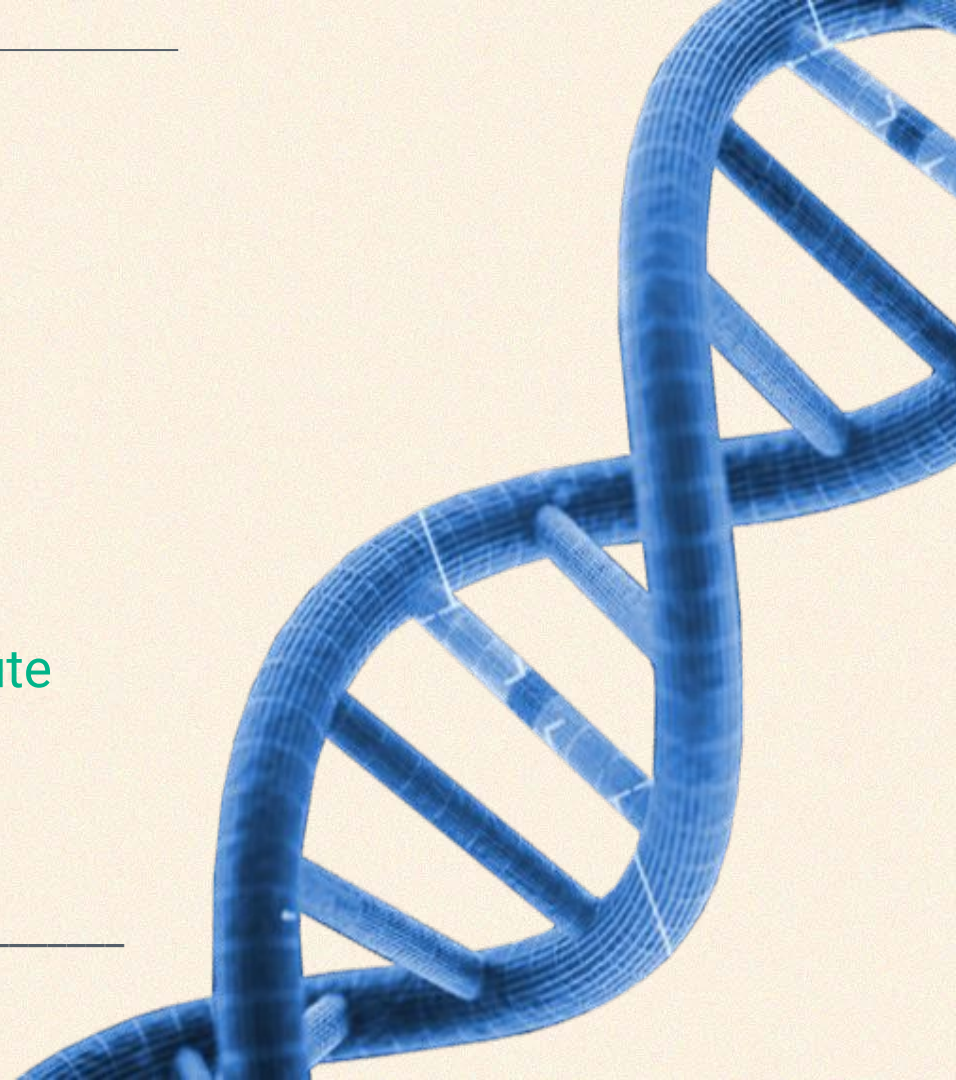


iTRAQ

Isobaric tags for relative and absolute
quantitation





Introduction

Protein relative quantification is an important aspect of many proteomics experiments. This type of work can be done in a variety of ways.
ex) (iTRAQ) which was developed by Applied Biosystems Incorporation in 2004.

