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Title: Enriching Subcellular Proteins in *Leptospira* Using a Triton X-114-Based Fractionation Approach

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

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

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

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **10/06/2025**

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Current Protocol Length

Number of Steps: 13
Number of Shots: 25

Introduction

1.1. **Homen Phukan:** We're isolating subcellular proteins in *Leptospira* to understand protein localization, which helps in identifying potential virulence factors in gram-negative bacteria.

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

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

1.2. **Homen Phukan:** Subcellular proteomics now employs high-throughput mass spectrometry and optimized fractionation to dissect pathogen protein architecture with enhanced precision. An example is virulence factor detection.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.6.1*

What technologies are currently used to advance research in your field?

1.3. **Homen Phukan**- We use Triton X-114-based phase separation, LC-MS/MS, SDS-PAGE, and immunoblotting to extract and identify compartment-specific proteins in bacteria.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.3*

What are the current experimental challenges?

1.4. **Homen Phukan:** Maintaining protein integrity during extraction, detergent optimization, and avoiding cross-contamination between cellular fractions remain key challenges.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.10.1*

What research questions will your laboratory focus on in the future?

1.5. **Homen Phukan:** We aim to map dynamic changes in the pathogenic bacteria proteome during host infection and find new virulence factors.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.1*

Protocol

2. Culturing *Leptospira* Strain and Harvesting Cells

Demonstrators: Suneetha Hariharan

2.1. To begin, grow *Leptospira interrogans* in Ellinghausen McCullough Johnson Harris medium supplemented with 1% BSA **[1-TXT].** After 7 days of incubation, the biological replicates of the *Leptospira* culture transfer into 50-milliliter centrifuge tubes **[2]**.

2.1.1. WIDE: Talent removing the culture flasks from the incubator. **TXT: *Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni strain Fiocruz L1-130**

2.1.2. Talent adding culture to two separate labeled centrifuge tubes.

2.2. Centrifuge the tubes at 2,500 *g* for 45 minutes at 4 degrees Celsius **[1]**. Decant the supernatant into a beaker containing disinfectant and discard it **[2]**. Reserve the cell pellets for the next step **[3]**.

2.2.1. Talent placing the tubes into a centrifuge and adjusting the settings.

2.2.2. Talent pouring supernatant carefully into a disinfectant beaker.

2.2.3. Close-up of the remaining cell pellet at the bottom of the tube.

2.3. Wash each cell pellet three times with 1 milliliter of PBS supplemented with 5 millimolar magnesium chloride **[1]**. After each wash, centrifuge at 10,000 *g* for 10 minutes at 4 degrees Celsius **[2]**.

2.3.1. Talent adding phosphate-buffered saline to each pellet and mixing gently.

2.3.2. Talent placing the tubes into a high-speed centrifuge and setting parameters.

3. Subcellular Protein Extraction from the Harvested Culture

Demonstrators: Homen Phukan, Suneetha Hariharan and Madathiparambil Gopalakrishnan Madanan

3.1. Treat the harvested *Leptospira* cell pellet with 1 milliliter of extraction buffer **[1-TXT]**. Below 25 degrees Celsius, gently mix the buffer with the pellet using a micropipette

until the solution becomes visibly turbid **[2]**.

3.1.1. Talent pipetting 1 milliliter of extraction buffer into tubes containing the harvested cell pellet. **TXT: Buffer components: 10 mM Tris-Cl (pH 8); 1% Triton X-114; 150 mM NaCl**

3.1.2. Close-up of talent mixing the pellet and buffer slowly with a micropipette as the solution becomes cloudy.

3.2. Incubate the mixture overnight at 4 degrees Celsius **[1]**.

3.2.1. Talent placing the sealed tubes in a refrigerator at 4 degrees Celsius.

3.3. Next, mix the overnight incubated extract with a micropipette **[1]**. Centrifuge the mixture at 15,000 *g* for 30 minutes at 4 degrees Celsius **[2]**. Carefully decant the supernatant into a clean vial **[3]** and retain the cell pellet for the next step **[4]**.

3.3.1. Talent pipetting to re-mix the extract in the tube.

3.3.2. Talent loading the tube into a centrifuge.

3.3.3. Talent decanting the clear supernatant into a labeled vial.

3.3.4. Talent keeping aside the pellet.

3.4. Now, add 50 microliters of buffer containing protease inhibitor cocktail to the retained pellet **[1-TXT]**.

3.4.1. Talent pipetting 50 microliters of freshly prepared buffer into the pellet tube. **TXT: Buffer: 10 mM Tris-Cl (pH 8); 8 M Urea; 1% SDS**

3.5. Vortex the tube continuously for 5 minutes **[1]**, then incubate at 4 degrees Celsius for another 5 minutes **[2]**.

