Genomics and Transcriptomics

High-throughput methods

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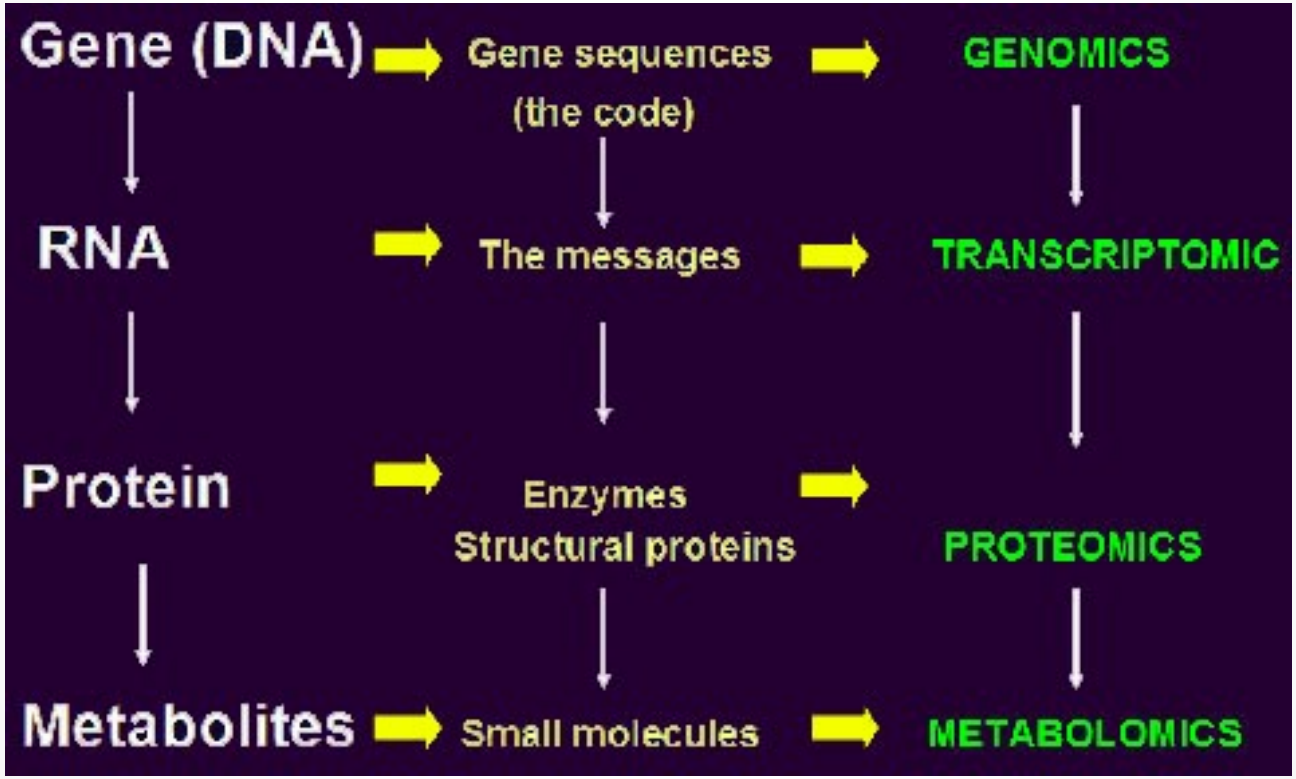
Thematics

- Genomics
  - Genomes, projects
  - Applications
  - Genome sequencing
    - de novo sequencing
    - re-sequencing
  - SNP analysis

Genomics

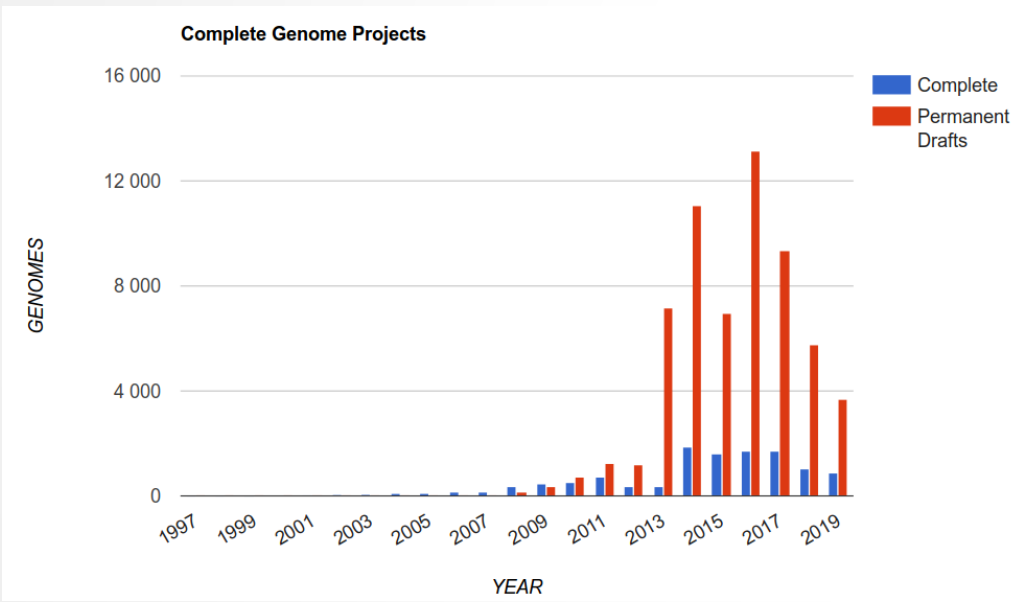
- Genome: complete set of genetic material within an organism
  - It is coded with DNA (or RNA in some viruses)
  - Genes and non-coding sequences
- Genomics investigates
  - whole genomes
  - intercations between genes and non-coding regions
  - genome structures
  - gene locations
  - similarities and differences between genomes
- In contrast: genetics usually investigate functions of a single gene.
- Bioinformatics is massively needed to investigate genomes.

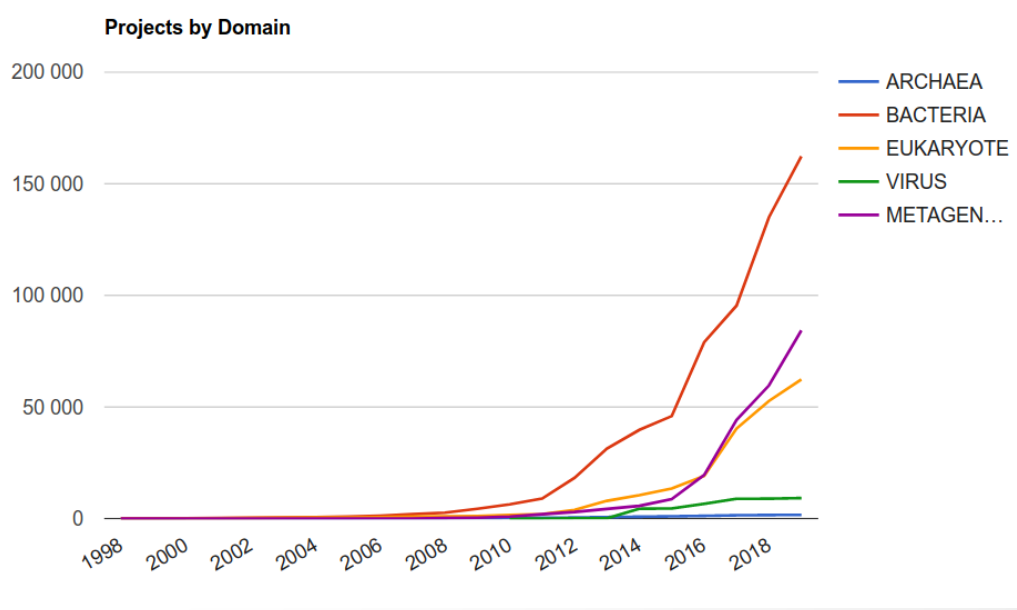
Omics


Gene (DNA)
Gene sequences
(the code)
GENOMICS
RNA
The messages
TRANSCRIPTOMIC
Protein
Enzymes
Structural proteins
PROTEOMICS
Metabolites
Small molecules
METABOLOMICS

