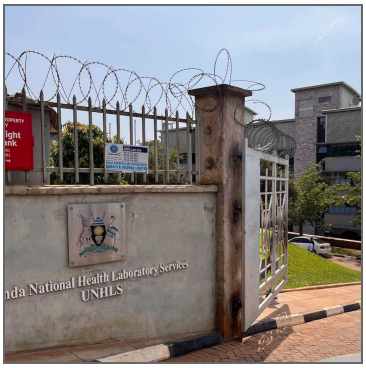

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Protocol status: Working

DOH Workshop Protocol Part 1: Purification of High- Molecular Weight Genomic DNA from Gram-Negative Bacteria (MagAttract HMW DNA)

In 2 collections

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ABSTRACT

This protocol enables extraction of high-molecular-weight DNA from gram-negative bacterial cultures.

ATTACHMENTS

pyxtbzpxx.pdf

Created: Mar 05, 2024

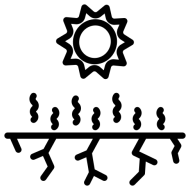
Last Modified: Mar 08, 2024

PROTOCOL integer ID: 96352

GUIDELINES

Important points throughout protocol:

Be gentle when pipetting, too much fast pipetting/ extended vortexing can shear the DNA which will result in poorer sequencing outcomes.



Do not let the beads dry after removal of supernatant. This can be avoided by keeping Eppendorf tubes closed if beads are not submerged.
Note: When bead pellet is moist, it appears shiny. As they start to dry, the shine reduces, and cracks start to form.

When working with beads ensure they are thoroughly mixed before using. This can be achieved by vortexing for at least 10 seconds before use and vortexing between use to prevent beads settling.

MATERIALS

Starting Materials

- 1.5 mL of overnight bacterial cell culture.

Equipment

- P1000 pipette (Micropipette with 100 µL – 1000 µL range)
- P200 pipette (Micropipette with 20 µL – 200 µL range)
- P20 pipette (Micropipette with 2 µL – 20 µL range)
- P10 pipette (Micropipette with 0.5 µL – 10 µL range)
- Thermomixer (for heating, cooling and mixing 1.5ml tubes)
- Benchtop centrifuge for 1.5 µL (up to 5000 Gs) OR Bento Lab
- Minicentrifuge (for brief spin downs of 1.5ml eppendorfs and 0.2ml PCR tubes) OR Bento lab with improvised adaptor made from 0.5ml eppendorf within a 1.5ml eppendorf with caps cut off.
- PCR thermoblock (21 °C - 80 °C required) OR Bento Lab
- DNA fluorometer (Promega Quantus or Themofisher Qubit)
- Vortex mixer

Consumables

- P1000 filter pipette tips (with 100 µL – 1000 µL range)
- P200 filter pipette tips (with 20 µL – 200 µL range)
- P20 filter pipette tips (with 2 µL – 20 µL range)
- P10 filter pipette tip (with 0.5 µL – 10 µl range)
- 1.5 ml Eppendorf DNA LoBind tubes
- 0.2 ml thin-walled PCR tubes
- Absolute ethanol (>96%)
- Distilled water (1400 mL per sample)
- HMW Magattract Kit

1. Nuclease Free Water
2. Buffer MW1 (Ethanol must be added)
3. Buffer PE (Ethanol must be added)
4. MagAttract Suspension G
5. Proteinase K

6. RNAase A (100 undetermined)
7. Buffer ATL

BEFORE START INSTRUCTIONS

- If precipitate is formed in Buffer ATL, dissolve by incubating at 37°C with occasional shaking. The presence of precipitate can result in incomplete lysis.
- Prepare (but do not start) the thermomixer to 56°C at 900rpm for 30min for the lysis step.

Purification of High- Molecular Weight Genomic DNA from Gram-Negative ...

59m 12s

1

Pellet 1.5 mL of the Gram negative bacterial cell culture by spinning at 5000 x g for 00:03:00 .

3m

Note

When removing tube from centrifuge, be careful not to shake the tube too much which can dislodge the pellet.

2 Remove and discard supernatant without disturbing the pellet. Use a P200/P20 pipette if there is a small amount of supernatant remaining.

Note

The cell pellet can be stored at -30 °C to -15 °C or -90 °C to -60 °C for future use, or can be used immediately.

3 Resuspend the bacterial pellet in 180 µL Buffer ATL, add 20 µL Proteinase K and mix by flicking/stirring the tube.

Note

Pipette gently, be careful not to shear DNA

4 Incubate for 00:30:00 on thermomixer at 56 °C shaking at 900rpm. 30m

Things to do while waiting:

- Ensure that Buffers MW1 and PE were prepared according to instructions on them (i.e. adding appropriate amount of Ethanol).

