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Title: Isolation of Histone from Sorghum Leaf Tissue for Top Down Mass Spectrometry Profiling of Potential Epigenetic Markers

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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. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

   ☒ Interviewees self-record interview statements. JoVE can provide support for this option.

4. **Filming location:** Will the filming need to take place in multiple locations? **No, all within walking distance**

Current Protocol Length

Number of Steps: 20
Number of Shots: 39

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Mowei Zhou:** Our protocol uncovers epigenetic markers for sorghum plant development and drought resistance. Such molecular understanding will help develop better solutions to adapt crops for extreme climates in the future.

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

1.2. **Mowei Zhou:** This protocol generates high purity histones from sorghum leaf tissue suitable for untargeted profiling of post-translational modifications by mass spectrometry.

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

Introduction of Demonstrator on Camera

1.3. **Mowei Zhou:** Demonstrating the procedure will be Shadan Abdali and Tanya Winkler, post-bachelor research associates from EMSL.

1.3.1. INTERVIEW: Author saying the above.

1.3.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Protocol

2. Nuclei isolation from Sorghum leaf sample

2.1. To begin, grind a few sorghum leaves with liquid nitrogen **[1]** and store them in a 50-milliliter centrifuge tube at minus 80 degrees Celsius **[2]**. Use approximately 4 grams of the leaf powder for histone analysis of each sample **[3]**.

2.1.1. Talent grinding sorghum leaves in liquid nitrogen. Author NOTE: poplar leaves were used to show the steps, which are identical to sorghum.

2.1.2. Talent transferring the powder in tube and storing.

2.1.3. Talent weighing 4 grams of cryo-ground powder for analysis.

2.2. Prepare extraction buffers 1, 2A and 2B as described in the text manuscript **[1]** and add a protease inhibitor tablet to extraction buffer-1 to make a final concentration of 0.2x **[2]**.

2.2.1. Prepared buffers. *Video editor: Show Table 1 on the screen.*

2.2.2. Talent adding protease inhibitor tablet to the extraction buffer-1.

2.3. Then, add 20 milliliters of the prepared extraction buffer-1 to the ground leaf powder **[1]** and gently mix or vortex for 10 minutes **[2]**. *Videographer: This step is important!*

2.3.1. Talent adding extraction buffer-1 to the leaf powder.

2.3.2. Talent mixing the solution till it dissolves completely.

2.4. Using mesh 100, filter the solution twice with 2 milliliters of extraction buffer-1 each time **[1]**. *Videographer: This step is important!*

2.4.1. Talent filtering the solution using mesh 100.

2.5. Next, centrifuge the filtrate at 3000 times g for 10 minutes at 4 degrees Celsius in a swinging bucket rotor to pellet the cell debris and large subcellular organelles **[1]**. Simultaneously, prepare extraction buffer-2A by adding protease inhibitor to a final concentration of 0.4x **[2]**.

2.5.1. Talent centrifuging the filtrate.

2.5.2. Talent adding protease inhibitor to extraction buffer-2A.

2.6. Discard the supernatant without disturbing the pellet **[1]**, then resuspend the pellet in 5 milliliters of the prepared extraction buffer-2A **[2]**. Incubate it on ice for 10 minutes with gentle mixing **[3]**. *Videographer: This step is difficult and important!*

2.6.1. Talent discarding the supernatant carefully.

2.6.2. Talent adding extraction buffer-2A to the pellet.

2.6.3. Talent incubating the solution on ice

2.7. Centrifuge the solution at 2100 times g for 15 minutes at 4 degrees Celsius in a swinging bucket rotor to pellet the debris and nuclei **[1]**. Decant the supernatant carefully without disturbing the pellet **[2]**. *Videographer: This step is important!*

2.7.1. Talent centrifuging the sample.

2.7.2. Talent decanting the supernatant carefully.

3. Isolation of nuclei from remaining cytoplasmic contaminants

3.1. Prepare extraction buffer-2B by adding the protease inhibitors to a final concentration of 1x **[1]**. Add 5 milliliters of this prepared buffer to the pellet obtained after the centrifugation **[2]**.

3.1.1. Talent adding protease inhibitor tablet to extraction buffer-2B.

3.1.2. Talent adding the extraction buffer-2B to the pellet.

3.2. Centrifuge at 2100 times g for 15 minutes at 4 degrees Celsius in a swinging bucket rotor to pellet debris and nuclei **[1]**. Simultaneously, prepare nuclei lysis buffer by adding a protease inhibitor tablet **[2]**.

3.2.1. Talent centrifuging the sample.

3.2.2. Talent adding a protease inhibitor tablet to the lysis buffer.

3.3. Carefully decant the supernatant without disturbing the pellet **[1]** and resuspend the pellet in 250 microliters of the lysis buffer **[2]**.

3.3.1. Talent decanting the supernatant.

3.3.2. Talent adding nuclei lysis buffer to the pellet.

3.4. Vortex the solution for 15 seconds at maximum speed to homogenize it and resuspend the material **[1]**, then sonicate it for 5 minutes at 4 degrees Celsius **[2]** and store it at minus 80 degrees Celsius **[3]**.

3.4.1. Talent vertexing the solution.

3.4.2. Talent sonicating the homogenized solution.

3.4.3. Talent storing the solution in minus 80 refrigerator.

4. Nuclei lysis and histone extraction

4.1. Thaw the frozen nuclei sample and add 750 microliters of 5% guanidine buffer **[1]**, then sonicate it for 15 minutes at 4 degrees Celsius **[2]**.

