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Precautions for use :
Because the *araB* promoter and *araC* gene derived from of *Salmonella typhimurium* are contained in Chaperone Plasmid pG-KJE8, pGro7, pKJE7, and pTf16, please follow the guideline for experiments using recombinant DNA issued by the relevant authorities and the safety committee of your organization of your country in using this product.
NOTICE :
The intellectual property of the plasmids supplied in this product is owned by TAKARA BIO INC. It is prohibited to use this product for any commercial purpose or to transfer this product to a third party. Individual license agreement must be concluded with TAKARA BIO INC. when this product is used for industrial purposes.
The modified plasmid may also be subject to the ownership of TAKARA BIO INC. Accordingly, to use the modified plasmid for any commmercial purpose or to transfer it to a third party requires a prior contact to TAKARA BIO INC.
The use of this product is limited for research purposes. It must not be used for clinical purposes or for *in vitro* diagnosis.

I. Description :

Proteins have been gaining attention as one of the challenges in the area of post-genomics, with emphasis being placed on analyses of their structures and functions. Large-quantity expression of recombinant proteins is essential for structural and functional analyses of interest and, to support this need, a variety of protein expression systems have been developed.

Escherichia coli is commonly used as a host for protein expression, since it represents a simple system that offers a wide choice in expression systems. However, expression of foreign proteins in *E. coli* often results in various problems, such as the formation of inclusion bodies and protease degradation of the protein. These issues often are a result of improper folding of the expressed proteins, and are problems frequently encountered in protein function research.

Molecular chaperones have been demonstrated to be involved in the protein folding process, and numerous studies have been conducted to elucidate the mechanisms of *in vivo* protein folding. HSP Research Institute, Inc. has made significant contributions in the area of chaperone research.

Takara's Chaperone Plasmid Set consists of five different types of "chaperone team" plasmids developed by HSP Research Institute, Inc., which are designed to enable efficient expression of multiple molecular chaperones known to work in cooperation in the folding process. It has been reported that coexpression of a target protein with one of these chaperone teams increases recovery of expressed proteins in the soluble fraction. Such proteins are often unrecoverable using conventional methods due to the formation of inclusion bodies (see Figure 1).

II. Component :

1. Plasmid pG-KJE8 :	10 ng/μl	100 μl
2. Plasmid pGro7 :	10 ng/μl	100 μl
3. Plasmid pKJE7 :	10 ng/μl	100 μl
4. Plasmid pG-Tf2 :	10 ng/μl	100 μl
5. Plasmid pTf16 :	10 ng/μl	100 μl

No.	Plasmid	Chaperone	Promoter	Inducer	Resistant Marker	References
1	pG-KJE8	dnaK-dnaJ-grpE groES-groEL	*araB* *Pzt-1*	L-Arabinose Tetracyclin	Cm	2, 3
2	pGro7	groES-groEL	*araB*	L-Arabinose	Cm	2
3	pKJE7	dnaK-dnaJ-grpE	*araB*	L-Arabinose	Cm	2
4	pG-Tf2	groES-groEL-tig	*Pzt-1*	Tetracyclin	Cm	3
5	pTf16	tig	*araB*	L-Arabinose	Cm	3

<Available *E. coli* Expression Systems>

These chaperone plasmids carry an origin of replication derived from pACYC and a chloramphenicol resistance gene (Cm^r). This system allows their use with *E. coli* expression systems that utilize ColE1-type plasmids containing the ampicillin resistence gene as a marker, which are the most commonly used. The chaperone genes are situated downstream of either the *araB* or *Pzt-1* (tet) promoter. Thus, expression of target proteins and chaperones can be induced individually if the target gene is placed under the control of another promoter (e.g. *lac*). These plasmids also contain either *araC* or *tetR* for each promoter. Note that this system cannot be used in combination with chloramphenicol-resistant *E. coli* host strains or expression plasmids that carry the chloramphenicol-resistance gene. For example, *E. coli* BL21 (DE3), which is often used with pET systems etc., is acceptable as a host strain, but *E. coli* BL21 (DE3) pLysS or BL21 (DE3) pLysE which contain either the pLysS or pLysE plasmids containing the pACYC replication origin and the Cm^r gene, cannot be used with this system. Other applicable hosts include *E. coli* JM109.

