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DNA extraction for HMW DNA

In 1 collection

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is intended for extraction of HMW DNA from bacterial or viral samples.

Guidelines

For mixing steps in this protocol, never mix by vortexing or pipettinig. Mix by inversion or gently flicking the tube.

Safety warnings

This protocol uses phenol:chloroform and chloroform. Read the SDS for these reagents before starting and always use them in a fume hood.

Before start

Ensure your sample is 500ul or less in volume.

Lyse cells/viruses

1 Add 600ul lysis buffer to less than 500ul of sample in a 2mL tube.

600 µL lysis buffer

Note

To make lysis buffer, add the following to 10mL of 1X TE:
1. 300ul 20% SDS
2. 60ul 20mg/mL protK

1.1 Incubate 1hr at 37 C.

37 °C

01:00:00

Extract DNA

2 Add 1 vol phenol:chloroform and mix by inversion.

Safety information

Always work with phenol:chloroform in a fume hood.

2.1 Spin at max speed 5 min at RT.

00:05:00

Room temperature

2.2 Remove aqueous layer to new 2mL tube.

3 Repeat step 2.

4 Add 1 vol chloroform and mix by inversion.

Safety information

Always work with chloroform in a fume hood.

4.1 Remove aqueous layer to new 2mL tube.

4.2 Spin at max speed 5 min at RT.

00:05:00

Room temperature

Precipitate DNA

5 Add ice cold absolute ethanol to fill tube (2.5-3 vol) and mix by inversion.

5.1 Incubate at -20C for >30 min.

00:30:00

-20 °C

Note

Shorter incubation time can be used, but is not recommended for low biomass samples. The longer the incubation the more DNA will precipitate, but note that more salts will precipitate as well.

5.2 Spin at max speed for 15 min at 4 C. Remove and discard supernatant.

00:15:00

4 °C

6 Rinse pellet with 1mL RT 70% ethanol.

1 mL 70% ethanol

6.1 Spin at max speed for 2 min.

00:02:00

Room temperature

6.2 Remove and discard supernatant.

7 Air dry DNA pellet.

Note

This can be done at room temperature, but will go faster at 40 C. Avoid over-drying the pellet as this will make it more difficult to resuspend.

Resuspend DNA

8 Resuspend DNA in 100ul warm nuclease-free water.

100 µL water

Note

Do NOT vortex or pipet to resuspend. Gently flick the tube and leave at room temperature for up to 4 hours. Store at 4 C overnight and flick again the next day. If your pellet is difficult to resuspend, incubate at 56 C flicking periodically until the pellet dissolves.

9 At this point you can check the concentration (we recommend Qubit) and purity (NanoDrop) of your DNA. Ensure the pellet is fully resuspended before you try.