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Rapid one-step construction of hairpin RNA

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ABSTRACT

Hairpin RNA (hpRNA) is commonly used for gene-function exploration and gene engineering. In this study, a novel method was developed to construct intron-containing hairpin RNA (ihpRNA) rapidly and efficiently based on Overlap Extension PCR (OE-PCR). This method, Mixed One-step OE-PCR (MOOE-PCR), can amplify two inverted repeats of DNA fragments and a spliceable intron in parallel, and then assemble them to generate ihpRNA constructs in the same tube without the purification of intermediate products. This method required a PCR process of 38–40 cycles and ordinary PCR reagents. A total of 10 ihpRNA constructs were amplified successfully using this method, with the stems ranging from 50 bp to 484 bp in length. Our results suggest that this novel method is a useful strategy for constructing ihpRNA.

Introduction

RNA interference (RNAi) is an efficient tool in gene-function exploration and gene engineering in a wide range of species [1–4]. Hairpin RNA (hpRNA) constructs are commonly used to induce degradation of target genes through RNAi machines [5,6]. In plants, intron-containing hairpin RNA (ihpRNA) with a spliceable intron as a spacer sequence shows the highest efficiency [7,8]. Currently, ihpRNA technology is a very powerful tool for gene discovery and gene engineering in plants [9].

To facilitate the generation of ihpRNA constructs, several methods have been used. For instance, a generic vector, pHANNIBAL, that contains a functional intron had been reported [8]. This method requires several rounds of restriction and ligation, and is tedious and time-consuming. Another construct, pHELLSGATE, takes advantage of Gateway technology, which facilitates easy cloning of PCR fragments [8]. However, the reagents used in this method are expensive, especially to researchers in developing countries. We designed various ihpRNAs to test the silencing efficiency, and included stem lengths as short as 50 bp and 100 bp. These small segments are difficult for gel extraction after restriction or amplification, and the method of restriction and ligation and the Gateway system are not suitable for these short ihpRNAs. Therefore, a more versatile and low-cost method for the rapid generation of ihpRNA is needed.

Overlap Extension PCR (OE-PCR) is a simple and versatile technique for gene splicing and site-directed mutagenesis [10–12]. OE-PCR has been used widely for gene synthesis and mutagenesis, and some improvements have been made [13–15]. One significant improvement had been reported for site-directed mutagenesis, which used asymmetric PCR in a one-step OE-PCR without any intermediate purification [15]. A method for ihpRNA construction based on OE-PCR was described by Xiao et al. [16]. This approach is simple and rapid, but requires a template including an extron and its adjacent intron in genomic DNA to generate the stem and intron of ihpRNA. Therefore, the position of the stem and the variety of introns are limited.

To develop a more versatile approach for rapid generation of hpRNAs, we describe a novel method that combines OE-PCR and asymmetric PCR to construct ihpRNA in one tube. This method has been successful in the generation of various ihpRNAs, with the stems ranging from 50 bp to 484 bp in length.

Materials and methods

Plasmid and enzymes. The TA clone vector, PMD-18T (Takara, Dalian, China), was used for the cloning of PCR products. The cp gene (860 bp) of papaya ringspot virus (PRSV) (GenBank Accession No. EF183499) cloned in the PMD-18T vector or the purified PCR product of the cp gene was used as a template for amplifying the stems of ihpRNAs. The intron region (266 bp) of Arabis pumila COR15α gene (GenBank Accession No. AY587559) cloned in the PMD-18T vector or the purified PCR product of the intron was used as template for amplifying the intron of ihpRNAs. Ex Taq, LA Taq

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and Pyrobest DNA Polymerase were purchased from Takara (Takara, Dalian, China), High Fidelity PCR Enzyme was purchased from Fermentas (Fermentas, Vilnius, Lithuania), and Tiangen Taq was purchased from Tiangen (Tiangen, Beijing, China).

