Mar 15, 2024

Isolation of HMW gDNA from a bioreactor biofilm for long read sequencing

Microbiology Resource Announcements

DOI
dx.doi.org/10.17504/protocols.io.q26g7yj4qgwz/v1

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Protocol Citation: Viola A Manning, Kristin Trippe 2024. Isolation of HMW gDNA from a bioreactor biofilm for long read sequencing . **protocols.io https://dx.doi.org/10.17504/protocols.io.q26g7yj4qgwz/v1**

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

Created: March 28, 2023

Last Modified: March 15, 2024

Protocol Integer ID: 79607

Keywords: bioreactor biofilm for long read, isolation of hmw dna, isolation of hmw gdna, bioreactor biofilm, hmw dna, hmw gdna, dna

Abstract

This protocol describes isolation of HMW DNA for long read sequencing from a bioreactor biofilm.

Materials

DNA/RNA Shield DNA-RNA_Shield.pdf

DNA-Quick HMW MagBead kit Zymo_Quick-DNA_HMW_MagBead...
PBS (Gibco #70013-032)
TE (Ambion #AM9861)
10% SDS (ThermoFisher #15553027)
2 and 5 mL eppendorf tubes
magnetic bead separator
microcentrifuge
Vortex Genie
Thermomixer
pipettes and filtered pipette tips

Troubleshooting

Storing bioreactor biofilm

1 Aliquot 750 uL of biofilm into 1.5 mL eppendorf tubes.

2 Spin in microcentrifuge (eppendorf 5424) at ~21,000 x g for 5 minutes. 00:05:00 5m

3 Resuspend pellet in 1 mL DNA-RNA_Shield.pdf by pipetting up and down multiple times.

4 Store resuspended pellet at -80 °C until ready to process.

Disrupting the biofilm

5 Transfer 1 mL of sample into 2mm bashing beads (Zymo S6003)

> Note
>
> Will have approximately 100 uL of biofilm in 1 mL of DNA/RNA Shield

6 Place on Vortex Genie with horizontal adapter and shake for 10 minutes, top speed.

7 Remove supernatant to a 2 mL tube.

8 Centrifuge 5000 x g for 1 minute. 00:01:00 1m

Extract the pellet 1m

9 Remove supernatant to a 5 mL eppendorf tube.

10 Resuspend pellet in 500 uL PBS and pipette mix until pellet is visibly resuspended.

11 The remaining steps of this protocol were adapted from the Microbial Lysis and DNA Purification section of Zymo_Quick-DNA_HMW_MagBead... . 1m

Centrifuge at 5000 x g for 1 minute and combine supernatant with the original supernatant. 00:01:00

12 Resuspend pellet in 1 mL PBS, spin, and discard supernatant.

13 Add 500 uL TE and 125 uL 100 mg/ml lysozyme in TE.

14 Pipette mix until pellet is visibly resuspended and incubate at 55 °C for 30 minutes, flicking occasionally. 00:30:00 30m

15 Combine with supernatants and aliquot ~ 1 mL into each of two 2 mL eppendorf tubes.

16 To each tube, add 50 uL 10% SDS and 25 uL Proteinase K, pipette mix, incubate at 55 °C for 30 minutes on a Thermomixer at 500 rpm. Incubate longer if it looks like lysis is incomplete. 00:30:00 30m

17 Centrifuge 5000 x g for 1 minute and transfer 1 mL supernatant into two each 5 mL eppendorf tubes. 00:01:00 1m

18 Add 2 mL Quick-DNA MagBinding Buffer.

19 Add 66 uL well resuspended magnetic beads, mix by pipetting up and down 5 times, put on rotating mixer for 10 minutes. 00:10:00 10m

20 Transfer tube to magnetic stand, let beads separate, remove and discard supernatant.

21 Remove from the magnetic stand, add 500 uL Quick-DNA MagBinding Buffer, transfer to 2 mL eppendorf tube.

22 Pipette 5 times to mix and then put on rotator for 5 minutes. 00:05:00 5m

23 Place on magnetic stand and remove supernatant.

24 Remove from magnetic stand, add 500 uL Pre-Wash Buffer, and pipette 10 times to reususpend.

25 Place on magnetic stand, let beads separate, and remove supernatant.

26 Remove from stand, and add 900 uL g-DNA wash buffer and pipette 10 times to resuspend.

27 Transfer to clean 2 mL Eppendorf tube, place on stand, let beads separate, and remove supernatant.

28 Repeat steps 26 and 27.

29 Dry beads in hood for 20 minutes.

30 Resuspend in 100 uL DNA elution buffer, pipette mix 20 times, and incubate on Thermomixer for 5 min at 25 °C while shaking at 1000 rpm. 00:05:00 5m

31 Transfer sample to magnetic stand, transfer solution to new tube.

32 Store at -20 °C .