Rapid and Robust Polysome Isolation and Fraction RNA Extraction for Studying the Seed Translatome

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Published in the Plant Biology section

Translation of mRNA into functional proteins is a fundamental process underlying many aspects of plant growth and development. Yet, the role of translational regulation in plants across diverse tissue types, including seeds, is not well known due to the lack of methods targeting these processes. Studying the seed translatome could unveil seed-specific regulatory mechanisms, offering valuable insights for breeding efforts to enhance seed traits. Polysome profiling is a widely used technique for studying mRNAs being translated. However, the traditional method is time-consuming and has a low polysome recovery rate; therefore, it requires substantial starting material. This is particularly challenging for species or mutants with limited seed quantities. Additionally, seed polysome fractions often yield low quality RNA due to the abundance of various compounds that interfere with conventional RNA extraction protocols. Here we present a robust polysome extraction method incorporating a size-exclusion step for polysome concentration streamlined with a rapid RNA extraction method optimized for seeds. This protocol works across multiple plant species and offers increased speed and robustness, requiring less than half the amount of seed tissue and time compared to conventional methods while ensuring high polysome recovery and yield of high-quality RNA for downstream experiments. These features make this protocol an ideal tool for studying seed translation efficiency and hold broad applicability across various plant species and tissues.

Basic Protocol 1: Robust polysome extraction for seeds
Basic Protocol 2: Rapid fraction total RNA extraction

Keywords: mRNA • polysome • RNA extraction • seed • translation

How to cite this article:
Duong, H. N., Ansaf, H., Cornish, P., Mendoza-Cozatl, D., Schenck, C., & Angelovici, R. (2024). Rapid and robust polysome isolation and fraction RNA extraction for studying the seed translatome. *Current Protocols*, *4,* e70007. doi: 10.1002/cpz1.70007

INTRODUCTION

Regulation of gene expression occurs through various levels, including epigenetic modifications, transcription, splicing, and translation. Among these, translation plays a

CURRENT PROTOCOLS
A Wiley Brand
Current Protocols e70007, Volume 4
Published in Wiley Online Library (wileyonlinelibrary.com).
doi: 10.1002/cpz1.70007

pivotal role in determining protein expression levels (Schwanhäusser et al., 2011; Urquidi Camacho et al., 2020). There is an increasing interest in elucidating these processes in plants across diverse tissue types, including seeds (Bai et al., 2017; Galland & Galland, 2015; Lokdarshi et al., 2020; Mustroph, Zanetti, et al., 2009); however, translational regulation is still largely unknown due the lack of accessible methods targeting these processes. Thus, a better understanding of translation processes can bridge the transcriptome-proteome gap and reveal novel regulatory mechanisms in gene expression dynamics, which ultimately may offer valuable insights for breeding targets aimed at improving seed viability and quality.

Polysome profiling is one of the most used techniques to investigate translation regulation among various assays. Traditional polysome profiling involves two key steps: polysome isolation followed by RNA extraction and sequencing (Chassé et al., 2017; Mustroph, Juntawong, et al., 2009; Pringle et al., 2019; Rivera et al., 2015). The first step involves pelleting polysomes over a 30% to 60% sucrose cushion, followed by their resuspension, then separation over a sucrose gradient into fractions containing actively translating mRNAs and ribosomes (Rivera et al., 2015). However, this process can be time-consuming with low polysome recovery yield and thus requires substantial amounts of starting biological material (usually of 500 to 1000 mg plant sample), posing challenges for species or mutants or biological stages producing small seed quantities. Next, the fractionated polysomes undergo an RNA extraction step. Common RNA extraction methods based on phenol-chloroform or Trizol often yields poor-quality RNA from polysomes due to high sucrose content from the sucrose gradients. Additionally, seeds are rich in various compounds, such as starch, polyphenols, polysaccharides, and lipids, making high-quality RNA extractions challenging with traditional methods (Mornkham et al., 2013; Siles et al., 2020; Vennapusa et al., 2020).