Experimental design. The rapid construction of ihpRNA by the Mixed One-step OE-PCR (MOOE-PCR) method is depicted in Fig. 1A and is analyzed in detail in Fig. 1B. This method consists of only 1 step, a PCR process of 38–40 cycles, and the ihpRNA structure is

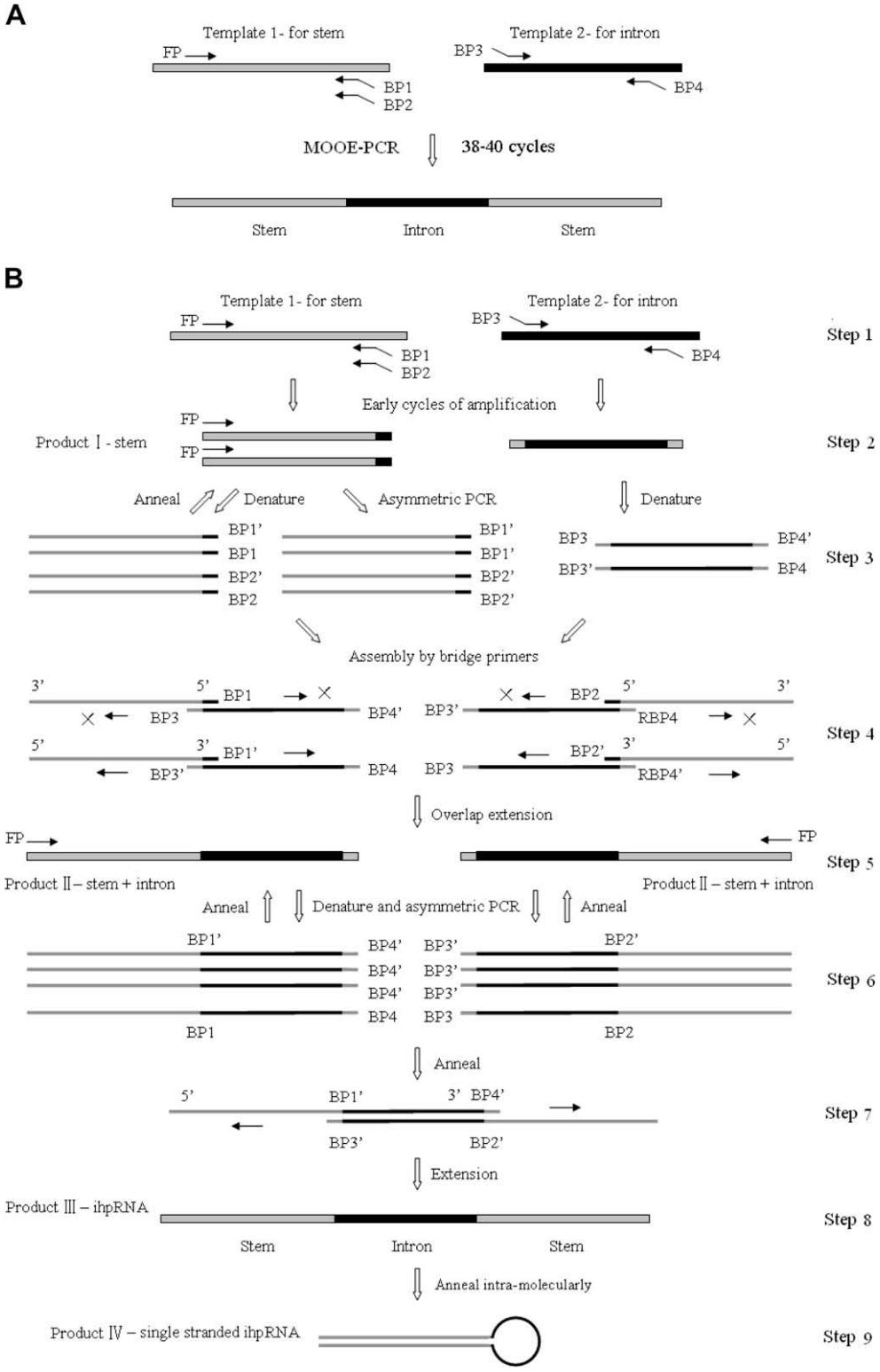

Fig. 1. Schematic diagram of the MOOE-PCR method. (A) The simple process of MOOE-PCR. Adding 2 templates, 1 FP and 4 BPs (BP1–4) (ratio of 20:1), and PCR reagents into a PCR tube, the ihpRNA structure is generated after a PCR process of 38–40 cycles. (B) The analysis of MOOE-PCR. Four amplicons were produced in the process of MOOE-PCR. BP1′–4′ are complementary sequences to BP1–4. The concentration ratio of BP to FP was 1:20, which resulted in asymmetric PCR. With the BPs exhausted in the late cycles of asymmetric PCR, the major amplification products were the single-stranded DNA of stem (step 3) and stem + intron (step 6) primed by FP, which enhanced the efficiency of overlap extension.