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Analyzing the Translatome of Lymphatic and Venous Endothelial Cells In Vivo via Translating Ribosome Affinity Purification (TRAP)

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Abstract

Zebrafish are a powerful model for investigating vascular and lymphatic biology due to their genetic tractability and optical transparency. While translating ribosome affinity purification (TRAP) has been widely applied in other systems, its application in zebrafish has remained limited. Here, we present an optimized TRAP protocol for isolating ribosome-associated mRNAs from endothelial cells in vivo, without the need for cell dissociation or sorting. Using a novel transgenic zebrafish line, which expresses HA-tagged Rpl10a under the *mrc1a* promoter, we enriched actively translating endothelial transcripts. Differential expression analysis revealed robust upregulation of vascular and lymphatic genes including *flt4*, *kdrl*, and *lyve1b*. This approach captures the endothelial cell translatome with high specificity and offers a robust platform for investigating the molecular mechanisms of endothelial biology under genetic, environmental, or toxicological perturbations.

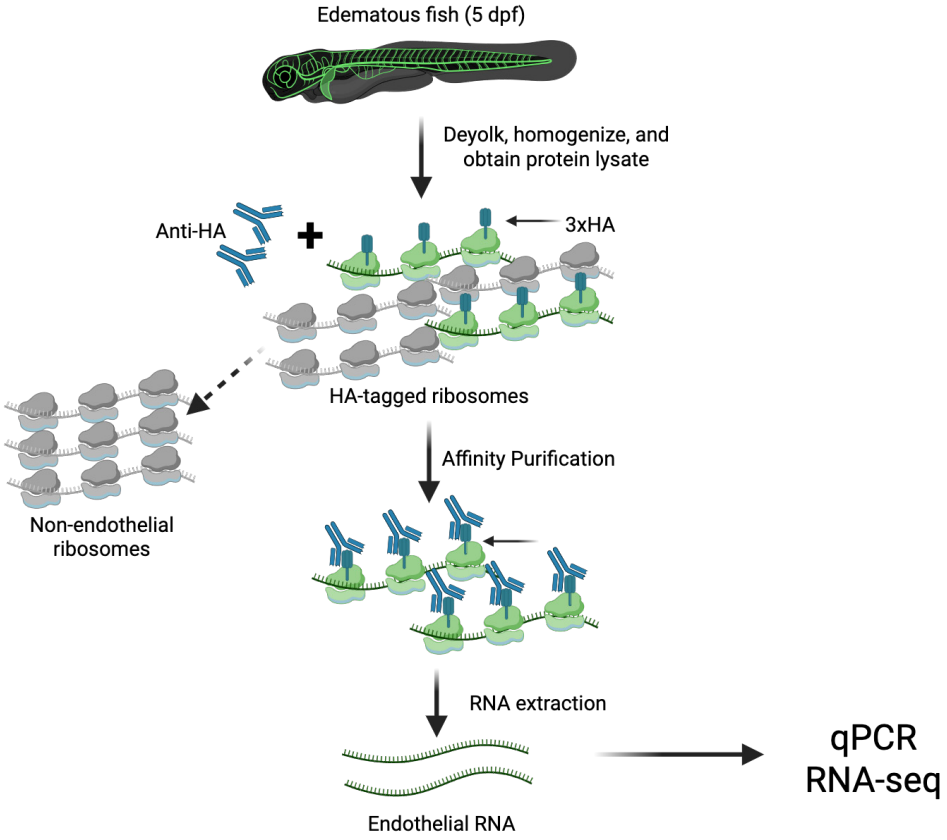
Key features

- Permits in vivo isolation of the endothelial ribosome without cell sorting.
- Optimized TRAP protocol to analyze lymphatic and venous endothelial gene expression in zebrafish.
- Utilizes a stable transgenic zebrafish line.
- Compatible with real-time quantitative PCR (qPCR) and next-generation sequencing (RNA-seq).

Keywords: TRAP, Endothelial, Translatome, Ribotag, Ribosome, Lymphatic, Venous, Zebrafish

This protocol is used in: Scientific Reports (2025), DOI: 10.1038/s41598-025-92970-1

Graphical overview



Graphical overview of the translating ribosome affinity purification (TRAP) protocol. Transgenic RiboTag zebrafish are deyolked and homogenized to generate whole-tissue lysates. Anti-HA antibodies are added to the lysate to selectively bind HA-tagged endothelial ribosomes. Magnetic Dynabeads conjugated to the antibody Fc domain enable affinity purification of the HA-tagged ribosomes using a magnetic stand. Unbound, non-endothelial ribosomes remain in the supernatant, while the Dynabead–αHA-ribosome–mRNA complex is subjected to RNA extraction to isolate actively translated endothelial RNA. This protocol provides an in vivo snapshot of cell type–specific translational activity.

