Tek-P2A-iCre Mouse Model Strategy -CRISPR/Cas9 technology

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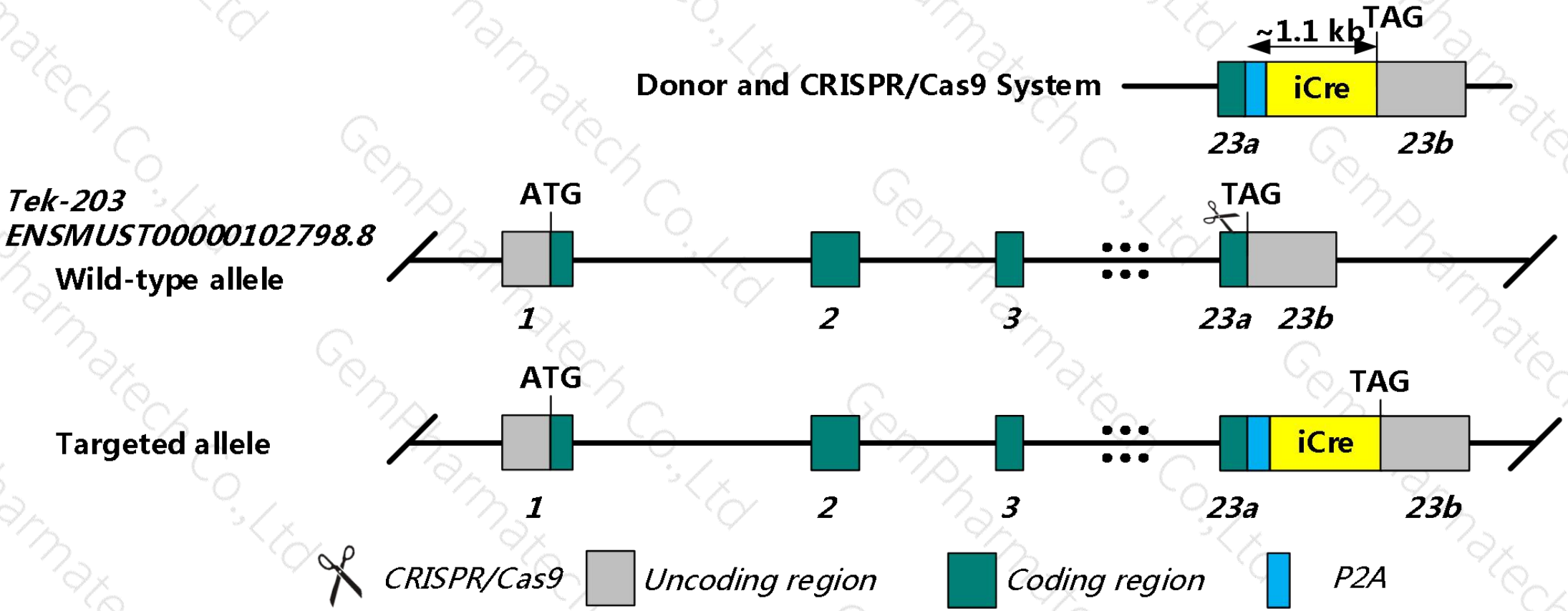
Project Overview

Project Name	***Tek-P2A-iCre***
Project type	**cas9-ki**
Strain background	**C57BL/6JGpt**

Knockin strategy

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



Technical routes

- The mouse *Tek* gene has 4 transcripts.According to the structure of *Tek* gene,*Tek-203* (ENSMUST00000102798.8)transcript is selected for this strategy.The transcript of *Tek-203* contains 23 exons,codes 1123aa,the ATG is located in exon1,and the TAG is located in exon23.

- We constructed CRISPR/Cas9 system targeting mouse *Tek* gene and donor vector,P2A-iCre will be introduced to near the TAG of mouse *Tek* gene.The iCre will be expressed under the direction of endogenous regulatory mechanism.

- The project will use CRISPR/Cas9 technology to modify *Tek* 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.

Notice

- According to the existing MGI data,homozygous mutation of this gene results in embryonic lethality during organogenesis, impaired vascular branching in the embryo and yolk sac, abnormal cardiac development, and in some cases hemorrhages.
- The P2A-linked genes drived expression in the same promoter and are cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide.
- Insertion of P2A-iCre may affect the regulation of the 3' end of the *Tek* gene.
- There may be 1 to 2 amino acid synonymous mutation in exon23 of *Tek* gene in this strategy.
- The *Tek* gene is located on the Chr4. If the knockin 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 gene transcription and translation, it is impossible to predict all of them at the existing technology level.

