SEQUENCING

Circular consensus sequencing with long reads

Wenger, A.M. et al. *Nat. Biotechnol.* http://doi.org/dbgr (2019).

Long-read sequencing technologies have advantages in genome assembly, structural variant detection and haplotype phasing, but are less suited for single-nucleotide variant (SNV) and insertion/deletion (indel) calling due to the high error rate in comparison with short-read sequencing. Wenger et al., from Pacific Biosciences, optimized the circular consensus sequencing (CCS) protocol to achieve long, high-fidelity reads, in which they selected the SMRTbell library with fractions tightly distributed at 15 kb for high-coverage sequencing. The CCS library preparation was inspired and optimized based on their findings that polymerases have a better survival on damage-free DNA molecules. They employed the CCS protocol in sequencing the human genome (HG002) and achieved an average length of 13.5 kb, and an average accuracy of 99.8%. The CCS performance is comparable to, or better than, that of short-read sequencing in SNV and indel calling. *LT*

https://doi.org/10.1038/s41592-019-0605-6

MICROSCOPY

The miCube open microscope

Martens, K. J. A. et al. *Nat. Commun.* **10**, 3552 (2019).

Single-molecule localization microscopy has become a powerful tool for imaging subcellular structures and for tracking the movements of single molecules within cells. Despite the power of these approaches, a lack of appropriate microscopes still limits widespread use. Martens et al. have developed the miCube, an open-source microscopy framework for doing single-molecule microscopy. The miCube is straightforward for users to build, with readily available and custom-made parts, and is run with freely available software. In this way, the miCube is poised to pave the way for broader uptake of localization microscopy. To demonstrate the performance of the miCube, the authors used their system to study the interactions between dCas9 and DNA in live bacteria using single-particle tracking in the presence or absence of DNA targets, which ultimately resulted in a model that predicts Cas9 cleavage efficiency in bacteria. *RS*

https://doi.org/10.1038/s41592-019-0607-4

STRUCTURAL BIOLOGY

Transplanting antihelix antibody epitopes

Kim, J. W. et al. *Proc. Natl Acad. Sci. USA* **116**, 17786–17791 (2019).

Antibodies can facilitate X-ray crystallography of target proteins. They facilitate crystallization by binding and freezing the conformations of proteins that tend to adopt multiple structural states. Generating conformation-specific antibodies for target proteins, however, is both time-consuming and challenging. Kim et al. show that antibodies that bind helices can be made to bind unrelated proteins by engineering a solvent-exposed helix to resemble the antibody epitope. This epitope 'transplanting' methodology was demonstrated by crystallizing eight engineered protein–antibody complexes using three antihelix antibody fragments. In each case, the overall structure of the mutated protein was not altered, and the antibody–helix interaction was both high-affinity and identical to the native antibody–helix complex. The technique could prove to be useful for studying transmembrane proteins and high-throughput drug design, among others, by accelerating X-ray crystallography, electron microscopy and nanotechnology. *AS*

https://doi.org/10.1038/s41592-019-0606-5

GENOMICS

High-fidelity nucleases

Miller, J. C. et al. *Nat. Biotechnol.* **37**, 945–952 (2019).

Genome engineering with any type of exogenous nuclease needs to improve on two aspects if it is to realize its full potential for therapeutic applications: the editing efficiency must be high, to allow for biallelic or even multiplexed editing; and editing at unintended sites must be eliminated. Much progress has been made to increase the fidelity of currently used engineered nucleases such as Cas9, but most efforts to date have focused on tuning the affinity of the nuclease for its target DNA. Lam et al. instead add a point mutation to Fok1, the catalytic domain of zinc finger nucleases, and thereby slow the cleavage kinetics. This, in turn, reduces off-target activity. After developing an assay to detect very rare insertion/deletions below a signal of 0.01%, the researchers document a reduction in cutting at known off-target sites by up to 3,000-fold. They are able to target a T-cell receptor locus with 98% efficiency and no off-target cutting. This modified Fok1 domain could also be fused to other nucleases, such as Cas9, and enhance their specificity. *NR*

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