Cat. # 3360_3364

For Research Use

TaKaRa

Cold Shock Expression System pCold™ DNA

Product Manual

v202003Da

Table of Contents

I. Description

Elucidation of protein structure and function maintains an important role in postgenomic obtaining large amounts of correctly folded recombinant protein. *E. coli* expression systems, which are used extensively for the production of recombinant proteins, offer two major advantages over other types of expression systems: (1) ease of use, and (2) low cost. However, some recombinant proteins do not fold correctly during expression in *E. coli*, and result in deposits of inactive insoluble protein termed "inclusion bodies".

In collaboration with Prof. Masayori Inouye (University of Medicine and Dentistry of New Jersey, USA), Takara Bio has developed the pCold DNA Vectors, a series of novel protein expression vectors. The pCold Vectors provide increased *in vivo* protein yield, purity, and solubility for expressed recombinant proteins using "cold shock" technology. More specifically, the *cspA* (cold shock protein A) promoter and related elements have been incorporated into these vectors to up-regulate target protein production at lowered incubation temperatures (37℃ - 15℃). This temperature drop also suppresses expression of other cellular proteins and temporarily halts overall cell growth. This process allows expression of target proteins at high yield, high purity (up to 60% of cellular protein), and increased solubility as compared with conventional *E. coli* expression systems.

All four cold-shock expression vectors, pCold DNA I - IV, contain a *cspA* promoter for efficient protein expression , as well a downstream, 5' untranslated region (5' UTR), *lac* operator, ampicillin resistance gene, ColE1 origin of replication, M13 IG fragment, and multiple cloning site (MCS). Some of these vectors also contain a translation enhancing element (TEE), and/or a his tag sequence and/or a factor Xa cleavage 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

【Plasmid storage buffer】
10 mM Tris-HCl, pH 8.0
1 mM EDTA

<Available *E. coli* host strains>
Most *E. coli* strains can be used as an expression host for the pCold DNA series, because these vectors utilize the *cspA* promoter, which is derived from *E. coli*.

III. Storage

-20℃

Note: Use within 2 years from date of receipt under proper storage conditions.

