Protein Engineering

Cold-Shock Vectors

manual cat info

Ideal for Efficient Soluble Protein Expression using *E. coli* promoter of *cspA*

- **Overexpression up to 60% of total protein.**
- **Vs. conventional systems**
  -Difficult proteins can be expressed.
  -Insoluble proteins are produced in soluble form; further improved with our Chaperone Plasmids.
- **Target protein up to 90% of newly synthesized.**
- **Useful for isotope labeling.**
- **Wide range of *E. coli* subspecies as host.**

Elucidation of protein structure and function is an important subject for post-genome study. An efficient protein production system is essential to study these subjects. Expression systems in *E. coli* are extensively used in the production of recombinant proteins. *E. coli* expression systems have advantages of ease to use and low cost. For some genes, however, expression is difficult and expressed proteins are insoluble.

In order to solve these problems, TaKaRa conducted a joint research with Professor Masayori Inouye [(1)] (University of Medicine and Dentistry of New Jersey, USA) to develop an efficient protein expression vector based on the low-temperature expression gene (**cold-shock gene**) of *E. coli*. This product has the above advantages and provides an important tool for functional and structural analyses as well as other areas in protein research.

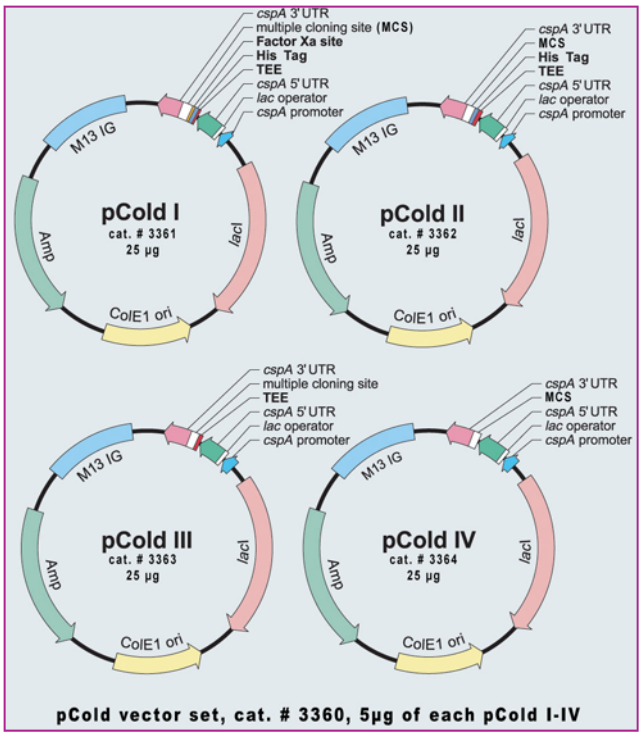

Figure 1. Cold Shock Vectors pCold I—IV

Product overview and features

When the culture temperature of *E. coli* is reduced sufficiently, bacterial growth is temporarily halted and expression of *E. coli* protein is decreased, while expression of a group of proteins, called “cold-shock proteins”, is specifically induced. This product is based on the promoter of a cold-shock gene *cspA*, downstream of which the *lac* operator, the 5’ untranslated region (*cspA* 5’ UTR), the translation enhancing element (TEE), the His Tag sequence, the Factor Xa cleavage sequence, and the multiple cloning site are located. The *lac* operator functions to regulate expression strictly and the TEE sequence acts to promote translation. The His Tag sequence and the Factor Xa cleavage sequence are useful for purification of protein and the subsequent removal of tags.
This product consists of 4 types (pCold I to IV) that have different sequences depending on the presence or absence of the TEE sequence, the His Tag sequence, and the Factor Xa cleavage sequence, allowing the selection of the best vector for each purpose.
Moreover, this product uses *cspA* promoter derived from *E. coli*, enabling almost all strains of *E. coli* to be used as host for expression.

