

***Slirp* Cas9-CKO Strategy**

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Reviewer: Xiaojing Li

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Project Overview

Project Name

Slirp

Project type

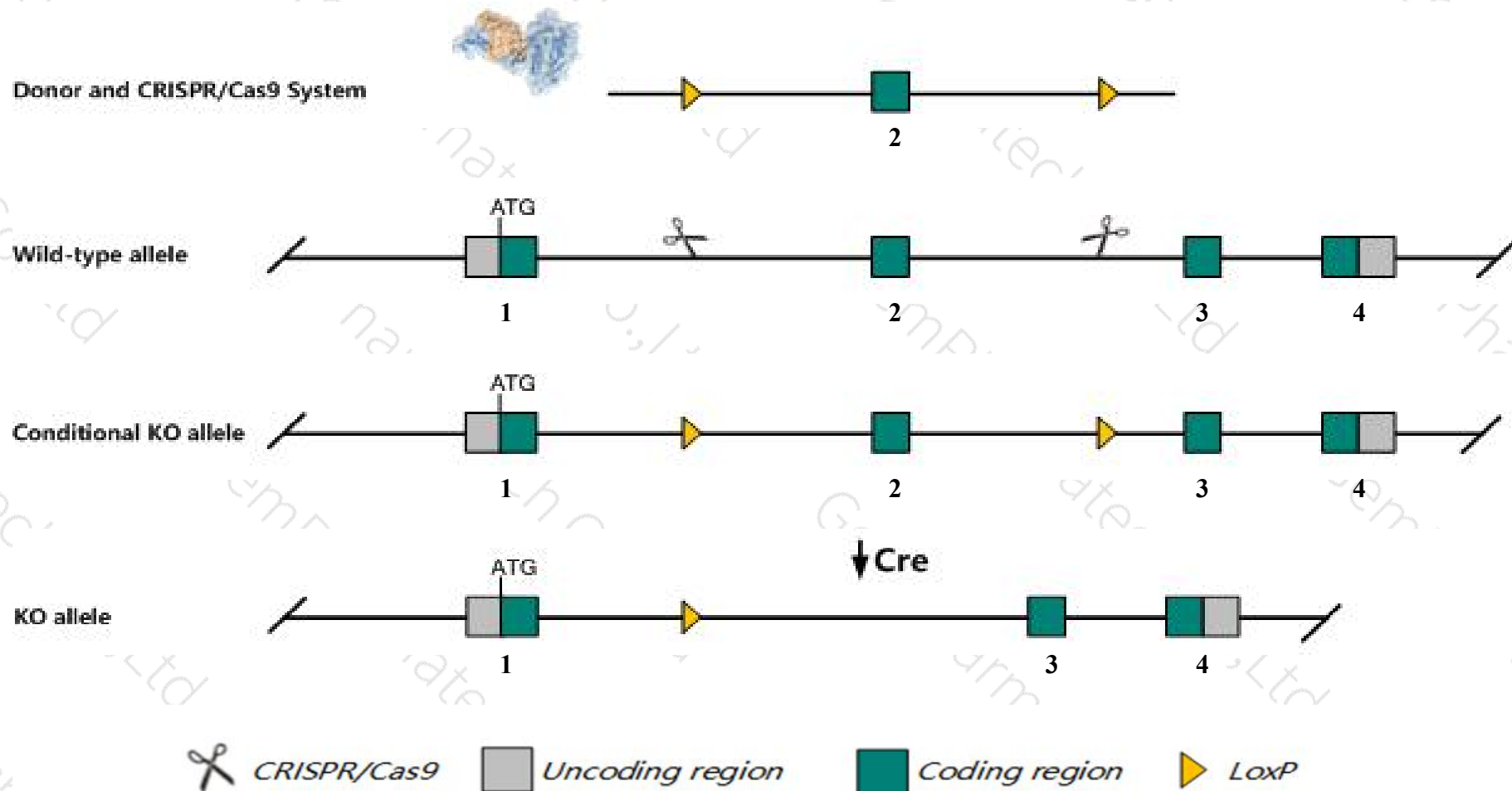
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



Technical routes

- The *Slirp* gene has 4 transcripts. According to the structure of *Slirp* gene, exon2 of *Slirp*-204(ENSMUST00000161023.7) transcript is recommended as the knockout region. The region contains 59bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Slirp* 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.
- The flox mice will be 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.

