An introduction to get started in genome assembly and annotation

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Updated: PURL (Persistent URL): gxaid.org/GTN:S00034

Plain-text slides

Requirements

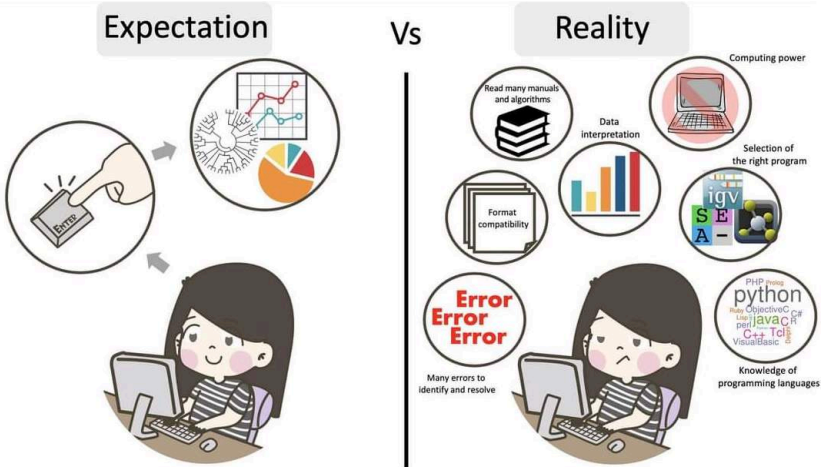
Before diving into this slide deck, we recommend you to have a look at:

- Introduction to Galaxy Analyses
- Sequence analysis
  - Quality Control: slides - hands-on

Questions

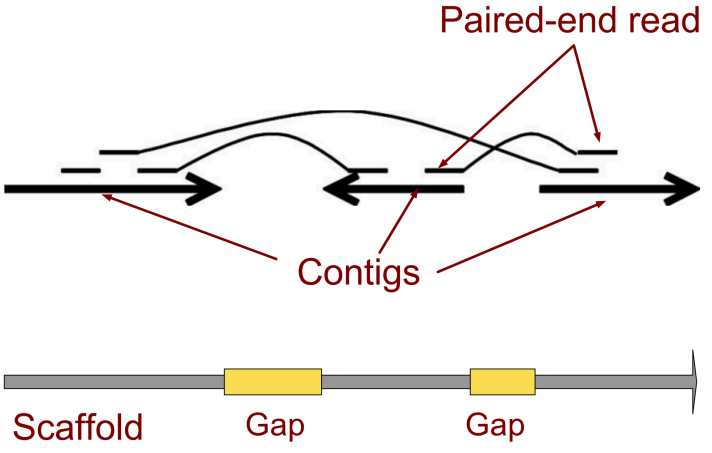
- Definitions of bioinformatics terms for assembly and annotation
- What are the guidelines before starting a Genome Assembly and Annotation project?
- What file formats are used for assembly and annotation?

