NGS and Genomes assemblies

Bioinformatics teachings
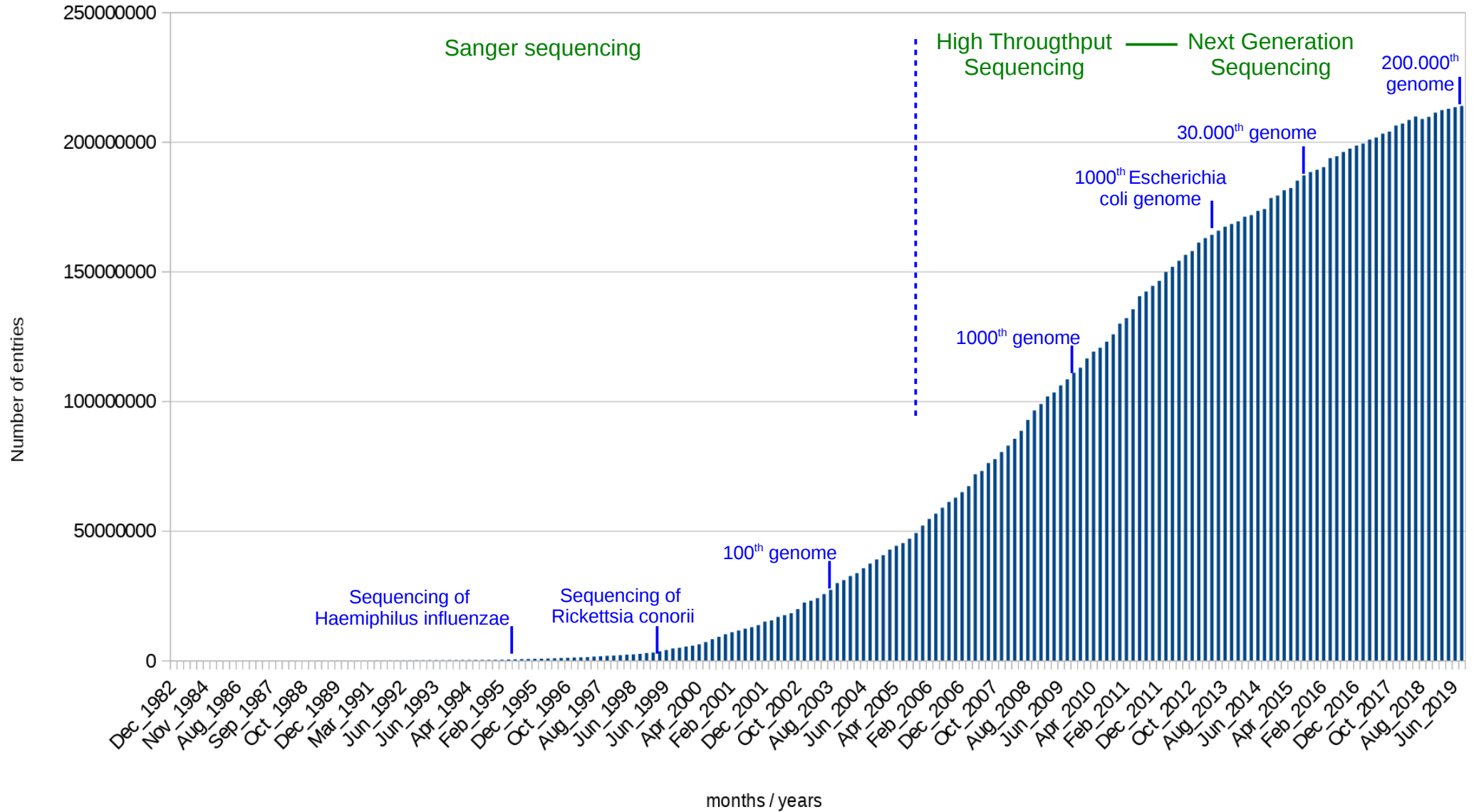
http://bioinfomed.fr - Olivier Croce (croce@unice.fr)

Data release

GenBank size

(GenBank.txt/gbrel.txt)

——— (in blue) : history of bacterial genomes sequencing

Data release

- Submission of the sequence on public databases
- Not always => publication

3 main public databases:

- EMBL-EBI - ENA (European Nucleotide Archive)
http://www.ebi.ac.uk/embl/

- GenBank (USA) – NCBI
http://www.ncbi.nlm.nih.gov/Genbank/

- DDBJ (DNA DataBank of Japon) – CIB
http://www.ddbj.nig.ac.jp/

They are associated (International Nucleotide Sequence Database Collaboration) and exchange the same data which is periodically duplicated together
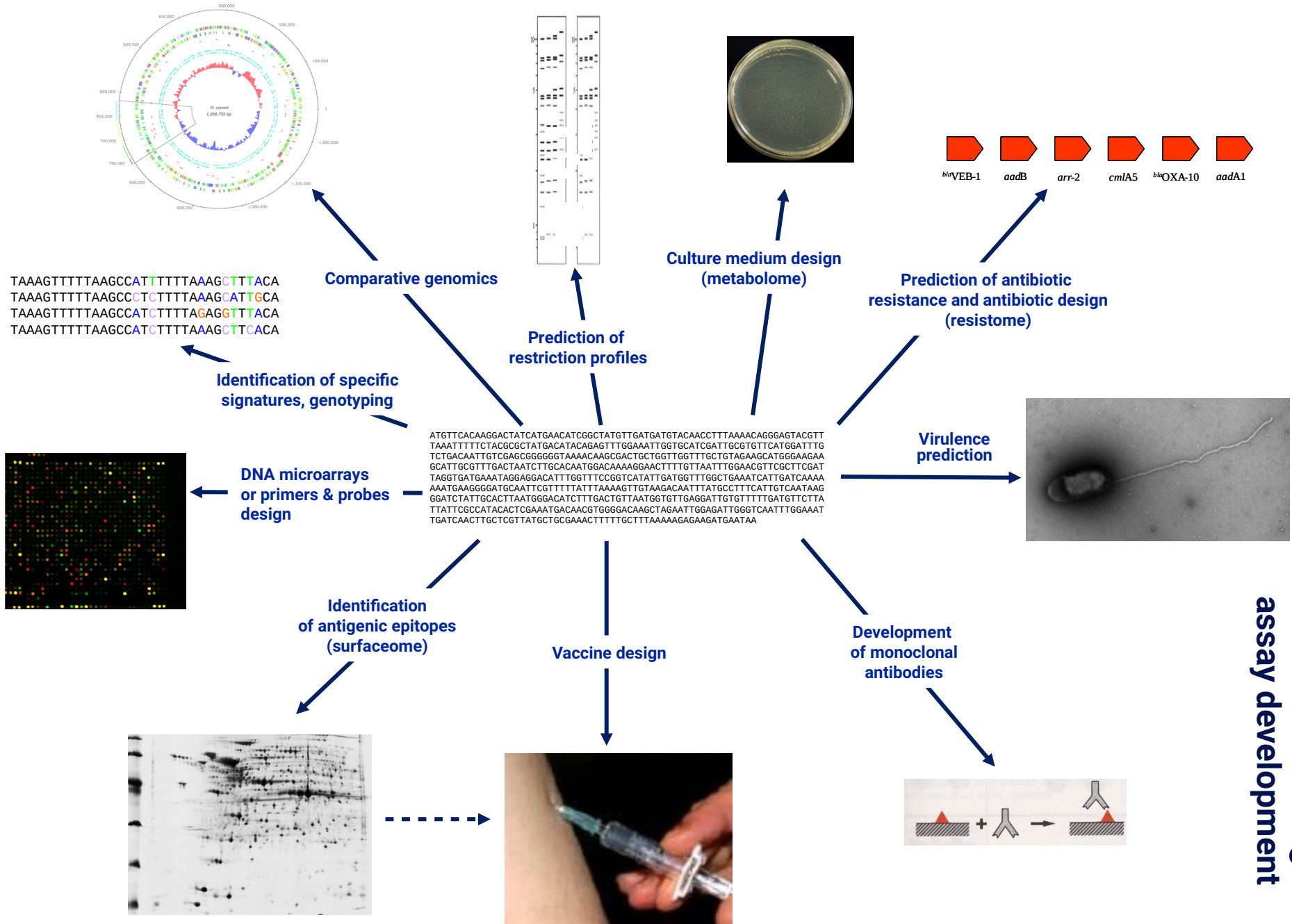
Contain:

- Sequences of DNA or RNA from various sequencers technologies and from many labs

