Module 1
Sequencing platforms and WGS terminology

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Overview

- Brief history of sequencing
- Sequencing platforms
  - Short reads
  - Long reads
- Pro's and con's
- Sequencing parameters for quality control

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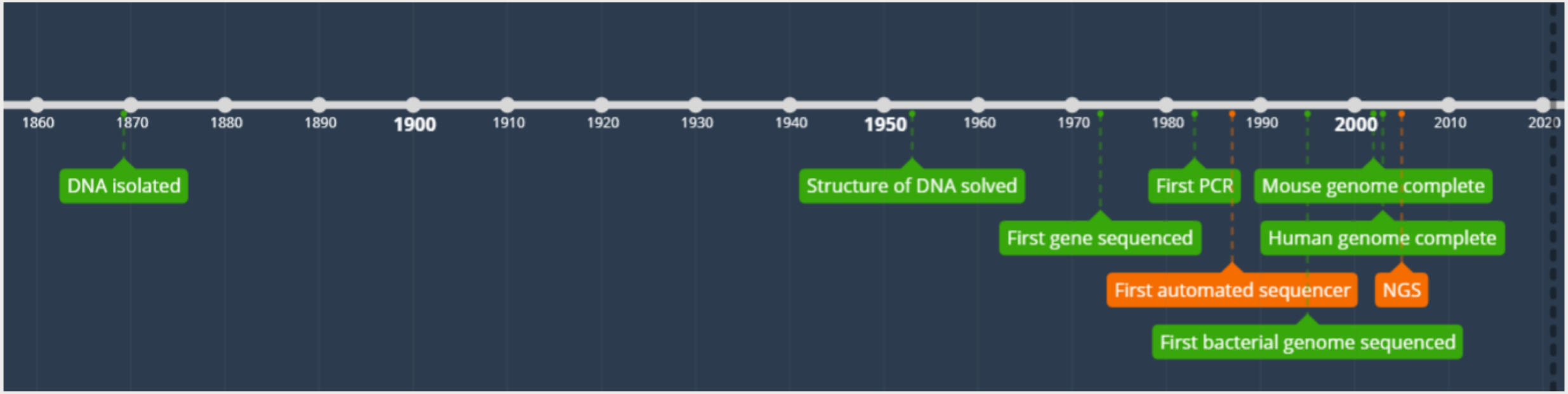
Brief history of sequencing

- First DNA isolation in 1869!
- The structure of DNA solved in 1953
- The first complete gene was sequenced in 1972



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Second (next) generation sequencing

- Possible to sequence an entire genome at once
- General process is:
  - Fragmenting the genome into many, smaller pieces
  - Randomly sampling for a fragment
  - Sequencing the fragment using one of many possible technologies

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Sequencing platforms

- **Short read technologies** (50 - 300 bases)
  - Illumina (MiSeq, HiSeq etc.)
  - 454
  - Ion Torrent

- **Long read technologies**
  - Pacific Biosciences (PacBio) (~20 kb)
  - Oxford Nanopore Technologies (MinION) (up to 200 kb)

