METHODS IN BRIEF

GENETICS

Site-specific gene insertion

The recent rise of custom-designed nucleases, such as zinc-finger nucleases or transcription activator–like effector nucleases, has enabled precise and targeted modification of the genome. The basic principle behind these nucleases is the same: DNA-binding modules are coupled to a nuclease and introduce double-strand breaks (DSBs) at the chosen site. DSBs are then repaired by error-prone nonhomologous end joining (NHEJ) or by less efficient homologous recombination (HR) to insert a sequence of choice. Maresca *et al.* now combine the efficiency of NHEJ with the precision of HR. They build on previous work that had shown that short stretches of linear DNA can be inserted at the DSB even if they share no homology with the target site. By cutting the target site and an insert of up to 15 kb in size with the same nuclease, Maresca *et al.* generate complementary overhangs that are efficiently joined.

Maresca, M. *et al. Genome Res.* published online (14 November 2012).

MICROSCOPY

Finding and correcting immobile fluorophores

The quality of super-resolution microscopy based on the localization of individual fluorophores to reconstruct an image with subdiffraction resolution depends on the accuracy of the localization estimates. These estimates are usually based on fitting an isotropic fitting function to each image spot. If the fluorophore dipole is freely rotating, this method provides accurate estimates. But if the dipole is fixed, the resulting anisotropic emission can result in serious localization errors and distorted images. Backlund *et al.* show how their previously reported double-helix point-spread function can be used to detect and correct mislocalizations caused by nonrotating fluorophores. They can also measure the orientation of the fluorophore. They hope to extend their analysis method to fluorophores that exhibit limited motion, which are probably more commonly observed in biological samples than nonrotating fluorophores.

Backlund, M.P. *et al. Proc. Natl. Acad. Sci. USA* **109**, 19087–19092 (2012).

BIOINFORMATICS

Quantifying cellular organization

How the shape and organization of cells are achieved is a question of both fundamental and potential clinical interest. It is typically addressed by analyzing overall cell morphology or by studying the location of specific intracellular structures with respect to other landmarks within the cell. Apte and Marshall now describe a general statistical method to quantify cellular organization. They define a test parameter that describes the distribution uniformity of intracellular structures—modeled as points located at their structure's centroid—within the cell. This parameter may theoretically be applied in two or three dimensions and can be compared among different populations of interest. The authors demonstrate the method on simulated data and quantitatively confirm cellular disorganization in *Chlamydomonas* mutants with disrupted centrioles.

Apte Z.S. & Marshall, W.F. *Proc. Natl. Acad. Sci. USA* published online (26 November 2012).

SMALL RNAs

Trapping microRNA targets

Individual microRNAs (miRNAs) can target up to hundreds of messenger RNAs (mRNAs) in the cell for silencing. To identify these targets, many methods capture miRNA as it interacts with mRNA by pulling down the gene-silencing machinery, such as by the immunoprecipitation of Argonaute proteins. But because targeted transcripts are subject to destabilization, isolation of these transient interactions can be problematic, especially for rarer transcripts. Cambronne *et al.* use a truncated and tagged version of the silencing protein GW182 that allows miRNA and transcripts to be recruited but prevents transcript destabilization. Using this 'RISCtrap' approach of pulling down the mutant GW182 and associated transcripts, they identify high-confidence neuronal target lists for three human miRNAs.

Cambronne, X.A. *et al. Proc. Natl. Acad. Sci. USA* published online (26 November 2012).