Phc1 Cas9-KO Strategy

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Reviewer: Daohua Xu

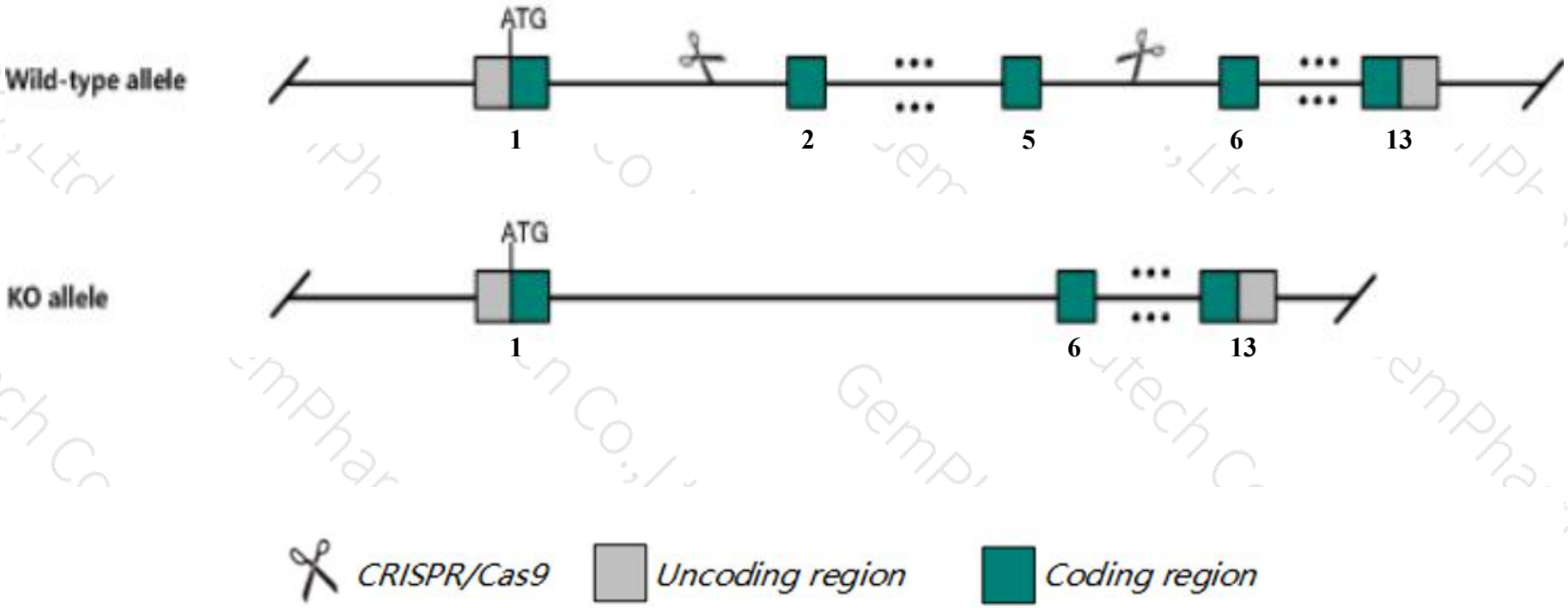
Design Date: 2020-7-21

Project Overview

Project Name	***Phc1***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Phc1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Phc1* gene has 16 transcripts. According to the structure of *Phc1* gene, exon2-exon5 of *Phc1-202*(ENSMUST00000081849.9) transcript is recommended as the knockout region.The region contains 835bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Phc1* gene. The brief process is as follows: CRISPR/Cas9 system 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.

Notice

➢ According to the existing MGI data,homozygous mutant mice exhibit perinatal lethality, posterior skeletal transformations and defects in neural crest derived tissues, including ocular abnormalities, cleft palate, parathyroid and thymic hypoplasia and cardiac anomalies. Hematopoiesis is impaired in fetal livers.

