

## Rapid Extraction of PCR-Competent DNA from Recalcitrant Environmental Samples

Michael R. Gillings

### Abstract

Advances in sequencing technologies have made the investigation of microbial ecology and community dynamics more tractable. The critical first step in such analyses is the efficient and representative recovery of PCR-competent DNA from complex environmental samples. All extraction protocols contain inherent biases, meaning that choice of method involves compromise between various factors, including efficiency, yield, universality, and representative extraction. Here, details are given for a routine method used in our laboratory to extract DNA from soils, sediments, biofilms, roots, and fungi.

**Key words** Microbial diversity, PCR, Microbial ecology, Soil, Sediment, Biofilm

---

### 1 Introduction

Our understanding of microbial ecology and diversity has rapidly expanded since the introduction of molecular methods for studying microbial communities [1], and the pace at which our knowledge accumulates will accelerate with the widespread use of next-generation sequencing technologies [2]. Because the majority of microorganisms are yet to be cultured [1], investigations must rely on direct analysis of environmental samples. A typical first step is the direct extraction of total DNA from environmental samples, which often contain diverse phyla and interfering substances in a complex matrix. The problems of representative extraction of DNA from complex substrates such as soil have been the subject of many studies.

Effective methods for DNA extraction must generate good yields of DNA, they must be unbiased in terms of recovering DNA from the diverse species that are present, and they must generate DNA that is suitable for downstream applications [3–5]. Many different methods for extraction of DNA from environmental samples have been published, but it appears that there is no one method that is free from bias [6–10]. Consequently, researchers can opt for

a specialized method tailored to their particular environment or can make a pragmatic choice based on a method's wide applicability to a number of systems.

Of available methods, those involving physical disruption by bead beating appear to have the broadest applicability and are readily available in kit form. Since the first description of this approach [11], various studies have shown that bead beating generates good yields of DNA [12] and that it retrieves DNA from a wide diversity of organisms, such that it is useful for comparative studies [13–15]. Bead beating can be optimized for particular applications [16] and is the basis of the lysis procedure used in the international standard soil extraction method ISO 11063 [17].

Here an outline is given for one rapid and adaptable DNA extraction method. It is based on a commercial kit (FastDNA, MP Biomedicals) that employs lysis with bead beating and DNA purification by absorption to silica. The protocol was originally described by Borneman et al. [11] and has been modified to speed up the extraction process and reduce reliance on proprietary reagents [18]. This method is rapid, generates DNA suitable for PCR analysis, and avoids the use of hazardous reagents. It is also adaptable and is able to extract DNA from a wide range of environmental samples including soils with different clay contents and soils polluted with heavy metals and aromatics [18]. The method has been used to extract DNA from soils for analysis of fungal diversity [19] and to extract DNA from fungal cultures [20], lichens, and mushrooms. It also works effectively on marine and freshwater biofilms [21–23] and on diverse sediments, charcoal filters and fecal samples [24, 25]. With minor modifications it can be used to extract DNA from plant roots and their associated microbiota and from bacterial spores and gram-positive organisms that are otherwise difficult to lyse [26].

---

## 2 Materials

Solutions are made with distilled water that has been 0.22  $\mu\text{M}$  filtered and then autoclaved. All user-prepared solutions are autoclaved again prior to addition of ethanol or SDS as required. All plasticware is also sterilized by autoclaving.

1. Environmental sample (*see Note 1*).
2. Lysing matrix E tubes (MP Biomedicals).
3. Balance.
4. FastPrep bead-beating machine (MP Biomedicals).
5. Sodium phosphate buffer (100 mM, pH 7.0).
6. MT buffer (MP Biomedicals proprietary reagent) (1 % sodium dodecyl sulfate, 1 % polyvinylpyrrolidone 40, EDTA plus proprietary inorganic salts) (*see Note 2*).

7. CLS-VF buffer (MP Biomedicals proprietary reagent) (sodium dodecyl sulfate, polyvinylpyrrolidone 40, Teepol 610S, and EDTA) (*see Note 2*).
8. CLS-TC buffer (MP Biomedicals proprietary reagent) (urea, sodium phosphate, SDS, and dithiothreitol) (*see Note 2*).
9. Microcentrifuge.
10. Protein precipitation solution (3 M potassium acetate, 4 % glacial acetic acid).
11. Vortex machine.
12. Binding matrix (MP Biomedicals glassmilk). Can be diluted 1:5 (v/v) with 6 M guanidine isothiocyanate. Store at 25 °C or above (*see Note 3*).
13. Wash buffer (100 mM sodium acetate, 70 % v/v ethanol).
14. Rotator wheel (optional).
15. TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA) (*see Note 4*).

