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TITLE:

Analysis of Translation in the Developing Mouse Brain Using Polysome Profiling

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SUMMARY:

The development of the mammalian brain requires proper control of gene expression at the level of translation. Here, we describe a polysome profiling system with an easy-to-assemble sucrose gradient-making and fractionation platform to assess the translational status of mRNAs in the developing brain.

ABSTRACT:

The proper development of the mammalian brain relies on a fine balance of neural stem cell proliferation and differentiation into different neural cell types. This balance is tightly controlled by gene expression that is fine-tuned at multiple levels, including transcription, post-transcription and translation. In this regard, a growing body of evidence highlights a critical role of translational regulation in coordinating neural stem cell fate decisions. Polysome fractionation is a powerful tool for the assessment of mRNA translational status at both global and individual gene levels. Here, we present an in-house polysome profiling pipeline to assess translational efficiency in cells from the developing mouse cerebral cortex. We describe the protocols for sucrose gradient preparation, tissue lysis, ultracentrifugation and fractionation-based analysis of mRNA translational status.

INTRODUCTION:

During the development of the mammalian brain, neural stem cells proliferate and differentiate to generate neurons and glia[1,2] . The perturbation of this process can lead to alterations in brain structure and function, as seen in many neurodevelopmental disorders[3,4]. The proper behavior of neural stem cells requires the orchestrated expression of specific genes[5]. While the epigenetic and transcriptional control of these genes has been intensively studied, recent findings suggest that gene regulation at other levels also contributes to the coordination of neural stem cell proliferation and differentiation[6–10]. Thus, addressing the

translational control programs will greatly advance our understanding of the mechanisms underlying neural stem cell fate decision and brain development.

Three main techniques with different strengths have been widely applied to assess the translational status of mRNA, including ribosome profiling, translating ribosome affinity purification (TRAP) and polysome profiling. Ribosome profiling uses RNA sequencing to determine ribosome-protected mRNA fragments, allowing the global analysis of the number and location of translating ribosomes on each transcript to indirectly infer the translation rate by comparing it to transcript abundance[11]. TRAP takes advantage of epitope-tagged ribosomal proteins to capture ribosome-bound mRNAs[12]. Given that the tagged ribosomal proteins can be expressed in specific cell types using genetic approaches, TRAP allows the analysis of translation in a cell type-specific manner. In comparison, polysome profiling, which uses sucrose density gradient fractionation to separate free and poorly-translated portion (lighter monosomes) from those being actively translated by ribosomes (heavier polysomes), provides a direct measurement of ribosome density on mRNA[13]. One advantage this technique offers is its versatility to study the translation of specific mRNA of interest as well as genome-wide translatome analysis[14].

In this paper, we describe a detailed protocol of polysome profiling to analyze the developing mouse cerebral cortex. We use a home-assembled system to prepare sucrose density gradients and collect fractions for downstream applications. The protocol presented here can be adapted easily to analyze other types of tissues and organisms.

PROTOCOL:

All animal use was supervised by the Animal Care Committee at the University of Calgary. CD1 mice used for the experiment were purchased from commercial vendor.

1. Preparation of solutions

NOTE**:** To prevent RNA degradation, spray workbench and all equipment with RNase decontamination solution. RNase-free tips are used for the experiment. All solutions are prepared in RNase-free water.

1.1. Prepare cycloheximide stock solution (100 mg/mL) in DMSO and store at -20 ˚C.

1.2. Prepare 2.2 M sucrose stock solution by adding 75.3 g of sucrose to RNase-free water and topping up the volume to 100 mL (for ~16 gradient preparation). The solution can be kept at -20 ˚C for long-term storage.

1.3. Prepare 10x salt solution (1 M NaCl; 200 mM Tris-HCl, pH 7.5; 50 mM $MgCl_2$).

1.4. Prepare 60% (w/v) sucrose chase solution, containing 30 g of sucrose, 5 mL of 10x salt solution and top the volume up to 50 mL with RNase-free water.

1.5. Optionally, add a speck of bromophenol blue or approximately 5 µL of 1% bromophenol blue in RNase-free water to the chase solution.

1.6. Store solutions at 4 ˚C.

2. Preparation of sucrose gradient

NOTE: Accuracy in preparation of sucrose gradients is critical in obtaining consistent and reproducible results.

2.1. To prepare six 10-50% sucrose gradients, dilute 2.2 M sucrose solution as in **Table 1**.

[Place **Table 1** here]

NOTE: Always prepare sucrose gradients in multiples of two to balance weight during ultracentrifugation.

2.2. Wipe the metal blunt end needle with RNase decontamination solution. Briefly rinse the ultracentrifuge tubes, tubing and the syringe using RNase-free water. Air-dry the tubes such that no water drop remains in the tubes as any remaining water will alter the sucrose concentration.

2.3. Place the metal needle on the clamp holder and the centrifuge tube on the motorized stage. To prepare gradients consistently, a home-assembled system was used that contains a motorized stage to hold the ultracentrifuge tube and a syringe pump to inject sucrose solutions (**Figure 1**, see step 9 for details).

2.4. Fill a 30 mL syringe with ~16 mL of 10% sucrose (enough for six gradients), place it on the syringe pump and connect it to the needle. Make sure that there are no air bubbles in the syringe, tubing, and needle. Wipe the tip off the needle to remove any residual solution.

2.5. Move the motorized stage up such that the tip of the needle touches the centre of the tube at the bottom.

2.6. Set the syringe pump at a flow rate of 2 mL/min for a volume of 2.3 mL.

2.7. After dispensing 2.3 mL of 10% sucrose solution, move down the motorized stage and repeat the process for all six gradients.

