IN THIS ISSUE

Protocol of the month

Hannon and colleagues describe a step-by-step protocol to clone short hairpin (sh) RNAs targeting a gene of interest into a retroviral vector. Expression of the shRNA sequence is driven by the RNA polymerase III promoter responsible for expression of small RNAs in the cell. Introduction of this construct into mammalian cells provides continuous expression of the shRNA and thereby achieves long-term knockdown of the target gene for both *in vitro* and *in vivo* experiments. In contrast to chemically synthesized short interfering (si) RNAs, this system facilitates lasting depletion of any gene of interest in primary or cultured cells as well as in whole organisms. The easy-to-follow protocol highlights critical steps of the procedure and provides tips for troubleshooting.
Protocol p163

New SNP tools make genotyping a snap

The abundant single nucleotide polymorphisms, or SNPs, make powerful genetic markers. Two groups present new high-throughput SNP platforms devised to allow rapid and high-quality genotyping. One platform, presented by Murray and colleagues, is specifically designed for genetic linkage analysis and is accompanied by a genetic map of the markers. The extremely low rates of genotyping errors attained with this platform promise linkage data of unprecedented quality. Matsuzaki *et al*., in turn, report on a pair of chips allowing the simultaneous genotyping of 100,000 SNPs, a great asset for association studies.
Article p113, Brief Communication p109, News and Views p104

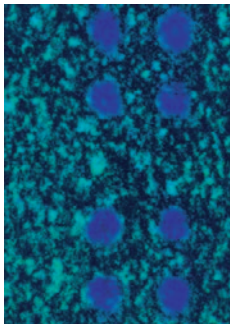
Analysis of protein methylation by mass spectrometry

Protein methylation is increasingly recognized as a crucial player in multiple cellular processes. The ability to identify new methylation sites and quantify the methylation status of individual proteins in various cellular conditions is essential for the understanding and characterization of the role of this post-translational modification. Mann and colleagues have adapted their SILAC technique (stable isotope labeling by amino acid in cell culture) to efficiently characterize protein methylation by mass spectrometry. Their method exploits the fact that natural protein methylation in the cell relies on a single primary methyl group donor. By supplying the cells with a labeled precursor in this metabolic pathway, they achieve direct labeling of the post-translational modification, increasing the confidence of its identification and quantitation.
Article p119

Fluorescence to build a better optical trap

Optical trapping and single-molecule fluorescence are two powerful tools to study the behavior of single molecules. While these two techniques have been widely used separately, their combined application in a defined location has been beset by technical challenges intrinsically linked to the enormous difference in the light levels involved, which can differ by 15 orders of magnitude. By carefully designing an instrument with optimized optical parameters, Block and colleagues have overcome some of these hurdles and now demonstrate the simultaneous use of an optical trap to apply force to a single labeled DNA molecule while recording the mechanistic consequences via fluorescence measurements.
Article p133

KO for the fly genome—all on a few slides



For a comprehensive analysis of gene function, a method in which every gene can be systematically targeted and knocked down is desirable. David Sabatini and colleagues developed an RNAi-based screen for *Drosophila* cells in which they arrayed 384 different dsRNAs on a microscope slide. The cells seeded on top of the dsRNAs showed localized depletion of the targeted genes, and any phenotype of interest could be easily evaluated by automated microscopy. Here, the authors identified genes involved in cell proliferation and cytoskeletal morphology. In a combinatorial screen, they transfected cells growing on the printed dsRNAs with an additional dsRNA targeting dPTEN and found novel genes involved in the dPTEN-independent regulation of dAkt phosphorylation. The density of dsRNA printing will allow them to array ~5,600 dsRNAs on a single slide so that the whole fly genome can be screened on only a handful of slides.
Article p127, News and Views p103