Cat. # 3366-3370

For Research Use

TaKaRa

Single Protein Production System (SPP System™)

Product Manual

v201909Da

Table of Contents

I. Description

A Single Protein Production system (SPP system) was proposed by Dr. M. Inouye's group at Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey. This system utilizes an *E. coli* protein MazF which was found by the same group and it was refered to as an mRNA Interferase. The protein was determined to be a sequence-specific endoribonuclease which cleaves single strand RNAs at ACA sequences. In this system, the transcript of interest which should not contain any ACA sequences (i.e. ACA-less) , and MazF are co-expressed in a host *Escherichia coli*. Therefore the MazF does not cleave the transcript of interest, but cleaves the ones derived from the host proteins or others at ACA sequences. So, only the transcript of interest is dominantly translated and only the protein of interest is dominantly expressed, with the SPP (Figure1). Instead of their dominant expression, some proteins of interest are expressed in lesser amount with this system than in other expression systems, for example, in Cold-shock expression vector system alone.

In order to construct SPP system, it is first necessary to prepare an ACA-less gene of interest by chemical synthesis or site-directed mutagenesis technology, etc. In designing the ACA-less gene, every ACA sequence in the gene of interest must be substituted to another keeping its amino acid sequence, and restriction sites for cloning must be also added to be in frame. Secondly the ACA-less gene is cloned into a ACA-less region of the pCold (SP-4) vector which has ACA-less transcription region. At this point the plasmid becomes ready for SPP of interest. Then a host *E. coli* is co-transformed with this expression plasmid for SPP and pMazF (Figure3) which is ready to express MazF in adequate quantity. The transformant with the both plasmids generates the SPP of interest.

Takara's SPP System ineludes four types of Cold-shock expression vectors for SPP, pCold I (SP-4), pCold II (SP-4), pCold III (SP-4) and pCold IV (SP-4), which vary in the existences of TEE (translation enhancing element), His-Tag, and Factor Xa cleavage site (Table1 & Figure2). pColdIV (SP-4) has no additional sequence at the upstream of the MCS.

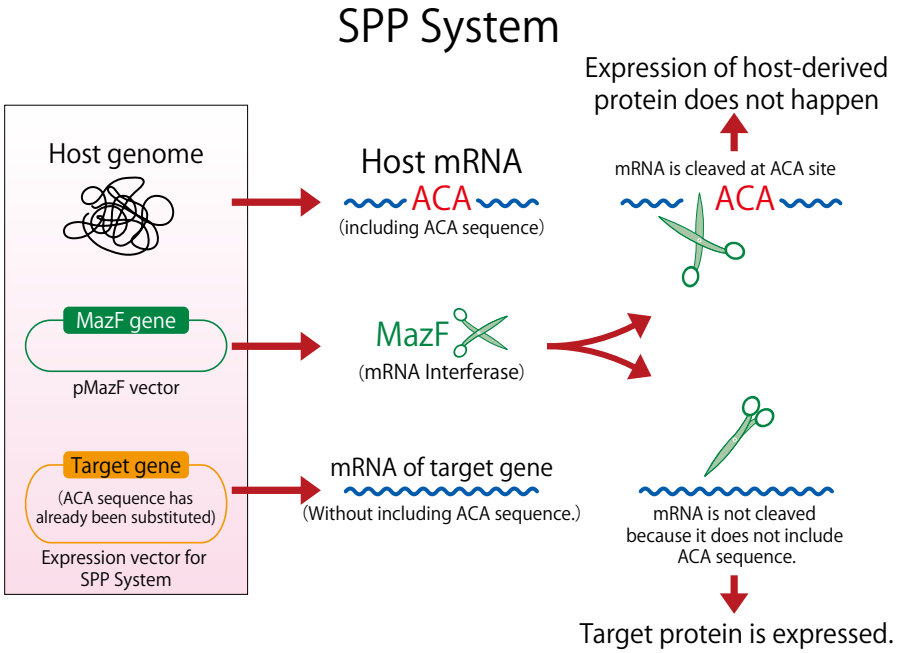


Figure 1. Principle of SPP System

II. Components

SPP System Set (Cat. #3366)

