Application of on-chip capillary electrophoresis to cell-free preparation of recombinant DNA

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ABSTRACT

An on-chip capillary electrophoresis-based DNA collection was applied to the isolation of target DNA species from a DNA mixture generated by a polymerase chain reaction (PCR), whose starting material was a ligation mixture of an insert and an expression vector. The collected DNA was then amplified by PCR and properly worked as template DNA in a coupled cell-free transcription/translation system. These results demonstrated that total operation in standard genetic engineering can be performed in a cell-free condition.

INTRODUCTION

DNA ligase-catalyzed DNA assembly is one of the key steps in the recombinant DNA technology. Because the reaction assembles DNA fragments randomly, it is required to separate the target DNA from the reaction mixture, where living cell-based selection of the target DNA has been widely utilized, which should be carried out in a biohazard facility. Although various recombinant proteins are prepared in cell-free expression systems outside the biohazard facility today, the template DNAs are still prepared by the living cell-dependent method. In the present report, we adopted an on-chip capillary electrophoresis (CE)-based DNA collection for the desired DNA selection, and demonstrated that the recovered DNA properly worked as the template for a coupled cell-free transcription/translation system (Scheme 1).

RESULTS AND DISCUSSION

We selected a green fluorescent protein (GFP) mutant GFPuv\(^1\) as a model gene, and tried to introduce the gene into an expression vector carrying T7 RNA polymerase-specific promoter and terminator. The insert coding GFPuv and the backbone of the expression vector were isolated from commercial vectors with a standard PCR using primers carrying restriction sites. After the digestion with restriction enzymes, the PCR-amplified fragments were assembled with T4 DNA ligase. From the mixture, the complete region that transcribed by T7 RNA polymerase was amplified with PCR (1st PCR). The amplified DNA was then collected with an on-chip CE-based DNA collection using a microfluidic device (Fig. 1, 2, Scheme 2). The operation was repeated twice and total 8 \(\mu\)L of sample was recovered, and without any purification, the recovered material was directly subjected to a PCR (2nd PCR) as a starting material. After the reaction, 1 \(\mu\)g of the amplified DNA was subjected to a coupled cell-free transcription/translation system, and after 4 h incubation at 30 \(^\circ\)C, the product eliminated green fluorescence (Fig. 2 b).

![Scheme 1](image)

**Scheme 1.** Cell-free preparation of a recombinant DNA as a template for cell-free transcription/translation by using an on-chip CE-based DNA collection.

![Fig. 1](image)

**Fig. 1.** (a) Design of the microfluidic device for the on-chip CE-based DNA collection. The device consists of a fluidic chip made of polydimethylsiloxane (PDMS) and a glass substrate on which Au/Cr electrodes are patterned.\(^1\) The size is in mm scale. (b) Structure of the PDMS fluidic chip. Total 6 ports were fabricated and the channel was filled with 1.2 % hydroxyethyl cellulose (HEC) in TBE buffer. (c) Layout of ports and channels. The volume of each port is less than 5 \(\mu\)L. The channel width and the channel depth are 90 \(\mu\)m and 30 \(\mu\)m, respectively.
It shows that the construction of the recombinant template DNA for the cell-free transcription/translation was successful in a cell-free manner.

In the traditional method using living cells for the construction of templates for protein expression, an overnight incubation is required for the growth after the transfection of the ligation mixture into the host cells, and for another night is required for the pre-culture for plasmid preparation. On the other hand, by using the present method, the comparable step can be carried out in a half day. Isolation of the target DNA from the mixture by using the on-chip CE-based DNA recovery requires less than 5 minutes, and the total time required for the separation is less than 5 hours including PCR amplification. Now, we are trying to separate the desired assembly from the ligation mixture directly by using the device with minor modifications, which makes the protocol free from PCR, and thereby the experimental time will be dramatically reduced, and also planning to install a cell-free transcription/translational part like ones we have reported previously downstream of the DNA recovery channel, in order to realize "on-chip genetic engineering". Further research is underway in our laboratory.

CONCLUSION

An on-chip CE-based DNA recovery was adopted to prepare the DNA carrying desired construction as a template for cell-free transcription/translation. By combining this method with a coupled cell-free transcription/translation system, total process of recombinant DNA technology was performed under a cell-free condition.

REFERENCES


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