

Hc Cas9-CKO Strategy

Designer:

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Design Date:

2019-7-18

Project Overview

Project Name

Hc

Project type

Cas9-CKO

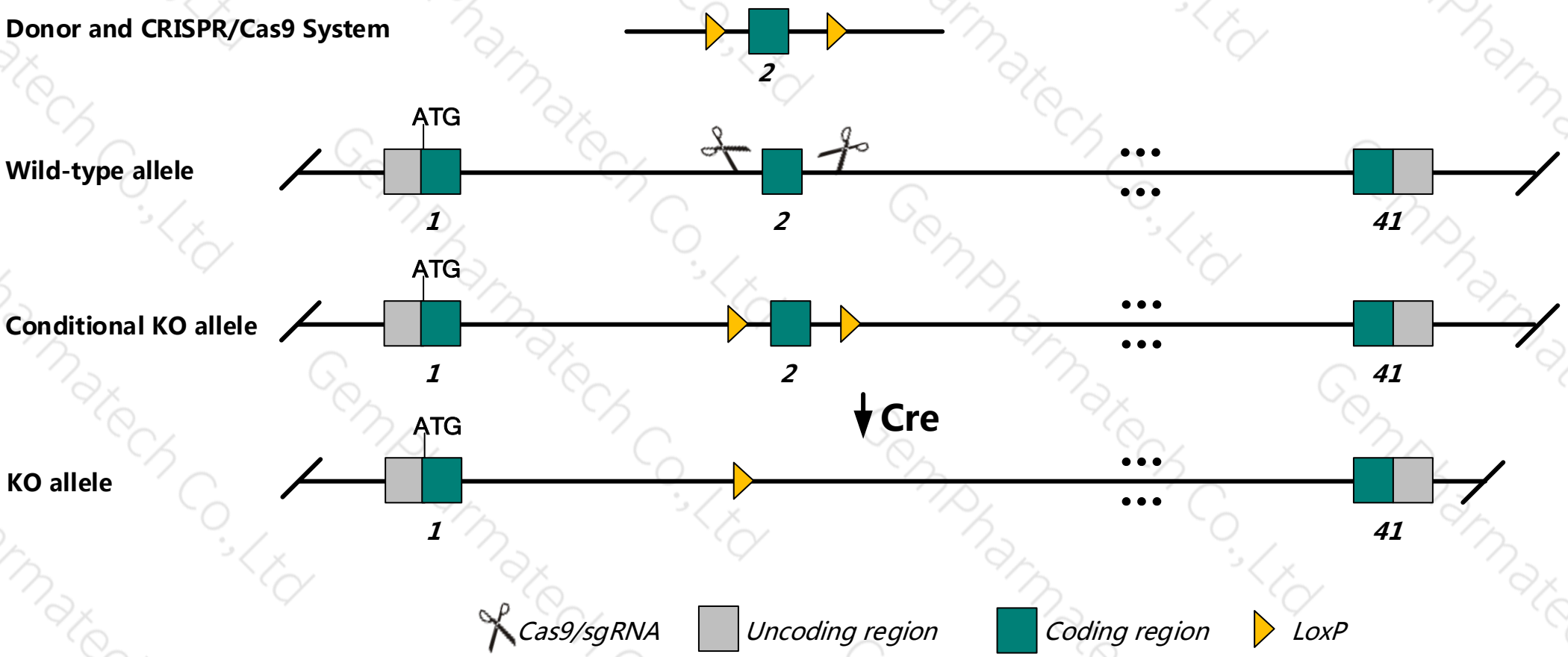
Strain background

C57BL/6JGpt

Conditional Knockout strategy

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

Donor and CRISPR/Cas9 System



- The *Hc* gene has 5 transcripts. According to the structure of *Hc* gene, exon2 of *Hc*-201 (ENSMUST00000028233.6) transcript is recommended as the knockout region. The region contains 193bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Hc* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed. Cas9, sgRNA 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 was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues or cell types.

