Cell-Free Synthesis of Fluorescent Proteins Using *ExiProgen*™ Automated Protein Synthesis System

Yousang Cho[1], Junho Jung[1], Nayoung Lee[1], and Jiwon Han[1*]
[1]Molecular Science Laboratory, Bioneer Corporation, 8-11Munpyeongseoro, Daedeok-gu, Daejeon 306-220, Korea
[*]Email: exiprogen@bioneer.co.kr, exiprogen-support@bioneer.co.kr

Introduction

In the post-genomics era, where vast DNA sequence information across many organisms has accumulated, identifying protein structure, function and protein-protein interactions is crucial to understand cellular and metabolic process as well as the cause of diseases at the molecular level. Protein expression is necessary to study the functional activity of proteins and the typical method for protein expression is *in vivo* expression using cells. This method involves the transfection of recombinant DNA into the host cell, cell culture and subsequent lysis and purification. Protein expression by cell culturing is a time-consuming process, so it is not easy to synthesize a number of proteins at the same time. To overcome this shortcoming, cell-free protein synthesis (*in vitro* transcription/translation) methods have been developed. Cell-free methods use cell extracts combined with ingredients essential for protein synthesis and synthesis reactions are performed *in vitro*. When recombinant DNA coding for the protein of interest is added and the mixture is maintained at a certain temperature, the desired protein is synthesized. Because this method does not require a separate cell line selection step, it is able to yield different types of protein in a short period of time, so it has potential advantages in increasing the throughput of protein synthesis. *In vitro* protein expression can also provide proteins that are difficult to over-express due to cellular toxicity effects (1-3).

ExiProgen Protein Synthesis System brings automation to *in vitro* protein expression and magnetic bead-based His-Tag affinity purification methods: the result is up to 16 highly pure proteins in less than 6 hours. *In vitro* protein synthesis using *ExiProgen* (Bioneer, Cat. No. A-5041) has a simple workflow; template DNA preparation, loading template DNA into *ExiProgen* EC1 Protein Synthesis Kit (Bioneer, Cat. No. K-7300), loading the kit components onto the deck of *ExiProgen*, and starting the system. *ExiProgen* EC1 Protein Synthesis Kit contains optimized *E. coli* extract which has T7 RNA polymerase and ribosomes, all other required components such as amino acids and an energy source for effective and efficient *in vitro* transcription and translation. The kit also contains Ni-NTA magnetic beads for fast and effective purification of expressed His-tagged proteins. With *ExiProgen* and *ExiProgen* EC1 Protein Synthesis Kit, up to 16 different kinds of highly pure proteins can be obtained at the same time within 6 hours of adding template DNA which can be in the form of a plasmid or linear PCR products.

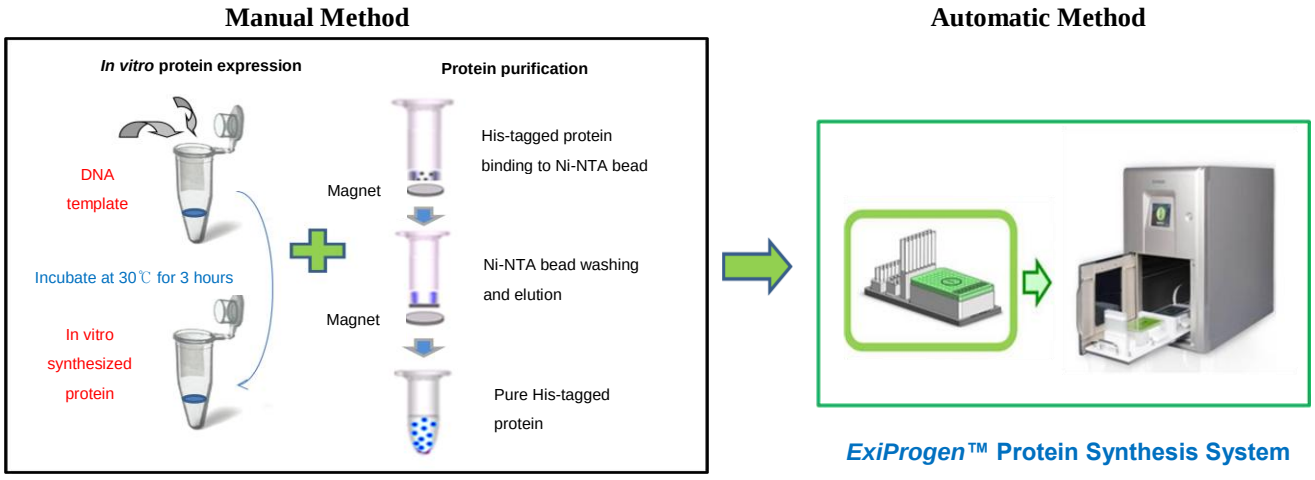

Figure 1. Comparison of manual and automated protein expression and purification
Left panel: Manual method of protein expression/purification – sequential and tedious
Right panel: Automated method of protein expression and purification – simply add DNA and run *ExiProgen*

Since the green fluorescent protein (GFP) was first discovered and purified from the jellyfish *Aequorea Victoria* in the 1960s, it has become one of the most useful and valuable protein in molecular and cellular biology, and several derivatives have been developed. Because fluorescent proteins emit fluorescence when excited at a specific wavelength of light, they are used as a marker of gene expression and can localize proteins of interest within living cells. After fusing DNA coding for GFP to a target protein gene and then transfecting into cells, the localization and dynamics of target protein in cells can be detected with fluorescence microscopy (4).

