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Title: Protein Extract Preparation and Co-Immunoprecipitation from *Caenorhabditis elegans*

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Author Questionnaire

1. Microscopy: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Anna Zinovyeva**: This protocol provides a step-by-step procedure for sample collection, extract preparation, and immunoprecipitation, for the confirmation of a successful protein pulldown and the detection of co-immunoprecipited proteins of interest **[1]**.

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

REQUIRED:

1.2. **Li Li**: The protein extract preparation can be performed for up to 24 samples and is compatible with a number of downstream applications, including immunoprecipitation and RNA pulldown experiments **[1]**.

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

OPTIONAL:

1.3. **Li Li**: This method can be adapted to facilitate the testing of interactions between two or more endogenous, endogenously tagged, or overexpressed *C. elegans* proteins in a variety of genetic backgrounds **[1]**.

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

OPTIONAL:

1.4. **Anna Zinovyeva**: Visual demonstration of this protocol is intended to make researchers comfortable with protein extract preparation and immunoprecipitation, hopefully encouraging those new to the techniques to use them in their research **[1]**.

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

Protocol

2. Worm Sample Collection

2.1. To collect a worm sample, first seed mixed stage or synchronized worms on solid nematode growth medium plates at the required temperature **[1-TXT]** and allow the worms to grow until the desired stage **[2]**.

2.1.1. WIDE: Talent placing worms onto plate(s)

2.1.2. Talent placing plate(s) at temperature **TEXT: See Stiernagle *et al*. and Porta-de-la-Riva *et al*. for basic *C. elegans* growth and maintenance details**

2.2. At the end of the incubation, use M9 buffer to wash the worms into a 15-milliliter conical tube **[1-TXT]** and pellet the worms by centrifugation **[2-TXT]**.

2.2.1. Talent washing worms into tube, with buffer container visible in frame **TEXT: See text for all medium and solution preparation details**

2.2.2. Talent placing tube(s) into centrifuge **TEXT: 2 min, 400 x g, RT**

2.3. At the end of the centrifugation, remove the supernatant **[1]** and wash the worms an additional 3-5 times in fresh M9 buffer per wash **[2]**.

2.3.1. Shot of cloudy supernatant, then supernatant being removed
2.3.2. Buffer being added to tube, with buffer container visible in frame

2.4. When the supernatant is no longer cloudy **[1]**, perform one final wash with double-distilled water **[2]** and transfer the loose worm pellet into a 1.5-milliliter microcentrifuge tube **[3]**.

2.4.1. Shot of non-cloudy supernatant
2.4.2. Talent adding water to tube
2.4.3. Shot of pellet, then pellet being collected, with microcentrifuge tube visible in frame

2.5. Pellet the worms with an additional centrifugation **[1]** and discard the remaining supernatant to obtain a packed worm pellet **[2]**.

2.5.1. Talent placing tube into centrifuge

2.5.2. Supernatant being discarded, then shot of pellet

3. Worm Pellet Extract Preparation

3.1. To prepare extract from the collected worm pellet, add an equal volume of ice-cold 2x lysis buffer to a recommended 300-microliter-volume pellet **[1]** and vigorously mix the resulting suspension **[2]**.

3.1.1. WIDE: Talent adding buffer to pellet, with buffer container visible in frame
3.1.2. Tube contents being pipetted or vortexed

3.2. Spin down the sample to collect the mixture at the bottom of the tube **[1]** and transfer the tube contents into a 1.5-milliliter RNase-free tube containing metal beads **[2]**.

3.2.1. Talent placing tube into centrifuge
3.2.2. Talent adding solution to tube, with bead container visible in frame *Videographer: Important step*

3.3. After capping the tube tightly, place the sample in a bead mill homogenizer at 4 degrees Celsius **[1]**, taking care that the sample is balanced inside the homogenizer **[2]**.

3.3.1. Talent placing tube into homogenizer *Videographer: Important step* NOTE: 3.3.1 – 3.4.1 all in one shot
3.3.2. Talent checking bead balance *Videographer: Important step*

3.4. Homogenize the sample at the highest speed for 4 minutes **[1]** before transferring the sample into a new 1.5-milliliter microcentrifuge tube without beads **[2-TXT]**.