Existing model information

http://www.informatics.jax.org/allele/summary?markerId=MGI:98664&alleleType=Endonuclease-mediated

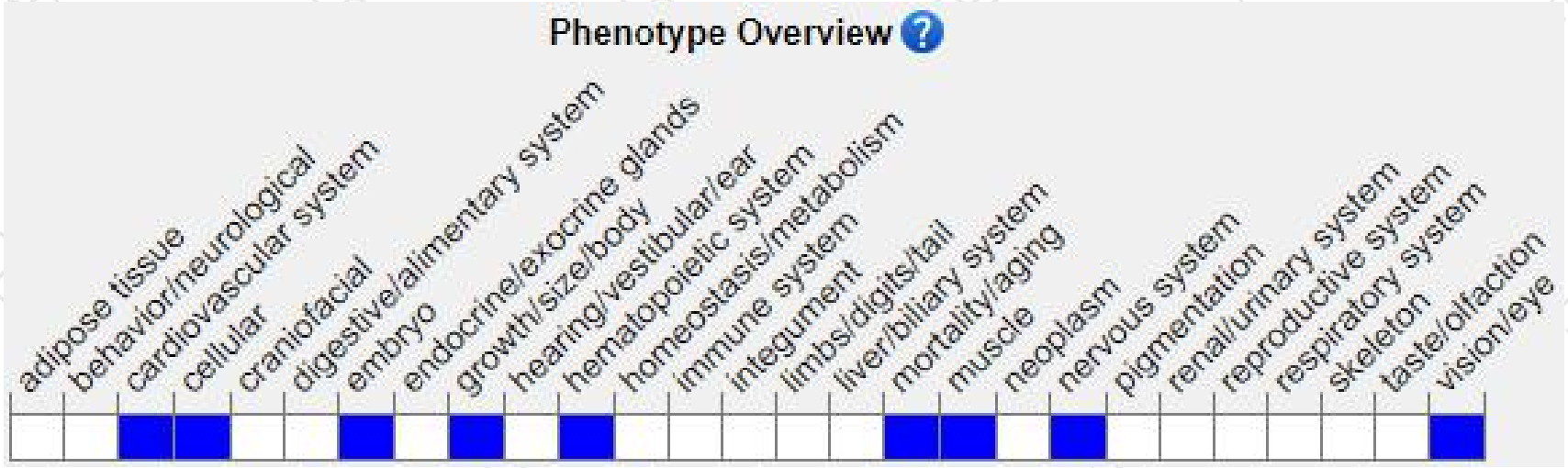
Allele Symbol Gene; Allele Name	Chr	Synonyms	Category	Abnormal Phenotypes Reported in these Systems	Human Disease Models
Tek$^{em1(cre)Smoc}$ TEK receptor tyrosine kinase; endonuclease-mediated mutation 1, Shanghai Model Organisms Center	4	Tek-(Cre)	Endonuclease-mediated (Recombinase)		
Tek$^{em1(dre)Bzsh}$ TEK receptor tyrosine kinase; endonuclease-mediated mutation 1, Bin Zhou	4	Tie2-Dre	Endonuclease-mediated (Recombinase)		
Tek$^{em2(cre/ERT2)Smoc}$ TEK receptor tyrosine kinase; endonuclease-mediated mutation 2, Shanghai Model Organisms Center	4	Tek2$^{Cre-ERT}$	Endonuclease-mediated (Inducible, Recombinase)		

Existing model information

http://www.informatics.jax.org/allele/summary?markerId=MGI:98664&alleleType=Targeted

