Phenol-chloroform extraction with ethanol precipitation

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Abstract

The traditional phenol extraction procedure, based on early work by Kirby (1957) and elaborated in most modern protocol compendia, yields very clean nucleic acids suitable for a variety of downstream applications. This extraction method is coupled with a routine alcohol precipitation step to allow buffer exchange, removal of trace amounts of chloroform, and concentration of nucleic acids (Sambrook and Russell 2001).

This is a protocol from:

Steward, G. F. and A. I. Culley. 2010. Chapter 16: Extraction and purification of nucleic acids from viruses. Manual of Aquatic Viral Ecology. Waco, TX:American Society of Limnology and Oceanography. doi:10.4319/mave.2010.978-0-9845591-0-7

Please see the published manuscript for additional information.

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Guidelines

Materials and equipment:
• fume hood
• microcentrifuge (refrigerated if possible)
• pipettes and sterile, disposable tips
• safety gear (gloves, lab coat, safety glasses)
• sterile microcentrifuge tubes
• TE buffer (10 mM Tris, 1 mM EDTA; pH 8)
• Tris-saturated phenol, pH 8 (*see* “Warning” below)
• CI (chloroform:IAA, 24:1, vol:vol) (*see* “Warning”)
• PCI (phenol:chloroform:IAA, 25:24:1) (*see* “Warning”)
• sodium acetate, 3 M, pH 5.2 (*see* Sambrook and Russell [2001] for a discussion of alternative salts that may be used for nucleic acid precipitation and their advantages and disadvantages)
• Optional: polyacryl carrier (Molecular Research Center)
• ethanol, 70% and 100%

Warning: Phenol can cause chemical burns if it comes in contact with bare skin. Phenol and chloroform are volatile and carcinogenic and must be used in a fume hood with proper protection (gloves, lab coat, and safety glasses). PCI and CI preparations that are ready to use can be purchased from a variety of scientific chemical suppliers. Details of how to prepare these solutions for oneself can be found elsewhere (Sambrook and Russell 2001).

Assessment: This traditional method of extraction is most commonly used to extract DNA. When phenol is saturated with alkaline buffer (e.g., Tris, pH 8), however, both RNA and DNA will partition to the aqueous phase, so the method can be used for total nucleic acid extraction. Either DNA or RNA can be specifically selected by digestion of the recovered total nucleic acids with RNase or DNase. If targeting RNA, an RNase inhibitor may be included to help ensure stability. If one wishes to isolate both RNA and DNA but in separate fractions by organic extraction, we recommend the use of the commercially available reagents TRI Reagent and TRIzol (see above).

Although still in use, the popularity of organic extraction has waned somewhat as new extraction procedures have been developed that do not require the use of toxic organic compounds. In addition to the extra precautions that must be taken when handling phenol and chloroform during extraction, the disposal of the resulting organic waste is costly.

Protocol

Step 1.
To the viral suspension (≤0.6 mL per 2-mL microcentrifuge tube, scale up for larger volumes) add an equal volume of PCI and shake to emulsify.

Step 2.
Centrifuge at 10,000g for 5 min to facilitate separation of the organic and aqueous phases.

DURATION
00:05:00

Step 3.
Transfer the DNA-containing aqueous phase (upper) to a new tube by aspiration with a pipette, being careful to avoid material at the interface.

Step 4.
Repeat steps 1–3 as needed until the interface appears to be free of extracted material (one extraction may suffice for relatively pure viral preparations).

Step 5.
Add an equal volume of CI to the aqueous phase and shake to emulsify.

Step 6.
Centrifuge as in step 2 to separate phases.

Step 7.
Transfer the aqueous phase (upper) to a new tube.

Step 8.
Add 1 µL polyacryl carrier.

NOTES
Vani Mohit 08 Sep 2015
This optional step is unnecessary when working with tens of nanograms or more of DNA, but can improve yields when working with nanogram to subnanogram quantities.

Step 9.
Add 1/10 of a volume of sodium acetate and invert tube or vortex to mix.

Step 10.
Add 2 volumes of ethanol and invert tube to mix.

Step 11.
Incubate sample on ice for 10 min.

DURATION
00:10:00

Step 12.
Centrifuge for 10 to 30 min, at 0–4°C if possible.

DURATION
00:30:00

Step 13.
Aspirate or decant the supernatant, being careful not to disturb the pellet.

NOTES
Vani Mohit 23 Sep 2015
A pellet may not be visible if the amount of DNA is low and no carrier has been added.

Step 14.
Add 500 µL ice-cold 70% ethanol.

Step 15.
Centrifuge at 10,000g for 10 min.

DURATION
00:10:00

Step 16.
Decant or aspirate supernatant as completely as possible, being careful not to disturb the pellet.

Step 17.
Allow residual liquid in the tube to evaporate by air-drying with the cap open and the tube upside down or by placing briefly in a centrifugal vacuum concentrator (e.g., SpeedVac concentrator, Thermo Scientific; Concentrator plus, Eppendorf).

NOTES
Vani Mohit 08 Sep 2015
Note that excessive drying will make the nucleic acid more difficult to dissolve.

Step 18.
Resuspend the dried pellet in a small volume of Tris (10 mM, pH 8) or TE buffer.

NOTES
Vani Mohit 08 Sep 2015
Note that some of the material will be on the side of tube, so the appropriate side of the tube should be exposed to the liquid used for resuspension to maximize recovery.

Step 19.
The purified, solubilized DNA may be stored at 4°C for short periods of time, at –20°C for long periods of time, and at –80°C indefinitely.

NOTES
Vani Mohit 08 Sep 2015
For long-term storage, one might also consider storing the dried DNA pellet, which should remain stable at room temperature or below if kept dry.

Warnings

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and safety glasses). PCI and CI preparations that are ready to use can be purchased from a variety of scientific chemical suppliers. Details of how to prepare these solutions for oneself can be found elsewhere (Sambrook and Russell 2001).