

Overview of DNA Purification for Nucleic Acid-Based Diagnostics From Environmental and Clinical Samples

Knut Rudi and Kjetill S. Jakobsen

Summary

Direct deoxyribonucleic acid (DNA)-based detection methods are crucial for future environmental monitoring and clinical diagnosis. In this chapter, we provide an overview of the various sample preparation approaches for bacteria for direct analyses (i.e., without culturing) in environmental and clinical samples. The issues of sampling, sample preservation, separation of the microorganisms from the environmental or clinical matrix, and DNA purification are covered. This chapter will focus on the advantages and the disadvantages of the methods available.

Key Words: NA purification; environmental/clinical analyses; direct DNA diagnostics; culture independent; polymerase chain reaction; PCR.

1. Introduction

Despite the fact that the analytical limitation in many cases is the result of the sample preparation step (e.g., separation of the cells/organisms from the environmental matrix and subsequent DNA purification), the development of new strategies in the field of sample preparation has been relatively limited (**1**). Microorganisms in their natural habitat may be present in low copy-numbers and in an environment that can degrade or chemically modify the nucleic acids and/or inactivate the enzymes that are used for the downstream nucleic acid analyses (**2**). Most sample preparation methods for DNA analyses are designed for defined materials, such as tissues and cultures (**3**). The challenges with natural samples are not only that the target nucleic acids are in low concentrations but also that the natural samples can be extremely heterogeneous and, in many cases, impossible to define. Finally, when analyzing nucleic acids from the environment, the issue concerning the origin of the nucleic acids is an impor-

From: *Methods in Molecular Biology*, vol. 345: *Diagnostic Bacteriology Protocols*, Second Edition
Edited by: L. O'Connor © Humana Press Inc., Totowa, NJ

tant one. It is often vital to determine whether the nucleic acids are from living or dead organisms or whether contaminating organisms or nucleic acids have been introduced during processing of the samples.

The particular problems with obtaining DNA for direct analyses of microorganisms from environmental or clinical matrixes will be addressed, how these problems are currently being solved, and some possible future solutions. Also discussed will be the issues of sampling, sample preservation, separation of the microorganisms from the environmental or clinical matrix and DNA purification (*see Fig. 1*). The focus will be treatment after sampling because the sampling procedures will be highly dependent on the applications (environmental or clinical).

2. Sampling

Crucial sampling issues are to obtain representative samples and to keep the samples sufficiently intact for analysis in the laboratory. Normally, microorganisms are not distributed uniformly in environmental or clinical samples. Precautions have to be taken at the site of sampling to avoid modification and/or degradation of the nucleic acid in the sample. For practical reasons, the pretreatment of the sample in the field or clinic should be kept to a minimum. However, any enzymatic activity that could degrade DNA should be inactivated, in addition to the prevention of chemical inactivation and/or degradation of the DNA. The aim is to stabilize the DNA and/or microorganisms until it reaches the analytical laboratory for further treatment (4,5).

The most frequently used methods for pretreatment are either drying, freezing, preservation using alcohol, fixation in formaldehyde, or combinations of the these (6). Alcohol, such as isopropanol or ethanol, is in many cases preferable as a preservative. Alcohol is easy to use, relatively nontoxic, kills most organisms, and in it DNA is stable. Using alcohol as a preservative also may reduce the risk of accidents with clinically infectious material. Drying of the samples may be an alternative for simple sample pretreatment. The problem with drying is that the sample is not immediately preserved. DNA may be damaged or chemically modified by enzymes or chemicals while water is still present. Furthermore, microorganisms may grow during the preservation phase. However, dried samples are relatively inert and can be stored for prolonged periods (7). An approach in which the sample is squeezed onto a special paper (FTA paper) and then dried also has been applied as a successful sample preparation method (8). Snap freezing in liquid nitrogen is probably the best way to preserve a sample (9). The advantage is also in the ability to grind the material while it still is frozen to ease the downstream DNA purification (9). However, it may not be practical to preserve the sample with liquid nitrogen freezing. Conservation with liquid nitrogen freezing requires that the sampling

