

Kit Cas9-KO Strategy

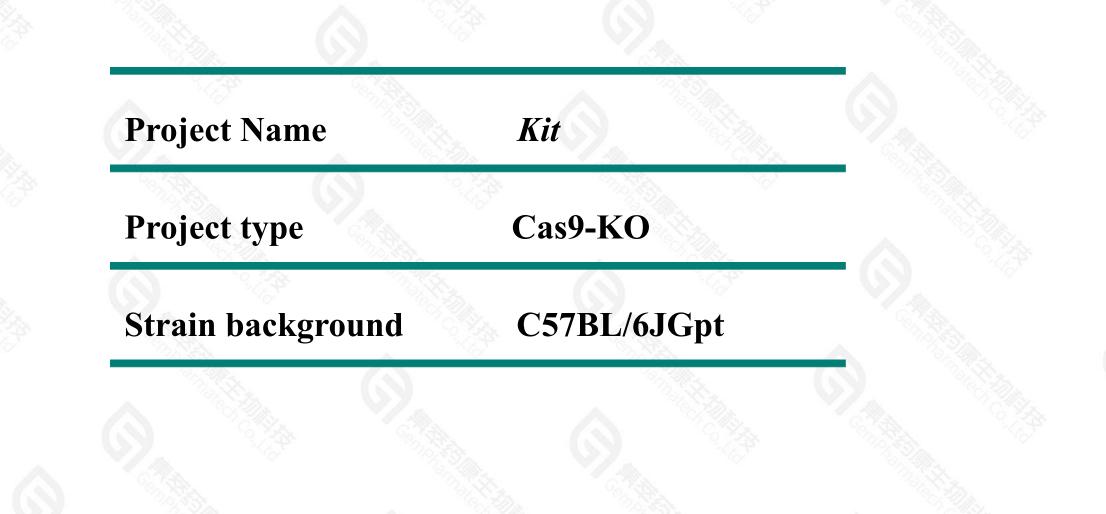
Designer: Ruirui Zhang

Reviewer: Zihe Cui

Design Date: 2021-4-19

Project Overview





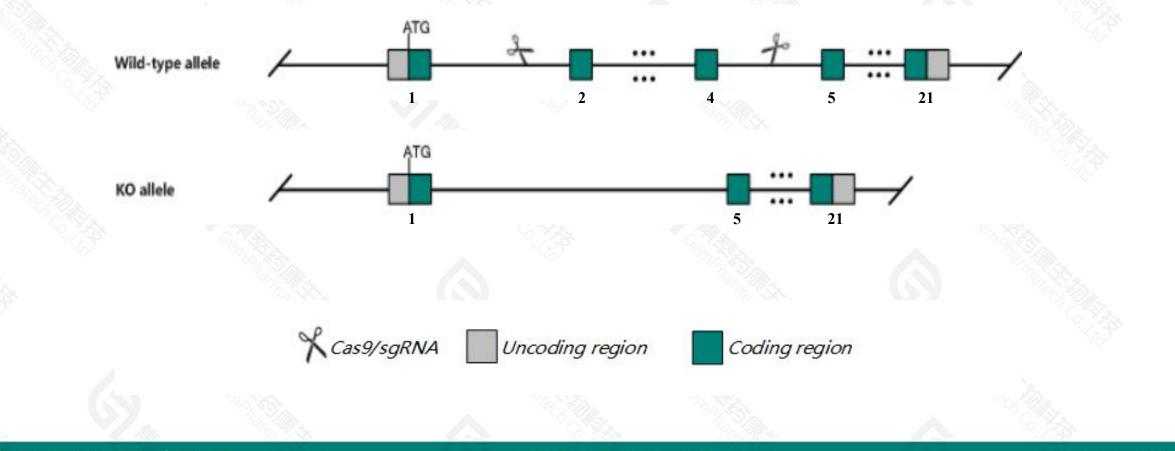
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Knockout strategy



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



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> The *Kit* gene has 8 transcripts. According to the structure of *Kit* gene, exon2-exon4 of *Kit-201*(ENSMUST0000005815.7) 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: 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.



> 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)



☆ ?

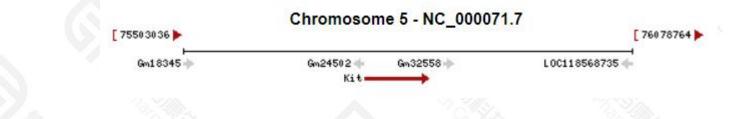
L Download Datasets

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

Gene ID: 16590, updated on 6-Apr-2021

Summary

Official Symbol Kit provided by MGI Official Full Name KIT proto-oncogene receptor tyrosine kinase provided by MGI Primary source MGI:MGI:96677 Ensembl:ENSMUSG0000005672 See related 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 W; Bs; SC; SO; Fdc; Ssm; Gsfs; SCO1; SCO5; SOW3; Tr-k; c-KI; CD117; belly; c-KIT; Tr-kit; Gsfsco1; Gsfsco5; Gsfsow3 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] Broad expression in lung adult (RPKM 39.0), cerebellum adult (RPKM 19.6) and 25 other tissues See more Expression Orthologs human all



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Transcript information (Ensembl)



