Protocol

Gel Purification of RNA

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For many applications, including size selection of RNAs and purification of in vitro transcription products, it is necessary to purify RNAs on a denaturing gel. This procedure describes how to purify transcripts that have been synthesized in vitro. It is useful for labeled or unlabeled RNAs when sufficient mass is present. It can also be used to isolate small RNAs. In general, RNA purification by denaturing gel electrophoresis is practical only when the size of the desired RNA is 600 nucleotides or less.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPES: Please see the end of this article for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Chloroform

Denaturing polyacrylamide gel (see Step 4)

EDTA (2 mM, pH 8.0)

Ethanol (100%)

Formamide gel-loading buffer <R>

Gel elution buffer <R>

Phenol:chloroform (1:1)

Sample of RNA

This procedure describes how to purify transcripts that have been synthesized using the protocol ***In Vitro Transcription of Labeled RNA: Synthesis, Capping, and Substitution*** *(Nilsen and Rio 2012). It can also be used for other cellular RNAs or small RNAs (e.g., those described in* ***Preparation of Small RNA Libraries for High-Throughput Sequencing*** *[Malone et al. 2012]).*

TBE electrophoresis buffer (10×) <R>

Equipment

Dry ice

Electrophoresis apparatus for denaturing polyacrylamide gel and power supply

Adapted from *RNA: A Laboratory Manual*, by Donald C. Rio, Manuel Ares Jr, Gregory J. Hannon, and Timothy W. Nilsen. CSHL Press, Cold Spring Harbor, NY, USA, 2011.

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Fluorescent markers (luminescent paint) also available commercially from art supply stores
Hand-held ultraviolet (UV) light (254 nm)
Ice
Microcentrifuge
Microcentrifuge tubes
Plastic wrap
Scalpel or single-edge razor blade
Temperature block (95°C)
X-ray film and developer
X-ray intensifying screen

METHOD

1. After phenol:chloroform:isoamyl alcohol (PCA) extraction and precipitation of the RNA with ethanol, resuspend RNA from a 50-µL transcription or 5′- or 3′-end-labeling reaction in 2.5 µL of 2 mM EDTA (pH 8), and add 5 µL of formamide gel-loading buffer.
2. Prepare a denaturing polyacrylamide gel as described in **Polyacrylamide Gel Electrophoresis of RNA** (Rio et al. 2010). Set up the gel in the gel box, add TBE electrophoresis buffer (diluted to 1×) to the upper and lower reservoirs, and prerun the gel for 15–45 min at a maximum of 1500 V/45 mA.

   If the RNA transcript is greater than 100 nucleotides, do shorter preruns (15–20 min).
3. Heat the sample from Step 1 for 1 min at 95°C and then place it on ice.
4. Load the sample. Use one lane for the RNA from one 50-µL transcription reaction (see **In Vitro Transcription of Labeled RNA: Synthesis, Capping, and Substitution** [Nilsen and Rio 2012]). If total cellular RNA is labeled, load it at 5–10 µg per lane. These amounts will not overload the lanes. (Refer to **Polyacrylamide Gel Electrophoresis of RNA** [Rio et al. 2010] to decide which gel to run.) Run the gel at a maximum of 1500 V/45 mA until the bromophenol blue dye is about halfway down the gel.

   If the sample is run too far, unincorporated radiolabel will be present in the lower buffer chamber, increasing the chances of unwanted contamination. This will not affect the RNA preparation, but it makes the cleanup process more time-consuming.

   If the RNA is not radiolabeled, proceed to Step 5; if the RNA is radiolabeled, proceed to Step 6.
5. If the RNA is not radiolabeled, visualize the band by UV shadowing.
   i. Remove one glass plate and cover the gel with plastic wrap.
   ii. Cut a wide area of the gel expected to contain the band of RNA and transfer it (plastic wrap side down) to a clean X-ray intensifying screen.
   iii. Shine the UV light on the gel piece.

      The exact location of the RNA band will appear purple in the gel under UV light.
   iv. Excise the band with a razor blade and then proceed to Step 7.
6. If the RNA is radiolabeled, visualize the band as follows:
   i. Remove one glass plate and cover the gel with plastic wrap. Place fluorescent ink markers on the corners of the gel and expose the gel to X-ray film.

      High-specific-activity transcripts should require 10–30 sec; trace-labeled transcripts may take 5–10 min.

ii. Identify the location of the radiolabeled RNA on the gel, using the fluorescent ink markers on the gel to line up the gel with the marks on the film.

iii. Use a razor blade to excise the pieces of the acrylamide gel that contain the RNA and then continue to Step 7.

7. Place the gel slices in a microcentrifuge tube with 400 µL of gel elution buffer (up to three slices from the same RNA in one tube).

8. Freeze the tube containing the buffer and gel pieces on dry ice for 15 min and then allow the RNA to diffuse from the gel by leaving the tube overnight at room temperature.

9. Centrifuge the tube at maximum speed for 10 min at room temperature, and draw off and save the supernatant in a clean microcentrifuge tube.

This supernatant should contain ≥70% of the RNA.

10. Extract the RNA with 1–2 volumes of 1:1 phenol:chloroform, then with 1 volume of chloroform. Precipitate the RNA with 2 volumes of ethanol.

11. Resuspend in 5–10 µL of H_2O and quantitate by incorporation of label or by A_{260} if necessary. Dilute to 500 ng/µL with H_2O.

RNA should be stored short term at –20°C or indefinitely at –80°C.

RECIPES

Formamide Gel-Loading Buffer

Reagent	Quantity (for 10 mL)	Final concentration
Deionized formamide	9.5 mL	95%
Bromophenol blue	2.5 mg	0.025% (w/v)
Xylene cyanol FF	2.5 mg	0.025% (w/v)
EDTA (0.5 M, pH 8.0)	100 µL	5 mM
H_2O	400 µL	

Store in aliquots at –20°C.

Gel Elution Buffer

Reagent	Quantity (for 500 mL)	Final concentration
Tris–HCl (1 M, pH 7.5)	10 mL	20 mM
Sodium acetate (2.5 M, prepared without pH adjustment)	50 mL	0.25 M
EDTA (0.5 M, pH 8.0)	1 mL	1 mM
SDS (10%, w/v)	12.5 mL	0.25%
H_2O	426.5 mL	

If the SDS precipitates, warm to 37°C until the precipitate disappears. Store indefinitely at room temperature.

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TBE Electrophoresis Buffer (10X)

Reagent	Quantity (for 1 L)	Final concentration
Tris base	121.1 g	1 M
Boric acid	61.8 g	1 M
EDTA (disodium salt)	7.4 g	0.02 M

Prepare with RNase-free H_2O. Dilute 100 mL to 1 L to make gel running buffer. Store for up to 6 mo at room temperature.

REFERENCES

Malone C, Brennecke J, Czech B, Aravin A, Hannon GJ. 2012. Preparation of small RNA libraries for high-throughput sequencing. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot071431.

Nilsen TW, Rio DC. 2012. In vitro transcription of labeled RNA: Synthesis, capping, and substitution. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot072066.

Rio DC, Ares M Jr, Hannon GJ, Nilsen TW. 2010. Polyacrylamide gel electrophoresis of RNA. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot5444.

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