Nbeal2 Cas9-CKO Strategy

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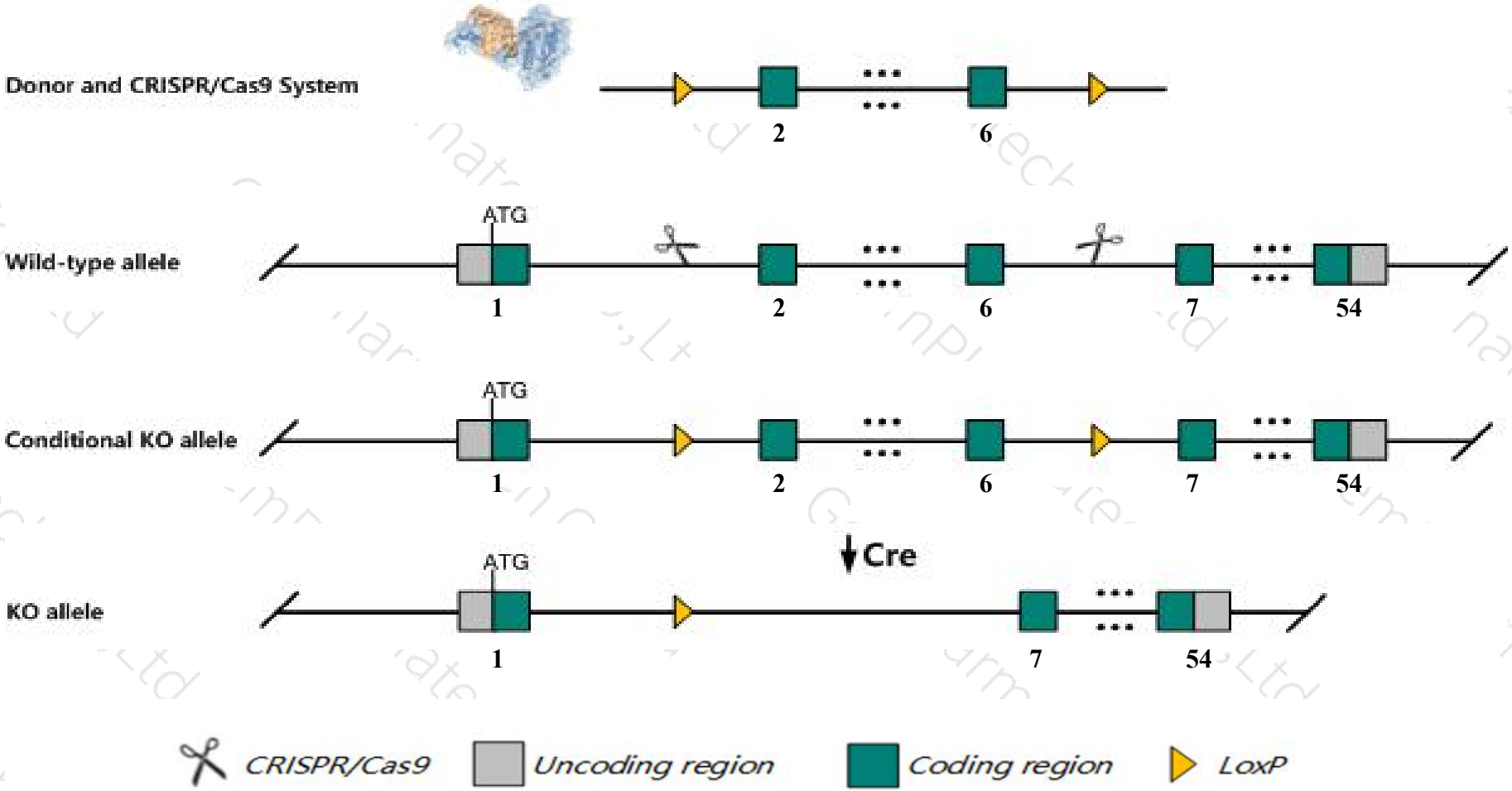
Project Overview

Project Name	***Nbeal2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Nbeal2* gene. The schematic diagram is as follows:



Technical routes

- The *Nbeal2* gene has 11 transcripts. According to the structure of *Nbeal2* gene, exon2-exon6 of *Nbeal2-210* (ENSMUST00000167320.7) transcript is recommended as the knockout region.The region contains 502bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Nbeal2* 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,Homozygous null mice exhibit megakaryocyte and platelet abnormalities resulting in impaired arterial thrombus formation and protection from infarction following cerebral ischemia. Wound repair is impaired. These abnormalities result in a bleeding disorder similiar to Gray Platelet Syndrome.

