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Modification of a commercial DNA extraction kit for safe and rapid recovery of DNA and RNA simultaneously from soil, without the use of harmful solvents

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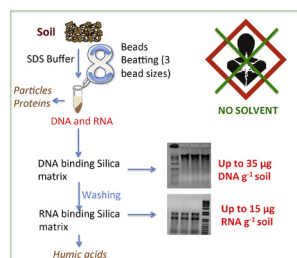
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GRAPHICAL ABSTRACT



ABSTRACT

An optimized method, based on the coupling of two commercial kits, is described for the extraction of soil nucleic acids, with simultaneous extraction and purification of DNA and RNA following a cascade scheme and avoiding the use of harmful solvents. The protocol can monitor the variations in the recovery yield of DNA and RNA from soils of various types. The quantitative version of the protocol was obtained by testing the starting soil quantity, the grinding parameters and the final elution volumes, in order to avoid saturation of both kits.

- A first soil-crushing step in liquid nitrogen could be added for the assessment of fungal parameters.

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- The protocol was efficient on different tropical soils, including Andosol, while their high contents of clays, including poorly crystalline clays, and Fe and Al oxides usually make the nucleic acid extraction more difficult.
- The RNA recovery yield from the previous tropical soils appeared to correlate better to soil respiration than DNA, which is positively influenced by soil clay content.

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1. Method details

We adapted a commercial DNA extraction kit (FastDNA SPINTM kit for soil, MP Biomedical, Santa Anna, CA), to recover DNA and RNA simultaneously, avoiding the use of hazardous solvents. This DNA kit, based on an SDS extraction buffer, has already shown its efficiency [1,2]. RNA recovery was allowed because special cares were taken to work under RNase-free conditions. All solutions and glassware were treated with diethyl pyrocarbonate (DEPC) to ensure that they were RNase free and only certified RNase- and DNase-free plastic tubes were used. Each centrifugation step was performed at 4 °C and tubes were placed on ice while waiting for the next step of the procedure. RNA could therefore be purified from the DNA washing solution at the step 8 of the protocol as shown on the flow chart presented in Fig. 1 and as further described. Modifications we brought to the initial protocol of the manufacturer are detailed in *italic character*.

1. Soil sample preparation: add 250 mg of soil sample (characteristics of soils used in this study are shown in Table 1) to a Lysing Matrix E tube and freeze overnight at −80 °C. *Initially the manufacturer preconized to use 500 mg of soil. We have tested 125, 250, 500 and 750 mg, and observed that 500 mg of soil samples led already to the maximum of DNA recovered, as 750 mg of soil did not improve the yield (Fig. 2). Therefore even if 500 mg of soil increased the RNA yield by a factor of almost 4, the soil samples being processed seemed to be limited to 250 mg to keep the extracted DNA below the saturation limit of the kit and to be able to follow variations of both molecules. Of course 500 mg has to be chosen if the objective is only to maximize the quantity of DNA and RNA recovered and not to relate their recovery yield to molecular biomass estimation.*
2. Cells lysing: add 978 µL sodium phosphate buffer, 122 µL of MT buffer. *When extracting nucleic acids from andosol, 20 mg caseine has to be added to the lysing mix in order to saturate the high adsorption capacity of the non-crystallized clays [3].*
3. Grinding: homogenize in the FastPrepTM instrument (MP Biomedical) for 40 s at a speed setting of 6.0. Cool sample on ice for 5 min and perform another grinding step under similar conditions (40 s, speed 6.0). *Initially the manufacturer preconized a single homogenization step. To increase the amount of nucleic acids recovered for further analyses, a second grinding step was introduced (Fig. 3).*
4. Centrifuge at 14,000 × g for 5 min at 4 °C. Transfer supernatant to a clean 2.0 mL microcentrifuge tube.
5. Protein precipitation: add 250 µL PPS (protein precipitation solution) and mix by shaking the tube by hand 10 times. Keep on ice before next step, when processing other samples.
6. Centrifuge at 14,000 × g for 5 min at 4 °C to pellet precipitate. Transfer supernatant to a clean 2 mL microcentrifuge tube.
7. DNA binding: re-suspend Binding Matrix suspension and add 1.0 mL to supernatant in the 2 mL tube. Place on rotary shaker for 12 min, 23 rpm at room temperature, to allow binding of DNA. *Initially the manufacturer preconized a 5 min binding time but we have observed that increasing the DNA binding time from 5 to 12 min avoided DNA contamination into the washing solution containing RNA. This was verified by PCR (data not shown).*
8. Centrifuge at 14,000 × g for 2 min at 4 °C to pellet DNA binding matrix. Remove the supernatant and transfer it into a clean 15 mL tube for further RNA purification. *Keep on ice before processing.*

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