Our diagram

Quantification strategies:

Quantification Proteomics

Relative quantification

Chemical isotopic labeling methods

In vitro

N-terminal peptide labeling

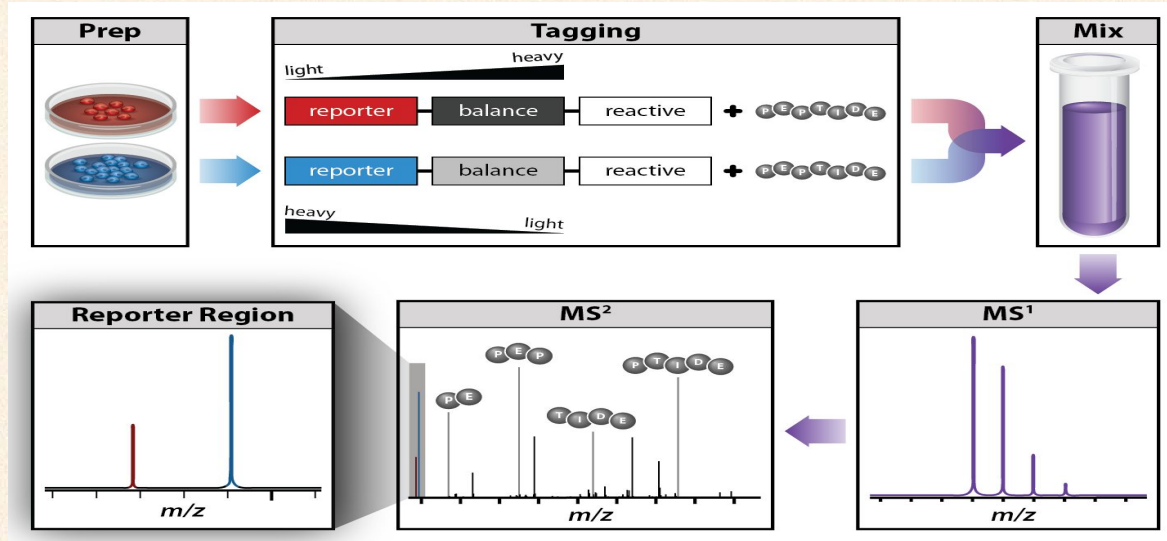
(iTRAQ)

Definition of iTRAQ

- Isobaric tags for relative and absolute quantitation (iTRAQ) is an isobaric labeling method in MS/MS applied to determine the proteins coming from different sources in a single experiment.
- Also referred to as **bottom-up approach** which allows the concurrent identification and relative quantification of hundreds of proteins in up to eight different biological samples in a single experiment .
- It is an isobaric labeling method to determine the amount of proteins from different sources in just one single experiment by mass spectrometry.

Isobaric tag

- a reporter group,
- a neutral balance portion
- a peptide reactive group to give an overall mass of 145.





iTRAQ

Advantages:

Advantages

- The iTRAQ proteomic technique has a high throughput and multiplexing capacity.
- It has higher sensitivity and specificity, Saves instrument time.
- The protein sequence coverage obtained with iTRAQ reagents is comparable to that obtained with other shotgun proteomic approaches.
- iTRAQ is useful for identifying and quantifying proteins across diverse MW and pI ranges, functional categories, cellular locations, and abundances.
- Increased analytical precision and accuracy



iTRAQ

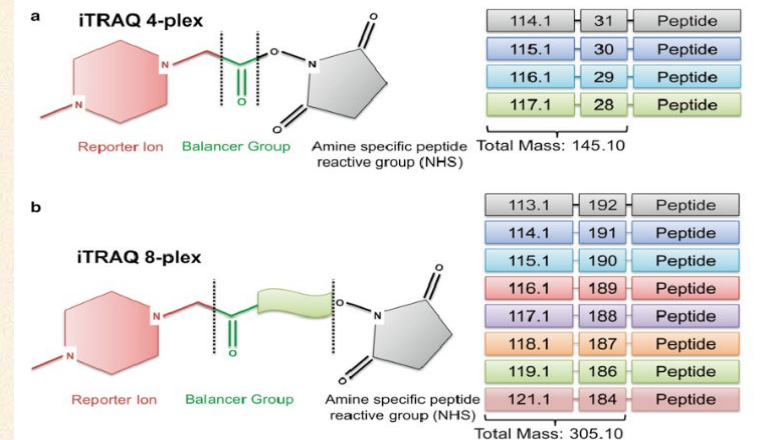
Disadvantages:

Disadvantages:

- Possible errors in quantitative due to
 - differences in efficiency of enzymatic digestion
 - peptide pre-fractionation step
- This technique is time-consuming, labor-intensive, and expensive
- Cannot be performed in most ion-trap instruments because they do not reach this low mass range.