    * Some genome fragments : one or more genes, intergenic sequences, parts of a genome
    * Completed genomes
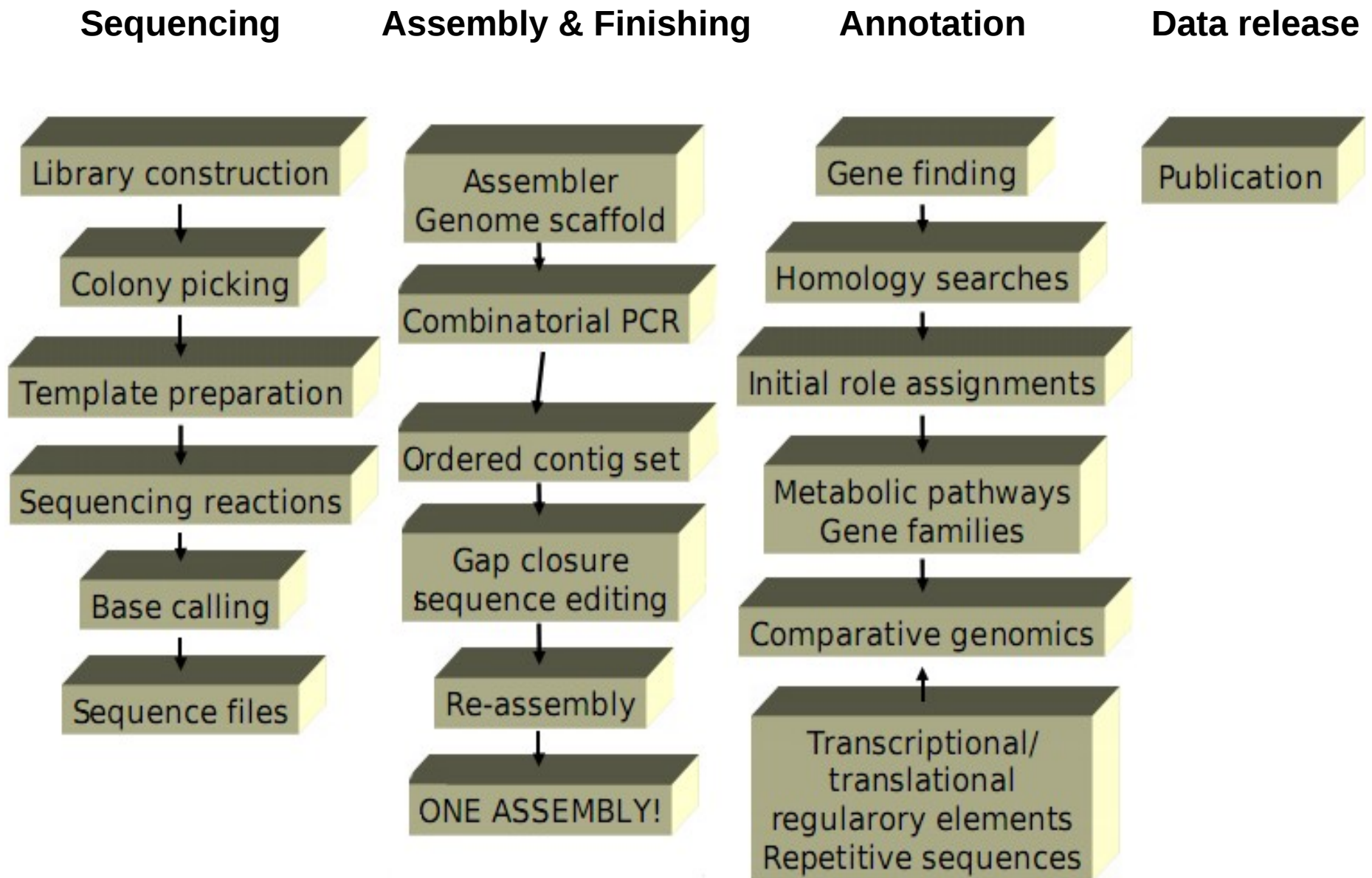   * mRNA, tRNA, rRNA (ie. 16s)

- Annotations

Aims

TAAAGTTTTTAAGCCATTTTTTAAAGCTTTACA
TAAAGTTTTTAAGCCCTCTTTTTAAAGCATTGCA
TAAAGTTTTTAAGCATCTTTTTAGAGGTTTACA
TAAAGTTTTTAAGCCATCTTTTTAAAGCTTCACA

