Hhat Cas9-KO Strategy

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Project Overview

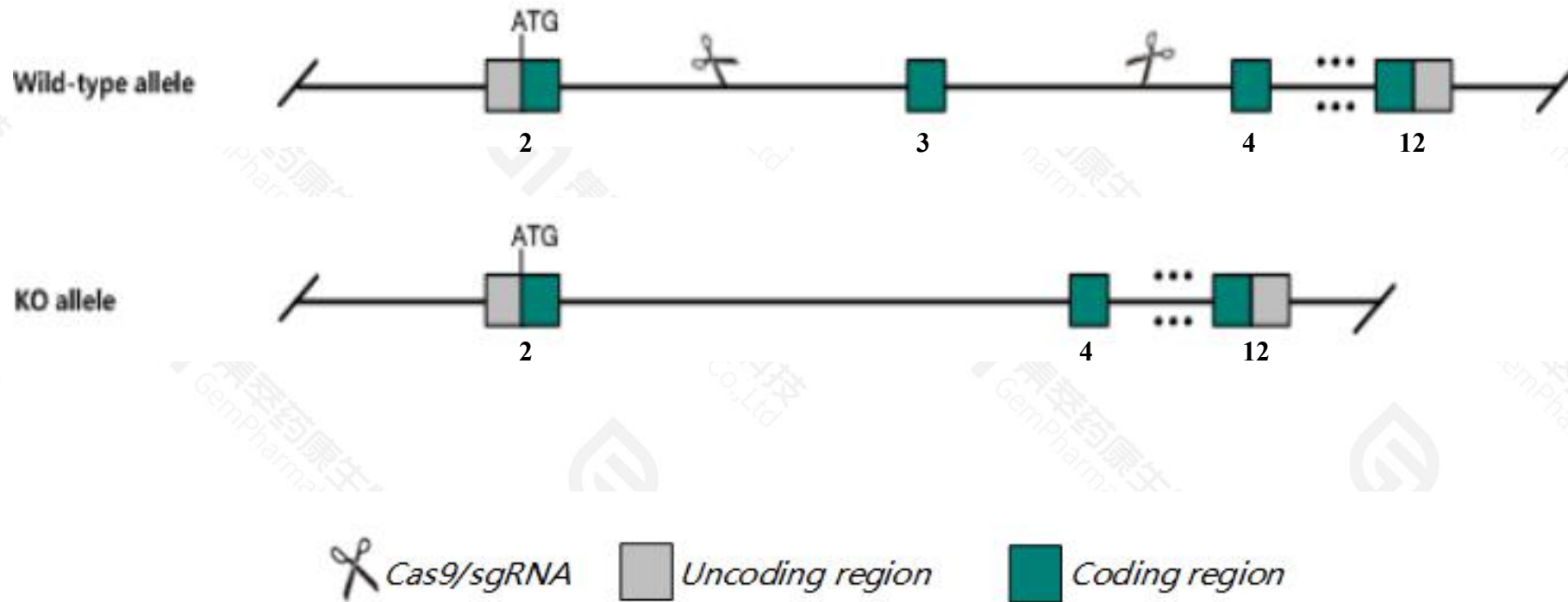|	
Project Name	*Hhat*
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

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

Technical routes

➤ The *Hhat* gene has 7 transcripts. According to the structure of *Hhat* gene, exon3 of *Hhat-201*(ENSMUST00000044190.12) transcript is recommended as the knockout region.The region contains 68bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Hhat* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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 null mice display neonatal lethality, holoprosencephaly, short-limb dwarfism, and oligodactyly.

