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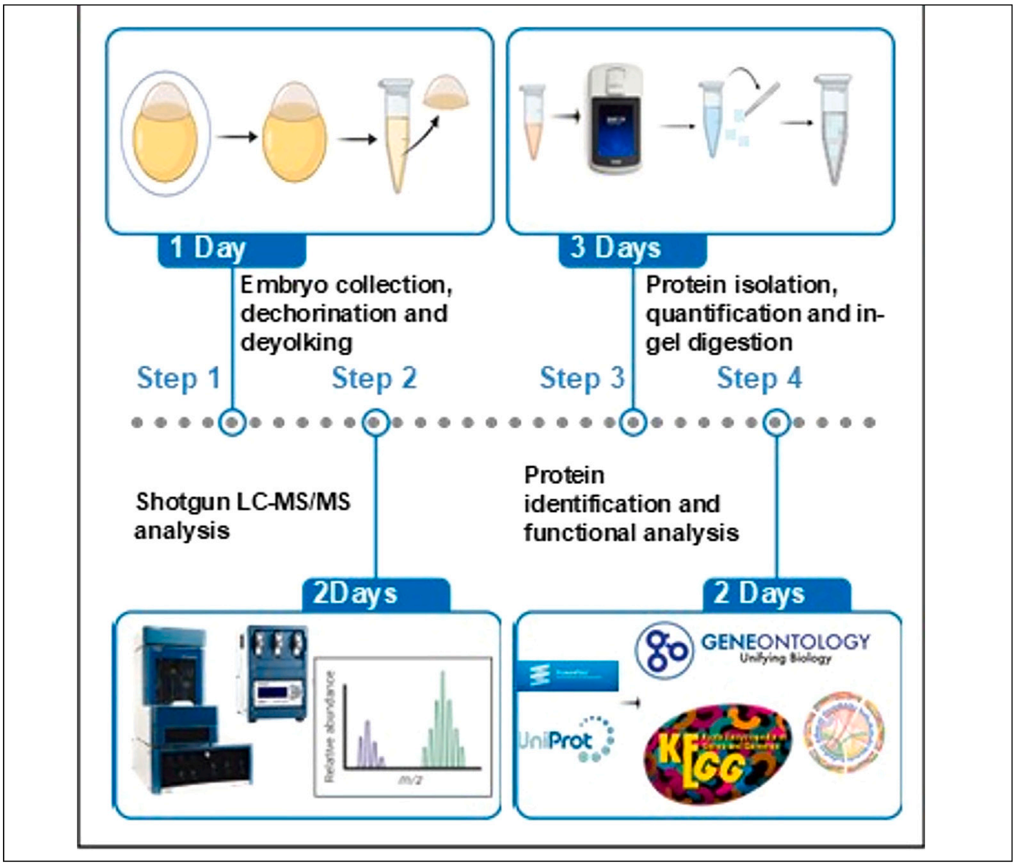
Protocol

Protocol for identification of proteins from deyolked zebrafish embryos



Zebrafish is a key model for studying vertebrate development and human diseases, but proteomic data during embryogenesis are limited due to interference from egg yolk proteins. Here, we present a protocol for the isolation, identification, and analysis of proteins from zebrafish embryos. We describe steps for dechorionation, deyolking, protein extraction, SDS-PAGE, and trypsin digestion. We then detail procedures for peptide separation using liquid chromatography-tandem mass spectrometry (LC-MS/MS), identification via ProteinPilot, and functional analysis with Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology.

Kathiresan Purushothaman, Saraphina Dianne Tneo Rwei Qing, Yong Xin Ee, ..., Shubha Vij, Qingsong Lin, Igor Babiak

purusothaman1981@gmail.com (K.P.)
igor.s.babiak@nord.no (I.B.)

Highlights

Steps for deyolking to remove yolk proteins for proteomic analysis

Procedures for isolating proteins from yolk-rich embryos of amphibians and birds

Guidance on analyzing protein expression during early embryogenesis in vertebrates

Instructions for identifying and quantifying proteins using LC-MS/MS

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Protocol

Protocol for identification of proteins from deyolked zebrafish embryos

Kathiresan Purushothaman,[1,2,3,4,8,9,*] Saraphina Dianne Tneo Rwei Qing,[2] Yong Xin Ee,[5] Nithiyakala Ananthakrishnan,[6] Shanmugam Harshiktha,[7] Shubha Vij,[2] Qingsong Lin,[5] and Igor Babiak[1,*]

[1]Genomics Group, Faculty of Biosciences and Aquaculture, Nord University, 8049 Bodø, Norway

[2]School of Applied Science, Republic Polytechnic, 9 Woodlands Avenue 9, Singapore 738964, Singapore

[3]Department of Preclinical Sciences and Pathology, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Ås, Norway

[4]Tropical Futures Institute, James Cook University Singapore, 149 Sims Drive, Singapore 387380, Singapore