Comparative genomics

**Culture medium design
(metabolome)**

^{bla}VEB-1 aadB arr-2 cmlA5 ^{bla}OXA-10 aadA1

**Prediction of antibiotic
resistance and antibiotic design
(resistome)**

**Prediction of
restriction profiles**

**Identification of specific
signatures, genotyping**

ATGTTCACAAGGACTATCATGAACATCGGCTATGTTGATGATGTACAACCTTTAAAACAGGGAGTACGTT
TAAATTTTTCTACGCGCTATGACATACAGAGTTTGGAAATTGGTGCATCGATTGCGTGTTCATGGATTTG
TCTGACAATTGTCGAGCGGGGGGTAAAACAAGCGACTGCTGGTTGGTTTGCTGTAGAAGCATGGGAAGAA
GCATTGCGTTTGACTAATCTTGCACAATGGACAAAAGGAACTTTTGTTAATTTGGAACGTTCGCTTCGAT
TAGGTGATGAAATAGGAGGACATTTGGTTTCCGGTCATATTGATGGTTTGGCTGAAATCATTGATCAAAA
AAATGAAGGGGATGCAATTCGTTTTTATTTAAAAGTTGTAAGACAATTTATGCCTTTCATTGTCAATAAG
GGATCTATTGCACTTAATGGGACATCTTTGACTGTTAATGGTGTTGAGGATTGTGTTTTTGATGTTCTTA
TTATTCGCCATACACTCGAAATGACAACGTGGGGACAAGCTAGAATTGGAGATTGGGTCAATTTGGAAAT
TGATCAACTTGCTCGTTATGCTGCGAAACTTTTTGCTTTAAAAAGAGAAGATGAATAA

**Virulence
prediction**

**DNA microarrays
or primers & probes
design**

**Identification
of antigenic epitopes
(surfaceome)**

Vaccine design

**Development
of monoclonal
antibodies**

From the bench to the publication

Sequencing	Assembly & Finishing	Annotation	Data release
Library construction	Assembler Genome scaffold	Gene finding	Publication
Colony picking	Combinatorial PCR	Homology searches	
Template preparation	Ordered contig set	Initial role assignments	
Sequencing reactions	Gap closure sequence editing	Metabolic pathways Gene families	
Base calling	Re-assembly	Comparative genomics	
Sequence files	ONE ASSEMBLY!	Transcriptional/ translational regularory elements Repetitive sequences	

Quality level of genomes

- The genome sequence must be completed with a high quality and annotated before the release

Of course the best, but very time consuming.
Actually, 90-95 % of a microorganism genome could be easy covered without finishing, but the 5-10 % remained can take many weeks or months to be ended
 => now easier using long reads sequening
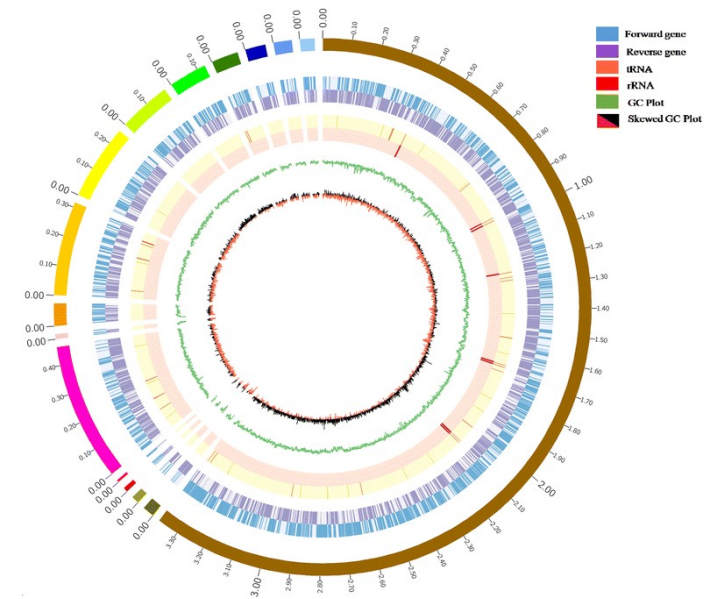
- The sequence should be uncompleted with a draft quality, whether we suppose most of the genes are sequenced (and identified)

Many eukaryote genomes are only draft genomes, because of the complexity of finishing

=> In general, fundamental research usually performs high quality genomes
and applicative research (industry, part of clinical) usually performs draft genomes

=> depending of the project : time and experience (bioinformatician), money (coverage of NGS), organisms, the question to answer

