

***Kit* Cas9-CKO Strategy**

Designer:

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Design Date:

2019-7-19

Project Overview

Project Name

Kit

Project type

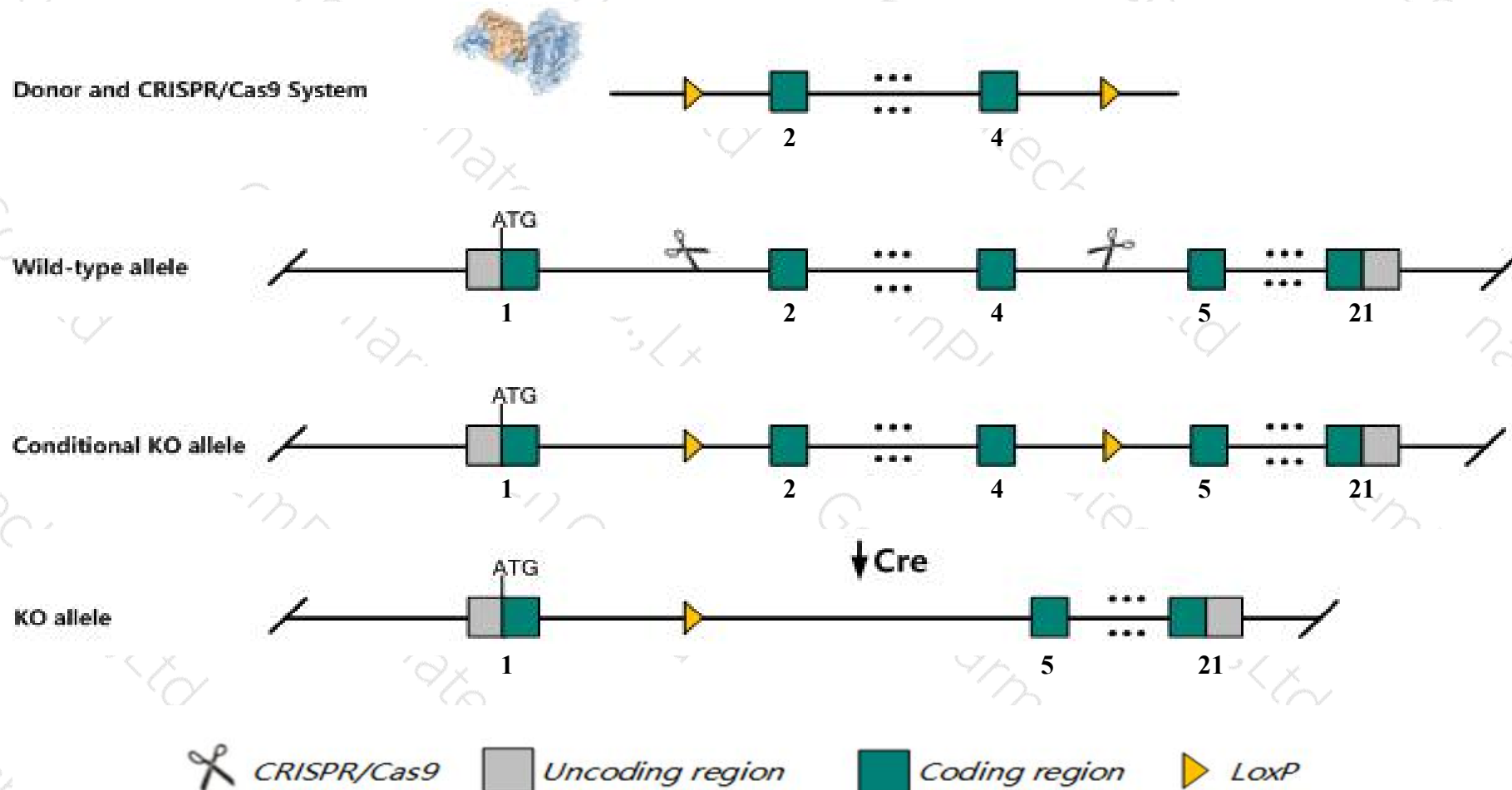
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



Technical routes

- The *Kit* gene has 8 transcripts. According to the structure of *Kit* gene, exon2-exon4 of *Kit-201* (ENSMUST00000005815.6) transcript is recommended as the knockout region. The region contains 692bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Kit* 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.

