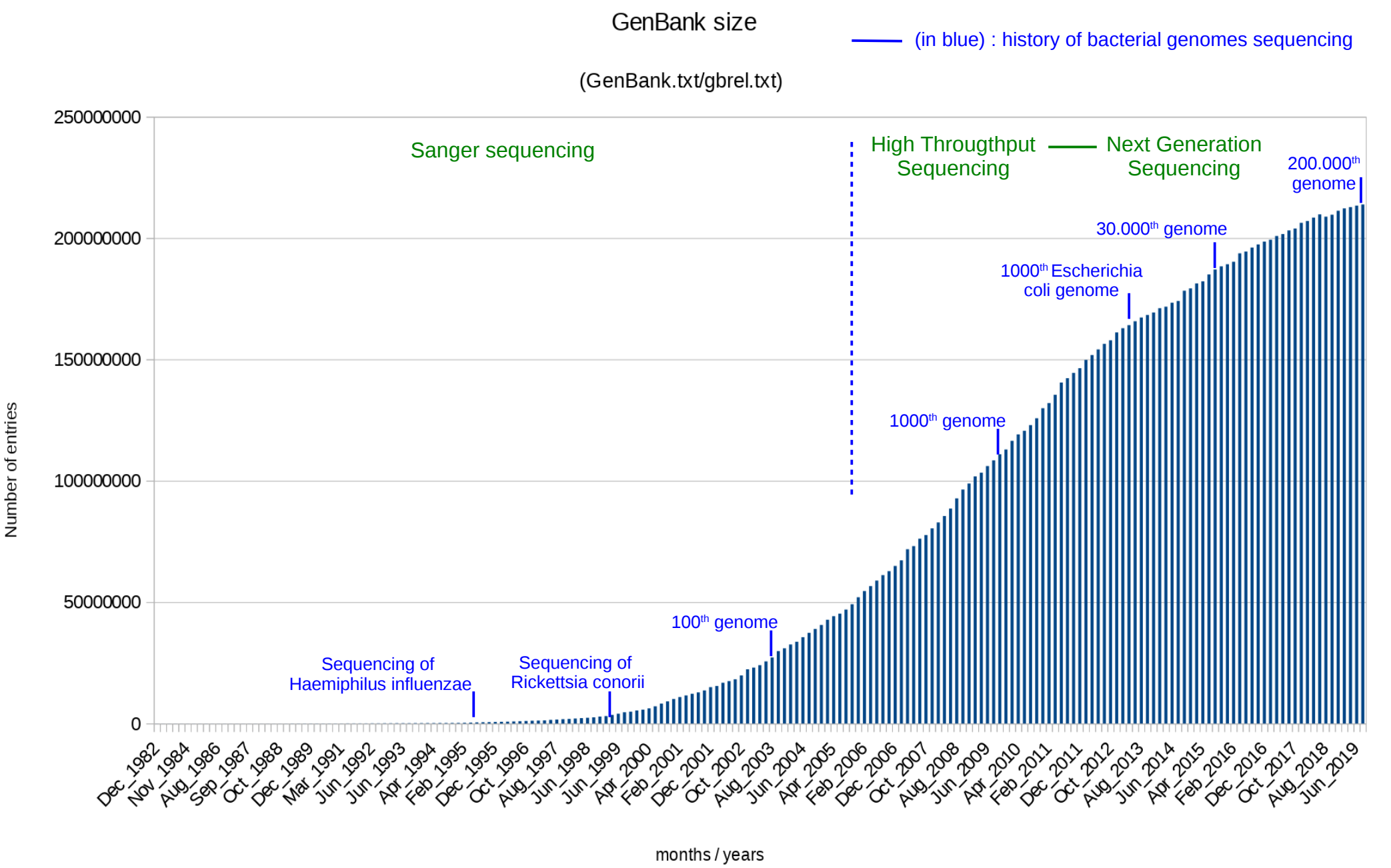
# NGS and Genomes assemblies

*Bioinformatics teachings*

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

# Data release



# Data release

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

**<u>3 main public databases</u>:**

- 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

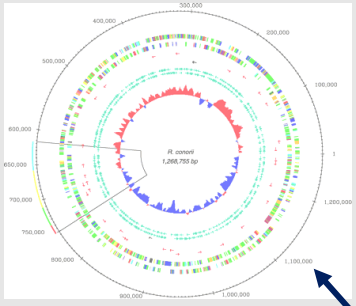
**<u>Contain</u>**:

