Polymerase Chain Reaction

EMD Team Fact Sheet—November 2011

This fact sheet, developed by the ITRC Environmental Molecular Diagnostics (EMD) Team, is one of 10 designed to provide introductory information about and promote awareness of EMDs. Please review the Introduction to EMDs Fact Sheet along with this one. A glossary is included at the end of this fact sheet.

Why is polymerase chain reaction relevant?

Polymerase chain reaction (PCR) is a technique that can test for the presence of the specific microorganism, family of microorganisms, or expressed genes in environmental samples such as soil, water, or sediment. When combined with traditional monitoring of contaminant concentrations over time, using PCR to identify microorganisms capable of degrading contaminants can provide project managers valuable information for site management and remedy selection. PCR therefore aids project managers (e.g., by providing a survey of specific microorganisms or developing targeted information for specific genes) in site conceptual model development, remedy selection and optimization, and determination of contaminant attenuation rates.

What does PCR do?

PCR techniques were originally described in the 1960s, were popularized during the late 1980s and early 1990s within the biotechnology industry, and today are routinely used in medical diagnosis and in environmental detection of microorganisms. PCR is a laboratory method that generates multiple copies of a specific (target) DNA sequence, if present in a sample, representing microorganisms or groups of related microorganisms known to biodegrade contaminants. Reverse transcriptase PCR (RT-PCR) is a laboratory method that transforms RNA associated with biodegradation into complementary DNA (cDNA) that is then detected by PCR. Additionally, PCR can be used to amplify DNA sequences for use in further analysis of the sequence by other EMD techniques such as quantitative polymerase chain reaction (qPCR), microarray analysis, and microbial fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE). For more information, see the qPCR, Microarrays, and Microbial Fingerprinting Methods Fact Sheets. PCR has been used to detect microorganisms capable of degrading contaminants such as petroleum hydrocarbons, pentachlorophenol, perchlorate, polychlorinated biphenyls (PCBs), metals, radionuclides, and chlorinated solvents. PCR also has potential applicability in other environmental monitoring efforts, such as tracking of fecal microorganisms, so-called "microbial source tracking," and identifying and tracking microorganisms potentially related to chemical compounds originating from industrial or energy-related activities.

How are the data used?

During biodegradation processes, microorganisms break down contaminants using enzymes. PCR can be used to detect the presence of either (a) a specific microorganism or group of microorganisms that are known to be able to biodegrade a specific contaminant or group of contaminants or (b) DNA sequences (genes) that regulate the production of enzymes (proteins) that biodegrade or partially biodegrade these contaminants. The genes a microorganism possesses not only enable identification of the microorganism but also determine which enzymes that microorganism can produce and therefore which contaminants it can biodegrade. Depending on which genes are analyzed, PCR can tell whether either a particular enzyme or a particular microorganism known to biodegrade specific contaminants is present in a sample. Detection of specific genes or microorganisms capable of biodegradation of a contaminant provides a direct line of evidence that bioremediation may be possible at a site. Additionally, this method can be used to analyze the diversity of the microbial community and relative changes in the community diversity in response to remedial activities.

Example Environmental Remediation Questions PCR Can Help Answer

- **Site Characterization**
  - What known organisms or functional genes are present?
  - How diverse is the microbial community?
- **Remediation**
  - Are the right microorganisms and/or genes present that are capable of degrading the contaminant? If so, how many (qPCR) and where?
  - Are the key microbial pathways active (RT-PCR)?
  - Is monitored natural attenuation (MNA) feasible?
  - Should an amendment be added (biostimulation)?
  - Is bioaugmentation necessary?

How does it work?

Microorganisms contain deoxyribonucleic acids (DNA) composed of long series of nucleotides represented by the letters A, T, C, and G. Some DNA sequences (the arrangement of the letters one after the other of this four-letter alphabet) are unique to specific organisms and can be used in PCR to determine whether that organism is present in a sample. PCR capitalizes on the ability of DNA polymerase (the enzyme that copies a cell's DNA before it divides in two) to synthesize new strands of DNA complementary to a template DNA strand. The PCR laboratory method selectively amplifies only the genes of interest (if present) in a sample. Sample preparation involves harvesting and concentrating microbial cells from the soil or groundwater sample (e.g., by filtration) and breaking these cells open to release their DNA (Figure 1). A typical reaction mixture contains template DNA (environmental DNA), short DNA primers specific to the target gene, DNA building blocks (e.g., deoxyribonucleotide triphosphates [dNTPs]) and a heat-stable DNA-synthesizing enzyme (DNA polymerase). This mixture is repeatedly cycled through a precise temperature sequence that leads to the exponential increase in the number of copies of the target gene (amplification) (see EPA 2004 for more information).

As shown in Figure 1, there are four steps in the PCR process: (a) In the first step the sample temperature is raised from room temperature (25°C) to 94–97°C, and the double-stranded DNA "unzips" into single strands (denaturation). (b) At 47–60°C the primers attach to the target sequence (annealing). (c) At 72°C DNA polymerase continues attaching dNTPs to each strand (elongation) until there are two double-stranded copies of the target sequence for each double-stranded copy available at the first step. (d) Finally, the sample temperature is raised to 94–97°C again, and the cycle is repeated 30–40 times to achieve the desired amplification of gene copies.