The truth about bioinformatics

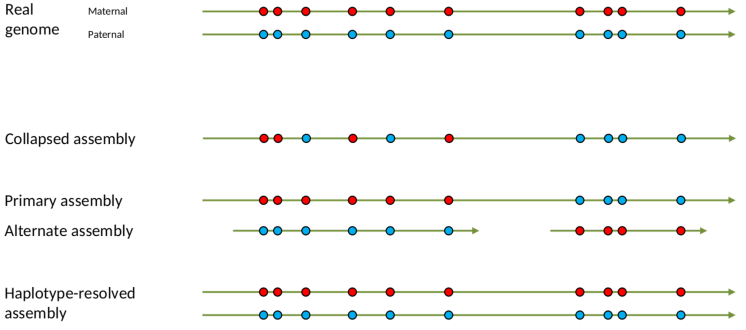


Let's start with some important definitions

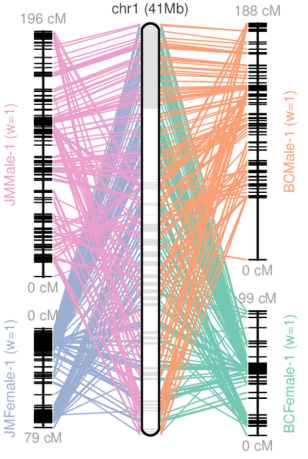
Contig: a contiguous sequence in an assembly. A contig does not contain long stretches of unknown sequences (aka assembly **gaps**).
Scaffold: a sequence consists of one or multiple contigs connected by assembly gaps of typically inexact sizes. A scaffold is also called a **supercontig**, though this terminology is rarely used nowadays.
Assembly: a set of contigs or scaffolds.



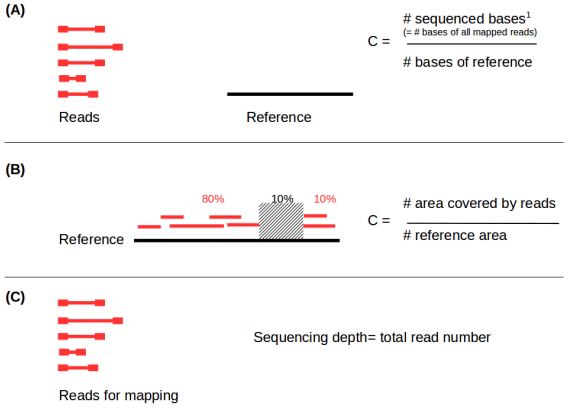
Haplotig: a contig that comes from the same haplotype. In an unphased assembly, a contig may join alleles from different parental haplotypes in a diploid or polyploid genome.
Primary assembly: a complete assembly with long stretches of phased blocks.
Alternate assembly: an incomplete assembly consisting of haplotigs in heterozygous regions. An alternate assembly always accompanies a primary assembly. It is not useful by itself as it is fragmented and incomplete.
Haplotype-resolved assembly: sets of complete assemblies consisting of haplotigs, representing an entire diploid/polyploid genome.



Telomere to telomere: An assembly where each chromosome is fully phased and assembled without gaps.
Linkage group: a set of contigs or scaffolds ordered and oriented using a collection of genes that are inferred to be located together on a single chromosome because of the pattern of their inheritance.

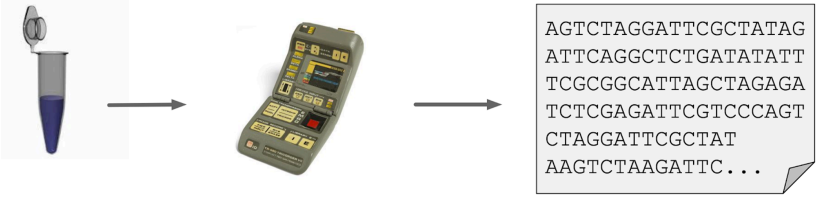


Coverage in terms of redundancy (A): number of reads that align to, or "cover," a known reference. It describes how often, in average, a reference sequence is covered by bases from the reads.
Coverage in terms of the percentage coverage of a reference by reads (B): E.g. if 90% of a reference is covered by reads (and 10% not) it is a 90% coverage.
Sequencing depth (C): total number of usable reads from the sequencing machine.



Assembly and annotation in a ideal world

In an ideal world ...