Genome programs

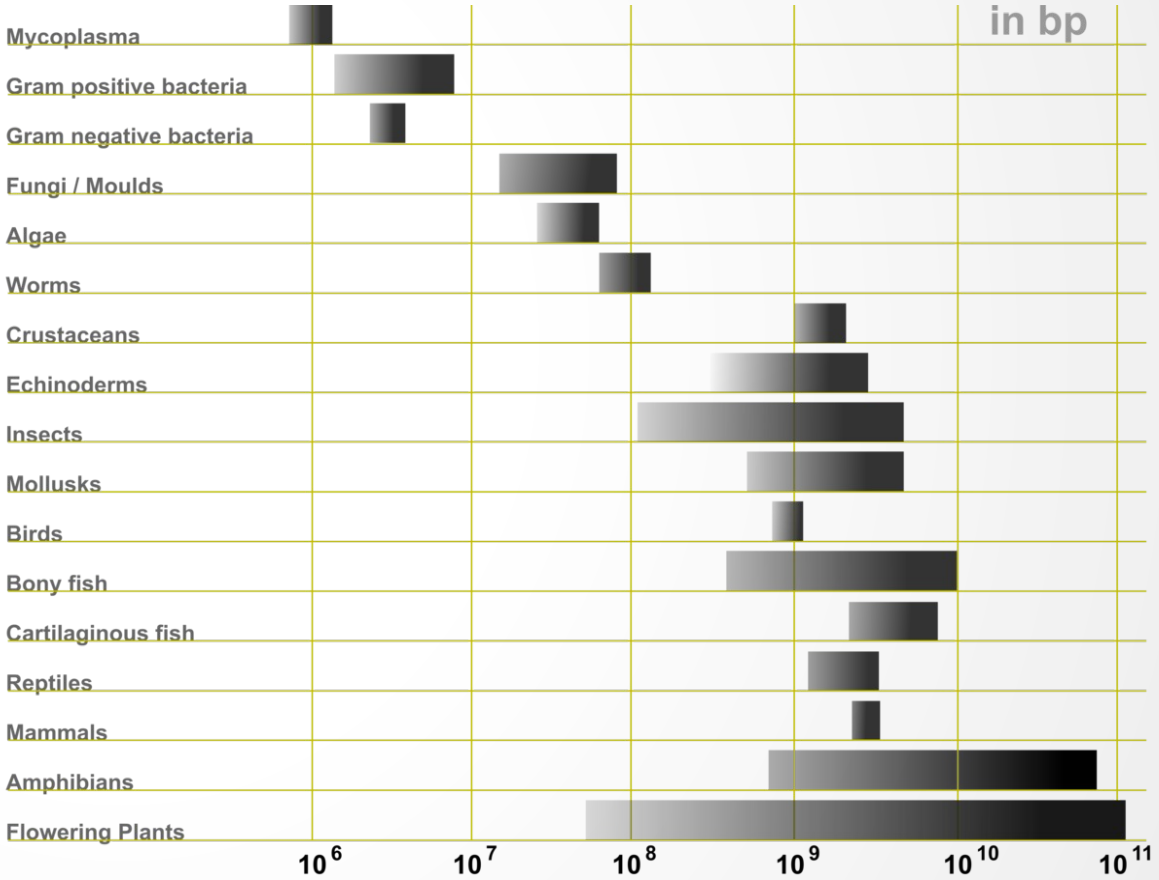
- GOLD, Genomes online database: https://gold.jgi.doe.gov/



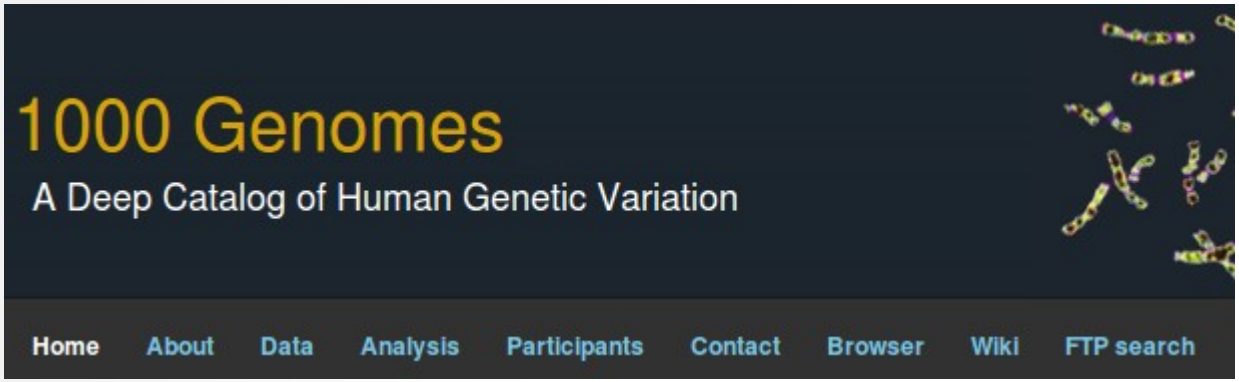


Genome size

- Virus
  (2 kb - 700 kb, kilobase = 1000 nt)
  - 1-2 stranded DNA or RNA
    - First sequenced genome:
      Phi-X174 phage, Fred Sanger, 1977
- Bacteria (139 kb - 13.000 kb)
  Archea (500 kb - 5.700 kb)
  - 2 stranded haploid chromosomes
    - plasmides
- Eucarya (8,2 Mb - 220.000 Mb,
  megabase = 1.000.000 nt)
  - diploid chromosomes - nuclear
  - Organelles with genome:
    mitochondria (16,6 kb)
    chloroplast (120 kb - 170 kb)
    - Human genome:
      June 2000 – Feb 2001



Genome programs



http://www.1000genomes.org/

http://www.uk10k.org/

- Aims of Beijing genomics Institute (BGI, China) sequencing center: million human genomes, million microbe genomes, million plant and animal genomes
  - The Million Human Genome Project
- 100,000 foodborne pathogen genome project
- Up to 100,000 NHS patients - human
- 50,000 Faroe Islanders Project - human
- 20,000 Global pneumococcal project - human
- 10,000 Genome 10k vertebrate sequencing project
- 10,000 autism genome projekt - human
- 5,000 arthropod genome sequencing project
- ...

Applications

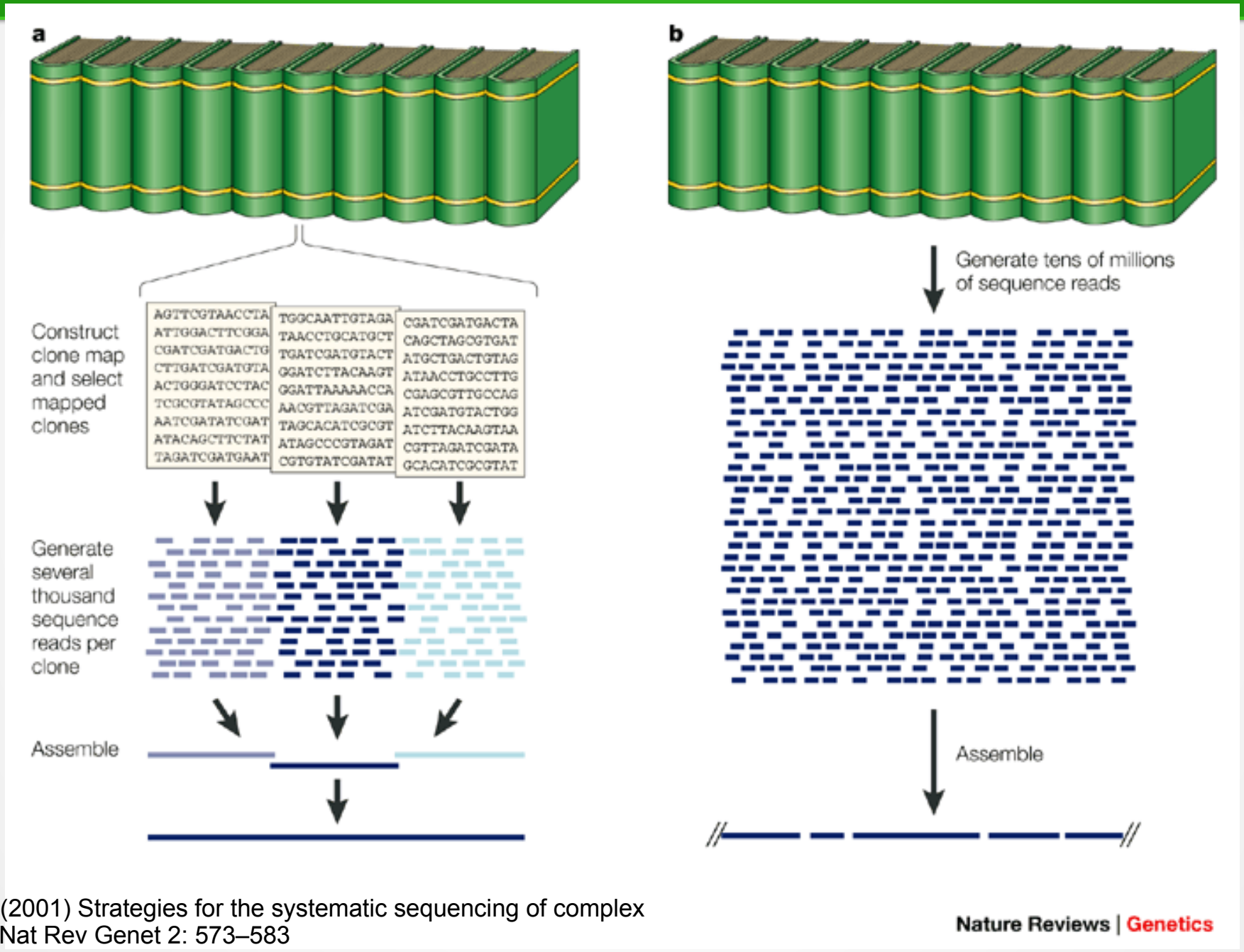
- Genetics
  - ie.: gene locations, environment, regulation, recombination hot-spots
- Populationgenetics
  - ie: explore the history of a population using SNP frequencies
- Evolutiongenetics
  - ie: investigate which part of the genome is under selection
  - phylogenomics
- Paleontology
- Medicine
  - diagnostics
  - personalized therapy, ie: genetherapy
  - ie. cancaer research
- Drug developement
- Agriculture (GMO)
- Food industry
- Forencinc science

How do we get the data?

