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A RAPID PCR BASED METHOD FOR GENE SYNTHESISTROL

Samir Naimov[1]*, Stefan Dukiandjiev[1], Ruud A. de Maagd[2]

[1]Department of Plant Physiology and Molecular Biology, University of Plovdiv,
24 Tsar Assen Street, Plovdiv, Bulgaria
[2]Business Biocides , Plant Research International B.V, P.O. Box 16, 6700 AA Wageningen, The Netherlands

ABSTRACT. Chemical synthesis of DNA sequences provides a powerful tool for modifying genes and for studying gene function, structure and expression. Here, we report a simple, high-fidelity and cost-effective PCR-based two-step DNA synthesis method for synthesis of long segments of DNA. The method involves two steps: 1) PCR assembling of chemically synthezyed DNA oligomers and 2) specific amplification of the full length DNA sequence . Compared with the previously published methods, the this method is rapid (2 days) and suitable for synthesizing long segments of DNA (2 kb) with high G + C contents, repetitive sequences or complex secondary structures.

KEY WORDS: SN19 hybrid, *Bacillus thuringiensis*, Synthetic genes, PCR

INTRODUCTION

Chemical synthesis of DNA sequences can provide a powerful molecular tool for modifying genes, elucidating gene functions and studying the structure–function relationship of proteins. The recent advances in the area of chemical synthesis of DNA molecules have made it possible to synthesize and assemble genes for improved or novel functions. Also, in many cases, it is highly desirable to use a chemical synthesis method to modify coding sequences to achieve high expression levels in desirable expression systems (1-5). Several methods for the synthesis and assembly of DNA sequences based on oligonucleotides have been described previously: a method for enzymatic ligation (6-11) of oligonucleotides reported in the 1980s, the *FokI* method published in 1988 (12), self-priming PCR developed in early 1990s(13-16), the PCR assembly method described in 1995 (17) and further improved in 2002 (19), and the template directed ligation (TDL) method published in 1996 (18). More recently, two methods of synthesis and assembly of long DNA sequences (5 kb) have been published. One is called the thermodynamically balanced

inside-out (TBIO) PCR-based gene synthesis method, which uses a novel method of primer design to achieve high-fidelity assembly of long gene sequences. Despite the intensive research done in this filed the gene synthesis is still time consuming procedure. In this paper we present a rapid PCR based method for gene synthesis, which allows an accurate assembling over of 1000 bp DNA sequences in two PCR steps.

MATERIAL AND METHODS

To demonstrate the possibility for rapid gene synthesis an artificially designed SN19 insecticidal protein encoding gene, described before, was optimized and subsequently resynthesized.

Bioinformatics recourses. Back-translation of the protein sequence and codon optimization were done using web based recourses. In order to express SN19 in potatoes, initially bacterial sequence was redesigned by back-translation of the protein sequence (fig.1) of SN19 insecticidal protein. For this purpose web-based recourses, such as back-translation tool (http://www.entelechon.com/eng/backtranslation.html) and codon usage tables (http://gcua.schoedl.de/ and http://www.kazusa.or.jp/codon/) were used.

Oligonucleotide design.

The 2000 bp sequence was split up first in two blocks A and B, 900 and 1100bp respectively and than additionally broken up to 50 bp long oligonucleotides, except 5' ends flanking ones (25 bp.). All of the primers used in this study were kindly provided by SynGen Inc. (Canada), and additionally purified on polyacrilamyde gel, as it is described elsewhere (maniatis).

PCR based oligo assembling.

The purified primers were diluted to final concentration of 0, 25 pmol-s per each, dNTPs (0.125 mM each) and 0.5 U Pfu-Turbo DNA polymerase (Stratagene) were added and primary PCR (fig.2) was performed using annealing temperature of 55C. A prorogated elongation time (from 30 sec up to 2 min) protocol and hot start at 2 min at 94C was used. When the PCR program completed two micro liters of the products were used as a template for second PCR with primers flanking the full length product. 30 cycles of a standard PCR protocol were performed using following program: 94C for 1 min, 55C for 45 sec., and 68C for 2.5 min.

Cloning in pGem-T easy and DNA sequencing.

Cloning and DNA were done according manufacture requirements. For DNA sequencing Big Dye termination mix was used. Cloning in pGem T easy was preceded by Taq DNA polymerase treatment for 5 min at 72C for additional adenilation of DNA ends.

