Jasper Decuyper

# BIOINFORMATICS FOR DUMMIES

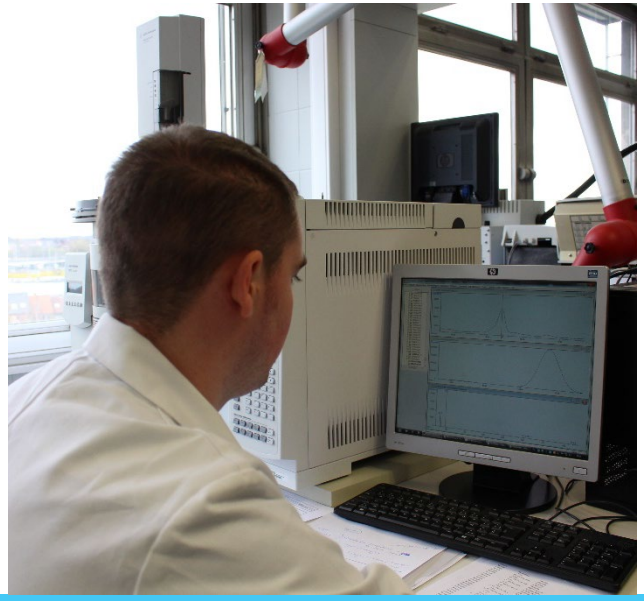
## MB&C2019 WORKSHOP

howest.be

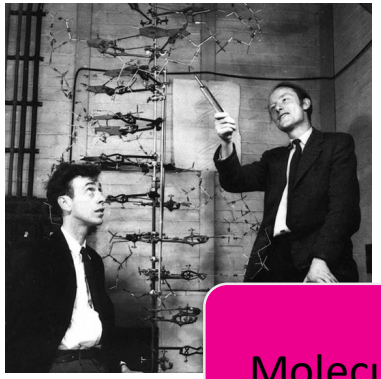
# INTRODUCTION

**Imagine your workspace without the computers…**

**Both in research laboratories and in hospitals…**



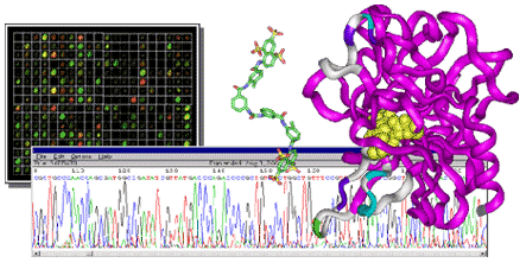
# INTRODUCTION



Molecular Biology


Information Technologies

**Bio-informatics**



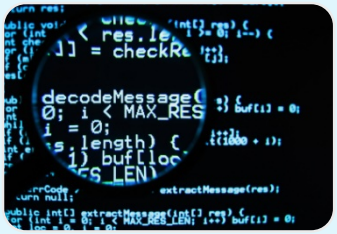
Combine:

- New insights and technologies in molecular biology
- Advances in information technologies

# INTRODUCTION

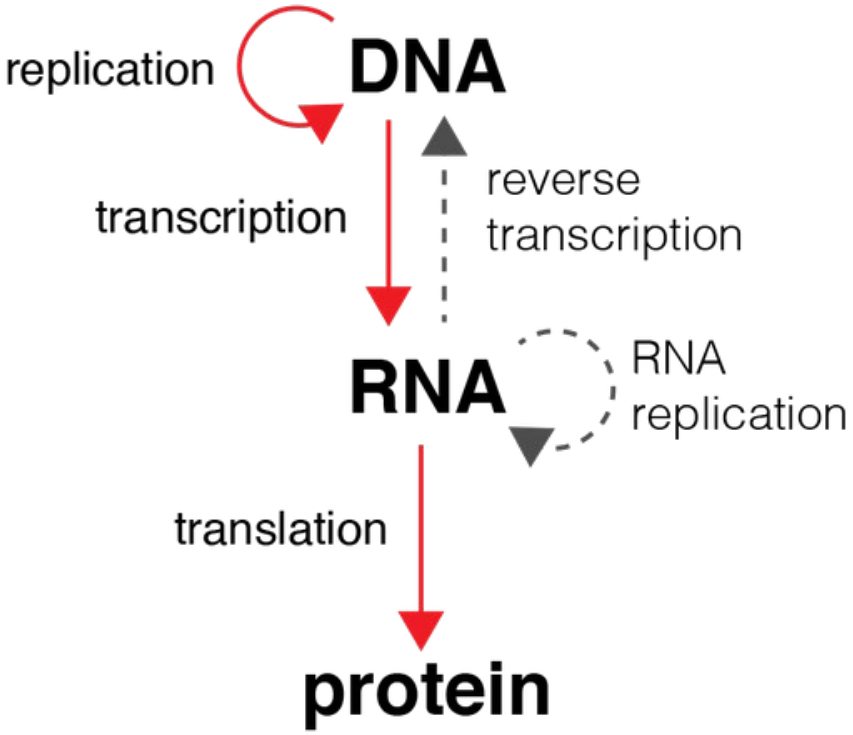
To store, organize and share molecular biological data in database systems

To process and analyse biological data by using bioinformatics tools in a “dry lab”



To integrate the different tools by means of scripting into a bioinformatics pipeline

