iJRASET
International Journal For Research in Applied Science and Engineering Technology

# INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

**Volume:** 8 **Issue:** XI **Month of publication:** November 2020

**DOI:** https://doi.org/10.22214/ijraset.2020.32168

www.ijraset.com

Call: 08813907089 | E-mail ID: ijraset@gmail.com

International Journal for Research in Applied Science & Engineering Technology (IJRASET)
*ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.429*
*Volume 8 Issue XI Nov 2020- Available at www.ijraset.com*

# ITRAQ TMT and SILAC: Proteomic Analysis and Advantages

Tapajyoti Routh
*Bengal Institution of Technology, Kolkata, India*

***Abstract:*** ***Label-based quantitation proteome analysis helps to understand the nature of these methods. iTRAQ utilizes isobaric reagents to label the primary amines of peptides and proteins. 4-plex and 8-plex, which can be used to label all peptides from different samples or treatments. It has high protein coverage and quantitative accuracy. Tandem Mass Tags (TMT) can be used to identify and quantify proteins in different kinds of samples. Each TMT tagging reagent is composed of an amine-reactive NHS-ester group, a spacer arm and an MS/MS reporter. Duplex, 6plex and 10plex are used to label different types of protein samples. The efficiency and accuracy are high for TMT. Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC) technique performs protein labeling by adding light, medium, or heavy stable isotope-labeled essential amino acids (lysine and arginine) to cell culture media. Efficiency of SILAC is higher. Its efficiency can be up to 100%. This review describes the above methods and their advantages.***

***Keyword: iTRAQ, Tandem Mass Tags, SILAC, Stable Isotope Labeling, Amino acids, Proteins, Peptides***

## I. INTRODUCTION

Label-based quantitation involves comparison of samples by labeling them with alternative differential mass tags thus allowing detection based on specific change in mass. This is a comparative approach which in general employs incorporation of chemically similar but isotopically different labels. Thus, the labeling strategy easily divulges both relative as well as absolute quantitation of proteins from individual samples within the same run (1). The dynamic changes of proteome abundance in cells have an important impact on various life processes.

For example, the occurrence and development of many diseases is often accompanied by abnormal expression of certain proteins. Quantitative proteomics is the accurate quantification and identification of all proteins expressed in a genome or all proteins in a complex mixed system.

At present, quantitative proteomics technology is mainly divided into labeling strategies and non-labeling strategies, in which the labeling strategies are divided into in vivo labeling (such as SILAC), and in vitro labeling (such as iTRAQ, TMT). Therefore, this review will be emphasized on current progress in quantitative proteomics techniques (mainly iTRAQ, TMT and SILAC) and their advantages.

## II. ISOBARIC TAGS FOR RELATIVE AND ABSOLUTE QUANTITATION (ITRAQ)

iTRAQ is multiplex protein labeling technique for protein quantification based on tandem mass spectrometry. This technique relies on labeling the protein with isobaric tags (8-plex and 4-plex) for relative and absolute quantitation. The technique comprises labeling of the N-terminus and side chain amine groups of proteins, fractionated through liquid chromatography and finally analyzed through MS. It is essential to find the gene regulation to understand the disease mechanism, therefore protein quantitation using iTRAQ is an appropriate method that helps to identify and quantify the protein simultaneously (2). iTRAQ has been applied for quantitative analysis of membrane and cellular proteins of Thermobifida fusca grown in the absence and presence of cellulose. About 181 membrane and 783 cytosolic proteins were quantified during cellulosic hydrolysis.

The quantified protein in cellulosic medium was involved in pentose phosphate pathway, glycolysis, citric acid cycle, starch, amino acid, fatty acid, purine, pyrimidine and energy metabolism. Consequently, these proteins have a functional role in cell wall synthesis, transcription, translation and replication (3).

The huge amount of oxidative and hydrolytic enzymes is secreted by Phanerochaete chrysosporium that degrade lignin, cellulose and mixture of lignin and cellulose. The secretory proteins were quantified from P. chrysosporium and 117 enzymes were quantified including cellulose hydrolyzing exoglucanases, endoglucanases, cellobiose dehydrogenase and β-glucosidases (4). The presence of soluble aluminum ions ($Al^{3+}$) in soil limits crop growth; however, Oryza sativa are highly aluminum tolerant; therefore, quantitative proteome analysis was carried out in response to $Al^{3+}$ in roots of O. sativa at early stages.

*ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.429*
*Volume 8 Issue XI Nov 2020- Available at www.ijraset.com*

Out of 700 identified proteins, the expression of 106 proteins was different in Al3+ tolerant and sensitive cultivars (5). The role of hydrogen peroxide (H2O2) in growth of wheat was identified through iTRAQ-based quantitative approach that showed that the increased concentration of H2O2 restrained the growth of roots and seedlings of wheat. Out of 3,425 identified proteins, 44 were newly identified H2O2- responsive proteins involved in detoxification/stress, carbohydrate metabolism and single transduction. Several proteins such as superoxide dismutase, intrinsic protein 1 and fasciclin-like arabinogalactan protein could possibly be involved in H2O2 tolerance (6).

iTRAQ was a useful tool for determination of molecular process involved in development and function of natural killer (NK) cells. Membrane bound proteins of NK cells from CD3-depleted adult peripheral blood cells and umbilical cord blood stem cells were quantified. Ontology analysis exhibited that many of these proteins were involved in nucleic acid binding, cell signaling and mitochondrial functions (7).

