otide tag that would allow them to orient the read and determine whether it came from the sense or the antisense strand.

To efficiently deal with the enormous amount of data generated by high-throughput sequencing technology, Jacobsen and Pellegrini developed algorithms that improved the quality of the base called during the actual sequencing procedure, allowed better mapping of the reads to the genome and filtered out any reads that still contained unconverted cytosines.

They installed filters in their analysis program that eliminated all reads that did not uniquely map to the genome and ended up with a DNA methylation map that comprised 84% of the plant genome.

The results speak to the increased sensitivity of this bisulphite-sequencing method over microarray-based techniques. The team at UCLA was able to find new methylation sites in genes previously classified as unmethylated; they mapped methylation across highly repetitive ribosomal DNA loci and accurately detected methylated promoters.

Of course such high-resolution methylation mapping is of interest not only to the plant community. Jacobsen is certain that their approach is transferable to higher organisms such as mouse and human. He sees the main limitation at this point in the high cost of sequencing for large genomes but adds confidently: "sequencing technologies are improving their throughput at a fast pace, so this technique will be practical quickly."

Detailed methylation patterns may soon be as self evident a resource as primary genomic sequences are at the moment.

Nicole Rusk

RESEARCH PAPERS

Cokus, S.J. *et al.* Shotgun bisulphate sequencing of the *Arabidopsis* genome reveals DNA methylation patterning. *Nature* **452**, 215–219 (2008).

locus "is inefficient for many studies because it does not drive uniformly high levels of expression in all tissues."

But how to counter these tissue-specific position effects to induce truly ubiquitous expression? The researchers flanked the transgenes with insulator elements. Addition of these insulators profoundly increased expression of the transgenes in all tissues and effectively blocked the tissue-specific position effects. So although the researchers did not find a 'golden locus' that could produce uniform, ubiquitous expression, they could create the same effect with insulator elements while making transgenic flies efficiently with site-specific integration. This particular insulator acts similarly to vertebrate CTCF insulators, suggesting the latter might also boost transgene expression in mice. But for flies, researchers at Janelia Farm are already using this strategy to develop a long hoped-for RNAi library.

These transgene landing site–containing fly lines have other uses as well. As Markstein proposes, "a series of sites with different levels of inducibility in a particular tissue may be targeted to create a controlled allelic series, or a single site may be selected because of its high or low inducibility in a specific tissue." Ultimately, though, she hopes to use these fly lines to study what makes them so capricious in the first place: position effects.

Katherine Stevens

RESEARCH PAPERS

Markstein, M. *et al.* Exploiting position effects and the gypsy retrovirus insulator to engineer precisely expressed transgenes. *Nat. Genet.*, published online 2 March 2008.

NEWS IN BRIEF

MICROSCOPY

High-speed super-resolution imaging

Methods for imaging fluorescent samples at resolutions much greater than possible with conventional imaging have only begun to be applied to living cells. Westphal *et al.* adapted one of the earliest super-resolution methods, stimulated emission depletion (STED) microscopy, for video-rate super-resolution imaging of fluorescently labeled synaptic vesicles in living cells. These structures were ideally suited for a first demonstration of this method but improvements should permit application to other systems.

Westphal, V. *et al. Science*, published online 21 February 2008.

PROTEOMICS

Cracking the histone H4 code

The post-translational modifications on histone tails known as 'codes' guide DNA-chromatin interactions. Phanstiel *et al.* describe a method using nanoflow high-performance liquid chromatography to separate intact histone tails, combined with high-resolution mass spectrometry–based sequencing, and applied it to decipher the combinatorial histone H4 codes in human embryonic stem cells undergoing differentiation.

Phanstiel, D. *et al. Proc. Natl. Acad. Sci. USA*, published online 7 March 2008.

IMAGING AND VISUALIZATION

Lighting up synapses

Assessing synaptic connectivity in the dense nerve bundles of the nervous system is very challenging. Feinberg *et al.* describe a method to label synapses in *Caenorhabditis elegans* by expressing complementary GFP fragments tethered to transmembrane proteins on different cells. With complementation of the GFP fragments, fluorescence is restored, and this signals the proximity of the presynaptic and postsynaptic plasma membranes.

Feinberg, E.H. *et al. Neuron* **57**, 353–363 (2008).

PROTEIN BIOCHEMISTRY

Counting disulfide bonds

Large-scale structural analysis of proteins containing multiple disulfide bonds has been difficult owing to the absence of methods for distinguishing their native forms from misfolded intermediates. Narayan *et al.* now describe a method that uses mild reduction to selectively reduce the less stable non-native disulfide bonds and chemical blocking of free cysteines, coupled with mass spectrometry to determine the number of disulfide bonds, thus allowing native forms of proteins in mixtures to be distinguished.

Narayan, M. *et al. Nat. Biotechnol.*, published online 17 February 2008.

BIOPHYSICS

Molecular cutting and pasting

Kufer *et al.* describe a method for assembling biomolecular structures in defined geometric patterns using atomic force microscopy. By taking advantage of the natural ability of DNA to hybridize and by applying different unbinding forces that act on different DNA geometries, they show that target molecules coupled to DNA oligomers can be picked from one area on a surface with an AFM tip, moved and deposited in a new location.

Kufer, S.K. *et al. Science* **319**, 594–596 (2008).