- According to the existing MGI data , Macrophage from mice homozygous for disruptions of this gene do not secrete complement C5. The 2 bp deletion found in A/J and AKR/J strains is associated with susceptibility to allergen-induced bronchial hyperresponsiveness and is a candidate for QTL Abhr2.
- Transcript *Hc-205* may not be affected.
- The *Hc* 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)

Hc hemolytic complement [*Mus musculus* (house mouse)]

Gene ID: 15139, updated on 23-Oct-2018

Summary

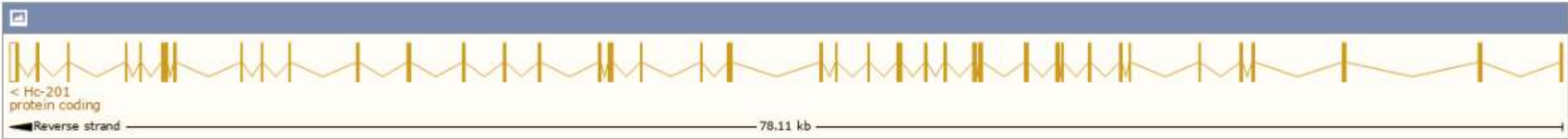
Official Symbol	Hc provided by MGI
Official Full Name	hemolytic complement provided by MGI
Primary source	MGI:MGI:96031
See related	Ensembl:ENSMUSG00000026874 Vega:OTTMUSG00000012274
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	C5; He; C5a; Hfib2
Summary	This gene encodes a component of the complement system, a part of the innate immune system that plays an important role in inflammation, host homeostasis, and host defense against pathogens. The encoded preproprotein is proteolytically processed to generate multiple protein products, including the C5 alpha chain, C5 beta chain, C5a anaphylatoxin and C5b. The C5 protein is comprised of the alpha and beta chains, which are linked by a disulfide bridge. Cleavage of the alpha chain by a convertase enzyme results in the formation of the C5a anaphylatoxin, which possesses potent spasmogenic and chemotactic activity, and the C5b macromolecular cleavage product, a subunit of the membrane attack complex (MAC). Mice with a homozygous mutation in this gene exhibit impaired bone fracture healing and an enhanced inflammatory response in an allergic lung disease model. [provided by RefSeq, Nov 2015]
Expression	Biased expression in liver E18 (RPKM 32.3), liver adult (RPKM 24.7) and 4 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

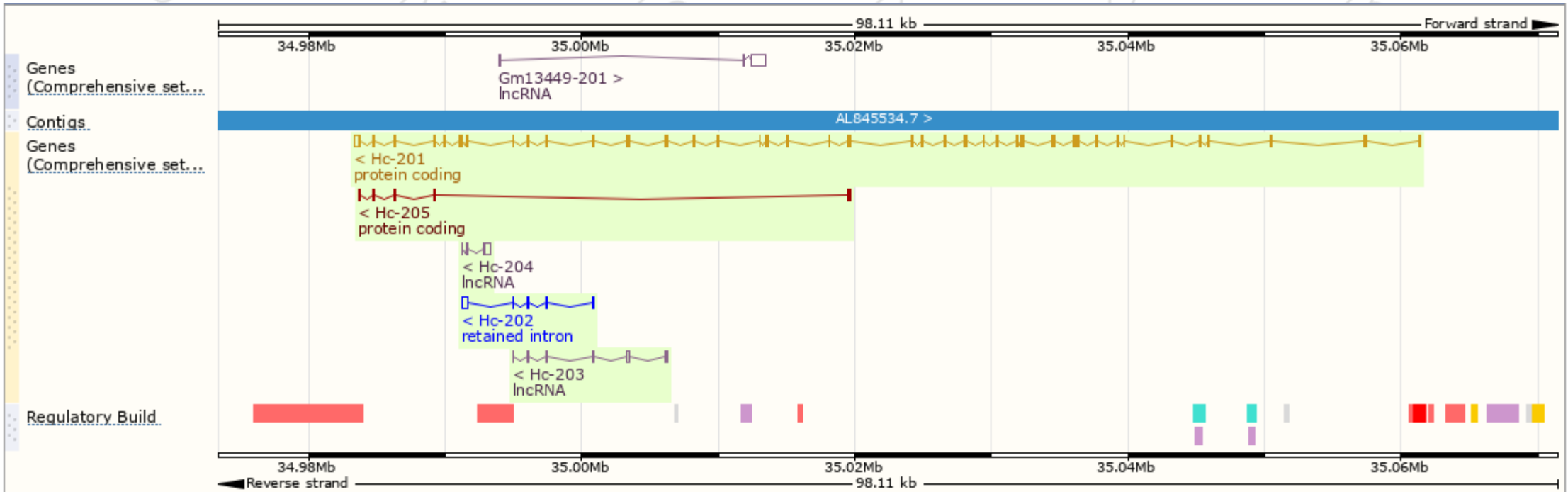
The gene has 5 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Hc-201	ENSMUST00000028233.6	5445	1680aa	<div><div></div>Protein coding</div>	-	P06684	TSL:1 GENCODE basic APPRIS P1
Hc-205	ENSMUST00000156412.2	579	193aa	<div><div></div>Protein coding</div>	-	A0A0J9YUU3	CDS 5' and 3' incomplete TSL:5
Hc-202	ENSMUST00000125549.7	694	No protein	<div><div></div>Retained intron</div>	-	-	TSL:2
Hc-204	ENSMUST00000156049.1	659	No protein	<div><div></div>lncRNA</div>	-	-	TSL:5
Hc-203	ENSMUST00000151628.1	655	No protein	<div><div></div>lncRNA</div>	-	-	TSL:3

