Mechanical Cell Disruption and Extraction of RNA from *B. subtilis*

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1. Cell disruption

- Grow a bacterial culture to the desired OD.
- Prepare killing ice by freezing centrifugation tubes containing 1/2 culture volume of killing buffer (*e.g.* use 15 ml of killing buffer for 30 ml culture volume to be harvested).
- Harvest cells quickly by centrifugation of 15 OD_{500} (3 min, 8000 rpm, 4°C).
- Discard the supernatant.
- Freeze pellet in liquid N_2 and store at -80 °C or continue immediately with RNA preparation.
- Resuspend bacterial frozen pellet in 200 µl of ice-cold killing buffer.
- Transfer cell suspension into a Teflon vessel filled with liquid N_2 and pre-cooled in liquid N_2.
- Close vessel containing cells and disruption ball, shortly submerge vessel in liquid N_2, insert vessel into the cell disruptor (Mikro-Dismembrator S from Sartorius).
- Mechanical cell disruption: 2 min, 2600 rpm.
- Resuspend resulting cell powder in 4 ml of lysis solution (pre-warmed to 50°C) by repeated pipetting.
- Transfer 1 ml aliquots of the lysate into microcentrifuge tubes (2 ml-tubes).
- Freeze microcentrifuge tubes in liquid N_2.
- Freeze lysates at -80°C or continue immediately with acid phenol extraction.

Lysis solution:

118.16 g guanidine-thiocyanate (final concentration: 4 M)
2.08 ml 3 M Na-acetate, pH 5.2 (final concentration: 0.025 M)
12.5 ml 10 % N-lauroylsarcosinate (final concentration: 0.5 %)
ad 250 ml with deionized water.

Killing buffer:

20 mM Tris-HCl, pH 7.5 (Stock solution: 1 M)
5 mM $MgCl_2$ (Stock solution: 1 M)
20 mM NaN_3 (Stock solution: 2 M)

2. Extraction of RNA (Acid phenol method)

- Add 1 volume acid phenol solution (e.g. 1 ml) to the frozen cell lysate, mix well on an Eppendorf tube shaker (e.g., Thermomixer from Eppendorf) until completely thawed, and then for 5 additional minutes.
- Centrifugation: 5 min, 12000 rpm, room temperature.
- Transfer supernatant into a fresh microcentrifuge tube; avoid carry-over of the DNA-containing interphase (e.g. transfer only 850 µl from 1 ml supernatant).
- Add 1 volume acid phenol solution (e.g. 850 µl), mix well for 5 min on the shaker.
- Centrifugation: 5 min, 12000 rpm, room temperature.
- Transfer supernatant into a fresh microcentrifuge tube; again avoid carry-over of the DNA-containing interphase (e.g. transfer only 700 µl from 850 µl supernatant).
- Add 1 volume Chloroform/IAA (e.g. 700 µl), mix well for 5 min on shaker.
- Centrifugation: 5 min, 12000 rpm, room temperature.
- Transfer total supernatant into a fresh microcentrifuge tube (e.g. 700 µl).
- Add 1/10 volume 3 M Na-Acetate, pH 5.2 (e.g. 70 µl), mix well, add 1 ml of isopropanol, mix and precipitate RNA overnight at -20°C.
- Centrifugation: 15 min, 15000 rpm, 4 °C.
- Carefully remove supernatant, wash pellet with 1 ml of 70 % Ethanol.
- Centrifugation: 15 min, 15000 rpm, 4 °C.
- Carefully remove supernatant, dry pellet in a vacuum centrifuge for 1 min (not longer!).
- Dissolve RNA-pellet in an appropriate volume (50-100 µl) of sterile water for 3 h on ice and then for 30 min at room temperature.
- Determine RNA concentration.
- Check quality of the RNA preparation using an Agilent Bioanalyzer.

Acid phenol solution:

Roti-Aqua-P/C/I obtained from Roth (phenol/ chloroform/ isoamyl alcohol; 25:24:1).

Chloroform/IAA:

Mix chloroform and isoamyl alcohol 24:1 volumes and equilibrate with Tris/HCl, pH 8.0.

Appendix A: Quality control of RNA preparations with the Agilent Bioanalyzer.

The quality control of the RNA preparations is performed with the RNA 6000 Nano LabChip Kit (Agilent Technologies) on the Agilent 2100 Bioanalyzer according to the manufacturer's instructions. As shown below in the synthetic gel image and the electropherograms, RNA preparations of high quality exhibit two bands above the 23S rRNA representing rRNA precursors not yet processed into the mature rRNAs. These bands indicate the high degree of integrity of the RNA preparations.