METHODS IN BRIEF

GENETICS

Shorter guide RNAs for cleaner editing

The recent widespread excitement around the use of the RNA-guided Cas9 endonuclease for targeted genome editing has been tempered by the realization that this tool can generate substantial off-target effects. Fu *et al.* now show that using slightly shortened guide RNAs (gRNAs), which target the Cas9 nuclease to the desired site in the genome, can help mitigate this problem. By reducing the region of gRNA-target complementarity from 20 nucleotides to 17 or 18 nucleotides, the researchers report that even 1- or 2-nucleotide mismatches can reduce cleavage to very low or even undetectable levels. They also show that truncated gRNAs can be used with paired Cas9 nickases to increase the fidelity of the platform even further.

Fu, Y. *et al. Nat. Biotechnol.* doi:10.1038/nbt.2808 (26 January 2014).

NEUROSCIENCE

Cell type–specific synaptic labeling

Several methods for labeling synaptic proteins and their interactions exist, but most of these strategies rely on overexpression of the tagged proteins. To avoid this overexpression, Chen *et al.* label synaptic proteins by modifying the endogenous genomic loci encoding synaptic proteins with bacterial artificial chromosomes. In addition, they make the expression of the labeled proteins dependent on recombination so that the labeling can happen in a cell type–specific manner. They name the method STaR, synaptic tagging with recombination, and apply it to characterize the number and distribution of synapses in the visual system of flies. Using two different recombination systems, one can also colabel presynaptic and postsynaptic partners. In principle, the system can be adapted to other model organisms such as zebrafish or mice.

Chen, Y. *et al. Neuron* **81**, 280–293 (2014).

GENE EXPRESSION

Unmixing gene expression in cell samples

Heterogeneous gene expression within tissues can be difficult to tease apart. Measuring expression in single cells is associated with high technical noise, and a lot of sampling is required to recover the features of the population. As an alternative, Bajikar *et al.* combine the robustness of gene expression measurements from random samples of ten pooled cells with computational deconvolution. They generate probabilistic models based on known features of transcription and use maximum-likelihood inference to estimate regulatory states at the single-cell level. Using the approach, they show that a cell state found in less than 3% of the population is needed for three-dimensional culture of breast epithelial spheroids. For limited sample sizes, the approach is more accurate than taking single-cell measurements.

Bajikar, S.S. *et al. Proc. Natl. Acad. Sci. USA* **111**, E626–E635 (2014).

MICROBIOLOGY

Screening for holes in Gram-negative bacteria

Penetrating the cell envelope of Gram-negative bacteria is not a trivial task, and most antimicrobial drugs fail to do so. To better understand the genes that make up the membrane, Paradis-Bleau *et al.* developed a high-throughput assay to screen mutant bacteria for the integrity of their envelope. They used an *Escherichia coli* deletion mutant library also expressing β-galactosidase and assessed the enzymatic conversion of the β-galactosidase substrate by a color readout. If the membrane were leaky, substrate would get into the cells, and this manifested as a red colony. The researchers confirmed the role of genes known to be essential for membrane biogenesis and found 102 further genes implicated in the process. Understanding the role of these genes may identify them as new drug targets.

Paradis-Bleau, C. *et al. PLoS Genet.* **10**, e1004056 (2014).