

Dr. Melinda Larsen
Department of Biological Sciences

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A High-Resolution in Situ Proteomics Atlas of Salivary Gland Development

This application addresses broad Challenge Area (06) Enabling Technologies and specific Challenge Topic, 06-DE-102 Structural and Molecular Atlases of Craniofacial Development. A critical issue for understanding organ development at a systems level is knowledge of temporal and spatial patterns of gene and protein expression throughout development. We have developed a novel fluorescence-based multiplexing technology for simultaneously tracking dozens of proteins within single formalin-fixed, paraffin-embedded tissue sections and novel software algorithms for quantifying and categorizing protein localization patterns for these markers at the subcellular level. This method involves direct immunohistochemistry (IHC) and automated imaging followed by complete inactivation of fluorophores that are directly conjugated to antibody probes. This direct IHC approach allows sequential multiplexed probing of the same tissue with multiple antibodies to dramatically increase the number of markers that can be simultaneously visualized in a single sample when compared with classical indirect IHC. This method eliminates the need for multiple spectrally compatible fluorophores and for serial tissue sections, thus allowing large amounts of data to be generated from a small amount of material. The dye-inactivation multiplexing procedure has been thoroughly characterized and tested through 100 rounds of cycling with no loss in tissue morphology or antigenicity. In this project, we will apply this innovative multiplexing technology for the first time to a developmental system to profile expression patterns of signaling proteins during mouse submandibular and sublingual salivary gland morphogenesis and differentiation in a developmental salivary gland tissue array. The end product will include a high-resolution in situ proteomics-based atlas, which categorizes and quantifies active signaling protein expression levels within specific cell types and subcellular compartments, throughout all key developmental time-points. This high-resolution anatomical systems-level analysis would be impossible to create using traditional genomics methods, which are done at the level of mRNA, or by traditional proteomic methods, which destroy the tissue context. This data set will complement the gene expression atlases developed through the intramural NIDCR program (Salivary Gland Atlas project, Drs. K. Yamada and M. Hoffman) and will integrate with other databases in FaceBase, and provide a truly unique data component for systems biology. The morphogenesis-related dataset will also inform a mathematical model of the developing salivary gland, (RO1DE0192444-01, M. Larsen,) and the differentiation-related dataset will identify new differentiation markers and pathway components for intelligent engineering of an artificial salivary gland (R21DE0192444-01, M. Larsen). This study will provide a foundation of basic developmental biology knowledge needed to interpret future studies in other normal and diseased mouse and human craniofacial tissues. Finally, the proposed studies will facilitate economic recovery through direct materials costs (i.e. microscope, fluidics, computers and controllers, and antibodies and supplies, which will all purchased from US vendors) and by providing two new positions to complete the antibody labeling and to become a key operator in Dr. Larsen's lab so that the technology can be applied to her future studies. The technology being applied to the atlas; however, is not currently commercially available to the public research community, but this project will facilitate the adaptation and transition of this powerful multiplexing method to mainstream research applications.