- The *Nbeal2* 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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Nbeal2 neurobeachin-like 2 [Mus musculus (house mouse)]

Gene ID: 235627, updated on 31-Jan-2019

Summary

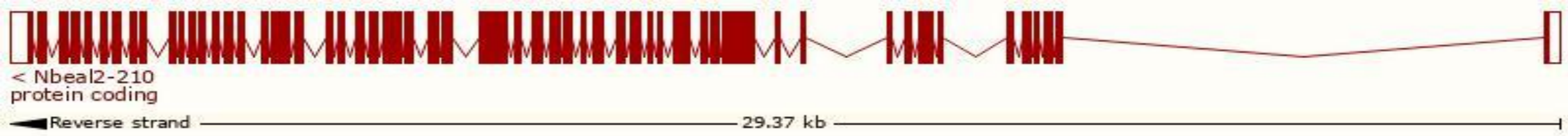
Official Symbol	Nbeal2 provided by MGI
Official Full Name	neurobeachin-like 2 provided by MGI
Primary source	MGI:MGI:2448554
See related	Ensembl:ENSMUSG00000056724
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1110014F23Rik, BC042396, mKIAA0540
Expression	Broad expression in thymus adult (RPKM 58.1), colon adult (RPKM 27.1) and 15 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

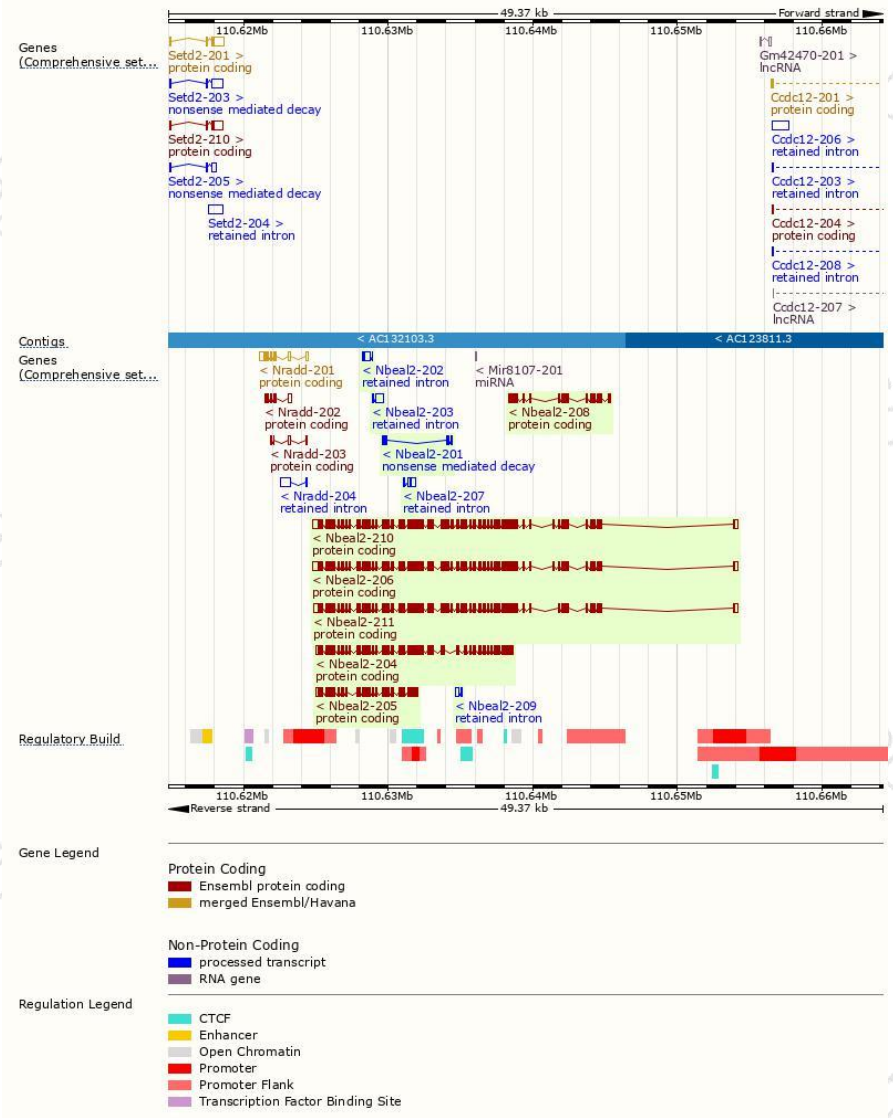
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nbeal2-210	ENSMUST00000167320.7	8803	2750aa	Protein coding	CCDS52939	E9Q9L6	TSL:1 GENCODE basic APPRIS P2
Nbeal2-206	ENSMUST00000133191.7	8782	2743aa	Protein coding	-	Q6ZQA0	TSL:5 GENCODE basic APPRIS ALT2
Nbeal2-211	ENSMUST00000196488.4	8599	2716aa	Protein coding	-	A0A0G2JFQ4	TSL:5 GENCODE basic APPRIS ALT2
Nbeal2-204	ENSMUST00000130024.1	6180	2032aa	Protein coding	-	F6VTL9	CDS 5' incomplete TSL:5
Nbeal2-205	ENSMUST00000131017.7	3303	1073aa	Protein coding	-	F6ZZB1	CDS 5' incomplete TSL:5
Nbeal2-208	ENSMUST00000149089.1	1869	593aa	Protein coding	-	D3Z2K6	CDS 3' incomplete TSL:5
Nbeal2-201	ENSMUST00000123996.1	457	96aa	Nonsense mediated decay	-	F7CGQ9	CDS 5' incomplete TSL:5
Nbeal2-203	ENSMUST00000129095.1	664	No protein	Retained intron	-	-	TSL:2
Nbeal2-207	ENSMUST00000138072.1	479	No protein	Retained intron	-	-	TSL:2
Nbeal2-202	ENSMUST00000126088.3	432	No protein	Retained intron	-	-	TSL:2
Nbeal2-209	ENSMUST00000153960.1	295	No protein	Retained intron	-	-	TSL:5

