CSI: TRITON

Crime scene DNA analysis is often critical for finding criminals, not to mention providing essential television viewing for millions of people on a weekly basis. When it comes to obtaining images of fingerprints, a number of techniques have been developed that have proven highly successful. But isolating DNA from fingermarks left at crime scenes is more challenging given the very low amount of DNA present on a fingermark. In this issue of *BioTechniques*, Jennifer Templeton and Adrian Linacre report a new methodology for generating DNA profiles from fingermarks with a greater than 70% success rate. The first key to this new method is the use of a swab moistened with the surfactant Triton-X to lift DNA from fingermarks. The authors found that the combination of positively charged fibers on the swab with Triton-X enabled greater uptake of DNA when touched to a surface. The second key feature of the technique is direct PCR amplification from the moistened swab, minimizing the possibility of contamination while avoiding sample preparation steps where DNA could be lost prior to profile generation. Using this approach, the authors obtained interpretable DNA profiles from 71% of 170 fingermarks tested, demonstrating the potential of this methodology to enhance DNA profiling in the future.

See “DNA profiles from fingermarks” on page 259.

RAINBOW FISH

Multi-color fluorescent in situ hybridization (FISH) has been used extensively to study and compare gene expression patterns in cells and tissues. Although effective, current two-color FISH approaches can prove problematic for detecting weakly expressed transcripts or monitoring signal strength against background fluorescence levels. In this issue of *BioTechniques*, Jennifer Schumacher and her colleagues from the Division of Developmental Biology at the Cincinnati Children's Hospital describe the development of a new FISH protocol using chromogenic substrates to provide high-resolution imaging. The authors used a combination of NBT/BCIP and Vector Red, both of which have fluorescent properties that do not overlap, in a protocol that enables chromogenic monitoring with long alkaline phosphatase reactivity. In proof-of-concept experiments, the researchers demonstrated that this chromogenic two-color FISH technique can be used to monitor developing reactions and improve the visualization of weak or variably expressed genes and transcripts. While the method was tested on zebrafish embryos, the authors suggest that it should be applicable to any model organism.

See “Two-color fluorescent in situ hybridization using chromogenic substrates in zebrafish” on page 254.

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

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GENOME-WIDE MAPPING OF 5-HYDROXYMETHYLCYTOSINE

Nearly six years ago, conventional wisdom about DNA methylation was upturned by the discovery of 5-hydroxymethylcytosine (5hmC) as an abundant epigenetic modification in mammalian genomes. Several 5hmC detection methods have since emerged, but they are hobbled by complex workflows and poor sensitivity. Now, Petterson et al. combine elements of existing epigenomic methods to create reduced representation 5hmC profiling (RRHP). First, genomic DNA is digested with (hydroxyl)methylation-insensitive *MspI* and ligated with two adapters, one of which recreates the *MspI* recognition site. The fragments are then incubated with an enzyme that glucosylates 5hmC, rendering 5hmC-containing *MspI* recognition sites resistant to cleavage. After a second digestion, only fragments in which the *MspI* sites contain 5hmC are left intact for subsequent amplification and sequencing. Unlike subtractive sequencing methods, only one library needs to be prepared for RRHP. In addition, the new method is more sensitive than a 5hmC enrichment approach and requires lower sequencing depth than other profiling techniques.

Petterson et al. 2014. RRHP: a tag-based approach for 5-hydroxymethylcytosine mapping at single-site resolution. *Genome Biol.* 15:456.

CONTACTLESS METHOD TO ESTABLISH A COCULTURE

Coculture techniques can help in vitro systems better mimic physiologically relevant cellular microenvironments. Usually, different cell types are positioned via micropatterning of adhesion molecules, or arranged by printing or mechanical manipulation. New work from Li et al. describes a noninvasive, contactless alternative based on standing surface acoustic waves. These “acoustic tweezers” confine particles in the cell culture medium using pressure fluctuations. Cells in a microchannel can be quickly lined up by these forces, then allowed to settle to the substrate for growth. By phase shifting the radiofrequency signal, cells of a different type can be placed in lines that interdigitate with rows of already-patterned cells. Using their platform, the authors show that they can monitor how HeLa cell migration is affected by coculture with endothelial cells. Key advantages of this method include the ability to use a minimal number of cells (<10,000) and avoidance of possibly confounding stimuli such as substrate heterogeneity or interference from physical barriers.

S. Li et al. 2014. Standing surface acoustic wave based cell coculture. *Anal Chem.* 86:9853-9.

Selected and written by Nijsje Dorman, Ph.D.

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