PandaPure™ Protein Systems and Reagent Kits

Synthetic Organelle Assisted Protein Expression (E. coli), Purification and Tag Removal

User Guide

PandaPure™ is a platform technology to simplify purification by targeting biologically synthetic organelles, instead of the external solid-phase supports used in traditional chromatography and other methods (e.g., resin matrices, columns, and magnetic beads).

PandaPure™ Protein Systems are particularly designed to streamline recombinant protein expression, purification, and tag removal into a “single-step” process. In a typical PandaPure workflow, the recombinant protein is expressed by host cells, compartmentalized into synthetic organelles intracellularly, and then released via tag removal mediated by PandaPure Protein Reagents.

By harnessing the autonomy of biology in constructing and organizing molecules, PandaPure provides a generic purification platform with minimal human intervention, and enables flexible, scalable, high-performance workflows, with the following core advantages:

- The Simplest Method: single-operation purification including tag removal, requiring no columns, beads, or proteases.
- Improved Expression: Targeting into synthetic organelles often improves expression of challenging targets, e.g., toxic, misfolding-prone, or aggregation-prone proteins.
- Scalability: Flexible for various scales, from high-throughput screening to process development.
- Compatibility: Suitable for both manual operation and lab automation.

Related Catalog Products:

- Systems: PK002, PK006
- Reagent Kits: RM001, RM002, RM003

Publication No.: PandaPure-202603-2.0

Ailurus

1 Introduction

Importance

Please read this User Guide carefully before using the product.

Safety

Refer to the Safety Data Sheet (SDS) for information regarding handling, storage, and disposal.

Intended Use

The product is intended for Research Use Only (RUO), and must not be used in any clinical or in vitro procedures for diagnostic purposes. The product is not designed for industrial-scale manufacturing. For any use beyond internal research, please contact Ailurus for further support via support@ailurus.bio.

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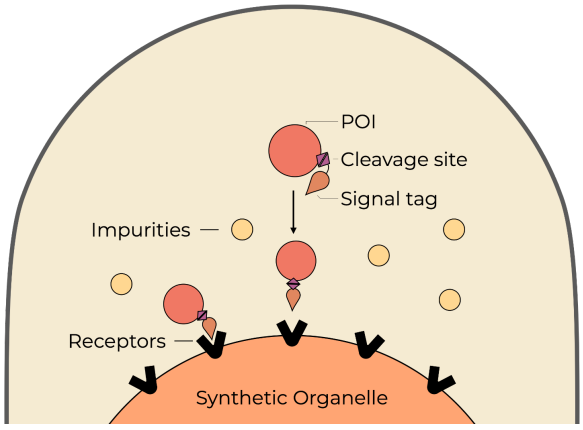
2 Product Description

Purification Principle

PandaPure™ Protein Systems combine DNA assets and reagent components for purification of recombinant proteins using synthetic organelles and a self-cleaving tag. During protein expression and targeting, the host cell is programmed to form synthetic organelles and compartmentalize target proteins. Then, triggered by **PandaPure™ Protein Reagent** (consisting of Tag Cleaver in a working Buffer), the protein is cleaved automatically from the tag, thereby released into the supernatants from the organelle.

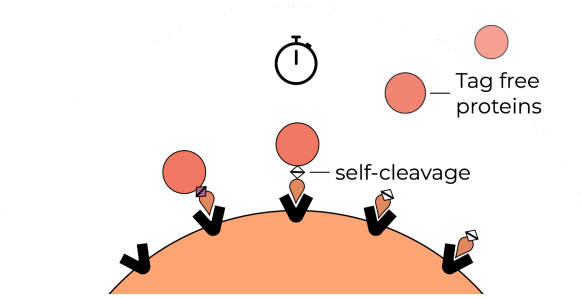
Together, PandaPure simplifies protein purification and eliminates the need for chromatographic resins, magnetic beads, or external protease treatments. The core workflow remains a two-phase process of intracellular targeting followed by reagent-driven release.

Phase 1: Protein Expression & Targeting



In a PandaPure™ expression system, tagged proteins are recruited by synthetic organelles through specific binding between signal tags and receptors; while impurities are excluded outside, and can be easily removed after cell lysis.

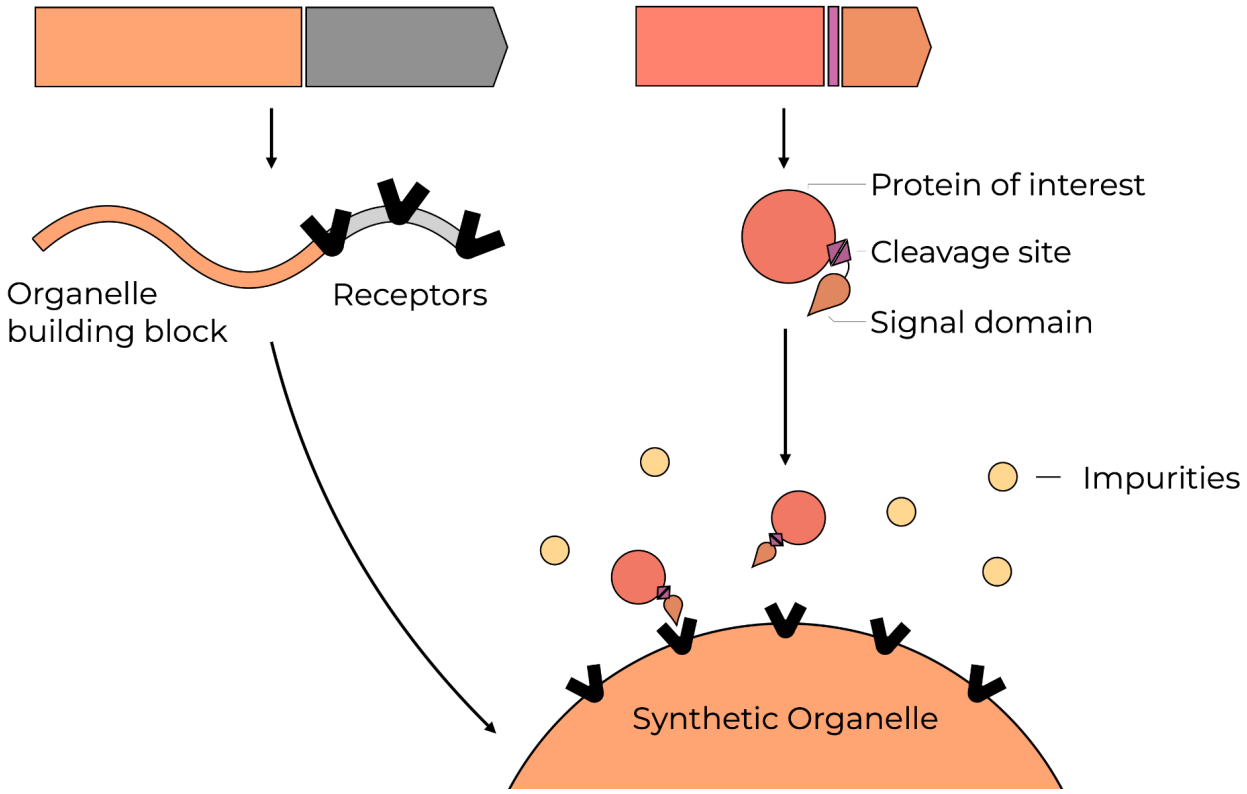
Phase 2: Protein Isolating and Releasing



Target-containing organelles can be easily isolated by cell lysis and simple separation. Then, with PandaPure™ Protein Reagent, self-cleavage occurs and tag-free proteins are released, allowing high purity in a single reaction with mild conditions.

