THE HEART OF THE MATTER

A picture is worth a thousand words—or so it is said. A picture might also be worth a thousand biochemical assays in some instances. When it comes to studying the dynamics of a beating heart, the ability to image the heart during all phases of contraction and relaxation and then extract meaningful physiological information from those images can be invaluable to understanding normal and abnormal cardiac function. In this issue, a team from Sanford-Burnham Medical Research Institute describes new advances for their semiautomatic optical heartbeat analysis (SOHA) application. The original SOHA program, which was described in *BioTechniques* in 2010, can be used to quantify a variety of functional parameters in the *Drosophila* heart. This latest version of the program allows for more precise quantification of cardiac contractions, increased signal to noise ratios, and reduced cost and time when analyzing the information contained in high-speed videos of beating hearts, all using an enhanced graphical interface. The latest SOHA program also makes possible the determination of various subphases of contraction and relaxation in the fly heart. Taken together, this new SOHA program will prove a welcome advance for researchers interested in exploring the cardiac dynamics of this well-studied model organism.

See “Enhanced assessment of contractile dynamics in *Drosophila* hearts” on page 77.

COLOR CHANGING AMPLIFICATION

Rapid detection of nucleic acid amplification products can enhance throughput in research labs as well as enable assays at field locations or in diagnostic settings. In this issue, scientists from New England Biolabs report a new technique to rapidly visualize nucleic acid amplification that takes advantage of the biochemistry of the reaction mixture. Upon incorporation of a new deoxynucleoside into a growing DNA chain, a pyrophosphate and a hydrogen ion are released into the media. Release of hydrogen ions causes a shift in the reaction pH that can be detected with the right methodology. Using a low buffer concentration and a pH-sensitive indicator dye, the authors showed that amplification products could be detected by a simple color change with high sensitivity (less than 10 copies) and in multiple assays (including loop-mediated isothermal amplification and PCR). This new pH-sensitive dye approach to amplification detection should provide a path towards rapid molecular diagnostic testing without the need for additional equipment or time.

See “Visual detection of isothermal nucleic acid amplification using pH-sensitive dyes” on page 59.

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

BioTechniques 58:51 (February 2015) doi 10.2144/000114251

ULTRASENSITIVE PROTEOMICS

Taking a cue from sample handling in genomics, Hughes et al. have devised a method for ultrasensitive proteomic analysis using paramagnetic beads. In the procedure, called single-pot solid-phase-enhanced sample preparation (SP3), cells or tissue samples are lysed, reduced, and alkylated in the presence of commercially available carboxylate-coated paramagnetic beads. Upon adding an organic solvent, the proteins are trapped in a layer around the hydrophilic beads. A series of 70% ethanol and acetonitrile washes efficiently removes detergent and chaotropes without dislodging the proteins. The same steps can be used to purify peptides from digestion buffer for analysis by mass spectrometry. As a single-tube method, SP3 minimizes sample loss, making it ideal for quantity-limited inputs such as single *Drosophila* embryos. The procedure is compatible with chemical labeling, and quantitative comparisons established the reproducibility of SP3 preparations from replicates of challenging (5000 cells) samples. Because paramagnetic beads are already used in high-throughput protocols, SP3 should prove as scalable as it is sensitive and cost-efficient.

C.S. Hughes et al. Ultrasensitive proteome analysis using paramagnetic bead technology. *Mol Syst Biol.* 10:757.

PREDICTING POTENT SHORT HAIRPIN RNAS

The increasing attention given to reproducibility in research is particularly pertinent to RNAi using short hairpin RNAs (shRNAs), as screens performed by different labs have produced widely varying results. To improve the potency and specificity of shRNAs, Knott et al. present a new design algorithm, shERWOOD. Using a method they previously developed for parallel assessment of shRNA potency, the authors collected data on a quarter-million shRNAs and performed an in-depth analysis of efficacy versus sequence to develop shERWOOD. The resulting shRNA prediction algorithm designed shRNA libraries that outperformed existing shRNA resources. In detailed analyses of selected individual targets, the majority of shERWOOD-predicted shRNAs reduced target mRNA levels by 80%. Importantly, off-target effects were limited. The shERWOOD web portal (<http://sherwood.cshl.edu:8080/sherwood/>) lists shRNAs for constitutive exons of all human, murine, and rat protein-coding genes. For custom shRNA prediction (e.g., for other model organisms or noncoding RNAs) shERWOOD is available as an online tool.

S.R. Knott et al. 2014. A computational algorithm to predict shRNA potency. *Mol Cell.* 56:796-807.

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BioTechniques 58:51 (February 2015) doi 10.2144/000114251