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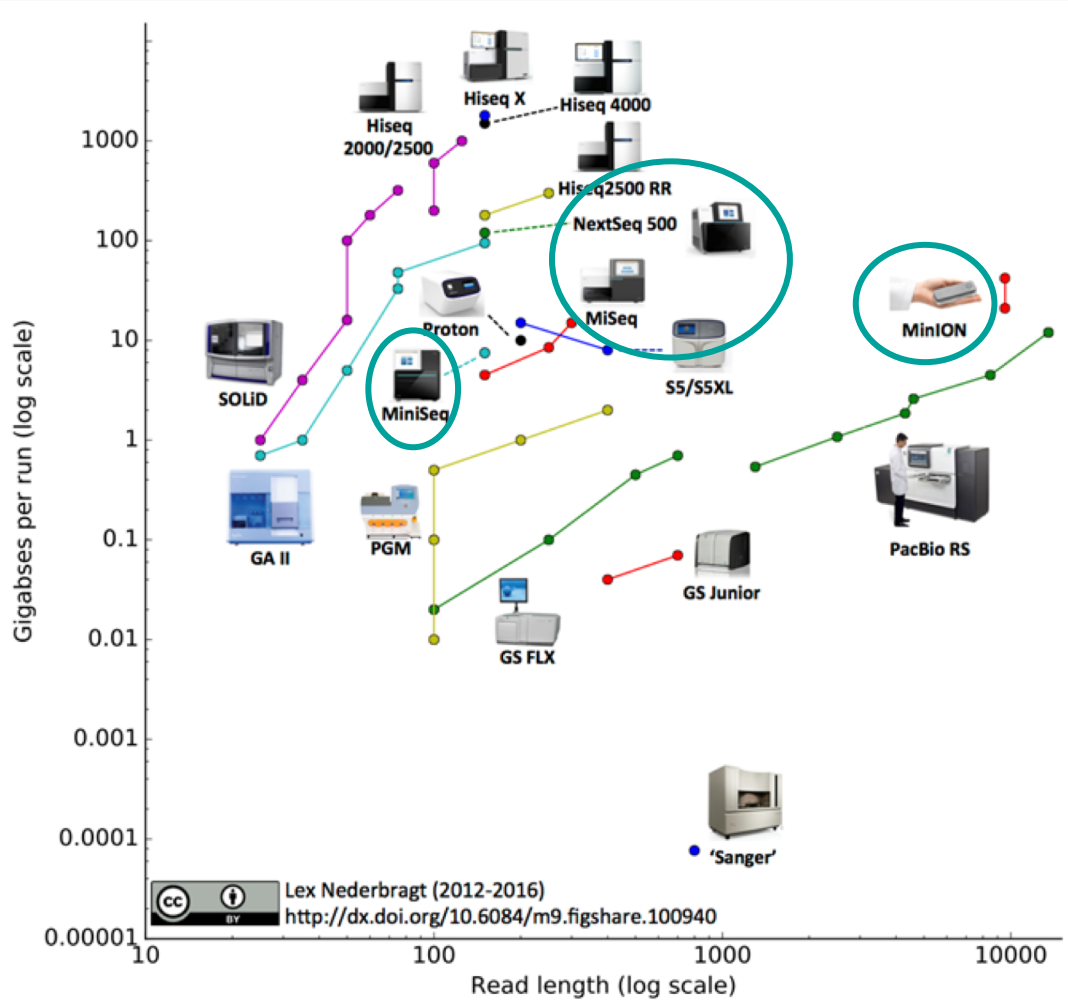
Sequencing platforms II



Some characteristics to consider:

Throughput/capacity

Run time

Instrumentation and service costs

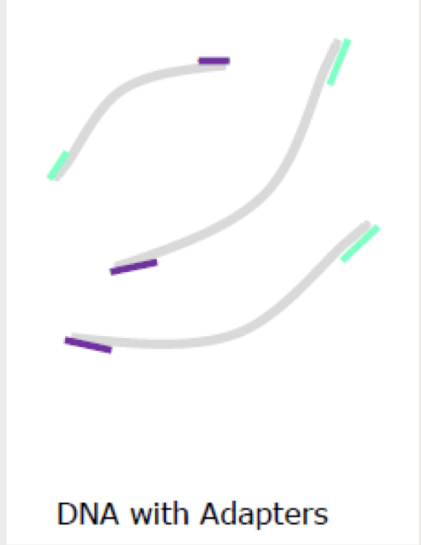
Reagent costs and availability

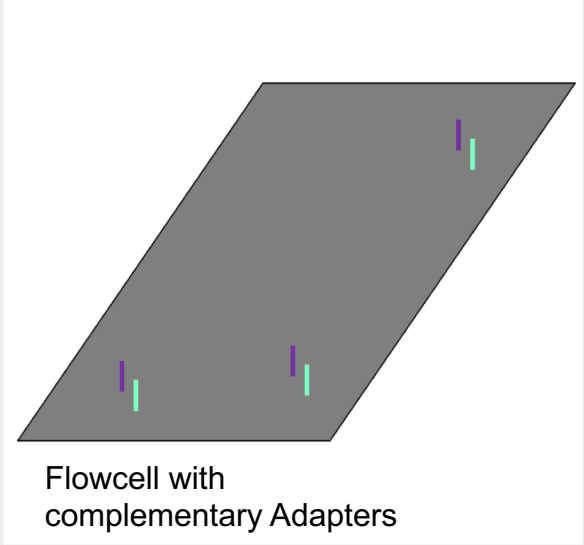
Infrastructure

Training of staff

Short read sequencers (Illumina)

