

***Humanized KITLG-PolyA* Cas9-KI Strategy**

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Reviewer:

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Design Date:

2019-9-27

Project Overview

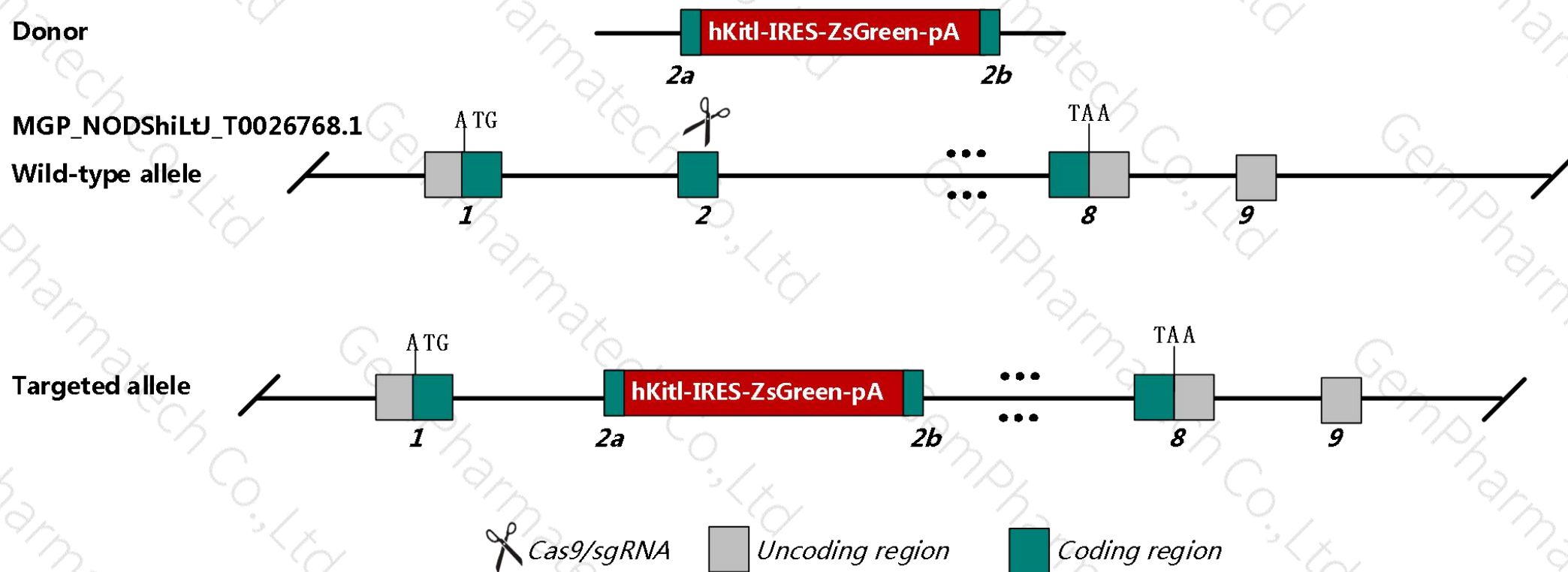
Project Name	<i>Humanized KITLG-PolyA</i>
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Project type	Cas9-KI
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Strain background	NODShiLtJ
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Knockin strategy