2.8. Repeat steps 2.4–2.7 to add 20% sucrose solution to the bottom of the tubes, followed by 30%, 40% and 50% sucrose solutions similarly.

2.9. After preparation of the gradient, seal the ultracentrifuge tube using a paraffin film.

2.10. Leave the tubes overnight at 4 ˚C such that the different sucrose layers diffuse together to give a continuous gradient.

3. Tissue dissection

NOTE: Pregnant mice were euthanized by cervical dislocation preceded by anesthesia with 5% isoflurane.

3.1. Collect CD1 mouse embryos at embryonic day 12, or other timepoints as needed, and place embryos in a Ø 10 cm plate containing ice-cold Hank's Balanced Salt Solution (HBSS) on ice to retain cell viability.

3.2. Under the dissection scope, transfer one embryo to a Ø 6 cm plate containing ice-cold HBSS.

3.3. Use 21-23 G needles to fix the position of the head by penetrating through eyes at an approximately 45° angle and apply force to make sure the needles are fixed on the plate (**Figure 2A**).

3.4. Use No.5 forceps to remove skin and skull, from the middle to the sides.

3.5. Use forceps to cut the olfactory bulbs and remove the meninges to expose the cortical tissues.

3.6. Use curved forceps to cut the cortical tissues into 2-3 mL of neurobasal medium on ice (**Figure 2B**). Pool cortical tissues from different embryos as needed. Tissues from 8-10 embryos usually give ~200 µg total RNA.

4. Cell lysis

4.1. On the day of tissue dissection, prepare fresh cell lysis buffer as described in **Table 2**.

[Place **Table 2** here]

NOTE: Supplement lysis buffer with protease and phosphatase inhibitors.

4.2. Add cycloheximide to the neurobasal medium with dissected tissues to a final concentration of 100 µg/mL and incubate at 37 ˚C for 10 min. Cycloheximide blocks translation elongation and, therefore, prevents ribosome run-off [15].

4.3. Centrifuge at 500 x *g* for 5 min at 4 ˚C and discard the supernatant.

4.4. Wash the tissues twice with ice-cold phosphate-buffered saline (PBS) supplemented with 100 µg/mL cycloheximide.

4.5. Add 500 µL of cell lysis buffer supplemented with 4 µL of RNase inhibitor. Pipette up and down to resuspend the tissue in lysis buffer. Use an insulin needle to gently lyse the tissue.

4.6. Incubate the tissues on ice for 10 min with brief vortexing every 2-3 min.

NOTE: To prevent tissue degradation, ensure that all steps of tissue lysis are carried out on ice.

4.7. Centrifuge at 2,000 x *g* for 5 min at 4 ˚C.

4.8. Transfer the supernatant to a new centrifuge tube on ice.

4.9. Centrifuge at ~13,000 x *g* for 5 min at 4 ˚C.

4.10. Transfer the supernatant to a new centrifuge tube on ice.

4.11. Measure the RNA concentration in the tissue lysate using a UV-Vis spectrophotometer.

NOTE: If multiple samples are included in the experiment, dilute samples to the same concentration with extra cell lysis buffer to minimize variation.

5. Sample loading and ultracentrifugation

5.1. Pre-cool the ultracentrifugation rotor and swing buckets at 4 ˚C, and set the temperature of the ultracentrifuge to 4 ˚C.

5.2. Keep 20 µL of the tissue lysate as total RNA input.

5.3. Load samples (50-300 µg RNA with an equal volume) on the top of the sucrose gradients by slowly dispensing the lysate to the walls of the ultracentrifuge tubes.

5.4. Gently place the ultracentrifuge tubes in the swing bucket. Ensure that all diametrically opposite buckets are balanced.

5.5. Load the swing buckets on the rotor. Set the ultracentrifuge to 190,000 x *g* (~39,000 rpm) at 4 ˚C for 90 min.

5.6. Gently place the gradients on ice after centrifugation.

6. Fractionation and sample collection

NOTE: A home-assembled fractionating, recording and collecting system is used for the analysis and collecting samples from the gradients (**Figure 3**, see Device components).

6.1. Place an empty ultracentrifuge tube on the tube piercer and gently penetrate the tube with the needle from the bottom.

6.2. Switch on the UV monitor, and open the digital signal recording software.

6.3. Fill a 30 mL syringe with 25 mL of 60% sucrose chase solution. Gently press the syringe such that the chase solution fills up the empty tube and go through the 254 nm UV monitor to set the baseline for detection.

6.4. Press auto-zero on the UV-monitor to register a baseline for detection. Press play on the software to begin recording.

6.5. Once the system establishes a baseline, pause recording on the software and retract the chase solution. Ensure that no residual chase solution remains in the system.

NOTE: Rinse the system with RNase-free water to remove residual sucrose solutions remaining from the previous run.

6.6. Load one sample tube to the tube piercer. Gently penetrate the tube with the needle from the bottom.

6.7. Begin recording on the software. Start the syringe pump (flow rate of 1 mL/min for a volume of 25 mL) and the fraction collector to collect polysome fractions. Set fractionator settings to 30 s.

NOTE: Before each run, ensure no air bubbles in the syringe or the tubing by gently pressing the syringe to let the chase solution flow continuously through the needle.