RESULTS AND DISCUSSION

To make a completely synthetic SN19 gene we have choose a oligonucloetides assembling by PCR. This approach is a time saving and gives an opportunity to produce a large amount of desired DNA product. In order to increase the expression levels of protein of interest in transgenic plants a condon optimized DNA sequence

was designed. Using internet based recourses a completely consistent to potato codon usage table gene construction was designed. All potential mRNA instability elements, poy A signals and really used codons were eliminated**.** In the same time during the oligonucleotide design process possibility for application of alternative methods such as primer ligation was taken in account. The resulted eukaryotic, 2000 bp in size DNA sequence was split up in 50 oligonucleotides, all of them except 5' ends flanking ones were 50 bp in length. Additionally the whole sequence was divided into two different blocks A and B respectively 900 bp and 1100 bp in size. To prevent incorporation of shorter olgonucleotides, 50 bp primers were mixed in 1:1 ratio (in molar basess) and purified by polyacrileamide gel electrophoresis. Because of the full overlap of the primers a prolongated elongation time PCR protocol was used. This method gives a great opportunity for semi-complete synthesis of DNA fragments and generates different n size intermediate PCR products. Each of them possibly can be used in the next PCR cycle for further elongation of the DNA product. Despite the fact that proves reading DNA polymerase was used, only 25% of the sequenced clones were containing less than 4 point mutations. This fact can be explained with the specificity of the amplified DNA sequence.

The method presented here allows de novo synthesis of long DNA sequences for relatively short time, with high rate of sequence accuracy and can be used for multi purpose custom gene synthesis.

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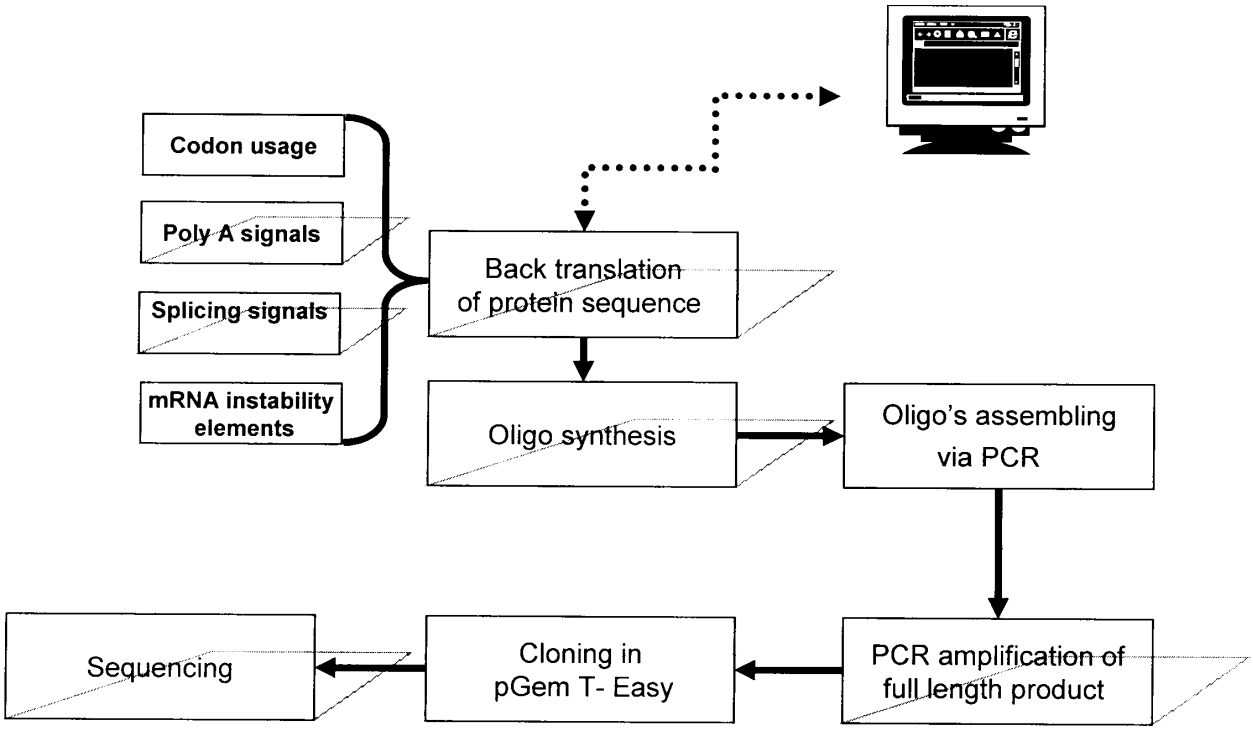

ig. 1. *Block scheme of back-translation, mRNA optimization and SN19 synthesis. Codon usage, splicing, poly A signals, and mRNA instability elements are taken in account.*

