BioSpotlight

Diving BAC into the Pool with Microarrays

Second-generation sequencing with its short read lengths is not practicable when determining whole-genome sequences from species possessing large genomes with a high content of repetitive DNA. Realistically, reference sequences for these species can only be obtained using physical map-based sequencing of BAC clones. A key step to this genome sequencing methodology is the ordering and orientation of BAC clones through their anchoring to a genetic map. This approach typically requires screening BAC libraries for clones with specific gene sequences using DNA hybridization or PCR, rendering it low-throughput and labor-intensive. In this issue, P. Hedley and colleagues at the Scottish Crop Research Institute (Dundee, Scotland) describe a new strategy for the high-throughput addressing of thousands of genes to thousands of BACs in parallel using a novel application of DNA microarray technology developed for the unsequenced 5.3-Gb genome of barley. Whole genome-amplified DNA derived from multi-dimensional pools of barley BAC clones was screened on a custom microarray of 42,000 expressed barley genes. The BACs were pooled using a previously developed “matrix” pooling strategy whereby the location of a clone along a

particular dimension (plate, row, or column of 384-well plates) of a Super Pool (SP) of seven 384-well plates could be determined by the presence of positive signals in two separate matrix pools in that dimension. As a proof of principle, the authors tested 2 SPs out of the 55 SPs from a barley BAC library with ~148,000 clones. All matrix pools from the first SP were labeled with Cy3 while those of the second SP were labeled with Cy5. Pairs of labeled matrix pools, one from each SP, were then cohybridized to a microarray. Signals extracted from the scanned images of the hybridized microarrays were analyzed using customized scripts to first identify those probes with a high signal in only two matrix pools in a particular dimension, and then to combine the information from all three dimensions for deconvolution and identification of specific BAC clones containing the microarray probe sequence. This cost-effective, high-throughput, and accurate method for anchoring BACs should be applicable to any species where a whole-genome sequence is lacking.

(See “Highly parallel gene-to-BAC addressing using microarrays” on page 165.)

Magnetic Attraction

Discovering the molecular mechanisms governing the differentiation of neural stem cells (NSCs) is essential before these cells can be used to develop therapies for neurodegenerative diseases. For those researchers wishing study specific genes that could induce or suppress differentiation of NSCs, an obstacle has been the difficulty in transfecting NSCs using conventional methods. Nucleofection and lipofection are commonly used for delivering genes to cells, but when applied to NSCs, they exhibit extensive toxicity soon after transfection and may interfere with studies of differentiation. While nucleofection does result in a reasonable percentage of transfected cells, it is costly and most cells die during electroporation. Writing in this issue of *BioTechniques*, Sapet et al. from OZ Biosciences (Marseille, France) along with colleagues from the National Center for Scientific Research in Paris demonstrate that magnetofection is a viable alternative method for efficiently delivering DNA to NSCs that minimizes undesired toxic effects and does not appear to affect differentiation. Magnetofection is accomplished by associating nucleic acid vectors carrying the desired sequences with super-

Neural stem cell differentiation. Images of neural stem cells acquired 4 days after NeuroMag transfection of a mix between a GFP vector together with either an empty vector (A) or a NeuroD1 expressing vector (B).

paramagnetic nanoparticles, which are added to a cell culture. When a magnetic field is then applied to the culture particles rapidly accumulate around the cells, leading to uptake of the DNA. The authors present an optimized protocol for transfecting NSCs using magnetofection, and show that the magnetofection method can be used to identify genes involved in the neuronal differentiation of NSCs. Because of the simplicity, low cost, reduced toxicity, and reliability of this method, the approach has the potential to be valuable for both small scale and high throughput projects seeking to identify new regulators of neuronal differenti-

ation, facilitating understanding of the differentiation of these cells and adding further insight into their potential for therapeutic development.

(See “High transfection efficiency of neural stem cells with magnetofection” on page 187.)

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