ADDING TO PCR

When it comes to the polymerase chain reaction, specificity and reaction efficiency are the keys to success. Amplifications need to be specific to minimize the generation of off-target amplicons, and efficiency is required to produce the maximum possible number of copied amplicons. PCR enhancers, such as bovine serum albumin (BSA), have been used in the past to improve PCR performance and thereby increase both specificity and efficiency. In the December issue of *BioTechniques*, Zhang et al. describe the use of a new PCR additive, bovine thrombin (BT), which is a coagulation protein. The authors were able to demonstrate the advantages of using BT in PCR by testing a variety of sample types under different conditions. When directly compared with BSA, BT was more effective; even when used at concentrations up to 178 times less than BSA, BT was still able to achieve similar levels of enhancement compared to BSA. This new PCR additive should prove a welcome addition in the PCR toolkit.

See “Bovine thrombin enhances the efficiency and specificity of polymerase chain reaction” on page 289.

A VARIABLE CITY OF BACTERIA

Biofilms have been described as microbial cities where bacteria can work together. So it should not be too surprising to learn that some aspects of bacterial biology change when bacteria are aggregated in a biofilm, including virulence. To explore these changes, researchers have turned to quantifying gene expression patterns from biofilm-associated and non-associated bacteria. However, this can prove very difficult with biofilm samples since there are a number of experimental steps where variability can be introduced when measuring mRNA levels. In this issue of *BioTechniques*, a team of researchers led by Nuno Cerca from the University of Minho examined these steps to locate where the majority of variability creeps into biofilm measurements. After carefully assessing the RNA extraction, cDNA synthesis, and qPCR steps, the authors found it was actually the biofilm growth conditions that led to the largest bias in gene expression quantification. Armed with this information, they asked a simple question—could pooling biofilm samples reduce this variation? Data from pooling experiments showed that when 20 biofilm samples were combined, gene expression variability was significantly reduced, leading to more accurate gene expression analysis from biofilm samples and a better picture of life within these bacterial communities.

See “Assessing and reducing sources of gene expression variability in *S. epidermidis* biofilms” on page 295.

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

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ANALYZING HISTONE MODIFICATIONS

Antibodies to histone post-translational modifications (PTMs) have been key to chromatin research, but lot-to-lot fluctuations and incomplete documentation of cross-reactivities hamper their usefulness. To improve reproducibility of chromatin studies, Kungulovski et al. examined histone modification interaction domains (HMIDs) as antibody alternatives. The researchers found that several HMIDs purified as GST fusions met quality control criteria established for histone PTM antibodies. The HMIDs were also able to functionally replace antibodies in a variation of the chromatin immunoprecipitation assay the authors named chromatin interacting domain precipitation (CIDOP). In combination with next-generation sequencing, CIDOP was capable of genome-wide chromatin analysis. While HMIDs can suffer from cross-reactivity, their nearly inexhaustible supply as recombinant proteins minimizes batch variations that compromise reproducibility. Further engineering of HMID proteins will allow addition of affinity tags, creation of site mutant negative controls, and, potentially, design of proteins with novel specificities.

G. Kungulovski et al. 2014. Application of histone modification-specific interaction domains as an alternative to antibodies. *Genome Res.* 24:1842-53.

IN-DEPTH PHOSPHOPROTEOMICS

The more completely the phosphoproteome can be defined, the better our understanding of intracellular signaling cascades. In a typical phosphoproteomic workflow, tryptic peptides from cell lysate are fractionated by strong cation exchange (SCX) chromatography to reduce sample complexity; phosphopeptides are then enriched by metal oxide affinity chromatography prior to liquid chromatography–tandem mass spectrometry. In general, high pH (HpH) reversed-phase chromatography, which separates peptides by hydrophobic interactions rather than charge, offers better resolving power than SCX, but its effects on phosphoproteomic coverage have not been rigorously investigated. Batth et al. describe the development of an optimized offline HpH fractionation protocol, followed by double titanium dioxide enrichment to recover multiphosphorylated proteins that tend to be found in the initial column flow-through. They showed 6-fold improved identification of phosphopeptide variants compared to the SCX-based approach, including detection of low-abundance tyrosine phosphorylation sites without the need for affinity purification using phosphotyrosine-specific antibodies.

T.S. Batth et al. Off-line high-pH reversed-phase fractionation for in-depth phosphoproteomics. *J Proteome Res.* [Epub ahead of print, November 4, 2014; doi: 10.1021/pr500893m].

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