- How does Illumina sequencing work?
  - Sequencing by synthesis





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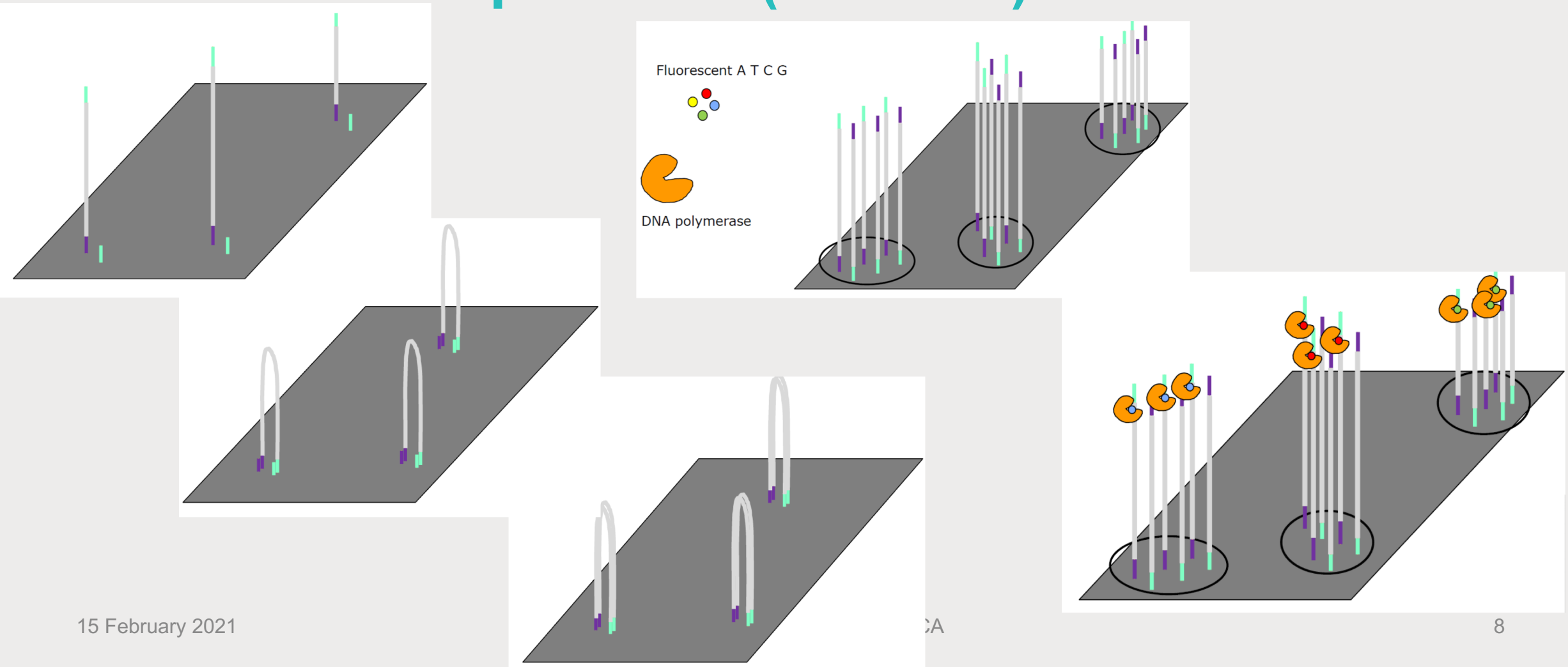
Short read sequencers (Illumina)



Different Illumina's

- HiSeq, NextSeq, MiSeq, …
- General chemistry is the same
- HiSeq gives more reads, takes more time and costs more
- MiSeq is faster, cheaper but gives less reads
- NextSeq uses two-dye system (faster cycle times and less expensive platform (than HiSeq))

Comparison of short reads technologies

Illumina
- Good accuracy
- Error rate ~0.1%
- Some underrepresentation in AT and GC rich regions
- High throughput

454
- Longer reads (up to 700 bp.)
- Longer insert size
- High cost per mega base
- Error rate ~1%
- Issues with homopolymers
- Discontinued

Ion Torrent
- Fastest runtime and work-flow
- More hands on time
- Error rate ~1%
- Issues with homopolymers

