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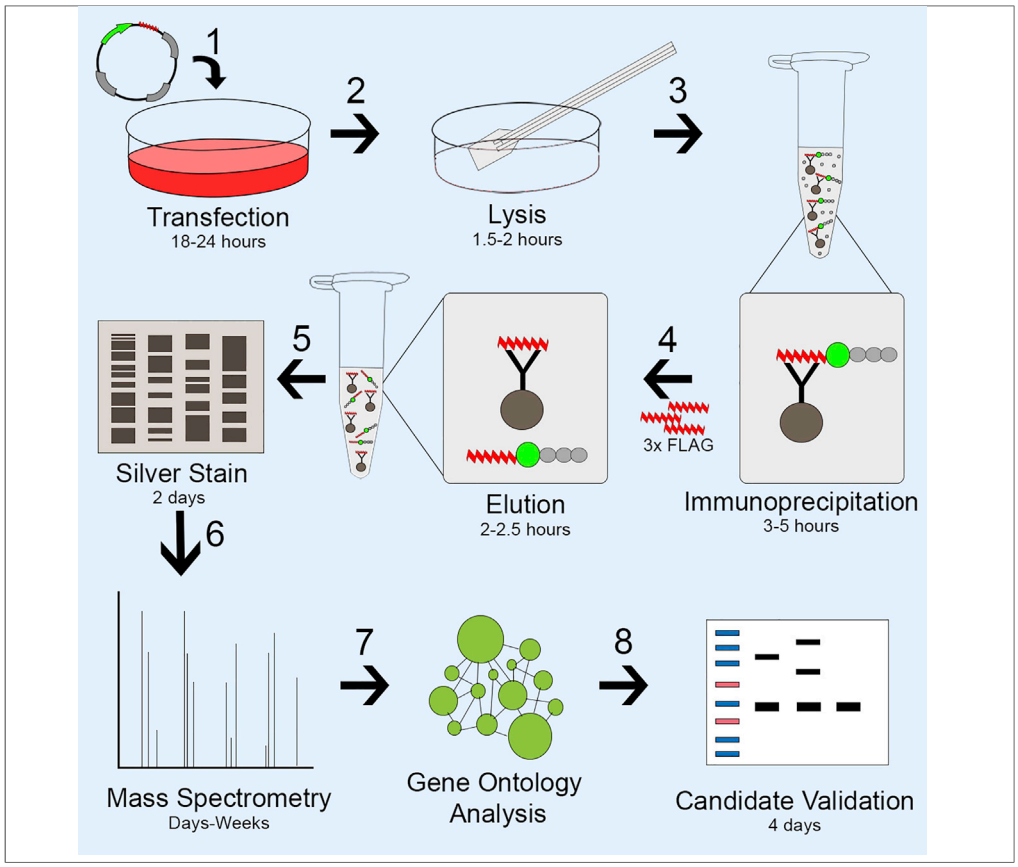
Protocol for Immuno-Enrichment of FLAG-Tagged Protein Complexes



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HIGHLIGHTS

FLAG-tagged proteins and their interactome can be immunopurified using FLAG antibodies

FLAG peptide elution enriches protein complexes and prevents elution of immunoglobulins

This protocol validated for ribonucleoprotein enrichment has broad application

Mass spectrometry and bioinformatics can reveal functions of a protein of interest

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SUMMARY

This protocol describes immunoprecipitation of proteins associated with FLAG-tagged recombinant proteins followed by mass spectrometry-based proteomics to identify the associated interactome components. FLAG epitope was chosen, because existing high-affinity monoclonal antibodies allow for sensitive immunoprecipitation and FLAG peptides permit efficient elution of protein complexes. With many commercially available FLAG tools, this protocol is highly versatile. This procedure reduces immunoprecipitation of nonspecific binding proteins. Gene ontology analyses performed following mass spectrometry-based proteomics may elucidate novel functions of proteins of interest.

