METHODS IN BRIEF

GENETICS

CRISPR-Cas9 for reporter insertions in mouse

The bacterial clustered, regularly interspaced, short palindromic repeats (CRISPR)-Cas9 system has previously been harnessed to efficiently mutate multiple genes in the mouse. Yang *et al.* now extend these studies to demonstrate the precise insertion of small epitope tags, fluorescent protein reporters and flanking *loxP* sequences into specific target sites in the mouse genome via homology-directed repair. This is achieved by injection of the CRISPR-Cas9 components, including either single-stranded oligonucleotides or double-stranded vectors as donor templates, directly into zygotes. The researchers further conducted an off-target analysis for multiple independent sites and, in contrast with the results of previous studies in human cells, found no modifications at sites with three or more mismatches with the target.

Yang, H. *et al. Cell* **154**, 1370–1379 (2013).

IMAGING

Faster imaging volumes

The fast imaging speed and low light doses associated with light-sheet fluorescence microscopy are making it the method of choice for volumetric imaging of living samples, and performance improvements continue to be made that open up new applications. Wu *et al.* describe an improvement to their previously reported inverted selective-plane illumination microscopy (iSPIM) light-sheet microscope design. By alternating illumination and collection between the two perpendicularly oriented objectives and fusing the resulting views, dual-view iSPIM (diSPIM) greatly improves on the speed and resolution of iSPIM. The capabilities of diSPIM were demonstrated by high-speed volumetric imaging of microtubules in living cells and developmental processes in *Caenorhabditis elegans* at the level of labeled nuclei and neurite extension in pre- and post-twitching embryos.

Wu, Y. *et al. Nat. Biotechnol.* doi:10.1038/nbt.2713 (13 October 2013).

BIOCHEMISTRY

Enzyme function discovery

Assigning functions to the millions of proteins discovered in genome sequencing projects remains a slow and tedious experimental process. Computational tools can expedite progress, but the results of computational predictions must be interpreted with care. Zhao *et al.* report an approach to predict enzyme substrate specificities using both structure knowledge and genome context, which they applied to annotate the known structure but unknown metabolic function of a marine bacterium protein, HpdD. They used homology modeling and *in silico* metabolite docking for several proteins in the genome neighborhood of HpdD to assign its function, and they confirmed their computational predictions with metabolomics and genetics experiments. The strategy can also be used to assign the functions of orthologs found in other organisms.

Zhao, S. *et al. Nature* doi:10.1038/nature12576 (22 September 2013).

SEQUENCING

Standardizing RNA-seq across laboratories

Large-scale RNA sequencing, or RNA-seq, is widely used to profile gene expression in cell and tissue samples. The Genetic European Variation in Health and Disease (GEUVADIS) medical sequencing consortium recently investigated the reproducibility of the results of this popular technique. Members of the consortium in seven sequencing centers sequenced the mRNA and small RNA from lymphoblastoid cell lines derived from 465 individuals, and they analyzed the sources of technical error. They identified some biases due to insert size and GC content, provided a set of quality measures for any RNA-seq project, and proposed suggestions on how to overcome technical biases. The news is good: technical variation in RNA-seq is small, and cross-laboratory comparisons are possible when standardized protocols are followed.

't Hoen, P.A.C. *et al. Nat. Biotechnol.* doi:10.1038/nbt.2702 (15 September 2013).