



Metagenomic library selection and validation by pyrosequencing

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Poster Presentation

Metagenomic library selection and validation by pyrosequencing

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Metagenomic libraries provide access to a wide range of non-culturable functional genes. After the creation of the libraries, clones need to be selected either based on phenotypic and/or genetic screening. Genetic screening of metagenomic DNA clone libraries provides several conceptual and technical challenges related to the development of the metagenomic approach. We present here a technique in which the 77 000 clones of a metagenomic library were spotted on high density membranes and hybridized with a probe solution consisting of a mixture of oligonucleotides complementary to several different genes. The pool of targeted genes included those associated with functions as diverse as denitrification, antibiotic resistance, and dehalogenation. Although 14 different genes were targeted, hybridization detected 134 positive clones out of the 77 000 tested, thus providing a drastic selection process. Positive clone DNA was pooled and pyrosequenced. Sequences were partially assembled in order to determine whether clone sequences could be reconstructed. The contigs were examined for the probe sequences and when present the genetic environment was analyzed. Clone sequences were compared (BLAST) to those obtained by 454FLX pyrosequencing of the original extracted metagenomic DNA. In addition, the probe sequences were BLASTed against the pyrosequences and the relative increase of probe occurrence was correlated with sequence conservation. This study demonstrates the potential of metagenomic approaches to uncover and quantify functional genes present in soil bacterial microbial community.