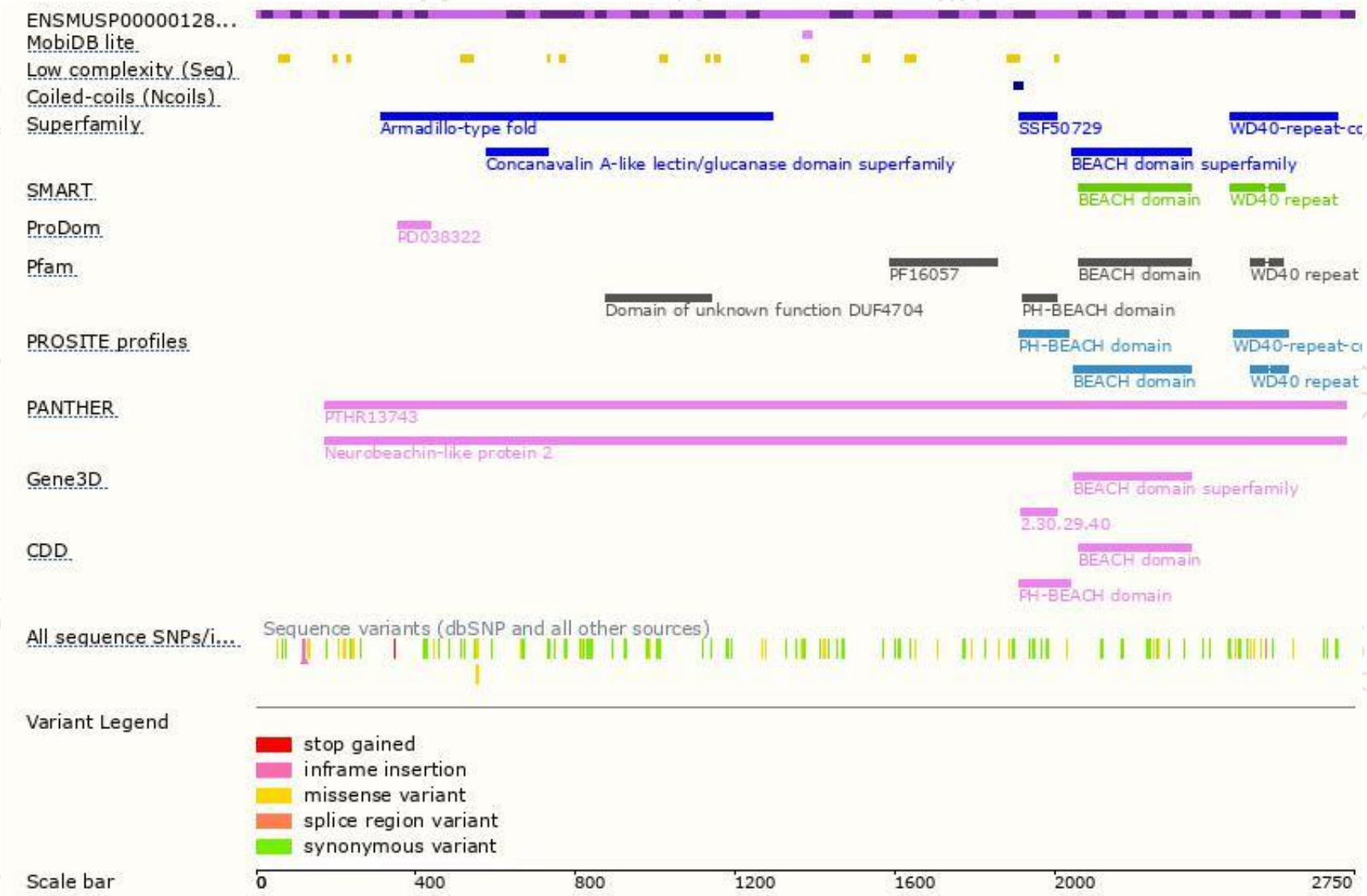
The strategy is based on the design of *Nbeal2-210* transcript,The transcription is shown below