Genome sequencing: in the past and today

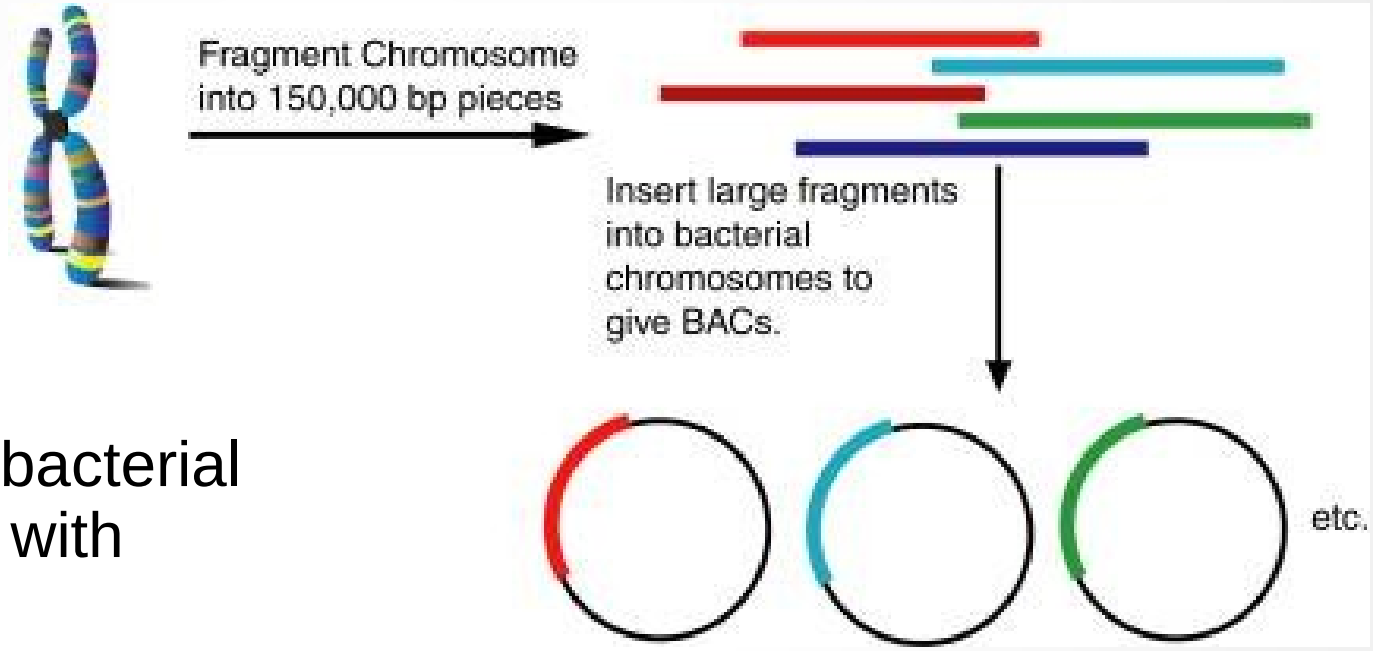
- Different strategies for genome sequencing:
  - In the past:
    - Clone based hierarchical sequencing (BAC – bacterial artificial chromosome – libraries)
    - Whole genom shutgun sequencing
  - Today:
    - Massively paralell Next Generation Sequencing (NGS)

Clone-by-clone vs. whole genome shotgun



Green ED (2001) Strategies for the systematic sequencing of complex genomes. Nat Rev Genet 2: 573–583

Clone based hierarchical sequencing (BAC to BAC)

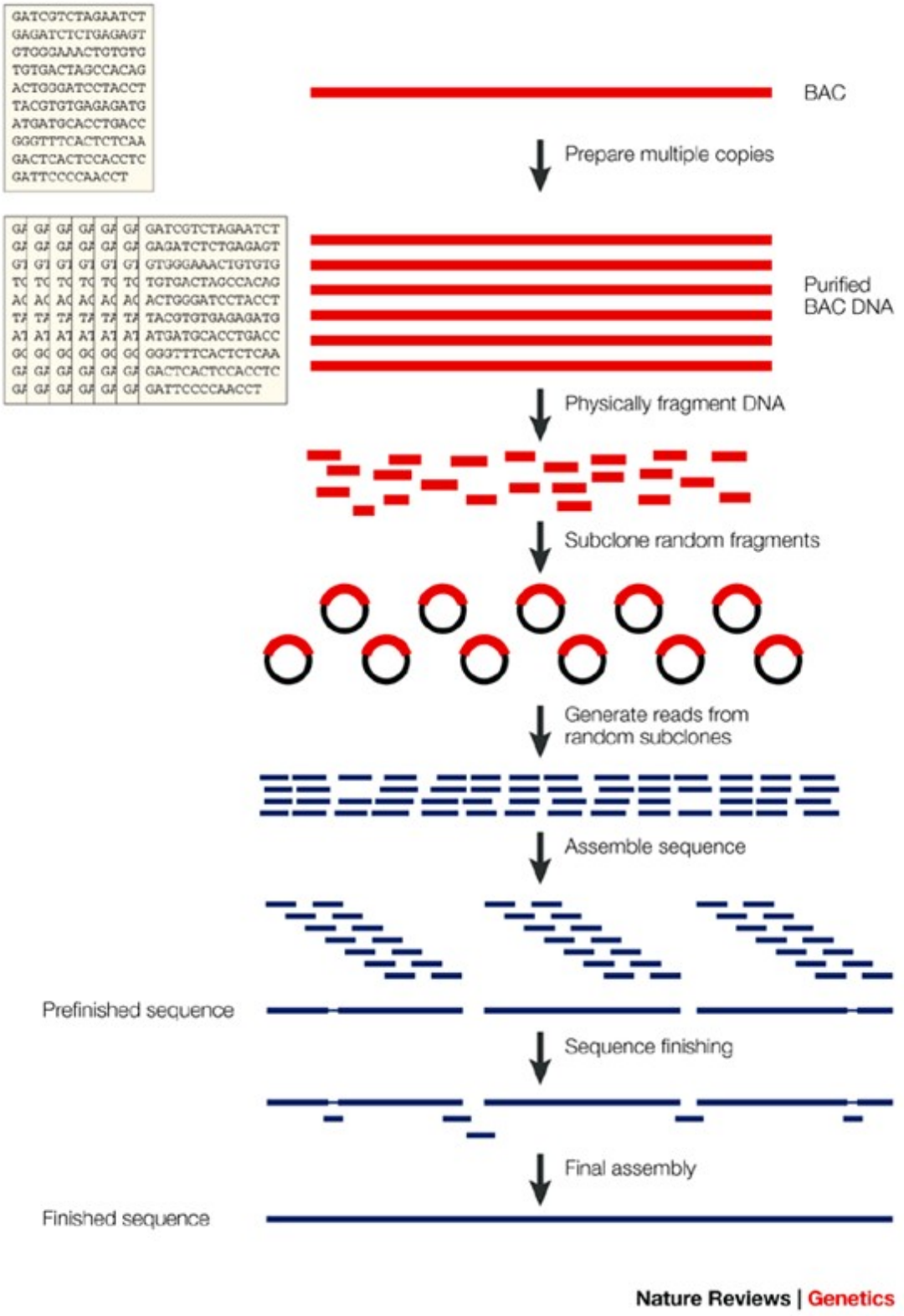


Sequencing viral and bacterial genomes was started with clone based method.

- The whole genome were cutted to ~40 - 150 kb overlapping pieces
  - Genomic location of each piece was determined (ie. using unique STS sties or FISH)
- Cloning – amplification (*E. coli*, BAC - Bacterial Artificial Chromosome - contigs)
  - BAC library: contains the whole genome of a species

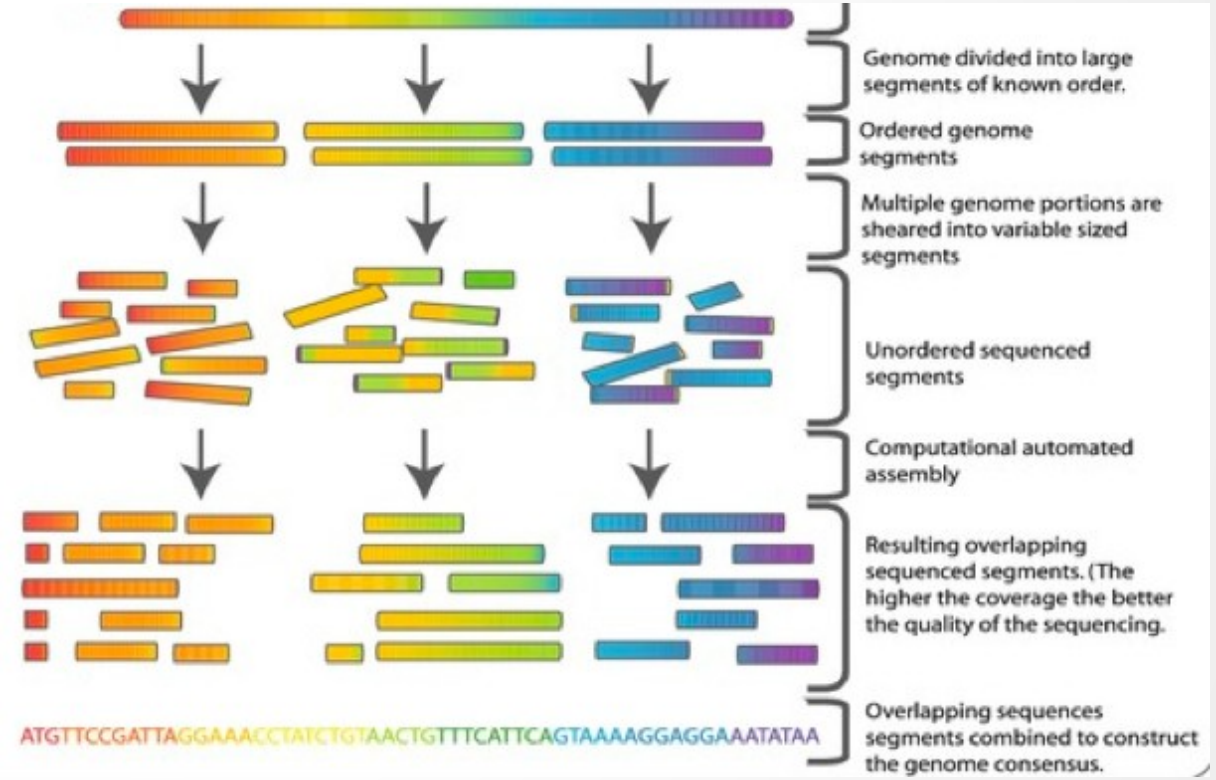
Clone based hierarchical sequencing

- Amplification
- Fragmentation
- Amplification: subclone libraries
- Reads from subclones



