Cat. # 3340

For Research Use

TaKaRa

Chaperone Plasmid Set

Product Manual

v201701Da

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Safety Precautions

Because the *araB* promoter and *araC* gene derived from *Salmonella typhimurium* are present on the Chaperone Plasmids pG-KJE8, pGro7, pKJE7, and pTf16, please follow all relevant guidelines for experiments using recombinant DNA as indicted by your organization when using this product.

I. Description

Large-scale expression of recombinant proteins is essential for structural and functional analyses of proteins. A variety of protein expression systems have been developed to produce high levels of protein. *Escherichia coli* is commonly used as a host for protein expression, since it is a simple system that can be used to express a wide variety of proteins. However, expression of protein in *E. coli* often results in various problems, such as the formation of inclusion bodies and protease degradation of the protein. These frequently encountered issues often are a result of improper folding of the expressed proteins.

Molecular chaperones are involved in protein folding, and numerous studies have been conducted to elucidate the mechanisms of *in vivo* protein folding. Takara Bio's Chaperone Plasmid Set consists of five different types of chaperone plasmids developed by HSP Research Institute, Inc. The plasmids are designed to enable efficient expression of multiple molecular chaperones known to work cooperatively in the protein folding process. It has been reported that coexpression of a target protein with one of these chaperone plasmids increases recovery of expressed proteins in the soluble fraction. Such proteins often form inclusion bodies using conventional methods (Figure 1).

II. Components

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	*dna*K-*dna*J-*grp*E *gro*ES-*groEL*	*araB* *Pzt-1*	L-Arabinose Tetracycline	Cm	2, 3
2	pGro7	*gro*ES-*gro*EL	*araB*	L-Arabinose	Cm	2
3	pKJE7	*dna*K-*dna*J-*grp*E	*araB*	L-Arabinose	Cm	2
4	pG-Tf2	*gro*ES-*gro*EL-tig	*Pzt-1*	Tetracycline	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 allows the plasmids to be used with *E. coli* expression systems that utilize ColE1-type plasmids containing the ampicillin resistance gene as a marker. The chaperone genes are 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 can be used 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.

