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Title: Efficient Purification of Elastin-Like Polypeptides (ELPs) from *E. coli* Using an Organic Solvent-Based Extraction and Precipitation Method

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

- 3. Filming location:** Will the filming need to take place in multiple locations? **NO**

- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 16

Number of Shots: 40

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

- 1.1. **David Thompson:** The scope of this research asks whether solvent extraction/precipitation can rapidly purify ELP fusion proteins while retaining fusion activity function.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What are the most recent developments in your field of research?~~

- 1.2. **David Thompson:** Recent developments include rapid solvent-based ELP purification with improved endotoxin control, enabling active, self-assembling nanoparticles for targeted nucleic acid delivery.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What significant findings have you established in your field?~~

- 1.3. **David Thompson:** Significant findings include demonstrating that organic solvent extraction–precipitation rapidly purifies ELP fusions with low endotoxin while preserving fusion protein activity.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What research gap are you addressing with your protocol?~~

- 1.4. **David Thompson:** This protocol addresses the lack of rapid, detergent-free methods to purify ELP fusions from *E. coli*, with or without inclusion bodies, without multi-step ITC.
 - 1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What advantage does your protocol offer compared to other techniques?~~

- 1.5. **David Thompson:** This protocol offers a single-step, rapid purification directly from pellets, yielding highly pure, low-endotoxin ELP fusions while preserving fusion protein function.
 - 1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

- 1.6. **Dr. David H. Thompson, Professor of Chemistry, Purdue University:** (authors will present their testimonial statements live)
 - 1.6.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

- 1.7. **Dr. David H. Thompson, Professor of Chemistry, Purdue University:** (authors will present their testimonial statements live)
 - 1.7.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

2. Organic Solvent-Based Extraction and SDS-PAGE Validation of Elastin-Like Polypeptides (ELPs)

Demonstrator: Joydeep Rakshit, Saloni Darji

2.1. To begin, weigh out 1 gram of the *E.coli* cell pellet [1]. Add 4 milliliters of freshly prepared lysis buffer to resuspend the cell pellet [2]. Vortex gently until the pellet is fully dispersed and mixed with the buffer [3].

2.1.1. Talent weighing 1 gram of cell pellet and transferring it to a tube.

2.1.2. Talent adding 4 milliliters of lysis buffer.

2.1.3. Talent vortexing the mixture gently until fully mixed.

Videographer's Note: 2.1.3 is in 2 parts

2.2. Add approximately 5 milligrams of lysozyme to the resuspended cells to assist in cell wall digestion [1]. Then place the tube in a refrigerator set at 4 degrees Celsius and incubate for 1 hour [2].

2.2.1. Talent adding 5 mg lysozyme to the resuspended sample.

2.2.2. Talent placing the tube into a 4 degrees Celsius refrigerator.

2.3. Following incubation, sonicate the sample using a microtip probe sonicator in 5-second intervals [1]. Keep the sample on ice and continue sonication until a uniform lysate free of visible particulates is obtained, typically for 5 to 8 minutes [2].

2.3.1. Talent sonicating the sample using specified pulse intervals.

2.3.2. Shot of the sample on ice during sonication showing transition to a uniform lysate.

2.4. Centrifuge the sonicated lysate at 10,000 *g* for 10 minutes at 20 degrees Celsius to clarify it [1]. Then carefully separate the supernatant containing the soluble fraction from the pellet containing the insoluble fraction [2].

