Université de Lille

Gene annotation

Adapted from the courses of the Bonsai team,

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Introduction

Challenge

- The constant **decrease** in **sequencing costs** makes it increasingly easy to obtain the sequence of the genome of a species

- However, in many respects, **genome annotation** has become more difficult!

  - The NGS **short reads** (Illumina) do not allow to obtain the **quality of assembly** of the first genomes (Drosophila, human, Arabidopsis...) obtained using Sanger technology

  - Genome sequencing projects with **unusual characteristics** and without prior data

  - Genome sequencing projects are now done "in house", by biologists who sometimes have **little bioinformatics skills**

Challenge

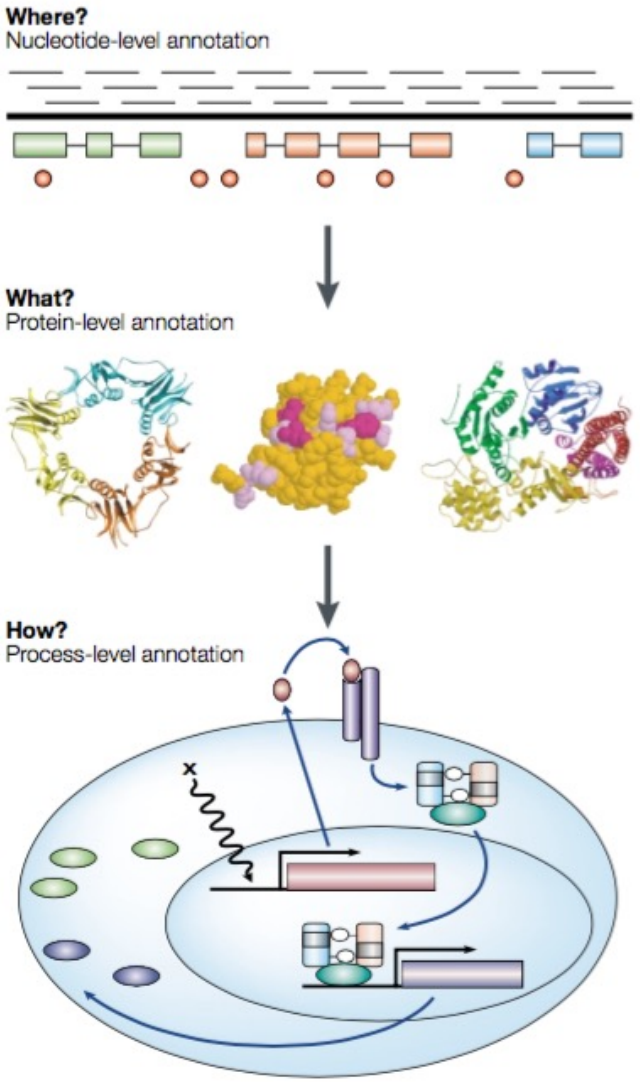


Figure 1 | **The three layers of genome annotation: where, what and how?**

- **Objective of the lesson: gene annotation**
  - background
  - main methods
  - application to bacteria
  - application to eukaryotes
- **Going further: protein annotation**
  - context
  - function prediction
  - prediction of cellular localisation
  - study of 2D and 3D structures

Stein L. Genome annotation: from sequence to biology. Nat Rev Genet. 2001 Jul;2(7):493-503.

Gene annotation

Quality of assembly

- The first step is to **validate the assembly**

- Observe the **metrics** (N50, L50..)

	A. halleri halleri	*A. halleri gemmifera*	*A.lyrata*	*A. thaliana*
Nb scaffolds	3 152	2 239	695	7
Total length	174 Mb	196 Mb	207 Mb	120 Mb
Genome cov.	68.3 %	76.9 %	89.9 %	88.9 %
Longest scaff.	1.5 Mb	4.3 Mb	33.1 Mb	30.4 Mb
N50	279 389	712 249	24 464 547	23 459 830
L50	177	71	4	3

Box 1 | Common statistics for describing genome assemblies

Genome assemblies are composed of scaffolds and contigs. Contigs are contiguous consensus sequences that are derived from collections of overlapping reads. Scaffolds are ordered and orientated sets of contigs that are linked to one another by mate pairs of sequencing reads.

Yandell M, Ence D. A beginner's guide to eukaryotic genome annotation. Nat Rev Genet. 2012 Apr 18;13(5):329-42.

Quality of assembly

Scaffold and contig N50s

By far the most widely used statistics for describing the quality of a genome assembly are its scaffold and contig N50s. A contig N50 is calculated by first ordering every contig by length from longest to shortest. Next, starting from the longest contig, the lengths of each contig are summed, until this running sum equals one-half of the total length of all contigs in the assembly. The contig N50 of the assembly is the length of the shortest contig in this list. The scaffold N50 is calculated in the same fashion but uses scaffolds rather than contigs. The longer the scaffold N50 is, the better the assembly is. However, it is important to keep in mind that a poor assembly that has forced unrelated reads and contigs into scaffolds can have an erroneously large N50.

Yandell M, Ence D. A beginner's guide to eukaryotic genome annotation. Nat Rev Genet. 2012 Apr 18;13(5):329-42.

Quality of assembly

- BUSCO http://busco.ezlab.org/
- Search for **universal single copy genes** in the assembly

	A. halleri halleri	*A. halleri gemmifera*	*A.lyrata*	*A. thaliana*
Complete universal single-copy orthologs	95.3%	97.6%	98.5%	98.2%
Fragmented universal single-copy orthologs	1.5%	0.3%	0.3%	0.5%
Missing universal single-copy orthologs	3.2%	2.1%	1.2%	1.3%

BUSCO v3

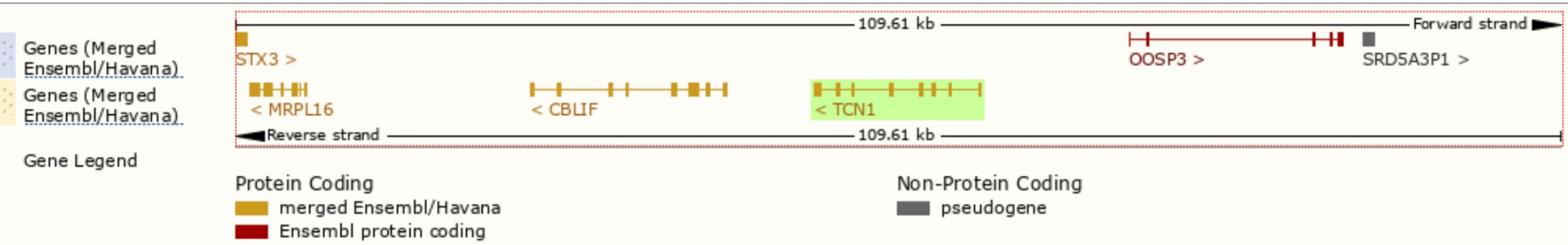
Assessing genome assembly and annotation completeness with
Benchmarking **U**niversal **S**ingle-**C**opy **O**rthologs

Identification of repeated sequences

- **Eukaryotic** genomes can be **rich in repeated sequences**: 47% of the human genome, and only 1-2% of the genome is coding!

- At first sight, the **human genome** seems to be **a model of inefficiency**: genes spaced by large regions (10-100 kb), introns

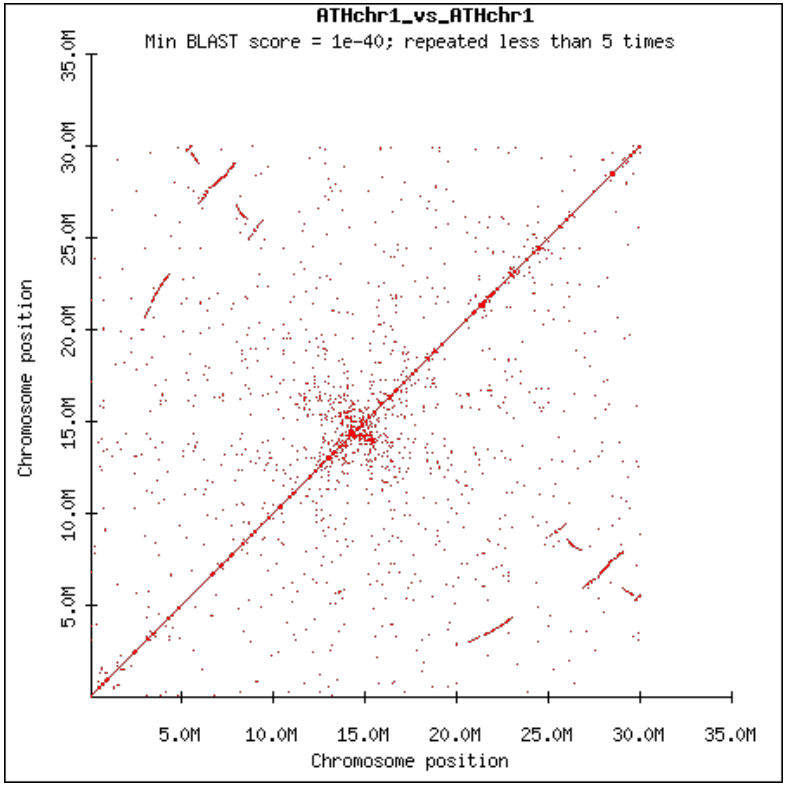
- **In yeast**: 60% of the genome encodes the 6000 proteins. The 35,000 human genes are encoded by a genome 300 x larger



Screenshot from Ensembl.org Human genome 11:59,804,864-59,914,472

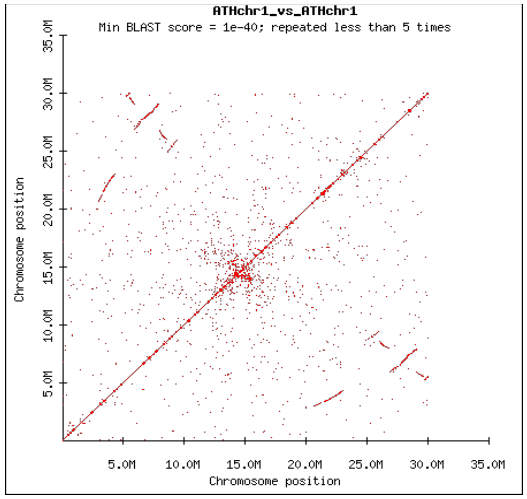
Identification of repeated sequences

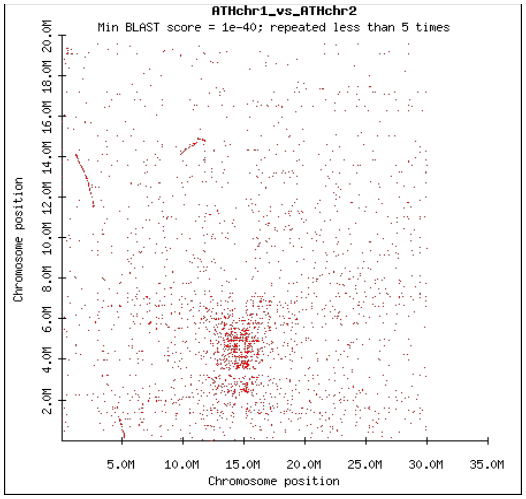
- **Dotplot** Chr1 *Arabidopsis thaliana* aligned against itself
- There are **many repeats**
- There are more repeats at the **centromere** in *At*

