FINAL SCRIPT: APPROVED FOR FILMING

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Title: Optimized Analysis of Proteins from *Xenopus* Oocytes and Embryos by Immunoblotting

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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? **Yes**

   There can be an optional shot of the film developer on the floor below the main filming location (step 4.4).

Current Protocol Length
Number of Steps: 26
Number of Shots: 55

Introduction

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

1.1. **Michael Sheets:** Over the course of an animal's lifetime, cell-fate decisions occur continually that allow for normal development and the health of the adult organism. These decisions depend on specific proteins referred to as cell-fate regulators. The goal of our research is to define the mechanisms that control the synthesis of these critical regulators.

1.1.1. 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?

1.2. **Charlotte Kanzler:** In addition to providing a detailed outline of immunoblotting in Xenopus oocytes and embryos, our protocol gives insight into challenges common to this model organism, such as antibody procurement.

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

How will your findings advance research in your field?

1.3. **Charlotte Kanzler:** Through in-depth study of the binding and repression mechanisms of Bicaudal-C, our research has helped establish a foundation of information by which to compare other translational regulators.

1.3.1. 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):

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

1.4. **Charlotte Kanzler:**

1.4.1. 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.)

1.5. **Charlotte Kanzler:**

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

Ethics Title Card

This research has been approved by the UW-Madison Institutional Animal Care and Use Committee

Protocol

2. Preparation of *Xenopus* Extract

Demonstrator: Charlotte Kanzler

2.1. To begin processing cells for immunoblotting, add 10 microliters of chilled cell lysis buffer, diluted to 1 times concentration with double deionized water, per embryo or oocyte **[1]**. Using a micropestle, homogenize the samples on ice **[2]**.

2.1.1. WIDE: Talent pipetting chilled, diluted lysis buffer into tubes containing embryos or oocytes.

2.1.2. Talent using a micropestle to homogenize the samples on ice.

2.2. Place the tubes in a benchtop centrifuge and spin the samples at 5,000 *g* at 4 degrees Celsius for 10 minutes to pellet debris, including yolk and insoluble pigments **[1]**. Transfer the supernatant to a clean 1.5-milliliter tube, ensuring that the pellet is not disturbed during transfer **[2]**.

2.2.1. Talent placing the tubes into the centrifuge and closing the lid.

2.2.2. Talent carefully pipetting the clear supernatant into a new 1.5 milliliter tube, avoiding the pellet at the bottom.

2.3. Add an equal volume of 2 times Laemmli sample buffer, supplemented with 5 percent weight by volume 2-mercaptoethanol, to the collected supernatant **[1]**. Heat the mixture at 95 to 100 degrees Celsius for 10 minutes to denature the proteins **[2]**.

2.3.1. Talent pipetting Laemmli buffer into the tube containing the supernatant.

2.3.2. Talent placing the tubes in a heat block or water bath set to 95 to 100 degrees Celsius.

2.4. Now, remove a 4 to 12 percent bis-tris SDS precast gel from its packaging **[1]** and peel off the sticker from the bottom of the gel **[2]**.

2.4.1. Talent unwrapping the precast gel from its packaging. NOTE: 2.4.1. and 2.4.2 are combined to a single shot (slated 2.4.1)

2.4.2. Talent removing the bottom sticker from the gel.

2.5. Insert the gel into the electrode assembly opposite a buffer dam **[1]** and place the entire

assembly into a vertical electrophoresis tank **[2]**.

2.5.1. Talent placing the gel into the electrode assembly, ensuring it is positioned opposite the buffer dam.

2.5.2. Talent placing the electrode assembly into the electrophoresis tank.

2.6. Then, fill both the electrode assembly and the bottom of the tank with Tris-MOPS-SDS running buffer **[1]**. Gently remove the gel comb **[2]** and pipette running buffer into the wells to eliminate bubbles and equilibrate the buffer **[3]**.

2.6.1. Talent pouring running buffer into the electrode assembly and bottom reservoir of the tank.

2.6.2. Talent carefully removing the comb from the top of the gel.

2.6.3. Talent pipetting running buffer into each well to remove bubbles.

2.7. Now, load 5 microliters of pre-stained protein standards into the first well and 10 microliters of each prepared sample into the remaining wells **[1]**.

