

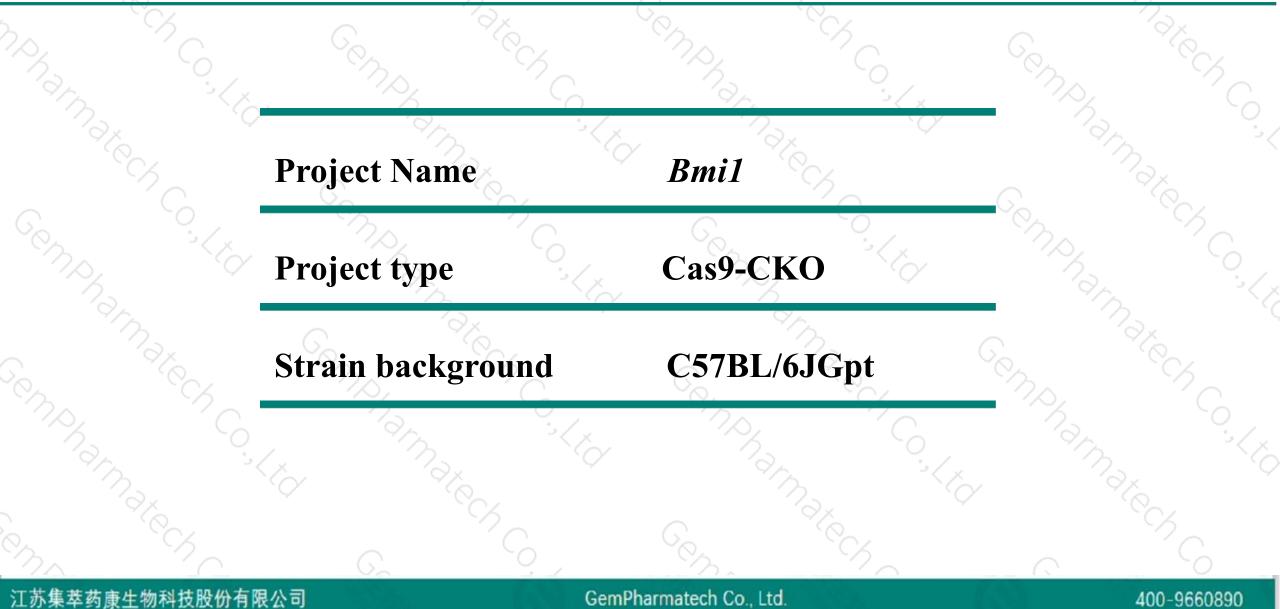
Bmil Cas9-CKO Strategy

Designer: Yupeng Yang Reviewer: Shilei Zhu Date: 2019/3/5

02

Project Overview



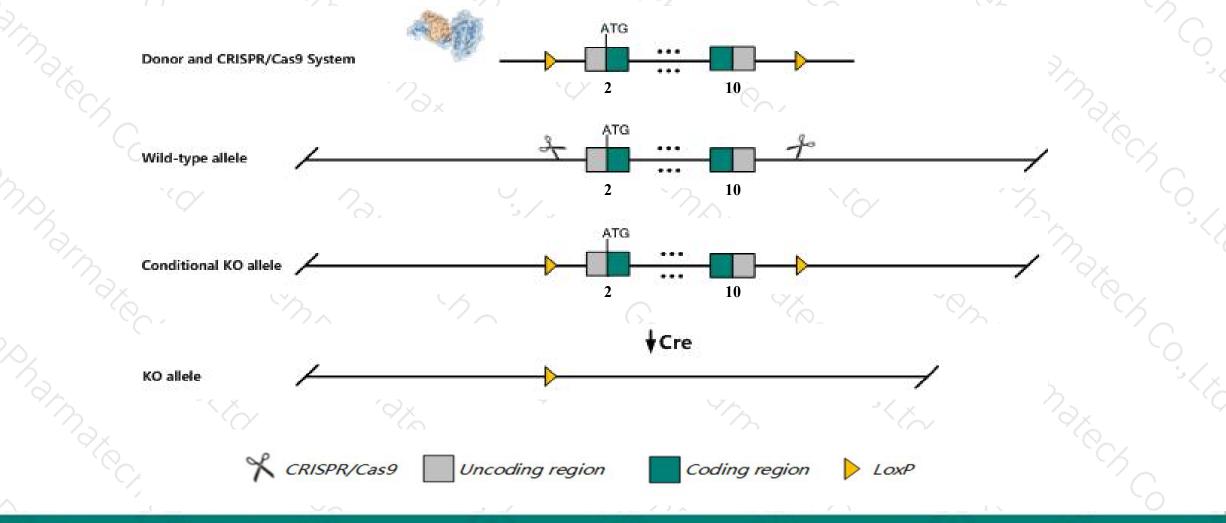


Conditional Knockout strategy



400-9660890

This model will use CRISPR/Cas9 technology to edit the *Bmi1* gene. The schematic diagram is as follows:



江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.



The Bmil gene has 7 transcripts. According to the structure of Bmil gene, exon2-exon10 of Bmil-201 (ENSMUST00000028071.12) 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 *Bmi1* 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 Bmil gene is located on the Chr2. 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)



\$?

Bmi1 Bmi1 polycomb ring finger oncogene [Mus musculus (house mouse)]

Gene ID: 12151, updated on 1-Mar-2020

Summary

Official Symbol	Bmi1 provided by MGI
Official Full Name	Bmi1 polycomb ring finger oncogene provided by MGI
Primary source	MGI:MGI:88174
See related	Ensembl:ENSMUSG00000026739
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
	Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AW546694, Bmi-1, Pcgf4
