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Desenvolvimento de uma estratégia de clonagem customizada de regiões promotoras do genoma da cana-de-açúcar

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RESUMO

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O controle da expressão gênica é operado em diferentes níveis. A nível transcricional este controle é determinado pela interação de fatores de transcrição com sequências *cis*-regulatórias que flanqueiam as regiões a serem transcritas. Esta interação estabelece a especificidade espaço-temporal da expressão. A obtenção de OGMs (organismos geneticamente modificados) baseia-se na inserção de um ou mais genes de interesse sob o controle de regiões promotoras, de modo a direcionar a expressão de tais genes. Portanto, a identificação de promotores constitutivos, bem como a de promotores específicos a determinados tecidos ou fases do desenvolvimento do organismo, ou induzidos por fatores externos como estresses bióticos ou abióticos, são de extrema importância para o desenvolvimento de novos produtos biotecnológicos. O objetivo deste trabalho foi desenvolver uma metodologia para identificação de regiões promotoras funcionais a partir de segmentos de um genoma qualquer. A inovação desta abordagem encontra-se na busca, e identificação de regiões com potencial de promover a expressão gênica, sem a necessidade de conhecimento prévio de um gene específico, além de possibilitar o isolamento de promotores não canônicos. O genoma da cana-de-açúcar foi escolhido como modelo para o desenvolvimento desta estratégia. Este híbrido é de grande interesse econômico e biotecnológico devido a demandas nos setores alimentício e energético. No entanto, oferece desafio considerável na identificação de promotores funcionais, pois o alto nível de ploidia eleva o risco de isolamento de promotores provenientes de cópias inativas do gene. Neste trabalho, apresentamos o estabelecimento desta estratégia por meio da clonagem customizada de fragmentos provenientes do genoma dos cultivares R570 e SP80-3280, a qual consistiu no fracionamento do DNA genômico e seleção de fragmentos na faixa de 2 a 4kb. Estes fragmentos foram clonados a montante de uma fusão de genes repórteres GFP:GUS, gerando bibliotecas de vetores. A triagem destas bibliotecas foi realizada através de ensaios de expressão transiente por biobalística em epitélio de cebola na qual foram analisados 142 clones distintos. Como resultado desta triagem foram isolados quatro clones que abrigam módulos regulatórios funcionais, designados como F6, B4, F7 e C5. Esses foram submetidos a novos ensaios de expressão transiente para detecção do gene GFP em outro sistema: transformação de protoplastos de arroz. Os quatro clones apresentaram expressão de GFP, confirmando a funcionalidade das regiões isoladas como promotores de expressão gênica. Subsequentemente, foi realizado um ensaio de transformação permanente em arroz através de *Agrobacterium tumefaciens* em que foram obtidas 12 plantas. Foi detectada expressão do marcador GUS em calos, folhas e raízes das plantas de arroz as quais abrigam construções sob regulação dos promotores F6, B4 e F7, comprovando sua funcionalidade. Desta maneira, o desenvolvimento do presente trabalho permitiu estabelecer uma metodologia

de recuperação de sequências regulatórias funcionais a partir de um genoma complexo como o da cana-de-açúcar e possibilitou a descoberta de quatro regiões genômicas distintas que abrigam módulos regulatórios funcionais com ampla possibilidade de serem explorados biotecnologicamente e no campo da ciência básica.

Palavras-chave: Promotores. Monocotiledôneas. Cana-de-açúcar. Transformação genética. Protoplastos.

ABSTRACT

KUROKI, M. A. **Customized promoter cloning strategy**. 2012. 137 p. Masters thesis (Biotechnology) - Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2012.

Gene expression is controlled at multiple levels. At the transcriptional level, control is determined by the interaction of transcriptions factors with *cis*-regulatory sequences flanking the regions to be transcribed. This interaction establishes the specificity of space-temporal expression. The basis of the construction of GMOs (Genetically Modified Organisms) is the insertion of one or more genes of interest under the control of promoter regions, which drive the expression of these genes. Therefore, the identification of constitutive promoters as well as specific, or inducible, promoters is extremely important to the development of new biotechnological products. The aim of this work is develop a strategy to identify functional promoter regions from any genome. The innovation of this approach relies on a screening method that identifies promoters without requiring previous knowledge of a specific gene, furthermore it allows the isolation of non canonical promoters. The modern sugarcane genome was chosen as a model for the development of this strategy. The modern sugarcane cultivar is a complex hybrid that is of major economic and biotechnological interest because of its importance to the agricultural and energy sectors. Notwithstanding, the sugarcane genome is a considerable challenge for identification functional promoters since the high level of ploidy increases the risk of isolating promoters from inactive copies of a gene. In this work we present the establishment of a strategy by means of customized cloning of fragments from the sugarcane cultivars R570 and SP80-3280. This strategy first involves fractionating genomic DNA and selecting fragments ranging from 2 to 4kb. Libraries were constructed by cloning these fragments upstream of a fusion of two gene reporters, GFP and GUS. The libraries were then screened by a transient expression assay using biolistic particle delivery of clones into epidermal onion cells. One hundred and forty two clones were analyzed and of these, four clones, F6, B4, F7 and C5, were isolated with putative functional regulatory modules. These clones were further analysed by another transient expression assay to detect GFP gene expression by transformation of rice protoplasts. All four clones showed GFP expression, confirming their activity in another monocot species. Three clones, F6, B4 and F7, were permanently transformed in rice, using *Agrobacterium tumefaciens,* and 12 plants were obtained. GUS expression was detected in the callus, leaves and roots of the rice plants with promoters from clones F6, B4 and F7, thus confirming the functionality of sequences in these clones. The present work has established a strategy to identify and extract functional regulatory sequences from a complex genome like sugarcane. It has also identified four genomic regions containing functional regulatory regions which show great potential of being useful in both the biotechnology field and in the field of basic science.

