Stem cell culture, further defined

Human pluripotent stem cells, both embryonic stem cells and induced pluripotent stem (iPS) cells, have traditionally been cultured on feeder cells or in otherwise undefined conditions. Several efforts to delineate defined culture conditions, some of them successful, have been reported in recent years. In this issue of *Nature Methods*, Thomson and colleagues take the process one step further. After systematic testing of which components of stem cell culture medium are needed and which are not, they report a simple, chemically defined mixture of just eight components for long-term culture of human pluripotent stem cells. They use it to derive human iPS cells with non-integrating vectors and culture them under defined conditions. Also in this issue, Yamanaka, Okita and colleagues report reprogramming with non-integrating vectors at increased efficiency and derive human iPS cell lines from individuals putatively homozygous at the major HLA loci.

*Article p424, Brief Communication p409, News and Views p389*

Rapid, high-resolution FTIR imaging

Fourier-transform infrared (FTIR) microspectroscopy is a chemical imaging technique that does not require stains or labels. Instead, biological molecules exposed to infrared light generate signature spectra based on molecular bond vibrations. For all of the interest in label-free imaging techniques, the broader application of FTIR microspectroscopy has been hampered by poor resolution and very long image acquisition times. Hirschmugl, Bhargava and colleagues now report that an instrumental configuration combining multiple synchrotron beams with wide-field detection substantially increases the spatial resolution of FTIR microspectroscopy and the size of the sample that can be imaged while substantially decreasing the time required for image collection. The spatial detail of the images possible with this technique should allow FTIR microspectroscopy to become competitive with optical microscopy for a number of biomedical imaging applications.

*Brief Communication p413, News and Views p385, The Author File p363*

RNAi in fly oogenesis

Long hairpin RNAs expressed from a microRNA scaffold potently trigger RNA interference (RNAi) in somatic cells in *Drosophila melanogaster*; but they are ineffective in silencing genes in the female germine. Perrimon and colleagues constructed modified microRNA-based expression vectors that allow generation of short hairpin RNAs (shRNAs), which are efficiently loaded into the RNAi machinery. These shRNAs not only potently trigger gene knock down during oogenesis, they are also effective silencers in somatic cells. The authors are also building up a resource targeting all 14,208 annotated protein-coding genes in the fruit fly with at least three shRNAs per gene. To date, 2,900 fly stocks are available and the anticipated rate of new construct release is 8,000 per year.

*Brief Communication p405*

A statistical validation tool for SRM

Selected reaction monitoring (SRM) is a targeted form of mass spectrometry, used to detect and quantify predefined sets of proteins with high sensitivity. Though the popularity of this method, especially for applications in systems biology and biomarker validation, has been rapidly growing, to date there has not been a convenient and objective tool for assessing error rates in SRM experiments. Aebersold and colleagues present mProphet, a software tool for automated data processing and statistical validation of SRM experiments. The tool allows more information to be gained from an SRM dataset and should especially facilitate large-scale targeted quantitative proteomics experiments.

*Article p430*

It’s in the beam

Light-sheet microscopy methods that illuminate a sample with a plane of illumination have made strong inroads into fluorescence imaging of large multicellular specimens by providing fast three-dimensional imaging with lower light exposure compared to point-scanning approaches. But the thickness of the beam has limited the utility for imaging single cells. Betzig and colleagues used a Bessel beam to generate a thinner light sheet and overcome this problem. Notably, they describe multiple methods to remove the side lobes of the Bessel beam that would otherwise compromise the thinness of the sheet. Their methods allowed them to image fast subcellular dynamics in single living cells with diffraction-limited three-dimensional isotropic resolution.

*Article p417*