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OG1RF_genomic_DNA

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Works for me

This protocol is published without a DOI.

Eadewunm

ABSTRACT

OG1RF genomic DNA isolation

PROTOCOL CITATION

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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 μ L 0.5M EDTA stock in 4.5 mL water).
- 4 Add 960 μ L of 50 mM EDTA to OG1RF pellet, then aliquot 480 μ L of 50 mM EDTA/cells to a 1.5 mL Eppendorf tube.
- 5 Add 120 μ L 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 μ L nuclei lysis solution
- 9 Incubate at 80°C for 5 min then cool to room temperature
- 10 Add 3 μ L of RNase solution
- 11 Incubate at 37°C for 60 minutes
- 12 Add 200 μ L 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 μ L 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 μ L 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 μ L of DNA hydration solution
- 22 Incubate at 65°C for 1 hour or leave at 4°C overnight