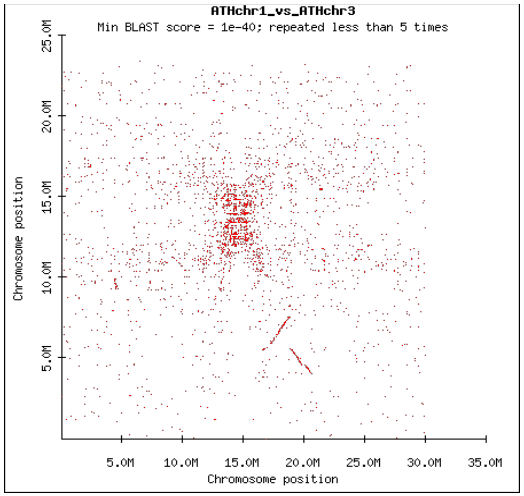


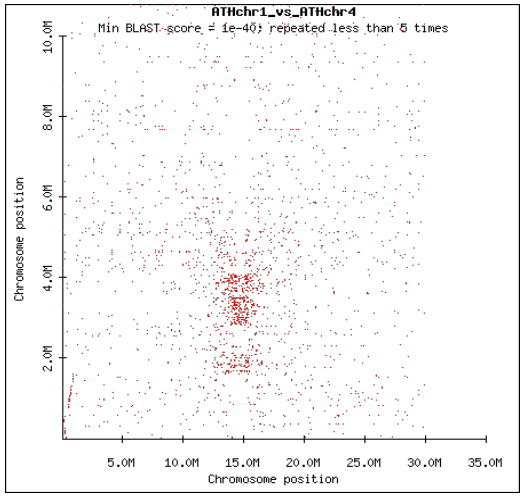
http://biolinx.bios.niu.edu/t80maj1/rice/arab_mega_dotplots.htm

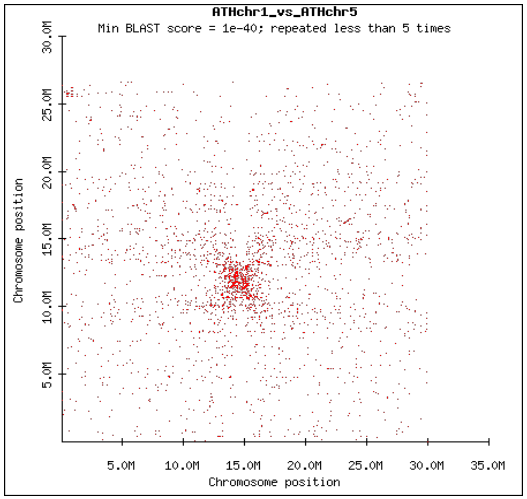
Identification of repeated sequences







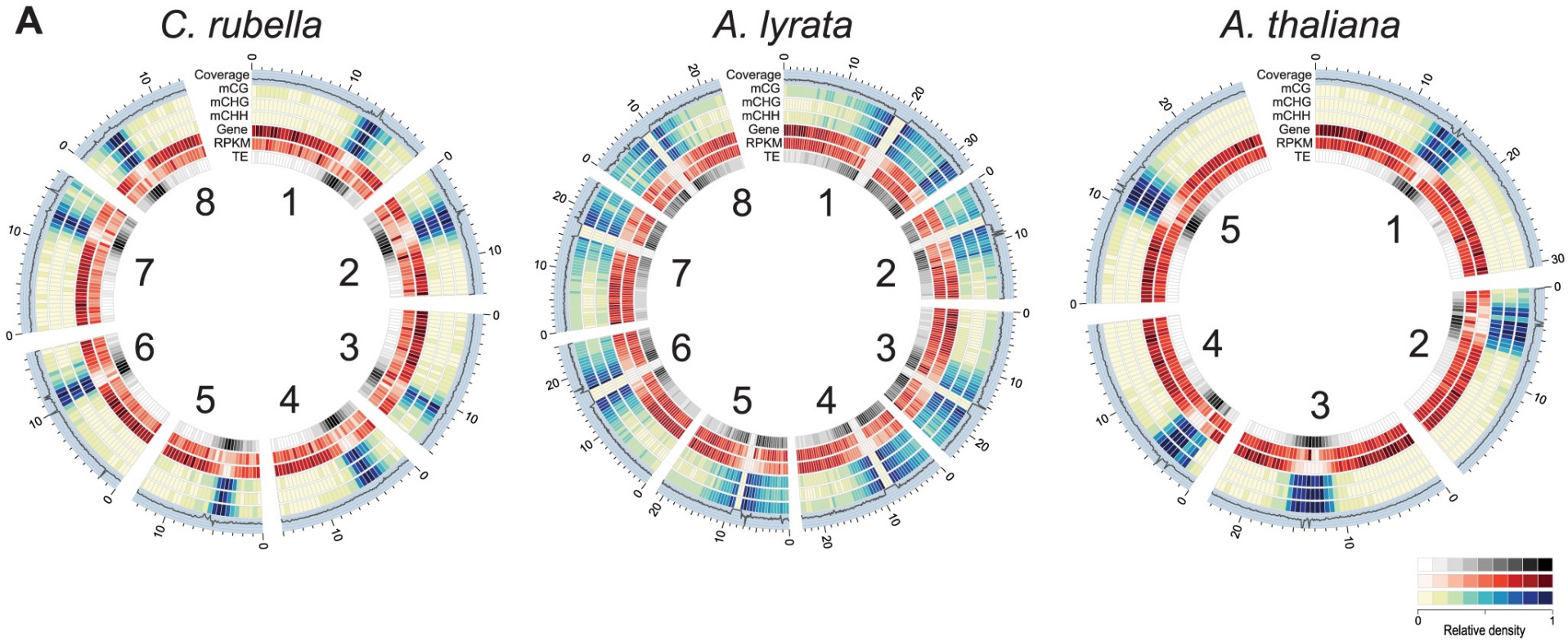




http://biolinx.bios.niu.edu/t80maj1/rice/arab_mega_dotplots.htm

Identification of repeated sequences

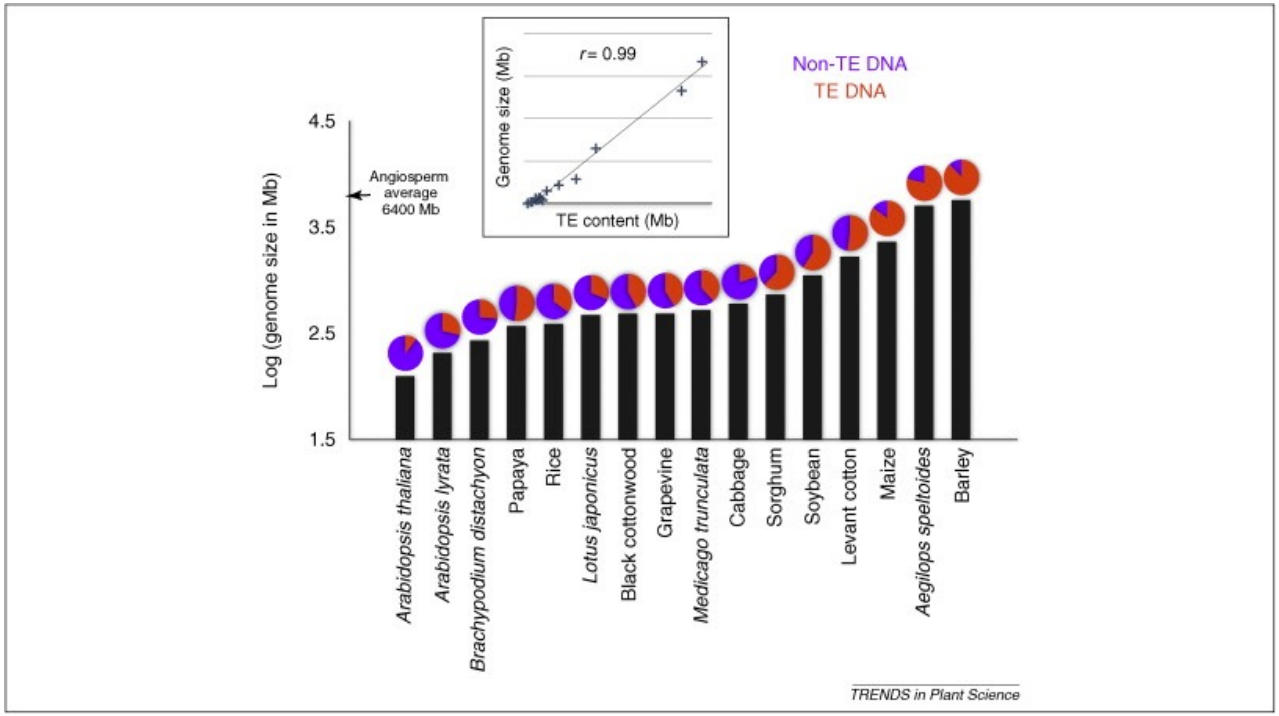
- Transposable elements can have **different locations** in the genome



Seymour et al., Plos Genet 2014

Identification of repeated sequences

- **Genome size** is **correlated** with **transposable element content**



Tenaillon MI, Hollister JD, Gaut BS. A triptych of the evolution of plant transposable elements. Trends Plant Sci. 2010 Aug;15(8):471-8.

Identification of repeated sequences

- **Repeated sequences interfere** with **genome assembly** and **gene prediction**: ORFs of transposable elements are identified as genes of the host organism. They can also produce errors in the annotation of neighbouring genes

- **Identification** of repeated sequences and their **masking** are usually **the first steps** in annotating a (eukaryotic) genome

- **Masking**: replace these regions with "N" or lower case letters (softmasking)

Identification of repeated sequences

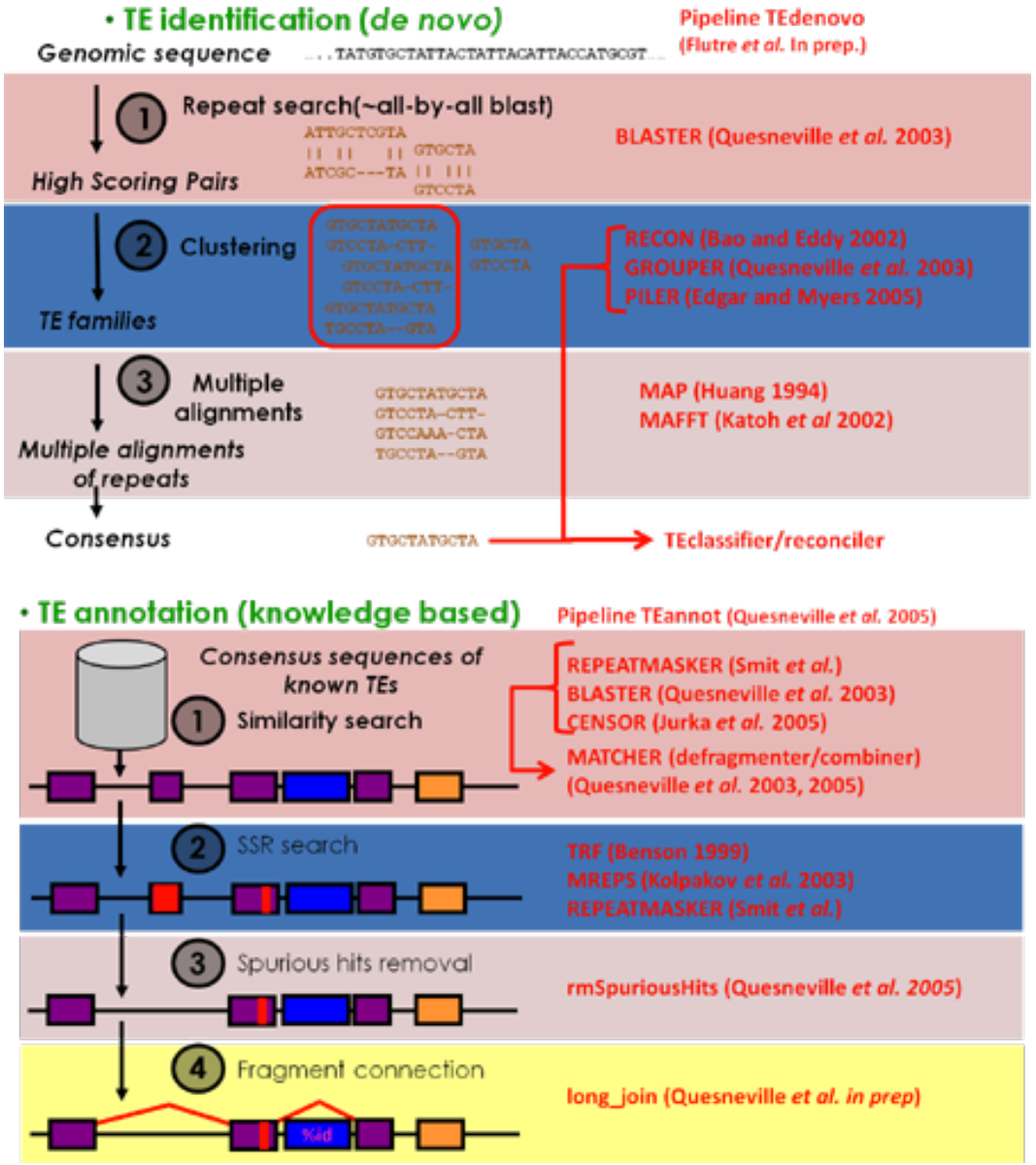
- Two types of analysis: **homology-based or *de novo***