5 Add 4 µL RNase A to the sample, mix by pulse vortexing or by tapping the tube several times, and incubate for 00:02:00 at Room temperature . 2m

Note

Pipette gently, be careful not to shear DNA

6 Set the thermomixer to 21 °C to give it time to cool for next steps.

7 DOH Workshop Protocol Part 1Fully mix MagAttract Suspension G. Vortex the MagAttract Suspension G vigorously until thoroughly mixed ~ 00:00:10 . 10s

Note

It is important that beads are fully mixed and re-mixed between every few samples.

Shake well before use

8 Add 15 µL MagAttract Suspension G and 280 µL Buffer MB to the sample. Mix by pulse vortexing 2s

(00:00:01 - 00:00:02 quick presses x 3).

Note

Pipette gently, be careful not to shear DNA

9 Place the tube onto the thermomixer and incubate at 21 °C for 00:03:00 at 1400 rpm.. 3m

10 Place tubes onto magnetic rack and wait until beads pellet (~ 00:01:00). 1m

11 While leaving tubes on the magnet, carefully remove supernatant without disturbing the beads using a P1000.

Note

Pipette gently, be careful not to shear DNA

12 Remove tube from magnetic rack and add 700 µL Buffer MW1 to the tube, flick the tube until bead pellet is unstuck from tube wall.

Note

MW1 Wash 1

13 Place the tube onto the thermomixer and incubate at 21 °C for 00:03:00 at 1400 rpm. 3m

14 Place tubes onto magnetic rack and wait until beads pellet (~ 00:01:00). 1m

15 While leaving tubes on the magnet, carefully remove supernatant without disturbing the beads using a P1000.

Note

Pipette gently, be careful not to shear DNA

16 For the second wash, remove tube from magnetic rack and add 700 µL Buffer MW1 to the tube, flick the tube until bead pellet is unstuck from tube wall.

Note

MW1 Wash 2

17 Place the tube onto the thermomixer and incubate at 21 °C for 00:03:00 at 1400 rpm. 3m

18 Place tubes onto magnetic rack and wait until beads pellet (~ 00:01:00). 1m

19 While leaving tubes on the magnet, carefully remove supernatant without disturbing the beads using a P1000.

Note

Pipette gently, be careful not to shear DNA

20 Remove tube from magnetic rack and add 700 µL Buffer PE to the tube, flick the tube until bead pellet is unstuck from tube wall.

PE Wash 1

21 Place the tube onto the thermomixer and incubate at 21 °C for 00:03:00 at 1400 rpm.. 3m

22 Place tubes onto magnetic rack and wait until beads pellet (~ 00:01:00). 1m

23 While leaving tubes on the magnet, carefully remove supernatant without disturbing the beads using a P1000.

Note

Pipette gently, be careful not to shear DNA

24 For the second wash, remove tube from magnetic rack and add 700 µL Buffer PE to the tube, flick the tube until bead pellet is unstuck from tube wall.

PE Wash 2

25 Place the tube onto the thermomixer and incubate at 21 °C for 00:03:00 at 1400 rpm. 3m

26 Place tubes onto magnetic rack and wait until beads pellet (~ 00:01:00). 1m

27 While leaving tubes on the magnet, carefully remove supernatant without disturbing the beads using a P1000. Then use a P200 to remove any traces of Buffer PE.

Note

Pipette gently, be careful not to shear DNA

28 While keeping the tube on the magnetic rack, slowly pipette 700 µL distilled water by pipetting down 1m the side of the tube opposite the pellet, without disturbing any of the beads/pellet. Leave for 00:00:30 - 00:01:00 and then remove the supernatant without disturbing any of the beads/pellet.

Note

Pipette gently, be careful not to shear DNA

29 Repeat step 28.

Note

If necessary, use a P20 to remove any remaining distilled water.

Pipette gently, be careful not to shear DNA

30

Remove the tube from the magnetic rack and add 50 µL of nuclease free water. Resuspend the beads by slowly pipetting or stirring with the pipette tip.

Note

Be as gentle as possible while ensuring that pellet is resuspended.

Pipette gently, be careful not to shear DNA

31

Place the tube onto the thermomixer and incubate at t 21 °C for 00:03:00 at 1400 rpm.

3m

32 Pellet beads on magnet and slowly pipette DNA eluate into a new lo-bind tube.

Note

Pipette gently, be careful not to shear DNA

Save the tube with magnetic beads in case of incomplete DNA elution, so you can repeat.