➢ The *Phc1* gene is located on the Chr6. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

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Phc1 polyhomeotic 1 [Mus musculus (house mouse)]

Gene ID: 13619, updated on 13-Mar-2020

Summary

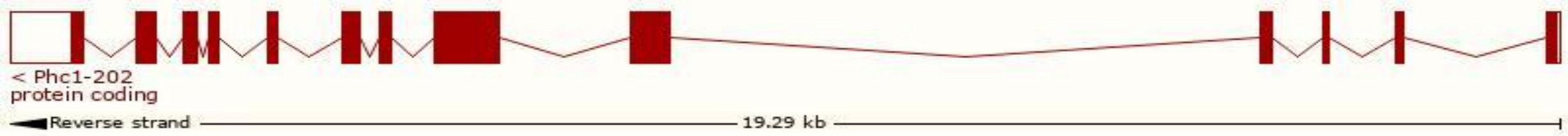
Official Symbol	Phc1 provided by MGI
Official Full Name	polyhomeotic 1 provided byMGI
Primary source	MGI:MGI:103248
See related	Ensembl:ENSMUSG00000040669
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AW557034, Edr, Edr1, Mph1, Rae-28, rae28
Expression	Broad expression in testis adult (RPKM 55.8), CNS E14 (RPKM 22.1) and 20 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

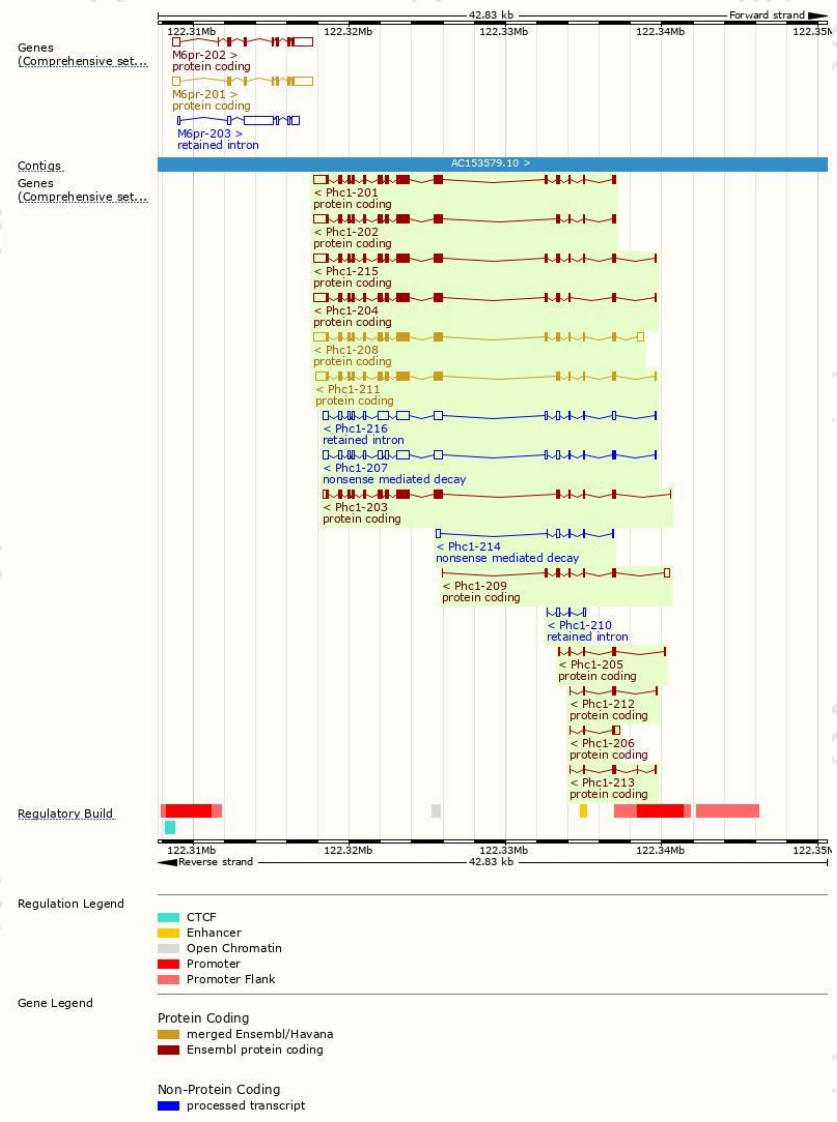
The gene has 16 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Phc1-208	ENSMUST00000160696.7	4213	1010aa	Protein coding	CCDS20494	Q7TT35	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P3
Phc1-215	ENSMUST00000161739.7	3893	1010aa	Protein coding	CCDS20494	Q7TT35	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P3
Phc1-201	ENSMUST00000079560.9	3839	1010aa	Protein coding	CCDS20494	Q7TT35	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P3
Phc1-202	ENSMUST00000081849.9	3683	958aa	Protein coding	CCDS39620	Q3V116	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Phc1-211	ENSMUST00000161054.7	3612	958aa	Protein coding	CCDS39620	Q3V116	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Phc1-203	ENSMUST00000112600.8	3127	958aa	Protein coding	CCDS39620	Q3V116	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Phc1-204	ENSMUST00000159252.7	3744	965aa	Protein coding	-	E0CXV8	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Phc1-209	ENSMUST00000160843.7	970	205aa	Protein coding	-	E0CXT0	CDS 3' incomplete TSL:5
Phc1-206	ENSMUST00000159657.7	566	80aa	Protein coding	-	E0CYR2	CDS 3' incomplete TSL:3
Phc1-205	ENSMUST00000159384.7	424	117aa	Protein coding	-	E0CXC9	CDS 3' incomplete TSL:3
Phc1-212	ENSMUST00000161149.7	377	80aa	Protein coding	-	E0CYR2	CDS 3' incomplete TSL:5
Phc1-213	ENSMUST00000161210.2	347	79aa	Protein coding	-	E0CXA6	CDS 3' incomplete TSL:5
Phc1-207	ENSMUST00000160163.7	3289	86aa	Nonsense mediated decay	-	E0CYV8	TSL:1
Phc1-214	ENSMUST00000161290.7	687	69aa	Nonsense mediated decay	-	F7D980	CDS 5' incomplete TSL:3
Phc1-216	ENSMUST00000161877.7	3530	No protein	Retained intron	-	-	TSL:1
Phc1-210	ENSMUST00000160863.1	410	No protein	Retained intron	-	-	TSL:5