Clone based hierarchical sequencing

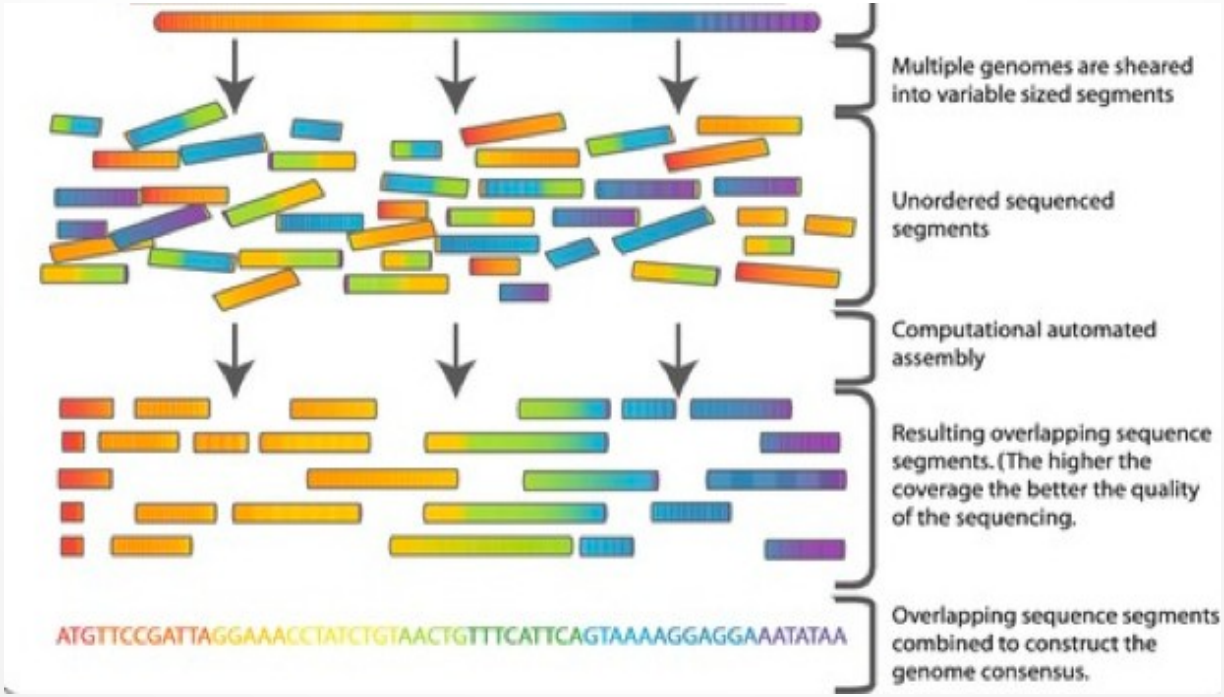
- Sanger sequencing
- Base calling:
  - Quality scores: PHRED
- Bioinformatics: genome assembly
  - PHARP software
  - Assembly the order of nucleotides of the BAC contigs based on the reads
  - Assembly the whole genome based on BAC contigs



http://www.phrap.org/phredphrapconsed.html

Whole genome shotgun sequencing - recent

- „Shutgun” breaking-up the whole genome (i.e pass through in a capillar)
  - 2 - 10 kilobase
  - Sequencing the pieces
- Assembly using computer
  - TIGR Assembler – first whole genome assembler software



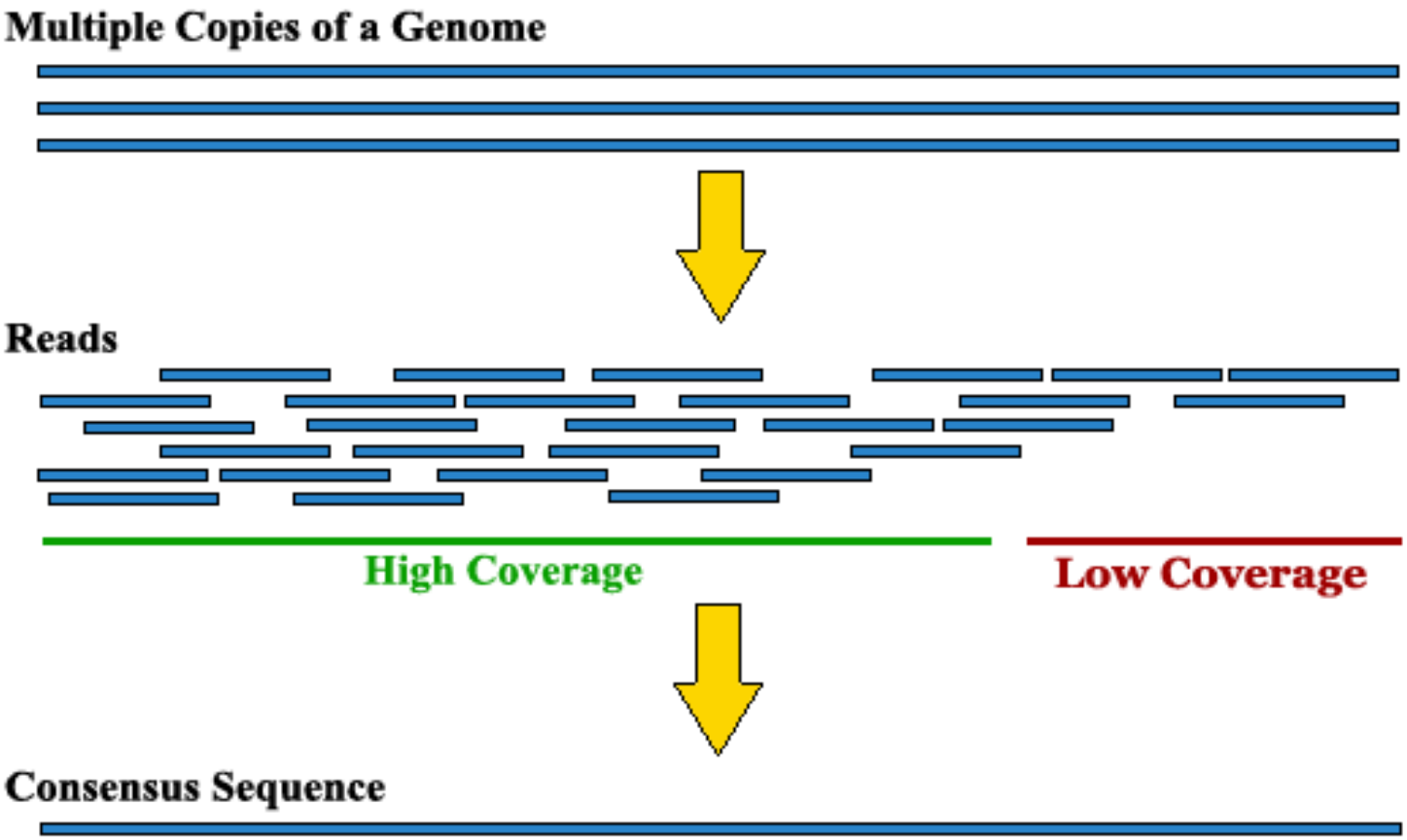
Celera
Craig Venter
1996

Comparison

- Clone based sequencing
  - Less chance to make errors during assembly
  - We know the place of the contigs for sure
  - Time consuming
  - Expensive
  - Less intensive computations: dealing with 100-200 Kb data at the same time
- Whole genom shutgun
  - More chance to make errors during assembly
  - We do not know the place of the comtigs
  - Fast
  - Less expensive
  - Computationally intensive: dealing with more Gb data at the same time