[5]Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543, Singapore

[6]Department of Biological Sciences, Mohamed Sathak Hamid College of Arts and Science for Women, Sakkarakottai 623536, India

[7]Madha Medical College & Research Institute, Chennai 600 128, India

[8]Technical contact

[9]Lead contact

*Correspondence: purusothaman1981@gmail.com (K.P.), igor.s.babiak@nord.no (I.B.)
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SUMMARY

Zebrafish is a key model for studying vertebrate development and human diseases, but proteomic data during embryogenesis are limited due to interference from egg yolk proteins. Here, we present a protocol for the isolation, identification, and analysis of proteins from zebrafish embryos. We describe steps for dechorionation, deyolking, protein extraction, SDS-PAGE, and trypsin digestion. We then detail procedures for peptide separation using liquid chromatography-tandem mass spectrometry (LC-MS/MS), identification via ProteinPilot, and functional analysis with Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology.

For complete details on the use and execution of this protocol, please refer to Purushothaman et al.[1]

BEFORE YOU BEGIN

This protocol outlines the steps for isolating and analyzing proteins from zebrafish embryos, specifically addressing the challenges posed by the presence of egg yolk proteins. To overcome the limitations imposed by the abundance of yolk proteins in zebrafish embryos, we developed a deyolking protocol that significantly improves protein detection. As demonstrated in our previous study, the deyolking procedure results in 2–4 times more proteins identified across developmental stages (cleavage, oblong, bud) compared to non-deyolked embryos (Figures 1A and 1B). This enhanced detection enables a clearer understanding of the proteome during zebrafish embryogenesis.

Aquaculture is vital for global food security, providing a sustainable way to meet growing seafood demand.[2–11] Zebrafish, *Danio rerio*, is commonly used in laboratory research due to its well-annotated genome, transparent embryos, small size, and short generation time.[12–14] While primarily focused on zebrafish, this protocol can also be adapted for other species with polylecithal eggs such as amphibians, reptiles, birds and other aquaculture species, where the removal of