Genetic Components & Expression System Descriptions

The PandaPure™ Protein Systems (E. coli) apply a modular two-plasmid system designed for bacterial E. coli strains for overexpression, including an Organelle Plasmid to encode the formation of synthetic organelles, and an Expression Plasmid to express the protein of interest with signal tags.



Synthetic Organelles: TEAR-2

The synthetic organelle is nano-to-micron-scale biological condensates produced under the genetic instructions. PandaPure uses a modular organelle system encoded by the organelle plasmid (pTEAR-2), which is a revised version modified from the RNA organelle class, Transcriptionally Engineered Addressable RNA (Guo H, et. al., Cell 2022).

In operation, the synthetic organelle acts as a programmable enrichment phase: target proteins are concentrated in the organelle while many host impurities remain outside, simplifying downstream recovery.

PandaPure™ Organelle Plasmid (pOrganelle): pTEAR-2

Organelle expression is controlled by the aTc-inducible PLtetO-1 system, allowing users to tune timing and expression strength for both analytical and production workflows (see Protocols for details). The plasmid contains p15A origin of replication (10 ~ 15 copies per cell), and chloramphenicol resistance (CmR).

Synthetic Organelle Properties and Compatibility

Synthetic organelles are generally compatible with common laboratory buffers, salts, and additives used in bacterial protein workflows, including typical non-ionic lysis conditions and routine helper enzymes. The organelle phase remains operational across normal process temperatures used for expression and recovery workflows, and is compatible with both manual and automation-friendly handling. However, freeze-thaw of bacterial cells or organelle-containing pellets should be avoided, as freezing can reduce compartment specificity and negatively impact final purity.

Cognate PandaPure Signal Tags

In PandaPure, each protein of interest is expressed as a fusion with a cognate capture-and-release tag that matches the organelle receptor logic. The recent release includes expanded tag options across both N-terminal and C-terminal cleavable architectures:

- Classic tag, tandem-dimeric binding protein of MS2 RNA aptamer
- Hi-affinity tag, monomeric binding protein of MS2 RNA aptamer with N55K mutant
- IEX tag (derived from poly-arginine), minimized positive-charge peptide enabling electrostatic binding.

Exact tag layouts are vector-defined and should be selected from the plasmid/vector table; all inserts must remain in-frame, with stop-codon handling aligned to the chosen vector design.

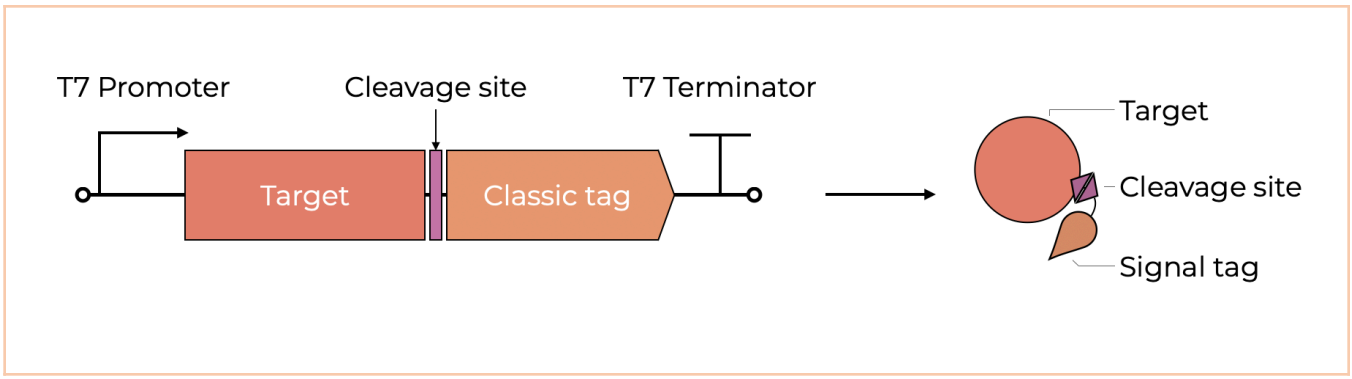
PandaPure™ Expression Vector: pPEV series

pPEV series include vectors to express the protein of interest (POI) with validated PandaPure Tags. PandaPure™ Protein Systems includes pPEV-1 to pPEV-4 (see below) for overexpression under T7/lac promoters. The plasmid contains ColE1 origin of replication (~ 40 copies per cell), and kanamycin resistance (KanR). More options are available at ailurus.bio.

Note: Starting in March 2026, PandaPure™ uses a unified naming system: pPEV for expression vector backbones and pPEX for expression plasmids carrying inserted target genes. Earlier names may still appear in some materials and refer to functionally compatible constructs.

Name	Tag type and location	Cloning method	Backbone	Available at
pPEV-1	C-term Classic tag	Gibson	pET-28a(+), KanR	PK006
pPEV-2	C-term Classic tag	Multi-cloning site (MCS)	pET-28a(+), KanR	PK002, PK006
pPEV-3	N-term Classic tag	MCS	pET-28a(+), KanR	PK006
pPEV-4	N-term IEX tag C-term Hi-affinity tag	MCS	pET-28a(+), KanR	PK006

Example diagram of expression unit pPEV-1 and pPEV-2


T7 Promoter
Cleavage site
T7 Terminator
Target
Classic tag
Target
Cleavage site
Signal tag

Cleavage Mechanism & PandaPure™ Protein Reagent Descriptions

PandaPure™ Protein Reagent and Kit Offerings

PandaPure™ Protein Reagent is provided as two sets of coordinate components:

- **PandaPure™ Protein Tag Cleaver**, includes the chemical agent and helper components that mediate tag cleavage. A 250x Tag Cleaver is available as an onboarding component of PandaPure™ Protein Systems (PK002, PK006).

- **Working buffer at pH 8-9.** Various buffers (e.g., Tris-HCl, HEPES, CHES), salts, and additives can be used after validation, with pPEX-GFP-1 as a recommended benchmark where applicable. Ailurus uses PandaPure Protein Buffer (HEPES) as the default choice for standard workflows where supplied with the product.

