



LEIDEN UNIVERSITY MEDICAL CENTER

# Bioinformatics practices

**Jeroen F. J. Laros**

**Leiden Genome Technology Center**

**Department of Human Genetics**

**Center for Human and Clinical Genetics**



General overview: from sample to data.

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First stage:

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- Make a plan.
- Make agreements.

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Second stage:

- The lab work.

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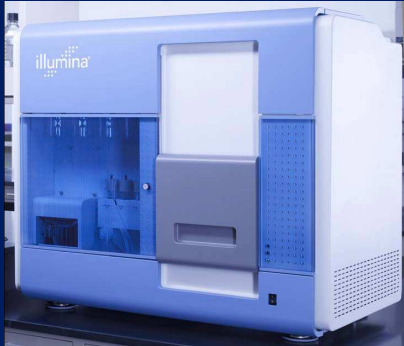
Second stage:

- The lab work.

Third stage (optional):

- Data analysis.

## The Illumina Genome Analyser II.



### Strong points:

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

## The Illumina HiSeq 2000.



### Strong points:

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

## The Roche / 454.



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### But:

- Read length:  $\pm 32$ .
- 5% “dark nucleotide” rate.

## The Ion Torrent.



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- Read length:  $\pm 350$ .
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- Difficulties with mono nucleotide stretches.
- Extremely low yield.

## The PacBio.



### Strong points:

- Read length: up to 10 kilobases.
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- Strobe sequencing.

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### But:

- Extremely high error rate.
- Dark nucleotides.

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.

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### Planning the sequencing run.

- Samples arrive (guaranteed by customer).
- Data into LIMS.
  - Customer.
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- Samples arrive (guaranteed by customer).
- Data into LIMS.
  - Customer.
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  - 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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Wait until we receive written confirmation before proceeding.

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SAGE/CAGE RNASeq:

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Specialised analysis:

- Hand work.
  - But not less structured.

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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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- Database to keep track of the versions of all used tools and custom scripts.
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- All individual commands are logged.

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When doing custom analysis, we are usually experimenting ourselves.

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Tip:

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

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