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RNA extraction with PGTX V.2

Lutz Berwanger[1]

[1]Institute of Synthetic Microbiology, Heinrich Heine University Düsseldorf

1 Works for me dx.doi.org/10.17504/protocols.io.78thrwn

Lutz LCB. Berwanger
Heinrich-Heine Universität Düsseldorf

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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STEPS MATERIALS

NAME	CATALOG #	VENDOR
Chloroform		
Isopropanol		
Ethyl alcohol, Pure 200 proof, for molecular biology	E7023	Sigma Aldrich

SAFETY 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
Centrifuge	Eppendorf	4°C
Thermo Mix	Eppendorf	95°C
Centrifuge	Heraeus	4°C
Freezer	Liebherr	-20°C

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

2 Centrifuge for 3 minutes at 4˚C and 4.000 g.
00:03:00

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

4 00:00:15

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

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

6 Incubate for 5 min at 95 °C, shaking at 250 rpm in Thermomixer (Eppendorf)
00:05:00

7 Rapidly chill 5 min on ice.
00:05:00

8 Add 700 µl Chloroform.

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

Chloroform

9 Let the samples incubate for 10 min at room temperature in a Thermomixer. Vortex from time to time.
00:10:00

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- C/I (Chloroform/Isoamylalcohol).
00:15:00

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

11 Thoroughly mix by vortexing. Centrifuge for 15 min at high speed. Transfer the upper aqueous phase to a 1.5 mL reaction tube.
00:15:00

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

12 Add 1 volume of isopropanol.

Isopropanol

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

00:30:00

14 Centrifuge at 14.000 g for 30 min.

00:30:00

15 Discard supernatant.

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

Ethyl alcohol, Pure 200 proof, for molecular biology
by Sigma Aldrich
Catalog #: E7023

17 Centrifuge at 14.000 g.

00:05:00

18 Repeat washing step with 75% chilled ethanol.

Remove excess of EtOH by using a pipet.

19 Air dry pellet at RT. Do not overdry!

00:10:00

20 Resuspend the pellet with 30 µL volume of ddH_2O.

Usually 40 µL DECP-treated H_2O