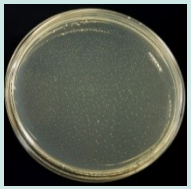
- Sequences of DNA or RNA from various sequencers technologies and from many labs

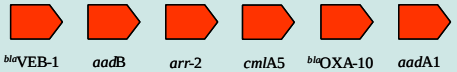
    * Some genome fragments : one or more genes, intergenic sequences, parts of a genome
    * Completed genomes
    * mRNA, tRNA, rRNA (ie. 16s)

- Annotations

# Aims








Phenotype prediction

Comparative genomics

Culture medium design (metabolome)

Prediction of antibiotic resistance and antibiotic design (resistome)

Prediction of restriction profiles

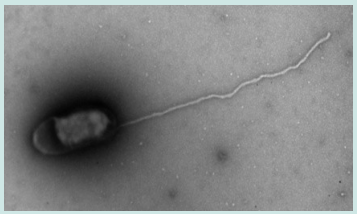
TAAAGTTTTTAAGCCATTTTTTAAAGCTTTACA
TAAAGTTTTTAAGCCCTCTTTTAAAGCATTGCA
TAAAGTTTTTAAGCCATCTTTTAGAGGTTTACA
TAAAGTTTTTAAGCCATCTTTTAAAGCTTCACA

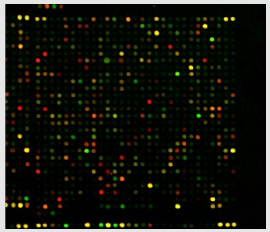
Identification of specific signatures, genotyping

ATGTTCACAAGGACTATCATGAACATCGGCTATGTTGATGATGTACAACCTTTAAAACAGGGAGTACGTT
TAAATTTTTCTACGCGCTATGACATACAGAGTTTGGAAATTGGTGCATCGATTGCGTGTTCATGGATTTG
TCTGACAATTGTCGAGCGGGGGGTAAAACAAGCGACTGCTGGTTGGTTTGCTGTAGAAGCATGGGAAGAA
GCATTGCGTTTGACTAATCTTGCACAATGGACAAAAGGAACTTTTGTTAATTTGGAACGTTCGCTTCGAT
TAGGTGATGAAATAGGAGGACATTTGGTTTCCGGTCATATTGATGGTTTGGCTGAAATCATTGATCAAAA
AAATGAAGGGGATGCAATTCGTTTTATTTAAAAGTTGTAAGACAATTTATGCCTTTCATTGTCAATAAG
GGATCTATTGCACTTAATGGGACATCTTTGACTGTTAATGGTGTTGAGGATTGTGTTTTTGATGTTCTTA
TTATTCGCCATACACTCGAAATGACAACGTGGGGACAAGCTAGAATTGGAGATTGGGTCAATTTGGAAAT
TGATCAACTTGCTCGTTATGCTGCGAAACTTTTTGCTTTAAAAAGAGAAGATGAATAA

Molecular detection and identification

Virulence prediction



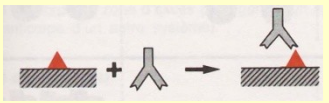


DNA microarrays or primers & probes design

Identification of antigenic epitopes (surfaceome)

