Construction of an *In Vitro* Gene Expression System, PURESYSTEM, and Its Application on Post Genomic Research

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Recent technological advance has deciphered the genomic sequence ranging from plants to mammalian species. The biological importance of these sequences, however, has yet to be discovered through functional and structural analysis of its coded proteins.

The present prevailing method for protein synthesis is the *in vivo* method, where cloned genes are introduced into living cells such as *Escherichia coli* and yeasts. Although this is the most widely used method of protein production from laboratory to industrial basis, the *in vivo* method has its limitations. It is inappropriate for high throughput expression, and purification of proteins which affects the cellular control mechanisms of the host cell. Cell free protein synthesis is a novel protein production method using cell lysate as the translational material. The known cell free lysate systems today are derived from *E.coli*, rabbit reticulocyte and wheat germ. Although the advantage of cell free synthesis is that it enables the production of cell toxic proteins, the cell lysate used in the system contains many unrelated or translation inhibitory factors and many junk proteins.

PURESYSTEM is a completely new *in vitro* gene expression system which adopts a new approach to protein synthesis. All the components essential for transcription and translation are extracted and purified from *E. coli*. These factors and enzymes are "reconstituted" within a test tube, forming a "pure" system without any inhibitory factors or junk proteins. The protein is synthesized by simply adding the template DNA into the system.

Unlike conventional cell free methods, PURESYSTEM does not require energy recycling system for protein synthesis. When the energy recycling system is added to this system, however, it results in a measured increase in protein yield, confirming that the energy recycling system can work efficiently in a batch reaction.

PURESYSTEM allows the target protein to be purified and separated easily from the factors and enzymes in simple affinity purification, because the components are fused with histidine tags. After transcription and translation, the target protein is purified in a simple two step reaction; the removal of the ribosome by cut off membrane of 100kDa, followed by the removal of histidine tagged factors by a nickel column. The target protein is detected in single band purity in SDS-PAGE.

Various genes from many origins were tested for protein production in PURESYSTEM, and the result indicated that the factor most affecting the protein production was each proteins characteristics rather than the genetic origin. Proteins ranging from 2kDa to 100kDa in size were successfully synthesized.

This *in vitro* expression system, PURESYSTEM, with its unique characteristics, can be applied to many areas of post genomic research.

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EMPLOYMENT

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05/98	Establishment of Pioneer Bioscience, President
08/98	Center for Advanced Science and Technology Incubation (CASTI), Senior Associate
10/98	Technomart Inc., Patent Distributor Advisor
06/99	Establishment of Effector Cell Institute Inc., President (06/99-08/01)
06/00 -	Establishment of Venture Mall Co., Ltd. (renamed Post Genome Institute), President
06/01 -	Post Genome Institute Co., Ltd.
12/01 -	Establishment of Japan Bio-venture Development Association (JBDA), Managing Director

RESEARCH PUBLICATIONS

Sharma S, Murai F, Miyanohara A, Friedmann T (1997) Noninfectious virus-like particles produced by Moloney murine leukemia virus-based packaging cells deficient in viral envelope become infectious in the prescence of lipofection reagents. *Proc Natl Acad Sci USA* 94: 10803-10808

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