

***H11-Zp3-iCre-ployA* Cas9-KI Strategy**

Designer:

Yanhua Shen

Reviewer:

Xiaojing Li

Design Date:

2019-08-12

Project Overview

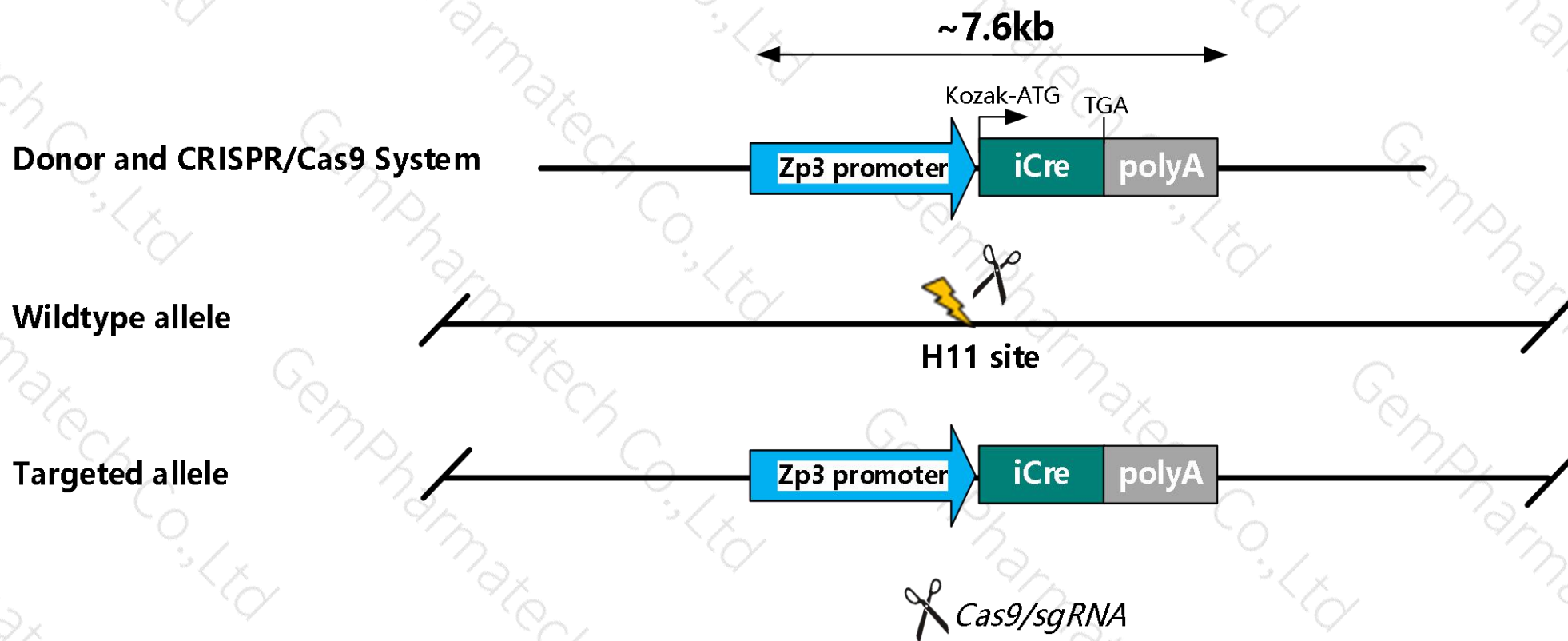
Project Name	<i>H11-Zp3-iCre-ployA</i>
---------------------	----------------------------------

Project type	Cas9-KI
---------------------	----------------

Strain background	C57BL/6J
--------------------------	-----------------

Knockin strategy

The *Zp3-iCre-ployA* fragment was inserted into H11 site of mice and the schematic diagram is as follows:



Summary of mouse *Zp3* promoter from JAX

Allele Symbol: Tg(Zp3-cre)3Mrt 	
Allele Name	transgene insertion 3, Gail R Martin
Allele Type	Transgenic (Recombinase-expressing)
Allele Synonym(s)	Zp3-cre; Zp3::Cre; delta-R
Gene Symbol and Name	Tg(Zp3-cre)3Mrt  , transgene insertion 3, Gail R Martin
Gene Synonym(s)	Zp3-cre; delta-R
Promoter	<i>Zp3</i> , zona pellucida glycoprotein 3, mouse, laboratory
Expressed Gene	<i>cre</i> , cre recombinase, bacteriophage P1
Site of Expression	prior to first meiotic division
Strain of Origin	FVB/N
Chromosome	UN
General Note	Homozygous transgenic mice are viable and fertile.
Molecular Note	This transgene expresses NLS-Cre recombinase under the control of a mouse zona pellucida 3 promoter. This promoter is active in oocytes. No expression was detected in somatic tissues.
Mutations Made By	Dr. Gail Martin, University of California San Francisco

