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Customer’s order of pCold DNAs will be accepted only when the Purchaser’s Agreement is signed by a customer and is attached with an order.

- pCold DNAs (hereinafter “PRODUCTS”) are covered by U.S. Patents No. 6479260, which are owned by TAKARA BIO, and the U.S. Patents, 5981280, 6686174, 6333191, which are owned by University of Medicine & Dentistry of New Jersey and are exclusively licensed to TAKARA BIO.
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Cold Shock Expression System pCold DNA

I. Description:

Elucidation of protein structure and function is an important subject for post-genome study and an efficient protein production system is an indispensable fundamental technology to study these subjects. Expression systems with *E. coli* as host 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 (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, pCold DNA Series has the above advantages and provides an important tool for functional and structural analyses as well as other areas in protein research.

Product overview and features
When the culture temperature of *E.coli* is reduced sufficiently, the growth is temporarily halted and almost of protein expression decrease, while expression of a group of proteins called “cold-shock proteins” is specifically induced.
Cold-shock expression vectors, pCold DNA I-IV, are designed to perform efficient protein expression utilizing the promoter derived from *csp*A gene, which is one of the cold-shock genes. At the downstream of the *csp*A promoter, *lac* operator is inserted so that the expression is strictly controlled. In addition, 5' untranslated region (5' UTR), translation enhancing element(TEE), His-Tag sequence, Factor Xa cleavage site, and multicloning site(MCS) are located at the downstream of the *csp*A promoter. As this product utilizes the promoter derived from *E.coli*, most *E.coli* strains can be utilized as an expression host. There are four kinds of pCold vectors, whose arrangements vary in the existence of TEE, His Tag Sequence and Factor Xa clevage site.

II. Components

Product Name	code	size
pCold Vector Set	3360	1 Set
1) pCold I DNA		5 µg
2) pCold II DNA		5 µg
3) pCold III DNA		5 µg
4) pCold IV DNA		5 µg
pCold I DNA	3361	25 µg
pCold II DNA	3362	25 µg
pCold III DNA	3363	25 µg
pCold IV DNA	3364	25 µg

<Available *E.coli* host strains>
Most *E.coli* strains can be used as an expression host for pCold DNA series, because these vectors utilize the promoter of a cold-shock gene, *csp*A, derived form *E.coli*.

