# *hAxl* Cas9-KI Strategy

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**Reviewer: Jinlong Zhao**

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# Project Overview

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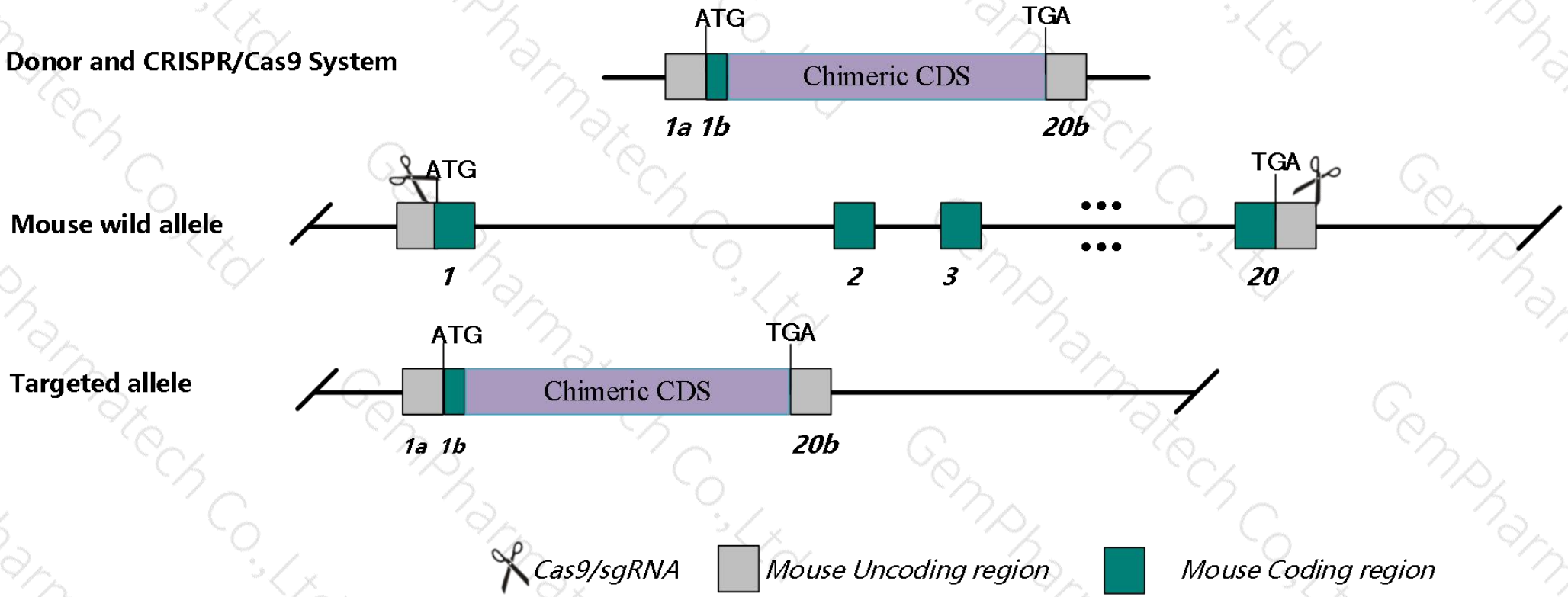
| **Project Name** | ***hAxl*** |
| **Project type** | **Cas9-KI** |
| **Strain background** | **C57BL/6JGpt** |

# Strategy

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



# Technical routes

➢ According to the available data of ensembl, there are 8 human AXL gene transcripts and 5 mouse Axl gene transcripts,select mouse transcript Axl-201(Ensmust00000002677.10) .

➢ In this project we use CRISPR/Cas9 technology to modify *Axl* 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.

➢ According to the genetic structure, it is recommended to select the transcript of Axl-201 (ENSMUS00000002677.10). After the signal peptide of Axl-201, the human and mouse chimeric CDS element and the transcription Stop signal are knocked in. The length of the knockin element is about 4.2kb, and the human and mouse chimeric CDS is shown under the transcription regulation of mouse Axl gene.