Protein profiling was carried out in mouse liver regeneration following a partial hepatectomy. A total of 827 identified proteins, 270 were quantified as well. Fabp5, Lactb2 and Adh1 were downregulated among these while Pabpc1, Mat1a, Oat, Hpx and Dnpep were upregulated (8).

### *A. Procedure*

The ITRAQ method is based on the covalent labeling of the N-terminus and side chain amines of peptides from protein digestions with tags of varying mass. There are currently two mainly used reagents: 4-plex and 8-plex, which can be used to label all peptides from different samples/treatments.

These samples are then pooled and usually fractionated by liquid chromatography and analyzed by tandem mass spectrometry (MS/MS). A database search is then performed using the fragmentation data to identify the labeled peptides and hence the corresponding proteins.

The fragmentation of the attached tag generates a low molecular mass reporter ion that can be used to relatively quantify the peptides and the proteins from which they originated. At the peptide level, the signals of the reporter ions of each MS/MS spectrum allow for calculating the relative abundance (ratio) of the peptide(s) identified by this spectrum. The abundance of the reporter ions may consist of more than one single signal in the MS/MS data and the signals have to be integrated in some way from the histogram spectrum.

At the protein level, the combined ratios a proteins' peptides represent the relative quantification of that protein. The MS/MS spectra can be analysed using software that is freely available: i-Tracker (9) and iTraqX (10,11)

### *B. Proteomic Analysis*

iTRAQ suited for unbiased untargeted biomarker discovery. Relative quantification of proteins for biomarker discovery in complex mixtures by mass spectrometry can easily and quickly be achieved using iTRAQ technology. iTRAQ is ideally suited for comparing normal, diseased, and drug-treated samples, time course studies, biological replicates and provides relative quantitation. iTRAQ technology utilizes isobaric reagents to label the primary amines of peptides and proteins. The iTRAQ reagents usually consist of an N-methyl piperazine reporter group, a balance group, and an N-hydroxy succinimide ester group that is reactive with the primary amines of peptides.

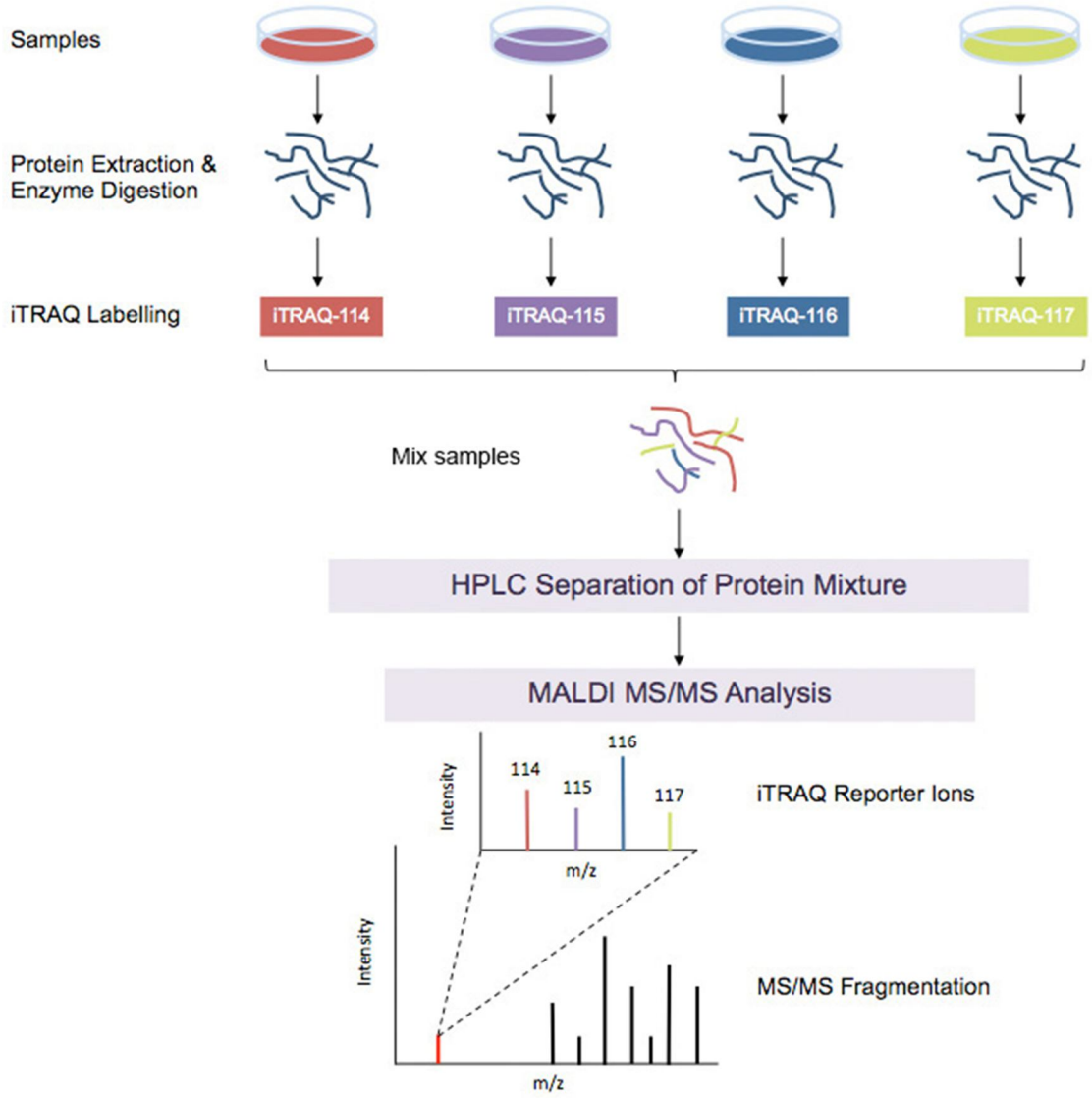
The balance groups present in each of the iTRAQ reagents function to make the labelled peptides from each sample isobaric and the quantification is facilitated through analysis of reporter groups that are generated upon fragmentation in the mass spectrometer. There are currently two mainly used reagents: 4-plex and 8-plex, which can be used to label all peptides from different samples/treatments.

