Slc34a1-CreERT2-EGFP Cas9-KI Mouse Model Strategy

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Project Overview

Project Name	***Slc34a1-CreERT2-EGFP***
Project type	**cas9-ki**
Strain background	**C57BL/6JGpt**

Technical routes

➢ The *Slc34a1* gene has 6 transcripts.

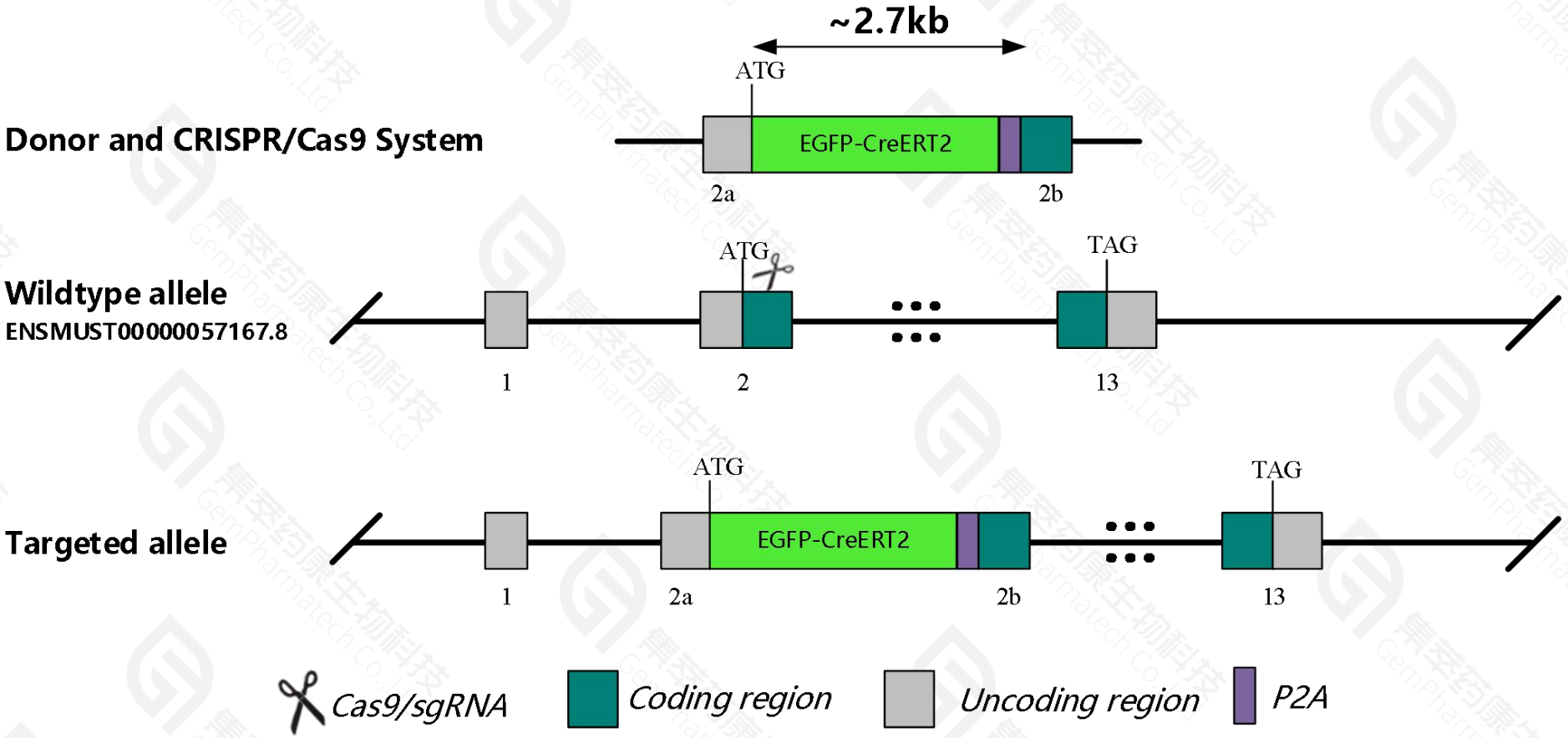
➢ According to the structure of *Slc34a1* gene, the element *EGFP-CreERT2-P2A* will be inserted at the translation start codon of *Slc34a1-201*(ENSMUST00000057167.8), the length of inserted fragment is about 2.7kb.