Sequencing technologies
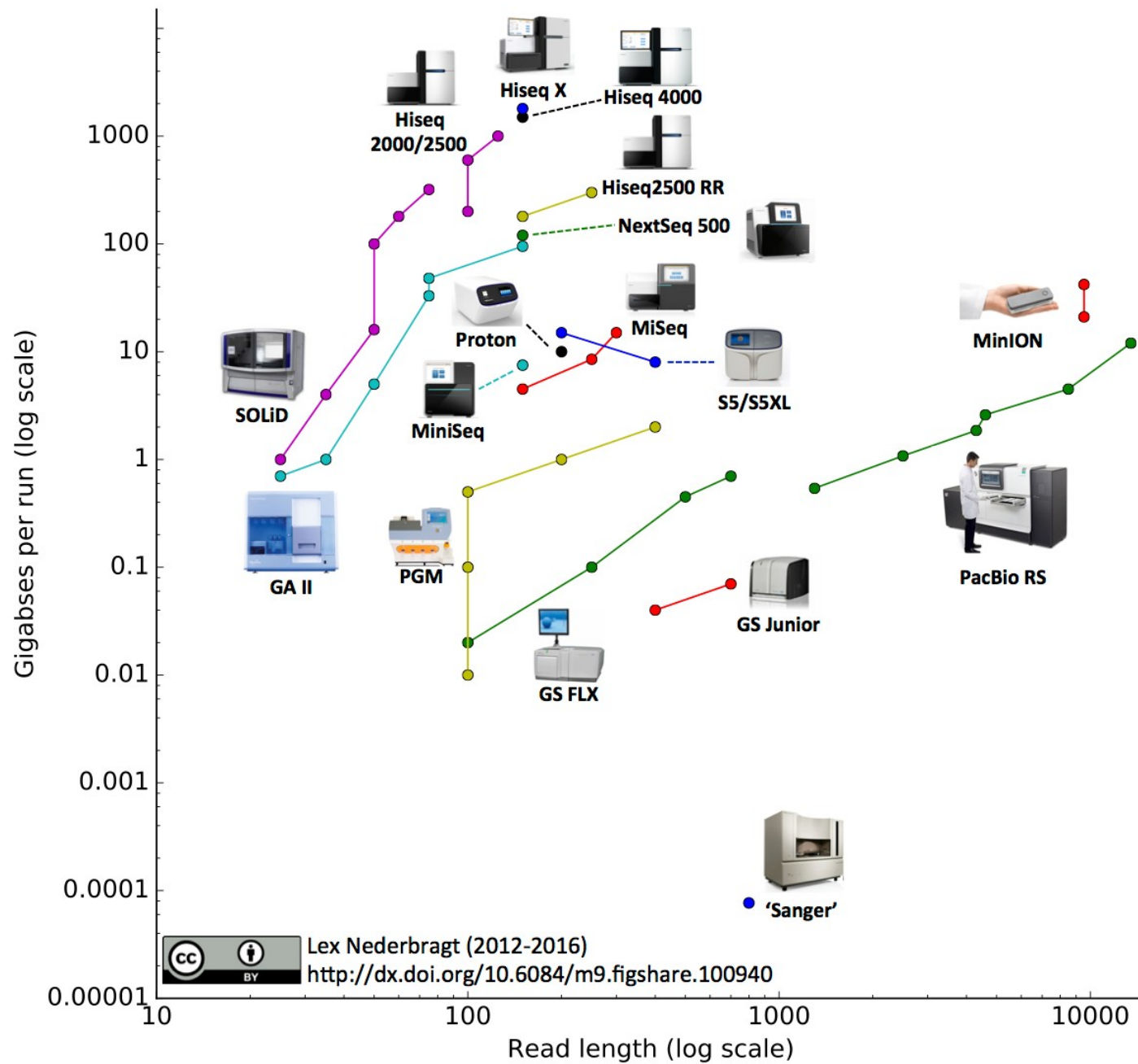
Principle of sequencing and assembly