How are the data reported?

When PCR is used to target a specific gene or microorganism, data from PCR are reported simply as present/absent for the target sequence. However, when the products from PCR are used in another EMD method, more detailed information is available. See the qPCR, Microarrays, and Microbial Fingerprinting Methods Fact Sheets for further information.

Variations on PCR

Quantitative PCR—qPCR estimates the number of specific sequences or genes and by inference the number of microorganisms or groups of microorganisms in a soil, sediment, or groundwater sample. (See the qPCR Fact Sheet for further information.) There are significantly fewer qPCR targets than PCR targets to date.

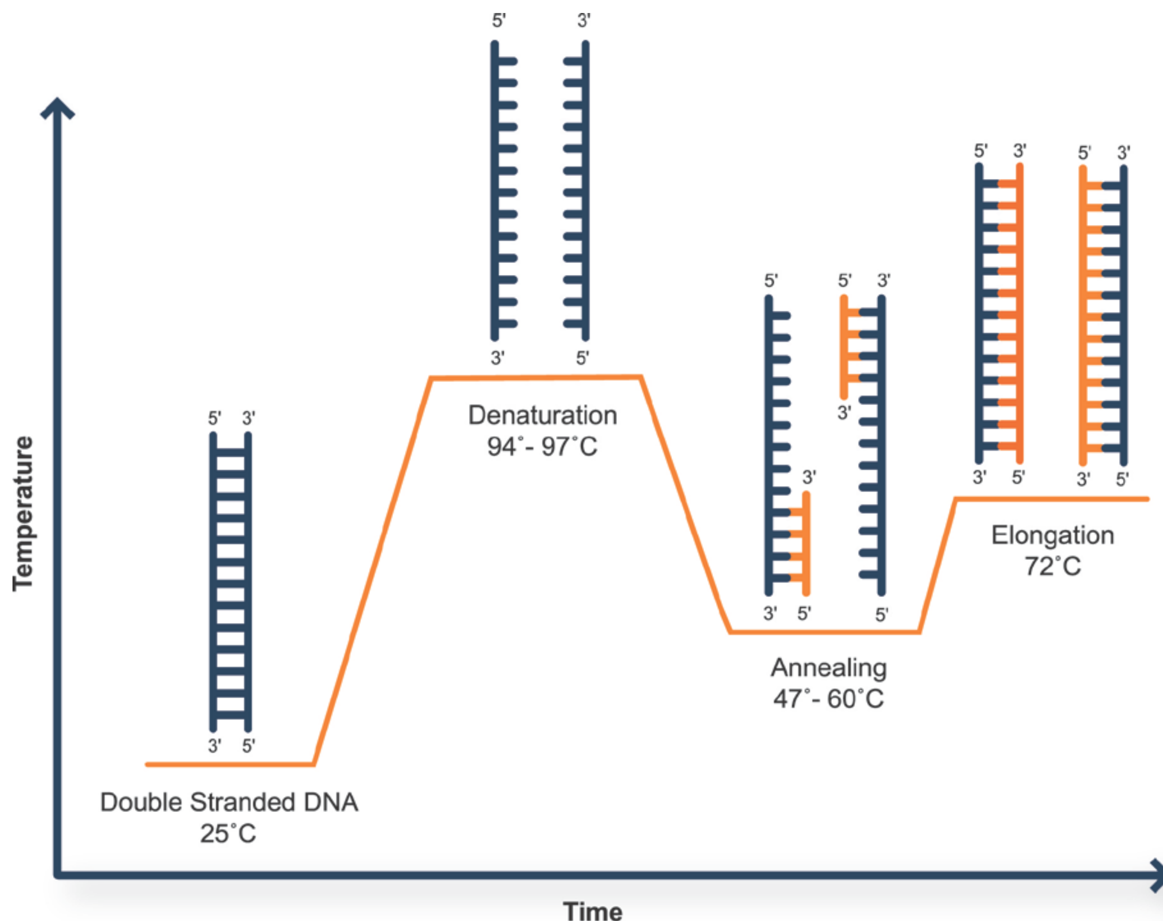

Figure 1. The PCR process. *Source:* EPA 2004.

Reverse transcriptase PCR—RNA is a nucleic acid, and the production of RNA is a necessary step to "read" genes and produce enzymes. If RNA related to biodegradation is detected in soil or groundwater, it can be inferred that microorganisms are actively making enzymes and thus biodegrading contaminants. This information allows the project manager to discriminate between microorganisms actively degrading contaminants from those microorganisms that may have the capability for biodegradation but may not be degrading contaminants for a variety of reasons. In RT-PCR, the RNA associated with biodegradation is transformed by a chemical reaction in the laboratory (so-called reverse transcription) into cDNA that can then be detected by PCR. When combined with qPCR (RT-qPCR), this method is quantitative (see the qPCR Fact Sheet).

