On-chip Capillary Electrophoresis Fractionation of DNA Construct for Cell-free Protein Expression

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An on-chip capillary electrophoresis-based DNA collection was applied to isolate desired DNA construct from a reaction mixture of DNA-assembly instead of traditional living cell-based screening. The isolated construct was amplified with polymerase chain reaction and subjected to a coupled cell-free transcription/translation system as a template, and the template-dependent protein expression was observed. It was demonstrated that total process of standard recombinant DNA technology was performed in a cell-free condition.

Cell-free translation is one of the powerful tools in the field of proteomics. Especially, the fact is attractive that target protein can be prepared without any usage of biohazard facilities. Thanks to the development of cell-free protein synthesis techniques for this decade, various kinds of proteins are now prepared in cell-free translation systems.[1] However, the template DNA subjected to the cell-free systems is still prepared with living cell-based method because the template is constructed with standard recombinant DNA technology,[2] in which DNA ligase-catalyzed DNA assembly and living cell-dependent screening of the construction are utilized. Because DNA ligase assembles DNA fragments randomly and complex mixture is generated,[3] it is required to isolate the target DNA species from the mixture, where living cell-based selection has been widely utilized, which should be carried out in a biohazard facility. In this report, we adopted an on-chip capillary electrophoresis (CE)-based DNA collection for the screening of desired DNA selection,[4] and demonstrated that the recovered DNA properly worked as the template in a coupled cell-free transcription/translation system (Figure 1).

We selected a green fluorescent protein (GFP) mutant GFPuv[5] as a model gene, and introduced the gene into an expression vector carrying T7 RNA polymerase-specific promoter and terminator (Figure 2).[6] When the construction is succeeded, transcription of the target gene will be under the control of T7 RNA polymerase, and green fluorescence will be observed after the translation. The insert coding GFPuv and the backbone of the expression vector were isolated from commercial vectors with a standard PCR[7] using primers carrying restriction sites. After the digestion with restriction enzymes, the PCR-amplified fragments were assembled with T4 DNA ligase.[8] From the reaction mixture, the complete region that T7 RNA polymerase transcribes was amplified with PCR (1st PCR). The amplified DNA was then collected with an on-chip CE-based DNA collection using

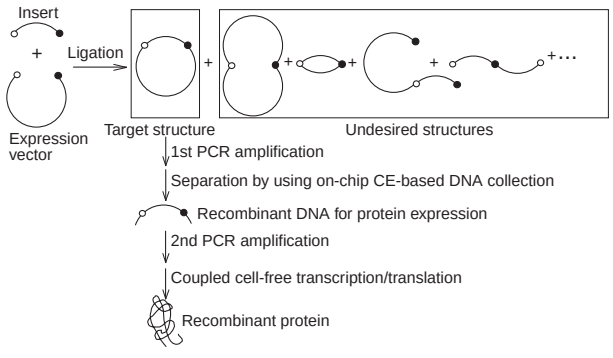

Figure 1. Construction of a template DNA for cell-free protein expression by using on-chip CE-based DNA collection.

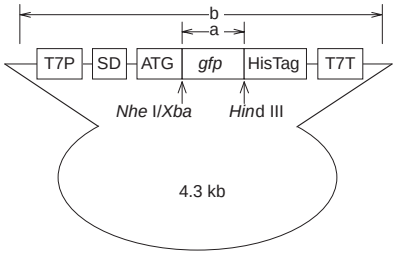

Figure 2. Schematic illustration of the DNA construction for coupled cell-free transcription/translation. T7P; T7 RNA polymerase-specific promoter, SD; ribosome binding site,[12] ATG; initiation codon, gfp; GFPuv gene, HisTag; hexahistidine-tag,[13] T7T; T7 RNA polymerase-specific terminator. "a" corresponds to "Insert" in Figure 1 and "b" is the region that amplified by the 1st and the 2nd PCR.

