Extl2 Cas9-CKO Strategy

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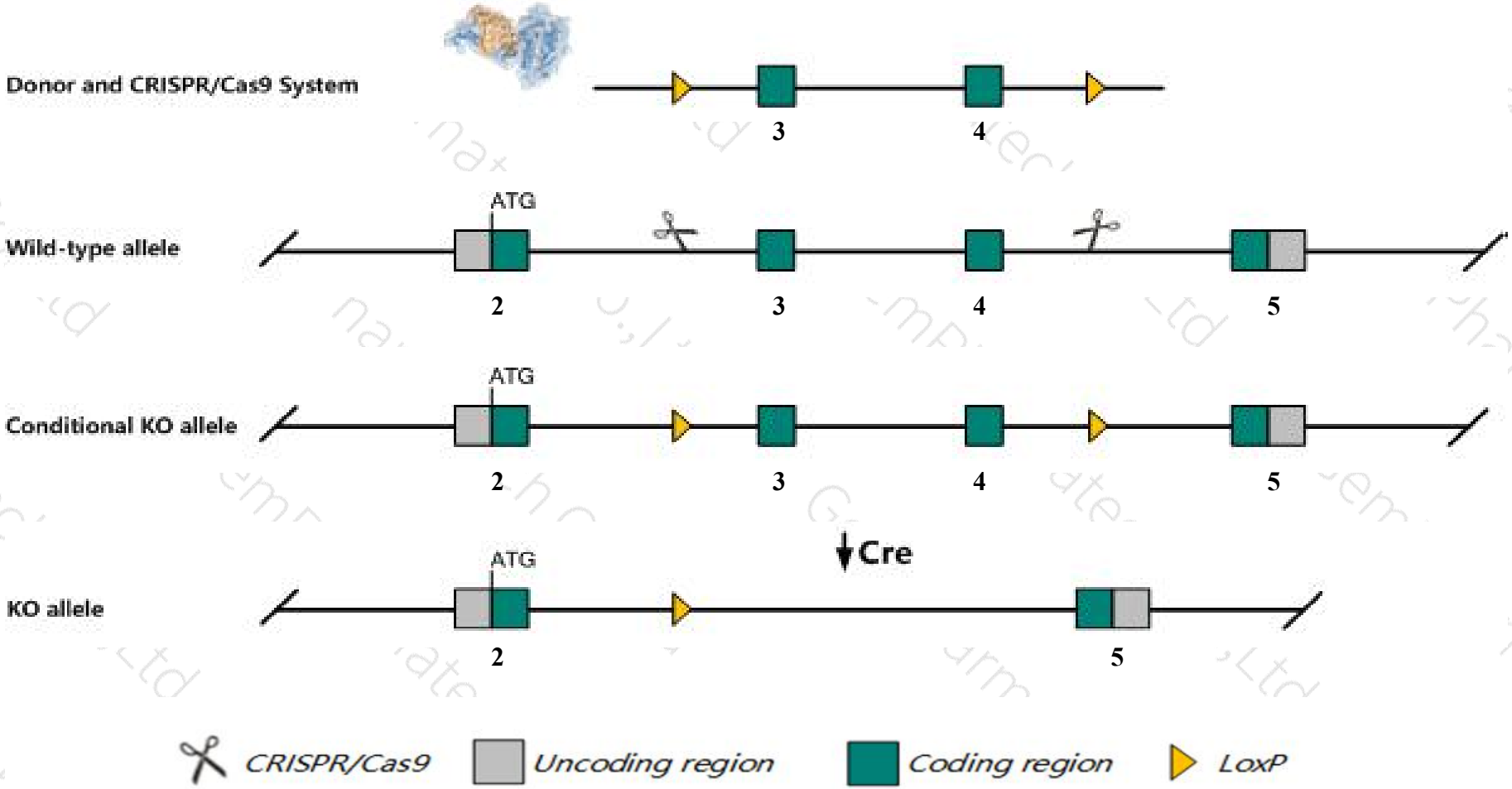
Project Overview

Project Name	***Extl2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Extl2* gene. The schematic diagram is as follows:



Technical routes

- The *Extl2* gene has 6 transcripts. According to the structure of *Extl2* gene, exon3-exon4 of *Extl2-201* (ENSMUST00000029575.11) transcript is recommended as the knockout region.The region contains 499bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Extl2* 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.

