Bacterial genomics DNA isolation using CTAB

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Summary
This scaled up CTAB method can be used to extract large quantities of large molecular weight DNA from bacteria and other microbes.

Materials & Reagents

<table>
<thead>
<tr>
<th>Materials/Reagents/Equipment</th>
<th>Vendor</th>
<th>Stock number</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Disposables</strong></td>
<td></td>
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<tr>
<td>1.5-mL microcentrifuge tube</td>
<td>Eppendorf</td>
<td>22 36 320-4</td>
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<tr>
<td>50-mL Nalgene Oak Ridge polypropylene tube</td>
<td>VWR</td>
<td>21010-568</td>
</tr>
<tr>
<td>10-mL pipette</td>
<td>Falcon</td>
<td>357551</td>
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<tr>
<td>1-mL pipette tips</td>
<td>MBP</td>
<td>3781</td>
</tr>
</tbody>
</table>

| **Reagents**                                 |        |              |
| CTAB (*see preparation notes at end)         | Sigma  | H-6269       |
| NaCl                                         | Sigma  | S-3014       |
| TE buffer (10mM Tris; 1 mM EDTA, pH 8.0)     | Ambion | 9858         |
| Lysozyme                                     | Sigma  | L-6876       |
| Proteinase K                                 | QIagen | 19131        |
| 5M NaCl                                      | Ambion | 9759         |
| 10% SDS                                      | Sigma  | L-4522       |
| Chloroform                                   | Sigma  | C-2432       |
| Isoamyl alcohol                              | Sigma  | I-9392       |
| Phenol                                       | Sigma  | P-4557       |
| Isopropanol                                  | VWR    | PX-1835-14   |
| Ethanol                                      | AAPER  | ------------ |
| DNase-free RNase I (100 mg/mL)               | Epicentre | N6901K    |
| Molecular biology grade DNase-free water     |        |              |

| **Equipment**                                |        |              |
| Hot Plate                                    |        |              |
| 250 mL glass beaker                         |        |              |
| Magnetic stir rod                           |        |              |
| Thermometer                                  |        |              |

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Automatic pipette dispenser
Sorval 500 Plus centrifuge (DuPont, Newtown, CT)
65°C water bath
37°C incubator/heat block
56°C heat block

Procedure
Cell preparation and extraction techniques.
(Modification of “CTAB method”, in Current Protocols in Molecular Biology)

Cell growth:
To minimize gDNA sampling bias (e.g., excess coverage of sequences around the origin of replication) please take precautions NOT to proceed with DNA isolation while most of the cell population is in the stage of active DNA replication. We recommend collaborators to check the cell growth prior to DNA isolation. DNA should be prepared from cell culture that is either in late log phase or early stationary phase. If the cells are in the early log phase, the culture should be placed on ice or 4°C to slow down the growth and allow DNA replication to complete prior to cell lysis and DNA isolation.

If at all possible, please produce more DNA from a single isolation event than is strictly required for library creation and freeze aliquots of the extra DNA. Then, should more DNA be required for finishing it will be available. If extra cells are available instead, please consider storing extra aliquots in 15-40% glycerol at -80°C.

1. Grow cells (see above) in broth and pellet at 10,000 rpm for 5 min or scrape from plate.
2. Transfer bacterial suspension to the appropriate centrifuge tube.
3. Spin down cells in microfuge or centrifuge at 10,000 rpm for 5 minute.
4. Discard the supernatant.
5. Resuspend cells in TE.
6. Adjust to OD$^{600}$ = 1.0 with TE buffer (10mM Tris; 1 mM EDTA, pH 8.0)
7. Transfer given amount of cell suspension to a clean centrifuge tube. -------
8. Add lysozyme (conc. 100mg/ml). Mix well. -------------------------------
   This step is necessary for hard to lyse gram (+) and some gram (-) bacteria.
9. Incubate for 30 min. at 37°C.
10. Add 10% SDS. Mix well. -----------------------------------------------
11. Add Proteinase K (10mg/ml). Mix well. -------------------------------
12. Incubate for 1-3 hr at 56°C. If cells are not lysed (as seen by cleared solution with increased viscosity) incubation can proceed overnight (16 hrs).
13. Add 5 M NaCl. Mix well. -----------------------------------------------

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15. Incubate at 65°C for 10 min.


