YENEPOYA
(DEEMED TO BE UNIVERSITY)
Recognized under Sec 3(A) of the UGC Act 1956

Multiomics Technology

Core course for Pre-PhD: 4 credits

Yenepoya Research Centre
Yenepoya (Deemed to be University)
University Road, Deralakatte
Mangalore – 575018

Course Name: Multiomics Technology

1.	Course Type	: Core
2.	Level	:Ph.D. (Pre-PhD course work)
3.	Credit Value:	:4 Credits
4.	Total Hours	:60 (L:P:S: 10:25:25)
5.	Total Marks:	:100 (IA= 40 + Final exam= 60)

6. Course Objectives

- To develop and create awareness about the importance of databases, tools, their versions, and their limitations in data analysis, for better visualization and evaluation of the experimentally derived biological datasets.
- To apply the knowledge of value-added applications and follow the evolving omics technologies towards diagnostics and therapeutic applications in the global scenario.
- To expose the research scholar to distinguish/prioritize the low-throughput technology platforms for secondary support/validation of specific molecules derived from high-throughput technology platforms.
- To develop skills to carry out real-time analysis of distinct biological samples using the global proteomics and metabolomics platforms following standard operating protocols.
- To develop skill and induce confidence/ self-motivation in the research scholars to formulate, plan and carry out experiments employing multi-omics technologies and subsequently, the data analysis, towards efficient management of time and productivity.
- To prepare the research scholars for their doctoral research with basic quest and query on the existing data in their research themes for enhanced evaluation and better demonstration of their research to the scientific community.

7. Learning outcome

This course will prime the research scholars to demonstrate enhanced research aptitude in employing omics technologies for quality research publications and patents.

8. Competencies

1. Describe methods used for visualization of genes from the sequence- and site-specific features at DNA, RNA, protein and post-translational modification level towards their integrated functions with each other
2. List the custom optimizable mass spectrometry-based data acquisition parameters, search engines and design experiment, analyze, and interpret data in the field of proteomics and metabolomics
3. Practice of care in sample preparation, operation and handling of mass-spectrometers following standard operating procedures
4. Identify the pros and cons of the existing data analysis and gene-set enrichment analysis tools for specific biological-context-dependent data interpretation.
5. Practice of safety procedures and appropriate waste disposal protocols while conducting research
6. Practice research ethics while performing research using biological samples

Content of the Course

Module 1: Bio-analytical skills and practices (12 h)

1.1. Initiate good laboratory practices-contaminations and spillages, value of the clinical and other biological samples with respect to quantity and availability, influence of volatile substances, and the use of hazardous chemicals in the laboratory environment; Assessment of the standard operating procedures on each of the instruments to safe guard the researchers, instruments and the biological samples.

1.2. High-throughput and low-throughput technologies: Impact of high-throughput technologies; low-throughput molecular level analysis and validation tools for sequence, variations and copy number; applications of PCR methodologies for research and clinical applications; clinical applications of FISH; Impact of cost and throughput on the biological analysis of samples for research and biomedical applications.

1.3. Applications of distinct bio-analytical instrumentation facility - Chromatography techniques- adsorption, partition, affinity, ion exchange and size exclusion; Spectroscopy and their applications (UV-Vis, Fluorescence and NMR), Different types of centrifuges, Separation Methods: Centrifugation-Sedimentation principle, differential centrifugation; Different microscopy techniques- Light, Fluorescence, Confocal, SEM and TEM, Image formation, image resolution and representation of images in reports

1.4. Cell biology techniques: Exposure to cell culture facility; Assessment of cellular viability, cell proliferation and migration assays, histopathological analysis of samples; immunocytochemistry method, grading, advantages, limitations and its applications in clinical research; Cell heterogeneity, markers and applications of flow cytometry; Evaluation of current single cell omics data.

1.5. Discovery and validation: Complementation applications of diverse bio-analytical methods and technologies currently available as primary and supporting validation platforms; Approaches for discovery of biomarkers and therapeutic targets for diseases/disorders; Stepping towards the validation and clinical trials; Statistical parameters for biomarker discovery and validation; Interactions with pathologists and clinical practitioners.

Module 2: Proteomics in practice and applications (12 h)

2.1. Mass Spectrometry – instrumentation: international service providers of mass spectrometry platforms, multiple ionization methods and their established applications (MALDI, electrospray), different types of mass analyzers, fragmentation modes (CID, HCD and ETD) and detectors.

2.2. Sample processing for proteomics: Different types of cell and tissue (plant and animal) lysis techniques used in proteomic sample preparations; Handling of biological fluids (clinical samples: blood/serum, urine, saliva etc.) in proteomic sample preparation; Methods available for protein and peptide estimation; Protease enzymes and their role in proteomic sample preparation; distinct methods for sample preparation across laboratories; preparation of cytoplasmic, membrane, and nuclear fractions; Label free and labeled peptide and protein quantification methods; Peptide (isobaric labeling, ICAT etc.) and protein labeling techniques (SILAC); protein and peptide level normalizations; removal of MS incompatible salts and chemicals from proteomic samples (C18 desalting, protein precipitations, buffer exchange etc.); protein and peptide level fractionation techniques (SDS-PAGE, bRPLC, SCX etc.).

2.4. Mass spectrometry based proteomic approaches and acquisition techniques: Difference between Top-Down; Bottom-Up/Shotgun proteomic analysis; Use of peptide mass fingerprinting; Different types of mass spectrometry based acquisition techniques (DDA, DIA, PRM, MRM/SRM, AIF, and SPS3), how they evolved over the years, pros and cons of DDA and DIA.

2.5. Proteomic data analysis: Database, de-novo sequencing and spectral library search methods, algorithms and tools (SequestHT, Mascot, X!Tandem, MS-Amanda, MaxQuant, PEAKS studio, deepnovo, Andromeda, Percolator, mProphet etc.), Difference between spectrum centric and peptide centric proteomic data analysis, Relative and absolute quantification methods in proteomics, Statistical validation techniques applied in proteomic database and spectral library searches (Xcorr, Ion score, Q-value, PEP score etc.).

2.6. Statistical analysis: Different types of protein/peptide abundance value normalization techniques used, data imputations; Identification of differentially expressed proteins and peptides techniques (Fold change, Student T-test, ANOVA etc.). Proteomic database/spectral library search result visualization techniques (Heat map, Bar plot, Box plot, Volcano plot).

Module 3: Metabolomics in practice and applications (12 h)

3.1 Mass spectrometry for metabolomics-mass spectrometry service providers for

metabolomics, SCIEX QTRAP Mass spectrometer- principles and applications of triple quadrupole ion trap mass analyzers, fragmentation techniques, ms/ms based metabolomics

3.2 Sample processing for metabolomics: an overview and applications in biology, extraction of metabolites, general work flow including quenching and sample preparation, different solvent systems for metabolite extraction, workflow for lipidomics

3.3 LC optimizations and modes of data acquisition: Positive and negative polarities, gradient selection, types of columns, column and pore size, data acquisition quality, QA/QC.

3.4 Data analysis- Data repositories, Introduction to Peak detection, retention time alignment; identification of molecular features and metabolites. Structural confirmation of metabolites. Introduction to data analysis tools Software- MZmine, XCMS, Metaboanalyst, Lipid Search, MS2 compound, Metabolic pathways and inborn errors of metabolism, statistical methods most applied for analysis of metabolomics data (PCA, PLS, PLS-DA)

3.5 Analytical methodologies: Choose methods of measurement and carry out basic experimental design for a given biological and biomedical problem; methods and their applications for pre-clinical, clinical trials, cell models and plant experiments; targeted vs. untargeted metabolomics; development of targeted assays for small molecules.

3.6 Integrative analysis of the metabolome with other omics such as genomics, transcriptomics and proteomics; integration of metabolites into biosynthetic pathways; Comparative analysis between various metabolite repositories and databases.

Module 4: Genomics in practice and applications (14 h)

4.1 Genomics: Sequencing technologies - Sanger sequencing-principle, methodology and applications; multiple generations of NGS; Sequencing strategies based on clonal amplification technology, bridge amplification, pyrosequencing, Illumina Sequencing by synthesis platform (SBS); PacBio (SMRT technology), Oxford Nanopore system etc.

4.2 Transcriptomics: Transcriptomics technologies; Mapping RNA- sequencing dataset onto reference genome; Concepts of RPKM and FPKM; Concepts on the analysis of differential gene expression across sample types; Different strategies for the analysis of mRNAs and non-coding RNA genes; Approaches for the identification and validation of potential biomarkers at transcript level.

4.3 Next Generation Sequencing (NGS): Applications of NGS analysis in oncology, Standard data formats, measures and steps in quality control-Phred score, FastQC and FastX tool kits; Open and commercial data analysis tools and pipelines; Impact of read length, read depth, and sequence coverage across multiple NGS

technology platforms; Multiple approaches to clustering; Tools for functional enrichment analysis of gene sets; Evolution of Galaxy platform; Impact of the Human Genome sequencing project; Concepts on polymorphisms; Implications of data derived from whole genome sequencing, whole exome and targeted exomes for clinical applications.

4.4 Integrative genomics, transcriptomics and proteomics: Integrating genomic variants with transcriptome; Impact of sequence variations and copy number alterations on transcriptome; Multiple levels of post-transcriptional regulations; Applications of proteogenomic analysis for refinement of genomes and gene models; Trends in proteogenomic analysis of cancers; Impact of sequence variations on phenotype; Current concepts on genotype-phenotype correlation; Tools for integrative omics analysis.

4.5 Comparative genomics: Comparative genomics approaches for sequence level and protein function prediction and other applications; Genome Wide Association Studies (GWAS); concepts and utility of phylogenetic analysis; Quantitative trait locus (QTL); QTL databases; Analysis of G-quadruplex structures on DNA and RNA; Topology-Associated Domains (TADs) in genomes and A/B Compartments.

Module 5: Signaling pathway for systems biology (10 h)

5.1 Protein-protein interactions: Different methods and tools to study protein-protein interactions; interaction networks; protein-protein interaction databases, their version and data content.

5.2 Enzyme-substrate reactions: analysis of the kinase sequences and their domains; enzyme-specific databases, site-specific modifications in proteins, PTM enrichment analysis and tools; PhosphoSite Plus and Phosida databases.

5.3 Localization of proteins: Subcellular compartmentalization of proteins; Specific sequence features, modifications and interactions in proteins associated with their subcellular compartmentalization; Membrane proteome and their characterization.

5.4 Signaling pathway: Signaling pathway databases; Current pathway enrichment analysis tools; Limitations of the current pathway enrichment tools.

Teaching-learning methods

Modules	**Teaching-learning**		
	Lecture	Practical/Hands on	Self-study
Module 1: Bio-analytical approaches	1.1		1.1
	1.2		1.2
		1.3	1.3 (seminar)
		1.4 (Group discussion)	1.4
	1.5		1.5

Module 2: Proteomics in practice and applications	2.1	2.1 (Team work)	2.1
			2.2 (seminar)
	2.3	2.3	2.3
	2.4	2.4 (Group discussion)	
	2.5	2.5 (Group discussion)	
	2.6	2.6 (Team work)	
Module 3: Metabolomics in practice and applications	3.1	3.1(Group discussion)	3.1
	3.2	3.2 (Hands on)	3.2
			3.3 (seminar)
	3.4	3.4 (Hands on)	3.4
		3.5 (Group discussion)	3.5
	3.6	3.6 (Group discussion)	
Module 4: Integrative Genomics and transcriptomics		4.1(Group discussion)	4.1
	4.2	4.2	4.2
	4.3	4.3 (Group discussion)	4.3
			4.4 (seminar)
	4.5		4.5
Module 5: Signaling pathway for systems biology		5.1 (Group discussion)	5.1
		5.2 (Group discussion)	5.2
	5.3		5.3
	5.4	5.4 (Hands on)	5.4

1. Assessments

Formative assessment: (40 Marks)

1	Internal Exams - 40 marks each (2)	20 M
2	Seminar (2)	8 M
3	Group discussion (2 Including ethical and regulatory issues)	6 M
4	Case studies (2)	6 M

Summative Assessment: (60 marks)

Sl. No.	Details	Q X M
Two Questions to assess the knowledge and problem solving abilities in the given context		4X10 M=40 M
1	Knowledge on application of any of the major instruments used in the In vitro and in vivo cancer research for generating data (E.g. Discuss on the potential low-throughput validation platforms for protein expression analyzed using mass spectrometry and sequence variations analyzed using NGS approaches.)	

2	Problem solving ability: Designing experimental protocol for a given research problem and interpretation of data (Discuss based on any specific applications, the comparative analysis of the peptide and protein separation techniques with their pros and cons.)	
3 4	Two questions to assess the analytical skills to solve a given hypothetical research problem Write the methodology for solving a given research problem (Discuss global and quantitative proteomics/DDA and DIA methods/method development for targeted metabolomics/NGS work flow.)	
5	Descriptive questions to assess knowledge from module 3, 4 and 5.	4X5=20 M

*A question bank will be maintained with multiple scenarios.

Learning Resources

Text Books

1. Baxevanis AD and Ouellette BFF (2005). Bioinformatics – A practical guide to the analysis of genes and proteins (3rd edition). Wiley India.
2. Brown TA (2010). Gene cloning and DNA analysis: An introduction. Wiley-Blackwell.
3. Bruce Albert, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, Peter Walter 2007. Molecular Biology of the cell- 5th edition. Garland Science, New York.
4. Fan TWM, Andrew N and Richard M (2012). The handbook of metabolomics. Humana Press.
5. Green MR and Sambrook J (2012). Molecular cloning – A laboratory manual. Cold Spring Harbor Laboratory Press.
6. Gross JH (2011). Mass spectrometry – A textbook. Springer.
7. Karp G (2009). Cell and molecular biology: Concepts and experiments, 7th edition. John Wiley & Sons.
8. Kulkarni S and Pfeifer J (2014). Clinical genomics. Academic Press.
9. Leung H-CE (2012). Integrative proteomics. In Tech.
10. Lindon, JC, Nicholson JK, Holmes E (2007). The handbook of metabonomics and metabolomics. Elsevier.
11. Lodish H, Berk A, Zipursky SL, Matsudaira P, Baltimore D and Darnell J (2008). Molecular cell Biology. W. H. Freeman.
12. Miller K and Levine J (2010). Biology. Pearson.
13. Primrose SB and Twyman RM (2006). Principles of gene manipulation and genomics. Blackwell Publishing.
14. Reece RJ (2004). Analysis of genes and genomes. John Wiley & Sons Ltd.
15. Simpson R (2002). Proteins and proteomics: A laboratory manual. Cold Spring Harbor Laboratory Press.
16. Wilson K and Walker J (2010). Principles and techniques of biochemistry and molecular biology, 7th edition. Cambridge University Press.

Suggested readings

1. Bourmaud A, Gallien S and Domon B (2016). Parallel reaction monitoring using quadrupole orbitrap mass spectrometer: Principle and applications. Proteomics.
2. Gallien S and Domon B (2015). Advances in high-resolution quantitative proteomics: implications for clinical applications. Expert Rev Proteomics, 12, (5): 489-98.
3. Goodwin S, McPherson JD and McCombie WR (2016). Coming of age: ten years of next-generation sequencing technologies. Nat Rev Genet, 17, (6): 333-51.
4. Harsha HC, Molina H and Pandey A (2008). Quantitative proteomics using stable isotope labeling with amino acids in cell culture. Nat Protoc, 3, (3): 505-16.
5. Harsha HC, Pinto SM and Pandey A (2013). Proteomic strategies to characterize signaling pathways. Methods MolBiol, 1007, 359-77.
6. Maiolica A, Junger MA, Ezkurdia I and Aebersold R (2012). Targeted proteome investigation via selected reaction monitoring mass spectrometry. J Proteomics, 75(12): 3495-513.
7. Mardis ER (2013). Next-generation sequencing platforms. Annu Rev Anal Chem (Palo Alto Calif), 6: 287-303.
8. Metzker ML (2010). Sequencing technologies - the next generation. Nat Rev Genet, 11(1): 31-46.
9. Parker CE, Domanski D, Percy AJ, Chambers AG, Camenzind AG, SmithDS and Borchers CH (2014). Mass spectrometry in high-throughput clinical biomarker assays: multiple reaction monitoring. Top CurrChem, 336: 117-37.
10. Picotti P and Aebersold R (2012). Selected reaction monitoring-based proteomics: workflows, potential, pitfalls and future directions. Nat Methods, 9(6): 555-66.
11. Pinto SM, Nirujogi RS, Rojas PL, Patil AH, Manda SS, Subbannayya Y, Roa JC, Chatterjee A, Prasad TS and Pandey A (2015). Quantitative phosphoproteomic analysis of IL-33-mediated signaling. Proteomics, 15(2-3): 532-44.
12. Rauniyar N (2015). Parallel Reaction Monitoring: A Targeted Experiment Performed Using High Resolution and High Mass Accuracy Mass Spectrometry. Int J MolSci, 16(12): 28566-81.

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