We have developed a simplified protocol to address these challenges and streamline the process based on the following considerations. First, ribosomes are large protein complexes of rRNA and ribosomal proteins consisting of small and large ribosomal subunits with sizes varying from 1.1 to 3.1 MDa (Cox & Arnstein, 2003), which makes them suitable for concentration using ultra-centrifugal filters separating protein complexes based on molecular weight. This method effectively removes detergents, thus expediting sample processing while ensuring maximum sample recovery, and offering efficient concentration capability (Chernokalskaya et al., 2004; Smejkal et al., 2006; Wiśniewski et al., 2011). Second, while the hot borate method stands out as an optimized RNA extraction

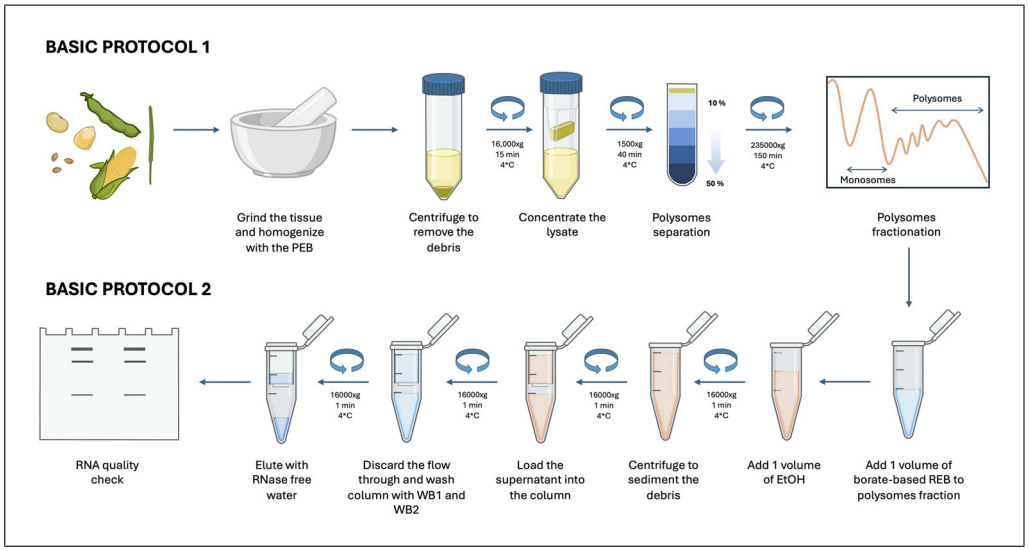

Figure 1 Schematic overview of Basic Protocols 1 and 2.

method for metabolite-rich samples like seeds due to its high yield and purity (Mornkham et al., 2013; Siles et al., 2020; Vennapusa et al., 2020), it is a lengthy protocol that can take up to 2 days. Therefore, to overcome this problem, we combined the borate buffer (Wan & Wilkins, 1994) with a commercially available silica column, which resulted in rapid RNA extraction with high yield and quantity.

Here we present a streamlined protocol incorporating the ultra-centrifugal filtration step for polysome concentration and seed-specific silica-column-based RNA extraction method, which requires less than half the starting biological material and less than half the time of conventional protocol while ensuring high-quality RNA yield ready for downstream experiments (Fig. 1). Overall, this protocol provides an ideal approach for studying seed translation efficiency and holds broad applicability across various plant species (model, non-model, and crop) and tissues.

ROBUST POLYSOME EXTRACTION FROM SEEDS

BASIC PROTOCOL 1

The maize seeds in this protocol were collected 48 hr after imbibition, which is the time when the seed is actively translating stored mRNAs for germination (Dommes & Van de Walle, 1983). While we demonstrate this protocol using maize seeds, this method can also be applied to seeds of various species and at different developmental stages. The seed samples were collected and frozen with liquid nitrogen prior the extraction. Here, we show how to extract and concentrate cellular lysate utilizing the 100 kDa Amicon filter-concentrator, followed by polysome fractionation using the 10% to 50% sucrose gradient.

