ONE TUBE FOR YOUR CLICK NEEDS

Copper-catalyzed azide-alkyne cycloaddition (CuAAC), a variant of so-called “click” chemistry, has seen wide adoption within the biomedical community for conjugation reactions. Although this azide-alkyne cycloaddition reaction is commonly used for labeling biomolecules, current protocols require removal of the copper catalyst through multiple purification steps. In the December issue of *BioTechniques*, Michael Hill and his colleagues from Occidental College describe a one-pot, heterogeneous bioconjugation and purification solution for selectively activated CuAAC that makes click bioconjugations less cumbersome and time-consuming. The conjugation solution, a mixture of ethynyl-labeled molecules and azide-bearing ligands, is layered onto a size-exclusion spin column containing zing amalgam in an ion-exchange resin; centrifuging and washing result in clean and efficient coupling using the single column. The authors demonstrate the effectiveness of their one-pot solution by labeling a membrane-penetrating peptide and glycosylating a DNA oligonucleotide.

See “Heterogeneous catalysis for azide-alkyne bioconjugation in solution via spin column: Attachment of dyes and saccharides to peptides and DNA” on page 329.

KARYOTYPING FOR THE MASSES

“Do-it-yourself” solutions, where lab equipment designed for one use is adapted for a new purpose, are on the rise. Multispectral karyotyping uses chromosome-specific probes with unique combinations of fluorophores along with specialized instruments and analysis programs to dissect chromosome composition. Although powerful, the need for specialized equipment has limited use of this technique. In this issue of *BioTechniques*, Tamara Potapova and her colleagues at the Stowers Institute for Medical Research report a method for multispectral karyotyping using conventional laser scanning confocal microscopes. Advances in spectral microscopes have made it possible to separate overlapping emission spectra. Armed with this knowledge, the authors generated sets of chromosome paints from mouse and human chromosomes that they used to image chromosomes from single cells on a spectral microscope. To analyze the data, they designed companion software compatible with many open source programs, including ImageJ. Taken together, the researchers have created an effective methodology for multispectral karyotyping that does not require specialized instrumentation, making the technique accessible to more labs.

See “Karyotyping human and mouse cells using probes from single-sorted chromosomes and open source software” on page 335.

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

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MICROWELL ARRAYS FOR EXTENDED CELL CULTURE

High-throughput single-cell analysis usually involves sophisticated microfluidic equipment. Microwell arrays are a simpler alternative, but entail tiny, rapidly depleted volumes of culture medium. To sidestep this problem, Ornoff et al. developed microwell arrays with a permeable base made from the cationic polysaccharide chitosan (micropatterned epoxy photoresist film is used to fabricate the well walls). Seeded cells can be overlaid with mineral oil to prevent well-to-well biochemical crosstalk and cell migration. Beneath the wells is a shared reservoir of cell culture medium, which cells can access through the chitosan, allowing cell growth for up to 4 days. Unlike commercially available permeable supports for cell growth, the chitosan layers have a molecular exclusion limit of 10 kDa, allowing metabolites and nutrients to easily diffuse while forming a de facto barrier for many signaling molecules. This platform will facilitate probes of cellular heterogeneity, particularly those involving extended culture or monitoring of dynamic responses to small molecule stimuli.

D.M. Ornoff et al. Co-fabrication of chitosan and epoxy photoresist to form microwell arrays with permeable hydrogel bottoms. *Biomaterials*. [Epub ahead of print, September 28, 2015; doi: 10.1016/j.biomaterials.2015.09.032].

IMPROVED GENE FINDING WITH RNA-SEQ

The explosion in RNA-seq data provides opportunities to identify previously unannotated genes. Although de novo transcript assembly methods can identify genes in the absence of a reference genome, greater accuracy is possible with ab initio algorithms that align short reads to reference sequences. Now, Watanabe et al. introduce what may be the most powerful ab initio program to date, Tiling Assembly. Using RNA-Seq data from rice, Tiling Assembly identified substantially more potential novel genes—that is, nonoverlapping with annotated genes—than Cufflinks, an established assembly program. Most of the predicted genes appeared to be protein-coding, and comparison to full-length cDNA data revealed a high level of accuracy in Tiling Assembly’s gene identification. Applying the Tiling Assembly workflow to four other model organisms yielded high-confidence predictions for novel genes, suggesting both the flexibility of this pipeline and the existence of undiscovered genes in highly studied organisms.

K.A. Watanabe et al. 2015. Tiling Assembly: a new tool for reference annotation-independent transcript assembly and novel gene identification by RNA-sequencing. *DNA Res.* 22:319-29.

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