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DNA extraction for human microbe samples. V.1

In 3 collections

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1 Works for me dx.doi.org/10.17504/protocols.io.bcmriu56

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ABSTRACT

This protocol is used to clarity the process of total DNA extration for human microbe samples.

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COLLECTIONS

The Female Urinary Microbiota Protocols Collection

Protocols for the female urinary microbiota in relation to the reproductive tract microbiota

Protocols for "The female urinary microbiota in relation to the reproductive tract microbiota."

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The Female Urinary Microbiota Protocols Collection

Protocols for the female urinary microbiota in relation to the reproductive tract microbiota

Protocols for "The female urinary microbiota in relation to the reproductive tract microbiota."

ABSTRACT

This protocol is used to clarity the process of total DNA extration for human microbe samples.

1 200µl samples were mixed with 500 µl TE buffer and 20 µl of 10 mg/ml lysozyme solution.

2 Incubate at 37 °C for 90min. Invert the tube several times to mix every 15 minutes. Centrifuge at 13,000 rcf/min for 10 min at 4 °C and discard the supernatant.

3 Add 300 µl of 20 mg/ml proteinase K and 5µl of 10% SDS. Vortex to resuspend cells.

4 Incubate at 55 °C in a shaking water bath for 120 min to effect complete lysis. Invert the tube several times to mix every 15 minutes.

5 Centrifuge at 13,000 rcf/min for 10 min at 4 °C, then transfer the supernatant to a nuclease-free 2 ml microfuge tube.

6 Add 700 µl phenol-chloroform-isoamylalcohol (25:24:1) and vortex to mix well, centrifuge at 13,000 rcf/min for 10 min at 4 °C, then transfer the supernatant to a nuclease-free 2 ml microfuge tube.

7 Add 700 µl chloroform-isoamylalcohol (24:1) and vortex to mix well. Centrifuge at 13,000 rcf/min for 10 min at 4 °C, then transfer the supernatant to a nuclease-free 1.5 ml microfuge tube.

8 Add 1/10 volume of 3M sodium acetate, 2 µl of 5 mg/ml glycogen and 800 µl cold isopropanol. Incubate at -20 °C for the night.

9 Centrifuge at 13,000 rcf/min for 10 min at 4 °C, then discard the supernatant.

10 Washed with 800 µl of 70% ethanol. Centrifuge at 13,000 rcf/min for 10 min at 4 °C, then discard the supernatant.

11 Wash the precipitate with a second 800 µl of 70% ethanol and centrifuge as above. discard the supernatant.

12 Let sit for 15 min to dry the precipitate. Add 1×TE buffer to resuspend the precipitate.

13 Add 1µl RNase A to samples and invert tube several times to mix. Incubate at 37 °C for 30 minutes.

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