➤ The *Hhat* gene is located on the Chr1. 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）

Hhat hedgehog acyltransferase [Mus musculus (house mouse)]

Gene ID: 226861, updated on 17-Dec-2020

▲ Summary

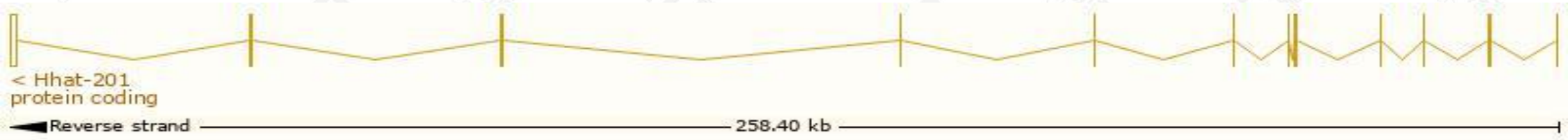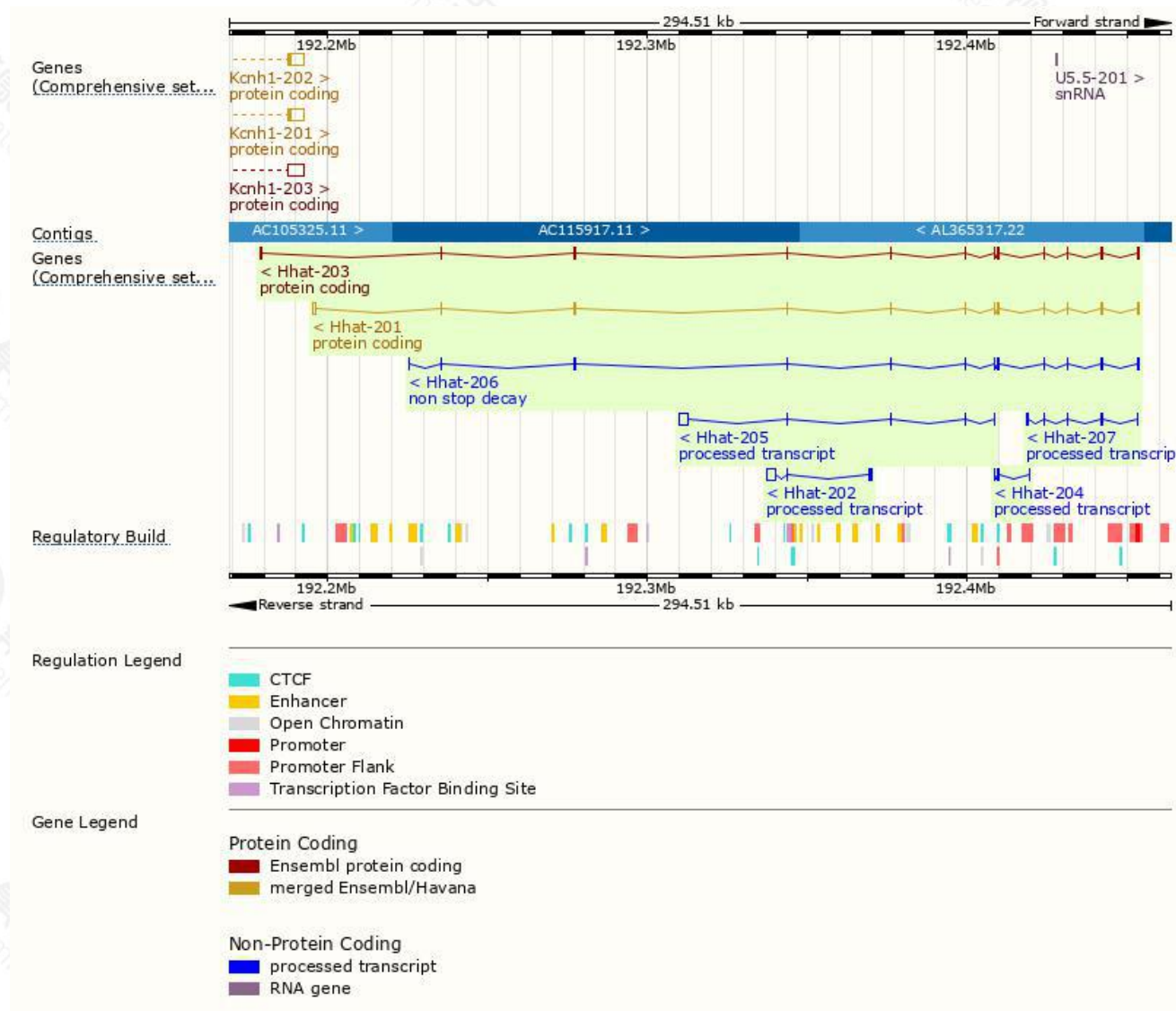|	
Official Symbol	Hhat provided by MGI
Official Full Name	hedgehog acyltransferase provided by MGI
Primary source	MGI:MGI:2444681
See related	Ensembl:ENSMUSG00000037375
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2810432O22Rik, AI462858, AP-2, AP-2CRE, S, Skn, Tg(TFAP2A-cre)1Will
Expression	Ubiquitous expression in ovary adult (RPKM 4.9), adrenal adult (RPKM 4.5) and 26 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Hhat-201	ENSMUST00000044190.12	2839	499aa	Protein coding	CCDS15628		TSL:1 , GENCODE basic , APPRIS P2 ,
Hhat-203	ENSMUST00000128619.8	1819	473aa	Protein coding	-		TSL:1 , GENCODE basic , APPRIS ALT2 ,
Hhat-206	ENSMUST00000192585.2	1800	507aa	Non stop decay	-		TSL:1 ,
Hhat-205	ENSMUST00000154755.7	3247	No protein	Processed transcript	-		TSL:1 ,
Hhat-202	ENSMUST00000123721.2	2892	No protein	Processed transcript	-		TSL:1 ,
Hhat-204	ENSMUST00000154203.3	673	No protein	Processed transcript	-		TSL:2 ,
Hhat-207	ENSMUST00000192946.2	408	No protein	Processed transcript	-		TSL:5 ,

