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.

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Guidelines

Keep RNA cold and in an RNase-free environment.

Before start

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

Protocol

Harvest

Step 1.

Grow cyanobacteria to an OD_{750} of 1.

Fill a 50 mL tube with ice. Add culture until full (approx. 25 mL)

Centrifuge tube 5 min at maximum speed.

Step 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.

Step 3.

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

REAGENTS

PGTX by Contributed by users

SAFETY INFORMATION

PGTX contains phenol; wear safety gear and gloves

Extract

Step 4.

Heat samples at 95˚C in a shaking heat block. Vortex samples from time to time to ensure complete lysis.

Extract

Step 5.

Place samples on ice for 5 min.

Extract

Step 6.

Add 700 µL Chloroform/IAA. Mix well. Incubate at RT for 10 min, vortexing from time to time.

SAFETY INFORMATION

Wear safety gear

Extract

Step 7.

Centrifuge samples at maximum speed for 10 min to separate phases.

Transfer aqueous phase to a fresh tube.

Extract

Step 8.

Add 1 vol Chloroform/IAA. Mix well by vortexing. Centrifuge 10 min at maximum speed.

Transfer aqueous phase to a fresh tube.

Precipitation

Step 9.

Add 3 vol. of 100 % EtOH + NaOAc 10:1 to the sample. Mix well.

Precipitate 1 h at -80˚C or over night at -20˚C.

Precipitation

Step 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.

Precipitation

Step 11.

Wash pellet with 70% EtOH.

Centrifuge for 15 min, 4˚C at maximum speed.

Completely remove supernatant.

Dry at RT for 5 min. Do not overdry!

Resuspend pellet in 40 µL pure, RNase-free water.

Warnings

Hazardous materials: PGTX, Chloroform

Wear safety gear and gloves at all times.