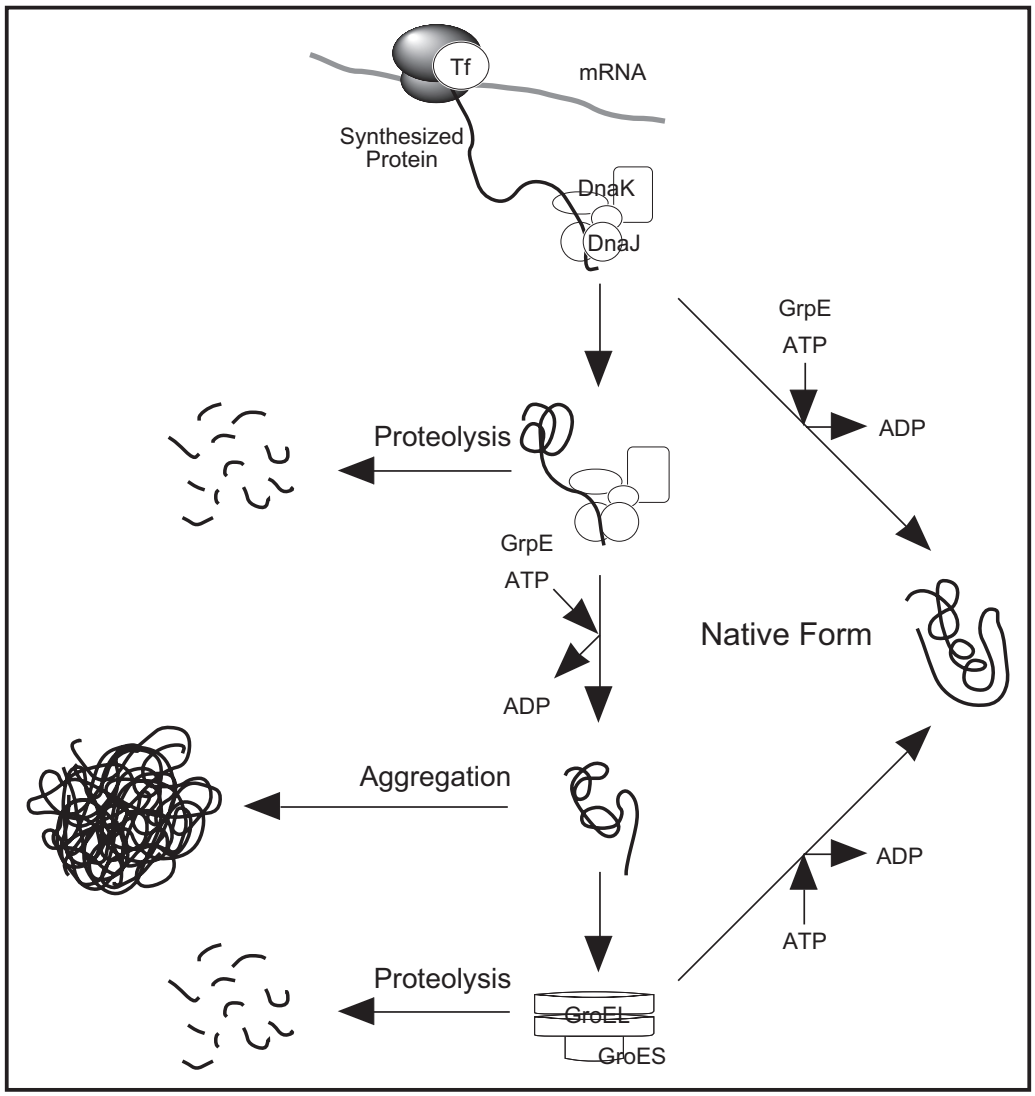

Fig. 1 Possible model for chaperone - assisted protein folding in *E. coli* (Reference 1).

III. Storage : − 20℃

IV. Usage method :

<Chaperone Co-expression>

The optimum chaperones and culture conditions (e.g. medium, culture temperature, aeration conditions, timing of induction, inducer concentration, and induction period) vary depending upon the target protein. A usage example is shown below. Optimum conditions should be determined independently for each target protein.

1. Construction of a System for Co-expression

An effective method for constructing a system for coexpression of target proteins and chaperones involves two steps: transformation of *E. coli* with a chaperone plasmid followed by transformation with an expression plasmid for the target protein.
One-step methods, i.e. simultaneous transformation with a chaperone plasmid and an expression plasmid for a target protein, and two-step methods involving transformation with an expression plasmid followed by transformation with a chaperone plasmid are not recommended, as they are known to result in very low transformation efficiency.

(1) Construct an expression plasmid for a target protein to be expressed in *E. coli*.

(2) Prepare competent cells of an *E. coli* expression host using a standard method. (Commercially available competent cells may be used instead.)

(3) Transform the competent cells prepared in (2) with one of the chaperone plasmids contained in this set, and select the transformants from selection medium plates containing 20 μg/ml chloramphenicol (use approximately 1 μl of plasmid for each transformation.)

