Isl2 Cas9-CKO Strategy

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Project Overview

Project Name	***Isl2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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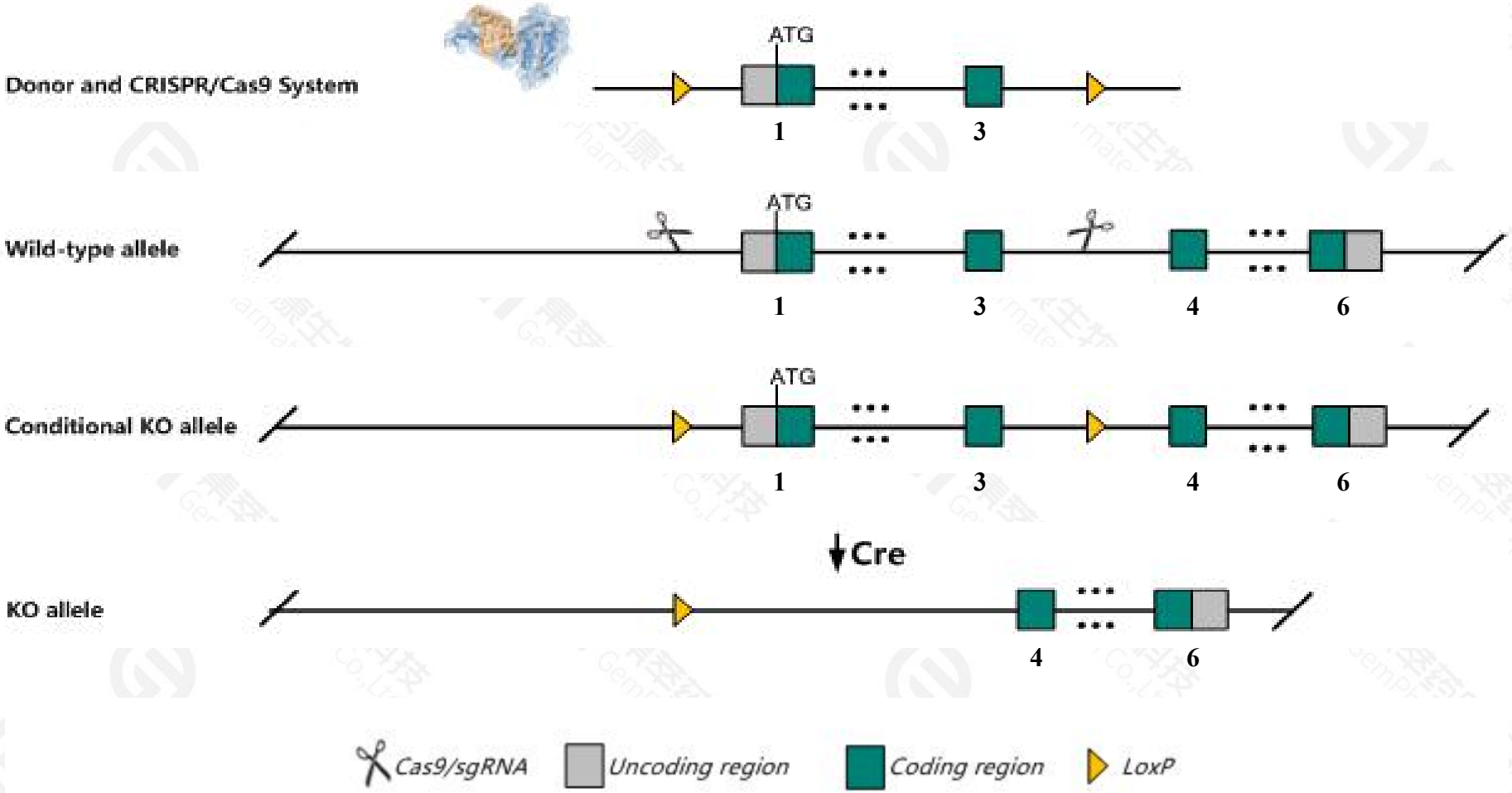
This model will use CRISPR/Cas9 technology to edit the *Isl2* gene. The schematic diagram is as follows:



Technical routes

The *Isl2* gene has 5 transcripts. According to the structure of *Isl2* gene, exon1-exon3 of *Isl2-201*(ENSMUST00000034869.11) transcript is recommended as the knockout region.The region contains start codon ATG.Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Isl2* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor 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.