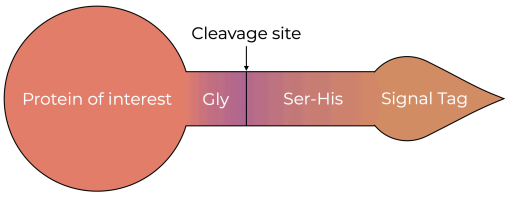
For standard use, PandaPure™ Protein Reagent is prepared by diluting PandaPure™ Protein Tag Cleaver stock solution into a compatible working buffer at the desired concentration. Freshly prepared reagent is generally preferred. Do not use the reagent if visible precipitation is observed.

Starting from March 2026, PandaPure™ Protein Reagent Kit (RM001, RM002, RM003) replaces the composite reagent offering (PR050, PR100, PR250) for improved flexibility and on-shelf stability.

Each kit includes 250x PandaPure™ Protein Tag Cleaver and a respective volume of PandaPure™ Protein Buffer (HEPES).

Cleavage Mechanism and Site Logic

The cleavage logic is designed to separate the target protein from the capture tag after organelle isolation, enabling recovery of tag-removed product in the supernatant after centrifugation. During reaction, hydrolysis occurs between glycine and serine, generating a free carboxyl group at glycine on the recovered C-terminus.



Final cleavage efficiency and terminal residue outcome depend on signal-tag placement and adjacent residues in the construct design. For example, pPEV-1 supports scarless C-terminal recovery in the standard design configuration. Further optimization can be pursued by removing glycine to target zero residual residues, although cleavage efficiency may decrease depending on sequence context.

Cleavage Characteristics (Temperature, pH, Time, Caveats)

PandaPure cleavage is typically run under mild alkaline conditions and moderate temperatures, with recommended starting ranges of pH 8-9 and 25-37°C (or up to 45°C when target stability allows).

For routine setup, users can expect detectable release within hours, while 16-24 hour incubations are commonly used to maximize practical yield and simplify experiment planning. Extension to longer incubations may be used during optimization.

Cleavage is strongly reduced at low temperature and near-neutral pH, so conditions below ~20°C or around pH 7 are not recommended for standard recovery.

As caveats, target-dependent optimization is expected, and non-standard additives should be screened case by case for effects on cleavage efficiency and protein integrity.

Required and Optional Materials Not Supplied

While PandaPure™ Protein Systems provide key onboarding components, other materials or their equivalent alternatives are required to perform PandaPure. Optional additives can be used to improve performance of PandaPure™ workflow in operational easeness, yield, and purity (See Protocols for details).

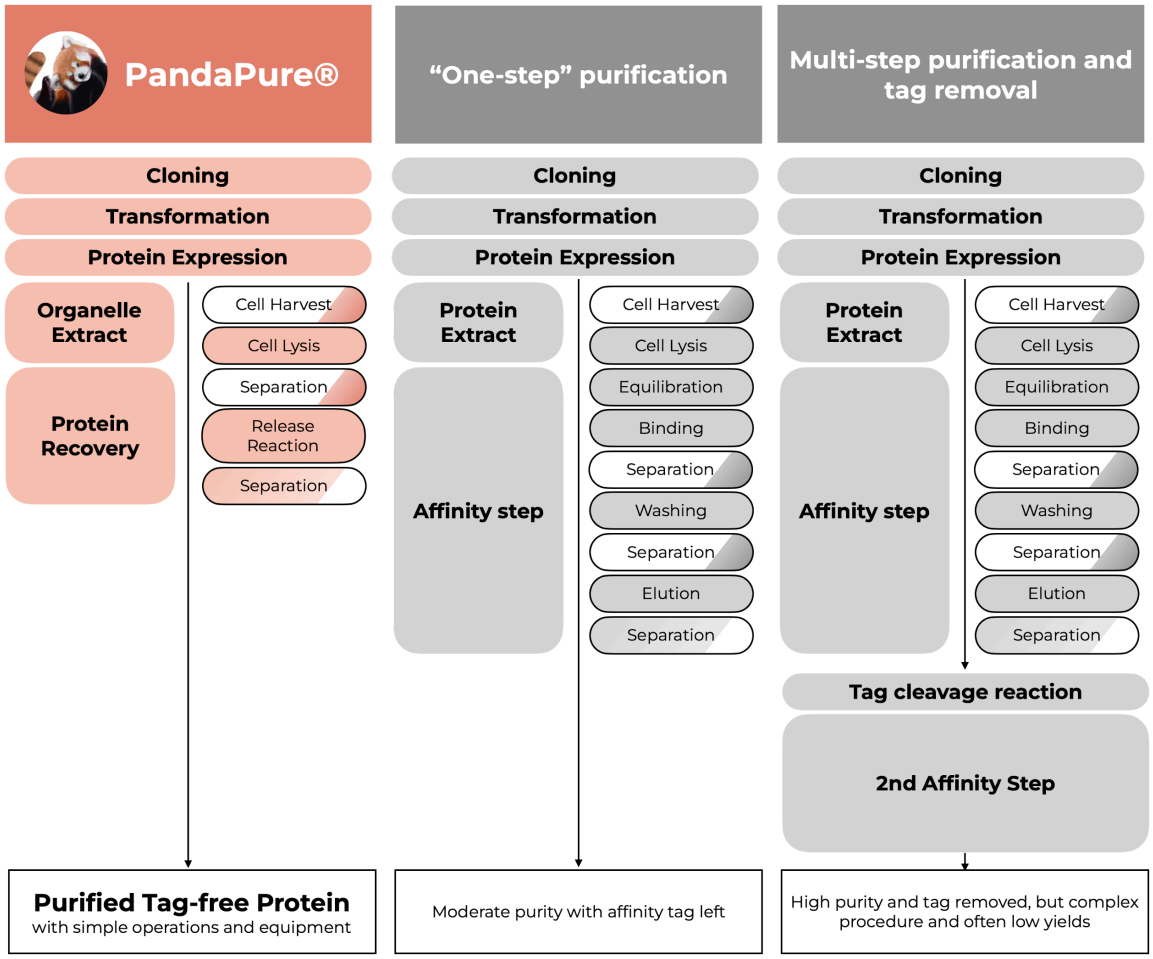
Items	Usage	Examples
Cloning enzymes	Molecular cloning of protein of interest into pPEVs	NcoI (NEB R3193V) to linearize pPEV-1 vector
		Gibson assembly kit (NEB E5520S)
		T4 DNA ligase (NEB M0202S)
Cloning competent cells	Cloning transformation and plasmid propagation	DH10B (Thermo Scientific EC0113)
T7 Expression competent cells	Strain for protein expression for T7 promoter driven pPEV-1, 2, 3 and 4 vectors	BL21(DE3) (Invitrogen C601003)
Growth medium	Bacterial medium for seed culture and protein expression	SOC (Sigma-Aldrich S1797) SOB (BD Difco 244310).
IPTG	Induce protein expression	Sigma-Aldrich I6758
Anhydrotetracycline (aTc)	Induce organelle formation	Sigma-Aldrich 37919
Bacterial lysis reagent (or equivalent)	Non-ionic detergent for mild-condition lysis of bacteria	Ailurus LR050, LR250, LR500 Thermo Scientific 78243 Millipore 70584
Centrifuge and rotors (or equivalent)	Separation of cells/organelles	Eppendorf 5810 Rotor FA-45-48-11 (2-ml) Rotor FA-45-6-30 (50-ml) Rotor A-2-DWP-AT (plates)
Lysozyme (optional)	Disrupt cell walls, improve purity	Sigma-Aldrich 1.05281
Dnase I (optional)	Degrade DNA, reduce viscosity	Thermo Scientific EN0521
Washing buffer (optional)	Remove remaining impurities	PBS, pH 7.2, 1 x 500 mL (Thermo Scientific 20012027)
0.22 µm filter (optional)	Product refinement	Millipore GSWP04700
Others	Basic labwares and consumables	

