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Research infrastructure

Basics of Sequencing Technologies

PřF:E4014 Projekt z Matematické biologie a biomedicíny - biomedicínská bioinformatika
FI:IV110 Project in Sequence Analysis
FI:IV114 Projekt z bioinformatiky a systémové biologie

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Sequencing

DNA Sequencing

DNA sequencing is the process of determining the nucleic acid sequence – the order of nucleotides in DNA.

Examples

Question: What’s it good for?

Sequencing Technology

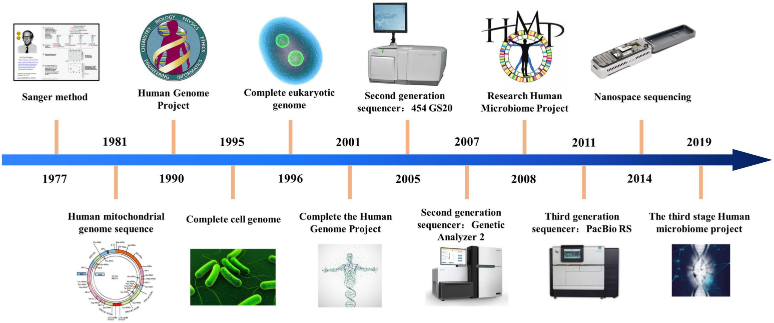

Sanger method
Human Genome Project
Complete eukaryotic genome
Second generation sequencer： 454 GS20
Research Human Microbiome Project
Nanospace sequencing
1981
1995
2001
2007
2011
2019
1977
1990
1996
2005
2008
2014
Human mitochondrial genome sequence
Complete cell genome
Complete the Human Genome Project
Second generation sequencer： Genetic Analyzer 2
Third generation sequencer： PacBio RS
The third stage Human microbiome project
HMP

Figure: History of sequencing technology[1]

Illumina sequencing

- NextGeneration sequencing technology
- Sequencing by synthesis
- Utilizing PCR
- Widely used

Principle

`https://youtu.be/fCd6B5HRaZ8?si=0Np6Q6pX4236HnvN`

Oxford Nanopore

- Third generation of sequencing technology
- Single molecule
- Long reads
- Real time

Principle

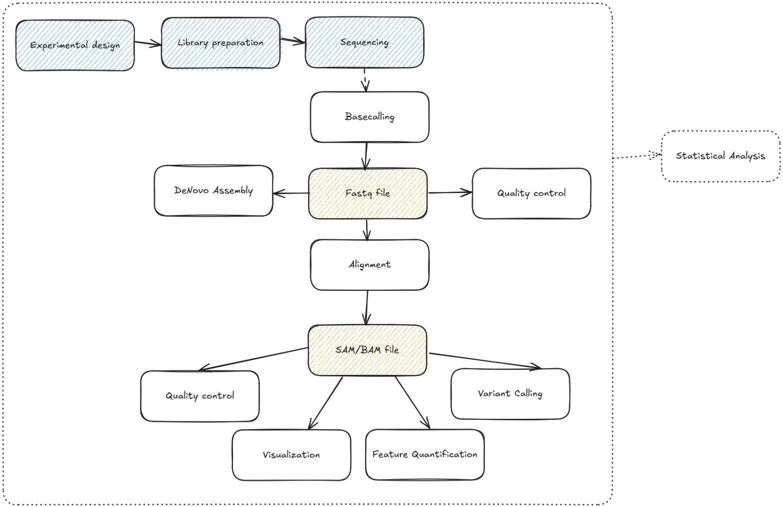
`https://youtu.be/RcP85JHLmnI?si=k732mK9liWV3gw5d`