IV. Vector Map

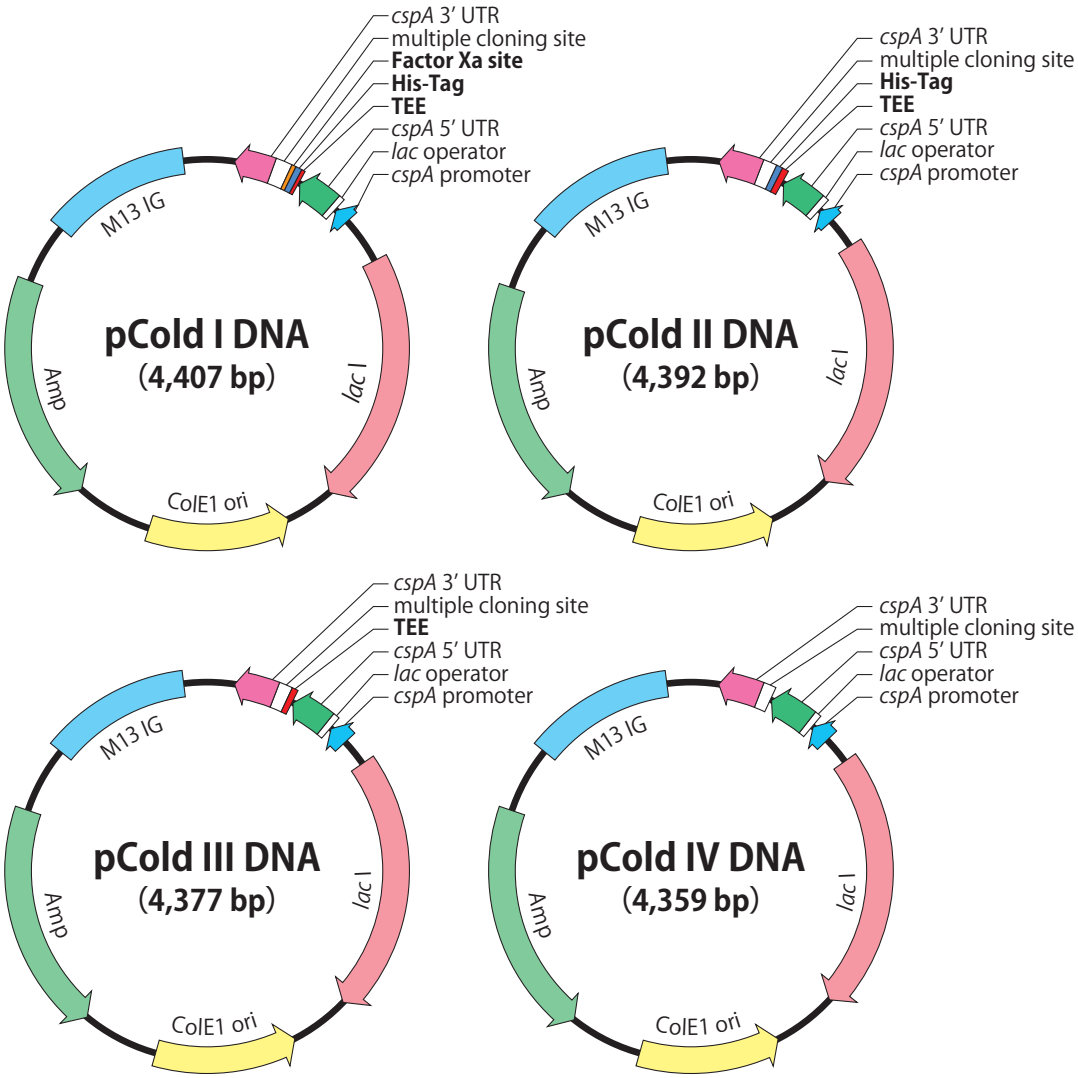


Figure 1. pCold Vector Map

	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

V. Protocol

How to express the target gene:

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

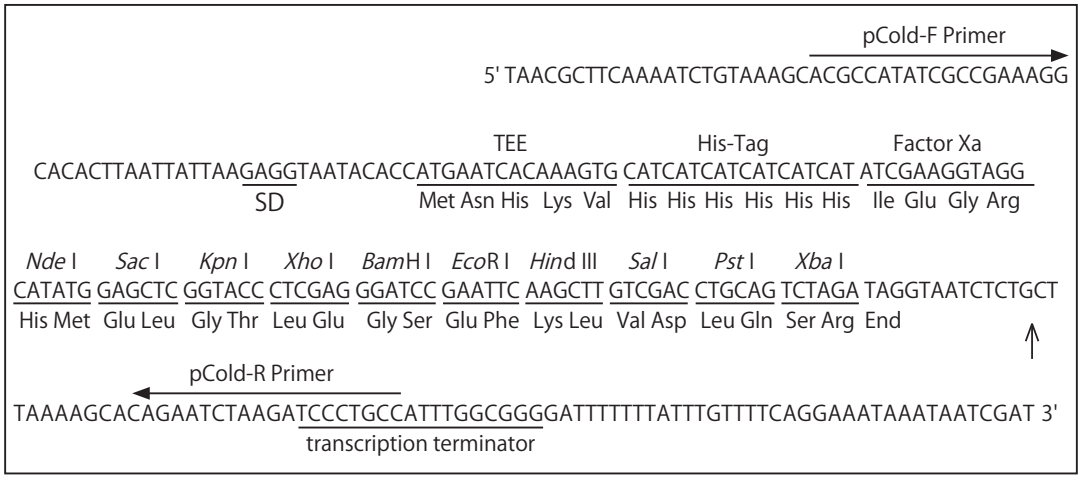
A general description of the protocol is shown below.

1) Insert the target gene fragment into the MCS of pCold DNA. Be sure that the sequence of the fragment is inserted in-frame with the fusion tag sequence.
2) Transform the *E. coli* host strain (e.g. BL21) with this plasmid, and select the transformants on an agar plate containing ampicillin.
3) Inoculate LB medium containing 50 - 100 μg/ml of ampicillin with Amp^+ transformant clones, and incubate with shaking at 37℃.
4) When the OD_{600} of the culture reaches 0.4 - 0.8, quickly cool the culture to 15℃ in ice water, and let stand for 30 minutes.
5) Add IPTG at a final concentration of 0.1 - 1.0 mM, and continue the culture with shaking at 15℃ for 24 hours.
6) Collect the cells, and confirm the presence, amount, and solubility of the target protein using SDS-PAGE or an activity assay.

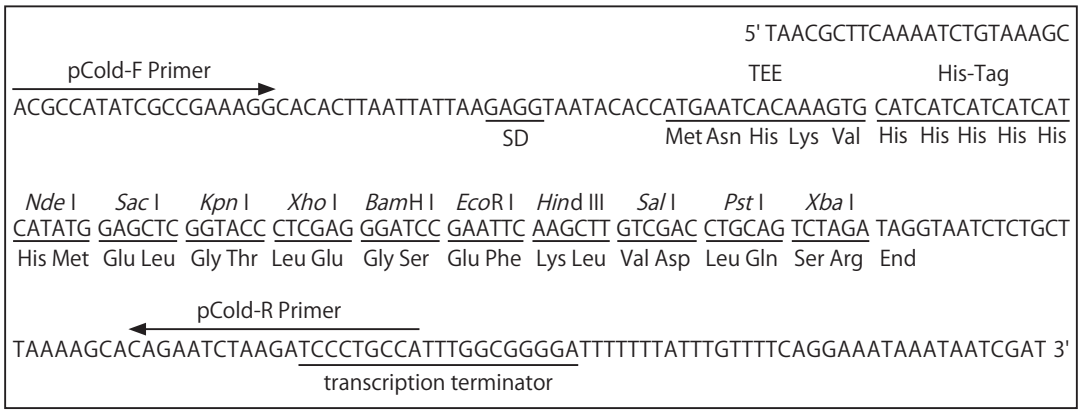
By optimizing the host strain, culture, and expression induction conditions (e.g., culture medium and temperature, degree of aeration and agitation, timing of induction, IPTG concentration, culture conditions after induction, etc.), it may be possible to increase the expression level and solubility of the target protein. Moreover, when the expressed protein is insoluble, the combined use of pCold DNA with Chaperone Plasmid Set (Cat. #3340) may be effective.