# MOLECULAR BIOLOGY AND BIOINFORMATICS

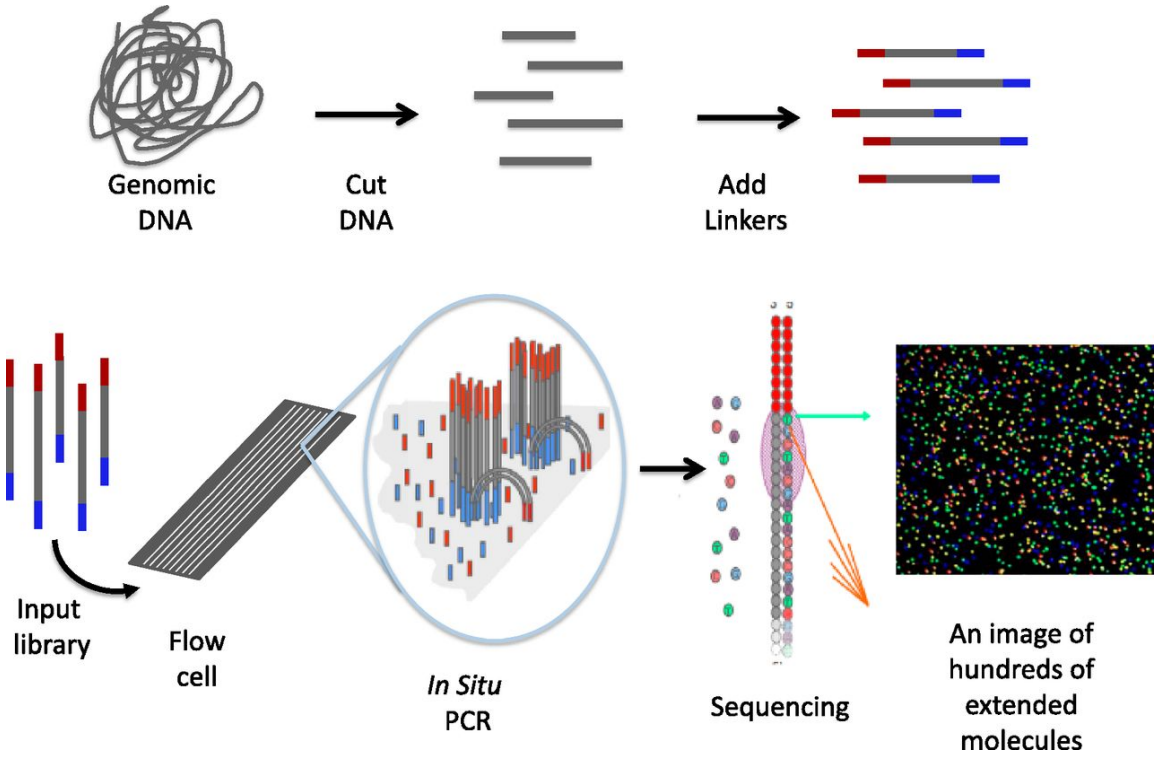



Important (high-throughput) technologies:

- Next Generation Sequencing
  - Sequencing and expression analysis
- Microarray
  - Expression and genetic variation analysis
- Mass spectrometry
  - Protein (sequence) identification

# NEXT GENERATION SEQUENCING

Johnsen, J. M., Nickerson, D. A. & Reiner, A. P. (2013). Massively parallel sequencing: the new frontier of hematologic genomics. *Blood*, *122*(19), 3268–3275.




## Short-read NGS

- 2 approaches:
  - → Sequencing by synthesis
  - → Sequencing by ligation
- 35-700 bp read length
- High accuracy (~ 99,99%)
- Complex assembly

NATURE REVIEWS | GENETICS VOLUME 17 | JUNE 2016 | 337

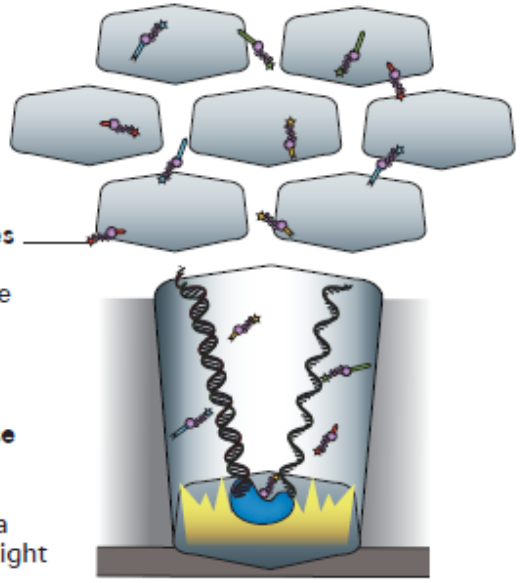
# NEXT NEXT GENERATION SEQUENCING

**SMRTbell template**
Two hairpin adapters allow continuous circular sequencing

**ZMW wells**
Sites where sequencing takes place

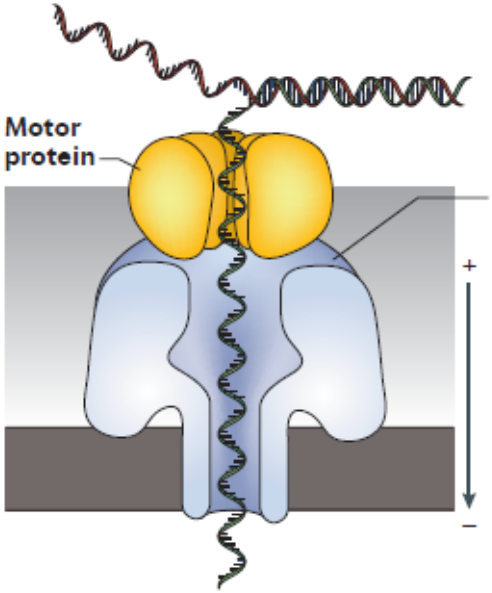
**Labelled nucleotides**
All four dNTPs are labelled and available for incorporation

**Modified polymerase**
As a nucleotide is incorporated by the polymerase, a camera records the emitted light



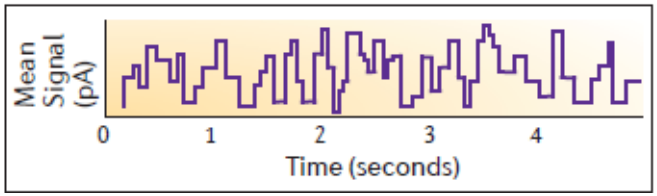
**PacBio output**
A camera records the changing colours from all ZMWs; each colour change corresponds to one base

**Leader–Hairpin template**
The leader sequence interacts with the pore and a motor protein to direct DNA, a hairpin allows for bidirectional sequencing



