BioSpotlight

Bait and Prey

Protein interactions are essential to nearly every cellular function, making studies to identify and characterize these associations crucial to understanding biological processes. While co-immunoprecipitation has proven a valuable technique for detecting protein-protein interactions, it requires one antibody to isolate the bait protein and another to detect an interaction. This dependence upon antibodies increases the costs associated with protein interaction studies and reduces reliability, since antibodies can vary in quality among manufacturing lots and aren't uniformly validated. In an effort to develop a method for probing protein-protein interactions that does not rely on antibodies, C. Huang and K. Jacobson of the University of North Carolina, Chapel Hill (Chapel Hill, NC, USA) turned to the high-affinity biotin-avidin interaction. Their technique, which is called the AviZZ system, uses a bait protein that has been fused to the synthetic IgG binding protein ZZ, while a potential interaction partner is labeled with the 15-amino acid Avitag, which can be recognized and biotinylated by BirA, a recently discovered *Escherichia coli* biotin ligase. Following co-expression of the two modified proteins, biotin treatment to activate BirA, and cell

lysis, the ZZ-fused bait protein can be easily precipitated using inexpensive, non-immune rabbit IgG-conjugated Sepharose beads while the biotinylated interacting protein can be detected by Western blot using fluorescent streptavidin. As a demonstration of the system, protein-protein interaction assays were done to test for the coprecipitation of paxillin and Git1, and a similar strategy was employed for GST pulldown assays. When compared with conventional antibody detection, the Avitag-BirA system exhibited up to 18-fold greater sensitivity with 5-fold greater efficiency. The use of IgG for precipitation and fluorescent streptavidin for detection also reduces the expense and time required to perform protein interaction studies, since antibodies are not required and the detection can be performed in a single step. Together, Huang and Jacobsen's data show that the AviZZ system is an advantageous alternative when it comes to protein interaction studies in cells that overexpress proteins of interest.

See "Detection of protein-protein interactions using nonimmune IgG and BirA-mediated biotinylation" on page 881.

Out of the Darkness

DNA amplification using isothermal approaches presents a wealth of possibilities when it comes to point-of-care diagnostics. Isothermal amplification methods, such as rolling circle amplification, strand displacement amplification, nucleic acid-based amplification (NASBA) and loop-mediated isothermal amplification (LAMP), are particularly attractive in remote settings and third-world countries since these approaches do not rely on complicated and expensive thermocyclers to rapidly change temperatures, as is required in traditional PCR amplification. But thus far isothermal amplification has remained a bit of a "black box;" it is not clear when certain steps occur in the amplification process, such as when intermediate products are generated and how important such products are to the overall amplification reaction. A clearer understanding of these steps could lead to improved reaction conditions and reagents, thereby enhancing the diagnostic potential of isothermal amplification methods. Usui et al. have taken a large step toward better understanding isothermal amplification in the current issue of *BioTechniques*. Using a novel method, the authors determined the actual time point of intermediate product generation during turn-back primer isothermal DNA amplification (TIA). Initially, the authors had to

Schematic model of two types of turn-back primer. After primary annealing and DNA extension, the primer "turns back" onto complementary sequences on the newly extended DNA.

answer the question of whether the generation of intermediate products affected the outcome of the isothermal amplification reaction at all. Reasoning that if intermediate products influenced the time to amplification in TIA then the reactions should be shorter in the presence of intermediate product, the authors mixed intermediate products they generated in the lab with plasmid templates. They found that

in the presence of an increasing concentration of intermediate product, the time to exponential amplification decreased. By then extrapolating dsDNA amplification curves to identify the time point of initial intermediate product generation, Usui et al. demonstrated that generation of this product is in fact a rate-limiting step for isothermal amplification using the TIA method, and that the design of the TIA primers can significantly influence the efficiency of intermediate product generation. The insights provided by Usui et al.'s method have the potential to expand the use of isothermal amplification by shining a light on one of the most critical steps in this amplification process.

See "Effect of turn-back primer for intermediate product generation on isothermal DNA amplification" on page 888.

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