These samples are then pooled and usually fractionated by nano liquid chromatography and analysed by tandem mass spectrometry (MS/MS). iTRAQ workflow (4-plex) is shown above.

Samples to be quantified are prepared under various treatment conditions followed by cell lysis to extract proteins. After using a standard protein assay to estimate the protein concentration of each sample, proteins are digested using an enzyme, such as trypsin, to generate proteolytic peptides.

Each peptide digest is labeled with a different iTRAQ reagent and then the labelled digests are combined into one sample mixture. The combined peptide mixture is analysed by LC-MS/MS for both identification and quantification.

A database search is then performed using the fragmentation data to identify the labelled peptides and hence the corresponding proteins.




The fragmentation of the attached tag generates a low molecular mass reporter ion that can be used to relatively quantify the peptides and the proteins from which they originated (20).

*C. Advantages*

*1)* iTRAQ can simultaneously mark 4 to 8 samples in one experiment. The operation is simple and fast. It is suitable for high-throughput detection of multiple samples, and the experiment design is more flexible.

*2)* iTRAQ is an in vitro labelling performed at the peptide level and is suitable for many types of biological samples.

*3)* iTRAQ has the characteristics of good repeatability and high sensitivity, and qualitative and quantitative can be performed simultaneously.

## III. TANDEM MASS TAG (TMT)

The Thermo Scientific TMT Isobaric and Isotopic Mass Tagging Kits and Reagents enable quantitative labeling of proteins extracted from cells and tissues. Each isobaric tagging reagent within a set has the same nominal parent (precursor) mass and is composed of an amine-reactive NHS-ester group, a spacer arm and an MS/MS reporter. The reagents label peptides prepared from cell-based or tissue samples, either two samples for the duplex kit or six samples for the sixplex kit. For each sample, a unique reporter mass results in the MS/MS spectrum (i.e., 126-127Da for TMT2 and 126-131Da for TMT6 Isobaric Label Reagents). These reporter ions are in the low mass region of the MS/MS spectrum and are used to report relative protein expression levels during peptide fragmentation.

