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DNA extraction from sputum

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1 Works for me dx.doi.org/10.17504/protocols.io.6enhbde



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Breaking the cells with liquid nitrogen

10m

- 1 The sputum samples were transferred to 1.5ml Eppendorf tubes for liquid nitrogen cell wall breakage, with a maximum of 600 µl of sputum per tube. Under full PPE conditions, the lid of the tube was left open, and a small plastic pestle placed to sit inside. Liquid nitrogen was transferred into a 100 ml cup. The tube, with the pestle inside, was placed into the liquid nitrogen, up until the lid opening, for three seconds. Once removed, the pestle was used to ground up and crush the sputum inside, turning it continuously for 60 seconds, and the process was repeated five times for each tube.

Following the manufacturer's instructions of Sputum DNA Isolation Kit (Cat. 46200, Norgen BioTek Corp., Thorold, ON, Canada)

2m

- 2 Add 4 mL of Slurry D for every 1 mL of sputum sample in a 15 mL conical tube. Mix well by inversion. (Note: Slurry D contains silicon carbide resin and must be mixed well before every pipeting).

- 3 Centrifuge for 5 minutes at 2,000 RPM. Discard the supernatant.

5m

- 4 Add 20 µL of both Proteinase K and Lysozyme (user supplied) to the precipitated slurry pellet resulting from the sputum sample. Vortex for 10 seconds.

10s

- 5 Incubate the mixture at 60°C for 20 minutes.

20m

- 6 After the 20 minute incubation, add 260 µL Solution WN, and mix well by pipetting up and down.

1m

- 7 Transfer the entire contents into a Mini Filter Spin column (provided) with collection tube.

1h

- 8 Centrifuge for 1 minute at 14,000 RPM, and discard the flow-through.

1m

- 9 Apply 500 µL of Wash Solution BE to the column and centrifuge for 1 minute. Discard the flowthrough and reassemble the spin column with its collection tube.

1m

- 10 Apply 500 µL of Wash Solution BE to the column and centrifuge for 1 minute. Discard the flow-through and reassemble the spin column with its collection tube.

1m

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| 11 | Apply 500 μ L of 96-100% Ethanol (user supplied) to the column and centrifuge for 1 minute. Discard the flow-through and reassemble the spin column with its collection tube. | 1m |
| 12 | Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube. | 2m |
| 13 | Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 100 μ L of Nuclease free water to the column and centrifuge for 2 minutes at 2,000 RPM, followed by 1 minute at 14,000 RPM. | 2m |
| 14 | The DNA concentration of the samples is determined using DeNovix dsDNA Broad Range Fluorescent Assay Kit (Denovix, Wilmington, USA) | 10m |



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