Materials

150 mg seed material
Polysome extraction buffer (PEB) (see recipe)
Sucrose gradient buffer (see recipe)

Mortar and pestle (incubated in 230°C oven for at least 12 hr and cooled to room temperature prior to use)
Liquid nitrogeon
Ice
50-ml conical tubes (Fisher, cat. no. 339652)
Sorvall Lynx 6000 ultracentrifuge (or tabletop centrifuge), 4°C
Amicon Ultra centrifugal filter, 100 kDa MWCO (Sigma, cat. no. UFC9100)
TH-641 ultracentrifuge rotor (Thermo Fisher)
Open-top ultra-clear centrifuge tube (Beckman, cat. no. 344059)
Sorvall WX+ Ultra Series centrifuge (Sorvall, cat. no. 75000100)
Gradient station/Triax full spectrum flow cell (BIOCOMP, model 108)

NOTE: Before the extraction, turn on all centrifuges and set the temperature to 4°C. Put the ultra-centrifuge rotors inside, vacuum, and let them cool down. Pre-chill the mortar and pestle and place the extraction buffer on ice.

1. Pulverize 150 mg seed material thoroughly with appropriate amount of liquid nitrogen and a mortar and pestle. Add 3 ml PEB and grind to achieve a homogenous mixture. Let the mixture sit on ice with gentle occasionally mixing with pestle for 10 min.

   Before adding PEB, samples need to be ground to a fine powder with no observable small particles. This is crucial for a good extraction.

   Do not let sample thaw at any time without PEB.

   For rich starch materials such as maize seed, more PEB is needed (≥5 ml) to get the final collected 3 ml extraction mixture since the starch will absorb some of the buffer.

