3Z2M5ENSMUST0000209688.140591aaProtein coding-A0A1B0GR88

The strategy is based on the design of *Bbc3-201* transcript, The transcription is shown below



## **Genomic location distribution**





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## **Protein domain**



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All sequence SNPs/i	Seque	nce variants	(dbSNP and	all other sou	rces)					
Variant Legend			0.00000		-					
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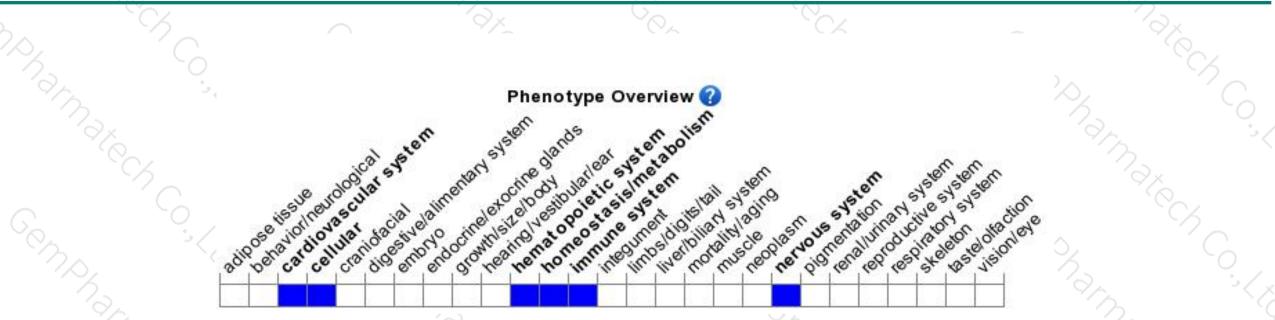
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## Mouse phenotype description(MGI)





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 disruptions in this gene display abnormalities in apoptosis but otherwise are phenotypically normal.



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



