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Construction of Simple Cosmid Library Derived from Oak silkworm (Antheraea yamamai) and Rapid PCR-based Screening Method

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A cosmid library has been constructed with genomic DNA isolated from oak silkworm, *Antheraea yamamai*. This library contained approximately 300,000 original clones. To estimate the quality of the library, as first step, a hundred of original cosmid clone were randomly picked up and cosmid DNAs from each clone were prepared and digested with *Eco*RI revealed that all the clones contain inserts and the average insert size is about 38 to 40 kb. Therefore, it was estimated that this cosmid library covers approximately 4.9 genome equivalents. To isolated rapidly and efficiently specific cosmid clones representing target gene from the library, the PCR-based screening system has been constructed. First, primary amplified cosmid clones were stored at about 20,000 colonies per well in 16 microtube X 10 and DNAs were prepared from cosmid clones of each well pooled in three-dimensions. The high sensitivity and specificity of the PCR allow the detection of target genes in three-dimensionally pooled clones. The PCR-based screening protocol consists of three successive PCR steps and colony hybridization. Three successive PCR steps efficiently limit the location of positive cosmid clone to a single well of 10X 16 microtubes. Final isolation of the positive clone is accomplished by colony hybridization using a single filter representing 2,000 cosmid from the positive microtube. To prove the quality of the constructed cosmid library and the rapidity and efficiency of the PCR-based screening, fibroin gene were used. The PCR-based screening results showed that the amplicons of fibroin gene tested present in simple three-dimensionally pooled cosmid clones. Southern hybridization and nucleotide sequences analysis was confirmed that the selected PCR-based clone is fibroin gene. These results demonstrate the establishment of a rapid and efficient PCR-based screening system for cloning and isolation cosmid clones containing silkworm-derived specific genes