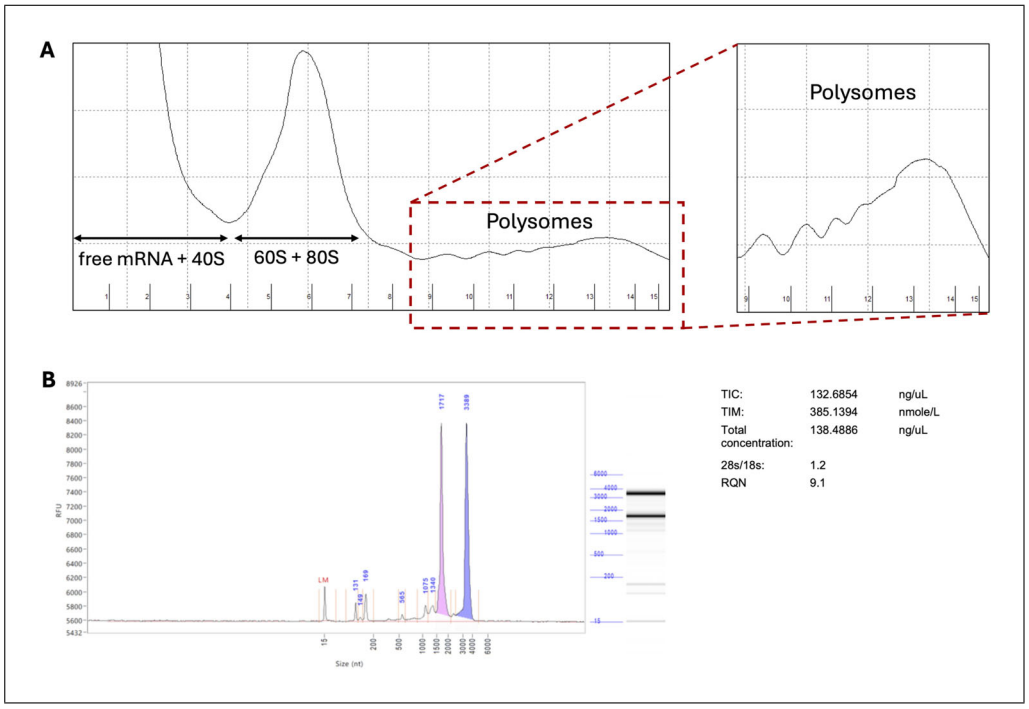

Figure 2 Representative polysome profile and RNA quality. (**A**) Polysome profile from maize/Arabidopsis seeds. (**B**) Quality of extracted RNA from polysome fragment measured by Agilent Fragment Analyzer System.

2. Pour the mixture into 50-ml conical tubes. Balance the tubes and centrifuge in the Sorvall Lynx 6000 ultracentrifuge 15 min at 12,000 × *g*, 4°C.
3. Load the supernatant without disturbing the pellet to an Amicon Ultra centrifugal 100 kDa filter. Centrifuge 40 min at 1500 × *g*, 4°C.
4. Prepare the 10% to 50% sucrose gradients with sucrose gradient buffer (Merchante et al., 2016) in open-top ultra-clear centrifuge tube, and put them on ice.

   Each sample lysate concentrate will be loaded onto a separate sucrose gradient. Therefore, the number of sucrose gradients needed is equal to the number of samples.

   Further details for making sucrose gradients using BIOCOMP system can be found at http://www.biocompinstruments.com.

5. Load 500 µl of the concentrated lysate on top of the sucrose gradient and balance with extraction buffer.
6. With the Sorvall WX+ Ultra centrifuge and TH-641 rotor (or similar), centrifuge 2.5 hr at 234,269 × *g*, 4°C.

   This set speed is based on Merchante et al. (2016).

7. Carefully remove the gradients and process them with the BIOCOMP gradient station/Triax full spectrum flow cell or similar fractionator.

   Pre-chill collection tubes by placing in ice or liquid nitrogen before collecting the fractionated polysomes.

8. Collect the tubes corresponding with the monosomes or polysomes (Fig. 2A).

   See Understanding Results for details on how to identify the desired fractions. In this protocol, fractions numbered from 10 to 15, which correspond to polysomes, are pooled together for Basic Protocol 2 total RNA extraction.

9. Freeze the fractions and store at −80°C until ready for fraction total RNA extraction.

RAPID FRACTION TOTAL RNA EXTRACTION

BASIC PROTOCOL 2

Below we describe the polysome fraction total RNA extraction incorporating hot borate extraction buffer and the Direct-zol miniprep plus kit for a rapid RNA extraction method. This method can be completed in <30 min, compared to days with traditional hot borate extraction methods.

Materials

Polysome sample prepared in Basic Protocol 1
2× RNA extraction buffer (REB) (see recipe)
100% ethanol
Direct-zol RNA miniprep plus kit (Zymo Research, cat. no. R2072) containing:
  Zymo-Spin IIICG columns
  RNA prewash buffer
  RNA wash buffer
  2-ml collection tubes
H_2O, DNase/RNase-free

15- or 50-ml conical tubes
Benchtop centrifuge, room temperature and 4°C

1. If previously frozen, thaw the polysome sample.
2. Add 1 volume of 2× REB (heated at 80°C with 10 mM DTT freshly added) into 1 volume of the polysome fraction. Mix by inverting tubes several time.

   If samples are pooled, use 15- or 50-ml conical tubes.
3. Add 1 volume of 100% ethanol. Mix by inverting tube several times and centrifuge 2 min at 16,000 × *g*, room temperature.