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



Human KITLG CDS

ATGAAGAAGACACAAACTTGGATTCTCACTTGCATTTATCTTCAGCTGCTCCTATTTAATCCTCTCGTCAAAACTGAAGGGATCT
GCAGGAATCGTGTGACTAATAATGTAAAAGACGTCCTAAATTGGTGGCAAATCTTCCAAAAGACTACATGATAACCCTCAAATA
TGTCCCCGGGATGGATGTTTTGCCAAGTCATTGTTGGATAAGCGAGATGGTAGTACAATTGTCAGACAGCTTGACTGATCTTCTG
GACAAGTTTTCAAATATTTCTGAAGGCTTGAGTAATTATTCCATCATAGACAAACTTGTGAATATAGTGGATGACCTTGTGGAGT
GCGTGAAAGAAAACCTCATCTAAGGATCTAAAAAAATCATTCAAGAGCCCAGAACCCAGGCTCTTTACTCCTGAAGAATTCTTTAG
AATTTTTTAATAGATCCATTGATGCCTTCAAGGACTTTGTAGTGGCATCTGAAACTAGTGATTGTGTGGTTTTCTTCAACATTAAGT
CCTGAGAAAGATTCCAGAGTCAGTGTCAAAAACCATTTATGTTACCCCCTGTTGCAGCCAGCTCCCTTAGGAATGACAGCAGTA
GCAGTAATAGGAAGGCCAAAAATCCCCCTGGAGACTCCAGCCTACACTGGGCAGCCATGGCATTGCCAGCATTGTTTTCTCTTAT
AATTGGCTTTGCTTTTGGAGCCTTATACTGGAAGAAGAGACAGCCAAGTCTTACAAGGGCAGTTGAAAATATACAAATTAATGAA
GAGGATAATGAGATAAGTATGTTGCAAGAGAAAGAGAGAGAGAGTTTCAAGAAGTGTA

Mouse Kitl CDS

ATGAAGAAGACACAAACTTGGATTATCACTTGCATTTATCTTCAACTGCTCCTATTTAATCCTCTCGTCAAAACC AAGGAGATCT
GCGGGAATCCTGTGACTGATAATGTAAAAGACATTACAAAACCTGGTGGCAAATCTTCCAAATGACTATATGATAACCCTCAACTA
TGTCGCCGGGATGGATGTTTTGCCTAGTCATTGTTGGCTACGAGATATGGTAATACAATTATCACTCAGCTTGACTACTCTTCTG
GACAAGTTCTCAAATATTTCTGAAGGCTTGAGTAATTACTCCATCATAGACAAACTTGGGAAAATAGTGGATGACCTCGTGTTAT
GCATGGAAGAAAACGCACCGAAGAATATAAAAGAATCTCCGAAGAGGCCAGAAACTAGATCCTTTACTCCTGAAGAATTCTTTAG
TATTTTCAATAGATCCATTGATGCCTTTAAGGACTTTATGGTGGCATCTGACACTAGTGACTGTGTGCTCTCTTCAACATTAGGT
CCCGAGAAAGATTCCAGAGTCAGTGTCACAAAACCATTTATGTTACCCCCTGTTGCAGCCAGCTCCCTTAGGAATGACAGCAGTA
GCAGTAATAGGAAAGCCGCAAAGTCCCCTGAAGACTCGGGCCTACAATGGACAGCCATGGCATTGCCGGCTCTCATTTCGCTTGT
AATTGGCTTTGCTTTTGGAGCCTTATACTGGAAGAAGAAACAGTCAAGTCTTACAAGGGCAGTTGAAAATATACAGATTAATGAA
GAGGATAATGAGATAAGTATGTTGCAACAGAAAGAGAGAGAATTTCAAGAGGTGTAA

- The *Kitl* gene has 3 transcripts. According to the structure of *Kitl* gene, *MGP_NODShiLtJ_T0026766.1* is selected for presentation of the recommended strategy.
- *MGP_NODShiLtJ_T0026766.1* gene has 11 exons, with the ATG start codon in exon1 and TAA stop codon in exon9.
- We make *Humanized KITLG-PolyA* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near exon2 of *Kitl* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in *Humanized KITLG-PolyA* into exon2 of *Kitl* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

- According to available data (MGI), Mutations in this gene affect migration of embryonic stem cells and cause similar phenotypes to mutations in its receptor gene (Kit). Mutants show mild to severe defects in pigmentation, hemopoiesis and reproduction.
- The Kitl gene is located on the 10# chromosome. If this gene is knocked into mice and matched with other mouse strains, please avoid the two genes on the same chromosome, otherwise the offspring of the double-gene homozygous positive will not be obtained.
- After humanization of the KITLG gene, the function of the mouse gene may be lost, resulting in the result of the gene KO.
- The scheme is designed according to the genetic information in the existing database. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Gene information (NCBI)