How to express the desired gene

Insert the desired gene to a cold-shock vector to prepare a plasmid for expression
↓
Transform host *E. coli* (e.g. BL21) with the plasmid for expression
↓
Inoculate the transformant in the medium containing ampicillin and culture at 37°C
↓
At OD_{600} = 0.4 - 0.5, refrigerate the culture solution at 15°C and leave to stand for 30 min
↓
Add IPTG to the culture solution and shake culture at 15°C for 24 hours.
↓
Harvest and homogenize
↓
Determine the presence and expression level of the desired product with SDS-PAGE in soluble and insoluble fractions

Actual examples

As described below, expression of genes that showed poor expression level or solubility in the T7 promoter expression system were attempted with the cold-shock vector. pCold I DNA was used as cold-shock vector and BL21 was used as host for expression. Expression from T7 promoter-driven vectors was conducted with the common procedure of adding IPTG and culturing at 37°C.

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(1) Example of expressed gene

For human gene A (estimated molecular weight: 31 kDa), expression was not observed in the T7 expression system, but was observed in the cold-shock expression system (Fig.2).

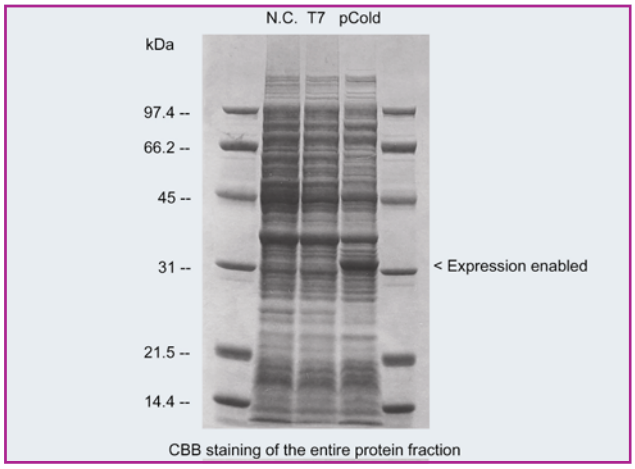

Figure 2. Expression of human gene A

(2) Example of gene with increased expression level

For thermophile gene B (estimated MW: 30 kDa), solubility was improved and expression level was increased compared to the T7 expression system (Fig.3).

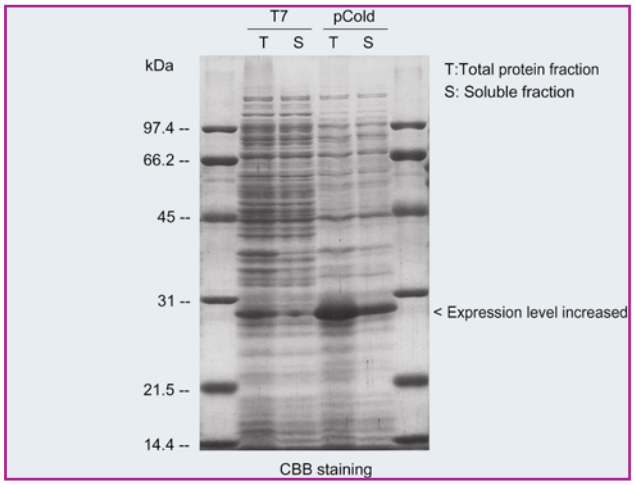

Figure 3. Expression of thermophile gene B

(3) Examples of gene with increased soluble expression level

For human gene C (estimated molecular weight: 80 kDa), expression is mostly insoluble in the T7 expression system. In the cold-shock expression system, however, the expression level of soluble fraction was increased remarkably (Fig.4).

Cold-shock expression systems are expected to improve the expression level and solubility of the desired product compared to the T7 expression system.

