Tutorial 4: Quantification Methods in Proteomics

Bio312

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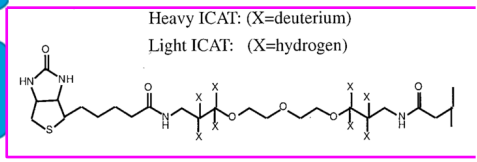
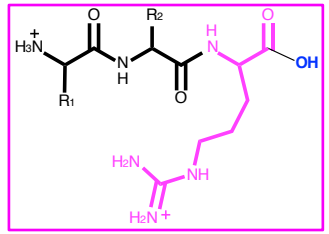
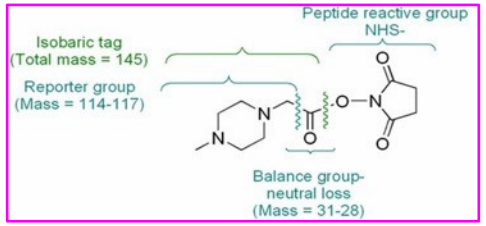
Quantitative Methods: Review

- **Quantitative proteomics**
  - 2-DE based methods
  - MS based methods
    - Stable isotope labeling
      - Absolute quantification
        - Chemically synthesized standard
        - Biologically synthesized standard
      - Relative quantification
        - MS1 quantification
          - Metabolic labeling (SILAC, ^{15}N)
          - Chemical labeling (ICAT, Dimethyl)
          - Enzymatic labeling (^{18}O)
        - MS2 quantification
          - Isobaric labeling (iTRAQ, TMT)
    - Label-free relative quantification
      - Spectral Counting
      - Peptide ion intensity

- In vivo labeling with isotope enriched media
- Cell culture







Count the number of peptides or sspectra assigned to a given protein

MS1 signal intensity

1. Which of the following is true about Proteomics?

A. Proteomics has enabled the identification of ever-increasing numbers of protein

B. Proteomics generally refers to the large-scale experimental analysis of proteins and proteomes

C. Proteome is the entire set of proteins that is produced or modified by an organism or system

D. All the above

2. **When quantifying proteins from an MS experiment, how do you work out what level a change is likely to be due to biology, and not experimental or technical variation?**

A. Use 2-fold as a generic cut-off

B. Use pathway analysis software

C. Look in the literature to see what other people use

D. Analyze replicates to measure experimental noise

3. Which of these is an advantage of difference in-gel electrophoresis (DIGE) compared to gel-free approaches?

A. More sensitive

B. Intact proteins allow detection of changes in protein modification

C. Allows a greater range of proteins to be analyzed

D. Proteins are identified as part of the quantification

4. In HPLC (high performance Liquid chromatography), the time taken for a particular peptide travel through the column to detector is called:

A. Retention time
B. Travel time
C. Average time
D. Performance time

5. Which parameter from LC-MS analysis is proportional to analyte concentration?

A. Chromatographic retention time

B. Total ion chromatogram

C. Mass spectral m/z value

D. Chromatographic peak area

6. Do the tryptic products of proteins have the same mass spectrometry intensity?

A. Yes

B. No

7. Which parameter from LC-MS or GC-MS analysis is proportional to analyte concentration?

A. Chromatographic retention time

B. Total ion chromatogram

C. Mass spectral m/z value

D. Chromatographic peak area

8. Isobaric tags are:

A. Molecules of equal charge

B. Molecules of equal mass

C. Fluorescent labels for proteins

D. Used in selected reaction monitoring

9. Which of the following is required when using a chromatographic system coupled to a mass spectrometer for absolute quantification of an analyte?

A. A very high resolution mass spectrometer

B. An authentic standard of the analyte

C. A high performance chromatography system

D. As many points on the calibration curve as possible

10. If you require a complex sample preparation workflow to enrich a particular population of proteins from cultured cells, an appropriate method of quantification would be what?

A. DIGE

B. SILAC

C. iTRAQ

D. Label-free methods

11. Proteins from clinical samples can be labelled with stable isotopes by which of the following?

A. SILAC

B. Cy dyes

C. iTRAQ

D. ^{18}O water

12. If your quantitative proteomics experiment contains a large number of samples, which of these would be a good method to choose?

A. iTRAQ

B. SILAC

C. Label-free quantification

D. Western blotting