# METHODS IN BRIEF

#### IMMUNOLOGY

#### Bacteria stand in for HIV

The outstanding challenge in creating an effective HIV vaccine is the design of an epitope that leads to antibodies that effectively neutralize HIV strains. It is not for a lack of candidates. The large glycoprotein subunit gp120, a key part of the viral envelope, is an obvious target for neutralizing antibodies. Unfortunately more than half of its glycans are contributed by the host and thus not seen as foreign by the immune system; they only differ in their dense clustering on gp120. Such a cluster of oligomannose has led to the creation of one neutralizing antibody, but subsequent efforts using linear or branched sugars, or engineered yeast cells displaying oligomannose have failed to produce antibodies that effectively neutralize HIV. Clark et al. discovered that a soil bacterium displays a lipooligosaccharide that contains tetramannose, and they show that heat-killed bacteria elicit the production of antibodies that bind HIV. These bacteria-derived glycoconjugates may prove effective triggers of neutralizing antibodies.

Clark, B.E. et al. Chem. Biol. 19, 254-263 (2012).

### GENOMICS

#### Sequencing broken DNA

Double-stranded breaks (DSBs) that occur in the DNA—for example, during recombination—can be mapped using chromatin immunoprecipitation followed by sequencing, but genomewide mapping of these 'recombination hotspots' is challenging in mammalian cells in part because of a low frequency of the hotspots. Inspired by approaches used in yeast, Khil *et al.* incorporated single-stranded DNA (ssDNA) computational detection methods and an ssDNA-enrichment step based on the faster annealing time of ssDNA compared to double-stranded DNA to the standard chromatin immunoprecipitation—sequencing protocol. The method—ssDNA sequencing or SSDS—improved the sensitivity and specificity of meiotic DSB hotspot detection in mouse samples. The method can also be extended to other systems in which identification of ssDNA or DSBs is desired.

Khil, P.P. et al. Genome Res. advance online publication (24 February 2012).

### CELL BIOLOGY

## **Quantifying RNA localization**

There are many transcripts that are not randomly localized in cells, as has been revealed by imaging of mRNA distribution in both fixed and living samples. Methods for robust, unbiased and quantitative image analysis of mRNA distribution are still needed. Park *et al.* now describe the use of two measures, the polarization index and the dispersion index to characterize mRNA distribution in cells, while taking cell size and geometry into account. Using these measures, the authors characterize mRNA distribution in fluorescence *in situ* hybridization images of budding yeast and chicken fibroblasts as well as in live time-lapse images of migrating mouse fibroblasts in which the beta-actin transcript carries GFP-labeled MS2 repeats. This approach should enable the discernment of subtle phenotypes and could possibly be amenable to automation in the future.

Park, H.Y. et al. Cell Reports 1, 179-184 (2012).

## PLANT SCIENCES

## Faster genetic mapping in plants

For traits that are difficult to map, such as quantitative trait loci that exert small phenotypic effects, geneticists often rely on recombinant inbred lines. Each line is a snapshot of the genomic reshuffling caused by meiotic recombination from a parental cross; inbred progeny are homozygous at nearly all loci so that the makeup of each line does not change in subsequent generations. Unfortunately, inbreeding to fixation takes many generations. Seymour et al. now apply centromere-mediated genome elimination in Arabidopsis thaliana to generate a similar outcome in only two generations. Crossing F1 plants to a centromeric histone mutant produces haploid plants from gametes, which spontaneously develop fertile doubled-haploid offspring. The group showed that the doubled-haploid lines are very similar to recombinant inbred ones and used them to map genes that control flowering time.

Seymour, D.K. et al. Proc. Natl. Acad. Sci. USA advance online publication (27 February 2012).