---

### 3 Methods

#### 3.1 DNA Extraction

Samples are homogenized by bead beating, proteins and polysaccharides selectively precipitated, and DNA purified by absorption onto glassmilk.

##### 3.1.1 DNA from Soils, Sediments, Biofilms, and Fungi

1. Weigh 200–400 mg of environmental sample (soil or sediment) into a lysing matrix E tube. For biofilms, fecal material, lichens, mushrooms, or fungal hyphae, a sample of 100–200 mg should be used. In the case of diffuse biofilms or planktonic cells, these can be collected by centrifugation and resuspended in 780  $\mu$ l of phosphate buffer before transfer into the lysing matrix tube.
2. Add 780  $\mu$ l of phosphate buffer and 122  $\mu$ l of MT buffer.
3. Tighten the screw-cap on the tube, making sure that no sample is trapped between the rim and the internal O-ring.
4. Load tubes into the FastPrep machine and process for 30 s at 5.5 m/s. If some material still looks unhomogenized, process for a further 30 s after waiting 1 min for the tubes to cool down. If the original samples were dry, leave the tubes to incubate at room temperature for 15 min to 2 h before proceeding to centrifugation. This improves extraction efficiency.
5. Centrifuge for 5 min at 14,000  $\times g$  to pellet beads and soil debris.
6. Recover 600  $\mu$ l of supernatant into a fresh 1.5 ml tube (*see Note 5*).
7. Add 150  $\mu$ l protein precipitation solution and gently vortex to mix. Stand at room temperature for a few minutes to allow the precipitate to form.

8. Centrifuge for 5 min at  $14,000\times g$  to pellet proteins and polysaccharides.
9. Recover 700  $\mu\text{l}$  of the supernatant into a fresh 1.5 ml tube (*see* **Note 5**).
10. Add 700  $\mu\text{l}$  of binding matrix and mix thoroughly with the supernatant. Tubes may be placed on a rotator for 5 min to increase the efficiency of DNA binding.
11. Pulse spin the tubes to pellet the glassmilk, now containing the bound DNA.
12. Decant the supernatant, and gently resuspend the pelleted glassmilk in 800  $\mu\text{l}$  of wash buffer. Tubes may be placed on a rotator for 5 min to increase the efficiency of washing.
13. Pulse spin the tubes to pellet the washed glassmilk, and decant the used wash buffer. If the wash buffer is brown or colored, **step 12** may be repeated.
14. Place the tubes back in the centrifuge and pulse spin to collect any remaining supernatant in the bottom of the tube.
15. Carefully remove the remaining supernatant with a micropipette.
16. Air-dry the pellet for a few minutes.
17. Resuspend the glassmilk pellet in 200  $\mu\text{l}$  of TE buffer. Ensure that the pellet is fully resuspended, and allow the TE to elute the DNA at room temperature for 5 min. Elution can be improved by incubation at  $50^\circ\text{C}$  in a water bath or heat block.
18. Centrifuge for 2 min at  $14,000\times g$ .
19. Recover 160  $\mu\text{l}$  of the supernatant, which now contains the environmental DNA.
20. Transfer to a fresh, labeled tube, and store at  $-20^\circ\text{C}$  until use.

This method works well for soil samples, marine and freshwater sediment samples, biofilms, and feces. It can also be used for extracting DNA from pure fungal cultures grown over cellophane on agar plates or from mushrooms, toadstools, and lichens.

### 3.1.2 DNA from Plant Roots and Leaves

For extracting DNA from leaves or plant roots (including plant symbionts) the following steps can be substituted for **steps 1–8** above:

1. Finely chop 200 mg of plant material with a sterile scalpel and add to a lysing matrix E tube.
2. Add 800  $\mu\text{l}$  of CLS-VF buffer and 200  $\mu\text{l}$  of protein precipitation solution.
3. Load tubes into the FastPrep machine and process for 30 s at 5.5 m/s. If some material still looks unhomogenized, process for a further 30 s after waiting 1 min for the tubes to cool down.
4. Centrifuge at  $14,000\times g$  for 5 min to pellet beads, cell debris, polysaccharides, and proteins.
5. Continue from **step 9** above.