Key words: Promoter, monocot, sugarcane, genetic transformation, protoplast.

1 INTRODUÇÃO

A biotecnologia tem se destacado nos últimos anos como uma inovação tecnológica capaz de agregar riquezas a diferentes áreas do setor produtivo, entre elas, o setor da saúde e da produção de alimentos. No setor agrícola e florestal, a engenharia genética de plantas tem contribuído substancialmente para o maior entendimento da regulação gênica e desenvolvimento de plantas por meio da geração de organismos transgênicos para amplo uso na agricultura e fins industriais e farmacêuticos. A produção de organismos transgênicos de interesse comercial se baseia no desenvolvimento de métodos capazes de controlar a expressão do transgene de maneira precisa.

Desta maneira existe um grande empenho para se identificar novos promotores, já que estes são conhecidos por serem os processadores centrais da regulação da expressão de um gene e, portanto, são considerados ferramentas moleculares na pesquisa e desenvolvimento da biotecnologia. O objetivo central deste trabalho é o estabelecimento de uma metodologia de identificação rápida de promotores em média escala, a qual poderá ser aplicada a qualquer genoma de interesse. A inovação contida nesta proposta se baseia em adotar uma estratégia de clonagem customizada de sequências regulatórias capazes de regular a expressão gênica, sem a necessidade do conhecimento prévio do gene de interesse. Trata-se, portanto, de um trabalho com enfoque acadêmico e aplicado, o qual está vinculado ao programa FAPESP de Pesquisa em Bioenergia (BIOEN). Esse programa objetiva estimular e articular atividades exploratórias que possam gerar novos conhecimentos sobre a cana-de-açúcar, com o intuito de aprimorar sua capacidade na indústria em tecnologias dirigidas ao bioetanol, assim como aumentar sua competitividade interna e externa. Para o desenvolvimento deste projeto, foi escolhido como modelo biológico, a cana-de-açúcar, um híbrido de elevado interesse econômico e de alta complexidade genética. Poucos promotores foram isolados para a cana-de-açúcar, principalmente devido ao elevado nível de ploidia e o alto risco de se identificar regiões regulatórias provenientes de cópias inativas dos genes. Desta maneira, o desenvolvimento desta estratégia inovadora em cultivares de alto interesse permitirá resgatar novos motivos regulatórios capazes de

dirigir a expressão gênica. Essas sequências irão permitir a construção de cassetes de expressão destinados a programas de biotecnologia cujo objetivo é o aumento da biomassa desta cultivar e de outras, assim como possibilitará estudos de genômica funcional os quais permitirão desvendar módulos regulatórios inéditos.

6 CONCLUSÕES

- A estratégia de clonagem customizada e geração de bibliotecas de vetores contendo fragmentos de DNA associados a genes repórteres GUS e GFP foi estabelecida com sucesso.

- O processamento do genoma através da utilização de endonucleases de restrição específicas foi adequado para o êxito na seleção de fragmentos de DNA com tamanho adequado para a identificação de potenciais regiões promotoras.

- A estratégia de triagem funcional associou os processos de identificação e ensaio funcional das regiões promotoras, acelerando a obtenção de regiões promotoras da expressão gênica.

- Quatro regiões regulatórias funcionais (F6, B4, F7 e C5) foram isoladas do genoma da cana-de-açúcar mediante expressão transitória em epitélio de cebola e confirmada em protoplastos de arroz.

- Foi possível observar diferenças na intensidade de indução da expressão do gene GUS dirigida pelos promotores F6, B4 e F7 em plantas transgênicas de arroz.

- A disponibilização de módulos regulatórios já com informação funcional abre amplas perspectivas a serem exploradas no campo da biotecnologia e da ciência básica.

- A utilização do genoma da cana-de-açúcar como modelo para a estratégia de clonagem customizada foi aplicada com êxito e pode ser estendida a qualquer genoma de interesse.

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