

***Rad18* Cas9-CKO Strategy**

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Design Date: 2020-1-8
Reviewer: Jia Yu

Project Overview

Project Name

Rad18

Project type

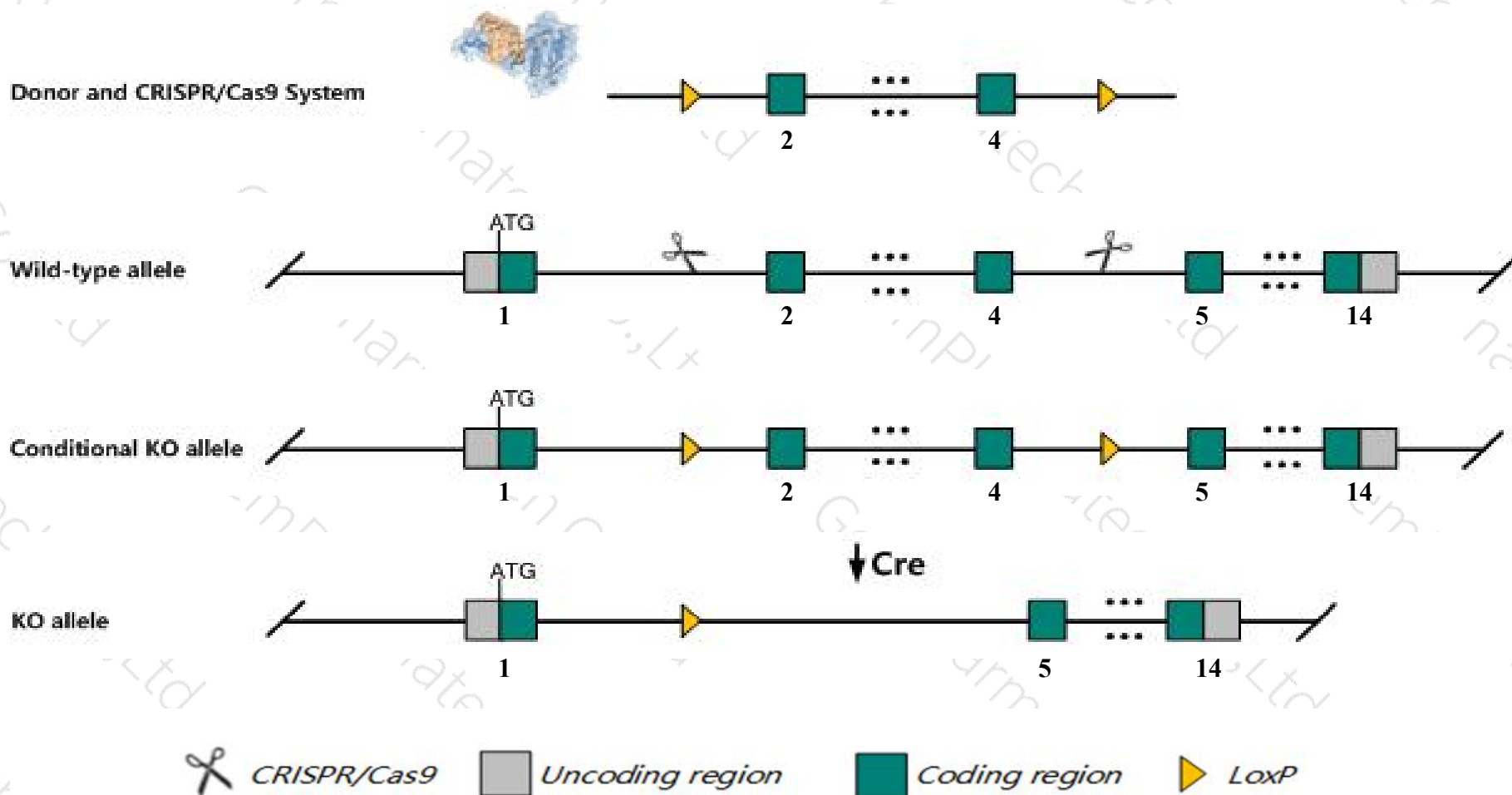
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rad18* gene. The schematic diagram is as follows:



- The *Rad18* gene has 8 transcripts. According to the structure of *Rad18* gene, exon2-exon4 of *Rad18-201* (ENSMUST00000068487.11) transcript is recommended as the knockout region. The region contains 215bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rad18* 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, Male mice homozygous for a null allele exhibit age-dependent decrease in fertility, germ cell number, and testes weight with progressive degeneration of seminiferous tubules.
- The *Rad18* gene is located on the Chr6. 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)