III. Vector map:

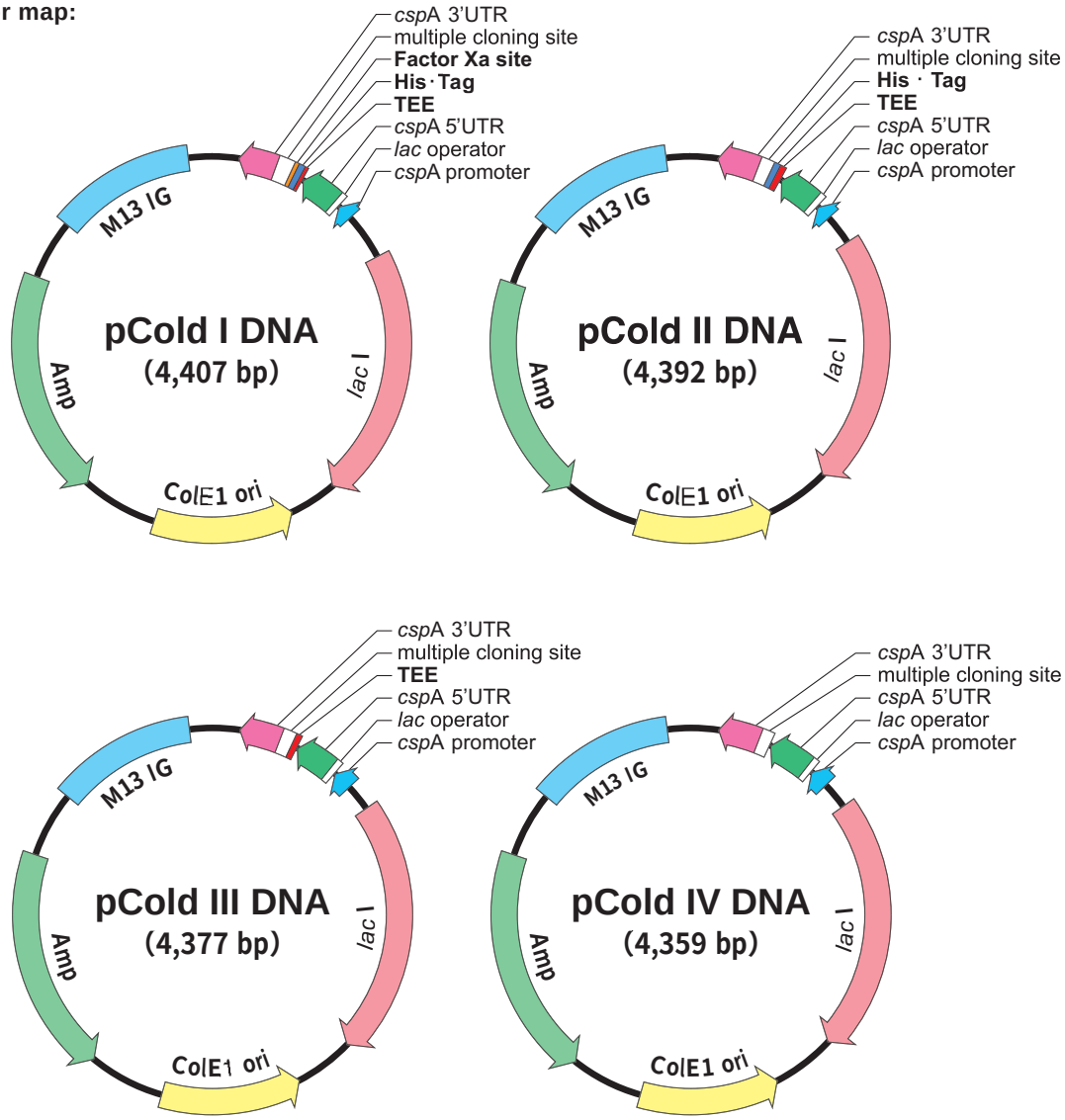


Fig.1 pCold Vector Map

	TEE	His Tag	Factor Xa Cleavage Site	GenBank Accession No.
pCold I DNA	+	+	+	AB186388
pCold II DNA	+	+	-	AB186389
pCold III DNA	+	-	-	AB186390
pCold IV DNA	-	-	-	AB186391

IV. Storage: -20°C (for shipping and storage)

V. Protocol:

How to express the target gene:

The cultivation / induction conditions (culture medium, culture temperature, aeration, timing of induction, concentration of an inducer, cultivation time after induction) should be examined for each target protein.

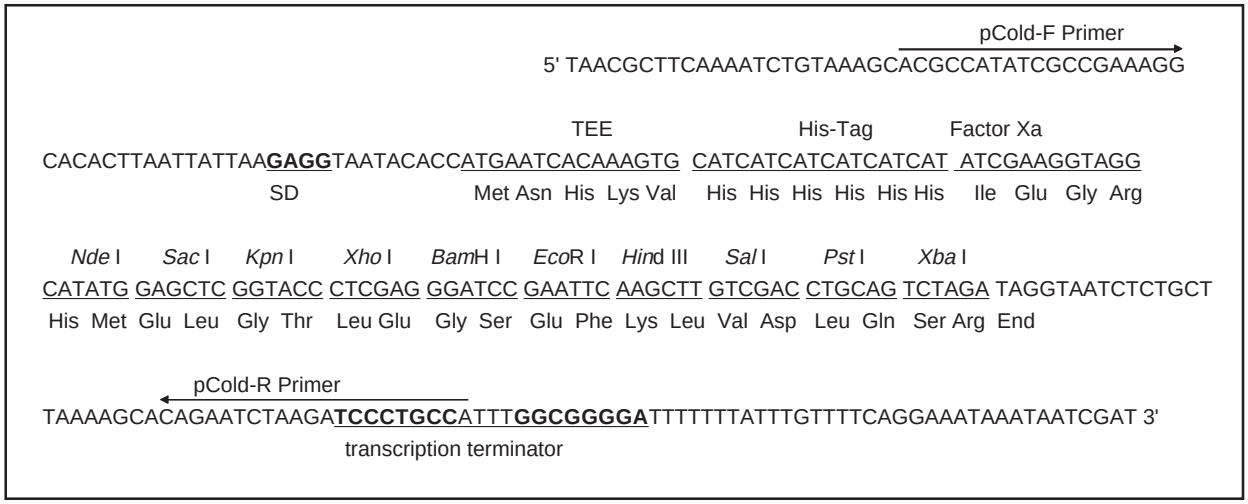
The example of general method is shown below.