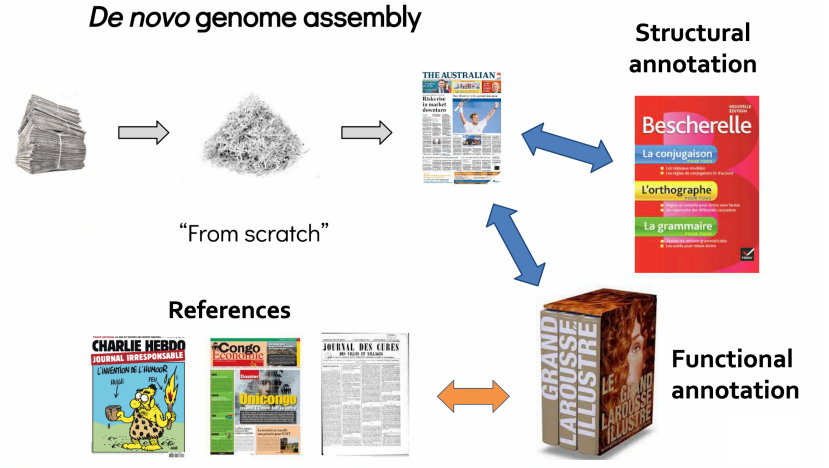


DNA

iSequencer ™

46 chromosomal
and
1 mitochondrial sequences

Key concepts for assembly and annotation



Steps before starting a genome project

- Step 1: Build a broad community of collaborators for the project, if possible
- Step 2: Gather information about the target genome
- Step 3: Select the best possible DNA source and an optimal extraction procedure
- Step 5: Choose an appropriate sequencing technology
- Step 6: Check the computational resources requirements and availability

Build a wide community for the project (*if it's possible*)

The aim of a genome project is to **sequence the entire target genome** for a wide **range of genomics applications**. **Analyses**, **reanalyses** and **integration** of genomic and other phenotype information are required:

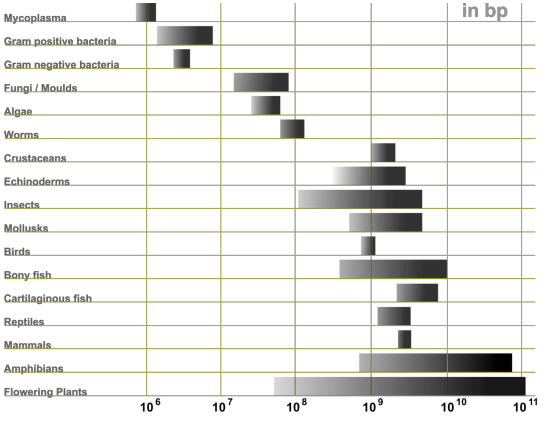
- **Facilities:** Wet lab, sequencing, bioinformatics,...
- **Personnel:** Highly skilled
- **Software:** Knowledge intensive

⚠ The cost of data storage, maintenance, transfer, and analysis are likely to be significant and will represent an increasing proportion of overall sequencing costs in the future.

Genome information: Genome size

How to collect informations?

- Experimentally : Flow cytometry
- Databases:
  - Fungi: http://www.zbi.ee/fungalgenomesize
  - Animals: http://www.genomesize.com
  - Plants: http://data.kew.org/cvalues
- Bibliography



https://commons.wikimedia.org/w/index.php?curid=19537795

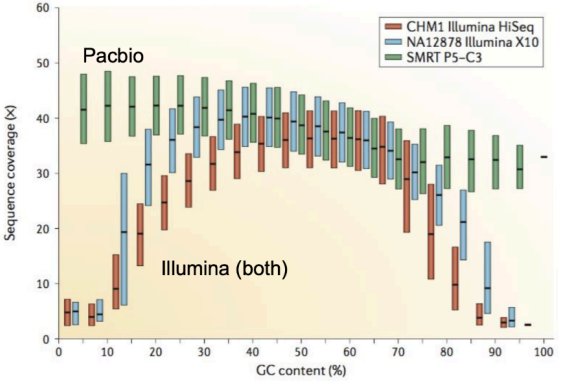
Genome information: GC content

Why?

Sequencing is not random! GC and AT rich regions are under-represented.

How to solve?

- Chemistry quirks
- Increase the sequencing depth
- Technologies combination (long and short reads)



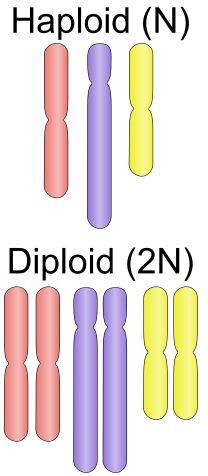
Chaisson et al. Genetic variation and the de novo assembly of human genomes. Nat Rev Genet 16, 627–640 (2015).