Notice

- According to the existing MGI data,Mice homozygous for a knock-out allele exhibit increased production of heparin sulfate and chondroitin sulfate in mouse embryonic fibroblasts, liver and brain.

- The *Extl2* gene is located on the Chr3. 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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Extl2 exostoses (multiple)-like 2 [Mus musculus (house mouse)]

Gene ID: 58193, updated on 31-Jan-2019

Summary

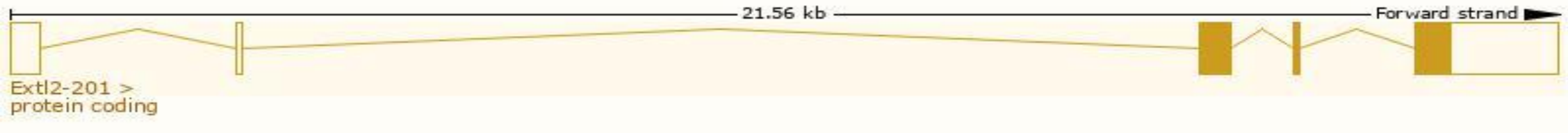
Official Symbol	Extl2 provided by MGI
Official Full Name	exostoses (multiple)-like 2 provided byMGI
Primary source	MGI:MGI:1889574
See related	Ensembl:ENSMUSG00000027963
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	3000001D04Rik, AW146439
Expression	Broad expression in cerebellum adult (RPKM 14.9), cortex adult (RPKM 7.8) and 24 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

