Environmental Biochemistry

DNA extraction 101 - v.1

A small collection of DNA extraction protocols and other notes

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DNA Extraction Protocol, Chloroform - type: "NZ"

modified after Taylor et al. (2004)

1. use 1.5 ml crytubes, add tissue/sponge material
2. add 1 ml extraction buffer
3. fill 200 μl PCR tube with sterile zirconium beads and add to the cryotube[1]
4. add 0.0015 g of PVPP (1 PCR tube cap-full)[2]
5. add 300 μl chloroform:isoamyl alcohol[3]
6. spin for 30 seconds in the Fastprep machine @ 5.5
7. spin for 30 seconds @ 14000 rpm
8. transfer supernatant to 1.5 ml Eppendorf tube (~1 ml worth)
9. spin for 30 minutes @ 14000 rpm
10. transfer supernatant to 2 ml tube (don't get any solids)
11. add 100 μl 3M sodium acetate and 600 μl isopropanol & shake
12. leave overnight @ -20°C, or for 1 hour at room temperature
13. spin for 30 minutes @ 14000 rpm (4°C)
14. remove and discard supernatant
15. add 1 ml or 70% ethanol, briefly mix
16. spin for 10 minutes @ 14000 rpm (4°C)
17. remove and discard supernatant
18. add 1 ml 70% ethanol, briefly mix
19. spin for 10 minutes @ 14000 rpm (4°C)
20. remove and discard supernatant
21. speedvac for 15 minutes at medium drying temperature & keep the tube lids open and facing towards
22. dissolve DNA in 30 μl dH_2O and run gel to check[4]

Notes:

1. always use a screw cap microcentrifuge tube for tissue homogenization, any other tube with a common lid (even safe-locks) can cause a serious phenol-chloroform contamination in your lab at this step
2. during DNA extraction polyphenol oxidation products can covalently bind to nucleic acids, which would make it impossible to remove them - common methods to prevent this reaction involves a broad use of different reagents: a) polymers (polyvinylpyrrolidone (PVP) & polyvinylpolypyrrolidone (PVPP) - byding up the polyphenols and preventing them from reacting with the DNA; b) antioxidants (bME, Ascorbic acid, & DTT) - denaturing and suppressing the activity of the polyphenol oxidases enzymes, and slowing down their breakdown of polyphenols; c) detergents (CTAB & SDS) - solubilizing lipids and enzymes that complex with DNA and make them easier to remove (see http://www.mobio.com/blog/2012/08/30/get-to-the-root-of-plant-dna-and-rna-isolation/)
3. you can also use Phenol:Chloroform:Isoamyl alcohol
4. any reasonable volume (~10 to 100 μl) of dH_2O or TE buffer can be used - the more volume you use, the lower the DNA concentration is in your final DNA extract - personally I prefer 1x TE buffer over dH_2O and go with 50 μl to 100 μl

Reference:

Taylor MW, Schupp PJ, Dahllöf I, Kjelleberg S & Steinberg PD (2004) Host specificity in marine sponge-associated bacteria, and potential implications for marine microbial diversity. *Environ. Microbiol.* 6: 121–130. DOI: 10.1046/j.1462-2920.2003.00545.x

DNA Extraction Protocol, Phenol-Chloroform - type: "ICBM"

1. transfer bacterial colonies to 500 µl PCR water / dH_2O[1]
2. spin for 5 minutes @ 13000 rpm and remove supernatant
3. add 0.25 g sterile zirconium beads, 500 µl phosphate buffer, 500 µl phenol:chloroform:isoamyl alcohol (25:24:1) (PCI) & 60 µl SDS 10%[2]
4. vortex for 5 minutes[3]
5. heat block or water bath for 10 minutes @ 60°C
6. spin for 6 minutes @ 10000 rpm (4°C)
7. transfer supernatant into a 1.5 ml clean tube
8. add 500 µl PCI
9. vortex for 2 minutes
10. spin for 6 minutes @ 10000 rpm (4°C)
11. transfer supernatant into a clean 1.5 ml tube
12. repeat the washing steps (from 7 to 10) until the interphase is free of any visible contamination, then stop after step 10 and goto step 13
13. transfer the supernatant to a 2 ml tube
14. add 30 µl sodium acetate (3M, pH 5.2), ~1 ml Isopropanol (-20°C) -> 2-2.5 volume[4]
15. leave @ -20°C for 3 hours min. or @ -80°C for 1 hour
16. spin for 30 minutes @ 13000 rpm (4°C)
17. remove supernatant and add 1 ml 80% ethanol (-20°C)
18. wait for 10 minutes (4°C to -4°C)
19. vortex briefly
20. spin for 15 minutes @ 13000 rpm (4°C)
21. remove supernatant and leave DNA pellet under a clean bench to allow the ethanol to vaporize[5]
22. dissolve DNA in 100 µl of PCR water[6]

1. instead of liquid colonies you can also use any kind of tissue, but you don't have to spin down its content @ step 2
2. see comment 2, page 2
3. if you extract DNA from tissue samples and not from liquid bacterial colonies, you can/should use a homogenizer instead of a vortex
4. this step is a bit contradicting and I'm not sure if this is totally correct - literature says that one should use 0.7 volume of Isopropanol or 2.5 volume of Ethanol. Hence, I assume that this protocol is based on Ethanol precipitation and not Isopropanol but someone changed it at some time - furthermore, Isopropanol precipitation at cold temperatures (e.g., -20°C to -80°C) increases the amount of salt in your final DNA extract. Therefore, incubate with Isopropanol at room temperature for a short time or chill the DNA with Ethanol overnight (as a rule of thumb) - (for further details see: http://bitesizebio.com/2839/dna-precipitation-ethanol-vs-isopropanol && Johannes Regenburg "Herstellung und Analyse normalisierter cDNA-Banken", Herbert Utz Verlag Wissenschaft, ISBN 3-89675-241-3, page 31)
5. I also assume that this protocol is used by a lab with no speedvac at hand - hence, if you have a speedvac in your lab, don't hesitate to use this instead of a clean bench ;)
6. see comment 4 page 2

Nanodrop - notes on nucleic acid purity

Table 1 Main absorbing compounds

Wavelength / nm	Main Absorbing Compound
230	Organic or carbohydrate contaminants
260	DNA and RNA
270	Phenol
280	Proteins / Enzymes

Table 2 260/280 ratio

260/280 Ratio	Sample Consistency
1.3	< 50 % contaminants
1.5	50 % nucleic acid & 50 % contaminants
1.8	pure DNA
2	pure RNA / Phenol contamination

Table 3 260/230 ratio

260/230 Ratio	Sample Consistency
< 2.0	Carbohydrat, Phenol, Guanidine, Glycogen contaminants
2.0 - 2.2	pure nucleic acid
> 2.2	bad blank or wrong blank solution

References

http://www.nanodrop.com/Library/T042-NanoDrop-Spectrophotometers-Nucleic-Acid-Purity-Ratios.pdf
http://www.nanodrop.com/Library/T009-NanoDrop 1000-&-NanoDrop 8000-Nucleic-Acid-Purity-Ratios.pdf