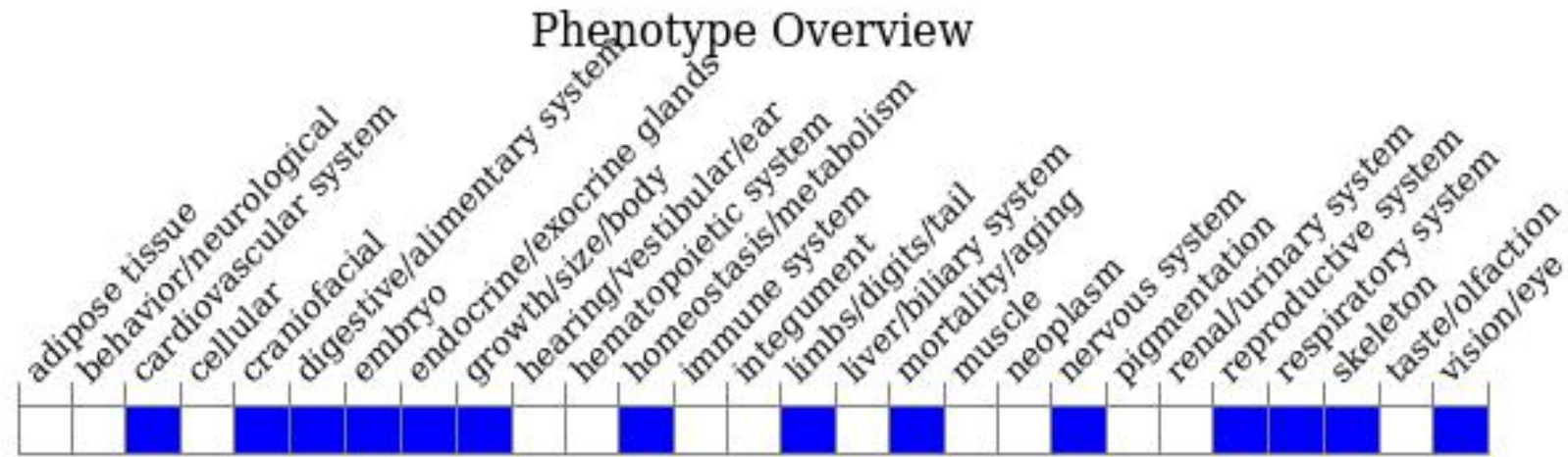The strategy is based on the design of *Hhat-201* transcript,the transcription is shown below:

< Hhat-201
protein coding

◀ Reverse strand ─────────────── 258.40 kb ───────────────

Genomic location distribution

Protein domain
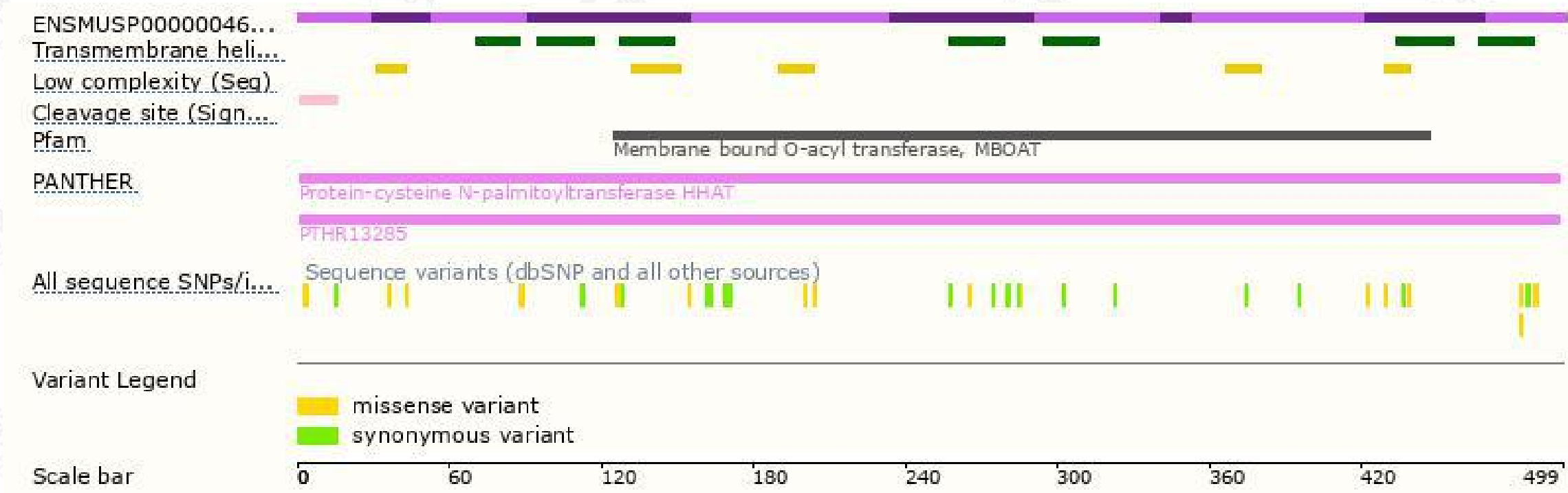
Mouse phenotype description(MGI)

Phenotype Overview

Phenotype categories: adipose tissue, behavior/neurological, cardiovascular system, cellular, craniofacial, digestive/alimentary system, embryo, endocrine/exocrine glands, growth/size/body, hearing/vestibular/ear, hematopoietic system, homeostasis/metabolism, immune system, integument, limbs/digits/tail, liver/biliary system, mortality/aging, muscle, neoplasm, nervous system, pigmentation, renal/urinary system, reproductive system, respiratory system, skeleton, taste/olfaction, vision/eye

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 neonatal lethality, holoprosencephaly, short-limb dwarfism, and oligodactyly.

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