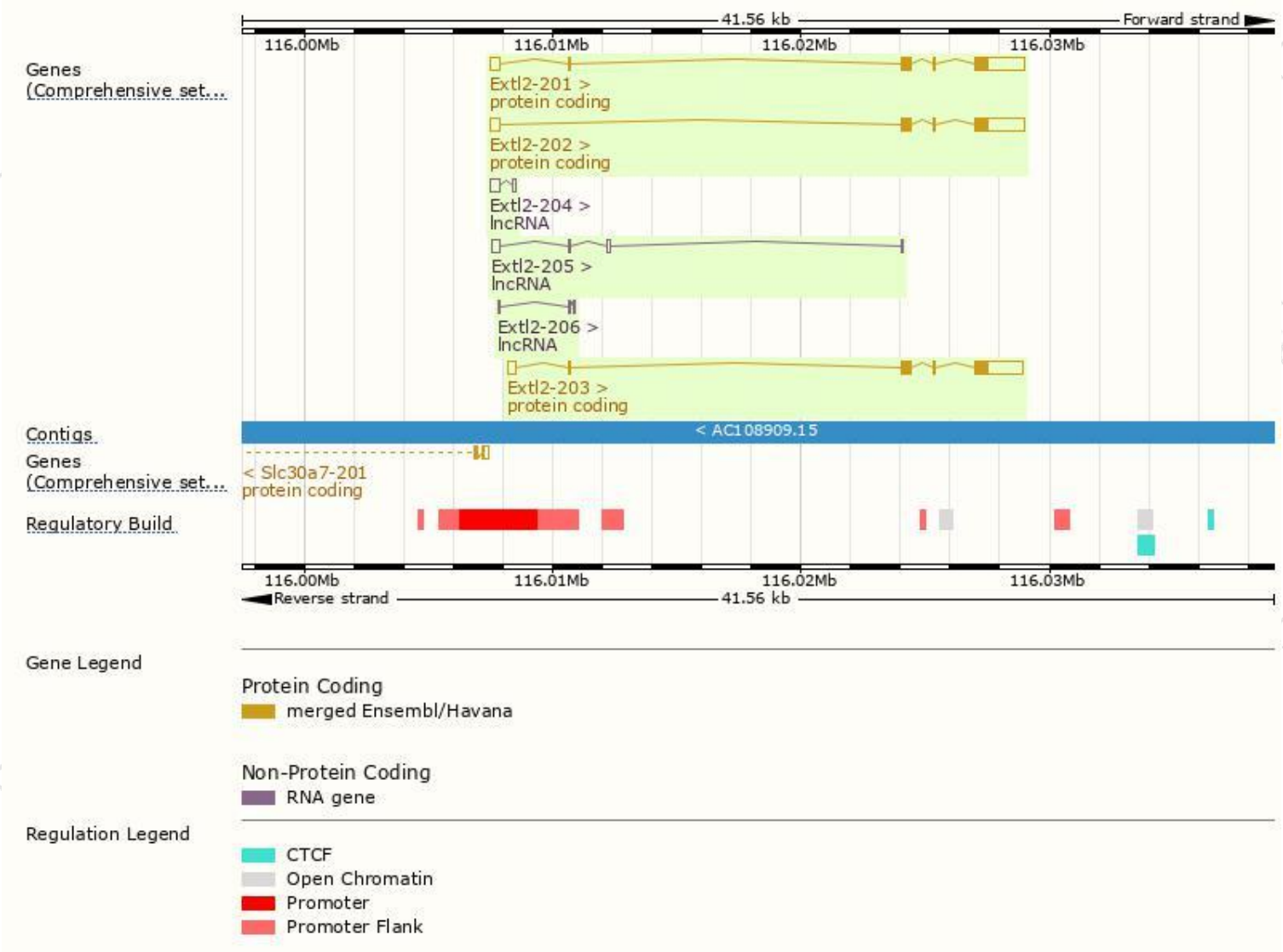
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Extl2-201	ENSMUST00000029575.11	3000	330aa	Protein coding	CCDS17784	Q9ES89	TSL:1 GENCODE basic APPRIS P3
Extl2-202	ENSMUST00000106501.7	2887	316aa	Protein coding	CCDS51057	Q3TSR0	TSL:1 GENCODE basic APPRIS ALT2
Extl2-203	ENSMUST00000106502.1	2840	330aa	Protein coding	CCDS17784	Q9ES89	TSL:1 GENCODE basic APPRIS P3
Extl2-205	ENSMUST00000145945.7	642	No protein	lncRNA	-	-	TSL:5
Extl2-204	ENSMUST00000128228.1	534	No protein	lncRNA	-	-	TSL:3
Extl2-206	ENSMUST00000149920.1	159	No protein	lncRNA	-	-	TSL:3

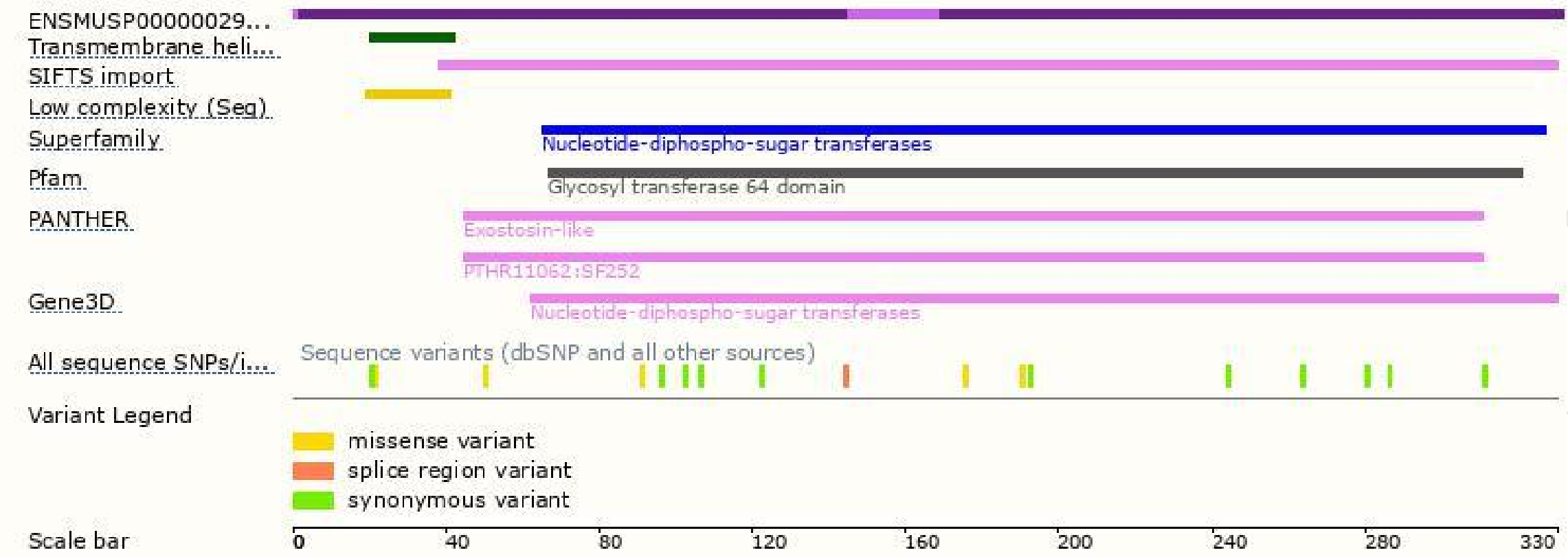
The strategy is based on the design of *Extl2-201* transcript,The transcription is shown below



