Research Center for
Pharmaceutical Nanotechnology
Tabriz University of Medical Sciences
RCPN

DNA extraction from bacteria

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Solutions

- The lysing buffer: 2% CTAB, 100 mM Tris_HCl, 1.4 M NaCl, 1% PVP, 20 mM disodium salt of ethylenediaminetetraacetic acid (Na_2EDTA), 0.2% LiCl. The pH should be adjusted at ~8 for the solution before autoclaving).

- TE buffer: 10 mM Tris_HCI (pH 8.0), 1 mM EDTA (pH 8.0)

Detailed procedure

1. Freeze and ground the bacterial pellet (from liquid culture) or selected colonies of bacteria (i.e., 2–3 colonies) into a fine powder in liquid nitrogen using precooled mortar and pestle.

2. Add 800 µL of lysis solution to the sample, mix vigorously and then transfer to a 1.5 tube.

3. Incubate the sample at 65°C (30 min for Gram_negative bacteria; 2 h for Gram-positive bacteria). Shack sample every 10 min gently.

4. Centrifuge the tube at 6160 *g* (10000 rpm) for 5 min at 4°C.

5. Transfer the supernatant into a new tube and add an equal volume of chloroform isoamilalchol (24:1 vol/vol). Flip the tube gently several times.

6. Centrifuge the tube at 8870.4 *g* (12000 rpm) for 8 min at 4°C. The centrifugation speed (*g*) should be adjusted to the designated value to avoid shearing stress and obtain intact DNA.

Transfer the upper phase into a new tube.

7. Add 100 µl of 5 M sodium acetate into the tube and mix by gentle flipping to increase the yield of extracted DNA.

8. Add equivalent volume of cold (–20°C) isopropanol to precipitate the DNA, then store the sample at –20°C for 30min. Add Isopropanol drop_wise and mix gently, since rapid addition may cause DNA fragmentation.

9. Centrifuge the sample at 12073.6 *g* (14000 rpm) for 10 min at 4°C.

10. For the first washing step, remove the supernatant and add 500 µL of 96% ethanol (4°C) then centrifuge at 8870.4 *g* (12000 rpm) for 5 min.

11. For the second washing step, remove the supernatant and add 500 µL of 70% ethanol (4°C) to the sample and centrifuge at 8870.4 *g* (12000 rpm) for 5 min.

12. Discard the supernatant and dry the pellet at room temperature.

Note: higher centrifugation speeds or excessive drying period may decrease the water solubility of the pellet.

13. Dissolve the pellet in 50 µl TE buffer and store DNA solution at –20°C.

Reference

Atashpaz, S., Khani, S., Barzegari, A., Barar, J., Zununi Vahed, S., Azarbaijani, R., *et al.* (**2010**) A robust universal method for extraction of genomic DNA from bacterial species. *Mikrobiologiia* 79: 562-6.

Good luck!

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