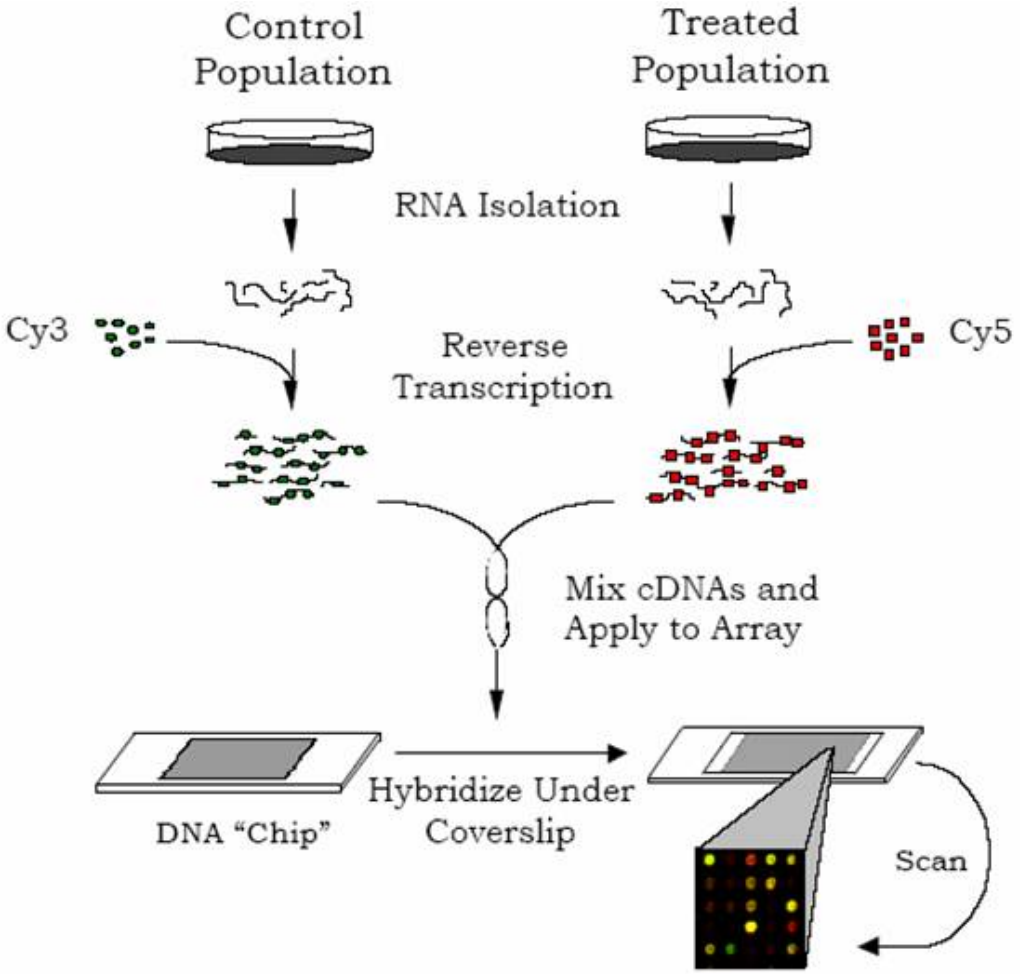

**Alpha-hemolysin**
A large biological pore capable of sensing DNA

**Current**
Passes through the pore and is modulated as DNA passes through

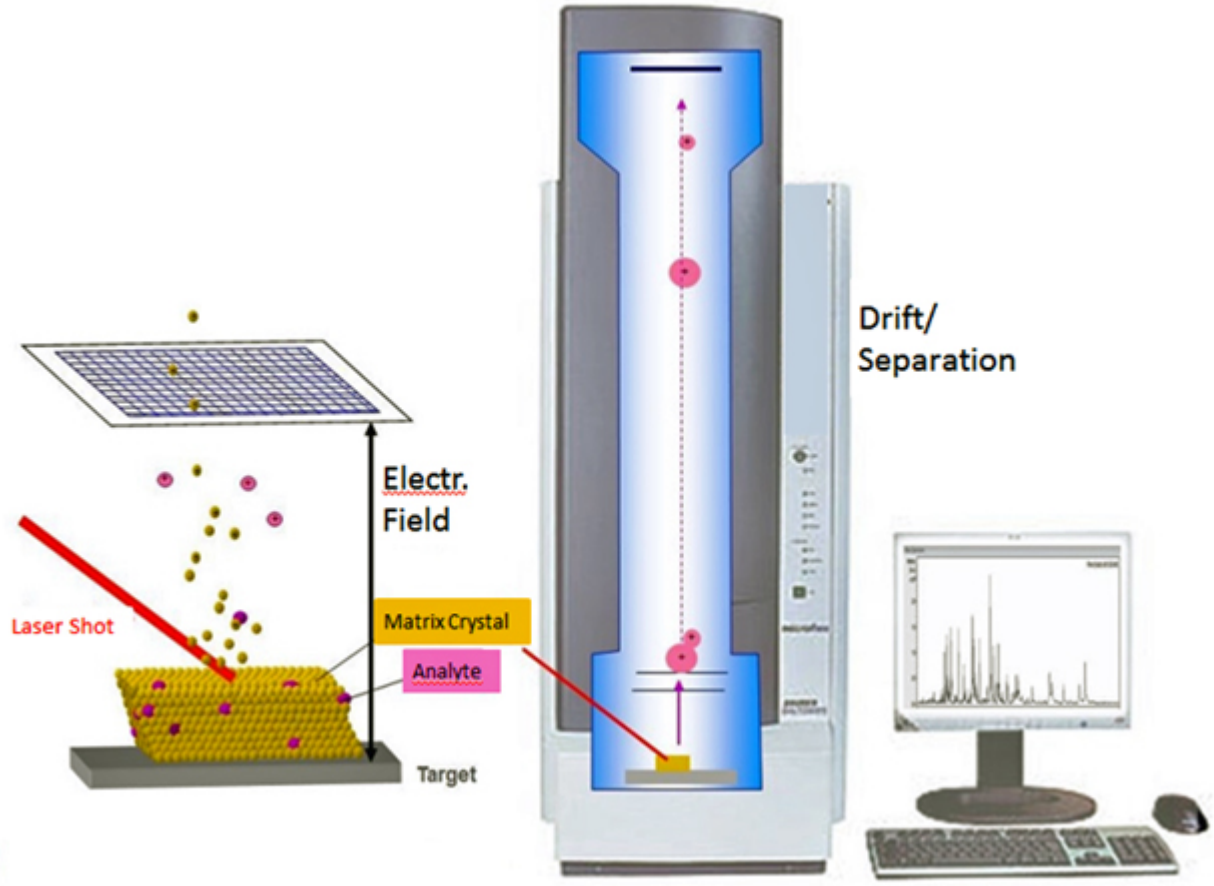



**ONT output (squiggles)**
Each current shift as DNA translocates through the pore corresponds to a particular k-mer

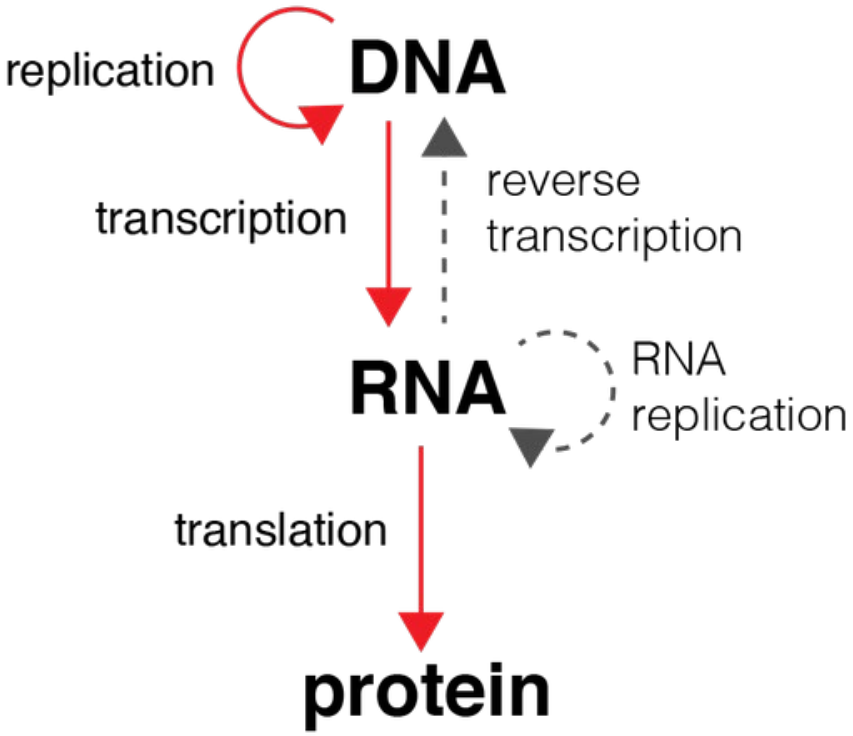
# MICROARRAYS



# MASS SPECTROMETRY



# MOLECULAR BIOLOGY AND BIOINFORMATICS

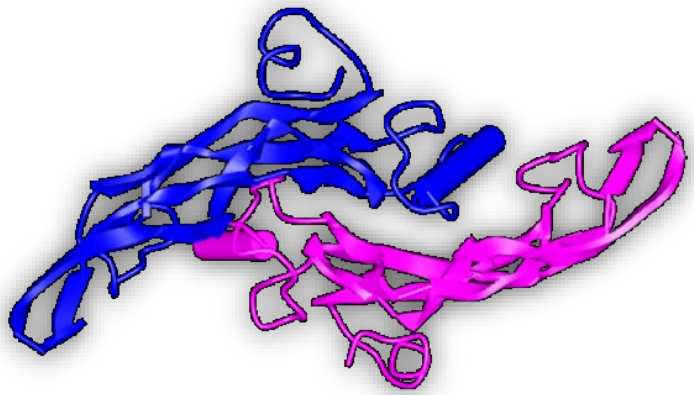



Biological databases:

- DNA
  - → Sequence and loci
  - → (Natural) genetic variation
- RNA
  - → Transcripts (and variants)
  - → Gene expression
- Protein
  - → Sequence and function
  - → Phenotype (and diseases)

