The identification and quantitation of complex protein mixtures have been facilitated by mass spectrometric methods based on differential stable isotope labelling. These tags, which can be recognized by MS, provides a basis for quantification. Stable Isotope Labeling by Amino acids in Cell culture (SILAC) incorporates specific labelled amino acids into proteins for differential analysis.

**Quantitative Proteomics - SILAC**

In this learning object, the learner will be able to:

- Describe SILAC.
- List out the Applications of SILAC
SILAC
SILAC is a simple method for in vivo incorporation of a label into proteins for quantitative proteomic purposes. Two groups of cells are cultured in media that are identical in all respects except that one contains a heavy, isotopic analog of an essential amino acid while the other contains the normal light amino acid.
**SILAC**

The essential amino acids which are obtained from the cell culture medium are incorporated into the corresponding newly synthesized proteins during cell growth and replication. Medium containing the heavy amino acids will give rise to heavy, isotopic proteins.
After a number of cell divisions, all instances of the particular amino acid will be replaced by its isotopic analog. The grown cells are then combined together and harvested. Centrifugation of the mixture will result in the pelleting of cells which can then be used for further analysis.
The grown cells are then lysed using a suitable lysis buffer and the proteins degraded using a proteolytic enzyme like trypsin. This results in a mixture of light and heavy peptide fragments which can be quantified suitably by MS.
The complex mixture of peptide fragments is further separated by SDS-PAGE to simplify the analysis. Each band of the gel is cut out and redissolved in a suitable buffer solution. These simplified peptide fragments are then used for further analysis.
Further purification is carried out by liquid chromatography wherein the sample is passed through a column containing a packed stationary phase matrix that selectively adsorbs only certain analyte molecules. Reverse phase and strong cation exchange chromatography are the most commonly used. The eluted fractions are further characterized by MS.
The purified peptide fragments are then analyzed by MS/MS. Peptides containing the heavy amino acid show higher m/z than the corresponding light peptide fragments. The pairs of identical peptides can be differentiated due to the mass difference and the ratio of peak intensities can be correlated to the corresponding protein abundance.
**SILAC**

The MS/MS data analysis shareware has some extra inputs such as Quantitation, MS/MS tolerance, peptide charge, instrument etc. in addition to the fields for PMF. They require inputs from the user regarding the experimental parameters used such as enzyme cleavage, protein name, modifications etc. and the desired search criteria like taxonomy, peptide tolerance etc. Commonly used protein databases against which the MS information is processed to retrieve sequence data include NCBI, MSDB and SwissProt. The data file generated from MS is uploaded and the search carried out.
Peptide bond formation

SILAC is a useful quantitative approach that has found applications for several proteomic studies.
The authors determined fold change of peptide pairs between haploid and diploid yeast cells using SILAC. Labeled lysine residues were used to grow the diploid yeast cells while haploid cells were grown in normal lysine medium. The cultures were mixed, proteins extracted and analyzed by LC-MS/MS. Protein ratios between haploid and diploid cells were determined with high accuracy. Comparison revealed that 97.3% of the proteome changes less than 50% in abundance.
1. **Stable Isotope Labeling by Amino acids in Cell culture (SILAC):** SILAC is a simple and convenient method for *in vivo* incorporation of a suitable label into proteins for quantitative MS-based proteomics. Two groups of cells are grown in cultures that are identical in all respects except that one contains a light medium with regular, unmodified essential amino acid while the other contains a heavy medium, in which a heavy isotopic form of the amino acid is present.

2. **Light medium:** Cell culture medium containing the regular, unmodified forms of all the amino acids.

3. **Heavy medium:** Cell culture medium in which labelled analogs of certain essential amino acids are supplied to cells (for eg. Leucine-D3, arginine-C13). These amino acids get incorporated into the proteins after a number of cell divisions and can be used to determine the relative protein abundance by measuring MS signal intensities between corresponding light and heavy peptides.

4. **Cell lysis & proteolysis:** The cells that have been grown in light or heavy medium are lysed using a suitable lysis buffer and the proteins then digested using enzyme such as trypsin. Peptide fragments of suitable length are generated for analysis by MS.
5. **Quantification by MS:** The peptide fragments obtained after proteolytic digestion are then subjected to analysis by suitable mass spectrometry techniques. The intensity of MS signals obtained for light and heavy peptides is directly related to the relative protein abundance.
1. **Haploid yeast cells:** The haploid number \((n)\) is the number of chromosomes in a gamete. A yeast having only ‘\(n\)’ chromosomes is said to be a haploid cell.

2. **Diploid yeast cells:** Yeast cells having two homologous copies of each chromosome \((2n)\) are said to be diploid cells.

3. **Peptide spectrum:** Once SILAC has been carried out on the haploid and diploid yeast cells using light and heavy media, the peptide spectrum is generated following LC-MS/MS analysis.
Which of the following type of amino acids are labeled during SILAC?

- Essential
- Non-essential
- Neutral
- Non-polar

Congratulations, you have chosen the correct answer.
The m/z difference between light and heavy Arginine is

- 2 Da
- 6 Da (Correct)
- 8 Da
- 10 Da

Congratulations, you have chosen the correct answer.
Which cell lines can be used for SILAC analysis

- HeLa
- C127
- HEK293
- none
- all

Congratulations, you have chosen the correct answer.
Question: 1 2 3 4 5 6

Reverse phase chromatography is based on which of the following interactions?

- Ionic
- Covalent
- Hydrophobic
- Hydrogen bonding

Congratulations, you have chosen the correct answer.
The function of DTT during in-gel digestion of proteins is:

- Oxidation of disulphide bonds
- Cleavage at N-terminal of amino acids
- Cleavage at C-terminal of amino acids
- Reduction of disulphide bonds

Congratulations, You have chosen the correct answer.
Which of the following statements concerning SILAC is incorrect?

- no chemical difference between labeled and natural amino acid isotopes
- cells behave exactly like control cell population grown in presence of normal amino acid
- incorporation of isotope label is 100%
- incorporation of isotope label is 50%

Congratulations, You have chosen the correct answer.
References

Research papers:


Websites:

http://www.silac.org