1.	Cold Shock Expression vector for SPP System pCold I (SP-4) DNA, pCold II (SP-4) DNA, pCold III (SP-4) DNA, pCold IV (SP-4) DNA	ea. 20 μg (0.5 μg/μl)
2.	MazF (mRNA Interferase) expression plasmid pMazF DNA	0.5 μg (20 ng/μl)
3.	Positive Control pCold I (SP-4) envZB DNA Expression plasmid prepared by inserting ORF of *E. coli*-derived protein envZB without ACA sequence into pCold I (SP-4) DNA (Estimated molecular weight of expressed protein 19.6 kDa)	0.2 μg (20 ng/μl)

SPP System I (Cat. #3367)

1.	Cold Shock Expression vector for SPP System pCold I (SP-4) DNA	20 μg (0.5 μg/μl)
2.	MazF (mRNA Interferase) expression plasmid pMazF DNA	0.5 μg (20 ng/μl)
3.	Positive Control pCold I (SP-4) envZB DNA	0.2 μg (20 ng/μl)

SPP System II (Cat. #3368)

1.	Cold Shock Expression vector for SPP System pCold II (SP-4) DNA	20 μg (0.5 μg/μl)
2.	MazF (mRNA Interferase) expression plasmid pMazF DNA	0.5 μg (20 ng/μl)
3.	Positive Control pCold I (SP-4) envZB DNA	0.2 μg (20 ng/μl)

SPP System III (Cat. #3369)

1.	Cold Shock Expression vector for SPP System pCold III (SP-4) DNA	20 μg (0.5 μg/μl)
2.	MazF (mRNA Interferase) expression plasmid pMazF DNA	0.5 μg (20 ng/μl)
3.	Positive Control pCold I (SP-4) envZB DNA	0.2 μg (20 ng/μl)

SPP System IV (Cat. #3370)

1.	Cold Shock Expression vector for SPP System pCold IV (SP-4) DNA	20 μg (0.5 μg/μl)
2.	MazF (mRNA Interferase) expression plasmid pMazF DNA	0.5 μg (20 ng/μl)
3.	Positive Control pCold I (SP-4) envZB DNA	0.2 μg (20 ng/μl)

< Available *E. coli* expression strains >

As Cold Shock Expression vectors for SPP System utilize *cspA* promoter derived from *E. coli*, almost all *E. coli* strains can be used as a host for expression. However, pMazF DNA used for co-expression utilizes chloramphenicol as a selection marker, chloramphenicol-resistant *E. coli* strains, e.g. Rossetta™ (Novagen), cannot be used as a host.