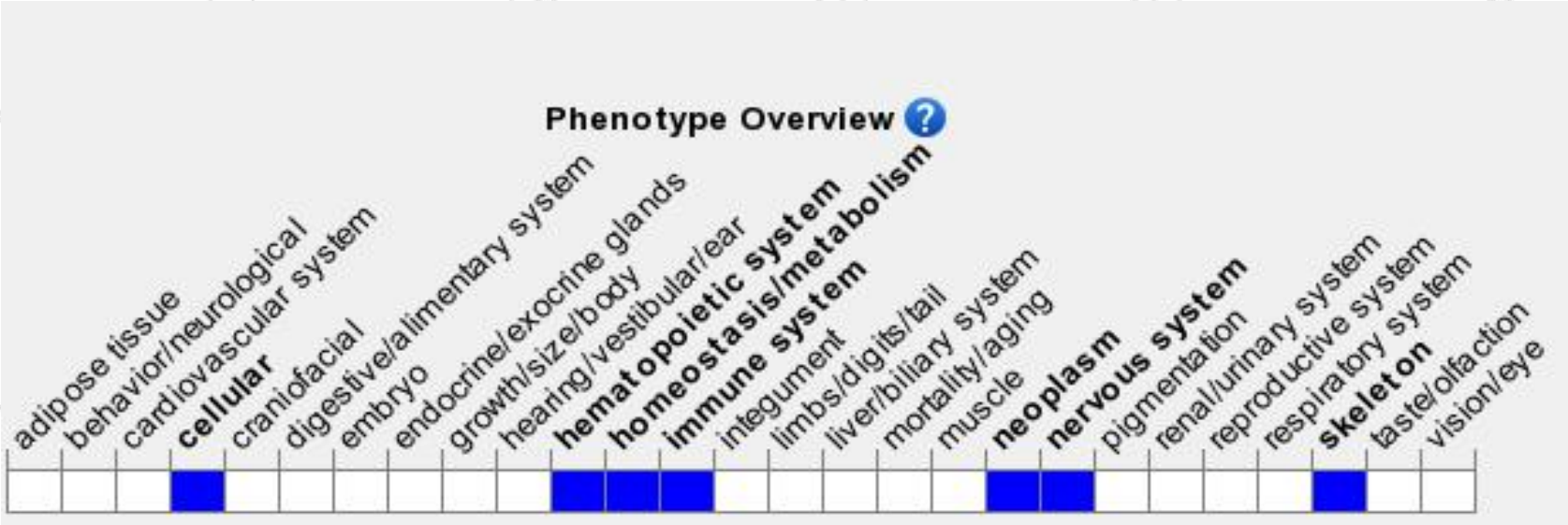
Genomic location distribution



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 null mice exhibit megakaryocyte and platelet abnormalities resulting in impaired arterial thrombus formation and protection from infarction following cerebral ischemia. Wound repair is impaired. These abnormalities result in a bleeding disorder similiar to Gray Platelet Syndrome.

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

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