A Practical Beginner's Guide to Proteomics

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Abstract

Introduction

[paragraph about what proteomics means today]

[history of proteomics? how we got here]

[paragraph about what proteomics can do] A wide range of questions are addressable with proteomics experiments, which translates to a wide range of variations of proteomics workflows. Sometimes identifying what proteins are present is desired, and sometimes the quantities of as many proteins as possible are desired.

Protein Extraction

Discussion of methods for protein extraction and solubilizaition.

1. Choice of Lysis buffer

- Urea can cause chemical modifications

2. chemicals to avoid
3. removal of contaminations, Protein Precipitation
4. protein alkylation

- choices of reduction and alkylation reagents, TCEP/DTT/2BME, Chloroacetamide/iodoacetamide, n-ethyl maleimide

Proteolysis

1. discussion of protein sequence coverage is determined by the choice of proteolysis
2. why trypsin is the most common choice (charge and length character)
3. theoretical studies of proteolysis and enzyme [1]
4. Challenges associated with alternative enzyme choices (non-specific and semi-specific enzymes)
5. Alternative enzyme choices (one paragraph each?) - LysC
6. GluC
7. AspN
8. Alpha-lytic protease [2] and how it enables mapping human SUMO sites [3].
9. others?

Peptide and Protein Labeling

Discussion of methods to isotopically label peptides or proteins that enable quantification

1. SILAC/SILAM
2. iTRAQ
3. TMT
4. dimethyl labeling

Peptide or Protein Enrichment

Protein enrichment (e.g. for protein protein interactions)

- coIP
- APEX
- bioID
- bioplex

Peptide enrichment

- antibody enrichments of modifications, e.g. lysine acetylation [4].
- TiO2 and Fe enrichment of phosphorylation

Experiment Design

This section should discuss trade offs and balancing them to design an experiment. 1. constraints: Each experiment will have different constraints, which may include the number of samples needed for analysis, or desire to quantify a specific subset of proteins within a sample. 2. sample size 3. statistics 4. costs

References

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3. **Site-specific identification and quantitation of endogenous SUMO modifications under native conditions.**
   Ryan J Lumpkin, Hongbo Gu, Yiying Zhu, Marilyn Leonard, Alla S Ahmad, Karl R Clauser, Jesse G Meyer, Eric J Bennett, Elizabeth A Komives
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