- As transposable elements are **poorly conserved** between species, *de novo* analysis has the advantage of being able to identify specific families of elements

- Once a database of transposable elements has been obtained, the elements can be **identified** using tools such as RepeatMasker, Crossmatch... It is also possible to combine different tools

- In addition to transposable elements, the identified repeats can also include regions of **low complexity** and **repeated genes**: histones, tubulins...

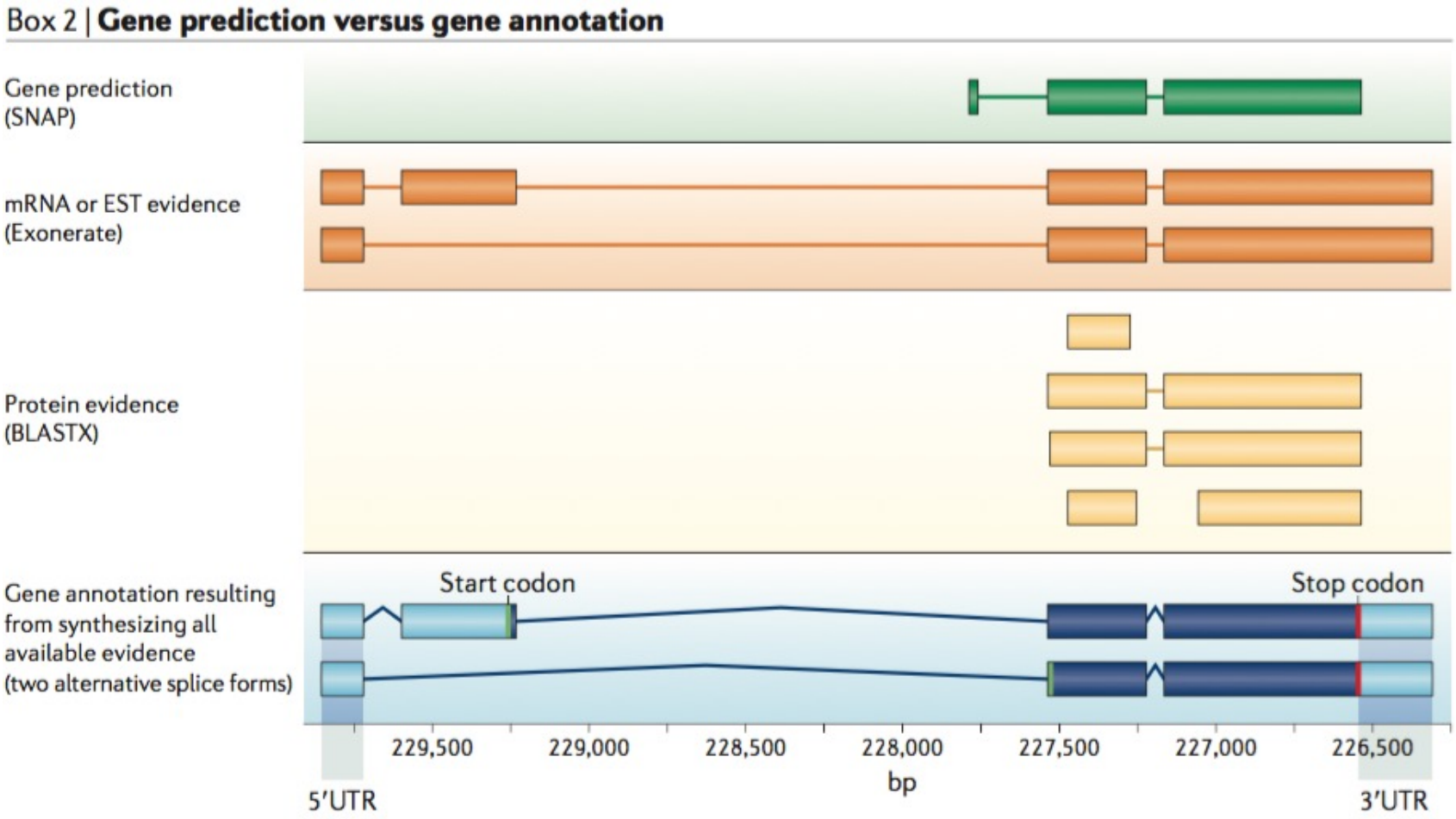
Identification of repeated sequences



The REPET package (URGI)

Flutre T. et al, 2011

Gene annotation



Yandell M, Ence D. A beginner's guide to eukaryotic genome annotation. Nat Rev Genet. 2012 Apr 18;13(5):329-42.

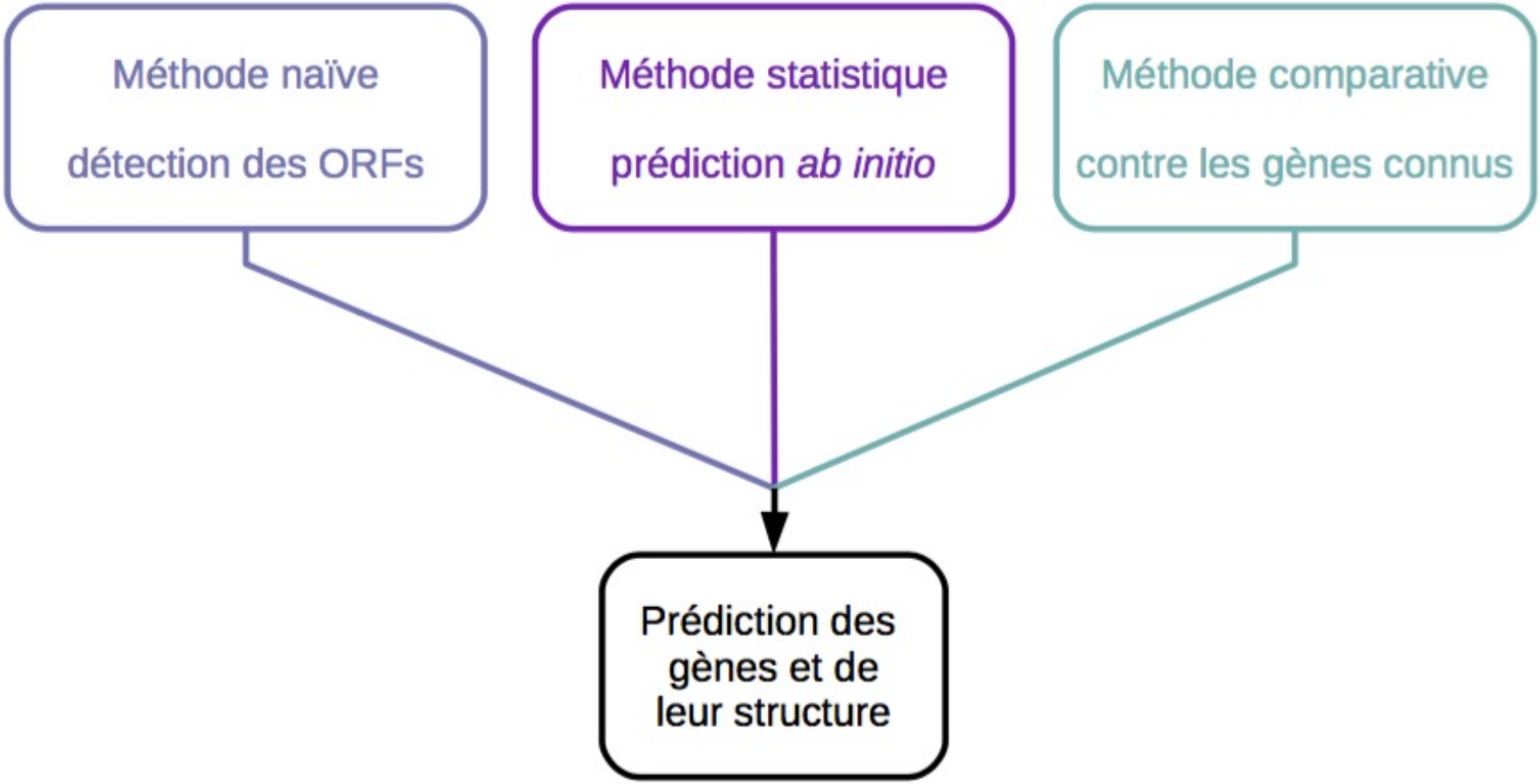
Gene annotation

- **Starting point**: raw nucleic acid sequences

- **Output**
  - Start and end positions of genes
  - Transcription, splicing and translation signals
  - Idea of the function of proteins encoded by genes

- **Limits**
  - Some genes are not predicted (false negatives)
  - Some predicted genes are not true genes (false positives)
  - Precise gene boundaries are sometimes wrong (wrong initiation codon choice...)