6.8. Collect the fractions into 1.5 mL tubes (500 µL each) using a fraction collector.

6.9. The fractions can be processed immediately or stored at -80 ˚C.

6.10. After fraction collection, rinse the system with RNase-free water to remove any remnant sucrose.

7. Extraction of RNA

7.1. To each fraction, add 10 ng of luciferase mRNA spike-in control.

7.2. Add three volumes of guanidium hydrocholride based commercial RNA isolation reagent to each fraction.

7.3. Vortex briefly for 15 s.

7.4. Extract RNA using an RNA extraction kit compatible with the solution used in 7.2.

8. Reverse transcription and real-time PCR

8.1. Measure the concentration of the RNA using UV-Vis spectrophotometer.

8.2. Subject RNA to reverse transcription using a cDNA synthesis kit, according to manufacturer's protocol.

8.3. Use quantitative real-time PCR (qPCR) to examine the polysomal distribution of *gapdh* mRNA as an example. qPCR was performed using a qPCR detection system and a qPCR mastermix reagent, with the following cycling conditions: 95 °C for 10 min, then 40 cycles of 95 °C for 15 s, 60 °C for 30 s, 72 °C for 40 s, followed by 95 °C for 60 s.

8.4. Obtain Threshold cycle (Ct) values from the amplification plots and used to calculate fold change using ΔΔCt method.

9. Sucrose gradient making system assembly

NOTE: Follow the steps to assemble each component (as described in **Table 3**) of the sucrose gradient maker (**Figure 1**).

9.1. Mount two vertical brackets (A2) on the base breadboard (A1).

9.2. Use two slim right-angle brackets (B2) to mount the linear stage actuator to the base breadboard (A1).

9.3. Use the setscrew on the Ø12.7 mm aluminum post (C2) to fix it on the tube holder base breadboard (C1) as a stand for the tube holder.

9.4. Assemble the right-angle Ø1/2" to Ø6 mm post clamp (C3) with the post (C2) and the mini-series optical post (C4).

9.5. Use the setscrew on the optical post (C4) to connect a small V-clamp (C5) as the tube holder.

9.6. Put a centrifuge tube in the tube holder and adjust the angle to make it vertical. Mark the position of the tube and mount the pedestal post holder (C6) on the base breadboard (C1) to support the tube.

9.7. On the other side of the breadboard (A1), use the setscrew of the Ø12.7 mm aluminum post (D1) to fix it and connect a mini-series optical post (D3) using a right-angle Ø1/2" to Ø6 mm post clamp (D2).

9.8. Connect the miniature V-clamp (D4) with blunt-end needle (D5) to the post (D3). Adjust the angle of the clamp to set the needle vertical, and adjust the length of post to ensure the needle meets the centrifuge tube.

9.9. Connect the actuator (B1) to the stepper motor driver (E1) and use the UNO R3 controller board and the joystick module from the UNO starter kit (E2) to control the actuator. A power adaptor (e.g., 9-24 V AC/DC adjustable power adaptor) can be used to drive the motor separately.

9.10. Place the syringe pump (F) next to the gradient making station and connect the syringe with the needle.

9.11. Control the tube holder stage up and down using the joystick.

[Place **Table 3** here]

10. Fractionating and detecting system assembly (Figure 2).

10.1. Use a regular round jaw burette clamp to mount the optics module of the UV monitor on the tube piercer. Attach one end of the tubing (1 cm long and 0.56 mm internal diameter) to the connecter of the tube piercer and the other end to the Fluid In port of the optics module. Connect the Fluid Out port to the fractionator.

10.2. Connect the optics module with the control unit of the UV monitor according the manual of the manufacturer.

10.3. Use a breakout cable to connect the signal output socket to the digital converter at the ground and analog input connections, according to the manufacturer's manual.

10.4. Connect the digital converter to a laptop using a regular USB cable, and record the converted digital signals using data acquisition software.

REPRESENTATIVE RESULTS

As a demonstration, the cortical lysate containing 75 µg RNA (pooled from 8 embryos) was separated by the sucrose gradient into 12 fractions. Peaks of UV absorbance at 254 nm identified fractions containing the 40S subunit, 60S subunit, 80S monosome and polysomes (**Figure 4A**). Analysis of fractions by western blot for the large ribosomal subunit, Rpl10 showed its presence in the 60S subunit (fraction 3), monosome (fraction 4) and polysomes (fractions 5-12) (**Figure 4B**). In contrast, cytoplasmic proteins Gapdh and Csde1 were not associated with ribosomes but were enriched in fractions containing free RNA (fraction 1) (**Figure 4B**). Consistent with the separation of proteins in different fractions, we found that *gapdh* and *sox2* mRNAs were highly enriched in the fractions containing heavy polysomes (more than three ribosomes in fractions 7-12), suggesting that *gapdh* and *sox2* mRNAs are efficiently translated in the developing cortex (**Figure 4C,D**). In contrast, *rpl7* and *rpl34* mRNAs were enriched in the fraction containing monosomes, suggesting repressed translation (**Figure 4E,F**)[16]. These results validated our polysome fractionation protocol.

FIGURE AND TABLE LEGENDS:

Table 1: Sucrose dilutions for preparations of sucrose gradients.

Table 2: Preparation of polysome lysis buffer.

Table 3: Gradient making system components.

Figure 1: Setup for sucrose gradient preparation. (**A**) Home-assembled sucrose gradient maker consisting of a linear stage actuator, a tube holder on the motorized stage, a stage controller set, a metal blunt-end needle mounted on a needle holder, and an automated

syringe pump with a syringe connected to the needle (see **Table 3**). (**B**) To prepare the sucrose gradient, the ultracentrifuge tube is placed in the tube holder. Sucrose solutions are dispensed through the blunt-end needle using a syringe pump.

