IN BRIEF

NEUROSCIENCE

Pose estimation with deep learning

Elife https://doi.org/10.7554/eLife.47994 (2019).
Elife https://doi.org/10.7554/eLife.48571 (2019).

An in-depth study of animal behavior at the individual level requires analysis of the animal's pose. Recent deep-learning-based approaches include DeepLabCut and LEAP. Graving et al. developed an optimized deep learning architecture called Stacked DenseNet to surpass DeepLabCut and LEAP in inference speed and accuracy. The researchers applied their DeepPoseKit framework to datasets of fruit flies, locusts and zebras. While DeepPoseKit is optimized to analyze posture in 2D in freely moving animals, Günel et al. developed a deep learning pipeline to monitor the pose of tethered fruit flies in 3D while they walked on a ball. DeepFly3D uses images from seven cameras to identify 38 landmarks on the animal's body and to generate a representation of a fruit fly's pose in 3D. The researchers used their pipeline to perform a behavioral classification task in control and optogenetically manipulated fruit flies. *NV*

https://doi.org/10.1038/s41592-019-0678-2

NEUROSCIENCE

Modeling neurulation

Nat. Biotech. **37**, 1198–1208 (2019).

As an early stage of human development, neurulation sees the formation of the main ectodermal lineages, including neural progenitors, neural crest, sensory placodes and epidermis. Ectodermal lineages are involved in a number of genetic diseases, underscoring the need for in vitro systems modeling the developmental processes involved. Haremaki et al. cultured pluripotent human embryonic stem cells (hESCs) on circular micropatterned substrates with dual-SMAD inhibition and subsequent BMP4 stimulation. The standardized neural structures generated ('neuruloids') are capable of self-organization and acquire fates representing the main lineages. Expression analysis of marker genes using single-cell RNA-sequencing data suggests neuruloids recapitulate development at the neural tube closure stages, from days 21 to 25. Applying this technology to generate neuruloids using isogenic Huntington's disease hESCs, the authors identified disease-specific phenotypes manifesting during early development. *LT**

https://doi.org/10.1038/s41592-019-0680-8

GENETICS

Vector integration at breaks

Nat. Commun. https://doi.org/10.1038/s41467-019-12449-2 (2019).

Adeno-associated virus (AAV) vectors hold great interest for encoding CRISPR–Cas nucleases for in vivo CRISPR delivery. Yet the potential consequences of long term expression of Cas9 from an AAV vector are largely unknown. Hanlon et al. analyzed the integration events of AAV vectors in differentiated cells of brain, muscle and cochlea. Across the three different organs, they observed similar, high levels of AAV integration at nuclease-induced breaks in therapeutically targeted genes. Detailed AAV mapping in mouse brain shows that the expression of Cas9 does not affect genome-wide integration outside the target locus. To learn the complete integration profiles, the researchers constructed a 465-bp miniature AAV vector for sequencing the entire integration. They observed that the integrants could be fragmented, full-length or concatemers. Therefore, AAV vector integration at target sites should be assessed, especially when clinical applications are considered. *LT*

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SENSORS AND PROBES

Detecting intracellular PPIs with CLEM

Cell Chem. Biol. **26**, 1407–1416 (2019).

Visualizing protein–protein interactions (PPIs) with correlative light and electron microscopy (CLEM) has mostly been restricted to extracellular interactions. To overcome this limitation, Boassa et al. have generated a split-miniSOG reporter. miniSOG has the attractive property of being both fluorescent and able to generate reactive oxygen species upon illumination. These species can then be harnessed to precipitate 3,3′-diaminobenzidine, which reacts with osmium tetroxide to form an EM-visible precipitate. To detect PPIs, the researchers split miniSOG and fused the resulting fragments to the proteins of interest. As the two fragments exhibit low affinity for each other, they only reconstitute in the presence of a PPI, and the reconstitution is reversible when the PPI is no longer present. The researchers illustrate the utility of their split-miniSOG by visualizing the interaction between Fos and Jun in cultured cells, as well as other PPIs, using both light and electron microscopy. *NV*

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