Sequencing step: reads have heterogeneous distribution

Assembly and finishing step
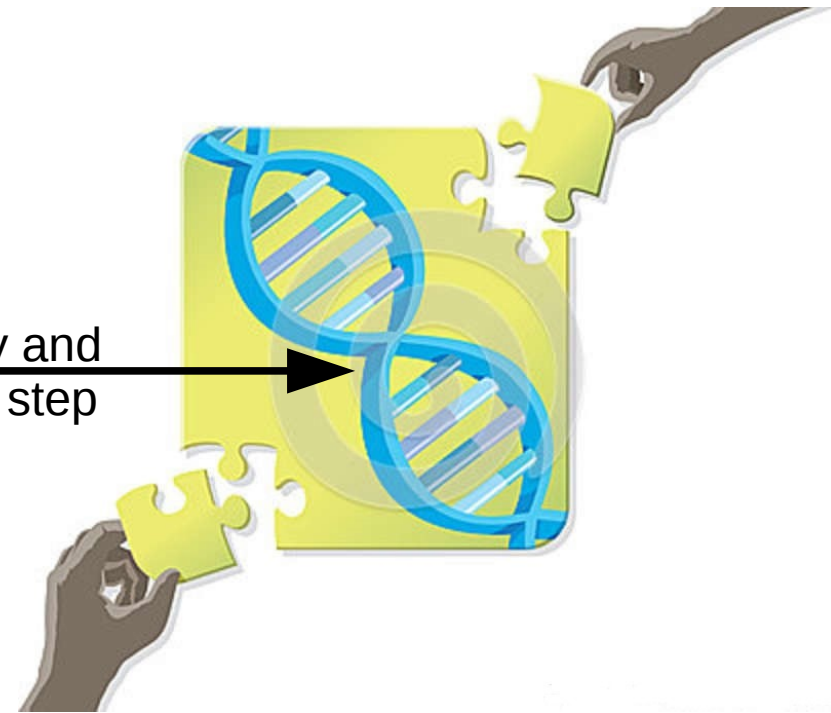
Principle of sequencing and assembly

Fragmentation + sequencing
=> sets of reads

Build of contigs with overlapping regions

Assembly :
=> alignements of reads + consensus

Principle of sequencing and assembly

Search for best pairings by comparing each sequence (and its reverse complement) against every others sequences to find the best overlapping

=> list of best candidates with similarities criteria
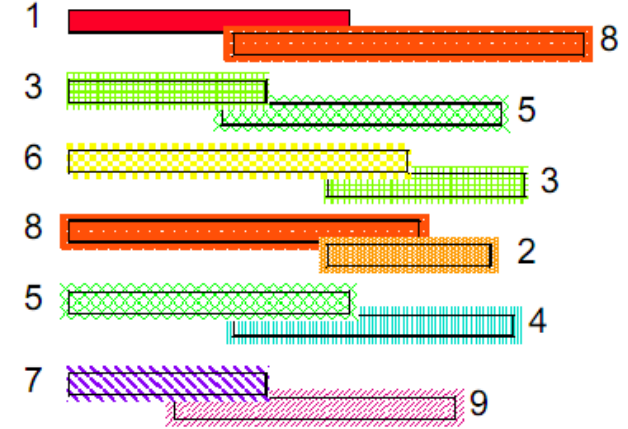
Best candidate is a compromise between :

- maximum overlap length - region of similarity between regions
- minimum overhang length - unaligned ends of the sequences
- maximum % identity in overlap region
- minimum repeat length

overlap (19 bases)   overhang (6 bases)

...AGCCTAGACCTACAGGATGCGCGGACACGTAGCCAGGAC
CAGTACTTGGATGCGCTGACACGTAGCTTATCCGGT...

overhang   % identity = 18/19 % = 94.7%
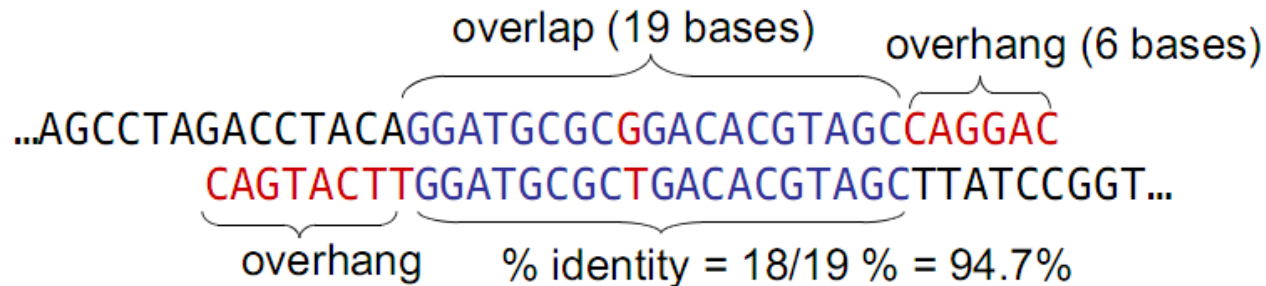
=> Many **assemblers** tools existed (depending of sequencings technologies, libraries, genomes size, etc..)
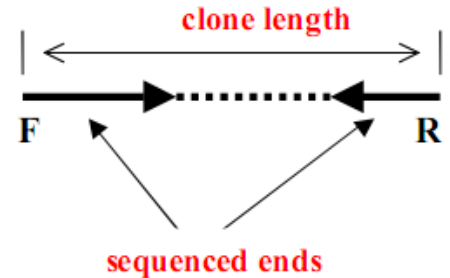
Constructions of library from genomes fragments

