METHODS IN BRIEF

GENE EXPRESSION

Bundled reporter gene assays

Reporter genes are common tools for revealing transcriptional states, regulatory circuitry and responses to stimuli. To scale up to study the activation of multiple pathways, O'Connell *et al*. switched to sequencing rather than a light- or color-based reporter readout. The researchers generated lentiviral vectors, each encoding synthetic DNA response elements for one of 40 signaling pathways with a corresponding sequence tag. They then transfected cells as a pool and used RNA sequencing to measure tag frequencies as well as global transcription. Using this transcription factor activity sequencing (TF-seq) approach, O'Connell *et al*. inferred pathway dynamics in bone-marrow-derived macrophages from *Myd88*-knockout mice upon stimulation by small molecules or pathogen-associated molecular pattern molecules (PAMPs). TF-seq pathway activity measurements could not be reproduced using RNA or ChIP sequencing data alone.

O'Connell, D.J. *et al*. *Cell Systems* **2**, 323–334 (2016).

PROTEOMICS

A viral trap for protein interactions

Affinity purification–mass spectrometry, wherein a bait protein is tagged with a purification handle, is widely used to capture and identify protein interactions. The method, however, is highly dependent on lysis and purification conditions, which can have a substantial impact on the results. As a way to avoid the potential pitfalls, Eyckerman *et al*. developed a method called Virotrap, which avoids cell lysis and helps preserve protein interactions during affinity purification. In this approach, a bait protein is fused to HIV-1 Gag, which results in its accumulation at a mammalian cell membrane and subsequent budding off of virus-like particles. The virus-like particles containing bait–prey complexes can be readily sorted and the prey proteins can be identified using western blotting or mass spectrometry. The authors show that Virotrap confirms known protein interactions and reveals novel interactions as well.

Eyckerman, S. *et al*. *Nat. Commun*. **7**, 11416 (2016).

STEM CELLS

In vitro model of human implantation

Blastocyst implantation in the uterus is a critical event in human development that initiates germ layer establishment as well as the generation of extra-embryonic yolk sac and placenta. It is also difficult to model *in vitro*. Shahbazi *et al*. have now adapted their protocol for modeling implantation in the mouse for use with human embryos. They plated human blastocysts in two successive media and used microscopy to identify embryos that adhered at day 7 and underwent changes associated with implantation. Normoxic culture and KnockOut Serum supplementation were important for robust implantation and epiblast survival up to day 13. Cultured embryos segregated lineages, formed bilaminar discs and developed extraembryonic tissues. Using the protocol on both human embryos and matrigel-grown pluripotent stem cells, the researchers discovered that lumen formation in pro-amniotic cavity development is preceded by cell polarization.

Shahbazi, M.N. *et al*. *Nat. Cell Biol*. **18**, 700–708 (2016).

LAB ON A CHIP

Counting proteins by slowing diffusion

Detecting individual proteins in cells is very challenging, especially when attempting to count those with very low copy numbers. To aid in the detection of these elusive molecules, Okumus *et al*. present microfluidics-assisted cell screening, a method that facilitates the counting of fluorescently tagged, low-abundance protein copies using total internal reflection fluorescence (TIRF) microscopy. In this approach, individual bacterial cells are squeezed through narrow microfluidic channels. This process of mechanical compression slows down protein diffusion in the cytoplasm and enhances the spatial separation of the target protein copies. The compression process also has the benefit of reducing autofluorescence and keeping all target protein copies within the objective depth of focus. The authors applied the method to study the effect of the low-abundance adaptor protein SprE on the stress-response regulator RpoS in *Escherichia coli*.

Okumus, B. *et al*. *Nat. Commun*. **7**, 11641 (2016).