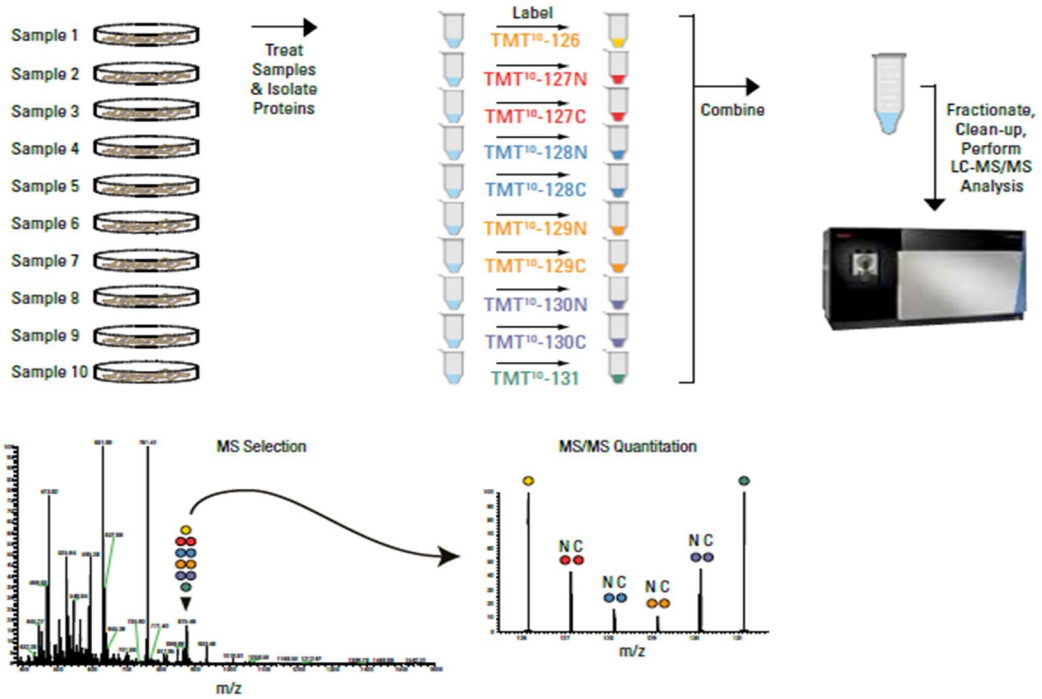
The TMT Reagents share an identical structure, allowing TMTzero and TMTsixplex Reagents to be used also as isotopic "light" and "heavy" duplex tags. These tags are used to quantitate protein expression changes in cell-based or tissue samples that may not be amenable to metabolic isotopic labeling strategies (e.g., SILAC). These isotopic pairs can also be used in targeted quantitation strategies, including selective reaction monitoring (SRM, see the Additional Information Section). Peptides and proteins labeled with all TMT Reagents can be enriched with the anti-TMT Antibody Resin and TMT Enrichment Kit (see the Additional Information and Related Thermo Scientific Products Sections).

### *A. Procedure*

Protein extracts are isolated from cells grown in culture or from tissue samples. After removing amine-based buffers and thiol reagents, samples are reduced, alkylated and digested overnight. Samples are labeled with the TMT Reagents and then mixed at the duplex or the sixplex level. Strong-cation exchange (SCX) fractionation simplifies complex samples before LCMS/MS analysis. Data analysis software is used to analyze the reporter ions in the low mass region. Peptides are typically labeled with TMT Reagents because it allows quantitation of every peptide, but intact proteins can also be labeled. There are several advantages to labeling intact proteins. For example, combining labeled samples earlier in the sample process will reduce sample variability. Also, mixed samples enable single processing for fractionation and digestion. Fractionation methods include ion exchange chromatography, 1D-PAGE and phosphoprotein enrichment.

### *B. Proteomics Analysis*

Isobaric tags labelling technique was firstly applied for the simultaneous identification and relative quantification of peptide pairs by Thompson et al. in 2003. Tandem Mass Tag (TMT) system was designed by Thermo Fisher Scientific for the identification and quantification of proteins in different kinds of samples. Each TMT tagging reagent is composed of an amine-reactive NHS-ester group, a spacer arm and an MS/MS reporter. And the isobaric tagging reagent within a set has the same nominal parent (precursor) mass. These reporter ions are in the low mass region of the MS/MS spectrum and are used to report expression changes in cell-based or tissue samples that may not be amenable to metabolic isotopic labelling strategies such as SILAC. The isobaric labels TMT (tandem mass tagging) are available in up to 11 tags that can be used for labelling practically any peptide or protein sample. TMT makes it possible to multiplex the analysis, to efficiently use the instrument time and exert further controls for technical variation. Due to its ability to multiplex up to 11 samples, TMT are widely used for quantitative protein biomarker discovery. TMTzero, TMTduplex, TMTsixplex, TMT10plex, and TMT11plex Reagents share an identical structure, allowing efficient transition from methods development to multiplex quantitation.



TMT quantification is performed by measuring the intensities of fragment reporter ions released from the labels in the tandem MS mode (MS2) during peptide fragmentation. Precursor ions are selected in the full scan mode (MS1) to be fragmented. Since ion selection step reduces the noise levels, it is advantageous. Ideally, only one selected precursor ion is fragmented during the precursor ion fragmentation. However, it is common that other precursor ions are caught within the specified m/z window and are fragmented together with the selected precursor in practice. This is precursor co-isolation or mixing. Isobaric Labelling works best with mass-spectrometers which allow MS3-level quantitation such as Thermo's Fusion Orbitraps. The additional filtering step allows almost complete correction of co-isolation caused by contamination of MS1 precursors (21).

### C. *Advantages*

1) *High Sensitivity:* low abundance protein can be detected.
2) *Strong Separation Ability:* can separate acid / alkaline protein, protein less than 10KD or greater than 200KD, insoluble protein, *etc.*
3) *Wide Scope of Application:* any type of protein can be identified, including membrane proteins, nuclear proteins, and extracellular proteins.
4) *High Throughput:* 10 samples can be analysed at the same time, especially suitable for differential protein analysis of samples with multiple processing methods or from multiple processing times.