## (SEQUENCE) REPOSITORIES

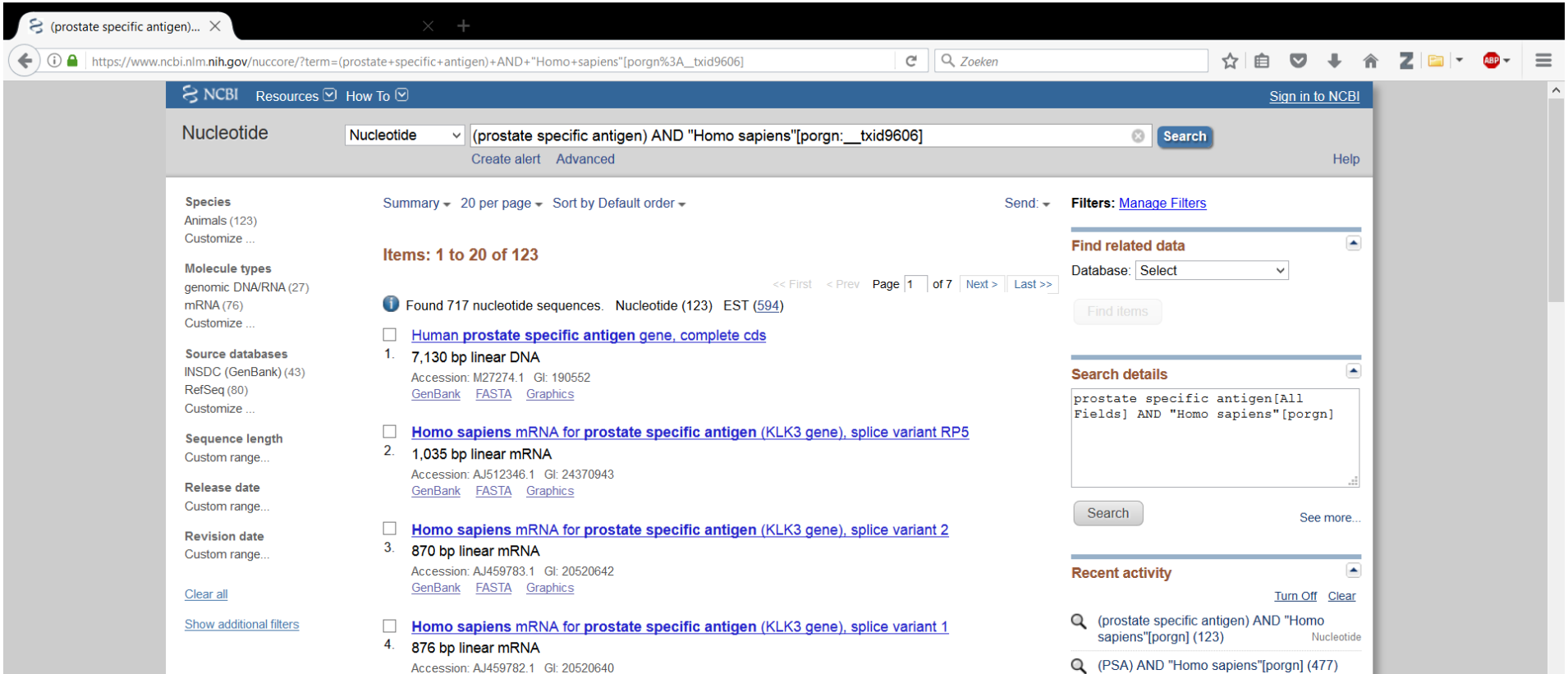
- Exploratory example: **TGF beta 1** – an important protein involved in cell proliferation, differentiation and growth



| | |
|---|---|
| NCBI Gene | • https://www.ncbi.nlm.nih.gov/gene/7040<br>• General and integrated sequence and locus information |
| NCBI Nucleotide | • https://www.ncbi.nlm.nih.gov/nuccore/?term=TGFB1+AND+"Homo+sapiens"[Organism]<br>• All available (partial) TGF beta 1 nucleotide sequences → ± 135 records (!) |
| NCBI UniGene | • https://www.ncbi.nlm.nih.gov/UniGene/clust.cgi?UGID=2394304<br>• Transcripts and gene expression (EST Profile) information |
| UniProt or NCBI Protein | • http://www.uniprot.org/uniprot/P01137<br>• High-quality recourse of protein sequence and functional information |