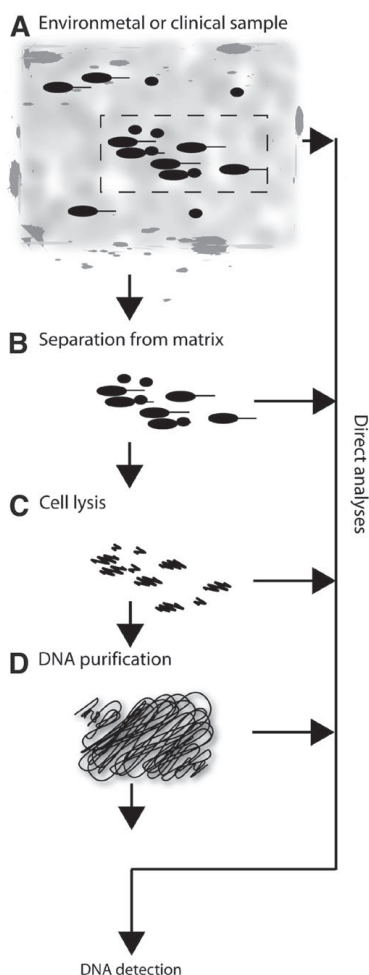


Fig. 1. Schematic representation of the process of analyzing environmental samples. The environmental sample could have a heterogeneous composition. It is important to obtain a representative sample (A) in the analysis of microbial communities. The bacteria are separated from the matrix (B) after the sampling. Then, the microorganisms are disrupted, and the DNA are released (C). Finally, the DNA is purified (D) and is ready for downstream applications such as PCR. Steps B and D can be omitted in special cases and the DNA detected directly.

site is close to the laboratory; in addition, the sample treatment is quite extensive. When immediate preservation and stability are important issues, liquid nitrogen could be an alternative. Unfortunately, formaldehyde fixation has been a common way for sample preparation. Unbuffered acidic formaldehyde

nearly immediately destroys DNA (*10*), whereas buffered formaldehyde does not inactivate DNA that rapidly. However, nucleic acids are not stable over a period of time in formaldehyde (*11*). As opposed to formaldehyde, the iodine containing microscope fixation solution Lugol does not interfere with DNA. Lugol has been used for preservation of environmental samples that have been successfully applied for DNA analyses (*12,13*).

3. Separation of Bacteria From Matrix

Normally, the process of separating the cells from the environmental or clinical matrix is conducted in a laboratory. This step is important, both because major enzymatic inhibitors can be located in the matrix (*14*) and because of the loss of sensitivity and specificity if the DNA is isolated directly from the matrix. The sensitivity issue is of particular importance in monitoring or diagnosis of harmful or pathogenic bacteria. Microorganisms may form biofilms that are tightly attached to a surface. Critical steps are the separation of the organisms from the matrix. For soil samples, the separation of the microorganisms from the matrix can be a particular problem. The microbial cells may be tightly associated with the soil matrix, as is the case for clay particles, where the microorganisms may be bound to the particles through ionic interaction (*15*). Most of the methods for sample preparation from soil are thus based on direct lysis approaches (*16*). Recently, there has been an increased focus on microorganisms in air. This focus is both related to the possibility of biological warfare and the recognition of airborne transmission of pathogens (*17*). Generally, sampling from air is performed either by filtration or centrifugation. The cells are then transferred to a liquid phase before further treatment (*18*).

Immunocapture is a common strategy for the separation of target cells/organisms from a matrix (*19*). Approaches based on paramagnetic beads are the most widely applied. The paramagnetic beads are mixed with the matrix and, after complex formation between the beads and the target microorganisms, these cells can be purified through the application of a magnetic force.

Microorganisms in water and other hydrophilic liquids have been isolated and/or concentrated through unspecific adsorption onto polymer beads by lowering the water activity by the addition of alcohol and salt. This assay has been successfully applied in the analyses of cyanobacterial communities in water (*20*). A physical separation based on general binding properties or common affinities among whole groups of microorganisms also may be used (*21*). Such unspecific adsorption methods involve coating surfaces with lecithin, carbon, or metal hydroxides (*22*). The advantage of these strategies is that a wide range of cells can be isolated simultaneously, whereas the disadvantage is that the approach used may not be completely selective with respect to cell binding.

Copurification of undesirable compounds, or compounds that prevent the microbial binding, is a potential problem.

Generally, bacteria are relatively dense compared with most biological material and tissues. Density gradient centrifugation may thus be applied to separate the microbial cells from a biological matrix (14). This separation can be beneficial both as a result of the removal of inhibitory compounds and the fact that DNA from other organisms also may be inhibitory to polymerase chain reaction (PCR). The limitation is that the approach is quite technically challenging.

