Imaging allele-specific SNPs

Individual cells, even those that have the same genetic background and are grown in the same environment, show differences in gene expression. Two research groups now independently extend single-molecule RNA fluorescence in situ hybridization (FISH) to not only quantify these differences but do so in an allele-specific way. Hansen and van Oudenaarden combine short allele-specific probes with probes that are specific for the gene but not the allele and are then able to distinguish allelic transcripts that differ in only 12 SNPs with high accuracy. Raj, Levesque and colleagues achieve even higher sensitivity, and are able to detect transcripts differing in only a single SNP, by using an allele-specific probe that is partially hybridized to a masking oligo before binding its target in combination with a non-allele specific probe.

Genetic tag for active neurons

Singling out the neurons that are active in the brain of an organism while it performs specific behaviors is currently of great interest. But doing so at cellular resolution and in the whole brain poses challenges for direct visualization. Another, more indirect, way of getting at active circuits is to use genetic tags that enable recently active neurons to be labeled permanently. The expression of immediate-early genes (IEGs) such as c-fos and Arch has been instrumental for this purpose in neuroscience. Improving the IEG toolbox, Bito and colleagues engineer a synthetic promoter, E-SARE, that drives neuronal activity-dependent gene expression more potently than other immediate-early gene promoters used for this purpose. They show the potential of this tool for identifying neuronal ensembles in the mouse brain that respond to specific sensory stimuli and for mapping their axonal projection patterns.

A database of worm behavioral phenotypes

In some contexts, impediments to understanding the relationship between genotype and phenotype occur predominantly at the phenotyping step. The study of behavior in the nematode Caenorhabditis elegans is an example. Many single-gene knockdowns have no reported effect on worm movement. One explanation for this is that it is difficult to reproducibly and quantitatively score movement at a large scale. Schafer, Brown and colleagues now describe a growing collection of segmented and feature-extracted videos recording locomotion in thousands of individual worms from hundreds of C. elegans mutant strains and make this resource available for analysis by other researchers.

Microbial genomes find their place

The genomes of bacteria and archaea are being sequenced at an accelerating pace, and researchers need a robust way to assign them to their species of origin. Classic methods that compare entire genomes at a time are considered the most accurate but are too difficult to implement at large scale, whereas the common approach of using ribosomal marker-gene sequence to define taxa gives poor resolution at the species level. An alternative method takes advantage of multiple markers rather than single genes or entire genomes. Bork and colleagues offer the specI software, which uses up to 40 universal single-copy genes for rapid, fully automated and highly accurate species assignment without the need to build a phylogenetic reference tree.

Steps toward in situ sequencing

Current RNA sequencing technologies use RNA transcripts extracted from cells and thus cannot easily connect gene expression data to information about the cells’ spatial organization within tissue. There is interest in the field in developing RNA sequencing methods that enable sequencing RNA molecules in situ. Taking steps in this direction, Nilsson, Wahlby and their colleagues adapt methods based on padlock probing, rolling-circle amplification and sequencing-by-ligation chemistry to work off of tissue samples. They sequenced stretches of up to four nucleotides in specific mRNAs in breast cancer tissue sections. This approach enabled the detection of specific mutations in targeted mRNAs as well as the expression levels of several transcripts by sequencing barcodes.