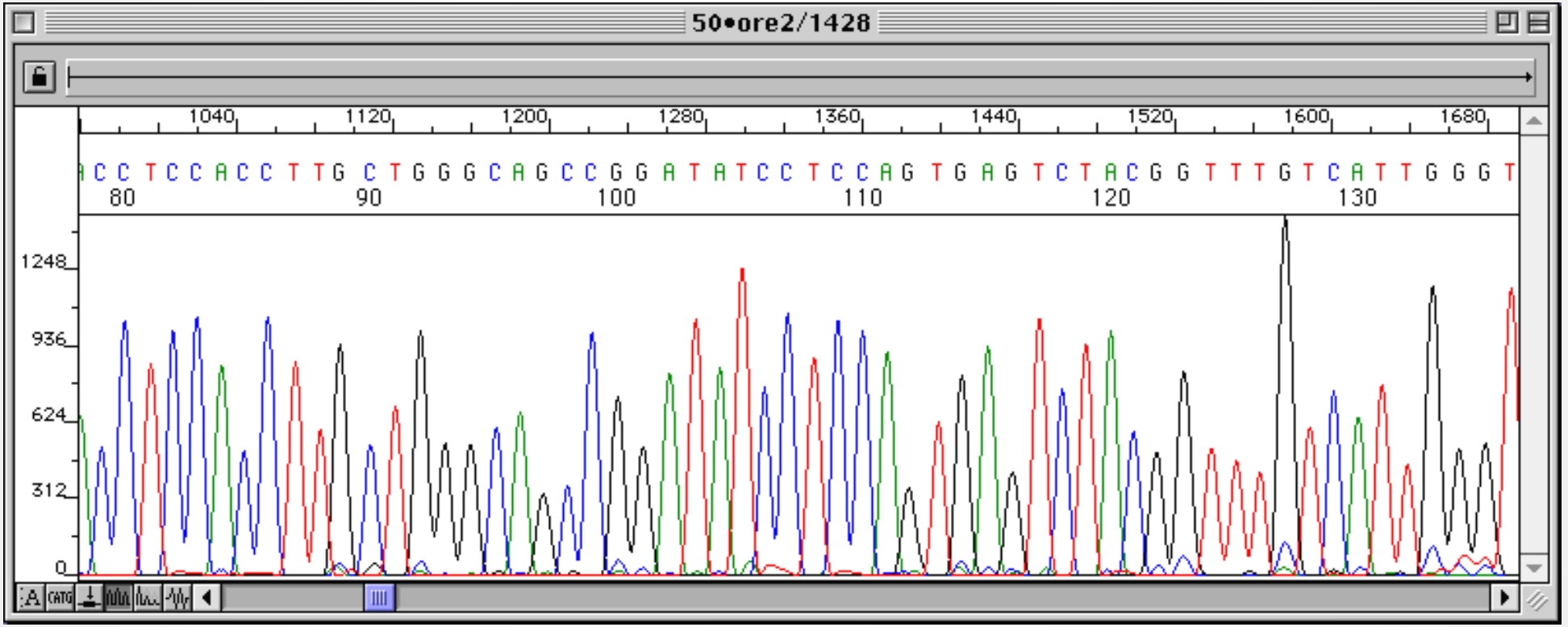
High coverage is needed

Coverage


Multiple Copies of a Genome
Reads
High Coverage
Low Coverage
Consensus Sequence

Chain-terminating Sanger sequencing

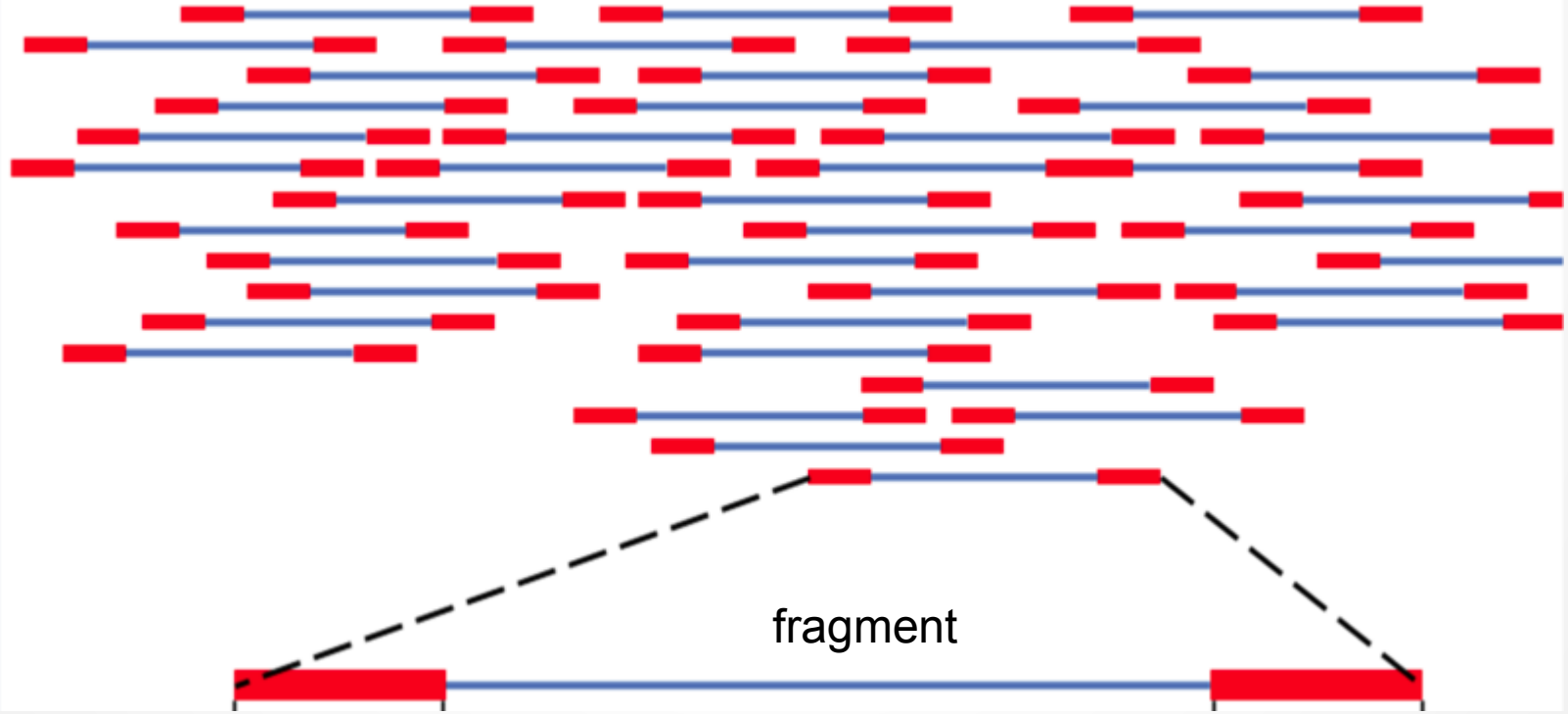
The dideoxynucleotides are fluorescently labeled for detection in automated sequencing machines. → Electropherogram



Read length: 900-1000 nucleotides

Next Generation Sequencing - NGS

- **High throughput (highly parallel), sequencing a lot of regions at the same time → fast, cheap**
- Sequencing the beginning (single end sequencing), or the beginning and the end (paired end seq.) of fragmnets.
- Sequencing 1 million DNA fragments at the same time