VI. Multiple Cloning Site

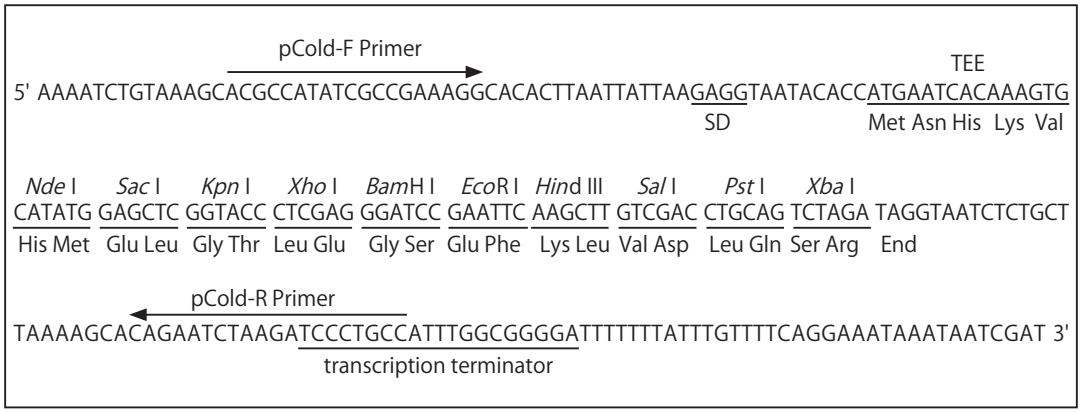
pCold I DNA (Cat. #3361)



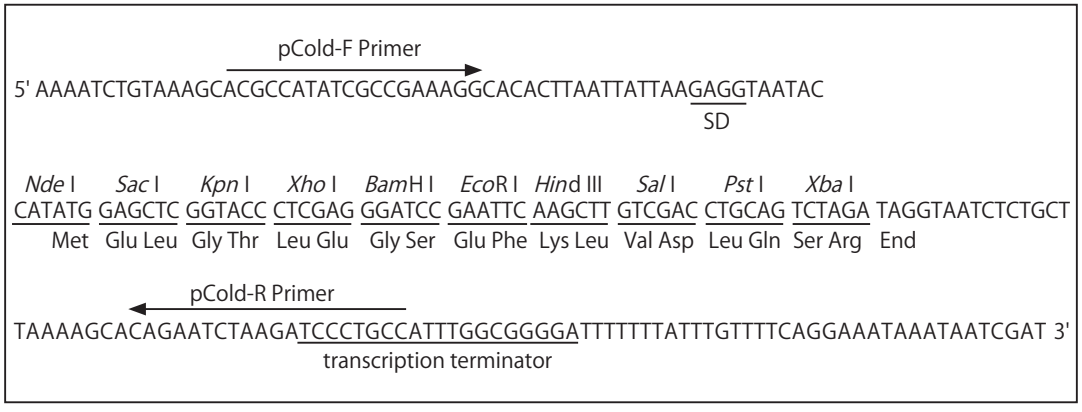
pCold II DNA (Cat. #3362)



pCold III DNA (Cat. #3363)



pCold IV DNA (Cat. #3364)



VII. Application

As described below, expression of genes that showed low expression levels or poor solubility in a T7 promoter expression system was 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 using the general method for adding IPTG and incubating the culture at 37°C.

(1) Expression of target protein was observed using the pCold system.
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 (Figure 2).

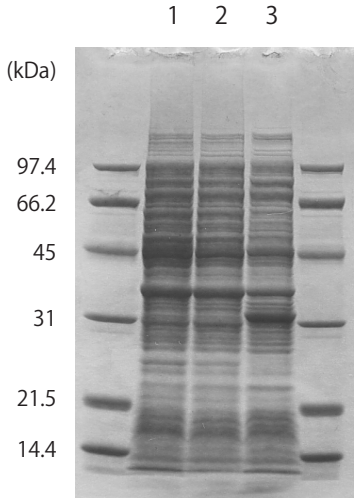


Figure 2. Expression of human gene A was compared using pCold and T7 Vector followed by CBB staining.

(2)The expression level increased using the pCold system.
For thermophile gene B (estimated molecular weight: 30 kDa), solubility was improved and the expression level was higher than that obtained using the T7 expression system (Figure 3).