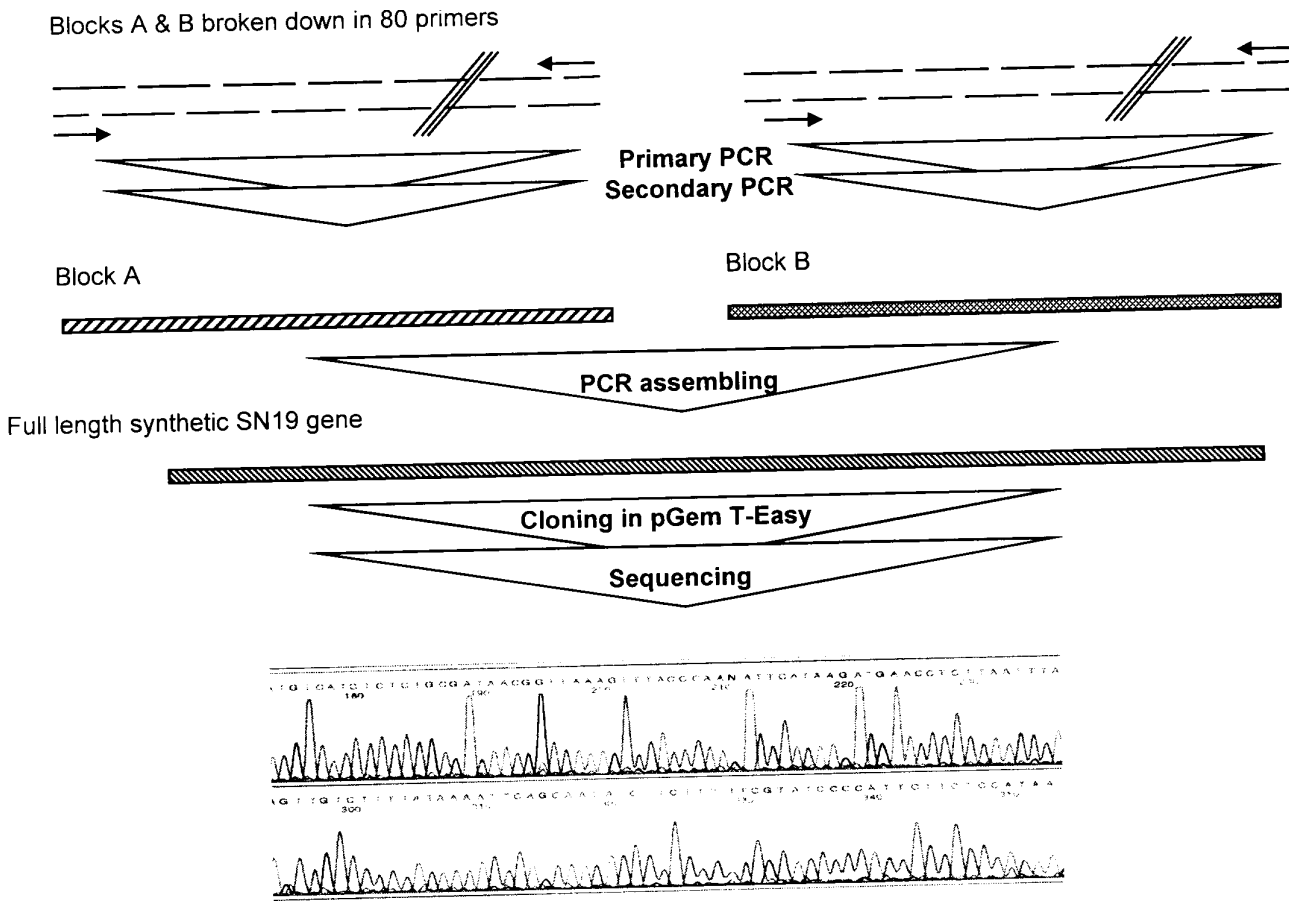

Fig. 2. *SN19 synthesis by PCR mediated oligonucleotids assembling.*