For complete details on the use and application of this protocol, please refer to Valdez-Sinon et al. (2020).

BEFORE YOU BEGIN

This protocol was used in a recent publication (Valdez-Sinon et al., 2020) to isolate and identify the interactome of a protein known as Cdh1 (Fzr1) for which we hypothesized to play a role in RNA granules and protein synthesis. The results yielded an enrichment of ribonucleoprotein complexes with 36.7% of the interactome being associated with translation function and regulation. The enrichment of ribonucleoprotein complexes in the published interactome of Cdh1 demonstrate the utility in using this protocol to investigate the interactome of proteins that are known or hypothesized to interact with translational complexes. Additionally, the interactome of Cdh1 included non-translation-related proteins, suggesting that this protocol has broad applicability for proteins interacting with a FLAG-tagged protein of interest.

Prior to the experiment, prepare the buffers to be used for lysis and immunoprecipitation and diluted 3× FLAG peptide. These may be done up to one month in advance with the buffers stored at 4°C and the diluted 3× FLAG peptide at −80°C. Protease and/or phosphatase inhibitors should be added to the buffers on the day of the experiments.

Prepare Diluted 3× FLAG Peptide

Timing: 10 min

1. Dilute 3× FLAG Peptide to 5 mg/mL in 0.1 M MOPS solution pH 7.4.
2. Separate into 100 µL aliquots into microcentrifuge tubes.
3. Snap freeze on dry ice for 10 min.
4. Store at −80°C and thaw on ice before use. Avoid multiple freeze/thaws.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse anti-FLAG	Sigma	F1804
Sheep Anti-Mouse Dynabeads	Invitrogen	11031
Protein G Dynabeads	Invitrogen	10007D
Chemicals, Peptides, and Recombinant Proteins		
3× FLAG Peptide	Sigma-Aldrich	F4799
COMPLETE 50× Protease Inhibitor	Roche	05056489001
4× Laemmli Buffer	Bio-Rad	1610747
Polymag Neo	OZ Biosciences	PG60200
Opti-MEM	Gibco	31985070
10× Phosphate Buffered Saline	EMD Millipore	EM-6505-4L
Calcium Chloride	n/a	n/a
Magnesium Chloride	n/a	n/a
HEPES	n/a	n/a
Sodium Chloride	n/a	n/a
EGTA	n/a	n/a
MOPS (3-morpholinopropane-1-sulfonic acid)	n/a	n/a
Triton X-100	Fisher Scientific	AC327372500
Experimental Models: Cell Lines		
Neuro2A Cells	ATCC	CCL-131
Recombinant DNA		
FLAG-Tagged Recombinant DNA	n/a	n/a
Other		
Magnetic Plate	OZ Biosciences	MF10096
Magnetic Separation Rack	ThermoFisher	12321D
Sonicator- Sonic Dismembrator Model 100	Fisher Scientific	
Screw Cap Microcentrifuge Tubes	Sarstedt	72.607
Screw Caps For Microcentrifuge Tubes	Sarstedt	65.716.999
Cell Lifter	VWR	76036-004

Materials and Equipment

Reagent	Final Concentration	Volume
PBS/Mg/Ca	1× Phosphate buffered saline with 0.1 mM Calcium chloride and 1 mM Magnesium chloride in double distilled H_2O	1,000 mL
10× Buffer A	100 mM HEPES, 1.5 M Sodium Chloride, 10 mM EGTA, 1 mM Magnesium Chloride in double distilled H_2O, pH 7.4	1,000 mL
IP Buffer	1× Buffer A with 0.1% Triton X-100 in double distilled H_2O	500 mL
Lysis Buffer	1× Buffer A with 0.5% Triton X-100 in double distilled H_2O with 1× Complete Protease inhibitors	100 mL
3× FLAG – Peptide	Resuspended to 5 mg/mL in 0.1 M MOPS solution	50 µL aliquots

STEP-BY-STEP METHOD DETAILS

Transfection of Cells

Timing: 18–24 h

Prior to immunoprecipitation, your cells need to be transfected with the FLAG-tagged plasmid of choice. It is recommended to perform initial confirmation assays of the plasmid before scaling up for this immunoprecipitation experiment. This protocol is optimized for purification of diverse protein complexes as well as ribonucleoprotein complexes(Gokhale et al., 2012), so it is a good approach when the protein of interest is known or hypothesized to be involved in translational or other processes of mRNA regulation.

