TOOLS IN BRIEF

BIOINFORMATICS

The ups and downs of transcripts

Turning raw RNA sequencing data into a genome-wide view of transcription requires overcoming a number of difficulties. Many unique but related transcripts can be produced by alternative exon splicing, promoters or termination, which makes it difficult to identify or quantify isoforms. To compare expression between samples, variability across replicates needs to be addressed. Trapnell *et al*. describe Cuffdiff 2, software that provides accurate quantification and differential expression analysis at gene and individual transcript resolutions. The approach models variability in the number of fragments associated with each transcript across replicates and can be used on data from a variety of experimental designs. Using Cuffdiff 2, the authors found transcripts that are differentially expressed between wild-type and HoxA1-deficient human cells.

Trapnell, C. *et al. Nat. Biotechnol.* **31**, 46–53 (2013).

MODEL ORGANISMS

A mouse model to study SUMOylation

Many cellular processes, from nuclear transport to neuronal synaptic transmission, are controlled by SUMOylation. SUMOylation is a conserved post-translational modification, akin to ubiquitylation, that can affect the localizations, interactions and function or stability of many different types of protein substrates. Key SUMOylation substrates or the SUMOylation process itself are often altered in many diseases as well. However, studying SUMOylation has been technically challenging, and mammalian models that allow the identification of SUMOylated proteins *in vivo* have not been available. To address this, Tirard *et al*. developed knock-in mice expressing a tagged version of the *Sumo1* gene, which encodes one of the main enzymes involved in SUMOylation. The knock-in mice, called His_6-HA-SUMO1, enabled the pulldown of several new SUMOylation substrates from mouse brains.

Tirard, M. *et al. Proc. Natl. Acad. Sci. USA* **109**, 21122–21127 (2012).

SYNTHETIC BIOLOGY

TAL effectors on demand

Transcription activator–like (TAL) effectors are modular proteins that can bind specific DNA sequences and cut them when coupled to the FokI DNA nuclease. TAL effector nuclease (TALEN) construction methods focus on assembling repeats containing two variable amino acids that determine binding specificity to a single nucleotide, but the most efficient methods are designed for high-throughput applications. Schmid-Burgk *et al*. use a variation of ligation-independent cloning to rapidly produce expression-ready TALENs. The approach uses a proofreading polymerase and a single supplied nucleotide to generate precise single-stranded overhangs that can specifically anneal with adjacent sequence fragments. The replication origin of each vector is also formed by correct annealing, making the process efficient enough that it does not require bacterial colony screening. The authors offer a large sequence library for easy hierarchical TALEN construction.

Schmid-Burgk, J.L. *et al. Nat. Biotechnol.* **31**, 76–81 (2013).

SMALL RNAs

RNA SHAPEs up

RNA molecules in the cell form complex secondary and tertiary structures that can provide clues to their specific functional roles. RNA secondary structures can be mapped on a broad scale with a method called selected 2′-hydroxyl acylation analyzed by primer extension, or SHAPE. In new work, Spitale *et al*. moved SHAPE into living cells, with the design and testing of new 2′-hydroxyl acylation reagents that are water soluble at high concentrations. The improved approach allowed them to characterize the secondary structure of 5S ribosomal RNA (rRNA) in living mouse embryonic stem cells, human cancer cells, fruit fly cells, yeast and *Escherichia coli*. A comparison of SHAPE results from probing 5S rRNA structure *in vivo* versus *in vitro* showed that RNA-RNA and RNA-protein interactions are important for docking the 5S rRNA into the ribosome.

Spitale, R.C. *et al. Nat. Chem. Biol.* **9**, 18–20 (2013).