Crybb2 Cas9-CKO Strategy

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Project Overview

Project Name	***Crybb2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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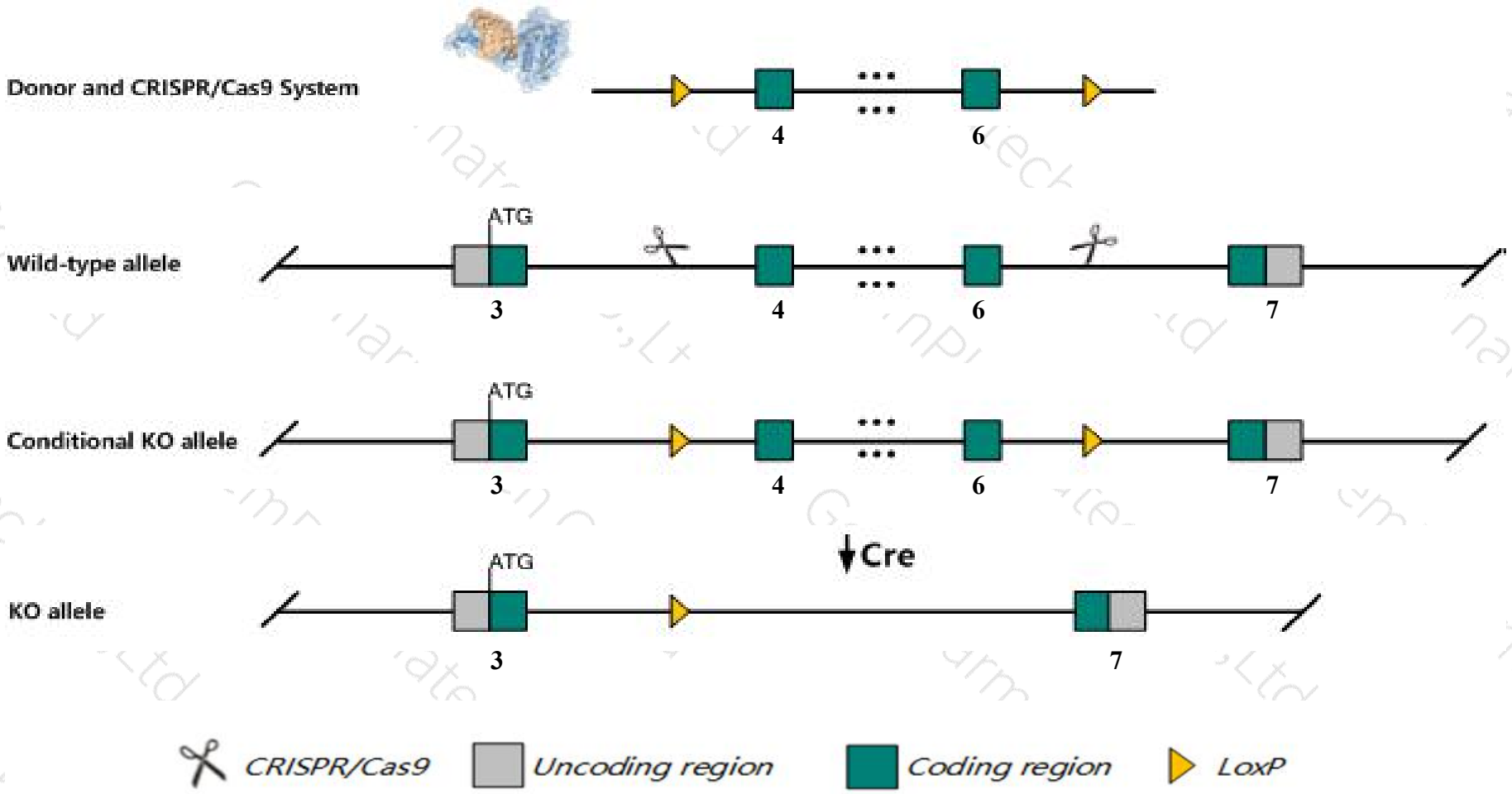
This model will use CRISPR/Cas9 technology to edit the *Crybb2* gene. The schematic diagram is as follows:



Technical routes

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- The *Crybb2* gene has 5 transcripts. According to the structure of *Crybb2* gene, exon4-exon6 of *Crybb2-202* (ENSMUST00000112336.7) transcript is recommended as the knockout region.The region contains 395bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Crybb2* 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,Mutations in this gene cause progressive lens cataracts discernable at 12-15 days of age as mild anterior or cortical opacity and progressing to total opacity by 6-12 weeks of age.

- Transcript *Crybb2*-203&204 may not be affected.

- The *Crybb2* gene is located on the Chr5. 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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Crybb2 crystallin, beta B2 [*Mus musculus* (house mouse)]

Gene ID: 12961, updated on 31-Aug-2019

Summary

Official Symbol	Crybb2 provided by MGI
Official Full Name	crystallin, beta B2 provided by MGI
Primary source	MGI:MGI:88519
See related	Ensembl:ENSMUSG00000042240
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Cryb-2
Summary	This gene is a member of the beta-crystallin family. Beta crystallins, along with alpha and gamma crystallins, are the major proteins found in the eye lens. These proteins maintain the refractive index of the lens whilst also maintaining its transparency. Since lens central fiber cells lose their nuclei during development, crystallins are made and then retained throughout life, making them extremely stable proteins. Beta and gamma crystallins are considered be a superfamily and have a similar domain architecture, including four Greek Key motifs. Beta-crystallins form aggregates of different sizes and are able to self-associate to form dimers or to form heterodimers with other beta-crystallins. The protein encoded by this gene may have Ca2+-binding activity and could be associated with potential functions in the hippocampus and in sperm. Targeted knockout of this gene in mouse induces age-related cataract. A chain-terminating mutation in a similar gene in human was found to cause type 2 cerulean cataracts. [provided by RefSeq, Feb 2015]
Expression	Low expression observed in reference dataset See more
Orthologs	human all