Rad18 RAD18 E3 ubiquitin protein ligase [*Mus musculus* (house mouse)]

Gene ID: 58186, updated on 10-Oct-2019

Summary

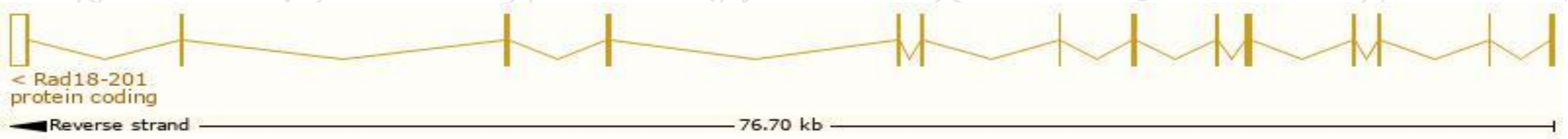
Official Symbol	Rad18 provided by MGI
Official Full Name	RAD18 E3 ubiquitin protein ligase provided by MGI
Primary source	MGI:MGI:1890476
See related	Ensembl:ENSMUSG00000030254
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Rad18sc; 2810024C04Rik
Expression	Broad expression in testis adult (RPKM 6.5), liver E14 (RPKM 4.9) and 19 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

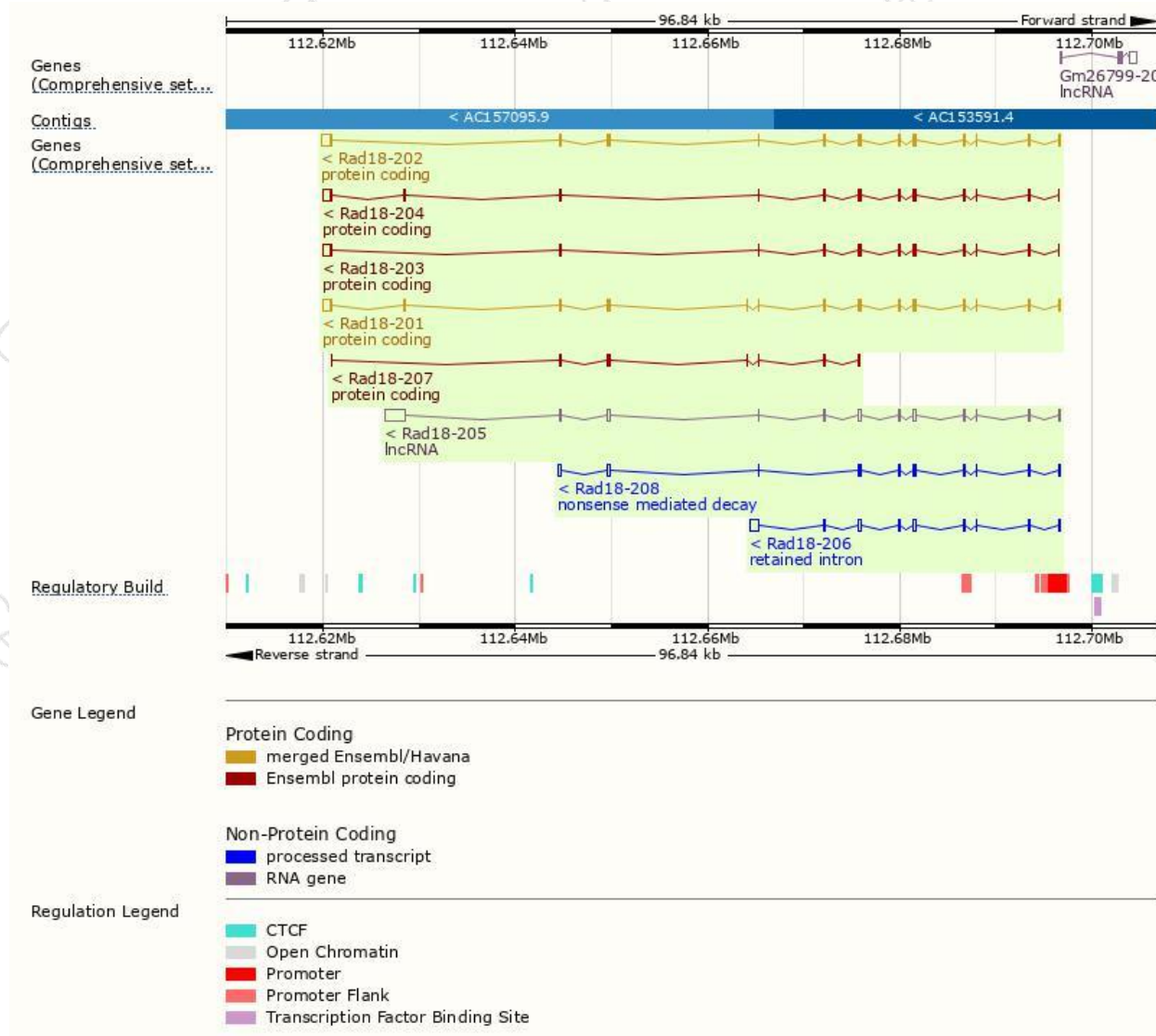
The gene has 8 transcripts,all transcripts are shown below:

Name ▲	Transcript ID ▲	bp ▲	Protein ▲	Biotype ▲	CCDS ▲	UniProt ▲	Flags ▲
Rad18-201	ENSMUST00000068487.11	2577	556aa	Protein coding	CCDS51870	E9Q392	TSL:1 GENCODE basic APPRIS ALT2
Rad18-202	ENSMUST00000077088.10	2577	509aa	Protein coding	CCDS39589	Q9QXK2	TSL:1 GENCODE basic APPRIS P3
Rad18-203	ENSMUST00000113180.7	2136	428aa	Protein coding	-	D3Z734	TSL:5 GENCODE basic APPRIS ALT2
Rad18-204	ENSMUST00000113182.7	2220	449aa	Protein coding	-	D3Z733	TSL:5 GENCODE basic APPRIS ALT2
Rad18-205	ENSMUST00000132590.7	3535	No protein	lncRNA	-	-	TSL:1
Rad18-206	ENSMUST00000135092.1	2004	No protein	Retained intron	-	-	TSL:1
Rad18-207	ENSMUST00000142079.1	724	241aa	Protein coding	-	F6ZKR0	CDS 5' and 3' incomplete TSL:5
Rad18-208	ENSMUST00000156063.1	1496	309aa	Nonsense mediated decay	-	W4VSP9	TSL:5

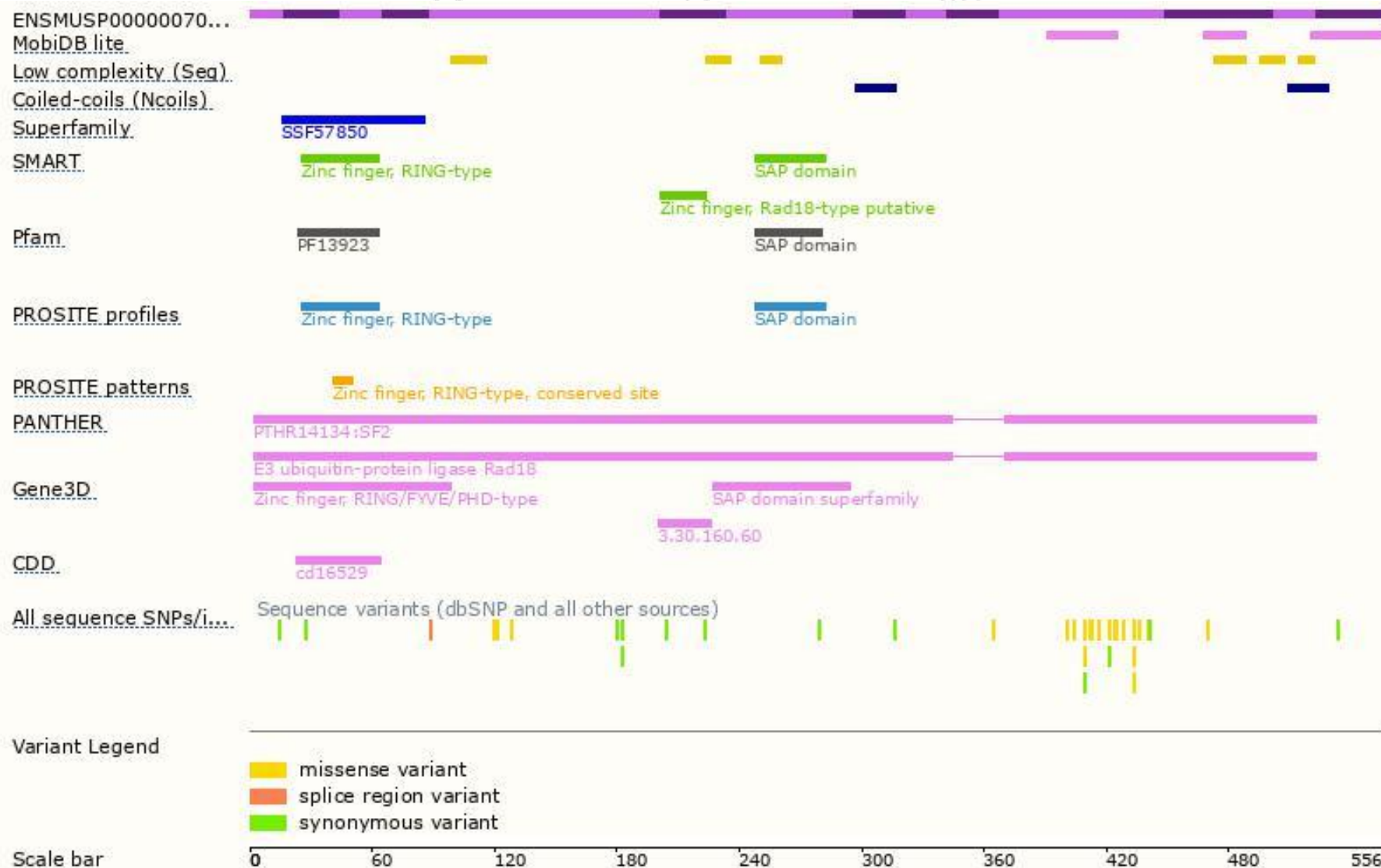
The strategy is based on the design of *Rad18-201* transcript,The transcription is shown below



