IMMUNOLOGY

Representation matters for T cell responses

Martin, M. D. et al. *Cell Rep.* **31**, 107508 (2020).

Recent studies report the failure of inbred mice to recapitulate the individuality of immune responses observed in genetically diverse populations. To map the diversity in the antiviral $CD8^+$ T cell response, Martin et al. infected 47 mouse strains from the Collaborative Cross (CC) with acute lymphocytic choriomeningitis virus (LCMV) Armstrong. CC is an established recombinant mouse panel generated from diverse founder strains to better model human diseases. The researchers showed that, in comparison to inbred strains, CC strains produced varied quantities of cytokines during the innate immune response. This variation was indicative of the scale of the effector response and the size of the memory pool, suggesting that genetic factors underlying innate responses could inform T cell immunity. The authors confirmed these results using quantitative trait linking experiments. This study highlights the use of CC to discover genetic factors that may modulate antiviral responses, thus offering insight into T cell response diversity at a population level. *MM*

https://doi.org/10.1038/s41592-020-0874-0

SEQUENCING

Mapping DNA single-strand breaks

Sriramachandran, A. M. et al. *Mol. Cell* https://doi.org/10.1016/j.molcel.2020.03.027 (2020).

Sequencing-based methods have been developed to detect DNA damage events and reveal their genome-wide distribution. Single-strand breaks (SSBs) are one of the most frequent types of DNA damage in the genome and have been profiled by methods involving poly(A) tailing or labeling during nick translation, which may reduce the resolution of the original position of break sites. To obtain a map with nucleotide resolution, Sriramachandran et al. developed genome-wide ligation of 3′-OH ends followed by sequencing (GLOE-Seq), which detects free 3′-OH termini resulted from SSBs, lesions or other repair intermediates. In GLOE-Seq, genomic DNA is heat-denatured and ligated to a biotinylated adapter with the assistance of a splinter oligonucleotide, followed by fragmentation and capture on streptavidin beads. GLOE-Seq has been applied in mapping DNA SSBs and lesions in yeast and human chromatin. The researchers also provide a software pipeline for annotating and visualizing strand breaks. *LT*

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NEUROSCIENCE

Optimized tissue clearing

Susaki, E. A. et al. *Nat. Commun.* **11**, 1982 (2020).

Rendering large tissue samples such as organs or whole organisms transparent yet suitable for antibody or dye staining remains challenging despite the wealth of tissue-clearing protocols. In particular, full tissue penetration of staining reagents merits further research. Susaki et al. have developed an improved clearing pipeline based on the established CUBIC technique. Their modified CUBIC-HistoVision approach optimizes ionic strength, temperature and chemical composition of the tissue-clearing cocktails, as well as antibody staining conditions and other steps in the pipeline. Furthermore, the researchers tested several nuclear stains as well as 44 antibodies relevant to the neuroscience community for their performance in CUBIC-HistoVision. The researchers primarily showcase the pipeline for immunostaining or nuclear labeling of mouse brains, but also demonstrate it on an intact infant marmoset specimen. *NV*

https://doi.org/10.1038/s41592-020-0875-z

SINGLE-MOLECULE

Fast analysis of single-molecule data

White, D. S. et al. *Elife* **9**, e53357 (2020).

Statistically robust single-molecule experiments can include large numbers of long recordings of single-molecule trajectories. However, methods for analyzing these large datasets in an unsupervised manner have lagged methods for generating the data. White et al. have developed DISC (Divisive Segmentation and Clustering), which provides fast and accurate unsupervised analysis of large datasets. In DISC, model-free statistical learning is merged with the Viterbi algorithm, yielding analysis results that are more accurate than commonly used algorithms and improving speeds by three orders of magnitude. The researchers validated the performance of DISC on simulated data and then demonstrated the algorithm's utility on experimental data looking at cooperativity of the binding of cAMP to cyclic nucleotide binding domains from cyclic nucleotide-gated ion channels. *RS*

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