1. DNA extraction protocol using UltraClean™ Soil DNA Isolation Kit (Catalog No. 12800-50).

All materials used that did not come in the kit were previously sterilized. The protocol was done in a PCR chamber after decontamination with UV light for 15 minutes. Forceps and micropipettes in use were also decontaminated under the UV light just before the procedure. The extraction was carried through the following steps:

i. Using clean flamed forceps take the filter from the sample tube (for sampling procedure and filtration details see protocol "Collection and Conservation of Microbial samples") and add it directly to the 2ml Bead Solution tubes provided. Gently vortex to mix;

ii. Using a 100µl micropipette add 60µl of Solution S1 and invert the tube several times;

iii. To retain as maximum DNA as possible, centrifuge the remaining solution in the original sample tube at maximum speed (12000rpm) for 2 minutes. Discard the supernatant, re-suspend the pellet with 200µl of Solution IRS (Inhibitor Removal Solution) and add this solution to the bead tubes;

iv. Secure bead tubes horizontally on the rotator (Grant Boekel model HIR10M) and use at maximum speed (20r/min.) for at least 15 minutes (at 20ºC);

v. Centrifuge tubes at 12000 rpm for 30 seconds (Eppendorf model 5702 R) and transfer the supernatant to a clean microcentrifuge tube;

vi. Add 250µl of Solution S2 and vortex for 5 seconds;

vii. Incubate the tubes at 4°C (refrigerator) for at least 5 minutes;

viii. After that time centrifuge for 1 minute at 12000 rpms;

ix. Avoiding the pellet, transfer entire volume of supernatant in to a clean microcentrifuge tube;

x. Add 1000µl plus 300µl of Solution S3 to the supernatant. Attention is needed at this step as the volume may touch the tube rim before loading all 300µl of Solution S3 (if that happens discard the remain volume which cannot be loaded);

xi. Vortex for 5 seconds;

xii. Load approximately 700µl onto a spin filter and centrifuge at 12000 rpms for 1 minute. Discard the flow through, add the remaining supernatant to the spin filter, and centrifuge at 12000 rpms for 1 minute.

xiii. Repeat the previous step three times, until all supernatant has passed through the spin filter

xiv. Add 300µl of Solution S4, centrifuge for 30 seconds at 12000 rpms and discard the flow through;

xv. Centrifuge again for 1 minute at 12000 rpms;

xvi. Carefully place spin filter in a new clean tube avoiding to splash any Solution S4 onto the spin filter

xvii. Add 30µl of Solution S5 to the center of the white filter membrane and centrifuge for 30 seconds at 12000 rpms;

xviii. Repeat the previous step loading 25µl of Solution S5 (does not contain EDTA);

xix. Discard the spin filter and store the DNA samples frozen (-20°C) until further analyses.