➢The mouse transcript of Axl-201 (Ensmust00000002677.10) contains 20 Exons, with the translation start site Atg at Exon1 and the translation stop site Tag at Exon20.

# Chimeric CDS

ATGGGCAGGGTCCCGCTGGCCTGGTGGTTGGCGCTGTGCTGCTGGGGGTGTGCAGCCCCCAGGGGCACGCAGGCTGAAGAAAGTCCCTTCGTGGGCAACCCAGGGAAT
ATCACAGGTGCCCGGGGACTCACGGGCACCCTTCGGTGTCAGCTCCAGGTTCAGGGAGAGCCCCCCGAGGTACATTGGCTTCGGGATGGACAGATCCTGGAGCTCGCGGACAG
CACCCAGACCCAGGTGCCCCTGGGTGAGGATGAACAGGATGACTGGATAGTGGTCAGCCAGCTCAGAATCACCTCCCTGCAGCTTTCCGACACGGGACAGTACCAGTGTTTGG
TGTTTCTGGGACATCAGACCTTCGTGTCCCAGCCTGGCTATGTTGGGCTGGAGGGCTTGCCTTACTTCCTGGAGGAGCCCGAAGACAGGACTGTGGCCGCCAACACCCCCTTCA
ACCTGAGCTGCCAAGCTCAGGGACCCCCAGAGCCCGTGGACCTACTCTGGCTCCAGGATGCTGTCCCCCTGGCCACGGCTCCAGGTCACGGCCCCCAGCGCAGCCTGCATGTT
CCAGGGCTGAACAAGACATCCTCTTTCTCCTGCGAAGCCCATAACGCCAAGGGGGTCACCACATCCCGCACAGCCACCATCACAGTGCTCCCCCAGCAGCCCCGTAACCTCCA
CCTGGTCTCCCGCCAACCCACGGAGCTGGAGGTGGCTTGGACTCCAGGCCTGAGCGGCATCTACCCCCTGACCCACTGCACCCTGCAGGCTGTGCTGTCAGACGATGGGATGG
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TGTGGCTGCCGCCTGCGTCCTCATCTTGGCCCTGTTCCTTGTCCATCGGAGGAAGAAGGAGACTCGATATGGGGAGGTGTTTGAGCCAACCGTGGAAAGAGGTGAACTGGTAG
TCAGGTACCGTGTCCGAAAGTCCTACAGCCGGCGGACCACTGAAGCCACCTTGAACAGTCTGGGCATCAGTGAAGAGCTGAAGGAGAAACTACGAGACGTCATGGTAGATCG
GCATAAGGTGGCCTTGGGGAAGACCCTGGGAGAAGGAGAATTTGGCGCTGTGATGGAAGGTCAGCTCAATCAGGATGACTCCATCCTCAAGGTCGCTGTGAAGACCATGAAAA
TTGCCATCTGCACAAGATCAGAGCTGGAGGATTTCCTGAGTGAAGCTGTCTGCATGAAGGAATTTGACCACCCCAACGTCATGAGGCTCATTGGCGTCTGTTTTCAGGGCTCTG
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TACCTGCGTCAAGGAAATCGGCTGAAACAGCCTGTGGACTGTCTGGACGGCCTGTATGCCCTGATGTCTCGGTGCTGGGAACTGAACCCTCGAGACCGGCCAAGTTTTGCGGA
GCTCCGGGAAGACTTGGAGAACACACTGAAGGCTCTGCCCCCTGCTCAGGAGCCAGATGAAATCCTCTATGTCAACATGGATGAGGGCGGAAGCCACCTTGAACCCCGTGGGG
CTGCTGGAGGAGCTGACCCCCCAACCCAACCTGATCCTAAGGATTCCTGTAGCTGTCTCACTGCAGCTGACGTCCACTCAGCTGGACGCTATGTCCTTTGTCCTTCTACAGCCCC
AGGACCCACTCTGTCTGCTGACAGAGGCTGCCCAGCACCTCCAGGGCAGGAGGACGGAGCCTGA

Human extracellular structure is shown in red , mouse intracellularand and transmembrane structure are shown in black，
mouse Signal peptide is shown in blue.

