TOOLS IN BRIEF

GENOMICS

Scaled-up functional tests of genetic variants

Our knowledge of the genome has been improving, but biochemical annotation and sequence conservation still cannot adequately predict the impact of genetic variation in noncoding regions. Two groups have now designed massively parallel reporter assays, each using pools of barcoded plasmids encoding variants and a minimal promoter, to scan for effects on gene expression that can be read out by sequencing. In lymphoblastoid and hepatocarcinoma cells, Tewhey *et al*. tested over 32,000 variants from expression quantitative trait loci and putative regulatory regions, finding over 800 variants that increase expression levels. In erythroid cells, Ulirsch *et al*. studied over 2,700 variants genetically linked to red blood cell traits, finding 32 functional variants and confirming the role of three using targeted genome editing.

Tewhey, R. *et al*. *Cell* **165**, 1519–1529 (2016).

Ulirsch, J.C. *et al*. *Cell* **165**, 1530–1545 (2016).

GENOMICS

RiboHMM finds translated sequences in ribosome footprints

To understand a genome, one needs to know which sequences can be translated. This is particularly important in light of recent findings that some annotated noncoding RNAs do indeed encode functional peptides. High-throughput sequencing of RNA protected by ribosomes is an efficient method that allows the identification of coding sequences by the presence of a three-base-pair periodicity reflecting the genetic code. Raj *et al*. developed RiboHMM, a probabilistic model that takes the abundance of protected fragments as well as their periodicity into account. RiboHMM joins tools such as RiboTaper by the group of Uwe Ohler, ORF-RATER by Jonathan Weissman's team and RibORF by Kevin Struhl and colleagues. The degree to which these tools complement each other will need to be determined in a direct comparison.

Raj, A. *et al*. *eLife* http://dx.doi.org/10.7554/eLife.13328 (2016).

IMMUNOLOGY

The whole repertoire (an estimate)

The human adaptive immune system can generate a staggering range of T and B cell clones that express different immune receptors and antibody sequences. Many sequencing-based methods seek to profile immune diversity, but the high complexity and predominance of rare clones make it difficult to guess at the full repertoire on the basis of sampling. Kaplinsky and Arnaout describe an algorithm based on expectation maximization that reconstructs the clone-size distribution of entire immune repertoires from limited samples. Their Recon software estimates different diversity indices such as species richness and entropy and enables measurement accuracy to be quantified. It also allows researchers to estimate the coverage needed to confidently predict differences between two samples.

Kaplinsky, J. & Arnaout, R. *Nat. Commun*. **7**, 11881 (2016).

MICROSCOPY

A panoramic yet detailed view of the mouse brain

A traditional trade-off of large-field-of-view microscopes is their low resolution, especially in the axial direction. Sofroniew *et al*. have now developed a microscope that is not afflicted by this trade-off. Their two-photon random access mesoscope has a field of view that covers several brain regions, lateral resolution that is close to the diffraction limit and axial resolution that is below the size of neuronal cell bodies. This enables the visualization of more than 200,000 neurons in the mouse brain at subcellular resolution. Furthermore, the researchers were able to image the activity of thousands of neurons in behaving animals sufficiently fast to discern action potentials and to correlate the activity of single neurons with the activity of whole brain areas.

Sofroniew, N.J. *et al*. *Elife* **5**, e14472 (2016).