GENOME WATCH

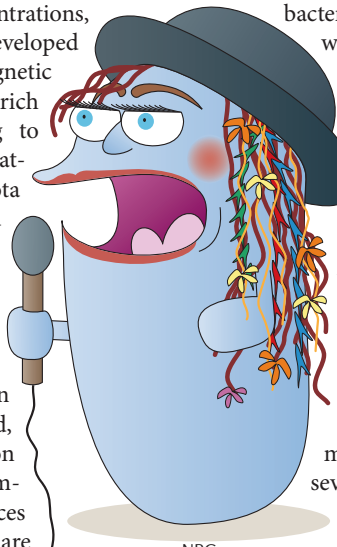
Culture-free club

Josephine M. Bryant

This month's Genome Watch highlights recent studies demonstrating that genomic analyses of pathogens in clinical samples are not limited to culture-friendly bacteria.

The use of bacterial genomics for the detection and characterization of pathogens in clinical samples has traditionally relied on the ability to culture bacteria. However, this is not possibile for all pathogens. Currently available culture techniques are also often technically challenging and time-consuming, which can be a barrier to high-throughput genomic analyses.

The obligate intracellular pathogen *Chlamydia trachomatis* must typically undergo tissue culture if researchers are to obtain sufficient DNA for whole-genome sequencing from clinical samples. However, a recent study demonstrates that it is possible to sequence whole genomes of *C. trachomatis* directly from vaginal swabs[1]. The authors used multiple displacement amplification (MDA), which allows for amplification of DNA from very low starting concentrations, combined with a newly developed antibody-based immunomagnetic separation technique to enrich for *C. trachomatis* (owing to the high levels of contaminating human and microbiota cells that are typically found in vaginal swabs). In conjunction with multiplexed Illumina sequencing, complete *C. trachomatis* genomes were obtained for five of the 18 swabs tested, with a mean coverage exceeding 38-fold, making SNP identification possible. Although the number of high-quality sequences obtained was modest, these are the first bacterial genomes generated directly from clinical samples, and refinement of the technique is predicted to enable sequencing of a wide range of difficult-to-cultivate bacteria from clinical samples.



NPG

One potential caveat of MDA is amplification bias, which is exacerbated by low levels of starting material and by contaminating DNA. However, a second study reports a method to circumvent this problem[2]. In this study, single bacterial cells were cultivated in gel microdroplets (GMDs) to generate more starting material for MDA. This technique is especially useful for studying microbial communities, as individual cells can be grown in separate GMDs and can be further manipulated and sorted by flow cytometry. Using this approach, near-complete genomes were obtained for *Streptococcus* and *Enterococcus* spp. from oral and stool microbiome samples, and the resultant assemblies were found to be substantially larger and more complete than those obtained using MDA of a single cell.

An alternative approach for obtaining a whole-genome sequence from an uncultivable bacterium is to use metagenomics, which can detect all the genetic material present in a complex sample. This also has the advantage of identifying previously undetected pathogens that might be contributing to disease. In a retrospective study, total DNA was analysed from faecal samples obtained during an outbreak of Shiga-toxigenic *Escherichia coli* O104:H4 in Germany[3]. In addition to the recovery of sequences that matched the outbreak strain, several previously undetected pathogens were found, such as *Clostridium difficile* and *Salmonella enterica*. These data demonstrate the diagnostic sensitivity of such an approach and also highlight the idea that it might be overly simplistic to assume there is only one pathogen causing a disease.

A similar approach was also used to examine vaginal swab samples[4]. Using MDA followed by ultra-deep Illumina sequencing, *C. trachomatis* was detected, in addition to a number of co-infecting bacteria. However, in both of these studies, only low genome coverage of the pathogens of interest was obtained. This suggests that although metagenomics can provide information on previously undetected pathogenic species, the low coverage that it yields might make downstream epidemiological analyses difficult.

These studies represent a recent drive to sequence pathogens without culture, but each approach comes with its own challenges. More work will be required to make these techniques more high throughput and more reliable to allow culture-free sequencing to become feasible in clinical settings.

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

doi:10.1038/nrmicro3052
Published online 10 June

1. Seth-Smith, H. M. *et al.* Whole-genome sequences of *Chlamydia trachomatis* directly from clinical samples without culture. *Genome Res.* **23**, 855–866 (2013).
2. Fitzsimons, M. S. *et al.* Nearly finished genomes produced using gel microdroplet culturing reveal substantial intraspecies genomic diversity within the human microbiome. *Genome Res.* **23**, 878–888 (2013).
3. Loman, N. J. *et al.* A culture-independent sequence-based metagenomics approach to the investigation of an outbreak of Shiga-toxigenic *Escherichia coli* O104:H4. *JAMA* **309**, 1502–1510 (2013).
4. Andersson, P. *et al.* Sequences of multiple bacterial genomes and a *Chlamydia trachomatis* genotype from direct sequencing of DNA derived from a vaginal swab diagnostic specimen. *Clin. Microbiol. Infect.* 6 Apr 2013 (doi:10.1111/1469-0691.12237).

Competing interests statement
The author declares no competing financial interests.