3.4.1. Sample being homogenized
3.4.2. Talent adding sample to new tube **TEXT: Alternative: Use magnet to remove beads**

3.5. Spin down the sample to clarify the protein extract **[1-TXT]** and transfer the supernatant to a new 1.5-milliliter tube on ice without transferring the white, cloudy precipitate at the top of the sample **[2]**.

3.5.1. Talent placing tube into centrifuge *Videographer: Important step* **TEXT: 20 min, 19,000 x *g*, 4 °C**
3.5.2. Talent adding supernatant to tube NOTE: Use take 2 or 3. *Videographer: Important step*

3.6. Set aside 10 microliters of the clarified extract to determine the total protein concentration according to standard protocols **[1]** and immediately dilute the sample

to a 5- or 10-milligram of protein/milliliter of ice-cold 1x lysis buffer on ice **[2-TXT]**.

3.6.1. Talent adding aliquot to tube
3.6.2. Talent adding lysis buffer to tube, with lysis buffer container visible in frame NOTE: Use take 3 **TEXT: Alternative: Flash freeze in LN_2 for -80 °C-storage**

4. Immunoprecipitation

4.1. For immunoprecipitation of the protein of interest, first resuspend the magnetic beads by inversion **[1]** and transfer 150 microliters of the 50% bead-suspension into a 1.5-milliliter tube **[2]**.

4.1.1. WIDE: Talent inverting tube
4.1.2. Talent adding beads to tube

4.2. Magnetize the beads on ice against a magnetic stand for about 1 minute **[1]**. When the solution is clear, discard the supernatant **[2]**.

4.2.1. Talent placing tube onto magnetic
4.2.2. Shot of clear solution, then supernatant being aspirated

4.3. Remove the tube from the magnetic stand **[1]** and wash the beads with three, 300-microliter volumes of 1x lysis buffer **[2]**.

4.3.1. Talent removing tube and adding buffer to tube, with buffer container visible in frame NOTE: This and next shot together
4.3.2. Talent adding buffer to tube, with buffer container visible in frame

4.4. After the last wash, resuspend the beads in 150 microliters of ice-cold lysis buffer **[1]** and transfer 75 microliters of the bead slurry to 2-milligrams of the protein extract sample **[2-TXT]**.

4.4.1. Buffer being added to beads, with buffer container visible in frame *Videographer: Important/difficult step*
4.4.2. Talent adding beads to extract *Videographer: Important/difficult step* **TEXT: Hold remaining beads on ice**

4.5. After 1-hour incubation at 4 degrees Celsius with gentle agitation **[1]**, place the sample tube in the magnet on ice for about 1 minute **[2]**.

4.5.1. Sample being agitated *Videographer: Important step*
4.5.2. Talent placing tube onto magnet *Videographer: Important step*

4.6. When the beads are fully magnetized and the sample is clear **[1]**, transfer the supernatant to a new 1.5-milliliter tube without disturbing the beads **[2]**.

4.6.1. Shot of clear sample, then sample being aspirated and then being transferred to tube *Videographer: Important step* NOTE: This and next shot together
4.6.2. Talent adding sample to tube *Videographer: Important step*

4.7. Set aside 10% of the sample for Western blot analysis **[1]** and add 20 micrograms of affinity purified antibody to the precleared lysate for a 1-hour incubation at 4 degrees Celsius with gentle agitation **[2]**.

4.7.1. Talent adding sample to tube
4.7.2. Talent adding antibody to lysate

4.8. At the end of the incubation, add the remaining 75 microliters of prewashed bead suspension to the antibody-lysate mixture **[1]** for an additional 1-hour incubation at 4 degrees Celsius with gentle agitation **[2]**.

4.8.1. Talent adding beads to tube
4.8.2. Sample being agitated

4.9. At the end of the incubation, place the sample back onto the magnet for about 1 minute **[1]**.

4.9.1. Talent placing tube onto magnet

4.10. When the beads are fully magnetized and the sample is clear, wash the beads containing the immunoprecipitate three times in 450 microliters of wash buffer on ice **[1-TXT]**.

4.10.1. Talent adding buffer to tube, with buffer container visible in frame **TEXT: Optional: Collect supernatant for Western blot analysis**

4.11. After the last wash, resuspend the bead pellet in 20 microliters of 2x SDS (S-D-S)-beta-mercaptoethanol protein gel loading buffer **[1-TXT]** and boil the sample at 95 degrees Celsius for 5 minutes **[2-TXT]**.

