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Cloning circadian promoters

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Introduction

Regulation of circadian clock genes is difficult because their expression levels alter during day and night. To control expression of CCA1 that is repressed during the day and upregulated at night[1] we overexpress CCA1 or cca1-RNAi using the TOC1[2] promoter that drives the expression during the day.

Subject terms: Plant biology

Keywords: chlorophyll starch EMSA ChIP circadian clock gene expression epigenetics hybrid vigor allopolyploid

Procedure

CCA1 overexpression construct: subclone TOC1::CCA1 into binary vector pEarlygate303 (obtained from the *Arabidopsis* Biological Resource Center, ABRC, #CD694).

1. Amplify the TOC1(At5g61380.1) promoter fragment from *A. thaliana* Columbia genomic DNA and using the primer pair (restriction sites lower case) 5'-GGgaattcCGTGTCTTACGGTGGATGAAGTTGA-3' (EcoRI) and 5'-GGggatccGTTTTGTCAATCAATGGTCAAATTATGAGACGCG-3' (BamHI) and a full-length CCA1 cDNA fragment using the primer pair: 5'-GCGGCCggatccATGGAGACAAATTCGTCTGGAG-3' (BamHI) and 5'-GGCCGCtctagaTCATGTGGAAGCTTGAGTTTC-3' (XbaI).
2. Clone the TOC1 promoter fragment and CCA1 cDNA into pBlueScript and validate the insert by sequencing.
3. For conventional cloning procedures, use pCAMBIA-based Gateway-compatible binary vectors system; Subclone the validated fragment into pEarlygGate303(CD694) using the primers 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTACGTGTCTTACGGTGGATGAAGTTGA -3' and 5-GGGGACCACTTTGTACAAGAAAGCTGGGTCTGTGGAAGCTTGAGTTTCCAACCG-3'.
4. Transform construct (ProTOC1::CCA1) into *A. thaliana* (Columbia) plants using floral dipping method[3].

cca1(RNAi) construct: Subclone TOC1: :cca1(RNAi) into binary vector pFGC5941 (CD3-447).

5. Clone inverted CCA1 cDNA fragments into pFGC5941 binary vector by a two-step cloning process using two pairs of restriction enzyme sites in the primer pairs. After subcloning the inverted repeat fragment, replace the original promoter with the TOC1 promoter (see above).
6. Amplify partial CCA1 fragments (~250-bp) using *A. thaliana* (Columbia) cDNA and primer pair (restriction sites lower case): F-RNAi CCA1 XbaI AscI 5'-GCGGCCtctagaggcgcgccTCTGGAAAACGGTAATGAGCAAGGA-3' and R-RNAi CCA1 BamHI SwaI 5'-GGCCGCcctaggtaaatttaCACCACTAGAATCGGGAGGCCAAA-3' (note: each primer introduces an "inner" restriction enzyme site, AscI or SwaI, and an "outer" restriction enzyme site, BamHI or XbaI that are located next to each other).
7. Clone the CCA1 PCR products from step 6 into pFGC5941 in a sense orientation using the "inner" restriction enzymes, AscI and SwaI and validate the insert by sequencing.
8. Digest the same PCR products from step 6 with the "outer" restriction enzymes, BamHI and XbaI, and clone the digested CCA1 fragment in an anti-sense orientation into the plasmid containing the sense fragment in step 7.
9. Amplify the TOC1 promoter fragment (ProTOC1) using the primer pair: F-EcoRI-ProTOC1 5'-GGGAATTCCGTGTCTTACGGTGGATGAAGTTGA-3' and R-ProTOC1-NcoI 5'-GCGGCCCCATGGGTTTTGTCAATCAATGGTCAAATTATGAGACGCG-3'.
10. Digest the TOC1 promoter fragment from step 9 with EcoRI and NcoI and clone it into the plasmid from step 8 to yield the pFGC5941-ProTOC1::cca1(RNAi) construct (note: this replaces the 35S promoter in pFGC5941 with the TOC1 promoter as described in the website: http://www.chromdb.org/rnai/vector_info.html Validate the insert by sequencing.

References

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2. Strayer, C., Oyama, T., Schultz, T. F., Raman, R., Somers, D. E., Mas, P., Panda, S., Kreps, J.A ., and Kay, S. A. Cloning of the *Arabidopsis* clock gene TOC1, an autoregulatory response regulator homolog. *Science* **289**, 768-771 (2000).

3. Clough, S. J., and Bent, A. F. Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J.* **16**, 735-743 (1998).

Associated Publications

This protocol is related to the following articles:

- **Altered circadian rhythms regulate growth vigor in hybrids and allopolyploids**

See other protocols related to this article

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Competing financial interests

The authors declare no competing financial interests.

Readers' Comments

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