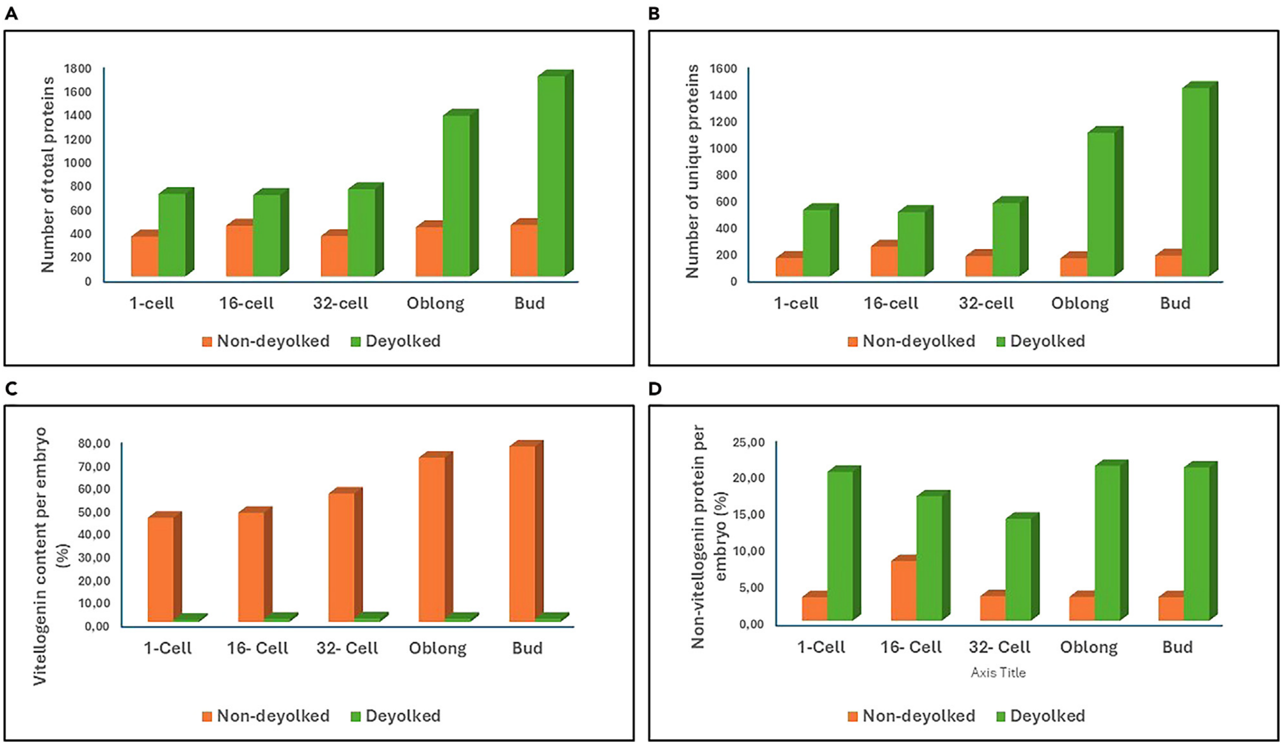

Figure 1. Impact of deyolking on protein identification and composition in zebrafish embryos
(A) shows the total number of proteins identified from both non-deyolked and deyolked embryos.
(B) presents the number of unique proteins identified in non-deyolked and deyolked embryos.
(C) compares the relative content of vitellogenins in non-deyolked versus deyolked zebrafish embryo samples across developmental stages.
(D) illustrates the relative content of non-vitellogenin proteins in deyolked versus non-deyolked zebrafish embryo samples, focusing on proteins shared between the two protocols.

yolk proteins is crucial for proteomic studies. Additionally, it may be useful in studying developmental stages in other vertebrates, offering insights into protein expression dynamics during early embryogenesis.

Institutional permissions

This experiment was done according to the Norwegian Regulation on Animal Experimentation (The Norwegian Animal Protection Act, No. 73 of 20 December 1974) which was certified by the National Animal Research Authority, Norway, General License for Fish Maintenance and Breeding no. 17.

Sample collection

Timing: Variable

1. Embryo collection (Figure 2).
   a. Collect embryos from natural spawning of adult zebrafish for two variants:
      i. Non-deyolked (intact) embryos.
      ii. Deyolked embryos.
   b. Stage and monitor embryos according to Kimmel et al.,[15] which describes the developmental stages of zebrafish.
   c. Collect embryos at five different developmental stages at 28.5°C:
      i. 1-Cell (0:30 h post-fertilization (hpf)).
      ii. 16-Cell (1:30 hpf).
      iii. 32-Cell (1:45 hpf).

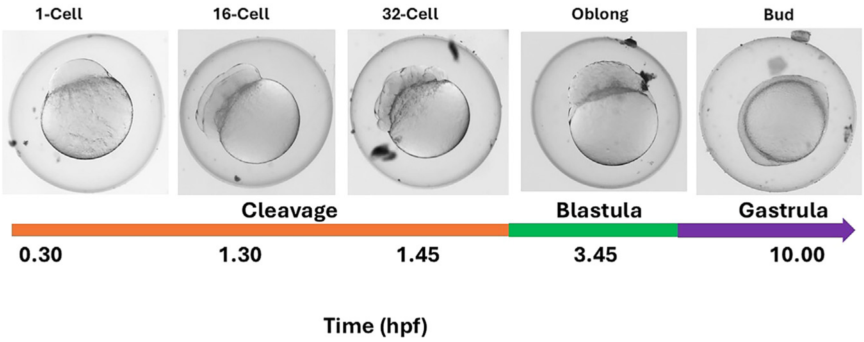

Figure 2. Developmental stages of zebrafish embryos sampled in this study
hpf, hours post-fertilization at 28.5°C.

  iv. Oblong (3:45 hpf).
  v. Bud (10:00 hpf).
d. Snap-freeze the non-deyolked (intact) embryos in liquid nitrogen.
e. Store embryos at −80°C.

Note: The zebrafish used in this experiment are of AB line.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Protease inhibitor cocktail	Thermo Scientific, Rockford, IL, USA	Cat#78429
2x SDS loading dye	Bio-Rad	Cat#1610737
Coomassie Brilliant Blue R-250	Bio-Rad	Cat#1610400
10% polyacrylamide gel	Bio-Rad	Cat#3450053
Pronase	Sigma-Aldrich, St. Louis, MO, USA	Cas#9036-06-0
Methyl methanethiosulfonate (MMTS)	Sigma-Aldrich, St. Louis, MO, USA	Cat#208795
Tris(2-carboxyethyl) phosphine (TCEP)	Sigma-Aldrich, St. Louis, MO, USA	Cat#C4706
Critical commercial assays		
Qubit Protein Assay Kit	Invitrogen	Cat#Q33211
Experimental models: Organisms/strains		
Zebrafish (*Danio rerio*)		ZFIN ID: ZDB-GENO-960809-7
Software and algorithms		
ProteinPilot 5.0 software Revision 4769	AB SCIEX	https://sciex.com/products/software/proteinpilot-software
UniProt	N/A	https://www.uniprot.org/
Panther	Panther14.0, 2018_04	https://pantherdb.org/webservices/go/overrep.jsp
KAAS - KEGG Automatic Annotation Server	N/A	https://www.genome.jp/kegg/kaas/
KEGG mapper	N/A	http://www.genome.jp/kegg/tool/map_pathway2.html
Other		
VirTis BenchTop K freeze dryer	Warminster, USA	
NanoLC Ultra −425	Eksigent, Dublin, CA, USA	N/A
TripleTOF 5600+	AB SCIEX	Part#5025900
ChiPLC-nanoflex system	Eksigent, Dublin, CA, USA	Part#950-00070
ProteoCol C18P	Trajan	Part#PDPN-1036-G
4%–20% Mini-PROTEAN TGX Precast protein gels	Bio-Rad, Hercules, California, USA	Cat#4561094

