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OG1RF_genomic_DNA

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[1]In-house protocol

1 Works for me This protocol is published without a DOI.

Eadewunm

ABSTRACT

OG1RF genomic DNA isolation

PROTOCOL CITATION

Elizabeth Fozo 2020. OG1RF_genomic_DNA. **protocols.io** https://protocols.io/view/og1rf-genomic-dna-bp4tmqwn

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ABSTRACT

OG1RF genomic DNA isolation

Steps

1 Generate an overnight culture in 10 mL of BHI

2 Spin down culture in morning for 10 min at 3500 RPM and pour off supernatant.

3 Make 50 mM EDTA (500 µL 0.5M EDTA stock in 4.5 mL water).

4 Add 960 µL of 50 mM EDTA to OG1RF pellet, then aliquot 480 µL of 50 mM EDTA/cells to a 1.5 mL Eppendorf tube.

5 Add 120 µL of 10 mg/mL lysozyme

6 Incubate at 37°C for 60 minutes

7 Spin at 13000 RPM for 2 minutes and pour off supernatant.

8 Add 600 µL nuclei lysis solution

9 Incubate at 80°C for 5 min then cool to room temperature

10 Add 3 µL of RNAse solution

11 Incubate at 37°C for 60 minutes

12 Add 200 µL of protein precipitation solution. Vortex vigorously for 20 seconds

13 Put on ice for 5 minutes

14 Spin at 13000 RPM for 3 minutes

15 Transfer supernatant to a clean Eppendorf tube containing 600 µL of isopropanol

16 Gently invert

17 Spin at 13000 RPM for 2 minutes and pour off supernatant. Be careful to not disturb pellet.

18 Add 600 µL of 70% ethanol to wash DNA pellet. Stream ethanol directly onto pellet without touching the pipette tip to the pellet.

19 Spin at 13000 RPM for 2 minutes and pour off supernatant carefully.

20 Invert and air dry

21 Add 100 µL of DNA hydration solution

22 Incubate at 65°C for 1 hour or leave at 4°C overnight