3.5.1. Talent holding the tube on a vortex mixer.

3.5.2. Talent placing the vortexed tube in a 4 degrees Celsius refrigerator for incubation.

3.6. Then, centrifuge the tube at 15,000 *g* for 30 minutes at 4 degrees Celsius **[1-TXT]**.

3.6.1. Talent loading the tube into the centrifuge, adjusting the speed and temperature. **TXT: Designate the resulting supernatant as "P"**
AUTHOR'S NOTE: 3.6.1 aligned with 3.5.2

3.7. Now, adjust the Triton X-114 concentration in the extracted supernatant to 2 percent **[1]**.

3.7.1. Talent pipetting Triton X-114 into the supernatant and mixing gently.

3.8. Incubate the mixture at 37 degrees Celsius for 1 hour **[1]**, then centrifuge at 2,000 *g* for 5 minutes at 30 degrees Celsius to promote phase separation **[2]**. Allow the mixture to settle for 1 minute to complete separation **[3]**.

3.8.1. Talent placing the tube in a 37 degrees Celsius incubator.

3.8.2. Talent loading the tube into a centrifuge set to 2,000 × g at 30 degrees Celsius.

3.8.3. Close-up of tube resting at bench as two phases become visible.

3.9. To isolate the top aqueous phase, pierce the interface with a sterile syringe and transfer it to a vial **[1]**. ~~Using a micropipette, isolate the bottom detergent phase into a separate vial **[2].**~~

AUTHOR'S NOTE: Skip shots 3.9.2 to 3.10.1. 3.9.3 not listed in the script

3.9.1. Talent inserting a syringe carefully through the interface to extract the upper phase.

~~3.9.2. Shot of using a micropipette to collect and transfer the lower phase.~~

~~3.10. Finally, label the vials as aqueous phase "A" and detergent phase "D" before storing them at minus 20 degrees Celsius for future use **[1-TXT].**~~

~~3.10.1. Talent placing the tubes in a -20 degrees Celsius freezer. **TXT: Perform SDS-PAGE and immunoblotting to assess the proteins**~~

Results

4. Results

4.1. SDS-PAGE followed by immunoblotting confirmed the presence of LipL41 *(Lip-L-41)* protein in Aqueous, Detergent, and Pellet fractions, indicating its distribution across all cellular compartments **[1]**.

4.1.1. LAB MEDIA: 2B *Video editor: Highlight the lanes T, A , D, P in the upper image LipL41*

4.2. FlaB *(F-L-A-B)* protein was detected exclusively in the Pellet fraction, confirming its localization to the inner membrane-associated periplasmic space **[1].**

4.2.1. LAB MEDIA: 2B *Video editor: Highlight the lane P in the lower image FlaB*

Pronunciation Guide:

1. *Leptospira*

- **Pronunciation link:** https://www.merriam-webster.com/medical/leptospira
- **IPA:** /ˌlep.təˈspaɪ.rə/
- **Phonetic Spelling:** lep-tuh-SPY-ruhmerriam-webster.com+7merriam-webster.com+7merriam-webster.com+7merriam-webster.com+2merriam-webster.com+2merriam-webster.com+2

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2. Triton

- **Pronunciation link:** https://www.merriam-webster.com/dictionary/triton
- **IPA:** /ˈtraɪ.tən/
- **Phonetic Spelling:** TRY-tunmerriam-webster.com+2merriam-webster.com+2merriam-webster.com+2merriam-webster.com+1merriam-webster.com+1

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3. SDS-PAGE

- **Pronunciation link:** No confirmed link found
- **IPA:** /ˌɛs.diː.ɛs ˈpeɪdʒ/
- **Phonetic Spelling:** ess-dee-ess PAGE

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4. Immunoblotting

- **Pronunciation link:** No confirmed link found
- **IPA:** /ɪˌmjuː.noʊˈblɑː.tɪŋ/
- **Phonetic Spelling:** ih-MYOO-noh-blot-ing

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5. LipL41

- **Pronunciation link:** No confirmed link found
- **IPA:** /lɪp ɛl ˈfɔːrti wʌn/
- **Phonetic Spelling:** lip-ell-FOR-tee-onemerriam-webster.com

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6. FlaB

- **Pronunciation link:** No confirmed link found
- **IPA:** /flæb/
- **Phonetic Spelling:** flabmerriam-webster.com+34merriam-webster.com+34merriam-webster.com+34merriam-webster.com+1merriam-webster.com+1