Third Generation Sequencing

- Long reads
- Single-molecule
- Real-time
- Platforms:
  - Pacific Biosciences (PacBio)
  - Oxford Nanopore Technologies (MinIon)

Comparison

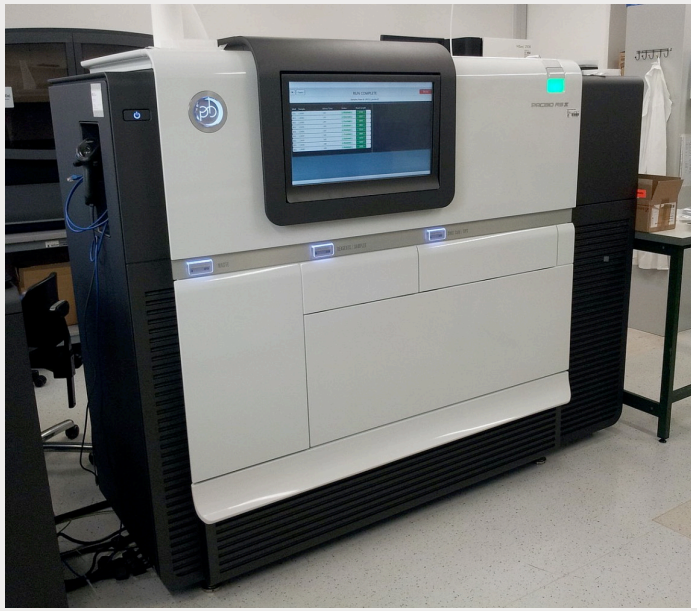
- Illumina
  - Short reads
  - High accuracy

Price:

- MiSeq: low instrument cost, higher cost per Gb data
- NextSeq: medium high instrument cost, lower cost per Gb
- HiSeq: high instrument cost, low cost per Gb data

Comparison (II)

- PacBio
- Long reads
- High error rates
- Price:
  - medium high instrument cost
  - very high cost per Gb data



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Comparison (III)

- Nanopore
  - Very long reads
  - High error rates
  - Portable
- Price:
  - Very low instrument cost
  - High cost per Gb data

MinIon

GridIon

Comparison of long reads technologies

Pacific Biosciences

- Long reads. (Max: 50 kb. Avg.: 10-15 kb)
- Error rate ~15% (single pass)
- Precision can be improved to 99.999%
- Low throughput
- Expensive (acquisition cost)

Oxford Nanopore

- Very long reads (up to 900 kb.)
- Large error rates (3-8%)
- Portable

Still some limitations in sequence processing by tools available

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Sequencing output

What is fastq?
Fasta + quality scores

Raw data – fastq files 1 read: 4 lines

```
@FCC0CD5ACXX:1:1101:1103:2048#ACCGT/1
ACNGTGTTTTTAGTTATTGTTTTGTTAAGTTGGGTTTTTTGTACCCAATAGCCAACAAGCCGCCTTTATGGCGGTTTTTTTGTGCCTGAAAAGTGGGCG
+
 BP`ccceggcegihiiighiifhihfddgfhi^efgfhhhhhegiiiiiiiiihiihihggeeccdddcccacWTT^acc[ab `]`[ b`^BBBBBBB
@FCC0CD5ACXX:1:1101:1165:2058#ACGTT/1
ACGTTAGCAGAATCGCTTTCTGTTCGTTTTCCACCTGCGACAGACGCACCGGACCACGGTTGGCGAGATCGTCGCGCAGAATATCGGCGGCACGCTGCG
+
bb_eeceefeggehhdagfghhiihfghighhffhifhhcghfdhiihafgdceba`a\aaccc^V]^baccaccXaaX^bbcccaac[_X]]a[aacX
@FCC0CD5ACXX:1:1101:1135:2082#AGCGT/1
AGCGTGACAAACATTTTATTGCGCCCGGTTTTATCCAGCTTGAATGCCTGACGAAAGAAGATGATGGTGACGACGATGGAGAGAACAATCAGCACCAGA
+
bbbeeeeefggfgiihgiigiiiiiiiffgifgeghiiihhfefffhhhfgh_fhggdgegeaceeacbdcbcc\^aa]``_^bb]bcccccbac_a^b
```

