FIXING THE FLUORESCENCE

In histological studies, tissues are often fixed in formalin and embedded in paraffin to preserve structural features for microscopy. But the use of formalin and paraffin does have drawbacks for scientists interested in molecular analyses. One of these challenges is detection of green fluorescent protein (GFP) as a cell or tissue marker, since natural protein fluorescence is inactivated during fixation. For researchers, this means a trade-off: Use different fixation conditions and lose histological resolution, or preserve resolution with the added need for immunological techniques to detect reporter proteins. In this issue of *BioTechniques*, Andrew Liss and his colleagues at the Massachusetts General Hospital provide a simple solution: an ethanol-based fixation protocol that enables direct visualization of natural protein fluorescence while at the same time preserving tissue histology. To demonstrate the robust nature of the technique, the authors examined multiple fluorescent proteins in murine tissue samples. As an added benefit, ethanol-based fixation is also compatible with immunohistochemistry studies, further enhancing the experimental possibilities for fixed tissue samples.

See “A method for fixing and paraffin embedding tissue to retain the natural fluorescence of reporter proteins” on page 153.

ONE-DIRECTION GENE SYNTHESIS

When synthesizing genes or constructing gene libraries using PCR, double-stranded DNA amplification, which is required in most cases to generate final products, can result in polymerase-induced errors or template amplification biases. In this issue, Brian DeDecker and his team from the University of Colorado Boulder propose a clever solution to these potential problems: unidirectional, single-stranded reactions for gene synthesis. The new technique, polymerase step reaction (PSR), takes advantage of standard non-modified forward primers in combination with multiple reverse template primers that are blocked with a 3' propyl group to prevent extension. The outcome is a reaction where the single-stranded DNA product grows in a unidirectional fashion with each cycle. After a final fill-in reaction, a double-stranded gene synthesis product results. DeDecker and his colleagues used PSR to assemble a 288-bp antibody library, finding that the final product possessed variability with minimal redundancy or error propagation. They also synthesized a 758-bp gene product without any variability. PSR is a welcome addition for researchers seeking a quick, efficient method to generate small gene products or libraries.

See “Gene and library synthesis without amplification: polymerase step reaction (PSR)” on page 163.

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

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GENOME EDITING OF PRIMARY CELLS

Although the CRISPR-Cas9 system works well in cell lines, genome editing of primary human cells presents a far greater challenge. Now Hendel et al. report a strategy that substantially improves the success rate when targeting human primary T cells and CD34+ hematopoietic stem and progenitor cells (HSPCs). The method uses nucleofection of a synthetic single guide RNA (sgRNA) containing three chemically modified nucleotides at each terminus. The modifications confer greater stability, and the use of RNA avoids potential toxicity from transfection of expression plasmids. In stimulated T cells, cotransfecting modified sgRNA with Cas9 mRNA achieved nearly 50% indel frequencies. In CD34+ HSPCs from peripheral blood, indel frequencies of 18%–23% were observed. Simultaneous use of 2 sgRNAs has been shown to enhance gene disruption, and this pattern also held for the chemically modified variety, with 74%–93% allele modification in stimulated T cells and frequencies of 38%–43% in CD34+ HSPCs.

A. Hendel et al., Chemically modified guide RNAs enhance CRISPR-Cas genome editing in human primary cells. *Nat Biotechnol.* [Epub ahead of print, June 29, 2015; doi:10.1038/nbt.3290].

LABELING MEMBRANE-LOCALIZED PROTEINS

Fluorescence microscopy of labeled membrane proteins gives unparalleled information about localization and trafficking, provided the tag doesn't alter these characteristics. As an alternative to the relatively bulky SNAP- and Halo-tags (181 and 295 amino acids, respectively), Bedbrook et al. propose the 13-amino acid “SpyTag” and its binding partner, a SpyCatcher–fluorescent protein fusion. A SpyTagged channelrhodopsin trafficked to the membrane more efficiently than a SNAP-tagged version and behaved identically to the unlabeled protein in patch-clamp recordings. Unlike synthetic probes, SpyCatcher is not cell permeable, so it can be used to screen for correct membrane localization. The covalent SpyTag/Catcher bond gives long-lived labeling—up to three days in cultured cells. The key advantage however is in vivo labeling. For instance, in *Caenorhabditis elegans* transgenic for a SpyTag channelrhodopsin and a heat-shock promoter-driven extracellularly secreted SpyCatcher, specific labeling of the membrane protein was visible 24 hours after SpyCatcher induction.

C.N. Bedbrook et al. Genetically encoded spy peptide fusion system to detect plasma membrane-localized proteins in vivo. *Chem Biol.* [Epub ahead of print, July 23, 2015; doi:10.1016/j.chembiol.2015.06.020].

Selected and written by Nijsje Dorman, Ph.D.

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