Background

The blood and lymphatic systems form an integrated vascular network that delivers oxygen and nutrients, removes metabolic waste, and maintains fluid balance and homeostasis. These networks are composed of endothelial cells, whose functions are tightly regulated under both physiological and pathological conditions [1,2]. Disruption of endothelial regulation can impair fluid balance, resulting in fluid accumulation and tissue edema. In response to edemagenic signals in the microenvironment, lymphatic and venous endothelial cells activate molecular programs that promote edema clearance [3].

Zebrafish have emerged as a valuable animal model for studying vascular development due to their optical transparency, genetic tractability, and evolutionary conservation of endothelial gene function [4–7]. In our recent study, we utilized live imaging of early-stage zebrafish larvae exposed to abrupt salinity changes to induce osmotic stress and tissue edema. This model revealed increased lymphangiogenesis and remodeling of the lymphatic vasculature during edema resolution, characterized by proliferation of lymphatic progenitors and expansion of the primary lymphatic network [3].

Although endothelial responses to edema are generally initiated at the transcriptional level, post-transcriptional mechanisms, including translational regulation, likely contribute to dynamic changes in gene expression [8,9]. Transcriptome profiling tools such as microarrays and RNA sequencing from total RNA provide insights into RNA abundance, but they do not reflect the state of translation activity [10,11]. Since protein synthesis is determined by mRNAs engaged with actively translating ribosomes, profiling the translatome offers a more accurate representation of functional gene expression.

Conventional transcriptome analyses rely on fluorescence-activated cell sorting (FACS) of labeled endothelial cells, which requires mechanical and chemical dissociation into single-cell suspensions [12,13]. However, this process can disrupt native cell–cell and cell–matrix interactions, potentially altering gene expression and introducing artifacts. Translating ribosome affinity purification (TRAP) using the RiboTag strategy provides a powerful alternative for cell type–specific isolation of ribosome-associated mRNAs in vivo, bypassing the need for cell sorting [14–20]. The rapid, non-disruptive nature of TRAP minimizes ex vivo artifacts and preserves in vivo gene expression profiles.

To enable endothelial-specific TRAP in zebrafish, we developed a transgenic line *Tg(mrc1a:egfp-2a-rpl10a-3xHA)*^{y723}, expressing hemagglutinin (HA)-tagged Rpl10a and eGFP via a viral 2A peptide under control of the endothelial-specific *mrc1a* promoter [3]. Following osmotic challenge, larvae display vascular remodeling and enable the isolation of actively translating mRNAs from lymphatic and venous endothelial cells. Translatome analysis revealed upregulation of key endothelial genes such as *flt4*, *kdrl*, and *lyve1b* [3]. Coupling TRAP with RNA sequencing allows for comprehensive identification of genes involved in endothelial responses to edema.

This protocol describes an optimized TRAP workflow for profiling the endothelial translatome in edematous zebrafish. It provides a robust, reproducible method for capturing dynamic gene expression changes during edema formation and resolution.