III. Storage

-20℃

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

IV. Vector Map

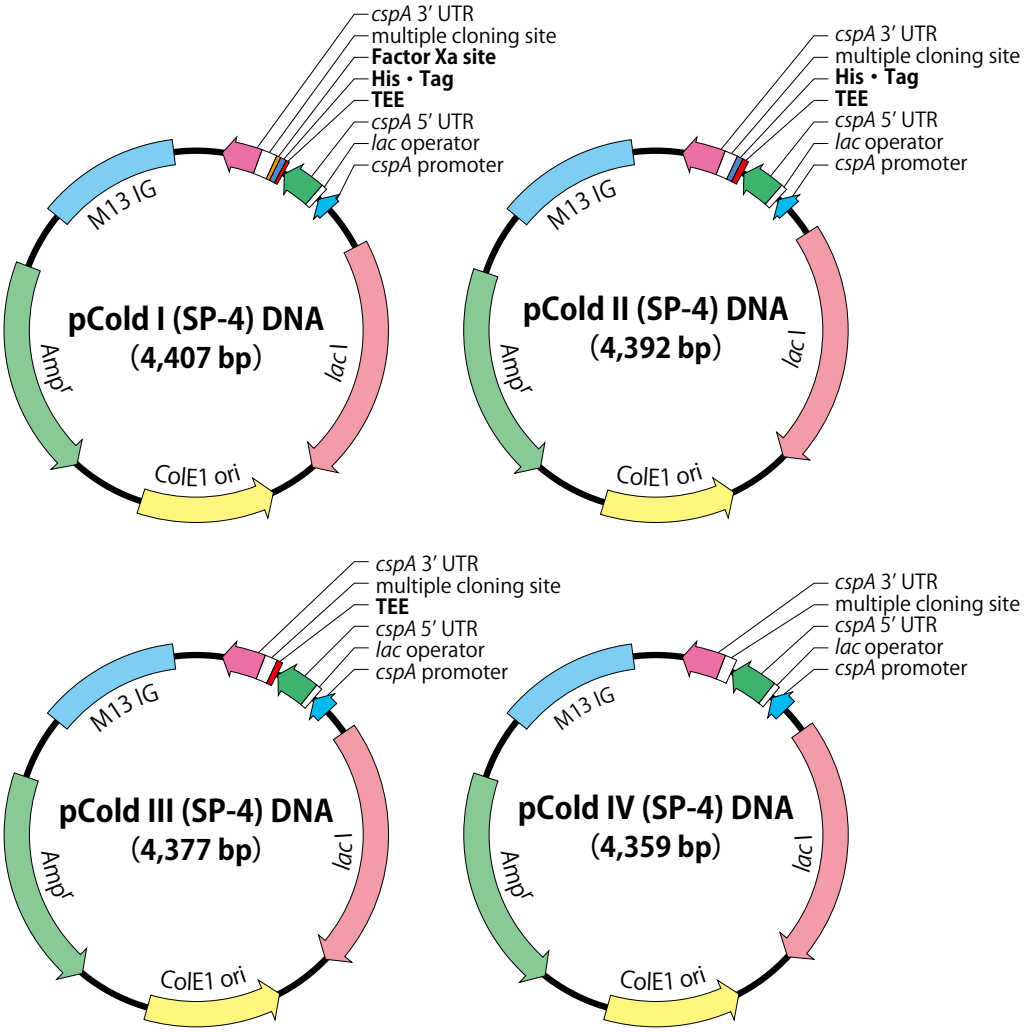


Figure 2. Vector map of pCold I - IV (SP-4) DNAs

Table1 : Various tags of pCold (SP-4) DNAs

Vector	TEE Sequence	His-Taq Sequence	Factor Xa cleavage site
pCold I (SP-4) DNA	○	○	○
pCold II (SP-4) DNA	○	○	—
pCold III (SP-4) DNA	○	—	—
pCold IV (SP-4) DNA	—	—	—

GenBank Accession No.

- pCold I (SP-4) AB248600
- pCold II (SP-4) AB248601
- pCold III (SP-4) AB248602
- pCold IV (SP-4) AB248603

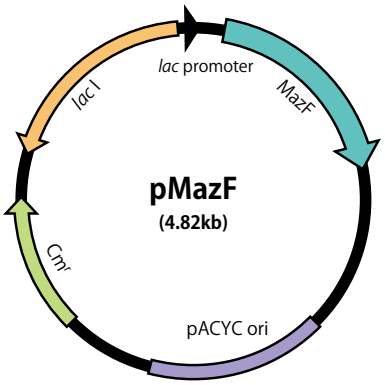


Figure 3. Vector map of pMazF

V. Form

10 mM Tris-HCl (pH 8.0), 1 mM EDTA solution

VI. Purity

For pCold (SP-4) DNA series :

1. Confirmed to maintain cloning sites by dideoxy sequencing method
2. Confirmed to be cleaved at a single site by restriction enzymes *Nde* I, *Sac* I, *Kpn* I, *Xho* I, *Bam*H I, *Eco*R I, *Hin*d III, *Sal* I, *Pst* I, and *Xba* I.