## IV. STABLE ISOTOPE LABELING WITH AMINO ACIDS IN CELL CULTURE (SILAC)

SILAC is an MS-based approach for quantitative proteomics that depends on metabolic labeling of whole cellular proteome. The proteomes of different cells grown in cell culture are labeled with "light" or "heavy" form of amino acids and differentiated through MS. The SILAC has been developed as an expedient technique to study the regulation of gene expression, cell signaling, posttranslational modifications. Additionally, SILAC is a vital technique for secreted pathways and secreted proteins in cell culture (12). SILAC was used for quantitative proteome analysis of B. subtilis in two physiological states such as growth during phosphate and succinate starvation. More than 1,500 proteins were identified and quantified in the two tested states. About 75% genes of B. subtilis were expressed in log phase. Moreover, 10 phosphorylation sties were quantified under phosphate starvation while 35 phosphorylation sites under growth on succinate (13). Highly purified mutant adenovirus deficient in protein V (internal protein component), wildtype adenovirus and recombinant virus were quantified through SILAC. Viral protein composition and abundance were constant in all types of viruses except virus deficient in protein V which also resulted in reduced amount of another viral core protein (14). SILAC was used by for quantitative proteome analysis of A. thaliana. Expression of glutathione S-transferase was analyzed in response to abiotic stress due to salicylic acid and consequent proteins were quantified (15). Salt stress response and protein dynamics in photosynthetic organism Chlamydomonas reinhardtii have been studied to establish the proteome turnover rate and changes in metabolism under salt stress conditions. RuBisCO was found as the most prominent protein in C. reinhardtii (16). The intracellular stability of almost 600 proteins from human adenocarcinoma cells have been analyzed through "dynamic SILAC" and the overall protein turnover rate was determined. Tissue regeneration is imperative in many diseases such as lung disease, heart failure and neurodegenerative disorders. The tissue regeneration and protein turnover rate were quantitatively analyzed in zebra fish. Proteome analysis showed that fin, intestine and liver have high regenerative capacity while heart and brain have the lowest. The proteins in tissue regeneration were mainly involved in transport activity and catalytic pathways (16,17).