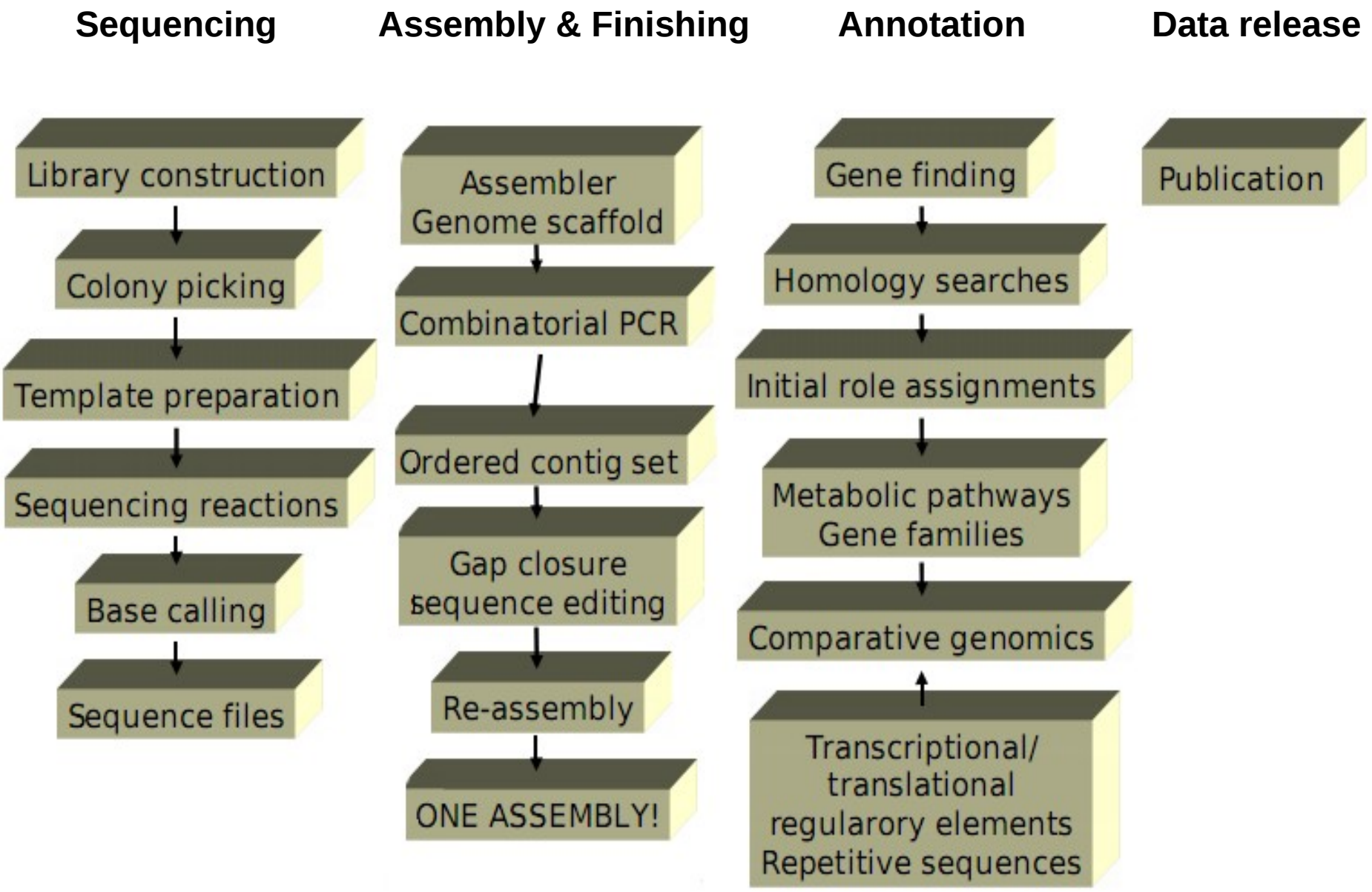
Vaccine design

Development of monoclonal antibodies



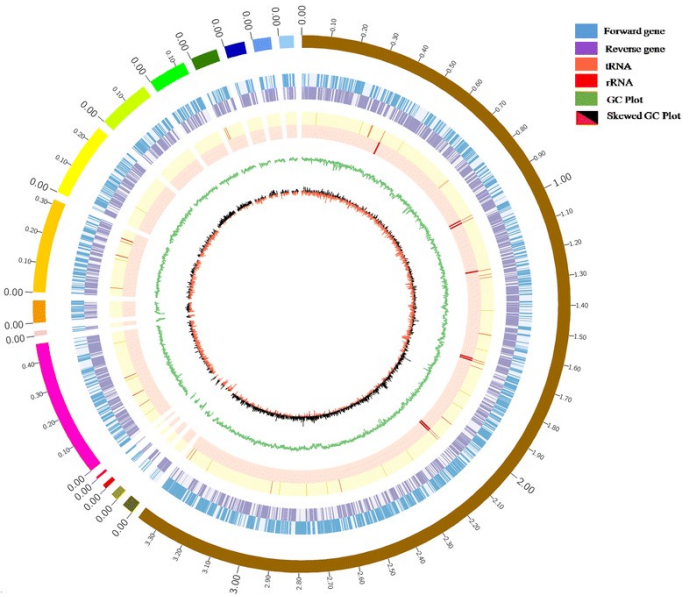
Vaccine & serological assay development

# From the bench to the publication



# 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

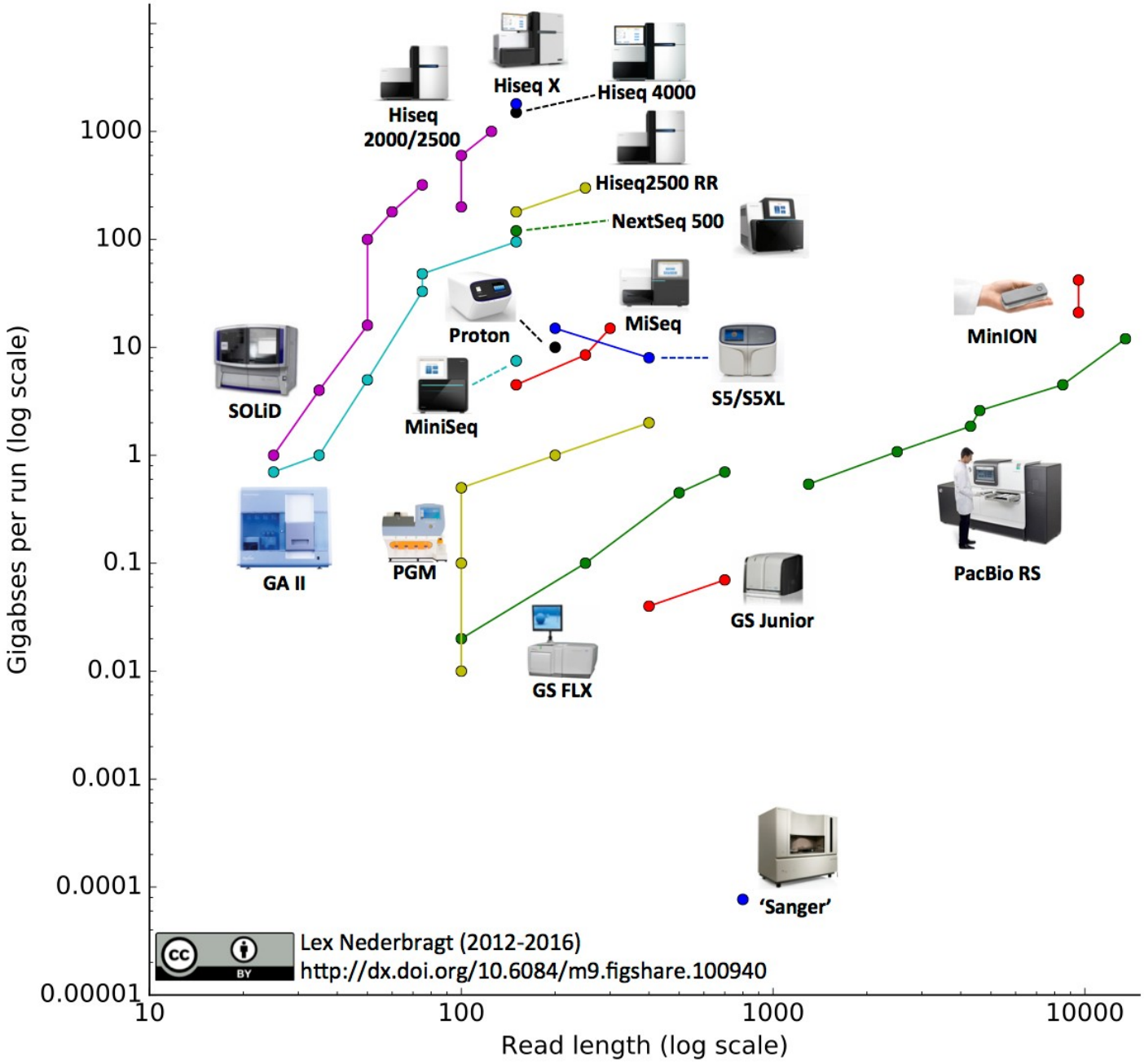
**- The sequence should be uncompleted with a draft quality, whether we suppose most of the genes are sequenced (and identified)**

