FADE RESISTANT CULTURING

When it comes to fluorescent proteins (FP) and their usage in cell biology, photostability is a key factor. If fluorescence decreases rapidly or is low to begin with, obtaining biologically relevant data from an experiment can be difficult. In an effort to better understand photostability issues associated with cells in culture, Konstantin Lukyanov and colleagues from the Institute of Bioorganic Chemistry in Moscow, Russia systematically evaluated the influence of growth conditions and media composition on green fluorescent protein (GFP) stability. The authors examined the components in Ham's F12 culture medium that might enhance GFP photostability, finding that decreased concentrations of riboflavin and pyridoxine but increased concentrations of other components compared to DMEM were key to generating greater FP photostability. They also showed that cell density and serum concentration have profound effects on photostability. Taken together, Lukyanov and his co-authors provide a set of guidelines for researchers looking to optimize FP photostability for their cell culture experiments and ultimately get the most meaningful data out of FP experiments.

See "Influence of cell growth conditions and medium composition on EGFP photostability in live cells" on page 258.

FINDING THAT BETTER MATE

Although the number of sequenced genomes is quickly rising, there are still many species for which a reference genome remains missing. When it comes to de novo genome sequencing in these cases, mate-pair reads are essential to obtaining the necessary scaffolding data that will ultimately result in a genome assembly, but generating good mate-pair libraries for this purpose remains a challenging task. In this issue of *BioTechniques*, a team of researchers from the RIKEN Center for Developmental Biology, led by Shigehiro Kuraku, describe a series of modifications for one of the most popular mate-pair library kits, the Nextera Mate Pair Sample Prep Kit, that should enhance both yield and efficiency. Through intensive DNA shearing to decrease insert sizes, sequencing with >150 cycles, and the use of variable tagmentation conditions, Kuraku and his colleagues were able to demonstrate clear increases in overall sequence continuity while at the same time reducing the costs associated with generating mate-pair libraries.

See "Optimization and cost-saving in tagmentation-based mate-pair library preparation and sequencing" on page 253.

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

BioTechniques 58:215 (May 2015) doi 10.2144/000114282

VISUALIZING PROTEOME-LEVEL METABOLIC DYNAMICS

Most methods for monitoring global protein metabolism in cell culture lack spatial information or require cell fixation. Previously, Wei et al. described a live imaging technique based on stimulated Raman scattering (SRS) detection of the carbon-deuterium bond in cells metabolically labeled with deuterated amino acids. While promising, the method was relatively insensitive and could only assay protein synthesis. Now the authors have improved the sensitivity and, by examining the vibrational signature of methyl groups in pre-existing protein pools, their approach can also monitor protein degradation. Even pulse-chase experiments are possible, by sequentially exposing cells to non-branched and then branched deuterated amino acids (the two subtypes can be distinguished by SRS). The new method works in cell culture, live brain tissue slices, zebrafish embryos, and mice. It should be especially well-suited for exploring protein synthesis related to memory formation and protein metabolism in animal models of disease.

L. Wei et al. 2015. Imaging complex protein metabolism in live organisms by stimulated Raman scattering microscopy with isotope labeling. *ACS Chem Biol.* 10(3):901-8.

TISSUE-SPECIFIC GENOME EDITING IN ZEBRAFISH

Loss-of-function studies in zebrafish have come a long way from random mutagenesis screens. However, CRISPR/Cas9-based genome editing results in global inactivation of the target gene. Ablain et al. now report a vector system for tissue-specific gene inactivation in zebrafish. In their integratable vector, which is microinjected into one-cell-stage embryos, the guide RNA is ubiquitously expressed, while Cas9 is driven by a tissue-specific promoter. Tests with an erythrocyte-specific promoter showed blood-lineage knockout of an enzyme involved in heme biosynthesis in both F0 embryos and F1 offspring selected for presence of the integrated vector. Moreover, experiments involving a vector targeting *p53* confirmed the system is also applicable to genetic suppressor screens. The authors anticipate that their vector will be compatible with targeting multiple genes (via incorporation of several guide RNA units) and that the overall approach may be transferable to other vertebrate models such as *Xenopus* and medaka.

J. Albain et al. 2015. A CRISPR/Cas9 vector system for tissue-specific gene disruption in zebrafish. *Dev Cell.* 32(6):756-64.

Selected and written by Nijsje Dorman, Ph.D.

BioTechniques 58:215 (May 2015) doi 10.2144/000114282