### 3.1.3 DNA from Gram-Positive Organisms and Spores

For cultures of gram-positive organisms and spore formers, the following modification can be used.

1. Resuspend 40 mg of bacterial culture in 1,000  $\mu\text{l}$  of CLS-TC buffer and transfer to a lysing matrix tube. If crystals have formed in the CLS-TC, warm the solution in a water bath at approximately 50 °C to redissolve the urea.
2. Load tubes into the FastPrep machine and process for 40 s at 6.0 m/s.
3. Centrifuge at 14,000 $\times g$  for 5 min to pellet beads, cell debris, polysaccharides, and proteins.
4. Continue from **step 9** above.

## 3.2 Quality Control

The yield and purity of DNA can be qualitatively tested by agarose electrophoresis and by performing PCR directed at universal target genes.

1. Load an aliquot (10  $\mu\text{l}$ ) of the DNA prepared in Subheading [3.1](#) onto a 1 % w/v agarose gel, and subject it to electrophoresis. Stain and photograph the gel according to standard procedures.
2. Examine the molecular weight of the extracted DNA in comparison with a molecular weight marker or DNA ladder. Extracted DNA should be larger than 10 kb and should be visible as a coherent band without smearing into low-molecular-weight regions. No RNA should be present in the extraction, but if it is, RNase A digestion can be performed concurrently with the PCR by adding 1  $\mu\text{l}$  of a 1 mg/ml pre-boiled RNase A solution to each reaction.
3. Estimate the amount of DNA present by comparison to known amounts present in the molecular weight ladder. It is often not possible to obtain accurate spectrophotometer readings from environmental DNA because of interfering substances.
4. Use 1  $\mu\text{l}$  of the DNA and 1  $\mu\text{l}$  of a 1:10 dilution in TE as templates for PCR. The test PCR should be directed at a high-copy-number DNA target that is known to be present in the original environmental sample. The 16S ribosomal RNA genes are a suitable target for bacterial DNA, and any set of universal 16S rDNA primers can be used in this test [[18](#)]. For eukaryotic targets, the internal transcribed spacer region (ITS) of the ribosomal RNA genes is also a good target for universal primers.
5. Run out the PCR on a 2 % (w/v) agarose gel according to standard methods. Most extractions will generate amplicons from both neat and diluted DNA; however, if PCR inhibitors are present, amplicons may only be generated from the 1:10 dilution. If no amplification is generated despite the presence of visible DNA, then more washing of the binding matrix (**step 12**) may be warranted. Otherwise further dilution or

cleanup of the DNA using ethanol precipitation may be needed. It is unusual for DNAs prepared using the technique in Subheading 3.1 to not be PCR competent.

---

## 4 Notes

1. Sampling strategies for soils, sediments, and biofilms should be carefully planned and tailored towards the hypothesis being tested. Consideration should be given to both small- and large-scale spatial variation as well as temporal variation and appropriate replicates taken. Ideally, samples should be processed with minimal delay to maintain the microbial assemblages present in the field. Storage of temperate samples at 4 °C is recommended to preserve communities prior to extraction.
2. While most reagents can be prepared by the user, the exact formulation of MT, CLS-VF, and buffers are not known, and these are best purchased from the supplier.
3. Because DNA yields from environmental samples are usually low, they fall far below the binding capacity of the glassmilk, which can be diluted without appreciable loss of yield. Warm the solution to redissolve any crystals that might form, and resuspend the glassmilk immediately before use.
4. We store TE buffer and sterile PCR water as 1 ml aliquots frozen at -20 °C. After thawing for use, any unused portions are disposed of. This procedure helps to manage potential contamination.
5. The volumes of supernatant that are harvested can be increased, but the relative proportions of the solutions added in the next step must be also proportionally increased.

