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RNA extraction from Synechocystis sp. PCC 6803 with Trizol reagent

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1 Label all required tubes and store on ice (or freezer, if not needed immediately)

2 Pre-cool all required centrifuges

3 Fill sterile 50 mL tube (Falcon) w/ ice and store in ice bath

4 Pour 20-25 mL cell culture (OD750 < 1.0) to ice-filled tube (up to ~45 mL mark)Note: avoid long transport of cell culture before harvest

5 spin down at 4000 - 5000 g and 4 ° C for 5 min

6 discard supernatant (w/ ice) into big beakerNote: depending on strain/mutant some cells will get lost at this step

7 resuspend cell pellet in residual water (~ 1 mL)

8 transfer suspension into 2 mL (safe lock!) tubes (work on ice!)

9 spin down at ~13.000 g and 4° C for 15 sec

10 discard supernatant w/ pipetNote: try to remove supernatant 'as quantitatively as possible'

11 resuspend pellet in 1 mL Trizol reagent

12 store at -20 °C or (better) -80 °C

13 incubate frozen samples at 65° C for 15 min under constant agitationNote: if no shaking thermoblock is available, vortex once per minute

14 add 200 µL (ice cold) chloroform-isoamylalcohol (24:1) per 1 mL Trizol and vortex for 30 sec

15 spin down at ~11.000 g and 4° C for 10 min

16 transfer upper, aqueous phase to fresh 1.5 mL tube

17 add 1 Vol. (ice cold) phenol-chloroform-isoamylalcohol (25:24:1)Note: for RNA preparation phenol solution/mixtures should not be Tris-buffered

18 transfer upper, aqueous phase to fresh 1.5 mL tube

19 add 1 Vol. isopropanol (2-propanol), 10 µL 3 M Na-Acetat (pH 5.2) and 1 µL glycogen (RNA grade, Thermo)

20 incubate o/n at -20 for precipitation

21 spin down at ~13.000 g and 4° C for 30 min

22 remove supernatant and wash pellet w/ 70% EtOH (ice cold)

23 spin down at ~13.000 g and 4° C for 10 min

24 repeat steps 24 and 25

25 discard supernatant and air-dry RNA pellet for ~10 min

26 resuspend RNA Pellet in ~30 µL ultra-pure water and store at -20 or (better) -80°C