2.4.1. Talent placing the sample into a centrifuge.

2.4.2. Talent pipetting out the supernatant from pellet.

2.5. Pipette out 10 to 20 microliters of the supernatant [1] and resuspend an equivalent mass of the pellet in lysis buffer [2-TXT]. Mix both fractions with 4X Laemmli buffer to a final 1X concentration [3], then heat the suspension at 95 degrees Celsius for 5 minutes [4]. ~~Load equal amounts to assess ELP expression in both fractions for SDS-~~

~~PAGE comparison [5-TXT].~~

2.5.1. Talent pipetting out 10 – 20 µL of the supernatant.

2.5.2. Talent resuspending the pellet in specified lysis buffer. **TXT: Lysis buffer: 50 mM Tris - HCl pH 8.0, 150 mM NaCl, 1 mM EDTA**

2.5.3. Talent mixing the pellet with Laemmli buffer.

2.5.4. Talent heating the suspension at 95 °C.

2.5.5. ~~Talent preparing the samples for gel loading. TXT: ELP: Elastin-Like Polypeptide~~
Videographer's Note: Shot deleted

~~2.6. If the ELP is predominantly soluble, proceed with organic-solvent extraction using the clarified supernatant [1]. If it is predominantly in the pellet, discard the supernatant and continue with organic-solvent extraction of the pellet [2].~~

Videographer's Note: 2.6.2-2.7.2 was deleted

~~2.6.1. Talent labelling the soluble fraction for extraction.~~

~~2.6.2. Talent discarding supernatant and preparing pellet for inclusion body workflow.~~

~~2.7. For insoluble proteins, invert the centrifuge tubes to allow residual lysis buffer to drain by gravity for 1 to 2 minutes [1]. Leave the pellet to air dry at room temperature for 5 to 10 minutes until free of excess moisture [2-TXT].~~

~~2.7.1. Talent inverting centrifuge tubes on a rack.~~

~~2.7.2. Shot of air drying pellet at room temperature. TXT: Process ELP-1 Cm2 soluble construct like other soluble constructs~~

2.8. To perform solvent extraction, add 4 milliliters of organic solvent or solvent mixture to the air-dried pellet or supernatant [1]. For binary solvent mixtures, use exact volumetric ratios such as 2 milliliters of each for a 1 to 1 combination [2].

Videographer's Note: 2.8.2 should go before 2.8.1

2.8.1. Talent adding 4.0 milliliters of solvent to the pellet or supernatant.

2.8.2. Talent measuring and mixing binary solvents in specified ratio.

2.9. Vortex the suspension vigorously for 1 minute to ensure proper solvent penetration into the pellet [1]. Now, incubate the mixture at 20 degrees Celsius for 5 minutes to allow aggregation of extracellular proteins and solubilization of ELP in the organic phase [2].

2.9.1. Talent vortexing the sample vigorously.

2.9.2. Talent ~~placing the tube in an incubator set to~~ incubates at 20 degrees Celsius or at room temperature for 5 minutes on a workbench.

2.10. Centrifuge the sample at 13,000 g for 10 minutes at 20 degrees Celsius to separate solubilized proteins from insoluble debris [1]. Carefully collect the supernatant without

disturbing the pellet, as it contains the solubilized ELP [2]. Measure the volume of the collected supernatant precisely to prepare for precipitation [3].

2.10.1. Talent placing sample in centrifuge.

2.10.2. Talent pipetting out the supernatant from the centrifuged sample.

2.10.3. Talent using a pipette or graduated tube to measure supernatant volume.

2.11. Next, add acetone or acetonitrile to the supernatant at 2.33 times its volume for protein precipitation [1]. Incubate the mixture at 20 degrees Celsius for 5 to 7 minutes [2].

2.11.1. Talent adding acetone or acetonitrile to the measured supernatant.

2.11.2. Talent ~~placing the tube in an incubator set to~~ incubates at 20 degrees or room temp for 10 minutes on workbench.

2.12. Centrifuge the sample at 10,000 *g* for 10 minutes at 20 degrees Celsius [1]. Then discard the supernatant [2] and air dry the pellet either under a gentle nitrogen gas stream for 10 to 15 minutes [3] or by placing uncapped centrifuge tubes upside down on lint-free wipes in a fume hood for 1 hour at 20 degrees Celsius [4].

