TAKING CHARGE OF EXTRACELLULAR VESICLES

Extracellular vesicles (EVs) play important roles in long-distance intercellular communication and are potentially useful as biomarkers. EVs have been isolated from body fluids by centrifugation, size exclusion chromatography, filtration, precipitation, and affinity purification; however, these methods vary in the extent of contamination of the isolated EVs with non-EV protein complexes and RNAs. In this issue of *BioTechniques*, Maja Kosanović and her colleagues at the University of Belgrade present a new method for isolating EVs that reduces such contamination. Given that they are negatively charged due to their surface molecules, the team tested ion-exchange chromatography (IEC) as an approach for purifying EVs. Using amniotic fluid as the source of EVs, they compared the gold-standard method, consisting of ultracentrifugation followed by sucrose density gradient centrifugation, with their novel method of ultracentrifugation followed by IEC. As demonstrated by protein electrophoresis and immuno- and lectin blotting, the use of IEC significantly reduced the contamination seen with the gold-standard method.

See “Ion-exchange chromatography purification of extracellular vesicles” on page 65.

RAPID AND NO-WASH LIVE-CELL STAINING

Live-cell fluorescent dyes are critical tools for cell biology research, allowing the visualization and tracking of cells in various assays. In this issue, Alexey A. Pakhomov and his colleagues at the Russian Academy of Sciences describe a new fluorescent dye for rapid, no-wash staining of live cells. This dye is derived from boron dipyrromethene (BODIPY), a molecule with numerous other derivatives that are used as fluorescent sensors and for conjugation to biomolecules. Some of these derivatives are lipophilic, binding to the plasma and subcellular membranes, and they tend to aggregate in polar media such as the cytosol and extracellular space, which results in aggregation-caused quenching. The team tested these derivatives for use in live-cell staining and demonstrated that one of them immediately stained the plasma and subcellular membranes of live mammalian cells with high contrast, without the need for washing. In fact, washing the stained cells with PBS removed the dye, allowing the cells to be stained with another dye of the same color.

See “BODIPY-based dye for no-wash live-cell staining and imaging” on page 77.

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

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TWO-TAILED miRNA QUANTIFICATION

The analysis of microRNAs (miRNAs) is challenging given the short sequence lengths of these molecules. In an attempt to design a more specific and sensitive assay to quantify miRNA expression patterns, Androvic et al. developed a new method they named Two-tailed RT-qPCR. This technique uses a unique primer design incorporating two specific hemiprobcs, which are complementary to the miRNA of interest and separated by a tether that can fold into a hairpin to prevent non-specific interactions. The reverse transcription reaction to generate cDNA is primed from the 3'-end hemiprobe, while the 5'-end hemiprobe is displaced. After reverse transcription, the resulting cDNA is quantified using two different target-specific primers. The authors showed that Two-tailed RT-qPCR has the sensitivity to detect as few as 10 target miRNA molecules while providing a dynamic range of 7 logs. This new approach should be of particular interest to those seeking highly accurate miRNA quantification.

Androvic, P. et al. 2017. Two-tailed RT-qPCR: a novel method for highly accurate miRNA quantification. *Nucleic Acids Res.* doi: 10.1093/nar/gkx588

MASSIVE CELL SEPARATION

Single-cell sequencing has become commonplace in life science research. By studying single cells, scientists are learning about cellular heterogeneity, lineage determination, and hierarchical relationships. In a new article in *BMC Genomics*, Goldstein et al. take single-cell sequencing high-throughput with their description of a massively parallel single-cell profiling pipeline. The authors took advantage of the ICELL8 system, which enables the separation of cell mixtures into 5184 nanowells and then uses imaging to locate wells possessing only a single cell. Once a nanowell containing a single cell is identified, in-well mRNA isolation and barcoded-cDNA generation is performed, after which the resulting cDNA from multiple wells is pooled for sequence analysis. Using the pipeline, the authors were able to sequence transcriptomes from more than 1000 cultured human and mouse cells, along with nearly 500 mouse pancreatic islet cells.

Goldstein, L.D. et al. 2017. Massively parallel nanowell-based single-cell gene expression profiling. *BMC Genomics*. 18:519.

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

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