Expression	Ubiquitous expression in whole brain E14.5 (RPKM 11.2), testis adult (RPKM 11.2) and 27 other tissues See more
Orthologs	human all

江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

400-9660890

Transcript information (Ensembl)



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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Bmi1-201	ENSMUST00000028071.12	3594	<u>324aa</u>	Protein coding	CCDS15711	P25916 Q2LC58	TSL:1 GENCODE basic APPRIS P1
Bmi1-202	ENSMUST00000051929.12	2747	<u>324aa</u>	Protein coding	CCDS15711	P25916 Q2LC58	TSL:1 GENCODE basic APPRIS P1
Bmi1-207	ENSMUST00000156284.7	713	<u>197aa</u>	Protein coding	45	A2ASR8	CDS 3' incomplete TSL:3
Bmi1-206	ENSMUST00000150834.7	521	<u>159aa</u>	Protein coding	20	A2ASR7	CDS 3' incomplete TSL:5
Bmi1-204	ENSMUST00000134734.7	426	<u>105aa</u>	Protein coding		A2ASR6	CDS 3' incomplete TSL:5
Bmi1-205	ENSMUST00000147365.1	359	<u>75aa</u>	Protein coding	.	A2ASR5	CDS 3' incomplete TSL:5
Bmi1-203	ENSMUST00000132014.1	464	No protein	IncRNA	10	-	TSL:2

The strategy is based on the design of *Bmi1-201* transcript, The transcription is shown below

