

Genomic DNA extraction with CTAB (Cetyl trimethylammonium bromide)

Notes: This protocol can be used on most gram-negative bacteria to obtain high-quality genomic DNA, which is both pure and very high molecular weight. The method needs to be modified for use on Gram-positives or yeast etc, by adding on extra lysis treatments at the 'front end' of the protocol. Remember that 'genomic DNA' means 'all the DNA', so this method extracts both chromosomes and plasmids. Note also that all waste chloroform and phenol arising from this procedure must be discarded as hazardous chemical waste (not down the sink!).

Hazards: CTAB* is a strong detergent, it will damage eyes and irritate skin
Chloroform is toxic by inhalation and will also damage eyes.
Phenol is toxic and corrosive to skin and eyes.

Protocol:

1. Grow culture in 5 ml broth, pellet cells (~3000 g, 10 min), discard supernatant.
2. Add 400 µl TE and 100 µl NaCl (5 M) to pellet, resuspend cells by vortexing.
<<put on safety glasses and gloves>>
3. Add 50 µl CTAB, vortex, incubate 60°C for 20 min, occasionally mixing by inversion of tube.
4. Add 500 µl chloroform, vortex and mix thoroughly, incubate on ice 30 min.
5. Spin at ~10,000 g in microfuge (cold if possible) for 10 min.
6. Slowly pipette out and retain supernatant – avoid interface material and lower phase.
7. Add 500 µl phenol:chloroform, vortex until milky solution obtained (~20 sec), spin at top speed in microfuge for 5 min, retain supernatant.
8. Add 500 µl chloroform, vortex, spin 5 min, retain supernatant.
9. Discard all waste phenol and chloroform into chemical waste bottle in fume hood.
<<take off safety glasses and gloves>>
10. Add 1/10 volume Na-acetate (50 µl) and 2 vol. ice-cold ethanol (1000 µl) to precipitate DNA – incubate at -20°C for at least 1 hour.
11. Spin at top speed in microfuge (cold if possible) 10 min, drain off ethanol, retain pellet.
12. Add 500 µl 70% ethanol, resuspend pellet by flicking, allow to sit for ~5 min at room temp, then spin and drain again.
13. Invert tube on paper towel with lid open for ~5 min to drain last bits of 70% ethanol, then transfer to 60°C incubator (OK if tube is lying on its side, but lid open) for 10 min to dry residual ethanol.
14. Redissolve pellet in 100 µl EB. Heating at 50-60°C (up to an hour, intermittent mixing) and/or addition of more EB may be required to dissolve all the DNA.
15. Store final DNA preparation at -20°C. If you will be using it a lot, consider making multiple smaller aliquots, since repeated freeze/thawing will deteriorate the quality.

CTAB GENOMIC DNA PREP SOLUTIONS

TE: 10 mM Tris-HCl, 1 mM EDTA. pH 8.0 Autoclaved.

NaCl: 5 M NaCl. Autoclaved.

CTAB: 10% w/v CTAB. Autoclaved. May need heating (~50°C) to dissolve before each use.

Chloroform: Mixture of chloroform and isoamyl alcohol (24:1 ratio) equilibrated with a layer of TE buffer (pH 8) on top. Not sterilized.

Phenol:chloroform: Mixture of phenol, chloroform, isoamyl alcohol (25:24:1 ratio) equilibrated with a layer of TE buffer (pH 8) on top. Not sterilized.

Na-acetate: 3M sodium acetate in RO water, sterilise by autoclaving (don't need to adjust pH)

EB (Elution buffer): 5 mM Tris-HCl. pH 8.0 Autoclaved.

CTAB GENOMIC DNA PREP – further notes and comments

1. Plate-grown cells are fine also – growth scraped from half of a streak-plate is about the same amount of biomass as a fully-grown 5 ml broth. The procedure can also be easily scaled up tenfold to accommodate 50 ml broths. In this case, it is recommended to add an isopropanol precipitation step after the first chloroform extraction, then do a 70% ethanol rinse, dry pellet, redissolve in 1-2 ml TE. Then the amount of phenol and chloroform used is greatly reduced (you can use 500 µl instead of 5 ml !), and the procedures can be done in microfuge rather than big centrifuge (faster!).
2. Wash cell pellet in TE (resuspend then pellet again) before lysis if nuclease activity is a problem.
3. A lysozyme incubation can be added if cells don't lyse well with CTAB alone. In this case, after adding TE and NaCl to cell pellet, add lysozyme to 1 mg/ml, and incubate 1 hour at 37°C.
4. A proteinase K step could be added at the same point as the lysozyme step, or immediately after the lysozyme step (add prot. K to 0.5 mg/ml, incubate 60 min @ 37°C)
5. Increasing the heat and duration of the CTAB step might help with tough cells (eg. 70°C, 1 hour).
6. Vortexing steps can be replaced or supplemented by inversion and flicking of tube.
7. RNase A can be added to remove RNA – either add this at the start of the prep, or to the dissolution buffer used at the end of the prep. Remember to boil RNase before use to kill any DNase in it (see Sambrook for method).
8. Overdrying of the final DNA pellet is BAD. Its better to leave a trace of ethanol and water in the pellet (and have it dissolve easily), than to remove all moisture, and then have great difficulty dissolving the pellet. However, too much ethanol is also BAD...DNA will float out of wells on gel.
9. Elution buffer (EB) is just Tris-HCl, which gives the DNA some protection due to its pH buffering. EB wont give as much protection as TE, which also protects against nucleases. However, TE can interfere with subsequent enzyme reactions (EB won't)
10. We have used this procedure successfully with *Pseudomonas stutzeri* strains which did not give good DNA preps with SDS-based DNA extraction methods due to polysaccharide production. The CTAB procedure would likely work with many gram-negative strains without modification, but gram positives would likely require the addition of lysozyme and proteinase K steps, and may also require modification of medium – eg. addition of glycine and/or ampicillin to weaken cells.