The best of two sequencing worlds

High-throughput pyrosequencing—either using Roche’s 454 bioluminescence detection or Ion Torrent’s electrochemical readout—is fast but limited in throughput; fluorophore-based sequencing allows for high throughput and sensitivity but is slower. Xie and colleagues now combine these two approaches in a method they call fluorogenic sequencing. They trap the target DNA with a DNA polymerase and a phosphatase in a microwell, serially add the four nucleotides, each labeled with the same dye, and seal the microreactor after addition of each nucleotide. If a nucleotide is incorporated into the growing DNA strand, the dye is cleaved and becomes fluorescent. As the signal is confined to the microwell, it does not need to be recorded in real time and high signal strengths ensure that the target DNA does not need to be highly amplified. Article p581

Renewable protein binders on a production scale

Despite the central importance of antibodies as tools for biomedical research, their quality and availability varies widely. Efforts such as the Human Protein Atlas strive to generate and characterize high-quality polyclonal antibodies, but these reagents are not renewable. Although technologies for generating renewable binders exist, they have not previously been put to the test on a large scale. Colwill, Gräslund and the Renewable Protein Binder Working Group now describe the results of a large-scale effort to produce and characterize renewable binders (including monoclonal antibodies generated by hybridoma technology and recombinant Fab or single-chain Fv reagents generated by phage display) to 20 closely related human SH2 domain proteins. Their results show that specific, high-affinity renewable protein binders can be efficiently produced in a reasonable time frame using existing technologies. The work suggests a way forward for producing renewable binders on a proteome scale. Analysis p551, News and Views p545

Microfluidics for stem cell culture

Microfluidic technology has much potential for revealing the heterogeneity of cell populations, but cells must be maintained in a physiologically relevant state. Hansen and colleagues describe a microfluidic array in which they clonally culture preleukemic and primary mouse hematopoietic stem cells over five days. An iso-osmotic bath and medium perfusion maintain desirable culture conditions, and the cells remain spatially unperturbed, permitting imaging, staining and lineageing. Cells can be recovered from the device for functional analysis; they retain their functional properties in vitro and in vivo. The authors use this setup to measure growth rate and to study the response to temporally controlled steel factor addition in hundreds of clonally cultured cells. Article p592

Which CLIP to use?

RNA is not an autonomous polymer; rather, it is regulated by many RNA-binding proteins that determine its function. An efficient way to decipher the role of any given RNA-binding protein is to map its genome-wide binding sites by cross-linking the protein to the transcript, immunoprecipitating the complex and sequencing the RNA. Although this cross-linking and immunoprecipitation (CLIP) method seems straightforward, several variants have emerged, and their respective merits have not yet been systematically compared. Zavolan and colleagues now report an approach for CLIP data analysis and compare two popular CLIP methods, highlighting bias in each and ways to reduce this bias. Analysis p559

Quantifying behavior in worms

Caenorhabditis elegans is an attractive organism for studying the biological basis of behavior as it has relatively simple neural circuitry, is genetically tractable and displays several behaviors that can be studied in the laboratory. A detailed dissection of worm behavior needs rapid and robust quantification. In this issue, two groups describe tools that will prove valuable in this effort. Kerr and colleagues describe the Multi-Worm Tracker open-source software for automated real-time tracking and quantitative analysis of behavior in dozens of freely moving worms. Albrecht and Bargmann describe structured arenas in which worms crawl in liquid environments and to which liquid-borne chemical stimuli are delivered, via microfluidics, with very precise spatial and temporal control. Both sets of tools have been applied, in these papers, to yield new insight into worm behavior. Articles p592, p599