

# **Bnc2** Cas9-CKO Strategy

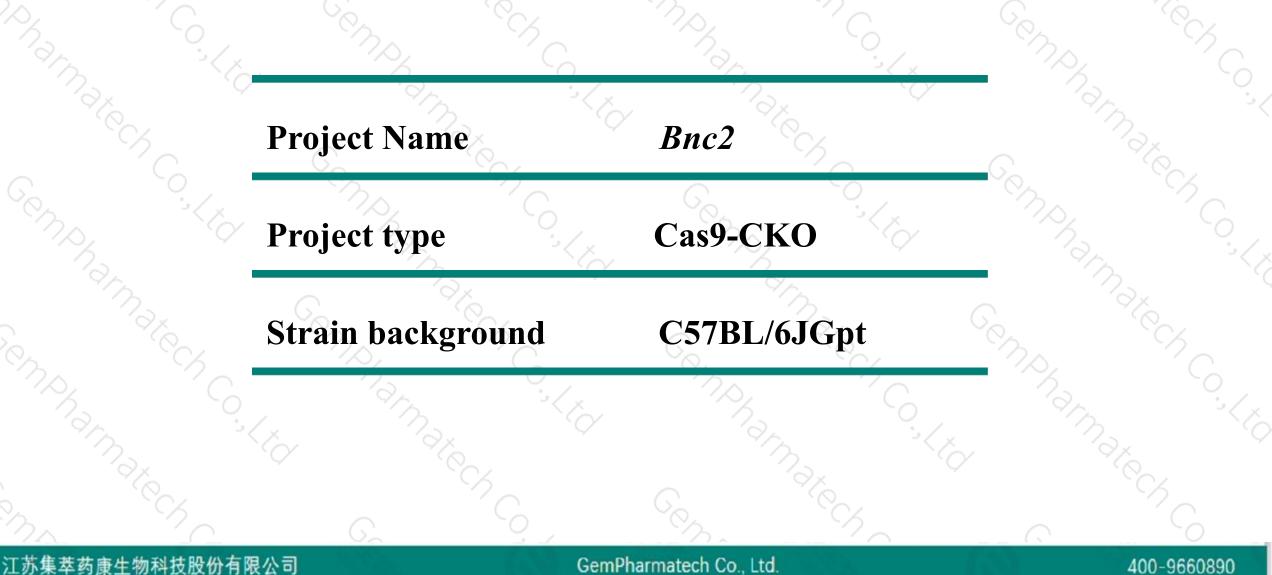
Designer: Reviewer:

**Design Date:** 

Daohua Xu Huimin Su 2019-9-6

# **Project Overview**





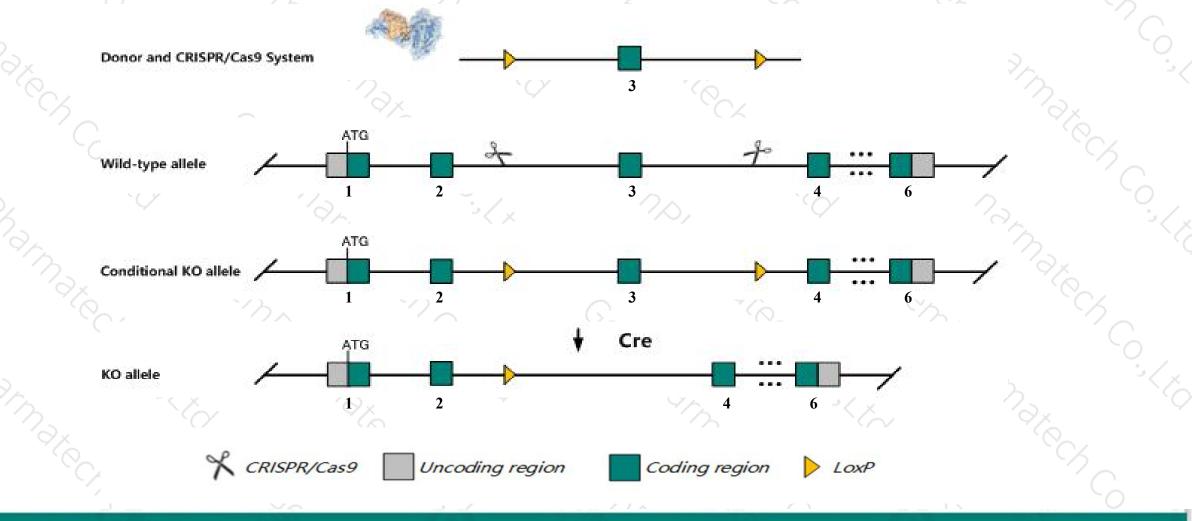
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# **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the *Bnc2* gene. The schematic diagram is as follows:



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The Bnc2 gene has 22 transcripts. According to the structure of Bnc2 gene, exon3 of Bnc2-215 (ENSMUST00000176691.7) transcript is recommended as the knockout region. The region contains 103bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Bnc2* 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 gene trap insertion die within 24 hrs of birth and display cleft palate, an overall size reduction of the head and tongue, and abnormal craniofacial bone development due to impaired multiplication of embryonic craniofacial mesenchymal cells.
- The Bnc2 gene is located on the Chr4. 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)



☆ ?

### Bnc2 basonuclin 2 [Mus musculus (house mouse)]

Gene ID: 242509, updated on 20-Mar-2019

#### Summary

Official SymbolBic2 provided by MGIOfficial Full Namebasonuclin 2 provided by MGIPrimary sourceMGI:MGI:2443805Primary sourceInsembl:ENSMUSG0000028487Gene typeprotein codingRefSeq statusVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;<br/>Muroidea; Murinae; Mus; MusAlso knownas5031434M05Rik, 8430420F16RikExpressionBiased expression in limb E14.5 (RPKM 4.5), bladder adult (RPKM 2.8) and 13 other tissuesSee more<br/>human all

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# **Transcript information (Ensembl)**



### The gene has 22 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	1			
Bnc2-215	ENSMUST00000176691.7	3385	<u>1032aa</u>	Protein coding	CCDS18301	3301 Q8BMQ3 TSL:1 GENCODE basic APPRIS P2					
Bnc2-201	ENSMUST00000102820.8	4133	<u>1127aa</u>	Protein coding	100	Q8BMQ3	TSL:5 GENCODE basic APPRIS ALT2				
Bnc2-214	ENSMUST00000176612.7	3963	<u>861aa</u>	Protein coding	640	H3BLG6	TSL:5 GENCODE basic				
Bnc2-202	ENSMUST00000107198.8	3948	<u>1099aa</u>	Protein coding	1020	H3BIU2	TSL:5 GENCODE basic APPRIS ALT2	×			
Bnc2-211	ENSMUST00000176418.7	1236	<u>405aa</u>	Protein coding	885	H3BKX5	CDS 3' incomplete TSL:5	Co.			
Bnc2-206	ENSMUST00000175800.7	1232	<u>294aa</u>	Protein coding	100	H3BJ03	CDS 3' incomplete TSL:5	$  \heartsuit \rangle$			
Bnc2-218	ENSMUST00000176971.1	1127	<u>239aa</u>	Protein coding	(12)	H3BL10	CDS 5' incomplete TSL:5				
Bnc2-217	ENSMUST00000176947.7	763	<u>237aa</u>	Protein coding	120	H3BKJ2	CDS 3' incomplete TSL:5				
Bnc2-216	ENSMUST00000176702.7	723	<u>241aa</u>	Protein coding		H3BIY6	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5				
Bnc2-207	ENSMUST00000175969.7	636	<u>181aa</u>	Protein coding	100	H3BL54	CDS 3' incomplete TSL:5				
Bnc2-220	ENSMUST00000177040.1	626	<u>126aa</u>	Protein coding	(in)	H3BJR1	CDS 5' incomplete TSL:5				
Bnc2-210	ENSMUST00000176370.1	566	<u>29aa</u>	Protein coding	325	H3BJE5	CDS 3' incomplete TSL:5				
Bnc2-222	ENSMUST00000177277.7	561	<u>85aa</u>	Protein coding	100	H3BKY6	CDS 5' incomplete TSL:5				
Bnc2-204	ENSMUST00000175756.7	364	<u>87aa</u>	Protein coding	1993	H3BKR9	CDS 3' incomplete TSL:5				
Bnc2-213	ENSMUST00000176601.1	280	<u>86aa</u>	Protein coding	627	H3BKQ4	CDS 5' incomplete TSL:5				
Bnc2-219	ENSMUST00000176998.7	276	<u>82aa</u>	Protein coding	325	H3BK78	CDS 5' incomplete TSL:5				
Bnc2-209	ENSMUST00000176346.7	692	<u>64aa</u>	Nonsense mediated decay	10.0	H3BJD5	TSL3	D .			
Bnc2-203	ENSMUST00000123276.7	2986	No protein	Retained intron	100	8.0	TSL2	$\sum_{i=1}^{n}$			
Bnc2-208	ENSMUST00000176264.1	845	No protein	IncRNA	627	2	TSL:5				
Bnc2-205	ENSMUST00000175757.7	792	No protein	IncRNA	325	12	TSL3				
Bnc2-221	ENSMUST00000177256.7	545	No protein	IncRNA	12		TSL:5				
Bnc2-212	ENSMUST00000176476.7	541	No protein	IncRNA	(H)		TSL5				