Main methods



Naive approach

- **Principle:** search for coding sequence signals
  - Start with an initiation codon ATG + other
  - End with a termination codon TAA, TAG or TGA
  - Have a size multiple of 3 (for genes without introns)

- **Implementation:** detect open reading frames
  - ORFs = Open Reading Frames
  - Frames that may contain a gene
  - >50 nt between an initiation codon and a termination codon
  - Blind translation in the 6 reading frames (3 frames per DNA strand)

Naive approach

- The **6 reading frames** of a nucleic sequence

Naive approach

- **Advantages**
  - *ab initio* method: without prior knowledge
  - Reduces the amount of data to be analysed for sequence comparison

- **Limits**
  - Not all ORFs are genes
  - Sensitive to sequencing errors
  - Not very useful for eukaryotic genes (presence of introns)

Statistical approach

- **Principle**: discriminate between coding and non-coding sequences
  - Based on code usage bias

- **Implementation**:
  - Learning the code usage for a given organism from reliable coding sequences
  - Calculation of the probability that a portion of a sequence is coding
  - Analysis of transcription and translation signals to determine gene boundaries

Bias in the use of the genetic code

- 1 amino acid is encoded by N codons → synonymous codons
- Non-uniform distribution of codons used

aa	codons	% par aa	Nb
A	GCA	0,65	11
	GCC	0	0
	GCG	0	0
	GCT	0,35	6
F	TTC	0,21	7
	TTT	0,79	27
G	GGA	0,50	11
	GGC	0	0
	GGG	0,05	1
	GGT	0,45	10

Exemple : gène cytB de *P. falciparum*
G+C = 27.59 % du génome

Statistical approach

- **Advantages**
  - *ab initio* approach: without prior annotation of genes
  - More reliable criteria than the naive approach

- **Limits**
  - Need for a training dataset: confirmed coding sequences
  - Does not detect small genes/exons (below detection threshold)
  - CDS identification only, no indentification of UTRs
  - No identification of alternative splicing

- Some tools such as TwinScan, FGENESH, Augustus, Gnomon, GAZE and SNAP, can use evidence (mRNA, proteins) to improve evidence-driven predictions (compared to ab initio)

Comparative approach

- **Principle**: locate annotations from databases on the genome sequence
  - Alignments with known proteins → location of CDS, including introns
  - Alignments with mRNAs (ESTs, cDNAs, etc.)→ location of CDSs + UTRs, including introns

- **Implementation**
  - Sequence comparison against libraries of mRNA or protein using Blast
  - Alignment of matched mRNAs or proteins using a specialised software

Comparaison with mRNAs

- **Comparison of the DNA sequence to nucleic acid databases** using Blastn (or equivalent)

  (- Detection of contaminating sequences (vectors...)
  Specialised blast: VecScreen)

  - Detection of mRNAs potentially derived from the DNA sequence → comparison with mRNAs obtained from the same species or from closely related species

- **Alignment** between the **DNA** sequence and the **matched mRNAs** using **specialised software**

  - Fine determination of 5' and 3' UTRs and exons
  - Software: EST2genome, Splign

Use of RNA-seq data

- This is the data that has the greatest potential to improve annotation
- Allows a better delimiation of exons, splice sites, and alternative splicing events
- But large amount of data, complex because often short Illumina reads
- 2 ways to use the reads
  - Genome-independent *de novo* assembly of reads (ABySS, SOAPdenovo, Trinity). The resulting transcripts are then aligned to the genome in the same way as seen for mRNA
  - Directly aligned to the genome (TopHat, GSNAP, Scripture), then the alignments are assembled using Cufflink

Comparison to proteins

- **Comparison** of the translated DNA sequence (in the 6 frames) **to protein databases** using BLASTX
  - Detection of proteins potentially encoded by the sequence

- **Alignment** of **matched proteins** using specialised software
  - Determination of initiation codon and intron/exon junctions
  - Software: GeneWise

Comparative approach

- **Advantages**
  - Validates potential genes by comparison with experimental data (mRNA, proteins)
  - Provides clues to protein function

- **Limits**
  - Requires *a priori* knowledge

  Does not find orphan sequences

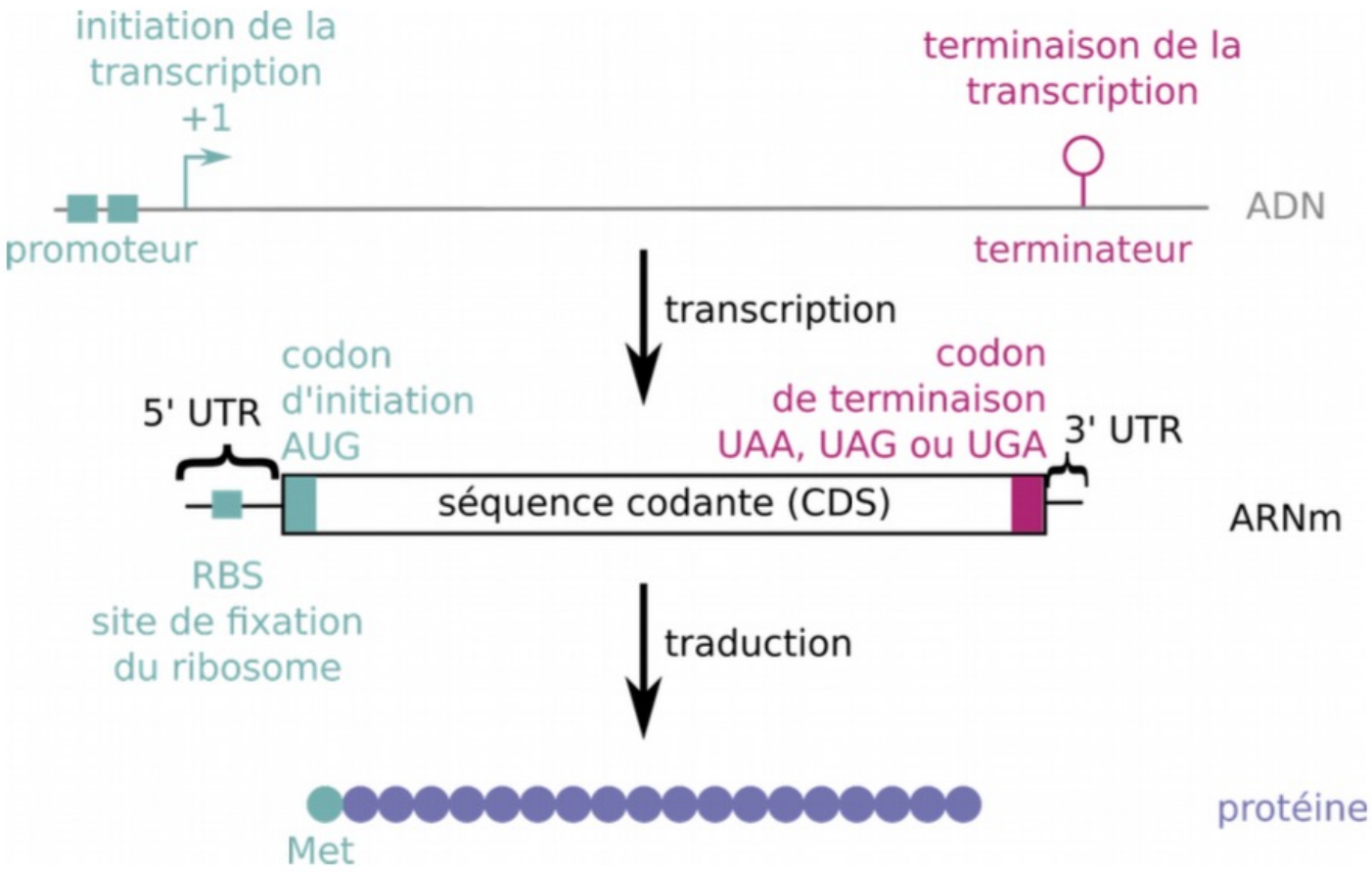
  Difficult with isolated genomes from a taxonomic point of view
  - Propagates errors in libraries

Structure of prokaryotic genes

- Over **80% of the genome is coding**
  - Short intergenic sequences
  - On average: one gene per 1,000 nucleotides (kb)

- **Simple gene structure**
  - Short transcribed but untranslated regions (3' and 5' UTR)
  - No intron (with some exceptions)

Structure of prokaryotic genes



Structure of prokaryotic genes

- Here is an extract from the **genome sequence** of Pseudoalteromonas sp.

>AB057417
aacgaaaagattaaaaatttatcatttttctcttggaatttttactctacccccatta
atgaatgcaaattagaaaagctttttctgtactgttcagaaactgttaggagaactaaa
aaacatgaacattcgtcctttacaagatcgcgtaatcgttaaacgtctagaagaagaaac
aaaatctgctggcggtattgtattaactggctctgcagctgaaaaatcaactcgcggaga
agtagtagccgtaggtaatggtcgtattttagataacggtgacgttagagctttagaagt
aaaagccggtgacactgtgttatttggctcatatgttgagaaaactgaaaagatcgaagg
tcaagagtacctgatcatgcgtgaagacaacattttaggcattgtaggctaagcctactt
ttcgtttaacacacatttaagaatttagagg

Proposed workflow

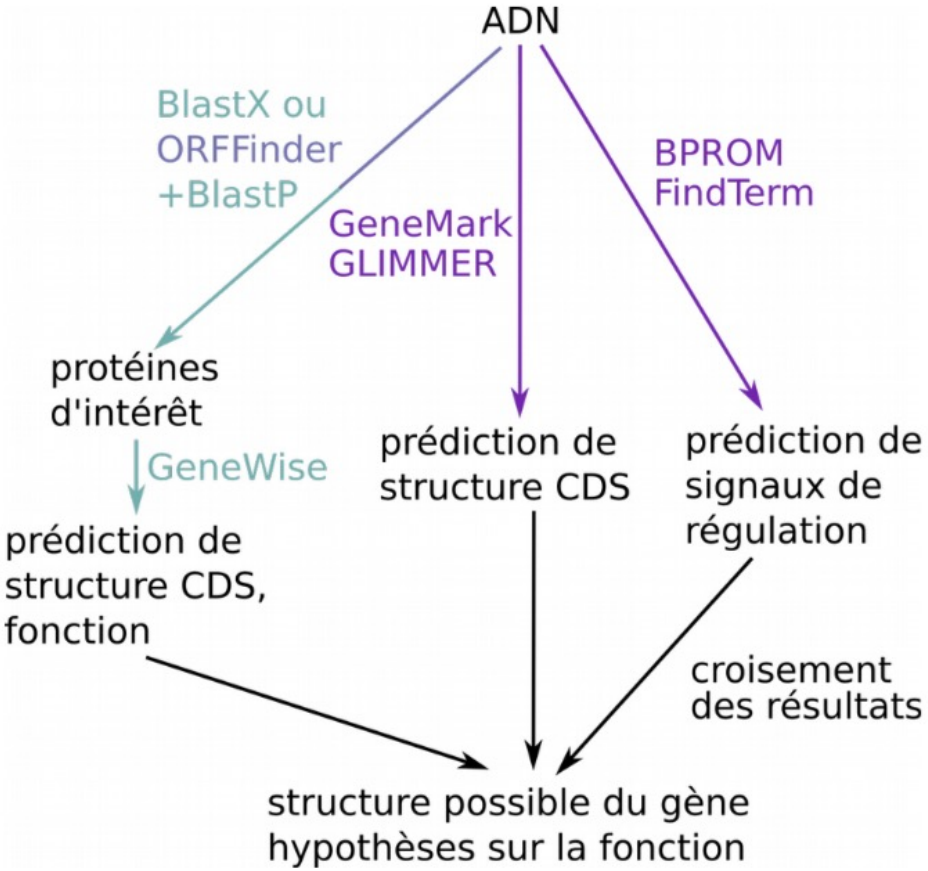
- **Analyse in 4 steps**

1. ORF identification
   - ORFFinder

2. ORF validation
   - SmartBlast (GeneWise if needed)

3. Statistical prediction of CDS
   - GeneMark, GLIMMER

4. Statistical prediction of regulatory signals
   - BPROM



ORFfinder

Open Reading Frame Finder

ORF finder searches for open reading frames (ORFs) in the DNA sequence you enter. The program returns the range of each ORF, along with its protein translation. Use ORF finder to search newly sequenced DNA for potential protein encoding segments, verify predicted protein using newly developed SMART BLAST or regular BLASTP.

This web version of the ORF finder is limited to the subrange of the query sequence up to 50 kb long. Stand-alone version, which doesn't have query sequence length limitation, is available for Linux x64.