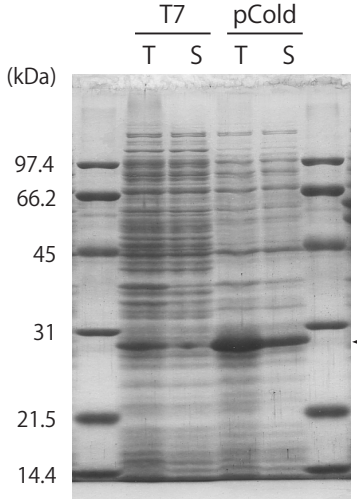


Figure 3. Expression of thermophile gene B was compared with total protein and soluble protein using pCold and T7 Vector followed by CBB staining.

(3) The level of soluble protein increased using the pCold system.

For human gene C (estimated molecular weight: 80 kDa), expressed protein was mostly insoluble in the T7 expression system. In the cold-shock expression system, however, the expression level of the soluble fraction increased remarkably (Figure 4). Cold-shock expression systems are expected to improve the expression level and solubility of the target protein compared to the T7 expression system.

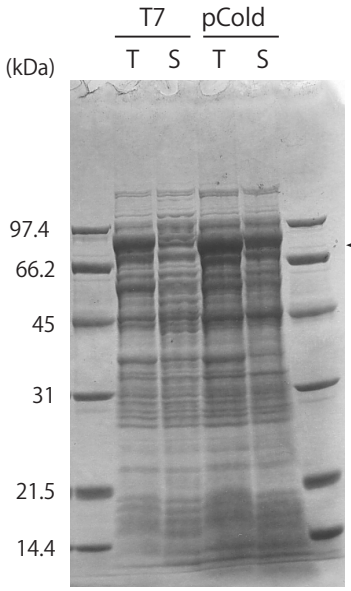


Figure 4. Expression of human gene C was compared using pCold and T7 Vector. Soluble and insoluble fractions were prepared and run on an SDS-PAGE gel, followed by CBB staining.

(4) Comparative pulse-labeling experiment

The expression of human gene D (estimated molecular weight: 12 kDa) was compared in both systems using a pulse-labeling experiment (Figure 5). In the T7 expression system, *E. coli* proteins other than the target protein were also labeled. In contrast, most of the labeled proteins expressed using the the cold-shock expression system were products of the target gene, indicating that target gene expression was specifically induced.

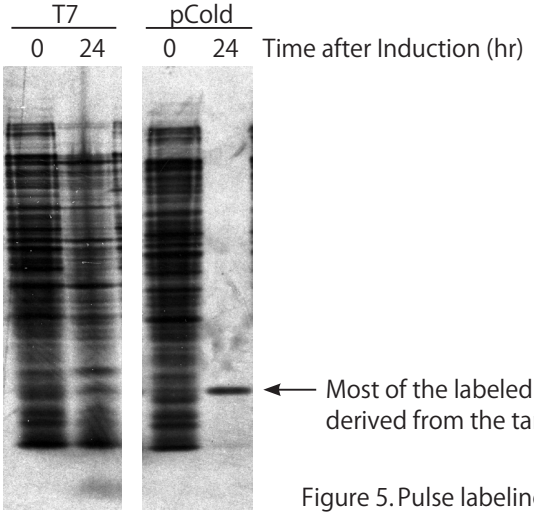


Figure 5. Pulse labeling of human gene D

VIII. Q&A

Q1: Why does pCold DNA allow efficient expression of proteins at low temperatures?

A1: pCold DNA, which was developed based on a *cspA* gene that encodes a cold shock protein, contains the *cspA* promoter, 5′ UTR, and the N-terminal region of *cspA*.

It is possible to achieve transcription from the *cspA* promoter at 37℃; however, translation is not efficient because of the extreme instability of the downstream 5′ UTR at 37℃. By lowering the temperature from 37℃ to 15℃, the 5′ UTR becomes highly stable. This results in improved translation efficiency, allowing extremely efficient protein synthesis at a low temperature (15℃)[1)].

Moreover, when the mRNA that encodes some of the N-terminus of *cspA* is transcribed, ribosomes are preferentially used for translation of the transcribed mRNA and rarely supplied for translation of other mRNAs (ribosome trapping)[2)].

As described above, pCold DNA enables extremely efficient protein expression at low temperatures by virtue of a *cspA* promoter that promotes increased transcriptional activity at low temperatures, structural stability of the 5′ UTR at low temperatures, and ribosome trapping. Although protein expression at low temperatures has been performed before, the innovative features of pCold DNA provide a suitable, unique expression vector for protein expression at low temperatures.

1) Mitta M, *et al. Mol Microbiol*. (1997) **26**: 321-335.
2) Xia B, *et al. J Biol Chem.* (2001) **276**: 35581-35588.

Q2: What are possible solutions if no expression is observed?

A2: Consider changing the type of vector, host, culture, and induction conditions.