➢ The mouse *Slc34a1*-201 transcript contains 13 exons. The translation initiation site ATG is located at exon2, and the translation termination site TAG is located at exon13, encoding 637aa.

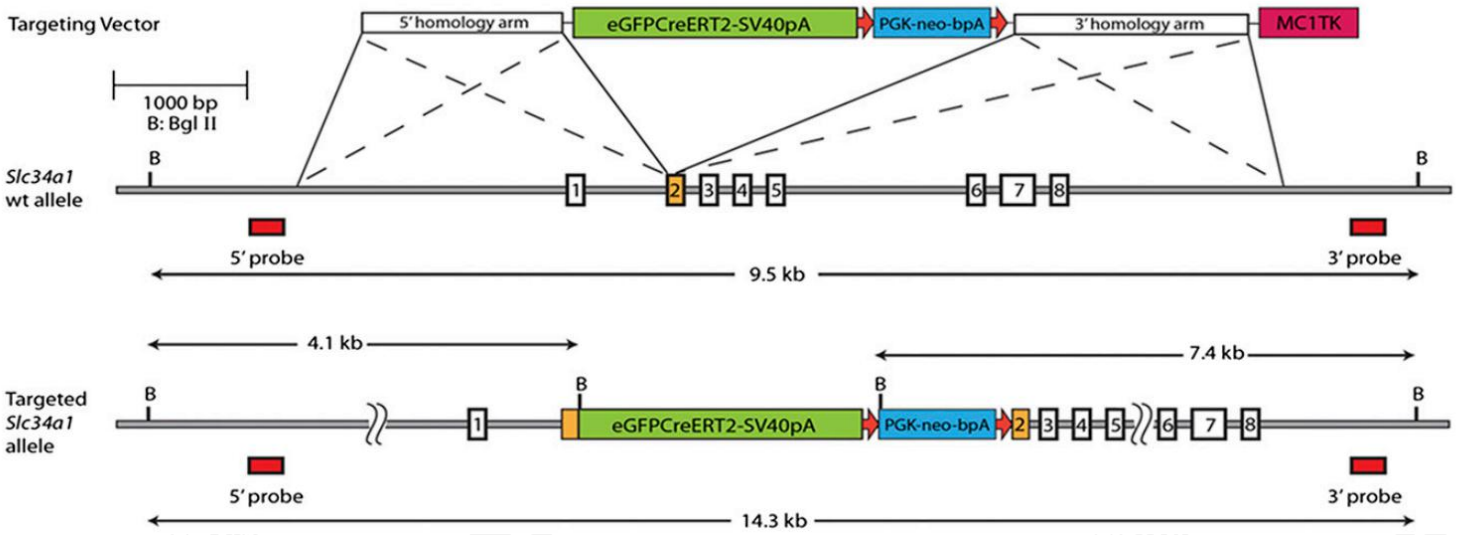
➢ In this project we use CRISPR/Cas9 technology to modify *Slc34a1* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed. Cas9, sgRNA and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice.Fertilized eggs were transplanted to obtain positive 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.

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Slc34a1* gene. The schematic diagram is as follows:



Reference



Creation of GFPCreERt2 Knock-in to the *SLC34a1* Locus. A targeting vector was constructed to insert the eGFPCreER^{T2} (GCE) transgene and a *frt*-flanked PGKneobpA selection cassette into the initiation codon of the *Slc34a1* gene. The GCE transgene comprises an enhanced green fluorescent protein (eGFP) and a tamoxifen inducible Cre-recombinase fusion gene (CreER^{T2}). A negative selectable marker thymidine kinase (MC1TK) cassette was also included in the targeting vector to select against

[1] Kusaba T , Lalli M , Kramann R , et al. Differentiated kidney epithelial cells repair injured proximal tubule[J]. Proceedings of the National Academy of Sciences of the United States of America, 2014, 111(4):1527-1532.