3 PandaPure Operation Workflow

Overview

To perform PandaPure, we need to

1. First establish the expression system (e.g. PandaPure *E. coli* strain) with both PandaPure Organelle and Protein Expression Plasmids.
2. Then, constructed strains can be used to express and purify proteins with simple operations.



Part I - Build the PandaPure Strain

Cloning of PandaPure POI Expression Plasmid pPEX-POI

PandaPure Protein Systems provide pPEVs to clone pPEX-POI by various cloning methods.

1. Insert protein of interest (POI) into pPEV.
2. Transform assembly products into competent cells for cloning.
3. Verify and amplify plasmids of pPEX-POI.

Several strategies can be used to generate genes compatible with PandaPure with other preferred expression vectors, e.g., changing inducible promoters or adding solubility tags.

Short PandaPure tags (e.g. IEX poly arginine tag) can be directly cloned together with POI into desired vectors by incorporating the tag sequence directly into the 5' overhang of the PCR primers.

For custom vectors, please submit your request at Ailurus Service for design assistance, gene synthesis, and cloning.

Transformation of PandaPure Organelle and POI Expression Plasmids

Then, the PandaPure Strain is constructed by transforming pTEAR-2 and pPEX-POI into an expression strain carrying T7 polymerase. Either chemical or electroporation method works.

Part II - PandaPure Your Protein

Protein Expression

PandaPure is compatible with various strategies to culture bacteria for protein expression.

The typical procedure includes:

1. Seed culture: Grow from a single colony of PandaPure strain.
2. Pre-culture: Grow seed culture into a fresh medium to mid-log phase.
3. Induction: Supplement 0.05-0.5 mM IPTG and 50-200 ng/ml aTc.

Protein Purification

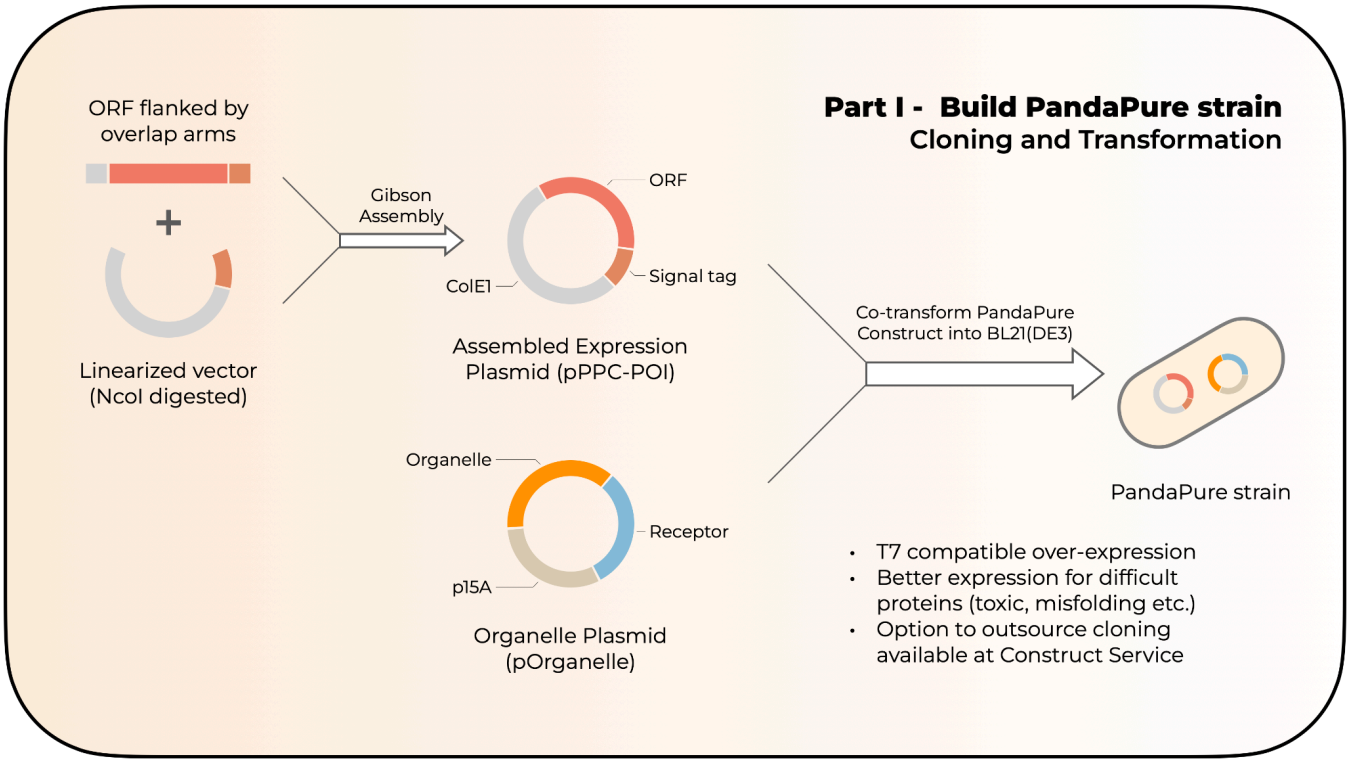
Purification includes the extraction of organelles in the pellet after cell harvests and lysis, then a protein recovery step using PandaPure Protein Reagent. As the following:

1. Harvest and lyse cells, by e.g., non-ionic detergent, or sonication.
2. Collect the pellet, by e.g., centrifugation at 1,000 g for 30 mins, or higher speed.
3. Incubate the pellets in PandaPure™ Protein Reagent at 25-37°C for 4 hours or longer.
4. Collect the protein extract, by e.g., centrifugation at 1,000 g for 30 mins, or higher speed.

Step-by-step Protocol Part I - Cloning of PandaPure Strain

To construct a PandaPure-compatible expression plasmid, you can either request Service at Ailurus to order a ready-to-use set of plasmids, or assemble your own using the Expression Vector provided.

The following diagram is a suggested workflow of cloning genes of interest into pPEV-1 using Gibson assembly. Other vectors can be used by traditional restriction enzymes and ligation methods in a similar logic. All inserts must remain in-frame, with stop-codon handling aligned to the chosen vector design.



