FINAL SCRIPT: APPROVED FOR FILMING

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Title: Overexpressing and Purifying a Toxic Nuclease from *Escherichia coli*

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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**

Current Protocol Length
Number of Steps: 19
Number of Shots: 41

Introduction

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

1.1. **Erik Daquilanea:** Our lab aims to structurally and biochemically characterize a conserved endonuclease found in nidoviruses, including coronaviruses, to develop an evolutionary model and provide a basis for therapeutic targets.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.3.1*

What are the current experimental challenges?

1.2. **Erik Daquilanea:** Nucleases can be difficult to express in *E. coli* systems due to enzymatic activity on cellular DNA or RNA, which can result in slow growth and poor protein yields.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

How will your findings advance research in your field?

1.3. **Erik Daquilanea:** Our finding will allow us the purify toxic nucleases from other nidoviruses for further downstream biochemical and structural studies.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1*

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

Protocol

2. Culturing the Cells and Inducing Protein Expression with IPTG

Demonstrator: Erik Daquilanea

2.1. To begin, use one 50-milliliter sterilized Erlenmeyer flask for each liter of culture, and prepare 2 to 3 additional flasks as starter cultures **[1]**. Label one of the flasks with a star to designate it for optical density checks, as its measurements will represent the entire growth unless there is a visual difference among flasks **[2]**.

2.1.1. WIDE: Talent placing sterilized 50 milliliter Erlenmeyer flasks on the workbench.

2.1.2. Talent labeling one flask with a star using a lab marker.

2.2. Using a graduated cylinder, prepare a master mix by combining 60 milliliters of 2x TY *(T-Y)* medium and 60 microliters of ampicillin stock solution thawed from minus 20 degrees Celsius **[1]**. Gently, swirl the mixture to combine **[2]** and aliquot 10 milliliters of the master mix into each Erlenmeyer flask **[3]**.

2.2.1. Talent pouring 2xTY medium into a cylinder and adding ampicillin stock with a micropipette.

2.2.2. Talent swirling the container to mix the master solution.

2.2.3. Talent pipetting 10 milliliters of the mix into each flask.

2.3. Now, remove the transformed agar plate from the incubator **[1]**. Using a sterile toothpick or pipette tip, pluck a single, isolated colony from the plate **[2]** and transfer it directly into a flask containing media **[3]**. Repeat this procedure until each flask contains a colony-inoculated toothpick **[4]**.

2.3.1. Talent opening the incubator and removing the bacterial plate.

2.3.2. Talent isolating a single colony using a sterile tool from the plate.

2.3.3. Talent dropping the tool into a flask containing media.

2.3.4. Shot of the remaining flasks with the toothpick.

2.4. Place the inoculated flasks into a shaking incubator set to 210 revolutions per minute and 37 degrees Celsius for approximately 5 to 7 hours **[1]**.

2.4.1. Talent loading the inoculated flasks into the incubator and adjusting the settings.

2.5. To check the optical density at 600 nanometers, use a serological pipette to remove 1 milliliter of media from the starred flask **[1]**. Continue checking periodically until the optical density reaches between 0.8 and 1.0 **[2]**.

2.5.1. Talent removing 1 milliliter of media from the starred flask using a serological pipette.

2.5.2. Talent placing the sample in a spectrophotometer.

2.6. Once the target optical density is achieved, add 1 milliliter of 1 molar IPTG solution thawed from minus 20 degrees Celsius to each flask to induce protein overexpression **[1]**.

2.6.1. Talent pipetting 1 milliliter of IPTG into each culture flask.

2.7. Then, transfer the 2-liter flasks to a shaking incubator set to 210 revolutions per minute and 16 degrees Celsius for overnight induction for 14 to 16 hours **[1]**.

2.7.1. Talent placing the flasks in a large incubator.

3. Cell Lysis and Lysate Clarification

3.1. Resuspend the bacterial pellet by adding 2 milliliters of lysis buffer for every 1.2 grams of pellet **[1]**. Add 100 microliters of 1 molar AEBSF *(A-E-B-S-F)* for every 10 milliliters of lysis buffer as a protease inhibitor **[2]**. If using a pellet combined from 4 liters of culture, mix it with 100 microliters of DNase I to achieve a final concentration of 200 units **[3]**.

