

Bach2 Cas9-CKO Strategy

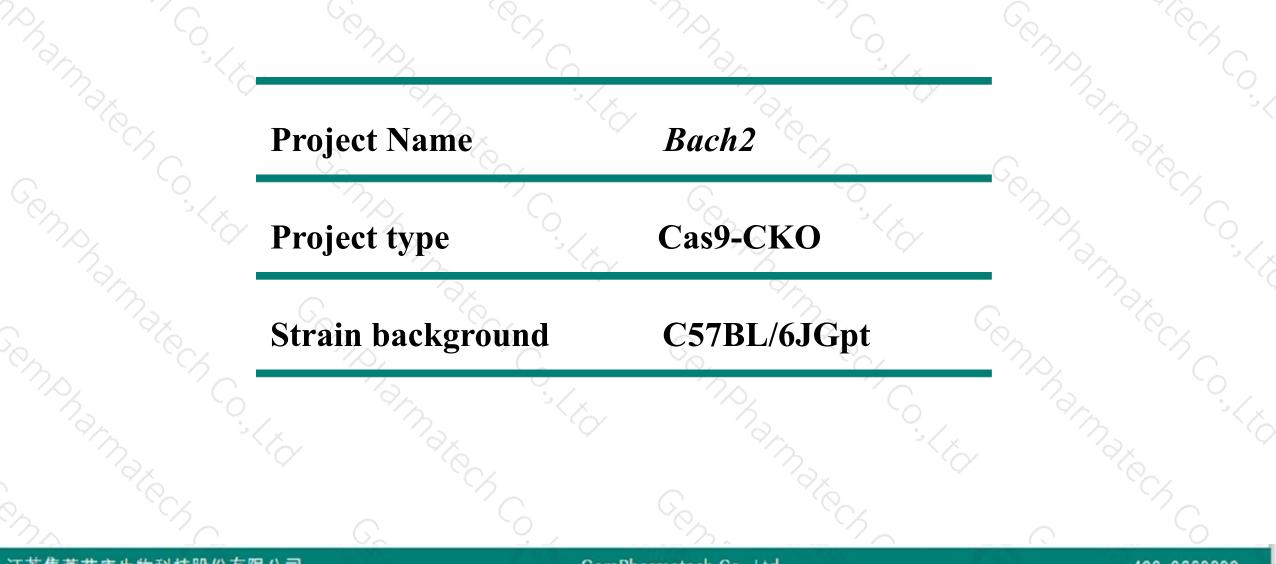
Designer: Reviewer:

Design Date:

Daohua Xu Huimin Su 2019-9-9

Project Overview





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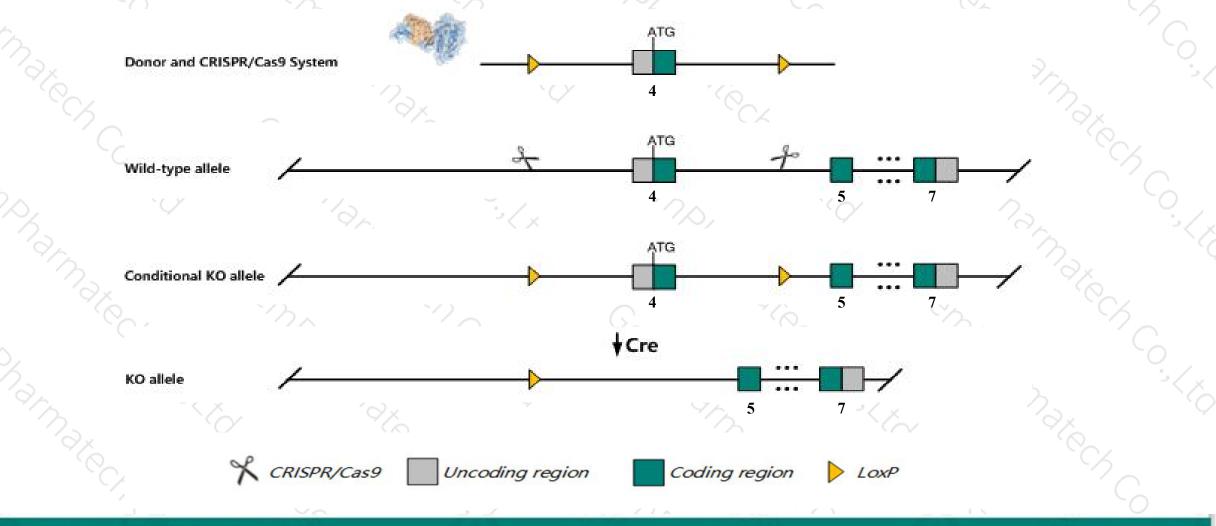
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Conditional Knockout strategy



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This model will use CRISPR/Cas9 technology to edit the Bach2 gene. The schematic diagram is as follows:



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The Bach2 gene has 7 transcripts. According to the structure of Bach2 gene, exon4 of Bach2-202 (ENSMUST00000108180.8) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Bach2* 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 mice display impaired B cell differentiation and reduced B cell numbers.
- The *Bach2* 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)



☆ ?