Figure 2: Dissection of the cortical tissues from the development mouse embryo. (**A**) Image showing an E12.5 CD1 embryo fixed to a Ø 6 cm plate using 21G-23G needles. (**B**) Image showing the dissected cortex after removal of the skin, skull and meninges (marked with white dashed lines).

Figure 3: Setup for fractionating, recording and sample collecting. Image depicting the home-assembled fractionation, recording and sample collection system comprising of an automated syringe pump, a tube piercer, a fraction collector and a UV monitor with a digital convertor connected to a laptop for the continuous monitoring of UV absorbance. Data acquisition is handled by the commercial data acquisition software.

Figure 4: Polysome profiling analysis of the developing mouse cortex. (**A**) UV absorbance showing fractions containing free RNA (fraction 1), 40S subunit (fraction 2), 60S subunit (fraction 3), 80S monosomes (fraction 4) and polysomes (fraction 5-12). 75 µg total RNA pooled from 8 embryos was used. (**B**) Western blots showing the distributions of Gapdh, Rpl10 and Csde1 proteins in fractions. qPCR analysis showing the distribution of *gapdh* (**C**), *sox2* (**D**), *rpl7* (**E**) and *rpl34* (**F**) mRNAs in fractions.

DISCUSSION:

Polysome profiling is a commonly used and powerful technique to assess the translational status at both single gene and genome-wide levels[14] . In this report, we present a protocol of polysome profiling using a home-assembled platform and its application to analyze the developing mouse cortex. This cost-effective platform is easy to assemble and generate robust, reproducible sucrose gradients and polysome profiling with high sensitivity.

It is worthy to note that the preparation of consistent and good quality sucrose gradients is critical to obtain reproducible polysome profiling results[17]. Changes in the volume of 10-50% sucrose solutions during gradient preparation could cause the shift of the polysome peaks and inconsistency of results in downstream analysis. Moreover, disturbing the gradient during the insertion and removal of the needle could contribute to the inconsistency of gradient preparation. Compared to other approaches and commercial devices, our home-made gradient making system used a motorized stage to reduce disturbance of gradients during preparation and a syringe pump to accurately dispense sucrose solutions, which offers a cheap and simple solution for gradient preparation.

Applying the polysome profiling platform and protocol present here to the developing mouse cortex, we found that the ribosomal protein Rpl10 and cytoplasmic proteins Gapdh and Csde1 were distributed in the correct fractions. Moreover, the highly translated gapdh and sox2 mRNAs were enriched in polysomal fractions, while translationally repressed rpl34 and rpl7 mRNAs showed less enrichement in polysomes, which validate our platform and protocol. With a similar approach, the translational status of other genes in the developing cortex can be determined individually by real-time PCR. Of note, polysome profiling has been used to analyse translational status in the developing brain at the genome-wide level. In this regard,

fractions containing polysomes can be combined and extracted RNA can be analysed using deep sequencing. The platform and protocol present here can be adapted and further optimized to suit translatomic studies using other cell types and tissues. Considering the availability of cortical tissues limited by experimental conditions (e.g., <50 µg RNA), additional optimization of the protocol may provide further increases in the efficiency and reproducibility of the experiments.

While polysome profiling provides insights into the translational status of mRNAs in the developing cortex, it has limitations to analyze specific cell types at later developmental stages, when different neural cell types are present. To address this question, polysome profiling can be integrated with TRAP by pull-down of epitope tagged ribosomal protein expressed under a tissue specific promoter[11].

In conclusion, the platform and protocol that we report here for sucrose gradient making and fractionation provide an economical solution to polysome profiling experiments in the context of brain development as well as other biological contexts.

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COMPETING INTERESTS

The authors declare no competing interests.