   If salt precipitation occurrs after adding absolute ethanol, centrifuge to sediment the salt and collect the supernatant without disturbing the precipitated pellet.
4. Load the supernatant onto a Zymo-Spin IIICG column (0.7 ml capacity) and centrifuge 30 s at 16,000 × *g,* room temperature. Discard the flow-through.
5. Repeat step 3 until all remaining supernatants are processed.
6. Add 400 µl RNA prewash buffer to the column. Close, and centrifuge 1 min at 16,000 × *g*, 4°C, to wash the column. Discard the flow-through.
7. Repeat step 6.
8. Add 700 µl RNA wash buffer to the column and centrifuge 1 min at 16,000 × *g*, 4°C, to wash the column. Discard the flow-through.
9. Repeat step 82.
10. Centrifuge 1 min at 16,000 × *g*, 4°C, to remove any remaining excessive buffer. Carefully transfer column to a new RNA-free collection tube.
11. Add 50 µl DNase/RNase-free water directly onto the column silica membrane. Wait 2 min then centrifuge 2 min at 16,000 × *g*, 4°C.
12. The eluted RNA can be used directly for downstream experiments or store at −80°C until use.

REAGENTS AND SOLUTIONS

Polysome extraction buffer (PEB)

Make the following stock solutions at the following concentrations for later preparation of PEB (Merchante et al., 2016). All stock solutions are made with

diethyl-pyrocarbonate-(DEPC)-treated water (or RNase-free water) and sterilized by autoclave unless otherwise stated. Store stock solutions up to 2 months at room temperature unless otherwise specified.

1 M Tris·HCl, pH 8.8 (Sigma-Aldrich, cat. no. 93352)
1 M sucrose (autoclave ≤10 min to prevent caramelizing sucrose) (Sigma-Aldrich, cat. no. S0389)
1 M KCl (Sigma-Aldrich, cat. no. P9541)
1 M NaCl (Sigma-Aldrich, cat. no. S9888)
1 M $MgCl_2$ (Sigma-Aldrich, cat. no. M8266)
0.5 M EGTA, pH 8.0 (Sigma-Aldrich, cat. no. 324626)
100 mM DTT (filter-sterilized and stored until use at −20°C) (RPI, cat. no. 578517)
100 mg/ml cycloheximide (filter-sterilized and stored up to 2 months at −20°C) (Sigma-Aldrich, cat. no. 1810)
50 mg/ml chloramphenicol (filter-sterilized and stored up to 2 months at −20°C) (Sigma-Aldrich, cat. no. 31667)
Detergent mix, 20% (v/v) of each of detergent in water:
Brij-35 (Sigma-Aldrich, cat. no. 8019620250)
Triton X-100 (Sigma-Aldrich, cat. no. T8787)
Igepal CA 630 (Sigma-Aldrich, cat. no. I8896)
Tween 20 (Sigma-Aldrich, cat. no. P1379)

Make PEB immediately before the start of the experiment by adding the volume indicated in Table 1 for 5 ml (one sample), fill up to working volume with DEPC-treated water (or RNase-free water).

RNA extraction buffer (REB), 2×

Make the stock solutions at the following concentration for later preparation of REB (Wan & Wilkins, 1994). All the stock solutions are made with DEPC-treated water (or RNase-free water) and sterilized by autoclave. Store stock solutions up to 2 months at room temperature.

1 M Na borate (Sigma-Aldrich, cat. no. SX0355)
500 mM EGTA (Sigma-Aldrich, cat. no. 324626)
10% sodium deoxycholate (Sigma-Aldrich, cat. no. D6750)
20% SDS (Sigma-Aldrich, cat. no. 436143)

Table 1 Polysome Extraction Buffer Recipe

Stock solution	Volume for one sample (5 ml)	Final concentration
1 M Tris·HCl, pH 8.8	550 µl	110 mM
1 M sucrose	500 µl	100 mM
1 M KCl	500 µl	100 mM
1 M NaCl	375 µl	75 mM
1 M $MgCl_2$	100 µl	20 mM
0.5 M EGTA	125 µl	12.5 mM
100 mM DTT	150 µl	3 mM
20% detergent mix	31.25 µl	6.25 µl/ml
Triton X-100	125 µl	25 µl/ml
100 mg/ml cycloheximide	1.875 µl	37.5 µg/ml
50 mg/ml chloramphenicol	2.5 µl	25 µg/ml
DEPC MilliQ H_2O	Up to final volume	