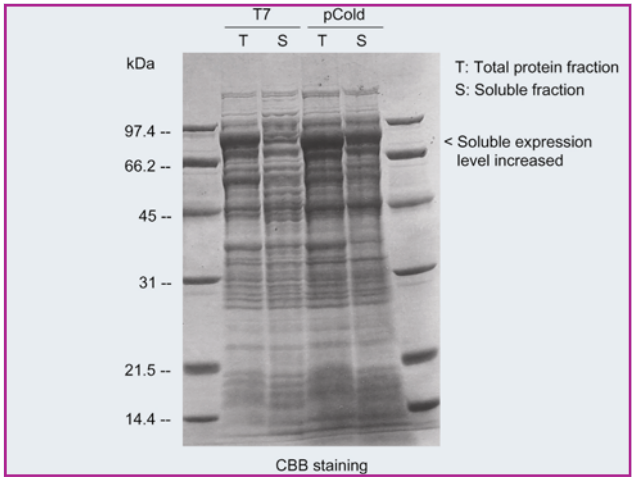

Figure 4. Expression of human gene C

(4) **Comparative study with pulse-labeling experiment**

Human gene D (estimated molecular weight: 12 kDa) was pulse-labeled to compare both expression systems (Fig.5). In the T7 expression system, *E. coli* proteins other than the desired protein were also labeled. In contrast, most of labeled proteins in the cold-shock expression system were the expression product of the desired gene, indicating that the expression of the desired gene was specifically induced.

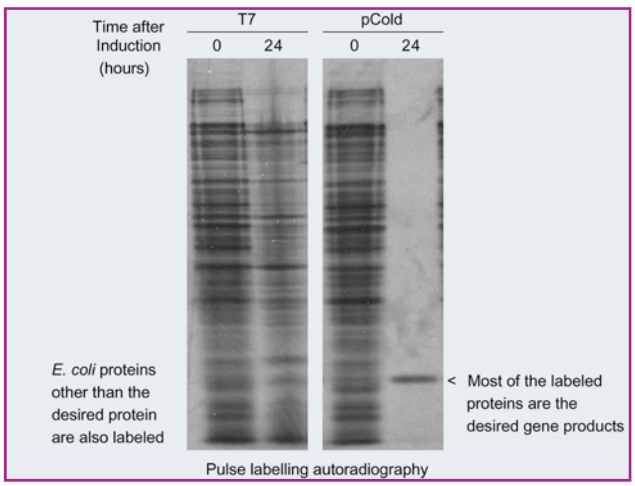

Figure 5. Pulse labeling of human gene D

Cold-shock expression systems are highly useful for preparation of isotope-labeled proteins and as expression systems of recombinant proteins.

TaKaRa provides a contract protein expression service using this technology. For more information, please contact us.

Note: pCold vectors are intended for research use only. In the case of protein production with commercial purpose, a special license from Takara Bio will be necessary.

(1)Guoliang Qing, Li-Chung Ma, Ahmad Khorchid, G V T Swapna, Tapas K Mal, Masanori Mitta Takayama, Bing Xia, Snagita Phadtare, Haiping Ke, Thomas Acton, Gaetano T Montelione, Mitsuhiko Ikura and Masayori Inouye (2004) *Nature Biotrchnology*, **22**, 7, 877-882

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Combined Effect of Cold Shock Expression System and Chaperone Plasmids

The pCold Vector Series is a new protein expression system based on the *E. coli* promoter of the cold shock gene *cspA*. The expression level of the target protein is up to 60% of bacterial proteins and 90% of nascent proteins. Cold shock expression system often allows to express more proteins than conventional T7 system. It also improves the solubilization rate of proteins.
However, some proteins do not achieve sufficient expression or solubilization when pCold vectors are used. In such cases, combination with the Chaperone Plasmid Set might help. The following sections provide some examples for which the co-expression of pCold vectors and chaperone plasmids significantly improved expression and solubilization of the target protein.

Cold Shock Expression Vectors

pCold I DNA used in this experiment contains *lacI* operator, *cspA* 5' untranslated region, Translation Enhancing Element (TEE), His_6 tag sequence, Factor Xa cleavage sequence and multicloning site downstream of the *cspA* promoter.

Chaperone Plasmid Set

The Chaperone Plasmid Set consists of 5 different plasmids, each of which is designed to express different combinations of *E. coli* molecular chaperones as chaperone teams, known to act in protein folding. Thus it is expected to improve the expression level and rate of solubilization of recombinant protein through co-expression of chaperone.
The chaperone plasmid pG-Tf2 used in this experiment expresses tig, groES, and groEL as a chaperone team, under regulation of *Pzt1* promoter, induced by tetracycline.

pCold and Chaperone plasmids

As pCold vectors and Chaperone plasmids carry different replication origin and selection marker, they can be co-transformed. In addition as expression of the protein of interest and chaperone team are not triggered by same promoters, inductions can be made separately.