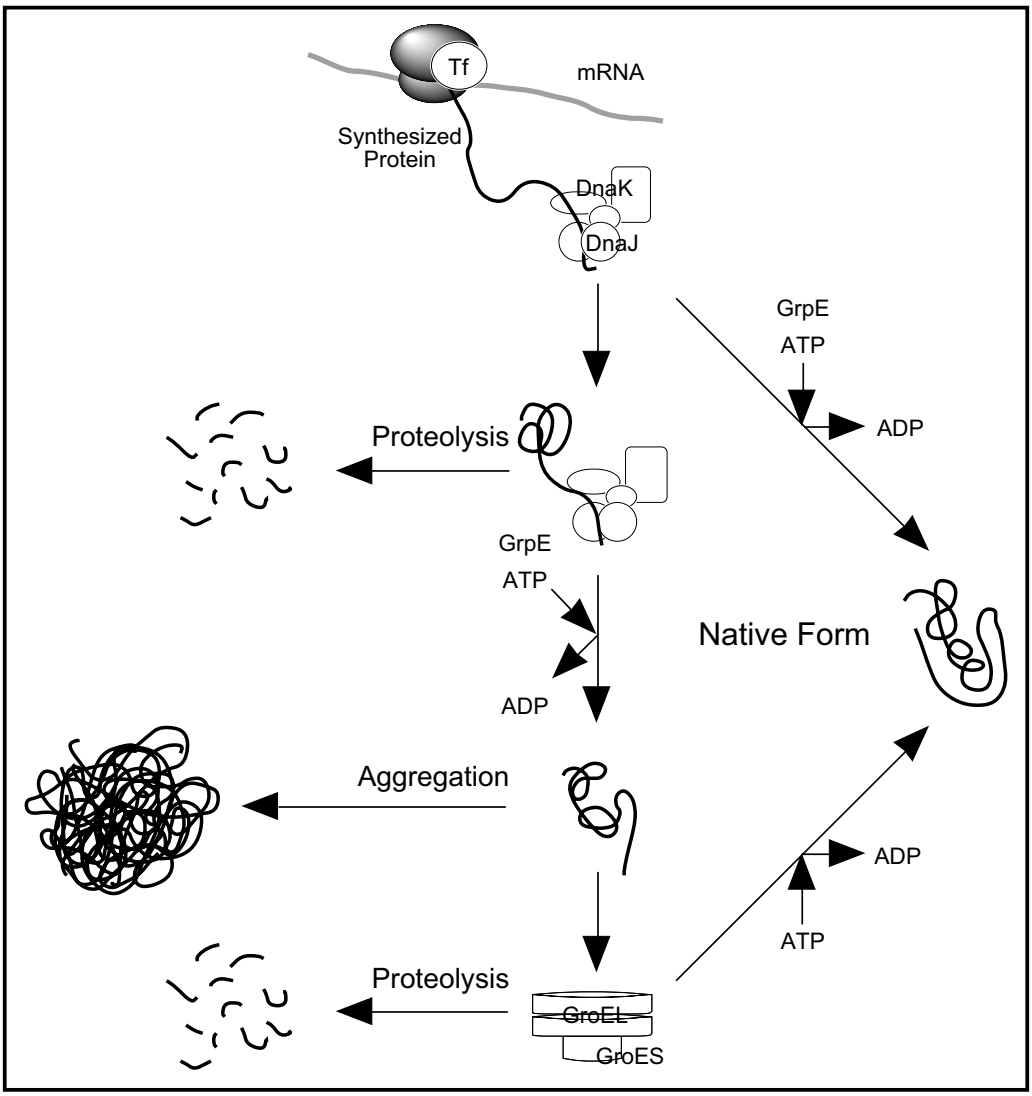

Figure 1. Possible model for chaperone - assisted protein folding in *E. coli* (Reference 1).

III. Storage

− 20℃

IV. Protocol

<Chaperone Co-expression>

The optimal chaperone plasmid and culture conditions (e.g., medium, culture temperature, aeration conditions, time of induction, inducer concentration, and induction period) vary depending upon the target protein. An example is provided below. Optimal conditions should be determined independently for each target protein.

1. Construction of a System for Coexpression

   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 (use ~1 μl of plasmid for each transformation), and select the transformants from plates containing 20 μg/ml chloramphenicol.

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

2. Coexpression Experiment
   The experiment shown below is an example of a coexpression experiment that uses a pUC plasmid carrying the ampicillin resistance marker and the target gene downstream of the *lac* promoter, and a chaperone plasmid from this set.

   (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.
   * Use 0.5 mg/ml of L-arabinose and/or 5 ng/ml of tetracycline at first. Low concentrations of tetracycline do not significantly affect the growth of *E. coli*.

