Cat. # 3372

For Research Use

TaKaRa

pCold™ GST DNA

Product Manual

v201810Da

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I. Description

Effective protein expression systems are essential for analyzing protein structure and function. Expression systems using *E. coli* as a host are widely used for recombinant protein production. Although *E. coli* expression systems are easy to work with, some genes cannot be efficiently expressed in *E. coli* because of protein insolubility and toxicity.

In collaboration with Professor Masayori Inouye (University of Medicine and Dentistry of New Jersey), Takara Bio has developed a system for improving protein expression in *E. coli* that is based on cold shock technology (pCold). With this system, the culture is shifted to a low incubation temperature, thereby halting bacterial growth and the expression of most *E. coli*-derived proteins. Simultaneously, the expression of cold shock proteins is specifically induced. With the pCold vector, target gene expression is driven by the promoter of *cspA*, an *E. coli* cold shock gene. Thus, the pCold expression system can significantly improve protein expression, purity, and solubility[1].

The expression vector pCold GST DNA was developed by incorporating glutathione S-transferase (GST) derived from *Schistosoma japonicum* as a soluble tag[2, 3]. The proteins expressed using this vector have an N-terminal GST tag, which can improve the stability and solubility of the fused protein.

The pCold GST vector includes a 5' untranslated region (5' UTR), translation enhancing element (TEE), his tag, GST tag, and multiple cloning site (MCS) downstream of the *cspA* promoter (Figure 1). In addition, a *lac* operator has been inserted downstream of the promoter to allow precise control of gene expression. Finally, because the pCold vector series uses an *E. coli* promoter, virtually any strain of *E. coli* can be used as a host for protein expression.

High affinity purification of the GST-tagged fusion proteins expressed using pCold GST is possible. Furthermore, a highly specific HRV 3C protease recognition sequence has been inserted between the GST tag and MCS, allowing removal of the GST tag from the recombinant protein. Because the optimum reaction temperature for HRV 3C protease is low (4 - 5℃), tag cleavage can be performed under moderate conditions.

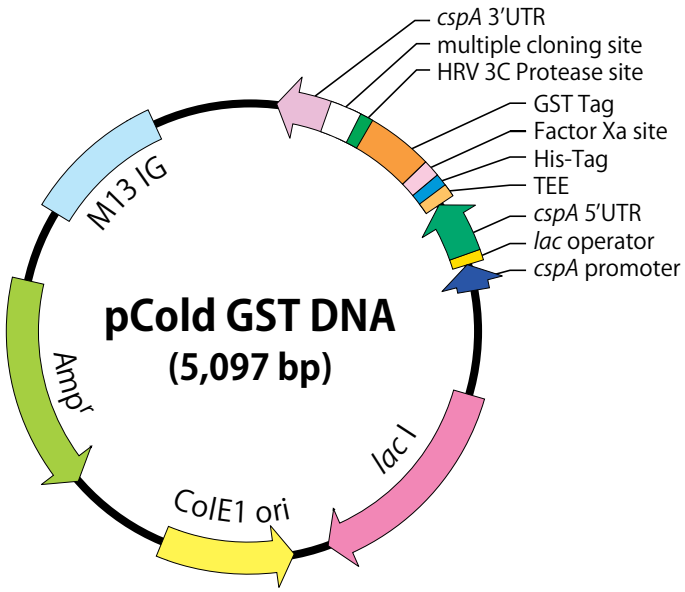


Figure 1. pCold GST DNA vector map

II. Components

pCold GST DNA 25 μg

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

[Suitable host *E. coli* strains]
The *cspA* promoter is derived from *E. coli*, therefore any *E. coli* strain can be used as host for protein expression.

III. Storage -20℃

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

IV. Protocol

Cloning and expression of a target gene:

(1) Insert the target gene fragment into the multiple cloning site of pCold GST vector. Be sure that the sequence of the fragment is inserted in-frame with the GST tag sequence.

