Oct 15, 2019 Version 2

RNA extraction with PGTX V.2

DOI
https://dx.doi.org/10.17504/protocols.io.78thrwn

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Protocol Citation: Lutz Berwanger 2019. RNA extraction with PGTX. **protocols.io** https://dx.doi.org/10.17504/protocols.io.78thrwn

Protocol status: Working
We use this protocol and it's working

Created: October 15, 2019

Last Modified: October 15, 2019

Protocol Integer ID: 28659

Keywords: rna extraction methodologies for cyanobacteria, rna extraction in cyanobacteria, rna extraction with pgtx, rna extraction, based rna extraction methodology, cyanobacteria, rna, bmc molecular biology, molecular biology, extraction, pgtx, alternative phenol

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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Materials

STEP MATERIALS

Chloroform

Isopropanol

Ethyl alcohol, Pure 200 proof, for molecular biology **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E7023

Protocol materials

Isopropanol

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Chloroform

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	39.6 g			See add.

					Info
	Glycerol	6.9 mL			
	8-hydroxyquinoline	0.1g			
	EDTA	0.58 g			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 Glycerol 8-hydroxyquinoline EDTA Sodium acetate Guanidine thiocyanate Guanidine hydrochloride	39.6 g 6.9 mL 0.1 g 0.58 g 0.8 g 9.5 g 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.

Safety information

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.

Safety information

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

Safety information

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

Safety information

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 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E7023

17 Centrifuge at 14.000 g.

00:05:00

18 Repeat washing step with 75% chilled ethanol.

Note

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.

Note

Usually 40 µL DECP-treated H_2O