3.1.1. Talent pipetting lysis buffer into the tube containing the bacterial pellet.

3.1.2. Talent adding AEBSF using a micropipette.

3.1.3. Talent inverting the tube to mix.

3.2. After vortexing for 30 seconds, transfer the vortexed mixture into a Dounce tissue grinder **[2]** and use a loose pestle to homogenize the sample with approximately 10 strokes **[3]**.

3.2.1. Talent pouring the vortexed solution into a Dounce grinder.

3.2.2. Talent performing about 10 strokes with a loose pestle.

3.3. Then, transfer the homogenized sample into a metal beaker for sonication **[1]**.

3.3.1. Talent pouring the homogenized solution into a clean metal beaker.

3.4. To maximize sample recovery, rinse the original pellet tube with 5 milliliters of lysis buffer **[1]** and vortex briefly **[2]**. Pour the rinse into the Dounce homogenizer, apply approximately 5 strokes with the pestle **[3]**, and transfer the contents to the sonication beaker **[4]**.

3.4.1. Talent adding lysis buffer to the pellet tube.

3.4.2. Talent holding the tube on a vortex mixer.

3.4.3. Talent transferring the rinse to the Dounce grinder and performing about 5 strokes.

3.4.4. Talent pouring the second homogenate into the metal beaker.

3.5. Add 75 microliters of Triton X-100 to the metal beaker to aid in membrane lysis and solubilization **[1]**. Place the metal beaker into an ice bath to keep the sample cold during sonication **[2]**.

3.5.1. Talent adding Triton X-100 to the beaker using a micropipette.

3.5.2. Talent placing the beaker into a pre-prepared ice bath.

3.6. Now, insert the sonication probe into the metal beaker **[1]** and sonicate the sample for 6 minutes and 30 seconds, with pulses every 2 seconds and amplification set to 70 percent **[1]**.

3.6.1. Talent positioning the sonication probe into the sample.

3.6.2. Talent operating the sonicator with specified parameters and starting the cycles.

3.7. Then, using a serological pipette, transfer the lysate into a centrifuge tube **[1]**. Add 1 molar AEBSF at a 1 to 100 dilution to the same tube as a fresh protease inhibitor **[2]** and centrifuge the sample at 26,915 *g* for 50 minutes to clarify the lysate **[3]**.

3.7.1. Talent pipetting the sonicated lysate into a centrifuge tube.

3.7.2. Talent adding AEBSF to the centrifuge tube using a micropipette.

3.7.3. Talent placing the tube in the centrifuge.

4. Protein Elution and Tag Cleavage

4.1. After performing affinity chromatography by gravity filtration, elute the His-tagged Nsp15 *(N-S-P-15)* protein in three stages, with the first and second elutions each using 2 milliliters of elution buffer, and the third using 1 milliliter **[1]**.

4.1.1. Talent adding the elution buffer to the column.

4.2. Prepare a 1 to 1 dilution of Bradford reagent in water for a quick qualitative protein assay **[1]**. Transfer 10 microliters from each elution into separate tubes and invert gently to mix **[2]**. Assess the color change to blue to estimate protein content and decide how to combine the elutions accordingly **[3]**.

4.2.1. Talent vortexing the reagent mixed with water.

4.2.2. Talent pipetting 10 microliters from each elution into separate tubes and inverting to mix.

4.2.3. Talent looking at tubes with blue colours.

4.3. Next, using a serological pipette, apply the cleavage reaction mix containing the previous eluate directly onto the resin **[1]** and collect the eluate into a 15-milliliter conical tube **[2]**.

4.3.1. Talent carefully pipetting the cleavage reaction onto the resin.

4.3.2. Talent collecting flow-through in a conical tube.

4.4. Wash the resin twice with 2 milliliters of cleavage buffer, collecting both washes into the same 15 milliliter tube **[1]**. Add AEBSF to this tube to quench thrombin and reach a final concentration of 10 millimolar **[2]**.

4.4.1. Talent pouring cleavage buffer on the resin.

4.4.2. Talent adding AEBSF to the combined eluate using a micropipette.

4.5. Finally, transfer the entire repass sample to a new 30-kilodalton molecular weight cutoff concentrator **[1]**. Centrifuge the sample at 3,000 *g* for 10 minutes to reduce the volume to 500 microliters or less **[2]** and transfer the concentrated protein sample into a 0.5-milliliter microcentrifuge tube **[3]**.