Bach2 BTB and CNC homology, basic leucine zipper transcription factor 2 [Mus musculus (house mouse)]

Gene ID: 12014, updated on 9-Apr-2019

Summary

Official Symbol	Bach2 provided by MGI								
Official Full Name	BTB and CNC homology, basic leucine zipper transcription factor 2 provided by MGI								
Primary source	MGI:MGI:894679								
See related	Ensembl:ENSMUSG0000040270								
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	E030004N02Rik								
Expression	Broad expression in whole brain E14.5 (RPKM 5.3), CNS E14 (RPKM 5.0) and 24 other tissues See more								
Orthologs	human all								

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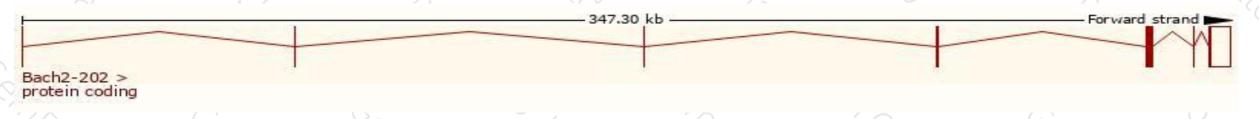
Transcript information (Ensembl)



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

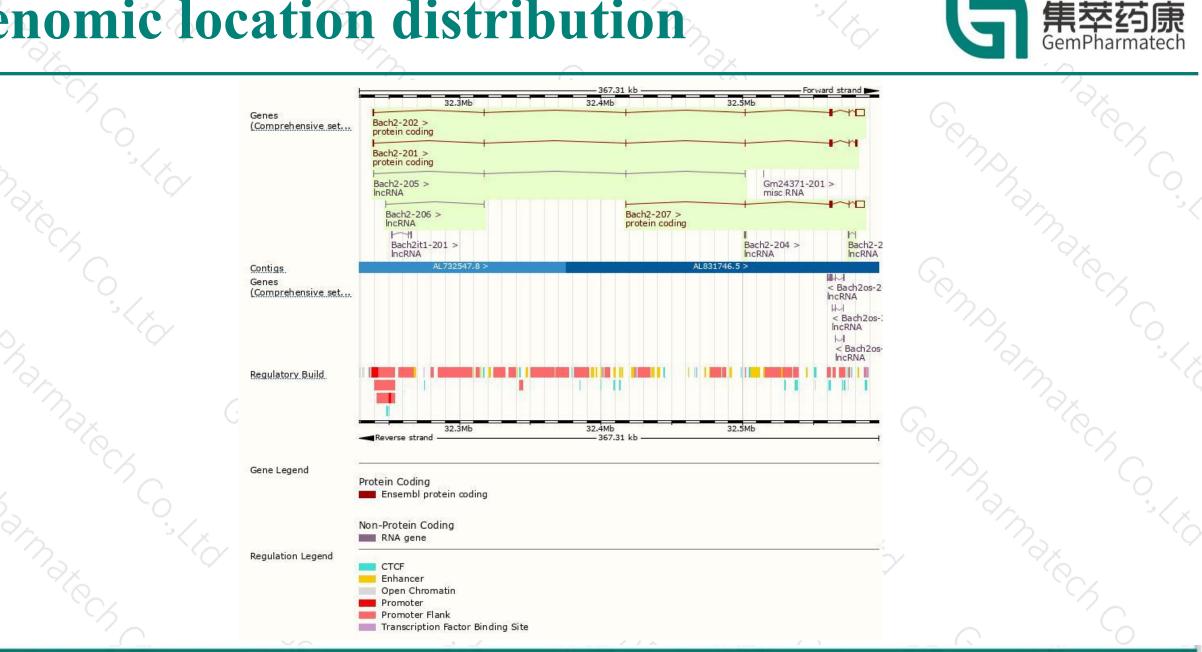
Name 🝦	Transcript ID	bp 👙	Protein 👙	Biotype 🍦	CCDS 👙	UniProt 🝦	Flags 🔶			
Bach2-202	ENSMUST00000108180.8	8882	<u>839aa</u>	Protein coding	<u>CCDS51135</u> &	<u>P97303</u> &	TSL:5	GENCODE basic		APPRIS P1
Bach2-207	ENSMUST00000171600.1	8493	<u>839aa</u>	Protein coding	CCDS51135	<u>P97303</u> &	TSL:1 GENCODE basic		APPRIS P1	
Bach2-201	ENSMUST0000037416.12	3462	<u>716aa</u>	Protein coding	- ×	<u>P97303</u> @	TSL:5 GENCODE basic			
Bach2-204	ENSMUST00000146748.1	658	No protein	IncRNA	i s	-	TSL:1			
Bach2-203	ENSMUST00000125263.1	538	No protein	IncRNA	2		TSL:5			
Bach2-205	ENSMUST00000149201.7	336	No protein	IncRNA	2	5 (143)	TSL:5			
Bach2-206	ENSMUST00000156430.1	3 <mark>31</mark>	No protein	IncRNA	2.2 2.2	124			TSL:3	