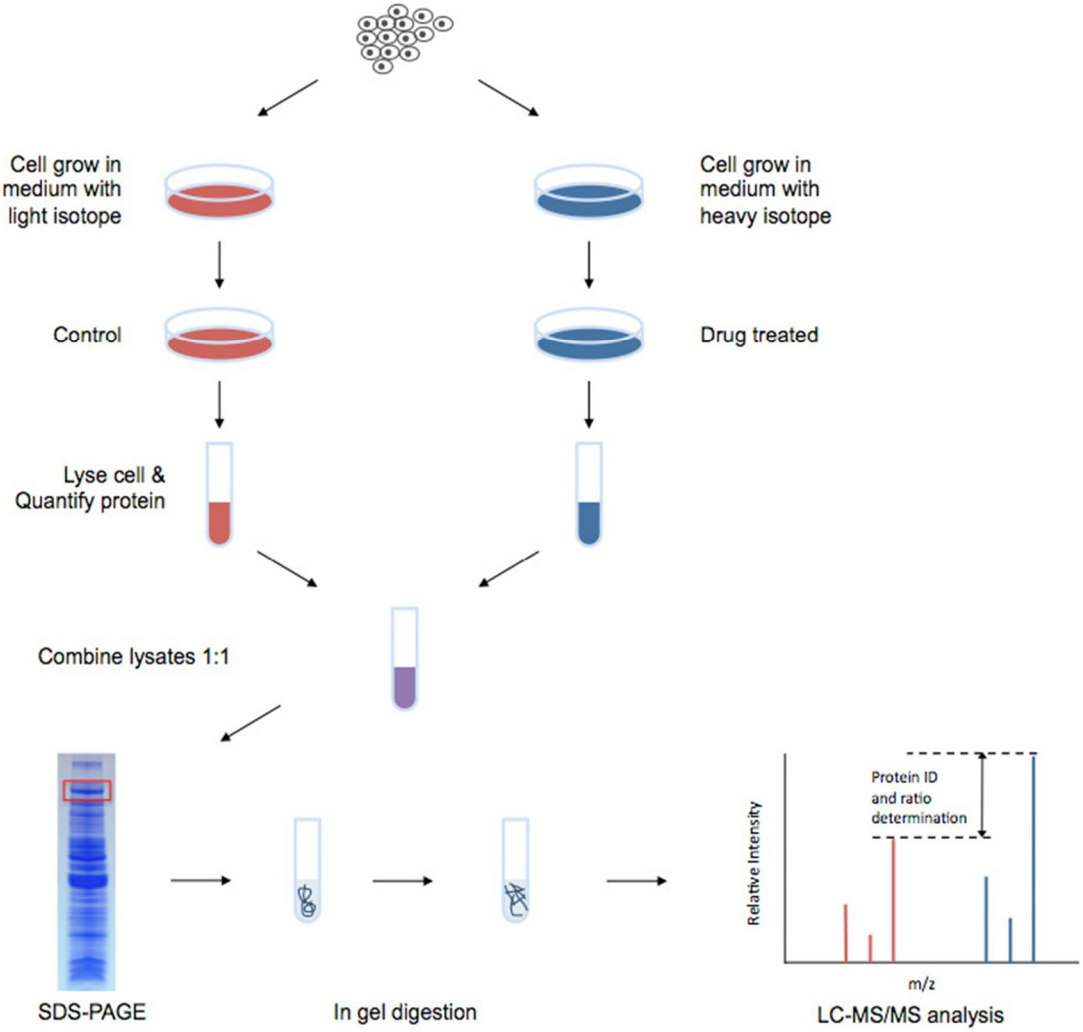
### A. *Procedure*

Two populations of cells are cultivated in cell culture. One of the cell populations is fed with growth medium containing normal amino acids. In contrast, the second population is fed with growth medium containing amino acids labeled with stable (non-radioactive) heavy isotopes. For example, the medium can contain arginine labeled with six carbon-13 atoms ($^{13}C$) instead of the normal carbon-12 ($^{12}C$).

When the cells are growing in this medium, they incorporate the heavy arginine into all of their proteins. Thereafter, all peptides containing a single arginine are 6 Da heavier than their normal counterparts. Alternatively, uniform labeling with $^{13}C$ or $^{15}N$ can be used. The trick is that the proteins from both cell populations can be combined and analyzed together by mass spectrometry. Pairs of chemically identical peptides of different stable-isotope composition can be differentiated in a mass spectrometer owing to their mass difference. The ratio of peak intensities in the mass spectrum for such peptide pairs reflects the abundance ratio for the two proteins (18,19).

*B. Proteomic Analysis*

Stable isotope labelling by amino acids in cell culture (SILAC) is a powerful method to study the relative proteomic change under differential treatments, which relies on the mass spectrometry and the metabolic incorporation of amino acids with substituted stable isotopic nuclei. In SILAC, a given 'light' or 'heavy' form of the amino acid is incorporated into two samples. Two cell populations are grown in culture media that are identical except that one of them contains a 'light' and the other contains a 'heavy' form of a particular amino acid (e.g. 12C and 13C labelled L-lysine, respectively). As the two isotopically labelled amino acids are essentially chemically identical, their incorporation does not interfere with normal cell growth, while leading to proteins/peptides that are distinguishable by mass and thus are ideal for mass spectrometric analysis. SILAC approaches are well suited for monitoring changes in post-translational modifications. At SILAC labelled including cell culture, treatment of cells, and proteomics analysis. Based on SLIAC and mass spectrometry, we can analyse the relative proteomic change under differential treatments. In addition, compare with other technologies, we can provide protein-protein interaction and post-transitional modification analysis. The ability to multiplex two or three samples per analysis allows for increased throughput and cost savings in quantitative proteomics experiments along with improved relative quantitation (22).




*C. Advantages*

*1)* SILAC is a living cell-level labelling technology. The labelling effect is stable and efficient, and its labelling efficiency is not affected by lysate.
*2)* SILAC requires less samples, usually only a few dozen micrograms of protein per sample is sufficient.
*3)* SILAC uses mass spectrometry to identify and quantify multiple proteins simultaneously.
*4)* SILAC uses in vivo labelling technology, which is close to the true state of the sample.

*ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.429*
*Volume 8 Issue XI Nov 2020- Available at www.ijraset.com*

