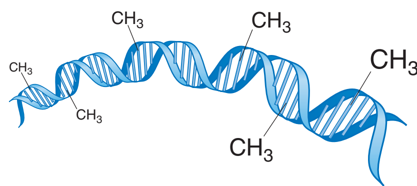
A direct view of the fifth base

Will some single molecule sequencing strategies be able to deliver on the promise of direct methyl cytosine sequencing?

The often-quoted 'four bases' that make up the code of life are really five. The occurrence of a methyl modification on cytosine has important implications for the regulation of DNA transcription; abnormal methylation can wreak havoc with gene regulation and can be the underlying cause of disease.

Given its importance, methods to systematically map the methylome—that is, all genome-wide occurrences of methylcytosine—have recently flourished. Joseph Ecker and colleagues just provided a complete map of all methylcytosines in human embryonic stem cells at base-pair resolution (*Nature* **462**, 315–322, 2009). What this and other sequencing-based



DNA double helix with methylcytosines.

methods, such as the strategy developed by Meissner *et al.* that uses reduced representation libraries (*Nature* **454**, 766–770, 2008), have in common is that they rely on bisulfite conversion, the chemical conversion of unmethylated cytosine to uracil that is then converted to thymine in subsequent amplification steps. Though effective, this approach requires some hands-on time, to ensure complete conversion, and computational effort to map sequences using a redundant code in which a thymine can represent either a thymine or an unmethylated cytosine in the original sequence.

Earlier this year, a report by scientists at Oxford Nanopore Technologies (*Nat. Nanotechnol.* **4**, 265–270, 2009) held out the tantalizing possibility that this labor-intensive technique could be replaced by a simple sequencing step with a sequencer that can directly distinguish unmodified cytosine from methylcytosine. In a nanopore, each base that traverses through it is identified by the current amplitude, the extent to which it blocks the current that is flowing through the pore. Methylcytosine produces a signal clearly distinguishable from those of the other four bases and can thus be read directly.

At present nanopores have only been shown to sequence short oligonucleotides, a far cry from the whole-genome sequencing achieved by bisulfite conversion, and some technical hurdles, such as making sure each base enters the nanopore in the right order and gets swept out on the other side, still need to be worked out—but once they are, the direct sequencing of the fifth base will have a big impact on the field. Let's see if it happens in 2010. **Nicole Rusk**

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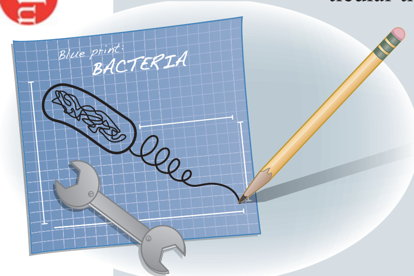
Synthetic life

Will new methods and an emerging understanding of the minimal requirements for cellular life be sufficient to construct a synthetic organism?

Last year we chose synthetic biology, in particular the creation of a synthetic, live organism with a minimal genome, as a method to watch. Although that task was not achieved in 2009, significant strides towards this ultimate goal were nevertheless reported.



Drawing up a blueprint for a synthetic bacterium.

One of the many aims of the broad field of synthetic biology is to harness biological processes to address such global challenges as producing biofuels, synthesizing complex pharmaceuticals and cleaning up polluted environments. Another major objective is to grasp the elusive nature of what is fundamentally required for cellular life.

Researchers at the J. Craig Venter Institute have made it a long-term goal to design a synthetic organism containing the bare minimum of genes required for life, both for practical applications and for understanding basic biology. This necessitated the development of new methods to synthesize and assemble genomes. In 2008, Venter and colleagues used *in vitro* recombination to join small synthetic fragments into larger DNA strands, but they needed to move into yeast for the final recombination step to assemble the 583-kb genome of *Mycoplasma genitalium* (*Science* **319**, 1215–1220, 2008). In 2009, they pushed this approach further, demonstrating all–*in vitro* assembly of the *M. genitalium* genome (*Nat. Methods* **6**, 343–345, 2009). This not only streamlines the method but also circumvents problems of incompatibility between the synthetic genome and the host.

Also in 2009, Venter and colleagues showed that they could manipulate the cloned *Mycoplasma mycoides* genome in yeast before transplanting it into *Mycoplasma capricolum* (*Science* **325**, 1693–1696, 2009). Besides providing a useful approach for manipulating genetically intractable organisms, the ability to engineer a bacterial genome in yeast will prove valuable in defining the minimal requirements for a viable organism. Perhaps in 2010 we may finally witness the emergence of a synthetic bacterium.

The possibility of creating synthetic life has, not surprisingly, raised security and bioethics concerns. Thus it is also a method, in 2010 and beyond, that society at large should watch. **Allison Doerr**

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