(2) Transform the host *E. coli* cells with the plasmids, and select 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 to a final concentration of 1 mM, and incubate with shaking at 15℃ for 12 - 18 hours.

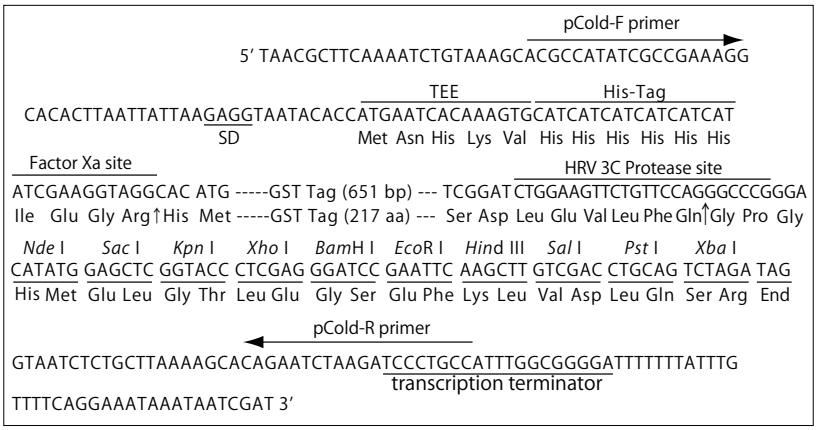
(6) Confirm the presence, amount, and solubility of the target protein using SDS-PAGE or activity measurement.

Notes:

1. 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.
2. GST affinity purification resins such as Clontech's Glutathione-Superflow Resin (Cat. #635607/635608) can be used to purify GST-tagged fusion proteins.
3. The GST tag can be cleaved using HRV 3C protease (Cat. #7360).

V. Multiple Cloning site map

pCold GST DNA



Primer sequence

pCold-F primer :
5'-ACGCCATATCGCCGAAAGG

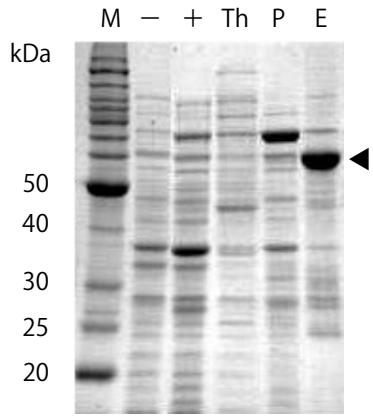
pCold-R primer :
5'-GGCAGGGATCTTAGATTCTG

VI. Experimental Examples

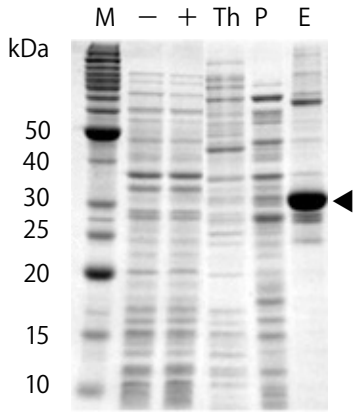
The following experimental data has been provided by Dr. Chojiro Kojima (Institute for Protein Research, Osaka University).

(1) Expression and purification of GST fusion proteins

After inserting the gene fragment into pCold GST DNA and introducing the vector into *E. coli* cells, protein expression was induced according to the recommended protocol (see section IV.) The GST fusion protein was subsequently purified using a GST affinity resin.



