METHODS IN BRIEF

PROTEOMICS

A proteome in an hour

Obtaining deep proteomic coverage by mass spectrometry has typically been a long, labor-intensive task involving extensive fractionation and lengthy instrument analysis times. Hebert *et al.* now report an approach for comprehensively analyzing the yeast proteome in just over 1 hour. Their highly optimized protocol includes improved sample preparation and chromatographic separation and, most importantly, the latest-generation Orbitrap Fusion mass spectrometer technology. This instrument possesses a high tandem-mass-spectral acquisition speed and a control environment with multiple independent processing units. The protocol was highly reproducible and yielded an average identification of nearly 4,000 proteins, about 90% of the expressed yeast proteome. This achievement shows that mass spectrometry technology can be an efficient, routine tool for profiling proteomes: the authors estimate that the expressed human proteome could be analyzed in a few hours with their approach.

Hebert, A.S. *et al. Mol. Cell. Proteomics* doi:10.1074/mcp.M113.034769 (19 October 2013).

MOLECULAR BIOLOGY

Cell type–specific ChIP

An organism's development depends on carefully orchestrated cell type–specific gene expression, which is regulated largely by chromatin structure. To fully appreciate the role that chromatin domains play in this process, Schauer *et al.* developed CAST-ChIP (chromatin affinity purification from specific cell types by chromatin immunoprecipitation). They focused on the fly brain and used the cell type–specific expression of a tagged transgene in combination with affinity-tag purification to enrich enough cells to profile polymerase II binding and histone H2A.Z positions in glia and neurons. The polymerase II data yielded ~1,500 genes expressed specifically in these cell types. The authors also saw that H2A.Z shows no cell type–specific distribution and thus marks universally active chromatin. CAST-ChIP will allow the exploration of chromatin structures and their effect on gene expression in complex tissues.

Schauer, T. *et al. Cell Rep.* **5**, 271–282 (2013).

CHEMICAL BIOLOGY

Hydrogel photopatterning

Hydrogels mimicking the natural environment of a cell are useful scaffolds for three-dimensional cell culture. Control over the spatial patterning of extracellular matrix proteins and growth factors within a hydrogel is important for replicating a physiological microenvironment. Although photopatterning is a promising technique for this purpose, photopatterning of delicate full-length proteins has not yet been broadly achieved owing to the lack of accessible approaches. Mosiewicz *et al.* harnessed photocaging to expose reactive lysine residues at defined positions within a hydrogel. This allows the covalent anchoring of peptide substrates attached to a protein of interest via a reaction catalyzed by the enzyme transglutaminase factor XIII. With this method, the authors were successful at directing mesenchymal stem cell migration, showing that the cells preferentially moved into the protein-patterned regions of the hydrogel.

Mosiewicz, K.A. *et al. Nat. Mater.* **12**, 1072–1078 (2013).

GENE EXPRESSION

Cellular hierarchies by single-cell RT-qPCR

Methods to measure gene expression in single cells are in high demand. Several such methods are currently in use, but higher throughput and coverage are still desirable. Guo *et al.* now improve reverse transcription–quantitative PCR (RT-qPCR) from single cells, using algorithms for primer design and optimizing experimental conditions to measure up to 280 markers in 1,500 single cells in a multiplex fashion. They implement this approach to quantitatively measure gene expression of selected markers in normal and leukemic mouse hematopoietic stem and progenitor cells as well as in mature blood cell types. They demonstrate that analysis of the resulting data can capture known lineage relationships between cells. This improved approach should enable the study of dynamic and heterogeneous cell populations beyond those in the bone marrow.

Guo, G. *et al. Cell Stem Cell* **13**, 492–505 (2013).