1.1. Design Your Insert

Design your DNA fragment containing the open reading frame (ORF) of your protein of interest flanked by overlapping arms. It is recommended to have overlap regions between 15-40 bp, with a melting temperature above 48 degrees. For example:

5'd[ctagaaataattttgtttaactttaagaaggagatatacc - ORF - GGTTCTCATCATTGGGGGAGCGGTGGCAGTATGGCATCCA]3'

Use *in silico* assembly tools, e.g., Benchling and Snapgene, to simulate Gibson assembly, to ensure IN-FRAME & NO STOP CODON.

1.2. Prepare Insert DNA Fragment

De novo synthesis of the design including the primer is recommended. Otherwise, use high-fidelity DNA polymerases to add overlaps and amplify the fragments.

1.3. Linearize Expression Vector

Use NcoI to digest the pPEV-1. And add alkaline phosphatase to prevent vector self ligation (optional but recommended). Otherwise, use PCR to amplify the plasmid into linear form. After, add DpnI to remove circular template (recommended).

1.4. Set up Gibson Assembly

Set up Gibson Assembly reaction. Following the manufacturer's manual, incubate samples at 50°C for 15 minutes. Or use another long-homology based cloning method.

1.5. Transformation & Verification of pPEX-POI

Transform 2 µl assembly reaction products into 50 µl competent cells of cloning strain, e.g., DH10B, DH5a, JM109, and plate the transformants on LB agar plate with 50 µg/ml kanamycin. Incubate at 37°C overnight (~16 hours).

Verify constructs by Sanger sequencing of 8 colonies using the T7 forward primer: 5'd[TAATACGACTCACTATAGGG]3'.

Note: If you are confident about the efficiency of expression strain competent cells, you can skip steps 1.5-1.6, and directly transform assembly reaction products in step 1.7.

1.6. Amplify pPEX-POI

Select the clone with confirmed sequences, and grow in 5 ml LB with 50 µg/ml kanamycin overnight (~16 hours). Purify the plasmid by, e.g., a commercial plasmid mini-prep kit.

1.7. Transformation of PandaPure Strain

To establish the bacterial strain for PandaPure workflow, both PandaPure Organelle Plasmid (pTEAR-2), and Protein Expression Plasmid (pPEX-POI) need to be introduced into a T7 polymerase carrying host, e.g. BL21(DE3), or BL21-AI.

Here are some of the suggested approaches. (continued on the next page)

A. Co-transformation

Ideal for the quick launch for one or several proteins.

Transform > 250 ng pTEAR-2 and > 250 ng pPEX-POI into 50 µl competent cells of expression strain.

Note: Competent cells of expression hosts, such as BL21(DE3), often have a low efficiency. Thus, high-amount DNAs are needed for two-plasmid co-transformation, especially when the protein of interest is large or toxic.

B. Sequential transformation (Multi-well version)

Ideal for high-throughput experiments for more than 24 proteins, especially for automated workflow by liquid handling robotics. As the following:

1. Transform ~ 100 ng pPEX-POI into 50 µl competent cells of expression strain, or assembly product if competent cells have high efficiency.
2. Make chemically competent cells of strains carrying pPEX-POI in 96-well format, by e.g., Hanahan or calcium chloride, Inoue, or TSS method. Note: TSS method is recommended here, for the lower requirement on bacterial growth phase.
3. Transform ~ 20 ng pTEAR-2 each well, and amplify the transformants under two antibiotic selection.

C. Sequential transformation (Single-tube version)

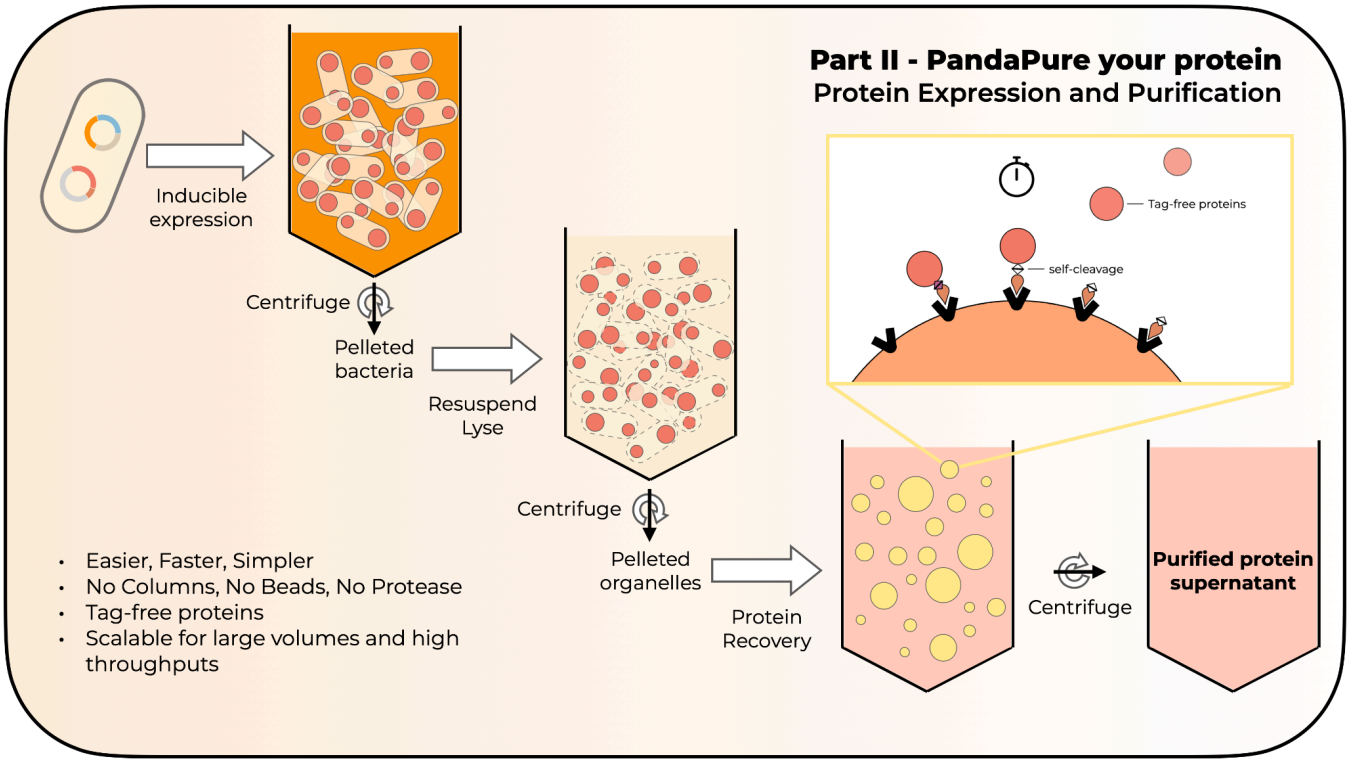
Ideal for randomized library screening. As the following:

1. Transform ~ 100 ng pTEAR-2 into 50 µl competent cells of expression strain.
2. Make high-efficiency competent cells of the transformant (PANDA strain). A chemical method using CCMB80 or electroporation method is recommended.
3. Transform pPEX-POI library, and isolate single colonies randomly.

