processes

MDPI

Supplementary Material

Construction of an Artificial Cell Capable of Protein Expression at Low Temperatures Using a Cell Extract Derived from *Pseudomonas fluorescens*

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Validation of protein expression capability of the cell-free protein expression system derived from the LacZ-deficient *E. coli* strain

In this study, the developed cell-free protein expression system derived from *P. fluorescens* was compared with that derived from *E. coli* the aspect of protein expression capability. To do so, protein expression capability of the cell-free protein expression system made from the LacZ-deficient *E. coli* strain was validated to be equivalent to that made from *E. coli* in the previous literature [1].

We chose a fluorescent protein, GFP, as a model protein to measure protein expression capability. Amount of GFP expressed in the cell-free protein expression system derived from the LacZ-deficient *E. coli* strain was compared with that in the literature (~ 26 μM) [1]. GFP was expressed in the cell-free protein expression system derived from the LacZ-deficient *E. coli* strain as below. For GFP synthesis in 10 μL of the cell-free protein expression system, 4.0 μL of Premix (S30 Premix Plus in S30 T7 High-Yield Protein Expression System purchased from Promega), 3.6 μL of the cell extract from the LacZ gene-deficient BL21-Gold (DE3) strain, 1.0 μL of DNA encoding GFP after T7 promoter, 1.0 μL of 50 U/μL T7 RNA polymerase (Takara), and 0.4 μL of 1670 ng/μL tRNA (tRNA from E. coli Mre 600; Sigma-Aldrich) were mixed. The fluorescent intensity of this solution was recorded at 29°C using a plate reader (Infinite F200; Tecan) with a GFP filter unit.

To quantify the concentration of GFP in the cell-free protein expression system, a calibration line was prepared as shown in Fig. S1. The calibration line was determined as below. First, we prepared GFP solutions with designated concentrations. In detail, we used a recombinant fluorescent protein eGFP (NOVUS biologicals, Recombinant eGFP Protein) and prepared 1 μM, 2 μM, 3 μM, and 4 μM of eGFP solutions. We measured their fluorescence intensities with a plate reader. Linear fitting of these values of the fluorescence intensities derived Equation S1. Where, F [a.u.] represents the measured fluorescence intensity, C [μM] represents the concentration of GFP.

$$F = 295.31C - 313.44 \quad \text{(S1)}$$

Figure S2 shows the fluorescence intensity of the cell-free protein expression system derived from the LacZ-deficient *E. coli* strain during GFP expression. At 8 h, the value of the fluorescence intensity is approximately 8500. Applying this value to Equation (S1), the GFP concentration is calculated to be 29.84 μM. This concentration is equivalent to that in the previous study, 0.75 mg/mL (= 25.86 μM) at 8 h [1]. Therefore, it was indicated that the cell-free protein expression system made from the LacZ-deficient *E. coli* strain in this study

had protein expression capability comparable to that in the previous literatures. Thus, it was concluded that the comparison between the cell-free protein expression system derived from *P. fluorescens* and *E. coli* in this study was valid.

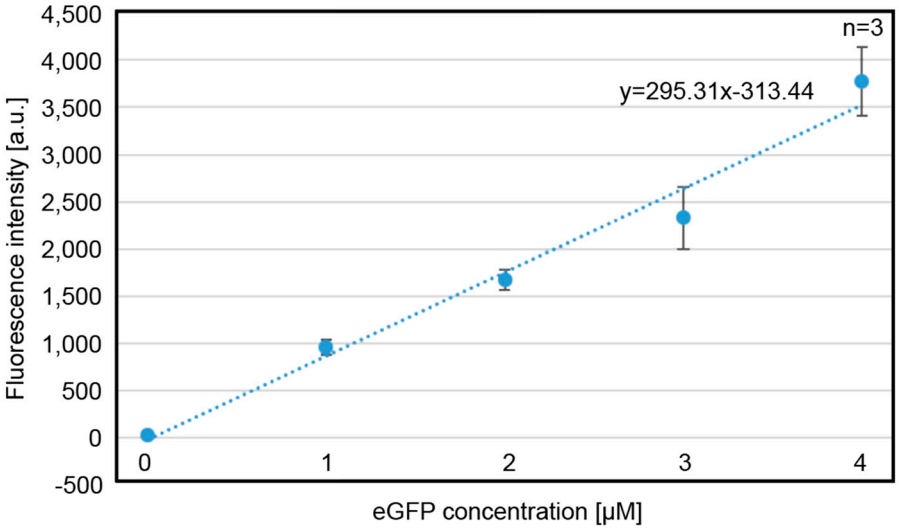

Figure S1. Relationship between concentration of eGFP and fluorescence intensity. Error bars represent standard deviations (n = 3). The dotted line represents the approximate straight line.

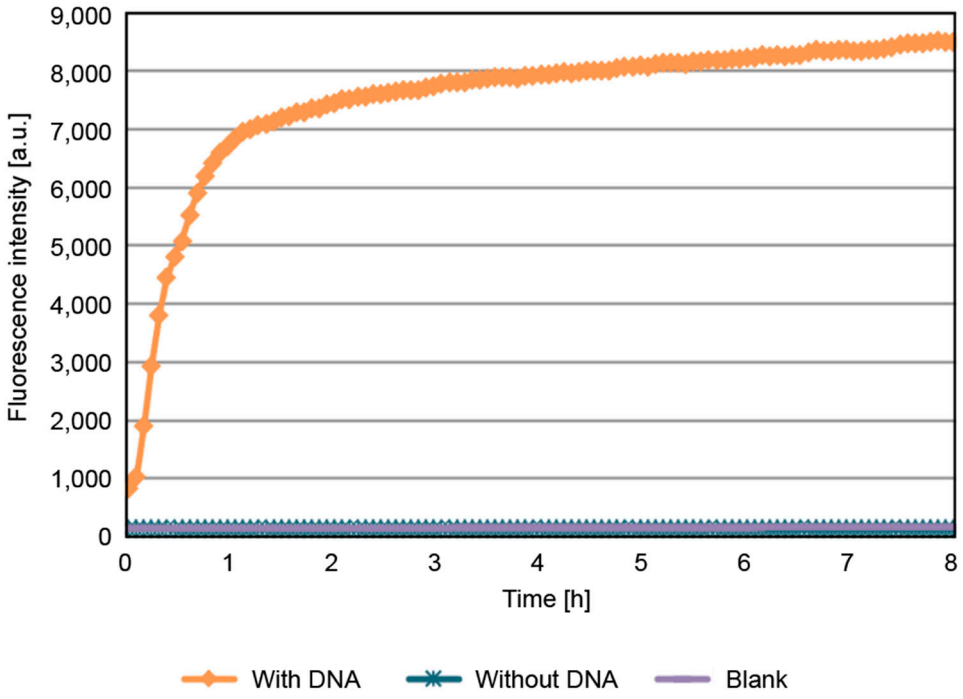

Figure S2. Time-course of fluorescence intensity of the GFP-expressing cell-free protein expression system derived from LacZ-deficient *E. coli* strain. With DNA: With final concentration of 63.2 ng/uL of DNA encoding deGFP under T7 promoter. Without DNA: Without the deGFP-encoding DNA. Blank: Without the solution (Background).

References

1. Sun. Z.Z.; Hayes. A.C.; Shin. J.; Caschera. F.; Murray. M.R. Protocols for implementing an *Escherichia Coli* based TX-TL cell-free expression system for synthetic biology. *J Vis Exp* **2013**, *79*, e50762.