- Change the type of vector (pCold I to IV DNA, pCold TF DNA, pCold ProS2 DNA, pCold GST DNA). In some cases, proteins can be expressed by attaching a TEE or his tag sequence to the N-terminus. Expressing the target protein as a fusion with a solubility-promoting tag such as TF, ProS2 or GST is also effective.
- Examine codon usage. Some genes are influenced by the frequency of codon usage. In some cases, the expression level can be improved by using commercially available strains of *E. coli* that support rare codons (such as Rosetta2 cells from Merck Millipore).
- Expression may be influenced by pre-culture and storage conditions of expression clones (see Q8).
- Change the timing of cold-shock induction. The expression level sometimes decreases if induction is late. In this case, early induction may increase the expression level.
- The cooling process prior to addition of IPTG should be sufficient (normally 30 minutes or longer at 15℃). Cultures should continue to be incubated at 15℃ following addition of IPTG.

Q3: What are possible solutions if expressed proteins are insoluble?

A3: Appropriate culture and induction conditions are different for each target protein. Consider changing culture and induction conditions, the strain of *E. coli* used as a host, and the extraction method, in accordance with the following recommendations:

- Change the timing of induction (Try starting induction at different times between the early and late logarithmic phases).
- Change the concentration of the inducer (IPTG) (0.1 to 1 mM).
- Change the temperature and incubation time of the culture following induction (15℃ for 24 hours is usually appropriate).

- Consider changing the *E. coli* host strain and trying chaperone co-expression using the Chaperone Plasmid Set or commercially available strains of host *E. coli* (such as Origami cells from Merck Millipore) that facilitate solubilization of expressed proteins.
- Change the extraction method. Some proteins are not sufficiently solubilized using a commercially available *E. coli* lysis agent. It is also effective to perform sonication with 0.1 to 1% of detergent (octylglycoside, NP-40, or Triton X-100).
- Change the type of vector. Expressing the target protein as a fusion with a solubility-promoting tag such as TF, ProS2 or GST is also effective.

Q4: What molecular weight range of proteins can be expressed?

A4: We have expressed proteins from several kDa to 100 kDa.

Q5: From which species have genes been expressed so far?

A5: We have expressed *E. coli*, thermophile, hyperthermophile, human, mouse, and plant genes.

Q6: What are the criteria for selecting pCold vectors?

A6: The TEE sequence, which is present in pCold I, II, and III DNA, facilitates translation of the target gene. Proteins expressed using vectors with a his tag sequence (pCold I, II DNA) can be purified with Ni^{2+} columns. If the presence of excess amino acids at the N-terminus of the target protein is undesirable, use pCold I DNA. This vector allows cleavage of the tag sequence with Factor Xa. Alternatively, use pCold IV DNA, which does not contain any TEE or tag sequences.
If the target protein was not expressed or was insoluble using pCold I - IV DNA, we recommend using fusion vectors that express soluble tags, such as pCold TF DNA, pCold ProS2 DNA, or pCold GST DNA.

Q7: How much protein can be expressed in 1 L of medium?

A7: The concentration of expressed protein usually ranges from several mg to several tens of mg/L, although expression levels vary for each target gene. Approximately 3 L of culture can yield mg scale amounts of protein (after purification). Expression of a target protein can be detected using SDS-PAGE followed by CBB staining.

Q8: Is it possible to store *E. coli* that was transformed with a pCold vector containing a target gene on a plate at 4℃?

A8: We do not recommend storage at 4℃ on a plate because it may cause the target protein to leak from the cells. Pick the colony from the plate promptly, prepare a glycerol stock, and store at -80℃.

Q9: How should one select from among the 5 chaperone plasmids when performing coexpression with the Chaperone Plasmid Set?

A9: Expression systems based on pCold vectors tend to produce better results when performing coexpression with chaperone teams containing the tag sequence. Begin by attempting coexpression with pG-Tf2 or pTf16.

Q10: To what extent does the OD_{600} increase when incubating cultures at 15℃ for 24 hours after induction?

A10: The OD_{600} values of cultures incubated for 24 hours after induction using this system are different for each *E. coli* host strain and each different inserted target gene. The OD_{600} value is typically about 1.2 using BL21 as a host strain.

Q11: Which strains of host *E. coli* have been used so far?

A11: We have expressed many proteins using BL21 as a host. Origami and Rosetta cells from Merck Millipore are also available. Although we recommend starting with BL21, most *E. coli* strains can be used as hosts for pCold vectors that use the *cspA* promoter derived from *E. coli*.

Q12: The pCold vector expression system induces expression of target proteins when cultures are incubated at low temperatures. Why is IPTG added prior to induction?

A12: Since pCold vectors use the promoter of a cold-shock protein, they have low intrinsic levels of target protein expression at 37℃. However, some inserts induce a small amount of leakage. Therefore, we employed a regulatory system based on the *lac* operon as a supplementary measure.

In the case of pET vectors whose expression is strictly controlled by the addition of IPTG, the effect of the absence or presence of IPTG on expression is significant. In the case of pCold vectors where induction is controlled by a shift in temperature, whether or not expression is regulated by the *lac* operon after the temperature is lowered is less consequential. Nevertheless, we recommend addition of IPTG.