Kitl kit ligand [*Mus musculus* (house mouse)]

Gene ID: 17311, updated on 12-Aug-2019

Summary

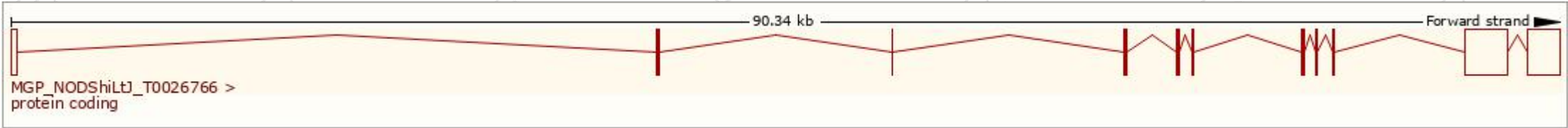
Official Symbol	Kitl provided by MGI
Official Full Name	kit ligand provided by MGI
Primary source	MGI:MGI:96974
See related	Ensembl:ENSMUSG00000019966
Gene type	protein coding
RefSeq status	VALIDATED
Organism	<i>Mus musculus</i>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Gb; SF; SI; Clo; Con; Mgf; SCF; SLF; blz; Kitlg; contrasted
Expression	Ubiquitous expression in lung adult (RPKM 2.7), CNS E18 (RPKM 2.6) and 27 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

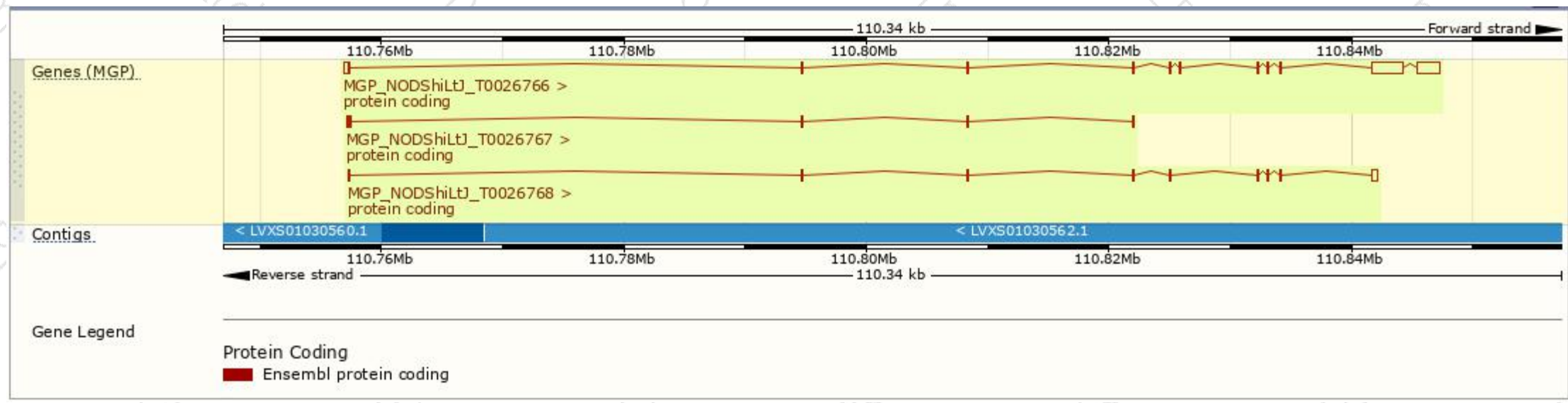
The gene has 3 transcripts, and all transcripts are shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
-	MGP_NODShiLtJ_T0026766.1	5642	273aa	 Protein coding	CCDS36046	P20826 Q8C9K1	-
-	MGP_NODShiLtJ_T0026768.1	1260	245aa	 Protein coding	-	-	-
-	MGP_NODShiLtJ_T0026767.1	675	160aa	 Protein coding	-	-	-