1) Insert the target gene to the multicloning site of pCold DNA to construct the plasmid for expression.
2) Transform the *E.coli* host strain (e.g. BL21) with the plasmid of expression, and select the transformants on the selection plate including ampicillin.
3) Inoculate the transformant in the medium including 50-100 ug/ml of ampicillin, and culture at 37°C with shaking.
4) At OD600= 0.4 - 0.5, refrigerate the culture solution at 15°C and leave to stand for 30 minutes.
5) Add IPTG at the final concentration of 0.1- 1.0 mM, and continue the culture with shaking at 15°C for 24 hours.
6) Collect the cells, and confirm the expression of target protein with SDS-PAGE in soluble and insoluble fractions or activity assay.

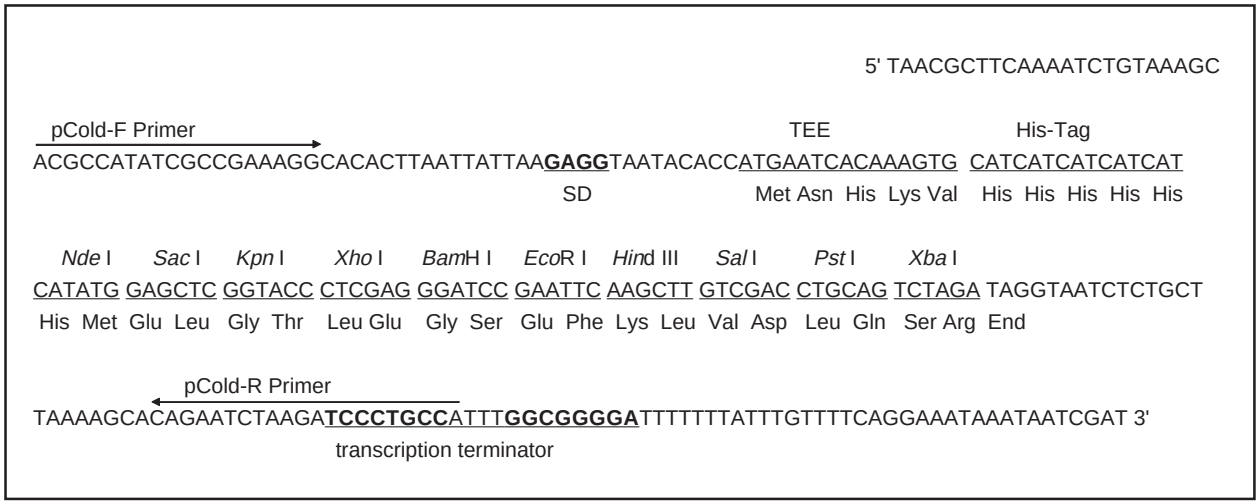
By selection of the *E.coli* host strains for expression and optimization of cultivation / induction conditions (culture medium, culture temperature, aeration, timing of induction, concentration of an inducer, cultivation time after induction), the expression level and the degree of soluble expression are improvable. Moreover, when the expressed protein is insoluble, the combined use with Chaperone Plasmid Set (TaKaRa Cat.# 3340) is effective.

VI. Multiple cloning site:

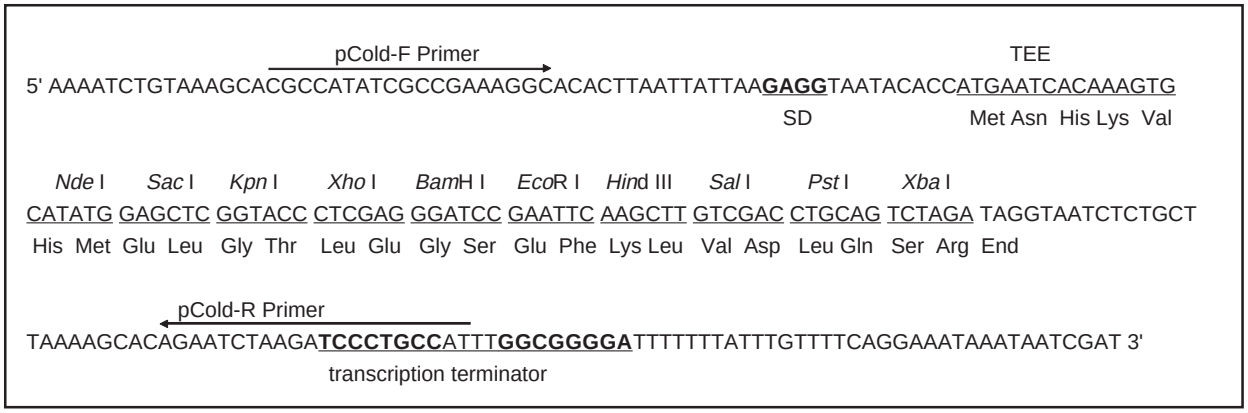
pCold I DNA (Code. 3361)