MATERIALS AND EQUIPMENT

Deyolking buffer (store at 37°C for up to 30 days)

Reagent	Final concentration	Stock concentration	Volume to add to 50 mL
NaCl	55 mM	5 M	550 μL
KCl	3.6 mM	2 M	90 μL
$NaHCO_3$	1.25 mM	1 M	62.5 μL
MilliQ water	–	–	49.43 mL

SDS lysis buffer (store at 37°C for up to 30 days)

Reagent	Final concentration	Stock concentration	Volume to add to 1 mL
Sodium dodecyl sulphate (SDS)	1%	10%	100 μL
Triethylammonium bicarbonate buffer (TEAB)	0.5 M	1 M	500 μL
Protease inhibitor cocktail	1 x	100 X	10 μL
MilliQ water	–	–	390 μL

Coomassie blue staining solution (store at 37°C for up to 30 days)

Reagent	Final concentration	Stock concentration	Volume to add to 1 mL
Coomassie Brilliant Blue R-250	–	–	0.25 g
Methanol	50%	100%	50 mL
Acetic acid	10%	100%	10 mL
MilliQ water	–	–	40 mL

STEP-BY-STEP METHOD DETAILS

Dechorionation and deyolking

Timing: 30–45 min

This step removes the chorion and yolk of the egg to allow better detection of less abundant proteins and improve Liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based shotgun proteomics analysis.

1. Removing the chorion from the eggs:
   a. Transfer the embryos into a Petri dish containing phosphate-buffered saline (PBS) supplemented with 1.0 mg/mL Pronase.
   b. Incubate the Petri dish content on a shaking incubator set at low speed for 5 min at 37°C.
   c. Wash embryos with PBS at least five times or until no visible chorion fragments remain.
2. Removing the yolk from the embryos:
   a. Transfer the dechorionated embryos to 1.5 mL tubes containing 1.0 mL of deyolking buffer.
   b. Disrupt the embryos by pipetting them repeatedly through a 100 μL pipette tip.
   c. Invert the tube several times to mix the contents gently.
   d. Centrifuge at 13,000 x g for 1 min at 4°C.
   e. Discard the supernatant containing the yolk.
   f. Re-suspend the pellet in fresh deyolking buffer and repeat steps 2b–2e for two additional times.
   g. Re-suspend the pellet in 10 mM Tris-HCl (pH 7.4).
   h. Centrifuge again at 13,000 x g for 1 min at 4°C.
   i. Discard the supernatant.
   j. Snap-freeze the pellet in liquid nitrogen.
   k. Store at −80°C.

Protein extraction of non-deyolked (intact) and deyolked embryos

Timing: 1 day

This step is crucial for isolating proteins from both intact and deyolked embryos, enabling downstream proteomic analyses. Proper extraction ensures that proteins are solubilized and preserved for accurate quantification and characterization.[16]

3. Lysis of embryos:
   a. Add 100 μL of SDS lysis buffer to the tubes containing embryos.
   b. Vortex-mix the tubes thoroughly to ensure complete mixing.
   c. Incubate the samples at 90°C for 30 min to facilitate protein denaturation.
   d. Cool the samples on crushed ice for 5 min.
   e. Incubate the samples at 37°C for 5 min to ensure complete solubilization of SDS, then cool on crushed ice for 5 min. Centrifuge at 13,000 x g for 20 min at 4°C to pellet any debris.
   f. Transfer the supernatant, which contains the solubilized proteins, to a new tube.
   g. Freeze-dry the samples using the VirTis BenchTop K freeze dryer at −80°C for 18 h to preserve the proteins for transport to our collaborator lab.
4. Protein quantification:
   a. Dissolved protein samples in MilliQ water.
   b. Quantify the protein concentration using the Qubit Protein Assay Kit and Qubit 3.0 Fluorometer, following the manufacturer’s instructions.

Polyacrylamide gel electrophoresis

⏱ Timing: 1 day

One-dimensional gel electrophoresis allows visualization of protein profiles, enabling researchers to evaluate protein separation and integrity (Figure 3).[1]

5. Sample preparation:
   a. Mix equal volumes of protein samples with 2x SDS loading dye.
   b. Denature the samples by incubating at 95°C for 10 min.

