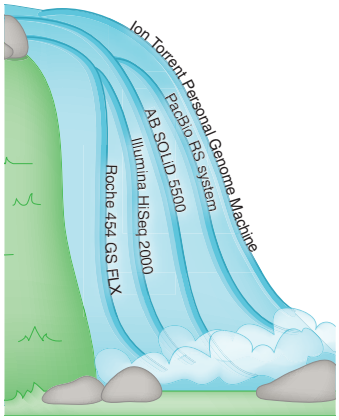
Torrents of sequence

In 2011, we will see the arrival of new and improved sequencing technologies.

It is hard to overstate the positive changes that high-throughput sequencing has brought to biological research in the last few years. Each year developments with the potential to change the sequencing landscape emerge, and 2010 was no different. Ion Torrent launched in February 2010 with a sequencer based not on dye-labeled oligonucleotides and expensive optics but on ion detection (the machine has been casually referred to as 'a pH meter that sequences'). The principle is straightforward: DNA to be sequenced is captured in a microwell, and unmodified nucleotides are floated across the wells, one at a time. The polymerase incorporates the appropriate oligonucleotide into the growing strand, and the hydrogen ion that is released changes the pH in the solution, which is detected by an ion sensor. This allows sequencing in real time. The read length of around 100 base pairs is comparable to that of other high-throughput sequencers, but the throughput is currently still lower than that of established short-read methods, although increasing the size of the semiconductor chips will change this. A technology that substantially enhances read length to 1 kilobase or more—Pacific Bioscience's SMRT (single-molecule, real-time) system—was commercially launched in November 2010. Longer reads will be advantageous when sequencing repeat-rich regions or regions with many structural variants.



Some of the new or improved sequencing platforms.

Of course, the more established sequencing technologies should not be written off. Development is happening everywhere: Life Technologies not only invested in Ion Torrent's new technology when it acquired the company in October 2010 for $375 million but also improved the SOLiD platform to ensure higher throughput, of 20–30 gigabases per day, with a maximum of 75-base-pair reads. Illumina's HiSeq 2000 reportedly has a throughput of 25 gigabases per day with a length of 100 base pairs or less. And for the 454 GSL-FLX, Roche is working on enhancing the current average read length of 500 base pairs; the company also plans to develop its own semiconductor-based sequencing system in partnership with DNA Electronics.

Notably, sequencers are not only being developed with ever-increasing throughput in mind, but some, such as Ion Torrent's Ion Personal Genome Sequencer and Roche's GS Junior, are benchtop machines that may put medium-size sequencing projects within reach for almost every laboratory.

The final word as to which sequencing technology, or combination thereof, will emerge as the dominant one is far from spoken, but 2011 will undoubtedly be an interesting year for people interested in all things sequencing. **Nicole Rusk**

Seamless delivery

The payoffs for efficient cargo delivery into living cells make the development of better methods worthwhile.

For decades, researchers have pursued methods to efficiently bring impermeant macromolecules into the cytosol and the nucleus of living cells. The list of established techniques is long. It goes from chemical loading (temporarily permeabilizing cells with a chemical agent), to vehicle-based loading (fusing cells to a cargo-carrying liposome or a red blood cell ghost), to mechanical loading (microinjection or wounding of the cell membrane), to the use of cell-penetrating peptides fused to cargo, to electroporation.

Speedy cargo delivery.

Surprisingly, none of these methods work universally well for large molecules other than DNA. The problems are manifold: the size of the delivered cargo is restricted, uptake can be inefficient, cargo may be trapped in endocytic vesicles or aggregate inside the cell, delivery can be restricted to a certain cell type, cells are often damaged in the process, and some methods are time- and labor-intensive, requiring special skill and equipment.

Constant improvements to delivery techniques aim to overcome these hurdles, and the potential applications are legion. To pick one example: the effective delivery of antibodies labeled with dyes or quantum dots would enable studies of their endogenous targets' subcellular distribution in live cells in real time. One could visualize chromatin modifications with antibodies to histone modifications as well as other chromatin-associated proteins and follow their dynamic changes. Hiroshi Kamura and colleagues recently showed the potential of such an approach (*J. Cell Biol.* **187**, 781–790; 2009) when they used mechanical loading to deliver dye-labeled Fab fragments against histone H3 phosphorylated on serine 10, a histone mark essential for chromosome segregation, to live cells and preimplantation mouse embryos.

The delivery of fluorescent antibodies would mean that it is no longer necessaryto express fluorescent fusion proteins or fuse the protein of interest to a peptide tag that binds a fluorophore-conjugated chemical. Instead, any endogenous proteins for which a good antibody exists could be localized, even at a resolution below the diffraction limit in the case of super-resolution microscopy. What stands between the universal realization of these and other applications are lipid bilayer membranes that prove surprisingly hard to penetrate. **Nicole Rusk**

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