2.7.1. Talent pipetting pre-stained protein standard into the first lane of the gel.

2.8. Place the lid on the electrophoresis tank and connect it to a power supply **[1]**. Then, set the voltage to 200 volts to initiate electrophoresis **[2]**. Stop electrophoresis once the sample buffer dye front exits the gel **[3]**.

2.8.1. Talent placing the lid on the electrophoresis tank and attaching the power cables to the power supply.

2.8.2. Show the power supply screen set to 200 V as the user initiates the run.

2.8.3. Show the dye front nearing the edge of the gel.

3. Wet Transfer of Proteins onto a Membrane

3.1. Before electrophoresis completes, begin assembling the transfer sandwich in the transfer clip **[1]**. Fill a glass baking dish with chilled transfer buffer and submerge all transfer materials in this buffer during assembly **[2]**.

3.1.1. Talent opening the transfer clip to begin assembly. NOTE: 3.1.1, 3.1.2, 3.2.1 and 3.2.2 are combined to a single shot (slated 3.1.1)

3.1.2. Talent pouring chilled transfer buffer into the glass dish and ensuring all components are submerged.

3.2. Place the transfer clip in the dish with the black half lying against the bottom, the white half pointed upward, and the clip open to the left **[1]**. Lay one or two fiber sponges flat on the black half of the transfer clip **[2]**. After cutting two pieces of 0.35-millimeter cellulose chromatography paper to size, place one on top of the sponges **[3]**.

3.2.1. Talent positioning the transfer clip inside the dish of chilled buffer.

3.2.2. Talent placing the fiber sponges flat on the black half of the clip.

3.2.3. Talent placing the first piece of cut chromatography paper over the sponges.

3.3. Once electrophoresis is complete, remove the gel from the electrophoresis tank **[1]**.

3.3.1. Talent lifting the gel cassette out of the tank and removing the gel carefully.

3.4. Using the gel cassette opening lever, carefully pry apart the gel plates while ensuring that the gel remains attached to one side **[1]**. Trim off the wells from the top of the gel using the gel releaser **[2]**.

3.4.1. Talent using the gel cassette opener to separate the gel plates gently.

3.4.2. Talent trimming off the gel wells cleanly with the gel releaser.

3.5. Place the gel, still attached to one half of the plastic cassette, onto the filter paper **[1]** and remove the remaining cassette half so the gel stays flat on the paper **[2]**.

3.5.1. Talent placing the cassette half with the gel onto the filter paper within the transfer clip. NOTE: 3.5.1 and 3.5.2 are filmed in a single shot (Slated as 3.5.1)

3.5.2. Talent lifting off the plastic cassette half, leaving the gel on the filter paper.

3.6. Next, cut a piece of 0.45-micrometer nitrocellulose membrane to match the gel's dimensions **[1]**. Remove the membrane from its protective paper, briefly wet it in transfer buffer, and carefully place it on top of the gel **[2]**.

3.6.1. Talent measuring and cutting a square of nitrocellulose membrane.

3.6.2. Talent wetting the membrane in buffer and positioning it directly on the gel surface.

3.7. Then, lay a second piece of filter paper on top of the nitrocellulose membrane **[1]**. Using a roller, gently but firmly press out all air bubbles **[2]**.

3.7.1. Talent placing a clean filter paper sheet on top of the membrane. NOTE: 3.7.1. and 3.7.2 are filmed in a single shot (slated as 3.7.1)

3.7.2. Talent rolling over the paper to remove any trapped bubbles from the stack.

3.8. Stack one or two additional fiber sponges on top of the filter paper to complete the sandwich **[1]**. Close the transfer cassette and clip it tightly to seal the assembled sandwich **[2]**.

3.8.1. Talent layering the final sponges over the assembled stack in the transfer clip. NOTE: 3.8.1 and 3.8.2 are filmed in a single shot (slated as 3.8.1)

3.8.2. Talent folding the white half of the clip over the top of the stack and locking the transfer cassette securely.

3.9. Then, insert the closed transfer sandwich into the transfer core with the clip facing upwards and ensure the black side of the clip faces the black side of the core **[1]**. Place the transfer core into the electrophoresis tank **[2]**, add an ice pack and a stir bar to the tank, ensuring the stir bar can rotate freely **[3]**.