Comparison

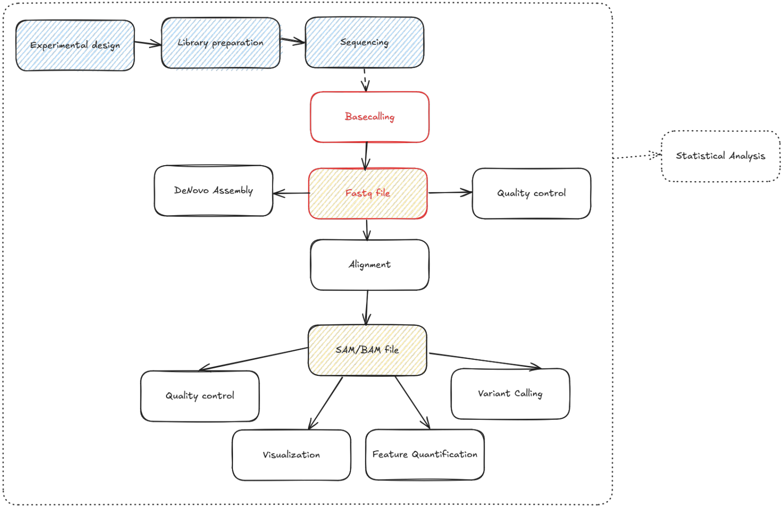
	Illumina	Oxford Nanopore
Read length	< 600 bp	< 2 Mbp
Accuracy	99 %	87-98 %
Price per Gbp	$ 40-60 (NextSeq) $ 10-35 (NovaSeq)	$ 50-200 (minION) $ 20-40 (PromethION)
Real-time		✓
Epigenomics	(Special chemistry)	✓

Table: Comparison of technologies

General workflow



Basecalling



Basecalling

Definition

Basecalling is the process of converting raw sequencing signals into a nucleotide sequence (A, T, C, G).

Examples

Question: How is basecalling done for Illumina and Oxford Nanopore?

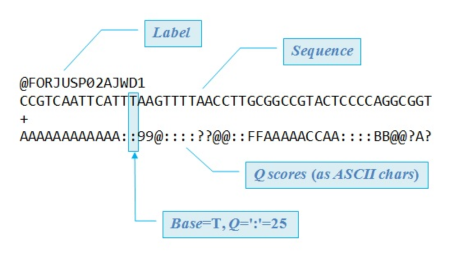
Fastq format

The FASTQ format is a text-based file format used to store both the raw sequence data and the corresponding quality scores from sequencing. Each entry consists of four lines:

1. Sequence identifier starting with @.
2. Raw nucleotide sequence (A, T, C, G).
3. + symbol, sometimes followed by the same identifier.
4. PHRED quality scores encoded as ASCII characters corresponding to each nucleotide in the sequence.

Fastq format

Examples

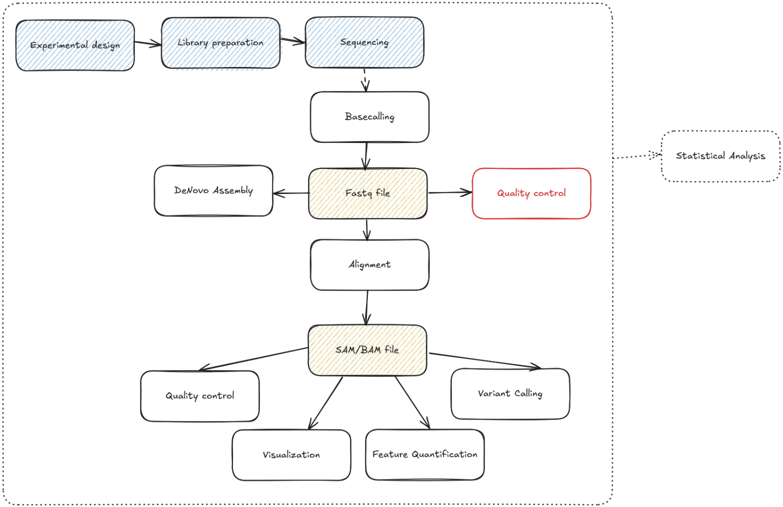


```
@FORJUSP02AJWD1
CCGTCAATTCATTTAAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGT
+
AAAAAAAAAAAA::99@::::??@@::FFAAAAACCAA::::BB@@?A?
```

PHRED Score

The PHRED score is a quality score that indicates the accuracy of a nucleotide base call in DNA sequencing, with higher scores representing higher confidence and lower error probabilities.

