MAXING OUT BARCODES

A major challenge when it comes to next-generation sequencing (NGS) is the high degree of throughput possible with current systems. For smaller labs, there is often not a need to generate so many sequencing reads from a single lane. Barcoding is one approach to overcome this situation; researchers can examine multiple samples in the same run by coding each with a different barcode and then deconvoluting. But even here, if the goal is to maximize the number of samples in a run, bar codes can still come up short. In this issue of BioTechniques, Jeppe Andersen and his colleagues from University of Copenhagen, Denmark describe a simple technique to increase the number of possible barcodes up to three-fold. The authors take advantage of restriction endonucleases to digest samples in specific patterns prior to barcoding, creating an additional level of discrimination between concurrently run samples. In their proof-of-concept experiment, Andersen and his colleagues used 24 barcodes along with 3 different restriction endonucleases to sequence and identify 72 different samples. The authors supply a deconvolution algorithm to decipher restriction endonuclease patterns, as well as instructions on selecting appropriate enzymes, to make it even easier for researchers to maximize their barcoding efforts.

See “Next generation sequencing of multiple individuals per barcoded library by deconvolution of sequenced amplicons using endonuclease fragment analysis” on page 91.

A NUT-Y IDEA

Post-translational modifications can influence protein function; thus, identifying these modifications is key to understanding human biology and disease. Currently, gel systems capable of analyzing protein modifications require either expensive reagents or two-dimensional electrophoresis. In this issue of BioTechniques, Christopher Buehl and his colleagues at Michigan State University describe a new neutral pH gel system for the analysis of acetylation and phosphorylation protein modifications by one-dimensional electrophoresis. The neutral pH urea Triton polyacrylamide gel electrophoresis system, which the authors call NUT-PAGE, can separate proteins based on their charges at pH 7.0, generating distinct banding patterns for acetylated and phosphorylated proteins. The authors illustrate the capabilities of NUT-PAGE by analyzing the phosphorylation state of alpha-synuclein as well as the acetylated and phosphorylated species of histone H3. Taking advantage of inexpensive and readily available lab reagents, NUT-PAGE provides a cost-effective approach for analyzing post-translational modifications.

See “Resolving acetylated and phosphorylated proteins by neutral urea Triton-polyacrylamide gel electrophoresis, NUT-PAGE” on page 72.

Selected and written by Nathan Blow, Ph.D.
BioTechniques 57:57 (August 2014) doi 10.2144/000114193

PEPTIDE-BASED KINASE INHIBITORS

SEE THE LIGHT

Although optogenetics seems minimally invasive, the technique usually relies on overexpression of the protein of interest fused with a light-responsive domain, a strategy that may lead to non-physiological effects. An emerging alternative is engineering photoswitchable peptides to inhibit the activity of the target protein. On this basis, Yi et al. incorporated a 17–amino acid sequence from the PKA inhibitory peptide PKI into LOV2, a light-sensitive domain from a plant phototropin. Upon proper placement of the PKI peptide within LOV2, expression of the LOV2-PKI fusion approximately halved PKA activity in a light-dependent manner in living cells (presumably as the light-induced conformational change in LOV2 exposed the inhibitory motif). Analogous placement of a myosin regulatory light kinase inhibitor peptide produced a construct that reduced phosphorylation of its target by more than two-thirds. This selective, reversible strategy for peptide-based inhibition should be applicable to other kinases and potentially other enzymes where peptide inhibitors have been identified.


MICROFLUIDIC PREPARATION OF REPLICA MICROBIAL CULTURES

While metagenomics is invaluable for probing microbial diversity in environmental samples, detailed genomic and functional studies require pure cultures. Genetic analysis can identify appropriate colonies for cultivation but results in destruction of the target cells. A solution is replica cultures, one for use in destructive assays, and the other for scale-up culture. Ma et al. show that SlipChip, a microfluidic platform in which liquid handling occurs by sliding etched plates across each other, can produce individually addressable replica arrays of microbial cultures. First, the microbial sample is “slipped” into tiny wells, dispersing no more than one cell per chamber. After incubation, the plates are teased apart, giving two identical arrays of microcolony-containing wells. One plate can be used for genetic analysis, and based on the results, selected wells on the other plate can be picked for further culture. The replica SlipChip works well with anaerobic culture conditions, which are difficult to maintain in traditional microfluidic systems.


Selected and written by Nijsje Dorman, Ph.D.
BioTechniques 57:57 (August 2014) doi 110.2144/000114193