

RNA extraction with PGTX

Lutz Berwanger

Abstract

This protocol is used for RNA extraction in cyanobacteria after Pinto et al. 2009.

Pinto, Fernando Lopes; Thapper, Anders; Sontheim, Wolfgang; Lindblad, Peter (2009): Analysis of current and alternative phenol based RNA extraction methodologies for cyanobacteria. In: *BMC molecular biology* 10, S. 79. DOI: 10.1186/1471-2199-10-79



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Protocol

Step 1.

Fill 50 mL Falcon tube with ice. Fill with bacterial liquid culture, up to a volume of 45 mL.

Step 2.

Centrifuge for 3 minutes at 4°C and 4.000 g.

 **DURATION**

00:03:00

Step 3.

Discard supernatant. Resuspend cell pellet in residual water (1 mL). Transfer to 2 mL 'safe lock' tube.

Step 4.

 **DURATION**

00:00:15

Step 5.

Resuspend the cell pellet in 1 ml of PGTX. Freeze in liquid nitrogen and store at -20 °C.

 **SAFETY INFORMATION**

Wear goggles, a lab coat and gloves when dealing with PGTX and liquid nitrogen.

Step 6.

Incubate for 5 min at 95 °C, shaking at 250 rpm in Thermomixer (Eppendorf)

 DURATION

00:05:00

Step 7.

Rapidly chill 5 min on ice.

 DURATION

00:05:00

Step 8.

Add 700 µl Chloroform.

 REAGENTS

✓ Chloroform by Contributed by users

 SAFETY INFORMATION

Wear goggles, a lab coat and gloves when dealing with phenol and chloroform.

Step 9.

Let the samples incubate for 10 min at room temperature in a Thermomixer. Vortex from time to time.

 DURATION

00:10:00

Step 10.

Centrifuge for 15 min at 14.000 g, 4 °C. Transfer the upper aqueous phase (**contains RNA**) to a fresh reaction tube and add the same volume (450 µL) of Aqua- P/C/I (Phenol/Chloroform/Isoamylalcohol).

 DURATION

00:15:00

 SAFETY INFORMATION

Wear goggles, a lab coat and gloves when dealing with phenol and chloroform.

Step 11.

Thoroughly mix by vortexing. Centrifuge for 15 min at high speed. Transfer the upper aqueous phase to a 1.5 mL reaction tube.

 DURATION

00:15:00

 SAFETY INFORMATION

Wear goggles, a lab coat and gloves when dealing with phenol and chloroform.

Step 12.

Add 1 volume of isopropanol.



REAGENTS



Isopropanol by Contributed by users

Step 13.

Mix and incubate for at least 30 min at -20 °C. (Can be left overnight.)



DURATION

00:30:00

Step 14.

Centrifuge at 14.000 g for 30 min.



DURATION

00:30:00

Step 15.

Discard supernatant.

Step 16.

Wash with 75% chilled ethanol. Avoid resuspending the pellet.



REAGENTS

Ethyl alcohol, Pure 200 proof, for molecular biology [E7023](#) by [Sigma Aldrich](#)

Step 17.

Centrifuge at 14.000 g.



DURATION

00:05:00

Step 18.

Repeat washing step with 75% chilled ethanol.



NOTES

Lutz Berwanger 19 Dec 2016

Remove excess of EtOH by using a pipet.

Step 19.

Air dry pellet at RT. Do not overdry!



DURATION

00:10:00

Step 20.

Resuspend the pellet with 30 µL volume of ddH₂O.

Usually 40 µL DECP-treated H₂O

Warnings

Additional Information

Always provide RNA work on ice and always wear Gloves.



GHS Label elements, including precautionary statements

Signal word	Danger
Hazard statement(s)	
H227	Combustible liquid.
H302	Harmful if swallowed.
H319	Causes serious eye irritation.
H311	Toxic in contact with skin.
H314	Causes severe skin burns and eye damage.
H330	Fatal if inhaled.
H341	Suspected of causing genetic defects.
H371	May cause damage to organs.
H373	May cause damage to organs through prolonged or repeated exposure.

H402 Harmful to aquatic life.

Precaution Statement(s)

P260 Do not breathe dust/ fume/ gas/ mist/ vapours/ spray.

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.

P284 Wear respiratory protection.

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P310 Immediately call a POISON CENTER or doctor/ physician.

Material list

Chemical	Volume/Mass	Company	Serial no.	Comments
Phenol	39.6g			See add. Info
Glycerol	6.9mL			
8-hydroxyquinoline	0.1g			
EDTA	0.58g			See add. Info
Sodium acetate	0.8g			
Guanidine thiocyanate	9.5g			
Guanidine hydrochloride	4.6g			
Chloroform				See add. Info
Isopropanol				See add. Info
EtOH	70%			
DEPC treated Water		Roth		

Buffer

Name	Ingredients	Comments
100 mL PGTX Solution:	Phenol	39.6 g
	Glycerol	6.9 mL
	8-hydroxyquinoline	0.1 g
	EDTA	0.58 g
	Sodium acetate	0.8 g
	Guanidine thiocyanate	9.5 g
	Guanidine hydrochloride	4.6 g

Equipment list

Device	Company	Comments
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Centrifuge	Eppendorf	4°C
Thermo Mix	Eppendorf	95°C
Centrifuge	Heraeus	4°C
Freezer	Liebherr	-20°C