METHODS IN BRIEF

GENOMICS

Timed CRISPR action enhances homology-driven repair

Cells use two strategies to repair a double-strand break (DSB) in their DNA: one, the joining of nonhomologous ends, leads to variable mutations; the other, homology-directed repair (HDR), uses a homologous donor to precisely repair the break. Targeted nucleases such as Cas9, which is part of the clustered, regularly interspaced, short palindromic repeats (CRISPR) system, induce such DSBs, but the rate of HDR is often low. Lin *et al.* reasoned that the simple synchronization of cell-cycle stages and the timed delivery of the Cas9–guide RNA complex would increase the rate of HDR. They transfected the Cas9 ribonucleoprotein complex into various cell types in late S or G2 phase—the only stages in which HDR occurs—and saw HDR-mediated editing levels of nearly 40%.

Lin, S. *et al. eLife* doi:10.7554/eLife.04766 (15 December 2014).

STEM CELLS

Reprogramming methods compared

Schlaeger *et al.* report a systematic comparison of three methods for reprogramming somatic cells to induced pluripotent stem cells that do not involve incorporation of exogenous DNA into the genome. They reprogrammed human fibroblasts using a Sendai virus–based system, an episomal system, or mRNA-based reprogramming, benchmarking these to integrating viral methods. They report no discernible difference between the methods in terms of pluripotency or differentiation potential of the resulting cells. RNA-based reprogramming is more efficient (when it succeeds), but it has an overall lower success rate than the other methods. Episomal reprogramming generates cells with a slightly higher incidence of karyotypic instability, but this is still lower than that seen with retroviral reprogramming. Cells reprogrammed with Sendai virus require a relatively long time until they are vector free. The most appropriate method will depend on the needs of each laboratory; this analysis should help researchers decide which reprogramming method best meets their needs.

Schlaeger, T.M. *et al. Nat. Biotechnol.* doi:10.1038/nbt.3070 (1 December 2014).

IMAGING

Bacterial ghosts for cryo-electron tomography

Cryo-electron tomography (cryo-ET) is a powerful tool for generating images of three-dimensional structures of biological specimens. However, it is limited to imaging thin structures. The study of membrane-protein complexes in intact bacterial membranes by cryo-ET remains challenging owing to the thickness of typical bacteria. To bypass this problem, Fu *et al.* generated bacterial 'ghosts' for use in cryo-ET by expressing the phage ΦX174 lysis gene E in *Escherichia coli*. Gene E expression leads to spot lysis, which allows the cytoplasm to escape while preserving the cell membrane and cell shape. By carrying out cryo-ET on these ghosts, the researchers were able to generate a high-resolution tomographic reconstruction of chemotaxis receptor signaling complexes within the cell membrane. This method holds promise for facilitating structural analysis of membrane-protein complexes in a native context.

Fu, X. *et al. Structure* **22**, 1875–1882 (2014).

SEQUENCING

RNA caps in bacteria stabilize transcripts

Some bacterial RNA was recently found to undergo covalent modification with nicotinamide adenine dinucleotide (NAD), but the function of this modification is not clear. Cahová *et al.* have now developed NAD captureSeq, a method to identify messages that bear this mark in the prokaryotic transcriptome. The method relies on chemical conversion of NAD to a moiety that can undergo copper-catalyzed cycloaddition, or 'click chemistry' with biotin azide, for subsequent enrichment with streptavidin beads and sequencing. The researchers found that NAD can stabilize transcripts analogously to the 5′ cap structure in eukaryotic cells. The mark is more prevalent in small regulatory RNAs, and the researchers were able to identify an enzyme with decapping activity.

Cahová, H. *et al. Nature* doi:10.1038/nature14020 (22 December 2014).