Bacterial genomic DNA isolation using CTAB

Version Number:	3
Start Production Date:	8-25-04
Stop Production Date:	(current)
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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

Materials/Reagents/Equipment	Vendor	Stock number
Disposables		
1.5-mL microcentrifuge tube	Eppendorf	22 36 320-4
50-mL Nalgene Oak Ridge polypropylene centrifuge tube	VWR	21010-568
10-mL pipette	Falcon	357551
1-mL pipette tips	MBP	3781
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		

Last Updated 10/23/12

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.

		1.5ml	**30ml**	**60ml**
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 $OD^{600} \cong 1.0$ with TE buffer (10mM Tris; 1 mM EDTA, pH 8.0)			
7.	Transfer given amount of cell suspension to a clean centrifuge tube. -------	740µl	14.8ml	29.6ml
8.	Add lysozyme (conc. 100mg/ml). Mix well. ----------	20µl	400µl	800µl
	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. ----------	40µl	800µl	1.6ml
11.	Add Proteinase K (10mg/ml). Mix well. ----------	8µl	160µl	320µl
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. ----------	100µl	2ml	4ml

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14. Add CTAB/NaCl (heated to 65°C). Mix well. ---------- 100µl 2ml 4ml
15. Incubate at 65°C for 10 min.
16. Add chloroform:isoamyl alcohol (24:1). Mix well. ---------- 0.5ml 10ml 20ml
17. Spin at max speed for 10 min at room temperature.
18. Transfer aqueous phase to clean microcentrifuge tube (should not be viscous).
19. Add phenol:chloroform:isoamyl alcohol (25:24:1). Mix well. ---------- 0.5ml 10ml 20ml
20. Spin at max speed for 10 min at room temperature.
21. Transfer aqueous phase to clean microcentrifuge tube.
22. Add chloroform:isoamyl alcohol (24:1). Mix well. ---------- 0.5ml 10ml 20ml
23. Spin at max speed for 10 min at room temperature.
24. Transfer aqueous phase and add 0.6 vol isopropanol (-20°C).
    (e.g. if 400 µl of aqueous phase is transferred, add 240 µl of isopropanol. ---- Add 0.6 vol. ----
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).
29. Resuspend in ~170 µl of DNase-free water. Proceed to RNAse treatment.

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

Note: RNase I @ 10U/µl, one unit digests 100 ng of RNA per second

DNA (in H_20)	170µl
10X RNase I buffer	20µl
RNase I	10µl
	200ul

1.2 Mix & Spin down.

1.3 Incubate tube at 37°C for 1 hr.

Checkpoint: Check a small aliquot (5ul) 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.

Last Updated 11/12/2012