A view of the steady-state distributions of proteins within a cell

Using hyperLOPIT to perform high-resolution mapping of the spatial proteome.

Mulvey CM, Breckels LM, Geladaki A, Britovšek NK, Nightingale DJH, Christoforou A, Elzek M, Deery MJ, Gatto L, Lilley KS (2017) *Nat Protoc* 12:1110–1135.

Eukaryotic cell health is dependent on the spatial and temporal organization of proteins that control cell growth, division, metabolism, and other vital processes. The specific subcellular localization of a protein can not only regulate its activity but also determine its function and downstream effects. Aberrant protein localization has been implicated in a diverse array of human diseases, including neuronal degeneration and cancer [1]. Protein localization has traditionally been studied using fluorescence microscopy in conjunction with GFP-tagged fusion proteins or fluorophore-labeled antibodies. However, these methods have limited throughput and are dependent on the generation of recombinant proteins or the availability of specific antibodies.

To obtain a more complete view of the spatial proteome, Dr. Kathryn Lilley and colleagues developed LOPIT (localization of organelle proteins by isotope tagging), which combines cell fractionation using density-gradient ultracentrifugation with multiplex quantitative mass spectroscopy [2]. In the LOPIT method, cellular proteins are first separated into various subcellular fractions based on their density. Each fraction is then labeled with stable isotope–encoded chemical tags that facilitate the measurement of abundance (and localization) of hundreds of proteins relative to organelle marker proteins.

In their 2017 article, Mulvey et al. describe their updated higher-multiplex LOPIT (hyperLOPIT) protocol that enables high-resolution mapping of thousands of proteins in a single experiment [3]. The hyperLOPIT protocol integrates improved fractionation strategies with technological advances in stable isotope tag labeling and mass spectrometry acquisition methods, increasing both the number of proteins analyzed per run and the granularity of protein localization to suborganelles and large protein complexes. The hyperLOPIT technique overcomes many of the limitations of the original LOPIT method, which only examined integral membrane proteins (not soluble or peripheral membrane proteins) in a few subcellular compartments, and resulted in suboptimal quantitation arising from the mass spectrometry analysis methods available at the time. In addition to optimized fractionation and mass spectrometry methods for higher-plex sample analysis, the hyperLOPIT workflow includes machine learning–based analysis of the spatial proteomics data (Figure 1).

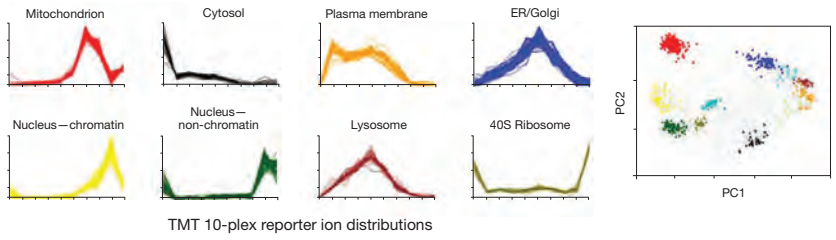

Figure 1. hyperLOPIT data analysis. Subcellular fractionation of E14TG2a murine embryonic stem cells using density-gradient ultracentrifugation coupled with isobaric mass tagging and synchronous precursor selection (SPS) MS^3 mass spectrometry produced spatial proteome maps of cellular proteins. Machine learning and data analyses are performed using pRoloc software (**bioconductor.org/packages/pRoloc**) to assign the protein profiles of unknown location to the profiles of well-known organelle markers. Line graphs (left) showing TMT reporter ion distributions for 8 organelles demonstrate that colocalized proteins exhibit similar profile patterns in the gradient. The multivariate data set can be visualized in 2D using principal component analysis (PCA, right) to provide a view of organelle separation and subcellular resolution; each point represents one protein and is colored according to its subcellular niche. Reproduced with permission from Christoforou A et al. (2016) *Nat Commun* 7:9992 and under the Creative Commons Attribution 4.0 International License (**creativecommons.org/licenses/by/4.0/**).

Mulvey et al. provide a visual step-by-step hyperLOPIT protocol with estimated time to complete each step, as well as a comprehensive list of reagents and equipment. Also included is a troubleshooting guide and links to a downloadable, open-source computational toolkit that includes pRoloc and pRolocGUI for the statistical analysis and interactive visualization, respectively, of spatial proteomics data. Although the hyperLOPIT method was developed using the mouse embryonic stem cell line E14TG2a [4], the authors state that it can be used to produce a spatial map of any cell line or homogeneous tissue, as long as the cells can be lysed without compromising the integrity of organelle membranes. Overall, the use of hyperLOPIT to create high-resolution maps of protein localization is a breakthrough in spatial proteomics that will contribute greatly to our understanding of cell function, organization, and disease states. ■

References

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3. Mulvey CM, Breckels LM, Geladaki A et al. (2017) *Nat Protoc* 12:1110–1135.
4. Christoforou A, Mulvey CM, Breckels LM et al. (2016) *Nat Commun* 7:9992.