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Figure 1

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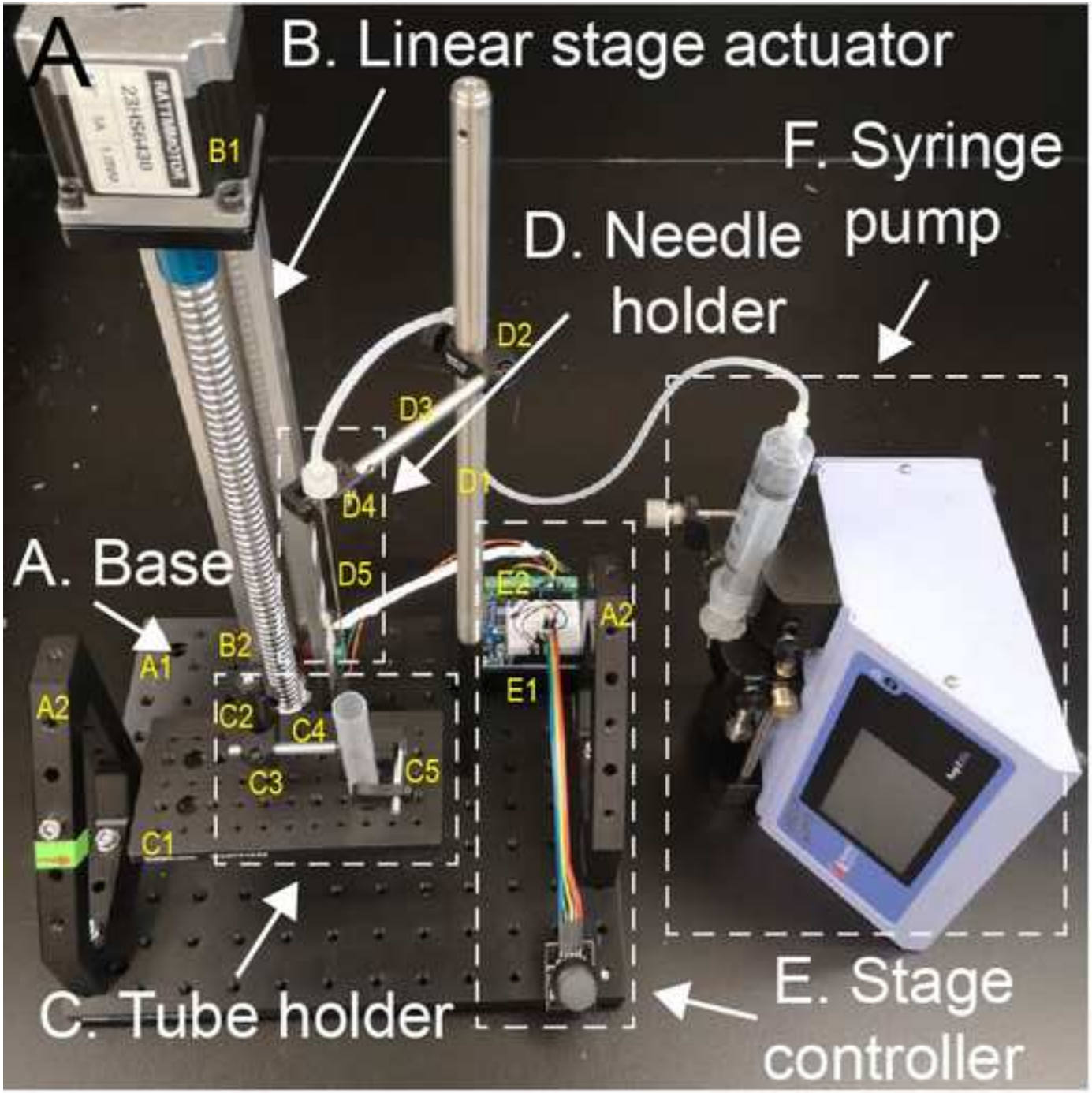