# (SEQUENCE) REPOSITORIES

- Example 1: **looking for the nucleotide sequence of PSA**
- https://www.ncbi.nlm.nih.gov/nucleotide/
- NCBI nucleotide query: “(prostate specific antigen)” restricted to humans



# (SEQUENCE) REPOSITORIES

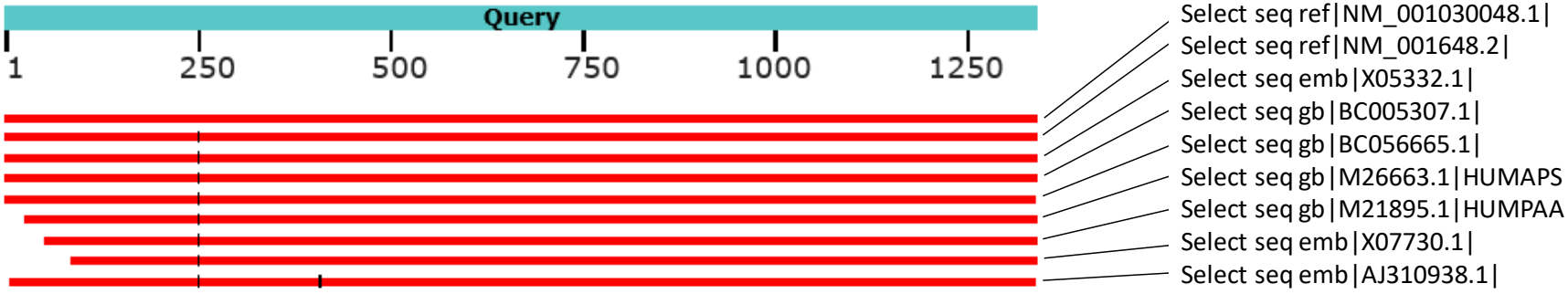
- Example 2: **the 1000 Genomes project** (2008-2015)
- Goal = to find most genetic variants with frequencies of at least 1% in the populations studied

- ACGTACGTACGTACGTACGTACGT
- ACGTACCTACGTACGTACGTACGT
- ACGTACCTACGTATGTTCGTACGT
- ACGTACGTACGTATGTTCGTACGT

## (SEQUENCE) REPOSITORIES

Solution for genetic and sequence diversity:

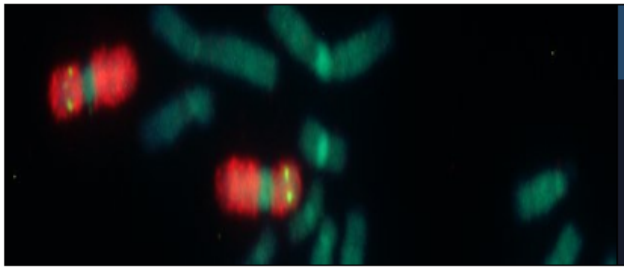
- **Genome Reference Consortium (GRC)**: to create the best possible reference assembly for human → latest major release: GRCh38
  - https://www.ncbi.nlm.nih.gov/grc/human
- **NCBI Reference Sequence Database (RefSeq)**: a non-redundant, well-annotated set of reference sequences incl. genomic, transcript, and protein
  - https://www.ncbi.nlm.nih.gov/refseq/
  - One gene – one sequence



NCBI Resources How To Sign in to NCBI

Gene Gene Search

Advanced Help



## Gene

Gene integrates information from a wide range of species. A record may include nomenclature, Reference Sequences (RefSeqs), maps, pathways, variations, phenotypes, and links to genome-, phenotype-, and locus-specific resources worldwide.

### Using Gene

- Gene Quick Start
- FAQ
- Download/FTP
- RefSeq Mailing List
- Gene News
- Factsheet

### Gene Tools

- Submit GeneRIFs
- Submit Correction
- Statistics
- BLAST
- Genome Workbench
- Splign

### Other Resources

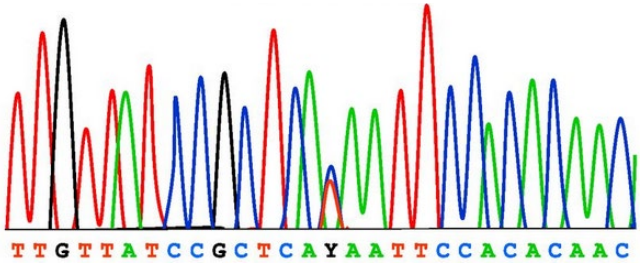
- HomoloGene
- OMIM
- RefSeq
- RefSeqGene
- UniGene
- Protein Clusters

### Representative queries

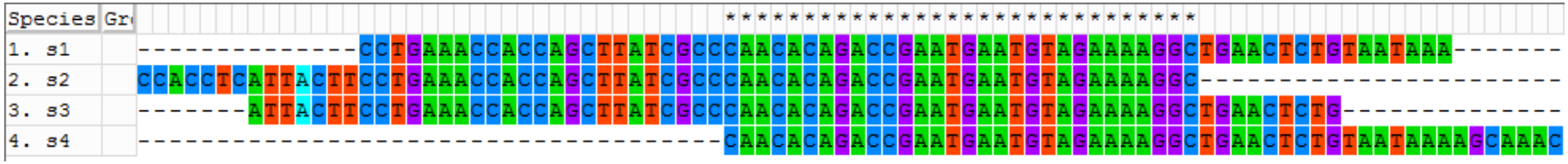
| Find genes by... | Search text |
|---|---|
| free text | human muscular dystrophy |
| chromosome and symbol | (II[chr] OR 2[chr]) AND adh*[sym] |