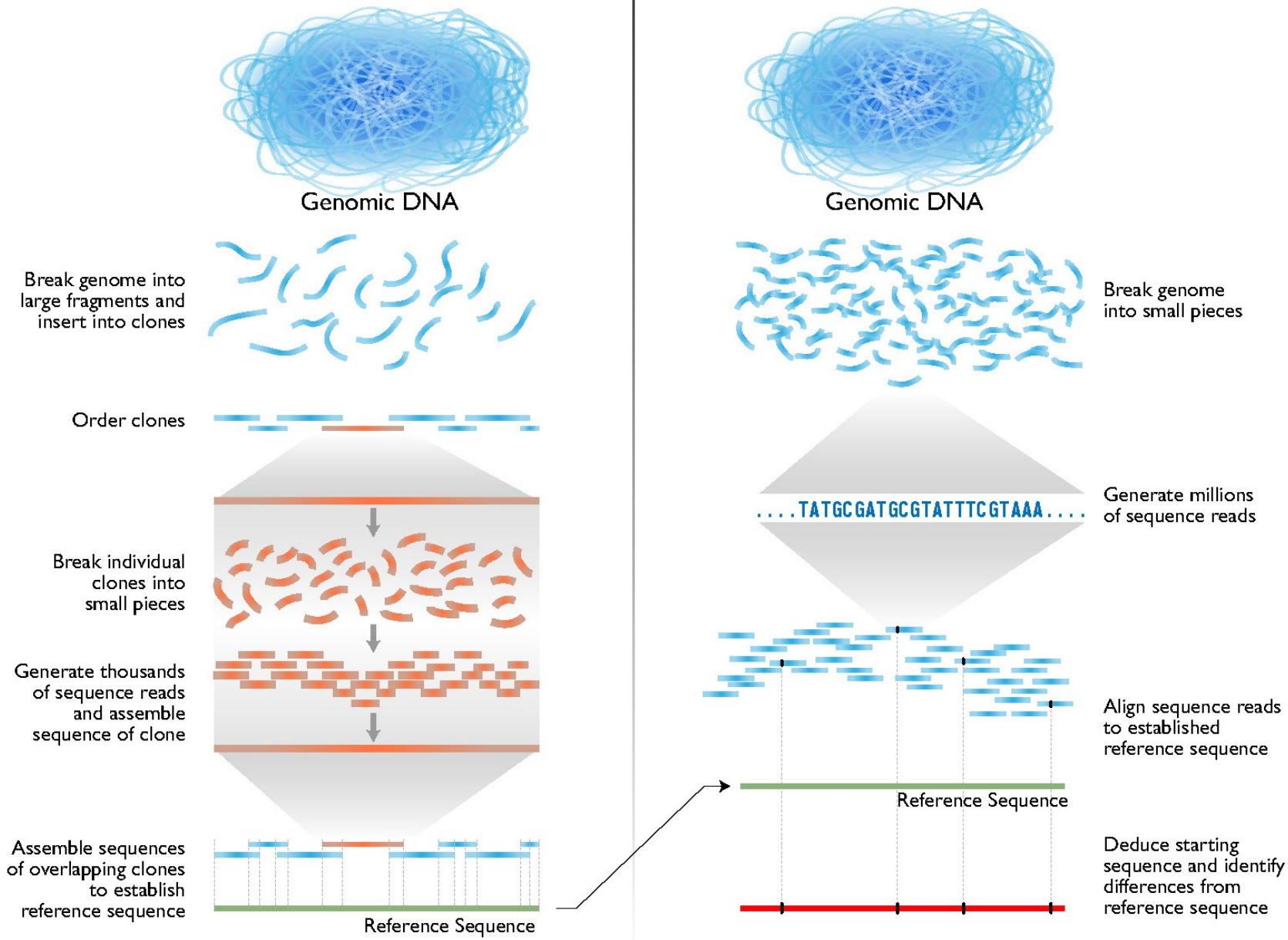


Next Generation Sequencing - NGS

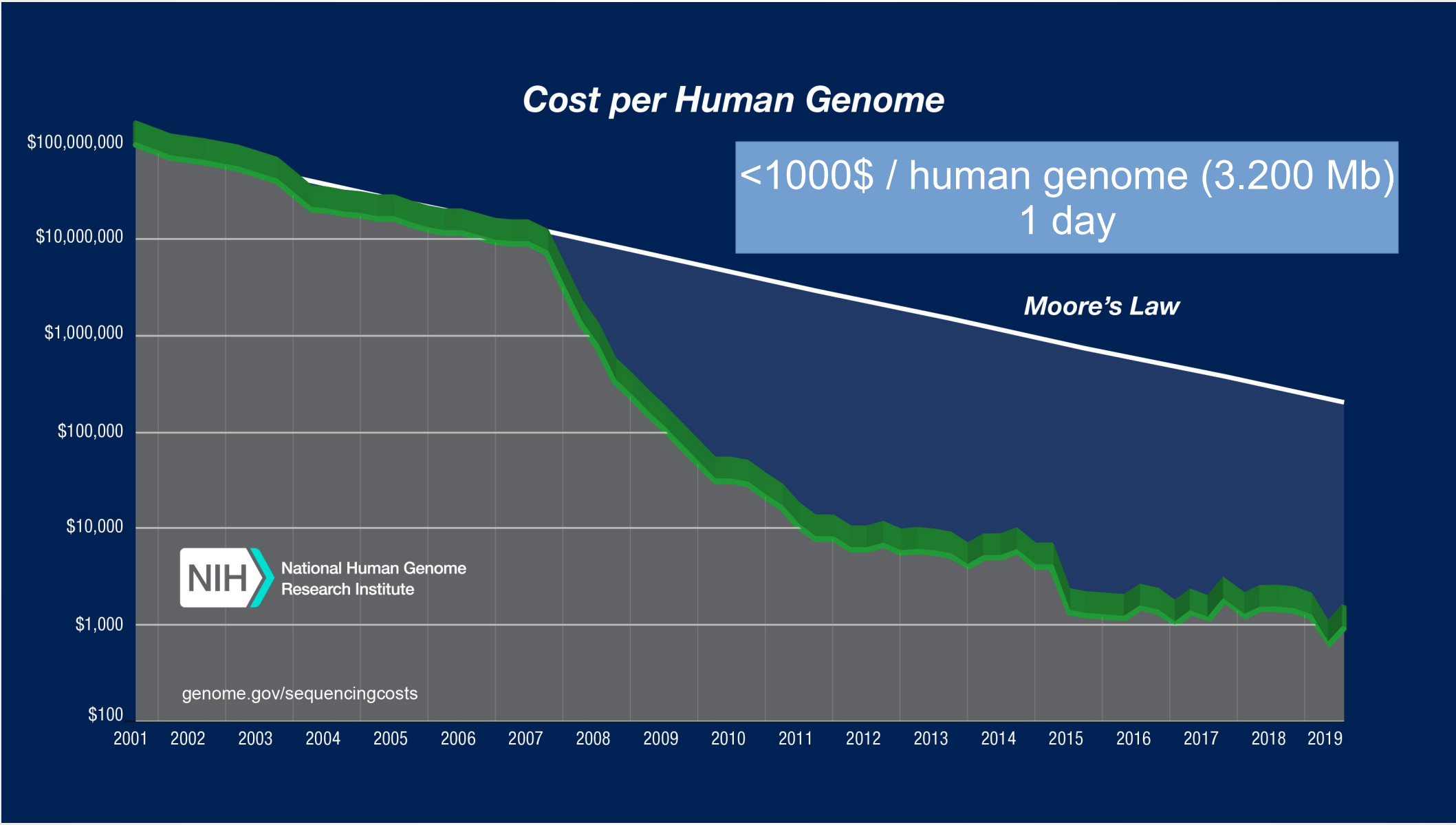
- Could be strand specific (forward, reverse)
- Methods (not based on Sanger sequencing):
  - Illumina (Solexa) sequencing
  - SOLiD sequencing
  - Ion Torrent sequencing
  - Pyrosequencing (454)
  - PacBio
  - Oxford nanopore
  - ...
- Read lengths: 50-700-thousands nts
- Million reads per day
  - Cost: 5 cent ~ 1 $ / 1.000.000 nt
- Sequnecing is fast (Human genome: a day), but the assembly is complicated and computationally intense

Human Genome Sequencing

1990-2006



Costs


Cost per Human Genome
<1000$ / human genome (3.200 Mb)
1 day
Moore's Law
$100,000,000
$10,000,000
$1,000,000
$100,000
$10,000
$1,000
$100
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
NIH
National Human Genome Research Institute
genome.gov/sequencingcosts

NGS instruments

Illumina HiSeq

Oxford Nanopore MinION

Illumina sequencing

Video
https://www.youtube.com/watch?v=HMyCqWhwB8E

Steps of genome analysis

1. Quality checking
2. Trimming: filter out low quality reads (or read parts)

3.a) Newly sequenced genome: *de novo* assembly

OR

3.b) Genome re-sequencing: mapping

4. Unfold genetic diversity: statistical analysis

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The reads

- Result of NGS: ie. fastQ file
  - quality checking (ie.: FastQC software)
  - trimming: filter out low quality reads (or read parts)

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De-novo genome assembly

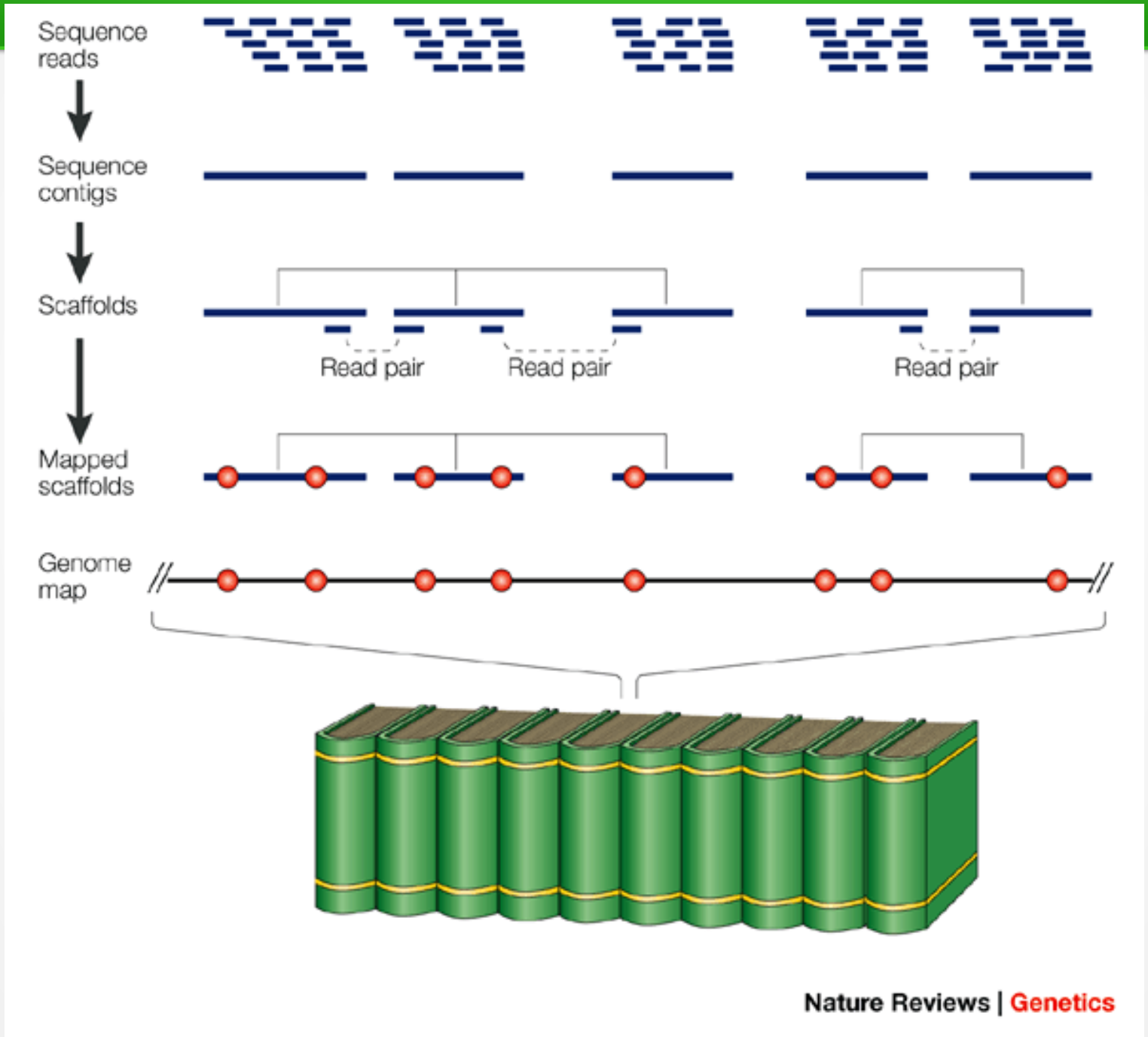
- Construction of the whole genome sequence based on reads
  - Among Eukaryotes the fruit fly genome was the first which was assembled purly by this method
  - Human genome: 2-3 billion reads (100X coverage)
- Gready algorithm:
  1. Pairwise alignment of all possible read pairs (based on sequence similarity)
  2. Merging the 2 reads that are the most similary – overlap the most
  3. Repeat step 2 till there are single reads
- Assembler softwares: ABySS, Celera WGA, Edna, Euler, MIRA, Newbler, SOAPdenovo, …
- Problem: we cannot check if the assembly was correcte – if the genome was newly sequenced
  - Causes of an incorrect assembly:
    - Repetitive regions – we should exclude these
    - Reads that aligned to a wrong place and/or in a wrong orientation

Aligning and non-aligning reads

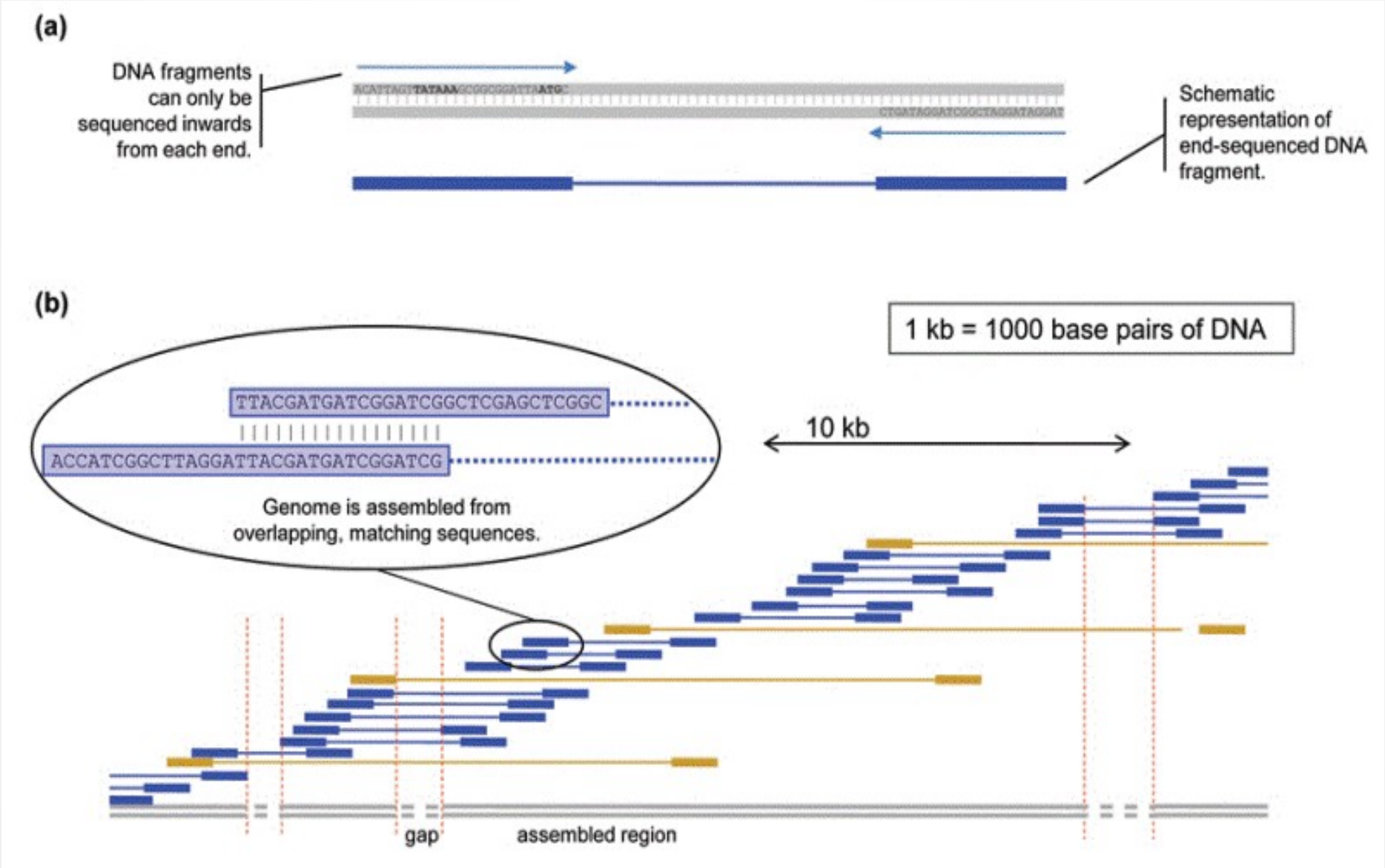
```
ATTGTGCTAGTCGTAGCTAGCT
          |||||||||||||||||||
          CTAGTCGTAGCTAGCTGTCAA

TGATGATGCTCTAAGATCTCAT
```

Genome assebly



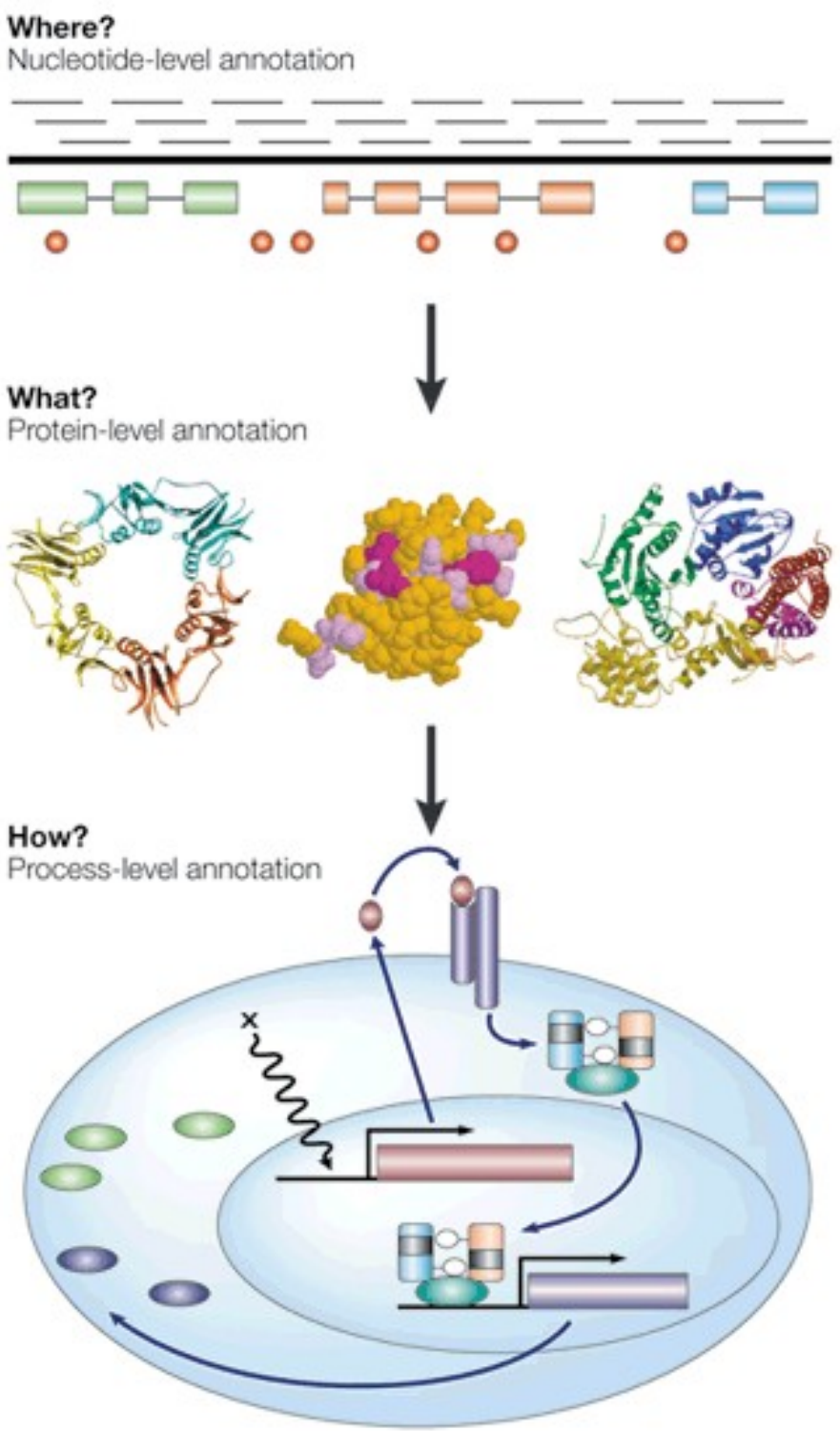
Genome assembly



Assemlby using different fragment sizes: blue - 5kb, yellow - 20kb

Genome annotation

- The process of finding and designating locations of individual genes and other features on raw DNA sequences
- Structural annotation:
  - Searching for ORFs
  - Gene structures (UTF, exon, intron...)
  - Promoter regions: based on motifs
- Functional annotation:
  - Biological functions of the ORFs (genes), ie. BLAST search
  - Gene expression data
  - Regulation networks...
- Annotation projects:
  - ENCyclopedia Of DNA Elements (ENCODE), Entrez Gene, Ensembl, GENCODE, Gene Ontology Consortium, GeneRIF, Uniprot, Vertebrate and Genome Annotation Project (Vega)



Stein L (2001) Genome annotation: from sequence to biology.
Nat Rev Genet 2: 493–503