Depending on the scale of your experiment, plan and plate the number of 10 cm dishes you will need. The amount of protein in the lysate is also dependent of the abundance of the protein of interest and the respective interactors in cells. Each immunoprecipitation reaction requires 500 μg of total protein lysate. Example: 1 × 10 cm plate of Neuro2A cells at 80% confluency will give a yield of over 2 mg of total protein lysate, so sufficient for protein concentration quantification, loading the input material on the gels, and 4 immunoprecipitation reactions.

1. Have 10 cm plates of your cell line at 50% confluency.
   a. For Neuro2A cells, we seeded 2 million cells on a 10 cm dish. 16 h after seeding, the cells were at 50% confluence.
2. For each 10 cm plate, prepare a microcentrifuge tube with 800 μL of Opti-MEM.
   a. Within each microcentrifuge tube, mix 8 μg of FLAG-tagged plasmid with 8 μL of Polymag (OZ Biosciences).
      i. If multiple plates are receiving same plasmid, can scale up volumes accordingly.
   b. Let tube(s) sit for 20 min at room temperature (20°C–25°C).
3. Add DNA-Polymag complexes to cells and place 10 cm plate(s) on magnetic plate (OZ Biosciences) in incubator and let sit for 20 min.
4. Remove cells from magnetic plate and leave in incubator until ready for lysis (~18–24 h).

Note: In our experience, magnetofection allows for a high transfection efficiency of ~80% of Neuro2A cells. While we suggest a magnetofection approach for transfection, you may utilize an equivalent approach (i.e. lipofection) if it achieves high-efficiency transfection (70%–90%).

Note: Use manufacturer instructions for transection reagent if need troubleshooting.

Note: If your plasmid is fluorescently tagged, confirm successful transfection after 12–24 h with fluorescent microscopy.

Prepare Immunomagnetic Beads

Timing: 2 h or 16–18 h

In certain protocols, antibodies are initially incubated with the protein extract before applying them to beads. However, we found that adding the antibody in excess (1 μg of antibody per 30 μL of Dynabeads) directly to the beads blocks the nonspecific sites on the beads. This step is done either on the day of or day before experiment (details are below). The antibody preincubation with beads also prevents excess antibody in solution that could keep antibody-antigen complexes from binding to already saturated beads.

5. Prepare IP Buffer + FLAG antibody (Sigma F1804) master mix in a 15 mL conical.

a. For each immunoprecipitation tube, will need 500 μL of IP Buffer + 1 μg FLAG antibody.
   i. The amount of antibody needed is proportional to the amount of protein lysate used during the immunoprecipitation. As will be detailed below, this protocol uses a fixed amount of 500 μg of protein lysate in each immunoprecipitation tube.
b. Scale up accordingly to experiment.
   i. (i.e. for 10 immunoprecipitation tubes, mix 5 mL IP Buffer with 10 μg of FLAG antibody).

6. For each IP reaction, add 30 μL Sheep Anti-Mouse Dynabeads (Invitrogen 11031) to a microcentrifuge tube. Alternately, protein G Dynabeads can also be used (Invitrogen 10007D).
7. Add 500 μL of IP Buffer/FLAG antibody master mix into each microcentrifuge tube.
8. Put on end-over-end rotator either at 4°C overnight (16–18 h) or 2 h at room temperature (20°–25°C).