Microorganisms in liquids also can be separated by dielectrophoresis. The approach is based on inducing an uneven charge distribution within a cell by an oscillating electrical field and using this as a criterion for separation (23). This technique, however, is both sensitive to the conductivity of the medium and to particulate contaminants because of the small size of the electrophoresis unit.

Currently, no single approach for separating microorganisms from environmental or clinical matrices fulfills the requirements for diverse range of environmental matrices that exist. There are still major challenges related both to the separation of microorganisms from the environmental matrix and in the processing of large sample volumes. There has, however, been progress recently in using common physical properties among groups of bacteria to develop more general sample preparation approaches (21).

DNA analyses of complex microbial samples require a rigid lysis procedure that does not introduce errors from the differential lysis of different microorganisms in the sample (24). Mechanical, chemical, and enzymatic approaches commonly are applied. The mechanical disruption methods involve grinding of the material—either fresh, freeze dried, or frozen in liquid nitrogen. Substances such as alumina or glass beads can be added to facilitate the mechanical grinding process. The advantage of grinding is that any type of material can be processed, whereas the disadvantage is the possibility of crosscontamination and that the process can be difficult to automate. Sonication (using ultrasound) to release nucleic acids also has been successfully applied to clinical samples (25). Enzymes can be used to selectively degrade certain types of biological material, for example, for tissues mainly containing proteins, proteases can be used to degrade the matrix. Nearly all cell disruption and lysis strategies are combined with chemicals such as detergents, chaotropic salts, and other denaturants that denature the biological material (26–28).

4. Analyses of Crude Lysates

In some special cases it is not necessary to purify the DNA from the samples. The presence of PCR inhibitors in these samples is so minimal that it will not

interfere with the PCR (29), or the samples can be diluted to prevent the inhibition of enzymatic reactions (30). When the amount of target material analyzed is very low, such as for the analysis of single cells or bacteria that have been concentrated by immunomagnetic separation, the DNA may actually be lost in the purification step (31).

However, most environmental and clinical samples may contain compounds that are potent inhibitors of the enzymes used for analyses of DNA (**Table 1**). The inhibitors can be in the form of proteases or nucleases that degrade the polymerase or nucleic acids, respectively. Substances that destabilize the enzymes (e.g., chaotropic salts) or polysaccharides that can interact with both the nucleic acids and/or enzymes also may be potent inhibitors (32). There are also compounds that may interfere directly with the polymerase activity or compounds that modify the nucleic acids (29).

By adding substances that facilitate the PCR in the presence of inhibitors, or by selectively removing inhibitors from the sample, recent developments have been achieved. The advantage of such approaches is the simplicity and speed (29). However, standardization of the protocols can be difficult because of the diverse nature of environmental samples.

5. DNA Purification

The classical way of purifying nucleic acids from complex-, inhibitor-, and protein-containing solutions is to apply organic solvents such as phenol/chloroform (33). Other organic solvents such as chloroform or ether can be used to separate, for instance, fat from the aqueous DNA-containing phase. For algal and plant materials where co-purification of polysaccharides together with DNA may be a problem, the polysaccharides can be selectively precipitated with cetyl trimethyl ammonium bromide (9). However, because of the toxicity as well as the complex handling involving centrifugation and removal of aqueous phase, DNA extractions with organic solvents are not ideal.

DNA can be bound to glass, silica particles, or other polymer surfaces in the presence of alcohol, high salt, or chaotropic agents and subsequently is released in low-salt buffers (26). Other approaches using detergents (27) or polyethylene glycol (28) to bind DNA onto polymer surfaces also have been developed. The solid-phase principle has been applied in several formats, such as cartridges, filters, and paramagnetic beads. Paramagnetic beads have the advantage over other solid phases that they can easily be manipulated by a magnet and thus eliminate the need for centrifugation steps and speeding up washing steps.

