SEQUENCING

Improving single-cell RNA counting

Hagemann-Jensen, M. et al. *Nat. Biotechnol.* **38**, 708–714 (2020).

In single-cell RNA sequencing, full-length transcriptome coverage is important for reconstructing the RNA molecule and assigning its allelic origin and isoform. Smart-seq2 offers a sensitive approach to obtaining full-length coverage of transcripts and enables the quantification of isoform-level expression from single cells. Yet Smart-seq2 lacks a unique molecular identifier (UMI), valuable for profiling isoform-level RNA counting. Hagemann-Jensen et al. describe Smart-seq3, which further improves the sensitivity of Smart-seq2. They examined hundreds reaction conditions for preparing transcriptome libraries and identified the optimized reverse transcriptase and buffer conditions. In addition, they introduce a UMI, a partial Tn5 motif and a tag sequence in the template-switching oligonucleotide, which allows them to sequence different parts of the full-length cDNA and thus to reconstruct the RNA molecules in silico. They show that Smart-seq3 can detect thousands more transcripts than Smart-seq2 per cell. *LT*

https://doi.org/10.1038/s41592-020-0901-1

NEUROSCIENCE

Benchmarked spike sorting

Magland, J. et al. *Elife* **9**, e55167 (2020).

Spike sorting involves the extraction of the activity of individual neurons from a sea of activity in extracellular recordings. While manual spike sorting is feasible for small datasets, automated spike sorting algorithms are a necessity for large-scale recordings. Magland et al. have established SpikeForest, a software suite and dedicated website for benchmarking spike sorting algorithms. The researchers ran ten popular spike sorting algorithms on 13 electrophysiology datasets that were associated with ground truth data. While most algorithms excelled for one or more of the datasets, none of the algorithms emerged as a clear winner across all datasets. This result shows that the choice of spike sorting algorithm should be guided by the characteristics of the dataset to be analyzed. For example, MountainSort4 performs well on tetrode and monotrode recordings, while Kilosort2 shines in recordings with low signal-to-noise ratio. Benchmarking results as well as Docker containers for the ten algorithms are available at https://spikeforest.flatironinstitute.org. *NV*

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SENSORS AND PROBES

A clearer view of mitophagy

Katayama, H. et al. *Cell* **181**, 1176–1187.e16 (2020).

Dysfunctional mitochondria are a hallmark of many diseases, but methods to study mitophagy, the process by which lysosomes remove these mitochondria, are limited. Katayama et al. have developed an improved indicator for mitophagy. They first engineered a cyan fluorescent protein called TOLLES (Tolerance of Lysosomal Environments) that is resistant to both acidic environments and proteases present in the lysosome. They then fused TOLLES to the yellow fluorescent protein YPet. This construct loses its yellow fluorescence in the lysosomal environment, as YPet is both acid- and protease-sensitive. The retained cyan fluorescence can be observed in fixed and living cells as a readout for autophagy. This probe, which the researchers call signal-retaining autophagy indicator (SRAI), was then specifically targeted to generate the mitophagy probe mito-SRAI. The researchers used mito-SRAI in a screen for compounds that induce mitophagy and to study mitophagy in neurons in a mouse model of Parkinson's disease. *RS*

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COMPUTATIONAL BIOLOGY

GemSpot allows modeling of ligands in cryo-EM maps

Robertson, M. J. et al. *Structure* **28**, 707–716.e3 (2020).

Recent developments in cryoelectron microscopy (cryo-EM) now routinely allow near-atomic and atomic-level resolution for biomolecules. Determining structures of protein–ligand complexes, however, remains a challenge, with the resolution of the bound ligand being significantly lower than that of the protein. Thus, understanding ligand binding position necessitates creative solutions, such as using computational chemistry methods in conjunction with experimental data. Robertson et al. have developed GemSpot, an automated computational docking pipeline for modeling and evaluating ligand binding poses in cryo-EM maps. It uses the tool GlideEM to for docking, taking into account the EM map potentials as restraints. Model refinement is done using the OPLS3e force field and quantum mechanics calculations, and water molecule sites are predicted using JAWS. The authors demonstrate the pipeline on a several proteins at varying levels of modeling complexity and EM map resolution. *AS*

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