BEST PRACTICE - on how to find a reference sequence

## HOMOLOGY SEARCHING

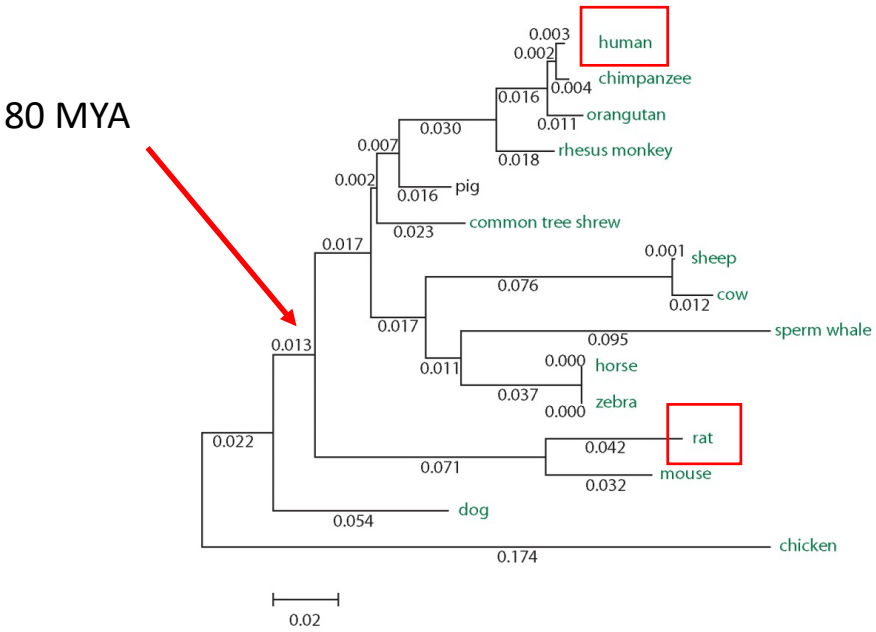



- Next Generation Sequencing
  - Result = **unknown nucleotide sequences**
- Determination of sequence identity ≠ simple keyword search strategy
- Instead: usage of <u>evolutionary model</u> to determine <u>homology</u> between nucleotide (or protein) sequences




- Based on sequence alignment
- **BLAST**: Basic Local Alignment Search Tool

# HOMOLOGY SEARCHING




## Homology

- Derived from a common ancestor
- 2 types:
  - → Orthologs = due to speciation event
  - → Paralogs = due to duplication event
- Typically based on morphological characteristics
- Making use of “molecular phylogeny” to determine homology

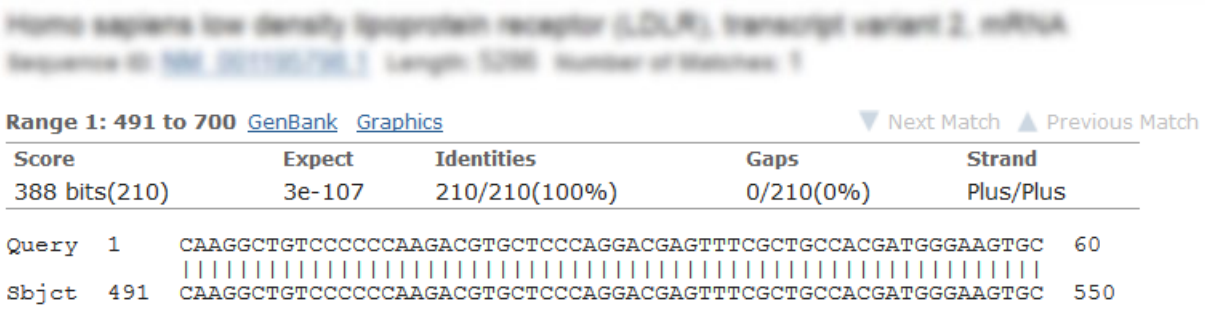
# HOMOLOGY SEARCHING

CAAGGCTGTCCCCCCAAGACGTGCTCCCAGGACGAGTTTCGCTGCCACGATGGGAAGTGCATCTCTCG
GCAGTTCGTCTGTGACTCAGACCGGGACTGCTTGGACGGCTCAGACGAGGCCTCCTGCCCGGTGCTCA
CCTGTGGTCCCGCCAGCTTCCAGTGCAACAGCTCCACCTGCATCCCCCAGCTGTGGGCCTGCGACAAC

- Given = an unknown human nucleotide sequence
  → https://www.bioit.be > "unknown human nucleotide sequence.fasta"

- To determine the identity → use BLAST
  - Against the *Homo sapiens* RefSeq RNA database, exclude models
  - https://blast.ncbi.nlm.nih.gov/Blast.cgi


- Identity?
- Bits score?
- Expect value?


Range 1: 491 to 700 GenBank Graphics ▼ Next Match ▲ Previous Match

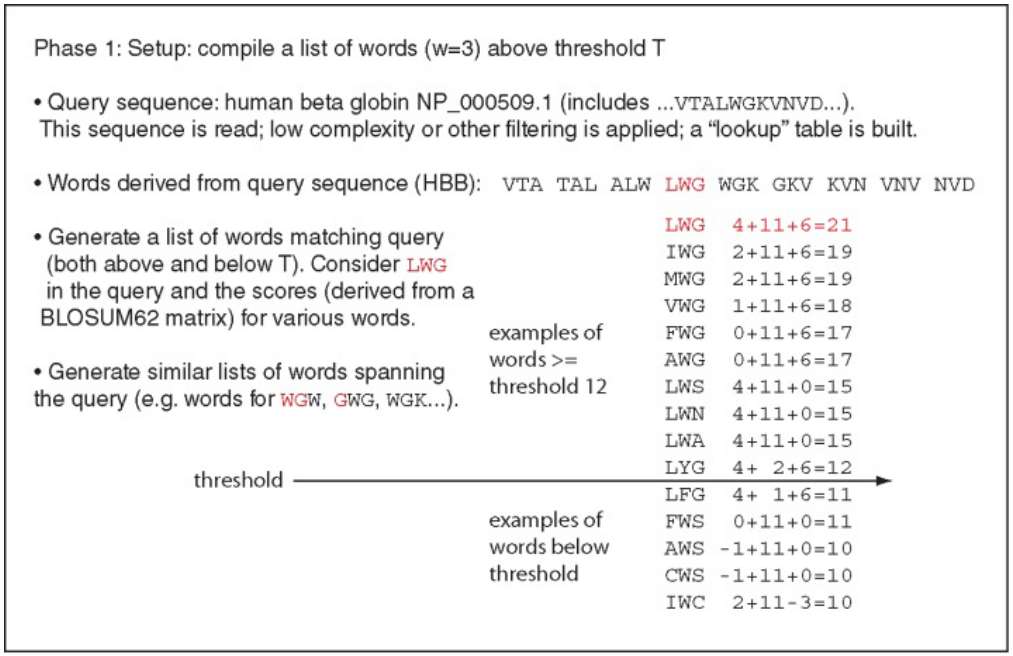
| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 388 bits(210) | 3e-107 | 210/210(100%) | 0/210(0%) | Plus/Plus |