Transcript information（Ensembl）

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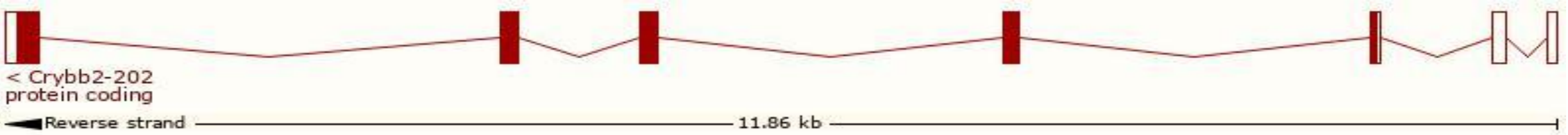
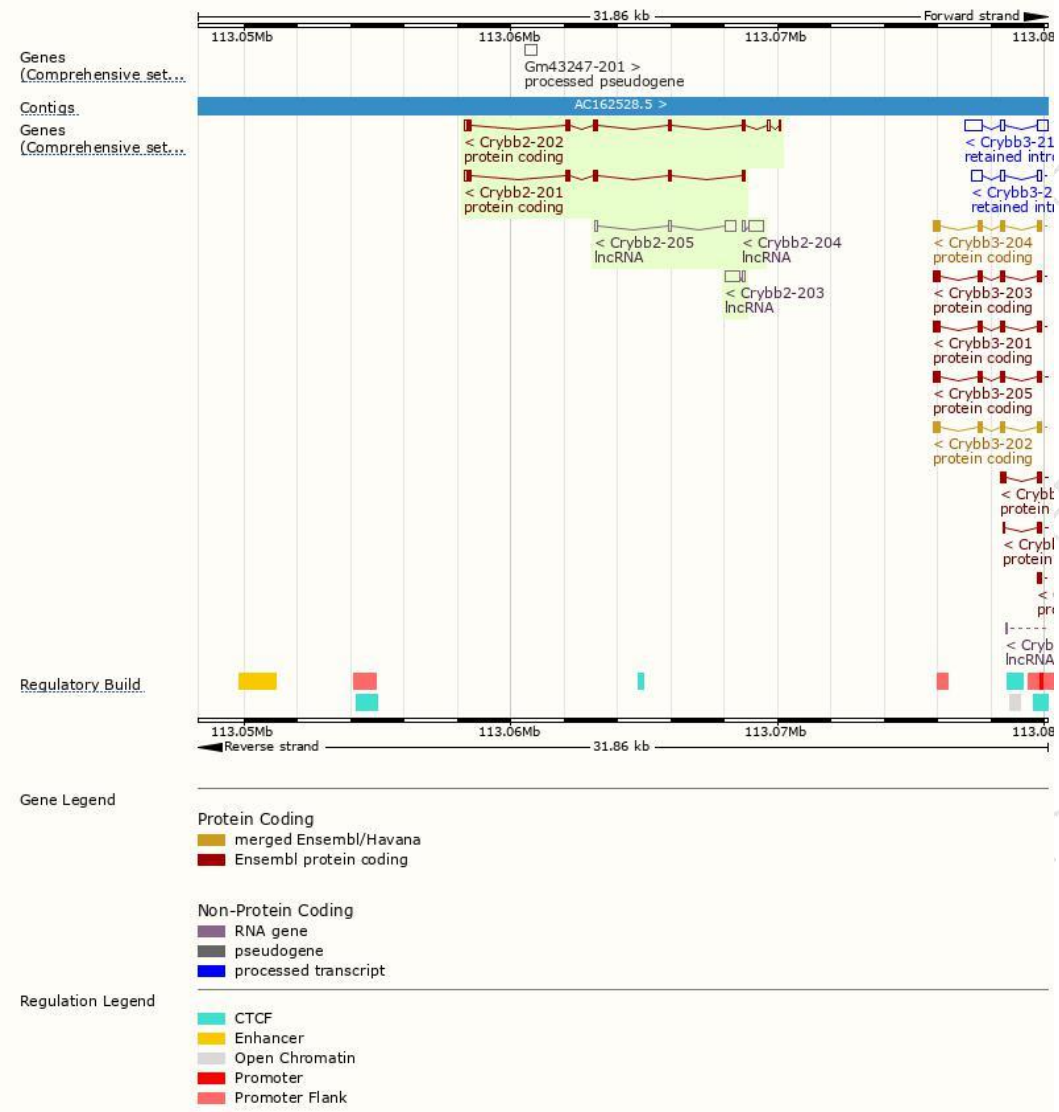
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Crybb2-202	ENSMUST00000112336.7	906	205aa	Protein coding	CCDS19546	P62696	TSL:1 GENCODE basic APPRIS P1
Crybb2-201	ENSMUST00000031295.7	724	205aa	Protein coding	CCDS19546	P62696	TSL:1 GENCODE basic APPRIS P1
Crybb2-204	ENSMUST00000144833.1	653	No protein	lncRNA	-	-	TSL:3
Crybb2-205	ENSMUST00000199639.1	641	No protein	lncRNA	-	-	TSL:3
Crybb2-203	ENSMUST00000137485.7	613	No protein	lncRNA	-	-	TSL:3

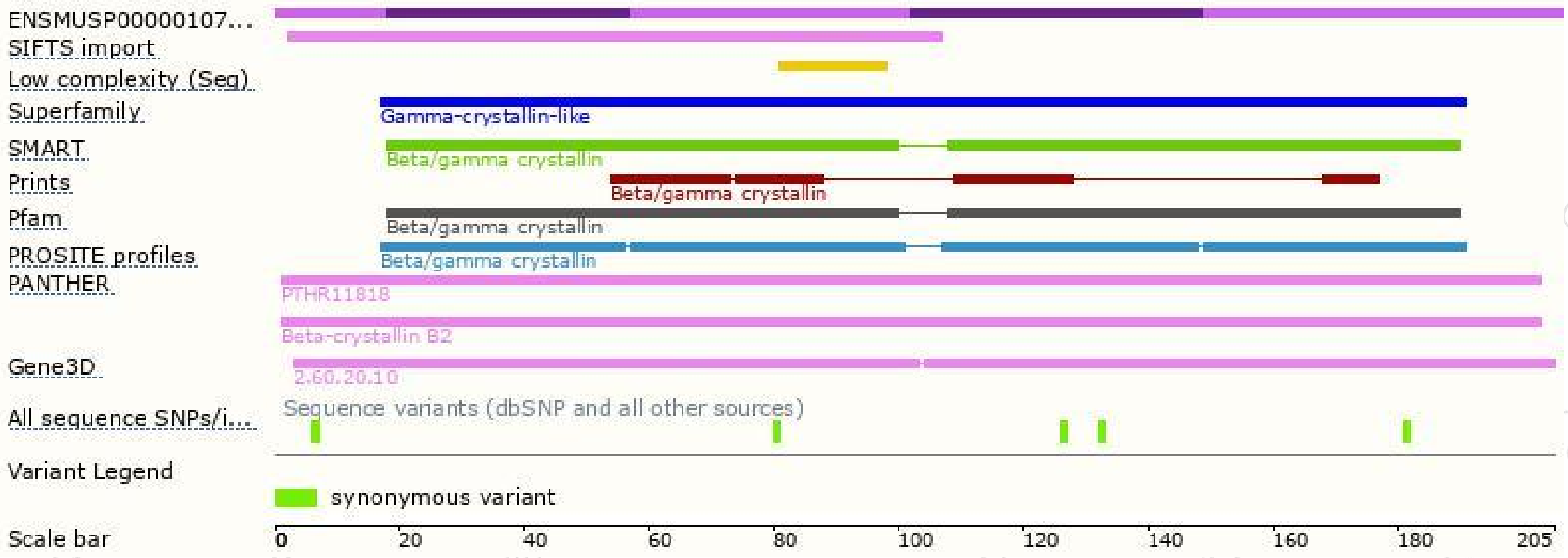
The strategy is based on the design of *Crybb2-202* transcript,The transcription is shown below



