Sp7-iCre TG Strategy

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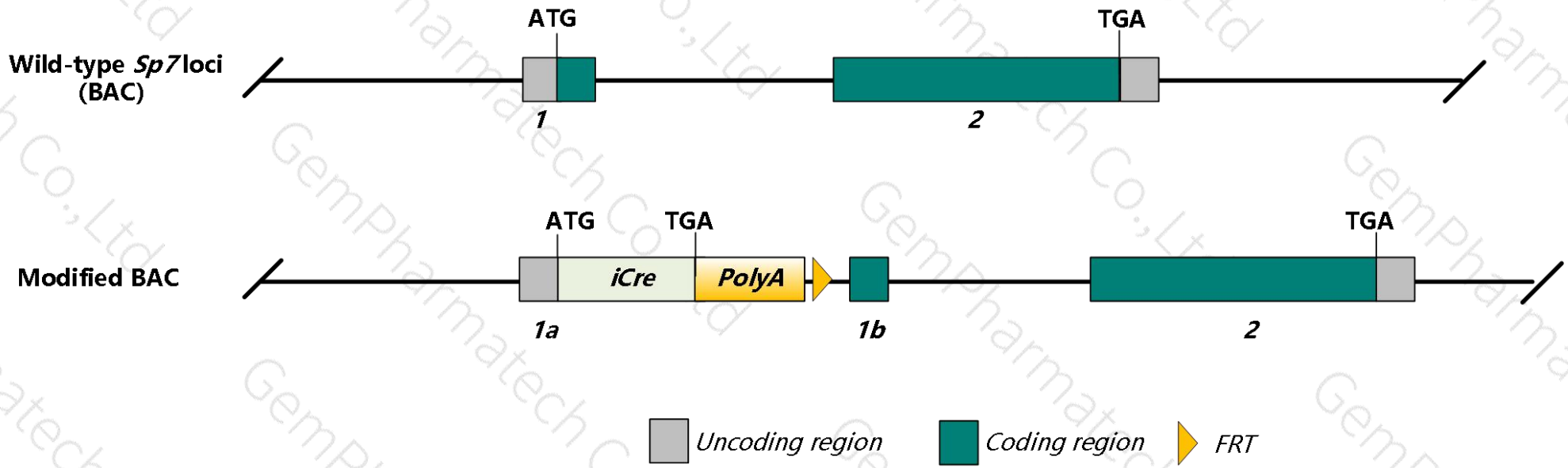
Project Overview

Project Name	***Sp7-iCre***
Project type	**TG**
Strain background	**C57BL/6J**

Knockin strategy

This model will use pronucleus injection technology to obtain the *Sp7-iCre* model. The schematic diagram is as follows:



Technical routes

- Transcript *Sp7*-201(ENSMUST00000078508.6) is selected for presentation of the recommended strategy.

- *Sp7*-201 gene has 2 exons, with the ATG start codon at exon 1 and TGA stop codon at exon 2.

- In this study, RP24-114P3 (~156kb) or RP24-362M3 (~170kb) of C57BL/6J mouse bacterial artificial chromosome (BAC) containing the entire *Sp7* locus (and other genes), was modified by targeting iCre-polyA sequence to the exon1 near the translation start codon of the *Sp7* locus, ensuring iCre is expressed from the endogenous promoter/enhancer elements of *Sp7*. transgenic fragments containing *Sp7-iCre-polyA* were micro-injected into the fertilized eggs of C57BL/6J mice, and obtained positive F0 generation (i.e., founder) mice.

Notice

- According to the MGI, Mice homozygous for a reporter allele die within minutes of birth displaying cyanosis, respiratory distress, arrested osteoblast differentiation, and failure of endochondral and intramembranous bone formation. Mice homozygous for a knock-out allele exhibit failure of bone ossification..

- Cre-mediated recombination is Specifically expressed in osteoblasts, which can be detected in embryonic phase.

- the vector construction needs to remove the LoxP site on the BAC backbone.

- Transgene fragment will be injected into the fertilized eggs, and randomly integrated into the genome, by the influence of insertion site and copy number, expression level of the transgenic mice may be different.