Note: For the immunoprecipitation tubes, it is recommended to use screw cap microcentrifuge tubes with O-rings to prevent loss of antibody solution. An end-over-end rotator ensures optimal incubation of beads with the antibody. Rotation should be gentle to prevent bead fragmentation. Avoid bead vortexing in between steps.

Note: Depending on the requirement, additional controls can be set up including: a no-antibody control (500 μL of IP Buffer with 30 μL of Dynabeads) or a control antibody against a protein that is not expressed in the lysate (such as a recombinant tag other than FLAG)(500 μL of IP Buffer, 30 μL of Dynabeads and 1 μg of nonspecific antibody).

Note: In order to obtain enough material to run a SDS-PAGE and silver stain from the same sample that will be analyzed by mass spectrometry, we suggest to obtain ~1 μg of eluted protein. This amount considers that the detection limit of silver stained protein bands is in the 5–10 ng range (Walker, 2005). Keep in mind that there may be between 20 to hundreds of proteins in the FLAG peptide eluate (Comstra et al., 2017; Gokhale et al., 2012). Moreover, the intensity of the band will be determined by the protein molecular weight and whether the protein has membrane domains or it is glycosylated. Thus, if you suspect abundant low molecular weight proteins it is better to load more material in the gel for silver stain. We can obtain 1 μg of material for silver stain analysis and mass spectrometry by having a minimum of 4–6 immunoprecipitation tubes for each experimental condition and negative control. We do not use silver stained material for band identification as depending of the silver stain protocol stained proteins do not perform well in the steps required for mass spectrometry analysis.

Cell Lysis: Preparation of Soluble Cellular Lysate

Timing: 1.5–2 h

Previously transfected cells will be lysed and protein concentration will be measured.

9. 18–24 h after cell transfection, move plates directly from the incubator onto ice.
   a. Ideally, cells will be ~80% confluency at time of lysis. Seeding confluency and/or number of plates initially seeded should be adjusted depending on doubling time of cells, expression and abundance of target proteins, and the goal of the experiment.
10. Wash plates 2× with 10 mL of PBS/Mg/Ca Buffer.
    a. For last wash, tilt plate to gather excess PBS/Mg/Ca and aspirate carefully to ensure no liquid is left on plate. This prevents dilution of the Lysis Buffer in the next step.
11. For each plate, lyse in 300 μL Lysis Buffer.
    a. Use cell scraper to lift cells off plate.
12. Move lysate into a microcentrifuge tube and sonicate for 3 × 5 s. This has been previously performed on a Fisher Scientific Sonc Dismembrator Model 100 on a power setting of 2. Ensure

tube in on ice during sonication and prevent incorporation of air bubbles. Both steps will help prevent protein degradation.

13. Keep lysates on ice for 30 min.
14. Spin lysate at 16,100 × *g* for 15 min at 4°C.
15. Move supernatant into new microcentrifuge tube.
    a. If multiple plates were transfected with same plasmid, can pool supernatant together.
16. Measure protein concentration using BCA, Bradford assay, or other suitable methods.
17. Determine the total volume of lysate and add additional Lysis Buffer to bring final concentration to 1 mg/mL.
18. Keep 50 μL of each lysate for input and keep on ice.

Pause Point: For step 13, Lysate may be left on ice for longer than 30 min. Additionally, while not ideal, lysate can be flash-frozen after step 15 and can be kept at −80°C until needed for immunoprecipitation.

⚠ CRITICAL: Ensure adequate amount of Complete protease inhibitor is added to the final lysate. Insufficient amount of protease inhibitor will result in protein degradation. Additionally, all buffers and centrifugation steps should be at 4°C to prevent protein degradation.

Immunoprecipitation of FLAG-Tagged Ribonucleoprotein Complexes and Associated Proteins

Timing: 3–5 h

Immunoprecipitation with the previously prepared immunomagnetic beads will be used to isolate your FLAG-tagged protein and its interactome.

