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Genefish: an alternate metagenomic approach for capturing targeted bacterial diversity in an engineered recipient *E. coli* strain

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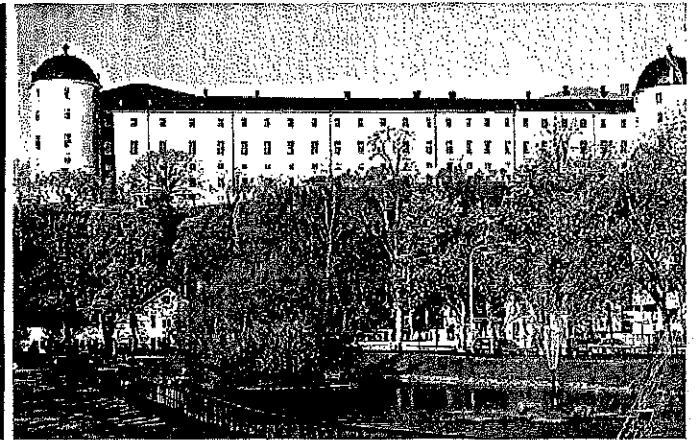
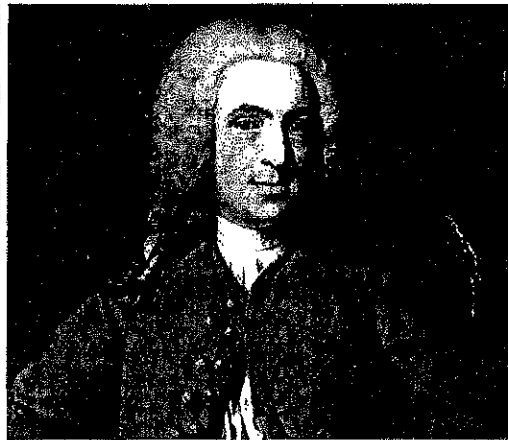
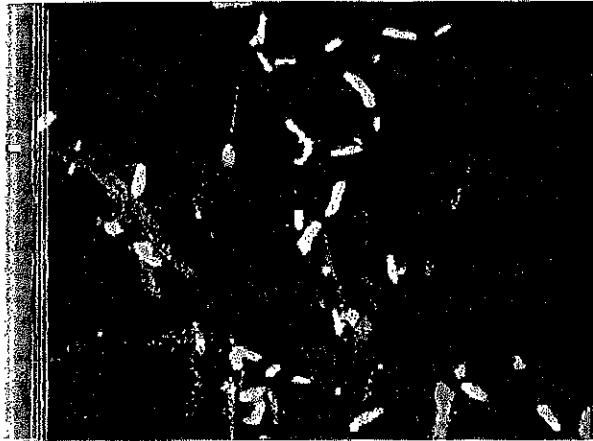
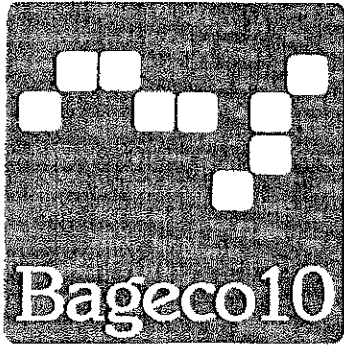
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GENEFISHING: AN ALTERNATE METAGENOMIC APPROACH FOR CAPTURING TARGETED BACTERIAL DIVERSITY IN AN ENGINEERED RECIPIENT *E. COLI* STRAIN

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The metagenomic approach, defined as the direct recovery and cloning of bacterial DNA from the environment in domesticated bacterial hosts has been widely used to study bacterial populations and their functional genes in numerous environments. The advantage of this approach over conventional culture based techniques is that it encompasses a wider range of bacteria by bypassing the bias of uncultivability of more than 99% of the bacteria in soil. However, in complex and rich environments such as soils, the huge level of bacterial diversity requires construction, handling and screening of several million clones in order to cover a significant proportion of bacterial genes in the indigenous community. These methods are time and money consuming, and require access to specialized robots that are unavailable to most microbial ecology laboratories. Our objectives were to develop an alternative metagenomic approach in which only bacterial recombinant clones harbouring inserts with sequence based selected genes could develop on growth media. This positive screening technology, called "Genefish" is based on homeologous recombination to extract specific genes from the metagenome into the specifically engineered recipient *E. coli* strain. The key characteristic of this approach is the use of two inducible lethal genes to kill non recombinant bacteria. We will present molecular details of this "Genefish" recipient *E. coli* strain and our first results of its *in vitro* and *in situ* use to extract denitrification related genes from the soil metagenome.