TAGGING ALONG WITH CARBOXYLATED PROTEINS

Carboxylation of proteins is a biomarker for oxidative stress that is traditionally studied using two complementary techniques: immunoblotting and tandem mass spectroscopy. For both of these methods, tagging of the carboxylated protein using probes that form a covalent bond with the reactive carbonyl plays an important role. In this month's issue, Timothy J. Griffin and colleagues from the University of Minnesota, along with researchers at Thermo Fisher, introduce a novel multifunctional tagging reagent for analysis of carboxylated proteins. This derivative of the tandem mass tag (TMT) isobaric labeling reagents, called aminoxyTMT, contains a carbonyl-reactive aminooxy group. The authors demonstrate that, in combination with the anti-TMT antibody, aminoxyTMT labeling of carboxylated proteins allows for their visualization in immunoblots and their enrichment from complex mixtures. When used for tandem mass spectroscopy, aminoxyTMT enables the identification of carboxylated proteins and the sites of their modification as well as the relative quantification of the carboxylation state.

See “AminoxyTMT: A novel multi-functional reagent for characterization of protein carboxylation” on page 186.

dPCR SAMPLES CAN'T STAND THE HEAT

Digital PCR (dPCR) has greatly facilitated the accurate quantification of DNA mutations in tissue samples. Fragmentation of intact genomic DNA samples is required prior to dPCR, but there has been no study of the effects of different fragmentation methods on the performance of dPCR point mutation assays. In this issue of *BioTechniques*, Muneesh Tewari and her group at the University of Michigan examined three methods of fragmentation: heating, sonication, and restriction digestion. Wild-type genomic DNA treated by each method was analyzed by droplet dPCR to determine the wild-type and mutant allele counts for the *KRAS* and *BRAF* genes. While heating to 95°C is the easiest fragmentation method, it resulted in a high background of mutation detection for particular *KRAS* mutations compared to the other two methods. This effect appeared to be due to heat-induced mutations and specifically affected dPCR assays detecting guanine to adenine (G>A) mutations. Fragmentation by heating also overestimated gene copy number. These results indicate that researchers should be careful about which DNA fragmentation method they select for their dPCR-based mutation detection assays.

See “Mutant DNA quantification by digital PCR can be confounded by heating during sample preparation” on page 175.

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MEASURING GENE REGULATION

Relating transcription factor binding to specific patterns of gene expression has proven to be difficult, particularly at the single-cell level. But now, Sepulveda et al. describe a workflow for measuring gene regulation in individual cells. Using the lysogeny maintenance promoter of phage lambda as a model system, the authors first simultaneously measured concentrations of the lambda repressor CI in single cells, as well as numbers of mRNA transcripts from the promoter. To measure CI concentrations, they used an immunofluorescence assay, while single-molecule fluorescence in situ hybridization (FISH) provided measurements of promoter activity. By then modeling the CI concentration and transcript data, the researchers were able to define activity patterns that corresponded to specific CI binding configurations and uncover unique insights into promoter state switching. While applied initially to the lysogeny maintenance promoter, this approach should be adaptable to other genetic systems for promoter binding and gene expression studies.

Sepulveda et al. 2016. Measurement of gene regulation in individual cells reveals rapid switching between promoter states. *Science*. 351:1218-1222.

TISSUE BIOPRINTING GETS THICK

Three-dimensional bioprinting advances have made it possible to create complex tissue architectures in the lab. But more development is needed to fully realize the potential of this technique and use it to faithfully mimic the structure and longevity of human tissue. Now, Kolesky et al. report a new technique for 3-D bioprinting that allows long-term perfusion of thicker tissues on chips. To generate vascularized tissues within perfusion chips, they first printed silicone ink on the glass surface. After this, a cell-laden ink along with ink composed of a triblock copolymer was printed on the chip. The result was a network of interconnected channels lined with cells, which could be perfused over an extended period using external pumps. The authors validated their printing method by creating a simple tissue construct composed of two parallel channels embedded in a fibroblast matrix and then a more complex, heterogeneous tissue microenvironment >1 cm thick. This new bioprinting approach is another step towards creating more complex tissue architectures in the lab that can be used for ex vivo and, eventually, in vivo applications.

Kolesky, D. et al. 2016. Three-dimensional bioprinting of thick vascularized tissues. *Proc. Natl. Acad. Sci.* 113: 3179-3184.

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

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