19. Centrifuge the previously prepared tubes of Dynabeads + FLAG antibody for 1 min 16,100 × *g* at 4°C.
20. Place tubes on magnetic rack and aspirate off liquid (preferably with a gel loading tip).
21. Add 1 mL IP Buffer to each tube and rotate on end-over-end rotator for 5 min at 4°C.
22. Repeat steps 20 + 21.
23. Centrifuge tubes for 1 min at 16,100 × *g* and aspirate off liquid.
24. Add in lysates to each prepared IP tube:
    a. For out-competition controls, add in 490 μL of lysate in 10 μL of 3× FLAG Peptide (final concentration 340 μM, Sigma) per tube.
    b. For experimental conditions, add in 500 μL (at 1 mg/mL) of lysate per tube.
    c. It is recommended to have an equal amount of control and experimental tubes per experiment.
25. Let tubes of lysate + beads rotate at 4°C for 2–4 h on end-over-end rotator.
26. Place tubes on magnetic rack, and aspirate off liquid (preferably with gel loading tip).
    a. The unbound fraction may be collected and kept to check IP efficiency.
27. Do one quick wash by adding 1 mL of IP Buffer.
    a. Turn tubes up and down 10–15 times.
28. Centrifuge tubes for 1 min at 16,100 × *g* at 4°C and aspirate on magnetic rack.
29. Add 1 mL IP Buffer to each tube and put on end-over-end rotator at 4°C for 5 min.
30. Centrifuge tubes 1 min at 16,100 × *g* at 4°C and aspirate on magnetic rack.
31. Repeat steps 29+30 four times.
32. Add 1 mL IP Buffer into each tube and put on end-over-end rotator at 4°C for 10 min.
33. Centrifuge tubes for 1 min at maximum speed at 4°C and aspirate on magnetic rack.

Note: 500 μL at 1 mg/mL of lysate solution is optimal for incubation with the beads. This volume and amount ensures adequate mixing of the lysate on the end to end rotor and prevents drying of beads.

Immunoaffinity Purification of FLAG-Tagged Ribonucleoprotein Complexes

Timing: 2–2.5 h

Elution of FLAG-tagged proteins and associated complexes can be performed either with 4× Laemmli buffer or the FLAG-antigenic peptide followed by heating at 75°C. We have systematically tested different concentrations of the FLAG peptide to elute FLAG-tagged proteins and their associated interactors from the Dynabeads. We found that the minimum final concentration of the peptide required for efficient elution was 340 μM (Comstra et al., 2017; Gokhale et al., 2012). Elution with Laemmli buffer after heating at 75°C is harsher whereas using the antigenic peptide prevents the elution of immunoglobulins (IgG)(Gokhale et al., 2012). IgGs appear as prominent bands at 50 kDa and 25 kDa, potentially obscuring identification of proteins of similar molecular weights by MS/MS.

34. Prepare master mix of elution solution.
    a. For each microcentrifuge tube, need 16 μL of Lysis Buffer mixed with 4 μL of 3× FLAG peptide (340 μM final concentration). This forms the elution buffer.
    b. Scale up based on the number of total tubes. Prepare enough for 1–2 extra tubes to ensure that there is enough elution solution.
35. Following aspiration of the last wash (Step 33), add 20 μL of elution solution to each microcentrifuge tube and put on ice for 2 h.
    a. Tap the tubes to mix approximately every 15 min.
36. Centrifuge tubes at maximum speed at 4°C for 1 min.
37. Place tubes on magnetic rack and move elution solution/protein mixture into a new labeled tube.
    a. If have multiple tubes for the same condition, pool elutants into one microcentrifuge tube.
38. Add 1:1 μL of 4× Laemmli buffer to input tube(s) and heat for 5 min at 75°C or 95°C. Denaturation at 75°C prevents aggregation of membrane proteins, if these are suspected in the sample.
39. Flash freeze elutants and heated input on dry ice and store at −80°C. It is important to flash freeze the proteins since proteins can continue to be modified/oxidized after denaturation and freezing at −20°C.