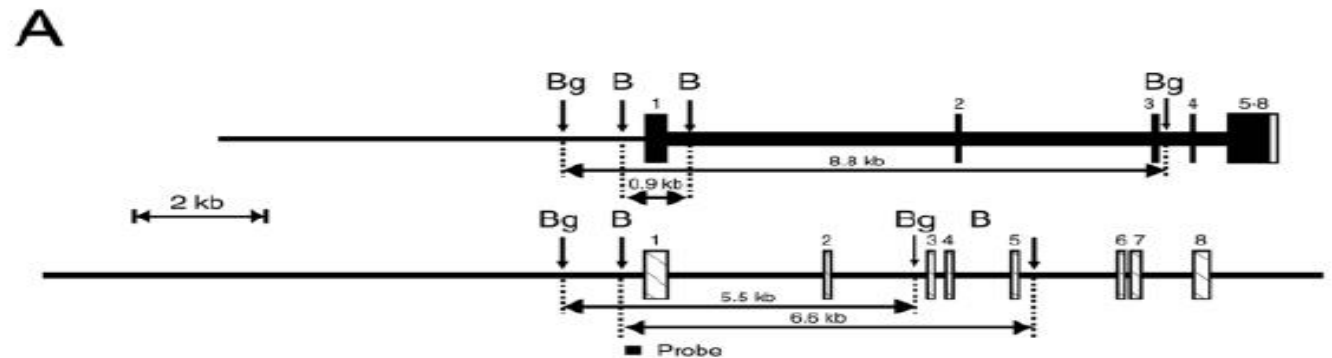
<https://www.jax.org/strain/003394>

Fig. 1. Human ZP3 transgene. (A) *Top*, human ZP3 transgene composed of 6 kb of the 5'-flanking region of the mouse *Zp3* gene (thin line), 9.5 kb human ZP3 minigene (thick line and boxes) and 310 bp of the bovine growth hormone (BGH) polyadenylation and termination signals (open box). *Bottom*, schematic representation of the mouse *Zp3* locus with 8 exons (numbered, hatched boxes) encompassing 8.6 kb on mouse chromosome 5. Vertical arrows indicate restriction enzyme sites for *Bam*HI (B) and *Bgl*II (Bg) and the sizes of the resultant fragments are indicated.

MATERIALS AND METHODS

Transgenic mouse lines

A 6 kb fragment of mouse *Zp3* extending from an *Eco*RI site in the



T.L. Rankin, Z.B. Tong, P.E. Castle, E. Lee, R. Gore-Langton, L.M. Nelson, J. Dean. Human ZP3 restores fertility in *Zp3* null mice without affecting order-specific sperm binding. *Development* 1998 125: 2415-2424.

集萃药康
GemPharmatech

1. Lan ZJ, Xu X, Cooney AJ. Differential oocyte-specific expression of Cre recombinase activity in GDF-9-iCre, Zp3cre, and Msx2Cre transgenic mice. *Biol. Reprod.* 2004 Nov ;71(5) .
2. T.L. Rankin, Z.B. Tong, P.E. Castle, E. Lee, R. Gore-Langton, L.M. Nelson, J. Dean. Human ZP3 restores fertility in Zp3 null mice without affecting order-specific sperm binding. *Development* 1998 125: 2415-2424.

- The *Zp3* gene has 2 transcripts. According to the structure of *Zp3* gene, *Zp3-201*(ENSMUST00000005073.12) is selected for presentation of the recommended strategy.
- *Zp3-201* gene has 8 exons, with the ATG start codon in exon1 and TAA stop codon in exon8.
- The *Zp3* promoter is from the references, the length is about 6kb.
- H11, located on mouse chromosome 11, is a safe site for foreign gene insertion. The foreign gene integrated into this site can be expressed stably and efficiently without destroying the function of endogenous gene.
- In this study, the *Zp3-iCre-ployA* gene fragment was inserted into H11 site of mice by CRISPR/Cas9 technology. The brief process is as follows: the donor vector and sgRNA were constructed in vitro, Cas9, donor and sgRNA were microinjected into the fertilized eggs of C57BL/6J mice, and F0 generation mice were obtained. The F0 positive mice were mated with C57BL/6J mice by PCR, sequencing, and southern blot, then the stable inheritance of F1 positive mice model was obtained.