Genome information: Ploidy level

Ploidy (N):

Number of sets of chromosomes in a cell

Organism	**Ploidy**
Bacteria	1N
Human, mouse, rat	2N
Amphibians (*Xenopus*)	2N to 12N
Plants (wheat)	6N
Autopolyploid	.
Hybrids	.



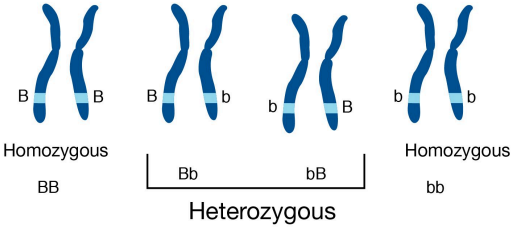
Higher ploidy -> harder to assemble => Increase of sequencing depth

Daniel Hartl. Essential Genetics: A Genomics Perspective. Jones & Bartlett Learning. p. 177. ISBN 978-0-7637-7364-9. (2011).

Genome information: Heterozygosity level

Heterozygous: Locus-specific, diploid (2N) organism has two different alleles of a particular gene at the same locus

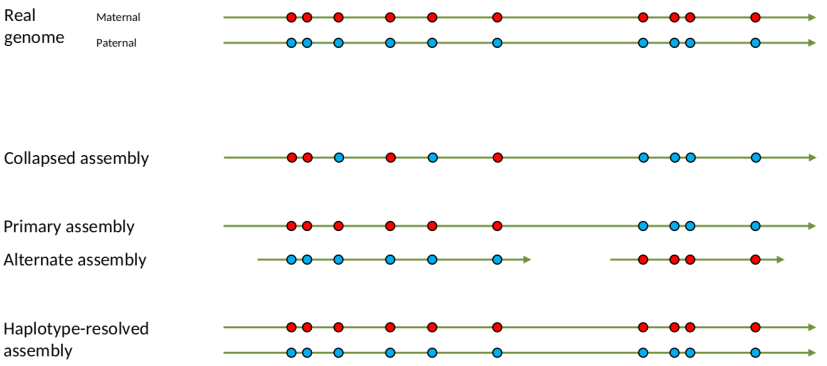
Heterozygosity is a metric used to indicate the probability that an individual is heterozygous for a particular allele



Higher heterozygosity -> harder to assemble => Increase of sequencing depth

https://www.genome.gov/genetics-glossary/heterozygous

Genome information: Heterozygosity level

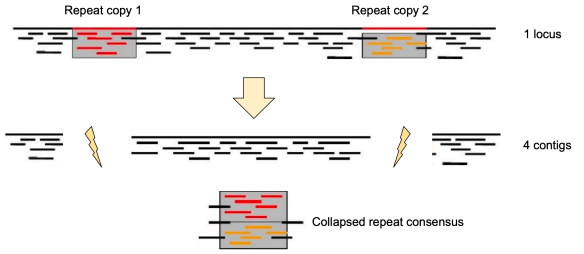


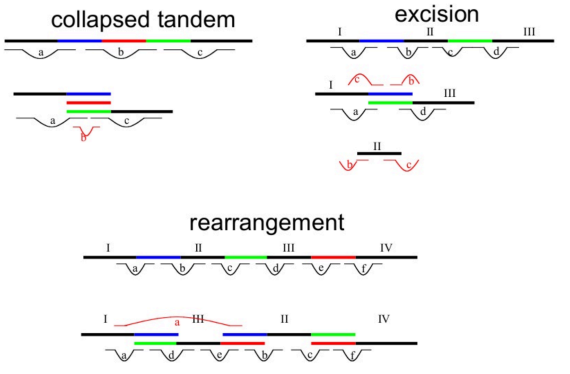
Heng Li's blog: lh3.github.io/2021/04/17/concepts-in-phased-assemblies

Genome information: Complexity aka repeats elements

It is impossible to resolve repeats of length L unless you have reads longer than L

Most common source of assembly errors:





Genome information: Others

- **Karyotype:** chromosome number
- **Sex chromosome system:** None, XY, ZW, UV,...
- **Purity:** possible presence of contaminants and/or symbionts?
- Is there any other **useful data (NCBI, SRA, ENA, etc)** that could improve my assembly?