17. Spin at max speed for 10 min at room temperature.

18. Transfer aqueous phase to clean microcentrifuge tube (should not be viscous).


20. Spin at max speed for 10 min at room temperature.

21. Transfer aqueous phase to clean microcentrifuge tube.


23. Spin at max speed for 10 min at room temperature.

24. Transfer aqueous phase and add 0.6 vol isopropanol (-20°C).

25. Incubate at -20°C for 2 hrs to overnight.

26. Spin at max speed for 15 min at 4°C.

27. Wash pellet with cold 70% ethanol (directly from -20°C freezer), spin at max speed for 5 min.

28. Discard the supernatant and let pellet dry at room temp. This may take some time (20 min. to several hours, depending on humidity).


1.1 Set up the following reaction in a 1.5ml microcentrifuge tube (multiple reactions can be done in different tubes):

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA (in H₂O)</td>
<td>170µl</td>
</tr>
<tr>
<td>10X RNase I buffer</td>
<td>20µl</td>
</tr>
<tr>
<td>RNase I</td>
<td>10µl</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>200ul</td>
</tr>
</tbody>
</table>

1.2 Mix & Spin down.

1.3 Incubate tube at 37°C for 1 hr.

**Checkpoint:** Check a small aliquot (5µl) on an agarose gel with a no treatment control. Run gel 10-15 min. If there is only a trace of RNA, proceed with next step, heat inactivation. If a large amount of RNA is still present, add another 10µl of RNase I and repeat the incubation.

1.4 Heat inactivate enzyme at 70°C for 15 min.

1.5 Place tube on ice to cool.

2. Ethanol Precipitation

2.1 Add 1/10 volume of 3M Sodium Acetate to your sample.

2.2 Add 2.5 volumes of 100% ethanol.

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2.3 Mix and spin down sample.
2.4 Place at -80°C for 30 min (-20°C 2 hrs to overnight).
2.5 Spin sample at 4°C for 20 min to pellet DNA.
2.6 Carefully, pour off supernatant.
2.7 Wash pellet with 70% ethanol (cold).
2.8 Spin sample at 4°C for 3-5 min.
2.9 Pull off all ethanol with pipet tip.
2.10 Air dry pellet (or vacuum dry for 5-15 min using no heat).
2.11 Resuspend pellet with 100 µl of TE.
2.12 If multiple reactions, combine them.
2.13 Run 1 µl in a 1% agarose gel to check quality.
2.14 Store DNA @ -80°C or -20°C.

Measure DNA concentration with fluorometer dsDNA assay (Qubit or equivalent) or UV absorption (Nanodrop). The 260/280 ratio should be approximately 1.8. The 260/230 ratio should be 1.8 – 2.2 for pure DNA. Note that residual phenol absorbs strongly at 270 nm and will inflate the apparent DNA concentration. If using Nanodrop check whether the peak (which should be at about 258 nm) is shifted toward 270 nm. Note that the JGI requires submission of a Qubit/fluorometric measurement. Nanodrop readings are not acceptable QC measurements for the JGI.

Notes and precautions.

-In step 1, do not use too many bacterial cells (an OD$_{600}$ of not more than 1.2 is recommended), or DNA does not separate well from the protein.

-Most of the time, inverting several times is sufficient to mix well. Shaking too hard will shear the DNA.

-Use any protocol for DNA precipitation, the one in this protocol works well.

Reagent/Stock Preparation

CTAB/NaCl (hexadecyltrimethyl ammonium bromide)

Dissolve 4.1 g NaCl in 80 ml of water and slowly add 10 g CTAB while heating (~65°C) and stirring. This takes more than 3 hrs to dissolve CTAB. Adjust final volume to 100 ml and sterilize by filter or autoclave.

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