- According to the existing JAX data, Cre is expressed prior to first meiotic division.
- Due to the insert region is large (~7.6 kb), the mouse offspring need a southern blot.
- H11 is located on Chr11. Please take the loci in consideration when breeding the Knock-in mice with other gene modified (e.g., iCre) strains, if the other gene is also on Chr11, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Gene information (NCBI)

Zp3 zona pellucida glycoprotein 3 [*Mus musculus* (house mouse)]

Gene ID: 22788, updated on 10-Aug-2019

Summary

Official Symbol	Zp3 provided by MGI
Official Full Name	zona pellucida glycoprotein 3 provided by MGI
Primary source	MGI:MGI:99215
See related	Ensembl:ENSMUSG00000004948
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	Zp-3
Expression	Restricted expression toward ovary adult (RPKM 68.2) See more
Orthologs	human all

Genomic context

Location: 5 G2; 5 75.62 cM

Exon count: 8

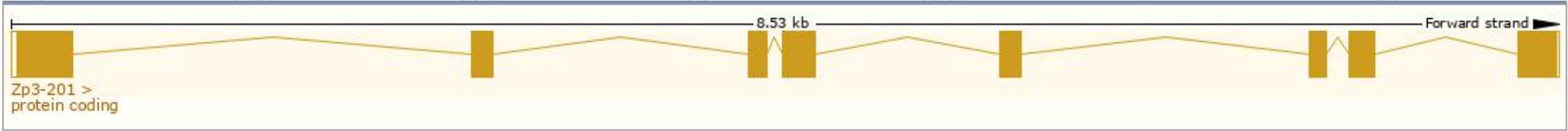
See Zp3 in [Genome Data Viewer](#)

Transcript information (Ensembl)

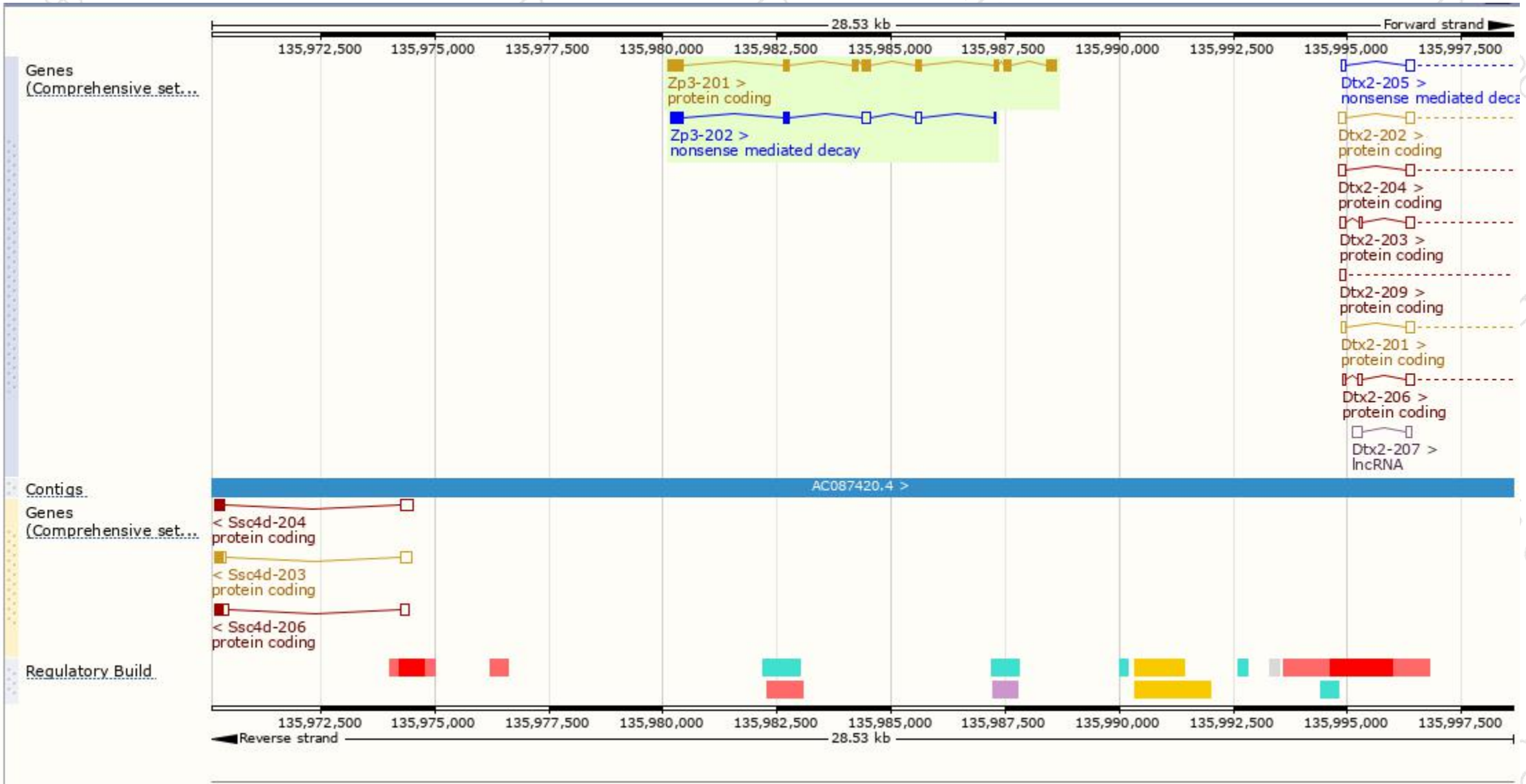
The gene has 2 transcripts, and the transcript is shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Zp3-201	ENSMUST00000005073.12	1323	424aa	Protein coding	CCDS19749	P10761	TSL:1 Gencode basic APPRIS P1
Zp3-202	ENSMUST00000131563.1	734	136aa	Nonsense mediated decay	-	F6VD35	CDS 5' incomplete TSL:5