The flox mice was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Notice

According to the existing MGI data,mutations of this gene result in neonatal lethality, motor neuron migration defects and impaired visceral motor neuron differentiation.

The *Isl2* gene is located on the Chr9. If the knockout 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 biological processes,all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information NCBI

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Isl2 insulin related protein 2 (islet 2) [Mus musculus (house mouse)]

Gene ID: 104360, updated on 12-Jan-2021

Summary

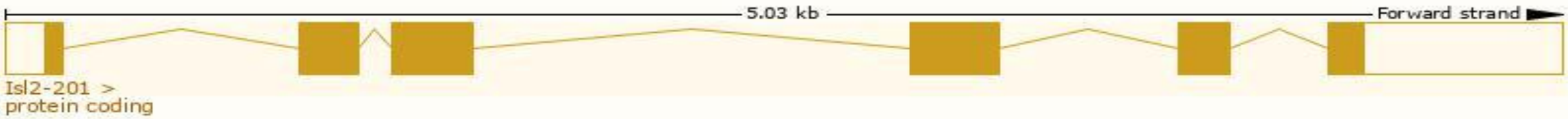
Official Symbol	Isl2 provided by MGI
Official Full Name	insulin related protein 2 (islet 2) provided by MGI
Primary source	MGI:MGI:109156
See related	Ensembl:ENSMUSG00000032318
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	3110001N10Rik, isle
Expression	Biased expression in CNS E11.5 (RPKM 2.3), colon adult (RPKM 1.4) and 11 other tissues See more
Orthologs	human all

Transcript information Ensembl

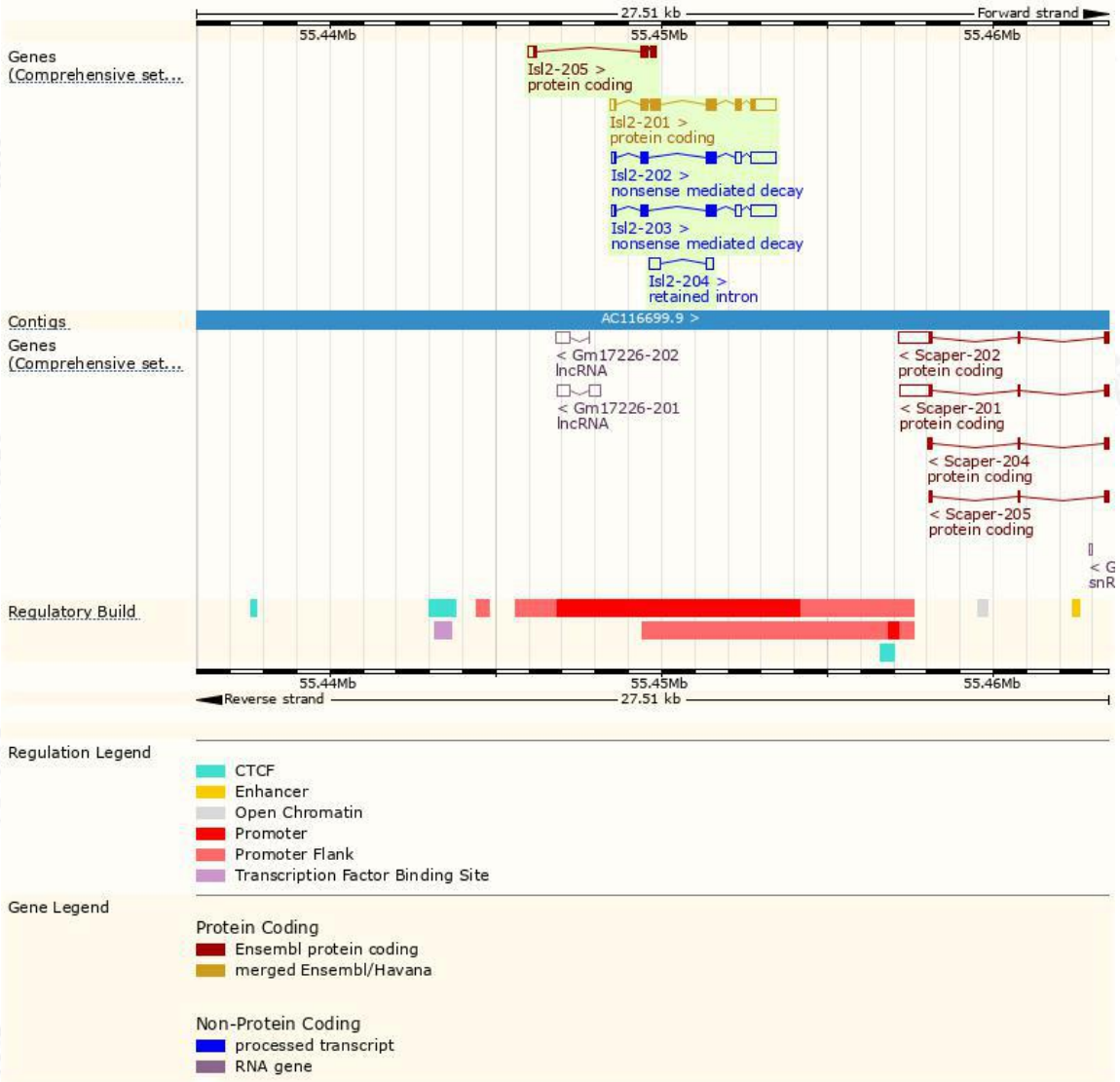
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Isl2-201	ENSMUST00000034869.11	1854	359aa	Protein coding	CCDS23205		TSL:1 , GENCODE basic , APPRIS P1 ,
Isl2-205	ENSMUST00000175950.8	637	151aa	Protein coding	-		CDS 3' incomplete , TSL:3 ,
Isl2-203	ENSMUST00000164373.8	1535	174aa	Nonsense mediated decay	-		TSL:1 ,
Isl2-202	ENSMUST00000114290.4	1532	174aa	Nonsense mediated decay	-		TSL:1 ,
Isl2-204	ENSMUST00000165302.2	529	No protein	Retained intron	-		TSL:3 ,