Genome information: Tips

- **Flow cytometry** :
  - Genome size
  - Ploidy level
- **k-mer frequency from Illumina reads** :
  - Genome size
  - Ploidy level
  - GC content
  - Heterozygosity
  - Repeats composition

The best possible DNA

Select the best possible DNA source and extraction method. The extraction of **high-quality DNA** is the **most important** aspect of a successful genome project
The **lack of a good starting material** will limit the choice of sequencing technology and **affect the quality of data obtained**

The best possible DNA: Chemical purity of DNA

Sample-related contaminants:

- Polysaccharides
- Proteoglycans
- Proteins
- Secondary metabolites
- Polyphenols
- Humic acids
- Pigments
- Etc,...

All these contaminants **can affect the efficiency of library preparation**, regardless of the technology, and this is especially true **for PCR-free libraries** (PacBio and ONT)

The best possible DNA: Quantity of DNA

Different technologies require different amount of DNA:

- Illumina and 10x > 3 ng
- BioNano > 200 ng
- ONT > 1 µg
- Hi-C > 5 µg
- PacBio > 15 µg

The best possible DNA: Structural integrity of DNA

High Molecular Weight (**HMW**) for Nanopore/PacBio (obtained mainly from fresh material)

The best possible DNA: Tips

- Many **DNA extraction protocol** are available for a wide range of species/taxa (VGP, Darwin Tree of Life, Nanopore, PacBio, etc)
- **Keep DNA samples** from the same individual in case of library preparation or sequencing failure, need more coverage, new sequencing technology, etc
- Use a **single individual** and sequence a **haploid, a highly inbred diploid** organism, or an **isogenic** individual

Appropriate sequencing technology

This mainly **depends on the quantity and quality of DNA as well as the cost of the experiment** but many parameters need to be considered before performing an NGS experiment:

- Short **versus** long reads or **both**
- Read length
- Read quality/error rate
- Genome read coverage/depth
- Library preparation
- Downstream applications

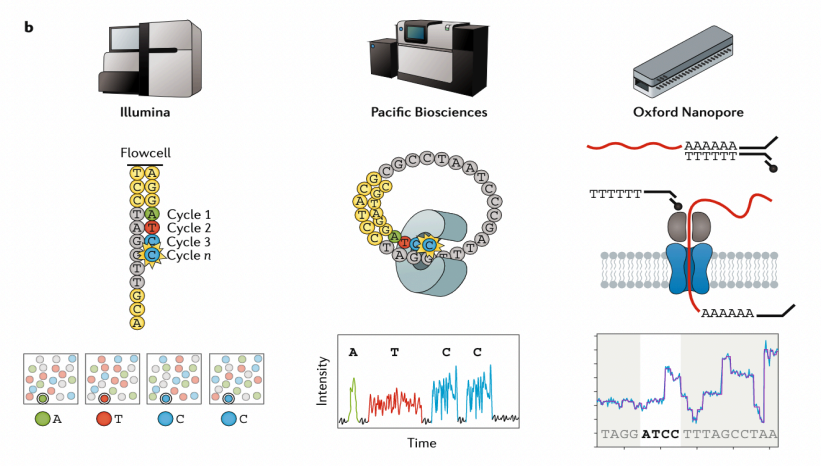
Appropriate sequencing technology: Assembly