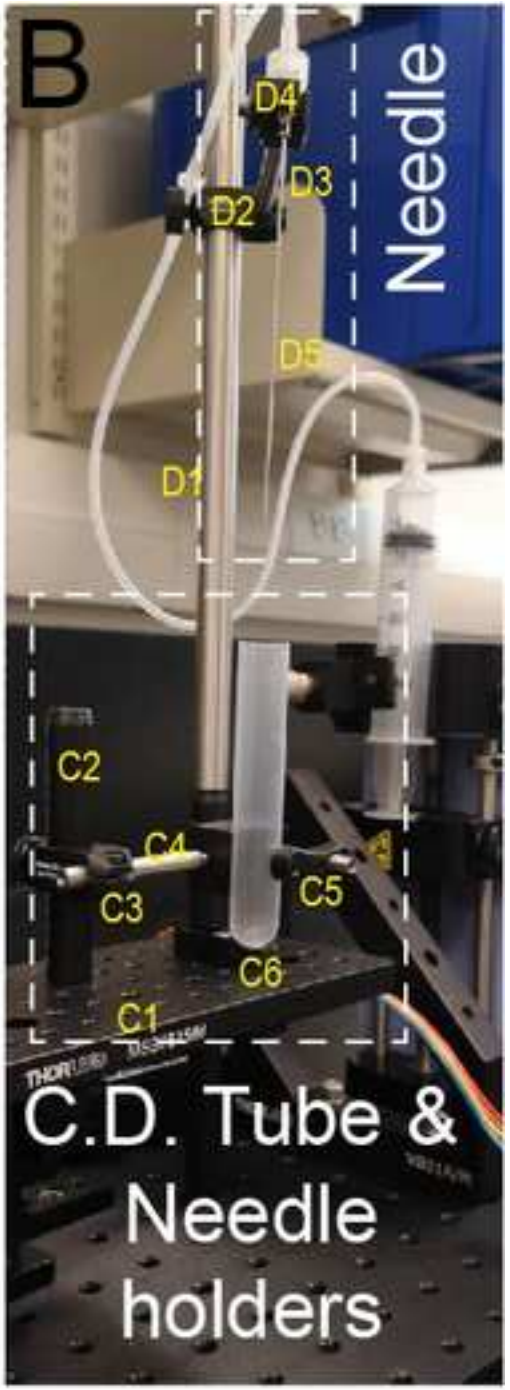

Figure 2

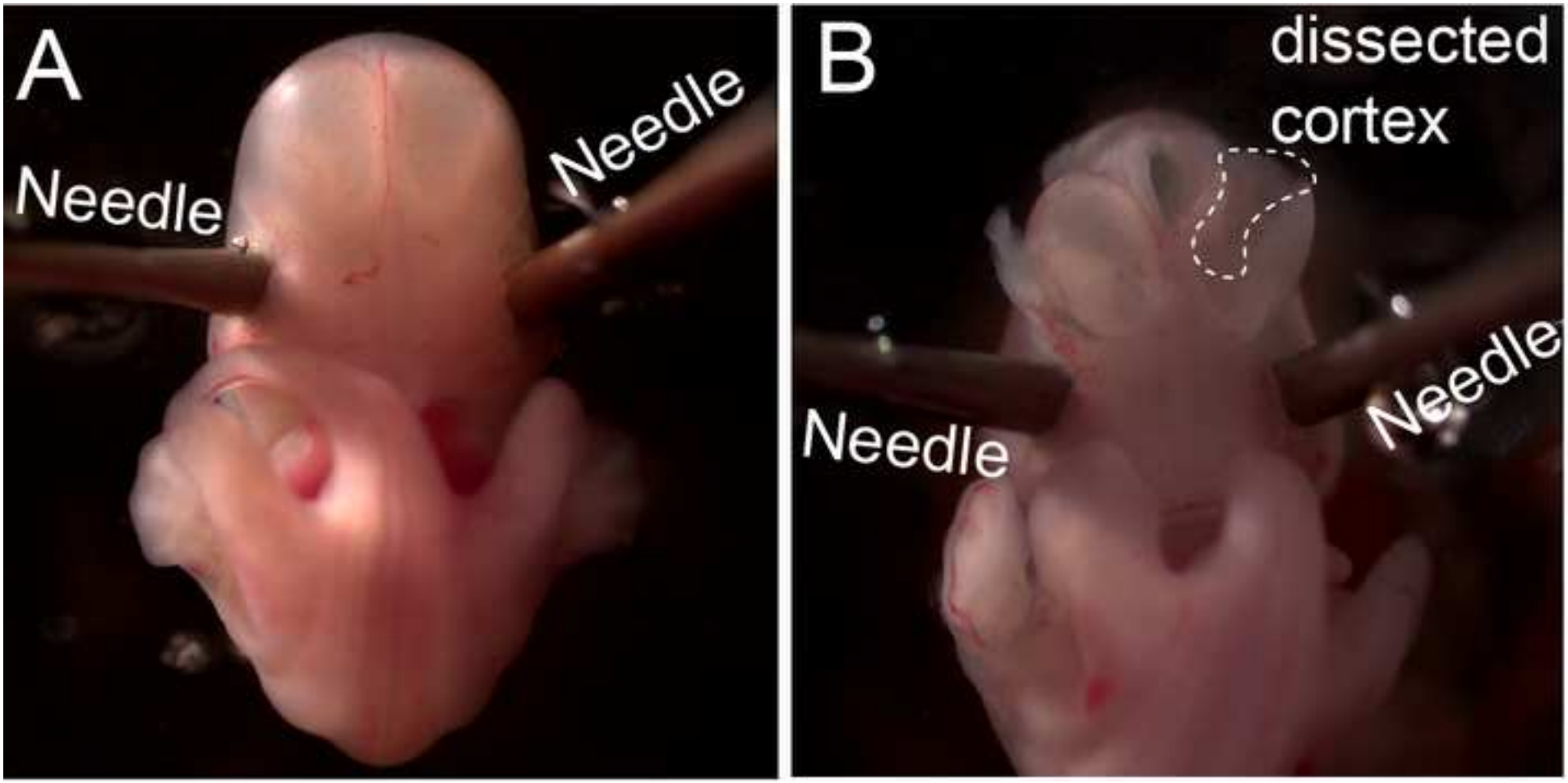

Figure 3

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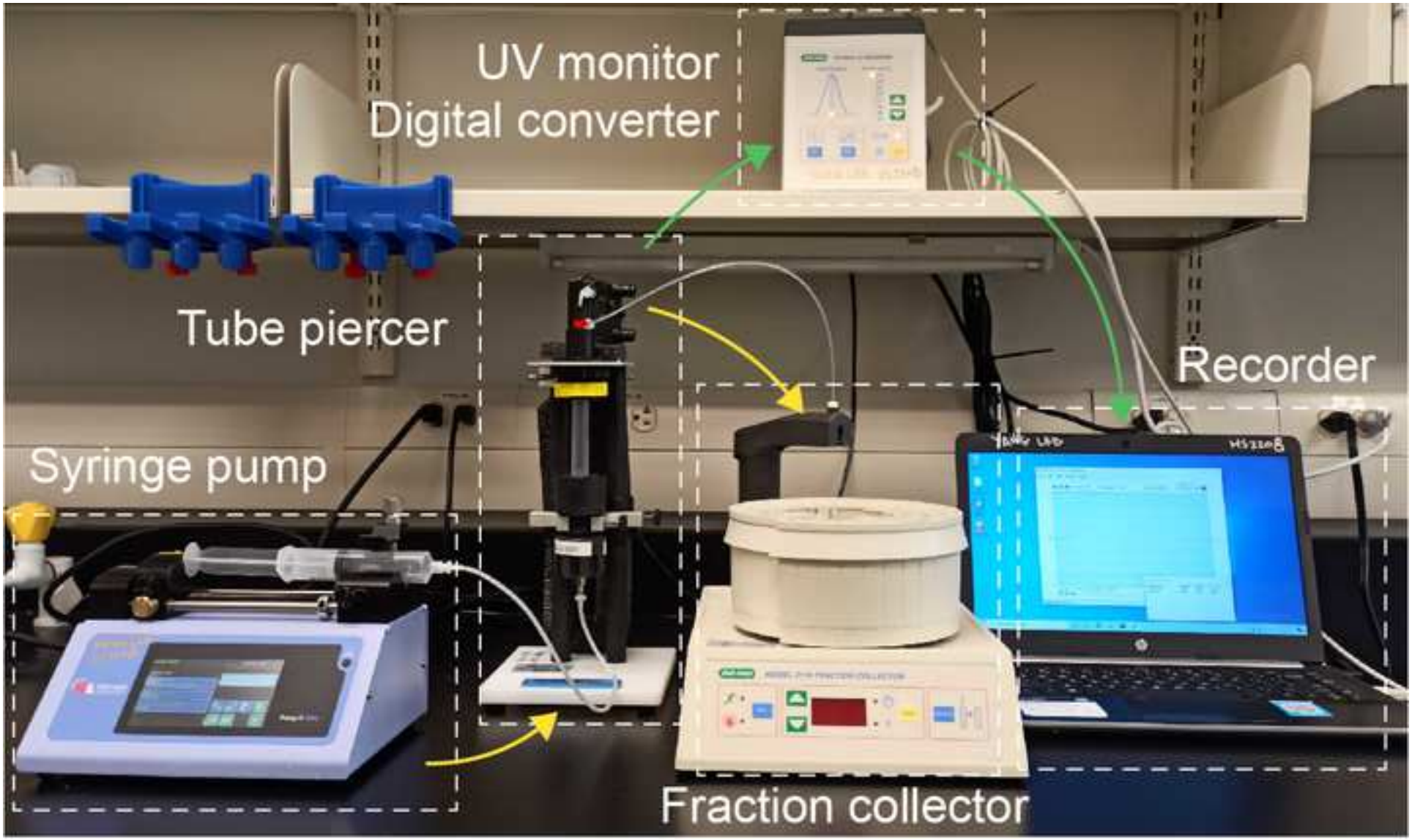

