SCORING BACTERIAL TUMBLES AND RUNS

Methods to study bacterial motility range from soft agar plate assays to microscopic tracking of bacterial movement. The latter method is much more precise and quantitative than the former, but it is also costlier and more complex due to need for high speed optical equipment, large amounts of data storage, and complex computation. In this month's issue of *BioTechniques*, William Bentley and his colleagues at the University of Maryland, College Park, describe TumbleScore, a MATLAB-based, freely available software for rapid tracking and quantification of bacteria swimming from movies with slow or fast frame-rates obtained from light or fluorescence microscopes. After the paths of the bacteria have been determined, software tools can be used to quantify complex motility behavior. For flagellated bacteria, the software provides various metrics for the phenomenon of tumbling, where the smooth path of the bacterium during a run propelled by the normal counterclockwise motion of the flagellum is interrupted by a sudden and random change in direction caused by a brief clockwise flagellar motion.

See “TumbleScore: Run and tumble analysis for low frame-rate motility videos” on page 31.

ANOTHER RECOMBINASE FOR RMCE

Site-specific recombinases such as Fip, Cre, and Φ C31 have been successfully exploited by researchers to rapidly and reliably modify the *Drosophila melanogaster* genome. One particularly useful application of site-specific recombinases is recombinase-mediated cassette exchange (RMCE), in which an acceptor cassette containing a transgenic construct flanked by sites specific for a recombinase is introduced into the *Drosophila* genome at a specific locus. In the presence of the site-specific recombinase, this acceptor cassette can be readily exchanged with a donor cassette containing a different transgenic construct flanked by the same recombinase-specific sites. This allows easy sequential introduction of any number of different cassettes into that specific genomic location. In this month's issue, Roumen Voutev and Richard S. Mann at Columbia University have adapted RMCE to work with the newer site-specific phage recombinase Bxb1. They demonstrate that Bxb1 can be efficiently used for RMCE, either alone or in parallel with other recombinases already in use for RMCE, further extending the versatility of RMCE in *Drosophila*.

See “Bxb1 phage recombinase assists genome engineering in *Drosophila melanogaster*” on page 37.

Selected and written by Patrick C.H. Lo, Ph.D.

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FASTER TISSUE CLEARING CHIPS

The ability to image deep inside tissues is important for basic biological research as well as for drug discovery. Methods such as CLARITY have made it possible to render opaque tissues transparent for deep imaging, but these clearing protocols are very time-consuming. Chen et al. now report a microfluidic chip-based strategy to clarify 3-D tissue samples for structural analysis that is 20 times faster than other approaches and also amenable to high-throughput screening. The key to this method lies in the 567-fold increase in the rate of exchange of interstitial fluids that can be achieved on the chip, resulting in a more rapid removal of light-scattering lipids from tissue samples. The authors used their new system to image subpopulations of cells during tumor spheroid formation and to monitor vascular degradation in murine pancreatic islets over time. This new microfluidic workflow should greatly enhance organ-on-a-chip studies as well as high-throughput drug screening efforts.

Chen et al. 2016. “Clarifying intact 3D tissues on a microfluidic chip for high-throughput structural analysis” *Proc. Natl. Acad. Sci.* [Epub ahead of print] doi:10.1073/pnas.1609569114

TEST-DRIVING NANOPORE SEQUENCING

Oxford Nanopore Technologies' MinION platform is the first nanopore-based single-molecule nucleic acid sequencing system to be released commercially. As more researchers begin to use nanopore sequencing, it's important that they understand the capabilities of this new technology. Oikonomopoulos et al. provide one such benchmark by testing the performance of the MinION platform for quantitative and qualitative assessment of cDNA populations. The authors sequenced a defined set of polyadenylated transcripts as well as a population of cDNAs from an HEK-293 cell line using the MinION platform, the Illumina HiSeq2500, and the PacBio RS II. Their MinION data were in good agreement with that of the other two most established platforms when it came to measuring cDNA abundance and length. The authors conclude that the MinION system can be used for reliable quantification of either long or short cDNA molecules in complex samples.

Oikonomopoulos et al. 2016. “Benchmarking of the Oxford Nanopore MinION sequencing for quantitative assessment of cDNA populations” *Scientific Reports* 6:31602. doi:10.1038/srep31602

Selected and written by Nathan S. Blow, Ph.D.

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