Constructed PandaPure strains can be used immediately for protein expression and purification, or stored in glycerol stock and revived afterwards.

Step-by-step Protocol Part II - Protein Expression and Purification

Various strategies can be applied for bacterial growth, inducible expression, and cell lysis. The following are two examples, a simple workflow at 1-ml culture scale, and a 5-day workflow for induced production at 50-ml or higher.



Simple production at 1-ml culture scale (shaking tube or deep-well plates)

Ideal for preliminary validation or high-throughput experiments within deep-well plates.

Prepare PandaPure strain

1. Transform 250 ng pTEAR-2 and 250 ng pPEX-POI into 50 µl competent BL21(DE3).
2. Grow transformants on LB agar plates added with 50 µg/ml kanamycin and 30 µg/ml chloramphenicol (antibiotics), 37°C overnight for ~16 hours.

Protein Expression

1. Isolate single colonies into 1 ml growth medium with antibiotics.
2. Grow at 25 ~ 37°C 220 rpm (tubes) or 900 rpm (deep-well plates) for 24 ~ 48 hours.

Optional: supplement 0.5 mM IPTG and 150 ng/ml aTc at 8 hr, to induce the expression.

Note: Accumulated expression in LB or richer SOB medium is often sufficient for small-scale validation, even without induction. Recovery medium for cloning (Sigma-Aldrich CMR0002) has a similar effect and may yield higher output than SOB. SOB can also be replaced by auto-induction media (e.g., ZYM-5052) for better control.

Protein Purification (operate under room temperature)

1. Centrifuge cultures at max speed for 10 mins. Discard the supernatant.
2. Resuspend the pellet by 200 µl lysis reagent
   Optional: supplemented with 10 µg/ml egg-white lysozyme and/or 10 unit/ml Dnase I, if higher purity and yields are needed. However, at this small scale, lysozyme should NOT be used alone, as the viscosity introduced by bacterial chromosomes will cause difficulty in resuspension of the pellets.
3. Incubate with shaking or rotating, for 20 ~ 30 mins.
4. Centrifuge cell lysates at max speed for 10 mins. Discard the supernatant.
5. Resuspend the pellet by 200 µl PandaPure™ Protein Reagent.
   Note: Standard PandaPure™ Protein Reagent is prepared by diluting PandaPure™ Protein Tag Cleaver (250x) stock solution into a compatible 1x working buffer (including PandaPure Protein Buffer) at the desired working concentration. Prepared reagents can be stored at ambient or 4°C for weeks, and for months at -20°C. Do not use the reagent if visible precipitation is observed.
6. Incubate overnight with shaking or rotating, for 16 ~ 24 hr.
7. Centrifuge cell lysates at max speed for 10 mins.
8. Transfer the supernatant, i.e. protein extract, in a clean tube.

Induced production at 50-ml culture scale

Ideal for lab-scale production for optimized yields, purity, and reproducibility.

Day 1 - Prepare Bacterial Strain

1. Grow transformants on LB agar plates added with 50 µg/ml kanamycin and 30 µg/ml chloramphenicol (antibiotics), 37°C overnight (~16 hours), to form single colonies.

   Use SOC agar plates and/or incubate at 25-30°C for toxic proteins.

Day 2 - Prepare Seed Culture

2. Isolate single colonies into 3 ml SOC medium with antibiotics.
3. Grow seed culture at 37°C 220 rpm overnight.

Day 3 - Pre-Culture & Induced Expression

4. Dilute 250 µl overnight cultures into 50 ml LB medium with antibiotics in 250-ml flask.
5. Grow pre-cultures at 37°C 220 rpm in a shaker incubator until OD600 reaches 0.3 ~ 0.4. Note: Approx. 2 ~ 5 hrs for non-toxic proteins.
6. Add 0.5 mM IPTG and 150 ng/ml aTc (inducers) to the culture.

   Note: Concentration can vary in a range of 0.05-0.5 mM IPTG and 50-200 ng/ml aTc.
7. Grow induced cultures at 37°C 220 rpm overnight.

Day 4 - Organelle Extraction & Protein Recovery Reaction

8. Centrifuge overnight cultures at 10,000 rpm for 10 mins.
9. Pour out liquid, and drain pellets.
10. Resuspend cell pellets with 10 ml pre-warmed lysis reagent per gram of cells by pipetting until homogenous.

    Optional: add 10 µg/ml egg-white lysozyme for higher purity and yield. 10 unit/ml Dnase I can be further supplemented to reduce viscosity of pellets.
11. Incubate cell lysis reaction at 37°C 220 rpm for 30 mins in the shaker incubator.

    Alternatively, sonication to lyse the cells.
12. Centrifuge cell lysates at 10,000 rpm for 10 mins, and removes supernatant.
13. Optional: wash the pellets by resuspension in 5 ml PBS.
14. Add 5 ml PandaPure Protein Reagent. Vortex to prevent pellet adherence.

Note 1: PandaPure™ Protein Reagent is prepared by diluting PandaPure™ Protein Tag Cleaver (250x) stock solution into PandaPure™ Protein Buffer (HEPES) at 1x working concentration. Prepared reagents can be stored at ambient or 4°C for weeks, and for months at -20°C. Do not use the reagent if visible precipitation is observed.

Note 2: complete homogenization by pipetting is optional here but not necessary.

15. Incubate the protein recovery reaction at 37°C 220 rpm for 24 hours.

Day 5 - Recovery of Protein Extract

16. Centrifuge the reaction mix at 10,000 rpm 10 mins.
17. Transfer the supernatant, i.e. protein extract, to a clean tube.

    Optional: clarify the extract through a 0.22 μm filter by syringe for refined purity.

18. Store the protein extract at 4°C for up to 1 month.

    For longer shelf life, add glycerol to 25-50% and store at −20°C.

    Optional: store remaining organelle structures at 4°C for up to 1 month, or −20°C for longer time, for future analysis.

For more protocols, visit ailurus.bio for the latest releases.

4 Guidelines to Process Optimization

Design & Cloning

- If plasmids are modified for specific needs, e.g., changing resistant markers or the origin of replication, it is highly recommended to design a modular two-plasmid system to avoid difficulty in cloning.
- Single-plasmid design containing both organelle and protein coding can be optimized for specific proteins, but is unlikely to be genetically stable across different targets.
- pPEX-POI is also compatible with other strains that contain T7 RNA polymerase, lacks kanamycin and chloramphenicol resistance, and p15A, ColE incompatible plasmids. Examples include BL21-AI, NovaBlue(DE3), T7 Express. Extra inducers to express T7 RNA polymerase may be needed according to the manufacturer's manual.
- If T7 promoter is not used, e.g., replaced by a Tet promoter, PandaPure systems can also work in other strains that are not commonly used for protein expression.
- Ensure Your insert ORF is IN-FRAME.
- AVOID STOP CODON in your insert fragment; signal tags should be located at the C-terminus of your protein of interest (POI).
- Multiple domains can spontaneously assemble into the vector, facilitating the construction of fusion proteins.
- Overlap regions of 15-40 bp with a melting temperature above 48°C are recommended.