Note: The next step will be concentrating the eluants in order to run on a SDS-PAGE gel. If you are skipping that step, you can instead add 1:1 μL of 4× Laemmli buffer to elutants and heat for 5 min at 75°C and flash freeze and store at −80°C.

⚠ CRITICAL: Avoid vortexing Dynabeads at any point as the process will fragment the beads.

Pause Point: Inputs/elutants may be kept at −80°C for up to a month before moving on to the next steps. Ideally, pooled elutants should be frozen for 16–18 h at −80°C before TCA precipitation (see below) to get optimum concentration.

Trichloroacetic acid (TCA)/Ethanol Precipitation

Timing: 1 h

As each immunoprecipitation tube will yield 20 μL of elutant, pooling together multiple replicates (>40 μL) will exceed the volume of standard SDS-PAGE gels. Furthermore, running larger volumes (>50 μL) on gels results in artifacts such as "smiling" that are visible by protein stains. TCA precipitation allows us to run the entire sample in a single well of a SDS –PAGE gel – either for a protein stain or an immunoblot.

40. Measure volume of each individual sample.
41. Add 1.96 μL sodium deoxycholate (5 mg/mL stock) per 100 μL of each sample.
    a. Example: for 200 μL sample, add 3.92 μL of sodium deoxycholate.
42. Add 10% TCA and incubate on ice for 20 min.
    a. Example: For above, would have 203.92 μL following step 41, so would then add 20.39 μL of TCA.
    b. Incubate on ice for 20 min
43. Centrifuge at 16,100 × *g* at 4°C for 15 min.
44. Carefully remove supernatant (should see very small, white pellet).
45. Add cold acetone (same volume as TCA added in step 42).
46. Centrifuge at 16,100 × *g* at 4°C for 10 min.
47. Carefully remove supernatant.
48. Leave tube open with a Kimwipe over the top and air dry for 5 min at room temperature (20°C–25°C).
49. Add 10 μL 4× Laemmli buffer.
50. Solution will most likely turn yellow due to acidity imparted by residual TCA in the pellet. Add 10 μL of 1 M Tris at pH 9.0. This should return the color of the solution to a dark blue.
51. Add another 10 μL of 4× Laemmli buffer.
52. Boil for 5 min at 75°C.

Note: Make sure you do not over-dry the pellet. This would make dissolution of the pellet extremely difficult. If the pellet is large and difficult to dissolve vortex the pellet and boil the sample repeatedly till a homogenous solution is obtained.

Biochemical Analysis of Immunopurified Complexes

Timing: 2 days

To confirm sufficient protein is enriched for mass spectrometry and that the immunoaffinity purification of FLAG-tagged protein complexes has worked, perform an immunoblot and a protein stain, such as with silver stain or Coomassie.

53. Load and run inputs and concentrated samples on an SDS-PAGE gel in duplicate.
    a. One set of gels are analyzed by protein stain (Silver stain or Coomassie).
        i. The protein stain confirms the amount of protein immunoprecipitated is sufficient for Mass Spectrometry analysis.
    b. The second gel is transferred to a PVDF membrane for immunoblot analysis using the FLAG antibody.
    c. An example of such an analysis is depicted in Figure 1 with confirmation of enrichment of FLAG-tagged protein and effective out-competition with 3× FLAG Peptide on Western blot (Figure 1A). Silver stain (Figure 1B) demonstrates sufficient enrichment of FLAG-protein complexes with nonspecific binding proteins identified in the 3× FLAG Peptide control. With Mass Spectrometry, the nonspecific binding proteins can be identified and excluded from the interactome of the protein of interest.

Mass Spectrometry

Timing: weeks

Mass Spectrometry Services were contracted from MS-Bioworks (https://www.msbioworks.com) to identify proteins enriched from the immunoprecipitation.