VII. Protocol

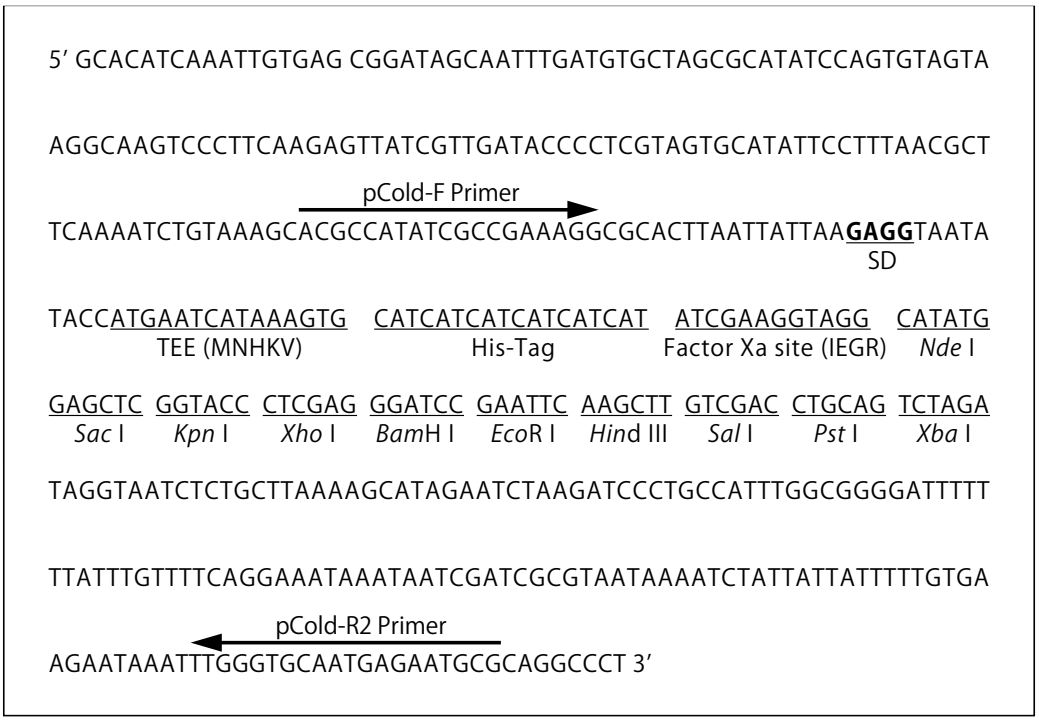
1. Construction of ACA-less gene expression plasmid
   1) Design an ACA-less gene of interest by substituting every ACA sequence in its original sequence to another while keeping its amino acid sequence. This ACA-less gene may also have restriction sites at the each end to make it in frame when cloned in pCold (SP-4).
   2) Synthesis and clone the ACA-less gene designed above by chemical synthesis or site-directed mutagenesis.
   3) Prepare the ACA-less gene fragment from the clone in 2) and clone it into a pCold I - IV (SP-4) DNAs to obtain the ACA-less gene expression plasmid.

2. Building up SPP system
   Transform a host *E. coli* with both components, pMazF and the ACA-less gene expression plasmid constructed above. The selection marker of for the pMazF, MazF expression plasmid is chloramphenicol resistance and this transformant is resistant to both ampicillin (100 μg/ml) and chloramphenicol (34 μg/ml).
   50 ng of each of the both plasmids is enough to use for transformation.
   Do not keep the transformant obtained under cool condition until use. A promoter of a cold-shock protein *csp*A is utilized on in pCold (SP-4) to express the protein of interest and this promoter functions under cool condition.
   Note that this system cannot be combined with chloramphenicol-resistant *E. coli* host strains or plasmids carrying chloramphenicol-resistance gene.
   E. coli BL21 is a good candidate host strain appropriate for this system.
   TaKaRa Competent Cells BL21(Cat. #9126) is available.

3. SPP of interest : Pulse-labeling in M9-glucose medium culture
   1) Inoculate the transformant obtained at Section 2 into M9-glucose Medium＊ previously warmed at 37℃ containing ampicillin (100 μg/ml) and chloramphenicol (34 μg/ml). Cultivate it shaking at 37℃ and monitor its OD_{600}.
   2) When the OD_{600} reaches about 0.5, cool down the culture to 15℃ quickly and leave it for 45 minutes under this condition.
   3) Add IPTG（Isopropyl-β-D-thiogalactopyranoside）into the culture to be a final concentration of 1mM. And then cultivate it shaking at 15℃ for 24 hours.
   4) Incubate by shaking at 15℃ for 24 hours.
   5) Add [^{35}S]-methionine to the culture and leave it at 15℃ for 15 min.
   6) Harvest these isotopic labeled cells and prepare them as a sample to be analyzed by autoradiography following SDS-PAGE.
      ＊ LB medium contains a slight amount of some kind of an inducer for expression controlled by lac operator. Minimum media or M9-glucose Medium is recommended to avoid this affect strictly.