Examples (click to set values, then click Submit button) :

- NC_011604 Salmonella enterica plasmid pWES-1; genetic code: 11; 'ATG' and alternative initiation codons; minimal ORF length: 300 nt
- NM_000059; genetic code: 1; start codon: 'ATG only'; minimal ORF length: 150 nt

Enter Query Sequence

Enter accession number, gi, or nucleotide sequence in FASTA format:

From: To:

Choose Search Parameters

Minimal ORF length (nt): 75

Genetic code: 1. Standard

ORF start codon to use:

- "ATG" only
- "ATG" and alternative initiation codons
- Any sense codon

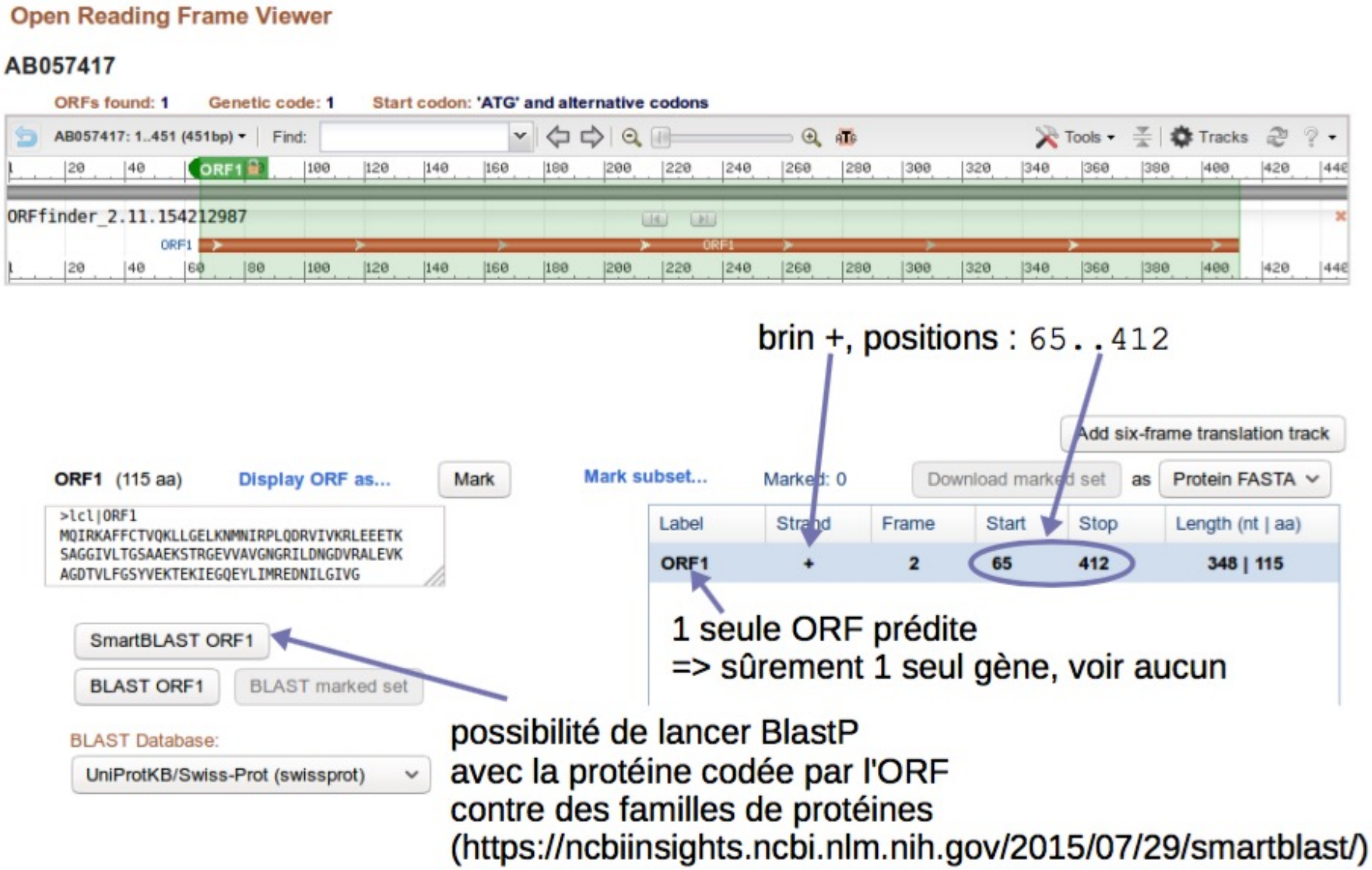
Ignore nested ORFs:

Start Search / Clear

Submit Clear

https://www.ncbi.nlm.nih.gov/orffinder/

ORFfinder results



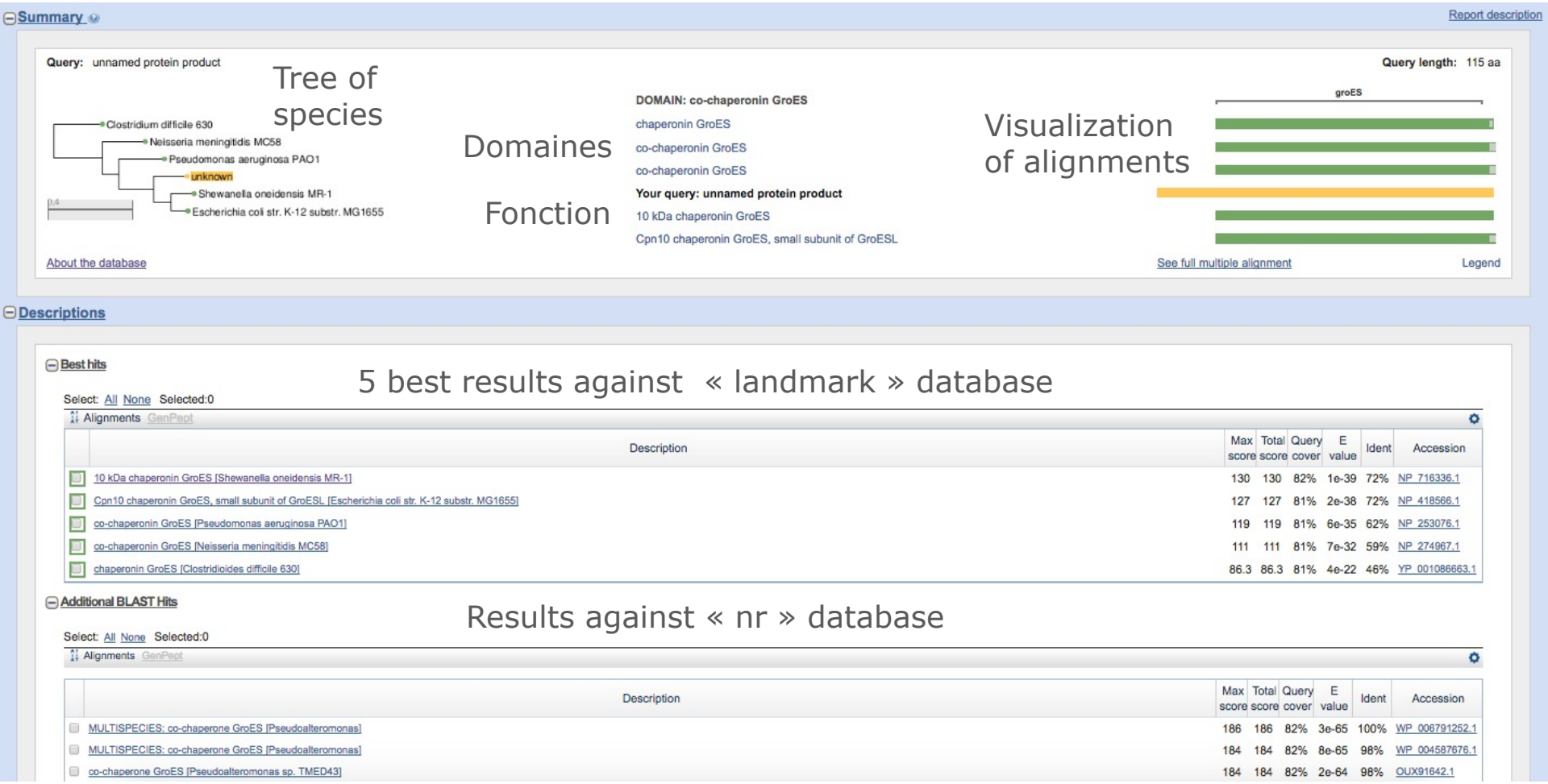
SmartBlast

- **SmartBlast** compares the ORF against database (« landmark database ») consisting of the proteomes of 27 species spread over a large phylogeny. Also compares against the nr database https://blast.ncbi.nlm.nih.gov/smartblast/smartBlast.cgi?CMD=Web&PAGE_TYPE=BlastDocs#searchSets

- It returns the 5 best results obtained against the « landmark database »

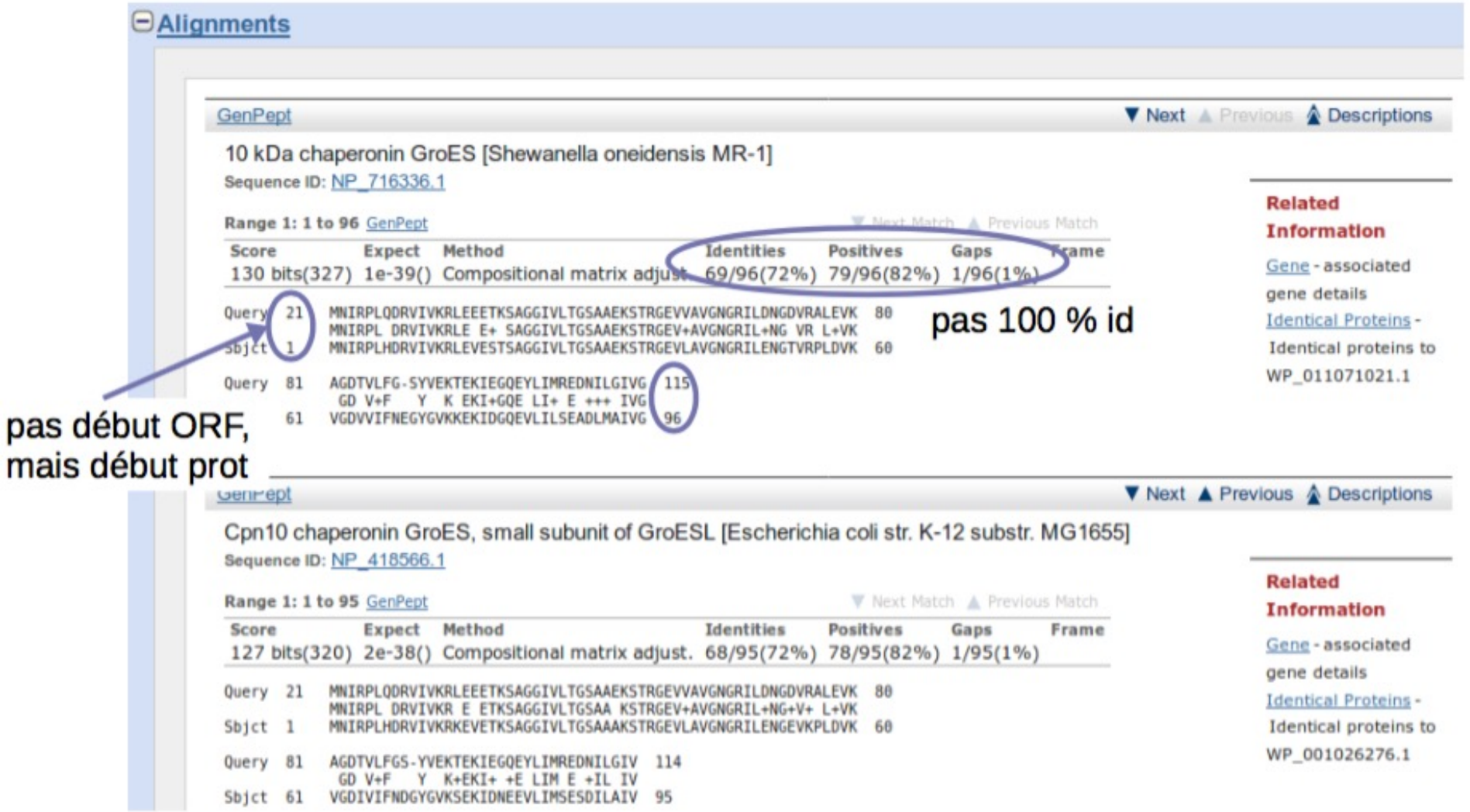
- He then returns the results obtained against the « nr » database