Nested PCR—Nested PCR is used to increase the specificity and/or the sensitivity of the PCR method. In nested PCR, two sets of primers are used: the first primer amplifies a specific region/gene in the microorganism's DNA, and the second primer amplifies a sequence within the original sequence.

Advantages

- PCR analyses are capable of detecting specific microorganisms or target genes within diverse microbial communities such as those present in environmental samples.
- PCR results are available within days, as the technique does not require growing the target microorganisms (which can be difficult, time-consuming, and not always possible).
- PCR analyses are sensitive (can detect as few as 100 cells or gene copies in a sample, e.g., 100 mg of soil or sediment or 1–10 L water).

- PCR analyses can be performed on a variety of sample types (e.g., water, soil, sediment).
- PCR analyses can be used to survey the general microbial community or target specific genes.

Limitations

- Although not particularly common, PCR results can be affected by the presence of some metals or humic acids in the environmental sample. Samples exhibiting PCR inhibition should be readily identified with basic quality assurance (QA)/quality control (QC) procedures. Furthermore, inhibition can often be eliminated with minor modifications to the DNA extraction procedure.
- Standardization of protocols for sample collection, storage, and extraction between laboratories is currently under way but is not yet complete. There is U.S. Environmental Protection Agency (EPA) guidance for QC for PCR analysis (EPA 2004).
- The development of PCR analysis is based on known biodegradation pathways and gene sequences. With ongoing research, additional PCR analyses will be developed to expand the applicability of the technique to other contaminants and newly identified biodegradation pathways.

Sampling Protocols

Sample matrices that can be analyzed by PCR include soil, sediment, groundwater, and filters. Sampling for microbiological samples can be easily incorporated into routine environmental monitoring programs. The following items are typical requirements for microbiological sampling: (a) use of aseptic sample collection techniques and sterile sample containers, (b) shipment of the samples to the laboratory within 24 hours of sample collection, and (c) maintenance of the samples at 4°C during handling and transport to the laboratory. Sample collection techniques and containers may vary depending on the matrix sampled and the laboratory analyzing the samples. Users should work with the analytical laboratory to ensure sampling protocols for collecting, handling, and transporting the samples are in place and understood.

Quality Assurance/Quality Control

To date, most EMDs do not have standardized protocols accepted by EPA or other government agencies. However, in 2004 the EPA published the *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples*, which is a useful guide for laboratories performing PCR. In addition, most laboratories work under standard operating procedures (SOPs) and good laboratory practices, which can be provided to the user (e.g., consultant, state regulator) on request.

Currently, users can best ensure data quality by detailing the laboratory requirements in a site-specific QA project plan (QAPP). This plan should include identification of the EMDs being used; the field sampling procedures, including preservation requirements; the SOPs of the laboratory performing the analysis; and any internal QA/QC information available (such as results for positive and negative controls). PCR analyses include both positive and negative controls to ensure that the PCR reaction occurred properly, i.e., that it was not inhibited by interfering substances in the reaction mixture and that it amplified only the target sequence and not other nontarget DNA. Both positive and negative controls should be included and undergo the exact same PCR protocol as the environmental samples.

Additional Information

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Glossary

bioaugmentation—The introduction of cultured microorganisms into the subsurface environment for the purpose of enhancing bioremediation of organic contaminants (EPA 2011).

biodegradation—A process by which microorganisms transform or alter (through metabolic or enzymatic action) the structure of chemicals introduced into the environment (EPA 2011).

DNA (deoxyribonucleic acid)—A nucleic acid that carries the genetic information of an organism. DNA is capable of self-replication and is used as a template for the synthesis of RNA. DNA consists of two long chains of nucleotides twisted into a double helix (EPA 2004).

deoxyribonucleotide triphosphates (dNTPs)—dNTPs are incorporated into DNA during elongation (EPA 2004).

enzyme—Any of numerous proteins or conjugated proteins produced by living organisms and facilitating biochemical reactions (based on EPA 2004).

gene—A segment of DNA containing the code for a protein, transfer RNA, or ribosomal RNA molecule (based on Madigan et al. 2010).

microbial community—The microorganisms present in a particular sample.

nucleic acid—A complex biomolecule consisting of a long "backbone" of organophosphate sugars with nucleotide bases attached.

primers—Short strands of DNA that are complementary to the beginning and end of the target gene and thus determine which DNA fragment is amplified during PCR or qPCR.

protein—Large organic compounds made of amino acids arranged in a linear chain and joined together by peptide bonds (U.S. Navy 2009).

RNA (ribonucleic acid)—Single-stranded nucleic acid that is transcribed from DNA and thus contains the complementary genetic information.

transcription—The first step in activation of a biochemical pathway where a complementary RNA copy is synthesized from a DNA sequence.

translation—The second step of gene expression where messenger RNA (mRNA) produced by transcription is decoded by the cell to produce an active protein.

EMD Team Contact

Robert Mueller, Team Leader

New Jersey Department of Environmental Protection

bob.mueller@dep.state.nj.us, (609) 984-3910

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