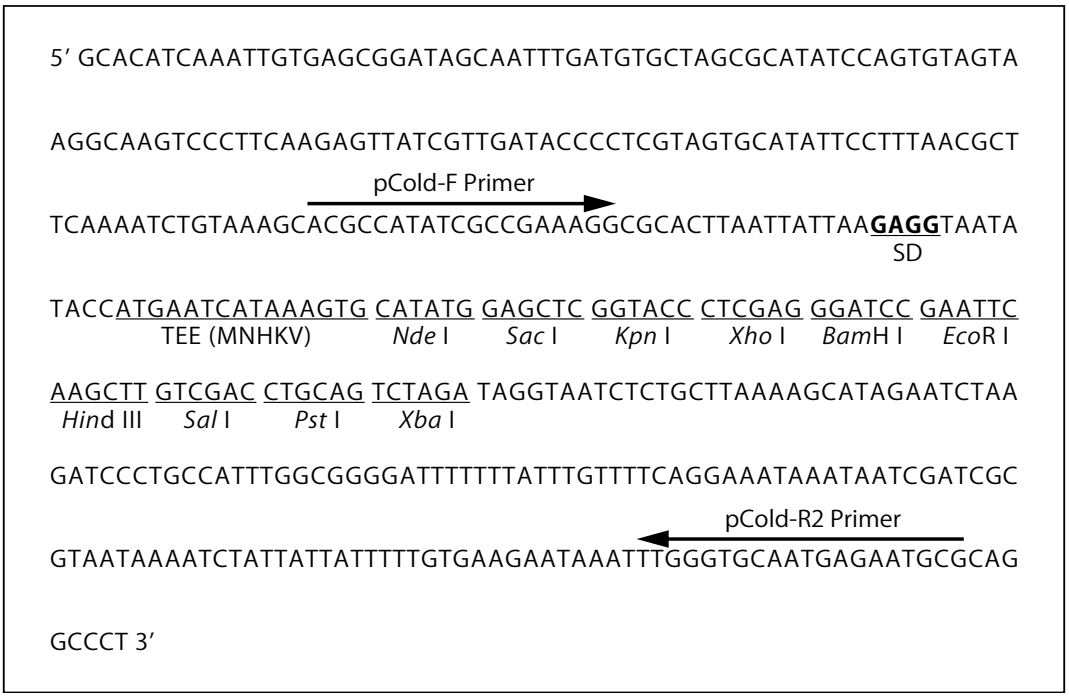
VIII. Multicloning Sites of pCold I ～ IV (SP-4) DNAs