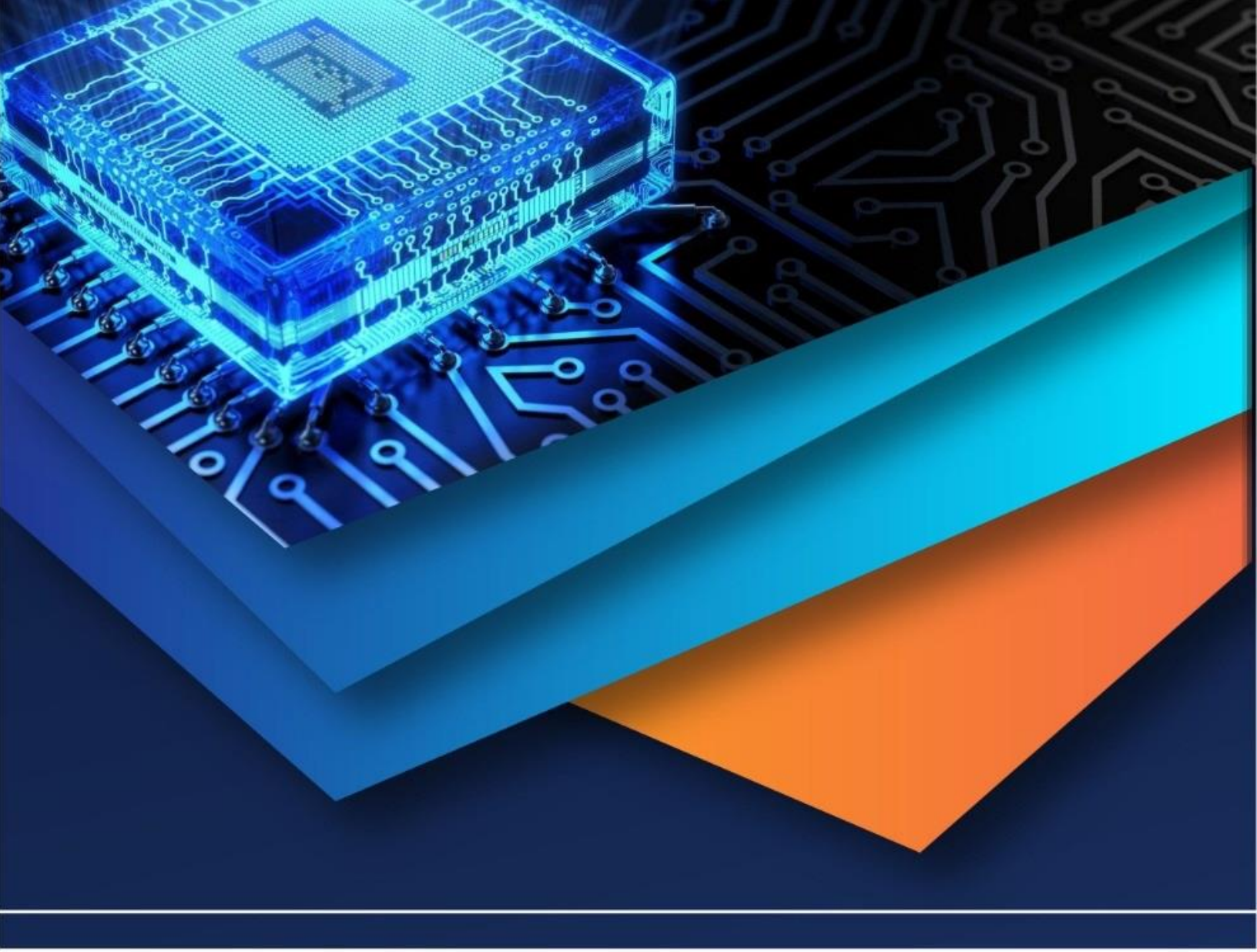
## V. CONCLUSION

The analysis of various proteomes using quantitative proteomics. We can see significant advantages of these methods. This helps us to figure out the effectiveness of different methods and their limitations. The method of choice depends to a large extent on the biological question, the researcher, the cost involved as well as the quality of the available MS instrument. The availability of high-quality data helps the researcher for the advancement of their studies. Comparison of the ITRAQ, TMT, SILCA are listed below.

| Characteristics | iTRAQ | TMT | SILAC |
|---|---|---|---|
| Labeling Method | In vitro | In vitro | In vitro |
| Label Specifications | 4plex/8plex | Duplex/6plex/10plex | - |
| Reporting group molecular mass | 113-121; The minimum molecular mass difference between labeled reagents was 1 Da | 126-131; The minimum molecular mass difference between labeled reagents was 6/1000 Da | - |
| Suitable mass spectrometer | TOF and Orbitrap mass spectrometry | The mass spectrometer has a resolution of at least 50,000 to distinguish different tags. Older versions of obtrap instruments can only distinguish 6plex | TOF and Orbitrap mass spectrometry |
| Advantages | High throughput<br>High protein coverage<br>Quantitative accuracy<br>High credibility<br>Data-rich | High throughput<br>High protein coverage<br>Quantitative accuracy<br>High credibility<br>Data-rich | A. Labeling efficiency can be up to 100%<br>B. Good quantitative repeatability, low protein consumption<br>C. Suitable for the identification and quantification of membrane proteins<br>D. Closer to the real state of the sample |
| Disadvantages | A. Sample preparation and enzymatic processes may cause discrepancies between parallel samples, resulting in biased results.<br>B. Occurrence of interference between co-screening and co-fragmentation of the precursor ion for complex samples. | A. Sample preparation and enzymatic processes may cause discrepancies between parallel samples, resulting in biased results.<br>B. Occurrence of interference between co-screening and co-fragmentation of the precursor ion for complex samples. | It is mainly suitable for passage able cells or bacteria. Other experimental materials are not applicable. Long turnaround time |