- This strategy is designed based on genetic information in existing databases. Due to the complexity of gene transcription and translation processes, all risks cannot be predicted under existing information.

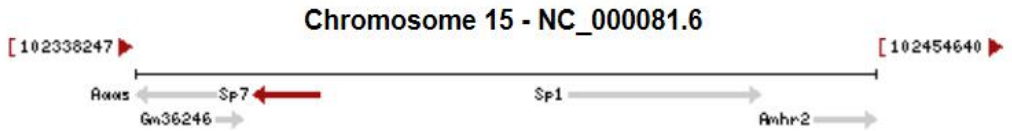
Gene information (NCBI)

Sp7 Sp7 transcription factor 7 [*Mus musculus* (house mouse)]

Gene ID: 170574, updated on 14-Aug-2019

Summary

Official Symbol	Sp7 provided by MGI
Official Full Name	Sp7 transcription factor 7 provided by MGI
Primary source	MGI:MGI:2153568
See related	Ensembl:ENSMUSG00000060284
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	C22; Osx; 6430578P22Rik
Expression	Biased expression in limb E14.5 (RPKM 8.9), frontal lobe adult (RPKM 5.1) and 5 other tissues See more
Orthologs	human all

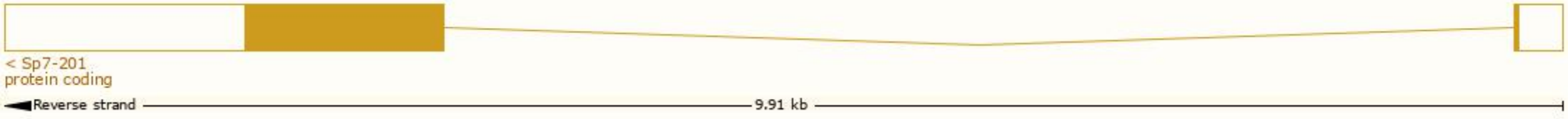


Transcript information （Ensembl）

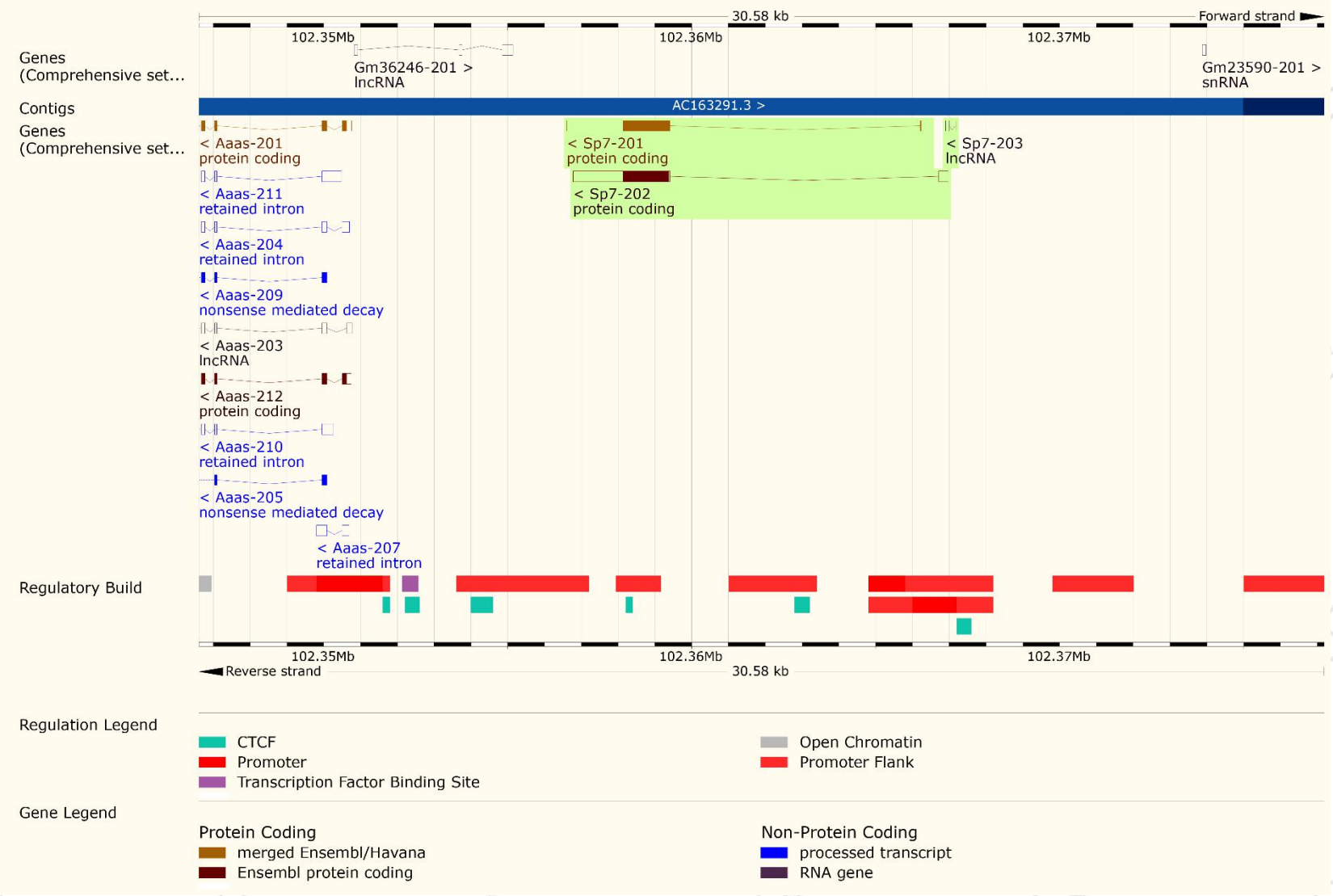
The gene has 3 transcripts, and all transcripts are shown below:

Name ▲	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Sp7-201	ENSMUST00000078508.6	3097	428aa	ENSMUSP00000077596.5	Protein coding	CCDS37228	Q2KHK9 Q8VI67	TSL:1 GENCODE basic APPRIS P2
Sp7-202	ENSMUST00000229464.1	2873	410aa	ENSMUSP00000154859.1	Protein coding	-	Q5RM08	GENCODE basic APPRIS ALT2
Sp7-203	ENSMUST00000231100.1	117	No protein	-	lncRNA	-	-	-

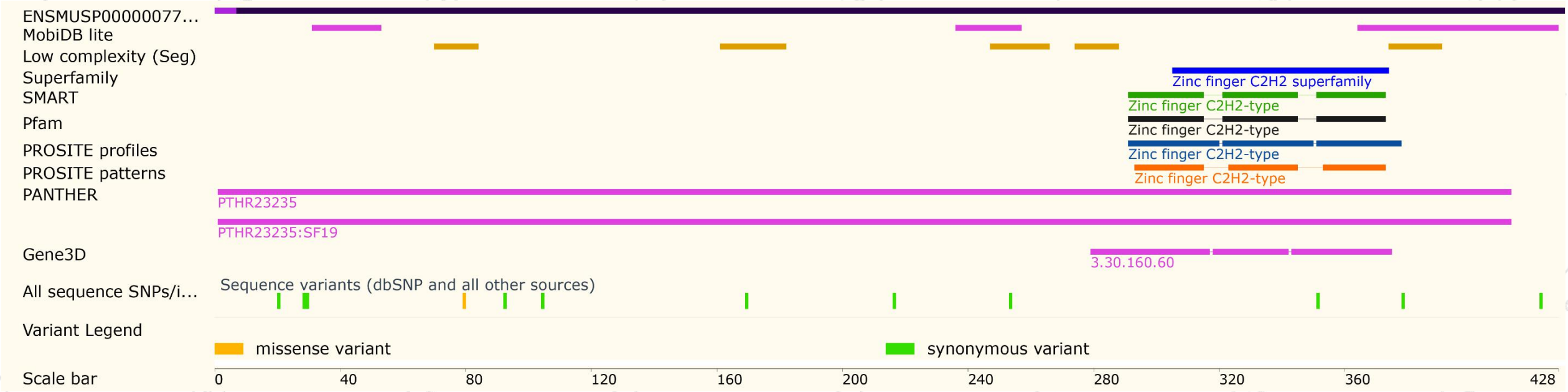
The strategy is based on the design of *Sp7-201* transcript, the transcription is shown below:



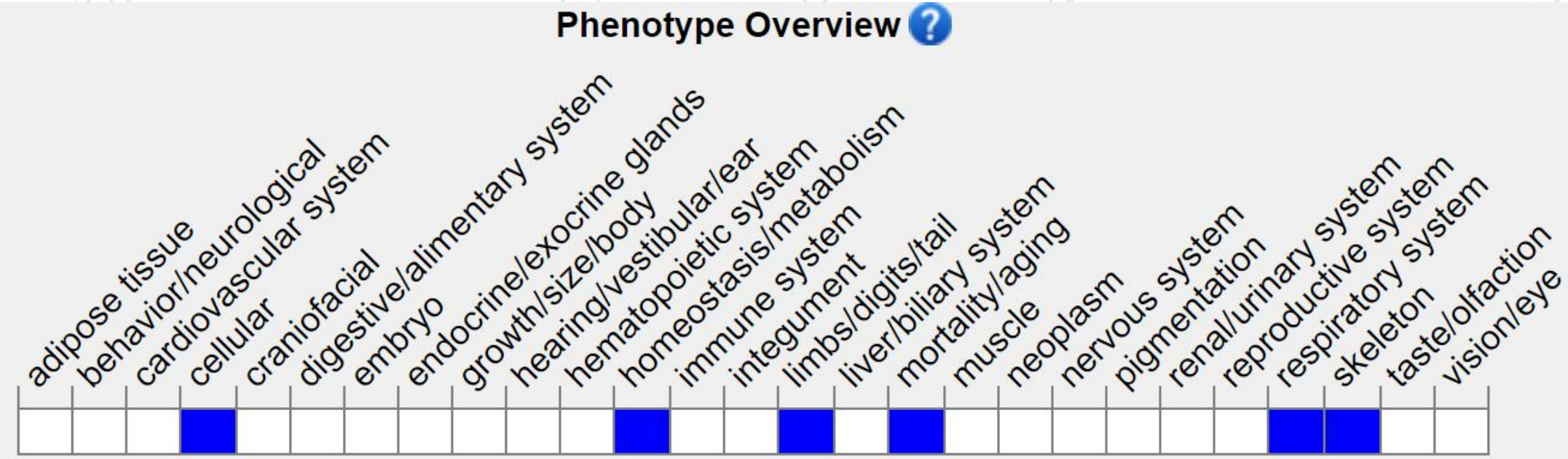
Genomic location distribution



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/marker/MGI: 2153568) .

According to the existing MGI data, Mice homozygous for a reporter allele die within minutes of birth displaying cyanosis, respiratory distress, arrested osteoblast differentiation, and failure of endochondral and intramembranous bone formation. Mice homozygous for a knock-out allele exhibit failure of bone ossification.

Coding Sequence of Codon-Optimized Cre Gene[1]