Notice

➢ According to the existing MGI data, homozygous null mice exhibit renal phosphate wasting, hyerpcalciuria, and skeletal abnormalities. Postnatal viability is reduced, putatively due to poor nutritional status.

➢ It is necessary to introduce 1-2 synonymous mutation in exon2.

➢ The insertion site may disrupt promoter of *Slc34a1*, the regulation of *Slc34a1* may be affected.

➢ The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide[2].

➢ The *Slc34a1* gene is located on the Chr13. Please take the loci in consideration when breeding this knockin mice with other gene modified strains, if the other gene is also on Chr13, it may be extremely hard to get double gene positive homozygotes.

➢ The scheme is designed according to the genetic information in the existing database. Inserting a foreign gene between the 5'UTR and the gene coding region may affect the expression of endogenous and foreign genes. Due to the complexity of biological processes, it cannot be predicted completely at the present technology level.

Gene information (NCBI)

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Slc34a1 solute carrier family 34 (sodium phosphate), member 1 [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 20505, updated on 12-Jan-2021

Summary

Official Symbol	Slc34a1 provided by MGI
Official Full Name	solute carrier family 34 (sodium phosphate), member 1 provided by MGI
Primary source	MGI:MGI:1345284
See related	Ensembl:ENSMUSG00000021490
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	N; Np; Npt2; Slc1; Npt2a; Slc17a2; NaPi-IIa
Expression	Restricted expression toward kidney adult (RPKM 2731.8) See more
Orthologs	human all

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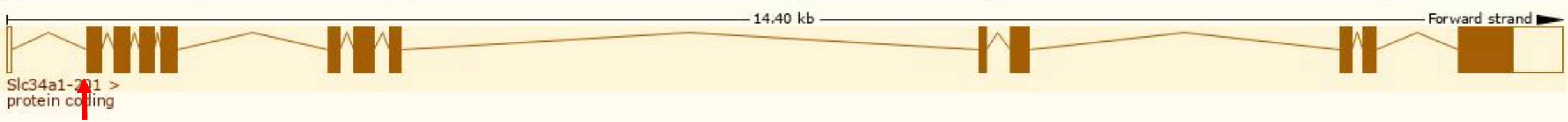
Transcript information （Ensembl）

The gene has 6 transcripts, and all transcripts are shown below:

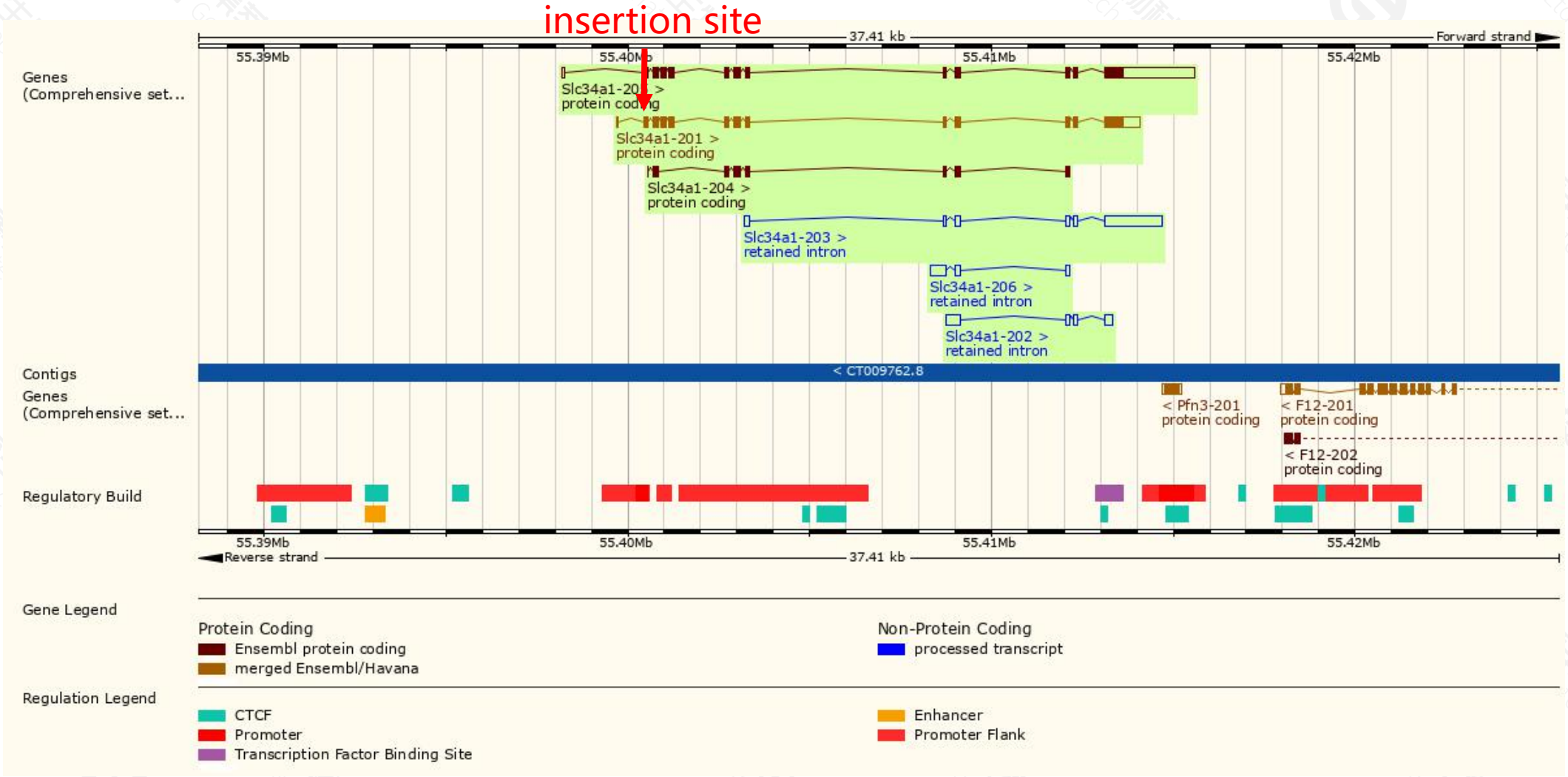
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Slc34a1-201	ENSMUST00000057167.8	2433	637aa	Protein coding	CCDS49271	Q9D2V6	TSL:1 GENCODE basic APPRIS P1
Slc34a1-202	ENSMUST00000223954.1	860	No protein	Retained intron	-	-	-
Slc34a1-203	ENSMUST00000224043.1	2168	No protein	Retained intron	-	-	-
Slc34a1-204	ENSMUST00000224925.1	928	309aa	Protein coding	-	A0A286YCG7	CDS 5' and 3' incomplete
Slc34a1-205	ENSMUST00000225259.1	3983	637aa	Protein coding	CCDS49271	Q9D2V6	GENCODE basic APPRIS P1
Slc34a1-206	ENSMUST00000225538.1	722	No protein	Retained intron	-	-	-

The strategy is based on the design of *Slc34a1*-201 transcript,the transcription is shown below:

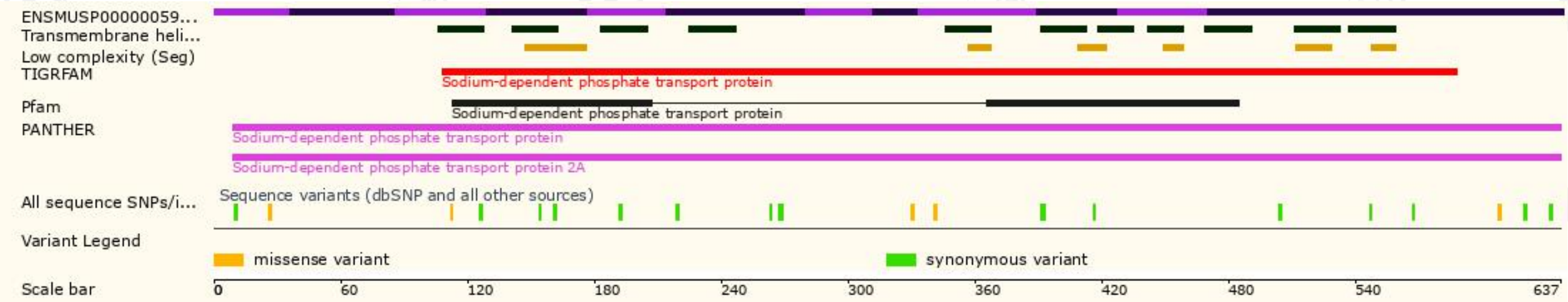


insertion site

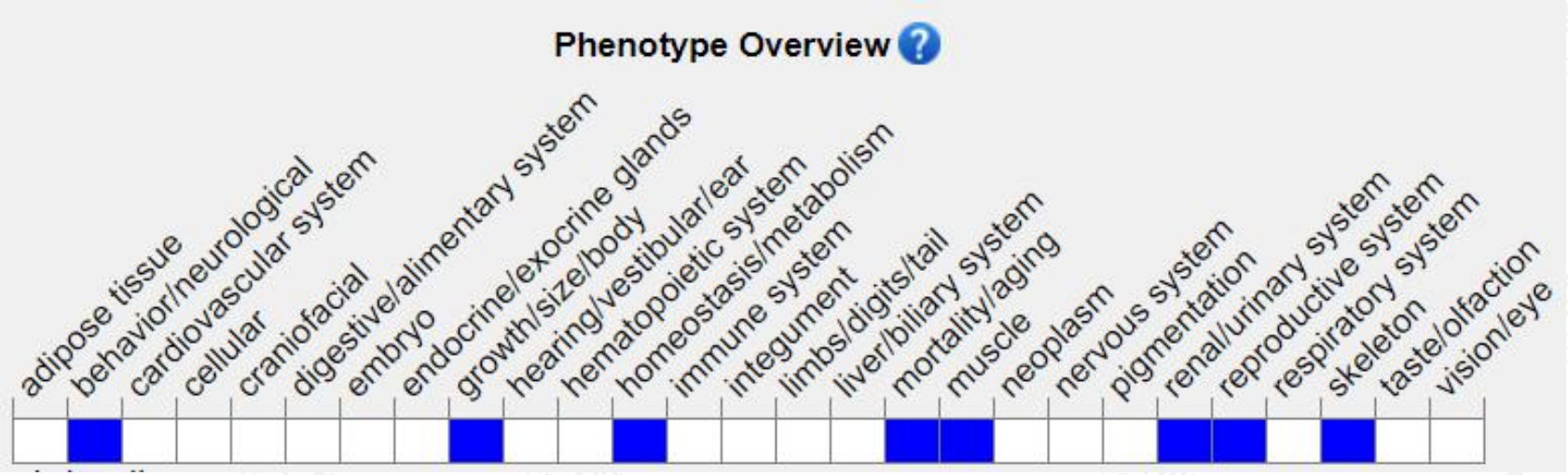
Genomic location distribution



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/) .

Homozygous null mice exhibit renal phosphate wasting, hyerpcalciuria, and skeletal abnormalities. Postnatal viability is reduced, putatively due to poor nutritional status.

References

[1] Kusaba T , Lalli M , Kramann R , et al. Differentiated kidney epithelial cells repair injured proximal tubule[J]. Proceedings of the National Academy of Sciences of the United States of America, 2014, 111(4):1527-1532.

[2] Jin Hee Kim1,Sang-Rok, et al. High Cleavage Efficiency of a 2A Peptide Derived from Porcine Teschovirus-1 in Human Cell Lines, Zebrafish and Mice. PLoS ONE 6(4): e18556.

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

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