B12 DNA Detergent: A Novel Technique to Remove PCR Inhibitors From Soil-Derived DNA

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After attending this presentation, attendees will know that it is possible to remove PCR inhibitors from environmental samples in a one-step procedure.

This presentation will impact the forensic community and/or humanity by demonstrating a simple procedure was developed for removal of humic and fulvic acids from forensic samples. Forensic scientists will be given a protocol that can be used to remove humic acids from environmental samples.

Procedures for purification of DNA extracted from bacteria in environmental samples are time-consuming and require expertise by the analyst. The extraction procedures must be complex, because environmental samples are contaminated with humic and fulvic acid substances, which often co-purify with the DNA. If the humic and fulvic acids are not removed from the DNA, they will prevent analysis by the PCR because the dissociation and enzymatic steps are inhibited. In addition, the compounds can influence the results of down stream procedures that utilize fluorescence detection, such as real-time PCR. Commercial kits are available for extraction of DNA from soil samples, but the yield and quality of the DNA is often poor.

An alternative to the commercial kits has been developed that has the potential to allow a high number of samples to be processed in a day. The new procedure uses a compound (hereafter referred to as PCR inhibitor remover, PIR) that is added to the crude extracts to initiate an immediate, selective reaction with humic acids and other PCR inhibitory materials. After mixing with the PIR compound, the DNA was precipitated, dissolved in buffer, and a portion tested in the PCR. To identify effective PIR compounds, the approach taken was to examine compounds known to be reactive with carbonyl groups because these moieties are highly present in humic acids and could act to sequester Mg2+ and form covalent bonds with primary amines of the polymerase. The PIR compounds were tested on known humic acid samples for their humic acid-removal capabilities by both fluorescence spectroscopy and PCR compatibility. A popular commercial kit was tested with the humic acid-containing samples in parallel experiments, and the results were compared with those obtained with the PIR compounds.

Thiamine, pyridoxamine (PDA), and phenylthiazolium bromide have been identified as promising PIR compounds. These compounds out perform the commercial kit by 10 to 100 fold, improving the PCR amplification 10-10,000 fold over untreated samples. PDA can alleviate PCR inhibition at concentrations exceeding natural levels of humic acids and was selected as the best PIR compound. To utilize PDA in a one-step reaction, it can be tethered to glass beads. An alternative approach is to use serial passage through 5mm and 0.2mm pore size filters to remove the large particles and aggregates of humic acids. A simple protocol will be presented for PCR-ready DNA from soil extracts using the PIR compound and filtration. Exploiting the fact that dilution of extracts often mitigates inhibition effects, it was found that the DNA extract without the PIR compound required up to a 1:10,000 dilution to obtain strong PCR products. In contrast, with the PIR compound, the DNA extract required only a 1:10 dilution to support the PCR. The purification procedure could be completed in less than 3 minutes.

Soil, Nucleic Acid Purification, Humic Acids

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