An insect cell-free protein synthesis system
昆虫培養細胞由来無細胞タンパク質合成系

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Introduction

The techniques of foreign gene expression systems are some of the most important technologies in the post-genome era. Cell-free protein synthesis systems are assumed to be powerful tools for such studies, because they are capable of translating exogenous mRNAs with high speed and they have the potential to synthesize any desired proteins, including both native and those that are toxic to cells. In this context, we developed a cell-free protein synthesis system from *Spodoptera flugiperda* 21 (Sf21) insect cells, and commercialized it as the Transdirect *insect cell*.

In this poster, we describe its basic performance and some applications of the insect cell-free system.

Transdirect *insect cell*

The Transdirect *insect cell* is a newly developed *in vitro* translation system for mRNA templates, which utilizes an extract from cultured Sf21 insect cells. An expression vector pTD1, which includes a 5'-untranslated region (UTR) sequence from a baculovirus polyhedrin gene as a translational enhancer, was also developed to obtain maximum performance from the insect cell-free protein synthesis system. This combination of insect cell extract and expression vector results in protein productivity of approx. 50 μg per mL of the translation reaction mixture (Fig. 1 and 2).

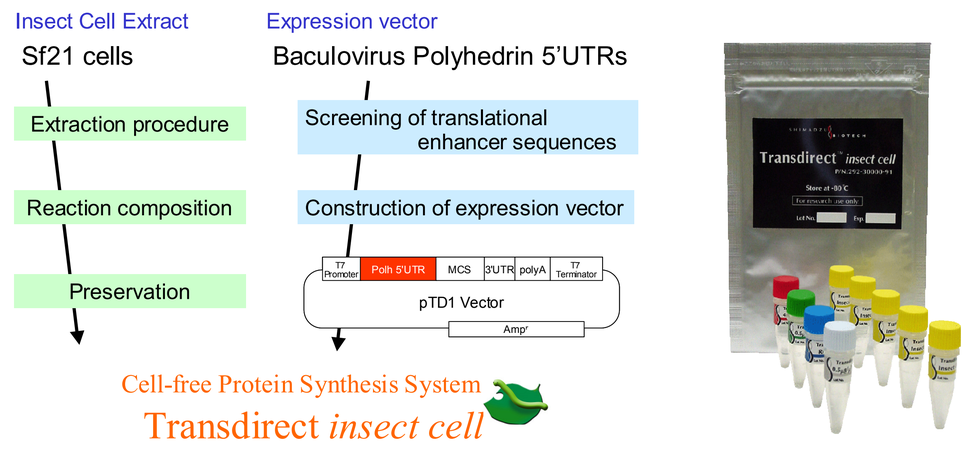


Fig. 1 Establishment of the insect cell-free protein synthesis system, Transdirect *insect cell*.

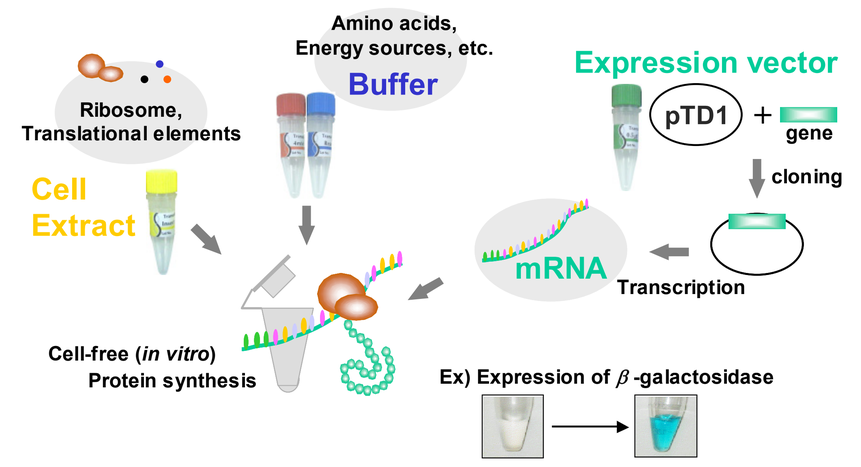


Fig. 2 Synthesis procedure.

Performance of the kit

The expected productivity of target proteins in the insect cell-free protein synthesis system is approximately 10-fold higher than that in rabbit reticulocyte lysate system (Fig. 3). This is the highest protein productivity yet noted among commercialized cell-free protein synthesis systems based on animal extracts.

Typically, about 20 μg of purified proteins were easily obtained by one step of affinity chromatography from 1 mL of translation reaction mixture (Fig. 4).