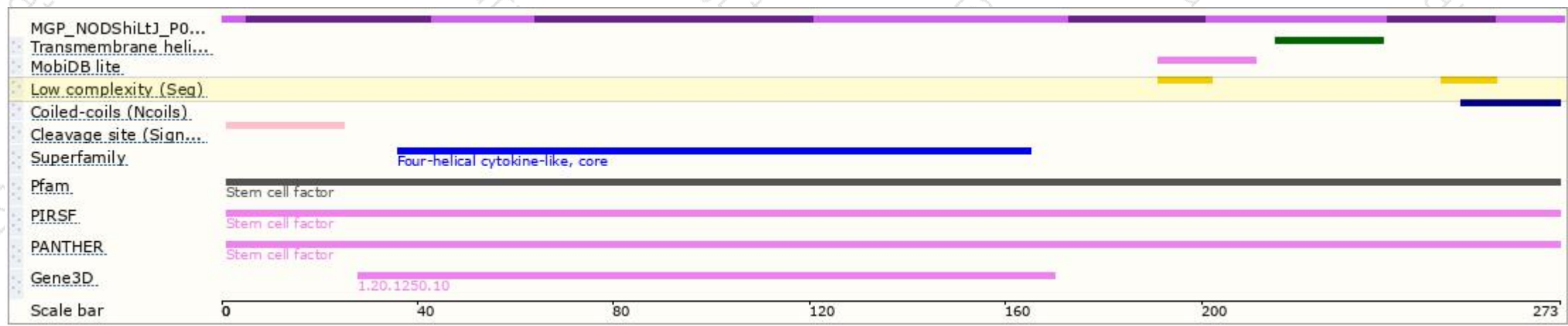
The strategy is based on the design of *MGP_NODShiLtJ_T0026766.1* transcript, The transcription is shown



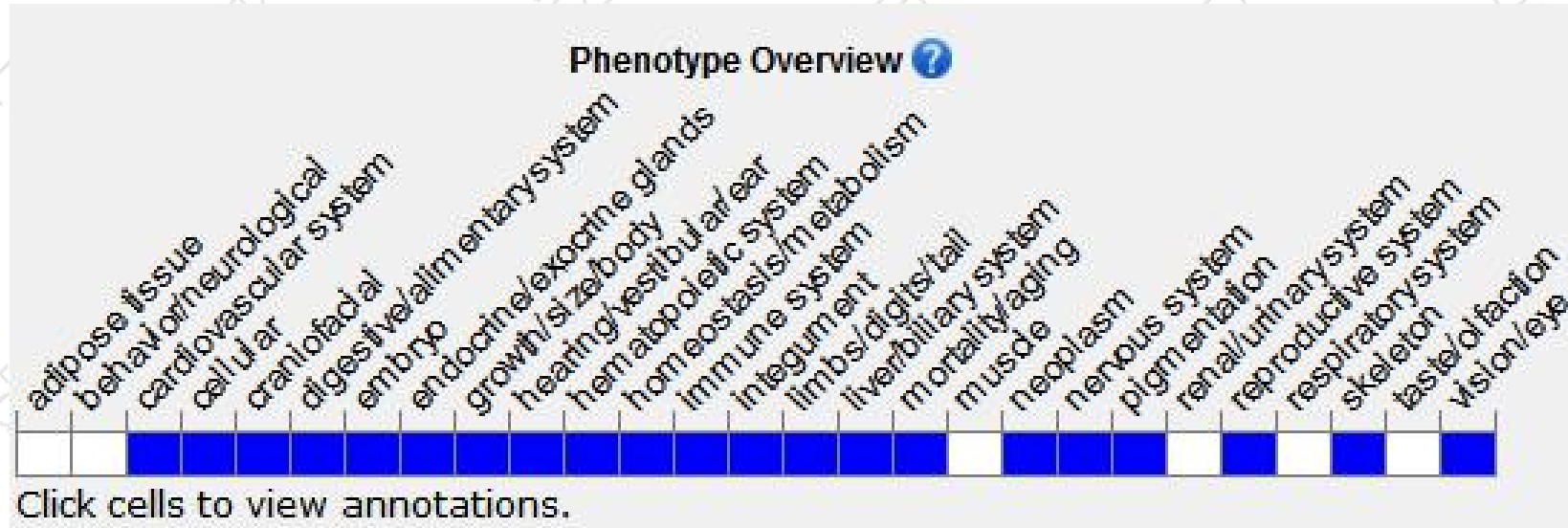
Genomic location distribution



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>) .

