Protein Engineering

pCold TF : Cold shock expression plasmid with folding capacity

For a strong promotion of soluble proteins expression

manual cat info

↓New *E. coli* cold shock expression vector developed from the findings of cold shock expression and chaperone coexpression

↓Strong tool for soluble expression using trigger factor as a solubilization tag

The cold shock expression vectors (pCold DNA series), based on the promoter and 5' UTR sequence of an *E. coli* cold shock gene *cspA,* can improve the rate of solubilization and soluble expression compared to conventional *E. coli* expression vectors[1)]. The solubilizing effect is further increased by the combination of these vectors with chaperone plasmids. Moreover, the desired protein can be obtained at high purity. Researchers are using these vectors as an important tool for protein research including functional and structural analyses.
Recently, Takara Bio developed a new fusion expression vector (pCold TF) using trigger factor (TF), an *E. coli* chaperone, as a solubilization tag. It is based on the findings of cold shock expression vectors and chaperone coexpression. TF is a protein with a molecular weight of 48 kDa that is associated with ribosomes, and is considered to be coupled with protein translation (co-translationally) to promote folding of nascent polypeptides[2)].
Using the pCold TF can correct insolubilization problem.

Product overview and features

(1) Substantial increase in the rate of solubilization

By inducing fusion expression of trigger factor (TF), which promotes folding and solubilization of proteins in a cell on the N-terminal side, the desired protein is expressed efficiently and the rate of soluble protein production is increased. The rate of solubilization is also increased substantially by the synergy of the chaperone function and cold shock expression system.

(2) Simple purification

The expressed protein has a His-tag sequence on the N-terminal side, allowing easy and efficient recovery and purification of the fusion protein by Ni-affinity chromatography.

(3) Easy removal of tag sequence

Amino acid sequences that are recognized by 3 different proteases (factor Xa, thrombin, and HRV 3C protease) are introduced in tandem, allowing reliable cleavage and removal of the TF region from the fusion protein after expression.

Solubilization and soluble protein production rates

Rate of solubilization: Probability of obtaining soluble proteins in an expression test of multiple genes, defined as follows: Number of solubilized genes/Number of genes.

Rate of soluble protein production: Proportion of soluble expressed protein in individual genes, defined as follows: Total amount of soluble protein/Total amount of desired protein.

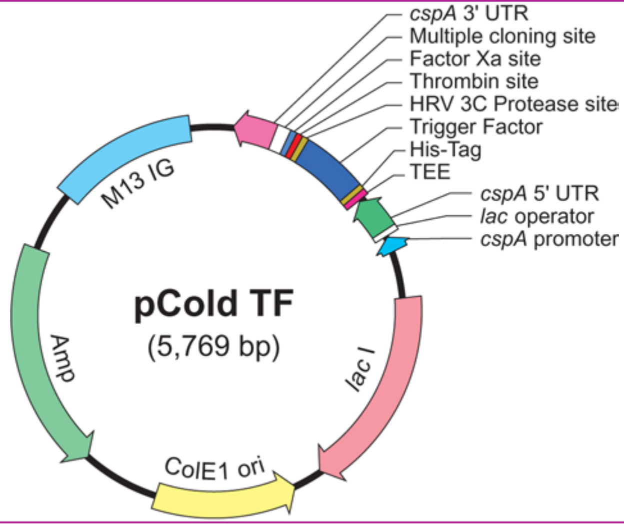


Fig. 1 **Schematic representation of a cold shock vector**

Inserting a gene encoding the desired protein into the pCold TF DNA, including TF gene, induces expression of target protein fused with TF. Expression level and rate of soluble protein production increase significantly, even for proteins that are difficult to express or difficult to solubilize.

The pCold TF DNA also exhibits the characteristics of conventional cold shock expression vectors (pCold series):

- High expression yield (more than 60 % of the total cellular proteins) outperforms regularly used expression vectors and facilitates protein purification.
- Expression at 15°C improves solubility and stability.
- Nascent protein synthesis up to 90% of total protein synthesis useful for efficient labeling or NMR without further purification.

The 5' untranslated region (5' UTR) and translation enhancing element (TEE) are located in the downstream of the promoter of the *cspA* gene, which is one of *E. coli* cold shock genes. The TEE sequence acts to promote translation. In the downstream of the promoter, the *lac* operator is inserted to regulate expression properly. Since transcription is induced by the cspA promoter derived from *E. coli*, there is the advantage that almost any *E. coli* strain can be used as a host for expression.

By using the pCold TF, genes that have been difficult to express so far can be expressed as soluble proteins, due to the highly efficient protein expression capacity of cold shock expression vectors and chaperone function of TF.