(4) Culture the transformants with the chaperone plasmid in liquid medium containing 20 μg/ml chloramphenicol and prepare the competent cells using a standard method.

(5) Retransform the competent cells prepared in (4) with the expression plasmid for the target protein, and select the transformants from selection medium plates containing 20 μg/ml chloramphenicol and a selection reagent for the expression plasmid.

2. Coexpression Experiment

The experiment shown below is an example of a coexpression experiment which uses a pUC plasmid carrying the ampicillin resistance marker as well as a target gene downstream of the *lac* promoter, with a chaperone plasmid from this set. Refer to additional relevant literature for further details as to the appropriate culture conditions to use in your application.

(1) To perform coexpression, inoculate the transformants into L medium containing 20 μg/ml chloramphenicol and 50 μg/ml ampicillin for plasmid selection and 0.5-4 mg/ml L-arabinose and/or 1-10 ng/ml tetracycline * for induction of chaperone expression. Incubate at 30-37℃ . Use both L-arabinose and tetracycline with pG-KJE8, L-arabinose only with pGro7, pKJE7, or pTf16, and tetracycline only with pG-Tf2.

* Please test with 0.5 mg/ml of L-arabinose and/or 5 ng/ml of tetracycline at first. Tetracycline at low concentrations does not significantly affect the growth of *E. coli*.

(2) When the OD_{600} of the culture reaches 0.4-0.6, add IPTG to be a final concentration of 0.1-1 mM, and culture at 30-37℃ for 1-4 h.

(3) After culturing, determine the amount of the target protein expressed and the amount of target protein in soluble form by SDS-PAGE and activity assay, Additionally, test various culture conditions (e.g. medium type, culture temperature, aeration conditions, timing of induction, inducer concentration, and induction period) to determine optimal conditions.

V. Q & A :

Q1. What size of chaperone proteins is expressed in this system?

A1. It is reported that the size of expressed chaperone proteins is as follows:

GroEL (around 60 kDa)
GroES (around 10 kDa)
DnaK (around 70 kDa)
DnaJ (around 40 kDa)
Tf (around 56 kDa)
GrpE (around 22 kDa)

Please note that you might have a different result in an actual gel image analysis. For instance, the GrpE band is usually identified above 29 kDa marker in the image.