The control of the yield and the purity of the isolated DNA are important parameters. The DNA quality can be measured empirically simply by evaluating the amplification efficiency of the subsequent PCR. However, such a mea-

Table 1
DNA Analysis of Bacteria in Different Matrixes

Sample type	Separation technique	Contaminants	Special Considerations
Air	Centrifugation, filtration (18)	Low amount. Particles mainly.	The microorganisms are transferred to a liquid phase
Liquids	Centrifugation, filtration, binding or affinity dielectrophoresis (23)	Many possible depending on liquid. However, relatively easy to define (20)	Heterogeneous low amount of particles
Soil	Ion exchange, affinity binding or density gradients (50)	Organic polymers, humic acids and ions (51)	Heterogeneous, strong binding of microorganisms to particles
Sediments	Centrifugation (12)	Similar to soil	Potential high content of dead cells
Feces	Affinity binding or density gradients (52)	Proteases, nucleases, and poly-saccharides (52)	High content of PCR inhibitors
Plant and animal tissues	Mechanical or enzymatic disruption in combination with affinity binding or density gradients (33)	Proteins, ion complexes, proteases, polysaccharides, and polyphenols	Very heterogeneous
Biofilms	Mechanical release from surface in combination with centrifugation, filtration or affinity binding	Polysaccharides	Difficult to obtain representative sample because of biofilm formation, and binding to the solid surface

surement does not give information about the kind of inhibitors present. Information about the inhibitors is crucial for the optimization of DNA extraction protocols. The main criterion for DNA purity has been measurements of protein contamination, and the most applied approach is to measure the adsorption of ultraviolet light with a wavelength of 260 nm (OD 260) and with a wavelength of 280 nm (OD 280). The OD260/OD280 ratio gives an indication of the DNA purity. For pure DNA, this ratio should be 1.7 (33). However, a ratio of 1.5 may indicate a 99% protein contamination. In addition, several pigments can interfere with the adsorption measurements (34). OD measurements do not give sufficient information for the investigation of PCR inhibitors in environmental samples. The DNA purity may be evaluated by more sensitive and specific methods to understand more about the DNA purification and the presence of potential inhibitors. Different standard methods in analytical chemistry such as matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) (35), high-pressure liquid chromatography (36), multispectral analyses, and liquid chromatography–mass spectrometry (LC–MS) yield accurate information about the different components in a sample (37). These methods, however, are not suited for routine applications but rather for optimization of the sample preparation approach.

6. Differentiation Between Viable and Dead Cells

There has been an increasing focus on the origin of the DNA purified from environmental sources. In particular, this relates to whether or not the DNA originates from viable or dead cells (38). Only approx 0.1 to 1% of the microorganisms in natural environments can be cultivated. Thus, it is not possible to determine cell viability by standard techniques (38). Soil samples, for example, often contain high amounts of free DNA in addition to DNA from dead microorganisms. Viability issues also are important in the investigations of pathogenic microorganisms in the environment, as well as in clinical settings.

DNA is, in most cases, too stable to be applied as a viable/dead marker. For instance, intact DNA has even been recovered from fossil material (39). Furthermore, the DNA stability may be dependent on both the strains and killing conditions (40). Thus, the current view is that DNA cannot be used as a viable/dead marker.

There have, however, been some recent advances in using DNA indirectly as a viable/dead marker (41–43). The principle applied is that DNA in living cells is protected by an intact cell wall/membrane, whereas these barriers are compromised in dead cells. The samples are treated with an agent that PCR inactivates the exposed DNA, resulting in a positive PCR amplification only from viable cells. There are also alternative methods being developed, such as measuring differences in the physical properties between viable and dead cells

or differences in DNA exposure (44). Separation based on physical properties can potentially be performed using the different density or dielectric properties between viable and dead cells (45). Development of methods for describing the different DNA fractions in environmental or clinical samples will be an important area for future understanding of microbial communities.

7. Future Automation

Few of the direct DNA-based methods applied for environmental or clinical analyses have been adapted for high-throughput purposes (46). For all kinds of routine diagnostic or detection purposes (usually associated with harmful or pathogenic microbes), automated protocols are likely to be the future choice. Automation of the process is a requirement for all large-scale screenings and/or to obtain reproducible results by eliminating human error.

For environmental analyses, handheld equipment that can be brought into the field is currently being developed (47). Because of the fear of biological warfare, the US army is a driving force in these developments (17). Advances also have been made in the field of pathogen control in animals used for food production (48). Future developments will be an integration of all steps into a single apparatus as in the concept of lab-on-a-chip. The current focus for lab-on-a-chip has changed from expensive silica-based to cheap plastic chips (49). These chips are gaining acceptability, mainly because they are affordable and because the liquid volumes that can be processed are in a practical range for most applications.

