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DNA extraction for human microbe samples. V.1

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We use this protocol and it's working

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Abstract

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

Troubleshooting

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.