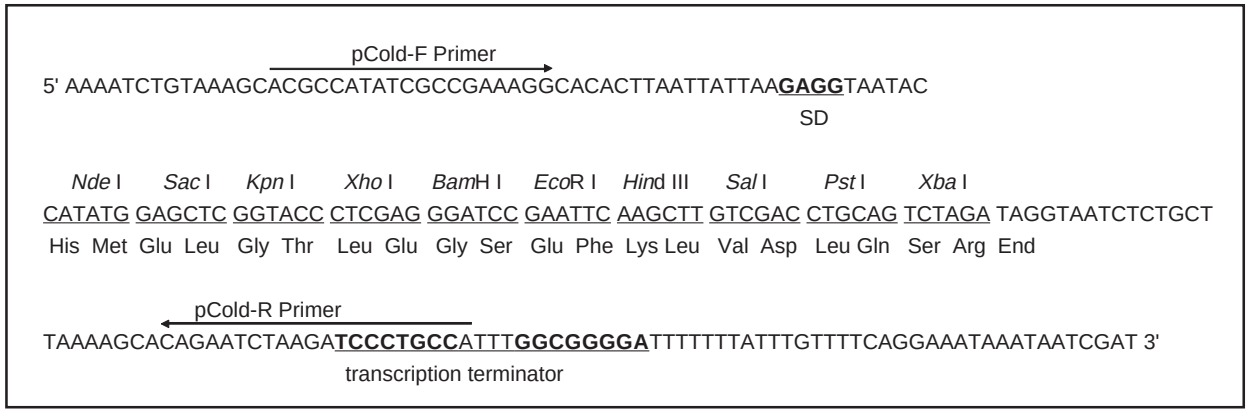
pCold II DNA (Code. 3362)



pCold III DNA (Code. 3363)



pCold IV DNA (Code. 3364)



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

(1) The expression became possible.

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).

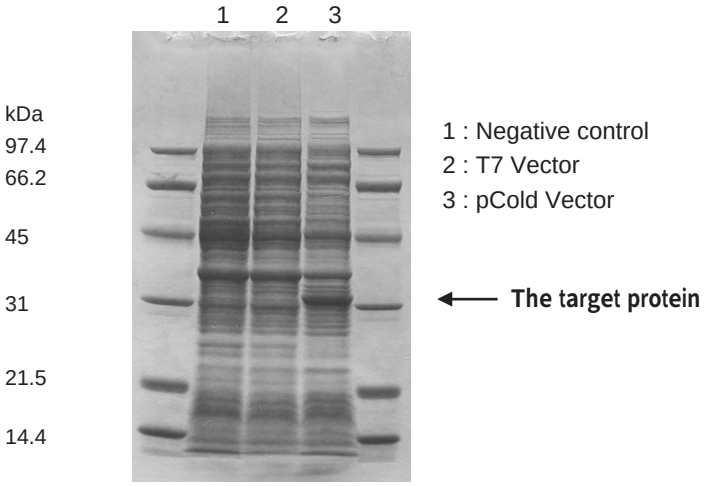


Fig.2 Expression of human gene A was compared using pCold and T7 Vector followed by CBB staining.

(2) The expression level increased.