References

1. Rudi, K. (2002) Application of nucleic acid probes for analyses of microbial communities, in *Rapid Analytical Microbiology. The Chemistry and Physics of Microbial Identification* (Olson, W., ed.), Davis Horwood International Publishing, Ltd., Surrey, UK, pp. 13–40.
2. Abu Al-Soud, W. (2000) *Optimization of Diagnostic PCR, a Study of PCR Inhibitors in Blood and Sample Pretreatment*. Department of Applied Microbiology. Lund University, Lund, p. 59.
3. Rudi, K., Kroken, M., Dahlberg, O. J., Deggerdal, A., Jakobsen, K. S., and Larsen, F. (1997) Rapid, universal method to isolate PCR-ready DNA using magnetic beads. *BioTechniques* **22**, 506–511.
4. Greenfield, L. and White, T. J. (1993) Sample preparation methods, in *Diagnostic Molecular Microbiology: Principles and Applications* (Persing, D. H., Smith, T. F., Tenover, T. C., and White, T. J., eds.), American Society of Microbiology, Washington, pp. 122–137.
5. Rogers, C. and Burgoyne, L. (1997) Bacterial typing: storing and processing of stabilized reference bacteria for polymerase chain reaction without preparing DNA- an example of an automatable procedure. *Anal. Biochem.* **247**, 223–227.

6. Bowman, J. P. and Sayler, G. S. (1996) Nucleic acid techniques in the developmental detection of microorganisms and their activities, in *Molecular Approaches to Environmental Microbiology* (Pickup, R. W. and Saunders, J. R., eds.), Ellis Horwood Limited, Chichester, UK, pp. 63–97.
7. Holst-Jensen, A., Kohn, L., Jakobsen, K. S., and TSchumacher, T. (1997) Molecular phylogeny and evolution of *Nonilinea* (Sclerotiniaceae) based on coding and noncoding rDNA sequences. *Am. J. Bot.* **84**, 686–701.
8. Rogers, C. D. and Burgoyne, L. A. (2000) Reverse transcription of an RNA genome from databasing paper (FTA(R)). *Biotechnol. Appl. Biochem.* **31**, 219–224.
9. Snead, M., Kretz, P., and Short, J. (1994) Methods for generating plant genomic libraries. *Plant Mol. Biol. Manual* H1:1–19.
10. Inoue, T., Nabeshima, K., Kataoka, H. and Koono, M. (1996) Feasibility of archival non-buffered formalin-fixed and paraffin-embedded tissues for PCR amplification: an analysis of resected gastric carcinoma. *Pathol. Int.* **46**, 997–1004.
11. Hamazaki, S., Koshiba, M., Habuchi, T., Takahashi, R., and Sugiyama, T. (1993) The effect of formalin fixation on restriction endonuclease digestion of DNA and PCR amplification. *Pathol. Res. Pract.* **189**, 553–557.
12. Bowers, H. A., Tengs, T., Glasgow, H. B., Burkholder, J. M., Rublee, P. A., and Oldach, D. W. (2000) Development of real-time PCR assays for rapid detection of *Pfiesteria piscicida* and related dinoflagellates. *Appl. Environ. Microbiol.* **66**, 4641–4648.
13. Jakobsen, K. S., Tengs, T., Vatne, A., et al. (2002) Discovery of the toxic dinoflagellate *Pfiesteria* in northern European waters. *Proc. Royal Soc. Lond. B.* **269**, 211–214.
14. Lantz, P., Matsson, M., Wadstrom, T., and Radstrom, P. (1997) Removal of PCR inhibitors from human fecal samples through the use of an aqueous two-phase sample preparation prior to PCR. *J. Microbiol. Methods* **28**, 159–167.
15. Hardarson, G. and Brough, W., eds. (1999) *Molecular Microbial Ecology of the Soil*, Kluwer Academic Publishers, The Netherlands.
16. McGregor, D. P., Forster, S., Steven, J., et al. (1996) Simultaneous detection of microorganisms in soil suspension based on PCR amplification of bacterial 16S rRNA fragments. *BioTechniques* **21**, 463–470.
17. Iqbal, S. S., Mayo, M. W., Bruno, J. G., Bronk, B. V., Batt, C. A., and Chambers, J. P. (2000) A review of molecular recognition technologies for detection of biological threat agents. *Biosens. Bioelectron.* **15**, 549–578.
18. Alvarez, A. J., Buttner, M. P., and Stetzenbach, L. D. (1995) PCR for bioaerosol monitoring: sensitivity and environmental interference. *Appl. Environ. Microbiol.* **61**, 3639–3644.
19. Swaminathan, B. and Feng, P. (1994) Rapid detection of food-borne pathogenic bacteria. *Annu. Rev. Microbiol.* **48**, 401–426.
20. Rudi, K., Skulberg, O. M., Skulberg, R., and Jakobsen, K. S. (2000) Application of sequence-specific labeled 16S rRNA gene oligonucleotide probes for genetic

