

***Carl-iCre-polyA* TG Strategy**

Designer:

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Reviewer

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Project Overview

Project Name

Car1-iCre-polyA

Project type

TG

Strain background

C57BL/6J

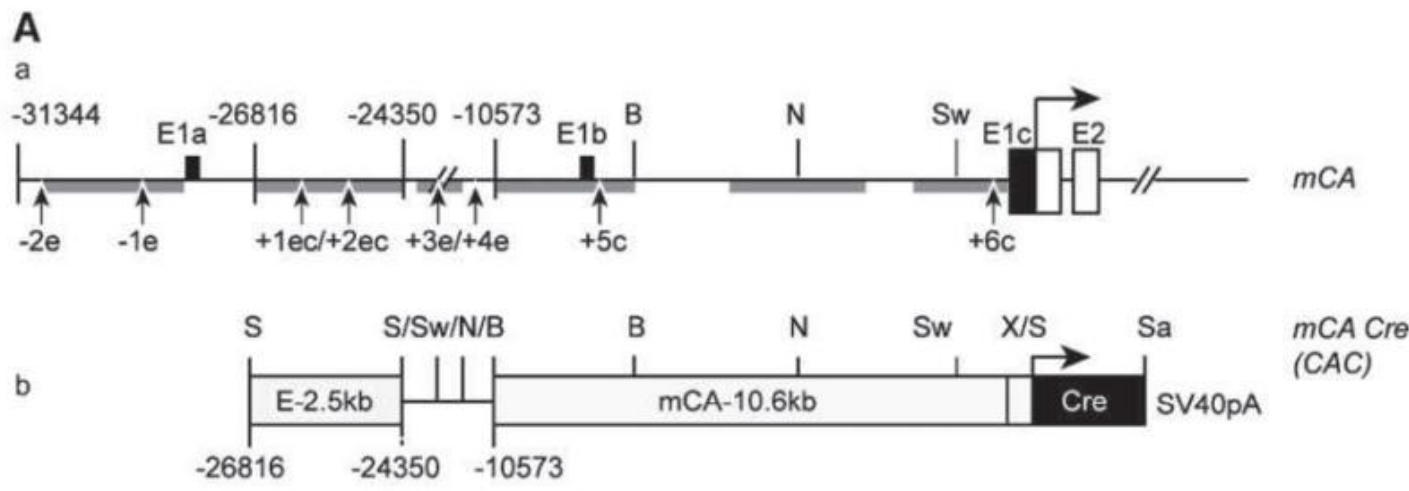
TG strategy

This mice model is made by transgenic technology, and the schematic diagram is as follows:



- Kozak : Kozak sequence (GCCGCCACC) will be added before the ATG , in order for the ribosome to recognize the initiation codon.

Summary of Mouse *Car1* Promoter^[1]



Transgene construction

The 10.6-kb colon-specific promoter and the shared 2.5-kb erythroid/colon enhancer fragment of the mouse CA1 (mCA1) gene were PCR amplified and assembled to generate the *pUC13kb-mCA-cre* plasmid. The mCA1 promoter/enhancer fragments were subcloned sequentially into *pUC12.4kb-villin* (a gift from Dr. Debra Gumucio, University of Michigan Medical School, Ann Arbor, MI) after removing the villin promoter sequence using *XhoI* and *SaII* to generate the *pUC13kb-mCA* plasmid. The Cre recombinase gene from *pPGK-cre-bpA* (a gift from Drs. Klaus Rajewsky and Marc Schmidt-Supprian, Harvard Medical School, Boston, MA) was subcloned into the *XhoI* and *SacII* sites of *pUC13kb-mCA* to generate the *pUC13kb-mCA-cre* plasmid.

FIGURE 1. Cre expression mediated by the mCA1 promoter/enhancer is restricted within the intestine to the large bowel. A, a, schematic of the mCA1 (mCA) locus with regions of DNA conserved between mouse and human (gray columns) and hypersensitive sites (numbers under the gene) identified. E, exon; e, erythroid cell; c, colon; ec, both erythroid cell and colon. b, schematic of mCA Cre (CAC) transgene construct. *BspEI* (B), *NsiI* (N), *SwaI* (Sw), *XhoI* (X), *SacII* (Sa), and *SaI* (S) restriction enzyme sites are indicated. B, a, Cre mRNA expression from the transgene measured in the proximal colon (CoP) of two

Mouse *Car1* Enhancer Sequence(2491bp)^[1]



