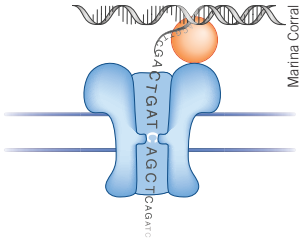
We present a selection of important methods and areas of methodological development worth watching in the coming years.

Disruptive nanopores

2013 will see the first commercial nanopore sequencers.

DNA sequencing technology has seen breathtaking improvements since we selected next-generation sequencing as Method of the Year in 2007. In 2011 we pointed to improved technologies that increased massive parallelization so that an entire human genome could be sequenced in a single run many times over, to a platform that no longer relied on optics for base reading but instead used ion sensing, and to improvements in single-molecule sequencing to yield very long reads. But even in a field as saturated with innovation as high-throughput sequencing, there is room for a yet more disruptive technology.

The announcement of the first commercial nanopore sequencers by Oxford Nanopore Technologies at 2012's Advances in Genome Biology and Technology meeting raised great expectation in the scientific community—and for good reasons. Nanopore sequencing promises to deal with most shortcomings of current sequencing platforms: reads are very long, with tens, if not hundreds, of kilobases; errors, currently between 1% and 4%, are random rather than bunched together at the end of a read; data can be read in real time; throughput is high (the GridION, the bigger of Oxford Nanopore's machines, promises the capacity to sequence the human genome at 30× depth in less than a day); input DNA is not destroyed in the process; and sample preparation is simple and cheap.



Marina Corral

Sequencing through a pore.

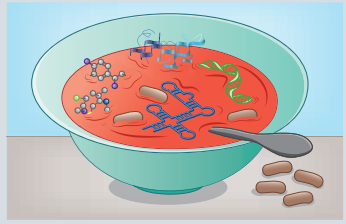
In the sequencer model that is soon to be commercialized, a single DNA strand winds its way through a protein pore, its transition speed controlled by a second protein attached to the pore. As the bases traverse the pore, they alter the current that runs through the pore in a way characteristic for particular base combinations. Ideally one would see a typical pattern for every base, but the present machines will produce patterns characteristic for base triplets that then need to be computationally deconvoluted. And these patterns are not limited to the four DNA bases: nanopores can also detect methylated and hydroxymethylated bases and can, in principle, sequence RNA directly.

No doubt the community will carefully scrutinize nanopore sequencing data as they are released from the first testing sites to see how well these expectations are met.

Nicole Rusk

Probing microbiome function

In studies of microbiome function, marker sequencing will be balanced by alternative profiling approaches.


Marina Corral

Integration of different molecular profiles can help characterize the microbial soup.

The large-scale sequencing of microbes taken directly from the environment began a land grab a few years ago. With metagenomic sequences pouring in from every imaginable habitat, where are the new frontiers in microbial ecology? Molecular profiling methods complementary to DNA sequencing promise major insights into the functions of the unseen biome.

Until now, the bulk of effort has been spent on sequencing phylogenetic markers to identify the number and type of microbes in a sample. But what do these microbes do? Shotgun DNA sequencing and a growing library of sequenced reference genomes help uncover function by highlighting which genes and metabolic pathways are present. Still, this information only outlines potential metabolic functions, and it is limited by the large fraction of poor annotations.

RNA sequencing, protein and metabolite profiling methods, on the other hand, pinpoint which pathways are active. They can lead to novel biochemical discoveries and mechanistic insights into environment-microbe interactions (for example, by sampling peptides and small molecules in the extracellular environment).

Combining complementary data such as transcript and protein expression can also provide better accuracy and support conclusions. Integration requires bringing together different expertise and may require new tools that can synthesize disparate data at the community level. Network modeling, adapted from traditional ecology and systems biology, is a promising approach that is finding use in microbial ecology.

In addition to large sequencing surveys, the future will see more targeted studies that use richer data from a number of approaches, making this an interesting area for methods development. Deciding when to 'go deep' rather than sample more broadly is an important consideration that will need to be driven by the research question. Ultimately, it will also be important to bring microbes back to the lab for in-depth functional studies using high-throughput culturing methods and artificial germ-free environments like gnotobiotic mice.

Tal Nawy