Allele Symbol Gene; Allele Name	Chr	Synonyms	Category	Abnormal Phenotypes Reported in these Systems
Tek$^{tm1.1(icre/Esr1*)Hrr}$ TEK receptor tyrosine kinase; targeted mutation 1.1, Hans-Reimer Rodewald	4	Tie2^{MCM} Tie2^{MCMdeltaNeo}	Targeted (Inducible, Null/knockout, Recombinase)	mortality/aging
Tek$^{tm1.1Aug}$ TEK receptor tyrosine kinase; targeted mutation 1.1, Hellmut Augustin	4	Tie2^{flox}	Targeted (Conditional ready)	
Tek$^{tm1.1Scba}$ TEK receptor tyrosine kinase; targeted mutation 1.1, Scott Baldwin	4	Tie2^{fl}	Targeted (Conditional ready)	
Tek$^{tm1.1Vlcg}$ TEK receptor tyrosine kinase; targeted mutation 1.1, Velocigene	4	Tek^{ex1COIN}	Targeted (Conditional ready, No functional change)	
Tek$^{tm1.1Yuhe}$ TEK receptor tyrosine kinase; targeted mutation 1.1, Yulong He	4	Tie2^{Flox}	Targeted (Conditional ready)	
Tek$^{tm1.2Scba}$ TEK receptor tyrosine kinase; targeted mutation 1.2, Scott Baldwin	4	Tie2^{-}	Targeted (Null/knockout)	
Tek$^{tm1a(EUCOMM)Hmgu}$ TEK receptor tyrosine kinase; targeted mutation 1a, Helmholtz Zentrum Muenchen GmbH	4		Targeted (Conditional ready, Null/knockout, Reporter)	
Tek$^{tm1b(EUCOMM)Hmgu}$ TEK receptor tyrosine kinase; targeted mutation 1b, Helmholtz Zentrum Muenchen GmbH	4		Targeted (Null/knockout, Reporter)	cardiovascular, mortality/aging, vision/eye
Tek^{tm1Ber} TEK receptor tyrosine kinase; targeted mutation 1, Alan Bernstein	4	Tie2^{F1100}	Targeted (Inserted expressed sequence)	cardiovascular, embryo, hematopoietic, mortality/aging, muscle
Tek$^{tm1c(EUCOMM)Hmgu}$ TEK receptor tyrosine kinase; targeted mutation 1c, Helmholtz Zentrum Muenchen GmbH	4	Tek^{lox}	Targeted (Conditional ready)	
Tek^{tm1Dmt} TEK receptor tyrosine kinase; targeted mutation 1, Daniel J Dumont	4	tek^{deltasp}, Tie2^{-}	Targeted (Null/knockout)	cardiovascular, embryo, hematopoietic, mortality/aging, muscle, nervous system
Tek^{tm1Sato} TEK receptor tyrosine kinase; targeted mutation 1, Thomas N Sato	4	tie-2 -	Targeted (Null/knockout)	cardiovascular, embryo, growth/size/body, mortality/aging, muscle
Tek$^{tm1e(EUCOMM)Hmgu}$ TEK receptor tyrosine kinase; targeted mutation 1e, Helmholtz Zentrum Muenchen GmbH	4		Targeted (Null/knockout, Reporter) **(Cell Line)**	

Mouse phenotype description(MGI)



mouse *Tek*:
Homozygous mutation of this gene results in embryonic lethality during organogenesis, impaired vascular branching in the embryo and yolk sac, abnormal cardiac development, and in some cases hemorrhages.

http://www.informatics.jax.org/marker/MGI:98664

Target gene

Gene name	mouse *Tek*
Gene ID(NCBI)	21687
Gene link(NCBI)	https://www.ncbi.nlm.nih.gov/gene/21687
Gene link(Ensembl)	http://asia.ensembl.org/Mus_musculus/Gene/Summary?g=ENSMUSG00000006386;r=4:94627526-94763213
chromosome location	Chr4

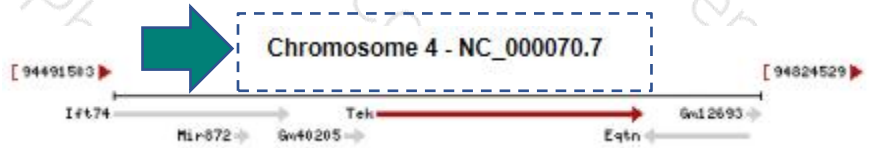
Gene information （NCBI）

Tek TEK receptor tyrosine kinase [*Mus musculus* (house mouse)]

Gene ID: 21687, updated on 17-Aug-2021

Summary

Official Symbol	Tek provided by MGI
Official Full Name	TEK receptor tyrosine kinase provided by MGI
Primary source	MGI:MGI:98664
See related	Ensembl:ENSMUSG00000006386
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	Hyk; Tie; STK1; Tie2; tie-; Tie-2; Cd202b; AA517024
Expression	Broad expression in lung adult (RPKM 28.4), subcutaneous fat pad adult (RPKM 7.5) and 18 other tissues See more
Orthologs	human all

