CELL MIGRATION ON MULTIPLE SUBSTRATES

Cell migration involves the interaction of cells with extracellular matrix (ECM) proteins. While there are numerous in vitro methods for studying the effect of ECM proteins on cell migration, many of these are limited to testing one substrate at a time. In this issue of *BioTechniques*, Ricardo A. Moreno-Rodriguez and his colleagues describe their new method, the linear array of multi-substrate cell migration assay (LAMA), which allows simultaneous analysis of the migration (as well as the adhesion and differentiation) of cells on multiple substrates under the same culture conditions. Specially constructed reaction tunnels are used to lay down up to 20 parallel lines of different ECM proteins in nanogram amounts on a glass slide. The ECM proteins are covalently attached to the aminosilanized glass surface using a bifunctional cross-linker, which helps to preserve native protein conformation. With its greater throughput and requirement for minimal amounts of expensive substrates and numbers of cells, LAMA should prove to be a powerful and flexible method for cell migration research.

See “Linear array of multi-substrate tracts for simultaneous assessment of cell adhesion, migration, and differentiation” on page 267.

SELF-ASSEMBLED PEPTIDE HYDROGELS AND WESTERN BLOTTING

The encapsulation of cells within hydrogels is a popular technique for 3-D cell culture and tissue engineering. Among the polymers used to form these hydrogels, self-assembling peptides (SAPs) have been of interest due to the ease of adjusting the properties of SAP hydrogels (SAPHs) by varying the concentration and composition of the SAPs during self-assembly. A potential concern with this approach, however, is whether the presence of SAPs could affect western blot analysis of proteins from the encapsulated cells. In this issue of *BioTechniques*, Alberto Saiani and his team demonstrate that a standard SDS-based homogenization buffer is unable to completely solubilize a specific SAP, resulting in the trapping of significant amounts of cellular protein in the insoluble SAP pellet. However, by using a urea-based homogenization buffer and multiple sonication cycles, they could completely solubilize the SAP, which prevented the loss of cellular proteins seen with the SDS-based buffer. Furthermore, the author showed that the solubilized SAP in the extract did not interfere with western blotting of the cellular proteins.

See “Western blot analysis of cells encapsulated in self-assembling peptide hydrogels” on page 253.

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

BioTechniques 63:247 (December 2017) doi 10.2144/000114615

A lncRNA PIPELINE

Long non-coding RNA (lncRNA) is a recently defined RNA class that has important regulatory functions in the cell. Identifying lncRNAs can be difficult, often requiring computational analysis of RNA-Seq data sets to pinpoint lncRNA candidates. Recently, Sun et al. reported a new pipeline aimed at speeding up lncRNA identification from RNA-Seq data sets. Their new tool, Ultrafast and Comprehensive lncRNA (UClncR), incorporates several previously developed tools into a workflow where scientists can directly import their RNA-Seq data sets for analysis. Initially, UClncR takes advantage of the StringTie program for rapid transcript assembly. From there, two modules are used to assess transcript coding potential, after which, predicted lncRNAs are listed and annotated. Other key enhancements offered by the UClncR tool include the ability to process both stranded and non-stranded RNA-Seq data sets and, also, to distribute jobs on a cluster of computers for processing multiple samples at the same time.

Sun, Z. et al. 2017. UClncR: Ultrafast and comprehensive long non-coding RNA detection from RNA-seq. *Sci Rep.* 7:14196.

END-LESS CLONING

DNA cloning remains a critical technique in molecular biology, especially as more and more researchers become interested in synthetic biology applications. When it comes to assembling large pieces of DNA from smaller fragments, several different approaches have recently been described that forgo the traditional cloning protocols using restriction enzymes and ligases. Zeng et al. add to this growing toolkit with a new approach they call Assembly of Fragment Ends After PCR (AFEAP) cloning. AFEAP cloning uses a two-step PCR methodology that generates overlapping DNA ends on each of the fragments to be assembled and then uses a second PCR step to generate single-stranded DNA that can be annealed to stitch together all of the DNA fragments. To demonstrate the utility of AFEAP cloning, the authors assembled an 8-kb plasmid from varying numbers of DNA pieces (up to 13 fragments) with high accuracy; they also assembled a bacterial artificial chromosome (BAC) clone containing a 200-kb insert. This new cloning method provides yet another option when it comes to precise and seamless DNA cloning and large-DNA sequence assembly.

Zeng, F. et al. 2017. AFEAP cloning: a precise and efficient method for large DNA sequence assembly. *BMC Biotechnol.* 17:81.

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

BioTechniques 63:247 (December 2017) doi 10.2144/000114615