pCold I (SP-4) DNA

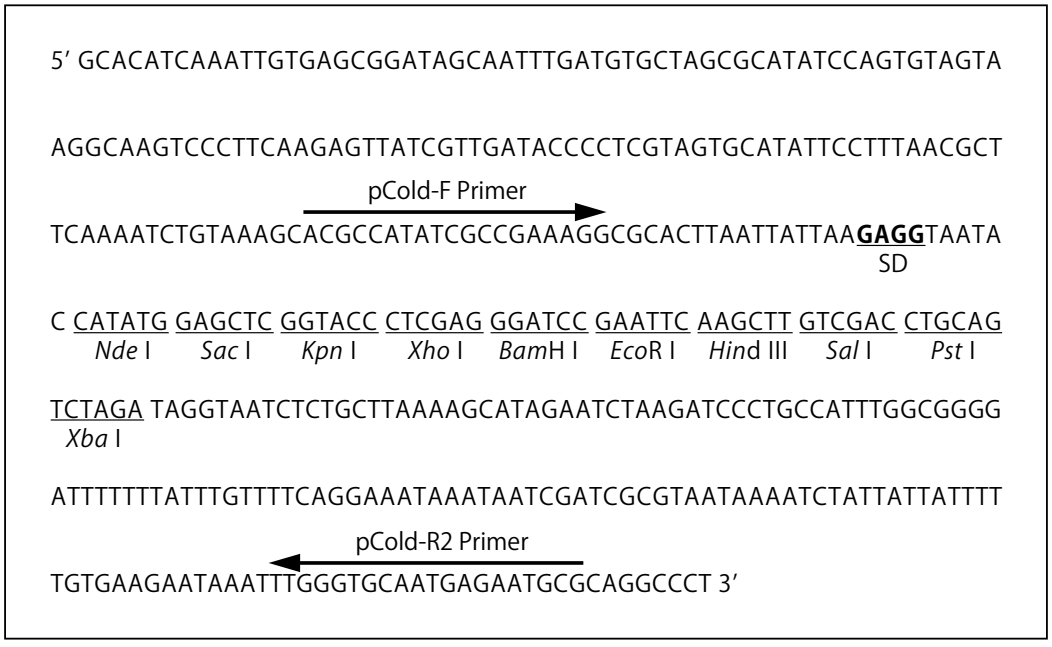


pCold II (SP-4) DNA

pCold III (SP-4) DNA



pCold IV (SP-4) DNA



IX. Application Example

Expression of *E. coli* envZB with SPP system

1. Pulse labeling in M9-glucose medium culture

E. coli BL21 transformant with Positive Control pCold I (SP-4) envZB was tested with or without the effect of MazF. The pulse labeling results much difference in the background level derived from the synthesis of host proteins. SPP system showed extremely low level of background. (Figure 4)

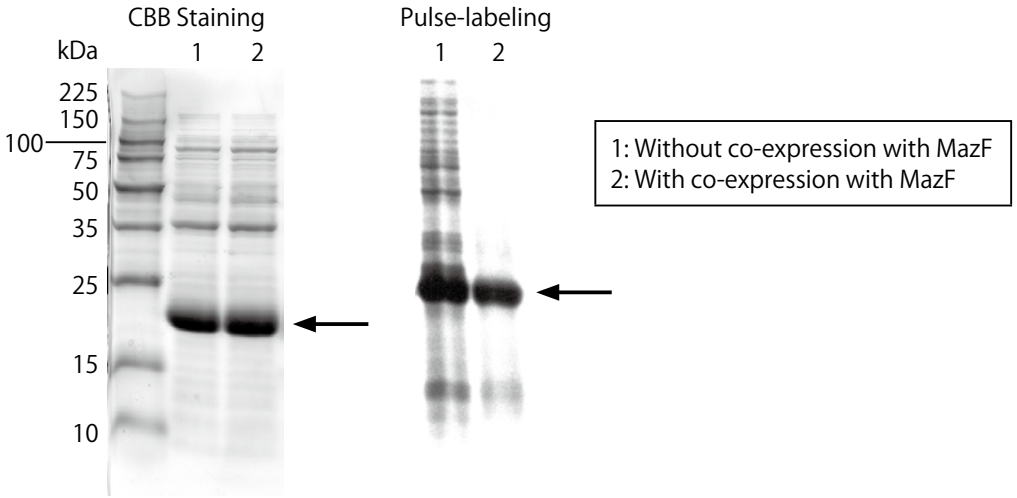


Figure 4. Expression of envZB protein
(CBB staining and pulse-labeling of soluble fraction derived from strain.)

2. *E. coli* envZB expression in LB medium culture

E. coli BL21 transformant with Positive Control pCold I (SP-4) envZB was tested with or without MazF co-expression.

The whole process was proceeded by following the protocol, and the amount of 0.1 at OD_{600} was applied to gel electrophoresis. There was not significant difference in the production level with the protein of of interest between the transformant with pMazF (SPP system) and the one with a plasmid carrying no MazF gene. (Figure 5)

In the system of MazF co-expression, the expression of new protein except target protein is suppressed. This may result in growth stress against *E. coli* itself. Accordingly the expression yield of a target protein may decrease compared than general expression with pCold DNAs.