Materials and reagents

Biological materials

1. Stable transgenic zebrafish line: *Tg(mrc1a:egfp-2a-rpl10a-3xHA)*^{y723}

Reagents

1. Invitrogen Dynabeads™ Protein G (ThermoFisher, catalog number: 10004D)
2. Direct-zol™ RNA MicroPrep (ZymoResearch, catalog number: R2060)
3. 100 µg/mL cycloheximide (Sigma, catalog number: C7698)
4. Protease inhibitor cocktail (Sigma, catalog number: P8340)
5. Pierce™ Bradford Protein Assay kit (ThermoFisher, catalog number: 23200)
6. 1 mM DTT (Sigma, catalog number: 646563)
7. 200 units/mL RNAsin (Promega, catalog number: N2115)
8. 1 mg/mL heparin (Sigma, catalog number: H3393-10KU)
9. 10% NP-40 (Roche, catalog number: 11-332-473-001)
10. Anti-HA antibody (Abcam, catalog number: ab9110)
11. TRIzol (Ambion Life Technologies, catalog number: 15596026)
12. Glycogen RNA-grade (ThermoFisher, catalog number: R0551)
13. High-Capacity cDNA Reverse Transcription kit (Applied Biosystem, catalog number: 4374966)
14. TaqMan Fast Advanced Master Mix (Applied Biosystem, catalog number: 4444557)
15. Phosphate-buffered saline (PBS), pH 7.4 (Fisher, catalog number: BP2438)
16. Pronase from *Streptomyces griseus* (20 mg/mL) (Sigma, catalog number: 10165921001)
17. Ethyl alcohol 200 proof (Sigma, catalog number: E7023-1L)
18. Chloroform HPLC-grade (Fisher, catalog number: C606SK-1)
19. Sodium chloride (NaCl) (Sigma-Aldrich, catalog number: S3014-1KG)
20. Potassium chloride (KCl) (Fisher Chemical, catalog number: P217-500)
21. Sodium bicarbonate ($NaHCO_3$) (Fisher Chemical, catalog number: S233-500)
22. Calcium chloride ($CaCl_2$) (MP Biomedicals, catalog number: 193819)
23. Magnesium chloride ($MgCl_2$) (Invitrogen, catalog number: AM9530G)
24. Calcium nitrate hydrate [$Ca(NO_3)_2{\cdot}xH_2O$] (Alfa Aesar, catalog number: 44515-14)
25. Magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H_2O$) (Sigma-Aldrich, catalog number: 230391-25G)
26. Tris pH 8.5 (Teknova, catalog number: 21327)
27. Tris-HCl pH 7.4 (Teknova, catalog number: T1074)
28. Molecular grade water (H_2O) (Corning, catalog number: 46-000-CM)
29. HEPES (Fisher Bioreagents, catalog number: BP310-500)

Solutions

1. Deyolking buffer (see Recipes)
2. Deyolking wash buffer (see Recipes)
3. Homogenization buffer (HB) (see Recipes)
4. Homogenization buffer+ (HB+) (see Recipes)
5. High salt buffer (HSB) (see Recipes)
6. High salt buffer+ (HSB+) (see Recipes)
7. Hypertonic salt solution (3× Danieau buffer) obtained from 10× Danieau buffer stock (see Recipes)
8. High-capacity RT mix for cDNA synthesis (see Recipes)
9. TaqMan Real-Time PCR (see Recipes)

Recipes

1. Deyolking buffer

Reagent	Final concentration	Quantity or volume
1 M NaCl	55 mM	5.50 mL
1 M KCl	1.9 mM	0.19 mL
1 M $NaHCO_3$	1.25 mM	0.125 mL
H_2O	n/a	94.185 mL
Total	n/a	100 mL

Store at 4 °C.

2. Deyolking wash buffer

Reagent	Final concentration	Quantity or volume
1 M NaCl	110 mM	11.00 mL
1 M KCl	3.5 mM	0.35 mL
1 M $CaCl_2$	2.7 mM	0.27 mL
1 M Tris (pH 8.5)	10 mM	1.00 mL
H_2O	n/a	87.38 mL
Total	n/a	100 mL

Store at 4 °C.

3. Homogenization buffer (HB)

Reagent	Final concentration	Quantity or volume
1 M Tris-HCl (pH 7.4)	50 mM	5.00 mL
1 M KCl	100 mM	10.00 mL
1 M $MgCl_2$	12 mM	1.20 mL
10% NP-40	1%	10 mL
H_2O	n/a	73.80 mL
Total	n/a	100 mL

Store at 4 °C.

4. Homogenization buffer+ (HB+)

Reagent	Final concentration	Quantity or volume
DTT	1 mM	10 µL
Protease inhibitor cocktail	0.01×	100 µL
RNAsin	200 units/mL	50 µL
Cycloheximide	100 µg/mL	50 µL
Heparin	1 mg/mL	100 µL
HB (see Recipe 3)	n/a	9.69 mL
Total	n/a	10 mL

Freshly prepared on ice.

5. High salt buffer (HSB)

Reagent	Final concentration	Quantity or volume
1 M Tris (pH 7.4)	50 mM	5.00 mL
1 M KCl	300 mM	30.00 mL
1 M $MgCl_2$	12 mM	1.20 mL
10% NP-40	1%	10.00 mL
H_2O	n/a	53.80 mL
Total	n/a	100 mL

Store at 4 °C.