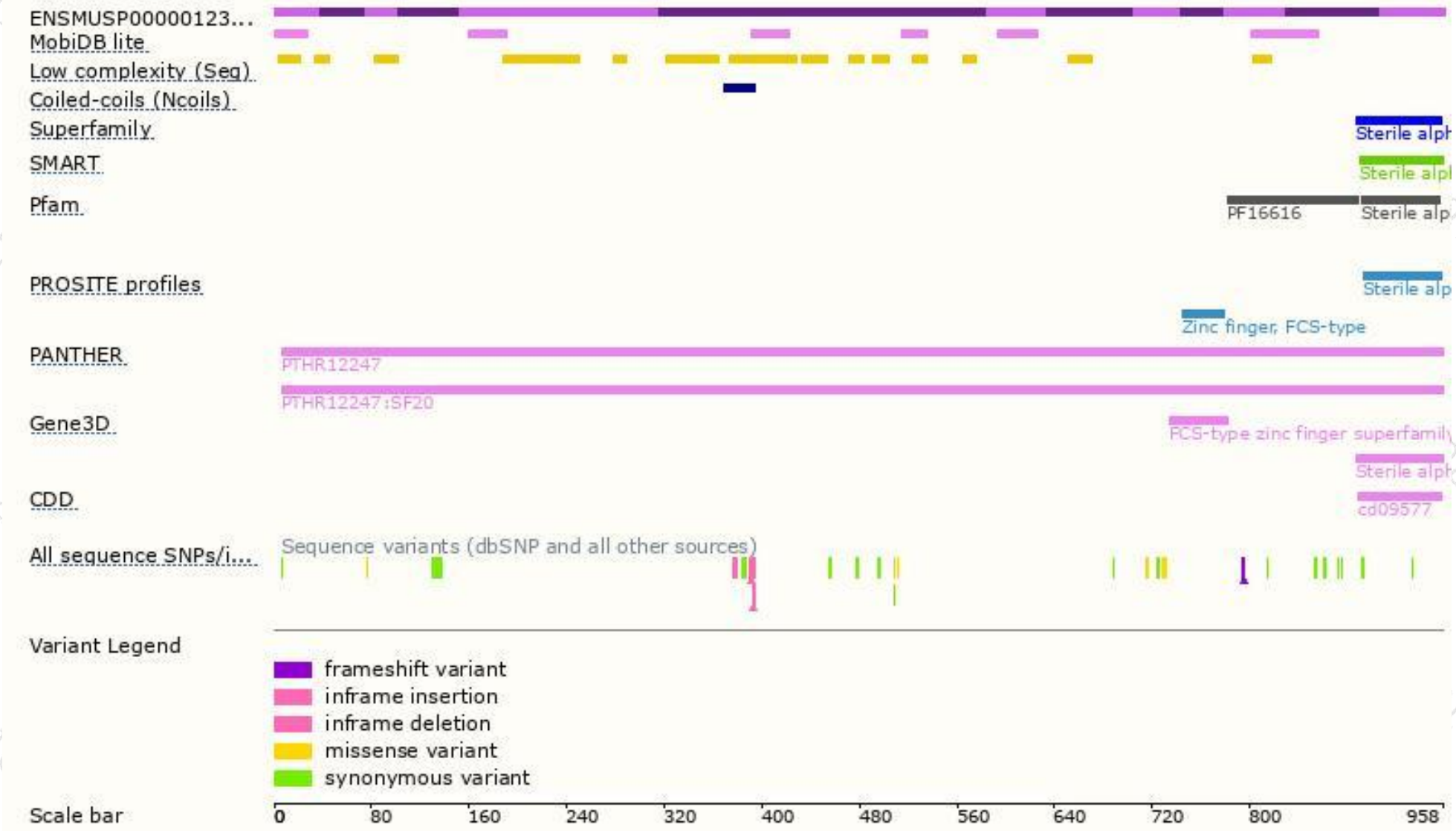
The strategy is based on the design of *Phc1-202* transcript,the transcription is shown below:



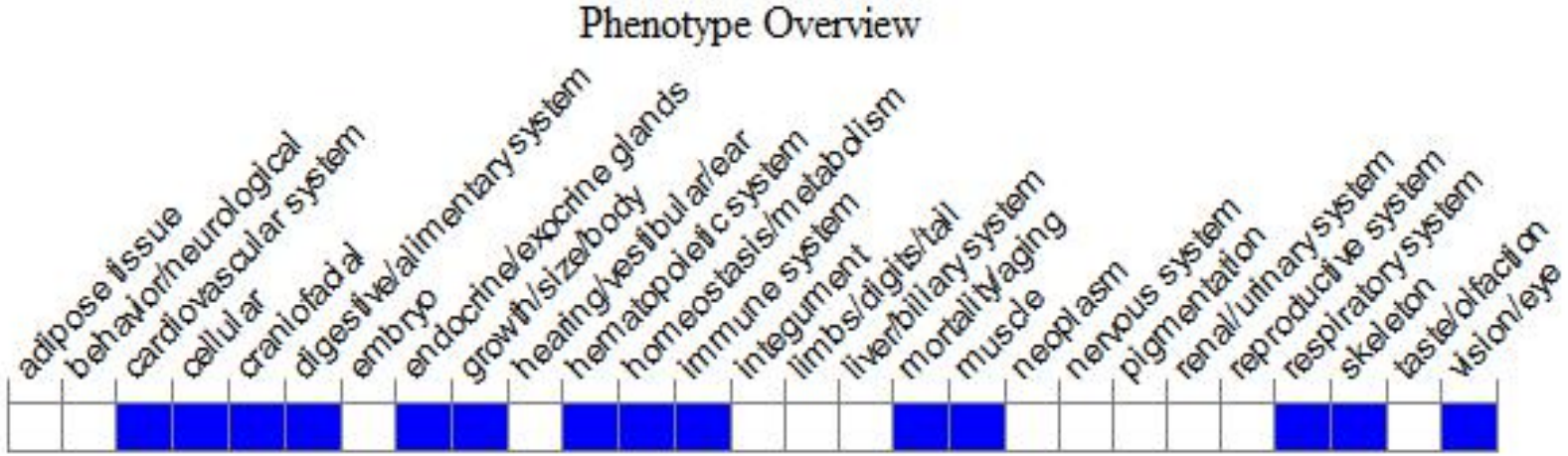
Genomic location distribution



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/).

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If you have any questions, you are welcome to inquire.

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