The strategy is based on the design of *Bnc2-215* transcript, The transcription is shown below

#### < Bnc2-215 protein coding

Reverse strand

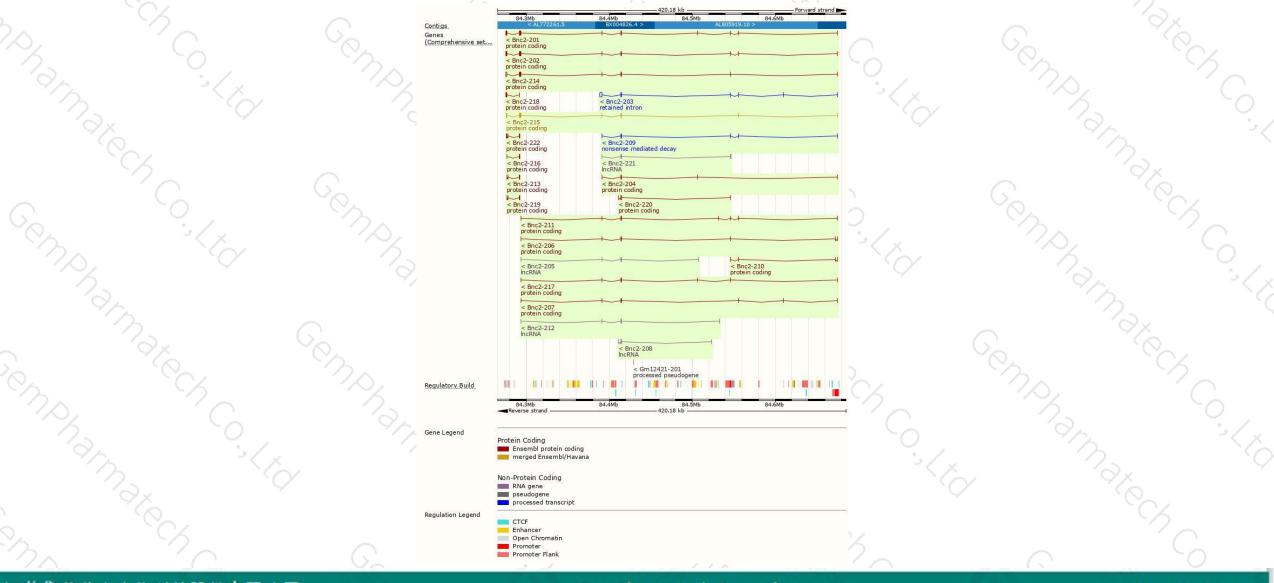
– 399.48 kb –

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### **Genomic location distribution**





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# **Protein domain**



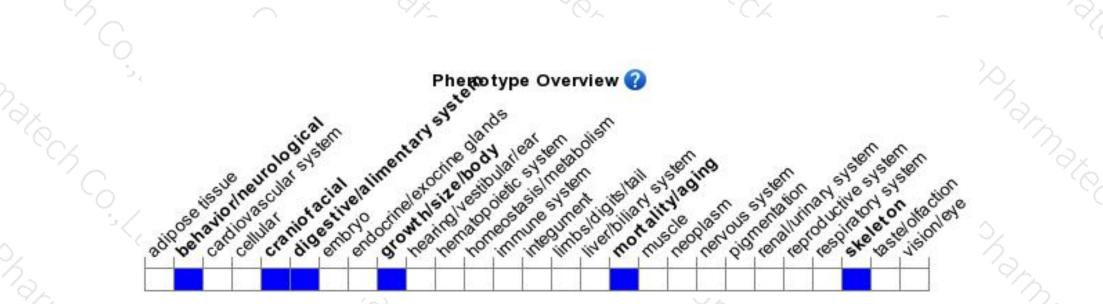
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# Mouse phenotype description(MGI)





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 a gene trap insertion die within 24 hrs of birth and display cleft palate, an overall size reduction of the head and tongue, and abnormal craniofacial bone development due to impaired multiplication of embryonic craniofacial mesenchymal cells.

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



