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Extraction of total RNA from *E. coli* cells

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We use this protocol and it's working

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Abstract

The protocol is used for the extraction of total RNA from *E. coli* cells. It is based on the method described by Chomczynski and Sacchi, 1987 (https://doi.org/10.1016/0003-2697(87)90021-2). Total RNA is isolated from cells expressing small regulatory RNAs (trRNAs) either constitutively (E. coli W3110) or after induction with anhydrotetracycline (E. coli W1310 Z1).

Materials

MATERIALS

- Roti-Aqua-P/C/I **Carl Roth Catalog #**X985.2
- Roti-C/I **Carl Roth Catalog #**X984.2
- NucleoZOL **Macherey-Nagal Catalog #**REF 740404.200
- Roti-Aqua-Phenol for RNA extraction **Carl Roth Catalog #**A980.1
- LB-Medium (Lennox) vegetal **Carl Roth Catalog #**0155.1
- IPTG **Carl Roth Catalog #**CN08.1
- Anhydrotetracycline **Catalog #**2-0401-001

additional solutions/buffers:

- Antibiotic stock solutions:
  Kanamycin (Km): 25 mg/ml
  Spectinomycin (Spec): 100 mg/ml
- Inducer stock solutions: 20 µg/ml Anhydrotetracycline (aTc) in EthOH, 10 mM Isopropyl-ß-D-thiogalactopyranosid (IPTG) in EthOH and a mixture of 20 µg/ml aTc and 10 mM IPTG in EthOH
- Ethanol
- Isopropanol
- 3 M Na-Acetat, pH 5.2
- RNAse free molecular grade water (DEPC-treated)

Troubleshooting

Safety warnings

Phenol is toxic! Work under the hood, wear protective gloves (Nitril) and change gloves immediately after contamination. Collect solid and liquid waste in special waste containers.

Before start

RNA is sensitive to degradation! Wear gloves, keep samples on ice when possible, use filter-tips and RNase free reagents. Pre-cool centrifuges and store isolated RNA-samples immediately at -20 or -80°C.

incubation of cells

1

- freshly transform *E. coli* cells with plasmids encoding trRNA sequences or empty vector
- inoculate 3 ml LB-vegetal medium + antibiotic (25 µg/ml Km for W1310, 25 µg/ml + 100 µg/ml Spec for W1310Z1) in culture tubes with a single colony
- incubate o/n at 37°C and 230 rpm

for constitutive RNA-synthesis (*E. coli* W3110):

- dilute 1:100 in fresh medium with 25 µg/ml Km and incubate until OD600 reaches 0.7 - 1.0 (in culture tubes or 6 well plates), proceed to step 2

for induced RNA-synthesis (*E. coli* W3110Z1):

- dilute 1:50 in fresh medium (200 µl o/n culture in 10 ml medium + antibiotic in 100 ml Erlenmeyer flask) and incubate until OD600 reaches 0.4 - 0.6
- if you have several different cultures that do not grow at same speed: store samples on ice until the last one reaches the defined OD (keep that one on ice as well for 10 min)
- meanwhile prepare 12 well plates with inducer: pipet in each well of a row 10 µl of either
  1) EthOH,
  2) aTC (= 200 ng/ml final concentration)
  3) IPTG (= 100 µM final concentration)
  4) aTc + IPTG (200 ng/ml, 100 µM final concentration)
- add 1 ml of culture and incubate at 37°C and 230 rpm for 1 h
- proceed with step 2

stop of RNA synthesis

2

- work under the hood
- mix 1 ml of cells with 200 µl 'stopmix'- solution (5 % phenol in ethanol) in a 2 ml safe lock tube tube → stops RNA production in the cells
- centrifuge for 5 min at 4°C and 14000 x g
- discard the supernatant and resuspend the pellet in 1 ml NucleoZOL (Macherey and Nagel), place on ice (better for rapid freezing: dry ice or liquid nitrogen)

→ store cells at -20 or -80 °C (only for a short time, maximum 2 weeks) or proceed to next step

RNA-isoloation

3

- incubate the sample at 65 °C and 250 rpm (Thermomixer) for 10 min

- mix with 400 µl Phenol-Chloroform/Isoamylalcohol (Roti®-)Aqua-P/C/I) by inverting for 10 s
- centrifuge at 4°C for 10 min at 14000 x g
- transfer aqueous (upper) phase to a fresh 1,5 ml safe lock reaction tube, work on ice
- mix with 450 µl Chloroform/Isoamylalcohol (Roti®-C/I)
- centrifuge at 4°C for 10 min at 14000 x g
- transfer aqueous (upper) phase to a new reaction tube and add 1 Vol. icecold Isopropanol + 1/10 vol (e.g. 20 µl for 200 µl Isopropanol) 3 M Na-Acetat (pH 5.2), mix and store at least 30 min at -20 °C or -80 °C
- centrifuge at 4°C for 30 min at 14000 x g
- remove the supernatant (take care of the RNA-pellet) and add 350 µl of icecold 75% Ethanol
- centrifuge for 5 min at 4°C and 15000 rpm
- add again 350 µl of icecold 75% etahnol and centrifuge for 5 min at 4°C and 15000 rpm
- remove the supernatant and dry the pellet at room temperature for ca.15 min
- resuspend the pellet in 30 µl Molecular Biology Grade Water and store at -80°C

4