The strategy is based on the design of Bach2-202 transcript, The transcription is shown below



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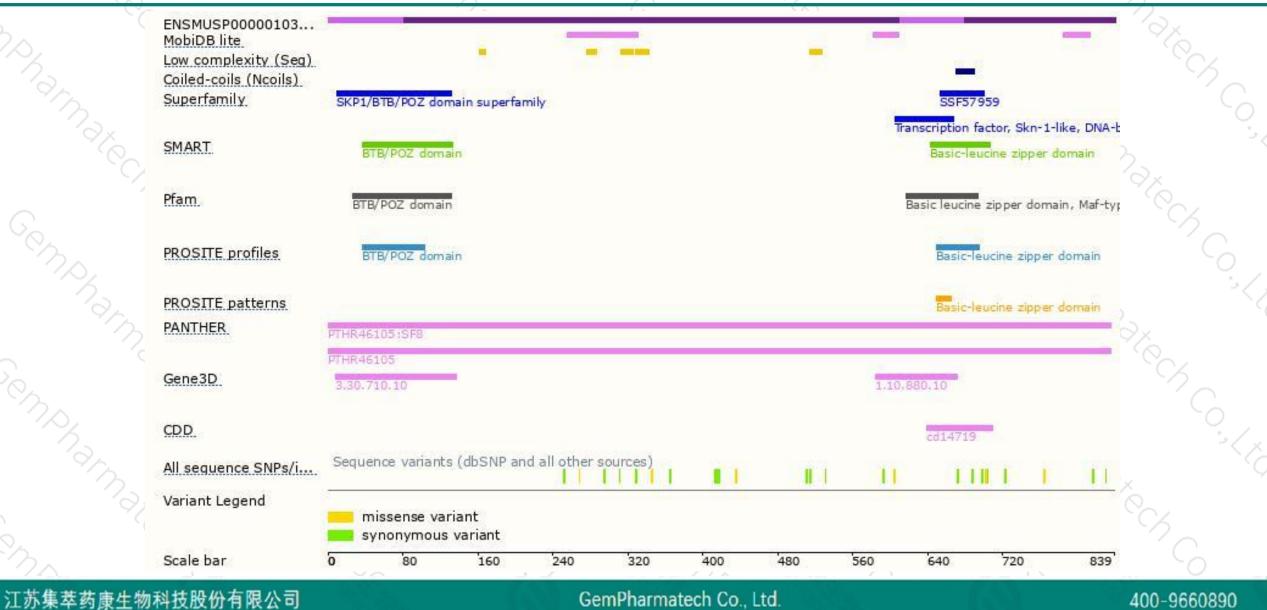
Genomic location distribution



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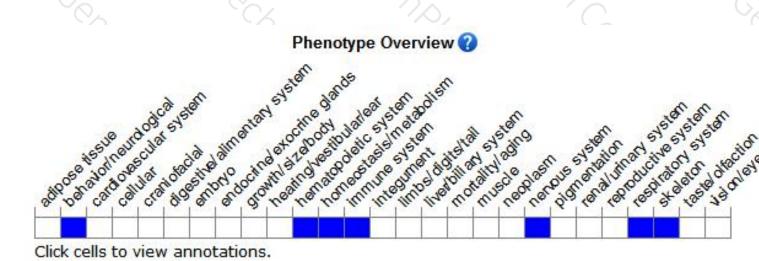
Protein domain





Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data, Homozygous null mice display impaired B cell differentiation and reduced B cell numbers.



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