Mutations in this gene affect migration of embryonic stem cells and cause similar phenotypes to mutations in its receptor gene (Kit). Mutants show mild to severe defects in pigmentation, hemopoiesis and reproduction.

Gene information (NCBI)

KITLG KIT ligand [*Homo sapiens* (human)]

Gene ID: 4254, updated on 7-Jul-2019

Summary

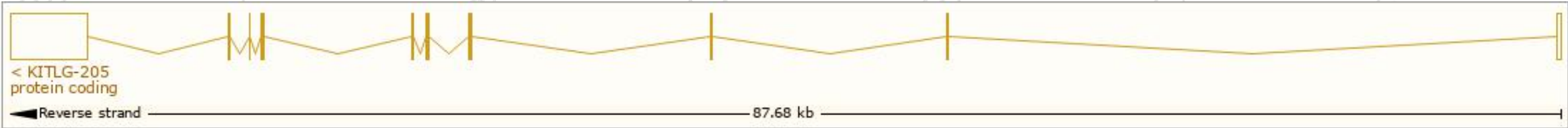
Official Symbol	KITLG provided by HGNC
Official Full Name	KIT ligand provided by HGNC
Primary source	HGNC:HGNC:6343
See related	Ensembl:ENSG00000049130 MIM:184745
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Homo sapiens
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo
Also known as	SF; MGF; SCF; SLF; DCUA; FPH2; FPHH; KL-1; Kitl; SHEP7; DFNA69
Summary	This gene encodes the ligand of the tyrosine-kinase receptor encoded by the KIT locus. This ligand is a pleiotropic factor that acts in utero in germ cell and neural cell development, and hematopoiesis, all believed to reflect a role in cell migration. In adults, it functions pleiotropically, while mostly noted for its continued requirement in hematopoiesis. Two transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Jul 2008]
Expression	Broad expression in lung (RPKM 24.5), colon (RPKM 14.0) and 22 other tissues See more
Orthologs	mouse all

Transcript information (Ensembl)