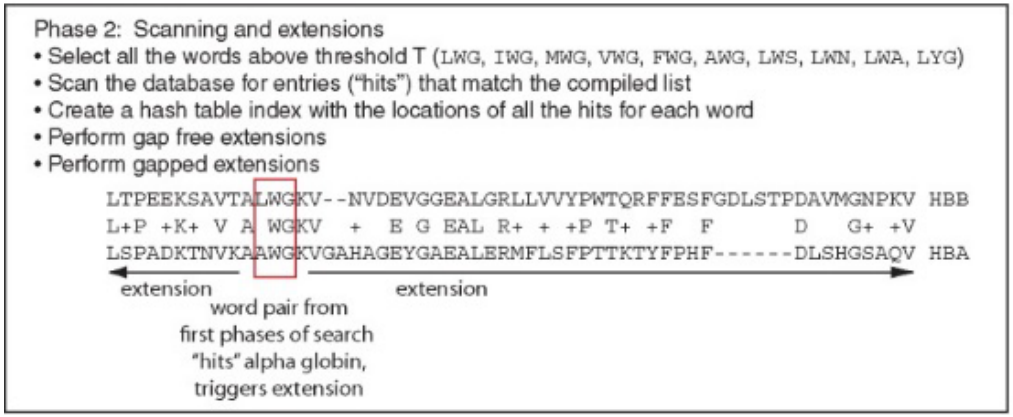
```
Query  1    CAAGGCTGTCCCCCCAAGACGTGCTCCCAGGACGAGTTTCGCTGCCACGATGGGAAGTGC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  491  CAAGGCTGTCCCCCCAAGACGTGCTCCCAGGACGAGTTTCGCTGCCACGATGGGAAGTGC  550
```

## HOMOLOGY SEARCHING

### BLAST

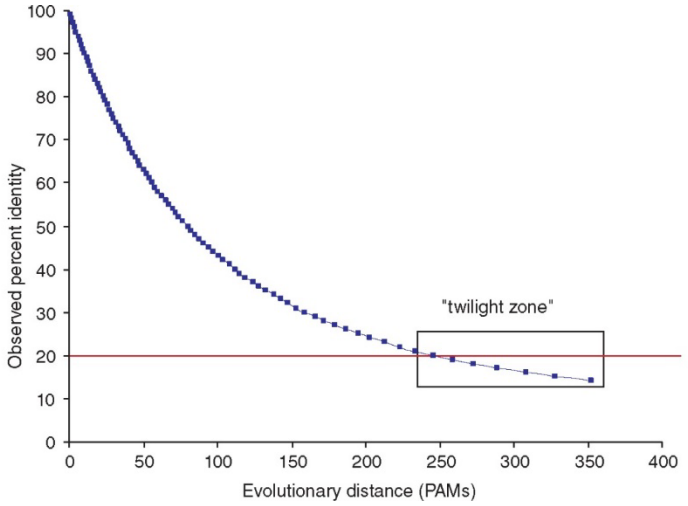
- ≠ simple keyword search strategy
- 3 steps:
  - → LIST
  - → SCAN
  - → EXTEND
- Based on a model of evolution and scoring system

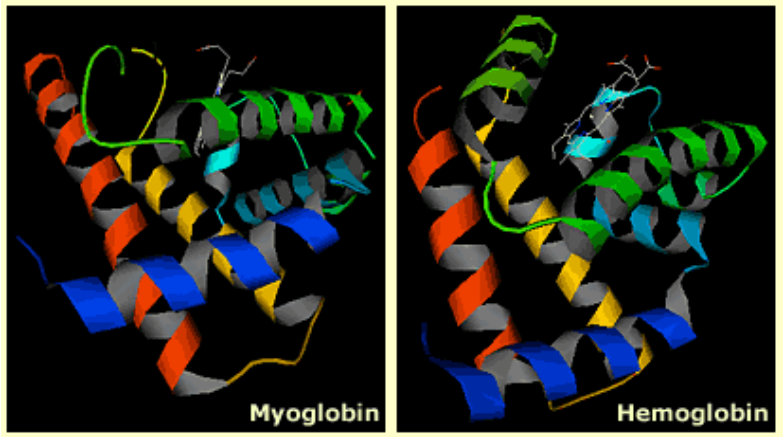


## HOMOLOGY SEARCHING

- Are two sequences homologous?
  - Percent identity (quantitative)
    + Expect value
- While homology = YES or NO question !!




Example: is it possible to predict that human **myoglobin** (NP_005359) and **beta hemoglobin** (NP_000509) are paralogs?

## DNA VARIANT ANALYSIS

Compare nucleotide sequence with a reference sequence

- **Nucleotide diversity** → DNA variant identification

- Example: nucleotide diversity in multiple hemoglobin beta variants
  - https://www.bioit.be > "HBB multiple sequence alignment.fasta"
  - Align sequences using MUSCLE software (www.ebi.ac.uk/Tools/msa/muscle/), output = HTML

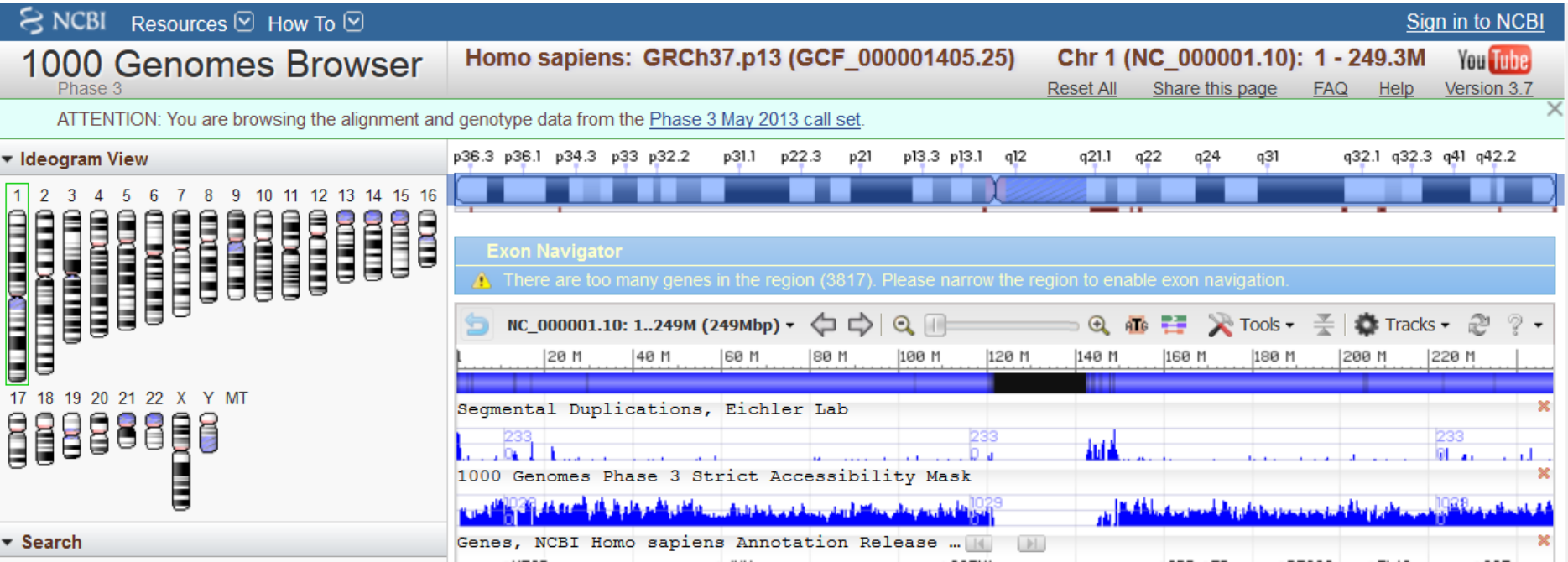
Multiple sequence alignment (MSA)