# Chimeric CDS(codon optimization)

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ATGGGCAGGGTCCCGCTGGCCTGGTGGTTGGCGCTGTGCTGCTGGGGGTGTGCAGCCCCACGAGGAACACAGGCCGAGGAGAGCCCTTTTGTCGGGAACCCCGGCAAT
ATTACAGGAGCACGAGGGCTCACGGGGACACTGCGCTGCCAGCTGCAGGTGCAGGGGGAGCCACCAGAAGTGCACTGGTTACGCGATGGGCAGATCCTCGAGCTTGCAGACT
CCACCCAGACTCAGGTTCCTCTAGGTGAAGATGAGCAGGATGACTGGATCGTGGTGTCTCAGCTCCGGATTACGTCCCTGCAGCTAAGTGACACTGGTCAGTACCAGTGCCTGG
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GCGTAGCCTGCACCTCTTCGCAAGGGCCTTCATCTTGGACACATTGGTTGCCAGTGGAGACACCCGAGGGAGTCCCACTTGGTCCTCCTGAGAACATTTCAGCTACCAGGAATG
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CTAGTGAAGTTCATGGCCGACATTGCCAGTGGTATGGAGTACCTGAGTACCAAGAGATTCATACATCGGGACCTGGCTGCCAGGAACTGCATGCTGAATGAGAACATGTCCGTG
TGTGTGGCAGACTTCGGGCTCTCCAAGAAGATCTACAACGGGGATTACTACCGCCAAGGGCGCATTGCCAAGATGCCAGTCAAGTGGATTGCTATTGAGAGTCTGGCAGATCGG
GTCTACACCAGCAAGAGCGATGTGTGGTCCTTCGGTGTGACAATGTGGGAGATCGCCACCCGAGGCCAAACTCCCTATCCAGGGGTGGAGAACAGTGAGATTTACGACTACCT
GCGTCAAGGAAATCGGCTGAAACAGCCTGTGGACTGTCTGGACGGCCTGTATGCCCTGATGTCTCGGTGCTGGGAACTGAACCCTCGAGACCGGCCAAGTTTTGCGGAGCTCC
GGGAAGACTTGGAGAACACACTGAAGGCTCTGCCCCCTGCTCAGGAGCCAGATGAAATCCTCTATGTCAACATGGATGAGGGCGGAAGCCACCTTGAACCCCGTGGGGCTGCT
GGAGGAGCTGACCCCCCAACCCAACCTGATCCTAAGGATTCCTGTAGCTGTCTCACTGCAGCTGACGTCCACTCAGCTGGACGCTATGTCCTTTGTCCTTCTACAGCCCCAGGA
CCCACTCTGTCTGCTGACAGAGGCTGCCCAGCACCTCCAGGGCAGGAGGACGGAGCCTGA

Human extracellular structure is shown in red , mouse intracellularand and transmembrane structure are shown in black，
mouse Signal peptide is shown in blue.The human extracellular domain sequence was optimized by mouse codon.

