BioSpotlight

Life on the Surface

The ability to study gene expression profiles in living cells en masse creates a powerful genetic approach to understanding cellular signaling pathways. One such high-throughput technique to examine expression patterns in living cells is live-cell microarrays, where spots consisting of genes or RNA interference (RNAi) cassettes are placed on glass slides that are then layered on top with live cells. The keys to the success of this method are (i) effectively attaching functional genes or RNAi cassettes to the glass surface, and (ii) delivering those biomolecules into the live cells. Non-viral delivery systems, such as those that complex small interfering RNAs (siRNAs) into lipoplexes, have shown promise in the past for effective gene delivery on live-cell microarrays. But viral vectors possess higher delivery efficiencies in many instances, so their possible use on live-cell arrays is of great interest to researchers. Reporting in the current issue of *BioTechniques*, McConnell et al. from Rice University document the use of adeno-associated virus (AAV) for effective gene delivery on a glass surface. AAV has benefits as a gene delivery vehicle since the virus genome is composed of only two genes and the virus itself is nonpathogenic with nearly 80% of the human population being seropositive. Although possessing beneficial characteristics, to actually work as a live-cell microarray platform the authors needed to define an optimal surface on the glass that first enables AAV attachment, but then later allows release of virus to transduce cells. Three different surface coatings were initially tested for spot size, virus patterning on the slide surface, cell adhesion, and gene expression on the slides, an indication of transduction efficiency. In the end, the authors found that nitrocellulose coatings yielded the best AAV deposition on their glass slides, and at the same time generated discrete pockets of highly transduced cells. The proof-of-concept data presented by McConnell et al. serves as a starting point for the further devel-

Schematic of patterning process. Recombinant AAV is spotted onto coated glass slides. Slides are backfilled with microarray stabilizer and then seeded with cells. Arrays are imaged 3 days later for gene expression.

opment of AAV-based live-cell microarrays, and their application in gene expression profiling in living cells.

See “Live – cell microarray surface coatings supporting reverse transduction by adeno-associated viruses” on page 255.

Understanding Nature’s Do-over Button

Mistakes happen in life and in DNA replication. In the case of DNA, a cellular error-correction system known as mismatch repair (MMR) is responsible for finding and fixing base mismatches which are initially missed by the proofreading functionality of DNA polymerase. The bacterial MMR system is composed of a complex of proteins (MutS, MutL, and MutH) that first act to nick and unwind the DNA, exposing the mismatch, and then remove the errant base and resynthesize the DNA. MMR is not the sole domain of bacteria though; the system is conserved from *E. coli* to humans. This conservation, along with the implications for mistakes in templates, has led to a great interest among scientists in deciphering how proteins in the MMR system interact with each other and how mismatch repair complexes function mechanistically. In order to study the process of MMR, researchers

need suitable DNA substrates that contain mismatches. And while approaches have been described to generate such molecules, they tend to be time-consuming and inflexible with respect to the sequence context of the mismatch. Xiao and colleagues describe a new approach for generating circular DNA templates containing mismatched bases in this issue of *BioTechniques* that could extend the range of MMR studies in the future. The authors initially generated linear PCR templates that were then nicked, resulting in 3' single-stranded overhangs that could be annealed to form ‘nanocircles’. Depending on the template, mismatched hemimethylated GATC sites (bacterial MMR substrates) or templates nicked on either the top or bottom strand (eukaryotic MMR substrates) could be generated. The authors demonstrated the robust nature of their method by generating nanocircles that varied

in size from 200 to 1000 bp and contained different mismatches. This new PCR-based approach should enable more detailed and accurate characterization of the proteins in the MMR complex as well as insights into the functional mechanics of these proteins during DNA mismatch repair.

See “Generation of DNA nanocircles containing mismatched bases” on page 259.

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