CHAPTER NINETEEN

RNA Purification – Precipitation Methods

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Abstract

When working with RNA, the need often arises to concentrate a sample or purify it from various salts, nucleotides, and proteins. RNA precipitation is an easy and cost-effective method for the concentration of RNA, leaving a pellet that can be resuspended in the buffer of choice.

Methods in Enzymology, Volume 530
ISSN 0076-6879
http://dx.doi.org/10.1016/B978-0-12-420037-1.00019-1

1. THEORY

RNA dissolves readily in water because both are highly polar substances in nature. In order to remove RNA from water, the charged backbone must be neutralized. This is generally achieved by the addition of monovalent cations in the form of salts (i.e., sodium acetate, ammonium acetate, lithium chloride) and in some cases alcohol. While the salt provides a positive charge to neutralize the phosphate backbone of RNA, the alcohol allows the salt to interact with RNA more effectively by changing the dielectric constant of the solvent, and enhancing electrostatic attraction between positively charged ions and the phosphate backbone. These net neutral particles can easily mass together and precipitate out of solution to be collected by centrifugation.

The choice of salt to be used depends on the application. Lithium chloride is efficient at precipitating RNA molecules of at least 100 nucleotides, but does not efficiently precipitate DNA, tRNA and other small RNA fragments, most proteins, and nucleotides, making it an ideal choice for the purification of mRNAs following *in vitro* transcription, or for the purification of ribosomal RNA. However, lithium chloride may not be as effective with low concentrations ($<400\ \mu g\ ml^{-1}$), so dilute RNAs may be more efficiently precipitated by ethanol and salt (Cathala et al., 1983).

Ammonium acetate is efficient for ethanol precipitating small and larger RNAs, but does not precipitate nucleotides, making it a good candidate for the purification of RNAs after reactions, but because it also precipitates proteins, phenol/chloroform extractions are usually performed first. Also, ammonium acetate can inhibit T4 polynucleotide kinase, so it is not a good choice for purifying RNAs that will be phosphorylated following purification.

Sodium acetate is highly efficient at ethanol precipitating nucleic acids, and as a result also precipitates nucleotides, DNA, and small RNA fragments. However, sodium acetate is the most versatile salt because in addition to being highly efficient at precipitating all nucleic acids, it does not inhibit many of the reactions that are often performed with purified RNAs.

When small amounts of RNA are to be precipitated, a carrier is often added to make the pellet more visible. Carrier RNA (yeast tRNA) or DNA (salmon sperm DNA) can be added, but for some purposes, these carriers would interfere with subsequent reactions or concentration measurements, so in these cases, glycogen can be added, as it readily precipitates in ethanol and makes RNA pellets visible.

2. EQUIPMENT

Microcentrifuge or floor centrifuge with appropriate rotors
Micropipettors
Micropipettor tips
1.5-ml microcentrifuge tubes
Beakers
1 l Graduated cylinders
Sterile 0.22-μm filter units
50-ml polypropylene conical tubes

3. MATERIALS

RNA to be precipitated
100% Ethanol
Lithium chloride (LiCl)
Sodium acetate (NaOAc)
Ammonium acetate (NH_4OAc)
Glacial acetic acid
Type I grade water (Molecular Biology grade, free of RNases)
Dry ice (optional)
95% Ethanol or Methanol (optional)
Glycogen

3.1. Solutions & buffers

7.5 M Ammonium acetate

Dissolve 578 g CH_3COONH_4 in a final volume of 1 l H_2O. Filter through a 0.22-μm bottle-top filter

3 M Sodium acetate

Dissolve 246 g CH_3COONa in 600 ml of H_2O. Adjust pH by adding acetic acid drop-wise until pH reaches 5.2. Adjust volume to 1-l with H_2O. Filter through a 0.22-μm bottle-top filter

7.5 M Lithium chloride

Dissolve 318 g LiCl in a final volume of 1 l H_2O. Filter through a 0.22-μm bottle-top filter

