RNA extraction with PGTX V.2

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


MATERIALS

STEP MATERIALS
- Chloroform Contributed by users
- Isopropanol Contributed by users
- Ethyl alcohol, Pure 200 proof, for molecular biology Sigma Aldrich Catalog #E7023

PROTOCOL MATERIALS
- Isopropanol Contributed by users Materials, Step 12
- Ethyl alcohol, Pure 200 proof, for molecular biology Merck MilliporeSigma (Sigma-Aldrich) Catalog #E7023 Materials, Step 16
- Chloroform Contributed by users Materials, Step 8

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** Combatible 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**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Volume/Mass</th>
<th>Company</th>
<th>Serial no.</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>39.6g</td>
<td></td>
<td></td>
<td>See add. Info</td>
</tr>
<tr>
<td>Glycerol</td>
<td>6.9mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Ingredients</td>
<td>Comments</td>
<td></td>
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<tr>
<td>-------------------</td>
<td>-----------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>100 mL PGTX Solution:</td>
<td>Phenol, Glycerol, 8-hydroxyquinoline, EDTA, Sodium acetate, Guanidine thiocyanate, Guanidine hydrochloride</td>
<td>39.6 g, 6.9 mL, 0.1 g, 0.58 g, 0.8 g, 9.5 g, 4.6 g</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Buffer

### Equipment list

<table>
<thead>
<tr>
<th>Device</th>
<th>Company</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifuge</td>
<td>Eppendorf</td>
<td>4°C</td>
</tr>
</tbody>
</table>
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.

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

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

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)

7. Rapidly chill 5 min on ice.
8 Add 700 µl Chloroform.

Safety information
Wear goggles, a lab coat and gloves when dealing with phenol and 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).

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

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.
15. Discard supernatant.

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

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

17. Centrifuge at 14,000 g.

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!

20. Resuspend the pellet with 30 µL volume of ddH₂O.

   Note
   Usually 40 µL DECP-treated H₂O