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Sequencing output

What is fastq?
Fasta + quality scores

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```
@FCC0CD5ACXX:1:1101:1103:2048#ACCGT/1
ACNGTGTTTTTAGTTATTGTTTTGTTAAGTTGGGTTTTTTGTACCCAATAGCCAACAAGCCGCCTTTATGGCGGTTTTTTTGTGCCTGAAAAGTGGGCG
+
_BP`cccegggegihiiighiifhihfddgfhi^efgfhhhhhegiiiiiiiihiihihggeeccdddcccacWTT^acc[ab_`]`[_b`^BBBBBBB
@FCC0CD5ACXX:1:1101:1165:2058#ACGTT/1
ACGTTAGCAGAATCGCTTTCTGTTCGTTTTCCACCTGCGACAGACGCACCGGACCACGGTTGGCGAGATCGTCGCGCAGAATATCGGCGGCACGCTGCG
+
bb_eeceefeggehhdagfghhiihfghighhffhifhhcghfdhiihafgdceba`a\aaccc^V]^baccaccXaaX^bbcccaac[_X]]a[aacX
@FCC0CD5ACXX:1:1101:1135:2082#AGCGT/1
AGCGTGACAAACATTTTATTGCGCCCGGTTTTATCCAGCTTGAATGCCTGACGAAAGAAGATGATGGTGACGACGATGGAGAGAACAATCAGCACCAGA
+
bbbeeeeefggfgiihgiigiiiiiiiffgifgeghiiihhfefffhhhfgh_fhggdgegeaceeacbdcbcc\^aa]``_^bb]bcccccbac_a^b
```

Header/ID

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Sequencing output

What is fastq?
Fasta + quality scores

Raw data – fastq files 1 read: 4 lines

DNA sequence

```
@FCC0CD5ACXX:1:1101:1103:2048#ACCGT/1
ACNGTGTTTTTAGTTATTGTTTTGTTAAGTTGGGTTTTTTGTACCCAATAGCCAACAAGCCGCCTTTATGGCGGTTTTTTTGTGCCTGAAAAGTGGGCG
+
_BP`ccceggcegihiiighiifhihfddgfhi^efgfhhhhhegiiiiiiiihiihihggeeccdddcccacWTT^acc[ab_`]`[_b`^BBBBBBB
@FCC0CD5ACXX:1:1101:1165:2058#ACGTT/1
ACGTTAGCAGAATCGCTTTCTGTTCGTTTTCCACCTGCGACAGACGCACCGGACCACGGTTGGCGAGATCGTCGCGCAGAATATCGGCGGCACGCTGCG
+
bb_eeceefeggehhdagfghhiihfghighhffhifhhcghfdhiihafgdceba`a\aaccc^V]^baccaccXaaX^bbcccaac[_X]]a[aacX
@FCC0CD5ACXX:1:1101:1135:2082#AGCGT/1
AGCGTGACAAACATTTTATTGCGCCCGGTTTTATCCAGCTTGAATGCCTGACGAAAGAAGATGATGGTGACGACGATGGAGAGAACAATCAGCACCAGA
+
bbbeeeeefggfgiihgiigiiiiiiiffgifgeghiiihhfefffhhhfgh_fhggdgegeaceeacbdcbcc\^aa]``_^bb]bcccccbac_a^b
```

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Sequencing output

What is fastq?
Fasta + quality scores

Raw data – fastq files 1 read: 4 lines

Name field (optional)

```
@FCC0CD5ACXX:1:1101:1103:2048#ACCGT/1
ACNGTGTTTTTAGTTATTGTTTTGTTAAGTTGGGTTTTTTGTACCCAATAGCCAACAAGCCGCCTTTATGGCGGTTTTTTTGTGCCTGAAAAGTGGGCG
+
_BP`ccceggcegihiiighiifhihfddgfhi^efgfhhhhhegiiiiiiiihiihihggeeccdddcccacWTT^acc[ab_`]`[_b`^BBBBBBB
@FCC0CD5ACXX:1:1101:1165:2058#ACGTT/1
ACGTTAGCAGAATCGCTTTCTGTTCGTTTTCCACCTGCGACAGACGCACCGGACCACGGTTGGCGAGATCGTCGCGCAGAATATCGGCGGCACGCTGCG
+
bb_eeceefeggehhdagfghhiihfghighhffhifhhcghfdhiihafgdceba`a\aaccc^V]^baccaccXaaX^bbcccaac[_X]]a[aacX
@FCC0CD5ACXX:1:1101:1135:2082#AGCGT/1
AGCGTGACAAACATTTTATTGCGCCCGGTTTTATCCAGCTTGAATGCCTGACGAAAGAAGATGATGGTGACGACGATGGAGAGAACAATCAGCACCAGA
+
bbbeeeeefggfgiihgiigiiiiiiiffgifgeghiiihhfefffhhhfgh_fhggdgegeaceeacbdcbcc\^aa]``_^bb]bcccccbac_a^b
```

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Sequencing output

What is fastq?
Fasta + quality scores

Raw data – fastq files 1 read: 4 lines

Quality scores