6. High salt buffer+ (HSB+)

Reagent	Final concentration	Quantity or volume
DTT	1 mM	10 µL
Protease inhibitor cocktail	0.01×	100 µL
RNAsin	200 units/mL	50 µL
Cycloheximide	100 µg/mL	50 µL
Heparin	1 mg/mL	100 µL
HSB (see Recipe 5)	n/a	9.69 mL
Total	n/a	10 mL

Freshly prepared on ice.

7. Hypertonic salt solution (3× Danieau buffer) obtained from 10× Danieau buffer stock

Reagent	Final concentration	Quantity or volume
580 mM NaCl	58 mM	10.00 mL
6.71 mM KCl	0.7 mM	10.43 mL
8.22 mM $MgSO_4$	0.4 mM	4.87 mL
8.53 mM $Ca(NO_3)_2$	0.6 mM	7.03 mL
50 mM HEPES	10 mM	20.0 mL
H_2O	n/a	47.67 mL
Total	n/a	100 mL

Store at 4 °C.

8. High-capacity RT mix for cDNA synthesis

Component	Quantity or volume
10× RT buffer	2.0 µL
25× dNTP Mix	0.8 µL
10× RT random primers	2.0 µL
MultiScribe™ reverse transcriptase	1.0 µL
RNase inhibitor	1.0 µL
Nuclease-free H_2O	3.2 µL
RNA template (100 ng)	10 µL
Total per reaction	**20 µL**

Volumes per reaction. Store at -20 °C.

9. TaqMan real-time PCR

Component	Quantity or volume
TaqMan Fast Advanced Master Mix (2×)	5.0 µL
TaqMan target gene primers FAM (20×)	0.5 µL
TaqMan reference gene primers VIC (20×)	0.5 µL
Nuclease-free H_2O	2.0 µL
cDNA (1:5)	2.0 µL
Total per reaction	**10 µL**

Volumes per reaction. Store at -20 °C.

Laboratory supplies

1. 1.5 mL microcentrifuge tubes (ABDOS, catalog number: P10202)
2. RNase-free elution tubes (Invitrogen, catalog number: AM12480)
3. VWR disposable pestles (VWR, catalog number: 47747-358)
4. SureOne sterile pipette tips (FisherBrand, catalog number: 02-707-442)

Equipment

1. Magnetic Eppendorf stand (ThermoFisher, catalog number: 12321D)
2. Sorvall™ Legend™ Micro 21R Microcentrifuge 4 °C microfuge (ThermoFisher, catalog number: 75002445)
3. 4 °C Gentle Eppendorf tube rotator (Fisher, catalog number: 11-676-342)
4. Zymo-DR Duet kit (ZymoResearch, catalog number: R2060)
5. Labnet centrifuge (Thomas Scientific, catalog number: 1160W29)
6. Vortex (Fisher, catalog number: 88-882-011)
7. Zeiss Stemi 305 microscope (Zeiss, catalog number: 435063-9020-100)
8. Biovortexer (BioSpec, model: 1083MC)
9. NanoDrop (Thermo Scientific, catalog number: 13400518)
10. Confocal laser scanning microscope (Zeiss, model: LSM880)
11. Quantitative PCR machine (Applied Biosystems, model: Quant Studio 7 Flex)

Software and datasets

1. GraphPad Prism Software, version 9.2.0

Procedure

Day 1
A. Experimental animal setup

1. Set up breeding tanks with an equal number of male and female *Tg(mrc1a:egfp-2a-rpl10a-3xHA)*^{y723} zebrafish. To synchronize embryo collection at a defined developmental stage, place a divider in the tank to separate males and females overnight. Although both homozygous and heterozygous transgenic embryos can be used, homozygous transgenic animals allow for better yield.

Day 2
B. Collect embryos/larvae

1. In the morning, collect fertilized eggs and maintain them at 28.5 °C under standard zebrafish husbandry conditions, in accordance with institutional animal care guidelines.
2. Collect embryos at the desired developmental stage. The mrc1a promoter drives vascular transgene expression prior to 24 h post-fertilization (hpf) and continues to adulthood [6].

Note: This protocol was optimized using embryos at 3 days post-fertilization (dpf).

C. Dechorionate embryos

1. Add pronase (20 mg/mL stock solution) to 0.3× Danieau solution at a final concentration of 2 mg/mL.
2. Incubate embryos at 28.5 °C until the chorions are digested and begin to disintegrate.
3. Wash three times with 0.3× Danieau solution and collect the dechorionated embryos.