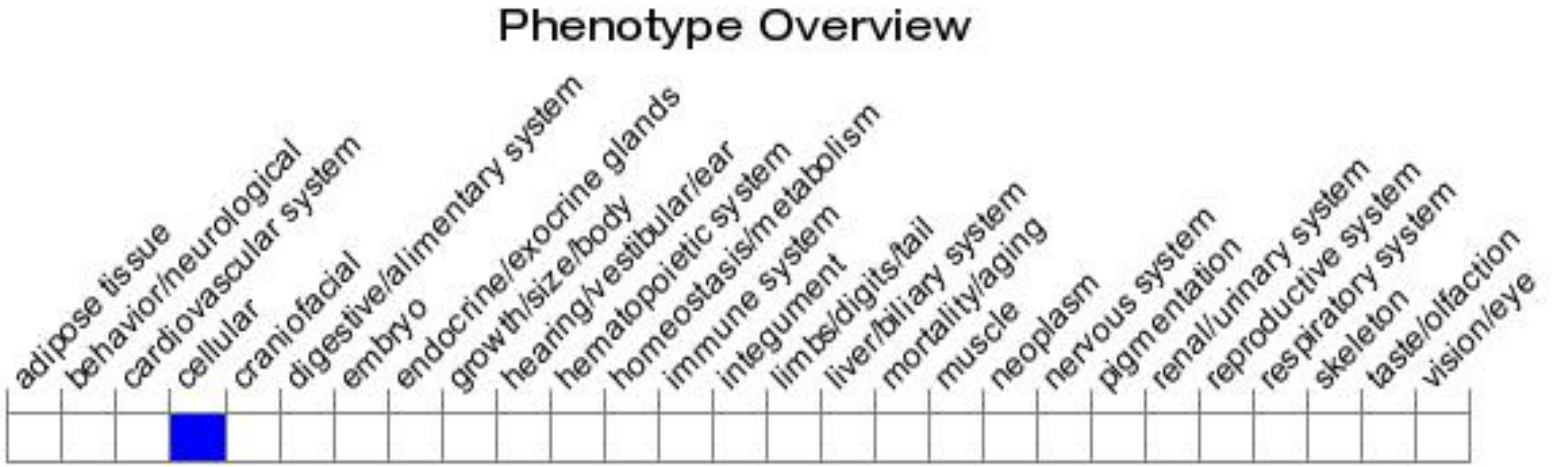
Genomic location distribution



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,Mice homozygous for a knock-out allele exhibit increased production of heparin sulfate and chondroitin sulfate in mouse embryonic fibroblasts, liver and brain.

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

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