Isolation of total RNA from Synechocystis

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1 Works for me

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ABSTRACT

Isolation of total RNA from Synechocystis sp. PCC 6803 Can be used for further work, like qPCR Always work fast, with ice and RNase-free (wear gloves!)

- 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
- PGTX contains phenol, wear safety gear and gloves

- Synechocystis culture
- Chloroform/IAA
- IPA + NaOAc (in a specific amount)
- 1 vol. IPA or 3 vol. EtOH and NaOAc 30:1 or 10:1

Start

Centrifuge 5 ml of culture (oD_{750} of~ 1) for 5 minutes at maximum speed (T = 4°C)
Discard supernatant. Resuspend cyanobacterial pellet in the remaining water (~1 ml). Transfer to a fresh 2 ml tube (RNase-free). Spin down 1 min at maximum speed.
Discard remaining supernatant. resuspend pellet in 1 ml PGTX solution. Flash freeze and store at -80°C for later extraction, or proceed with the next step.

Extract

Heat samples at 95°C for 5 minutes in a shaking heat block. Vortex samples from time to time to ensure complete lysis.
Place samples on ice for 5 minutes.
Add 700 µl Chloroform/IAA. Vortex sample until it is opaque. Incubate at RT for 10 min, vortexing from time to time.
Centrifuge samples at maximum speed for 10 minutes to seperate phases. Transfer aqueous phase (600 µl) to a fresh tube (RNase-free).
Add 1 vol. (600µl) Chloroform/IAA. Mix well by vortexing. Centrifuge 10 minutes at maximum speed. Transfer aqueous phase (500µl) to a fresh tube (RNase-free).

Precipitation

Add 517 µl IPA + NaOAc to the samples. Mix well. Precipate over weekend at -20°C.
Also possible: 1h at -80°C or over night at -20°C.
Centrifuge precipitated sample at 4°C and maximum speed for at least 30 min. Remove supernatant, making sure not to disrupt the RNA pellet.
Wash pellet with 70% EtOH (300µl). Centrifuge for 15 minutes, 4°C at maximum speed. Completely remove supernatant. Dry at RT for ~5 minutes. Do not overdry! Resuspend pellet in 40 µl pure, RNase-free water. Store at -80°C.