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



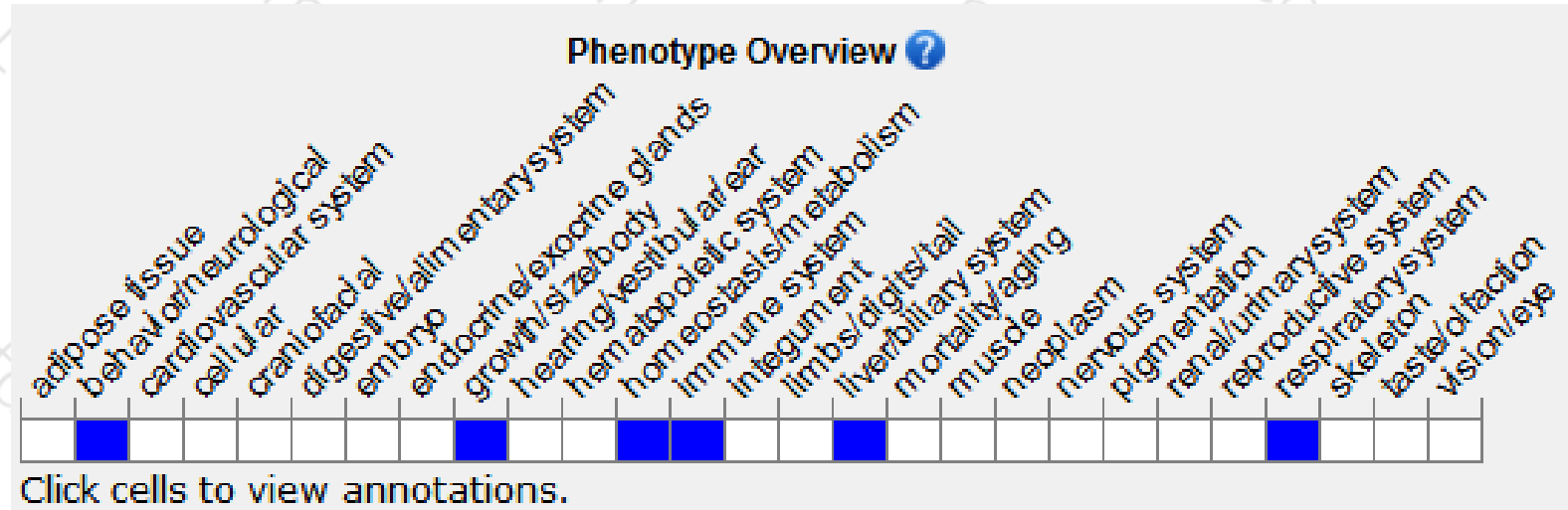
Genomic location distribution



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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