Figure 4

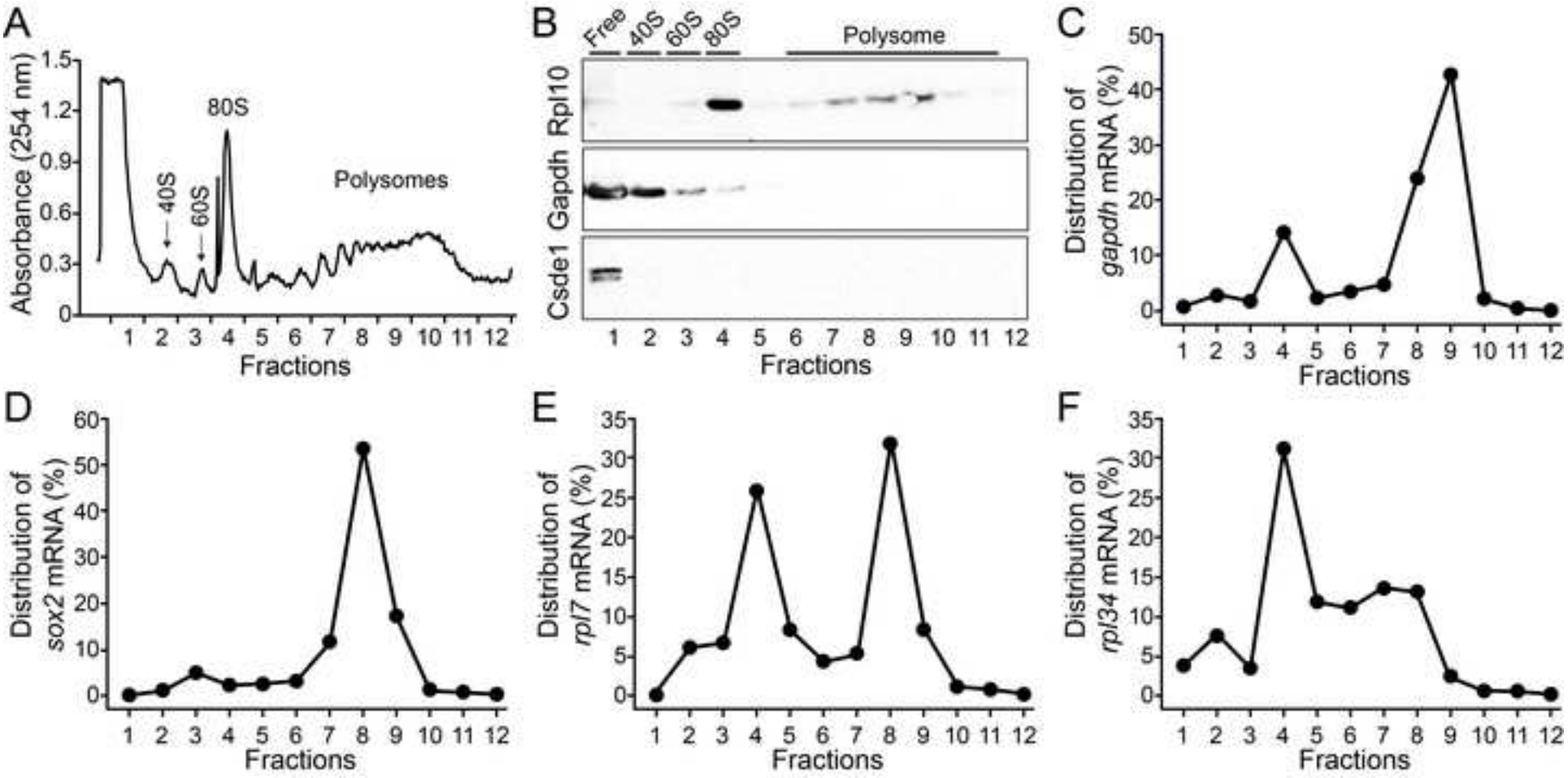

Figure 1


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Figure 4

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Table 1

Sucrose solution	**10%**	**20%**	**30%**	**40%**	**50%**
2.2 M sucrose	2 mL	4 mL	6 mL	8 mL	10 mL
10X salt solution	1.5 mL	1.5 mL	1.5 mL	1.5 mL	1.5 mL
Cycloheximide	15 µL	15 µL	15 µL	15 µL	15 µL
Water	11.5 mL	9.5 mL	7.5 mL	5.5 mL	3.5 mL
Total volume	15 mL	15 mL	15 mL	15 mL	15 mL