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

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

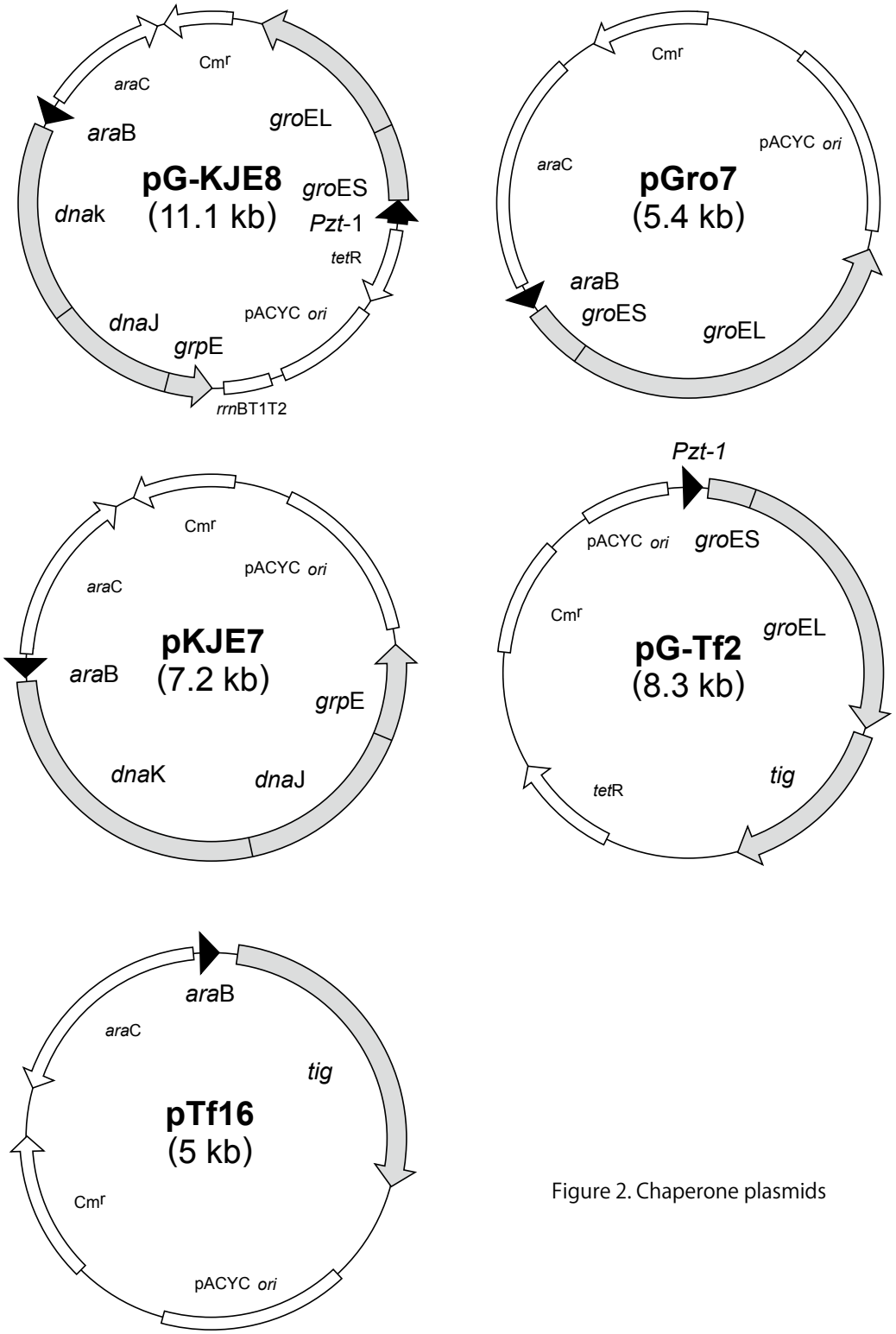

Figure 2. Chaperone plasmids

V. FAQ

Q1. What size of chaperone proteins is expressed in this system?
A1. It is reported that the size of the expressed chaperone proteins is:

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 slightly different results in an actual SDS-PAGE analysis. For example, the GrpE band is usually identified above the 29 kDa marker.

Q2. How should the target protein be purified?
A2. We recommend Ni affinity purification using His-Tag.
If the target protein is purified using a GST-Tag, there is a possibility that the purified target protein may contain some residual chaperone proteins, due to non-specific absorption with the glutathione resin. This can be detected as bands by SDS-PAGE. If this occurs, it is reported that each of the following steps can be used to improve purity:

- 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. References

Reviews

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

Expression of soluble recombinant proteins through coexpression with chaperones

2. Nishihara, K., *et al.* 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. (1998) **64**: 1694-1699.
3. Nishihara, K., *et al.* Overexpression of Trigger Factor Prevents Aggregation of Recombinant Proteins in *Escherichia coli.*
Appl Environ Microbiol. (2000) **66**: 884-889.

VII. Related Products

pCold™ I DNA (Cat. #3361)*2
pCold™ II DNA (Cat. #3362)
pCold™ III DNA (Cat. #3363)
pCold™ IV DNA (Cat. #3364)
pCold™ Vector Set (Cat. #3360)*2
pCold™ TF DNA (Cat. #3365)*2

<Chaperone Competent Cell BL21 Series*1>
Chaperone Competent Cells BL21 Set (Cat. #9120)*2
Chaperone Competent Cells pG-KJE8/BL21 (Cat. #9121)*2
Chaperone Competent Cells pGro7/BL21 (Cat. #9122)*2
Chaperone Competent Cells pKJE7/BL21 (Cat. #9123)*2
Chaperone Competent Cells pG-Tf2/BL21 (Cat. #9124)*2
Chaperone Competent Cells pTf16/BL21 (Cat. #9125)*2
TaKaRa Competent Cells BL21 (Cat. #9126)*2

*1 Competent cells prepared from *E. coli* BL21 strain including one of the chaperone plasmids are useful for protein expression with pCold DNA series. This product is not intended for protein expression systems using a T7 promoter, such as the pET system, because the BL21 strain does not express T7 RNA polymerase.

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

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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