The strength of regulation by the *lac* operon is different for each type of *E. coli* host. In general, those strains that contain *lac I*^{q} (such as JM109) and those that are regulated only with the *lac I* gene which is inherent to *E. coli* (such as BL21) vary in their strength of regulation. However, it may not be necessary to select bacterial strains containing *lac I*^{q}, since the strength of regulation after lowering the temperature is not important.

Q13: How should one remove the chaperone proteins from the chaperone co-expression system?

A13: Use pCold I or II DNA, which each include a his tag sequence, and purify the target protein by affinity chromatography that uses the his tag to remove the chaperone proteins.

Q14: For cold shock induction, should one rapidly chill the culture to be induced?

A14: Shifting the temperature from 37℃ to 15℃ should be performed as quickly as possible. For example, we recommend soaking the culture vessel in a water-ice bath until its temperature is reduced to 15℃, and maintaining this temperature for an additional 30 minutes. Make sure that the temperature is consistent throughout the culture, especially when working with large culture volumes.

Q15: There are no useful restriction enzyme sites in the MCS. What can I do?

A15: We recommend using the In-Fusion® HD Cloning Plus (Cat. #638909, etc.). It enables directional, seamless cloning of any PCR fragment into any linearized vector in a single 15-minute reaction. The In-Fusion Enzyme premix fuses PCR-generated sequences and linearized vectors efficiently and precisely, utilizing a 15 bp overlap at their ends. This 15 bp overlap can be engineered by designing custom primers for amplification of the desired sequences.

Q16: How should I purify his-tagged proteins?

A16: Use TALON® Metal Affinity Resin (Cat. #635501, etc.). TALON is an immobilized metal affinity chromatography (IMAC) resin charged with cobalt, which binds to his-tagged proteins with higher specificity. As a result, TALON resin yields his-tagged proteins of the highest purity. We also recommend His60 Ni Superflow Resin (Cat. #635659, etc.), which is a high-capacity Ni-IDA resin (up to 60 mg/ml of his-tagged protein) that allows one-step protein purification.

IX. Appendix

Expression Plasmid Construction - Example using the thioredoxin gene

1) Overview of pCold expression vector construction
   a) Select a restriction enzyme site such that the DNA fragment to be inserted will be in-frame with the pCold Vector.
   b) Prepare the DNA fragment.
   c) Digest the vector with the desired restriction enzymes.
   d) After ligating the digested vector with the insert DNA, transform it into an appropriate *E. coli* strain.
   e) Prepare purified plasmid from the appropriate transformants containing the target insert.
   f) The purified plasmid may be used for protein expression experiments.

   The insert DNA may be prepared by PCR amplification, excision of a cloned gene by restriction enzyme digestion, or gene synthesis. Alternatively, In-Fusion Cloning Kits can be used for easy and rapid directional cloning, in cases where appropriate restriction enzyme sites are not present in a target gene. An example using PCR amplification for insert DNA preparation is provided below.

2) Example : Plasmid preparation for expression of the *E. coli* thioredoxin gene

   a) Guidelines for primer design

      Protocol and points to consider when designing primers:

      i) Select two restriction enzymes that are absent in the insert DNA but present in the MCS of pCold TF DNA.
      ii) Construct PCR primers for the target sequence, adding the restriction sites selected above to the 5' terminus of each primer. Adjust the nucleotide number between the insert DNA sequence and N-terminal restriction sites such that the reading frame of the insert matches the reading frame of the pCold vector. A restriction site can be directly added to a stop codon at the C-terminus.
      iii) Add four or more nucleotides to sequences directly flanking the restriction sites. Most restriction enzymes require the presence of several bases outside of the recognition site for efficient digestion to occur. Without the presence of this extra sequence, digestion efficiency will decrease.

      [Example - Primer Design]

      Insertion of the thioredoxin gene into the pCold *Nde* I/*Xho* I MCS restriction enzyme cloning sites

      Nde I site: Primer 1 (forward primer)

      Nde I
      5'-GCCGCATATGAGCGATAAAATTATTCAC
      thioredoxin derived sequence＊1

      Xho I site: Primer 2 (reverse primer)

      Xho I
      5'- GCCGCTCGAGTTAGGCCAGGTTAGCGTC
      thioredoxin derived sequence＊2

      ＊1 When using the *Nde* I site, adjust the position of the thioredoxin gene start codon (ATG) to correspond with the ATG site of *Nde* I.
      ＊2 Complementary thioredoxin sequence with stop codon

b) Insert DNA Preparation

[Example - PCR amplification of the thioredoxin gene (350 bp)]

i) PCR amplification of the insert DNA.

Prepare the reaction mixture by combining the following reagents. (Use of a high fidelity PCR enzyme, such as PrimeSTAR® HS DNA Polymerase (Cat. #R010A), is recommended.)

