In vitro Protein Synthesis with *ExiProgen*™ Automated Protein Synthesis System Using in vivo Protein Expression pET Vectors

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Introduction

In order to better understand protein function, protein expression systems have been developed over the years that allow researchers to generate recombinant proteins at will. One of the best known and highly used expression systems is the pET Expression System developed in 1986 by W. F. Studier and B. A. Moffatt[1]. The pET system uses T7 polymerase for transcription of RNA and allows for control of protein expression via the lac Operon (Lac operator/Lac repressor system).

Protein expression using the pET expression system is typically accomplished *in vivo* using *E. coli* cells that express T7 polymerase. This method involves the transfection of the pET recombinant DNA into the host cell, selecting a cell line, cell culture and subsequent lysis and purification. Protein expression by cell culture is a time-consuming process; thus, it is not easy to synthesize a number of proteins at the same time. On the other hand, cell-free protein synthesis (*in vitro* protein transcription and translation) is a method to synthesize the desired protein in an efficient manner by adding template DNA containing the coding region of the protein of interest into a single tube which contains cell extracts and ingredients essential for protein synthesis. Because this method does not require a separate cell line selection step, it is able to yield diverse types of protein in a short period of time compared to *in vivo* protein expression. Hence, cell-free protein synthesis has potential advantages in high-throughput synthesis of proteins[2,3,4]. *ExiProgen* Protein Synthesis System brings automation cell-free protein synthesis and magnetic bead-based His-Tag affinity purification methods: the result is up to 16 highly pure proteins in less than 6 hours. The *ExiProgen* EC1 Protein Synthesis Kit has 16 reaction wells so that up to 16 different kinds of highly pure proteins can be obtained simultaneously within 6 hours of adding template DNA which can be in the form of an Expression vector or linear PCR products.

A major issue that has cropped up with labs using both *in vivo* and *in vitro* expression systems is this: *in vitro* protein expression vectors do not work well *in vivo*, and *vice versa*. In this study, we demonstrate that at least some of the vectors in the pET series can be used as template DNA for the cell-free protein synthesis with *ExiProgen* Protein Synthesis System.

Methods and results

We used pET15b, pET22b, pET23a and pET28a to test whether *ExiProgen* EC1 Protein Synthesis Kit is able to synthesize proteins when pET protein expression vectors are used as template DNA. 10 µg of pET15b-poly(A) polymerase, 10 µg of pET22b-MMLV RTase, 3.4 µg of pET23a-RNase H, and 6 µg of pET28a-PK16 (PTP) were used for the synthesis of each protein. Each DNA was added into a reaction well of the protein expression cartridge (cartridge 2) of *ExiProgen* EC1 Protein Synthesis Kit. The kit contents, including protein purification cartridge (cartridge 1), *E.coli* cell extract, elution tubes, and filter tips as well as cartridge 2, were then placed in the correct position on the deck of the *ExiProgen*. Next, *ExiProgen* was 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. To check the synthesis of the target proteins, the expression samples from 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 all four proteins we tested were expressed and purified with *ExiProgen* (Figure 1-4). The amount of each synthesized/purified poly(A) polymerase, MMLV RTase, RNase H, and PK16 (PTP) protein isolated was approximately 80 µg per reaction.

In summary, we successfully expressed and purified proteins from four pET vectors with *ExiProgen*, indicating that *ExiProgen* is able to synthesize proteins using these pET protein expression vectors as template DNA. As we generate more data, we will publish which pET vectors work well and which are not as robust, or do not work at all. Due to the automation, simplicity of use, and rapidness of protein synthesis, *ExiProgen* has the potential in applying to various research fields including identification of protein function, protein-protein interaction study, high throughput synthesis as well as enzyme engineering. *ExiProgen* will provide itself as a valuable tool for protein experimentation.

