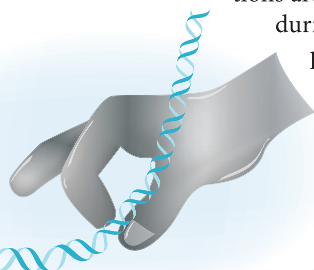
What methods could be in the running for Method of the Year next year? Read on for a subjective selection of some of the possibilities.

Zinc-finger nucleases

Genome-engineering tools with improved design and efficiency will become widely used.

The ability to make controlled changes in the genome is a very powerful tool. Several technologies exist for this purpose, but most have drawbacks. For instance, transgenes introduced into mammalian cells with viruses are subject to position effects. Homologous recombination does not work well in some systems, such as human cells.

Zinc-finger nucleases (ZFNs) can be used to create targeted double-stranded breaks in the genome and have thus generated much excitement. These enzymes consist of a DNA-binding portion, which can in principle be designed to target a particular location in the genome, and a Fok1 nuclease domain, which introduces the break. A donor DNA can be exogenously supplied to effect repair of the break by a homology-driven process, resulting in gene replacement with a desired sequence. Alternatively, mutations are introduced during an error-prone repair process.



ZFNs target specific sites in the genome.

ZFNs have by now been used to target the genome in several different systems: in mouse and human cells, in zebrafish, in plants, in fruit flies and in the rat. ZFNs can be used to create deletions of up to 15 megabase pairs in human cells and have been used recently to disrupt or tag genes in human pluripotent stem cells, a system of tremendous interest for genome engineering. No doubt the application of these tools will continue.

One of the ongoing difficulties is that it is not trivial to design ZFNs. Current methods are either relatively inefficient at generating enzymes that function well, or they are laborious and require dedicated screening systems. Many users depend on commercial services, at relatively high cost. Methods developers, however, continue to both improve approaches to design ZFNs (for instance, *Nat. Methods* **8**, 67–69; 2011) and to engineer enzymes with better properties (for instance, *Nat. Methods* **8**, 74–79; 2011). An efficient and user-friendly method to generate ZFNs that perform well is likely to spread rapidly among researchers in many fields.

Natalie de Souza

Targeted proteomics

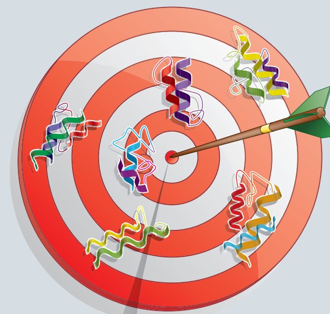
Targeted analysis of proteins on a broad scale with mass spectrometry is becoming a reality.

Based on rapid methodological developments in 2009, last year we selected targeted proteomics analysis by mass spectrometry as a Method to Watch. Progress in 2010 continued at a quickening pace.

The targeted proteomics approach differs fundamentally from the more familiar 'shotgun' approach in which the spectra generated from all detectable proteins in a sample are interpreted by database searching. In a targeted analysis, the mass spectrometer is programmed to analyze a preselected group of proteins. This can be achieved using a technology called selected reaction monitoring (SRM; also referred to as multiple reaction monitoring, MRM), whereby assays are developed on a triple quadrupole instrument to detect fragment-ion signals arising from unique diagnostic peptides representing each of the targeted proteins.

The SRM approach has proved to be highly sensitive, quantitatively accurate and highly reproducible. Compared to shotgun proteomics (still largely the domain of experts), protein detection is relatively rapid and straightforward with SRM. Though not yet quite as sensitive as immunological assays, SRM has the clear edge for multiplexed detection.

The main bottleneck to applying this technology on a broad scale has been the



Proteins are detected with high sensitivity and reproducibility via targeted proteomics.

difficulty of generating high-quality SRM assays. Over the last couple of years, methods for selecting appropriate diagnostic peptides and for generating SRM assays in high throughput using crude peptides have been developed. These developments have allowed Ruedi Aebersold's group to greatly expand their SRM Atlas database (*Nat. Methods* 5, 913–914; 2008); as a result of an intensified joint effort with Rob Moritz, this resource will soon feature SRM assays for about 95% of proteins from humans and yeast, and about 55% of mouse proteins.

As the availability of SRM assays grows, so will the biological applications of the technology. Many researchers see great potential in applying SRM for biomarker validation and systems biology studies. Recently, SRM was used to validate computationally predicted microRNA targets in worms (*Nat. Methods* **7**, 837–842; 2010). This work likely represents just a taste of interesting applications to come.

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