- profiling of cyanobacterial abundance and diversity by array hybridization. *Appl. Environ. Microbiol.* **66**, 4004–4011.
21. Rudi, K., Høidal, H. K., Katla, T., Johansen, B. K., Nordal, J., and Jakobsen, K. S. (2004). Direct PCR based detection and quantification of *Campylobacter jejuni* in chicken fecal and cecal samples through the application of the same paramagnetic beads for cell isolation and DNA purification. *Appl. Environ. Microbiol.* **70**, 790–797.
 22. Lucore, L. A., Cullison, M. A., and Jaykus, L. A. (2000) Immobilization with metal hydroxides as a means to concentrate food-borne bacteria for detection by cultural and molecular methods. *Appl. Environ. Microbiol.* **66**, 1769–1776.
 23. Cheng, J., Sheldon, E. L., Wu, L., et al. (1998) Preparation and hybridization analysis of DNA/RNA from *E. coli* on microfabricated bioelectronic chips. *Nat. Biotechnol.* **16**, 541–546.
 24. Field, K. G., Gordon, D., Wright, T., et al. (1997) Diversity and depth-specific distribution of SAR11 cluster rRNA genes from marine planktonic bacteria. *Appl. Environ. Microbiol.* **63**, 63–70.
 25. Millar, B. C., Jiru, X., Moore, J. E., and Earle, J. A. (2000) A simple and sensitive method to extract bacterial, yeast and fungal DNA from blood culture material. *J. Microbiol. Methods* **42**, 139–147.
 26. Boom, R., Sol, C. J., Salimans, M. M., Jansen, C. L., Wertheim-van Dillen, P. M., and van der Noordaa, J. (1990) Rapid and simple method for purification of nucleic acids. *J. Clin. Microbiol.* **28**, 495–503.
 27. Deggerdal, A. and Larsen, F. (1997) Rapid isolation of PCR-ready DNA from blood, bone marrow and cultured cells, based on paramagnetic beads. *BioTechniques* **22**, 554–557.
 28. Hawkins, T. L., O'Connor-Morin, T., Roy, A., and Santillan, C. (1994) DNA purification and isolation using a solid-phase. *Nucleic Acids Res.* **22**, 4543–4544.
 29. Abu Al-Soud, W. and Radstrom, P. (1998) Capacity of nine thermostable DNA polymerases to mediate DNA amplification in the presence of PCR-inhibiting samples. *Appl. Environ. Microbiol.* **64**, 3748–3753.
 30. Grevelding, C. G., Kampkotter, A., Hollmann, M., Schafer, U., and Kunz, W. (1996) Direct PCR on fruitflies and blood flukes without prior DNA isolation. *Nucleic Acids Res.* **24**, 4100–4101.
 31. Tengs, T., Dahlberg, O. J., Shalchian-Tabrizi, K., et al. (2000) Phylogenetic analyses indicate that the 19'hexanoyloxy-fucoxanthin-containing dinoflagellates have tertiary plastids of haptophyte origin. *Mol. Biol. Evol.* **17**, 718–729.
 32. Monteiro, L., Bonnemaison, D., Vekris, A., et al. (1997) Complex polysaccharides as PCR inhibitors in feces: *Helicobacter pylori* model. *J. Clin. Microbiol.* **35**, 995–998.
 33. Sambrook, J., and Russel, D. (2000) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
 34. Glasel, J. A. (1995) Validity of nucleic acid purities monitored by 260nm/280nm absorbance ratios. *BioTechniques* **18**, 62–63.

