Slit2 Cas9-KO Strategy

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Project Overview

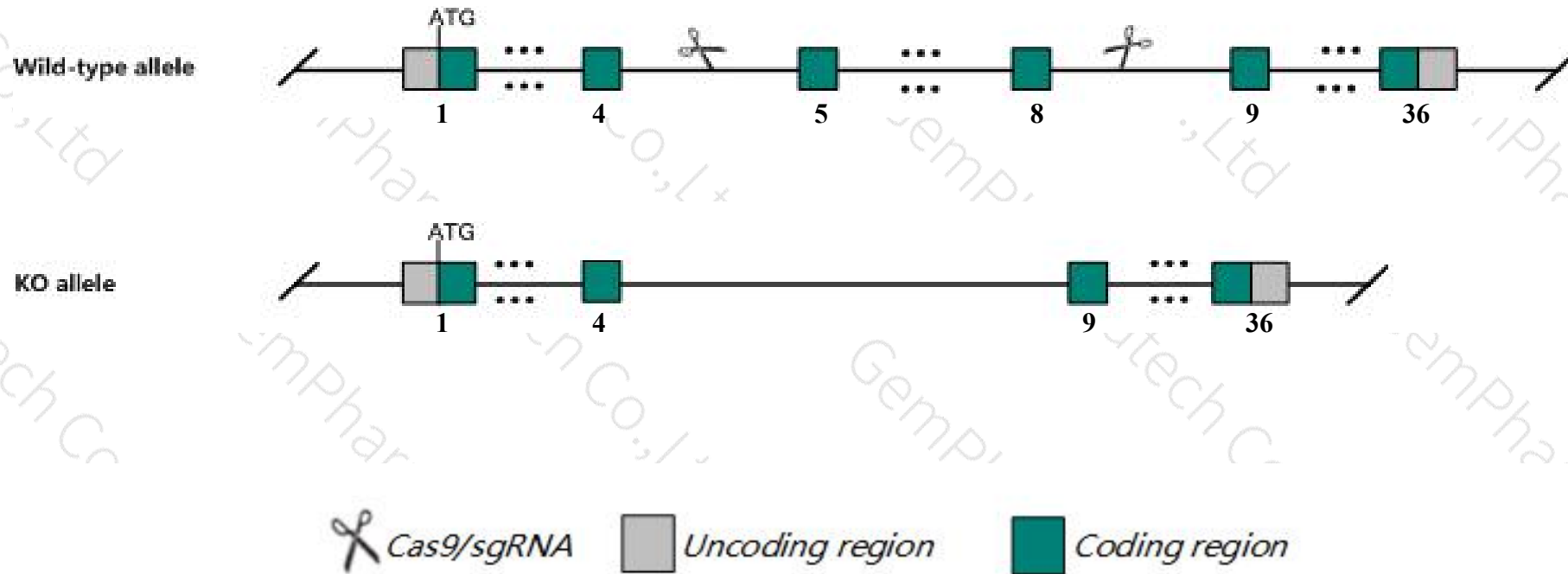|	
Project Name	*Slit2*
Project type	**Cas9-KO**
Strain background	**C57BL/6J**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Slit2* gene. The schematic diagram is as follows:

Technical routes

➢ The *Slit2* gene has 23 transcripts. According to the structure of *Slit2* gene, exon5-exon8 of *Slit2-208* (ENSMUST00000173107.7) transcript is recommended as the knockout region.The region contains 380bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Slit2* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.

Notice

➢ According to the existing MGI data,Homozygous mutants display perinatal lethality, abnormal ureteric bud development, multiple fused kidneys, multiple ureters, and hydroureter.

➢ The *Slit2* 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）

Slit2 slit guidance ligand 2 [Mus musculus (house mouse)]

Gene ID: 20563, updated on 16-Feb-2019

▲ Summary

|	
Official Symbol	Slit2 provided by MGI
Official Full Name	slit guidance ligand 2 provided by MGI
Primary source	MGI:MGI:1315205
See related	Ensembl:ENSMUSG00000031558
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	Drad-1, E030015M03Rik, E130320P19Rik, Slil3, b2b1200.1Clo, mKIAA4141, slit-2
Summary	The protein encoded by this gene is a member of the Slit family of secreted glycoproteins, which function as ligands for the Robo family of immunoglobulin receptors. Slit proteins play highly conserved roles in axon guidance and neuronal migration and may also have functions during other cell migration processes including leukocyte migration. In mammals, members of the slit family are characterized by an N-terminal signal peptide, four leucine-rich repeats, nine epidermal growth factor repeats, and a C-terminal cysteine knot. Mice deficient for this gene exhibit abnormal axonal projections in the embryonic forebrain and develop supernumerary uretic buds that maintain improper connections to the nephric duct. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Sep 2015]
Expression	Broad expression in CNS E11.5 (RPKM 6.3), limb E14.5 (RPKM 5.2) and 18 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 23 transcripts,all transcripts are shown below:

| Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Slit2-208	ENSMUST00000173107.7	9960	1521aa	Protein coding	CCDS19280	Q9R1B9	TSL:1 GENCODE basic APPRIS P3
Slit2-217	ENSMUST00000174421.7	7785	1542aa	Protein coding	CCDS71586	G3UYX7	TSL:1 GENCODE basic
Slit2-216	ENSMUST00000174313.7	7734	1525aa	Protein coding	CCDS71588	G3UY21	TSL:1 GENCODE basic APPRIS ALT1
Slit2-202	ENSMUST00000170109.8	7758	1533aa	Protein coding	-	A0A140T8T2	TSL:5 GENCODE basic APPRIS ALT1
Slit2-204	ENSMUST00000172493.2	767	255aa	Protein coding	-	G3UZW0	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Slit2-201	ENSMUST00000033967.14	6564	851aa	Nonsense mediated decay	-	G3X909	TSL:1
Slit2-212	ENSMUST00000173686.2	878	150aa	Nonsense mediated decay	-	G3UWQ3	CDS 5' incomplete TSL:3
Slit2-219	ENSMUST00000174658.7	633	118aa	Nonsense mediated decay	-	G3UZ61	CDS 5' incomplete TSL:5
Slit2-222	ENSMUST00000197737.1	4798	No protein	Retained intron	-	-	TSL:NA
Slit2-215	ENSMUST00000174061.7	3350	No protein	Retained intron	-	-	TSL:1
Slit2-207	ENSMUST00000172998.7	3265	No protein	Retained intron	-	-	TSL:1
Slit2-203	ENSMUST00000172484.7	3015	No protein	Retained intron	-	-	TSL:1
Slit2-223	ENSMUST00000199024.1	2877	No protein	Retained intron	-	-	TSL:NA
Slit2-220	ENSMUST00000196024.1	2492	No protein	Retained intron	-	-	TSL:NA
Slit2-211	ENSMUST00000173671.7	1945	No protein	Retained intron	-	-	TSL:1
Slit2-209	ENSMUST00000173303.5	854	No protein	Retained intron	-	-	TSL:3
Slit2-210	ENSMUST00000173646.2	518	No protein	Retained intron	-	-	TSL:3
Slit2-205	ENSMUST00000172561.1	415	No protein	Retained intron	-	-	TSL:5
Slit2-221	ENSMUST00000197400.4	3210	No protein	lncRNA	-	-	TSL:1
Slit2-218	ENSMUST00000174487.7	1915	No protein	lncRNA	-	-	TSL:1
Slit2-206	ENSMUST00000172824.7	826	No protein	lncRNA	-	-	TSL:3
Slit2-214	ENSMUST00000173926.7	620	No protein	lncRNA	-	-	TSL:3
Slit2-213	ENSMUST00000173774.7	454	No protein	lncRNA	-	-	TSL:5

