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Environmental DNA (e-DNA) extraction from soil

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We use this protocol and it's working

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Abstract

Soil harbors diverse microorganisms, including fungi that play crucial environmental roles but can also harm plants, animals, and humans through infections or spore exposure. In 2022, the WHO released several pathogenic fungi, including Fusarium group, Mucorales and Eumycetoma agents, which are prevalent in Thailand's agricultural soils and have caused significant human infections. Despite this, the genetic mechanisms behind their infections and antifungal resistance remain poorly understood, particularly in Southeast Asia. Antifungal resistance to major drug classes, such as azoles, echinocandins, and polyenes, is increasing, driven by specific resistance genes. To address these challenges, we propose establishing a Southeast Asian (SEA) network of mycologists to study fungal pathogens, focusing on collecting soil samples, extracting fungal genomic DNA, and creating a comprehensive genome database. This initiative will improve detection and management of drug-resistant fungi, enhance clinical treatments, and strengthen agricultural biosecurity. By leveraging genomic technologies and fostering international collaboration, the project aims to develop targeted strategies against fungal infections and resistance, benefiting public health, food security, and sustainable agriculture across SEA. Establishing a fungal biobank and expert network will support long-term efforts to combat antifungal resistance, providing critical resources for swift and accurate responses to fungal threats.

Attachments

PDF

Hand book DNeasy Pow...

470KB

Materials

1. Soil Sample: 0.5 g
2. Micropipette and Tips: 1000-10 µL
3. DNeasy PowerSoil Pro Kit (QIAGEN)
4. Eppendorf Tubes
5. Agarose and Electrophoresis Set
6. TBE Buffer
7. DNA Ladder

Soil Sample Preparation:

1 Weigh 0.5 g of soil (remove debris like stones or large particles).

DNA Extraction Using the DNeasy PowerSoil Kit:

2 Add the soil sample into the PowerBead Pro Tube.

3 Add Solution CD1 to the tube as per kit instructions.

4 Homogenize the sample using one of the following Vortex Adapter method.

Cell Lysis

5 Ensure proper mixing and cell lysis through homogenization.

Inhibitor Removal

6 Add Solution CD2 to remove impurities.

7 Add Solution CD3, then load the sample into the MB Spin Column.

Washing

8 Wash the column with Solution EA followed by Solution C5 to ensure purity.

Elution

9 Elute the DNA using Solution C6 to collect the extracted DNA.

DNA Qualification and Quantification:

10 Perform electrophoresis on an agarose gel to confirm DNA quality.

11 Use a Nanodrop spectrophotometer to measure DNA concentration and purity.

Submission of Environmental DNA:

12 Send the extracted e-DNA to the FAILSAFE Project Team at Chulalongkorn University for further analysis.