Table 2 Seed 2× RNA Extraction Buffer Recipe

Stock	Volume for 10 ml	Final working concentration
1 M sodium borate decahydrate	4000 µl	400 mM
500 mM EGTA	1200 µl	60 mM
20% SDS	1000 µl	2% (w/v)
10% sodium deoxycholate	2000 µl	2% (w/v)
PVP40	0.4 g	4% (w/v)
DTT	30.85 mg	20 mM
Nonidet-40	200 µl	2% (v/v)
DEPC MilliQ H_2O	up to final volume	

Table 3 Sucrose Gradient Buffer Recipe

	Volume for one sample (14 ml in total)		
Stock	7 ml of 10% SGB	7 ml of 50% SGB	Working concentration
Sucrose	0.7 g	3.5 g	10% and 50%, respectively
1 M Tris·HCl, pH 8.4	280 µl	280 µl	40 mM
1 M KCl	140 µl	140 µl	20 mM
1 M $MgCl_2$	70 µl	70 µl	10 mM
100 mg/ml cyclohexamide	3.5 µl	3.5 µl	50 µg/ml
50 mg/ml chloramphenicol	7 µl	7 µl	50 µg/ml
Superase In RNase Inhibitor (20 U/µl)	7 µl	7 µl	20 U/ml
DEPC MilliQ H_2O	Up to final volume	Up to final volume	

Add the following components fresh when making REB:
PVP40 (Sigma-Aldrich, cat. no. P0930)
DTT (RPI, cat. no. 578517)
Nonidet-40 (RPI, cat. no. N59000)

Since polysome fractions are in sucrose gradient solution, make the 2× REB by adding the volume indicated in Table 2 for one sample, fill up to working volume with DEPC-treated water (or RNase-free water).

Sucrose gradient buffer (SGB)

Make the stock solutions for SGB at the following concentrations. All the stock solutions are made with DEPC-treated water (or RNase-free water) and sterilized by autoclave unless otherwise stated. Store stock solutions up to 2 months at room temperature unless otherwise specified.

1 M Tris·HCl, pH 8.4 (Sigma-Aldrich, cat. no. 93352)
1 M KCl (Sigma-Aldrich, cat. no. P9541)
1 M $MgCl_2$ (Sigma-Aldrich, cat. no. M8266)
100 mg/ml cycloheximide (filter-sterilized and stored up to 2 months at −20°C) (Sigma-Aldrich, cat. no. 1810)
50 mg/ml chloramphenicol (filter-sterilized and stored up to 2 months at −20°C) (Sigma-Aldrich, cat. no. 31667)
Superase-In RNAase inhibitor (Thermo Fisher, cat. no. AM2694)

Make the SGB by adding the volume indicated in Table 3 for one sample, fill up to working volume with DEPC-treated water (or RNase-free water) (Merchante et al., 2016).

Table 4 Troubleshooting Table

Problem	Possible cause	Solution
Substantial volume of lysate after concentration step	Depends on seeds, the lysis can be dense/viscous and therefore might interfere with the filter	Additional centrifuge time can be applied until the volume of the supernatant is ∼500 µl
Low RNA quality	Salt or ethanol contamination	Make sure the supernatant from Basic Protocol 2, step 2, is taken without disturbing the precipitated pellet; after washing step, briefly centrifuge the column and blot the bottom to remove any remaining buffer

COMMENTARY

Critical Parameters

It is critical to maintain the working space RNAse-free and keep equipment at low temperature (4°C).

All consumables should be cleaned by DEPC-treated water (or RNase-free water) and autoclaved.

Only autoclaved DEPC-treated water (or RNase-free water) should be used for this protocol.