3.9.1. Talent aligning and inserting the transfer clip into the core with the correct orientation.

3.9.2. Talent lowering the transfer core into the electrophoresis tank.

3.9.3. Talent placing an ice pack and stir bar into the tank, positioning them for optimal flow.

3.10. Fill the tank with chilled transfer buffer until the apparatus is fully submerged **[1]** and secure the lid on top **[2]**. Connect the apparatus to the power supply, match electrode colors, and set the conditions to 100 volts for 1 hour with the stir bar rotating **[3]**.

3.10.1. Talent pouring chilled transfer buffer into the tank until full.

3.10.2. Talent placing and locking the lid on top of the tank.

3.10.3. Show power supply setup with electrodes connected and settings displayed as 100 V, 1 h

4. Post-Transfer Membrane Processing and Membrane Imaging

4.1. Once the transfer is complete, carefully open the cassette and disassemble the sandwich **[1]**. Remove the nitrocellulose membrane and mark the side that was in contact with the gel **[2-TXT]**.

4.1.1. Talent unclipping and opening the transfer cassette.

4.1.2. Talent removing the membrane, marking the contact side. **TXT: Keep this side facing up for the following steps**

4.2. Then, place the nitrocellulose membrane in a small container so that it lies flat against

the bottom **[1]**. Cover the membrane completely with Ponceau stain and rock gently on a rocker for 5 to 10 minutes **[2]**.

4.2.1. Talent positioning the membrane in a small flat-bottomed container.

4.2.2. Talent pouring Ponceau stain over the membrane and placing the container on a rocker.

4.3. After pouring the Ponceau stain, rinse the membrane several times with double-deionized water until the excess stain is removed and protein bands become visible in the lanes **[1-TXT].**

4.3.1. Talent rinsing the membrane repeatedly with double deionized water while gently swirling the container. **TXT: Perform blocking, washing, and overnight antibody incubation**

4.4. Once the secondary antibody incubation and washing are complete, the membrane is ready for imaging. In a darkroom, turn on the film developer and allow it to warm up **[1]**. Cut two squares of plastic wrap large enough to fully cover the membrane **[2]**. Using forceps, place the nitrocellulose membrane face up onto the first square of plastic wrap **[3]**.

4.4.1. Talent powering on the film developer.

4.4.2. Talent cutting two large sheets of plastic wrap.

4.4.3. Talent using forceps to transfer and position the membrane face up on one sheet of wrap.

4.5. In a 1.5-milliliter tube, mix equal volumes of the enhanced chemiluminescence Luminol and Enhancer reagents **[1]**. Then, use approximately 1 milliliter of the mixture to cover most membranes **[2]**.

4.5.1. Talent pipetting and mixing equal amounts of Luminol and Enhancer reagents in a labeled 1.5 milliliter tube.

4.5.2. Talent pipettes the prepared ECL mix onto the membrane.

4.6. ~~Next, pipette the ECL reagent mixture onto the nitrocellulose membrane **[1]**.~~ Using the plastic wrap, gently manipulate the membrane to ensure the entire surface is evenly coated, and incubate for 1 minute **[1]**. NOTE: The video is edited for the deleted shot

4.6.1. ~~Talent pipetting the mixed ECL solution directly onto the membrane~~. NOTE: Not filmed

4.6.2. Talent gently folding and pressing the plastic wrap to distribute the reagent

across the membrane surface.

4.7. Then, remove excess ECL reagent by grasping the membrane with forceps and lightly shaking off the liquid **[1]**. Place the membrane face up onto the second square of plastic wrap, fold it loosely over the membrane, and tape the wrap securely into an autoradiography cassette **[2-TXT]**.

4.7.1. Talent using forceps to gently shake or blot excess reagent from the membrane. NOTE: 4.7.1 and 4.7.2 are filmed in a single shot (slated as 4.7.1)

4.7.2. Talent positioning the membrane on the second plastic wrap sheet, folding it, and taping it securely into the cassette. **TXT: Use autoradiography film in the dark room to reveal protein bands**

4.8. After desired protein visualization is achieved, align the developed film with the glow-in-the-dark markers and use them as a guide to mark the position of the pre-stained protein standards onto the film **[1]**. Once imaging is complete, carefully remove the membrane from the plastic wrap and immerse it in TBSTw to wash off residual ECL reagent **[2]**.

