TRIzol DNA Extraction

Introduction

LucidChart Overviews | DNA | Combined Procedures List
https://static-content.springer.com/esm/art%3A10.1038%2Fncomms11179/MediaObjects/41467_2016_BFncomms11179_MOESM181_ESM.pdf

Materials

- overnight culture
- centrifuge
- microcentrifuge tubes
- heat block with 50 mL conical adapter (VWR digital heatblock)
- TRIzol LS Reagent
- isopropanol
- ethanol, 75%
- RNAse A

Procedure

1. Centrifuge 250 mL of overnight culture in five 50 mL tubes at 5000xg for 7 min at 4C
2. Remove the supernatant
3. Add additional 250 mL of the remaining cells to the same five 50 mL tubes and centrifuge at 5000 xg for 7 min at 4C
4. Repeat steps 2-3 until all the cells are centrifuged in the same five 50 mL tubes
5. During centrifugation, preheat 5 mL of Max Bacterial Enhancement Reagent (MBER) to 95C using a heat block with 50 mL conical adapter
6. After centrifugation, resuspend the cell pellets by adding 1 ml of preheated MBER to each 50 mL tube. Mix well and incubate at 95C for 4 minutes.
7. Add 7 mL of TRIzol to each tube containing the cell lysate. Homogenize the sample by pipetting up and down several times (if needed, samples can then be stored overnight at 4C or -20C)
8. Incubate the tubes at room temp for 5 minutes. Meanwhile, prepare twenty-four 2 mL tubes. Add 320 µL of cold chloroform to each tube.
9. After incubation, add 1.6 mL of the TRIzol-lysate mixture to each one of the pre-preared tubes. Then, mix by shaking the tube vigorously by hand for 15 seconds.

10. Incubate at room temperature for 3 minutes.

11. Centrifuge the samples at 12000 xg for 15 minutes at 4C. The mixture should separate into a lower red, phenol-choloroform stage, an interphase, and a colorless aqueous phase containing the RNA and ssDNA. The volume of the aqueous phase is ~1 mL.

12. During the centrifugation, prepare 24 new 2 mL tubes. Add 1 ml of cold isopropanol to each tube.

13. After centrifugation, transfer carefully the 1 mL resulting colorless aqueous phase from each tube to one of the pre-prepared tubes. Note that each tube should contain 1 mL of colorless aqueous phase and 1 mL of cold isopropanol. Then, mix by inverting the tubes.

14. Incubate at room temperature for 10 minutes.

15. Centrifuge at 15,000 xg for 10 minutes at 4C.

16. Remove the supernatant carefully without disturbing the pellet.

17. Resuspend the pellet in 1 mL 75% ethanol. Mix by vortexing.

18. Centrifuge at 7,500 xg for 5 minutes at 4C. Discard the supernatant.

19. Air-dry the pellet for 30 mins at room temp.

20. Resuspend the pellet from each 24 tubes with 50 µL water by pipetting up and down.

21. Collect the 24 50 µL solutions to a single new 2-mL tube ~1.2 mL and add 1.2 µL of RNAse A (100 mg per mL)

22. Incubate the resulting solution at 37C for overnight to allow RNA degredation.

23. The sol'n is now ready for concentration and PAGE analysis. Proceed to downstream applications, or store the DNA at 4°C overnight. For longer-term storage at –20°C, adjust the pH to 7–8 with HEPES and add 1 mM EDTA.