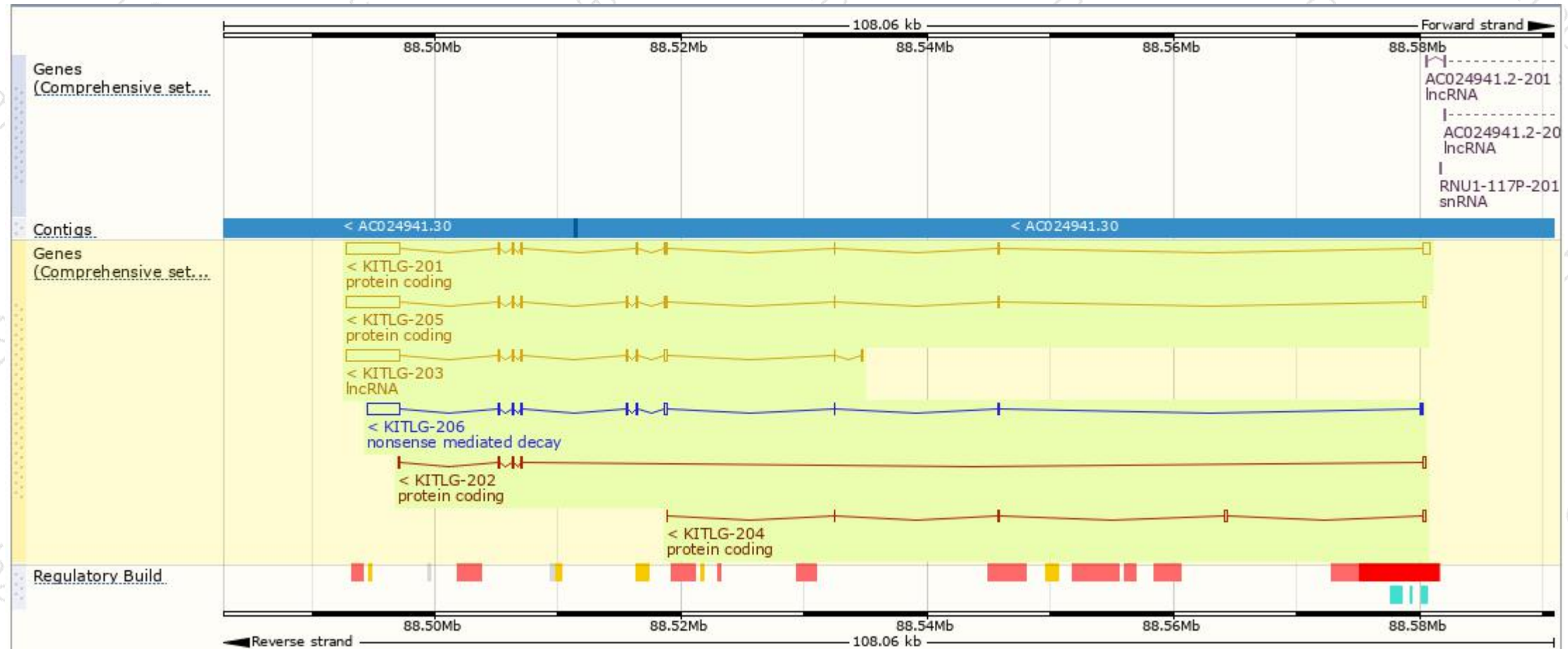
The gene has 6 transcripts, and all transcripts are shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq Match	Flags
KITLG-201	ENST00000347404.10	5737	245aa	 Protein coding	CCDS31867	A0A024RBF5 P21583	-	TSL:1 GENCODE basic
KITLG-205	ENST00000644744.1	5441	273aa	 Protein coding	CCDS31868	A0A024RBC0 P21583	NM_000899.5	GENCODE basic APPRIS P1 MANE Select v0.5
KITLG-202	ENST00000357116.4	565	56aa	 Protein coding	-	S4R384	-	TSL:4 GENCODE basic
KITLG-204	ENST00000552044.1	555	24aa	 Protein coding	-	S4R442	-	CDS 3' incomplete TSL:4
KITLG-206	ENST00000646633.1	3768	57aa	 Nonsense mediated decay	-	A0A2R8Y515	-	-
KITLG-203	ENST00000378535.4	5191	No protein	 lncRNA	-	-	-	TSL:1