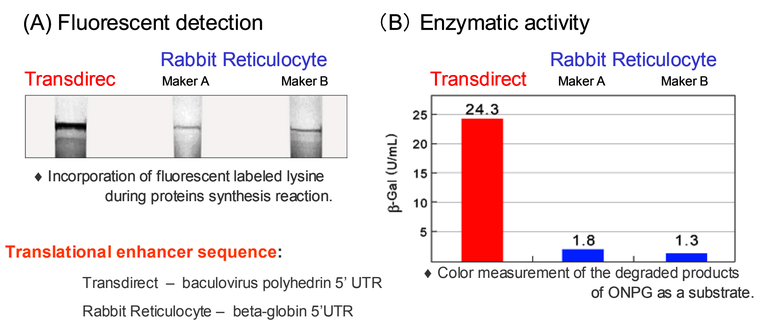
The pTD1 vector contains all factors involved in mRNA and protein synthesis, including the T7 promoter sequence required for mRNA synthesis, the polyhedrin 5'- UTR which enhances the translation reaction, and multiple cloning sites (MCS). The complete DNA sequence of the pTD1 vector is registered in the following DNA Databank: DDBJ/GenBank®/EMBL Accession Number AB194742.

The translation efficiency of mRNAs transcribed from the pTD1 vector was about 50-fold higher than those of mRNAs without an enhancer sequence (data not shown).

Moreover, the pTD1 vector functioned as an effective expression vector not only in the insect cell-free protein synthesis system but also in wheat germ extract and rabbit reticulocyte lysate systems (Fig. 5).

Basic performance

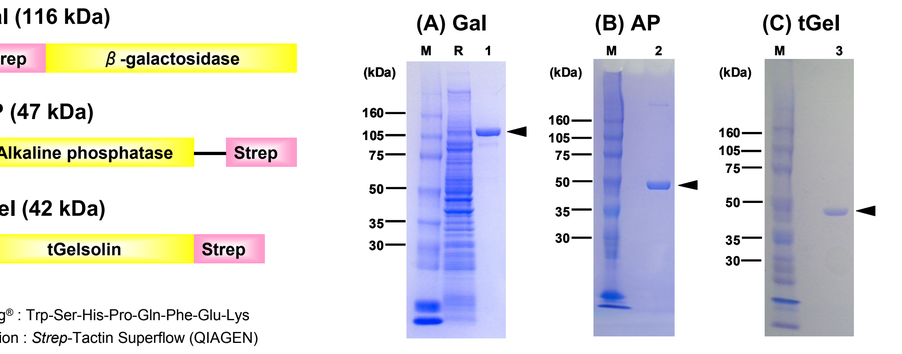
♦ **Model protein:** b-galactosidase (116kDa)



Translational enhancer sequence:
Transdirect – baculovirus polyhedrin 5' UTR
Rabbit Reticulocyte – beta-globin 5'UTR

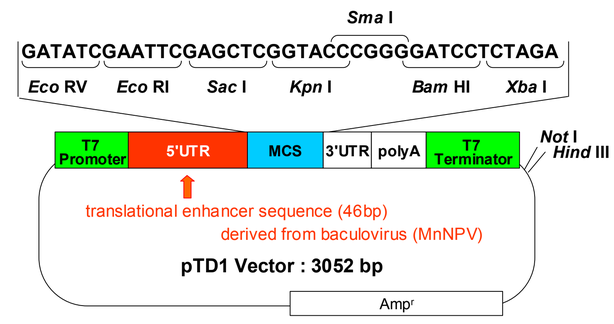
♦ **The productivity of Transdirect is applox. 10-fold higher than Rabbit system.**

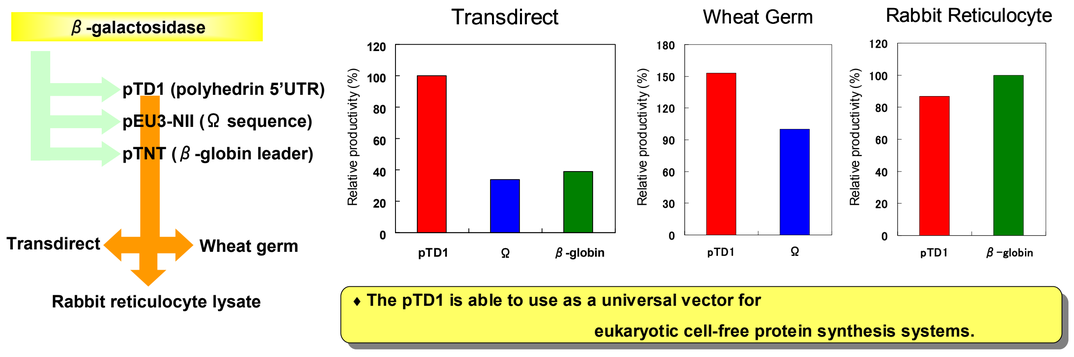
Fig. 3 Comparison of protein productivities.