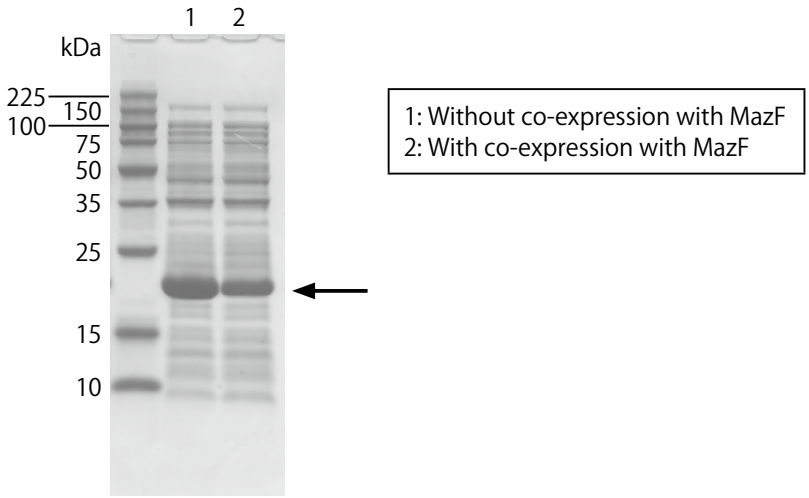


Figure 5. Expression of envZB protein
(CBB staining of soluble fraction derived from strain)

X. Q&A

Q1. What should be considered if expressed proteins are insolubilized?
A1. Possible strategies are:
- Changing the timing of cool-down and IPTG addition to induce the expression (Some examination may be needed between early and late logarithmic phases of the culture.)
- Reducing the concentration of IPTG (down to 0.1mM)
- Changing the strain of host *E. coli*
- Extracting the cultured cells by sonication in a buffer which contains 0.1 to 1% of detergent (for example, octylglycoside, Nonidet P-40, Triton X-100, etc.)

Q2. What are the criteria for selecting Cold-shock expression vectors for SPP System?
A2. TEE facilitates translation of interest when using pCold I (SP-4), pCold II (SP-4) and pCold III (SP-4). Proteins expressed using pCold I (SP-4) and pCold II (SP-4) can be purified with Ni or Co columns by means of its affinity to His-Tag. If you do not desire to attach any excess amino acid sequences to the N-terminus of the protein of interest, it is recommended to use pCold I (SP-4) that allows cleavage of the Tag sequence with Factor Xa, or pCold IV (SP-4) that does not possess TEE nor Tag sequences.

Q3. What quantity of the protein of interest is gained for from 1 L of culture?
A3. The expression level usually ranges from several mg to several tens of mg/l, although it is different for each protein of interest. An approximately 3-L culture can recover purified proteins in the mg scale, if the protein of interest can be detected by SDS-PAGE followed by CBB staining In the system of MazF co-expression, the expression of new protein except target protein is suppressed. This may result in growth stress against *E. coli* itself. Accordingly the expression yield of a target protein may decrease compared than general expression with pCold DNAs.

XI. References

1. Suzuki M, Mao L, and Inouye M. Single protein production (SPP) system in *Escherichia coli*. *Nature Protocols*. (2007) **2**: 1802-1810.
2. Suzuki M, Roy R, Zheng H, Woychik N, and Inouye M. Bacterial Bioreactors for High Yield Production of Recombinant Protein. *J Biol Chem*. (2006) **281**: 37559-37565.
3. Suzuki M, *et al*. Single Protein Production in Living Cells Facilitated by an mRNA Interferase. *Molecular Cell*. (2005) **18**: 253-261.
4. Zhang Y, *et al*. Insights into the mRNA cleavage mechanism by MazF, an mRNA interferase. *Journal of Biological Chemistry.* (2004) **280**: 3143-3150.
5. Zhang Y, *et al*. MazF cleaves cellular mRNAs specifically at ACA to block protein synthesis in *Escherichia coli*. *Molecular Cell*. (2003) **12**: 913-923.
6. Qing G, *et al*. Cold-shock induced high-yield protein production in *Escherichia coli*. *Nature Biotechnology*. (2004) **22:** 877-882.

XII. Related Products

mRNA Interferase™-MazF (Cat. #2415A)
pCold™ Vector Series (Cat. #3360 - 3364)
TaKaRa Competent Cells BL21 (Cat. #9126)
IPTG（Isopropyl-β-D-thiogalactopyranoside）(Cat. #9030)

Phone: +81-77-543-7247 Fax: +81-77-543-9254