- **Illumina or MGI:** short reads (up to 2x250bp) with high quality reads. Sequencing bias with AT/GC rich regions
- **IonTorrent:** short reads (up to 500bp) with medium quality reads
- **Nanopore:** long reads (average ~15kbp) with low quality reads. Errors are not randomly distributed!
- **PacBio:**
  - CLR: long reads (average ~20kbp) with low quality reads
  - HiFi: long reads (average ~15kbp) with high quality reads

Appropriate sequencing technology: Scaffolding

- **Hi-C: restriction enzyme fragmentation** (single, multiples sites or DNAse). Need huge amount of coverage.
  - Phase Genomics
  - Dovetail Genomics
  - Arima Genomics
- Optical mapping: technique to physically locate specific enzymes restriction sites or sequence motifs to produce DNA sequence fingerprints.
  - BioNano
  - BGI
- Mate pair (deprecated)
- BAC/YAC/Fosmids (deprecated)

Appropriate sequencing technology



Stark, R., Grzelak, M. & Hadfield, J. RNA sequencing: the teenage years. Nat Rev Genetics 20, 631–656 (2019).

Appropriate sequencing technology: Short vs long reads

Short reads platforms: Highest sequencing depth but shorter reads

Technology	Sequencing platform	Read length (bp)	Data output	Run time
Illumina	NovaSeq 6000 System		2.4–3.0 Tb	44 h
	NextSeq 550 System	150 PE*	100–200 Gb	29 h
	HiSeq 3000/4000 System		up to 1.5 Tb	4 days
	HiSeq X Series		1.6–1.8 Tb	3 days
Ion Torrent	Ion GeneStudio S5 System	200 SE	10–15 Gb	19 h
	Ion GeneStudio S5 Plus System	400 SE	20–30 Gb	10 h
	Ion GeneStudio S5 Prime System	200 SE	40–50 Gb	12 h
	Ion PGM 314 System	400 SE	30–50 Mb	2.3 h
	Ion PGM 316 System	600 SE	60–100 Mb	3.7 h
	Ion PGM 318System	200 SE	300–600 Mb	3.0 h
	Ion Proton System (Ion PI Chip)	200 SE	600 Mb–1 Gb	4.9 h
		400 SE	600 Mb–1 Gb	4.4 h
		200 SE	1.2–2 Gb	7.3 h
		400 SE	up to 15 Gb	2.5 h
		200 SE		
		400 SE		
		up to 200		
BGI/MGI	DNBSEQ-T7	100 PE, 150 PE	6 Tb	24 h
	DNBSEQ-G400/MGISEQ 2000/BGISEQ 500	400 SE, 100 PE, 150 PE,	18.75–1,080 Gb	~78 h
	DNBSEQ-G400 FAST	200 PE	330 Gb	12–13 h
	DNBSEQ-G50/MGISEQ 200/BGISEQ 50	100 SE, 150 PE	10–150 Gb	10–64 h
		50 SE, 100 SE, 50 PE, PE100		

Long reads platforms: Longer reads but less sequencing depth

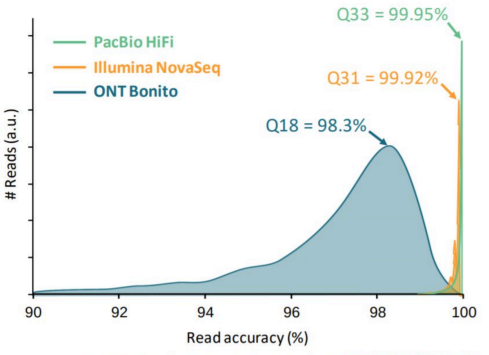
Technology	Platform	Read length (bp)	Data output	Run time
PacBio SMRT	RS II	~20 Kb	up to 1 Gb	4 h
	Sequel	8–12 Kb	3.5–7 Gb	30 Min–6 h
	Sequel II 2.0	~15 Kb	160 Gb/ SMRTcell	
Oxford NanoPore	Flongle		1.8 Gb	
	MinION	5–200 Kb▲	30 Gb	Real time+
	GridION Mk1	2 Mb longest▲	250 Gb	
	PromethION		up to 4 Tb	

Kanzi, A. M. et al. Next Generation Sequencing and Bioinformatics Analysis of Family Genetic Inheritance. Frontiers Genetics 11, 544162 (2020).