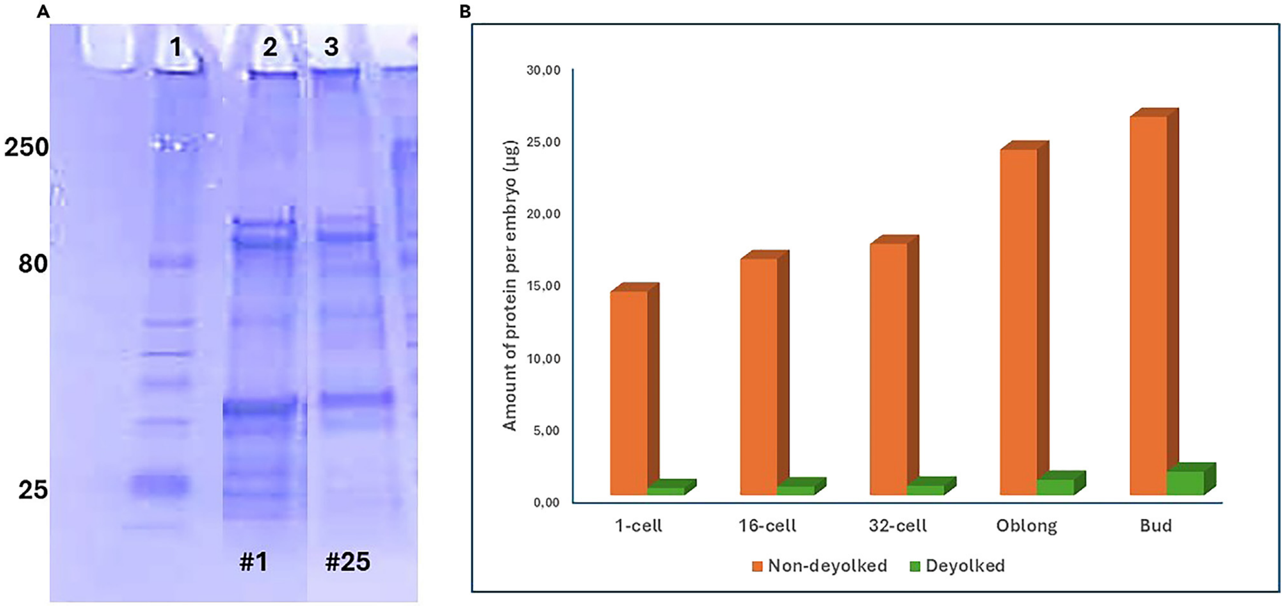

Figure 3. SDS-PAGE analysis of protein separation and yield from non-deyolked and deyolked zebrafish embryos across developmental stages
(A) show the SDS-PAGE protein separation, with lane 1 containing the protein ladder, lane 2 containing protein from a 1-cell stage non-deyolked embryo (1 embryo used), and lane 3 containing protein from a 1-cell stage deyolked embryo (25 embryos used).
(B) presents the protein yield comparison between non-deyolked and deyolked embryos across five developmental stages.

6. Gel electrophoresis:
   a. Load the samples onto 4%–20% Mini-PROTEAN TGX Precast Protein Gels in SDS running buffer.
   b. Run for 1 h at 90 V.
7. Gel staining:
   a. Wash the gel with deionized water for 10 min to remove excess buffer.
   b. Stain the gel with Coomassie Brilliant Blue R-250 for 20 min to visualize proteins.
   c. Prepare a de-staining solution by mixing 40% methanol and 10% acetic acid.
   d. De-stain the gel for 18 h in the de-staining solution at 22°C to enhance protein visibility.

In-gel protein digestion for protein identification using mass spectrometry

Timing: 2 days

The protein lysate is first polymerized in gel before the gel is processed for proteomics analysis.[17,18]

8. Gel polymerization and protein fixation:
   a. For each sample, prepare the polyacrylamide gel mixture by combining (10% APS, TEMED and 0.375 M Tris-HCl, pH 8.8 buffer).
   b. The APS and TEMED initiate the polymerization reaction.
   c. Take 30 μg of protein sample and add it to the polyacrylamide solution prepared in Step 8.a in a 1.5 mL microcentrifuge tube. Make a total volume of 100 μL with water.
   d. Immediately mix the solution to ensure homogeneity and avoid premature polymerization.
   e. Allow gel to harden completely in a 1.5 mL microcentrifuge tube.
   f. Fix the gel with 50% methanol and 12% acetic acid, for 30 min at 22°C to stabilize the proteins within the gel.
   g. Using a clean scalpel, cut the gel into small pieces (1 mm^3) and place the gel pieces into a 1.5 mL microcentrifuge tube.
9. Gel washing and dehydration:
   a. Add 500 μL of 50 mM ammonium bicarbonate (ABC)/50% acetonitrile (ACN) to immerse the gel pieces.
   b. Vortex-mix and incubate for 5 min.
   c. Using a fine micropipette tip, remove the solution without removing the gel pieces.
   d. Repeat steps a-c 3 times.
   e. Add 500 μL of 100% ACN to the gel pieces and incubate for 3 min.
   f. Using a fine micropipette tip, remove the solution without removing the gel pieces.
10. Reduction and alkylation of proteins:
   a. Add 400 μL of freshly prepared 5 mM Tris(2-carboxyethyl) phosphine (TCEP) in 50 mM ABC, making sure to cover the gel fully. Incubate for 1 h at 57°C.
   b. Using a fine micropipette tip, remove the TCEP solution without removing the gel pieces.
   c. Add 500 μL of 100% ACN to the gel pieces and vortex-mix.
   d. Using a fine micropipette tip, remove the ACN.
   e. Add 120 μL of 10 mM methyl methanethiosulfonate (MMTS) in 50 mM ABC. Incubate for 1 h at 22°C with occasional vortex-mixing.
   f. Using a fine micropipette tip, remove the MMTS solution without removing the gel pieces.
   g. Wash the gel pieces by adding 500 μL of 50 mM TEAB and vortex-mixing. Incubate for 5 min at 22°C.
   h. Using a fine micropipette tip, remove the solution.

