Worm embryos sorted out

Collecting precisely staged Caenorhabditis elegans embryos requires painstaking manual selection, posing a problem for analysis by techniques requiring large amounts of material. In this issue of Nature Methods, Rajewsky, Piano and colleagues collected tens of thousands of precisely staged 1-cell worm embryos by fluorescence-activated cell sorting (FACS), using a transgenic strain expressing a fluorescent marker only at the 1- and 2-cell stage. A single sort yielded 1-cell stage embryos at 70% purity, and the authors achieved 98% purity upon two rounds of sorting, though fixation was required in this case. They demonstrate the power of this approach by profiling small RNA populations in early worm embryos by high-throughput sequencing.

Article p745, News and Views p727

Single can be fatal

A cell’s survival depends on its ability to cope with DNA damage. The DNA repair machinery detects assaults on the genome from endogenous and exogenous sources and either fixes them or, failing that, sends the cell on the path to apoptosis. Most investigations into this repair process involve the use of irradiation or drugs to induce genome-wide damage. Bjerbaek and colleagues now present an alternative. They very selectively introduced damage by targeting a mutant Flp recombinase to a single genomic locus where it forms a covalent protein-DNA complex. Unless repaired, this complex is fatal for the cell. With this Flp-nick system the authors dissected the repair pathways needed to repair a single insult to the genome.

Article p753

Quantifying protein interactions

For a complete understanding of signal transmission or drug action in a cell, quantitative analysis is necessary for determining which interactions are the most relevant in a physiological context. Daub and colleagues report an affinity purification–mass spectrometry–based proteomics approach to determine dissociation constants for all proteins in a cell lysate that interact with a ligand immobilized on a bead. By using a triple stable isotope labeling by amino acids in cell culture (SILAC) strategy, they identified proteins that specifically bound to the ligand and obtained the dissociation constants for the interactions. They demonstrate the generality of the method by testing an immobilized kinase inhibitor, a phosphorylated peptide and an antibody as the ligand ‘bait’.

Brief Communication p741

Genome-wide genetic studies in bugs

A more complete understanding of the biology of microorganisms would benefit from methods to interrogate their genomes at a large scale. Camilli and colleagues applied high-throughput sequencing to study genetic interactions in the pathogen Streptococcus pneumoniae. They generated a saturated insertion library using the Mariner transposon, modified such that the insertion site in each mutant could be precisely identified by sequencing. Fitness of each mutant or double mutant strain could be determined from the number of reads after growth. The method does not require preexisting microarrays or arrayed knockout collections and should be applicable to several bacterial species.

Article p767

Profiling cellular subpopulations

Cultured cells are not homogenous, either in morphology or in marker expression or localization, and this is likely to be relevant for cellular function. Imaging cells with many markers would aid in profiling cellular subpopulations, but fluorescence microscopy is limited to 4 or 5 channels. Altschuler and colleagues now tackle this problem computationally. By identifying features of subpopulations that can replace the information in a given fluorescence channel, it is possible to ‘drop’ that channel, thus freeing it for examination of another marker of interest. They carried out this process iteratively and combined information for several markers in silico to generate virtual profiles of cellular subpopulations.

Article p759