Appropriate sequencing technology: Short vs long reads

Reads accuracy differs depending on the sequencing technology:

- Illumina and PacBio HiFi: more accurate
- ONT and PacBio CLR: less accurate (but longer)



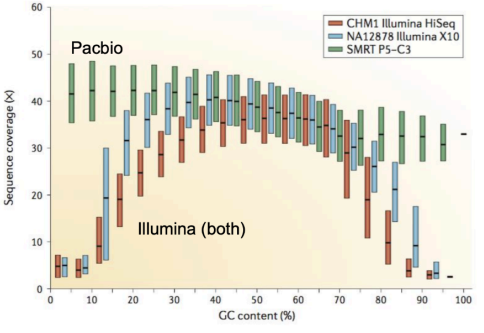
PacBio HiFi: HG003 18 kb library, Sequel II System Chemistry 2.0, precisionFDA Truth Challenge V2
Illumina: HG002 2×150 bp NovaSeq library, precisionFDA Truth Challenge V2
ONT: Bonito NCM Nanopore Tech. Update Dec. 2020 and Bonito Basecalling with R9.4.1

Appropriate sequencing technology: Coverage versus depth

Coverage in terms of redundancy

Coverage in terms of the percentage coverage of a reference by reads

Intuitively, increase sequencing depth should increase both types of coverage.



Chaisson et al. Genetic variation and the de novo assembly of human genomes. Nat Rev Genet 16, 627–640 (2015).

Computational resources and requirements

To be successful, you must have **sufficient computing resources (CPUS, RAM, walltime and storage)**.

- The resources needed are **different** for each step:
  - Assembly
  - Annotation
  - Other analysis tools
- For genome **assembly:**
  - Running times and RAM increase with data **type** and **amount**
  - More data for large genomes, increase **runtime/RAM/Storage**
  - Most of tools **run** on a **single node**: they are parallelized but not distributed
- For genome **annotation:**
  - Mapping/alignment of external data (RNA-seq, proteins) can be parallelized and distributed
  - Annotation process can be parallelized and distributed

Typical sequencing strategies: Bacterial genomes

- PacBio CLR or Oxford Nanopore reads at 40-50x coverage, self-correction and/or hybrid correction (using Illumina data)
- Illumina 2x250bp paired-end reads from MiSeq

Typical sequencing strategies: Larger genomes

- PacBio CLR or Oxford Nanopore reads at 100x coverage, hybrid correction using Illumina data and scaffolding using Hi-C
- PacBio HiFi reads at 30x coverage and scaffolding using Hi-C
- PacBio HiFi reads at 30x coverage, 120x Oxford Nanopore ultra long reads

Bioinformatics data formats

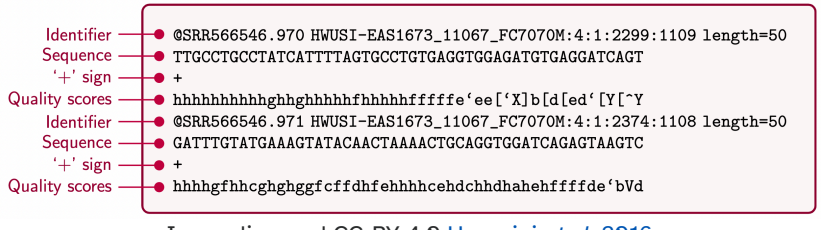
FASTA: a text-based format for representing either nucleotide sequences or amino acid (protein) sequences, in which nucleotides or amino acids are represented using single-letter codes.