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

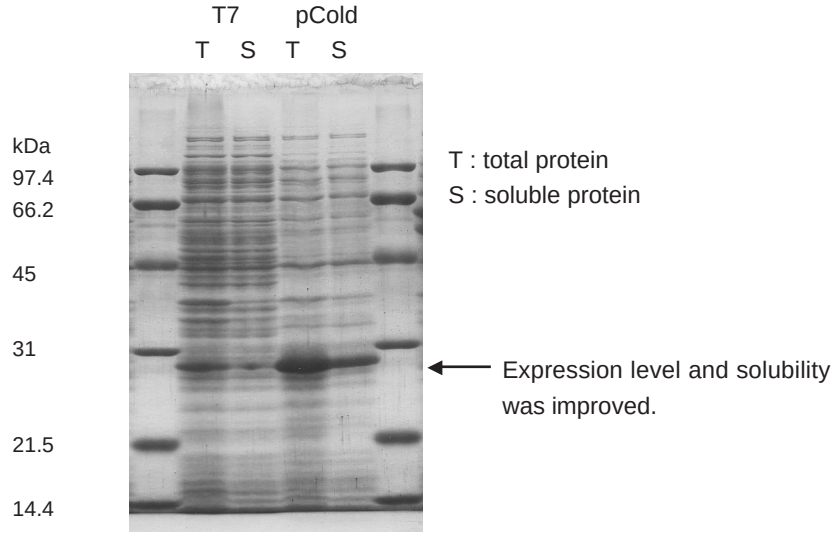


Fig.3 Expression of thermophile gene B was compared with total protein and soluble protein using pCold and T7 Vector followed by CBB staining.

(3) The soluble expression level was increased.

For human gene C (estimated molecular weight: 80 kDa), expression was 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 target protein compared to the T7 expression system.

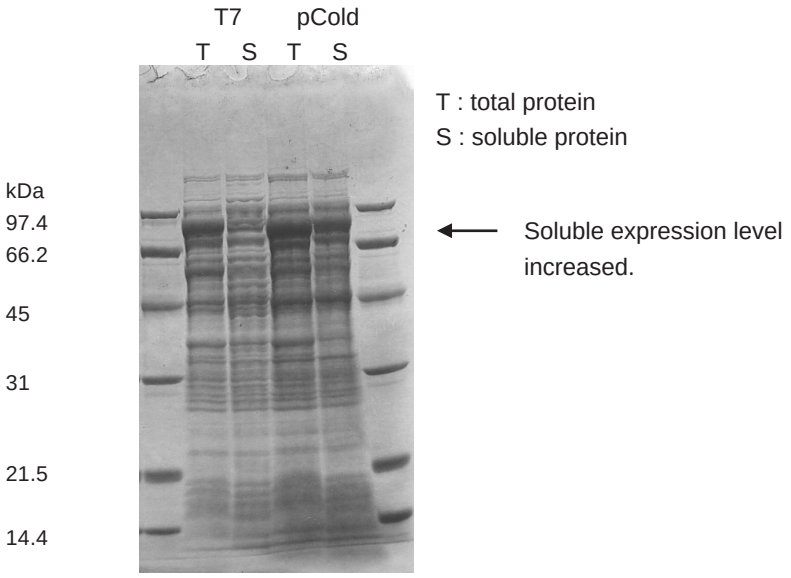


Fig.4 Expression of human gene C was compared using pCold and T7 Vector in soluble or insoluble fraction.

(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 target protein were also labeled. In contrast, most of labeled proteins in the cold-shock expression system were the expression product of the target gene, indicating that the expression of the target gene was specifically induced.

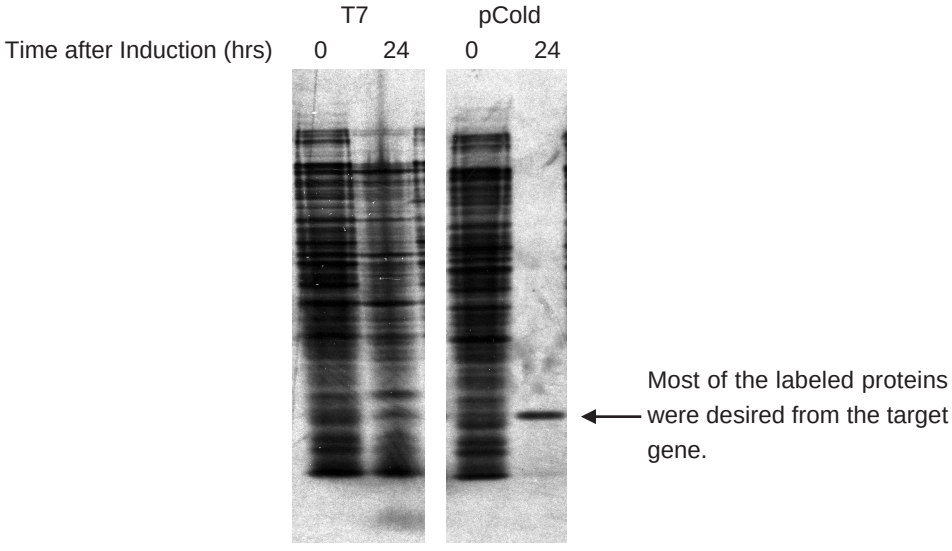


Fig.5 Pulse labeling of human gene D

VIII. Q&A

Q1: What parameters should be examined when the expressed protein is insoluble?