Template DNA (5 ng)＊1	1 μl
5X PrimeSTAR Buffer＊2	10 μl
dNTP Mixture (2.5 mM each)＊2	4 μl
Primer 1 (10 - 50 pmol/μl)	1 μl
Primer 2 (10 - 50 pmol/μl)	1 μl
PrimeSTAR HS DNA Polymerase (5 U/μl)	0.5 μl
Sterile purified water	32.5 μl
Total	50 μl

＊1 For plasmid DNA, use 10 pg - 1 ng; for cDNA or genomic DNA, use 5 - 200 ng.

＊2 5X PrimeSTAR Buffer and dNTP Mixture is supplied with PrimeSTAR HS DNA Polymerase (Cat. #R010A).

Amplify the insert DNA using the following PCR cycling parameters when using the TaKaRa PCR Thermal Cycler Dice™ *Touch*/Gradient (Cat. #TP350/TP600).＊

98℃	10 sec	┐
55℃	5 - 15 sec	├ 30 cycles
72℃	1 min	┘

＊ Not available in all geographical locations. Check for availability in your area.

ii) Verification of amplified product

Verify that the amplified insert DNA fragment is a single band of the expected size by performing agarose gel electrophoresis using 5 μl of the PCR product.

iii) PCR product purification

When amplified DNA appears as a single band, phenol/chloroform extraction or PCR clean-up with NucleoSpin Gel and PCR Clean-Up (Cat. #740609.10/.50/.250) is recommended for removing PrimeSTAR HS DNA Polymerase. When multiple PCR products are generated, first isolate the band of interest from the agarose gel and then further purify.

iv) Restriction enzyme digestion of amplified products

Digest the purified insert DNA with *Nde* I and *Xho* I restriction enzymes.

1) Prepare the following restriction enzyme digest mixture:

Insert DNA (0.5 - 1 μg)	x μl
10X K Buffer	3 μl
Nde I (10 U/μl)	1 μl
Xho I (10 U/μl)	1 μl
Sterile purified water	y μl
Total	30 μl

2) Incubate at 37℃ for 1 hour.

3) Purify the digested DNA by ethanol precipitation etc.*
4) Verify fragment purity using agarose gel electrophoresis or by measuring absorbance (OD_{260}).

* Both *Nde* I and *Xho* I can be inactivated by ethanol precipitation. However, when working with restriction enzymes that are not completely inactivated by ethanol precipitation, the digestion reaction should be extracted with phenol. In addition, further purification and recovery of digested DNA by agarose gel electrophoresis can completely remove short fragments generated by the digestion.

[Ethanol precipitation protocol]

1) Add 3 M sodium acetate, pH 5.2, to the restriction enzyme reaction mixture in a 1 : 10 ratio (e.g. 3 μl of 3 M sodium acetate to 30 μl reaction mixture), and mix well.
2) Add 2 - 2.5 times the volume of 100% cold ethanol to the above solution (e.g. add 66 μl 100% cold ethanol to 33 μl sodium acetate-digest mixture), and mix well. Chill at -20℃ for 30 minutes.
3) Centrifuge at 4℃, 12,000 rpm, for 10 - 15 minutes. Discard the supernatant.
4) Add 70% cold ethanol and centrifuge again at 4℃, 12,000 rpm, for 5 minutes.
5) Discard the supernatant and allow the pellet to air dry.
6) Dissolve the precipitate in 10 - 50 μl of TE buffer.

c) Restriction Enzyme Digestion of pCold DNA

Digest pCold with the same restriction enzymes that were used for the digestion of amplified insert DNA, and purify. Dissolve the purified DNA in TE buffer, and measure the DNA concentration by measuring absorbance.

i) Prepare the following reaction mixture:

pCold Vector	1 μg
10X K Buffer	3 μl
Nde I (10 U/μl)	1 μl
Xho I (10 U/μl)	1 μl
Sterile purified water	x μl
Total	30 μl

ii) Incubate at 37℃ for 1 - 2 hours.
iii) Purify the digested DNA by ethanol precipitation etc.*
iv) Dissolve the precipitated vector DNA pellet in TE buffer.
v) Measure the absorbance (OD_{260}) and calculate the DNA concentration. For dsDNA (double-stranded DNA), calculate the DNA concentration assuming 1 OD_{260} = 50 μg/ml.
vi) Adjust the DNA concentration to 100 ng/μl.

* After digestion with restriction enzymes, the vector DNA may be de-phosphorylated with *E. coli* Alkaline Phosphatase (BAP)(Cat. #2120A), or Calf Intestinal Alkaline Phosphase (CIAP) (Cat. #2250A). Note that de-phosphorylation is essential if only a single restriction enzyme was used for digestion. In addition, complete removal of short fragments generated by restriction enzyme digestion is recommended. Purify the vector from any short fragments using agarose gel electrophoresis, then further isolate and gel-purify the vector.

d) Ligation and transformation

i) Ligation reaction

Mix together the digested pCold and the insert DNA fragment, and use this mixture for performing a ligation reaction using DNA Ligation Kit <Mighty Mix> (Cat. #6023). A 1 : 3 -1 : 10 molar ratio of vector: insert DNA is recommended.

