A REALITY CHECK FOR dsRNAs

A number of popular methods and commercial kits for single-step isolation of high-quality RNA use a concentrated solution of guanidinium thiocyanate (GuSCN)—a powerful chaotropic agent—to denature cellular proteins and inactivate RNases. Besides acting as a robust protein denaturant, a less well-known effect of concentrated GuSCN solutions is to promote DNA–DNA, RNA–DNA, and RNA–RNA hybridization. In this issue of *BioTechniques*, Triin Mölder and Mart Speek at the Tallinn University of Technology provide evidence suggesting that use of concentrated GuSCN during isolation of cellular RNA causes the formation of double-stranded RNAs (dsRNAs) from complementary single-stranded RNAs (ssRNAs). The authors demonstrated that incubation in a concentrated GuSCN solution of low concentrations of two in vitro–transcribed RNAs with a region of complementarity catalyzed their hybridization into dsRNA, in the absence or presence of total cellular RNA. This suggests the need to carefully verify that any putative novel dsRNAs identified from RNA samples isolated by GuSCN extraction are not actually artifacts caused by this effect.

See “Accelerated RNA–RNA hybridization by concentrated guanidinium thiocyanate solution in single-step RNA isolation” on page 61.

INDENTING MULTIPLE CELLS

Studies of the response of cells to mechanical force transduction have relied on instruments such as atomic force microscopes (AFMs) or cytoindentors, but these tools can only indent single cells one-by-one, which makes the collection of data from a sufficient number of cells a tedious and time-consuming affair. To overcome this problem, researchers at McGill University led by Claire Brown and Sabrina Leslie describe in this month’s issue their development of a high-throughput, parallelized cytoindenter for the simultaneous local compression of multiple live cells. They modified their previously described convex lens–induced confinement (CLiC) set-up by adhering a film patterned with microposts to a microscope lens. This lens is then gradually lowered onto multiple cells maintained in a stage-top incubator to indent micrometer-sized areas on each cell while allowing imaging of those cells. By functionalizing the micropost arrays with an extracellular matrix protein such as fibronectin, it was also possible to pull on the cells. This novel parallelized cytoindenter will greatly facilitate studies of mechanobiology in living cells.

See “Parallelized cytoindentation using convex micropatterned surfaces” on page 73.

Selected and written by Patrick C.H. Lo, Ph.D.

BioTechniques 61:55 (August 2016) doi 10.2144/000114439

PROTEIN INTERACTIONS: LIMITING DIFFUSION ON ARRAYS

Protein arrays are important tools for those exploring protein–protein interactions on a large scale. These arrays can be spotted with purified proteins, or cell-free protein synthesis can be performed on the arrays. Protein synthesis approaches such as the nucleic acid programmable protein array (NAPPA) streamline the building of large arrays, but they also produce large, diffuse protein spots that limit array density. Yazaki et al. solve this problem with their newly devised HaloTag-NAPPA technology, which produces higher-density protein spotting. To accomplish this, they exchanged the C-terminal GST fusion used in the original NAPPA for a HaloTag fusion that can be irreversibly bound to ligands co-spotted on the array. HaloTag-NAPPA captured synthesized proteins with significantly less diffusion compared to GST fusion proteins. To test their method, the authors spotted 12,000 *Arabidopsis* clones onto arrays, synthesized proteins, and then examined protein–protein interactions with 38 plant transcription factors. The new approach identified novel interactions with high sensitivity and low background.

Yazaki, J. et al. 2016. Mapping transcription factor interactome networks using HaloTag protein arrays. *Proc. Natl. Acad. Sci. USA*. DOI: 10.1073/pnas.1603229113

GENE EXPRESSION PROFILING: IT’S ALL ABOUT LOCATION

Profiling gene expression is a powerful approach for understanding basic biology and human disease. Now, Stahl et al. introduce a new method that could significantly expand the scope of gene expression profiling by adding spatial resolution within tissue samples. In “spatial transcriptomics,” histological samples are placed onto slides with reverse transcription primers possessing positional barcodes immobilized on the slide surface. Following permeabilization, reverse transcription reagents are placed onto the tissues, where cDNA is synthesized and left coupled to the oligonucleotides on the slide. Once the tissue is enzymatically removed, the cDNAs are collected and sequenced, akin to traditional gene expression profiling. After sequencing, the locations of cDNA transcripts can be mapped back to the tissue using the incorporated positional barcodes. By adding only a few additional steps, scientists will now be able to obtain spatial information by transcriptional profiling, thus greatly enhancing gene expression data sets.

Stahl, P.L., et al. 2016. Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. *Science*. 353(6294):78–82.

Selected and written by Nathan S. Blow, Ph.D.

BioTechniques 61:55 (August 2016) doi 10.2144/000114439