How to express the desired soluble protein

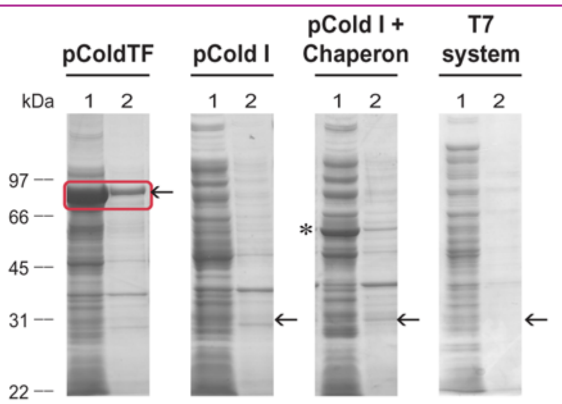
Insert a gene encoding the desired protein into the pCold TF.
↓
Transform *E. coli* host with the plasmid for expression.
↓
Inoculate the transformant in the medium containing ampicillin and culture at 37°C.
↓
At OD_{600} = 0.4 - 0.5, refrigerate the culture solution at 15°C and leave to stand for 30 min.
↓
Add IPTG to the culture and shake it at 15°C for 24 hours.
↓
Determine the presence, expression level, and rate of soluble protein production of the desired protein by SDS-PAGE.

Actual examples

An expression system based on the pCold TF DNA was compared with a pCold I DNA single expression system, a chaperone coexpression system based on pCold I DNA and chaperone plasmid pTF16 that expresses TF, and a T7 promoter expression system (including fusion with other soluble tags). For cold shock expression, BL21 was used as a host for expression. For T7 promoter expression, BL21 (DE3) was used as a host for expression and treated with the common procedure of adding IPTG and culturing at 37°C.

(1) Example of expressed gene

In this experiment, enzyme protein A (estimated molecular weight: 31 kDa), which is difficult to express, was used for investigation. When the pCold I DNA (single expression, chaperone coexpression) was used or an expression system based on T7 promoter was applied, no apparent bands were observed near the estimated molecular weight of the desired protein, 31 kDa. Only when the pCold TF DNA was used, the expression of the desired protein was observed and most of the expression was soluble (Fig. 2). This suggests that even proteins that are difficult to express can be expressed easily using the pCold TF DNA. For the enzyme protein A, it has been confirmed that the fusion protein maintains the enzyme activity.



* : Coexpressed Trigger Factor ← : Target Protein

When the pCold TF DNA was used, there was a greater amount of protein (protein fused with TF) compared with other expression systems; most of the protein was soluble.

Fig. 2 Expression of the enzyme protein A
Lane 1: soluble fraction; 2: insoluble fraction

(2) Example of gene with increased soluble expression level

In this experiment, the enzyme protein B (estimated molecular weight: 63 kDa) was used for investigation as an example of hardly soluble protein expression. The enzyme protein B showed almost no soluble expression when the T7 expression vector that induces the fusion expression of the Trx tag (approximately 12 kDa), Nus tag (approximately 55 kDa), or GST tag (approximately 26 kDa), known as solubilization tags, was used.

Moreover, almost no soluble expression was observed when the pCold I DNA (single expression, chaperone coexpression), with its excellent soluble expression capacity, was used.

In contrast, when the pCold TF DNA fused with trigger factor was used, most of the desired protein was obtained in the soluble fraction and the rate of soluble protein production was significantly higher than with other tags (Fig. 3). This result shows promise that soluble expressed proteins can be obtained by using the pCold TF DNA, even for proteins that have so far been difficult to express as soluble.

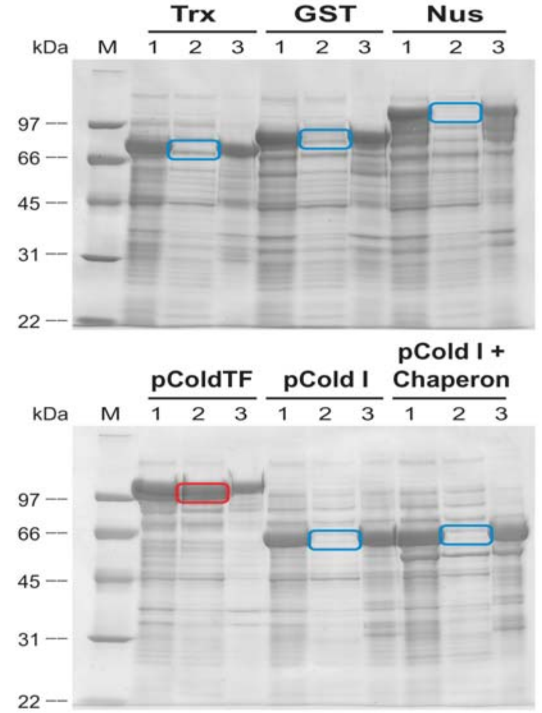


Fig. 3 Expression of the enzyme protein B
M: molecular weight marker 1: cell extract
2: soluble fraction 3: insoluble fraction