In this study, we synthesized green and red fluorescent proteins *in vitro* using *ExiProgen* Protein Synthesis System to demonstrate *ExiProgen*'s capability to automatically express and purify proteins after adding template DNA into reaction wells.

Methods and results

For the protein synthesis with *ExiProgen*, we constructed *in vitro* protein expression vector pBIVT (Bioneer, Cat. No. K-7350), which contains a T7 promoter, ribosomal binding site (RBS), T7 terminator, multi cloning site, and His-tag coding sequences at either the N-terminus or C-terminus. The AcGFP, (a GFP derived from the jellyfish *Aequorea coerulescens*), encoding gene was incorporated to pBIVT-1. SfGFP (super folder green fluorescent protein) and RFP (red fluorescent protein) expression vectors were kind gifts from Dr. Dong-Myung Kim at Chungnam National University.

For the expression of fluorescent proteins, 10 µg of pBIVT1-AcGFP, 7 µg of SfGFP expression vector, and 10 µg of RFP expression vector were added to three different reaction wells of the protein expression cartridge (cartridge 2) of the *ExiProgen* EC1 Protein Synthesis Kit. Next, the protein expression cartridge (cartridge 2), the protein purification cartridge (cartridge 1), cell extract, elution tubes, and filter tips (all provided in the kit) were placed in the correct position on the deck of *ExiProgen*. *ExiProgen* was then run after selecting the protocol number 902 as described in the kit manual. The run was finished in less than 6 hours and 250 µl of purified protein samples were collected in the elution tubes. Synthesized proteins in the elution tubes showed their characteristic colors which are visible with the naked eye. The fluorescence emitted from the fluorescent proteins was detected with UV illuminator, indicating that the fluorescent proteins synthesized with *ExiProgen* are properly folded into three-dimensional structures with functional activities (Fig. 2).

To check the synthesis of the target proteins, the unpurified expression samples from the "J" section of cartridge 2 and purification samples in the elution tubes were run in the SDS-PAGE gel. Samples were prepared for SDS-PAGE gel analysis as described in the manual. The SDS-PAGE result showed that AcGFP, SfGFP, and RFP were expressed and purified with *ExiProgen* (Fig. 3). The amount of synthesized AcGFP, SfGFP, and RFP is about 100 µg per reaction.

In this study, we successfully expressed and purified fluorescent proteins using *ExiProgen*, which makes use of cell-free protein expression (*in vitro* transcription and translation) and automatically purifies expressed His-tagged proteins with Ni-NTA magnetic beads. Since it is automated, easy-to-use, and provides rapid protein synthesis, *ExiProgen* has the potential applications several research fields including identification of protein function, protein-protein interaction study, protein structure labs as well as enzyme engineering and in Biofuel research labs. *ExiProgen* is proving to be a valuable tool for protein scientists.

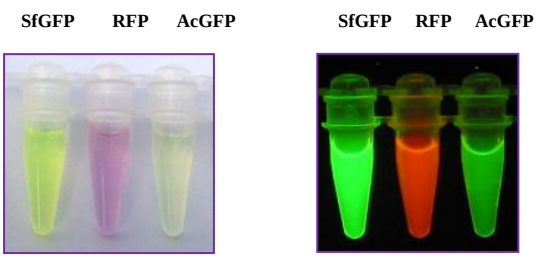

Figure 2. SfGFP, RFP, and AcGFP synthesized with *ExiProgen* emitted fluorescence.
Left panel: Color of each eluted protein sample detected with naked eyes. Right panel: Fluorescence from eluted protein samples detected with UV illuminator

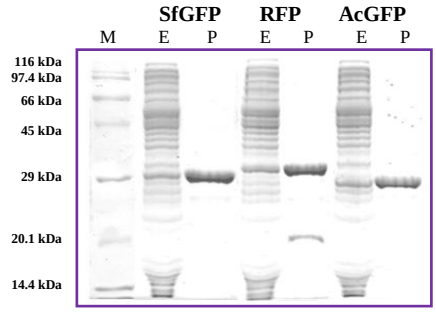

Figure 3. SfGFP, RFP and AcGFP were expressed and purified with *ExiProgen*.
Each expression sample and purification sample was run on 12% SDS-PAGE gel. Top of the lane: Protein name, Lane M: *AccuLadder*™ Protein Size Marker (Broad) (Bioneer, Cat. No. D-2010), Lane E: Unpurified expression sample, Lane P: Purified sample

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