4.8.1. Talent using glow markers to line up the developed film and marking the protein standard positions on the film.

4.8.2. Talent removing the membrane from the plastic wrap and submerging it into a container of TBSTw.

Results

5. Results

5.1. Ponceau staining of the membrane confirmed successful protein transfer **[1].**

5.1.1. LAB MEDIA: Figure 3A.

5.2. Endogenous Bicc1 *(Bicc-One)* protein was not detected in oocyte samples but was clearly detected in stage 7 and stage 10.5 embryo samples at approximately 107 kilodaltons **[1].**

5.2.1. LAB MEDIA: Figure 3B (α-Bicc1 panel). *Video editor: Highlight the absence of bands in lane 1, and then highlight the dark bands in lanes 3 and 5 at the 100 kDa marker level*.

5.3. A smaller 70-kilodalton protein band was detected in injected samples across all stages using the Bicc1 antibody, indicating successful expression of the HA-Bicc1 C-terminal fusion **[1].**

5.3.1. LAB MEDIA: Figure 3B (α-Bicc1 panel). *Video editor: Highlight top panel, black arrowhead, compare lanes 2, 4, and 6 with lanes 1, 3, and 5*

5.4. The HA antibody detected the same 70-kilodalton band only in injected samples, confirming the specificity of the Bicc1 antibody for the fusion protein **[1].** Two high molecular weight bands, approximately 250 and 270 kilodaltons, were detected in all samples using the Cnot1 *(C-Not-One)* antibody **[2].**

5.4.1. LAB MEDIA: Figure 3B (α-HA panel). *Video editor: Highlight the black arrowhead*

5.4.2. LAB MEDIA: Figure 3B (α-Cnot1 panel). *Video editor: Highlight both thick bands around the 250 kDa marker in all six lanes.*

5.5. Expression of the 54-kilodalton Ddx6 *(D-D-X-Six)* protein was detected in all samples but was visibly reduced in stage 10.5 embryos **[1].**

5.5.1. LAB MEDIA: Figure 3B (α-Ddx6 panel). *Video editor: Highlight the strong bands in lanes 1–4 around the 50 kDa mark, and then highlight the fainter bands in lanes 5 and 6*.

1. **Immunoblotting**
   Pronunciation link: https://www.howtopronounce.com/immunoblotting
   IPA (American): /ˌɪm.jə.noʊˈblɑːtɪŋ/
   Phonetic Spelling: *im-yuh-noh-BLAH-ting*

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2. **Micropestle**
   Pronunciation link: https://www.howtopronounce.com/micropestle
   IPA (American): /ˈmaɪ.kroʊˌpɛsəl/
   Phonetic Spelling: *MY-kroh-pes-uhl*

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3. **Centrifuge**
   Pronunciation link: https://www.merriam-webster.com/dictionary/centrifuge
   IPA (American): /ˈsɛntrəˌfjuːdʒ/
   Phonetic Spelling: *SEN-truh-fyooj*

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4. **Laemmli** (Laemmli buffer)
   Pronunciation link: https://www.howtopronounce.com/laemmli
   IPA (American): /ˈlɛm.li/
   Phonetic Spelling: *LEM-lee*

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5. **Electrophoresis**
   Pronunciation link: https://www.merriam-webster.com/dictionary/electrophoresis
   IPA (American): /ɪˌlɛktrəfəˈriːsɪs/
   Phonetic Spelling: *ih-LEK-truh-fuh-REE-sis*

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6. **Nitrocellulose**
   Pronunciation link: https://www.merriam-webster.com/dictionary/nitrocellulose
   IPA (American): /ˌnaɪtroʊˈsɛljəˌloʊs/
   Phonetic Spelling: *NY-troh-sell-yuh-lohs*

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7. **Chromatography** (as in cellulose chromatography paper)
   Pronunciation link: https://www.merriam-webster.com/dictionary/chromatography
   IPA (American): /ˌkroʊməˈtɑːgrəfi/
   Phonetic Spelling: *kroh-muh-TAH-gruh-fee*

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8. **Chemiluminescence** (as in enhanced chemiluminescence reagent)
   Pronunciation link: https://www.merriam-webster.com/dictionary/chemiluminescence
   IPA (American): /ˌkɛmiˌluːməˈnɛsəns/
   Phonetic Spelling: *keh-mee-LOO-muh-nes-uhns*