		9.61	kb/	Forw	ard strand 🗩
Bmi1-201 > protein coding				W.	1000
	1.1		101	6.2	

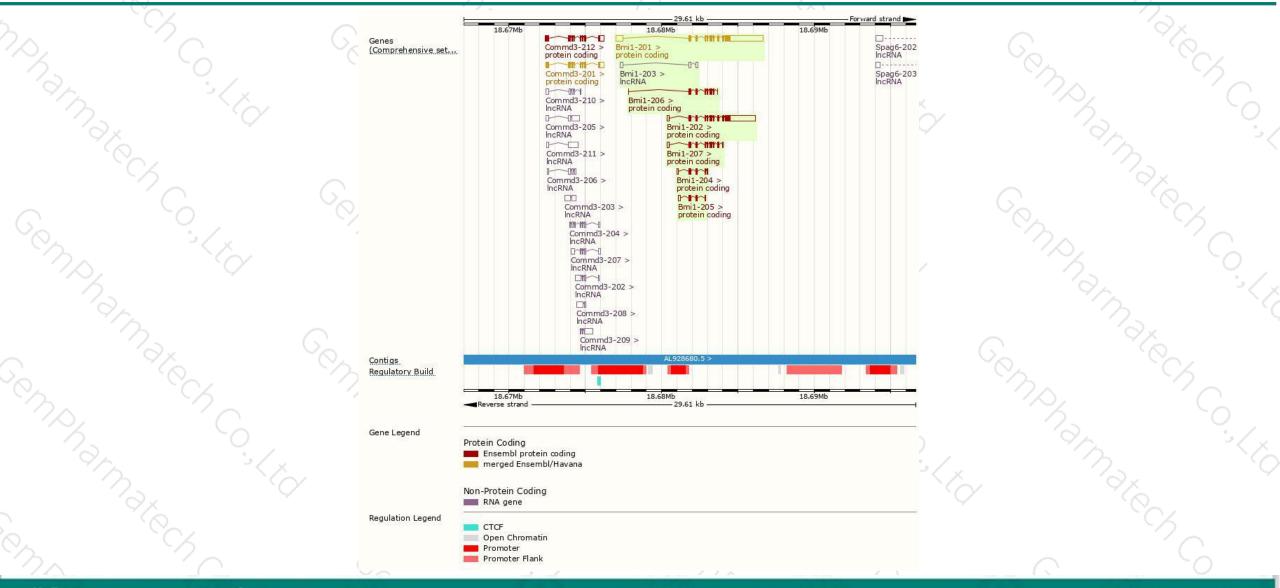
江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

400-9660890

Genomic location distribution





江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

400-9660890

Protein domain

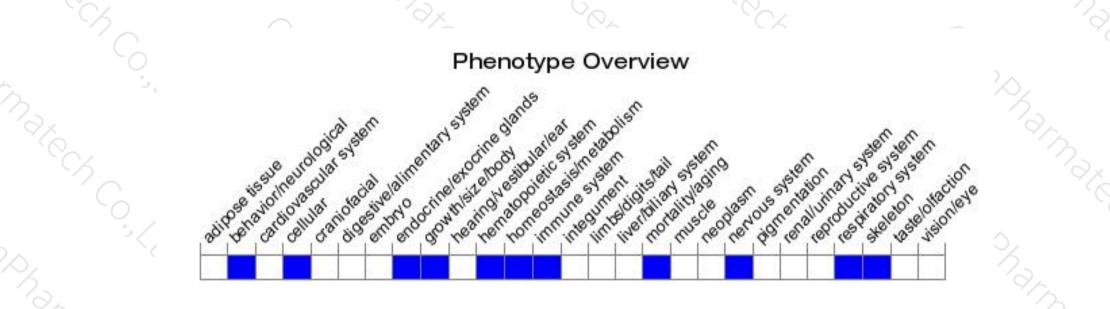
江苏



\triangle		、 司		127_3	GemPharmate	I A	10000			(N (17)	1001	00-966	
	Scale bar	100 Cost 200	ense variant nymous varia 40	ant 80	120	160	200	240		28	30	324	
	All sequence SNPs/i Variant Legend		vananca (abe	and an arrest of the			101	1	1	1	I	Ш	
	CDD	cd1673 Sequence		SNP and all ot	her sources)								· Ò.
	Gene3D	PTHR10825 Zinc finger,	ISF21 RING/FYVE/PH	(D-type	3,10,20,90								
3	PROSITE profiles PROSITE patterns PANTHER	PTHR10825		ype RING-type, con	served site								
	ENSMUSP0000028 SIFTS import MobiDB lite Low complexity (Seg) Superfamily SMART Pfam	SSF57 Zinc PF13	finger, RING-t	уре		RAWUL	domain		1				X Co.

Mouse phenotype description(MGI)





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



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



