DNA+RNA extraction from peat **HJ 27.09.2011**

Molecular Environmental Microbiology group
Department of Biosciences, University of Helsinki, Finland

This protocol has been used to extract DNA and RNA from peat samples for analysis of DNA, rRNA and mRNA for analysis of archaeal 16S rRNA gene and *mcrA* for methanogens. The protocol is a modification of a protocol developed at Finnish Forest Research Institute for analysis of fungi in soil and peat (see Korkama-Rajala et al. and Pennanen et al.). The final extracts contain both DNA and RNA. To analyze RNA, the extracts are treated with DNase.

To cite this protocol:
Korkama-Rajala et al. (2008) Microbial Ecology 56:76-89
Pennanen et al. (2004) Soil Biology and Biochemistry 36:841–848 (original protocol that was modified in Korkama-Rajala et al.)
Putkinen et al. (2009) FEMS Microbiology Ecology 70:87-98 (RNA extraction from peat, archaeal 16S rRNA)
Kotiaho et al. (2010) Biology and Fertility of Soils 46:567-575 (RNA extraction from peat, *mcrA* mRNA)

Contact: heli.juottonen@helsinki.fi or kim.yrjala@helsinki.fi

General, preparations before beginning:

- Keep the samples always on ice
- Materials (tubes, tips etc.) are autoclaved at 121^{o}C for 1h before use
- DEPC-treated water is used in preparation of solutions
- Pipettes and surfaces can be cleaned with RNAseAWAY or similar products

A. Preparation of PVPP columns

1. Pack columns (Micro-Spin, Bio-Rad) with PVPP (polyvinylpolypyrrolidone).
2. Add 500 µl sterile DEPC-treated H_2O.
3. Centrifuge 1300 *g* 3 min.
4. Discard flow-through, add 200 µl H_2O, centrifuge as above. Continue until the column has been stabilized 4-5 times or the volume of flow-through is the same as the added volume.
5. Place the column in a new tube. Store at +4 ^{o}C if prepared in advance.

B. Lysis of cells in FastPrep machine

1. In a 2-ml screw cap tube, add 0.5-1 cm quartz sand (1-2 mm), 400 µl of lysis buffer (75mM Tris-HCl, pH 7.4; 25mM EDTA, pH 8.0; 4.5% sodiumdodecylsulfate; 1.5% b-mercaptoethanol) and peat sample (0.4 g fresh weight).
2. Lyse cells in FastPrep machine at 4.5 m/s for 30 s.
3. Incubate at 65 °C for 30 min.

C. DNA and RNA purification

Phenol extraction and PVPP purification

1. Add 400 µl phenol-chlorofom-isoamylalcohol (50:49:1).
2. Vortex 1 min (half speed) , centrifuge 2 min (full speed/13 000 rpm).
3. Transfer upper phase into a new tube.
4. Add 400 µl chlorofom-isoamylalcohol (24:1).
5. Vortex 2 min, centrifuge 3 min.
6. Transfer ≤ 200 µl of upper phase into a PVPP column (stabilized and prepared as above) and centrifuge 1300 *g* 3 min.

PEG precipitation

1. Add 0.6 V of PEG solution (20% PEG in 2.5 M NaCl), mix well.
2. Keep on ice for 20 min.
3. Centrifuge 20 min at +4°C, remove supernatant.
4. Wash the pellet with 800 µl of cold 70% ethanol (prepared in DEPC-H_2O).
5. Centrifuge 5 min, remove supernatant, air dry or vacuum dry the pellet.
6. Suspend the pellet in 50 µl TE buffer, incubate at 50 °C 1 h, store at – 70 °C.