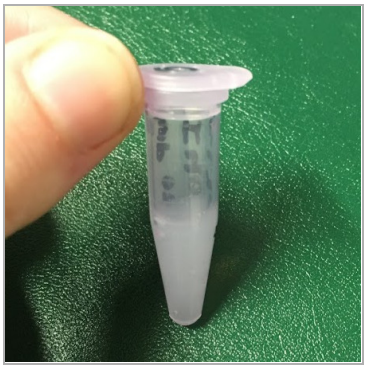

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We use this protocol and it's working

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Periplasmic Bacterial Expression and Purification of Elastin-like Polymers V.2

PLOS One

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ABSTRACT

This protocol describes the expression and purification procedures used in the Balog lab at the University of New England for producing recombinant elastin-like polymers (ELPs) in *E. coli*. It has been adapted from protocols used in the laboratory of Jennifer S. Martinez at Los Alamos National Laboratory, where it was originally developed based on the method of Hassouneh, Wafa et al. “Elastin-like polypeptides as a purification tag for recombinant proteins” *Current protocols in protein science* vol. Chapter 6 (2010): Unit 6.11.

GUIDELINES

The protocol workflow is as follows:

Day 1. Transformation
Day 2. Growth
Day 3. Harvest cells and begin purification
Day 4. Finish purification and dialyze protein
Day 5. Freeze-dry protein
Day 6. Store dried protein

A note about scale:
This protocol is written for the growth of 1 L of final culture. Volumes can generally be scaled up or down proportionally.

A note about expression conditions:
The expression conditions reported here are generally a useful starting point, but each combination of ELP gene, vector backbone, and bacterial strain is unique. When starting work on a new construct, we always recommend performing small-scale test expressions to optimize choice of media, expression time, growth temperature, and IPTG induction methods. We have seen reproducibly good results using fresh transformants and 2-4 h starter cultures. Others have adapted this protocol to use glycerol stocks and overnight starter cultures.

A note about keeping your protein happy:
While there are many good "pause points" in this protocol, I recommend only leaving protein overnight for one night at most at 4 °C before the dialysis step. Highly concentrated protein has been known to aggregate irreversibly even at 4 °C.

A note about SDS-PAGE:
If your ELP has no basic residues, it will not absorb Coomassie-based stains well. Image your gel soon after staining and destaining. For constructs that fail to bind Coomassie entirely, copper (II) chloride staining is recommended.

MATERIALS

BL21(DE3) competent cells
2XYT + agar plates with carbenicillan
Sterile glass plating beads
Sterile, nutrient-rich liquid media (for transformation outgrowth step)
1000X carbenicillan
15 mL SuperBroth, autoclaved in 100 mL flask (for starter culture)
1 L SuperBroth, autoclaved in 2 L flask
Pipette tips, autoclaved
Glass pipettes (5, 10, 25 mL), autoclaved
1XPBS + 20% w/v sucrose, sterile filtered, pre-chilled
1XPBS, sterile filtered, pre-chilled
Deionized water, sterile filtered, pre-chilled
5 M NaCl, sterile filtered
Dry NaCl
Eppendorf tubes, autoclaved
Round bottom Oak Ridge-style centrifuge tubes (~30-40 mL vol)
250-750 mL centrifuge bottles

Transformation

1 In a pre-chilled Eppendorf tube, mix 10-100 ng plasmid (typically 1-2 µL of mini-prep DNA) with 50-100 µL BL21(DE3) competent cells on ice. Let sit on ice for 30 min.

2 Heat shock at 42 ºC in a water bath for 1 min.

3 Allow cells to recover on ice for 2-3 min.

4 Add 1 mL sterile nutrient-rich liquid medium such as SOC or 2XYT.

5 Incubate 1 h at 37 ºC.

6 Centrifuge at 7000 rpm (~4600 x g) for 1 min at room temperature.

7 Remove all but ~50-100 µL of supernatant. Resuspend pellet in remaining supernatant.

8 Pipette resuspended cells on 2XYT + agar + carbenicillan plate and use sterile glass beads to spread.

9 Remove beads and store plate at 37 ºC overnight.

Expression

10 Inoculate 15 mL starter culture using a sterile loop to harvest several swipes of colonies from the transformation plate. Add 15 µL 1000X carbenicillan. Place flask in incubated shaker and shake at 200 rpm at 37 ºC until visibly cloudy (typically 2-4 h).

11 Inoculate 1 L SuperBroth with full volume of starter culture. Add 1 mL 1000X carbenicillan. Return 1 L culture to shaking at 200 rpm at 37 ºC for 24 h.

12 Harvest cells by centrifugation at 4000 rpm (~3000 x g) for 20 min at 4 ºC.

> **Note**
>
> At this point, cell pellets may be frozen at -80 ºC for subsequent purification.

Purification

13 Thaw cell pellets in water and immediately transfer to ice. Resuspend cell pellets thoroughly by pipetting and vortexing in 60 mL of ice-cold 1XPBS + 20% sucrose. Ice for 15 min.