4.11.1. Talent adding SDS-BME to pellet, with SDS-BME container visible in frame **TEXT: SDS: sodium dodecyl sulfate**
4.11.2. Talent placing tube at 95 °C **TEXT: Optional: Store denatured samples at -20 °C ≤several months**

4.12. For detection of the immunoprecipitated proteins by western blot analysis, load the

denatured protein sample onto an SDS-PAGE (page) gel without transferring the beads **[1-TXT]** and perform western blot analysis according to standard protocols, using the appropriate antibodies for the proteins of interest **[2-TXT]**.

4.12.1. Talent loading sample onto gel **TEXT: PAGE: polyacrylamide gel electrophoresis**
4.12.1B Added CU shot
4.12.2. Talent adding antibody to blot, with antibody container(s) visible in frame **TEXT: See text for western blot details**

4.13. Then detect the bands using horseradish peroxidase-based chemiluminescence **[1]**.

4.13.1. Talent combining the two HRP chemicals for the future detection of bands using HRP chemiluminescence

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
3.2., 3.3., 3.5., 4.4.-4.6.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
4.4. It is important to ensure that equal amounts of total protein are used for each immunoprecipitation when multiple IPs are performed simultaneously.

Results

5. Results: Representative *Caenorhabditis elegans* Protein Extract Co-Immunoprecipitation

5.1. The demonstrated bead mill homogenizer protocol **[1]** is comparable in total protein extraction to dounce-based methods **[2]** for the efficient extraction of nuclear **[3]** and cytoplasmic proteins **[4]**.

5.1.1. LAB MEDIA: Figures 2-4
5.1.2. LAB MEDIA: Figures 2-4 *Video Editor: please emphasize Bead Mill row in Figure 2*
5.1.3. LAB MEDIA: Figures 2-4 *Video Editor: please emphasize Dounce row in Figure 2*
5.1.4. LAB MEDIA: Figure 2-4 *Video Editor: please emphasize Figure 2*
5.1.5. LAB MEDIA: Figures 2-4 *Video Editor: please emphasize Figures 3 and 4*

5.2. In this analysis, argonaute proteins were determined to interact with members of the GW182 (G-W-one-eighty-two) protein family **[1]**, forming the microRNA-induced silencing complexes that bind to the target messenger RNAs and repress their expression **[2]**.

5.2.1. LAB MEDIA: Figure 3
5.2.2. LAB MEDIA: Figure 3 *Video Editor: please emphasize alg-1(0) lane*

5.3. The demonstrated extract and immunoprecipitation protocols can also be used to successfully recover ALG-1 (A-L-G-one) in HRPK-1 (H-R-P-K-one)-specific co-immunoprecipitates **[1-TXT]**.

5.3.1. LAB MEDIA: Figure 4 *Video Editor: please emphasize HRPK-1 band in wt IP lane* **TEXT: ALG-1: argonaute (plant) like-gene-1**

5.4. In addition, testing of the the ALG-1-AIN (A-I-N)-1 interaction in a variety of genetic backgrounds **[1-TXT]** revealed that HRPK-1 is unnecessary for the ALG-1-AIN-1 (A-I-N-one) microRNA-induced silencing complex assembly **[2-TXT]**.

5.4.1. LAB MEDIA: Figure 3 *Video Editor: please emphasize wt-hrpk-1(0) labels at top of gel* **TEXT: AIN-1: ALG-1 interaction protein**
5.4.2. LAB MEDIA: Figure 3 *Video Editor: please emphasize IP hrpk-1(0) bands* **TEXT: HRPK-1: heterogenous nuclear ribonucleoprotein (HnRNP) K homolog-1**

Conclusion

6. Conclusion Interview Statements

6.1. **Li Li**: It is important to make sure that the protein extraction and immunoprecipitation are performed at 4 degrees Celsius or on ice to maintain the stability of the samples **[1]**.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (3.3.-3.5., 4.4.-4.10.)

6.2. **Anna Zinovyeva**: This total protein extract preparation is compatible with protein immunoprecipitation and microRNA pull-down experiments using a microRNA complementary oligonucleotide and facilitates the downstream collection of both protein and RNA components **[1]**.

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