MARKING MUTATIONS

Determining whether a mutation is germline or somatic can be a challenge. One approach is using matched tissue from the same individual for comparison. However, if tissue is lacking, researchers can also look for the target mutation in databases to estimate the ratio of the new change to the normal sequence. But this approach can lead to the identification of rare mutations as somatic in some instances. In the current issue of *BioTechniques*, a team of researchers from the University of Navarra report a simple method for classifying these mutations. This new approach takes advantage of heterozygous polymorphisms as “landmarks” near the location of the target mutation. By amplifying a short sequence containing both a heterozygous polymorphism and the target mutation, and then sequencing the resulting products, the researchers were able to determine if a variant was always associated with the polymorphism (thus a germline variant) or only occasionally associated (thus a somatic variant). The authors provide experimental data demonstrating the ability of their method to differentiate germline versus somatic mutations, as well as suggesting that the approach could be used for studying loss of heterozygosity. One cautionary note is that the distance between the polymorphism and target mutation should be short to avoid possible recombination affecting the assay.

See “A simple approach for classifying new mutations as somatic or germinal in DNA samples lacking paired tissue” on page 327.

TOXICITY AVOIDANCE

The ability to control transgene expression is crucial for genetic analysis as well as in gene therapy applications. When it comes to lentiviral-mediated gene transfer and expression studies, toxic proteins can present serious problems for researchers. While several studies have detailed strategies for controlling toxic gene expression in target (transduced) cells, much less effort has been directed at controlling expression in the viral vector—producing precursor cells where toxic proteins can lead to reduced transfection efficiencies. In this issue, researchers from Marseille, France describe the construction of a new lentiviral vector using a backbone that inhibits transgene polyadenylation and therefore transgene expression in vector-producing cells, but reactivates the polyadenylation signal upon reverse transcription, enabling gene expression in transduced cells. This new lentiviral vector should create new opportunities for scientists to express and study toxic transgenes.

See “Off-on polyadenylation strategy as supplemental mechanism for silencing toxic transgene expression during lentiviral vector production” on page 311.

Selected and written by Nathan Blow, Ph.D.

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NANORULER-BASED SPLICE VARIANTS DETECTION

Most human genes produce multiple mRNA isoforms via alternative splicing, and perturbations in the relative concentrations of these splice variants can lead to disease. RT-PCR quantifies alternative transcripts at a point in time, but can't report on their fluctuations in living cells. Gold nanoparticles (GNPs), which easily conjugate to DNA probes, excel at live-cell imaging and generate a spectral shift when in close proximity. This “molecular ruler” functionality has given rise to a new method for detecting splice variants where a pair of DNA-conjugated GNPs are designed to anneal on either side of exons that are skipped in certain mRNA isoforms. Dark-field hyperspectral imaging of the GNPs measures interparticle distances of up to 30 nm, allowing inference of which splice variant is being detected. This technique, which was capable of distinguishing three BRCA1 splice variants in breast cancer cell lines, should help reveal unprecedented spatial and temporal details on mRNA isoforms.

K. Lee et al. Quantitative imaging of single mRNA splice variants in living cells. *Nat Nanotechnol.* [Epub ahead of print, April 20, 2014; doi:10.1038/nnano.2014.73].

SUBCELLULAR PROTEOLYSIS IMAGING

Proper regulation of protein degradation is crucial to cell viability. Defects in the proteolytic machinery of the cell may contribute to aging and neurodegeneration. Stable isotope labeling combined with mass spectrometric analysis is able to monitor proteomic changes over time, but this methodology averages out the contributions of many cells. Fluorescent reporter-based imaging can probe protein half-life in individual living cells, but requires fusion constructs. A new method combining metabolic labeling with stimulated Raman scattering (SRS) microscopy offers noninvasive visualization of protein degradation. The label is ^{13}C -phenylalanine, which can be picked up by SRS microscopy due to a vibrational frequency shift, differentiating the nascent proteome from the original. The method measures degradation by following the decay and increase, respectively, in the ^{12}C and ^{13}C channels after incubation of cultured cells in ^{13}C -Phe-substituted medium. This approach can also be used to measure degradation kinetics under stimuli such as oxidative stress, and provides subcellular resolution.

Y. Shen et al. Live-cell quantitative imaging of proteome degradation by stimulated Raman scattering. *Angew Chem Int Ed Engl.* [Epub ahead of print, April 15, 2014; doi:10.1002/anie.201310725].

Selected and written by Nijsje Dorman, Ph.D.

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