- According to the existing MGI data, Mutations at this locus affect migration of embryonic stem cell populations, resulting in mild to severe impairments in hematopoiesis, and pigmentation. Some alleles are homozygous lethal, sterile, or result in the formation of gastrointestinal tumors.
- The *Kit* gene is located on the Chr5. 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)

Kit KIT proto-oncogene receptor tyrosine kinase [Mus musculus (house mouse)]

Gene ID: 16590, updated on 9-Apr-2019

Summary



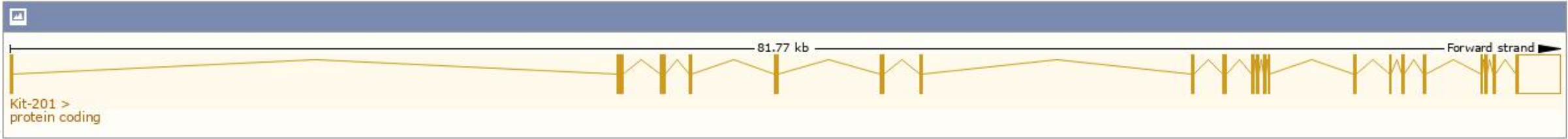
Official Symbol	Kit provided by MGI
Official Full Name	KIT proto-oncogene receptor tyrosine kinase provided by MGI
Primary source	MGI:MGI:96677
See related	Ensembl:ENSMUSG000000005672
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Bs, CD117, Fdc, Gsfsc01, Gsfsc05, Gfsow3, SCO1, SCO5, SOW3, Ssm, Tr-kit, W, c-KIT
Summary	The c-Kit proto-oncogene is the cellular homolog of the transforming gene of a feline retrovirus (v-Kit). The c-kit protein includes characteristics of a protein kinase transmembrane receptor. Alternatively spliced transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Jul 2008]
Expression	Broad expression in lung adult (RPKM 39.0), cerebellum adult (RPKM 19.6) and 25 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

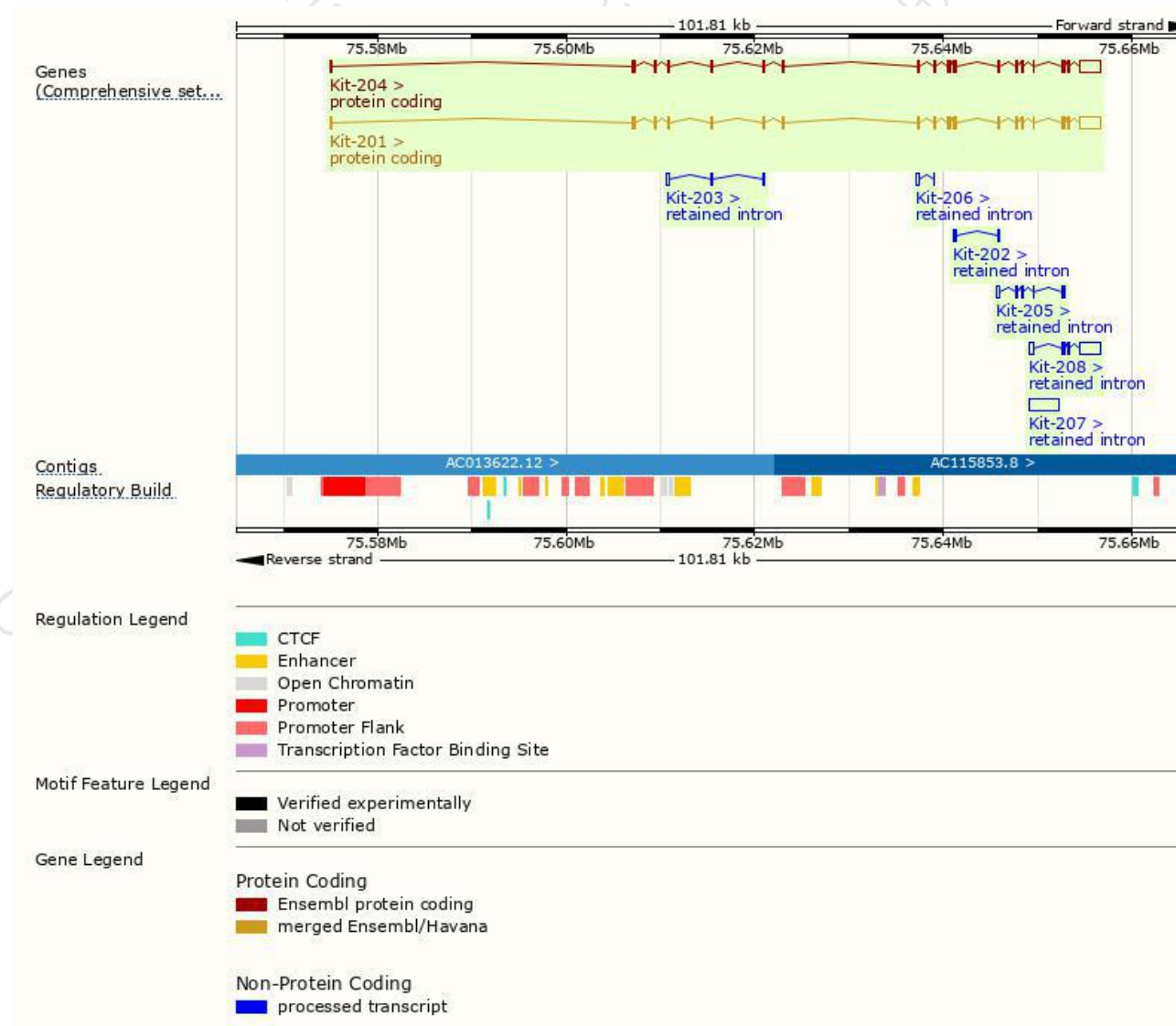
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Kit-204	ENSMUST00000144270.7	5241	975aa	Protein coding	CCDS80302	P05532	TSL:1 GENCODE basic APPRIS ALT2
Kit-201	ENSMUST00000005815.6	5214	979aa	Protein coding	CCDS51525	P05532	TSL:1 GENCODE basic APPRIS P3
Kit-208	ENSMUST00000202167.1	3175	No protein	Retained intron	-	-	TSL:1
Kit-207	ENSMUST00000201240.1	3104	No protein	Retained intron	-	-	TSL:NA
Kit-205	ENSMUST00000148993.4	842	No protein	Retained intron	-	-	TSL:5
Kit-203	ENSMUST00000143221.1	648	No protein	Retained intron	-	-	TSL:2
Kit-202	ENSMUST00000136002.1	392	No protein	Retained intron	-	-	TSL:5
Kit-206	ENSMUST00000151357.1	354	No protein	Retained intron	-	-	TSL:3