Transformation

- Co-transformation of two plasmids can be low efficiency, and require highly-competent host cells, high amounts of DNAs, and plating of all the transformants.
- For experiments that require high efficiency or when co-transformation is challenging, apply sequential transformation. This strategy to prepare a pTEAR-2 carrying strain is also recommended if you have adapted PandaPure™ as a routine protocol in your lab.
- If target protein is highly toxic, it is recommended to add aTc inducer during transformation and seed culture, to initiate synthetic organelles as early as possible to compartmentalize the protein; apply high-concentration glucose, e.g., using SOC medium for seed culture, to repress leaky expression; and use bacterial strains with lower leaky expression such as BL21-AI.
- Although it is common practice to isolate single colonies on solid agar plates, it has been shown that directly growing transformed and recovered cells in liquid medium without isolation of monoclonal can also yield good results in protein production, especially when a high-quality, pure plasmid is transformed.
- The PandaPure system provided is also compatible with other strains that contain T7 RNA polymerase, lacks kanamycin and chloramphenicol resistance, and p15A, ColE incompatible plasmids. Examples

include BL21-AI, NovaBlue(DE3), T7 Express. Extra inducers to express T7 RNA polymerase may be needed according to the manufacturer's manual.

Expression culture

- SOC or other medium with glucose is recommended to suppress potential leaky expression from pPEX-POI. Alternatively, inoculate single colonies into rich growth media such as LB and TB.
- Scale of cultures and chosen containers can be adapted according to your purpose, from 200 µl culture in 1 ml deep-well 96-well plate, to 200 ml culture in 1 L flasks.
- Protein yield and purity are dependent upon expression, conformation, solubility characteristics of recombinant protein. It is recommended to
    - Set up the process using GFP control first.
    - Perform a small scale, low throughput test to estimate the expression level.
    - Perform desired large-scale production or high-throughput experiment.
- Optimizing the culture condition is critical for the best use of PandaPure.
    - To start with, we recommend induction of 0.5 mM IPTG and 150 ng/ml aTc when the optical density at 600 nm (OD600) reaches 0.3-0.4 at 37°C overnight.
    - To adapt your goals, from high-throughput analysis to bulk production, you can adapt the following strategies:
        - Change induction procedures.
        - Vary induction levels from 0.05-0.5 mM IPTG and 50-200 ng/ml aTc. Optionally, 0.01-1 mM IPTG, 0-300 ng/ml aTc.
        - Optimize growth medium, e.g. YT media, TB media
        - Change culture, time and temperature.
- For GFP, expression for 3 hours after induction at 37°C is enough for downstream analyses. Recommended early-harvest times for expression are 37°C for 3 hours, 30°C for 5 hours, or 23°C for 16 hours overnight.Culture time can be prolonged to 48 hours or more depending on the process.

Purification and Recovery

- DO NOT freeze-thaw your cells, lysed pellets, or reacted pellets. Synthetic organelle TEAR-2 is NOT compatible with frozen temperature.
- Centrifugation to harvest cells, lysed pellets, and reacted pellets are recommended to be 10,000 × g for 10 min or higher speed or longer time. If the max speed is limited, e.g. for microplate centrifugation, prolonged time of centrifugation is necessary, e.g., 3,000 rpm for 30 mins.
- For overnight-induced culture of GFP control, cells may differentiate into two or multiple subpopulations carrying different amounts of GFP, visible after centrifugation, which is a normal feature for success expression.

- Common lysis reagents are tested compatible with PandaPure™, including commercial products for protein extraction, e.g., Thermo Scientific B-PER, Millipore Bugbuster, and nonionic surfactant, e.g., n-Octyl-β-D-glucopyranoside (OGP).
- Optimizing the lysis procedure is critical for protein yields and purity.
    - Add 25% extra volume of lysis reagent than using it for normal protein extraction.
    - Avoid excessive lysis reagents to prevent premature extraction of target proteins and lower final product yield.
    - For higher purity, 10 µg/ml lysozyme is recommended to remove large molecular weight impurity. However, it will notably lead to high viscosity, increasing the difficulty of operation, especially for small volumes. Additional DNase I is recommended when lysozyme is used.
    - Extend incubation for through lysis up to 1 hour if needed.
    - Apply multi-round cell lysis if needed.
    - Apply mechanical methods, e.g., sonicaction.
- Optimizing the protein recovery reaction is critical for protein yield.
    - It's recommended to use 1/10 volume of Protein Reagent relative to the culture.
    - Perform the protein recovery reaction at 37°C in a shaker incubator, rotating mixer, or other platforms, adjusting time based on protein stability.
    - It is not necessary to resuspend the pellet until homogenous when incubated at high speed overnight. Otherwise, it is highly recommended.
    - For unstable proteins at 37°C, perform the recovery reaction at 25 - 30°C, but avoid temperatures below 20°C.
    - Shorten or extend the reaction time as needed, from 2 hours to up to 3 days.
- DO NOT add RNase and/or protease into PandaPure™ Protein Reagent.

Storage and Downstream applications

- Storage conditions for proteins can be varied. Common practices include:
    - Solution in 25-50% glycerol or ethylene glycol at -20°C, shelf life for up to 1 year. Requiring sterile conditions.
    - Frozen at -20° to -80°C, in liquid nitrogen, or lyophilized, shelf life for years, one-time use only (repeated freeze-thaw cycles degrade proteins).
- For downstream applications, buffer exchanging, refinement, ultra-centrifuge concentration can be applied.
- Apply SDS-PAGE analysis to assess protein purity and integrity.
    - For protein extract: Mix 20 µl with 5 µl 5x SDS loading buffer, and denature at 95°C for 10 min.
    - For remaining organelle: resuspend in desired buffers, e.g., PBS, mix 20 µl with 5 µl 5x SDS loading buffer, and denature at 95°C for 10 min.
- Apply Bradford assay, or equivalent, to assess protein concentration.