Note

Perform the resuspension rapidly (~5 min) or decrease incubation time on ice to account for longer resuspension time. You should try to get the solution as homogenous as possible, but there may still be cell debris that does not go into solution.

14 Centrifuge at 7000 rpm (~4500 x g) for 10 min at 4 ºC. Remove supernatant to a fresh pre-chilled beaker or bottle on ice. This sample is **Periplasmic Fraction 1**.

15 Resuspend pellets thoroughly by pipetting and vortexing in 40 mL cold, sterile deionized water. Ice for 15 min.

Note

Perform the resuspension rapidly (~5 min) or decrease incubation time on ice to account for longer resuspension time. You should try to get the solution as homogenous as possible, but there may still be cell debris that does not go into solution.

16 Centrifuge at 7000 rpm (~4500 x g) for 10 min at 4 ºC. The supernatant after this spin is **Periplasmic Fraction 2**.

17 Pool **Periplasmic Fraction 1** and **Periplasmic Fraction 2** and measure the volume (which should be ~130 mL). This is your **Pooled Periplasmic Fraction**.

18 Allow the pooled periplasmic fraction to warm to room temperature. Add dry NaCl to a final concentration of 3 M. For 100 mL of pooled periplasmic fraction this is ~17.5 g of NaCl.

Note

Upon the addition of NaCl, the solution should turn cloudy, like milk. This is your first indication that ELP expression has been successful. Ensure that the NaCl has dissolved fully - it can be difficult to tell if the solution is very cloudy.

19 Centrifuge at 14,000 rpm (~17,000 x g) for 15 min at room temperature.

Note

Sticky, grey-white pellets should form at the bottom of the tubes. You may also observe floating white "pancakes". This is all ELP, this is the good stuff!

20 Carefully remove the supernatant. This is your **Salt Supernatant** sample, which you expect should **not** contain ELP. Resuspend pellets in 15 mL ice cold, sterile 1XPBS. Incubation on ice and gentle vortexing will assist with resuspension. Pipetting is not recommend as the protein is quite sticky and will be lost by sticking to pipettes.

Note

There are several critical considerations at this step. First, inspect the pellets to confirm that they consist entirely of ELP and cellular debris, **not undissolved NaCl**, which will interfere with the next step. Next, after resuspending in 15 mL 1XPBS, inspect the solution to determine whether ELP has fully dissolved and is **not in the coacervated state**. This can be difficult to ascertain because contamination at this stage also gives the solution a cloudy apperance. If the solution looks like any variation of dairy or non-dairy milk that you would consider drinking, the ELP is probably still coacervated and you should add another 15 mL cold 1XPBS. If the cloudiness is more brown, yellow, or translucent, like a milk you definitely would not consider drinking, the ELP has probably dissolved and is in monomer form. Consider seeking a second opinion at this step you are not sure.

21 While still keeping resuspended protein cold, split volume into an even number of pre-chilled 1.5 mL Eppendorf tubes (~1 mL per tube).

22 Centrifuge Eppendorf tubes at 14,000 rpm (~18,000 x g) for 10 min at 4 ºC. A brown contamination pellet should form.

23 Transfer supernatants to fresh Eppendorf tubes. This sample is **Cold Supernatant 1**, representing protein purity after **one round** of inverse transition cycling.

Purification - Temperature Cycling

24 Return protein to room temperature and add ~100 µL 5 M NaCl to each tube. Mix by inversion. If solution does not turn cloudy, add another 100 µL 5 M NaCl.

25 Centrifuge at 14,000 rpm (~18,000 x g) for 10 min at room temperature. Grey-white protein pellets should form.

26 Add 600 µL cold, sterile 1XPBS to each tube. Resuspend by alternating vortexing and incubating on ice or by rotating at 4 ºC.

Note

Complete resuspension at this step is very important. If protein is not completely resuspended, a substantial loss of product may be lost. Keep an eye on the pellet, which may become transparent when cooled but has not fully resuspended. If necessary, leave tubes rotating at 4 ºC overnight.

27 Centrifuge at 14,000 rpm (~18,000 x g) for 10 min at 4 ºC. A brown contamination pellet may form.

28 Transfer supernatants to **half** the number of fresh Eppendorf tubes by pooling two supernatants per fresh tube. The supernatant at this step is **Cold Supernatant 2**.

29 Repeat steps 24-28 until a contamination pellet is no longer visible after step 27 (cold spin), plus one more time for good measure. Following the final round of temperature cycling, keep cold

supernatant samples cold.

Post-purification

30 Analyze samples by SDS-PAGE. Suggested samples:

1. Periplasmic Fraction 1
2. Periplasmic Fraction 2 or Pooled Periplasmic Fraction
3. Salt Supernatant
4. Cold Supernatant 1
5. Cold Supernatant 2
6. Cold Supernatant (...)

31 Dialyze purified protein against 4 L of pre-chilled deionized water overnight at 4 ºC.

32 Store dialyzed protein at -80 ºC or flash freeze in liquid nitrogen. Lyophilize for long-term storage at -20 ºC.