QUICK CLEAN-UP OF DYE-LABELED DNA

Fluorescent dye-labeled DNAs are important reagents used in various molecular biology methods. A critical final step in fluorescent dye labeling of DNA is purification of the labeled DNA from unreacted dye. This is routinely carried out by chromatography, gel electrophoresis, or ethanol precipitation, but these methods can be time-consuming, labor-intensive, and result in a significant loss of labeled DNA. In this issue of *BioTechniques*, Seong Keun Kim and colleagues describe a quick, simple, and efficient method for purifying fluorescent dye-labeled DNA. By lowering the pH of the completed dye labeling reaction, the unreacted free dye is converted from an anionic form to a neutral form, which can then be easily extracted out by mixing the low-pH aqueous reaction solution with an organic solvent such as butanol. The neutral free dye partitions into the organic phase, while the hydrophilic, dye-labeled DNA remains in the aqueous phase. This method takes <10 minutes to perform, has a 97% recovery yield, and is more effective than ethanol precipitation when used to purify dye-labeled DNA oligonucleotides.

See “General and facile purification of dye-labeled oligonucleotides by pH-controlled extraction” on page 21.

TRAPPING OF METABOLIC GASES

Metabolic studies are increasingly being carried out in high-throughput formats using cell cultures in multiwell plates. This can be a challenge, however, for collecting gaseous metabolites, since a separate, sealed collection vessel is required for each well of the plate to prevent the free exchange of gases. In this month's *BioTechniques*, researchers led by David E. James have devised a simple and inexpensive method to collect metabolic gases from cell cultures grown in multiwell plates by repurposing the caps of 15-mL centrifuge tubes to create gas traps. A hole is drilled into the center of the cap, which fits perfectly when placed upside down into the top of each well of a 12-well plate; scintillation vial caps can be used for 24-well plates. The emitted gasses are then collected by adding the appropriate gas trapping solution to the caps and sealing the entire plate with Parafilm for a gas-tight seal. This method was shown to work for collecting radiolabeled CO₂ produced by both adherent cells and cells grown in suspension, as well as for collecting H₂S using a solid gas trapping medium in the trap.

See “A gas trapping method for high-throughput metabolic experiments” on page 26.

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

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EVOLVING A BASE EDITOR

Changing a single nucleotide within a target genomic region represents a powerful approach for genome engineering. For making G/C to A/T changes, base editing systems using cytidine deaminases along with a mutated, non-cleaving version of CRISPR/Cas9 for sequence targeting have been shown to be effective in a wide range of cell lines and animals. However, there are no known enzymes that can induce the opposite change: A/T to G/C. Using a directed evolution approach, David Liu and his colleagues at Harvard University have described for the first time a functional A/T to G/C base-editing system. Starting with the enzyme tRNA adenosine deaminase, which in nature converts A to G in RNA, the authors evolved an enzyme possessing adenosine deaminase functionality for DNA. Liu's new adenosine base editor, which also uses the non-active CRISPR/Cas9 complex for targeting, could efficiently make targeted, single-base A/T to G/C conversions in human cells.

Gaudelli, N.M. et al. 2017. Programmable base editing of A/T to G/C in genomic DNA without DNA cleavage. *Nature*. 55:464-471.

SEEING MEMBRANES COME TOGETHER

Contacts between the endoplasmic reticulum (ER) membrane and the mitochondrial outer membrane are involved in calcium signaling events and stress responses. In addition, several neurodegenerative diseases have been associated with changes in contacts between these membranes. Visualizing such interactions is challenging, since the distances between the membranes are below the resolution limits of conventional light microscopes (5–30 nm). To study these interactions, Mark Harmon and his colleagues at the The University of Edinburgh turned to their new bi-fluorescence complementation (BiFC) method. For BiFC, the authors used a split YFP Venus protein—one-half of which was expressed on the mitochondrial membrane, while the other half was expressed on the ER. When the two halves are in close proximity, YFP is reconstituted, and its signal can be visualized. Using this BiFC strategy, the authors showed that membrane associations changed as expected in response to serum deprivation and following the administration of a drug known to induce ER stress. These results clearly demonstrate the suitability of BiFC for studying ER/mitochondrial membrane interactions.

Harmon, M. et al. 2017. A Bi-fluorescence complementation system to detect associations between the endoplasmic reticulum and mitochondria. *Scientific Reports*. 7:17467.

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

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