5 Troubleshooting

Observation	Possible causes	Action
Failed transformation	Low-efficiency of competent cells	Use higher-efficiency cells
	Not enough plasmids	Increase used amount
	Challenging co-transformation	Transform sequentially
	Leaky expression and the protein is toxic	• Replace LB by SOC • Add aTc in agar plates • Choose hosts with lower leakiness, e.g., BL21-AI
Induced cells did not grow	Protein is toxic	• Choose hosts with lower leakiness, e.g., BL21-AI • Reduce IPTG, increase aTc • Induce at higher OD • Use auto-inducing medium
Cell is not lysed	Reagent is too cold	Pre-warm reagents
	Reagent and enzymes are inactive	Purchase new ones
	Reagent is not effective	Apply sonication
Protein stays in cell lysates	Failed to form organelles	Verify aTc induction.
	Insufficient organelles	Reduce IPTG induction, and increase aTc induction.
	Cells were frozen before purification	Redo the experiment without freeze-thaw cycle.
Low protein yield	Protein did not express	• Verify inducers • Optimize expression systems and conditions
	Lysis was not complete	Optimize lysis procedure
	Tag cleavage was not complete	Increase the incubation time Apply extra rounds of reaction
	Protein did not leave organelles	Extract proteins from the remaining pellets with a different buffer

	Protein is water-insoluble	Engineer protein solubility
	Extracted protein precipitates	• Add glycerol to stabilize protein • Exchange buffer for improved solubility
Poor protein purity	Cell were frozen before purification	• Redo the experiment without freeze-thaw cycle. • Try to extract proteins from the remaining pellets again at 25°C
	Incomplete removal of cytosolic impurity	• Optimize lysis procedure • Wash the pellet after lysis
	Contaminants from remained pellets	• Centrifuge at a higher speed for a longer time. • Clarify the sample through a 0.22 µm filter
Multiple bands	Pull-down of host components	Wash by non-ionic detergent
	Non-specific cleavage	• Reduce incubation time • Ask support@ailurus.bio for custom methods

6 Ordering Information and Related Offerings

Products

Products covered by this User Guide: PK002, PK006, and RM001-RM003. Components not listed are not included or are provided separately as add-ons, refills, or services. Other compatible PandaPure™ products are listed below for reference.

Product	Content & Quantity	Catalog Number
PandaPure™ Protein System (*E. coli*) Lesser Panda Edition II	PandaPure Organelle Plasmid pTEAR-2, 5 µg PandaPure Expression Vector pPEV-2, 5 µg PandaPure GFP Expression Plasmid pPEX-GFP-1, 5 µg [1] Onboarding offering: PandaPure™ Protein Tag Cleaver (250x), 0.2 mL [2]	PK002
PandaPure™ Protein System (*E. coli*) Standard Edition II	PandaPure Organelle Plasmid pTEAR-2, 5 µg 4 PandaPure Expression Vectors pPEV-1, pPEV-2, pPEV-3, pPEV-4, 5 µg each PandaPure GFP Expression Plasmid pPEX-GFP-1, 5 µg Onboarding offering: PandaPure™ Protein Reagent Kit, 250 mL reaction [3] Free Construct Service, 1 Gene x 5 Configurations [4]	PK006
PandaPure™ Protein Reagent Kit, Tag Cleavage and Protein Recovery	Components for 50, 100, or 250-mL reactions: PandaPure™ Protein Tag Cleaver (250x), 0.2 mL, 0.4 mL, or 1 mL PandaPure™ Protein Buffer (HEPES), 50 mL, 100 mL, or 250 mL	RM001, RM002, RM003

[1] PandaPure expression plasmid expressing sfGFP fused with C-terminal PandaPure Classic tag, as a positive control for system validation and process optimization.

[2] Working buffers are not included in PK002. Use a compatible buffer (pH 8-9) or supplement RM001/2/3 as needed.

[3] Same as RM003, including PandaPure™ Protein Tag Cleaver (250x), 1 mL, and PandaPure ™ Protein Buffer (HEPES), 250 mL.

[4] Complimentary Onboarding Construct Service applies to eligible PK006 orders, is valid for 6 months from the purchase date, and is activated after full payment for the associated PK006 order is received.

Scope includes gene synthesis for 1 target protein ≤ 600 aa (1.8 kb), and cloning into up to 5 construct designs for the same target gene using pPEV vectors included in PK006.

Non-standard or high-complexity sequences may require feasibility review, revised timelines, additional charges, or may be out of scope. Additional genes, longer sequences, additional constructs, and other custom options are handled as custom work subject to quotation and payment terms.

[5] RM001, RM002, and RM003 replace the previous reagent offerings PR050, PR100, and PR250, respectively. PK002 and PK006 replace the previous system offerings PK001 (PK-LP001) and PK005 (PK-HP001), respectively. Previous offerings PK001, PK005, PR050, PR100, and PR250 are discontinued. Where applicable, the current offerings are designed for technical continuity with previous PandaPure™ workflows.

Storage

All DNA molecules should be stored at -20°C to -80°C for up to 12 months.

Components of PandaPure Protein Reagent Kits can be stored at room temperature (15-25°C) for up to 12 months. Prepared PandaPure Protein Reagent can be stored for weeks at ambient or 4°C, and for months at -20°C. Do not use the reagent if visible precipitation is observed.

Products are shipped at ambient temperature.

Related products and services

Product	Content & Quantity	Catalog Number
PandaPure™ Protein Vector Set No.1 N-term Cleavable Tag	5 vectors with Classic tags at N-terminus in pET-21a, pET-30a, pET-32a, pCold II, pBADHisA, 5 ug each	VP003
PandaPure™ Protein Vector Set No.1 C-term Cleavable Tag	5 vectors with Classic tags at C-terminus in pET-28a, pET-21a, pGEX-4T-1, pMAL-c5X, 5 ug each	VP004

Visit ailurus.bio for additional vectors, expression plasmids, reagents, consumables, and accessories available from Ailurus to extend PandaPure workflows. Technical details for related offerings are provided in separate product documents.

For DNA synthesis and custom projects, submit your need to Ailurus Service to request a quote.

Note: Non-standard or high-complexity sequences may require feasibility review, revised timelines, additional charges, or may be out of scope.

Document Information

Visit ailurus.bio for the latest service and support information.

For further assistance, please contact our support team at: support@ailurus.bio

References

Please include Ailurus in the Materials and Methods section of future publications.

We recommend citing the following articles where the PandaPure™ method and synthetic organelles used in this user guide are described.

Guo, H., & Chen, J. (2024). Synthetic organelles enable protein purification in a single operation. bioRxiv, 2024-05.
DOI: https://doi.org/10.1101/2024.05.17.594729

Guo, H., Ryan, J. C., Song, X., Mallet, A., Zhang, M., Pabst, V., ... & Lindner, A. B. (2022). Spatial engineering of E. coli with addressable phase-separated RNAs. Cell, 185(20), 3823-3837.
DOI: 10.1016/j.cell.2022.09.016

Revision History: PandaPure-202603

Version	Date	Description
2.0	Mar 15th, 2026	Major release version for March 2026 launch alignment. Updated product-scope language, construct-service terms, and reagent/buffer wording for external consistency.
1.02	Feb 5th, 2025	Toolkit-version branch. Major revision for protocol updates, compatibility, better quality and accuracy.
1.01	June 16th, 2024	Major revision about user manual contents. Information of new Toolkit offerings were added. Existing information was updated and reformatted, for compatibility, better quality and accuracy.
1.0	Feb 8th, 2024	Initial release of PandaPure™ user manual for Construct Service and Reagents offerings.