```
@FCC0CD5ACXX:1:1101:1103:2048#ACCGT/1
ACNGTGTTTTTAGTTATTGTTTTGTTAAGTTGGGTTTTTTGTACCCAATAGCCAACAAGCCGCCTTTATGGCGGTTTTTTTGTGCCTGAAAAGTGGGCG
+
_BP`ccceggcegihiiighiifhihfddgfhi^efgfhhhhhegiiiiiiiihiihihggeeccdddcccacWTT^acc[ab_`]`[_b`^BBBBBBB
@FCC0CD5ACXX:1:1101:1165:2058#ACGTT/1
ACGTTAGCAGAATCGCTTTCTGTTCGTTTTCCACCTGCGACAGACGCACCGGACCACGGTTGGCGAGATCGTCGCGCAGAATATCGGCGGCACGCTGCG
+
bb_eeceefeggehhdagfghhiihfghighhffhifhhcghfdhiihafgdceba`a\aaccc^V]^baccaccXaaX^bbcccaac[_X]]a[aacX
@FCC0CD5ACXX:1:1101:1135:2082#AGCGT/1
AGCGTGACAAACATTTTATTGCGCCCGGTTTTATCCAGCTTGAATGCCTGACGAAAGAAGATGATGGTGACGACGATGGAGAGAACAATCAGCACCAGA
+
bbbeeeeefggfgiihgiigiiiiiiiffgifgeghiiihhfefffhhhfgh_fhggdgegeaceeacbdcbcc\^aa]``_^bb]bcccccbac_a^b
```

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Post-sequencing steps

- Quality control
  - Trimming of adaptors and low quality reads
  - Error correction
