High Throughput BAC Transgenesis for Gene Regulation Analysis in Zebrafish

Shuo Lin
Department of Molecular, Cell & Developmental Biology
University of California, Los Angeles
Los Angeles, CA 90095-1606

Email: shuolin@ucla.edu

The completion of genome sequence from multiple vertebrate species has enabled the identification of a large fraction of our gene catalogue and their physical chromosomal position. However, current efforts lag at defining the cis-regulatory sequences that control specific gene expression. Transgenesis with bacterial artificial chromosomes (BAC) offers greater fidelity in directing desirable expression of foreign genes. Application of this technology in the optically transparent zebrafish with the fluorescent protein reporters enables unparalleled visual analysis of gene expression in a living organism. Here have developed a streamlined procedure allowing direct selection of multiple BAC clones based on sequence database and rapid modification with GFP/RFP followed by transient transgenic analysis in zebrafish. The entire process can be achieved in less than three weeks. By comparing overlapping BACs for a gene of interest, critical regulatory regions can be quickly defined. This information, coupled with comparative bioinformatics employing sequences from other species, allows rapid identification and functional validation of multi-species conserved sequences (MCSs) in regulatory regions. As examples, data for modifying BACs for 20 genes and analysis of hematopoietic regulation of the zebrafish GATA-2 expression will be presented. These studies demonstrate the feasibility for large-scale high throughput generation and analysis of BAC-derived transgenic zebrafish.