1 mg ml^{-1} Glycogen
Dissolve 50 mg glycogen in 50 ml H_2O in a conical tube. Store at −20 °C in 1 ml aliquots

70% Ethanol
Mix 35 ml 100% ethanol and 15 ml water

4. PROTOCOLS

4.1. Preparation

Prepare RNA by isolating it from cells, polysomes (see Analysis of polysomes from bacteria, or Polysome Profile Analysis - Yeast, or Polysome analysis of mammalian cells or Polysome analysis for determining mRNA and ribosome association in Saccharomyces cerevisiae), or by *in vitro* transcription (see *In Vitro* Transcription from Plasmid or PCR amplified DNA). Phenol/chloroform extraction is recommended prior to ethanol precipitation.

4.2. Duration

Preparation	1 day
Protocol	1 h–1 day

4.3. Tip

RNAses are a ubiquitous component of skin. Always wear gloves when handling RNA and materials used for processing RNA.

5. METHOD 1 ETHANOL PRECIPITATION OF RNA

5.1. Overview

Precipitate RNA by incubating with a salt of choice and ethanol. Collect RNA by centrifugation, rinse pellet with 70% ethanol, and dissolve RNA pellet.

5.2. Duration

1 h to overnight

1.1 Add 0.1 volumes of 7.5 M ammonium acetate or 3 M sodium acetate and mix. Then add 2.5 volumes of 100% ethanol.

1.2 Incubate for 25 min in a dry ice/ethanol bath or for 2 h to overnight at −20 °C.

1.3 Pellet RNA at 12 000 × g for 15 min (maximum speed in a microcentrifuge).

1.4 Carefully remove the supernatant and wash the RNA pellet (do not try to resuspend pellet) by adding 2.5 volumes of 70% ethanol and allowing the pellet to soak for 2 min. Pellet at 12 000 × g for 2 min and remove all the ethanol.

1.5 Allow the pellet to dry in open tube covered with a Kimwipe for 5 min to 1 h at room temperature and dissolve RNA in H_2O or desired buffer.

5.3. Tip

To make a dry ice/ethanol bath, fill the bottom of a polystyrene container with 1–2 inches of dry ice and add 95% ethanol (or methanol) to just cover the dry ice. Use ethanol-resistant markers to label tubes (e.g., VWR lab markers) so that labels do not rub off. To dispose of dry ice/ethanol, leave the vessel on a bench top to sublimate/evaporate. Do not pour dry ice/ethanol into a sink as it can crack the sink basin and cause serious damage to plumbing. Ethanol/dry ice baths are at −72 °C and Methanol/dry ice ones at −78 °C.

5.4. Tip

The protocols described here are for small volumes, using 1.5-ml microcentrifuge tubes, and centrifugation can be done in a microcentrifuge. For larger volumes, use 14-ml Sarstedt tubes and centrifuge adaptors to pellet RNA in a Sorvall SS-34 rotor or equivalent for 45 min at 9000 rpm. The resulting RNA pellets are larger and generally need to dry longer (can be left to dry overnight).

5.5. Tip

When precipitating small amounts of RNA from dilute samples, glycogen can be added as a carrier molecule to increase yield, and will help form a more visible pellet. Add 1 μg μl^{-1} glycogen and mix before adding ethanol in Step 1.1 and proceed.

5.6. Tip

Step 1.2 is a good stopping point. RNA can be stored under ethanol at −20 °C for several months.

See Fig. 19.1 for the flowchart of Method 1.

