LEIDEN UNIVERSITY MEDICAL CENTER

Bioinformatics practices

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Introduction

General overview: from sample to data.

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First stage:

- Research question.
- Make a plan.
- Make agreements.

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- The lab work.

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Second stage:

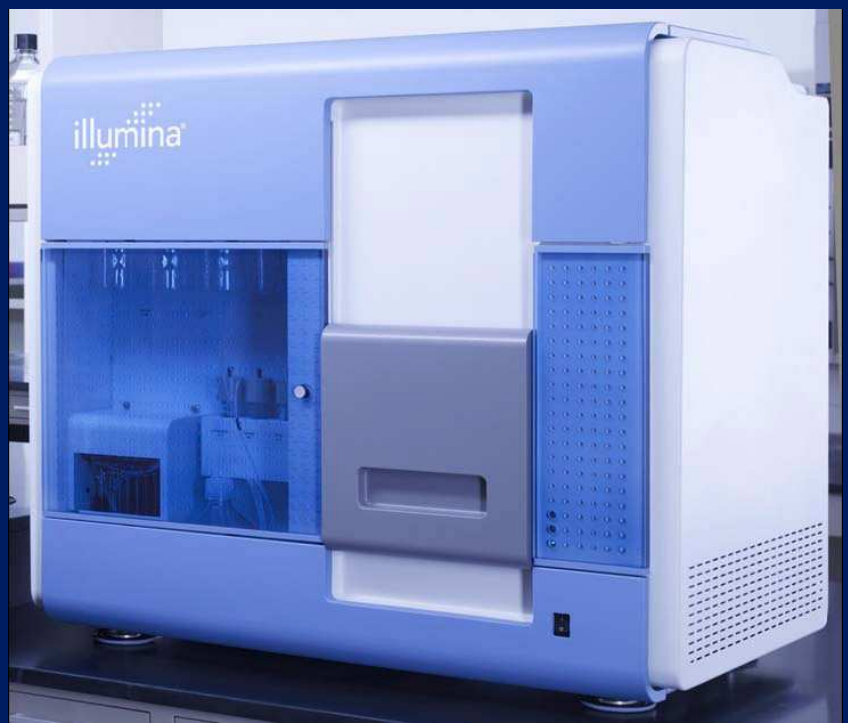
- The lab work.

Third stage (optional):

- Data analysis.

Introduction

The Illumina Genome Analyser II.



Strong points:

- Read length: $\pm 100 \times 2$.
- Runtime: 8 days.
- Output: ± 40 Giga bases.

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The Illumina HiSeq 2000.

Strong points:

- Read length: $\pm 150 \times 2$.
- Runtime: 8 days.
- Output: ± 150 Giga bases.

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The Roche / 454.

Strong points:

- Read length: ±500.
- Forensic science (STR markers).

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But:

- Difficulties with mono nucleotide stretches.
- Low yield.

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But:

- Read length: ± 32.
- 5% “dark nucleotide” rate.

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The Ion Torrent.

Strong points:

- Read length: ±350.
- Runtime: 2 hours.
- Able to pool over 100 amplicons in one run.

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Strong points:

- Read length: ±350.
- Runtime: 2 hours.
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- Difficulties with mono nucleotide stretches.
- Extremely low yield.

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The PacBio.

Strong points:

- Read length: up to 10 kilobases.
- True single molecule sequencing.
- Circular DNA sequencing.
- Strobe sequencing.

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- Read length: up to 10 kilobases.
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But:

- Extremely high error rate.
- Dark nucleotides.

First stage

The requirements and feasibility:

- Research question.
- Discuss the experiment.
- Discuss the technical possibilities.
- Budget.
- Discuss data analysis.

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- Discuss analysis in general.
- Make sure that the boundaries are clear.
- Preliminary decision about who will do the work.

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Make a tender offer.

Second stage

Planning the sequencing run.

- Samples arrive (guaranteed by customer).
- Data into LIMS.
  - Customer.
  - Date.
  - Type of experiment.

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Planning the sequencing run.

- Samples arrive (guaranteed by customer).
- Data into LIMS.
  - Customer.
  - Date.
  - Type of experiment.
- Sample preparation.
- Sequencing.
- Updating LIMS.
  - Flowcell id / lane number / barcode.
  - Type: paired end / single end.

Third stage

This is optional, mainly depending on the expertise of the client.

- A second discussion.
  - Details.
    - Which tools, how do they work.
    - Explain the report they will receive.
    - Answer any other questions.

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  - What will be delivered.
  - When will the analysis be done.

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Wait until we receive written confirmation before proceeding.

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Specialised analysis:

- Hand work.
    - But not less structured.

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Emphasis on quality control and information disclosure.

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In principle, we do not do any biological interpretation, but if we get a suggestion that helps clients guide them in their interpretation, we incorporate it.

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  - Stand alone scripts written in other languages (Perl, Python, . . .).

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- Database to keep track of the versions of all used tools and custom scripts.
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  - The version number of the pipeline is incremented.
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- All individual commands are logged.

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Tip:

- Some terminals (e.g., xterm) have a log functionality.

Questions?

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