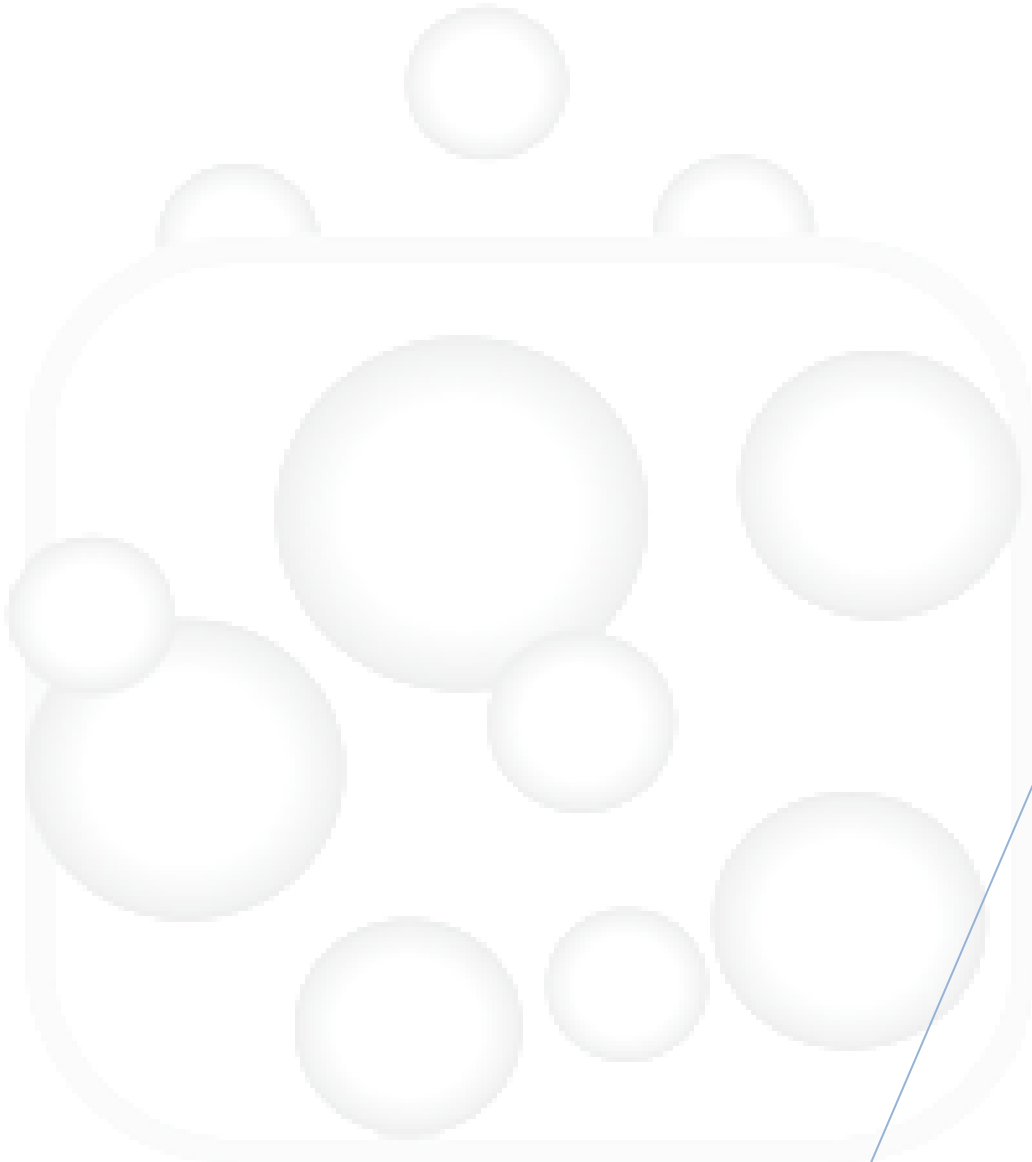




Research Center for
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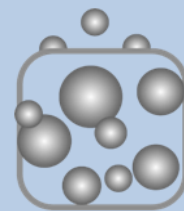
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DNA extraction from bacteria
PSR

Dr. Zonuoni. S
6/12/2016



RCPN

DNA extraction from bacteria

Solutions

- The lysing buffer: 2% CTAB, 100 mM Tris_HCl, 1.4 M NaCl, 1% PVP, 20 mM disodium salt of ethylenediaminetetraacetic acid (Na₂EDTA), 0.2% LiCl. The pH should be adjusted at ~8 for the solution before autoclaving).
- TE buffer: 10 mM Tris_HCl (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). Shake 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 isoamylalcohol (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 μ l 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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Document arrangement	Mohanna Osali, Rahimeh Mousavi
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Author / translator	Dr. Zonuoni. Sepideh