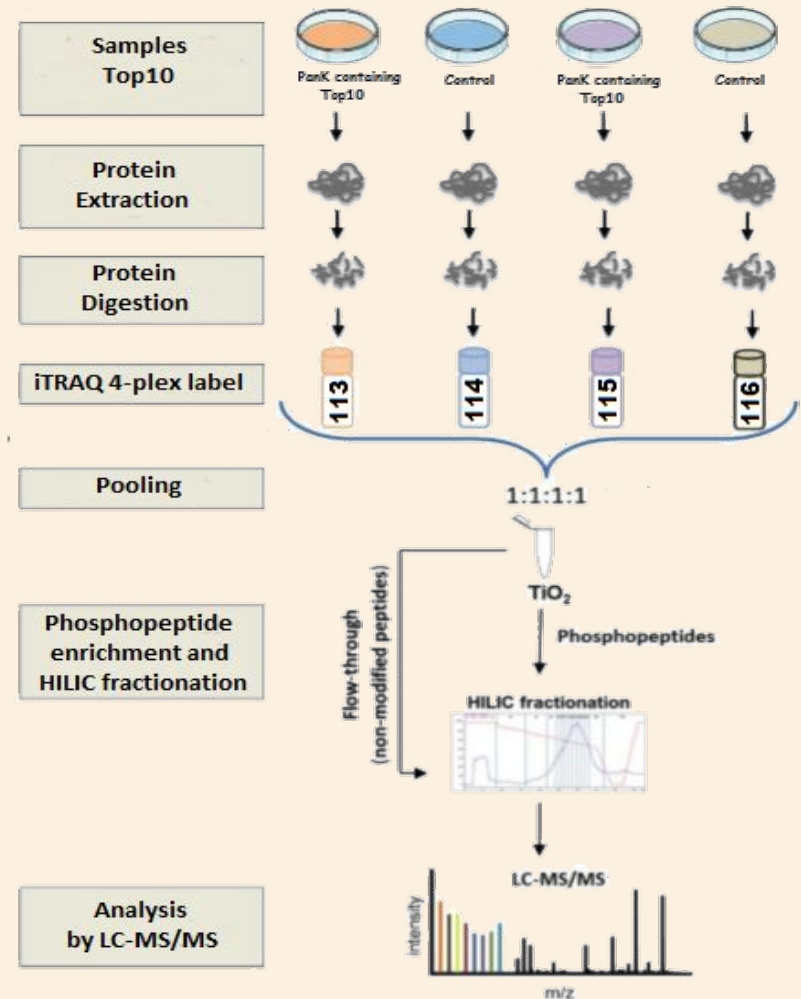
iTRAQ reagents

- The iTRAQ reagents are a collection of multiplexed, amine-specific stable isotope reagents.
- It enables simultaneous identification and quantitation, both relative and absolute.
- There are two types of iTRAQ Reagents:
 - 4-plex - for processing up to 4 samples
 - 8-plex - for processing up to 8 samples



iTRAQ Experiment Steps

1. Protein reduction and cysteine blocking
2. Protein Digestion "Add trypsin solution ,
Clean-up samples using ZipTip"
3. labeling "Add iTRAQ reagent to digested protein
sample"
4. pooling labeled samples
"Combine labeled samples in one tube"
5. purification "This step facilitates sample clean-up"





iTRAQ

and Bioinformatics

iTRAQ and Bioinformatics

Retinoblastoma is An eye cancer that begins in the back of the eye (retina).

- It causes red eye and eye swelling. Commonly occurs in young children, but can rarely occur among adults.

- Proteins in retinoblastoma were studied using **iTRAQ-based quantitative proteomic analysis and bioinformatics**. "The study's goal was to analyse proteins in patients' retinoblastoma (RB) aqueous humour (AH) and investigate their potential role in RB using the comparative proteomic technique of isobaric tags for relative and absolute quantitation (iTRAQ) coupled with offline two-dimensional liquid chromatography-tandem mass spectrometry."

iTRAQ-based quantitative proteomic analysis and bioinformatics study of proteins in retinoblastoma - PubMed (nih.gov)

Thanks!

A decorative vertical bar on the right side of the slide, composed of three stacked rectangular segments: a dark grey top segment, a teal middle segment, and a dark blue bottom segment.