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

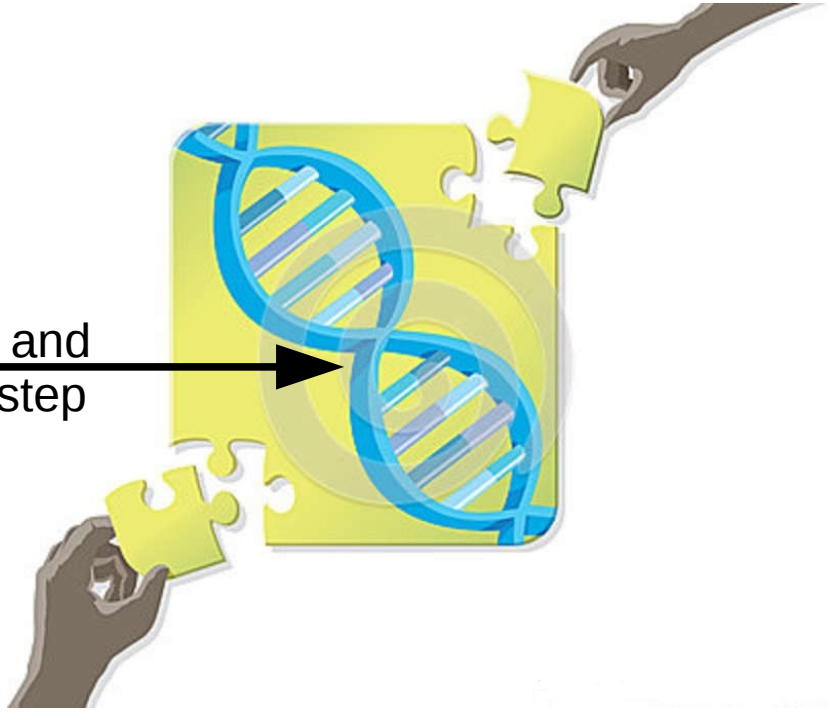
# Sequencing technologies



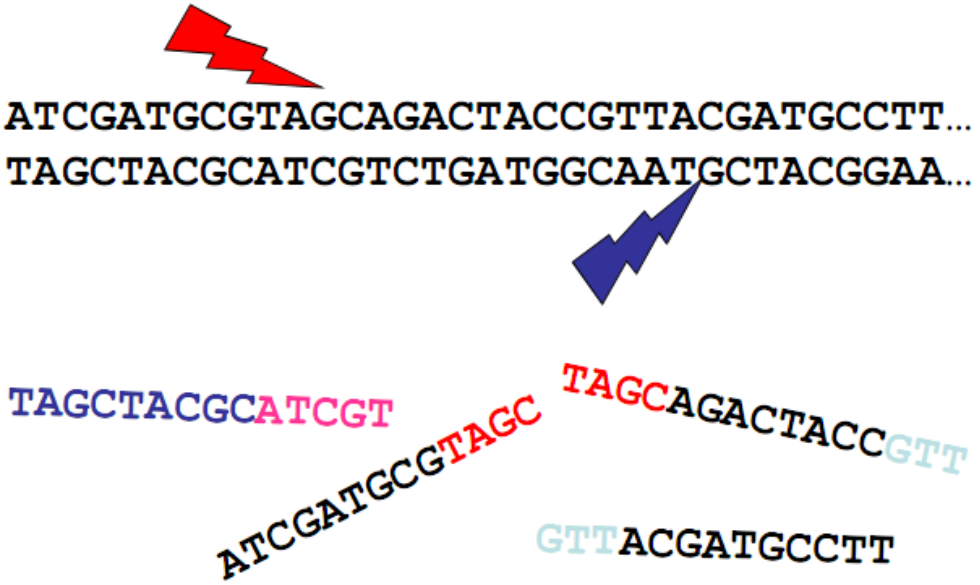
# Principle of sequencing and assembly

Sequencing step: reads have heterogeneous distribution

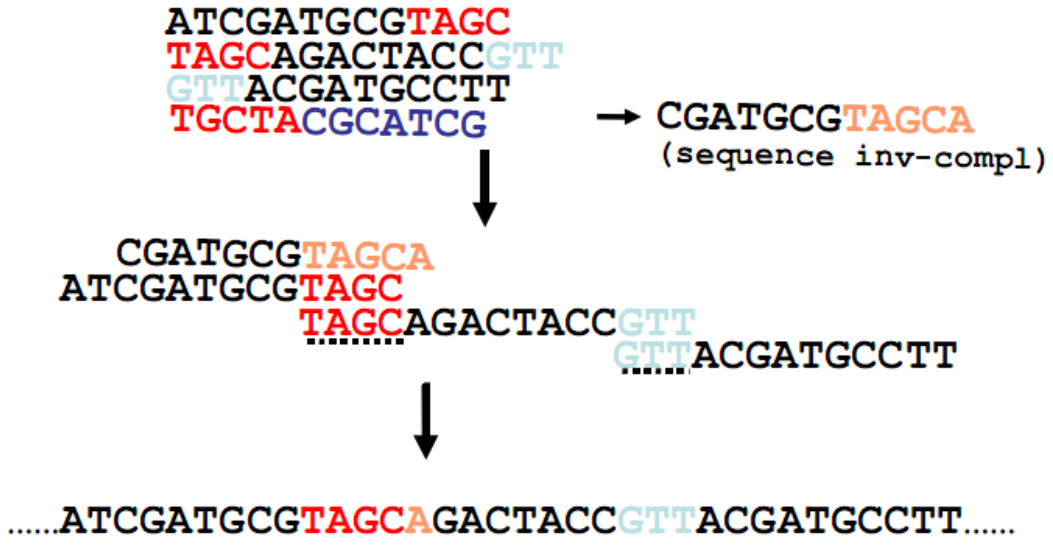
Assembly and finishing step



# Principle of sequencing and assembly




Fragmentation + sequencing
=> sets of reads

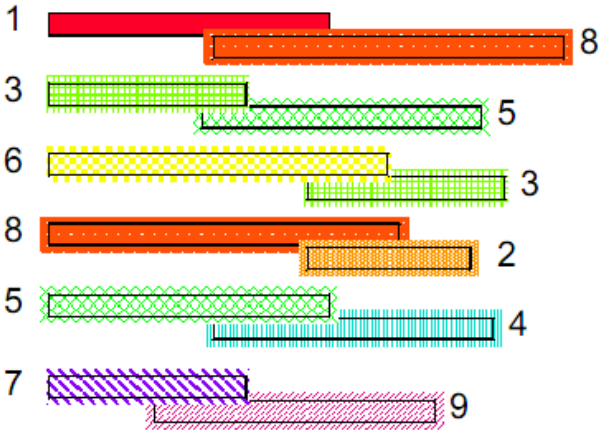



Build of contigs with overlapping regions

Assembly :
=> alignements of reads + consensus

# Principle of sequencing and assembly

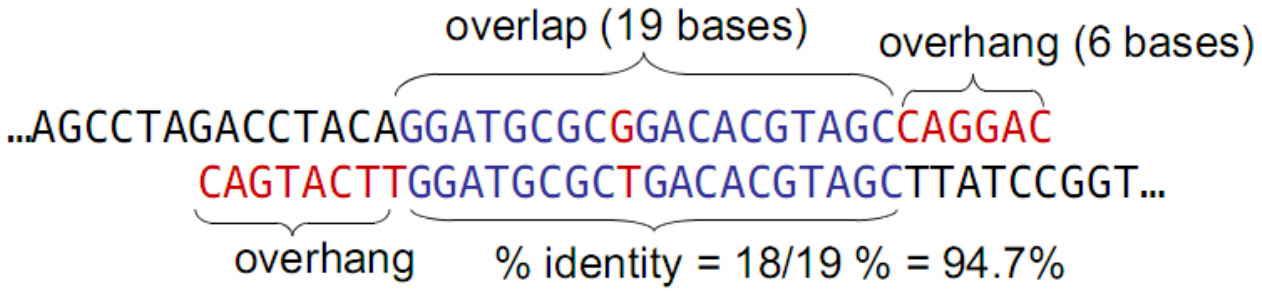
Search for best pairings by comparing each sequence (and its reverse complement) against every others sequences to find the best overlapping




=> 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




=> Many **assemblers** tools existed (depending of sequencings technologies, libraries, genomes size, etc..)

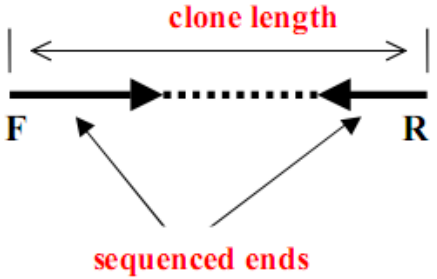
# Constructions of library from genomes fragments

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

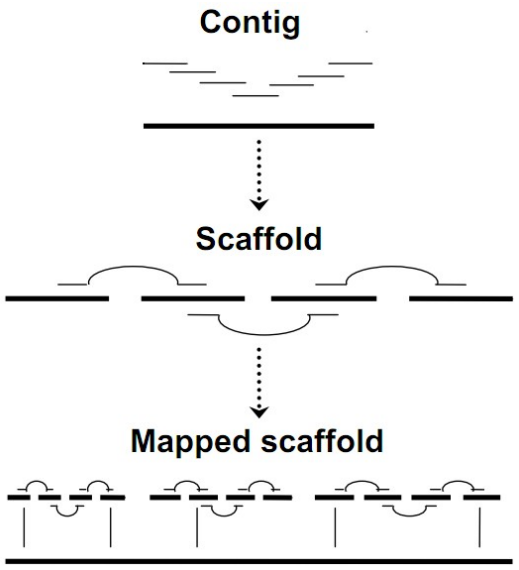
*** paired-end** (similar to **mate-pair**) : reads are sequenced by pairs

- 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”