35. Hung, K. C., Rashidzadeh, H., Wang, W., and Guo, B. (1998) Use of paraffin wax film in MALDI-TOF analysis of DNA. *Anal. Chem.* **70**, 3088–3093.
36. Rubsam, L. Z. and Shewach, D. S. (1997) Improved method to prepare RNA-free DNA from mammalian cells. *J. Chromatogr. B. Biomed. Sci. Appl.* **702**, 61–68.
37. Mock, K. (1993) Routine sensitive peptide mapping using LC/MS of therapeutic proteins produced by recombinant DNA technology. *Pept. Res.* **6**, 100–104.
38. Torsvik, V., Daae, F. L., Sandaa, R. A., and Ovreas, L. (1998) Novel techniques for analysing microbial diversity in natural and perturbed environments. *J. Biotechnol.* **64**, 53–62.
39. Lindahl, T. (1997) Facts and artifacts of ancient DNA. *Cell* **76**, 49–99.
40. Nogva, H., Bergh, A., Holck, A., and Rudi, K. (2000) Application of the 5'-nuclease PCR assay in the evaluation and development of methods for quantitative detection of *Campylobacter jejuni*. *Appl. Environ. Microbiol.* **66**, 4029–4036.
41. Rudi, K., Naterstad, K., Dromtorp, S. M., and Holo, H. (2005) Detection of viable and dead *Listeria monocytogenes* on gouda-like cheeses by real-time PCR. *Lett. Appl. Microbiol.* **40**, 301–306.
42. Rudi, K., Moen, B., Dromtorp, S. M., and Holck, A. L. (2005) Use of ethidium monoazide and PCR in combination for quantification of viable and dead cells in complex samples. *Appl. Environ. Microbiol.* **71**, 1018–1024.
43. Nogva, H. K., Dromtorp, S. M., Nissen, H., and Rudi, K. (2003) Ethidium monoazide for DNA-based differentiation of viable and dead bacteria by 5'-nuclease PCR. *BioTechniques* **34**, 804–813.
44. Nishino, T., Nayak, B. B., and Kogure, K. (2003) Density-dependent sorting of physiologically different cells of *Vibrio parahaemolyticus*. *Appl. Environ. Microbiol.* **69**, 3569–3572.
45. Lapizco-Encinas, B. H., Simmons, B. A., Cummings, E. B., and Fintschenko, Y. (2004) Dielectrophoretic concentration and separation of live and dead bacteria in an array of insulators. *Anal. Chem.* **76**, 1571–1579.
46. Palmer, C. J., Bonilla, G. F., Roll, B., Paszko-Kolva, C., Sangermano, L. R., and Fujioka, R. S. (1995) Detection of *Legionella* species in reclaimed water and air with the EnviroAmp *Legionella* PCR kit and direct fluorescent antibody staining. *Appl. Environ. Microbiol.* **61**, 407–412.
47. Belgrader, P., S. Young, B. Yuan, M. Primeau, L.A. Christel, F. Pourahmadi and M.A. Northrup. (2001) A battery-powered notebook thermal cycler for rapid multiplex real-time PCR analysis. *Anal. Chem.* **73**, 286–289.
48. Rudi, K. (2001) Direct DNA based campylobacter diagnostics: rapid methods enable new logistics in the production of *Campylobacter* free poultry. *New Food* **4**, 25–29.
49. Bruin, G. J. (2000) Recent developments in electrokinetically driven analysis on microfabricated devices. *Electrophoresis* **21**, 3931–3951.

50. Rochelle, P. (2001) *Environmental Molecular Microbiology: Protocols and Applications*. Horizon Scientific Press, Wymondham, UK, p. 264.
51. Watson, R. J. and Blackwell, B. (2000) Purification and characterization of a common soil component which inhibits the polymerase chain reaction. *Can. J. Microbiol.* **46**, 633–642.
52. Monteiro, L., Gras, N., Vidal, R., Cabrita, J., and Megraud, F. (2001) Detection of *Helicobacter pylori* DNA in human feces by PCR: DNA stability and removal of inhibitors. *J. Microbiol. Methods* **45**, 89–94.



<http://www.springer.com/978-1-58829-594-1>

Diagnostic Bacteriology Protocols

O'Connor, L. (Ed.)

2006, X, 226 p. 51 illus., Hardcover

ISBN: 978-1-58829-594-1

A product of Humana Press