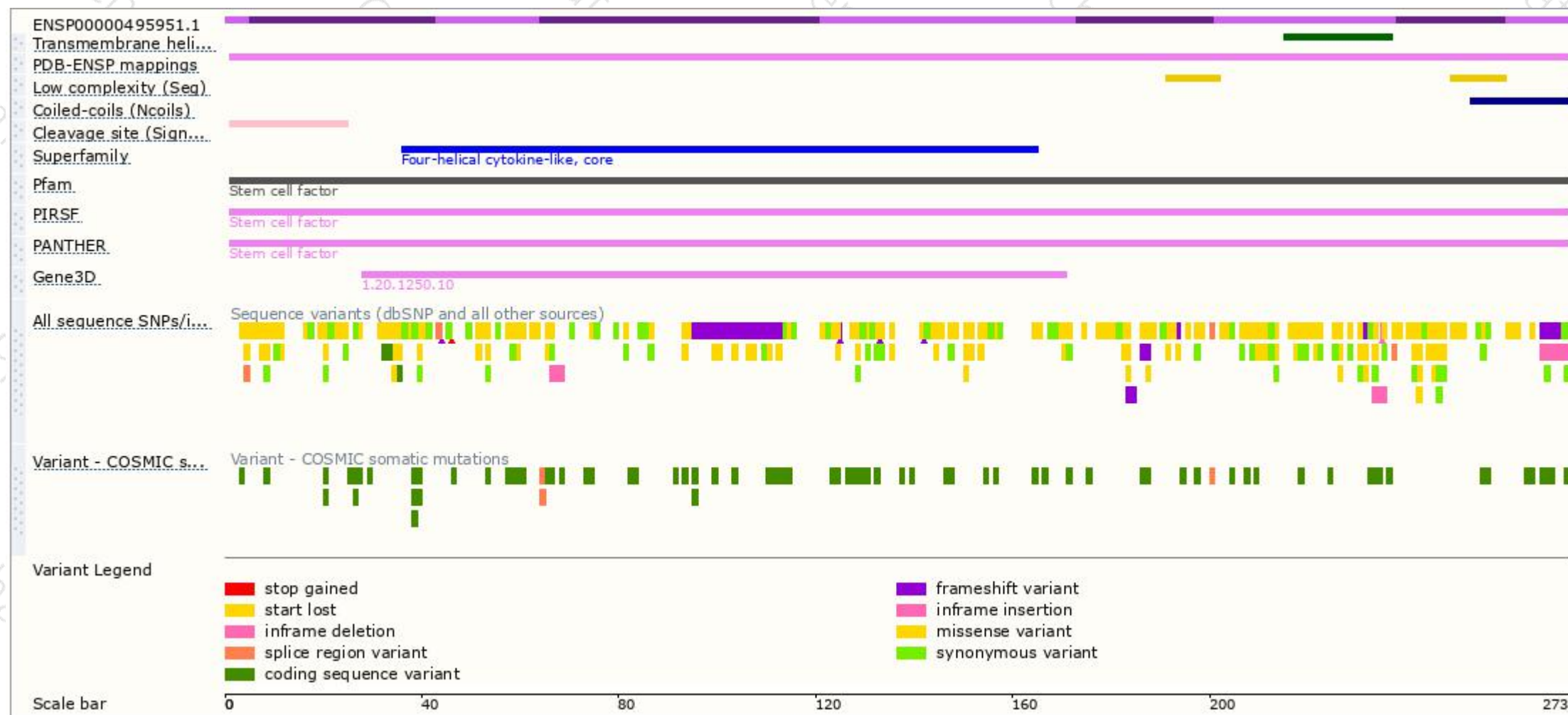
The strategy is based on the design of *KITLG-205* transcript, The transcription is shown below



Genomic location distribution



Protein domain



Human KITLG domain

Topology

Feature key	Position(s)	Description
Topological domain ⁱ	26 – 214	Extracellular Sequence analysis
Transmembrane ⁱ	215 – 237	Helical Sequence analysis
Topological domain ⁱ	238 – 273	Cytoplasmic Sequence analysis

Molecule processing

Feature key	Position(s)	Description
Signal peptide ⁱ	1 – 25	
Chain ⁱ (PRO_0000031913)	26 – 273	Kit ligand
Chain ⁱ (PRO_0000403391)	26 – 190	Soluble KIT ligand

Mouse KITL domain

Topology

Feature key	Position(s)	Description
Topological domain ⁱ	26 – 214	Extracellular Sequence analysis
Transmembrane ⁱ	215 – 237	Helical Sequence analysis
Topological domain ⁱ	238 – 273	Cytoplasmic Sequence analysis

Keywords – Cellular componentⁱ

Cell membrane, Cell projection, Cytoplasm, Cytoskeleton, Membrane, Secreted

PTM / Processingⁱ

Molecule processing

Feature key	Position(s)	Description
Signal peptide ⁱ	1 – 25	3 Publications
Chain ⁱ (PRO_0000031914)	26 – 273	Kit ligand
Chain ⁱ (PRO_0000403392)	26 – 190	Soluble KIT ligand By similarity

Kitl protein has both secreted form and transmembrane protein form. For this model, we need to secrete the form of protein, so we have a humanized strategy choice: 1-25aa using Mouse sequence (included in exon1-2), 26 -273aa replaced with Human 26-273aa

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