Image licensed CC-BY 4.0 Hosseini *et al.* 2016

Hosseini, M., Pratas, D. & Pinho, A. J. A Survey on Data Compression Methods for Biological Sequences. Information 7, 56 (2016).

Bioinformatics data formats

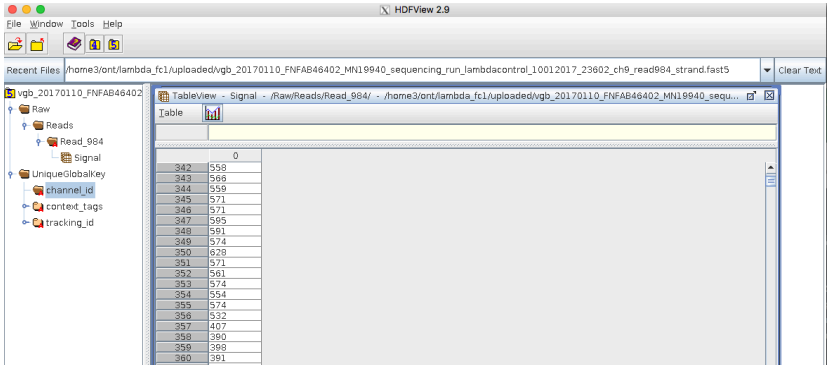
FASTQ: a text-based format for storing both a biological sequence (usually nucleotide sequence) and its corresponding quality scores (Phred). Both the sequence letter and quality score are each encoded with a single ASCII character for brevity. It's the standard sequencing output for Illumina and MGI sequencers.



Hosseini, M., Pratas, D. & Pinho, A. J. A Survey on Data Compression Methods for Biological Sequences. Information 7, 56 (2016).

Bioinformatics data formats

FAST5: the standard sequencing output for Oxford Nanopore sequencers. It is based on the hierarchical data format HDF5 format which enables storage of large and complex data. In contrast to fasta and fastq files a FAST5 file is binary and can not be opened with a normal text editor. Data stored in nanopore FAST5 files can contain the sequence of a read in fastq format (after basecalling), the raw signal of the pore as well as several log files and other information



Bioinformatics data formats

SAM (Sequence Alignment Map): a text-based format originally for storing biological sequences aligned to a reference sequence developed by Heng Li and Bob Handsaker et al.

BAM (Binary Alignment Map): the comprehensive raw data of genome sequencing; it consists of the lossless, compressed binary representation of the SAM format. It's the standard sequencing output for PacBio sequencers.

CRAM (Compressed Reference-oriented Alignment Map): a compressed columnar file format for storing biological sequences aligned to a reference sequence.

```
@HD VN:1.5 SO:coordinate
@SQ SN:ref LN:45
r001   99 ref  7 30 8M2I4M1D3M = 37  39 TTAGATAAAGGAT *
r002    0 ref  9 30 3S6M1P1I4M *  0   0 AAAAGATAAGG   *
r003    0 ref  9 30 5S6M       *  0   0 GCCTAAGCT     * SA:Z:ref,29,-,6H5M,17,0;
r004    0 ref 16 30 6M14N5M    *  0   0 ATAGCTTCA     *
r003 2064 ref 29 17 6H5M       *  0   0 TAGGC         * SA:Z:ref,9,+,5S6M,30,1;
r001  147 ref 37 30 9M         =  7 -39 CAGCGGC       * NM:i:1
```


Hosseini, M., Pratas, D. & Pinho, A. J. A Survey on Data Compression Methods for Biological Sequences. Information 7, 56 (2016).

🔑 Key points

- We learned the definitions of bioinformatics terms used in genomes assembly and annotation
- We have seen the bioinformatics file formats used for these analyses
- We learned the importance of preparing the project to ensure its success
- We learned the importance of surrounding ourselves with all the people who have knowledge of the different parts of the project (wet lab, sequencing, bioinformatics,...)

Thank You!

This material is the result of a collaborative work. Thanks to the Galaxy Training Network and all the contributors!

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