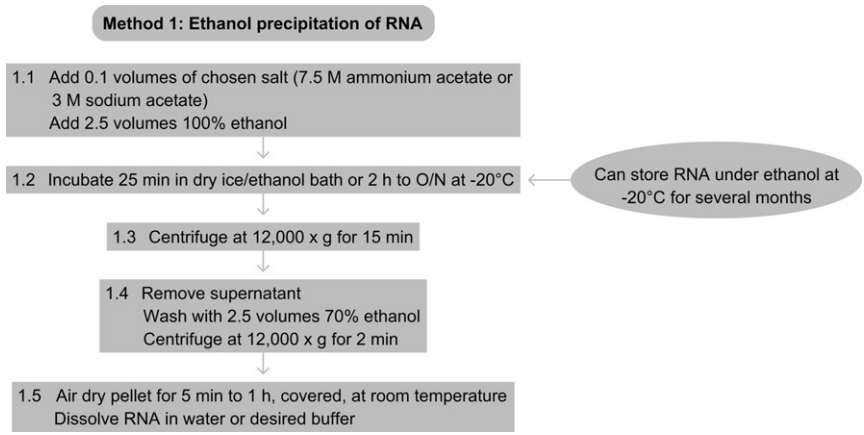

Figure 19.1 Flowchart of Method 1.

6. METHOD 2 LITHIUM CHLORIDE PRECIPITATION

6.1. Overview

Precipitate RNA using lithium chloride. Lithium chloride does not precipitate tRNA and may not work as well for RNAs under ~100 nucleotides or very dilute RNAs. Lithium chloride precipitation removes nucleotides and most proteins.

6.2. Duration

1 h

2.1 Add an equal volume of 7.5 M lithium chloride.

2.2 Incubate in dry ice/ethanol for 15 min or for at least 30 min at −20 °C.

2.3 Pellet RNA at 12 000 × g for 15 min (maximum speed in a microcentrifuge).

2.4 Carefully remove the supernatant and wash the RNA pellet (do not try to resuspend pellet) by adding 2.5 volumes of 70% ethanol and allowing the pellet to soak for 2 min. Pellet at 12 000 × g for 2 min and remove all the ethanol.

2.5 Allow the pellet to dry and resuspend in water or in a buffer of choice.

6.3. Tip

As little as 0.5 M LiCl in a solution precipitates 1 μg ml^{-1} RNA efficiently (see http://www.ambion.com/techlib/tb/tb_160.html for further reference).

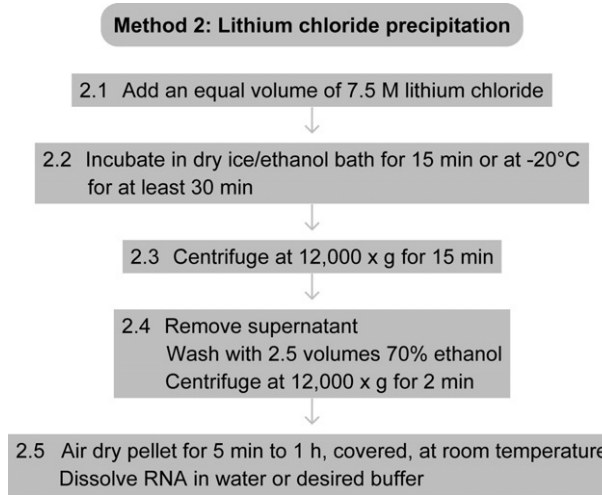

Figure 19.2 Flowchart of Method 2.

6.4. Tip

RNA pellets need to dry long enough so that residual ethanol will not interfere with subsequent reactions. However, overdrying RNA pellets can make pellets difficult to dissolve.

See Fig. 19.2 for the flowchart of Method 2.

REFERENCES

Referenced Literature

Cathala, G., Savouret, J. F., Mendez, B., et al. (1983). A method for isolation of intact, translationally active ribonucleic acid. *DNA*, *2*, 329–335.
http://www.ambion.com/techlib/tb/tb_160.html.

Referenced Protocols in Methods Navigator

Analysis of polysomes from bacteria.
Polysome Profile Analysis - Yeast.
Polysome analysis of mammalian cells.
Polysome analysis for determining mRNA and ribosome association in Saccharomyces cerevisiae.
In Vitro Transcription from Plasmid or PCR amplified DNA.