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 We use this protocol and it's working

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RNA extraction with PGTX

Forked from [RNA extraction with PGTX](#)

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M4455 - Synthetische Biologie und Biotechnologie

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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

MATERIALS

STEP MATERIALS

☒ Chloroform **Contributed by users**

☒ Isopropanol **Contributed by users**

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

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

C h e m i c a l	V o l u m e / M a s s	C o m p a n y	S e r i a l n o .	C o m m e n t s

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

Guanidines hydrochloride	4.6 g			
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	
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Equipment list

D e v i c e	C o m p a n y	C o m m e n t s
C e n t r i f u g e	E p p e n d o r f	4° C
T h e r m o M i x	E p p e n d o r f	9 5° C
C e n t r i f u g e	H e r a e u s	4° C
F r e e z e r	L i e b h e r	- 2 0° 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 **Contributed by users**

- 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 μ L) of Aqua- P/C/I (Phenol/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 **Contributed by users**

- 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 ddH₂O.

Note

Usually 40 μL DECP-treated H₂O