EASY QUANTIFICATION OF PROTEIN GLYCAN PHOSPHORYLATION

Phosphorylation of proteins, which is critical for their proper function and subcellular localization, can take place either directly on amino acid side chains or on the glycans of glycosylated proteins. Quantifying protein phosphorylation using current methods can be difficult, indirect, and time-consuming, especially for phosphorylated glycans. In this issue of *BioTechniques*, Chikkathur Madhavarao and colleagues at the Food and Drug Administration describe a rapid and direct approach using simple lab equipment for quantifying the relative amounts of phosphorylated glycans and phosphorylated amino acid side chains in proteins. Protein samples are separated by SDS-PAGE, and the gel is then stained with the fluorescent phosphate-specific binding dye Phos-Tag followed by a protein-binding fluorescent dye. Quantification of the binding of these two dyes provides the number of phosphates per protein molecule. Phosphorylated glycans are distinguished from phosphorylated amino acid side chains by treating the protein samples before gel separation with either peptide-N-glycosidase F (PNGase F) to remove N-linked glycans or with alkaline phosphatase to remove all phosphate groups.

See “Direct quantification of protein glycan phosphorylation” on page 117.

OPTIMIZED ONE-STEP SLIC

Sequence- and ligation-independent cloning (SLIC) is a seamless cloning method that exploits the exonuclease activity of T4 DNA polymerase to expose single-stranded regions at the ends of a linearized vector and a PCR-amplified insert to allow the complementary annealing of these regions. In one-step SLIC, the insert and linearized vector are mixed together with the T4 DNA polymerase in a single tube, incubated for 2.5 min at room temperature, and then transformed into competent bacteria. While this simple and cost-effective method works well, it can be inefficient for certain inserts. To overcome this issue, researchers led by Jung-Hyun Lee and Jae-Yeon Jeong, the developers of one-step SLIC, have now optimized the method. The team tested reducing the duration of T4 DNA polymerase treatment down to 5 s over a range of temperatures and found that a 30 s treatment at 50 °C resulted in high cloning efficiency for inserts with homology regions that are <20 bases and form strong secondary structures.

See “Optimizing T4 DNA polymerase conditions enhances the efficiency of one-step sequence- and ligation-independent cloning” on page 125.

Selected and written by Patrick C.H. Lo, Ph.D.

BioTechniques 63:95 (September 2017) doi 10.2144/000114582

MINIMUM INFORMATION FOR SINGLE CELLS

Advances in sequencing technology make it possible to reconstruct genomes from single cells or even from microbial metagenomes. Working with such samples is challenging, especially since both single cells and complex microbial communities (on a per-species basis) provide very little starting material, making DNA amplification prior to sequencing almost essential. However, whole-genome amplification methods can introduce contamination and sequence bias, diminishing the quality of the final genome/metagenome assembly. To address these issues, the Genomic Standards Consortium (GSC) presents new guidelines for the minimum information that should be reported when depositing into a database either single-cell genome assemblies or individual microbial genomes derived from metagenome assemblies. According to the GSC, the most critical criteria for assessing such genome assemblies are (i) assembly quality, (ii) completeness, and (iii) a measure of contamination. While single-cell genome studies are still at an early stage, the GSC's new standards provide a framework for reporting these criteria and will help guide the research community on how best to interpret and report data from single cells and metagenome assemblies.

Bowers, R.M. et al. 2017. Minimum information about single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea. *Nature Biotechnology*. 35:725-731.

ANIMALS IN THE VIRTUAL WORLD

Virtual reality (VR) isn't just creating unique experiences for computer gaming. It is also enabling new types of scientific research. One of the latest VR advances in the life sciences comes from Stowers et al., who constructed a new VR system for free-moving animals: FreemoVR. The authors validated FreemoVR for use with *Drosophila*, mice, and even zebrafish, demonstrating behavioral responses from the animals that would be expected in the real world. In a particularly interesting experiment, “virtual teleportation” was used to assess decision making in zebrafish. A special region (portal) would appear in the VR environment that, once the fish entered it, would teleport them to a new environmental scene. In another experiment, Stowers and his colleagues created a virtual fish that interacted with the real fish. This new system advances the use of immersive VR in the biology lab, creating new and intriguing opportunities for studying the impact of different environmental factors on living organisms using cutting-edge technology.

Stowers, J.R. et al. 2017. Virtual reality for freely moving animals. *Nature Methods*. DOI: 10.1038/NMeth.4399

Selected and written by Nathan S. Blow, Ph.D.

BioTechniques 63:95 (September 2017) doi 10.2144/000114582