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ration between Aberdeen and Essex Universities. At Aberdeen, a package of detection methods has been devised based on the genetic marking of test or "model" organisms—Gram-negative *Pseudomonas fluorescens* and Gram-positive *Bacillus subtilis* with *lux* genes from species of marine vibrios, which confer the property of bioluminescence. Stable constructs have been generated with both plasmid-borne and chromosomally incorporated genes leading to the expression of the luciferase system. The luminescence-based detection package formulated at Aberdeen has been complemented with the development of immunofluorescent cell detection technology at Essex.

Luminescence-based detection of GMOs has proved to have a number of distinct advantages over marker systems. The lack of significant background luminescence in the soil environment means that bacteria genetically modified by the introduction of *lux* genes can be identified selectively and with high sensitivity using methodologies which do not require extraction and enrichment and thus avoid the well-recognised theoretical and practical disadvantages associated with these traditional microbiological techniques. In addition, the activity of the luciferase system is dependent on the metabolic state of the cells. This association of luminescence with cellular metabolic activity enables determination not only of the presence of *lux*-tagged strains but also of their actual and potential metabolic activity, by measuring light output before and after substrate addition.

Light output from *lux*-modified bacteria can be detected and quantified in a number of ways: (1) For colonies on agar plates, detection can be made by eye in the darkroom, photographic and X-ray film, and CCD imaging; (2) for populations in the soil, detection can be made by luminometry; and (3) for single cels in soil, detection can be made by CCD-enhanced microscopy.

The unique power of *lux*-based bacterial detection in environmental samples has been particularly enhanced by the use of charge coupled device (CCD) technology. CCDs were developed as high density shift resisters or serial memories and, more recently, have been used for the magnification of extremely low light signals in astronomical observations. They equally have application in microbiology, however, for direct observation of plate cultures or in association with microscopic analysis of slide cultures, or even of actual soil samples. For viable cell enumeration of luminescent strains, CCD imaging can both reduce the time of incubation necessary for colony development and, more importantly, give greatly enhanced selective identification of light emitting colonies against high relative backgrounds of non-luminescent indigenous soil microbes. In fact, the sensitivity of CCD is such that, coupled with bright and dark field light microscopy, single cell detection of individual luminescent cells is possible, even in the presence of soil.

Under PROSAMO, luminescence-based detection has been integrated into a package of detection techniques for risk assessment. The currently available array of nucleic acid probe techniques, for example, apply equally to the recognition of the *lux* genes and other specific genetic material so that the detection of light emission to track luminescent cells can be complemented with established probe technology to track the *lux* genes themselves. Gene-specific probe technology is now itself open to considerable enhancement by the polymerase chain reaction (PCR), such that direct analysis of soil systems is feasible without the need for selective enrichment culture. Here the limitation is on the extraction of the target DNA from the environmental samples (usually soil) in a sufficiently clean state for amplification by PCR. This has been achieved successfully within PROSAMO using soils inoculated with *lux*-modified bacteria.

Detection of GMOs based on fluorescent antibody labeling has been developed at Essex to complement luminescence-based detection since the former does not require metabolically active cells, and GMOs persisting in soil for long periods may otherwise be difficult to reactivate.

Production of monoclonal and polyclonal antibodies which selectively recognise vegetative cells and endospores of *Bacillus subtilis* has demonstrated the potential of immunofluorescence as a powerful technique in risk assessment. The technique has been enhanced by the innovative development of bacterial cell sorting using flow cytometry. This enables fluorescently labelled cells to be rapidly (the flow cytometer can analyse up to 10,000 particles per second) separated from other soil microbes, as well as soil particles, and quantitatively recovered with high efficiency.

The detection technologies developed under the PROSAMO microbial programme link with existing detection techniques to provide a robust package for effective and thorough risk assessment by GMOs. The final phase of the programme is now involving the application of this detection package to answer key questions of risk assessment, particularly with respect to GMO survival and gene transfer in the soil environment.

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Errata

Figure 3 was inadvertently omitted from "Interactions in the Fourth Dimension" by Russ Granzow and Robert Reed (Bio/Technology **10**:*390-393). The missing figure is reproduced below.*

FIGURE 3. The effect of amino acid changes in the Z domain of Protein A on the on-rate and off-rate of its interaction with IgG.