SmartBlast results



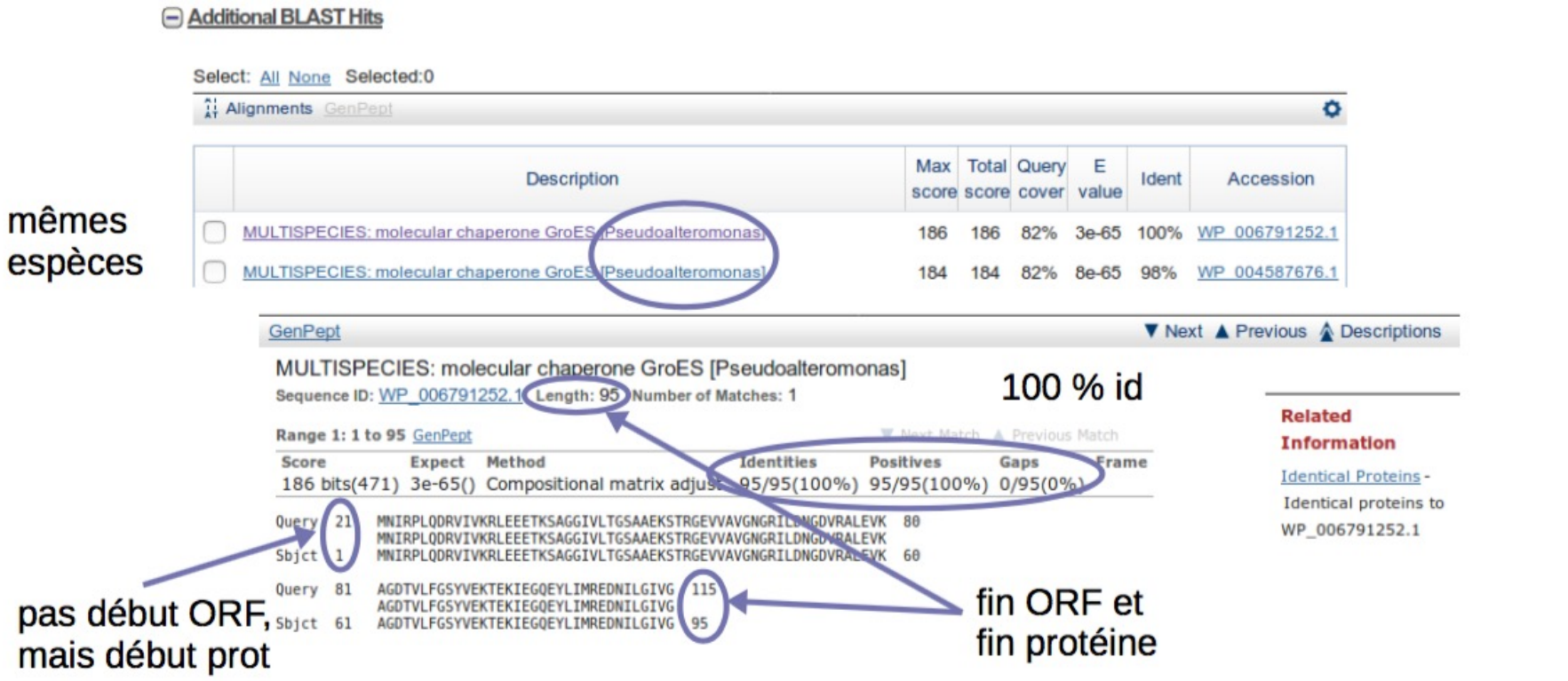
SmartBlast results

- Best hit against « landmark » database



SmartBlast results

- Best hit against « nr » database



SmartBlast results

- **ORF : 65..412** on the strand + of the DNA sequence
  - Codes a 115 aa protein + stop codon

- Alignments provided by SmartBLAST
  - Query 21..115 : only a part of the ORF protein
  So the **ORF is not fully coding**
  The alignement starts at 21 => the CDS starts at
  65+(21-1)*3 = 125
  End of the CDS at 412
  - Sbjct 1..95 : The protein from the database is complete
  The predicted coding sequence is complete

- The alignments obtained with different sequences are good
  - Prediction is **reliable**, no need for GeneWise

GeneMark

A family of gene prediction programs developed at
Georgia Institute of Technology , Atlanta, Georgia, USA.

Gene Prediction in Bacteria, Archaea, Metagenomes and Metatranscriptomes

Novel genomic sequences can be analyzed either by the self-training program **GeneMarkS** (sequences longer than 50 kb) or by **GeneMark.hmm with Heuristic models**. For many species pre-trained model parameters are ready and available through the **GeneMark.hmm** page. Metagenomic sequences can be analyzed by **MetaGeneMark** , the program optimized for speed.

Gene Prediction in Eukaryotes

Novel genomes can be analyzed by the program **GeneMark-ES** utilizing unsupervised training. Note that GeneMark-ES has a special mode for analyzing fungal genomes. Recently, we have developed a semi-supervised version of GeneMark-ES, called GeneMark-ET that uses RNA-Seq reads to improve training. For several species pre-trained model parameters are ready and available through the **GeneMark.hmm** page.

Gene Prediction in Transcripts

Sets of assembled eukaryotic transcripts can be analyzed by the modified **GeneMarkS** algorithm (the set should be large enough to permit self-training). A single transcript can be analyzed by a special version of **GeneMark.hmm with Heuristic models**. A new advanced algorithm GeneMarkS-T was developed recently (manuscript sent to publisher); The GeneMarkS-T software (beta version) is available for download .

Gene Prediction in Viruses, Phages and Plasmids

Sequences of viruses, phages or plasmids can be analyzed either by the **GeneMark.hmm with Heuristic models** (if the sequence is shorter than 50 kb) or by the self-training program **GeneMarkS**.

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http://exon.gatech.edu/GeneMark/

GeneMark results

- **GeneMark.hmm with Heuristic models**
- Same result as ORFfinder; in contradiction with the start identified by SmartBlast

```
GeneMark.hmm PROKARYOTIC (Version 3.26)
Date: Tue Jan  2 06:16:26 2018
Sequence file name: seq.fna
Model file name: GeneMark_hmm_heuristic.mod
RBS: false
Model information: Heuristic_model_for_genetic_code_11_and_GC_36

FASTA definition line: AB057417
Predicted genes
   Gene     Strand     LeftEnd     RightEnd        Gene      Class
    #                                             Length
    1         +           65          412           348         1
```

GeneMark results

- **GeneMark.hmm** model adapted for *Pseudoalteromonas sp.*
- Result **in agreement** with SmartBlast
- **Best results with a model** defined for the studied species

```
GeneMark.hmm PROKARYOTIC (Version 3.26)
Date: Tue Jan  2 06:25:38 2018
Sequence file name: seq.fna
Model file name: /home/genemark/parameters/prokaryotic/Pseudoalteromonas_atlantica_T6c/
RBS: true
Model information: Pseudoalteromonas_atlantica_T6c

FASTA definition line: AB057417
Predicted genes
   Gene     Strand     LeftEnd     RightEnd        Gene      Class
    #                                             Length
    1         +          125          412           288         1
```

Bprom

BPROM

Used in more than 800 publications.

Reference: V. Solovyev, A Salamov (2011) Automatic Annotation of Microbial Genomes and Metagenomic Sequences. In Metagenomics and its Applications in Agriculture, Biomedicine and Environmental Studies (Ed. R.W. Li), Nova Science Publishers, p. 61-78

BPROM - Prediction of bacterial promoters

BPROM is bacterial sigma70 promoter recognition program with about 80% accuracy and specificity. It is best used in regions immediately upstream from ORF start for improved gene and operon prediction in bacteria.

Paste nucleotide sequence here (plain or in fasta format):

```
>AB057417
aacgaaaagattaaaaatttatcatttttttctcttggaatttttttactctacccccatta
atgaatgcaaattagaaaagctttttttctgtactgttcagaaactgttaggagaactaaa
```

Alternatively, load a local file with sequence:

Local file name:

Choisissez un fichier | Aucun fichier choisi

Process | Reset

[Help]
[Example]

Return to page with other programs of group: Operon and gene finding in bacteria

http://www.softberry.com/berry.phtml?topic=bprom&group=programs&subgroup=gfindb

Bprom results

```
Threshold for promoters -  0.20
Number of predicted promoters -      2
Promoter Pos:    418 LDF-  4.85
-10 box at pos.    402 ttgtaggct Score    44
-35 box at pos.    381 gtgaag    Score    21
Promoter Pos:     80 LDF-  2.31
-10 box at pos.     65 atgcaaatt Score    29
-35 box at pos.     43 tttact    Score    42

Oligonucleotides from known TF binding sites:
For promoter at    418:
       fnr:  TCAAGAGT at position     361 Score -  13
      purR:  TTTTCGTT at position     419 Score -   5
      purR:  TTTCGTTT at position     420 Score -   6
    rpoD15:  TTAACACA at position     426 Score -  12
       crp:  ACACACAT at position     429 Score -  12
      glpR:  CACACATT at position     430 Score -   6
For promoter at     80:
      soxS:  TATCATTT at position      20 Score -   9
       fur:  ATCATTTT at position      21 Score -   8
```

proche des vraies positions

cf. page suivante

Summary of the analysis

- The methods lead to the **same conclusion**: the sequence contains a single CDS 125...412 (including the termination codon)
- Additional information given by SmartBLAST: the CDS encodes a **chaperone** of the type **Cpn10 / GroES**
- Glimmer (statistical prediction) finds no CDS

Prediction in bacteria: some pitfalls

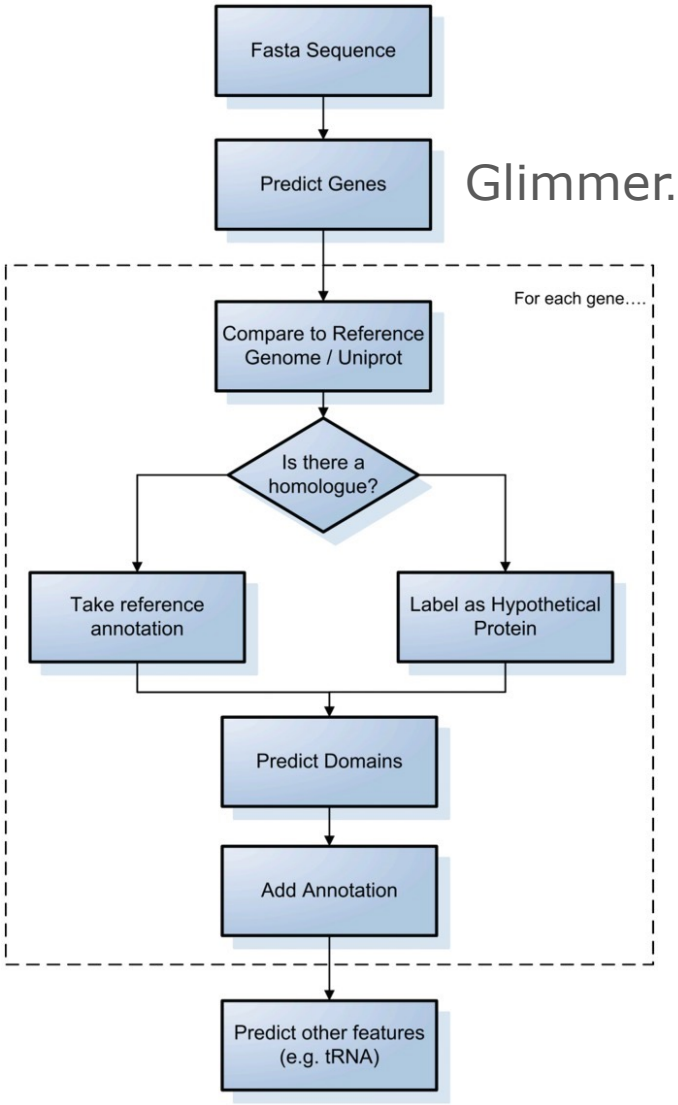
- **Several initiation codons** (AUG) on the sequence: Which is the right one?

