Sox10-c.32A>T&c.579G insert Mouse Model Strategy -CRISPR/Cas9 technology

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Project Overview





Technical Description



- The mouse *Sox10* gene has 4 transcripts.
- This project produced Sox10-c.32A>T&c.579G insert point mutation on exon3&exon4 of the transcript of Sox10-203(ENSMUST00000230532.1), C. 32A>T mutation was produced in exon3 of mouse Sox10-203, and the corresponding amino acid at position 11 was mutated from E to V. After the production of C. 579G by exon4, G base was inserted, resulting in the backward shift mutation of amino acid at position 193.
- In this project, *Sox10* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: In vitro, sgRNA and donor vectors were constructed. Cas9, sgRNA and donor were injected into the fertilized eggs of C57BL/6JGpt mice for homologous recombination, and obtained positive F0 mice identified by PCR and sequencing analysis. The stable inheritable positive F1 mice model was obtained by mating F0 mice with C57BL/6JGpt mice.

Mutation Site



Before mutation

After mutation

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| | M A E E Q D L S E V E L S P V G S E E P R C L S P G S A P S L G P |
| 1 | ATGGCCGAGG AACAAGACCT ATCAGAGGTG GAGCTGAGCC CTGTGGGCTC GGAGGAACCC CGCTGCCTGT CCCCAGGCAG CGCGCCGTCG CTGGGACCC |
| +1 | 20 G G G G S G L R A S P G P G E L G K V K K E Q Q D G E A D D D I |
| 101 | ACGGCGGCGG CGGTGGCTCG GGCTTGCGAG CCAGCCCGGG GCCCGGTGAA CTGGGCAAGG TCAAGAAGGA ACAGCAGGAC GGCGAGGCGG ACGATGACA |
| +1 | % FP V C I R E A V S Q V L S G Y D V T L V P M P V R V N G A S K S |
| 201 | GTTCCCCGTG TGCATCCGCG AGGCGGTCAG CCAGGTGCTC AGCGGCTACG ACTGGACGCT GGTGCCCATG CCCGTGCGCG TCAACGGTGC CAGCAAGAC |
| +1 | KPH V K R P M N A F M V W A Q A A R R K L A D Q Y P H L H N A E |
| 301 | AAGCCGCACG TCAAGAGGCC CATGAACGCC TTCATGGTGT GGGCACAGGC GGCACGCAGA AAGCTAGCCG ACCAGTACCC TCACCTCCAC AATGCTGAC |
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| 401 | TCAGCAAGAC ACTAGGCAAG CTCTGGAGGT TGCTGAACGA AAGTGACAAG CGCCCCTTCA TTGAGGAGGC TGAGAGGCTC CGGATGCAGC ACAAAAAGG |
| +1 | 20 H P D Y K Y Q P R R K N G K A A Q G E A E C P G G E A E Q G G A |
| 501 | CCATCCGGAC TACAAGTACC AACCTCGGCG GCGGAAGAAC GGGAAGGCAG CCCAGGGGGA GGCAGAATGC CCAGGCGG G AAGCCGAGCA AGGAGGGGG |
| +1 | A A I Q A H Y K S A H L D H R H P E E G S P M S D G N P E H P S G |
| 601 | SCTSCTATTC ASSCTCACTA CAASAGTSCC CACCTGSACC ACCSSCACCC ASAAGAAGSC TCCCCCATST CASATSSGAA CCCASASCAC CCCTCASS |
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The green region is exon3&exon4 of Sox10-203, and the yellow region and red region represents the c.32A > T&c.579G

insert mutation site.

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Strategy



This model uses CRISPR/Cas9 technology to edit the Sox10 gene and the schematic diagram is as follow:



Notice



- According to the data of MGI, Homozygotes for null mutations lack peripheral glial cells, melanocytes, and autonomic and enteric neurons, and die neonatally or sooner. Heterozygotes exhibit white spotting and megacolon.
- ➤ Transcript *Sox10-202* and *Sox10-204* may not be affected.
- > The phenotype of this model cannot be guaranteed to be completely consistent with that of the reference literature.
- > One to two synonymous mutations of amino acids will be intronduced on exon3&exon4 of Sox10.
- > The mutation was located close to the 5' terminal of *GM10863* gene, and its effect on it was unknown.
- Mouse Sox10 gene is located on Chr15. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr15, it may be extremely hard to get double gene positive homozygotes.
- 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 name and location (NCBI)



☆ ?

☆ ?

Sox10 SRY (sex determining region Y)-box 10 [Mus musculus (house mouse)]

Gene ID: 20665, updated on 3-Jan-2021

Summary



Genomic context



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400-9660890

Transcript information (Ensembl)

The gene has 4 transcripts, and all transcripts are shown below:

| Name 🖕 | Transcript ID 💧 | bp 🍦 | Protein 🖕 | Biotype 🖕 | CCDS 🔺 | UniProt Match | Flags | | |
|-----------|----------------------|------|--------------|-----------------|--------------------|-----------------|-------------------------------|--|--|
| Sox10-203 | ENSMUST00000230532.1 | 2780 | <u>466aa</u> | Protein coding | <u>CCDS49668</u> @ | <u>Q04888</u> @ | GENCODE basic APPRIS P1 | | |
| Sox10-201 | ENSMUST0000040019.4 | 2713 | <u>466aa</u> | Protein coding | CCDS49668@ | <u>Q04888</u> | TSL:1 GENCODE basic APPRIS P1 | | |
| Sox10-202 | ENSMUST00000230261.1 | 863 | <u>205aa</u> | Protein coding | | A0A2R8V124 | CDS 3' incomplete | | |
| Sox10-204 | ENSMUST00000230891.1 | 982 | No protein | Retained intron | | - | H (| | |

The strategy is based on the design of *Sox10-203* transcript, the transcription is shown below:



Genomic location distribution





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Protein domain



| ENSMUSP00000155 MobiDB lite Low complexity (Seg) Superfamily | | Hig | gh mobility group box d | omain superfamily | - | - . | | | _ | | _ |
|---|----------------|-----------------------|-------------------------|-------------------|-------|----------------|-----|-----|-----|-----|-----|
| SMARI | | | High mobility grou | o box domain | | | | | | | |
| Pfam. | Sox develo | pmental protein N-ter | minal | | | | | | | | |
| - | | | High mobility grou | p box domain | | | | | | | |
| PROSITE profiles | | | High mobility grou | p box domain | | | | | | | |
| PANTHER | PTHR45803:SF6 | | | | | | | | | | |
| | PTHR45803 | | | | | | | | | | |
| Gene3D | | | High mobility group | box domain superf | amily | | | | | | |
| CDD | | | cd01388 | | | | | | | | |
| All sequence SNPs/i | Sequence varia | ants (dbSNP and all | other sources) | 11 | | | | 0 | 11 | | 10 |
| Variant Legend | synonym | ous variant | | | | | | | | | |
| Scale bar | 0 | 40 80 | 120 | 160 | 200 | 240 | 280 | 320 | 360 | 400 | 466 |
| | | | | | | | | | | | |

Mouse phenotype description(MGI)



URL link is as follows: http://www.informatics.jax.org/marker/MGI:98358



Click cells to view annotations.

Homozygotes for null mutations lack peripheral glial cells, melanocytes, and autonomic and enteric neurons, and die neonatally or sooner. Heterozygotes exhibit white spotting and megacolon.

If you have any questions, please feel free to contact us. Tel: 025-5864 1534





