Citations

Parallel processing

Extracting both RNA and DNA from rare or tiny samples can be nerve wracking on account of the danger of sample loss and bias from uneven splitting of limited material. Writing in *Analytical Chemistry*, Strotman et al. present SNARE (Selective Nucleic Acid Removal via Exclusion), a microfluidic device designed to sequentially isolate RNA and DNA from samples with fewer than 100 cells. SNARE consists of two parallel channels that exit a sample input chamber, run through a middle well, and terminate in separate output wells. A cell suspension is added to the first well along with mRNA lysis buffer and oligo(dT) paramagnetic particles (PMPs), while silicon oil and nuclease-free water are placed in the second and third wells, respectively. After a brief lysis, a magnet is dragged alongside the first channel, pulling the mRNA-bound PMPs through the oil and into the connected elution chamber. DNA binding buffer and silica PMPs are then added to the input well, and after a short incubation, the magnet is used to pull the DNA-bound PMPs through the second channel. In SNARE, the oil-filled middle chamber, immiscible with aqueous solution, separates the upstream and downstream chambers. Unlike thiocyanate-phenol-chloroform extraction, no pipetting, precipitation, or washes are required, simplifying the workflow and minimizing sample loss. Like SNARE, spin column-based methods avoid toxic chemicals and

can work well for small samples. However, centrifugation steps are time consuming, consumables costs can add up, and transfer by pipetting risks sample loss. In quantitative PCR of GAPDH mRNA isolated by SNARE or a spin column kit for nucleic acid extraction from small samples, the relative signals were comparable for serial dilutions representing an input of 1 to 1000 cells, but variability was lower for the SNARE-isolated samples. In terms of DNA, the two techniques performed similarly in the 10–1000 cell range, but only SNARE-isolated DNA was detected at the single-cell level, albeit in only 50% of samples. Since characterizing circulating tumor cells is an important application of dual RNA/DNA isolation from small samples (e.g., identifying driver genes for disease progression), Strotman et al. performed SNARE on such cells from prostate cancer patients and confirmed expression of a cancer-related gene. These results suggest that labs needing to extract RNA and DNA from small samples should consider manufacturing a SNARE device to snag genomic and transcriptional data with unprecedented ease and speed.

L. Strotman et al. Selective Nucleic Acid Removal via Exclusion (SNARE): Capturing mRNA and DNA from a single sample. *Anal Chem.* [Epub ahead of print, September 26, 2013; doi:10.1021/ac402162r].

More ups and downs

Small molecule-based manipulation of intracellular protein levels works by fusing the protein of interest to a domain to which the small molecule binds and either stabilizes or triggers degradation. For example, an FK506-binding protein with a cryptic degron becomes destabilized upon binding with an FK506 analog, resulting in fusion protein degradation. A different FK506-binding domain can induce stabilization upon FK506 analog binding, raising fusion protein levels. However, a single domain that enables modulation of protein level in both directions would be preferable, and that is just what Neklesa et al. report in an article in *ACS Chemical Biology*. The new strategy uses a variant of HaloTag, which is commonly used for protein labeling. The authors previously developed a hydrophobic tag (HyT36) that binds HaloTag2 and triggers proteasome-mediated degradation. The goal of the new study was to identify a different small molecule that would stabilize HaloTag2. Neklesa et al. screened a 35,000-member library and found an aminotetrazole that stabilized a luciferase-HaloTag2 fusion.

Bidirectional modulation of protein level with the HALTS1 and HyT36 small molecule binders of HaloTag.

This compound, HaloTag stabilizer 1 (HALTS1), was tested in EGFP-HaloTag2 expressing cells; a concentration of 1 μ M had noticeable effects on protein accumulation at 6 hours, with a 4-fold increase by 72 hours. Higher concentrations could raise protein levels nearly 5-fold without evidence of toxicity. Together with HyT36-mediated degradation, HALTS1-based stabilization produces a 42-fold dynamic range in fusion protein modulation. HyT36 and HALTS1 can act sequentially, as the authors demonstrated in an experiment where EGFP levels were doubled by the latter and then reduced

to baseline by the former within three hours. To show bidirectional control of a physiologically relevant protein, the authors tested a HaloTag2 fusion with oncogenic Ras in a murine cell line. With HALTS1 stabilization, the cells transform and grow in foci; after HyT36 is added, Ras levels decline and an untransformed phenotype re-emerges. Future work may combine the HaloTag2 system with an FK506-based approach to control multiple proteins and accelerate the delineation of protein function and signaling pathways.

T.K. Neklesa et al. A bidirectional system for the dynamic small molecule control of intracellular fusion proteins. *ACS Chem Biol.* [Epub ahead of print, August 26, 2013; doi:10.1021/cb400569k].

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