

454 sequencing of rRNA genes from extant and Holocene Black Sea microbiota

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Filtered particulate organic matter from the Black Sea water column.

Simultaneous DNA and RNA extraction

Bing frozen (-80 °C or colder) filter in frozen (-80 °C), pre-combusted (8h 225 °C) mortar wrapped in alu foil and quickly slice the filter using a flamed scalpel. Add 4 ml of denaturing buffer after Hurt et al., 2001 and 50 µl Mercaptoethanol as well as 2 ml of zirconium beads and grind the **thawing** filter.

Transfer the sample into 50 ml sterile Corning tube and

1. Add an equal volume (~4 ml) of **Phenol:Chloroform:IAA, 25:24:1, pH 8**
2. Shake or vortex vigorously for 1 min. Incubate on ice for 15 min. This step ensures the complete disassociation of nucleoprotein complexes.
3. Centrifuge at $\geq 10,000 \times g$ for 15 min, preferably at 4 °C.
4. Without disturbing the interface, carefully transfer the aqueous phase (~4 ml) to a new tube.
5. Add an equal volume of chloroform, shake, and centrifuge at $> 10,000 \times g$ for 15 min at 4 °C.
6. Precipitate the DNA and RNA by adding 1/10 aqueous phase volume (~440 µl) **5 M RNase free NaCl**. Shake and add an equal volume (~4 ml) of ice cold **100% isopropanol**. Invert tube to mix and incubate at -20 °C for at least 1 hour.
11. Centrifuge at $\geq 10,000 \times g$ for 20 min at 4 °C to pellet the RNA.
12. Carefully remove the supernatant solution and wash the pellet with 4 ml of cold **70% ethanol** by vortexing.
13. Centrifuge at low speed (~5,000 $\times g$) for 5 min at 4 °C. Remove the supernatant and pulse spin to bring down residual ethanol. Remove residual ethanol with a fine-tipped pipette and allow the pellet to air dry for 1 to 5 min. Do not let the pellet dry completely as this will greatly decrease the solubility of the nucleic acids.
14. Resuspend the pellet in an appropriate amount (~100 µl) of **RNase-free water**.
15. Store the nucleic acids sample at -80 °C.

Holocene Black Sea sediments

Total DNA extraction

Total DNA was extracted from 2 to 5 gram of wet weight sediment using the PowerMax™ Soil DNA Isolation Kit following the descriptions of the manufacturer (Mobio, Carlsbad, CA, USA).