Opn1mw(M-opsin)-iCre-polyA TG Strategy

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Project Overview

Project Name	***Opn1mw-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 *M-opsin* Promoter[1][2]

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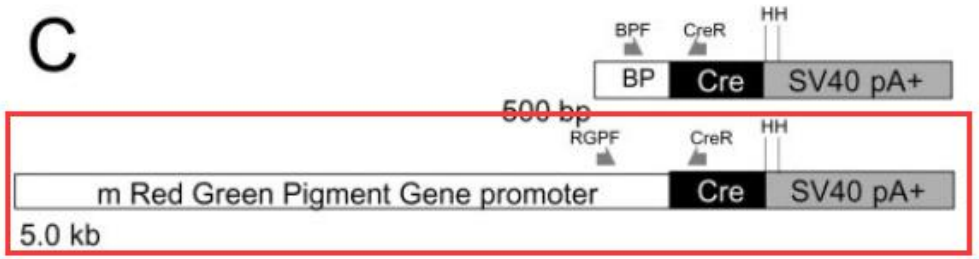

METHODS. Five kilobases of 5′ upstream sequence of the mouse red-green (M) opsin gene or 0.5 kb of the mouse blue (S) opsin gene was cloned into a Cre-expression plasmid. Transgenic

Transgene Vectors and Generation of Transgenic Mice

We generated a promoterless pCI vector (pCIpl) by excision of the cytomegalovirus (CMV) promoter from the pCI expression vector (Promega, Madison, WI) by *Msc*I and *Nbe*I endonuclease digestion, followed by blunt-ending and self-religation. A promoterless Cre vector (pCICre) was generated by insertion of a 1.1-kb *Mlu*I fragment from pMCCre[23] into the *Mlu*I site of pCIpl. We then amplified mouse red-green (GenBank accession S44742; http://www.ncbi.nlm.nih.gov/Genbank; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD) or blue (GenBank accession L27831) pigment gene promoter region by PCR using genomic DNA from the R1 mouse strain. The primers used were: forward 5′-CTAGCTAGCATACCTTGAAACCCACA-3′ and reverse 5′-CGCCTCGAGGCTGTAGAAAACTG-3′ for the mouse red-green pigment gene (mRGP) promoter; and forward 5′-GGCAGGATGCAGTTGTTTCT-3′ and reverse 5′-TCCCGCTTGGGATGCCCT-3′ for the mouse blue pigment gene (mBP) promoter. The 5.0-kb PCR product of the mRGP promoter and the 500 bp of the mBP promoter were subcloned into the pGEM-T easy vector (Promega, Madison, WI), digested by *Eco*RI, and then cloned into the pCICre vector. We excised the mRGP promoter driving Cre-recombinase or the mBP promoter driving the Cre-expression cassette from recombinant plasmids by *Nbe*I and *Nae*I digestion or *Xho*I and *Nae*I digestion, respectively (Fig. 1C). After purification (NucleoSpin; Clontech, Palo Alto, CA), each fragment was injected into the pronuclei of (C57BL/6 X SJL) F2 mouse eggs, which were implanted into pseudopregnant foster mothers using standard techniques. Transgenic founder mice and their progeny were identified by PCR using the following primers: forward RGPF: 5′-AATGGGAACAGTGGTGTGTG-3′; BPF: 5′-AGGAGGGTGCTGTAGGGAAG-3′; reverse (CreR): 5′-GAACGAACCTGGTCGAAATC-3′. Southern blot analysis of *Bam*HI- or *Hinc*II-digested genomic DNA or dot blot analysis was performed by hybridization with a 1.1-kb Cre gene probe,

https://www.ncbi.nlm.nih.gov/nuccore/S44742

1. Akimoto M, Filippova E, Gage PJ, Zhu X, Craft CM, Swaroop A. Transgenic mice expressing Cre-recombinase specifically in M- or S-cone photoreceptors. *Invest Ophthalmol Vis Sci.* 2004 Jan;45(1):42-7.
2. Srinivas M, Ng L, Liu H, Jia L, Forrest D. Activation of the blue opsin gene in cone photoreceptor development by retinoid-related orphan receptor beta. *Mol Endocrinol.* 2006 Aug;20(8):1728-41. Epub 2006 Mar 30.

Mouse *M-opsin* Promoter Sequence(5012bp)[1]

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TACCTTGAAACCCACATAAAAGTGTAAAAAGAAAACCAACTCCATTAATTTGTCATGAAGTCCAAGGGTGTAGCTTTGTAGTAGAATGCTTGCCCAGTATGTATAAGTTCCACCCAAGCCTCAATATCCATTTCAACTTCAGCC
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We choose 5012bp fragment of *M-opsin* promoter to drive *iCre* expression according to the reference described.

Technical routes

- The *Opn1mw* promoter[1-2] is from references, the length is about 5.0kb.

- In this study, the transgenic vector was constructed in vitro, and transgenic fragments containing *Opn1mw-iCre-polyA* were micro-injected into the fertilized eggs of C57BL/6J mice, and pcr-positive F0 generation (i.e., founder) mice were obtained.

Notice

- 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.

如您有任何疑问，欢迎垂询。

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