METHODS IN BRIEF

SYNTHETIC BIOLOGY

Making yeast

Following viruses and bacteria, the yeast is the latest organism to be equipped with a fully functional synthetic chromosome. Researchers in the Synthetic Yeast Genome Consortium (Annaluru *et al.*) engineered a leaner chromosome III of *Saccharomyces cerevisiae* with only 273 kilobase pairs instead of the native 317 kilobase pairs. The first step in the assembly, going from small nucleotides to 750-base-pair building blocks, was done by undergraduate students at Johns Hopkins University. The researchers then transformed the building blocks into yeast, where they successively replaced native sequence by homologous recombination. After 11 rounds, the entire chromosome III was made up of the synthetic sequence that no longer carried sequences such as subtelomeres and introns but instead had non-essential genes flanked with *loxP* sites to allow subsequent scrambling of the genome. Strains carrying the synthetic chromosome showed no reduction in fitness.

Annaluru, N. *et al. Science* **344**, 55–58 (2014).

GENOMICS

Phasing at any level of relatedness

Going from single-nucleotide polymorphism (SNP) genotype to a haplotype (the SNPs specific to one chromosome) requires statistical methods. Previous approaches to phasing, the determination of a haplotype, for individuals in a cohort have required knowledge about the relatedness of the individuals. Most methods assume no relatedness; some can deal with a predefined level of connections such as family trios. O'Connell *et al.* now present an approach that allows phasing at any level of relatedness. First they used SHAPEIT2 to infer a haplotype while ignoring all information about familial connections. Then they used a hidden Markov model (duoHMM) to infer the inheritance pattern from the SHAPEIT2 data and any available family information. Not only is this combined method highly accurate, it can also detect genotyping errors and infer recombination events in duos and trios.

O'Connell, J. *et al. PLoS Genet*. **10**, e1004234 (2014).

SENSORS AND PROBES

Cellular imaging of single-splice variants

Alternative splicing is an important form of post-transcriptional regulation, and disease states have been linked to incorrectly spliced transcripts. Methods are needed to quantitatively assess splicing in living cells. Lee *et al.* functionalized gold nanoparticles with either of two oligonucleotides that target opposite sides of a splice junction. Binding of both primers at a splice junction brings the nanoparticles into close proximity, resulting in a spectral shift that can be distinguished by hyperspectral plasmon resonance imaging. The method can detect smaller splicing differences than similar methods that use two fluorophores. The researchers showed that specific splice forms of breast cancer susceptibility gene 1 (*BRCA1*) can be detected and quantified down to the single-molecule level *in vitro* and in living cells.

Lee, K. *et al. Nat. Nanotechnol.* doi:10.1038/nnano.2014.73 (20 April 2014).

CELL BIOLOGY

Profiling tumors with sugar

Tumors are known to be heterogeneous at the genomic and protein levels. Veiseh *et al.* took heterogeneity profiling a step further, using fluorescent hyaluronic acid (HA), a glycan, to identify stable subpopulations within triple-negative breast cancer cell lines. HA binding to the cells is heterogeneous, and although the overall binding levels correlate with those of the HA receptors CD44 and RHAMM, this heterogeneity cannot be entirely explained by receptor expression level. The researchers made use of the heterogeneous HA signal to sort subpopulations of cells by flow cytometry. The subpopulations were stable and phenotypically different in both *in vitro* and *in vivo* assays: cells with high HA binding proliferated more slowly but appeared to be more invasive in three-dimensional cultures than cells with low HA binding. Sugar profiling thus uncovers or stabilizes previously unknown functional heterogeneity in even well-studied tumor cell lines.

Veiseh, M. *et al. Proc. Natl. Acad. Sci. USA* **111**, e1731–e1739 (2014).