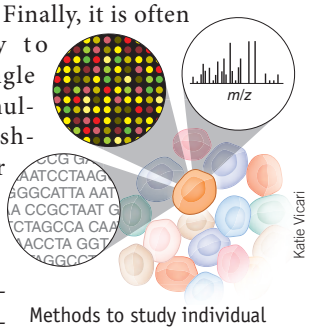
We present a selection of important methods and areas of methodological development worth watching in the coming years.

Single-cell methods

Improved single-cell methods are helping to unravel biological complexity.

The heterogeneity of cells in culture and in organisms poses a challenge for many experimental measurements. Population measurements are necessarily averages, masking the behavior of minority subpopulations and effectively blinding researchers to possibly interesting differences between cells.

The alternative is to make measurements on single cells. Methodologically speaking, this, too, is challenging on several fronts. Molecular analyses, whether on a particular macromolecule or at an 'omic' scale, can be difficult (or even impossible) to accomplish on the amount of material extracted from one cell. Methods with increased sensitivity are therefore in demand. Throughput is also a bottleneck. Basing firm conclusions on single-cell measurements means that one must be able to quickly and accurately analyze many cells. Finally, it is often necessary to analyze single cells in a multiplexed fashion, either because the cells exist in a heterogeneous population or because one wants to measure many parameters at the same time.



Methods to study individual cells are needed in the face of cellular heterogeneity.

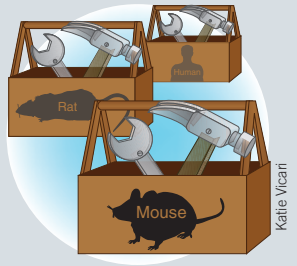
There continue to be methodological advances on all of these fronts. Mass cytometry, for instance—in which isotopes are used as antibody labels instead of fluorescent probes—considerably extends the multiplexing capabilities of flow cytometry (*Science* **332**, 687–695; 2011). In the measurement of gene expression, digital reverse-transcriptase PCR in a microfluidics device makes it possible to simultaneously monitor the expression of hundreds of genes in hundreds of single cells. As demonstrated in a recent study of tumor heterogeneity, this can be combined with single cell sorting and with statistical clustering methods to begin to dissect the cellular subpopulations that constitute a tissue (*Nat. Biotechnol.* **29**, 1120–1127; 2011). Microfluidics has also been at the heart of recent advances in molecular haplotyping, a measurement that is inherently made on a single cell.

As single-cell analysis is increasingly applied to ask biological questions, the demands for sensitivity and throughput will only increase, in particular for methods to read out macromolecules other than DNA and RNA. Biology is complex enough to keep single-cell methods developers in business for a while yet. **Natalie de Souza**

Functional genomic resources

Tools to manipulate murine genes on a genome-wide scale and to phenotype their effects in animals are maturing.

Thanks to sequencing and annotation efforts, we now know the sequence of next to all mouse genes. What we are lacking is a comprehensive understanding of what these ~20,000 genes actually do. Because a task of this magnitude cannot be tackled in piecemeal fashion, several years ago the mouse community started large-scale efforts, brought together under the umbrella of the International Knockout Mouse Consortium. Its goal is to mutate every protein-coding gene in an inbred mouse strain, C57BL/6, and to make the vectors to do so, as well as the resulting embryonic stem cells and mouse strains, available to the community. Although this work is ongoing, 2011 marked a considerable expansion of these tools. Researchers at the Wellcome Trust Sanger Institute led an effort to create conditional knockout alleles that can be switched back to wild type and subsequently selectively knocked out in a tissue-specific or time-specific manner (*Nature* **474**, 337–342; 2011). This resource currently encompasses roughly half of all mouse genes, with a pipeline set up to tackle the other half. Notably, a large percentage of the C57BL/6N embryonic stem cells with these conditional alleles are germline-competent and mice carrying them can thus be bred easily and are ready for phenotyping. Consortia such as The European Mouse Disease Clinic apply standardized phenotypic tests to various mouse strains generated by their partners at the International Knockout Mouse Consortium. Before long the (inbred) mouse will be in the fortunate position of having functional data on each of its genes. The rat will be next, with the Knock Out Rat Consortium following in the footsteps of the International Knockout Mouse Consortium. **Nicole Rusk**



Resources to functionally test mouse genes are nearing completion but others lag behind.