Table 2

Solution	**Final concentration**	**Volume**
Tris-HCl (pH 7.5)	20 mM	100 µL
KCl	100 mM	250 µL
$MgCl_2$	5 mM	25 µL
Triton X-100	1% (v/v)	500 µL
Sodium deoxycholate	0.5% (w/v)	500 µL
Dithiothreitol (DTT)	1 mM	5 µL
Cycloheximide	100 µg/mL	5 µL
RNase free water		Top up to 5 mL
Total	5 mL	5 mL

Component	Item
B1	Linear stage actuator
E1	Stepper motor driver
E2	UNO project super starter kit
A1	Breadboard
A2	Vertical bracket
B2	Slim right-angle bracket
C1	Mini-series breadboard
C5	Small V-clamp
D4	Miniature V-clamp
C2	Ø12.7 mm aluminum post
C4, D3	Mini-series optical post
D1	Ø12.7 mm aluminum post
C3, D2	Right-angle Ø1/2" to Ø6 mm post clamp
C6	Mini-series pedestal post holder base
D5	Blunt end needle
F	Syringe pump

Name of Material/Equipment	Company	Catalog Number
1.5 mL RNA free microtubes	Axygen	MCT-150-C
10 cm dish	Greiner-Bio	664160
1M MgCl2	Invitrogen	AM9530G
21-23G needle	BD	305193
2M KCl	Invitrogen	AM8640G
30 mL syringe	BD	302832
Blunt end needle	VWR	20068-781
Breadboard	Thorlabs	MB2530/M
Bromophenol blue	Sigma	115-39-9
CD1 mouse		Charles River Laboratory
Curved tip forceps	Sigma	#Z168785
Cycloheximide	Sigma	66-81-9
Data acquisition software TracerDA	Measurement Computing	
Digital converter	Measurement Comp	USB-1208LS
Direct-zol RNA miniprep kit	Zymo	R2070
Dithiothreitol (DTT)	Bio-basic	3/12/3483
DMSO	Bioshop	67-68-5
Dumont No.5 forceps	Sigma	#F6521
Fraction collector	Bio-Rad	Model 2110
HBSS	Wisent	311-513-CL
Linear stage actuator	Rattmmotor	CBX1605-100A
Luciferase control RNA	Promega	L4561
Maxima first strand cDNA synthesis kit	Themo Fisher	M1681
Miniature V-clamp	Thorlabs	VH1/M
Mini-series breadboard	Thorlabs	MSB7515/M
Mini-series optical post	Thorlabs	MS2R/M
Mini-series pedestal post holder bas	Thorlabs	MBA1
NaCl	Bio-basic	7647-14-5

Neurobasal media	Gibco	21103-049
Ø12.7 mm aluminum post	Thorlabs	TRA150/M
Parafilm	Bemis	PM992
PerfeCTa SYBR green fastmix	Quanta Bio	CA101414-274
Phosphate buffered saline (PBS)	Wisent	311-010-CL
Puromycin	Bioshop	58-58-2
Right-angle clamp	Thorlabs	RA90/M
Right-angle Ø1/2" to Ø6 mm post c	Thorlabs	RA90TR/M
Rnase AWAY	Molecular BioProducts	7002
RNase free tips	Frogga Bio	FT10, FT200, FT1000
RNase free water	Wisent	809-115-CL
RNasin	Promega	N2111
Slim right-angle bracket	Thorlabs	AB90B/M
Small V-clamp	Thorlabs	VC1/M
Sodium deoxycholate	Sigma	302-95-4
Stepper motor driver	SongHe	TB6600
Sucrose	Bioshop	57501
SW 41 Ti rotor	Beckman Coulter	331362
Syringe pump	Harvard Apparatus	70-4500
Syringe pump	Harvard Apparatus	70-4500
Triton-X-100	Bio-basic	9002-93-1
Trizol	Thermofisher Scientific	15596018
Tube piercer	Brandel	BR-184
Ultracentrifuge	Beckman Coulter	L8-70M
Ultracentrifuge tubes	Beckman Coulter	331372
UltraPure 1M Tris-HCl pH 7.5	Invitrogen	15567-027
UNO project super starter kit	Elegoo	EL-KIT-003
UV monitor	Bio-Rad	EM-1 Econo
Vertical bracket	Thorlabs	VB01A/M

Name	Primer
GAPDH-f	5'-ACC ACA GTC CAT GCC ATC AC-3'
GAPDH-r	5'-CAC CAC CCT GTT GCT GTA GCC-3'
Luciferase	5’-ACG TCT TCC CGA CGA TGA -3’
Luciferase	5’-GTC TTT CCG TGC TCC AAA AC -3’

Dear Editor,

Thank you for the editorial comments, and attached please find our revised manuscript "Analysis of translation in the developing mouse brain using polysome profiling" (JoVE62088).

In this version, we have included new results for both translationally activated and repressed mRNAs in addition to gapdh mRNA (Fig 4C-F). We have also addressed other specific comments marked in the manuscript. We hope that this version of the manuscript is now suitable for publication on JoVE.

Sincerely,
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