Deep quantitative proteomics of North American Pacific coast star tunicate (*Botryllus schlosseri*)

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Abstract

Botryllus schlosseri, is a model marine invertebrate for studying immunity, regeneration, and stress-induced evolution. Conditions for validating its predicted proteome were optimized using nanoElute® 2 deep-coverage LCMS, revealing up to 4,930 protein groups and 20,984 unique peptides per sample. Spectral libraries were generated and filtered to remove interferences, low-quality transitions, and only retain proteins with >3 unique peptides. The resulting DIA assay library enabled label-free quantitation of 3,426 protein groups represented by 22,593 unique peptides. Quantitative comparisons of a laboratory-raised with two field-collected populations revealed (1) a more unique proteome in the laboratory-raised population, and (2) proteins with high/low individual variabilities in each population. DNA repair/replication, ion transport, and intracellular signaling processes were unique in laboratory-cultured colonies. Spliceosome and Wnt signaling proteins were the least variable (highly functionally constrained) in all populations. In conclusion, we present the first colonial tunicate's deep quantitative proteome analysis, identifying functional protein clusters associated with laboratory conditions, different habitats, and strong versus relaxed abundance constraints. These results empower research on *B. schlosseri* with proteomics resources and enable quantitative molecular phenotyping of changes associated with transfer from *in situ* to *ex situ* and from *in vivo* to *in vitro* culture conditions.

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