A1: The optimal conditions for cultivation and induction vary depending on a kind of expressed protein. The cultivation and induction conditions should be determined by referring to the following points:

- Change the induction timing. It should be examined within early and late logarithmic growth phase.
- Change the concentration of inducer (IPTG) within 0.1-1 mM.
- Examine the cultivation time after induction (Generally 15°C, 24 hours is the most appropriate.)
- Change the conditions for aeration.

Moreover, it is recommended to try the co-expression with Chaperone Plasmid Set (TaKaRa Cat.# 3340) for soluble expression.

Q2: What remedial procedures should be taken in case that no protein is expressed or the expression level is low?

A2: It is recommended to examine again the conditions for cultivation and induction (Refer to the above Q1.), or change the host *E.coli* strain. When pCold IV was used, the use of other vectors from pCold I, II or III which contains TEE sequence can work to improve the expression level.

Q3: What host strains have been confirmed to work with pCold Vectors?

A3: BL21, Rosetta™, Origami™ from Novegen, Inc. BL21 is most commonly used as host. Origami™ lacks the trx/gor gene and allows the formation of disulfide bond in cytoplasm at high level. Accordingly, the solubility and refolding of expressed protein are facilitated. Rosetta™ contains a plasmid which supplies tRNAs corresponding to the codons that are rarely used in *E.coli*. It enables the universal transcription of the genes which are restricted by the codon usage of *E.coli*.

Q4: How to select a pCold Vector appropriate for one's application?

A4: The TEE sequence is useful for expression of a target protein. When pCold I or II DNAs including His • Tag sequence are used, expressed protein can be purified through Ni column. When expressed protein should not be linked with excess amino acid sequence at N-termini, pCold I DNA or pCold IV is recommended. That is because pCold DNA I contains Factor Xa cutting site, and also because pCold IV has the most simple structure.

Q5: Can *E. Coli* retaining a pCold vector that contains target gene be stored at 4°C on a plate?

A5: We don't recommend the 4°C storage on a plate because it causes a possible leak of target protein in the cell. Pick the colony from the plate promptly, prepare a glycerol stock and store at -80°C.

IX. Related Products

< For soluble expression of recombinant protein >

Chaperone Competent Cells BL21 Set (TaKaRa Cat.#9120)
Chaperone Competent Cell pG-KJE8/BL21 (TaKaRa Cat.#9121)
Chaperone Competent Cell pGro7/BL21 (TaKaRa Cat.#9122)
Chaperone Competent Cell pKJE7/BL21 (TaKaRa Cat.#9123)
Chaperone Competent Cell pG-Tf2/BL21 (TaKaRa Cat.#9124)
Chaperone Competent Cell pTf16/BL21 (TaKaRa Cat.#9125)
TaKaRa Competent Cell BL21 (TaKaRa Cat.#9126)
Chaperon Plasmid Set (TaKaRa Cat.#3340)

< *E.coli* Competent Cells >

E. coli DH5α Competent Cells (TaKaRa Cat.#9057)
E. coli HB101 Competent Cells (TaKaRa Cat.#9051)
E. coli JM109 Competent Cells (TaKaRa Cat.#9052)
E. coli DH5α Electro-Cells (TaKaRa Cat.#9027)
E. coli HB101 Electro-Cells (TaKaRa Cat.#9021)
E. coli JM109 Electro-Cells (TaKaRa Cat.#9022)

< Other >

IPTG (Isopropyl-β-D-thiogalactopyranoside) (TaKaRa Cat.#9030)

X. References

1) Masayori Inouye et. al. Nature Biotechnology, in press
2) Xia B, Etchegaray JP and Masayori Inouye (2001) *J. Biol. Chem.* **276**, 38, 35581 - 35588.
3) Kunitoshi Yamanaka, Masanori Mitta and Masayori Inouye (1999) *J. Bacteriology* **181**, 20, 6284 - 6291.
4) Li Fang,Yan Hou and Masayori Inouye (1998) *J. Bacteriology* **180**, 1, 90-95.
5) Li Fang,Weining Jiang, Weonhye Bae and Masayori Inouye (1997) *Molecular Microbiology* **23**(2), 355 - 364.
6) Weining Jiang,Li Fang and Masayori Inouye (1996) *J. Bacteriology* **178**, 16, 4919 - 4925.
7) Hiroyuki Tanabe, Joel Goldstein, Maozhou Yang and Masayori Inouye (1992) *J. Bacteriology* **174**, 12, 3867 - 3873.