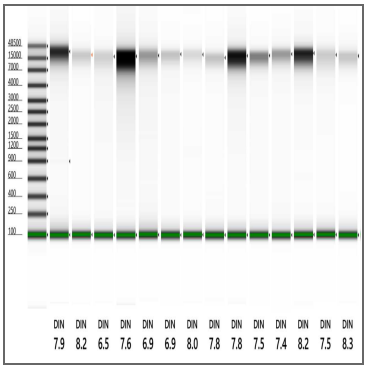

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We use this protocol and it's working

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Extraction of high molecular weight DNA from nasal lining fluid

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ABSTRACT

Assessing the microbiome in respiratory samples is often difficult due to the low biomass of microbes often present in these samples. While there are published methods for efficient extraction of DNA from samples such as bronchoalveolar lavage fluid for 16s rRNA sequencing or metagenomic sequencing (Saladie *et al.,* 2020), these methodologies are usually optimised for traditional short-read based sequencing technologies. With the advent of accessible and affordable long-read sequencing technologies for full length 16s rRNA sequencing (PacBio) and whole metagenomic sequencing (Oxford Nanopore), there is increased importance in extracting high quality, high molecular weight fragments of DNA from metagenomic samples. This protocol can be used to extract DNA from both low biomass (nasal lining fluid, bronchoalveolar lavage, nasal swabs) and high biomass (pure bacterial culture) samples.

GUIDELINES

This protocol can be used to extract DNA from both low biomass (nasal lining fluid, BALf, nasal swab) and high biomass samples (bacterial culture)

MATERIALS

The following materials were utilised for this protocol:

- Puregene tissue kit (Qiagen, #158023) containing cell lysis solution, protein precipitation buffer, proteinase K, RNase A, and DNA hydration buffer
- Ethanol, molecular grade (Sigma, #E7023)
- MetaPolyzyme (Sigma, #MAC4L)
- Phosphate buffered saline, sterile filtered (ThermoFisher, #10010023)
- 30% polyethylene glycol (PEG) 8000 solution in 1.6 M NaCl pH 6.7 (Bioworld, #41620040-1)
- UltraPure molecular grade water (ThermoFisher, #10977015)
- GenElute linear polyacrylamide (Sigma, #56575)
- 0.1mm silica/zirconia beads (Biospec, #11079101z)
- Isopropanol, molecular grade (Sigma, #I9516)

SAFETY WARNINGS

While this protocol does not utilise common hazardous chemicals used for DNA extraction (phenol/chloroform), care should be taken to follow the recommendations in the MSDS provided which each reagent, and ensure proper storage for the dangerous goods utilised in this protocol (flammable liquids etc)

BEFORE START INSTRUCTIONS

This protocol extracts DNA from samples stored in 45µL of PBS - if the sample is in a larger volume, the volumes of all following reagents can be scaled up, ensuring consistent ratios throughout the protocol. Otherwise, centrifuge sample at high speed for ~5 minutes and remove supernatant, resuspending pellet in 45uL of PBS

Preparation of reagents

1 MetaPolyzyme is recieved as a lypholysed powder. Reconstitute following manufacturers instructions to a final concentration of [M] 5 mg/mL, aliquot into 0.6mL microtubes, and store at -20°C until use

2 Create a [M] 70 % (v/v) solution of molecular grade ethanol with UltraPure water (approx. 1 mL per sample)

3 Add a small volume (up to the line of the conical portion on the tube) of 0.1mm silica/zirconia beads to a 2.0mL screw cap microtube suitable for bead beating (one per sample)

DNA extraction

1h

4 Add 5uL of MetaPolyzyme ([M] 5 mg/mL) to 45 µL of sample in PBS in a 1.5mL microtube

5 Incubate for 01:00:00 at 35 °C using a Thermomixer at 500 rpm 1h

6 Add sample (~50uL) to the prepared 2.0mL screw cap microtube containing beads

7 Add 250 µL of Cell Lysis Solution containing [M] 0.6 % (v/v) Proteinase K and [M] 0.6 % (v/v) RNase A solution to the sample

8 Bead beat sample for 00:00:30 in the Precellys24 bead beater 30s

9 Place sample on ice for 00:01:00 1m

10 Bead beat sample for 00:00:30 in the Precellys24 bead beater 30s

11 Centrifuge the sample after bead beating at 10000 x g for 00:01:00 1m

12 Remove supernatant (~300uL) and place in a new 1.5mL microtube

13 Incubate for 00:30:00 at 37 °C in a thermomixer at 500 rpm 30m

14 Increase the temperature to 56 °C and incubate for 01:00:00 in a thermomixer at 500 rpm 1h

15 Briefly place samples on ice to cool down to room temperature

16 Add 100 µL of protein precipitation solution containing [M] 1 % (v/v) GenElute linear polyacrylamide to the sample and mix thoroughly by inverting the tube 25 times

17 Incubate samples on ice for 00:05:00 5m

18 Centrifuge tube at 16000 x g for 00:03:00 3m

19 Add supernatant to a new 1.5mL LoBind tube, careful not to disturb the protein pellet

DNA precipitation

2h 47m

20 For low biomass samples where expected DNA recovery is low

20.1 Add 1.5 volumes of PEG solution to the sample and incubate in the dark at Room temperature for 02:00:00 2h

20.2 Centrifuge at 14000 x g for 00:30:00 at 24 °C 30m

21 For high biomass samples where expected DNA recovery is high

21.1 Add 1 volume of isopropanol to the sample and incubate at Room temperature for 00:05:00 5m

21.2 Centrifuge at 16000 x g for 00:05:00 at Room temperature 5m

22 Discard the supernatant by slowly drawing with a pipette at the air-liquid interface to avoid disturbing the pellet

> Note
>
> Pellets when precipitated with PEG will be near invisible, and easily detached from the tube, so care must be taken

23 Add 400 µL of [M] 70 % (v/v) ethanol to the sample and invert several times to wash the pellet

24 Centrifuge at 16000 x g for 00:01:00 1m

25 Discard the supernatant and add 400 µL of 70% [M] 0 % (v/v) ethanol to the sample and invert several times to wash the pellet

26 Centrifuge at 16000 x g for 00:01:00 — 1m

27 Discard the supernatant, and allow the pellet to air dry for 00:05:00 — 5m

28 Add 30 µL of DNA hydration solution or nuclease free water to the pellet

29 Incubate in the fridge at 4 °C Overnight

30 After this step, DNA is ready to be quantified and used downstream. DNA resuspended in water should be stored at -20 °C, while DNA resuspended in DNA hydration solution can be stored for up to one month at 4 °C