JUST ONE STEP

When yeast cells are deprived of nutrients, they enter stationary phase (SP) and differentiate into quiescent (Q) or nonquiescent (NQ) cells. Q cells are arrested in the G₀ cell cycle phase but can re-enter the cell cycle upon nutrient replenishment; they survive longer than NQ cells (which fail to enter G₀) and are more resistant to environmental stresses. These characteristics make yeast Q cells a useful model for the study of cellular aging, cell cycle re-entry, and self-renewal of cells. The most common approach to isolating yeast Q cells is by Percoll-based density gradient centrifugation, but this is a laborious and time-consuming method. In this issue of *BioTechniques*, Stephen M. Fuchs and his colleagues at Tufts University describe a simple, inexpensive, and rapid method for Q-cell isolation using single-step density gradient centrifugation with iodixanol. SP cells are layered on top of a cushion of 30% iodixanol in a microfuge tube and centrifuged for 5 minutes. The Q cells are pelleted, while NQ cells are trapped at the interface between the cell buffer and the iodixanol.

See “Density separation of quiescent yeast using iodixanol” on page 169.

USING AQUEOUS DNA TO GENOTYPE FISH

Fish species are important model organisms in areas such as development, population genetics, and aquatic toxicology. Nonlethal genotyping of fish larvae is critical for many of these studies, but tissue sampling methods such as fin clips or skin swabs are laborious and technically demanding, while other noninvasive methods are unsuitable for high-throughput applications. In this month's issue, Jaime R. Alvarado Bremer and his team at Texas A&M University, Galveston describe a noninvasive method for genotyping fish larvae based on collecting the aqueous environmental DNA (eDNA) that they shed. Hatchlings of sheepshead minnows, which are used for ecotoxicology studies, were individually placed into artificial seawater in microfuge tubes and held for 30 min, after which eDNA was isolated from the seawater using a spin-column-based kit. Genotyping of four loci following PCR amplification was highly successful and allowed the assignment of parentage for each hatchling. Survival of the larvae was very high, and the method should be amenable to high-throughput protocols and applicable to model species such as zebrafish.

See “Genotyping live fish larvae: non-lethal and noninvasive DNA isolation from 3–5 day old hatchlings” on page 181.

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

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CRISPRi-PROTi: WORKING TOGETHER

Gene knockdown is a commonly used technique for studying gene function; however, it is not without challenges, especially given that the approach does not provide a means to control existing protein levels and cannot be used for the study of essential genes. Martinez et al. provide a potential solution to these problems with their new CRISPRi-PROTi method, which enables simultaneous gene expression knockdown and protein degradation. The approach is actually a combination of two techniques. First, CRISPRi using a mutant Cas9 protein targets a specific gene for transcriptional knockdown or interference. At the same time, protein interference (PROTi) is achieved through an N-terminal protein degradation tag with a tobacco etch virus (TEV) cleavage site that is attached to the target protein. Upon chemical induction and TEV cleavage, the protein is destabilized and degraded. By combining CRISPRi and PROTi in a single system, Martinez et al. demonstrate gene expression inhibition and protein degradation at the same time.

Martinez, V. et al. CRISPR/Cas9-based genome editing for simultaneous interference with gene expression and protein stability. *Nucleic Acids Research*. [Epub ahead of print] Sept 2017. doi: 10.1093/nar/gkx979

TWO TIMES THE DATA

While either genomic or transcriptomic data can give crucial information on a cell's status, the combination of these data from the same source has the potential to provide unique insights not available from either alone. Van Strijp et al. demonstrate this with their new on-chip, microfluidic approach for differential extraction of DNA and RNA from the same single cell. First, single cells are trapped in picoliter-volume wells. Cell membranes are then lysed in a buffer containing the dye YOYO-1, releasing cellular RNAs for collection while maintaining genomic DNA in the nucleus. Following RNA isolation, the nuclear membrane is lysed, releasing genomic DNA that becomes stained with YOYO-1 and is then extracted. Using different dyes, the progress of the dual extraction can be monitored. To validate the system, the authors identified the tumor-driving pathway in a colorectal cell line by looking at expression patterns and correlating these with mutations in the genomic DNA.

Van Strijp, D. et al. Complete sequence-based pathway analysis by differential on-chip DNA and RNA extraction from a single cell. *Scientific Reports*. [Epub ahead of print] Sept 2017. doi: 10.1038/s41598-017-10704-4

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

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