Quality control



Quality control

- Describe the quality of sequencing data
- Set parameters of preprocessing (Trimming & Filtering)

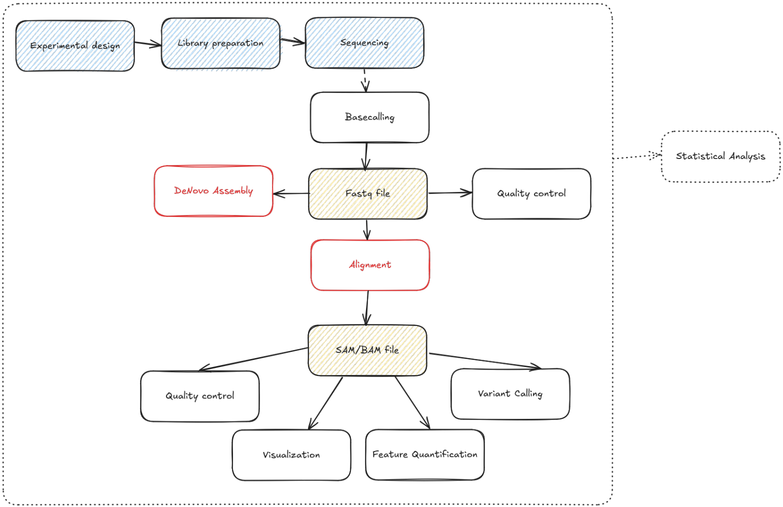
Examples

Question: What quality parameters to assess?

Examples

- Fastqc
- Nanoplot
- Fastp

Assembly & Alignment



Assembly & Alignment

DeNovo Assembly

De novo assembly is the process of constructing a genome sequence from short DNA fragments without the use of a reference genome, by assembling overlapping reads into longer contiguous sequences (**contigs**).

Alignment

Mapping is the process of aligning sequencing reads to a **reference genome** to determine the origin of each read and identify variations or similarities.

Examples

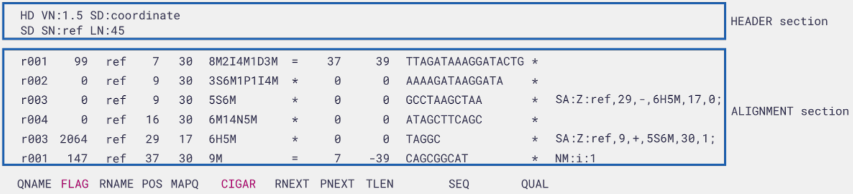
- DeNovo: SPADes
- Mappers: Bowtie2, BWA
- RNA Mappers: STAR (splice-aware mapping)

SAM/BAM format

SAM/BAM format

SAM (Sequence Alignment/Map) and BAM (Binary Alignment/Map) are file formats used to store aligned sequencing reads. Both include information about the read sequences, their alignment positions, mapping quality, and optional metadata.

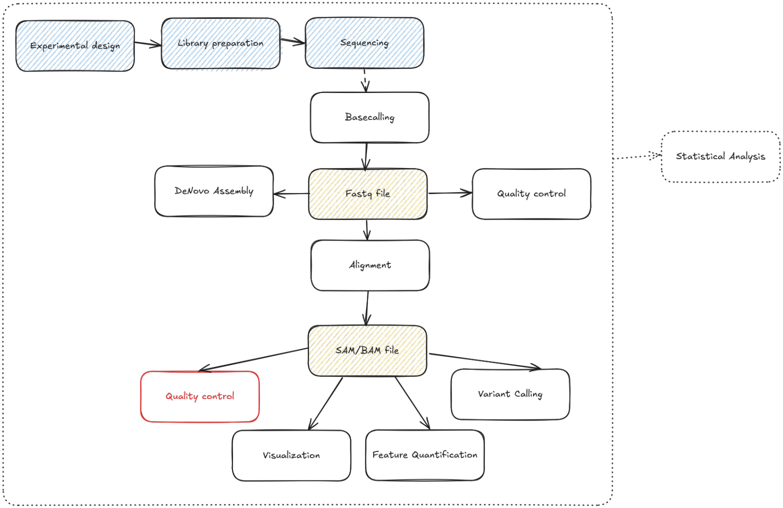
Examples



```
HD VN:1.5 SD:coordinate
SD SN:ref LN:45

r001    99  ref   7  30  8M2I4M1D3M  =   37   39  TTAGATAAAGGATACTG *
r002     0  ref   9  30  3S6M1P1I4M  *    0    0  AAAAGATAAGGATA    *
r003     0  ref   9  30  5S6M        *    0    0  GCCTAAGCTAA       *  SA:Z:ref,29,-,6H5M,17,0;
r004     0  ref  16  30  6M14N5M     *    0    0  ATAGCTTCAGC       *
r003  2064  ref  29  17  6H5M        *    0    0  TAGGC             *  SA:Z:ref,9,+,5S6M,30,1;
r001   147  ref  37  30  9M          =    7  -39  CAGCGGCAT         *  NM:i:1
```