→ Phylogenetic analysis

# DNA VARIANT ANALYSIS

Browsing genetic variations:

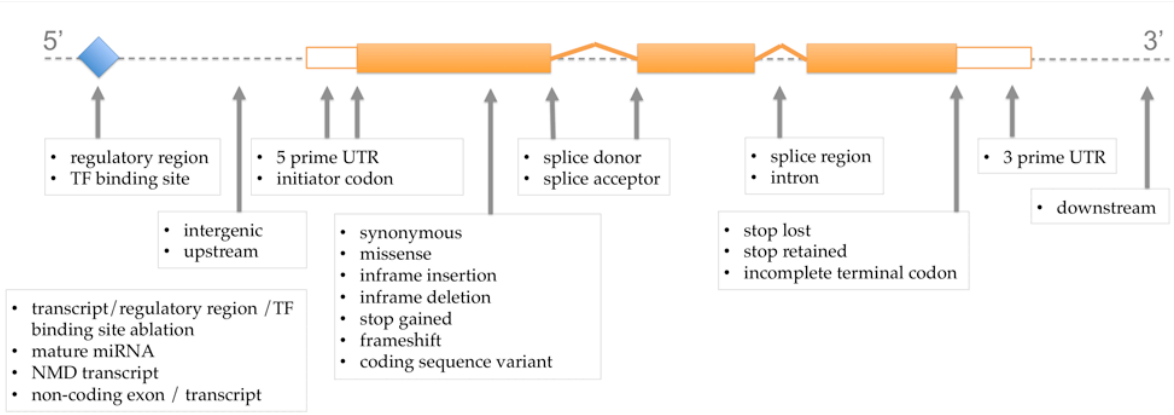
- Natural genetic variation → the 1000 Genomes Browser and/or Variation Viewer *(→ BRCA1?)*
  - https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/
  - https://www.ncbi.nlm.nih.gov/variation/view/
- The database of short genetic variation → NCBI dbSNP *(→ BRCA1?)*



# DNA VARIANT ANALYSIS

Genetic variation → **effect** on protein structure/function?

- Depends on the location of the mutation/variation:




- Make use of PROVEAN or SIFT (**s**orts **i**ntolerant **f**rom **t**olerant) score for amino acid substitutions:

| Uploaded variant | Location | Allele | Consequence | Impact | Symbol | Gene | Biotype | Exon | Intron | Amino acids | Codons | SIFT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| rs33958637 | 11:5225717-5225717 | C | missense_variant | MODERATE | HBB | 3043 | protein_coding | 3/3 | - | N/D | AAC/GAC | 0.85 |
| rs33958637 | 11:5225717-5225717 | G | missense_variant | MODERATE | HBB | 3043 | protein_coding | 3/3 | - | N/H | AAC/CAC | 0 |
| rs576852971 | 11:5226131-5226131 | G | intron_variant | MODIFIER | HBB | 3043 | protein_coding | - | 2/2 | - | - | - |

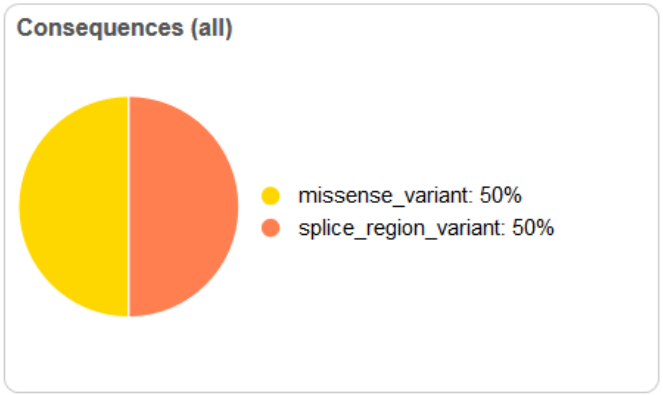
*Variant table @ Ensembl genome browser*

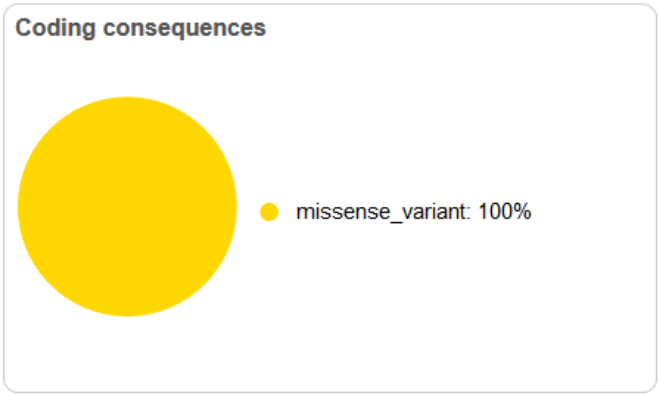
# DNA VARIANT ANALYSIS

Genetic variation → **effect** on protein structure/function?

- Variant Effect Predictor (https://www.ensembl.org/Homo_sapiens/Tools/VEP)

| Category | Count |
|---|---|
| Variants processed | 1 |
| Variants filtered out | 0 |
| Novel / existing variants | 0 (0.0) / 1 (100.0) |
| Overlapped genes | 1 |
| Overlapped transcripts | 1 |
| Overlapped regulatory features | - |




- Example: investigate rs13306510
  - Look up the SNP in the dbSNP database
  - Examine the SNP with the Variant Effect Predictor

## CONCLUDING REMARKS

- Bioinformatics is more than sequence alignment, BLAST and variant calling …

- Interested in more?



**@home**

The programme of the advanced bachelor training in **distance training** is the same as in full-time daytime education, but is built up differently and spread over **two academic years**.

Jasper Decuyper

# BIOINFORMATICS FOR DUMMIES

## MB&C2019 WORKSHOP

howest.be