- According to the existing MGI data, homozygous inactivation of this gene results in partial preweaning lethality and reduced male fertility characterized by asthenozoospermia and abnormal sperm annulus and sperm mitochondrial sheath morphology.
- The flox region is about 3.5 kb away from the 5th end of the *Alkbh1* gene, which may affect the regulation of this gene.
- The *Slirp* gene is located on the Chr12. 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)

Slirp SRA stem-loop interacting RNA binding protein [Mus musculus (house mouse)]

Gene ID: 380773, updated on 13-Mar-2020

Summary



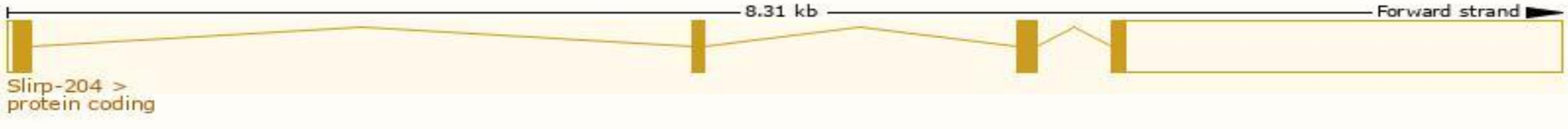
Official Symbol	Slirp provided by MGI
Official Full Name	SRA stem-loop interacting RNA binding protein provided by MGI
Primary source	MGI:MGI:1916394
See related	Ensembl:ENSMUSG00000021040
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1810035L17Rik
Expression	Ubiquitous expression in placenta adult (RPKM 41.2), liver E14 (RPKM 38.2) and 23 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

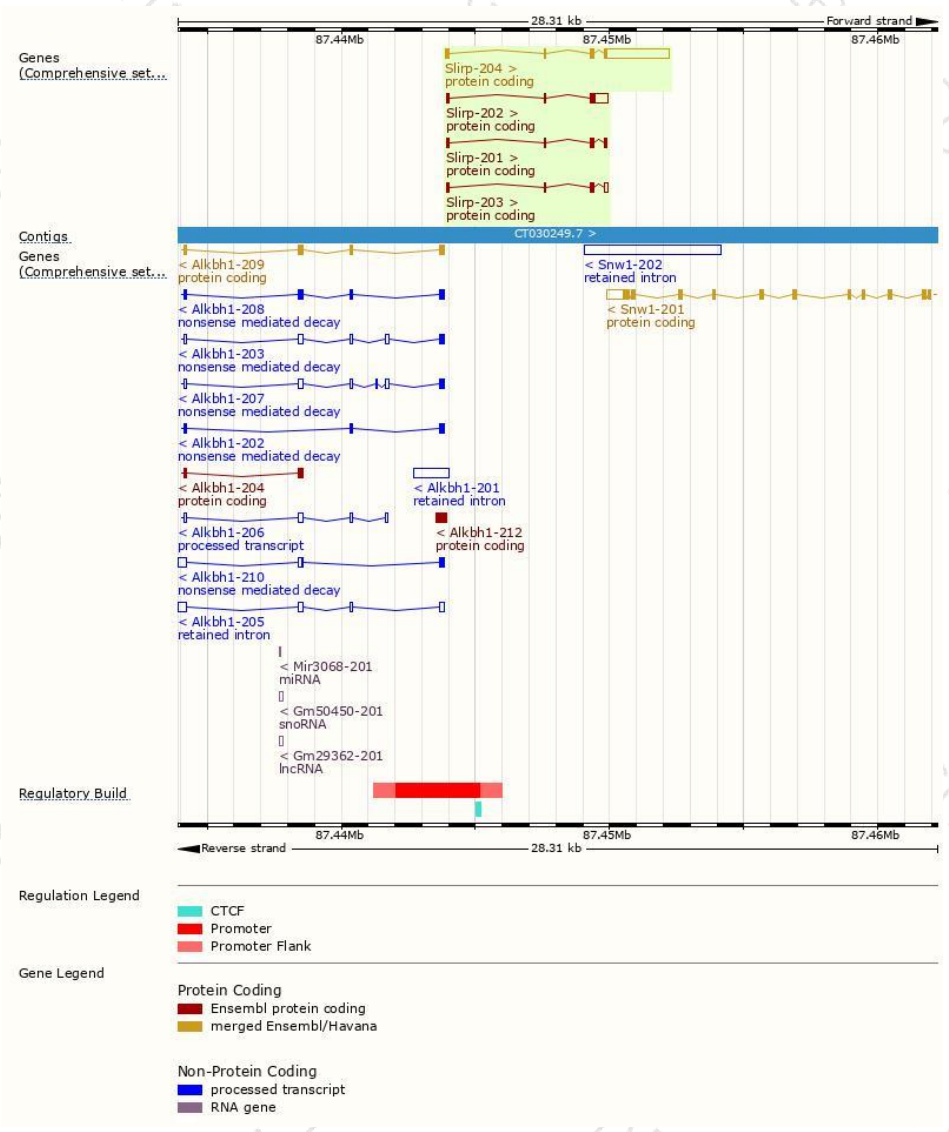
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Slirp-204	ENSMUST00000161023.7	2701	112aa	Protein coding	CCDS36503	Q14AR0 Q9D8T7	TSL:1 GENCODE basic APPRIS P2
Slirp-202	ENSMUST00000160488.7	802	99aa	Protein coding	-	Q9D8T7	TSL:1 GENCODE basic APPRIS ALT2
Slirp-203	ENSMUST00000160880.1	362	79aa	Protein coding	-	F7DE82	CDS 5' incomplete TSL:5
Slirp-201	ENSMUST00000077462.7	335	102aa	Protein coding	-	F8WHU8	CDS 5' incomplete TSL:5