Alignment QC



Alignment QC

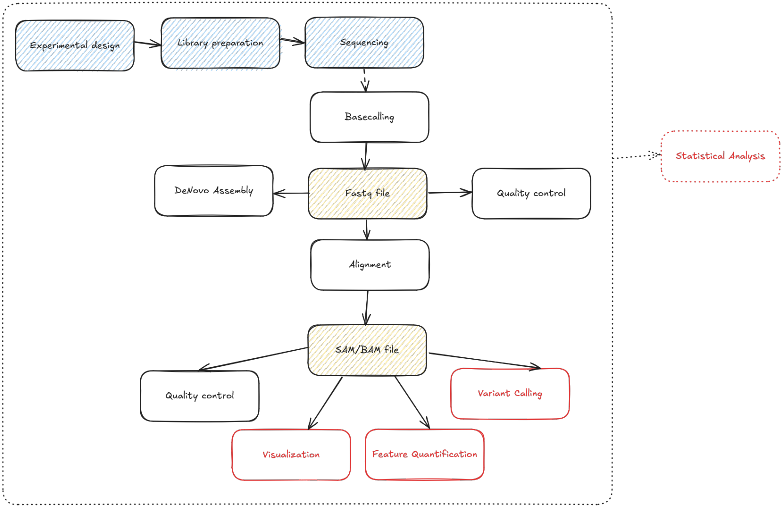
Examples

Question: What parameters to collect?

Examples

- Samtools
- QualiMap
- Picard tools

Postprocessing



Postprocessing

Depends on type of the experiment, quality of data, study design, hypotheses, ...

Visualization

Integrated Genome Browser (IGV)

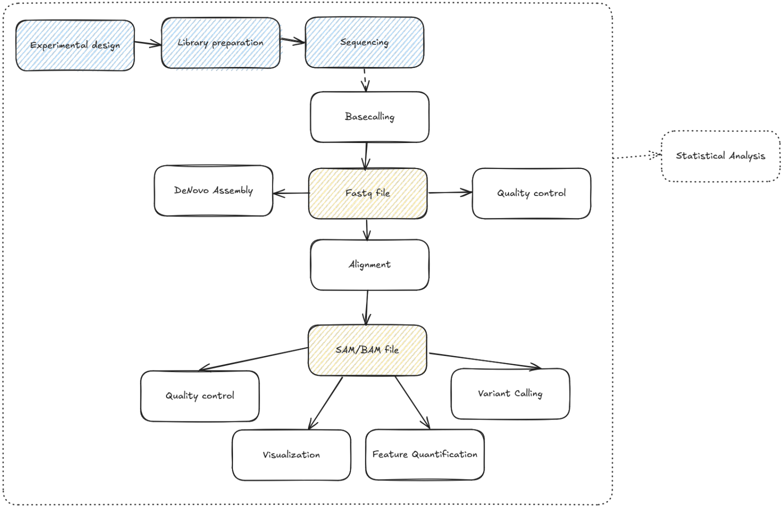
Feature Quantification

RNA-sequencing (genes), Metagenomics (bacteria)

Variant calling

Mutations, SNP, CNV

Summary


Experimental design
Library preparation
Sequencing
Basecalling
Statistical Analysis
DeNovo Assembly
Fastq file
Quality control
Alignment
SAM/BAM file
Quality control
Variant Calling
Visualization
Feature Quantification

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