Expressing target proteins through the co-expression of pCold I DNA and pG-Tf2

Using the BL21 strain as an *E. coli* host, a co-expression experiment was conducted in accordance with the following procedure:

1. Preparation of the co-expression system

(1) Transform BL21 Competent Cells with the chaperone plasmid pG-Tf2 and select with chloramphenicol.
(2) Liquid culture the transformant (BL21/pG-Tf2) and prepare competent cells.*

*** Ready to use BL21 Chaperone competent cells are now available. These cells are applicable to all expression vectors compatible with chaperone plasmids.**

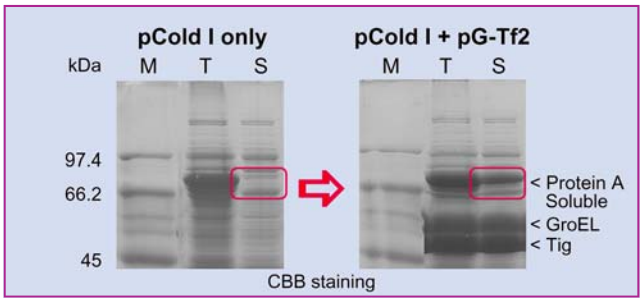
(3) Transform the BL21/pG-Tf2 strain with the recombinant pCold I DNA and select with chloramphenicol and ampicillin.
(4) Obtain co-expressed *E. coli*.

2. Co-expression experiment

(1) Incubate *E. coli* co-transformed with pCold I DNA and pG-Tf2 in the selective L medium and chaperone inducer (1 ng/ml tetracycline) at 37°C.
(2) At OD_{600} = 0.4 - 0.5, refrigerate the culture at 15°C and leave to stand for 30 min.
(3) Add 0.5 mM IPTG to the culture solution and shake culture at 15°C for another 24 hours.
(4) Harvest, homogenize and confirm the expression level of the target protein in the total protein fraction and the soluble fraction by SDS-PAGE.
For comparison of expression by recombinant pCold I alone, transformed BL21 strain was cultured in L medium containing ampicillin until OD_{600} 0.4 - 0.5 was reached. Then culture solution was left to stand at 15°C for 30 min, 0.5 mM IPTG was added and the resultant solution was shake cultured at 15°C for another 24 hours.

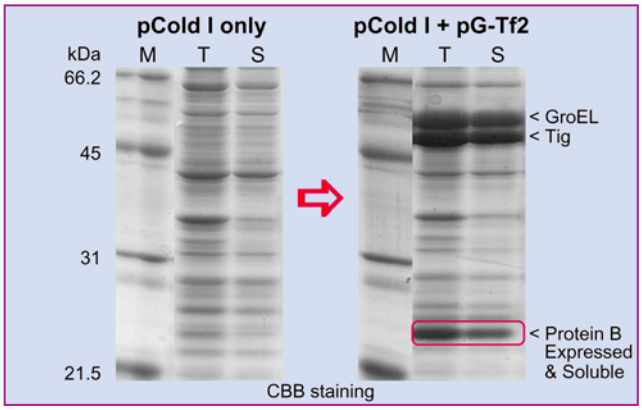
Example of promoted solubilization

Human gene A (~ 70 kDa) was insolubly expressed by pCold I only. However, the expression level in the soluble fraction increased significantly in the system in which the chaperone plasmid pG-Tf2 was co-expressed.



Example of successful expression and solubilization

Human gene B (estimated molecular weight 24 kDa) was hardly expressed in the expression system using pCold I DNA only. However, the co-expression with the chaperone plasmid pG-Tf2 resulted in the solubilization of most of the target protein as well as the expression of the target protein.



Conclusions

Combination of Cold Shock Expression Vectors and Chaperone Plasmid Set often leads to significant improvement of the expression level and the rate of solubilization of the target protein. If sufficient expression or solubilization can not be achieved using pCold vectors alone, we suggest to try co-expression with chaperone plasmids. Furthermore, pCold vector-based expression systems may produce better results by co-expressing the chaperone team carrying the tig sequence included in the Chaperone Plasmid Set (data not shown). For combination with pCold vectors, we recommend to consider starting with co-expression with pG-Tf2 or pTf16.