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



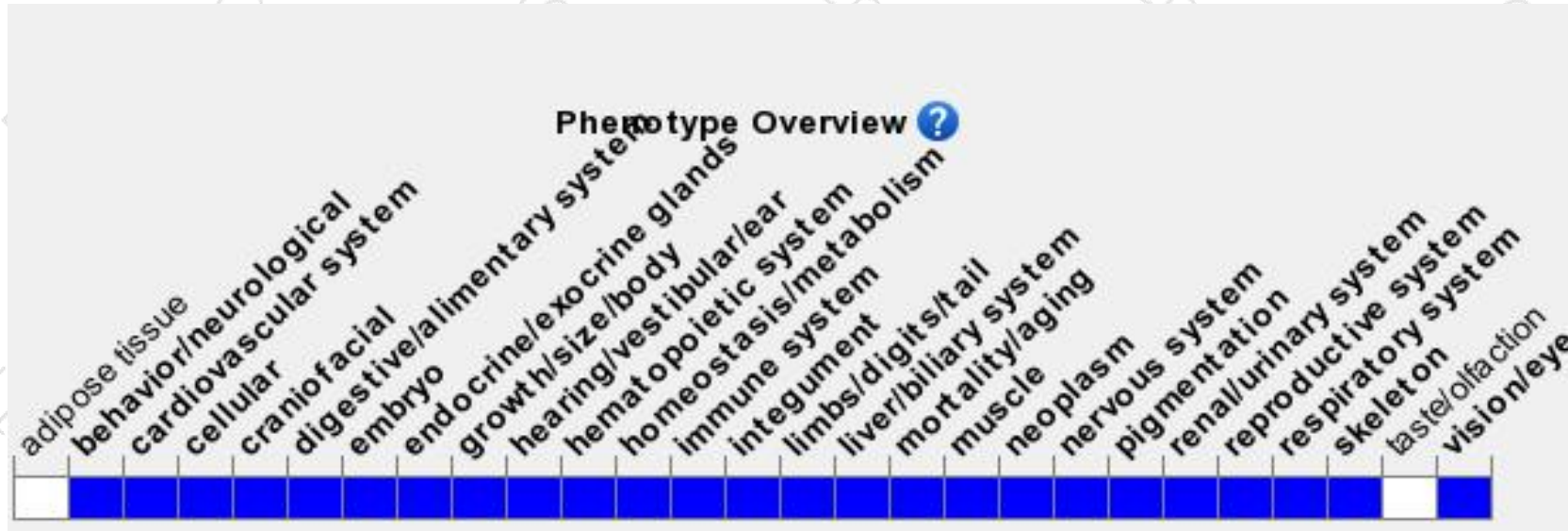
Genomic location distribution



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/>).

According to the existing MGI data, Mutations at this locus affect migration of embryonic stem cell populations, resulting in mild to severe impairments in hematopoiesis, and pigmentation. Some alleles are homozygous lethal, sterile, or result in the formation of gastrointestinal tumors.

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

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