gives information on the reactivity of the active-site cysteine.

This method does have limitations. An active-site cysteine with a pK_a several orders of magnitude lower than the other cysteine residues is required. At the moment, this value of pK_a is mainly found in tyrosine phosphatases and cysteine proteases. Moreover, because the difference in reactivity between the active site and other cysteines is only about 150-fold, the method cannot be applied to crude cell extracts (with contaminating proteins), but only to purified protein that is soluble at micromolar concentrations. Finally, reducing agents (β-mercaptoethanol or dithiothreitol) should not be present, because they will interfere with the assay. So, the enzyme must be stable in the absence of such reducing agents, at least for the duration of the assay.

In this era of expensive laboratory equipment, commercial kits, and premixed buffers, it is refreshing to run into an experiment in which researchers have applied the trusty spectrophotometer and routine laboratory chemicals to test a clever hypothesis.

–Richard Peters and Robert Sikorski

References

1. M. Pregel and A. Storer, *J. Biol. Chem.* **272**, 23552 (1997).
2. M. Pregel, T. Sulea, E. O. Purisima, A. Storer, in preparation.

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"Lighting" Rods

SIGHTINGS

Clever applications of new molecular technologies seem to come along in waves. A decade later, the tsunami launched after the development of the polymerase chain reaction technique has yet to crest. Now, brace yourself for the rising tide of papers showing novel applications of the green fluorescent protein (GFP). Isolated from jellyfish, GFP is a sensitive and versatile molecular tag that can be used in many biological studies.

A new GFP-based technology was recently published in *Science* by Valdivia and Falkow (*1*). The question was relatively straightforward. The scientists were interested in knowing the identity of the genes that are responsible for the virulence of the organism *Salmonella typhimurium*. Humans infected with *Salmonella* can suffer from gastroenteritis; mice will develop murine typhoid fever. The investigators made the assumption that *Salmonella* virulence genes would be turned on during bacterial infection, specifically when the bacteria were phagocytized by macrophages, a host defense that occurs early in vivo. Once internalized, *Salmonella* can evade destruction and survive as intracellular parasites.

The investigators designed a clever and robust selection scheme using GFP as a marker to tag those genes turned on only when *Salmonella* were in an intracellular environment. A library of plasmid clones was made that linked random sequences of the *Salmonella* genome upstream of a promoterless GFP gene. By chance, some of these sequences contained bacterial promoters capable of driving GFP expression. This library was introduced into a *Salmonella* host where plasmids exist as episiomes. Next, they selected bacterial clones that expressed GFP after the bacteria were phagocytized in culture. For this they used a macrophage-like cell line called RAW 264.7 that can phagocytose *Salmonella* in vitro. Bacteria were collected after lysis of the RAW 264.7–infected cultures and the light-producing strains were collected by fluorescence-activated cell sorting (FACS). Next, they took this sorted bacterial population, grew it in culture, and counterselected against clones that could express GFP outside of the RAW 264.7 cells by FACS sorting and collection of the nonfluorescing bacteria.

The alternating positive/negative selection

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served to isolate plasmids containing *Salmonella* promoters that were turned on specifically by phagocytosis. By infecting mice with such bacterial clones, they could show easily that the spleens and livers of the mice contained bacteria in the form of fluorescent rods. Sequence analysis of the natural *Salmonella* genes downstream of the GFP-tagged promoters revealed that several clones were already known to be involved in *Salmonella* virulence. In addition, several new genetic loci were also linked in this organism to the virulence process.

Clearly, the powerful sorting made possible by alternate positive/negative selection with GFP can be extended further in both bacterial and eukaryotic systems. It may be possible, for instance, to use GFP as a marker in mammalian cells in cultures to sort high-complexity plasmid libraries after transfection. Given an in vitro selection scheme, regulated eukaryotic promoters could be identified much like the *Salmonella* promoters. In addition, the use of GFP protein fusion libraries may provide a visual means to identify novel components in subcellular structures, as has been done previously with fusion proteins in yeast.

–Robert Sikorski and Richard Peters

Reference

1. R. H. Valdivia and S. Falkow, *Science* **277**, 2007 (1997).

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Internet Photoshopping

NET TIPS

The presentation of scientific data these days requires the use of ever more sophisticated graphics tools for Web site design and photo editing. The workhorse program in most labs is usually Adobe's Photoshop. Computer images can be cut, pasted, layered, and beautified in endless ways with just the standard Photoshop set of tools. However, the program itself can be easily tailored to adopt new functionalities in the form of plug-ins or filters. These small software modules will give the user the power to create breathtaking visual effects. Moreover, many of these modules are free on the Internet. Here is a sampling of Web sites to help with your graphic needs.

Photoshop Web Reference
www.adscape.com/eyedesign/photoshop/
This site is extremely well designed and can serve as the starting point for anyone new to Photoshop. It gives key background for using the basic tools, palettes, and filters.

Macworld Top 20
www.macworld.com/pages/september.96/Feature.2587.html
This article covers their list of 20 best Photoshop plug-ins. The detailed descriptions of each will give you an idea of what these tools can do for your images.

Ultimate Photoshop
www.sas.upenn.edu/~pitharat/photoshop/filters/plugins.free.html
As the name implies, this may be one of the most comprehensive collections of Photoshop add-ons on the Net today. If you are interested in free filters and plug-ins, you'll find them here.

–Robert Sikorski and Richard Peters

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Cookie Monster?

Cookies are small data structures sent from a Web server to your browser and saved on your hard drive in a text file. They are nothing more than a string of characters (letters