D. Induce edema

1. Subject dechorionated 3 dpf embryos to hypertonic conditions by incubating them in 3× Danieau solution for 24 h at 28.5 °C (Figure 1A).
2. Maintain control embryos in 0.3× Danieau solution under the same conditions.
3. After 24 h, transfer the treated embryos to 0.3× Danieau solution and incubate for an additional 24 h. Induced edema is visible with a brightfield microscope at 4 dpf (Figure 1B).
4. Collect both edematous and control larvae at desired developmental stages and proceed with TRAP protocol. This protocol used 5 dpf larvae. Samples are processed simultaneously to minimize batch effects.

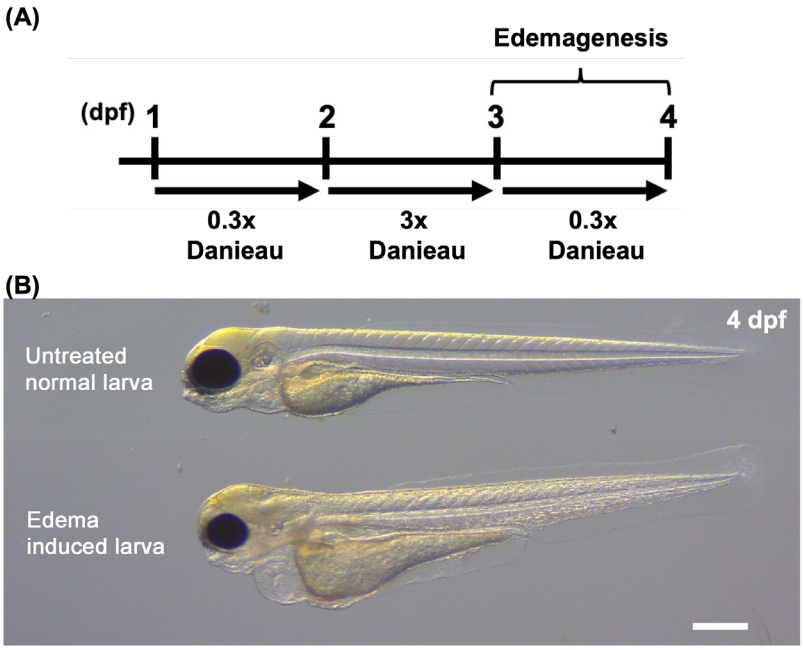


Figure 1. Schematic of edema induction. (A) Timeline of the edema induction protocol. Larvae were treated with 3× Danieau solution from 2 to 3 dpf. Edemagenesis was induced by replacing the medium with 0.3× Danieau solution at 3 dpf to impose osmotic stress. (B) Brightfield image of a control (untreated) larva and edema-induced larva at 4 dpf. Scale bar, 500 µm. More detailed information on the edema induction protocol is available in Olayinka et al. [3].

E. Buffer preparation

1. Chill all buffers on ice or at 4 °C prior to use.
2. Thaw all necessary inhibitors for HB+ buffer preparation.
Note: Cycloheximide may require warming to 30 °C to thaw completely.
3. Prepare HB+ buffer by adding inhibitors to the homogenization buffer (HB) immediately before use. Keep the HB+ buffer on ice.

F. Deyolk embryos

1. Transfer 200–500 embryos from each embryo group (edematous and control) into separate 1.5 mL microcentrifuge tubes and remove all remaining 0.3× Danieau solution.
2. Add 1 mL of deyolking buffer supplemented with 10 µL of protease inhibitor cocktail.
3. Gently disrupt the yolk by pipetting up and down using a 200 µL pipette tip. Confirm yolk separation under a stereomicroscope.
4. Centrifuge at 300× *g* for 30 s at 4 °C and discard the supernatant.
5. Wash the embryos three times with 1 mL of cold deyolking wash buffer, centrifuging at 300× *g* for 30 s at 4 °C each time. Repeat until the supernatant is clear.
6. Perform a final wash with cold PBS. Centrifuge at 300× *g* for 30 s, then discard the supernatant.

G. Embryo homogenization

1. Add 2 µL of HB+ buffer per embryo to the deyolked pellet for each group (edematous and control).
2. Homogenize thoroughly using a Biovortexer with pestles. Avoid introducing bubbles during homogenization.
3. Incubate the homogenate on ice for 10 min to ensure complete cell lysis.
4. Centrifuge at 3,000× *g* for 10 min at 4 °C. Transfer the supernatant to a new tube and discard the pellet.
5. Repeat the centrifugation of the supernatant phase at 10,000× *g* for 10 min at 4 °C to remove cellular debris.
6. Collect the final supernatant in a new 1.5 mL tube and keep it on ice before proceeding to immunoprecipitation.