- Possibility of **alternative initiation codons** (GUG, UUG)
  Confirmation by:
  - Presence of RBS (Ribosome Binding Site)
  - Comparative analysis with other species
  - Statistical prediction

- **Incomplete genes** (early stop codon, phase shift)
  - Real (corrected during translation, pseudogenes)
  - Sequencing errors
  - Detection by: BlastX reports inconsistencies (different frames); comparison + prediction

- **Overlapping genes**
  - Common in viruses, sometimes in bacteria (gene ends)

Alternative pipeline



Richardson EJ, Watson M. The automatic annotation of bacterial genomes. Brief Bioinform. 2013 Jan;14(1):1-12.

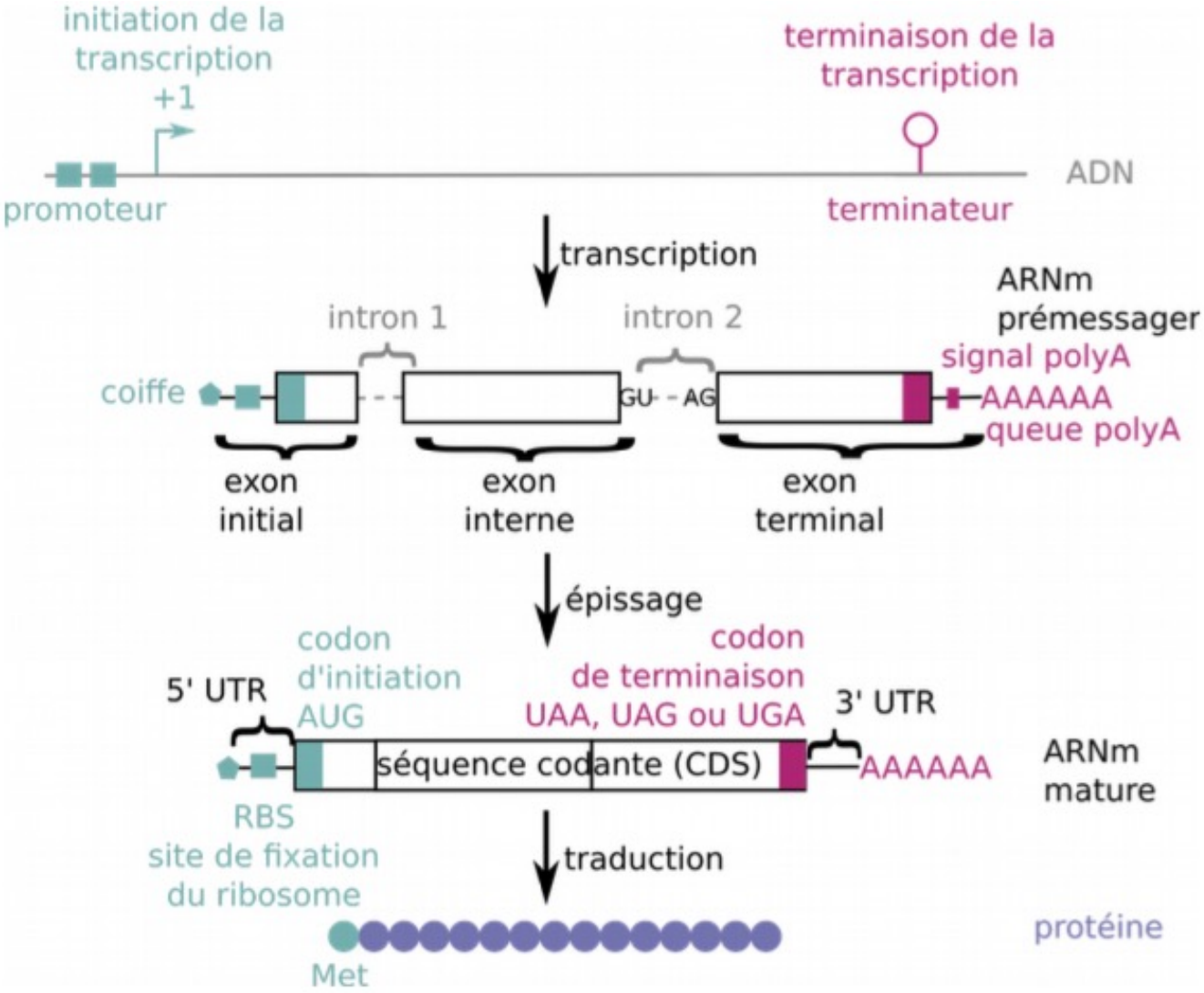
Eukaryotic gene structure

- **Low proportion** of protein coding sequences in genomes
  - About 2% of the human genome

- Presence of a very large number of **repeated sequences**
  - ~ 50% of the human genome

- **Complex gene structure**
  - Long 3' and 5' untranslated regions (non-coding exons)
  - Presence of introns, alternative splicing

Eukaryotic gene structure



Intron consequences

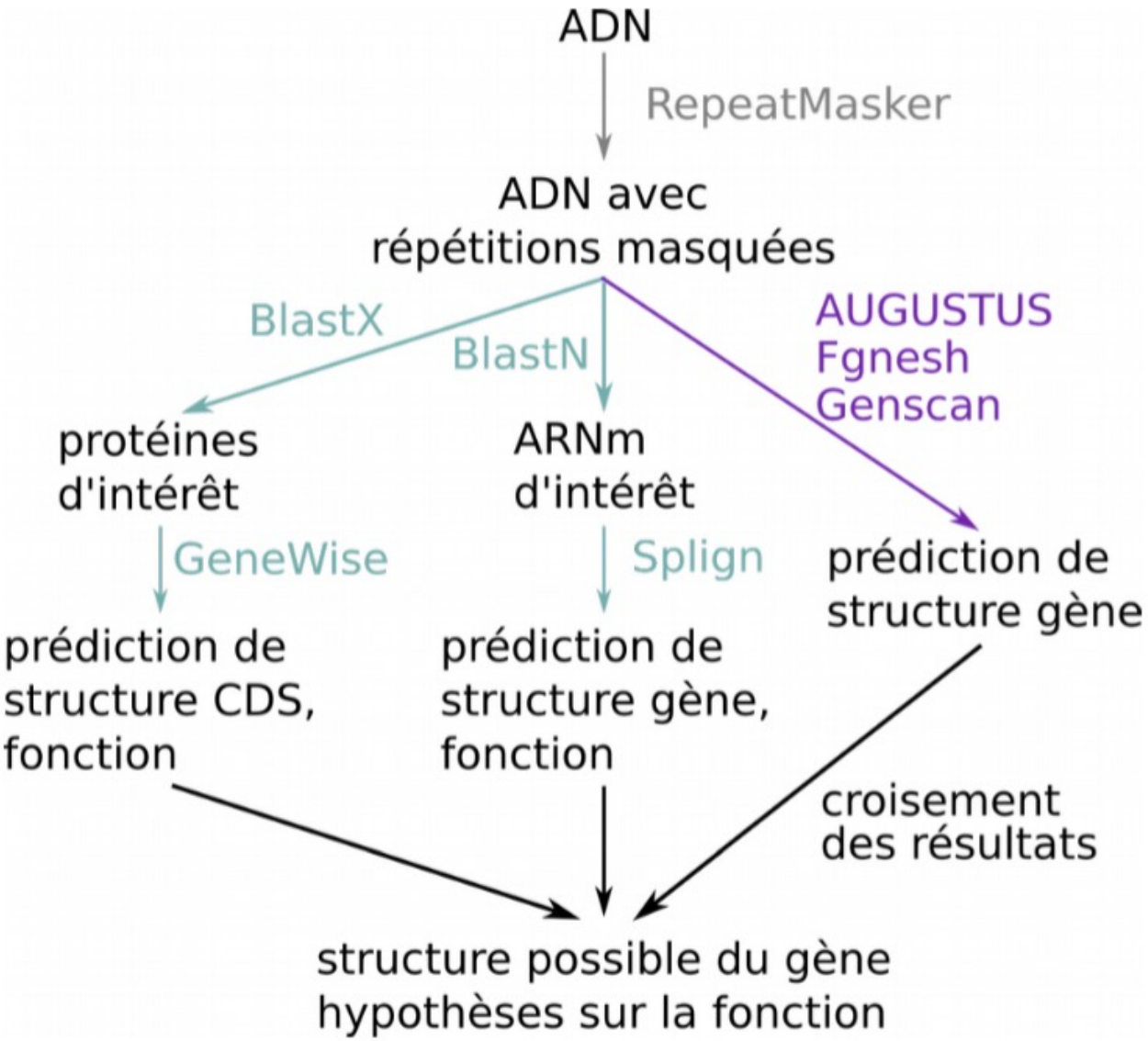
- **Exon size not a multiple of 3**
  - Codons cut by an intron
  - Frame shift from one exon to another
  - No strand change

- Existence of **short exons** (~ 10 nt)
  - Above the resolution limits of software

