TOOLS IN BRIEF

GENETICS

Improved guide RNA design

The efficiency with which particular single guide RNAs (sgRNAs) effect gene knockout via the clustered, regularly interspaced, short palindromic repeats (CRISPR)-Cas9 system can vary greatly. Doench *et al.* systematically examined the activity of sgRNAs targeting nine genes in human and mouse to identify sequence features of the target site that can be used to predict sgRNA activity. They delivered sgRNAs to cells using lentiviral vectors such that the sgRNAs were tiled over the target genes and were delivered at one sgRNA per cell. The cells were then screened by flow cytometry for knockdown of the targeted proteins, and the most efficient sgRNAs were thus identified. Analysis of target-site features of close to 2,000 sgRNAs yielded a model that Doench *et al.* implemented in a web tool that can be used to design highly effective sgRNAs.

Doench, J.G. *et al. Nat. Biotechnol.* doi:10.1038/nbt.3026 (3 September 2014).

SENSORS AND PROBES

A mass cytometry activity-based probe

Mass cytometry offers single-cell multiparametric analysis of a much higher order than that of traditional flow cytometry by utilizing high–molecular weight stable isotopes as tags that are read out by inductively coupled plasma mass spectrometry. This avoids the problems of spectral overlap that limit the number of parameters observable by fluorescence-based flow cytometry. Edgar *et al.* now demonstrate the potential for expanding the applications of this powerful technology to read out results from activity-based probes. They designed a probe for cellular hypoxia, dubbed Telox, by linking 2-nitroimidazole, a reporter for hypoxia, with a tellurium-based mass tag that can be quantified by mass cytometry. They demonstrated that Telox could identify hypoxic tumor cells in a mixture as well as discriminate populations of cells by the amount of tellurium labeling, reflecting differential oxygen exposure.

Edgar, L.J. *et al. Angew. Chem. Int. Ed. Engl.* doi:10.1002/anie.201405233 (4 September 2014).

GENETICS

Seeking genetic variants that affect splicing

Expression quantitative trait loci (eQTL) methods are used to associate genetic variants with changes in gene expression. Although RNA sequencing can measure the expression of individual splice forms, existing tools do not describe the abundance of individual isoforms relative to expression of the entire gene. Monlong *et al.* introduce sQTLseekeR, statistical software that represents splice forms as multivariate isoform ratios to detect splicing QTLs (sQTLs), genetic variants that are associated with altered splicing ratios. In analyzing 465 lymphoblastoid cell lines with genotype data from the 1000 Genomes Project and RNA sequence data from the GEUVADIS project, sQTLseekeR identified over 1,900 sQTLs (at a 1% false discovery rate), including a handful of '*trans*' sQTLs that affect the splicing of multiple genes and are enriched for RNA processing and splicing genes.

Monlong, J. *et al. Nat. Commun.* **5**, 4698 (2014).

SENSORS AND PROBES

A universal voltage indicator

Voltage sensing in humans has so far been impossible with fluorescent molecules such as organic dyes or genetically encoded voltage sensors. Treger *et al.* discovered that the dye indocyanine green (ICG), which is approved for use in humans, changes its fluorescence in response to voltage fluctuation. They showed that ICG can follow up to 100 action potentials per second in synthetic neurons. ICG reports voltage increases of 100 mV with a drop in fluorescence of about 2%. The researchers applied ICG to measure electrical activity in cultured neurons and rat hippocampal slices. Furthermore, they detected defects in synthetic neurons expressing a mutated sodium channel. Although ICG has not yet been applied to human tissue, conceivably this dye will be used to monitor electrical activity in humans in the future.

Treger, J.S. *et al. Biophys. J.* **107**, L09–L12 (2014).