# Amino acid sequences

MGRVPLAWWLALCCWGCAAPRGTQAEESPFVGNPGNITGARGLTGTLRCQLQVQGEPPEVHWL
RDGQILELADSTQTQVPLGEDEQDDWIVVSQLRITSLQLSDTGQYQCLVFLGHQTFVSQPGYVGL
EGLPYFLEEPEDRTVAANTPFNLSCQAQGPPEPVDLLWLQDAVPLATAPGHGPQRSLHVPGLNKT
SSFSCEAHNAKGVTTSRTATITVLPQQPRNLHLVSRQPTELEVAWTPGLSGIYPLTHCTLQAVLSDD
GMGIQAGEPDPPEEPLTSQASVPPHQLRLGSLHPHTPYHIRVACTSSQGPSSWTHWLPVETPEGVP
LGPPENISATRNGSQAFVHWQEPRAPLQGTLLGYRLAYQGQDTPEVLMDIGLRQEVTLELQGDG
SVSNLTVCVAAYTAAGDGPWSLPVPLEAWRPGQAQPVHQLVKEPSTPAFSWPWWYVLLGALVA
AACVLILALFLVHRRKKETRYGEVFEPTVERGELVVRYRVRKSYSRRTTEATLNSLGISEELKEKL
RDVMVDRHKVALGKTLGEGEFGAVMEGQLNQDDSILKVAVKTMKIAICTRSELEDFLSEAVCMK
EFDHPNVMRLIGVCFQGSDREGFPEPVVILPFMKHGDLHSFLLYSRLGDQPVFLPTQMLVKFMAD
IASGMEYLSTKRFIHRDLAARNCMLNENMSVCVADFGLSKKIYNGDYYRQGRIAKMPVKWIAIE
SLADRVYTSKSDVWSFGVTMWEIATRGQTPYPGVENSEIYDYLRQGNRLKQPVDCLDGLYALMS
RCWELNPRDRPSFAELREDLENTLKALPPAQEPDEILYVNMDEGGSHLEPRGAAGGADPPTQPDP
KDSCSCLTAADVHSAGRYVLCPSTAPGPTLSADRGCPAPPGQEDGA

Human extracellular structure is shown in red , mouse intracellularand and transmembrane structure are shown in black，
mouse Signal peptide is shown in blue.

# Homology analysis of AXL amino acids in human and mouse


➢The amino acid length encoded by human AXL was 894aa, and the amino acid length encoded by mouse Axl was 888aa. After comparison, the amino acid homology was about 90.3%.

➢The amino acid homology between human and mouse transmembrane region and intracellular region was up to 96.4%.

# Notice

➢ According to the existing MGI data,homozygous mutant mice are phenotypically normal, however in conjunction with mutations in other related receptor tyrosine kinases, mutations of this gene results in fertility defects, autoimmunity abnormalities, and aberrant apoptosis.

➢ The *Axl* gene is located on the Chr7. If this gene is knocked into the mouse and matched with other mouse strains, please avoid the two genes located on the same chromosome, otherwise it will not be able to get homozygous positive mouse offspring.

➢ This strategy is designed based on genetic information in the existing database.Due to the complexity of gene transcription and translation, it is impossible to predict all of them at the current technical level.

# Gene information （NCBI）

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## Axl AXL receptor tyrosine kinase [Mus musculus (house mouse)]

Gene ID: 26362, updated on 30-Mar-2019

### Summary

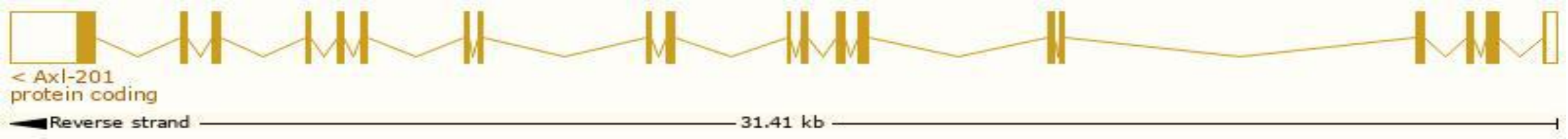
| | |
|---|---|
| **Official Symbol** | Axl provided by MGI |
| **Official Full Name** | AXL receptor tyrosine kinase provided by MGI |
| **Primary source** | MGI:MGI:1347244 |
| **See related** | Ensembl:ENSMUSG00000002602 |
| **Gene type** | protein coding |
| **RefSeq status** | VALIDATED |
| **Organism** | Mus musculus |
| **Lineage** | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| **Also known as** | AI323647, Ark, Tyro7, Ufo |
| **Expression** | Broad expression in ovary adult (RPKM 53.4), mammary gland adult (RPKM 42.5) and 28 other tissues See more |
| **Orthologs** | human all |