54. Submit eluted immunopurified complexes for Mass Spectrometry analysis.

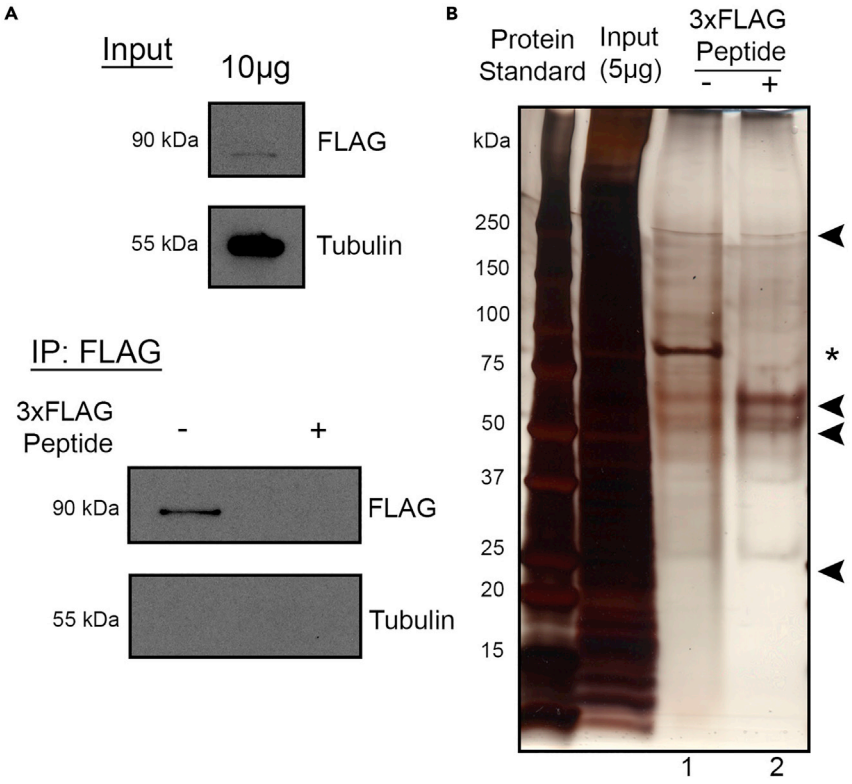

Figure 1. Immunoaffinity Enrichment of FLAG-Tagged Complexes

(A) Western blot demonstration of FLAG-protein into N2A cells (input, top) and enrichment of FLAG-tagged protein following immunoprecipitation (bottom). Out-competition control with 3X FLAG Peptide co-incubation prevents immunopurification of FLAG-tagged protein complexes.

(B) Silver Stain from same experiment as panel A shows an enrichment of putative FLAG-interacting proteins. Asterisk indicates major FLAG-tagged species (lane 1) that was out-competed by FLAG peptide (lane 2). Arrowheads indicate non-specific binding proteins in out-competition control (lane 2).

a. Label free or isobaric tagging approaches can be used to identify hits from background.

55. Peptides enriched two-fold compared to negative control can undergo DAVID Analysis (https://david.ncifcrf.gov) to categorize the interactome based on gene ontology.
    a. In previous publications, the DAVID analysis was run with medium classification stringency and threshold p value of $\leq$0.005 (Valdez-Sinon et al., 2020).
    b. For additional guidance on DAVID analysis and selecting parameters, utilize the following references: Huang et al., 2007, 2009.

EXPECTED OUTCOMES

This protocol will allow for the enrichment of FLAG-protein complexes and was optimized for the purification of ribonucleoprotein complexes. The use a FLAG-tagged construct permits the use of FLAG peptide elution of immunoprecipitated complexes, which reduces nonspecific interactions that may be falsely identified with elution by Laemmli buffer.

Mass spectrometry following immunoprecipitation will identify the interactome of your FLAG-tagged protein of interest. DAVID Functional Annotation Bioinformatics Microarray Analysis (https://david.ncifcrf.gov) on the interactome will classify interacting proteins based on biological processes, molecular function, or cellular compartment. Identification of interacting proteins as well as shared functions/localization among interactors may give insight into novel functions of a

protein of interest. Use of this protocol will allow for the robust identification of translational complexes, including initiation factors and ribosomes.