4.5.1. Talent loading the repass sample into a fresh 30 kilodalton concentrator.

4.5.2. Talent placing the concentrator in the centrifuge and setting time and speed.

4.5.3. Talent pipetting the final concentrated sample into a microcentrifuge tube.

Results

5. Results

5.1. Expression of wild-type Nsp15 in *Escherichia coli* resulted in slow cell growth with an approximate doubling time of 1 hour **[1]**, whereas the catalytic-dead mutant showed a normal doubling time of approximately 20 minutes **[2]**.

5.1.1. LAB MEDIA: Figure 1A. *Video editor: Highlight the blue curve labelled "WT Nsp15 PI"*.

5.1.2. LAB MEDIA: Figure 1A. *Video editor: Highlight the green curve labelled "H223A Nsp15 PI"* .

5.2. During affinity purification, a clear band corresponding to wild-type Nsp15 appeared in the elution lane, indicating successful isolation despite low expression **[1]**.

5.2.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the dark band in lane 7 labelled "Elution 1" at approximately between 50 and 37 marks*.

5.3. The catalytic-dead Nsp15 also eluted as a strong single band, indicating high yield **[1]**.

5.3.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the intense band in lane 7 labelled "Elution 1" at approximately 42 kilodaltons*.

5.4. Thrombin cleavage reduced the molecular weight of Nsp15 by approximately 2 kilodaltons, confirming successful His-tag removal **[1]**.

5.4.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the lane 3 "Post-Cleave*".

5.5. Size exclusion chromatography of wild-type Nsp15 showed two peaks, with the 11-milliliter peak corresponding to active hexameric form **[1]** and the 15-milliliter peak to inactive monomeric form **[2]**.

5.5.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the first peak with the "hexamer" icon*.

5.5.2. LAB MEDIA: Figure 4A. *Video editor: Highlight the second peak with the "monomer" icon*.

5.6. In a fluorescence-based RNA cleavage assay, only the hexameric wild-type Nsp15

showed visible degradation of RNA over time **[1]**, while the monomeric wild-type and all forms of catalytic-dead Nsp15 remained inactive **[2]**.

5.6.1. LAB MEDIA: Figure 5. *Video editor: Highlight the long lanes in "WT" hexamer (0 to 60 min) in 5A*.

5.6.2. LAB MEDIA: Figure 5. *Video editor: Highlight B*.

1. **nuclease**
   - IPA: /ˈnuːkliˌeɪs/
   - Phonetic: noo-klee-ace
2. **endonuclease**
   - IPA: /ˌɛn.doʊˈnuː.kli.eɪs/
   - Phonetic: en-doh-noo-klee-ace
3. **nidoviruses**
   - IPA: /ˌnaɪdoʊˈvaɪɹəsɪz/
   - Phonetic: nigh-doh-vye-rus-iz
4. **coronaviruses**
   - IPA: /ˌkoʊɹoʊvəˈvaɪɹəsɪz/
   - Phonetic: koh-roh-vuh-vye-rus-iz
5. **IPTG**
   - No standard entry; common pronunciation: **I-P-T-G**
   - Phonetic: eye-pee-tee-gee
6. **AEBSF**
   - No standard entry; common pronunciation letter-by-letter: **A-E-B-S-F**
   - Phonetic: ay-ee-bee-ess-eff
7. **Dounce**
   - IPA: /daʊns/
   - Phonetic: douns (rhymes with "bounce")
8. **sonication**
   - IPA: /ˌsɑniˈkeɪʃən/
   - Phonetic: soh-nih-kay-shun
9. **Thrombin**
   - IPA: /ˈθɹɒmbɪn/
   - Phonetic: throm-bin
10. **hexameric**
    - IPA: /ˌhɛgzəˈmɛɹɪk/
    - Phonetic: hex-uh-mer-ik
11. **monomeric**
    - IPA: /ˌmɑnoʊˈmɛɹɪk/
    - Phonetic: mah-no-mer-ik
12. **Escherichia coli**

- IPA: /ˌɛʃəˈrɪkiə ˈkoʊlaɪ/
- Phonetic: esh-uh-rik-ee-uh koh-lye