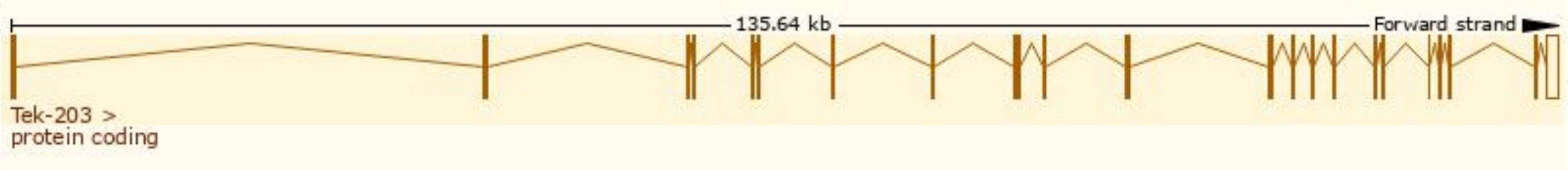


Transcript information（Ensembl）

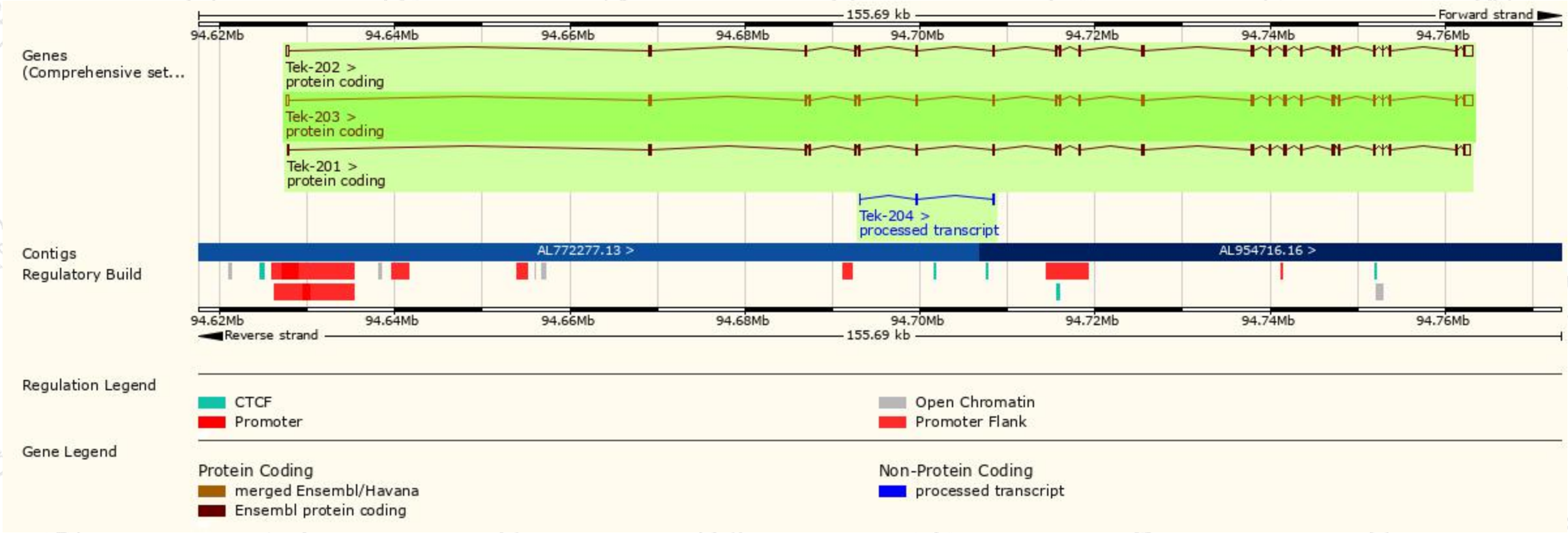
The gene has 4 transcripts, all transcriptional information is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS ▲	UniProt Match	Flags
Tek-203	ENSMUST00000102798.8	4654	1123aa	Protein coding	CCDS18361	B1AWS8	GENCODE basic APPRIS P3 TSL:1
Tek-201	ENSMUST00000071168.6	4176	1122aa	Protein coding	CCDS71421	Q02858	GENCODE basic APPRIS ALT1 TSL:1
Tek-202	ENSMUST00000073939.13	4549	1072aa	Protein coding	CCDS80122	Q80YS4	GENCODE basic TSL:1
Tek-204	ENSMUST00000131958.2	361	No protein	Processed transcript	-	-	TSL:3