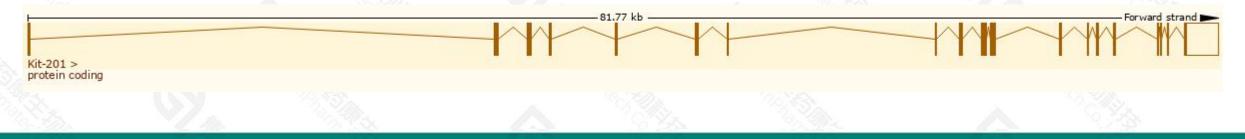
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The gene has 8 transcripts, all transcripts are shown below:

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Name 🍦	Transcript ID 👙	bp 🖕	Protein 🖕	Biotype 🍦	CCDS 🖕	UniProt Match A	Flags
Kit-201	ENSMUST0000005815.7	5214	<u>979aa</u>	Protein coding	CCDS51525	P05532-1 @	TSL:1 GENCODE basic APPRIS P3
Kit-204	ENSMUST00000144270.8	<mark>5241</mark>	<u>975aa</u>	Protein coding	CCDS80302	P05532-2 @	TSL:1 GENCODE basic APPRIS ALT2
Kit-208	ENSMUST0000202167.2	3175	No protein	Retained intron	-	-	TSL:1
Kit-207	ENSMUST00000201240.2	3104	No protein	Retained intron	<u>1</u>	4 <u>0</u>	TSL:NA
Kit-205	ENSMUST00000148993.5	842	No protein	Retained intron	-	-	TSL:5
Kit-203	ENSMUST00000143221.2	648	No protein	Retained intron	2 <u>2</u>	4 <u>0</u>	TSL:2
Kit-202	ENSMUST00000136002.2	392	No protein	Retained intron	-	-	TSL:5
Kit-206	ENSMUST00000151357.2	354	No protein	Retained intron	2013 2 <mark>-</mark>	<u>12</u>	TSL:3

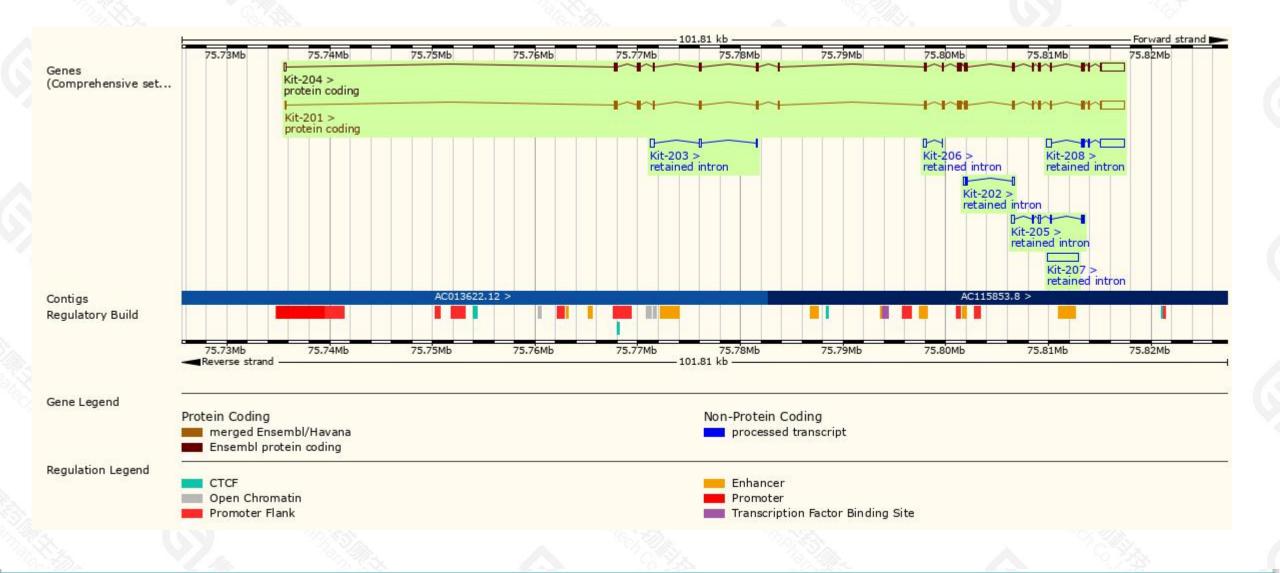
The strategy is based on the design of *Kit-201* transcript, the transcription is shown below:



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Genomic location distribution





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Protein domain



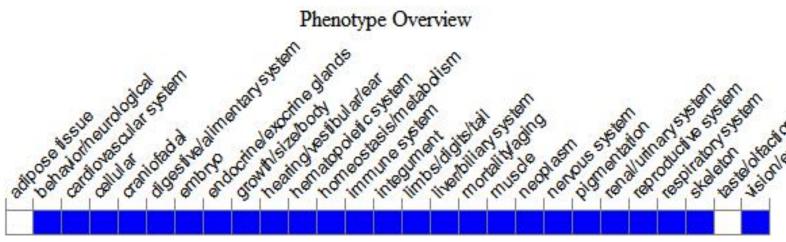


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Mouse phenotype description(MGI)





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.

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If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



