May 15, 2022

DNA extraction from dermatophytes using the Macherey-Nagel NucleoSpin™ Blood QuickPure kit (REF: 740569)

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dx.doi.org/10.17504/protocols.io.e6nvwkbpzvmk/v1

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This protocol describes the steps necessary to extract and purify genomic DNA from dermatophytes (and more specifically from dermatophytes of the genus Trichophyton).

DOI

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Khalid El Moussaoui 2022. DNA extraction from dermatophytes using the Macherey-Nagel NucleoSpin™ Blood QuickPure kit (REF: 740569). **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvwkbpzvmk/v1

dermatophytes, dna extraction, nucleospin

protocol ,

Mar 25, 2022

May 15, 2022

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Perform the DNA dosage directly after extraction and not after a freeze/thaw cycle. Be careful when preparing the medium : work under sterile conditions as much as possible to avoid contaminating the liquid medium.

Macherey-Nagel NucleoSpin™ Blood QuickPure kit (REF: MN 740569)
Sabouraud Dextrose Broth (REF : Merck S3306)
Cell Lysis Solution for genomic purification (REF : Promena A7933)

:

Medium preparation 4h 30m

1 Dissolve **30 g** of Sabouraud dextrose broth **Merck Millipore Sigma Catalog #S3306** in **1 L** of MilliQ Water **Contributed by users** and let mix on the heated magnetic stirrer for **00:05:00** (temperature and mixing speed knob at mid-step). 10m

2 Cover the flask with glass wool and aluminium foil. Autoclave it at **121 °C** for **00:30:00** . 4h

Cultivation of the strains 4d

3 After allowing to cool, transfer **25 mL** of this medium into a tube. Label the tube with the 1h

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strain number.

4 Using a sterile swab (or a sterile inoculation loop), gently collect the primary culture and dip the swab (or the sterile inoculation loop) into the tube containing the culture medium (prepared in the previous step). Close the tube halfway to allow gas exchange. 1m

5 Allow to grow in the incubator at **30 °C** until a sufficient flocculate is formed (requires at least 96 hours). Incubation time varies from strain to strain but flocculate should be visible after 5 days. If this is not the case, repeat the cultivation step. 4d

Preliminary steps 25m

6 Preheat the elution buffer to **70 °C** 1m

7 Using a Pasteur pipette, carefully remove the flocculate from the tube containing the previously cultured dermatophyte strain. Transfer this flocculate to a sterile tube containing glass beads, let's call it primary tube. Add **500 µL** of Cell Lysis Solution for Genomic Purification **Promega Catalog #A7933** to the primary tube. 2m

8 Cool this tube to **-20 °C** on the ice block for **00:01:00** . Then, heat this tube in a water bath at **70 °C** for **00:01:00** . Finally, run this tube through the cell disruptor at maximum speed for **00:01:00** . This constitutes 1 cycle of 3 steps. You must repeat this cycle 5 times. The recovered mixture is referred to as primary lysate in the following steps. 20m

DNA extraction 25m

9 Take **200 µL** of the primary lysate (from the preliminary steps) and transfer to a clean tube. Add **25 µL** of proteinase K and **200 µL** of lysis buffer BQ1. Homogenize with a vortex for **00:00:15** and then incubate the mixture for **00:15:00** at **70 °C** (in the water bath). 20m

10 Add **200 µL** of absolute ethanol (96-100%), vortex for **00:00:15** and then short-spin centrifuge for MAXIMUM **00:00:10** at **11000 x g** to accelerate protein precipitation. Do not centrifuge any longer. This may cause the DNA to be lost from the supernatant in the pellet. 1m

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11 Gently collect the supernatant of the solution (approximately **600 µL**) then apply it in the chromatographic column with the silica membrane. 1m

DNA purification 6m

12 Centrifuge the column at **11000 x g** for **00:01:00** to allow absorption of DNA onto the silica membrane and removal of contaminants at the same time. Keep the column and discard the flows-through. 1m

13 Place the column in a new collection tube and add **350 µL** of Buffer BQ2.Then centrifuge at **11000 x g** for **00:03:00** 3m

14 Place the column in a new collection tube (or discard the flows-through) and add **200 µL** of Buffer BQ2. Then centrifuge again at **11000 x g** for **00:01:00** 2m

15 Discard the flows-through or change the collection tube and centrifuge the column at **11000 x g** for **00:01:00** without adding any buffer. 1m

DNA elution 8m

16 Place the column in a clean collection tube. Add **50 µL** of elution buffer pre-heated at 70°C to the dried column. 1m

17 Incubate for **00:05:00** at room temperature and then centrifuge for **00:01:00** at **11000 x g** Discard the column and keep the flows-though which is the purified DNA. Store DNA at **-80 °C** to ensure stability. 7m

Spectrophotometric dosage

18 To determine the purity and concentration of the DNA, a NanoDrop dosage was performed. For this purpose, a negative control was prepared beforehand. This control will have undergone all the extraction steps but will not contain any material from dermatophytes.

19 Launch the computer program and select the "nucleic acid" mode. Make sure the sample deposit spot is clean and dry. If necessary, clean it with the wipes provided for this purpose. Then drop **2 µL** of the negative control and click on the "blank" box.

Proceed in the same way to measure the sample containing the DNA, but click on "measure"

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20 instead of "blank". There is no need to redo a blank between measurements.

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