*** single - end (= shotgun)** : reads sequenced independently

* paired-end (similar to **mate-pair**) : reads are sequenced by pairs

- The distance between the reads is known (length of the insert), with some experimental uncertainty

- Distance of insert depends of technology (ie. Illumina ~150 nt for paired-end, ~1-5 kb mate-paired)

Why using PE/MP ? length of reads is limited => assemble repeatitive regions by using reads as "anchors"
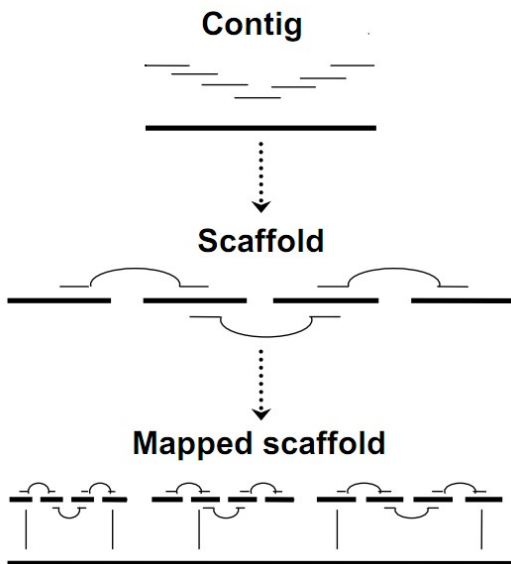
**Contigs** : group of overlapping reads, without gap

Scaffold : group of contigs order and in the same sens. Gap ("NNN") could existed and their length are known. Scaffolds exists only if a paired-ends (or mate pairs) sequencing was performed !

Mapped scaffolds : scaffolds mapped along a reference. Order, orientation and length of gaps are estimated, but not sure !

Main remaining problems
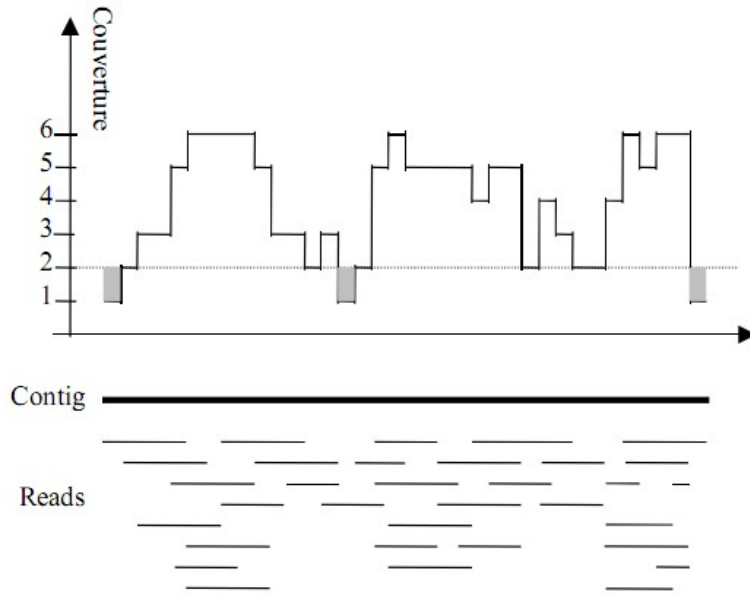
- Bad assembly of reads
- Low coverage of reads