The strategy is based on the design of *Slit2-208* transcript,The transcription is shown below
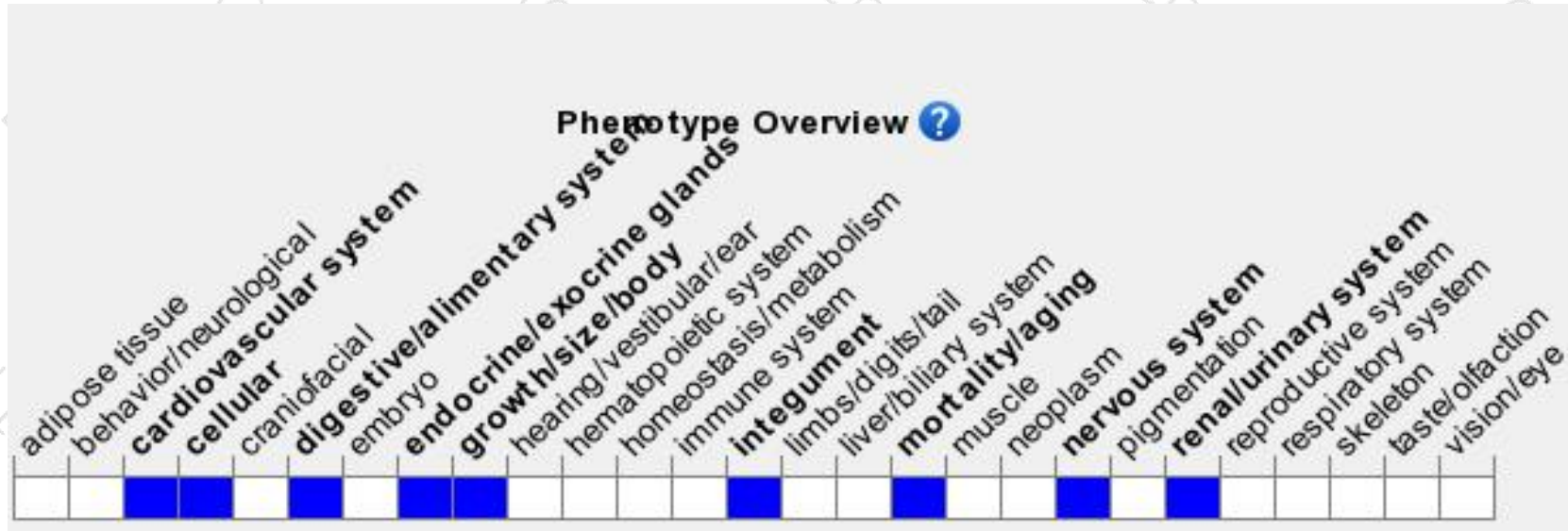
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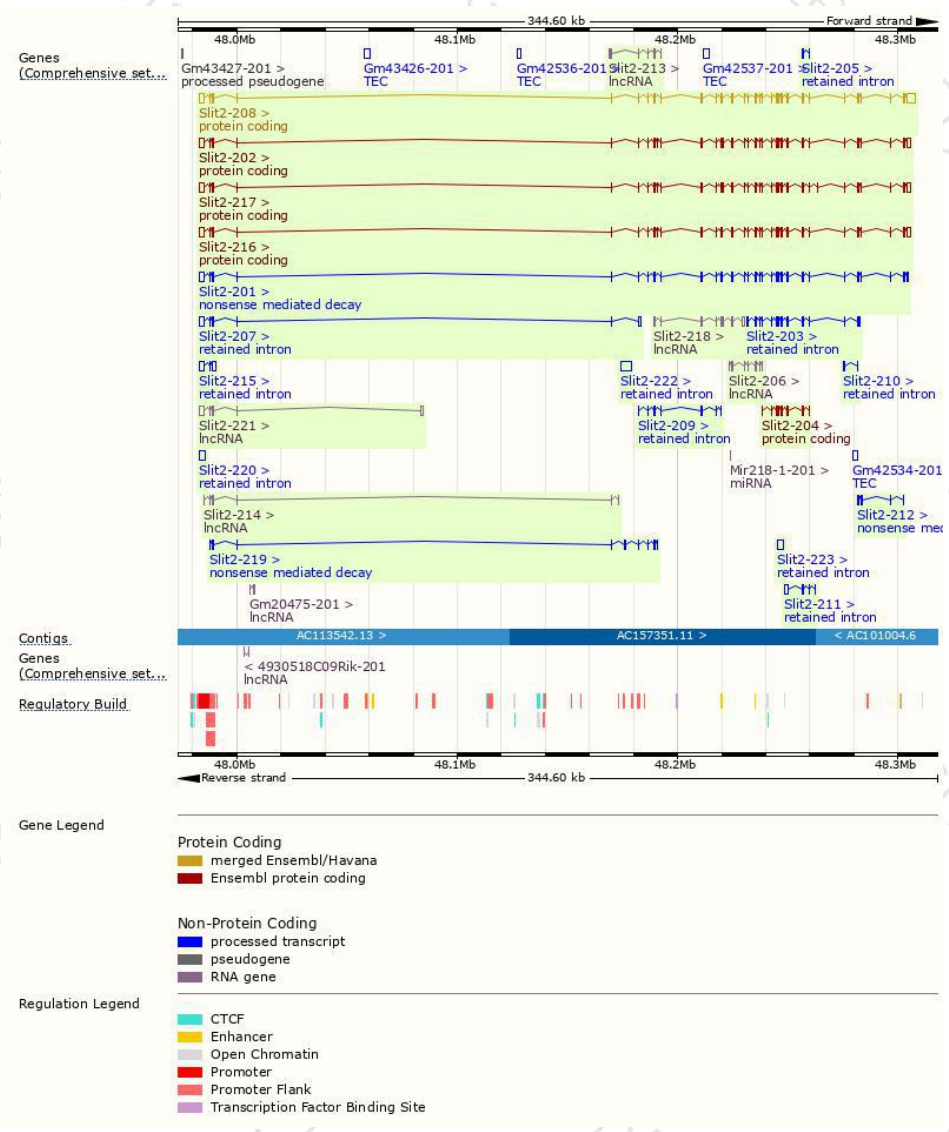
Protein domain
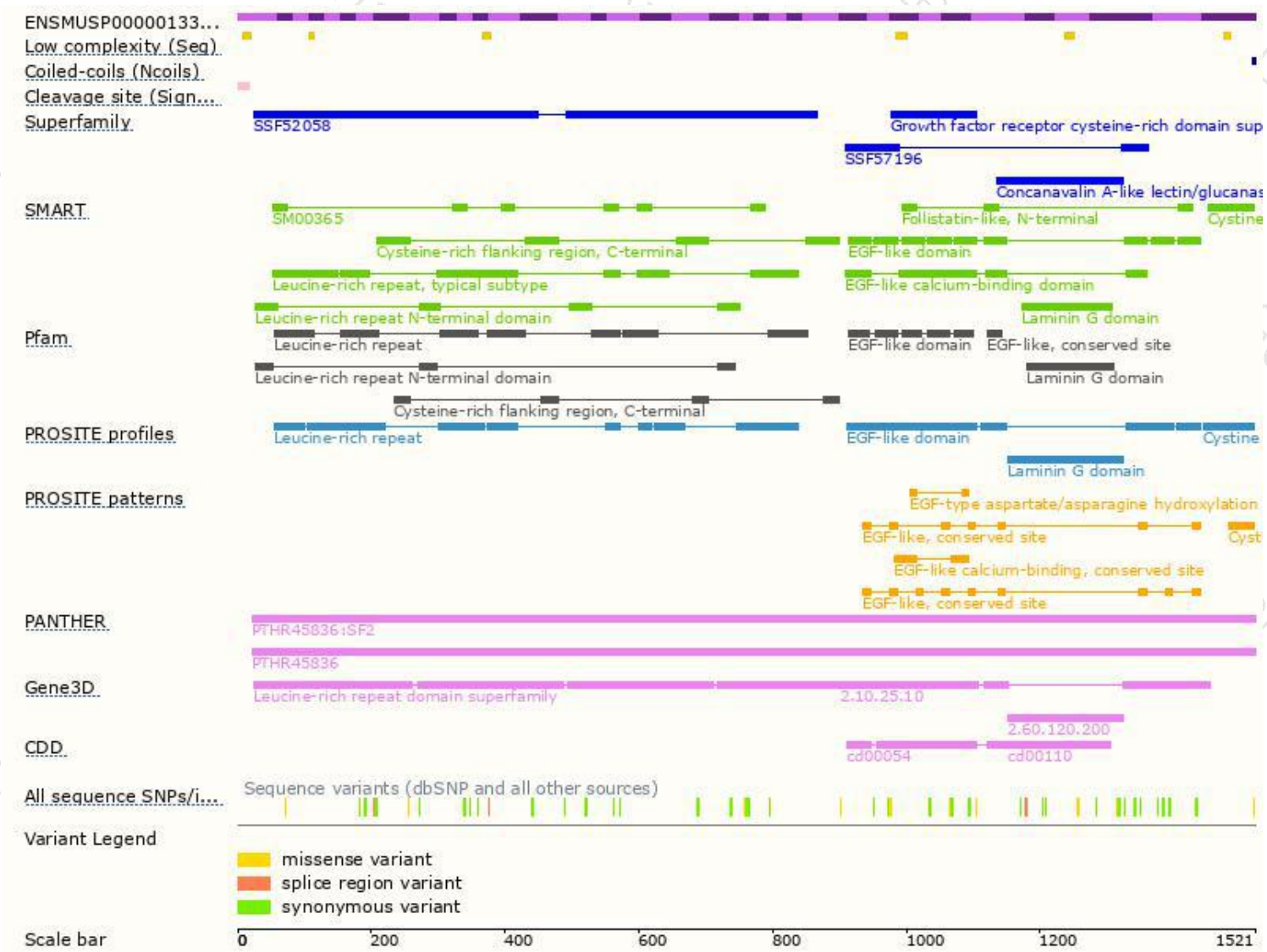
Mouse phenotype description(MGI)

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,Homozygous mutants display perinatal lethality, abnormal ureteric bud development, multiple fused kidneys, multiple ureters, and hydroureter.

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