BioSpotlight

Breaking point

Chromosomal rearrangements often disrupt specific genes, potentially leading to genetic disorders or syndromes. Defining the molecular nature of these syndromes often requires mapping the breakpoint locations, but traditional methods for mapping breakpoints such as fluorescence in situ hybridization (FISH) lack the resolution to precisely pinpoint a break at the nucleotide level. Multiplex ligation-dependent probe amplification (MLPA) and Sanger sequencing offer that single nucleotide resolution, but are laborious and time consuming. In the search for a more effective way to localize chromosomal breakpoints, Okoniewski et al. from the University of Zurich, Switzerland tested the capabilities of two next-generation sequencing platforms in identifying breakpoints, and present their conclusions in this issue of *BioTechniques*. The authors selected the Illumina HiSeq 2000 and PacBio RS platforms, which offer a significant number of short 100 bp reads and long 2 kb reads, respectively, and compared their abilities to characterize large deletions previously identified by MLPA and microarray analyses. The authors found that both platforms provided sufficient read depths to identify precise breakpoint locations at the nucleotide level with comparable sample preparation costs. The approaches

differed in preparation time -- with the Illumina methodology requiring more time for sample preparation and sequencing -- and in the approach to breakpoint detection. With the Illumina system, breakpoints were seen as an increase in mismatches, in part because the short reads were able to map across the breakpoints since the mapping software allowed several mismatches. In contrast, PacBio data showed a clear drop of read depth in the deleted region. During this study, the authors found data analysis particularly challenging since few dedicated computational techniques were available for characterizing large deletions using next-generation sequencing. Therefore, Okoniewski et al. decided to create a new script to count matches at putative breakpoint sites. This script, along with the validating data for the Illumina HiSeq and PacBio platforms, comprise a new alternative for rapid breakpoint identification that requires fewer resources and less time than traditional Sanger sequencing.

See “Precise breakpoint localization of large genomic deletions using PacBio and Illumina next-generation sequencers” on page 98.

Functional miRNA screens using qPCR

MicroRNAs (miRNAs) are involved in the regulation of developmental, physiological, and pathophysiological processes. Observing the differential expression of an miRNA during development or pathogenesis, however, does not prove a direct role for that miRNA in regulating the process since changes in abundance could be the consequence and not the cause. Functional miRNA screens can determine whether candidate miRNAs with altered expression levels are in fact regulating a specific biological process, and high-throughput functional assays are critical for screening large numbers of candidate miRNAs to identify clinically relevant targets. Such high-throughput screens typically take advantage of large libraries of miRNA mimics or lentiviral miRNA overexpression constructs that are individually arrayed in microplates, but this approach requires expensive robotics and is subject to the variations and errors inherent in large-scale, multiwell HTP screens. A simpler alternative is to use pooled lentiviral or retroviral miRNA libraries to infect a population of cells such that each infected cell only has a single virus overexpressing a unique miRNA or miRNA cluster. Specific miRNAs in the pool whose overexpression in cells then causes either over- or under-

Experimental design of the miR-HTS method.

representation of a specific phenotype can be identified by quantitating unique miRNA expression cassette “barcodes”. Methods to quantify barcodes include next-generation sequencing, bead-based assays, and custom-made microarrays, but all of these approaches require expensive instrumentation and complicated bioinformatic analyses. In this issue of *BioTechniques*, C. Civin and colleagues at the University of Maryland School of Medicine demonstrate how quantitative PCR can be used as a simpler means to assay for specific miRNA barcodes in functional screens of pooled lentiviral miRNA libraries. As a proof of principle, the authors used their

“miR-HTS” method to screen for miRNAs regulating cell growth in the IMR90 human lung fibroblast cell line using a pooled human lenti-miRNA library and custom qPCR assays. Fifty nine growth-inhibitory miRNAs were isolated, of which only four were previously identified as inhibiting growth in human lung cells. Nine of twelve miRNAs randomly selected from among the 55 novel candidate growth-inhibitory miRNAs were validated in follow-up experiments to inhibit IMR90 cell growth. This miR-HTS method is simpler, less expensive, and presents a more flexible approach for functional miRNA screening using pooled lenti- or retro-viral miRNA libraries.

See “A simple high-throughput technology enables gain-of-function screening of human microRNAs” on page 77.

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