Focus on RNAi in mammalian systems

You are studying a mammalian system, and you are interested in knocking down your favorite gene: of course you are thinking about RNA interference. But then you might ask, ‘What is the best way to select for effective and specific siRNAs? What are the best experimental approaches to maximize their specificity? What controls should be included to validate RNAi results?’ And perhaps you are interested in using RNAi selectively in certain tissues or conditionally in culture or in vivo. Turn to page 669 to find a set of Perspectives loaded with practical advice. In addition, you will also find a comparative analysis of siRNA and shRNA libraries that are currently available for functional genomics screens. Happy RNAi!

Quantitative phosphotyrosine profiling

Tyrosine phosphorylation of signaling proteins has a central role in many important biological processes. Nollau and colleagues have demonstrated previously that analysis of SH2 domains can be used to generate profiles of the global tyrosine phosphorylation state of cells. They now describe a clever improvement on this technique that results in a substantial advance in sensitivity and quantitative ability of the method. This was achieved by attaching oligonucleotide tags to the SH2 domains, allowing multiplex detection and quantification of phosphotyrosine substrates by real-time PCR. The assay can be easily expanded to other SH2 domains or even other protein binding domains.

Truly digital molecular detection

Quantitative PCR has proven to be a powerful method for quantifying numbers of DNA molecules or, by extension, any molecule tagged with a piece of DNA. Unfortunately the method relies on enzymatic amplification, and therefore the readout is actually an ensemble average. By combining proximity probe ligation and rolling-circle amplification in a solution-based reaction format, Nilsson and colleagues describe a method that permits true digital quantification of DNA or protein molecules. This has the potential to substantially improve the simplicity and accuracy of single-molecule detection.

GFP imaging in STED

As biologists examine biological processes in ever greater detail, there is increasing interest in ultrahigh-resolution fluorescence microscopy. Ideally, researchers would like to be able to follow individual fluorescent molecules in living cells. Stimulated emission depletion (STED) fluorescence microscopy has shown that it can resolve details with a resolution well below 20 nm, but until now it has been restricted to use with organic dyes. Hell and coworkers now show how this microscopy technique can be used with fluorescent proteins. Using this method they were able to obtain the first ~20 nm resolution images of GFP-labeled proteins in mammalian cells. This development brings ultrahigh-resolution imaging in living cells one step closer to reality.

A third base pair for DNA

Several research groups have been working toward expanding the DNA alphabet to include base pairs not found in nature. It is not a simple pursuit, however, to design unnatural base pairs that can be recognized by DNA and RNA polymerases for practical biological applications beyond the realm of ‘science fiction’. Hirao and colleagues now describe a new, hydrophobic DNA base pair that can be amplified by PCR with very high fidelity and that is transcribed into RNA with high efficiency. Additionally, the new system allows site-specific labeling of RNA with a small chemical tag such as biotin or a fluorophore.