## References

1. Hugenholtz P, Goebel BM, Pace NR (1998) Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *J Bacteriol* 180:4765–4774
2. Sogin ML et al (2006) Microbial diversity in the deep sea and the underexplored “rare biosphere”. *Proc Natl Acad Sci USA* 103:12115–12120
3. Frostegard A et al (1999) Quantification of bias related to the extraction of DNA directly from soils. *Appl Environ Microbiol* 65: 5409–5420
4. Krsek M, Wellington EMH (1999) Comparison of different methods for the isolation and purification of total community DNA from soil. *J Microbiol Methods* 39:1–16
5. Roose-Amsaleg CL, Garnier-Sillam E, Harry M (2001) Extraction and purification of microbial DNA from soil and sediment samples. *Appl Soil Ecol* 18:47–60
6. Feinstein LM, Sul WJ, Blackwood CB (2009) Assessment of bias associated with incomplete extraction of microbial DNA from soil. *Appl Environ Microbiol* 75:5428–5433
7. Inceoglu O et al (2010) Effect of DNA extraction method on the apparent microbial diversity of soil. *Appl Environ Microbiol* 76: 3378–3382
8. Martin-Laurent F et al (2001) DNA extraction from soils: old bias for new microbial diversity analysis methods. *Appl Environ Microbiol* 67:2354–2359

9. Robe P et al (2003) Extraction of DNA from soil. *Eur J Soil Biol* 39:183–190
10. Thakuria D et al (2008) Importance of DNA quality in comparative soil microbial community structure analyses. *Soil Biol Biochem* 40:1390–1403
11. Borneman J et al (1996) Molecular microbial diversity of an agricultural soil in Wisconsin. *Appl Environ Microbiol* 62:1935–1943
12. Lakay FM, Botha A, Prior BA (2007) Comparative analysis of environmental DNA extraction and purification methods from different humic acid-rich soils. *J Appl Microbiol* 102:265–273
13. Carrigg C et al (2007) DNA extraction method affects microbial community profiles from soils and sediment. *Appl Microbiol Biotechnol* 77:955–964
14. de Liphay JR et al (2004) Impact of DNA extraction method on bacterial community composition measured by denaturing gradient gel electrophoresis. *Soil Biol Biochem* 36:1607–1614
15. Luna GM, Dell’Anno A, Danovaro R (2006) DNA extraction procedure: a critical issue for bacterial diversity assessment in marine sediments. *Environ Microbiol* 8:308–320
16. Bürgmann H et al (2001) A strategy for optimizing quality and quantity of DNA extracted from soil. *J Microbiol Methods* 45:7–20
17. Petric I et al (2011) Inter-laboratory evaluation of the ISO standard 11063 “Soil quality—method to directly extract DNA from soil samples”. *J Microbiol Methods* 84:454–460
18. Yeates C, Gillings MR (1998) Rapid purification of DNA from soil for molecular biodiversity analysis. *Lett Appl Microbiol* 27:49–53
19. Green JL et al (2004) Spatial scaling of microbial eukaryote diversity. *Nature* 432:747–750
20. Stow A et al (2010) Differential antimicrobial activity in response to the entomopathogenic fungus *Cordyceps* in six Australian bee species. *Aust J Entomol* 49:145–149
21. Gillings MR, Holley MP, Selleck M (2006) Molecular identification of species comprising an unusual biofilm from a groundwater treatment plant. *Biofilms* 3:19–24
22. Holmes AJ et al (2001) Phylogenetic structure of unusual aquatic microbial formations in Nullarbor caves, Australia. *Environ Microbiol* 3:256–264
23. Wilson GS et al (2010) Heterogeneity of surface attached microbial communities from Sydney Harbour, Australia. *Marine Genomics* 3:99–105
24. Gillings M et al (2008) The evolution of class I integrons and the rise of antibiotic resistance. *J Bacteriol* 190:5095–5100
25. Gillings MR et al (2008) Recovery of diverse genes for class I integron-integrases from environmental DNA samples. *FEMS Microbiol Lett* 287:56–62
26. Young A et al (2006) Genetic uniformity among international isolates of *Leifsonia xyli* subsp. *xyli*, causal agent of ratoon stunting disease of sugarcane (*Saccharum* interspecific hybrids). *Aust Plant Pathol* 35:503–511

Environmental Microbiology

Methods and Protocols

Paulsen, I.; Holmes, A.J. (Eds.)

2014, X, 242 p. 35 illus., 19 illus. in color., Hardcover

ISBN: 978-1-62703-711-2

A product of Humana Press