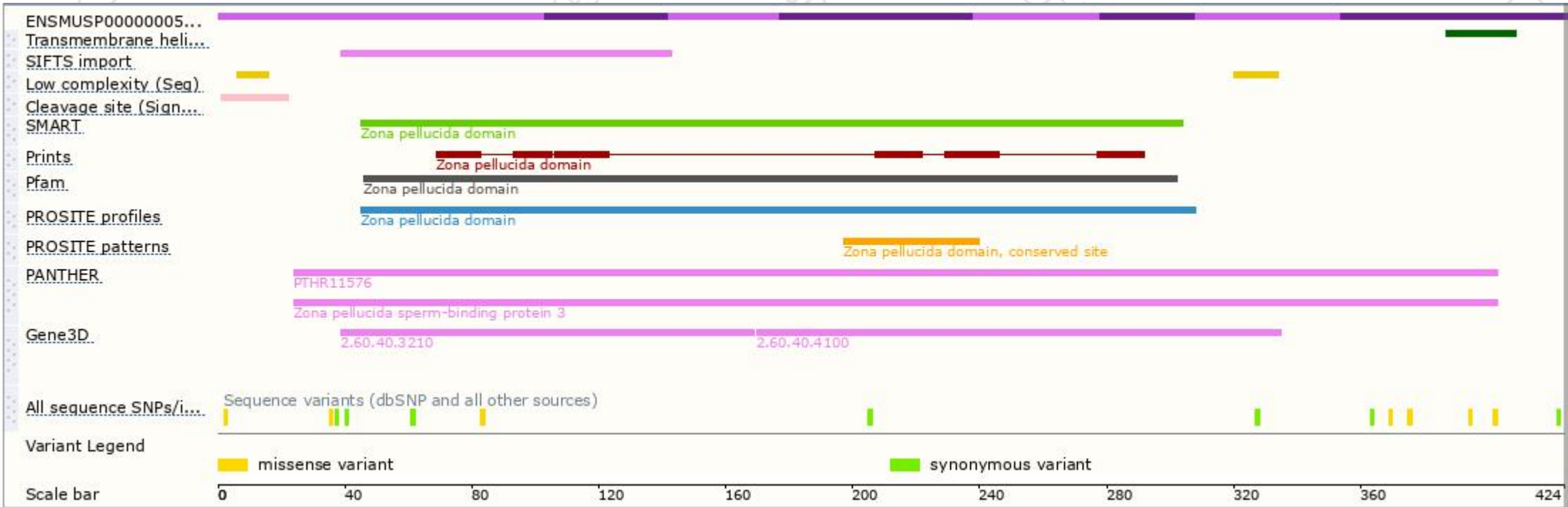
The strategy is based on the design of *Zp3-201* transcript, The transcription is shown below



Genomic location distribution



Protein domain



If you have any questions, you are welcome to inquire.
Tel: 025-5864 1534



集萃药康生物科技
GemPharmatech Co.,Ltd

