Isolation of total RNA from *Synechocystis* (PGTX method)

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ABSTRACT

Isolation of 40-200 µg of total RNA from *Synechocystis* sp. PCC 6803. Can be used for standard applications such as Northern Blot and qPCR.

GUIDELINES

Keep RNA cold and in an RNase-free environment.

SAFETY WARNINGS

Hazardous materials: PGTX, Chloroform
Wear safety gear and gloves at all times.

BEFORE START INSTRUCTIONS

PGTX solution (100 mL): 39.6 g phenol, 6.9 mL glycerol, 0.1 g hydroxyquinoline, 0.58 g EDTA, 0.8 g NaOAc, 9.5 g guanidine thiocyanate, 4.6 g guanidine hydrochloride

Harvest

- 1 Grow cyanobacteria to an OD₇₅₀ of ~1.
Fill a 50 mL tube with ice. Add culture until full (approx. 25 mL)
Centrifuge tube 5 min at maximum speed.
00:05:00
- 2 Discard supernatant. Resuspend cyanobacterial pellet in the remaining water (~1mL)
Transfer to a fresh 2 mL tube. Spin down 1 min at maximum speed.

⌚ 00:01:00

- 3 Resuspend pellet in 1 mL PGTX solution. Flash freeze and store at -80°C for later extraction, or proceed with the next step.

⊗ PGTX Contributed by users

Safety information

PGTX contains phenol; wear safety gear and gloves

Extract

- 4
- Add 0.2 g acid washed glass beads to the tube -> e.g: use a 'calibrated' 0.2 mL PCR tube for measuring

- Shake **3 x** in 'Precellys24' bead beater: **6800rpm** for **30 sec**
- Chill on **ice** for **2 min** between the runs.

- 5 Place samples on ice for 5 min.

⌚ 00:05:00

- 6 Add 200 μL Chloroform/IAA. Mix well. Incubate at RT for 10 min, vortexing from time to time.

⌚ 00:10:00

Safety information

Wear safety gear

- 7 Centrifuge samples at maximum speed for 10 min to separate phases.
Transfer aqueous phase to a fresh tube.

00:10:00

- 8 Add 1 vol Chloroform/IAA. Mix well by vortexing. Centrifuge 5 min at maximum speed.
Transfer aqueous phase to a fresh tube.

00:10:00

Precipitation

- 9
- add 0.7 vol. of 100 % 2-propanol to the sample. Mix well.
 - precipitate 1 h at -20°C.

-20 °C

01:00:00

- 10 Centrifuge precipitated sample at 4°C and maximum speed for at least 30 min.
Remove supernatant, making sure not to disrupt the RNA pellet.

00:30:00

Washing

- 11
- add 500 µL 70% EtOH
 - centrifuge for 5 min, 4°C at maximum speed (e.g. 14,000 g)
 - repeat washing step
 - completely remove supernatant with a pipet
 - air-dry pellet at RT for ~5 min in the fume hood
!Do not overdry!
 - resuspend pellet in 40 µL pure, RNase-free water.