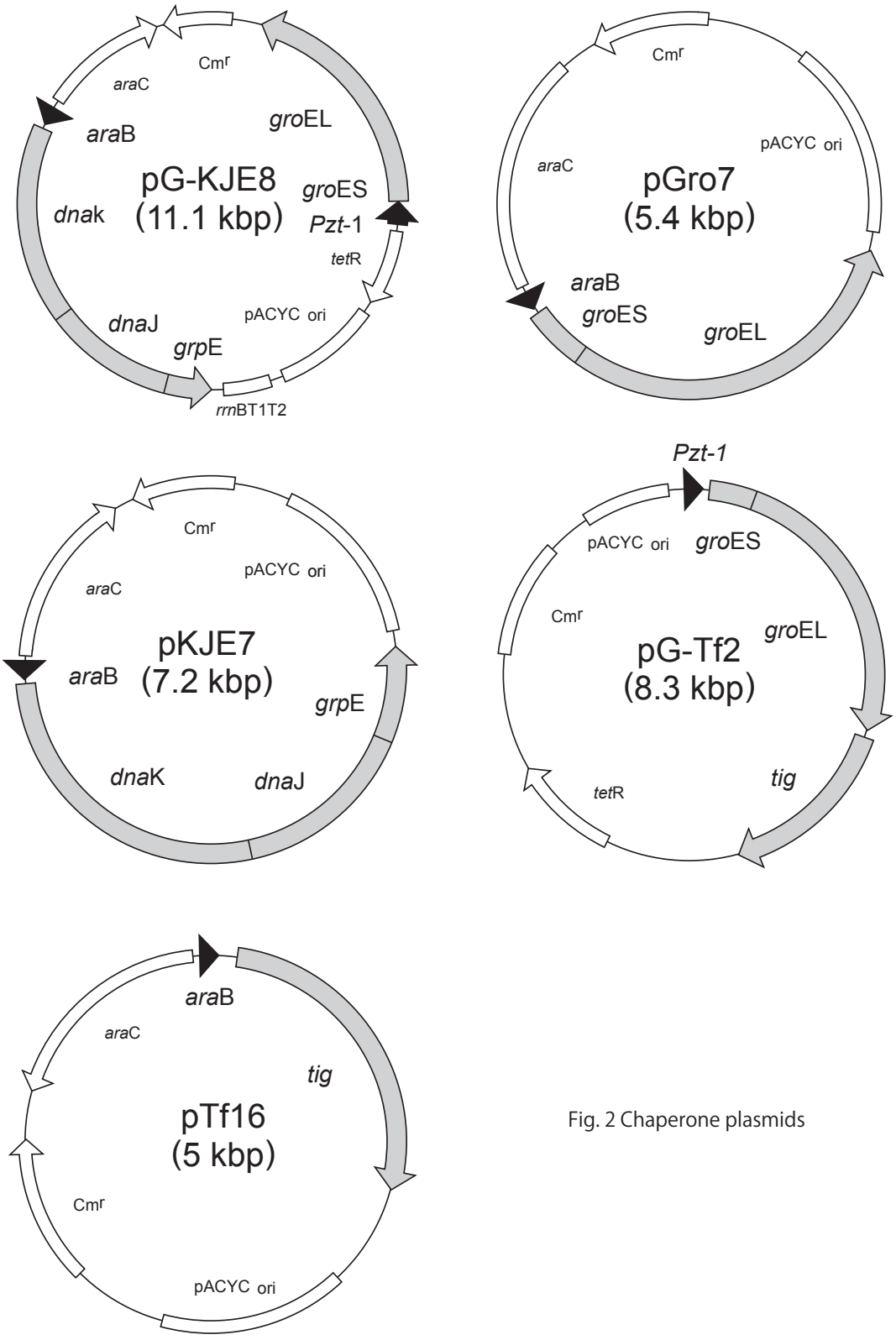

Fig. 2 Chaperone plasmids

Q2. What kind of option do we have in purification of the target protein?

A2. We recommend Ni affinity purification using His-Tag.

In case that the target protein is purified utilizing GST-Tag, there is a possibility that the purified target protein contains some chaperone proteins, which can be detected as bands by SDS-PAGE.

The reason that the residual protein is found is the chaperone proteins still stay in glutathione resin via target protein or by non-specific absorption even after washing and are eluted together with the target protein.

In that case, it is reported that each of the following steps can be applied for improvement :

- Separate by ion-exchange resin [*Proc. Natl. Acd. Sci. USA* (1995), **92**, 1048]
- Separate by ATP-Agarose substrate [*J. Biol. Chem.* (1984), **259**, 8820]
- Wash the glutathione resin bound with the GST-Tag fused target protein with a buffer including 3 mM Mg-ATP.
- Wash the glutathione resin bound with the GST-Tag fused target protein with a buffer including 10 mM Mg-ATP and 5 mg/ml casein and incubate at room temperature for 20-40 min.

VI. Reference :

Reviews

1. Thomas, J. G., *et al.* (1997) Molecular Chaperones, Folding Catalysts, and the Recovery of Active Recombinant Proteins from *E.coli*. *Appl. Biochem. Biotech*, **66**: 197-238

Expression of soluble recombinant proteins through coexpression with chaperones

2. Nishihara, K., *et al.* (1998) Chaperone Coexpression Plasmids: Differential and Synergistic Roles of DnaK-DnaJ-GroE and GroEL-GroES in Assisting Folding of an Allergen of Japanese Cedar Pollen, Cryj2, in *Eschericia coli. Appl. Environ. Microbiol*. **64**: 1694-1699
3. Nishihara, K., *et al.* (2000) Overexpression of Trigger Factor Prevents Aggregation of Recombinant Proteins in *Escherichia coli. Appl. Environ. Microbiol*. **66**: 884-889

VII. Related Products :

pCold™ I DNA (Cat. #3361)
pCold™ II DNA (Cat. #3362)
pCold™ III DNA (Cat. #3363)
pCold™ IV DNA (Cat. #3364)
pCold™ Vector Set (Cat. #3360)
pCold™ TF DNA (Cat. #3365)
<Chaperone Competent Cell BL21 Series>
Chaperone Competent Cells BL21 Set (Cat. #9120)
Chaperone Competent Cell pG-KJE8/BL21 (Cat. #9121)
Chaperone Competent Cell pGro7/BL21 (Cat. #9122)
Chaperone Competent Cell pKJE7/BL21 (Cat. #9123)
Chaperone Competent Cell pG-Tf2/BL21 (Cat. #9124)
Chaperone Competent Cell pTf16/BL21 (Cat. #9125)
TaKaRa Competent Cell BL21 (Cat. #9126)

∗ Each competent cell is prepared from *E. coli* BL21 strain including one of chaperone plasmids. It is useful for protein expression with pCold DNA series. This product is not intended for protein expression system utilizing T7 promoter, such as the pET system, because the BL21 strain used as a host doesn't expres T7 RNA polymerase.

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