Metagenomic BAC Libraries from Environmental DNA

Ena Urbach
eMetagen Corporation, Madison, WI 53704

eMetagen is a molecular prospecting company: we mine the genomes of uncultivated soil bacteria to discover new pharmaceuticals. These molecular gold nuggets are produced by biochemical pathways encoded by linked genes in hard-to-cultivate microbes. We access their potential by cloning very long segments of soil microbial DNA into Bacterial Artificial Chromosomes (BACs) and screening the resulting meta-genomic libraries with functional assays and genetic probes.

Large-insert clones are difficult to make from DNA in environmental samples. Even after enriching cells from the bulk of the soil matrix, remnant materials dilute the samples and bedevil molecular manipulations. Multiple rounds of electrophoretic size selection and in-gel enzymatic and chemical treatments yield low concentrations of precious, high molecular weight insert DNA. We use Lucigen's CopyRight™ cloning kit and Replicator™ competent cells to maximize our yield of large-insert clones (Figure 1).

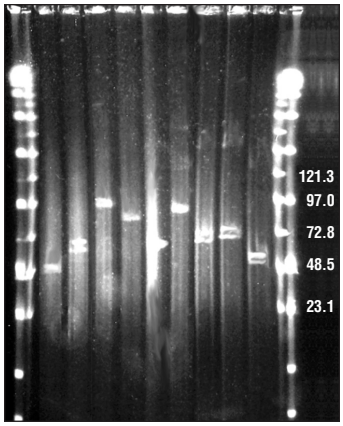

Figure 1. Soil metagenome BAC clones cut with PmeI reveal inserts >50 kb in length. Clones made with the CopyRight™ pEZ™ BAC vector (7.2 kb) were analyzed by pulsed-field gel electrophoresis.

Lucigen's CopyRight cloning system reduces the number of DNA manipulations required for large-insert cloning experiments. Most cloning protocols require ligation mixtures to be dialyzed before electroporation into competent *E. coli*. However, the Lucigen ligase system works efficiently under low salt conditions, so that ligated clones can be directly transformed into cells. Every step in a large-insert cloning protocol can cause grave losses in DNA size and overall yield. Skipping the dialysis step with Lucigen's rapid ligation system makes the difference between good and not-so-good yields of large-insert clones.

One of the most inefficient steps in BAC cloning is electroporation: the larger the DNA clone, the harder it is to introduce selectively into a bacterial host. We use special electroporation conditions to favor clones with highest molecular weight. Again, Lucigen's cloning system makes the difference: high efficiency Replicator competent cells enable us to tune the insert size of our BACs while maintaining sufficient yield to build industrial-sized clone libraries.

eMetagen's genomic prospecting benefits from Lucigen's CopyRight cloning system. The better-than-homemade features of the ligation mix and high efficiency competent cells complement eMetagen's technologies to unearth high-value drug products from soil microbes. ■