- Assembly
- Validation
- Data analysis

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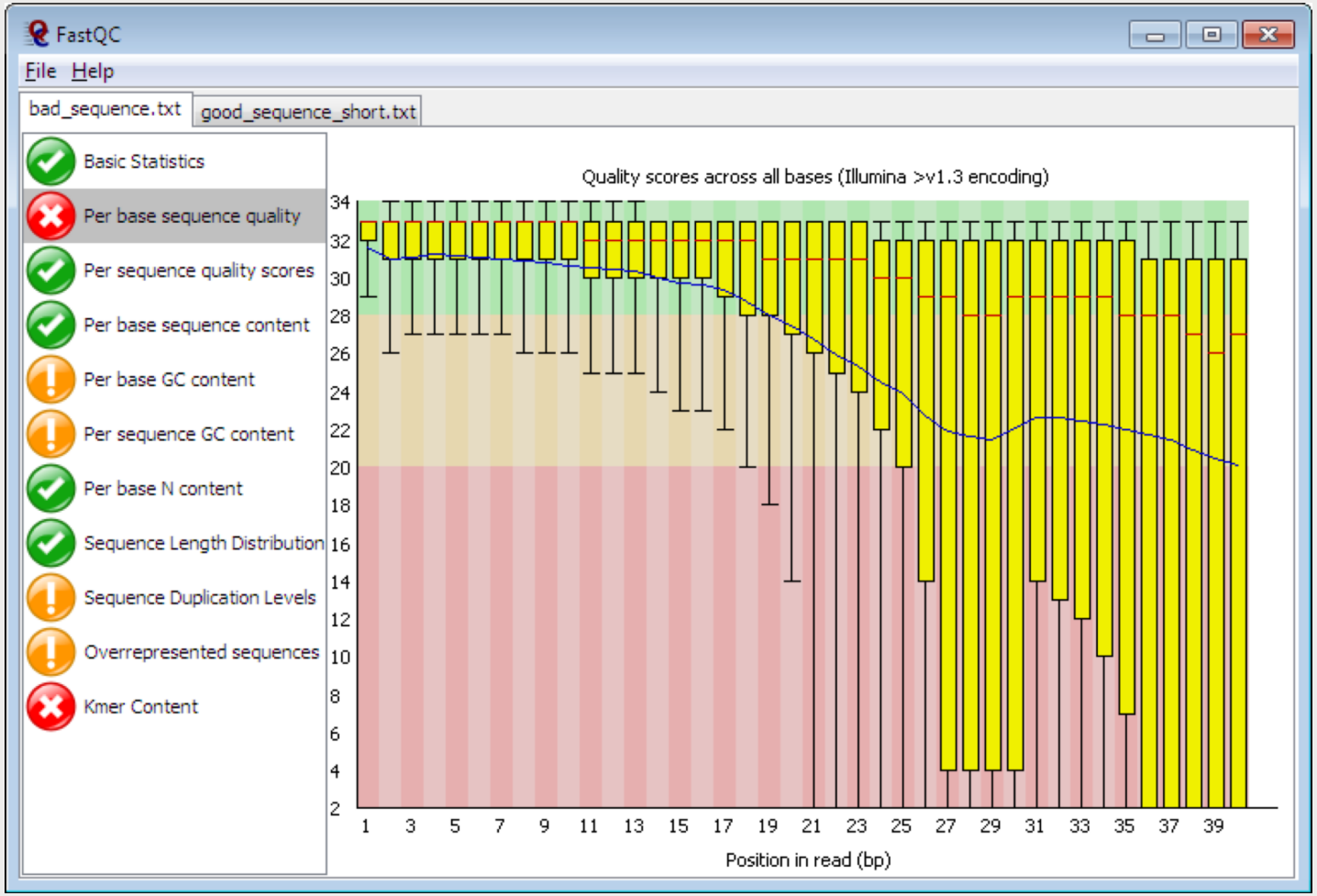
Quality control (QC)

- Different tools available
- QC on raw reads
  - % of quality bases
  - # of reads
- QC on assembly
  - QC parameters
    - Genome size
    - # of contigs
    - N50
    - Coverage



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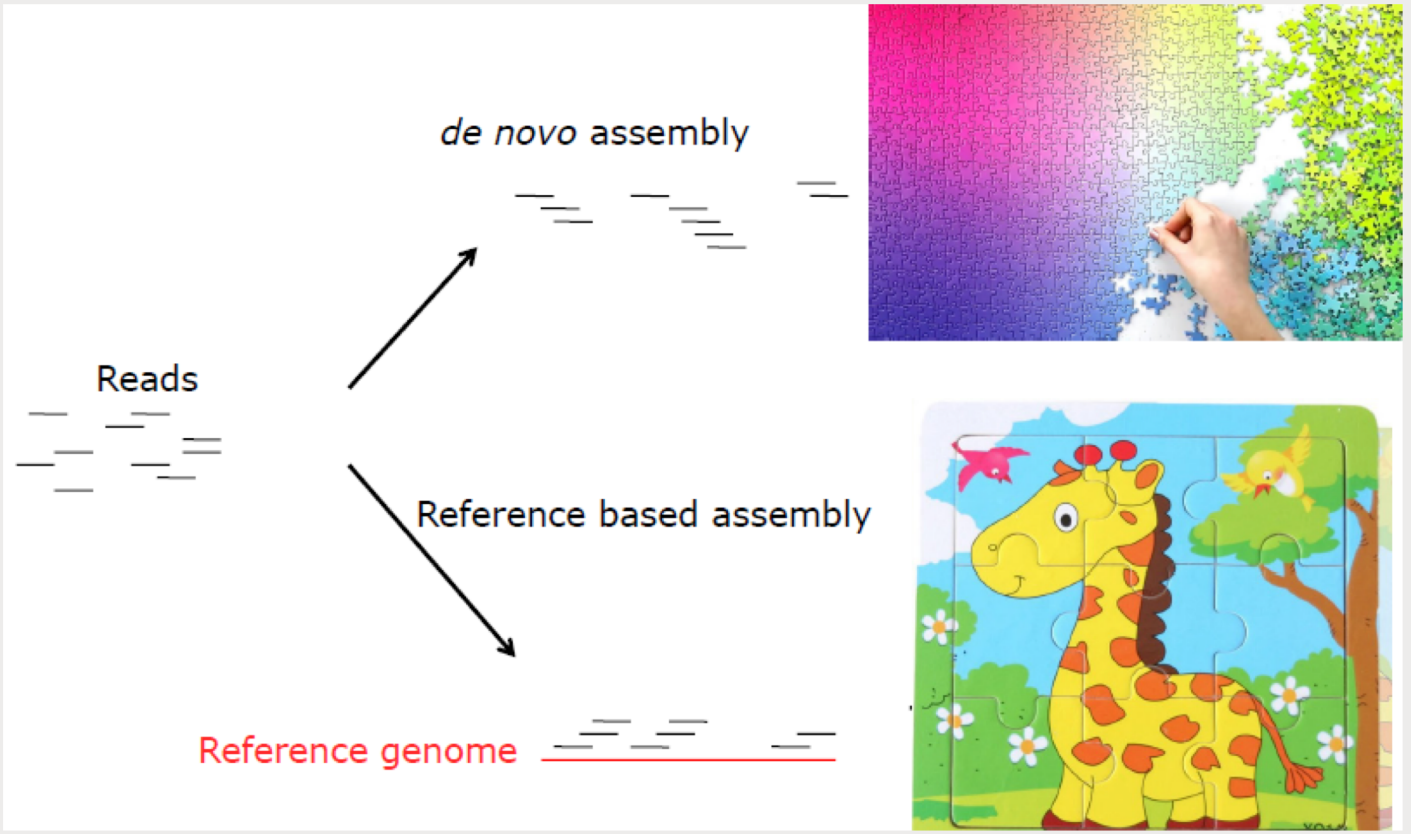
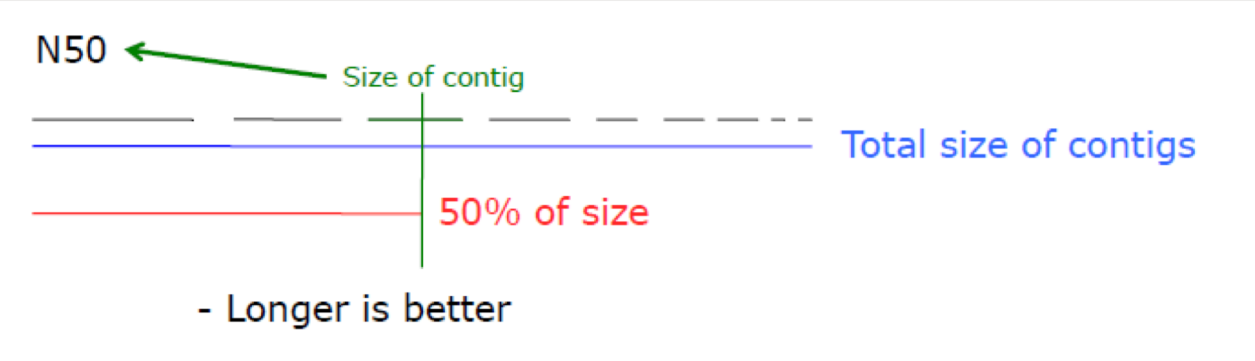
What is assembly?

- Assemble raw reads into larger stretches of DNA: contigs
  - Mapping to reference
  - *De novo assembly*



QC parameters- guidelines

- Number of contigs
  - < 500 contigs (the fewer, the better)
- Assembled genome size (Total size of contigs)
  - Close to expected size (+/- 20 %)
- N50 – higher is better (> 30.000)



QC parameters - guidelines

- Coverage
  - The number of times the genome is covered by the read data
  - Preferably > 20 X

$$C = N \cdot \frac{L}{G}$$

- N: Number of read
- L: Read length
- G: Genome size (target **or** assembly)

Example:
N = 5 mill
L = 100 bp
G = 5 Mbp

C = 5*100/5 = 100X

On average, 100 reads covers each position in the genome.

- Depth
  - The number of reads that covers a particular nucleotide in each position in the genome

$$\frac{\text{reads}}{\text{site}} = \text{depth}$$

The sequence equipment depend on your needs

- High variety in equipment
  - Throughput
  - Availability of reagents
  - Convenience in sequence analysis

Quality control is important!

- Ensure validity of your sequences
- Ensure adequate quality for further genome analysis
- Ensure comparability of results

QC
Genome
analysis

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Thank you

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