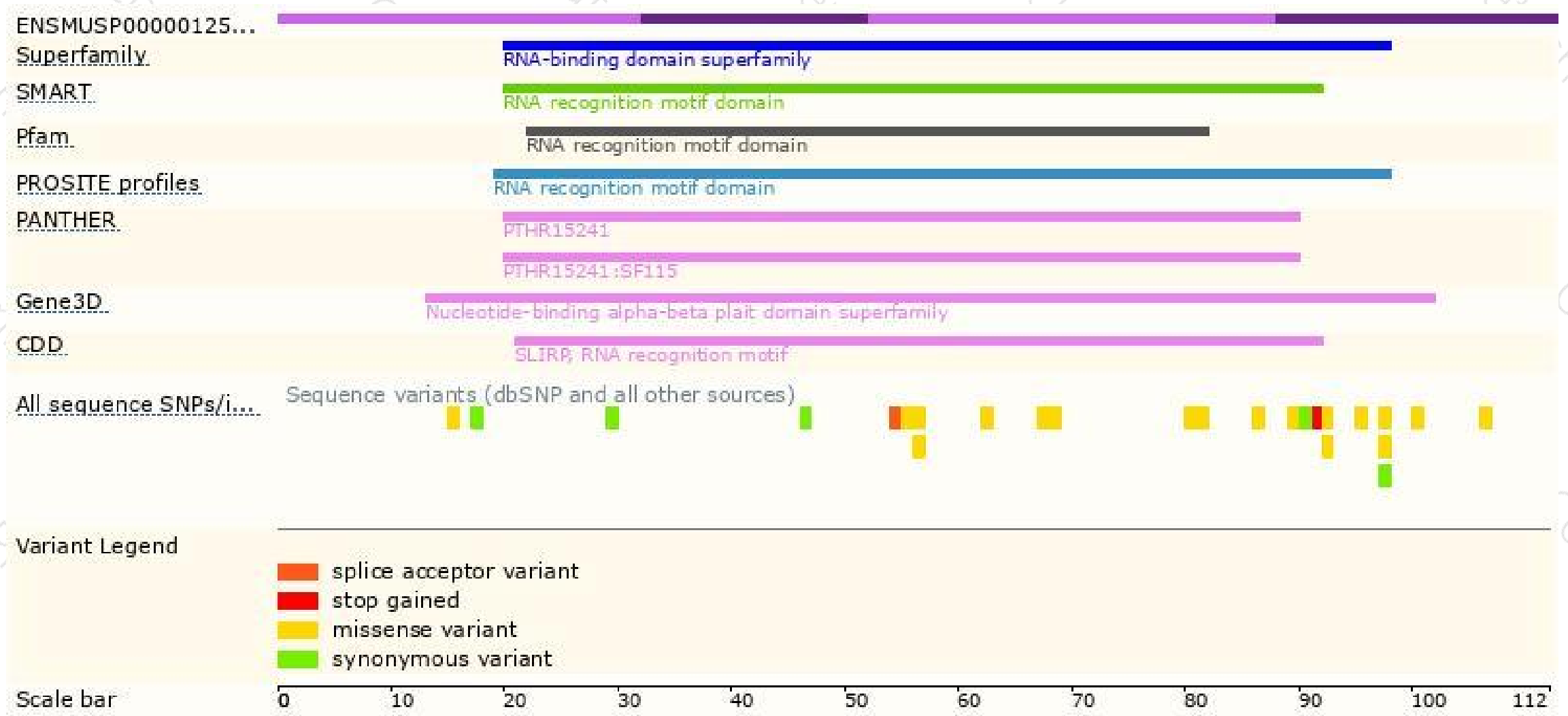
The strategy is based on the design of *Slirp-204* transcript,the transcription is shown below:



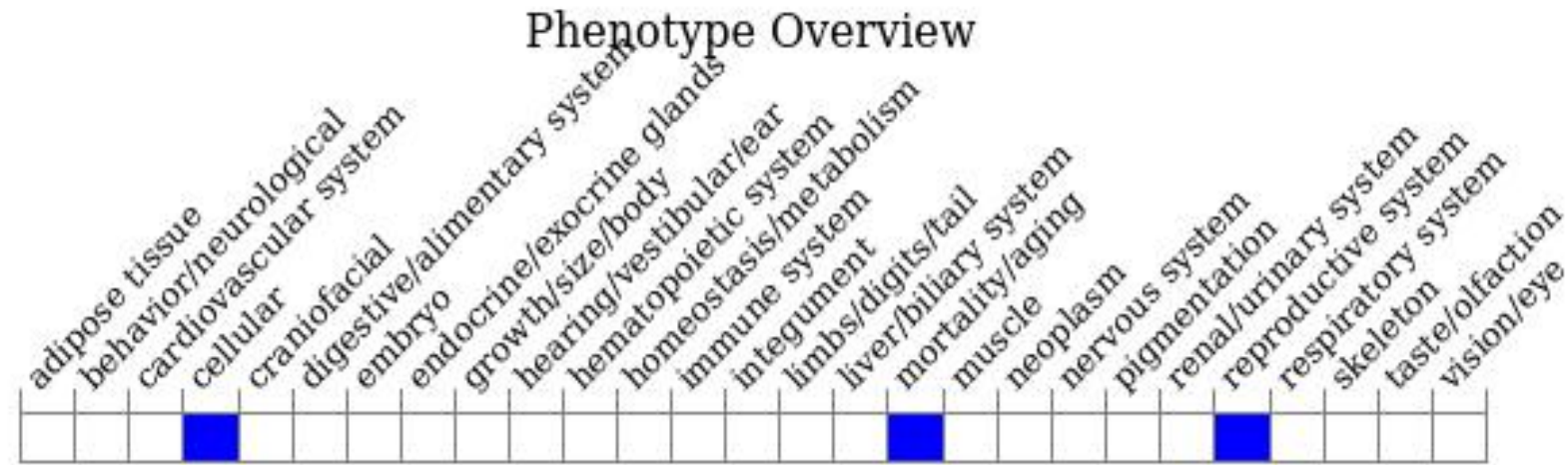
Genomic location distribution



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 inactivation of this gene results in partial preweaning lethality and reduced male fertility characterized by asthenozoospermia and abnormal sperm annulus and sperm mitochondrial sheath morphology.

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

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