# Transcript information（Ensembl）

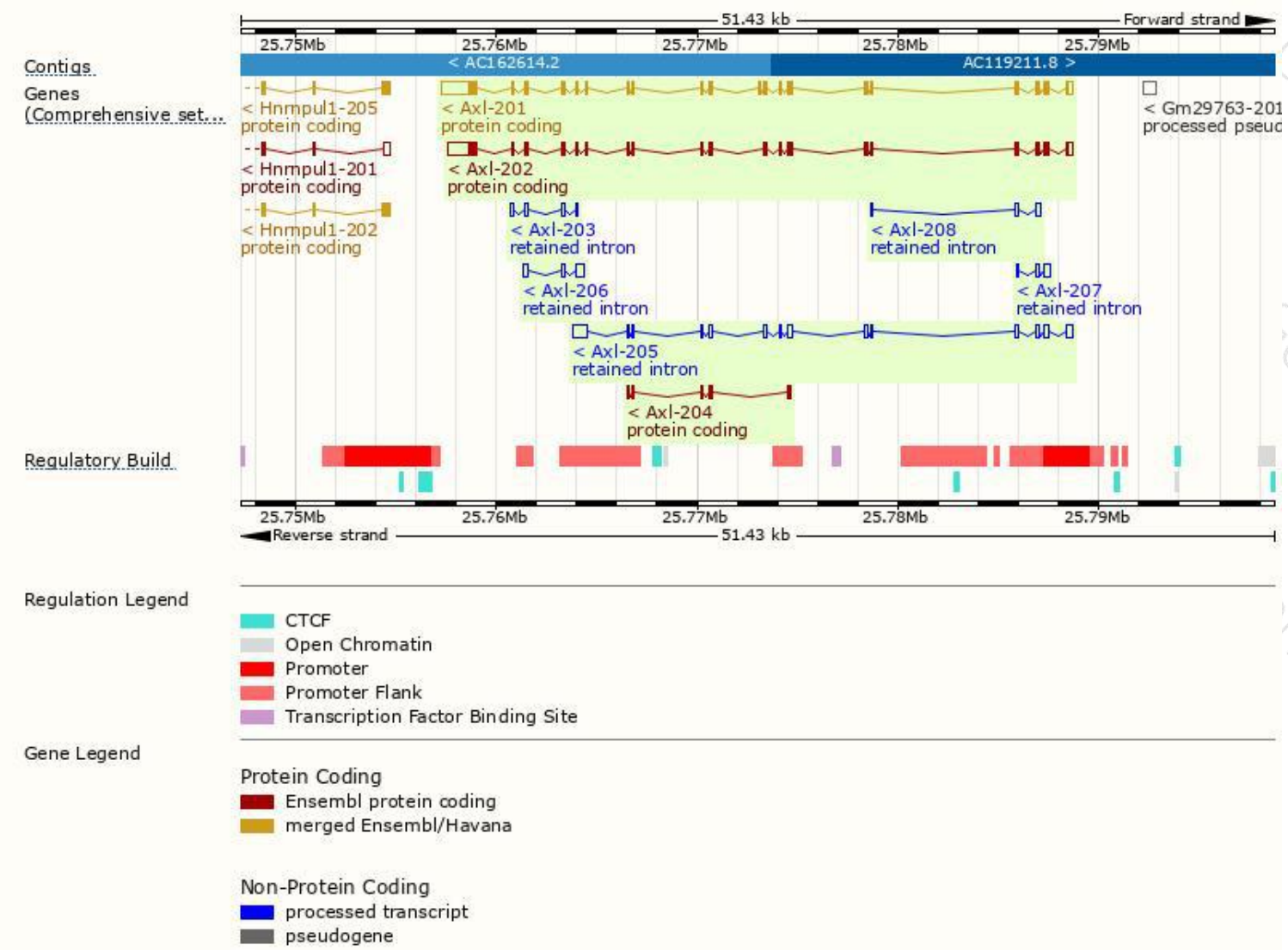
The gene has 8 transcripts,all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|---|---|---|---|---|---|---|---|
| Axl-201 | ENSMUST00000002677.10 | 4265 | 888aa | Protein coding | CCDS20996 | Q00993 | TSL:1 GENCODE basic APPRIS P3 |
| Axl-202 | ENSMUST00000085948.10 | 3899 | 879aa | Protein coding | CCDS57528 | Q6PE80 | TSL:1 GENCODE basic APPRIS ALT2 |
| Axl-204 | ENSMUST00000132038.2 | 563 | 188aa | Protein coding | - | F6YPR4 | 5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3 |
| Axl-205 | ENSMUST00000132989.7 | 2609 | No protein | Retained intron | - | - | TSL:2 |
| Axl-206 | ENSMUST00000137211.1 | 777 | No protein | Retained intron | - | - | TSL:3 |
| Axl-203 | ENSMUST00000124442.7 | 602 | No protein | Retained intron | - | - | TSL:2 |
| Axl-207 | ENSMUST00000137383.1 | 455 | No protein | Retained intron | - | - | TSL:3 |
| Axl-208 | ENSMUST00000147680.1 | 382 | No protein | Retained intron | - | - | TSL:3 |

