MICROBIAL RIBOSOME PROFILING

Understanding protein synthesis at the genome scale allows researchers to gain unique insights into the functions of cells and tissues. One particularly clever molecular technique for exploring protein synthesis in cells is ribosome profiling. Here, a cell lysate is treated with endonuclease, and mRNA fragments associated with ribosomes, which are protected from endonuclease digestion, are recovered and sequenced. Ribosome profiling has been employed in a number of recent studies, but the technique can be challenging to implement at first. To overcome this barrier to entry, Latif et al. detail a streamlined ribosome profiling protocol for microorganisms in this issue of *BioTechniques*. The authors present simplified alternatives for harvest, lysis, and ribosome recovery, as well as techniques for reducing the abundance of rRNAs and tRNAs in the sequencing library. This new protocol should lead to greater adoption of ribosome profiling in the microbiology community and hopefully provide new and unique insights into microbial protein synthesis and function.

See “A streamlined ribosome profiling protocol for the characterization of microorganisms” on page 329.

TAGGING TRANSLATIONAL PROFILING

Ribosome profiling using endonucleases, as described above, is not the only approach to measure the translational status of a cell. The RiboTag system employs an expressed ribosomal subunit tag that can be used to isolate and analyze cell-specific RNAs. This approach has been used extensively in transgenic mice with great success, but the technique has yet to be adapted for analysis of cells in heterogeneous cultures. In this issue, Lesiak et al. describe their adaptation of the RiboTag system for use with cultured cells. Using RiboTag immunoprecipitation, they recovered high-quality neuronal RNA from cultures of mixed neuronal and glial cells that could be used in a number of downstream assays (qPCR, RNA-seq, PCR). To demonstrate the potential of their modified RiboTag protocol, the authors showed how co-transfection of RiboTag with constructs bearing shRNAs can be used to assess gene expression knockdown and how RiboTag can measure receptor-mediated gene regulation with expressed DREADD receptors. This report, along with the new approach for ribosome profiling described by Latif et al., demonstrates the importance and utility of protein expression profiling.

See “RiboTag is a flexible tool for measuring the translational state of targeted cells in heterogeneous cell cultures” on page 308.

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BioTechniques 58:279 (June 2015) doi 10.2144/000114295

SINGLE-CELL ANALYSIS OF PROTEIN-PROTEIN INTERACTIONS

Single-molecule imaging of antibody-functionalized surfaces reliably quantifies protein binding affinities, kinetics, and stoichiometries. However, to date, single-molecule pulldown has been based on analysis of lysate from multiple cells. Now, Wedeking et al. describe single cell pulldown (SiCPull). Cells containing GFP- and RFP-tagged bait proteins are cultured on slides micropatterned with alternating bands of cell attachment peptides and high-affinity anti-GFP nanobodies. Adding mild detergent releases the bait proteins, whose interactions can be monitored by dual-color total internal reflection (TIRF) microscopy upon pulldown by the surface-anchored nanobodies. The alternating micropattern provides an internal control, as the regions without nanobodies reveal background noise. Since cytosolic proteins are captured before they have a chance to diffuse far, protein interactions can be analyzed in select, discrete cells. By monitoring decay of the red signal, dissociation of the RFP-tagged protein from its GFP-containing partner is measurable. For stoichiometric analysis, single-molecule photobleaching studies can be conducted.

T. Wedeking et al. Single cell GFP-Trap reveals stoichiometry and dynamics of cytosolic protein complexes. *Nano Lett.* [Epub ahead of print, April 27, 2015; doi:10.1021/acs.nanolett.5b01153].

SINGLE NUCLEUS EXOME SEQUENCING

Despite the success of single-cell RNA sequencing methods, analysis of genomic DNA from isolated cells is often thwarted by limited physical coverage and frequent technical errors. The challenge is copy number: To get adequate sequence coverage, early single-cell sequencing methods overamplified the template, causing high error rates. One workaround is to start with G2/M cells, but this option is only available with highly proliferative cell populations. In response, Leung et al. have developed single nucleus exome sequencing (SNES). DAPI-stained nuclei are isolated from cell culture (or, potentially, archival frozen tissue), separated by flow sorting, then lysed and subjected to time-limited isothermal multiple-displacement amplification. Samples that pass quality control—successful PCR amplification of a target on each chromosome—proceed to library construction, exome capture, and paired-end sequencing. The procedure can achieve 90% sequence coverage, with 87% and 82% detection efficiencies for single-nucleotide variants and indels, respectively.

M.L. Leung et al. SNES: single nucleus exome sequencing. *Genome Biol.* 25;16:55.

Selected and written by Nijsje Dorman, Ph.D.

BioTechniques 58:279 (June 2015) doi 10.2144/000114295