ATGGTGCCCAAGAAGAAGAGGAAAGTCTCCAACCTGCTGACTGTGCACCAAAACCTGCCTGCCCTCCCTGTGGATGCCACCTCTGATGAAGTCAGGAAGA
ACCTGATGGACATGTTCAGGGACAGGCAGGCCTTCTCTGAACACACCTGGAAGATGCTCCTGTCTGTGTGCAGATCCTGGGCTGCCTGGTGCAAGCTGAA
CAACAGGAAATGGTTCCCTGCTGAACCTGAGGATGTGAGGGACTACCTCCTGTACCTGCAAGCCAGAGGCCTGGCTGTGAAGACCATCCAACAGCACCTG
GGCCAGCTCAACATGCTGCACAGGAGATCTGGCCTGCCTCGCCCTTCTGACTCCAATGCTGTGTCCCTGGTGATGAGGAGAATCAGAAAGGAGAATGTGG
ATGCTGGGGAGAGAGCCAAGCAGGCCCTGGCCTTTGAACGCACTGACTTTGACCAAGTCAGATCCCTGATGGAGAACTCTGACAGATGCCAGGACATCAG
GAACCTGGCCTTCCTGGGCATTGCCTACAACACCCTGCTGCGCATTGCCGAAATTGCCAGAATCAGAGTGAAGGACATCTCCCGCACCGATGGTGGGAGA
ATGCTGATCCACATTGGCAGGACCAAGACCCTGGTGTCCACAGCTGGTGTGGAGAAGGCCCTGTCCCTGGGGGTTACCAAGCTGGTGGAGAGATGGATCT
CTGTGTCTGGTGTGGCTGATGACCCCAACAACTACCTGTTCTGCCGGGTCAGAAAGAATGGTGTGGCTGCCCCTTCTGCCACCTCCCAACTGTCCACCCG
GGCCCTGGAAGGGATCTTTGAGGCCACCCACCGCCTGATCTATGGTGCCAAGGATGACTCTGGGCAGAGATACCTGGCCTGGTCTGGCCACTCTGCCAGA
GTGGGTGCTGCCAGGGACATGGCCAGGGCTGGTGTGTCCATCCCTGAAATCATGCAGGCTGGTGGCTGGACCAATGTGAACATTGTGATGAACTACATCA
GAAACCTGGACTCTGAGACTGGGGCCATGGTGAGGCTGCTCGAGGATGGGGACTGA

References

[1] Shimshek DR, Kim J, Hübner MR, Spergel DJ. Codon-improved Cre recombinase (iCre) expression in the mouse. GeAlbis.2002 Jan.32(1):19-26.

[2] Rodda SJ, et al. Distinct roles for Hedgehog and canonical Wnt signaling in specification, differentiation and maintenance of osteoblast progenitors. Development. 2006 Aug;133(16):3231-44.

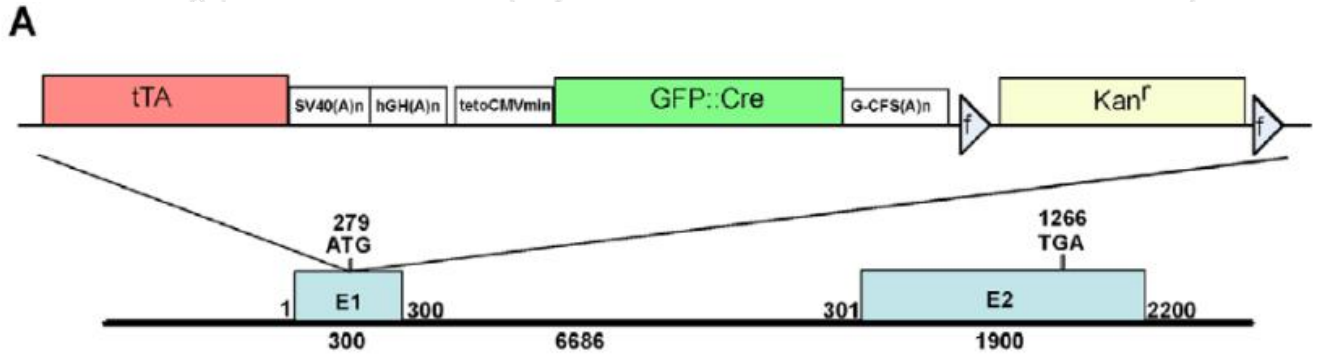


Fig. 2. Generation of an *Osx1-GFP::Cre* transgenic mouse line. (**A**) Schematic outlining the linear configuration of the pTGCK cassette used for homologous recombination to the first exon of the osterix1 locus contained within the BAC: RP23-399N14. The correctly targeted BAC was subsequently used to generate a transgenic mouse line by pro-nuclear injection. (**B-K**) Founder *Osx1-GFP::Cre* male transgenic mice were crossed to the female *Rosa26lacZ* reporter line and activity of the transgenic line was observed by way of whole-mount *lacZ* assay at (B) E14.5, and by *lacZ* assay or direct fluorescence microscopy on 15 μm cryosections of tibia from (C,D) E14.5, (F,G) E18.5 and (I,J) postnatal day 10 mice. (E,H,K) Negative control specimens for littermates, at each corresponding age, that do not carry the transgene.

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

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