(3) Purification of fusion protein and removal of tag sequence

The enzyme protein C (estimated molecular weight: 13 kDa) was hardly expressed as soluble when the pCold I DNA (single expression, chaperone coexpression) and T7 expression vector were used (experimental data are omitted). In contrast, when the pCold TF DNA was used, most of the desired protein with enzyme activity was obtained in the soluble fraction. By adsorbing the soluble fraction to a Ni-affinity column and eluting with imidazole, the fusion protein could be purified (Fig. 4).

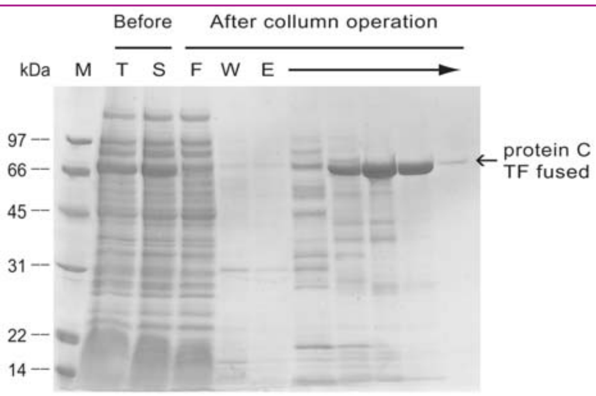


Fig. 4 Purification of the enzyme protein C fused with TF
M: molecular weight marker T: cell extract S: soluble extract
F: column passing fraction W: wash fraction E: eluates

Protein Engineering

The TF tag of the purified fusion protein can be cleaved and removed by cleaving the protein with factor Xa, thrombin, and HRV 3C protease. After treating the fusion protein with protease, the desired protein C was recovered in the supernatant of centrifugation. The desired protein C could be recovered as a soluble protein even after removal of the TF tag (Fig. 5). It was also confirmed that the desired protein C has an enzyme activity after removal of the TF tag.

[References]
1) Quing, G., *et al.* (2004) *Nat. Biotechnol.*, **22**, 877-882.
2) Gerlind, S., *et al.* (1995) *EMBO J.*, **14**, 4939-4948.

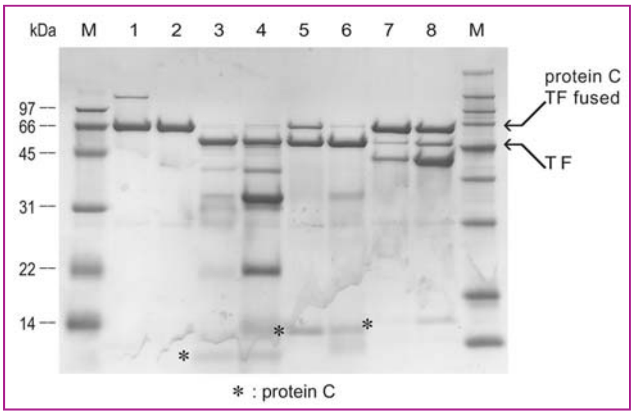


Fig. 5 Removal of TF tag by protease digestion

M: molecular marker
1: untreated
2: enzyme protein C fused with TF
3: factor Xa 0.5 U
4: factor Xa 5 U
5: thrombin 0.5 U
6: thrombin 5 U
7: HRV 3C protease 0.5 U
8: HRV 3C protease 5 U

Ordering information

Product	Cat. # (click for catalogue information)	Size
pCold TF DNA	3365	25 µg
pCold Vector Set	3360	1 set (5µg each of 3361-3364)
pCold I DNA	3361	25 µg
pCold II DNA	3362	25 µg
pCold III DNA	3363	25 µg
pCold IV DNA	3364	25 µg
Chaperone plasmid set	3340	10ng/µl each (100µl)
Chaperone Competent Cells BL21 Set	9120	1 set (3x100 µl of each 9121-9126)
Chaperone Competent Cells pG-KJE8/BL21	9121	1 set (100 µlx10)
Chaperone Competent Cells pGro7/BL21	9122	1 set (100 µlx10)
Chaperone Competent Cells pKJE7/BL21	9123	1 set (100 µlx10)
Chaperone Competent Cells pG-Tf2/BL21	9124	1 set (100 µlx10)
Chaperone Competent Cells pTf16/BL21	9125	1 set (100 µlx10)
Takara Competent Cells BL21	9126	1 set (100 µlx10)