Steps of genome analysis

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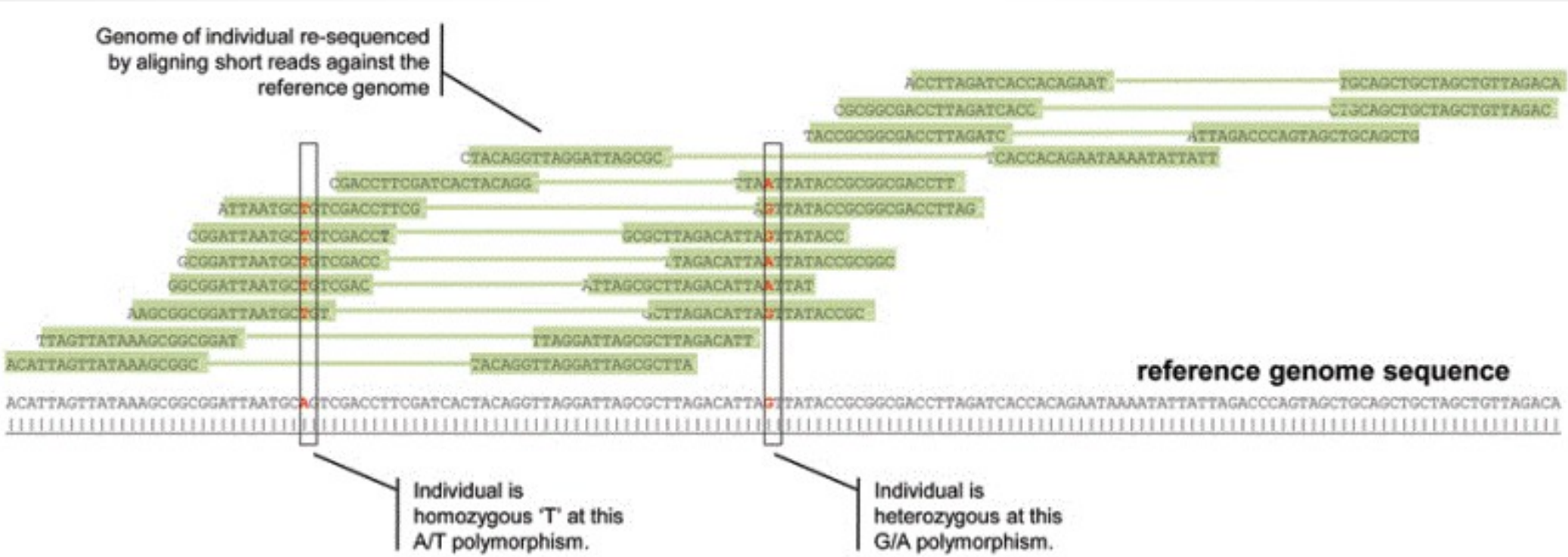
4. Unfold genetic diversity: statistical analysis

Re-sequencing



Re-sequencing

- Aim: Exploration of genetic diversity
- We map the reads to a known reference geneome
  - Less (but still intense) computation demand
  - genome variability can couse problems
  - Or even remain unobserved – ie. Chromosomal translocations
  - There can be biased or missing regions in the reference genome as well
- Mapping softwares: BWA (Burrow's Wheeler Transform Algorithm), Bowtie, GSNAP, SOAP2, …

Steps of genome analysis

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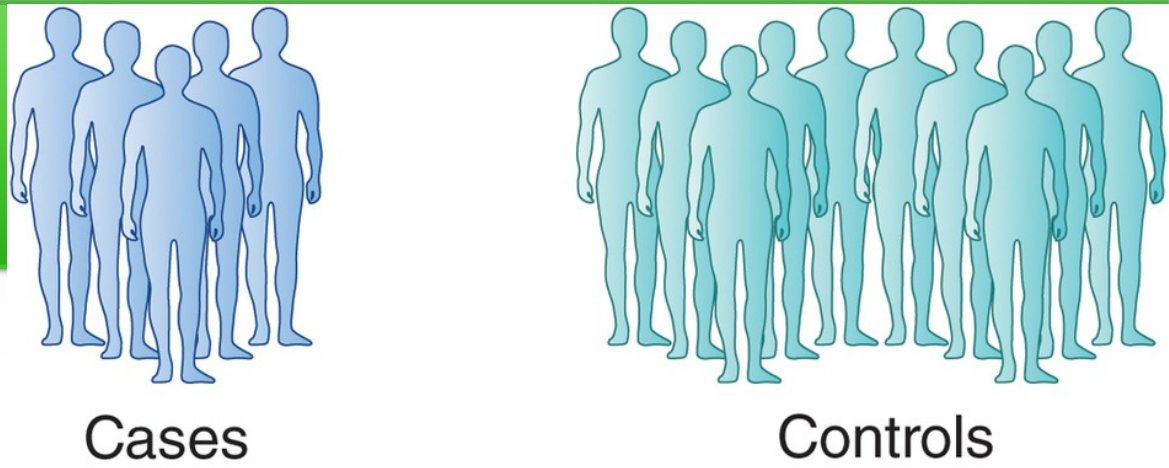
Exploring the genetic variability

- Genome differences between two individuals: ie. SNPs, in/dels, copy number variations, chromosome translocations
  - These can cause different phenotypes or diseases
- SNP analysis / GWAS: genome-wide association study
  - Study a genome-wide set of genetic variants in different individuals to see if any variant is associated with a trait (phenotype)
  - Mostly based on SNPs → allele frequencies
  - Traits: different phenotypes (ie. size or eye color of individuals) or genetic disorders

- Exploring the genetic variability
- SNP analysis
- GWAS: genome-wide association study



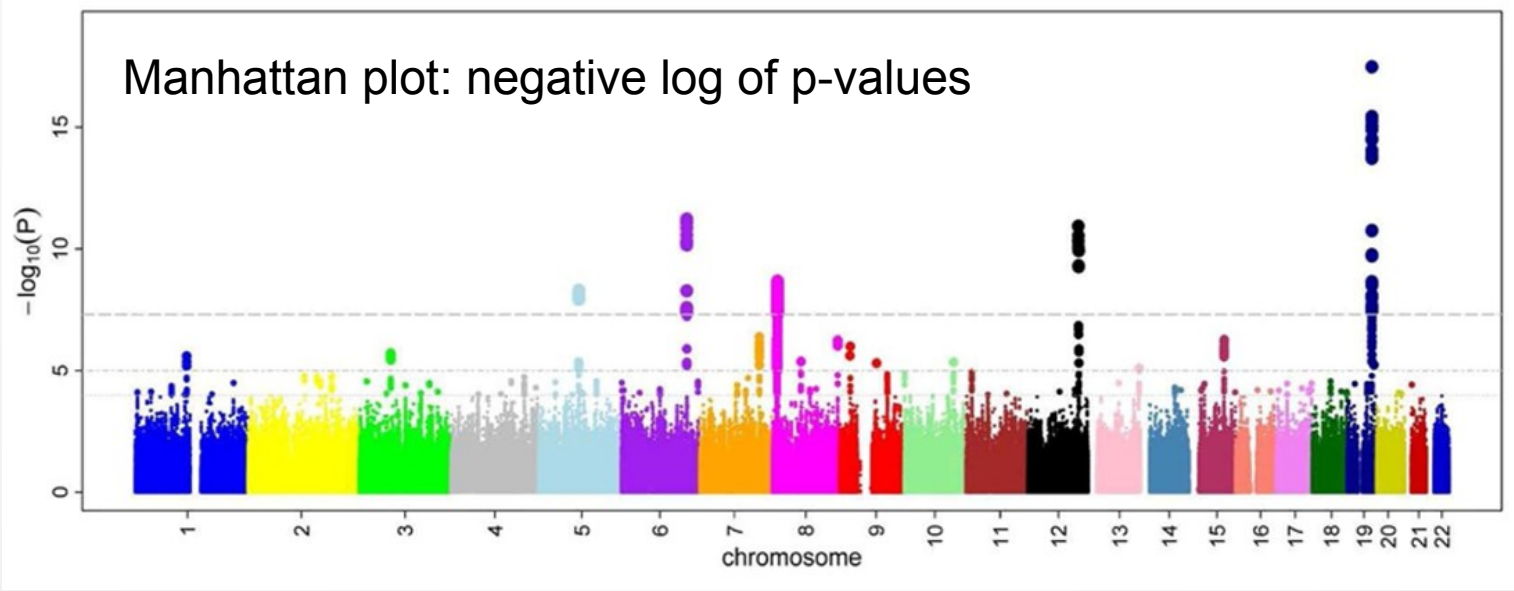
Register study

Collect saliva and blood for DNA extraction

GWAS and sequencing

Exploring the genetic variability

- If the phenotype is caused by a single SNP → it is easy to unfold
- If more than 1 SNP is playing some role to create the phenotype → we should involve many individuals
- We should choose individuals very carefully to exclude possible cofounding factors that would influence our investigation:
  - ie. gender, age, race of individuals, history of populations



Replicates

- Statistical definition: a fully repeated experiment or set of test conditions
- To calculate statistical tests we need more replicates
  - Replicates: samples got the same “treatment”
  - Depending on the investigation we need 2-3-100 replicates / treatment groups

Genome browsers

- Online, general:
  - http://www.ensembl.org/ 
  - https://genome.ucsc.edu/ 
  - http://www.ncbi.nlm.nih.gov/genome/ 
- Online, species specific:
  - Flybase, WormBase, …
- Offline:
  - Integrative Genomics Viewer (IGV)
  - Golden Helix GenomeBrowse, ...

Offline genome browser