- Bad insert size estimation
- Different orientation of contigs
- Error of sequencing
- Repeat sequence ambiguities
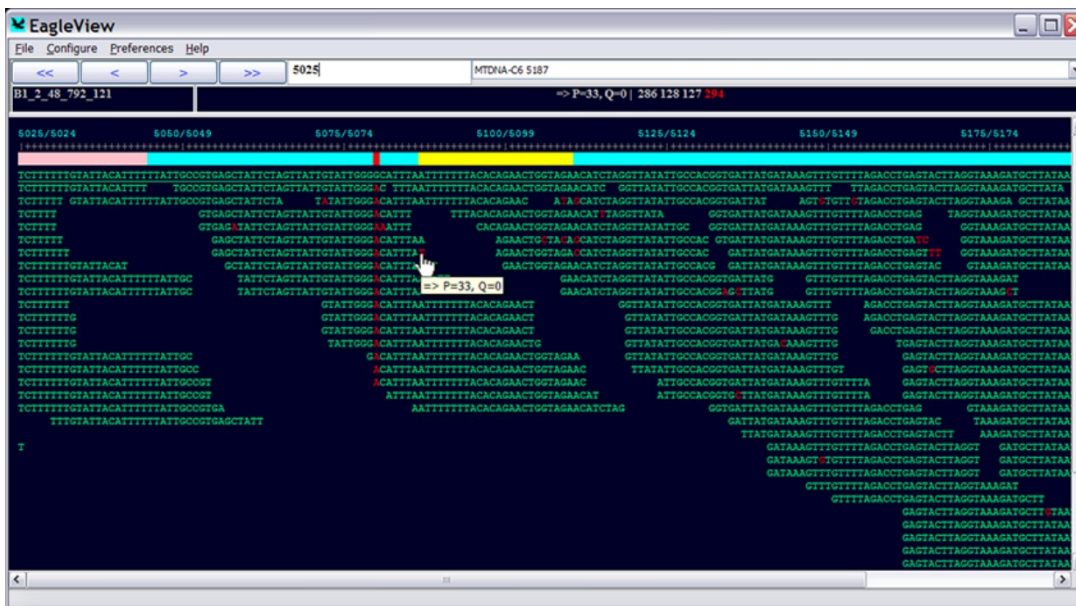
Re-Mapping, to see coverage, SNP and potential errors

Finishing

- (re)Mapping of reads along the assembled genome (or/and a reference)

- help to correct the low quality/coverage areas

- Check the order of contigs
- Check the redundancy of contigs (false contigs or true repeat contigs like rRNA operons)

- Compare syntheny between multiple assemblers (global alignement)

- Fill the gaps by extending the boundaries of each gap using ends of mapping reads (or use PCR)
- Order (or reorder) contigs
- Desassemble some areas if they seem to be false
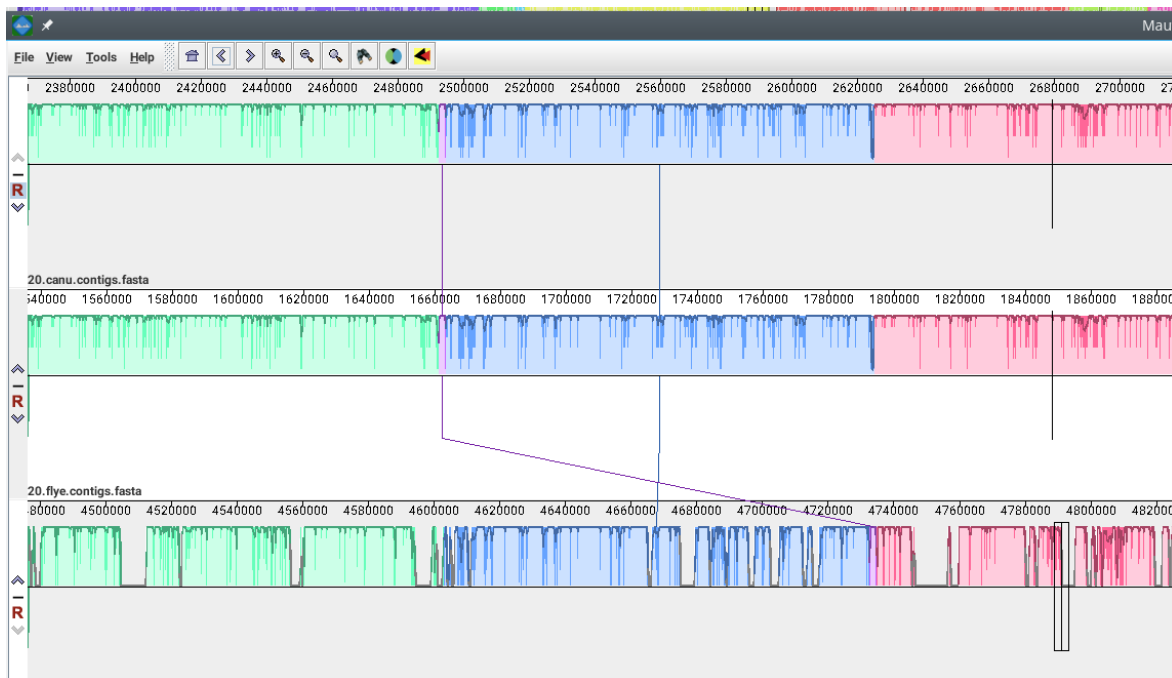
Bacillus cereus assemblies using 3 assemblers tools.
2 first genomes are very similar, the third show many differences

=> High improvement with new long-reads technology (MinIon Nanopore, PacBio)