

location coordinates and angular orientation coordinates for a mobile pill transmitter (PT), or other similar device that is introduced into, and moves within, a GI tract of a human or animal body. A set of as many as eight nonlinear equations has been developed and applied, relating propagation of a wireless signal between either two, three, or more transmitting antennas located on the PT, to four or more non-coplanar re-

ceiving antennas located on a signal receiver appliance worn by the user.

The equations are solved exactly, without approximations or iterations, and are applied in several environments: (1) association of a visual image, transmitted by the PT at each of a second sequence of times, with a PT location and PT angular orientation at that time; (2) determination of a position within the body at which a drug or

chemical substance or other treatment is to be delivered to a selected portion of the body; (3) monitoring, after delivery, of the effect(s) of administration of the treatment; and (4) determination of one or more positions within the body where provision and examination of a finer-scale image is warranted.

This work was done by John F. Schipper for Ames Research Center. Further information is contained in a TSP (see page 1). ARC-15810-1

Molecular Technique to Reduce PCR Bias for Deeper Understanding of Microbial Diversity

This technique has applications in medical manufacturing, food processing, and municipal water treatment.

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Current planetary protection policies require that spacecraft targeted to sensitive solar system bodies be assembled and readied for launch in controlled cleanroom environments. A better understanding of the distribution and frequency at which high-risk contaminant microbes are encountered on spacecraft surfaces would significantly aid in assessing the threat of forward contamination. However, despite a growing understanding of the diverse microbial populations present in cleanrooms, less abundant microbial populations are probably not adequately taken into account due to technological limitations. This novel approach encompasses a wide spectrum of microbial species and will represent the true picture of spacecraft cleanroom-associated microbial diversity.

All of the current microbial diversity assessment techniques are based on an initial PCR amplification step. However, a number of factors are known to bias PCR amplification and jeopardize the true representation of bacterial diversity. PCR amplification of a minor template

appears to be suppressed by the amplification of a more abundant template. It is widely acknowledged among environmental molecular microbiologists that genetic biosignatures identified from an environment only represent the most dominant populations. The technological bottleneck overlooks the presence of the less abundant minority population and may underestimate their role in the ecosystem maintenance.

DNA intercalating agents such as propidium monoazide (PMA) covalently bind with DNA molecules upon photolysis using visible light, and make it unavailable for DNA polymerase enzyme during polymerase chain reaction (PCR). Environmental DNA samples will be treated with suboptimum PMA concentration, enough to intercalate with 90–99% of the total DNA. The probability of PMA binding with DNA from abundant bacterial species will be much higher than binding with DNA from less abundant species. This will increase the relative DNA concentration of previously “shadowed” less abundant species

available for PCR amplification. These PCR products obtained with and without PMA treatment will then be subjected to downstream diversity analyses such as sequencing and DNA microarray. It is expected that PMA-coupled PCR will amplify the “minority population” and help in understanding microbial diversity spectrum of an environmental sample at a much deeper level.

This new protocol aims to overcome the major potential biases faced when analyzing microbial 16S rRNA gene diversity. This study will lead to a technological advancement and a commercial product that will aid microbial ecologists in understanding microbial diversity from various environmental niches. Implementation of this technique may lead to discoveries of novel microbes and their functions in sustenance of the ecosystem.

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