2.12.1. Talent placing the sample in a centrifuge.

2.12.2. Talent pipetting out the supernatant.

2.12.3. Talent drying pellet using nitrogen gas.

2.12.4. Shot of the uncapped centrifuge tubes being placed upside down on lint-free wipes in a fume hood.

2.13. Resuspend the dried protein pellet in 50 microliters of PBS [1]. Then pipette up and down gently to ensure complete dissolution [2]. Store the resuspended protein at minus 20 degrees Celsius for short- to medium-term use [3-TXT].

Videographer's Note: 2.13.1-2.13.2 are combined

2.13.1. Talent adding 50 μ L PBS to the pellet.

2.13.2. Talent pipetting the suspension up and down to resuspend pellet.

2.13.3. Talent placing sample in a minus 20 degrees Celsius freezer. **TXT: Avoid repeated freeze-thaw cycles to maintain protein integrity**

2.14. To perform validation using SDS-PAGE, first mix the purified protein with 4X SDS-PAGE loading buffer [1-TXT]. Vortex the solution briefly for homogeneity [2].

2.14.1. Talent mixing protein with loading buffer. **TXT: Protein: Buffer: 3:1**

Video Editor: Please note that the on-screen text has a ratio

2.14.2. Talent placing the solution in a vortex mixer.

2.15. Load the sample onto a 10 percent SDS-PAGE gel for ELP-I-Cm2 (*E-L-P-Intein-fused-to-Chorismate-mutase*) [1]. Run the gel at 100 to 120 volts until the tracking dye reaches the bottom [2].

2.15.1. Talent loading gel.

2.15.2. Shot of the gel electrophoresis unit being switched on.

2.16. Now, stain the gel using Instant Blue Coomassie stain or a suitable alternative for 2 hours at 20 degrees Celsius [1]. Rinse with deionized water until a clear background is achieved [2].

Videographer's Note: The first take of 2.16.2 was slated incorrectly. It is meant to be the second part of 2.16.1

2.16.1. Talent placing the gel in a dye solution.

2.16.2. Talent placing the stained gel in deionized water to reveal bands against clear background.

Results

3. Results

3.1. A prominent ELP-I-Cm2 band appeared at 100 kilodaltons following a lysis step prior to organic extraction [1].

3.1.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the band labeled "ELP-I-Cm2" at 100 kDa in the "Lysis" lane.*

3.2. Different organic solvent combinations resulted in varied extraction efficiencies and purities of ELP-I-Cm2 [1]. The AG (A-G) and BG (B-G) 1 to 1 mixtures yielded the highest protein recovery [2-TXT].

3.2.1. LAB MEDIA: Figure 3B.

3.2.2. LAB MEDIA: Figure 3B.

TXT: AG: isopropanol:acetonitrile;

BG: butanol:acetonitrile

Video editor: Emphasize the bands in the "AG" and "BG" lanes

3.3. The AG solvent-extracted ELP-I-Cm2 retained approximately 80 percent of its enzymatic activity compared to ITC (I-T-C) -purified protein [1], while BG retained around 50 percent activity [2], and the V40 (V-Forty) control showed almost no activity [3].

3.3.1. LAB MEDIA: Figure 3C. *Video editor: Highlight the bar labeled "AG"*

3.3.2. LAB MEDIA: Figure 3C. *Video editor: Highlight the bar labeled "BG".*

3.3.3. LAB MEDIA: Figure 3C. *Video editor: Highlight the bar labeled "V40".*

Pronunciation Guide:

❏ Elastin-Like Polypeptides

Pronunciation link: <https://www.merriam-webster.com/medical/elastin>

IPA: /ɪˈlæstɪn laɪk ˌpɑːliˈpeptɑɪdz/

Phonetic Spelling: ih-las-tin like pah-luh-pep-tydz

❏ Polypeptides

Pronunciation link: <https://www.merriam-webster.com/dictionary/polypeptide>

IPA: /ˌpɑːliˈpeptɑɪd/

Phonetic Spelling: pah-luh-pep-tyde

❏ Escherichia coli

Pronunciation link: <https://www.merriam-webster.com/dictionary/Escherichia%20coli>