Since the PEB contains detergents, avoid generating bubbles while mixing with sample.

For 2× REB, it is required to wait until all the buffer components dissolve.

Troubleshooting

See Table 4 for a list of potential problems, causes and solutions.

Understanding Results

Using sucrose gradients, smaller and less dense RNA and protein components, such as non-ribosomal mRNAs and 40S ribosomal subunits, migrate shorter distances into the gradient and accumulate near the top. Conversely, larger components like 60S and 80S, as well as polysomes, travel further into gradient, settling at a lower position. A traditional polysome profile will have peaks for 40S, 60S, and 80S ribosomal subunits as well as polysomes (Merchante et al., 2016). For this robust extraction method, we will see 40S and free ribosomal RNA merge into one peak, and 60S and 80S peaks come together as one big peak followed by the polysome peaks (Fig. 2A).

The RNA extracted from polysome fractions using the rapid extraction method yield ∼10 µg with distinctive peaks (bands) for 28S and 18S, and an RNA Quality Number (RQN) of 9.1, which indicates intact and high-quality RNA (Fig. 2B) ready for RNA sequencing or downstream experiments.

Time Considerations

Basic Protocol 1 can take ∼4 hr. Basic Protocol 2 can take ∼30 min.

Author Contributions

Ha Ngoc Duong: Conceptualization; data curation; formal analysis; investigation; methodology; visualization; writing—original draft. **Huda Ansaf:** Investigation; writing—review and editing. **Peter Cornish:** Conceptualization; writing—review and editing. **David Mendoza-Cozatl:** Conceptualization; writing—review and editing. **Craig A. Schenck:** Conceptualization; funding acquisition; project administration; writing—review and editing. **Ruthie Angelovici:** Conceptualization; funding acquisition; project administration; supervision; writing—review and editing.

Acknowledgments

We would like to acknowledge Dr. Abou Yobi for providing valuable comments on this protocol. This work was supported by the National Science Foundation (grant no. NSF-IOS 1754201).

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Data are available from the authors upon request.

Literature Cited

Bai, B., Peviani, A., van der Horst, S., Gamm, M., Snel, B., Bentsink, L., & Hanson, J. (2017). Extensive translational regulation during seed germination revealed by polysomal profiling. *New Phytologist*, *214*(1), 233–244. https://doi.org/10.1111/nph.14355

Chassé, H., Boulben, S., Costache, V., Cormier, P., & Morales, J. (2017). Analysis of translation using polysome profiling. *Nucleic Acids Research*, *45*(3), e15. https://doi.org/10.1093/nar/gkw907

Chernokalskaya, E., Gutierrez, S., Pitt, A. M., & Leonard, J. T. (2004). Ultrafiltration for proteomic sample preparation. *Electrophoresis*, *25*(15), 2461–2468. https://doi.org/10.1002/elps.200405998

Cox, R. A., & Arnstein, H. R. V. (2003). Translation of RNA to protein. In *Encyclopedia of physical science and technology*. Academic Press. https://doi.org/10.1016/b0-12-227410-5/00788-2

Dommes, J., & van de Walle, C. (1983). Newly synthesized mRNA is translated during the initial imbibition phase of germinating maize embryo. *Plant Physiology*, *73*(2), 484–487. https://doi.org/10.1104/pp.73.2.484

Galland, M., & Galland, M. (2015). Regulation of mRNA translation controls seed germination and is critical for seedling vigor. *Frontiers in Plant Science*, *6*(APR), 284. https://doi.org/10.3389/fpls.2015.00284

Lokdarshi, A., Guan, J., Urquidi Camacho, R. A., Cho, S. K., Morgan, P. W., Leonard, M., Shimono, M., Day, B., & von Arnim, A. G. (2020). Light activates the translational regulatory kinase GCN2 via reactive oxygen species emanating from the chloroplast. *Plant Cell*, *32*(4), 1161–1178. https://doi.org/10.1105/TPC.19.00751

