**MBB 291: Long Read Sequencing Applications in Plant and Animal Pathogen Genomics and Metagenomics**

**Course Overview**

This course provides comprehensive training on long read sequencing technologies and their applications in the study and identification of plant and animal pathogens, with emphasis on viruses and bacteria. Participants will learn the principles, techniques, and bioinformatics tools required to carry out long read sequencing projects, with a focus on practical applications in plant, and veterinary and zoonotic diseases, and metagenomics.

**Course Format**

3 h lecture per week with individual hands-on activities via Google Classroom

BYOD (Bring your own device: preferably laptop with at least 16 GB RAM, 500 GB SSD);

**Prerequisite(s):**

MBB 211 or COI

**Class size:** limited slots only

**Course Credit:** 3.0 units

**Instructor:** Andrew Montecillo (with invited lecturers and resource persons); for more information please contact [admontecillo@up.edu.ph](mailto:admontecillo@up.edu.ph)

**Schedule:** **MW: 8:30 - 10:00 am, IBS Bioinformatics Laboratory, 2nd Floor MBB Wing**

**Please fill out this course survey form:** [**https://forms.gle/KRWS8Ud6SzUKjoMc7**](https://forms.gle/KRWS8Ud6SzUKjoMc7)

**Course objectives:**

At the end of this course, the student will be able to:

1. Explain the fundamentals of long-read sequencing and its advantages over short-read sequencing in pathogen genomics and metagenomics applications.
2. Utilize long-read sequencing platforms and tools for accurate assembly of complex genomes of plant and animal pathogens.
3. Analyze long-read sequencing data to identify genomic variations, structural variants, and pathogenicity or virulence factors.
4. Utilize long-read sequencing platforms and tools for accurate assembly and analysis of near full-length 16S / ITS amplicons for microbial community studies.
5. Evaluate recent research and case studies demonstrating the impact of long-read sequencing on understanding and managing plant and animal pathogens and metagenomics.

**Course Materials**

- Lecture notes and slides

- Protocol guides and standard operating procedures (SOPs)

- Bioinformatics software tutorials (select tools and applications)

- Access to sequencing data and computational resources

- Datasets for each step of analysis

**Practical Sessions and Project Work**

- Hands-on practice with long read sequencing protocols

- Data analysis workshops using real-world datasets

- Group projects: design and execute a sequencing-based pathogen study / microbial community analysis

- Presentation of project findings and course wrap-up

**Evaluation**

- Weekly quizzes and assignments

- Practical lab reports and assessments

- Online exams and assessments

- Final project presentation and report

**Proposed Course Outline**

**Introduction to Long Read Sequencing**

* Overview of sequencing technologies (Sanger, short read, long read)
* Advantages and limitations of long read sequencing
* Applications of long read sequencing in plant and animal pathogen research
* Key platforms: Oxford Nanopore Technologies (ONT) and Pacific Biosciences (PacBio)

**Hands-on Activities: Basics of Unix Terminal Commands and Command Line interface, Conda Environments, and Jupyter Lab**

**Long-read Sequencing Protocols, Instrumentation, and Software and Hardware Requirements**

- Introduction to Long-read sequencing platform

- Sequencing protocols: flongle, MinION,

- Run setup and monitoring

- Troubleshooting common issues

**Library Preparation for Long-read Sequencing**

- Introduction to Long-read sequencing kits and library preparation protocols

- Techniques for preparing high-molecular-weight DNA

- Adapters and barcoding strategies

- Quality control steps during library preparation

**Bioinformatics for Long-read Data Analysis**

- Introduction to bioinformatics pipelines for long-read data analysis

- Basecalling, quality control, and error correction

- Tools for *de novo* assembly and reference-based mapping

- Variant calling and structural variant analysis

- Tools for full-length 16S and ITS amplicon sequencing data analysis

**Pathogen Detection and Characterization**

- Identifying pathogens from sequencing data

- Metagenomic approaches for pathogen detection

- Comparative genomics and phylogenetic analysis

- Introduction to Genome-based identification and phylogeny

- Case studies: outbreak investigations and surveillance

**Genome Annotation: Antimicrobial Resistance and Virulence Factors**

- Detecting antimicrobial resistance genes

- Analysis of virulence factors and pathogenicity islands

- Functional annotation of pathogen genomes

- Case studies: AMR in veterinary pathogens or plant pathogens virulence factor analysis