LIMITATIONS

While this protocol aims to minimize false identification of nonspecific proteins as interacting with a protein of interest (i.e. with FLAG peptide-co incubation as a negative control and FLAG peptide elution), it is possible that some nonspecific proteins are still misidentified. It is critical to follow up on any mass spectrometry results with confirmatory immunoprecipitation followed by western blotting. Additionally, as this protocol relies on transfection of an exogenous FLAG-protein, results from the mass spectrometry may not represent endogenous biological conditions. It is important to note that this method is primarily used to identify potential interactors of a given protein in a high throughput and efficient manner and there is a likelihood of obtaining false-positive results. Therefore, we highly recommend following up this protocol with a workflow paradigm involving independent verifications involving biochemical methods (immunoblots, fractionations, and reciprocal immunoprecipitations), alternate genetic tools, immunofluorescence, and activity assays.

TROUBLESHOOTING

Problem 1

Transfection efficiency is low.

Potential Solution

Increase the amount of DNA used during initial transfection or refer to manufacturer instructions of transfection agent for additional steps. Alternatively, try another transfection method such as lipofection or transduction with virus.

Problem 2

Protein concentration from lysed cells is less than 1 mg/mL.

Potential Solution

Confirm cells are at 80% confluency prior to lysis. If not, cells may need to be plated at a higher confluency prior to transfection.

If cell confluency is not the issue, try use less Lysis Buffer OR do not dilute lysate prior to taking protein concentration.

Problem 3

Insufficient protein eluted for mass spectrometry.

Potential Solution

This issue can be addressed by scaling up the preparation and its controls. It is advised to maintain the ratio of beads, to antibody, to cellular extract added to the beads, to wash volumes, and to elution. We recommend to scale up by increasing the number of microtubes for mass spectrometry. For example, one microcentrifuge tube reaction may be sufficient for silver stain, but 10 identical tubes may be needed for mass spectrometry. We pool these after the elution step. We discourage scaling up reactions into one large reaction with bigger volumes, reagents, and samples as this approach increases background and loss of material.

Problem 4

There is no protein visualized by western blot or silver stain following TCA precipitation.

Potential Solution

This might happen due to a) insufficient amount of protein used for immunoprecipitation or b) transient nature of interactions. In this case, insufficiency may be due to low or poor expression of the

bait protein and/or low expression of the corresponding interacting protein(s). In case there is insufficient protein, we can scale up the process using more than 20 IP reactions (Gokhale et al., 2012). Alternately if the interaction between the FLAG-tagged protein and its associated complexes is transient, we can use a chemical crosslinker (for example DSP (Gokhale et al., 2016; Zlatic et al., 2010)) to stabilize the interactions prior to immunoaffinity purification.

Additionally, the FLAG-epitope approach may not work for certain proteins of interest. This protocol may be then adapted and optimized for other recombinant epitope tags, such as myc, HA, His, or GFP along with a corresponding peptide or protein for elution. Alternatively, this approach can be attempted without an exogenous tag through the use of an antigenic peptide of your protein of interest allowing the identification of endogenous interactomes (Comstra et al., 2017; Gokhale et al., 2016; Ryder et al., 2013; Salazar et al., 2009).

Problem 5

After mass spectrometry there are too many potential background proteins.

Potential Solution

This problem can be addressed with curated databases of contaminant proteins associated to magnetic bead isolations (Comstra et al., 2017). See www.crapome.org (Mellacheruvu et al., 2013).

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Gary J. Bassell (gbassel@emory.edu).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

The study did not generate any unique datasets or code.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

A.N.V. and A.G. contributed to the writing of the manuscript; V.F. and G.J.B. edited the manuscript. Figures generated by A.N.V. Initial optimization by A.G. Lab infrastructure and resources by G.J.B. and V.F.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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