Merchante, C., Hu, Q., Heber, S., Alonso, J., & Stepanova, A. (2016). A ribosome footprinting protocol for plants. *Bio-Protocol*, *6*(21). https://doi.org/10.21769/bioprotoc.1985

Mornkham, T., Wangsomnuk, P. P., Fu, Y. B., Wangsomnuk, P., Jogloy, S., & Patanothai, A. (2013). Extractions of high quality RNA from the seeds of Jerusalem artichoke and other plant species with high levels of starch and lipid. *Plants*, *2*(2), 302–316. https://doi.org/10.3390/plants2020302

Mustroph, A., Juntawong, P., & Bailey-Serres, J. (2009). Isolation of plant polysomal mRNA by differential centrifugation and ribosome immunopurification methods. In *Methods in molecular biology* (Vol. *553*). Humana Press. https://doi.org/10.1007/978-1-60327-563-7_6

Mustroph, A., Zanetti, M. E., Jang, C. J. H., Holtan, H. E., Repetti, P. P., Galbraith, D. W., Girke, T., & Bailey-Serres, J. (2009). Profiling translatomes of discrete cell populations resolves altered cellular priorities during hypoxia in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(44), 18843–18848. https://doi.org/10.1073/pnas.0906131106

Pringle, E. S., McCormick, C., & Cheng, Z. (2019). Polysome profiling analysis of mRNA and associated proteins engaged in translation. *Current Protocols in Molecular Biology*, *125*(1), e79. https://doi.org/10.1002/cpmb.79

Rivera, M. C., Maguire, B., & Lake, J. A. (2015). Isolation of ribosomes and polysomes. *Cold Spring Harbor Protocols*, *2015*(3), 293–299. https://doi.org/10.1101/pdb.prot081331

Schwanhäusser, B., Busse, D., Li, N., Dittmar, G., Schuchhardt, J., Wolf, J., Chen, W., & Selbach, M. (2011). Global quantification of mammalian gene expression control. *Nature*, *473*(7347), 337–342. https://doi.org/10.1038/nature10098

Siles, L., Eastmond, P., & Kurup, S. (2020). Big data from small tissues: Extraction of high-quality RNA for RNA-sequencing from different oilseed Brassica seed tissues during seed development. *Plant Methods*, *16*(1), 80. https://doi.org/10.1186/s13007-020-00626-0

Smejkal, G. B., Li, C., Robinson, M. H., Lazarev, A. V., Lawrence, N. P., & Chernokalskaya, E. (2006). Simultaneous reduction and alkylation of protein disulfides in a centrifugal ultrafiltration device prior to two-dimensional gel electrophoresis. *Journal of Proteome Research*, *5*(4), 983–987. https://doi.org/10.1021/pr050439w

Urquidi Camacho, R. A., Lokdarshi, A., & von Arnim, A. G. (2020). Translational gene regulation in plants: A green new deal. *WIREs RNA*, *11*(6), e1597. https://doi.org/10.1002/wrna.1597

Vennapusa, A. R., Somayanda, I. M., Doherty, C. J., & Jagadish, S. V. K. (2020). A universal method for high-quality RNA extraction from plant tissues rich in starch, proteins and fiber. *Scientific Reports*, *10*(1), 16887. https://doi.org/10.1038/s41598-020-73958-5

Wan, C. Y., & Wilkins, T. A. (1994). A modified hot borate method significantly enhances the yield of high-quality RNA from cotton (Gossypium hirsutum L.). *Analytical Biochemistry*, *223*(1), 7–12. https://doi.org/10.1006/abio.1994.1538

Wiśniewski, J. R., Zielinska, D. F., & Mann, M. (2011). Comparison of ultrafiltration units for proteomic and N-glycoproteomic analysis by the filter-aided sample preparation method. *Analytical Biochemistry*, *410*(2), 307–309. https://doi.org/10.1016/j.ab.2010.12.004