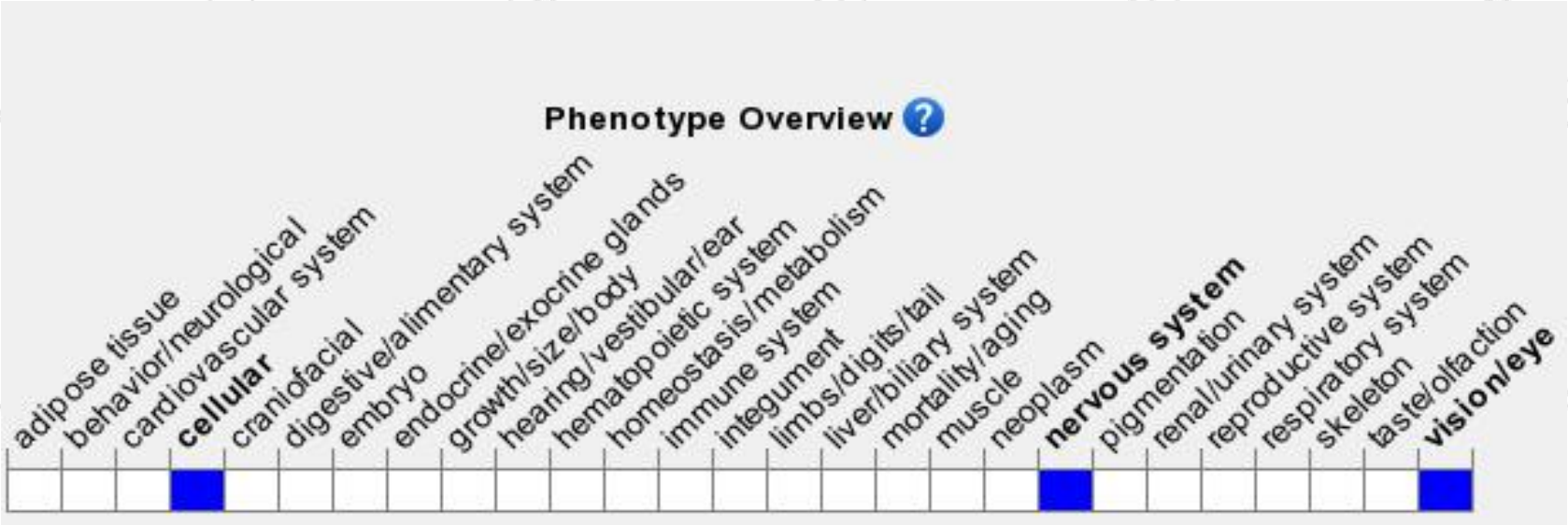
Genomic location distribution



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 in this gene cause progressive lens cataracts discernable at 12-15 days of age as mild anterior or cortical opacity and progressing to total opacity by 6-12 weeks of age.

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