H. Protein quantification

1. Quantify the total protein concentration from each embryo lysate (edematous and control) using the Bradford protein assay, following the manufacturer's instructions.
Note: Use BSA as a standard and perform measurements in triplicate to ensure accuracy.

I. Set up indirect immunoprecipitation (IP)

1. Add 1 µL of anti-HA antibody per 400 µg of protein into the lysate supernatant collected from section G.
2. Incubate the mixture on a tube rotator at 4 °C for 4 h to allow antibody-ribosome complex formation.

J. Prepare Dynabeads Protein G

1. Vigorously shake the Dynabeads Protein G tube to resuspend the beads evenly in the storage buffer.
2. Dispense 57 µL of Dynabeads per 400 µg of protein to a 1.5 mL tube.
3. Place the tube on a magnetic stand and allow the beads to collect on the tube wall; carefully remove and discard the storage buffer (Figure 2).
4. Take out the tube from the magnetic stand and add 800 µL of HB+ buffer to the beads.
5. Use a tube rotator to equilibrate beads to HB+ buffer by rotating at 4 °C for 1 h.

K. Affinity purification

1. Remove the HB+ buffer from the equilibrated Dynabeads using a magnetic stand (Figure 2).
2. Add 800 µL of the HA-antibody and lysate mixture to the beads.
3. Incubate the mixture overnight (~16 h) at 4 °C on a tube rotator to allow affinity binding.

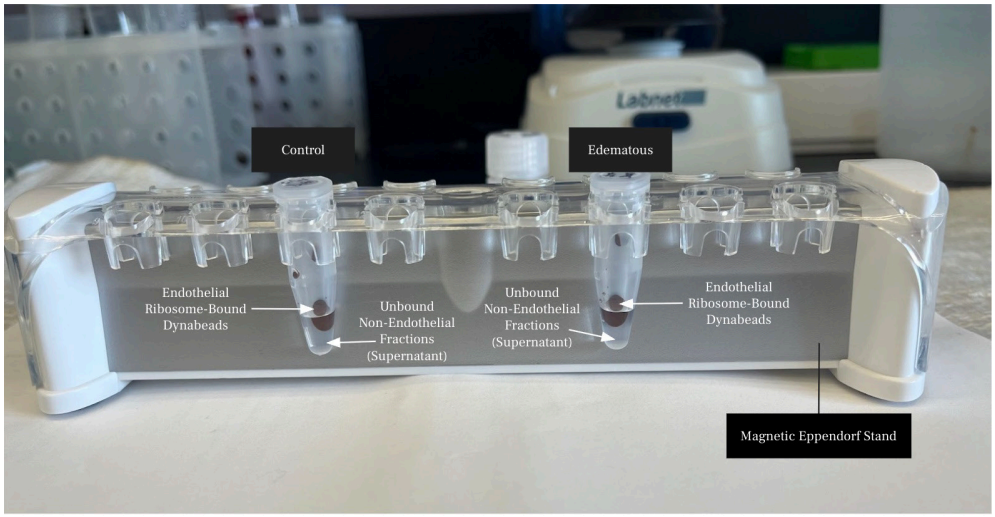


Figure 2. Image illustrating Dynabead-bound endothelial ribosome lysates and unbound protein lysates on a magnetic Eppendorf stand

Day 3
L. Wash beads

1. Remove and save the supernatant (unbound protein lysate) from each sample on ice (Figure 2).
2. Wash the beads three times with 800 µL of HSB+.
a. Rotate at 4 °C for 5 min.
b. Use a magnetic stand to collect the beads for 3 min and carefully remove the wash buffer (Figure 2).

3. After the second wash, transfer the beads to a fresh tube to reduce background before the final wash.

M. RNA isolation from the bead–antibody complex

1. Add 500 µL of TRIzol reagent directly to the antibody-bound beads.
2. Incubate on ice for 5 min to ensure thorough lysis.
3. Add 100 µL of chloroform and then vortex vigorously for 30 s.
4. Incubate the mixture at room temperature for 3 min.
5. Centrifuge at 12,000× *g* for 15 min at 4 °C. The mixture separates into a lower phenol-chloroform phase, an interphase, and a colorless upper aqueous phase.
6. Carefully transfer the upper aqueous phase to a fresh RNase-free tube.
7. Add 1.5× volume of 100% ethanol and 1 µL of glycogen as a carrier.
8. Purify RNA using the Direct-zol™ RNA MicroPrep kit according to the manufacturer's instructions.
9. Quantify RNA concentration by nanodrop.
10. Store RNA at -80 °C or continue immediately.