4.1.1. Talent adding guanidine buffer to the thawed nuclei sample.

4.1.2. Talent sonicating the solution.

4.2. Transfer the sample to a 2-milliliter tube **[1]** and spin it at 10,000 times g for 10 minutes at 4 degrees Celsius **[2]**.

4.2.1. Talent transferring the sample to 2 mL tube.

4.2.2. Talent spinning the sample.

4.3. Simultaneously prepare an ion exchange chromatography column by rinsing it with 2 milliliters of acetonitrile and 4 milliliters of water to minimize contamination on the surface **[1]**.

4.3.1. Talent rinsing ion exchange column with acetonitrile and water.

4.4. Load approximately 200 to 300 microliters of weak cation exchange resin on to the chromatography column and let the resin settle **[1]**. Wash the resin 4 times with guanidine buffer **[2]** and place the tube and column on ice **[3]**.

4.4.1. Talent adding resin to the column.

4.4.2. Talent washing the column with guanidine buffer.

4.4.3. Talent keeping the tube and column on ice.

4.5. Put the column on a 2-milliliter collection tube **[1]** and load the supernatant from the prepared sample slowly onto the resin bed without disrupting the resin **[2]**. As the solution is flowing through, load the eluent back to the top of the column 6 to 8 times to allow maximum binding to the resin **[3]**. *Videographer: This step is important!*

4.5.1. Talent putting the column on the 2 mL collection tube

4.5.2. Talent loading supernatant of the sample on the column.

4.5.3. Talent loading the eluent back to the column.

4.6. Then, load 2 milliliters of 5% guanidine buffer to wash the non-histone proteins off the column **[1]**. Elute the histone proteins with 1 milliliter of 5% guanidine buffer and collect the eluent **[2]**.

4.6.1. Talent washing non-histone proteins from the column using guanidine buffer.

4.6.2. Talent eluting the histone proteins using guanidine buffer.

4.7. Clean a 3 kilo Dalton cut-off spin filter with 500 microliters of wash solvent **[1]**, then desalt the remaining eluent using the precleaned spin filter **[2]**.

4.7.1. Talent cleaning the spin filter with wash solvent.

4.7.2. Talent desalting the remaining eluent through spin filter.

Results

5. Qualitative & quantitative analysis of the identified histones

5.1. Major histone proteins were observed in specific regions of the LC-MS feature map, indicating the success of the experiment **[1]**. This representative map shows intact, full-length histones in dashed boxes **[2]**.

5.1.1. LAB MEDIA: Figure 1a.
5.1.2. LAB MEDIA: Figure 1a. *Video editor: Emphasize the dashed boxes*

5.2. The LC-MS feature map of H2B and 16 kilo Dalton H2A proteoforms shows that both have multiple homologs with similar sequences as noted by the UniProt accession numbers **[1]**. Another group of H2A histones without the extended tails can be seen here **[2]**.

5.2.1. LAB MEDIA: Figure 1b.
5.2.2. LAB MEDIA: Figure 1c.

5.3. The N-terminal acetylation, additional lysine acetylations, and methionine oxidations in H4 histone proteins can be observed by examining the mass differences in the LC-MS feature map shown here **[1]**. There were two protein sequences identified for H3 histone proteins, H3.3 and H3.2 **[2]**.

5.3.1. LAB MEDIA: Figure 1d.
5.3.2. LAB MEDIA: Figure 1e. *Video editor: Emphasize on dense parallel lines.*

5.4. Electron transfer dissociation fragmentation was used to generate the fragmentation spectrum of the identified H3.2 proteoform **[1]**. The precursor ions in the previous and next MS1 spectra are shown here **[2]**, with their matched isotope peaks highlighted in purple **[3]**.

5.4.1. LAB MEDIA: Figure 2a.
5.4.2. LAB MEDIA: Figure 2b and c.
5.4.3. LAB MEDIA: Figure 2b and c. *Video editor: Emphasize the purple peaks*

5.5. Post-translational modifications can be localized using the sequence coverage map **[1]**.

5.5.1. LAB MEDIA: Figure 2d.

5.6. By comparing the relative abundance of the proteoforms, changes of truncated histone proteoforms specific to sample conditions were discovered **[1]**. C-terminal truncation of H4 was observed only in weeks 3 and 9 for some of the samples **[2]**.

5.6.1. LAB MEDIA: Figure 3.
5.6.2. LAB MEDIA: Figure 3 a and b. *Video editor: Emphasize weeks 3 and 9 in b.*

5.7. For H3.2, N-terminal truncated proteoforms were generally more abundant in week 10 **[1]**. In contrast, C-terminal truncated H3.2 were seen in earlier time points **[2]**. The H4 C-terminal truncated proteoforms were significantly more abundant in BTx642 *(prounce 'B-T-6-4-2')* than in RTx430 *(pronounce 'R-T-4-3-0')* **[3]**.

5.7.1. LAB MEDIA: Figure 3 c and d. *Video editor: Emphasize week 10 in d.*
5.7.2. LAB MEDIA: Figure 3 c.
5.7.3. LAB MEDIA: Figure 3 b. *Video Editor: Emphasize the red triangles.*

Conclusion

6. Conclusion Interview Statements

6.1. **Shadan Abdali:** When attempting this protocol, pay close attention to the colors of the supernatant and pellets. The color changes help identify potential problems in the grinding or lysis of the chloroplasts.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.6.1, 2.6.2.*

6.2. **Mowei Zhou:** We hope that this robust protocol for histone isolation from sorghum leaf will enable epigenetic research for other similar plants.

6.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.