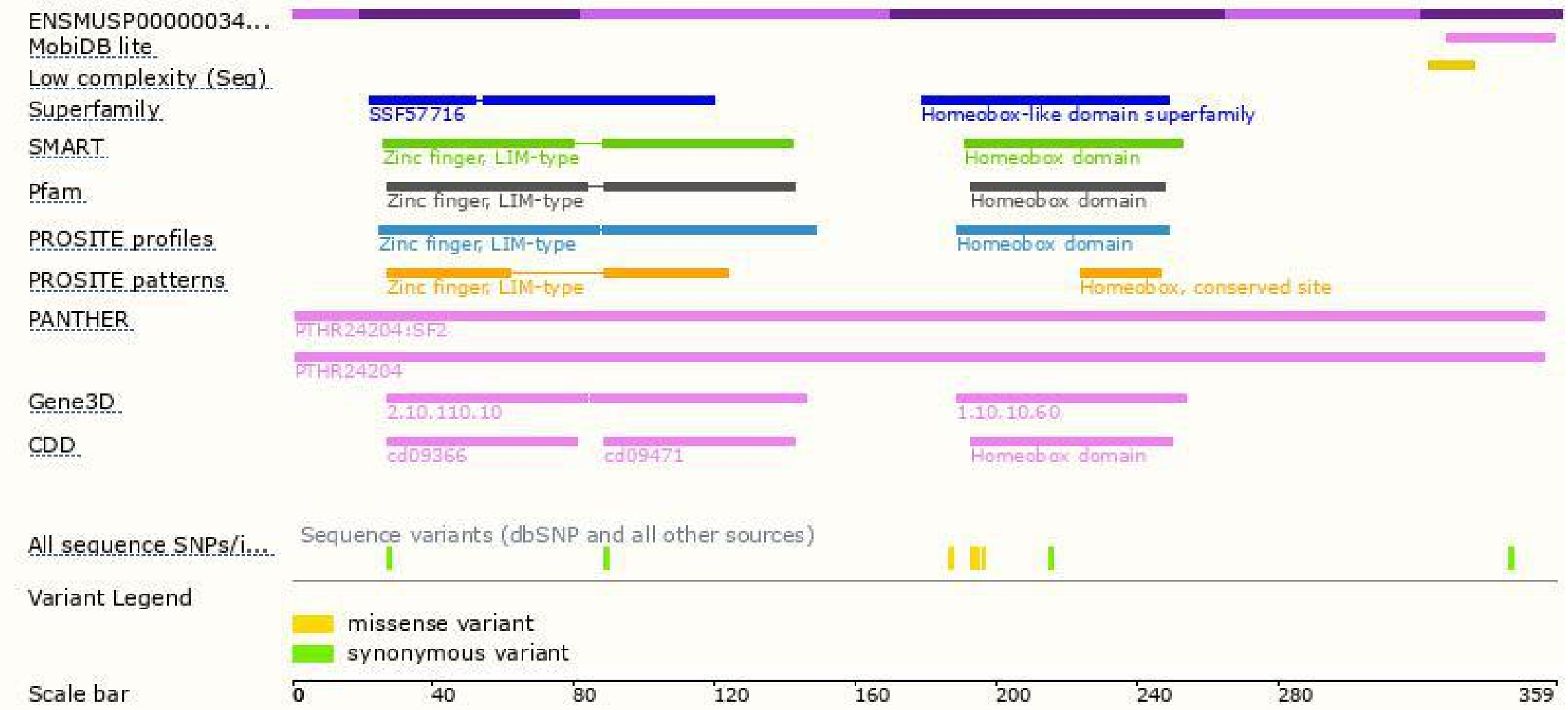
The strategy is based on the design of *Isl2-201* transcript,the transcription is shown below:



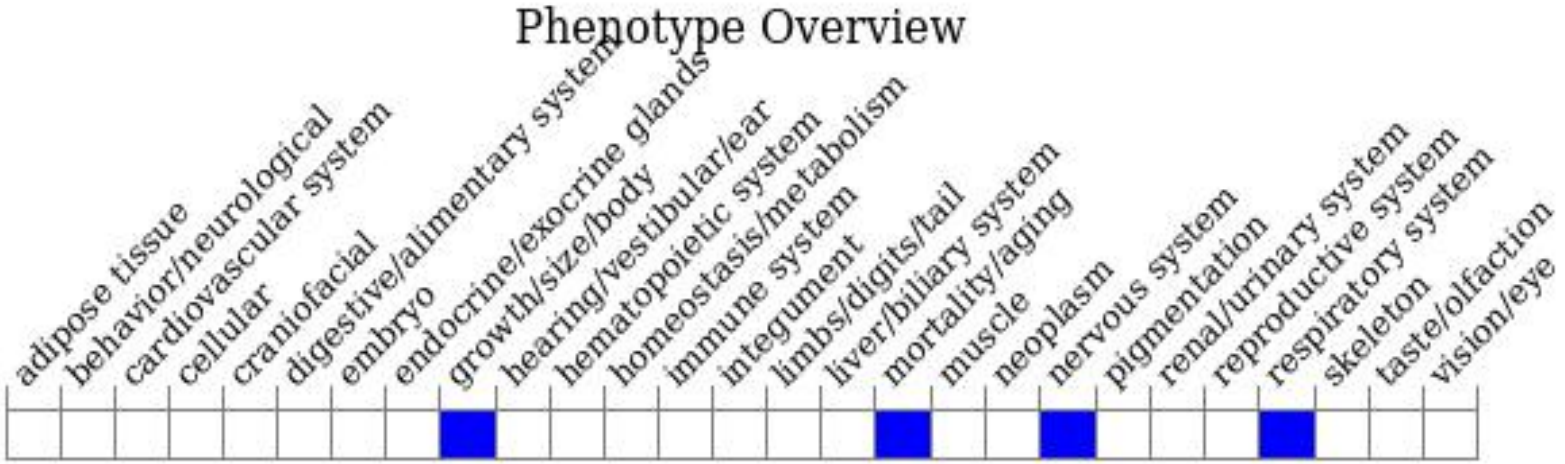
Genomic location distribution



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,mutations of this gene result in neonatal lethality, motor neuron migration defects and impaired visceral motor neuron differentiation.

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

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