♦ **About 20μg of purified proteins can obtain by affinity purification from 1mL of the reaction mixture.**

Fig. 4 Affinity purification.





♦ **The pTD1 is able to use as a universal vector for eukaryotic cell-free protein synthesis systems.**

Fig. 5 Performance of expression vector, pTD1.

Applications

Synthesis of proteins containing disulfide bonds.

Escherichia coli alkaline phosphatase (AP) which contains two disulfide bonds, was expressed in a soluble and active form using the insect cell-free system under non-reducing conditions. The efficiency of protein synthesis approached that measured under reducing conditions (normal kit) (Fig. 6). Human lysozyme (h-LYZ), which contains four disulfide bonds, was expressed under non-reducing conditions after addition of reduced glutathione, oxidized glutathione, and protein disulfide isomerase (data not shown).

Site-directed protein labeling.

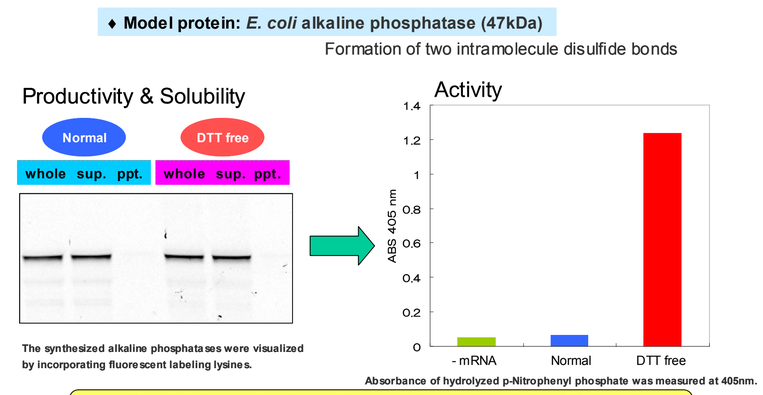
Four base codon (CGGG) or amber codon (TAG) was introduced into the N-terminal region of CAT (chloramphenicol acetyltransferase) coding sequence. In the both strategies, position-specific incorporation of fluorescent labeled amino acid was observed using the insect cell-free protein synthesis system.

Analysis of post-translational modifications.

Protein *N*-myristoylation is the important eukaryote specific lipid modification. To confirm whether the insect cell-free system has the ability to generate *N*-myristoylation, we chose tGelsolin (truncated human gelsolin) as a myristoylated model protein. Cell-free protein synthesis was carried out with or without addition of myristoyl-CoA. The wild-type tGelsolin was found to be *N*-myristoylated when myristoyl-CoA was added to the translation reaction mixture. Myristoylation did not occur on the G2A mutant, in which the myristoylation motif was disrupted, whereas this mutant was found to be *N*-acetylated after removal of the initiator Met.

Applications

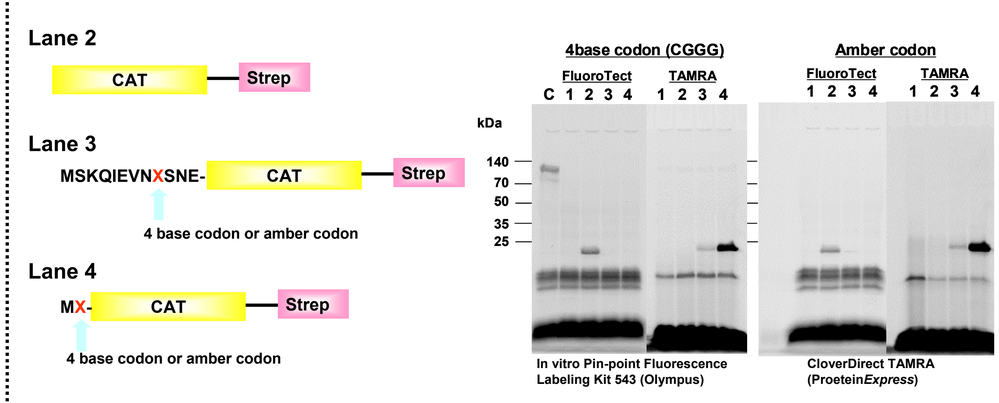
♦ **Model protein:** *E. coli* alkaline phosphatase (47kDa)



♦ **Possible to synthesize active protein which includes S-S bonds by removing reducing agent (DTT).**

A DTT free Transdirect is provided as a custom product.
Please inquire to t-direct@shimadzu-biotech.jp

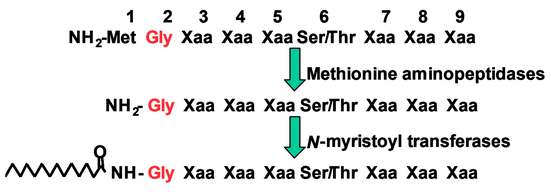
Fig. 6 Synthesis of a protein containing disulfide bond .