Prepare the following ligation reaction mixture on ice:

Digested pCold DNA : 100 ng (approx. 0.03 pmol)	1 μl
Insert DNA fragment (0.1 - 0.3 pmol)	4 μl
Ligation Mix (from DNA Ligation Kit <Mighty Mix>)	5 μl
Total	10 μl

Incubate at 16℃ for 1 hour.

ii) Transformation

1) Thaw *E. coli* HST08 Premium Competent Cells (Cat. #9128) on ice just before use.
2) Add 10 μl ligated DNA mixture to 100 μl competent cells, and mix gently.
3) Chill on ice for 30 minutes.
4) Incubate at 42℃ for 45 sec.
5) Chill on ice for 1 - 2 minutes.
6) Add warm (37℃) SOC Medium to a final volume of 1 ml.
7) Shake at 37℃ for 1 hour.
8) Plate on LB-ampicillin agar (100 μg/ml ampicillin) and incubate at 37℃ overnight.

e) Plasmid preparation and verification

Inoculate a colony obtained in Step d)-ii) above into LB-ampicillin broth (100 μg/ml ampicillin) and incubate with gentle shaking at 37℃ overnight. Purify the plasmid from the culture.

Digest the purified plasmid with the restriction enzymes *Nde* I and *Xho* I. Verify insertion of the correct DNA fragment by checking insert DNA fragment size using agarose gel electrophoresis.

When the vector construct has been verified, confirm the sequence of the inserted DNA fragment using the following sequencing primers. This plasmid can be used as an expression plasmid for subsequent experiments.

Upstream primer :	pCold-F	5'-ACGCCATATCGCCGAAAGG
Downstream primer :	pCold-R	5'-GGCAGGGATCTTAGATTCTG

This primer pair cannot be used for PCR because doing so results in an extra band of approximately 500 bp.

X. References

1) Mitta M *et al. Mol Microbiol*. (1997) **26**: 321-335.
2) Xia B, Etchegaray JP, and Masayori Inouye. *J Biol Chem*. (2001) **276(38)**: 35581-35588.
3) Masayori Inouye *et al*. *Nature Biotechnology.* (2004) **22(7)**: 877-882.
4) Kunitoshi Yamanaka, Masanori Mitta, and Masayori Inouye. *J Bacteriology*. (1999) **181(20)**: 6284-6291.
5) Li Fang, Yan Hou, and Masayori Inouye. *J Bacteriology.* (1998) **180(1)**: 90-95.
6) Li Fang, Weining Jiang, Weonhye Bae, and Masayori Inouye. *Molecular Microbiology.* (1997) **23(2)**: 355-364.
7) Weining Jiang, Li Fang, and Masayori Inouye. *J Bacteriology.* (1996) **178(16)**: 4919-4925.
8) Hiroyuki Tanabe, Joel Goldstein, Maozhou Yang, and Masayori Inouye. *J Bacteriology*. (1992) **174(12)**: 3867-3873.

XI. Related Products

Protein expression and purification-related products:

[Induction of target protein expression]
TaKaRa Competent Cells BL21 (Cat. #9126)
IPTG (Isopropyl-β-D-thiogalactopyranoside) (Cat. #9030)

[His-tagged fusion protein purification]
TALON® Metal Affinity Resin (Cat. #635501 - 635504/635652/635653)
TALON® Superflow Metal Affinity Resin (Cat. #635506/635507/635668 - 635670)
HisTALON™ Superflow Cartridge Purification Kit (Cat. #635649/635681)
His60 Ni Superflow Resin (Cat. #635659 - 635664)

[pCold vector series]
pCold™ TF DNA (Cat. #3365)*
pCold™ ProS2 DNA (Cat. #3371)*
pCold™ GST DNA (Cat. #3372)*

Cloning-related products:

[PCR amplification of target genes]
PrimeSTAR® Max DNA Polymerase (Cat. #R045A)
PrimeSTAR® GXL DNA Polymerase (Cat. #R050A/B)
PrimeSTAR® HS DNA Polymerase (Cat. #R010A/B)
Tks Gflex™ DNA Polymerase (Cat. #R060A/B)*

[Purification of target gene fragments]
NucleoSpin Gel and PCR Clean-up (Cat. #740609.10/.50/.250)

[Insertion of a DNA fragment into a vector and transformation]
In-Fusion® HD Cloning Plus (Cat. #638909)
In-Fusion® HD EcoDry™ Cloning Plus (Cat. #638912)
E. coli HST08 Premium Competent Cells (Cat. #9128)
E. coli DH5α Competent Cells (Cat. #9057)*
E. coli JM109 Competent Cells (Cat. #9052)
E. coli HST08 Premium Electro-Cells (Cat. #9028)
E. coli DH5α Electro-Cells (Cat. #9027)
E. coli JM109 Electro-Cells (Cat. #9022)

[Plasmid preparation from *E. coli*]
NucleoSpin Plasmid EasyPure (Cat. #740727.10/.50/.250)

* Not available in all geographic locations. Check for availability in your area.

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