QUANTIFICATION OF CHEMILUMINESCENT DNA PROBES USING LIQUID SCINTILLATION COUNTING

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ABSTRACT

This project involved the development of a method to quantify small amounts of specific sequences of DNA, in order to examine possible sources of dissolved DNA in aquatic ecosystems. Radioactively-labelled probes have traditionally been used to quantify such ecosystem processes. An approach was sought that employed non-radioactive probes because of disadvantages (e.g., shelf-life, safety, regulatory impediments) associated with the use of ^{32}P. Prior to this work, existing methods using non-radioactive probes were semi-quantitative, or relied on specialized and expensive instrumentation not available in most oceanographic research laboratories. The method developed and described in this thesis involves the use of the liquid scintillation counter to quantify luminescence from an enzyme-catalyzed reaction, where the enzyme is present in proportion to the amount of DNA in a sample. Seventeen attomoles (30 pg pUC18 DNA) of homologous DNA can be detected with this method, a detection limit comparable to radioactively-labelled DNA probes and low enough to be of use for field applications. Further, use of the liquid scintillation counter, a commonly available instrument to ecosystem researchers, makes this method easily accessible.