**Focus on fluorescence imaging**

Fluorescence imaging is becoming an increasingly important methodology in the biological sciences. The relatively noninvasive nature of the technique and its many applications have prompted many biologists to buy expensive equipment and take advantage of the power of the technique. Thus it is important that new and experienced users fully understand the underlying principles of the different fluorescence imaging modalities.

Beginning on page 901, pioneers and experts review this important topic. In a commentary, Yuste discusses the impact of recent advances in fluorescence imaging, and in a perspective, Tsien and colleagues provide practical advice on choosing appropriate fluorescent proteins. The core reviews of the focus contain discussions of the basic principles of fluorescence imaging and practical advice for using different imaging modalities including epifluorescence, optical sectioning methods such as confocal and two-photon microscopy, and fiber-optic fluorescence imaging.

**FOCUS p 901–949**

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**Invasion of siRNA**

The determination of effective siRNA molecules that efficiently knock down expression of a target gene can be a time-consuming trial-and-error task. If one desires to obtain a range of different siRNA potencies in order to study a gradient of expression of a gene of interest, this task becomes even more arduous. Shen and colleagues describe a clever method to quickly screen large numbers of siRNA molecules. Their procedure exploits the ability of bacteria to invade mammalian cells and introduce shRNA molecules capable of knocking down a target gene of interest. Using a luciferase–target gene fusion and a library of shRNA expressed in bacteria, they demonstrate that this method can be used to find many effective siRNA molecules.

**Article p967**

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**In rapid pursuit of mutations**

Pathogenic bacteria have mechanisms to invade eukaryotic cells and/or modulate their function. Many Gram-negative bacteria have evolved a secretion system that delivers bacterial proteins directly into the host cell. Despite the importance of this type III secretion system in mediating bacterial pathogenicity, there is little information concerning the details of how this system works. In particular, methods to examine its spatiotemporal function. Enninga and colleagues have developed a method based on the FlAsH reagent—a molecule that becomes fluorescent upon binding to a tetracysteine motif on a target protein—to directly visualize the real-time kinetics of type III secretion in *Shigella flexneri*.

**Article p959, News and Views p898**

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**Custom-made frogs**

The South African clawed frog better known as *Xenopus laevis* has long been a model organism for the study of vertebrate development. The more recent use of transgenic frogs, expressing exogenous genes or reporter genes under the control of regulatory sequences have greatly accelerated these studies. Although conceptually simple, the generation of transgenic *X. laevis* is a technical bottleneck for many laboratories. Allen and Weeks now present a method that, with the aid of the bacteriophage φC31 integrase, widens this bottleneck. They show that plasmids with an integrase recognition site can successfully recombine with the genome of frog embryos. When the genes to be studied are insulated from chromosome position effects, the result is a large number of healthy, transgenic embryos that may yield important clues as to how genes are regulated during frog development.

**Article p975, News and Views p897**

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Robust, high-throughput sequencing should allow rapid identification of these mutations. The groups of Albert and Berg now introduce ‘comparative genome sequencing’, based on hybridization of genomic DNA to high-density DNA microarrays, allowing rapid genome resequencing and mutation identification. This will be of interest in every area of research where mutation discovery is needed, including understanding the evolution of drug resistance and characterization of new infectious pathogen strains.

**Brief Communication p951**

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**Visualize the virulence**

Pathogenic bacteria have mechanisms to invade eukaryotic cells and/or modulate their function. Many Gram-negative bacteria have evolved a secretion system that delivers bacterial proteins directly into the host cell. Despite the importance of this type III secretion system in mediating bacterial pathogenicity, there is little information concerning the details of how this system works. In particular, methods to examine its spatiotemporal function. Enninga and colleagues have developed a method based on the FlAsH reagent—a molecule that becomes fluorescent upon binding to a tetracysteine motif on a target protein—to directly visualize the real-time kinetics of type III secretion in *Shigella flexneri*.

**Article p959, News and Views p898**

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