AAAAAGAAAAAATACACGTAATGCTGTTAATCCACACGTA AACACATGCCTTTCACTCCTCCCTGTTTTTATACCTGTTTTTATTCTCTCTTGCATAGTTTTATCTGCTACATCTCATGGC
CAAACACACTGTCAGTATTTTATGACTGCTGTGCTATGTAAAAAATCTGTAAACCTGGGGCACATTCTTGTGACCATAGTAATCCTTGGGGTACCCGTCCTGAAGAGGAAC
TGAAGGTCATCATCAGAGCCTTTCTGCTTCGTAATCCACTATATGCAATGAAATTCTTATCAATGTGGTAAACCTTTATTGTTCTCATTCCATGAAGCAGCTTCATGTGTGCTCAGAAAAT
GACTTTTTTTCCTTATAAAGTTATAAATTACAGTATCTCCATGGAAAAGGCTCTAGAAAAATATTTGCATTGGGTGGAACTTTCTGAACGCTGCCATAGAATTTAAACACTTGGGATGTTA
GTTCTTCATGTGCCATGAAAACCTTCATTGTCTAGAAAGTGAATTCATTCTAGTTGTATCTAGATCCAGACATGCAAAGATGATACAATTATGACCCAAATTAAGTTCCCTAGAGTAGTAT
GAAAACATAAACATAATAGCTATATACCTTTTCATTTTCTTTATTTTAATGATTCTTTCTATTAACTAATAAAATTTATTTTAAAAATATACAACCCAGTATTGATATTTTAAAGTCATCAAA
ATAATTCATAGTAGTTAAATGACTCTTAAATATACATGATAATTTCTGAAGCTAAAAGTAATATGCACTCAACCAGTTTTTAAATCTATTTGGAACATTAAACATGATAGAAGTAGAAAA
TATCTGTTATGAAGCCCTCAATGAAAGAAAATTGTGACAAGTTTTTGAATAGACAGAGACAACTTTTTATATATCATTTATACTTTTGTGTTTTTATCTGATTAAAAGAAGTTTGAAATTAT
GTATCTTCCATGTAGGTCAGGTTAAAATAATTTATGAGAAATCAGTAAAAACAAGTATTGACCAAGAAAAGAAAAGAAAGAACTCATGAGCGACTGCATACTTACACAGTAACAGTACAGTG
TCTTATATTTCTAAATAGTTTATTACATGAATTTGTTTATTTCTGTGTGTGGTAGGGGTTGTGTTTCATGCCTGGAATATAAGTATCTGTGACAGATAGCTTGTAAGAGTTGTTTCTCTCCT
TCCACCATGTGAGCTCCAGAAATCAAACCTCAGGTTGTCAAGAGTTGGTGACAACTACTCTTACCCACTGAGCCACCCAGCCCAATTGGCATGAATTTGACAGTAACTGTTGTTTTCAAATGT
TGAAGTTACTTTCTATTTATTCTATATGAAGAAATATATCTCCTCTCCTGGATAGGTCAAGCCAGAGGGATACACTGGGCAGTTTTTACTGAGTCCAGAGTGTCTACAAGGCAGGCAGGATG
TTTTGCTTTCTGCCTGCAGCAGTCTCCAGAGGTGACTGCAGGGGCTCCAGATCCCCTTGTTTAAAAAATAAAAAATAAAGCACTGTCCAGAACGTTGTTTGAGTCTTGCTAAAGACATTT
TTGAGTCTAGAGGCTTGATGTGACTTCCTCTGAATTATTGTCCCCAGATGGTCATGGTCCTTACCATTTTTATCCCTAGATATCTAATGCAGTATGAGCAAACTAATTTCTATCCTTTCTA
TTGCCAAAGCTAATATGGTCTTGATTTTTCAAAGGCAAGGGAATCAGTATAAATGAACATATTTATAATTCCTTAAGAAATATAATATAAGAAATATGAAACCTGACTGAATTATAAATTG
TAGATTGTAAGATAGTTAGTTTTATAAAACAATCTATTTATCCAGGCAGCTAGACTTCTAGGCCTGATGCAAAAGGGGGGCACCCTGTTATTTCTCTATCAGAATTGGTCAGCTAGAGCCCT
ACCCCTGATCTAGCCACAAAGCCCCAGAACCTCACTTAGCCATGTTTCATTGGCTATTTGTTGAACTTTAACCCTGTTCTCTGCTTACAGAGCTAGAACCAAGGGCTTCTTAACCGTAAC
TAAATGTTACTTCTAGTCTGTATGAAATAGTCCAGCAGGAGTCCAACCTGTTGGGGCAACAACGTAAGAGGAGTATTTACTGTGCAGGGTCACAATCACTCCCTCTTAGGAAGGGATTATAT
GGAGTCTTTCAAAGGACAATATTGCGCACATCCTCACAAGCACGACTCTCACAGCATCTTAAGTTGATGTACTACAGAGAGATGCTGGGCAACAATCTACTCCAAGACTATCTGCCTGGACT
ATAACTGAGTCTAACAGTCCACTGCTGGAATATCTTCCCCTACATAAAGGAATTTTGAGTTTAAATCTCCATATAAACTGATTAAATCAGAAGAAATCATCTTCTTAAGTTGTTTCCTT
TGACAAGTTGCTGTGAATTTGTTTCAAATGGTTAAATATTCAAATTCAG

Mouse *Carl* Promoter Sequence(10597bp)^[1]

[illegible]

Technical routes

- The *Car1* promoter^[1] is from references, the length is about 11.0kb.
- In this study, the transgenic vector was constructed in vitro, and transgenic fragments containing *Car1-iCre-polyA* were micro-injected into the fertilized eggs of C57BL/6J mice, and pcr-positive F0 generation (i.e., founder) mice were obtained.

- Transgenic fragments injected into the prokaryotes will be randomly integrated into the mouse genome. Affected by the insertion site and copy number of transgenic fragments, the expression level of transgenic mice may be different.
- 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.

[1] Xue Y; Johnson R; Desmet M; Snyder PW; Fleet JC. 2010. Generation of a transgenic mouse for colorectal cancer research with intestinal cre expression limited to the large intestine. Mol Cancer Res 8(8):1095-104.

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