♦ **Possible to synthesize site-specific fluorescent labeled protein.**

Fig. 7 Site-directed protein labeling .

Protein *N*-myristoylation



Protein *N*-myristoylation.
In general, protein *N*-myristoylation results from the co-translational addition of myristic acid to a Gly residue after removal of the initiating Met. The requirement for Gly is absolute, and Ser or Thr at position 6 is preferred.

Expression and purification of tGelsolin

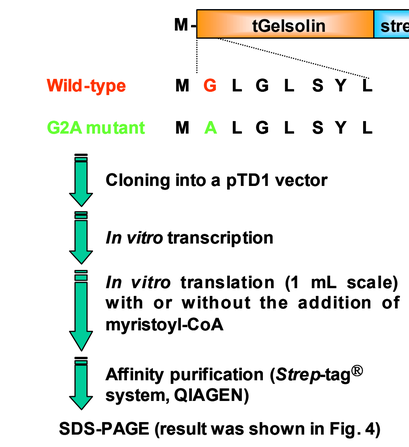
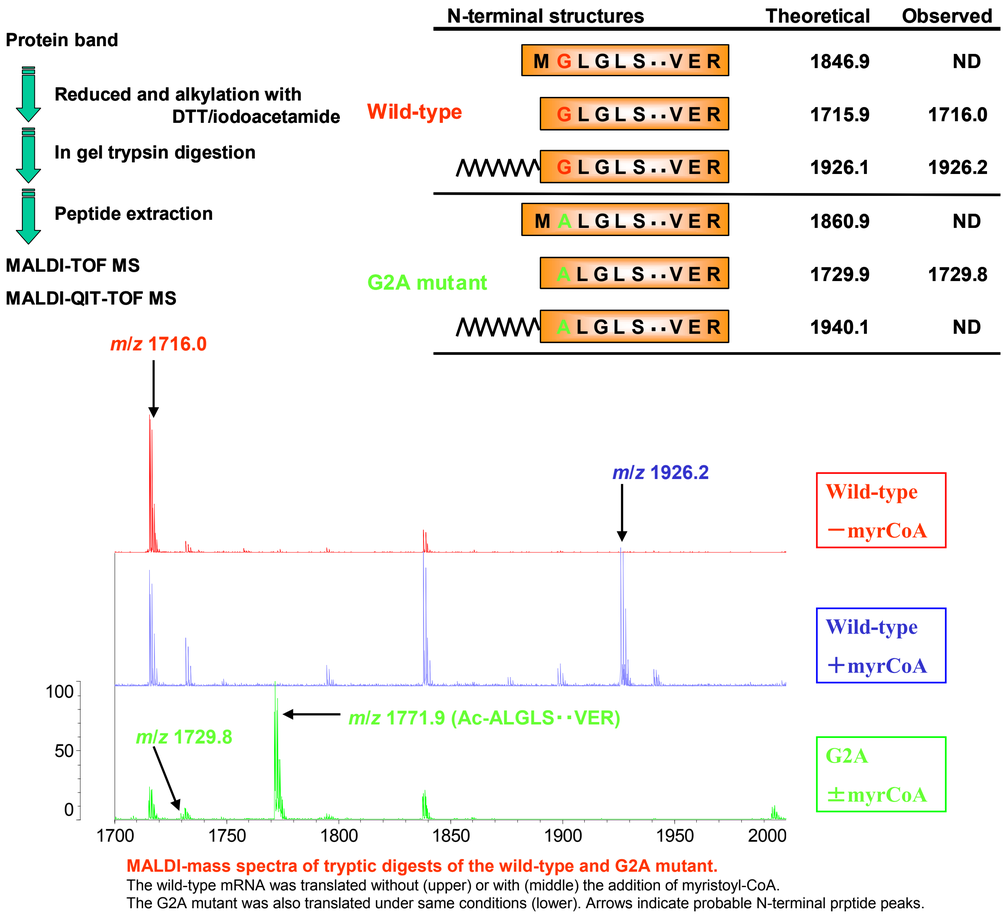


Fig. 6 Analysis of protein *N*-myristoylation .

PMF analyses of the tryptic digests of tGelsolin proteins



MALDI-mass spectra of tryptic digests of the wild-type and G2A mutant.
The wild-type mRNA was translated without (upper) or with (middle) the addition of myristoyl-CoA.
The G2A mutant was also translated under same conditions (lower). Arrows indicate probable N-terminal prptide peaks.

♦ **Transdirect has the potential to generate protein *N*-myristoylation ana *N*-acetylation.**

Conclusion

The insect cell-free protein synthesis system could offer a promising tool to perform gene expression analyses including not only the measurement of enzymatic activity but also investigation of post-translational modifications.

References

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