Note: We use methyl methanethiosulfonate (MMTS) for cysteine alkylation due to its fast reactivity and ability to form stable methylthio groups, preventing disulfide bond reformation. MMTS also reduces the risk of overalkylation artifacts compared to iodoacetamide, making it a suitable choice for our proteomic analysis.

11. Dehydration and re-swelling of gel pieces:
    a. Add 500 µL of 100% ACN to the gel pieces and vortex-mix. Incubate for 5 min at 22°C.
    b. Using a fine micropipette tip, remove the ACN without removing the gel pieces.
    c. Add 500 µL of 50 mM TEAB. Incubate for 5 min at 22°C.
    d. Using a fine micropipette tip, remove the TEAB solution without removing the gel pieces.
    e. Dehydrate the gel again by adding 500 µL of 100% ACN to the gel pieces and vortex-mix. Incubate for 5 min at 22°C.
    f. Using a fine micropipette tip, remove the ACN without removing the gel pieces.
    g. Speedvac dry the gel pieces in a vacuum centrifuge for 5 min.
12. Trypsin digestion of proteins in gel pieces:
    a. Add 12.5 ng/µL trypsin in 50 mM TEAB to completely cover the gel and incubate for 30 min at 4°C.
    b. Using a fine micropipette tip, remove excess trypsin solution.
    c. Add 40 µL of 50 mM TEAB. Incubate for 18 h at 37°C.
13. Extraction of peptides:
    a. Cool the gel sample to 22°C.
    b. Collect the peptide solution in a microcentrifuge tube by centrifuging at 6000 x g for 10 min and taking the supernatant.
    c. Add 200 µL of 50 mM TEAB, vortex-mix and incubate for 5 min at 22°C.
    d. Centrifuge at 6000 x g for 10 min and combine the supernatant into the same microcentrifuge tube with the peptide solution from step b.
    e. Add 200 µL of 5% formic acid (FA)/50% ACN, vortex-mix and incubate for 5 min at 22°C.
    f. Centrifuge at 6000 x g for 10 min and combine the supernatant into the same microcentrifuge tube with the peptide solution from step b.
    g. Add 200 µL of 100% ACN, vortex-mix and incubate for 5 min at 22°C.
    h. Centrifuge at 6000 x g for 10 min and combine the supernatant into the same microcentrifuge tube with the peptide solution from step b.
    i. Speedvac dry the peptide solution in a vacuum centrifuge.
    j. Reconstitute the peptides with 30 µL of 0.1% formic acid.
    k. Estimate the peptide concentration by measuring the absorbance at 214 nm, using Nanodrop.

LC-MS/MS setup

Timing: ~2 h per sample run

This step is essential for the separation, identification, and quantification of peptides from complex biological samples. By using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), researchers can obtain detailed information on the peptide composition of the samples, aiding in protein identification and quantification.

14. Peptide separation using Eksigent nanoLC Ultra and ChiPLC-nanoflex system in Trap-Elute configuration:
    a. Lyophilize the samples and add 30 µL of the dissolution buffer to rehydrate.
    b. Desalt the samples with a Sep-Pak tC 18 µL Elution Plate to remove impurities.
    c. Reconstitute the samples with 20 µL of 2% acetonitrile and 0.05% formic acid to prepare for loading.
    d. Load 5 µL of each sample on a 20 µm × 0.5 mm trap column for initial separation.
    e. Elute the samples on a 75 µm × 15 cm analytical column for further separation.
15. Online LC parameters:
    a. For routine protein profiling of gel samples, a 60-min gradient is sufficient for separating the peptides. For reverse-phased LC, the mobile phase A and B are 0.1% FA in water and 0.1% FA in ACN respectively.