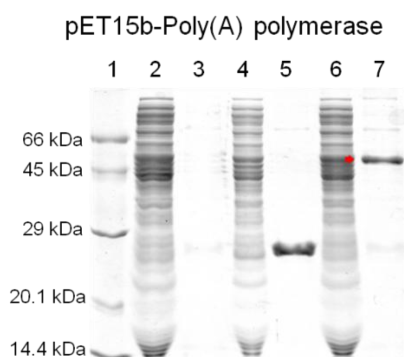

Figure 1. pET15b-poly(A) polymerase was used as a template DNA for the synthesis of poly(A) polymerase with *ExiProgen* EC1 Protein Synthesis Kit. Lane 1: *AccuLadder*™ Protein Size Marker (Low), Lane 2: Expression sample of negative control, Lane 3: Purified sample of negative control, Lane 4: Expression sample of positive control (pBIVT2-CAT), Lane 5: Purified sample of positive control (pBIVT2-CAT), Lane 6: Expression sample of poly(A) polymerase, Lane 7: Purified sample of poly(A) polymerase

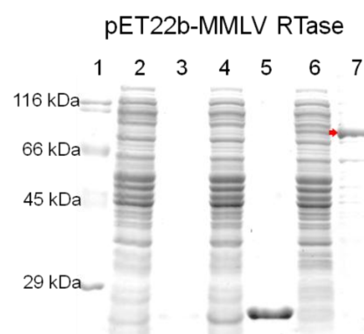

Figure 2. pET22b-MMLV RTase was used as a template DNA for the synthesis of MMLV RTase with *ExiProgen* EC1 Protein Synthesis Kit. Lane 1: *AccuLadder* Protein Size Marker (Broad), Lane 2: Expression sample of negative control, Lane 3: Purified sample of negative control, Lane 4: Expression sample of positive control (pBIVT2-CAT), Lane 5: Purified sample of positive control (pBIVT2-CAT), Lane 6: Expression sample of MMLV RTase, Lane 7: Purified sample of MMLV RTase

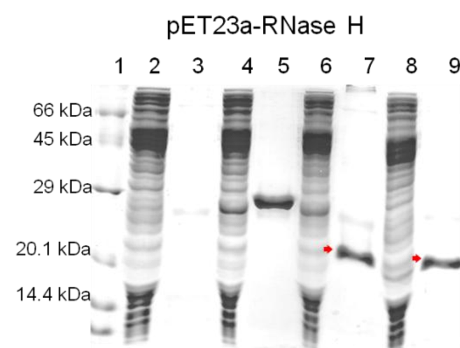

Figure 3. pET23a-RNase H was used as a template DNA for the synthesis of poly(A) polymerase with *ExiProgen* EC1 Protein Synthesis Kit. Lane 1: *AccuLadder* Protein Size Marker (Low), Lane 2: Expression sample of negative control, Lane 3: Purified sample of negative control, Lane 4: Expression sample of positive control (pBIVT1-AcGFP), Lane 5: Purified sample of positive control (pBIVT1-AcGFP), Lane 6: Expression sample of RNase H (C-His), Lane 7: Purified sample of RNase H (C-His), Lane 8: Expression sample of RNase H (N-His), Lane 9: Purified sample of RNase H (N-His)

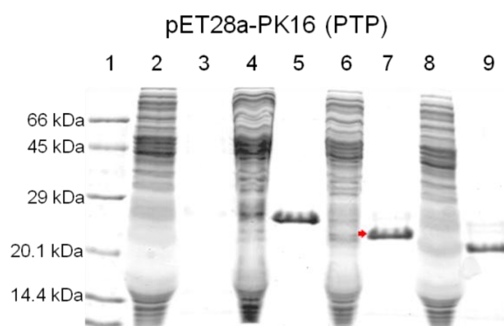

Figure 4. pET28a-PK16 (PTP) was used as a template DNA for the synthesis of PK16 (PTP) with *ExiProgen* EC1 Protein Synthesis Kit. Lane 1. *AccuLadder* Protein Size Marker (Low), Lane 2: Expression sample of negative control, Lane 3: Purified sample of negative control, Lane 4: Expression sample of positive control (pBIVT2-CAT), Lane 5: Purified sample of positive control (pBIVT2-CAT), Lane 6: Expression sample of PK16 (using pET22b-PK16), Lane 7: Purified sample of PK16 (using pET28a-PK16), Lane 8: Expression sample of PK16 (using pBIVT1-PK16), Lane 9: Purified sample of PK16 (using pBIVT1-PK16)

References

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