Comparing over 40 monomeric FPs

Fluorescent proteins (FPs) have been discovered or engineered that span the visible region and beyond. However, not all FPs are equal in terms of their photophysical properties and performance in fusions, and choosing the best FP for a given experiment can be daunting. Piston and colleagues tested more than 40 monomeric FPs in a unique, head-to-head comparison of photophysical properties such as brightness, photostability and pH. They also determined which FPs are the most monomeric using the organized smooth endoplasmic reticulum (OSER) assay. On the basis of these results, they were able to determine which blue, green, yellow, orange and red FPs are likely to be the best suited for cellular imaging applications, and which may require further optimization.

Better hypothesis tests for genomics

As the number of statistical tests applied to a set of data increases, so too does the probability of randomly achieving a significant result. To address this multiple testing problem in genome-scale data, corrections such as the popular Benjamini–Hochberg (BH) procedure are used to control the false discovery rate. The BH and other tests are based on significance $P$ values from individual tests that are all treated equally, despite their having different statistical properties. Huber and colleagues now introduce independent hypothesis weighting (IHW), a method that assigns weights to $P$ values in a data-driven manner, using independent covariates that inform each test’s prior probability of the null hypothesis. Open-source software provides an IHW implementation for improving power while controlling the false discovery rate in a broad range of multiple testing scenarios such as differential expression, genome-wide association and molecular quantitative trait loci analyses.

Somatic structural variants in healthy cells

Finding variants in healthy somatic cells is difficult. These mutations are rare and hard to distinguish from errors introduced during library preparation and sequencing. Maslov and colleagues developed a two-step strategy to profile large structural variants (SVs) in normal somatic cells. They combine a library-preparation method that is not contaminated with chimeric reads with an algorithm called Structural Variant Search (SVS) that identifies SVs on the basis of split read alignment and elimination of germline variants. Using a cell line with known viral integration sites, which serve as a surrogate for SVs, they determined that SVS has a specificity of 95% at a sensitivity of 36%. The authors also show that the number of SVs increases after treatment of healthy primary cells with two clastogenic compounds.

Testing performance of Cas9 activators

The CRISPR/Cas9 system has been found to function in a broad range of biological applications outside of gene editing. For example, mutations can be introduced into Cas9 that eliminate its nuclease activity but do not affect targeting. These ‘dead’ mutants of Cas9 can be fused to transcriptional activators for site-specific activation of target genes. Several such Cas9 activators have been described, yet their relative performances have not been compared in a detailed manner. Chavez, Church and colleagues carried out a rigorous characterization of the performance of multiple Cas9 activators across a broad range of target genes in human, mouse and fly cell lines, providing users with valuable guidance and validation for choosing reagents to use in their own studies.

A view into the fly brain at work

Neural activity in the brain of Drosophila melanogaster has been routinely imaged in immobilized animals. However, it is more difficult to monitor how the brain works in situations that are more dynamic, such as in freely moving animals or animals that engage in social interactions. Katsuki and colleagues developed an imaging system consisting of three cameras that tracks a fly in an arena and provides high-resolution images of the fly head and of the activity of calcium indicators expressed in neurons. The researchers use their imaging system to visualize brain activity in animals that explore the arena and in animals engaged in a courtship ritual. The approach will make imaging of neural activity in the fly brain possible during behaviors such as mating and acts of aggression, which were previously not amenable to such analyses.