Note: This protocol used 200–500 larvae at 5 dpf, collecting an RNA yield ranging from 400 to 600 ng. Samples should be pooled for RNA isolation to achieve sufficient yield for certain downstream applications (e.g., RNA-seq).

Data analysis

qPCR amplification curves and threshold cycle data were exported in .xlsx format for processing in Microsoft Excel. For each vascular gene, the ΔCT value was calculated by subtracting the cycle threshold (CT) of the reference gene (eef1a1) from the corresponding CT of the target (Ct_target–Ct_eef1a1). The ΔΔCT values were then obtained by subtracting the mean ΔCT of the supernatant control sample from each ΔCT value in both supernatant (sup) and purified endothelial (Endo) RNA. Fold changes in gene expression were calculated using the $2^{-\Delta\Delta CT}$ method. Replicate fold changes were averaged for each vascular gene and compared between endothelial and supernatant samples (Figure 3). The results show that the TRAP protocol, using the RiboTag strategy, efficiently enriches for specific endothelial gene markers such as *lyve1b*, *flt4*, and *cdh5*, while not enriching for ubiquitous genes like *n4bp1* (Figure 3A). This technique enables targeted analysis of gene expression for selected cell types within complex tissue samples and can be applied in a range of clinical and research contexts to profile cell type–specific gene expression (Figure 3B).

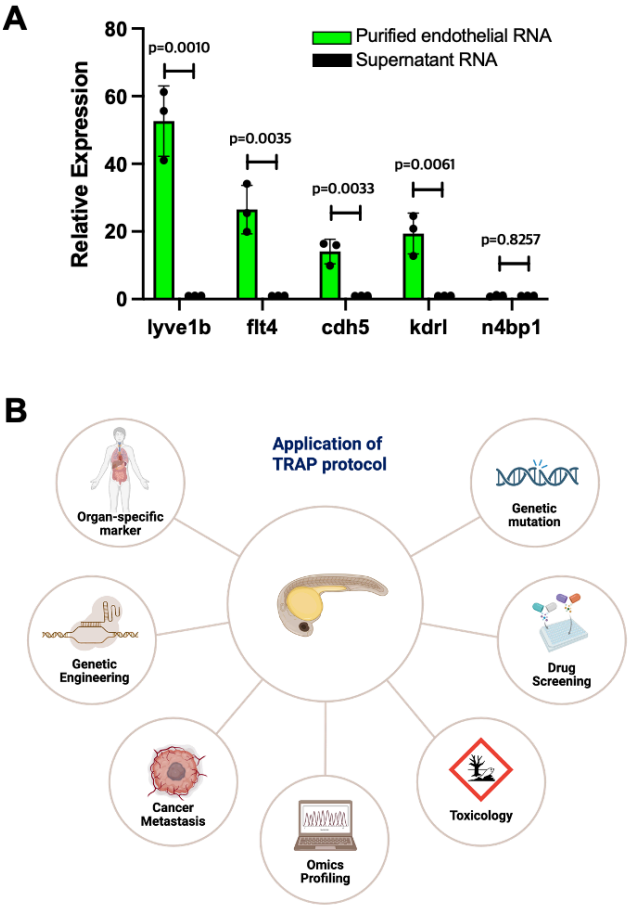


Figure 3. Analyzing endothelial translatome using the translating ribosome affinity purification (TRAP) assay. (A) Real-time qPCR showing specific enrichment of endothelial genes *lyve1b*, *flt4*, *cdh5*, and *kdrl* in the purified endothelial RNA, compared to non-endothelial RNA from the supernatant. *n4bp1*, a ubiquitous gene, served as a negative control. Fold change values were imported into GraphPad Prism version 9.2.0 for visualization and statistical analysis. Data were plotted as mean ± SD. An unpaired two-tailed Student's t-test was used to compare the groups. Significance was defined as p-value < 0.05. (B) Diverse clinical and biomedical research themes where the TRAP protocol can be employed.