**Microbial Community Analysis using Long-read 16S and ITS Amplicon Sequencing**

- Benchmarking and comparison of Long-read 16S amplicon sequencing vs short-read sequencing

- Case studies: Applications of long-read 16S amplicon sequencing on microbial community analysis

**Metagenome Assembled Genomes (MAGs) from Long-read Shotgun Sequencing**

- Bioinformatic pipelines on long-read sequencing for MAGs

**Emerging Technologies and Future Directions**

- Advances in long-read sequencing technologies

- Emerging applications in pathogen research

- Potential future developments and their implications

- Ethical, legal, and social considerations

**Proposed Schedule of Course Activities**

**Week 1: Introduction to Long-Read Sequencing Technologies**

- Overview of Long-Read Sequencing Platforms

- Presentation on technologies (e.g., PacBio, Oxford Nanopore).

- Demonstration of instrument setup and sample preparation.

**Weeks 2 - 3: Hands-on Activities: Basics of Unix Terminal Commands and Command Line interface, Conda Environment, Jupyter Lab, and Installation of Packages and Applications**

**Week 4: Long-read Sequencing Protocols, Instrumentation, and Software and Hardware Requirements**

- Introduction to Long-read sequencing platform

- Sequencing protocols: flongle, MinION,

- Run setup and monitoring

- Troubleshooting common issues

**Week 5: Library Preparation for Long-read Sequencing**

- Introduction to Long-read sequencing kits and library preparation protocols

- Techniques for preparing high-molecular-weight DNA

- Adapters and barcoding strategies

- Quality control steps during library preparation

**Weeks 6 - 7: Bioinformatics for Long-read Data Analysis**

- Introduction to bioinformatics pipelines for long-read data analysis

- Basecalling, quality control, and error correction

- Tools for *de novo* assembly and reference-based mapping

- Variant calling and structural variant analysis

- Tools for full-length 16S and ITS amplicon sequencing data analysis

**Week 8: Pathogen Detection and Characterization**

- Identifying pathogens from sequencing data

- Metagenomic approaches for pathogen detection

- Comparative genomics and phylogenetic analysis

- Introduction to Genome-based identification and phylogeny

- Case studies: outbreak investigations and surveillance

**Week 9: Genome Annotation: Antimicrobial Resistance and Virulence Factors**

- Detecting antimicrobial resistance genes

- Analysis of virulence factors and pathogenicity islands

- Functional annotation of pathogen genomes

- Case studies: AMR in veterinary pathogens or plant pathogens virulence factor analysis

**Week 10: Microbial Community Analysis using Long-read 16S and ITS Amplicon Sequencing**

- Benchmarking and comparison of Long-read 16S amplicon sequencing vs short-read sequencing

- Case studies: Applications of long-read 16S amplicon sequencing on microbial community analysis

**Week 11: Metagenome Assembled Genomes (MAGs) from Long-read Shotgun Sequencing**

- Bioinformatic pipelines on long-read sequencing for MAGs

**Week 12: Reporting on Emerging Technologies and Future Directions**

- Advances in long-read sequencing technologies

- Emerging applications in pathogen research

- Potential future developments and their implications

- Ethical, legal, and social considerations

**Weeks 12-14: Reporting and Presentations**

- Project Reports and Presentations

- Preparation of comprehensive lab reports.

- Presentations summarizing findings and practical applications.

**Week 15: Final Project and Assessment**

- Final Project Submission and Assessment

- Presentation of final projects.

- Peer and instructor assessment and feedback.

**Week 16: Review and Future Directions**

**- Course Review and Future Perspectives**

**Course requirements and assessments:**

| **2 Exams** | **50%** |
| --- | --- |
| **Quizzes, homeworks, class activities** | **25%** |
| **Individual Oral reporting and presentation** | **10%** |
| **Individual final project** | **15%** |
| **Total** | **100%** |