Nov 25, 2020

Isolation of RNA form Enterococcus faecalis

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[1]In-house protocol

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

Eadewunm

ABSTRACT

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PROTOCOL CITATION

Elizabeth Fozo 2020. Isolation of RNA form Enterococcus faecalis. **protocols.io** https://protocols.io/view/isolation-of-rna-form-enterococcus-faecalis-bp4rmqv6

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CREATED

Nov 25, 2020

LAST MODIFIED

Nov 25, 2020

PROTOCOL INTEGER ID

44913

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ABSTRACT

Isolation of RNA form Enterococcus faecalis

Steps

1 *E. faecalis* is stored at -80. Please see the appropriate personnel (i.e., Dr. Fozo) to pull the culture from frozen.

2 Begin a 10 ml liquid broth overnight (ON) of E. faecalis; grow without aeration in 37°Cincubator.

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3 The following morning, dilute culture to be 0.01in fresh media. Note, the general rule of thumb is to never remove more than 1/3 of cells from the initial starting culture.

4 For harvesting: have 15 ml ice blocks within labeled 50 ml conicals. Add 12 ml of culture (CAREFULLY—AVOID SPLASHING) to the conical. Pipettes must go into the biohazard pipette buckets (orange, lidded buckets)

5 Spin conicals at 4°C for 10 minutes. Decant the spent media into the labeled biohazard container "spent media." Treat spent media as noted in the "biohazard disposal protocol."

6 Resuspend cell pellets in 600 µl Solution GP (50 mM Tris-HCl, 10 mM EDTA, 1% SD, 30 mM sodium acetate). Please make sure the bench is covered in clean bench paper!

7 Carefully transfer the cell suspension to a 2 ml screw-capped tube containing 0.5 g (average diameter ≤ 106 µm; Sigma) and 650 µl **acid phenol**: chloroform (Ambion).

8 Bead beat cell suspension twice, for 45 seconds each in the cold (place tubes on ice about 2 minutes in between bead beating intervals). Make sure the hood is down over the bead beater and the lids are on tight to prevent phenol spills!

9 Spin the cell suspension at 10000 rpm, 4°C for 10 minutes.

10 Transfer the supernatant to a tube containing 500 µl **acid phenol**: chloroform (Ambion), preheated to 65°C. Vortex to mix. Let it sit at 65°C for10 minutes. Vortex to mix.

11 Spin the sample at 13000 rpm, 10 minutes. Transfer the supernatant to a clean tube

12 Add 400 µl **"regular" phenol:** chloroform, vortex, and spin 5 minutes, 13000 rpm, room temperature. Perform this step a minimum of two times until clean in appearance.
Note: Do NOT use acid phenol: chloroform.

13 Transfer to a clean tube, and add 400 µl chloroform. Spin as above.

14 Transfer supernatant to a clean tube. Add 400 µl of 95 (or) 99% ethanol. Place in -80 20 minutes or overnight.

15 Precipitate RNA: spin at 13,000 rpm, 4°C for 20 minutes. Carefully remove EtOH.

16 Wash pellets with 70% ethanol. Spin 13,000 rpm, 4°C for 10 minutes.

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17 Aspirate pellets and allow to air dry. Resuspend in RNAse-free water.

18 Check integrity on the gel and by 260/280 ratio. If need be, the sample can be treated with Turbo DNAse by Ambion.
DNAse treatment:

- 50 µg RNA in 85ul RNase-free water
- 10 ul10X Buffer
- 5 µl of Turbo DNAse

- 37°C for 20 minutes. Bring volume to 200 µl.
- Add 200 µl of phenol: chloroform and extract as described above (1 time).
- To supernatant, add 20 µl 3 M sodium acetate and 750 µl 95 or 99% ethanol. Precipitate RNA as above.

Checking the Optical Density of cultures:
When the culture OD 600 gets too high, the spec readings are inaccurate. I prefer to dilute my sample 1:5 (i.e., 200 µl culture within 800 µl media). Following readings, remove cell dilution to spent the BSL-2 liquid waste and treat with bleach. Cuvettes, like tips, must be treated as solid BSL-2 waste.

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