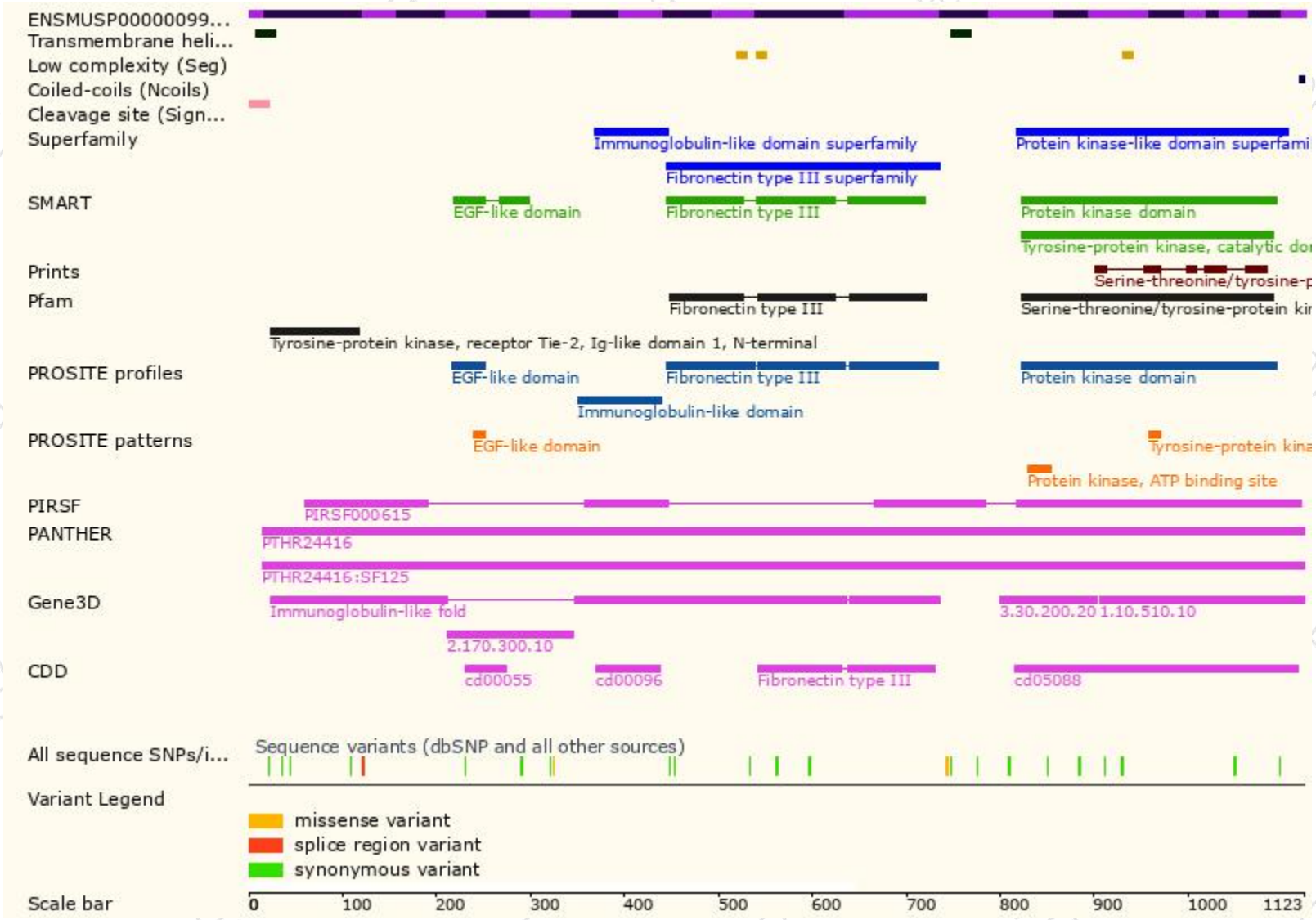
The strategy is based on *Tek-203* transcript design,it contains 23 exons,the length of transcript is 4654bps,and encodes 1123 amino acids.



Genomic location distribution



Protein domain



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

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