METAGENOMICS

Human gut bacterial genome reference

Zou, Y. et al. *Nat. Biotechnol.* **37**, 179–185 (2019).
Forster, S. C. et al. *Nat. Biotechnol.* **37**, 186–192 (2019).

The human gut microbiota harbors dynamic and complex populations of microorganisms that influence human health and disease. Advanced metagenomics sequencing in combination with computational tools enables analyses of genomic content and taxonomic classification of this microbiome. However, high-quality reference genomes are still needed for precise taxonomic classifications. Two recent studies independently report genome references of cultivated human gut bacteria. Zou et al. present the Culturable Genome Reference (CGR), which reports about 1,500 microbial genomes (264 new genomes) from 155 donors, representing more than 300 bacterial species. In addition, Forster et al. present the Human Gastrointestinal Bacteria Culture Collection (HBC), which provides genome references for 737 bacterial isolates, representing 168 known species and 105 novel species. Both research groups purified and cultivated bacterial isolates from human fecal samples, although they used different culturing media. The increasing number of references will improve mapping of metagenomics reads and functional characterizations. *LT*

https://doi.org/10.1038/s41592-019-0384-0

GENOMICS

Human immunity to Cas9

Charlesworth, C. T. et al. *Nat. Med.* **25**, 249–254 (2019).

Genome editing with the CRISPR–Cas9 system has inspired great hopes for the seamless correction of disease-causing mutations. The ease of the process—delivery of Cas9 and a guide RNA that directs the nuclease to its cleavage site, where the repair process creates or removes mutations—has raised hopes that deleterious human mutations can be fixed with similar ease. Charlesworth et al. now raise the important caveat that Cas9 nucleases often elicit an immune response in humans. The two most commonly used sources of Cas9 are the species *Streptococcus pyogenes* (Sp) and *Staphylococcus aureus* (Sa), bacteria that often infect humans. The researchers tested the blood of 125 adult donors for antibodies against the Cas9 species and found that 78% of donors react with SaCas9, and 58% with SpCas9. Furthermore, they showed that 78% and 67% of donors, respectively, showed positive T cell reactivity to the two Cas9 species. These preexisting adaptive immune responses to Cas9 in humans need to be taken into consideration for therapeutic applications of CRISPR. *NR*

https://doi.org/10.1038/s41592-019-0385-z

NANOBIOTECHNOLOGY

Improved exosome detection

Zhang, P. et al. *Nat. Biomed. Eng.* https://doi.org/10.1038/s41551-019-0356-9 (2019).

Exosomes are a subset of extracellular vesicles that play critical roles in normal and disease physiology. Their roles are currently the subject of intense interest; however, their small size (typically less than 150 nm in diameter) makes them challenging to study. Thus, improved methods for sensing, isolating, and analyzing exosomes and, more broadly, extracellular vesicles are badly needed. Zhang et al. have developed a device for extremely sensitive detection of exosomes, enabling the detection of as few as ten exosomes per microliter. Their microfluidic device uses three-dimensional herringbone nanopatterns, which promote exosome–surface interactions for improved binding. They used their device to study exosomes from people with ovarian cancer and from healthy controls. Their results identified a potential biomarker for early detection of ovarian cancer and highlight the benefits of such a sensitive platform. *RS*

https://doi.org/10.1038/s41592-019-0386-y

EPIGENETICS

Bisulfite-free epigenetic sequencing

Liu, Y. et al. *Nat. Biotechnol.* https://doi.org/10.1038/s41587-019-0041-2 (2019).

Bisulfite sequencing uncovers DNA modifications. However, it requires the conversion of unmodified cytosine to uracil, which can reduce DNA sequence richness, and when paired with harsh reaction conditions, it can substantially degrade DNA. To address these limitations, Liu et al. attempted to sequence 5mC and 5hmC directly and to retain unmodified cytosine intact. To this end, they used TET enzymes to oxidize 5mC and 5hmC to 5-carboxylcytosine (5caC), and then induced conversion to dihydrouracil (DHU) via borane reduction. A subsequent PCR reaction enabled the conversion of DHU to thymine. The researchers termed this 5mC/5hmC-to-T method TET-assisted pyridine borane sequencing (TAPS). An advantage is that the mild reaction conditions can preserve DNA fragments more than 10 kb long. To sequence 5mC alone, they used β-glucosyltransferase to protect 5hmC from TET oxidation and borane reduction so that only 5mC was converted to T. Alternatively, one could use potassium perruthenate to specifically oxidize 5hmC, thus enabling 5hmC-to-T conversion. *LT*

https://doi.org/10.1038/s41592-019-0387-x

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