Institute of Food Research

Protocol from the *Salmonella* group

RNA extraction and purification

1) Harvest 2.0 OD_{600} units of bacterial culture (for example 4 mls of a culture with an OD_{600} = 0.5)

2) Prepare a 50 ml Falcon tube containing 2/5 of the culture volume ice-cold 5% ($^v/_v$) phenol pH 4.3, 95% ($^v/_v$) ethanol (Phenol Sigma order code: P-4682). Add the appropriate volume of culture e.g. add 4 mls of culture to 1.6 mls of phenol/ethanol. Stand on ice for at least 30 min but no longer than 2 hours to stabilise the RNA and prevent degradation.

3) Centrifuge samples at 3220 X g 4°C for 10 min. Discard supernatant, resuspend bacterial pellets using residual liquid in tubes and transfer to 1.5 ml microcentrifuge tubes.

4) Spin tubes 60 secs at maximum speed in a microfuge and discard remaining liquid.

5) **Optional:** Freeze pellets at -80°C. Pellets can be kept for up to 1 month before continuing with RNA prep.

6) Resuspend pellets in 100 µl TE buffer containing 50 mg/ml lysozyme. Incubate at room temperature for 5 min.

7) Add 75 µl lysis reagent (Promega SV Total RNA Purification kit. Cat: Z3100) and mix by inversion several times.

8) Add 350 µl RNA dilution buffer from kit (Promega SV, Cat: Z3100). Mix well by inversion.

9) Heat samples at 70 °C for 3 min and then centrifuge for 10 min at full speed (13000 rpm).

10) Transfer supernatant to clean tubes supplied with the kit. Add 200 µl ethanol and mix by pipette. Transfer to spin columns (Cat: Z3100) and centrifuge columns for 30 secs at full speed. Discard eluate.

11) Wash columns with 600 µl wash buffer from the kit. Spin 30 secs at full speed.

12) Prepare DNase mix (all reagents supplied with the kit):
    a) 5 µl 90 mM $MnCl_2$
    b) 40 µl DNase core buffer
    c) 5 µl DNase

13) Apply 50 µl of DNase mix to column matrix and incubate at room temperature for 15 min.

14) Add 200 µl DNase stop mix (kit) and centrifuge 30 secs at full speed.

15) Wash columns with 600 µl wash buffer, by centrifugation for 30 secs at full speed. Discard eluate.

16) Wash columns with 250 µl wash buffer, by centrifugation for 30 secs at full speed. Discard eluate.

17) Transfer columns to sterile microcentrifuge tubes, add 100 µl RNAse-free distilled H_2O and allow to stand for 1 min.

18) Centrifuge at 4500 X g for 2 min. Discard column.

19) Check the RNA concentration using a spectrophotometer. From exponential phase cultures, you should typically obtain 50-60 µg RNA. Stationary phase and near-stationary phase samples will yield less, but nevertheless plenty to use. Quality of RNA should also be checked by the Aligent Bioanalyzer or a similar method to ensure the RNA is not degraded.

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