IPA: /ˌɛʃəˈrɪkiə ˈkɒʊlaɪ/

Phonetic Spelling: esh-uh-rik-ee-uh koh-lye

❏ Endotoxin

Pronunciation link: <https://www.merriam-webster.com/medical/endotoxin>

IPA: /ˌɛndəʊˈtɑːksɪn/

Phonetic Spelling: en-doh-tok-sin

❏ Inclusion bodies

Pronunciation link: <https://www.merriam-webster.com/medical/inclusion%20body>

IPA: /ɪnˈkluːʒən ˈbɑːdɪz/

Phonetic Spelling: in-kloo-zhuhn bod-eez

❏ Sonication

Pronunciation link: <https://www.merriam-webster.com/dictionary/sonication>

IPA: /ˌsɑːnɪˈkeɪʃən/

Phonetic Spelling: sah-nih-kay-shuhn

❏ Microtip

Pronunciation link: <https://www.merriam-webster.com/dictionary/microtip>

IPA: /ˈmaɪkrəʊˌtɪp/

Phonetic Spelling: my-kroh-tip

❏ Lysate

Pronunciation link: <https://www.merriam-webster.com/medical/lysate>

IPA: /ˈlaɪseɪt/

Phonetic Spelling: lye-sayt

❏ Centrifuge

Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>

IPA: /ˈsentrɪˌfjuːdʒ/

Phonetic Spelling: sen-trih-fyooj

❏ Supernatant

Pronunciation link: <https://www.merriam-webster.com/medical/supernatant>

IPA: /ˌsuːpərˈneɪtənt/

Phonetic Spelling: soo-per-nay-tuhnt

❏ Laemmli

Pronunciation link: <https://www.howtopronounce.com/laemmli>

IPA: /'ləmli/

Phonetic Spelling: lem·lee

❑ Polyacrylamide

Pronunciation link: <https://www.merriam-webster.com/medical/polyacrylamide>

IPA: /,pɑːliə'krɪləˌmaɪd/

Phonetic Spelling: pah·lee·uh·kril·uh·myde

❑ Electrophoresis

Pronunciation link: <https://www.merriam-webster.com/dictionary/electrophoresis>

IPA: /ɪˌlɛktroʊfə'riːsɪs/

Phonetic Spelling: ih·lek·troh·fuh·ree·sis

❑ Acetonitrile

Pronunciation link: <https://www.merriam-webster.com/dictionary/acetonitrile>

IPA: /,æsɪtoʊ'nɪtraɪl/

Phonetic Spelling: ass·ih·toh·nye·tryle

❑ Isopropanol

Pronunciation link: <https://www.merriam-webster.com/dictionary/isopropanol>

IPA: /,aɪsoʊ'proʊpən,ɔːl/

Phonetic Spelling: eye·soh·proh·puh·nawl

❑ Chorismate

Pronunciation link: <https://www.merriam-webster.com/medical/chorismate>

IPA: /'kɔːrɪzˌmeɪt/

Phonetic Spelling: kor·ihz·mayt

❑ Dystopia

Pronunciation link: <https://www.merriam-webster.com/medical/dystopia>

IPA: /dɪs'toʊpiə/

Phonetic Spelling: dis·toh·pee·uh

❑ Kilodaltons

Pronunciation link: <https://www.merriam-webster.com/dictionary/kilodalton>

IPA: /,kɪləʊ'dæltən/

Phonetic Spelling: kil·oh·dal·tuhn

❑ Precipitation

Pronunciation link: <https://www.merriam-webster.com/dictionary/precipitation>

IPA: /prɪˌsɪpɪ'teɪʃən/

Phonetic Spelling: prih·sip·ih·tay·shuhn

❑ Coomassie

Pronunciation link: <https://www.howtopronounce.com/coomassie>

IPA: /'kuːməsi/

Phonetic Spelling: koo·mas·see