   b. On an Eksigent NanoLC-425, inject 1 µg of peptides into a ProteoCol C18P (3 µm 120 Å, 300 µm × 10 mm; Trajan) trap column, and load at a flow rate 4 µL/min for 6 min using 100% mobile phase A.
   c. To separate and elute the peptides, use the following gradient:
      i. 5–15% B for 30 min.
      ii. 15–30% B for 30 min.
      iii. 30–90% B for 3 min.
      iv. 90 B for 10 min.
      v. 90–5% for 2 min.
      vi. Hold at 5% B for 15 min.
   d. Run solvent blank between sample injections to prevent and assess sample carry-over.
16. Mass spectrometry parameters:
   a. Acquired Information-dependent acquisition (IDA) MS using a TripleTOF 5600+ system (AB SCIEX, Massachusetts, US) under positive ionization mode, in the mass range of 350–1250 m/z at high sensitivity mode with an accumulation time of 250 ms.
   b. Select the 20 most abundant precursors between charge +2 to +5 for fragmentation and MS/MS analysis in the mass range of 100–1800 m/z, with an accumulation time of 100 ms.
   c. Use a dynamic exclusion of 12 s and rolling collision energy for MS/MS analysis.

Data analysis

Timing: ~2 h

This step facilitates the identification and quantification of proteins through computational analysis of the mass spectrometry data.

17. Software and database search:
   a. Use ProteinPilot 5.0.2 software (AB SCIEX) for protein identification.
   b. Use Paragon database search algorithm (5.0.0.0.4767) and the integrated false discovery rate (FDR) analysis function.
   c. Download the species-appropriate protein database in FASTA format from Uniprot. Add the common Repository of Adventitious Proteins (cRAP) into the protein.fasta file.
   d. Set the search parameters as follows:
      i. Sample Type: Identification.
      ii. Cysteine Alkylation: Methylthio (MMTS).
      iii. Digestion: Trypsin.
      iv. Instrument: TripleTOF 5600.
      v. Special Factors: Gel-based ID.
      vi. Species: None.
      vii. ID Focus: Biological Modification.
      viii. Database: Uniprot Zebrafish proteome.
      ix. Search Effort: Thorough ID.
      x. FDR Analysis: Yes.
   e. Conduct the MS/MS spectra search against a decoy database to estimate FDR for peptide identification.
   f. Ensure the decoy database consists of reversed protein sequences from the UniProt zebrafish database.
   g. Perform FDR analysis on the dataset, considering only peptides identified with a confidence interval ≥95%.
   h. Document the identified peptides for subsequent analysis and verification.

KEGG and Gene Ontology functional pathways analysis

Timing: Approximately 1–2 days (depending on the number of proteins and complexity of the analysis)

This step involves analyzing the functional pathways associated with the identified proteins to understand their biological roles. KEGG and Gene Ontology (GO) analyses provide insights into the metabolic pathways and biological processes the proteins are involved in, facilitating the interpretation of experimental data.

18. Protein identification from deyolked and non-deyolked samples:
    a. Perform KEGG analysis on proteins identified from deyolked and non-deyolked samples.
    b. Submit FASTA files to the KEGG Automatic Annotation Server (KAAS) at https://www.genome.jp/kegg/kaas/ for KEGG Orthology (KO) assignments.
    c. Map KEGG pathways by submitting obtained KO numbers to the KEGG mapper web server at http://www.genome.jp/kegg/tool/map_pathway2.html.
19. Gene Ontology (GO) Annotation:
    a. Obtain GO annotation results and pathways for differentially expressed proteins using Panther.
    b. Use the web conversion tool (https://biodbnet-abcc.ncifcrf.gov) to convert unmapped UniProt Accession IDs to ZFIN IDs.
    c. Utilize the Biomart web tool to convert unmapped ZFIN IDs to Gene stable IDs and manually identify unmapped IDs by gene names.

EXPECTED OUTCOMES

The expected outcomes from this zebrafish protein isolation and analysis protocol include a significant increase in protein identification. Our deyolking procedure significantly increased protein yield, with a 3.1-fold higher protein count in the 1-cell stage and a 2.5-fold higher count in the high stage, compared to the previous method. Deyolking increases the number of proteins across all developmental stages compared to non-deyolked samples; approximately 2-fold increase in the cleavage stages and over 3-4-fold in the later stages. This process eliminates 10%–30% of proteins Figures 1A and 1B).

When comparing proteins shared between non-deyolked and deyolked samples, vitellogenin (yolk protein in vegetal pole) representation in the deyolked samples reduced (36–58 times) Figure 1C and 3B), while non-vitellogenin proteins (proteins from animal pole) increased (2–6 times) (Figure 1D), depending on the developmental stage.[1] This improvement in data quality is anticipated to provide a clearer profile of the embryonic proteome by reducing interference from abundant yolk proteins, thereby allowing for a more accurate representation of less abundant proteins.