Validation of protocol

Purified endothelial and supernatant RNA samples were normalized to equal concentrations and reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit following the manufacturer's instructions. Each reaction contained an equal volume (10 µL) of master mix (Recipe 6) and normalized RNA in a PCR tube and was incubated in a thermal cycler under the following conditions: 25 °C for 10 min, 37 °C for 120 min, 85 °C for 5 min, and then held at 4 °C. The resulting cDNA was stored at -20 °C or used immediately for qPCR.

cDNA samples were diluted 1:5 with nuclease-free water and kept on ice. Real-time multiplex PCR reactions were performed using the TaqMan Fast Advanced Master Mix (Recipe 9), with reaction volumes adjusted according to the number of target genes and the number of technical replicates. Gene-specific FAM-labeled TaqMan probes were used for vascular targets, while a VIC-labeled probe targeting the reference gene eef1a1α served as the internal control. Multiplex reactions were prepared in a 96-well fast optical plate, sealed, and briefly centrifuged to ensure homogeneity. Plates were loaded into the Quant Studio 7 Flex Real-Time PCR System and amplified under the following conditions: hold at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 30 s.

This protocol (or parts of it) has been used and validated in the following research article:

- Olayinka et al. [3]. Compensatory lymphangiogenesis is required for edema resolution in zebrafish. *Scientific Reports.*

General notes and troubleshooting

General notes

1. Screen all embryos for transgenic expression prior to TRAP to ensure sample consistency. Both homozygous and heterozygous embryos can be used; however, a larger sample pool is recommended when embryos are heterozygous.
2. Perform all steps following edema induction on ice to preserve RNA integrity and protein complexes.
3. Prepare all supplemented buffers fresh on the day of the experiment to maintain reagent activity.
4. Handle samples gently throughout the protocol to minimize material loss and maximize yield.

Troubleshooting

Step	Problem	Possible cause	Solution
Embryo collection	Low fertilization or embryo yield	Suboptimal breeding conditions, unhealthy or aged fish	Use healthy breeding pairs; optimize tank setup and timing; separate breeding pairs overnight; avoid overcrowding.
Edema formation	Mild edema or normal appearance	Incorrect hypertonic solution composition or timing	Verify hypertonic solution composition; shake well before use; induce edema by 3 dpf at the latest.
Deyolking	Embryo damage or lysis	Aggressive pipetting or narrow pipette tips	Use wide-bore pipette tips (200 µL) and pipette gently to minimize mechanical stress.
Homogenization	Incomplete tissue lysis	Insufficient grinding or short homogenization and incubation time	Use a motorized pestle; homogenize thoroughly without air bubbles; incubate lysate on ice for at least 10 min.
Lysate	Low protein yield	Too few embryos or inadequate lysis	Use approximately 2 µL of HB+ buffer per embryo; verify lysis visually under a microscope.
IP setup	Nonspecific binding	Poor antibody specificity or inadequate washing	Use validated anti-HA antibody; prepare fresh cold HSB+ for washes; increase washes to 5 times.
IP setup	Bead clumping or loss	Prolonged incubation or harsh pipetting	Use gentle rotation; avoid foam formation; minimize bead handling during pipetting.
IP setup	Weak binding to HA-Rpl10a	Insufficient antibody concentration or low transgene expression	Confirm antibody activity; use 1 µL of antibody per 400 µg of protein; verify transgene expression at 2 dpf.
Washing	High background in eluate	Incomplete wash steps	Perform all washes with cold HSB+; transfer beads to a fresh tube after the second wash.
RNA isolation	Poor RNA yield	Loss of aqueous phase during phase separation	Use phase-lock gel tubes and pipette carefully to collect the aqueous phase.
RNA integrity	RNA degradation characterized by a RIN score under 7 or smearing	RNase contamination	Use RNase-free tubes, tips, and reagents; clean surfaces with RNaseZap; include DNase treatment. Avoid multiple freeze-thawing of RNA samples.
RNA quality	Ineffective inhibitors	Inhibitors degraded or thawed improperly	Always thaw inhibitors on ice; prepare HB+ fresh; avoid repeated freeze-thaw cycles.
RNA precipitation	Poor RNA yield	Missing carrier or low RNA concentration	Add 1 µL of glycogen and 1.5× volume 100% ethanol for precipitation; concentrate RNA using cleanup kits.

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The following figures were created using BioRender: Graphical overview, https://BioRender.com/vs4grkh; Figure 3B, https://BioRender.com/44gaqew.

Competing interests

The authors declare no conflicts of interest.

Ethical considerations

All procedures followed the required guidelines and regulations, conforming to the ARRIVE guidelines. Zebrafish husbandry and research protocols were approved by the University of Illinois Animal Care and Institutional Biosafety Committee (Animal Care and Use Protocol 23–112).

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