## REFERENCES

[1] Domon B, Aebersold R (2010) Options and considerations when selecting a quantitative proteomics strategy. Nat Biotechnol 28(7): 710–721
[2] Wiese, S., Reidegeld, K.A., Meyer, H.E., Warscheid, B.; Protein labeling by iTRAQ: A new tool for quantitative mass spectrometry in proteome research; Proteomics, (2007); 7(3): 340–350
[3] Adav, S.S., Ng, C.S., Sze, S.K.; iTRAQ-based quantitative proteomic analysis of Thermobifida fusca reveals metabolic pathways of cellulose utilization; Journal of Proteomics, (2011); 74(10): 2112–2122.
[4] Manavalan, A., Adav, S.S., Sze, S.K.; iTRAQ-based quantitative secretome analysis of Phanerochaete chrysosporium; Journal of Proteomics, (2011); 75(2): 642–654.
[5] Wang, Z.Q., Xu, X.Y., Gong, Q.Q., Xie, C., Fan, W., Yang, J.L., et al.; Root proteome of rice studied by iTRAQ provides integrated insight into aluminum stress tolerance mechanisms in plants; Journal of Proteomics, (2014); 98: 189–205.
[6] Ge, P., Hao, P., Cao, M., Guo, G., Lv, D., Subburaj, S., et al.; iTRAQbased quantitative proteomic analysis reveals new metabolic pathways of wheat seedling growth under hydrogen peroxide stress; Proteomics, (2013); 13(20): 3046–3058.
[7] Lund, T.C., Anderson, L.B., McCullar, V., Higgins, L., Yun, G.H., Grzywacz, B., et al.; iTRAQ is a useful method to screen for membranebound proteins differentially expressed in human natural killer cell types; Journal of Proteome Research, (2007); 6(2): 644–653.
[8] Hsieh, H.-C., Chen, Y.-T., Li, J.-M., Chou, T.-Y., Chang, M.-F., Huang, S.-C., et al.; Protein profilings in mouse liver regeneration after partial hepatectomy using iTRAQ technology; Journal of Proteome Research, (2009); 8(2): 1004–1013
[9] Shadforth IP, Dunnley PJ, Lilley KS, Bessant C (2005). "i-Tracker: For quantitative proteomics using iTRAQ". BMC Genomics. **6**: 145. doi:10.1186/1471-2164-6-145. PMC 1276793. PMID 16242023.
[10] Muth, T., et al., jTraqX: a Free, Platform Independent Tool for Isobaric Tag Quantitation at the Protein Level, Proteomics, 2010, 10(6): 1223-1225, doi:10.1002/pmic.200900374
[11] protein-ms - Browse /jTraqX at SourceForge.net
[12] Ong, S.-E., Mann, M.; Stable isotope labeling by amino acids in cell culture for quantitative proteomics. In Quantitative Proteomics by Mass Spectrometry. Humana Press, Totowa, NJ, USA, (2006), pp. 37–52
[13] Soufi, B., Kumar, C., Gnad, F., Mann, M., Mijakovic, I., Macek, B.; Stable isotope labeling by amino acids in cell culture (SILAC) applied to quantitative proteomics of Bacillus subtilis; Journal of Proteome Research, (2010); 9(7): 3638–3646.
[14] Alqahtani, A., Heesom, K., Bramson, J.L., Curiel, D., Ugai, H., Matthews, D.A.; Analysis of purified Wild type and mutant adenovirus particles by SILAC based quantitative proteomics; Journal of General Virology, (2014); 95(Pt_11): 2504–2511.
[15] Gruhler, A., Schulze, W.X., Matthiesen, R., Mann, M., Jensen, O.N.; Stable isotope labeling of Arabidopsis thaliana cells and quantitative proteomics by mass spectrometry; Molecular and Cellular Proteomics: MCP, (2005); 4(11): 1697–1709.
[16] Mastrobuoni, G., Irgang, S., Pietzke, M., Aßmus, H.E., Wenzel, M., Schulze, W.X., et al.; Proteome dynamics and early salt stress response of the photosynthetic organism Chlamydomonas reinhardtii; BMC Genomics, (2012); 13(1): 215.
[17] Westman-Brinkmalm, A., Abramsson, A., Pannee, J., Gang, C., Gustavsson, M.K., von Otter, M., et al.; SILAC zebrafish for quantitative analysis of protein turnover and tissue regeneration; Journal of Proteomics, (2011); 75(2): 425–434.
[18] Ong SE, Blagoev B, Kratchmarova I, Kristensen DB, Steen H, Pandey A, Mann M (May 2002). "Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics". *Mol. Cell. Proteomics*. **1** (5): 376–86. doi:10.1074/mcp.M200025-MCP200. PMID 12118079.
[19] Ong SE, Blagoev B, Kratchmarova I, Kristensen DB, Steen H, Pandey A, Mann M (May 2002). "Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics". *Mol. Cell. Proteomics*. **1** (5): 376–86. doi:10.1074/mcp.M200025-MCP200. PMID 12118079.
[20] Zieske Lynn R. A perspective on the use of iTRAQ™ reagent technology for protein complex and profiling studies. *Journal of Experimental Botany*, 2006(7):7.
[21] Lichao Zhang, Joshua E. Elias. Relative Protein Quantification Using Tandem Mass Tag Mass Spectrometry. *Methods in Molecular Biology*, 2017, 1550:185.
[22] Esthelle Hoedt, Guoan Zhang, Thomas A. Neubert. Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC) for Quantitative Proteomics.



10.22214/IJRASET

45.98

IMPACT FACTOR: 7.129

IMPACT FACTOR: 7.429

# INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY