

Title of Skill Course: Next Generation Sequencing (NGS) Analysis

1. Department: Department of Bioinformatics
2. Title: **Introduction to NGS and Data Handling (Basics)**
3. Sector: Bioinformatics
4. Year of implementation: 2022

Course Structure

Skill Level	Theory Hours	Practical Hours	Total Hours	Credits	No. of students in batch
4	10	05	15	01	30

Syllabus**Course Objectives: Students should be to**

1. Learn new techniques in NGS
2. Know about NGS platforms, different sequencing methods.

Theory Syllabus**Contact Hrs: 10****Unit I: Introduction to NGS technologies**

Introduction to next generation sequencing. Advantages and disadvantages of first generation sequencing. Next (second)-generation sequencing–difference between first and next generation sequencing, NGS platforms –Roche 454, ABI SOLiD, Ion torrent, Illumina.

Unit II: Technical Knowledge

Introduction to NGS technologies: DNA-seq, RNA-seq, ChIP-seq, Hi-C, Metagenomics, Single cell sequencing. Index, Barcode. Library preparation methods -Bridge amplification, Emulsion PCR. Sequencing methods –sequencing by synthesis, ion semiconductor, SMRT, nanopore.

Course Outcomes:

1. Illustrate the different platforms for next generation sequencing.
2. Identify types of sequencing methods used in different platforms

Reference Books:

1. Veena Kumari, Analytical Techniques in DNA Sequencing.
2. Stuart M. Brown, Next-Generation DNA Sequencing Informatics* (Cold Spring Harbor Laboratory Press, 2013).

1. Study NGS technologies: significance in genomics research and medical diagnostics.
2. Study NGS applications, such as whole-genome sequencing, RNA-seq, ChIP-seq, and metagenomics.
3. Introduce & describe the sequencing principles and technologies used by the major NGS platforms: Illumina, Ion Torrent, PacBio, and Oxford Nano pore.
4. Analyze Hi-C data to identify chromatin interactions and visualize 3D genome structures using HiCExplorer.
5. Study concept of unique molecular identifiers (UMIs) and their role in reducing PCR duplicates and increasing accuracy.
6. Provide an overview of library preparation methods, including bridge amplification and emulsion PCR.
7. Demonstrate the process of indexing and barcoding using simulation software.
8. Discuss different indexing strategies (e.g., inline, combinatorial indexing) and their applications.
9. Taxonomic classification of metagenomic sequences using Kraken2 or MetaPhlAn.
10. Align ChIP-seq reads to a reference genome using Bowtie2 or BWA.

BOS Sub Committee:

Sr. No.	Name of Member	Designation	Address
1	Dr.N.N.Bendre	Chairman	Y.C.I.S,Satara
2	Ms.S.N.Sanglikar	Member	Y.C.I.S,Satara
3	Mr.P.M.Bhosale	Member	Y.C.I.S,Satara
3	Dr.Rahul Jamdade	Academic Expert	Y.C. College Karad
4	Mr.BajarangKumbhar	Industrial Expert	

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Syllabus

Course Objectives:

1. Students able to learn techniques in NGS
2. They know about NGS platforms, different sequencing methods.

Theory Syllabus

Contact Hrs: 10

❖ **Unit I: NGS data formats**

Data formats overview –FASTQ, subreads, nanopore data, and single cell data. Single-end, Paired-end, Mate-pair. NGS Data sources –NCBI SRA, EBI-ENA, DDBJ-SRA, GEO; retrieving data from data sources

Unit II: - Data quality control Read trimming and preprocessing

SRA, GEO; Retrieving data from data sources -SRA toolkit; Aspera connect. Sequence quality measures –Phred quality score. Quality check –tool –FASTQC, Pre-processing: Trimmomatic, Fastx-toolkit.

Course Outcomes:

1. Understand the different platforms for next generation sequencing.
2. Understand the types of sequencing methods used in different platforms

Reference Books:

1. Analytical Techniques In DNA Sequencing by Veena Kumari
2. Stuart M. Brown, “Next-Generation DNA Sequencing Informatics”, Cold Spring Harbor Laboratory Press, 2013.

Practicals

05 Hours

1. Demonstrate how to search for and retrieve NGS datasets from NCBI SRA using the SRA Toolkit command-line tools.
2. NGS data formats and data quality check (QC) FastQC;
3. GEO –Direct download, using SRA toolkit, using Aspera Connect
4. Demonstrate basic operations on FASTQ files, such as quality assessment, read trimming, and adapter removal using FastQC and Cutadapt.
5. Explore visualization tools (e.g., IGV, Bandage) to visualize subreads and nanopore data.
6. Introduce single-cell sequencing data formats and applications.
7. Demonstrate tools like Cell Ranger or Scanpy for preprocessing and analyzing single-cell data.
8. Demonstrate how to use Trimmomatic for adapter removal, quality trimming, and read length filtering.
9. to perform quality assessment using FastQC and pre-processing using Trimmomatic or Fastx-toolkit.

10. Study Aspera Connect for faster and more efficient data transfer from SRA.

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2	Ms.S.N.Sanglikar	Member	Y.C.I.S,Satara
3	Mr.P.M.Bhosale	Member	Y.C.I.S,Satara
3	Dr.Rahul Jamdade	Academic Expert	Y.C. College Karad
4	Mr.BajarangKumbhar	Industrial Expert	