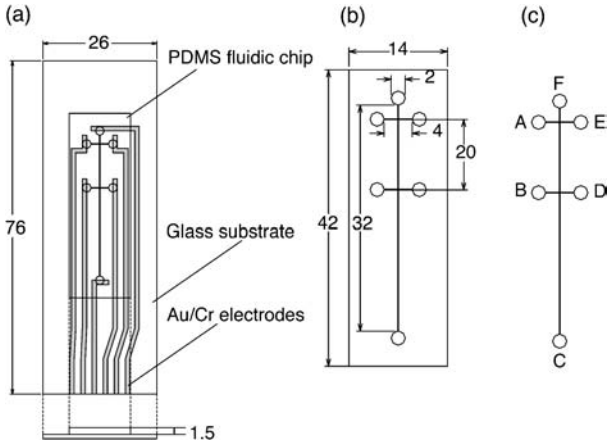

Figure 3. (a) Design of the microfluidic device for the on-chip CE-based DNA collection. The size is described in mm scale. (b) Structure of the PDMS[14] fluidic chip. (c) Layout of ports and channels. The volume of each port is less than 5 μL. The channel width is 90 μm and the channel depth is 30 μm. The channel was filled with 1.2% hydroxyethyl cellulose[15] in 1xTBE buffer.

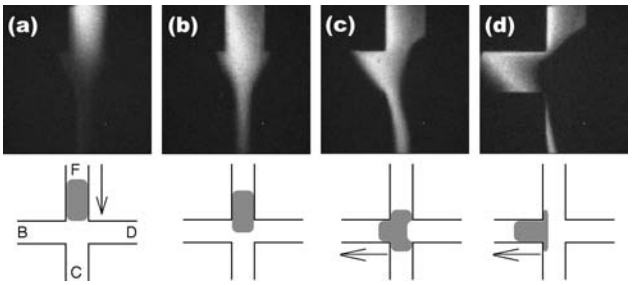

Figure 4. Captured video images of the recovery of the target DNA fraction from an enzymatic reaction mixture. (a, b) The target fraction came down to BD-FC intersection in vertical direction. (c, d) The electrophoresis was switched to horizontal direction and target fraction goes to the recovery port (Port B). The subjected amount of DNA was 100 ng, which was stained with SYBR Green I. The electrophoretic operations were implemented by a computer-controlled high voltage supply. The applied voltages were 500 V (a, b) and 100 V (c, d). The operation was monitored by an inverted fluorescence microscope and a CCD camera, and recorded by a video recorder.

Figure 5. Fluorescence of the GFPuv synthesized in a coupled cell-free transcription/translation system where the reaction was conducted by the recombinant DNA prepared by using the on-chip CE-based DNA collection. After the reaction, the mixture was stored for 24 h at 4 °C for maturation of GFP.[16]

a microfluidic device (Figure 3). The fabrication process of the device and the operation of the collection were carried out according to our previous reports.[9] The collection of target DNA fraction is shown in Figure 4. It was repeated twice and total 8 μL of sample was recovered. Without any purification, the recovered material was directly subjected to a PCR (2nd PCR) as a starting material. After the reaction, 1 μg of the amplified DNA was subjected to a DNA-directed coupled cell-free transcription/translation system[10] containing T7 RNA polymerase (Roche Diagnostic, RTS 100 *E. coli* HY Kit), and after 4 h incubation at 30 °C, the product eliminated green fluorescence (Figure 5). 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 recombinant DNA technique based on living cell-dependent DNA screening, an overnight incubation is required for the growth of the cells after the transformation, and another night is required for the pre-culture for plasmid preparation.[2] On the other hand, by using the present method, the comparable step can be performed in a half day: less than 5 min is enough for the on-chip CE-based DNA collection, and the total time required for the separation is less than 5 h including two PCR amplification. Now, we are trying to separate the desired assemble from the ligation mixture directly by using the device, 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/translation part[11] downstream of the DNA recovery channel, in order to realize "on-chip genetic engineering." Further research is underway in our laboratory.

In conclusion, an on-chip CE-based DNA collection was adopted to prepare the template DNA for coupled cell-free transcription/translation. By combining this method with a coupled cell-free transcription/translation system, total process of recombinant DNA was performed under a cell-free condition.

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