Name: Ava Holmes
Email: Ava.Holmes@nih.gov
PI Name: Bruce Hope
PI Email: bhope@intra.nida.nih.gov

# Multiplexed Population Selection and Enrichment Single Nucleus RNA Sequencing (Xpose-Seq) Enables Sample Identity Retention During Transcriptional Profiling of Target Populations

Ava R. Holmes[1], Katherine E. Savell[1], Rajtarun Madangopal[1], Padmashri Saravanan[1], Ryan G. Palaganas[1], Kareem D. Woods[1], Drake J. Thompson[1], Olivia R. Drake[1], Megan B. Brenner[1], Sophia J. Weber[1], Elise Van Leer[1], Jae H. Choi[1], Toni L. Martin[1], Jody C. Martin[2], Mia K. Steinberg[2], James W. Austin[2], Chloé I. Charendoff[2], Bruce T. Hope[1]

[1]Intramural Research Program, National Institute on Drug Abuse, Baltimore, MD 21224
[2]BD Biosciences, San José, CA 95131

Single cell and nucleus RNA-sequencing (sc/sn-RNAseq) approaches disentangle the complexity of biological tissues at unprecedented resolution and have been particularly beneficial in the central nervous system. High-throughput snRNA-seq methods excel in broad characterization, but they typically require capturing all nuclei, combining biological replicates, and post-hoc identification of populations of interest. Existing targeted snRNA-seq is technically challenging, expensive, and low throughput, limiting their practicality in complex, multifactor experimental designs common in neuroscience. To address these limitations, we developed XPoSE-seq, which combines flow cytometry based population enrichment with an antibody-based multiplexed snRNA-seq strategy for high-throughput targeted transcriptomic profiling of target populations.

The key component in XPoSE-seq is a novel tri-functional reagent (XPoSE-tag) that leverages 1) an antibody against the ubiquitous nucleoporin complex protein (Nup62), found in nuclei from all cell types, with dual conjugations of 2) a small molecule far-red fluorochrome (R718) to identify labeled nuclei in flow cytometry and 3) a distinct oligo-based Sample Tags (ST) to uniquely barcode nuclei from each sample. We verified successful nuclei tagging and multiplexing with XPoSE-tags, and combining XPoSE-seq and an activity-dependent transgenic rat, we enriched for neurons active during novel context exploration. This isolated active population included multiple cell types, and differential gene expression (DEG) analysis was more sensitive when analyzing the isolated active nuclei compared to the total capture. Overall, XPoSE-seq enhances targeted snRNA-seq sensitivity, enabling discovery of cell-type-specific changes in normal and disease states within the central nervous system.