GST-plant HY2 (286 aa) fusion protein:
Theoretical molecular weight ~ 61.9 kDa



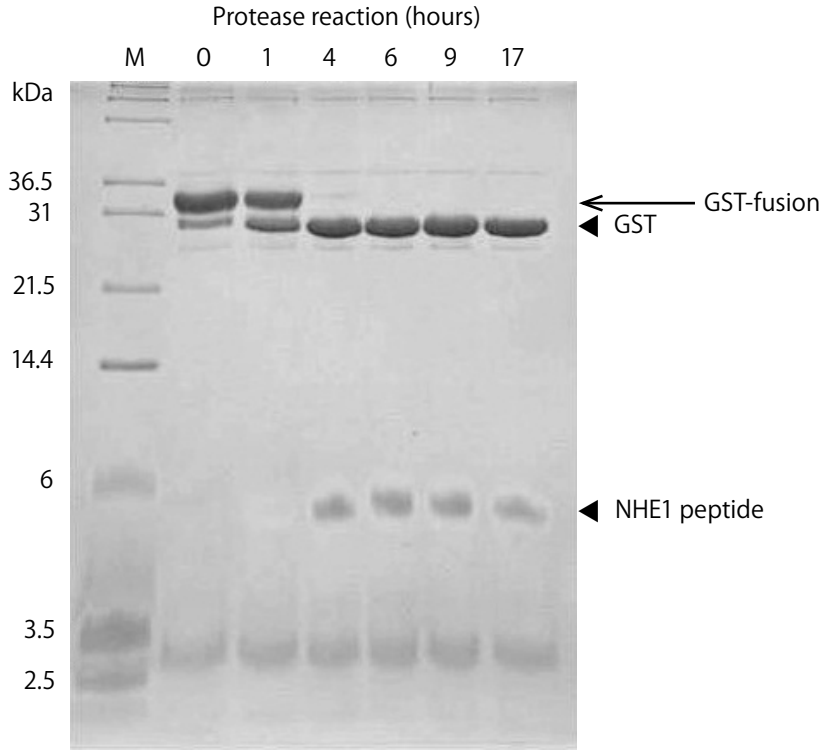
GST-mammalian NHE1 peptide (42 aa) fusion protein:
Theoretical molecular weight ~34.8 kDa

Lanes:
− : *E. coli* extract prior to expression induction
\+ : *E. coli* extract after expression induction
Th : Fraction not adsorbed to the GST affinity resin
P : Insoluble fraction (pellet)
E : GST affinity purification elution fraction
◀ : GST fusion protein

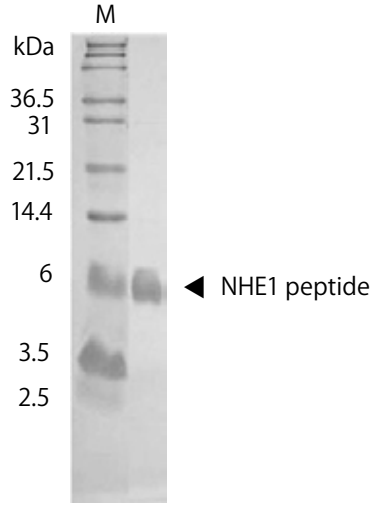
Conclusion: In each case, the induced GST fusion protein was obtained from the soluble fraction of the recombinant *E. coli* extract.

(2) GST tag cleavage and purification of fusion proteins

To remove the GST tag from GST-NHE1 expressed using pCold GST DNA, the protein was digested with HRV 3C protease at 4℃ and tag cleavage was monitored over time.



Next, the NHE1 peptide was purified from the Ni affinity-purified fraction of the above protease digest by gel filtration chromatography.



Conclusion: Highly purified target peptide was obtained after tag cleavage by protease digestion and purification of the digest.

VII. References

1) Qing G, *et al*. *Nat Biotechnol*. (2004) **22**: 877-882.
2) Hayashi K and Kojima C. *Protein Expr Purif*. (2008) **62**: 120-127.
3) Hayashi K and Kojima C. *J Biomol NMR*. (2010) **48**: 147-155.

VIII. 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)

[Purification of GST fusion proteins]
Glutathione-Superflow Resin (Cat. #635607/635608)
GST Purification Kit (Cat. #635619)

[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)

[pCold vector series]
pCold™ DNA Series (Cat. #3360 - 3364)*
pCold™ TF DNA (Cat. #3365)*
pCold™ ProS2 DNA (Cat. #3371)*

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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