GENOME WATCH

Singled out

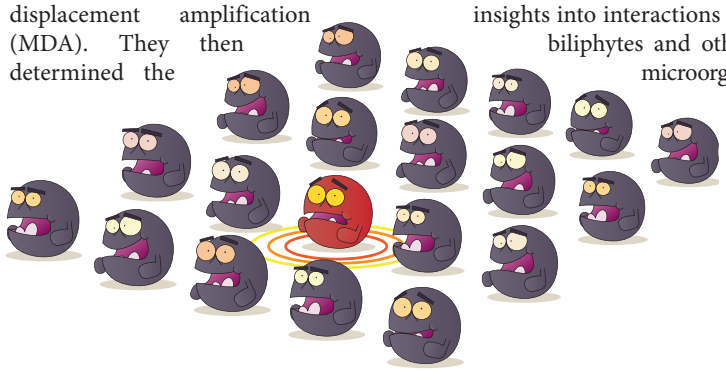
Alan Walker

This month's Genome Watch reviews a recent article that demonstrates the use of single-cell genomics as a means of characterizing uncultivated microorganisms.

The vast majority of microorganisms are not amenable to cultivation in the laboratory, and as a result we have a substantially incomplete and skewed knowledge of microbial life. Insights from small-subunit ribosomal RNA gene surveys and, more recently, metagenomic analyses have revealed the enormous extent of unknown microbial species and gene diversity. However, these techniques do not readily permit the study of direct interactions between organisms *in situ* and cannot define the coding potential of individual cells within a given environment. A complementary approach is the burgeoning field of single-cell genomics (see REF. 1 for a brief overview), in which individual cells are isolated from environmental samples and their genomic DNA is amplified and sequenced.

A recent study by Yoon *et al.*[2] used single-cell genomics in an attempt to characterize novel heterotrophic protists from a 50 ml sample of sea water taken off the coast of Maine, USA. To this end, the authors used fluorescence-activated cell sorting to specifically separate cells that lacked chlorophyll fluorescence, and amplified the genomes of these cells using multiple displacement amplification (MDA). They then determined the taxonomies of these isolates by sequencing their 18S rRNA genes. This revealed that six out of the 35 single-cell amplified genomes (SAGs) were derived from picobiliphytes, a distinct group of marine plankton that was initially identified from environmental 18S rRNA gene surveys and which currently has no cultivated members[3]. Previous work had indicated that picobiliphytes could have a photosynthetic lifestyle, owing to the apparent presence of plastid-like organelles with orange fluorescence indicative of phycobilin proteins[3]. The authors therefore shotgun sequenced three picobiliphyte SAGs using the 454 FLX Titanium platform in the hope that the genomes would reveal whether these cells were heterotrophic or photosynthetic. This generated ~90 Mb of individual reads and ~5 Mb of assembled contigs per SAG. The authors found no genomic evidence for the presence of plastids in any of the three SAGs, suggesting that these picobiliphytes are in fact heterotrophs. To rule out the possibility that plastid-related genes were simply missed owing to MDA bias, deep Illumina sequencing was carried out on two of the SAGs, but such genes were not detected. By contrast, in an experimental control the authors were able to recover more than 50% of the plastid-related genes from MDA-amplified DNA from the photosynthetic amoeba *Paulinella chromatophora*.

The SAGs also provided fascinating insights into interactions between the picobiliphytes and other environmental microorganisms. One of the SAGs contained a large number of sequence reads belonging to a previously uncharacterized single-stranded DNA virus, indicating that the authors had captured a cell in the midst of an infection by the viral invader. The assembled genome of this virus was compared with metagenomic data from the Global Ocean Survey[4], and this revealed that related viruses may be abundant in the ocean. The other two picobiliphyte cells did not appear to contain the single-stranded DNA virus. Instead, non-eukaryotic sequences seemed to correspond to DNA from marine Bacteroidetes, Proteobacteria and large DNA viruses. Although the authors concede that these sequences may result from contamination or attachment of foreign DNA to the surface of the isolated cells, they speculate that these microorganisms may be a food source for the picobiliphytes.



Although the single-cell approach suffers from complications such as amplification bias and contamination issues, this work hints at the future possibilities for single-cell genomics as the technology improves and the cost of sequencing continues to fall. We are approaching the era in which we can at last begin to understand the part that any cell plays in a given environment, without the requirement for prior cultivation.

Alan Walker is at the Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, UK.
e-mail: microbes@sanger.ac.uk

doi:10.1038/nrmicro2603

1. Walker, A. & Parkhill, J. Single-cell genomics. *Nature Rev. Microbiol.* **6**, 176–177 (2008).
2. Yoon, H. S. *et al.* Single-cell genomics reveals organismal interactions in uncultivated marine protists. *Science* **332**, 714–717 (2011).
3. Not, F. *et al.* Picobiliphytes: a marine picoplanktonic algal group with unknown affinities to other eukaryotes. *Science* **315**, 253–255 (2007).
4. Williamson, S. J. *et al.* The Sorcerer II Global Ocean Sampling Expedition: metagenomic characterization of viruses within aquatic microbial samples. *PLoS ONE* **3**, e1456 (2008).

Competing interests statement
The author declares no competing financial interests.