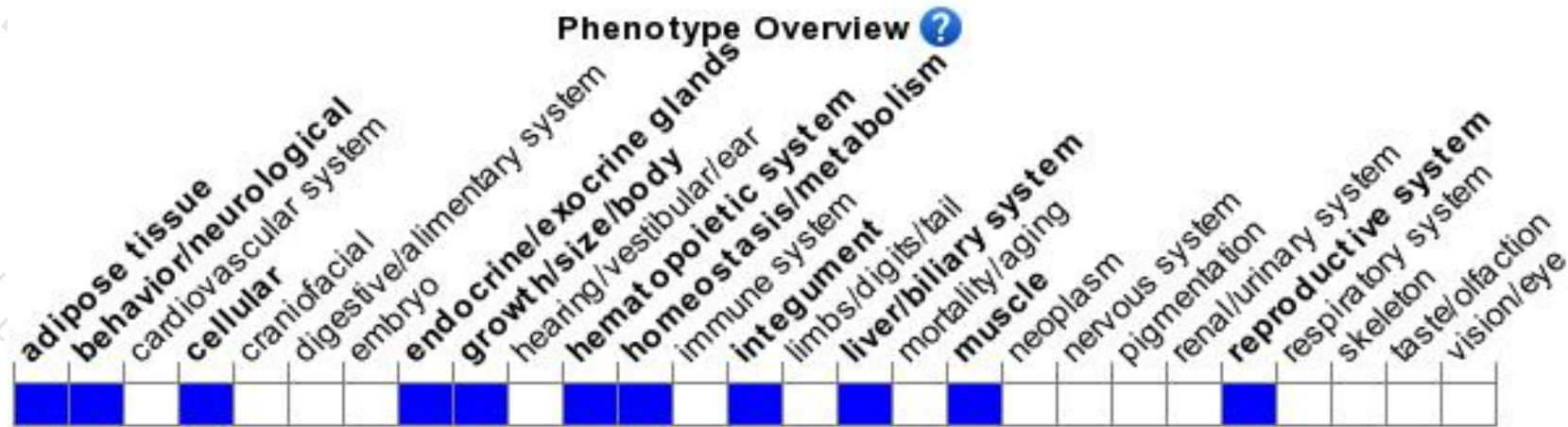
Genomic location distribution



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, Male mice homozygous for a null allele exhibit age-dependent decrease in fertility, germ cell number, and testes weight with progressive degeneration of seminiferous tubules.

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

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