Additionally, removing the yolk proteins and analyzing Gene Ontology (GO) and KEGG pathways, the protocol yields valuable insights into functional pathways and biological processes active during zebrafish embryogenesis, particularly those related to cellular organization, cell cycle regulation, and mitochondrial functions. Gene Ontology analysis revealed unique enrichments in RNA splicing, nuclear transport, cell surface receptor signaling pathway, DNA replication, and mitochondrial organization (Refer Figure 4, Table 2, Supplementary File S4, S5 and S6 at Purushothaman et al. 2019).[1] SDS-PAGE results demonstrate the effectiveness of the deyolking protocol, yielding a cleaner gel profile with more distinct protein bands from deyolked samples compared to non-deyolked ones (Figure 3).

Moreover, researchers can adapt the methods for other polylecithal species, such as amphibians, reptiles, and birds, providing a versatile tool for proteomic studies across various vertebrate models.

Ultimately, this protocol aims to enhance understanding of the molecular mechanisms governing vertebrate development, facilitating further investigations into human diseases modeled in zebrafish.

LIMITATIONS

The protocol for zebrafish protein isolation and analysis, while effective, has several limitations that should be considered. Firstly, although the dechorionation and deyolking process generally improves the identification of proteins by reducing yolk contamination, it results in the loss of specific proteins. For example, our study found that approximately 30% of proteins were lost at cleavage stages and 1% at oblong and bud stages.[1] This issue has been noted in other research, where the impact of protein depletion from deyolking was not adequately addressed, potentially skewing results.[1] Gene Ontology (GO) analysis revealed that these lost proteins are involved in critical biological processes, such as translation, protein folding, and mitochondrial organization, as well as molecular functions.[1]

Additionally, the overall procedure, involving multiple steps from deyolking to proteomics analysis, is time-consuming. This may limit the throughput of experiments, especially when large sample sizes are required. Furthermore, the protocol primarily focuses on early developmental stages, which may not capture the full spectrum of the proteome throughout zebrafish development. Lastly, while LC-MS/MS is a powerful tool for protein identification, it may not always achieve complete coverage of the proteome, particularly for low-abundance proteins or those that are challenging to ionize.

In summary, while the protocol enhances protein identification and provides valuable insights into zebrafish embryogenesis, researchers should be cautious about the potential loss of proteins and consider validating findings with parallel analyses of both deyolked and non-deyolked samples.

TROUBLESHOOTING

Problem 1

Protein precipitation during de-yolking.

Possible reasons

- Inadequate removal of yolk proteins or incomplete solubilization of proteins.

Potential solution

- Ensure thorough and careful centrifugation during the de-yolking step. If protein precipitation continues, increase the centrifugation time or adjust the speed to further remove yolk proteins.

Optimize the lysis buffer composition, ensuring it is suited for zebrafish embryos and effectively solubilizes proteins.

Problem 2

Inconsistent protein recovery post-de-yolking.

Possible reasons

- Incomplete separation of yolk proteins from the embryo extract.

Potential solution

Ensure the proper technique for separating the yolk proteins. For optimal yolk removal, use a higher-speed centrifugation step, or apply sequential washing with a denaturing buffer to remove excess yolk proteins.

Problem 3

Reduced protein yield after extraction.

Possible reasons

- Inadequate lysis conditions or insufficient disruption of the tissue.

Potential solution

- Confirm that the lysis buffer is prepared correctly and applied at the recommended temperature. Use a homogenizer or bead mill for more effective tissue disruption, especially for challenging tissues such as zebrafish embryos.

Problem 4

Contamination from yolk proteins affecting proteomic analysis.

Possible reasons

- Incomplete removal of yolk proteins during de-yolking.

Potential solution

- Ensure proper removal of yolk proteins during the de-yolking step. Use a more thorough centrifugation protocol or additional purification steps such as filtering or protein precipitation.

Problem 5

Incomplete cysteine alkylation.

Possible reasons

- Insufficient MMTS concentration or inadequate reaction time.

Potential solution

- Double-check the MMTS concentration and reaction time. If alkylation is still incomplete, increase the reaction time or adjust the reagent concentration based on the protocol.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources or reagents should be directed to and will be fulfilled by the lead contact, Kathiresan Purushothaman (purusothaman1981@gmail.com).

Technical contact

Technical questions on executing this protocol should be directed to and will be answered by the technical contact, Kathiresan Purushothaman (purusothaman1981@gmail.com).

Materials availability

This study did not generate new unique reagents.

Data and code availability

The raw data supporting the current study have not been deposited in a public repository because further studies are in progress but are available from the corresponding author upon request: Kathiresan Purushothaman (purusothaman1981@gmail.com).

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AUTHOR CONTRIBUTIONS

Conceptualization, I.B. and Q.L.; methodology, K.P., Y.X.E., N.A., and S.H.; writing, K.P., S.D.T.R.Q., Y.X.E., and N.A.; funding acquisition, I.B., K.P., and Q.L.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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