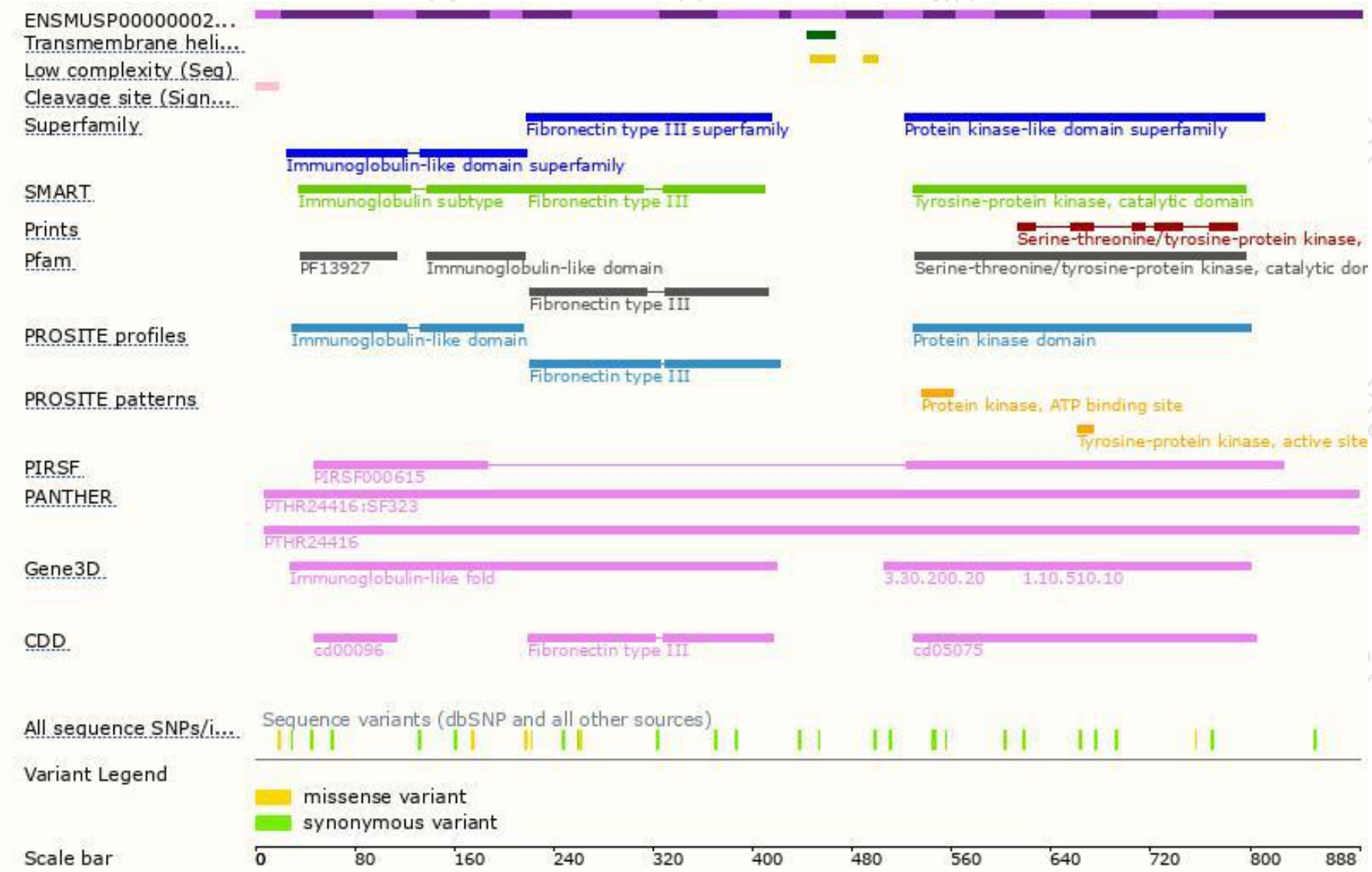
The strategy is based on the design of *Axl-201* transcript,the transcription is shown below:



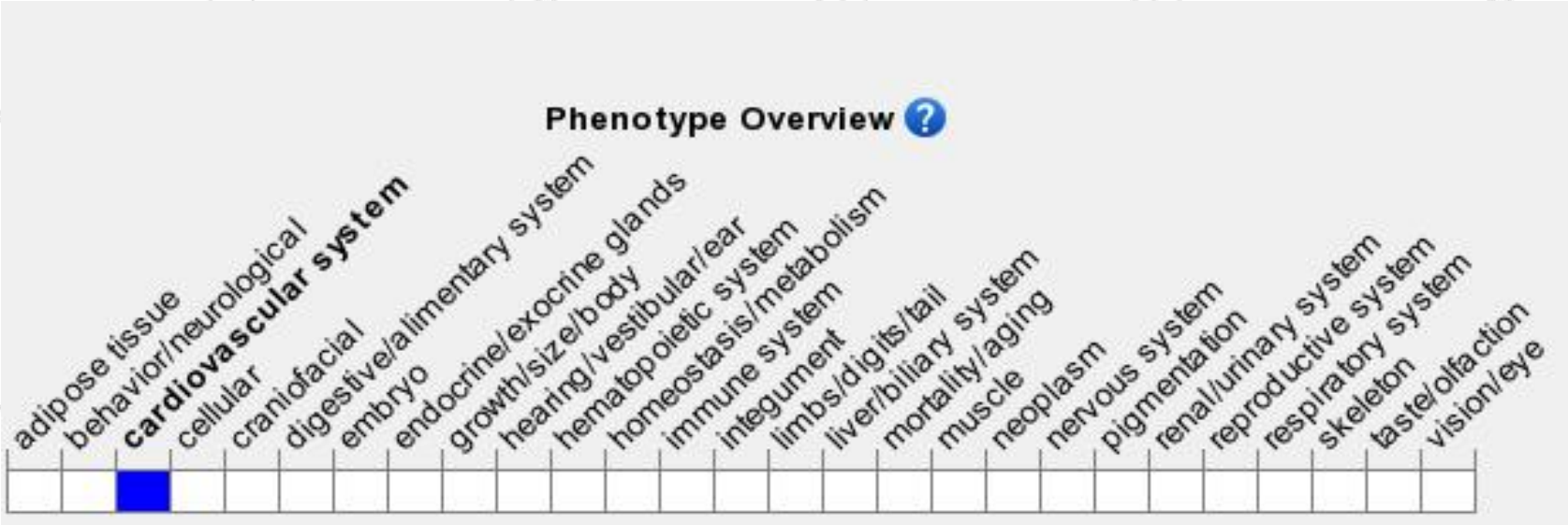
# Genomic location distribution



# 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/).*

According to the existing MGI data,homozygous mutant mice are phenotypically normal, however in conjunction with mutations in other related receptor tyrosine kinases, mutations of this gene results in fertility defects, autoimmunity abnormalities, and aberrant apoptosis.

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

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