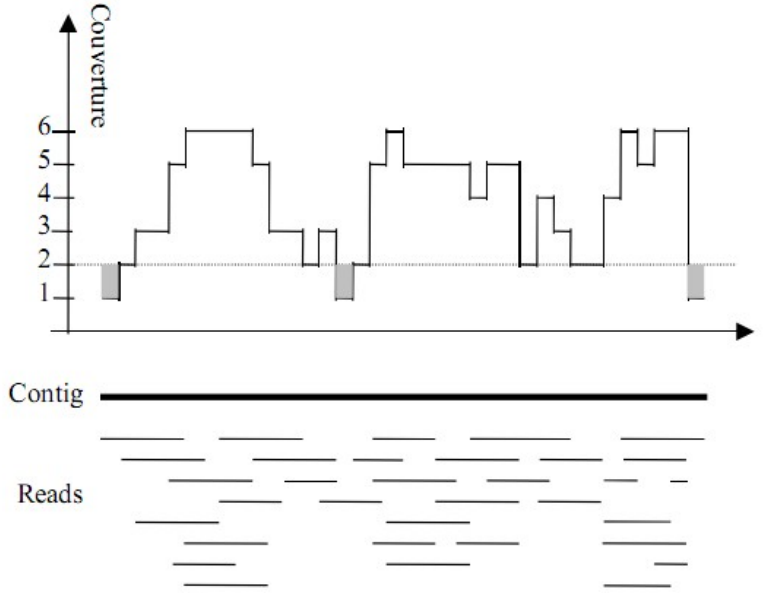



**Contigs** : group of overlapping reads, without gap

**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 !

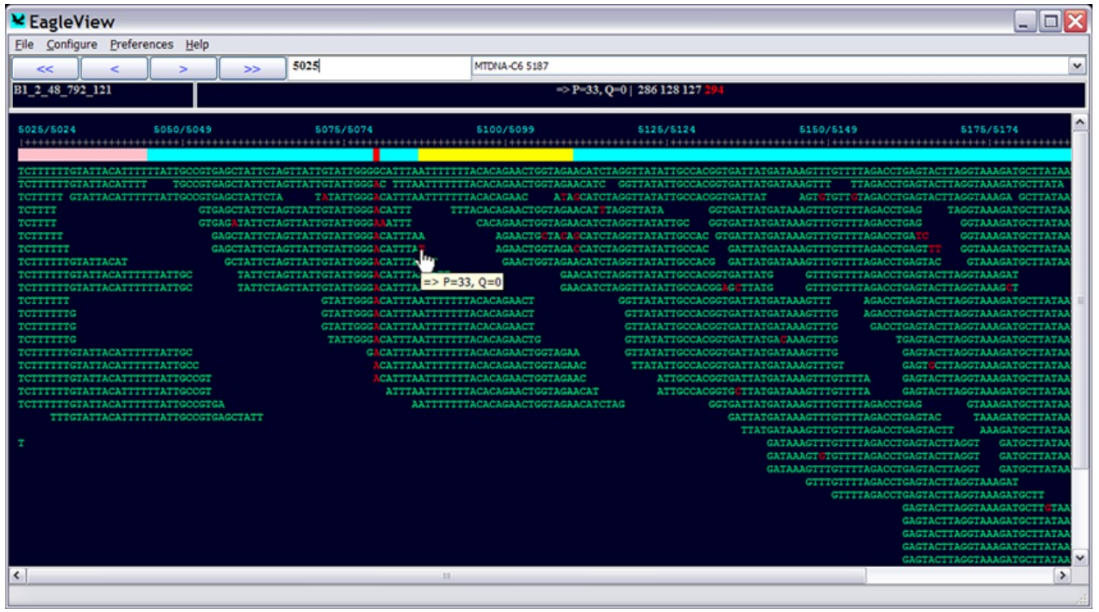
**Mapped scaffolds** : scaffolds mapped along a reference. Order, orientation and length of gaps are estimated, but not sure !

# 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

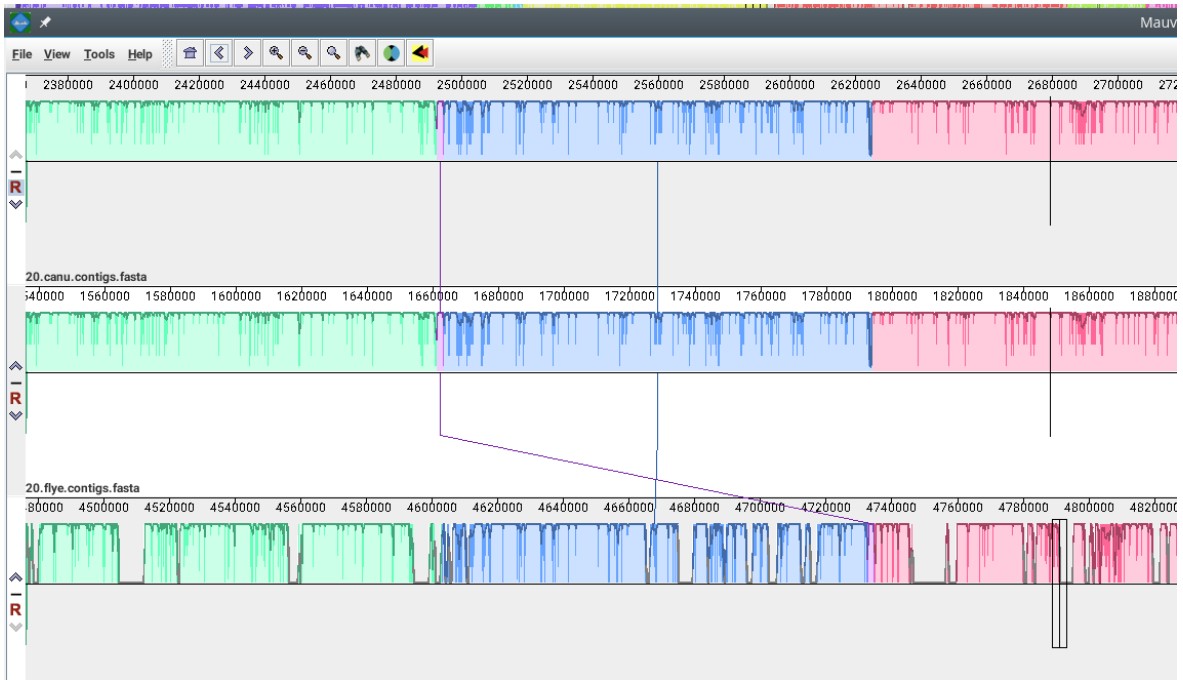
- (re)Mapping of reads along the assembled genome (or/and a reference)

- 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)