- Existence of **very long introns** (> exons)
  - Difficulty in locating exons

- **Alternative splicing**
  - Concerns > 50% of human genes

Suggested workflow



Example: study of an mRNA

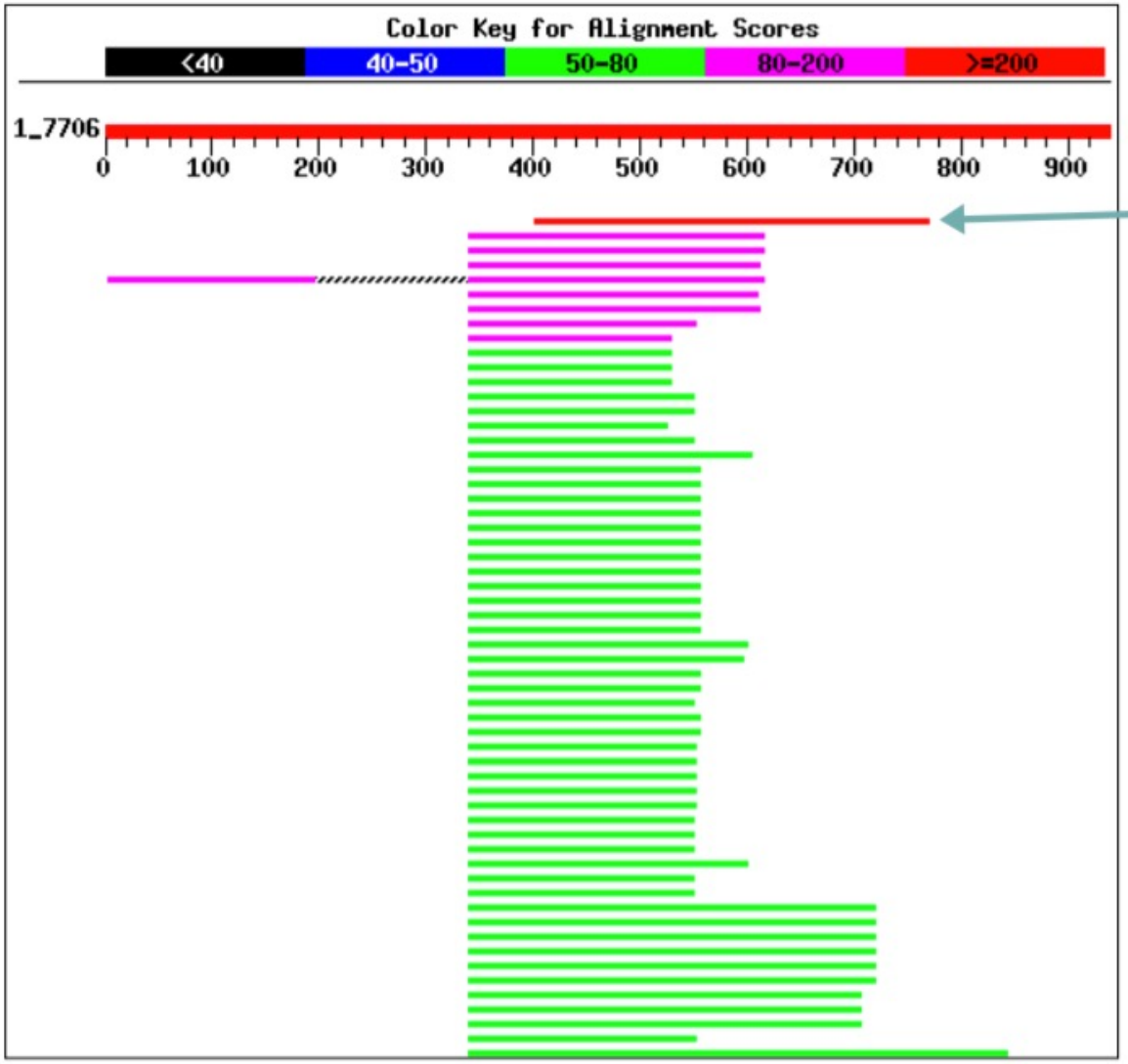
- 905 bp **mRNA** from a human cell

- **Three steps** analysis
  1. Search for CDS in mRNA
     BlastX + GeneWise

  2. Localization of the gene corresponding to the mRNA
     Blast "Genomes" + Est2genomes or Splign

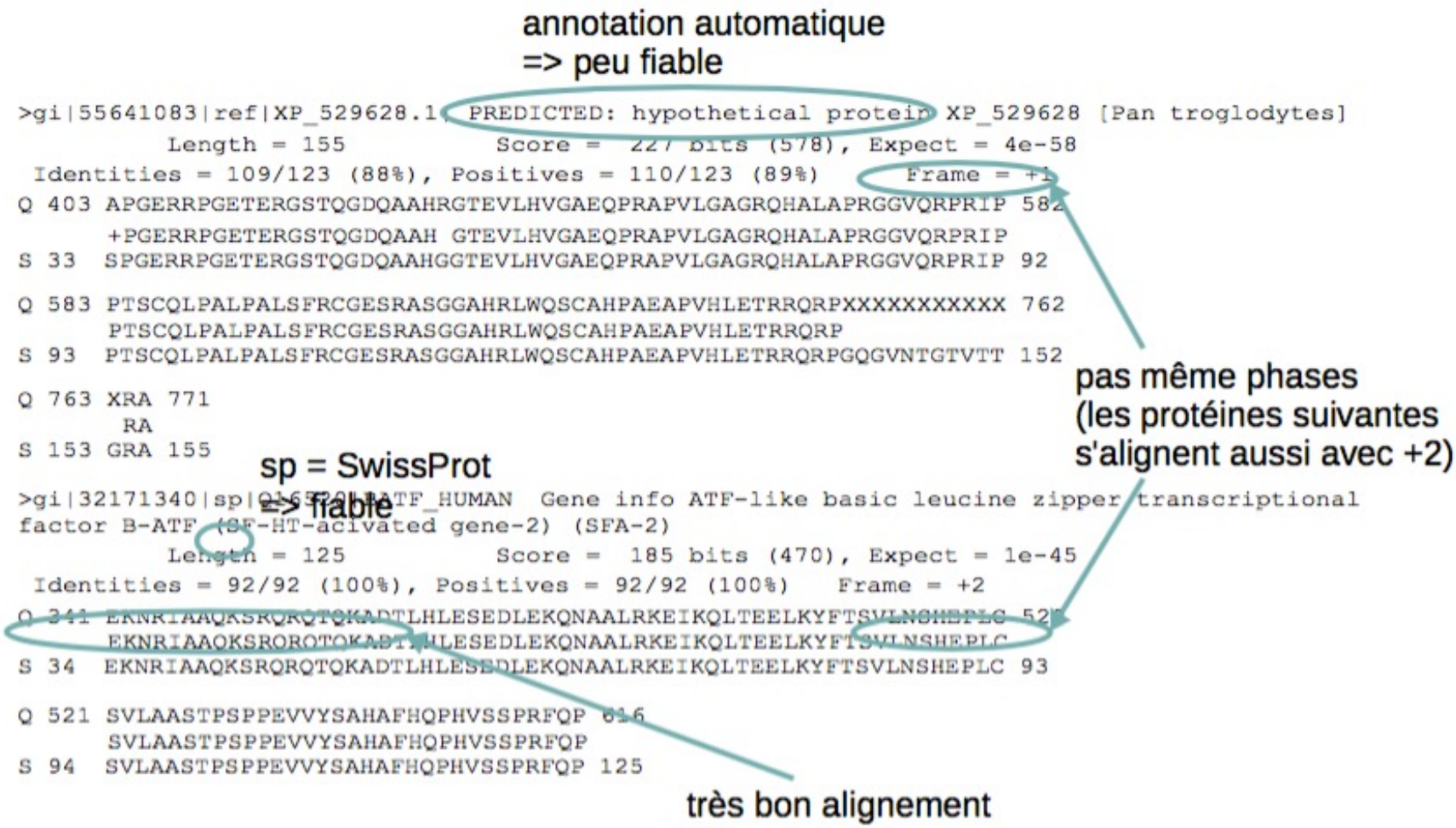
  3. Testing of statistical methods on the genome sequence
     FGENESH, AUGUSTUS, GeneScan

Blastx results, graphic



bon score mais seule protéine s'alignant avec cette région

Blastx results, alignments



Study of the alignment with the 2nd protein (1st is not relevant)

- BATF_HUMAN

Human protein, 100% identity => protein of interest

- Frame = +2: Coding sequence is on the + strand

- Query 341..616 / Sbjct 34..125
  - Need a specialised software to align this protein to the mRNA

- ATF-like basic leucine zipper transcriptional factor
  - May be a bZIP-type transcription factor

Wise

Pairwise Sequence Alignment

GeneWise compares a protein sequence to a genomic DNA sequence, allowing for introns and frameshifting errors.

STEP 1 - Enter your sequences

Enter or paste your **protein** sequence in any supported format:

Or, upload a file: Choisissez un fichier Aucun fichier choisi

AND

Enter or paste your **DNA** sequence in any supported format:

https://www.ebi.ac.uk/Tools/psa/genewise/

Wise results

```
BATF_HUMAN          1 MPHSSDSSDSSFSRSPPPGKQDSSDDVRRVQRREKNRIAAQKSRQRQTQ
                      MPHSSDSSDSSFSRSPPPGKQDSSDDVRRVQRREKNRIAAQKSRQRQTQ
                      MPHSSDSSDSSFSRSPPPGKQDSSDDVRRVQRREKNRIAAQKSRQRQTQ
ARNm_hsp          243 accatgaagtatactcccgacgttgggaagcaagaacaggcaaccacac
                      tcagcaggacgtggccccgaaaccaatggtaggaaagtccaaggagaca
                      gtccccctcccccttctcagcatttgaatggggatttccggcagggag

BATF_HUMAN         50 KADTLHLESEDLEKQNAALRKEIKQLTEELKYFTSVLNSHEPLCSVLAA
                      KADTLHLESEDLEKQNAALRKEIKQLTEELKYFTSVLNSHEPLCSVLAA
                      KADTLHLESEDLEKQNAALRKEIKQLTEELKYFTSVLNSHEPLCSVLAA
ARNm_hsp          390 aggacccgaggcgacaggccagaaccaggcattatgcaacgccttgcgg
                      acactatagaataaaacctgaataatcaataatccttagaactgcttcc
                      gcccgcggcacggagcgtacggcggcagaggccggggcccgcgcgggcc

BATF_HUMAN         99 STPSPPEVVYSAHAFHQPHVSSPRFQP
                      STPSPPEVVYSAHAFHQPHVSSPRFQP
                      STPSPPEVVYSAHAFHQPHVSSPRFQP
ARNm_hsp          537 aactccgggtagcgtccccgatcctcc
                      gcccccattagcactaacatgccgtac
                      cgcgccgggccccaccattcccgccgc

FT          CDS     243..617
```

protéine d'intérêt

prot codée par ARNm

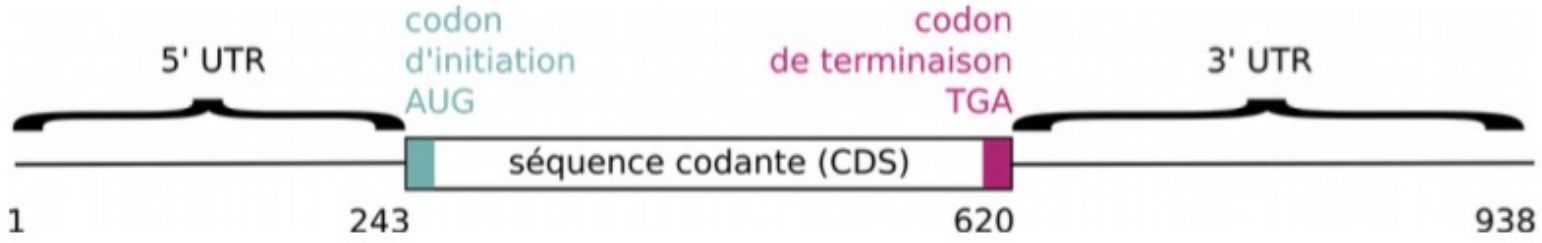
codons en colonne

début et fin de la CDS
sans le codon de terminaison

Wise results report

- Comparison with the protein of interest (BATF_HUMAN)
  - BlastX does not align the whole protein with the mRNA because the beginning of the protein contains an low complexity region that has been masked by BlastX

- GeneWise gives a CDS at position 243..617+3 on the mRNA
  - The protein is fully aligned with the mRNA

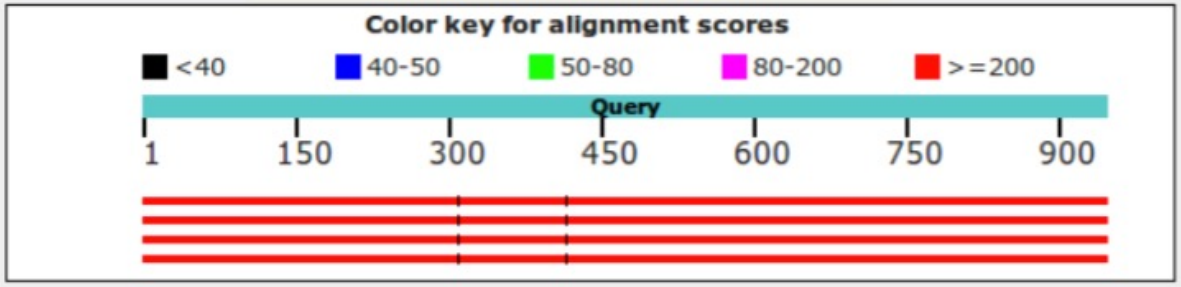


Blastn result against the genome

Graphic Summary

Distribution of the top 12 Blast Hits on 4 subject sequences

Mouse over to see the title, click to show alignments



Descriptions

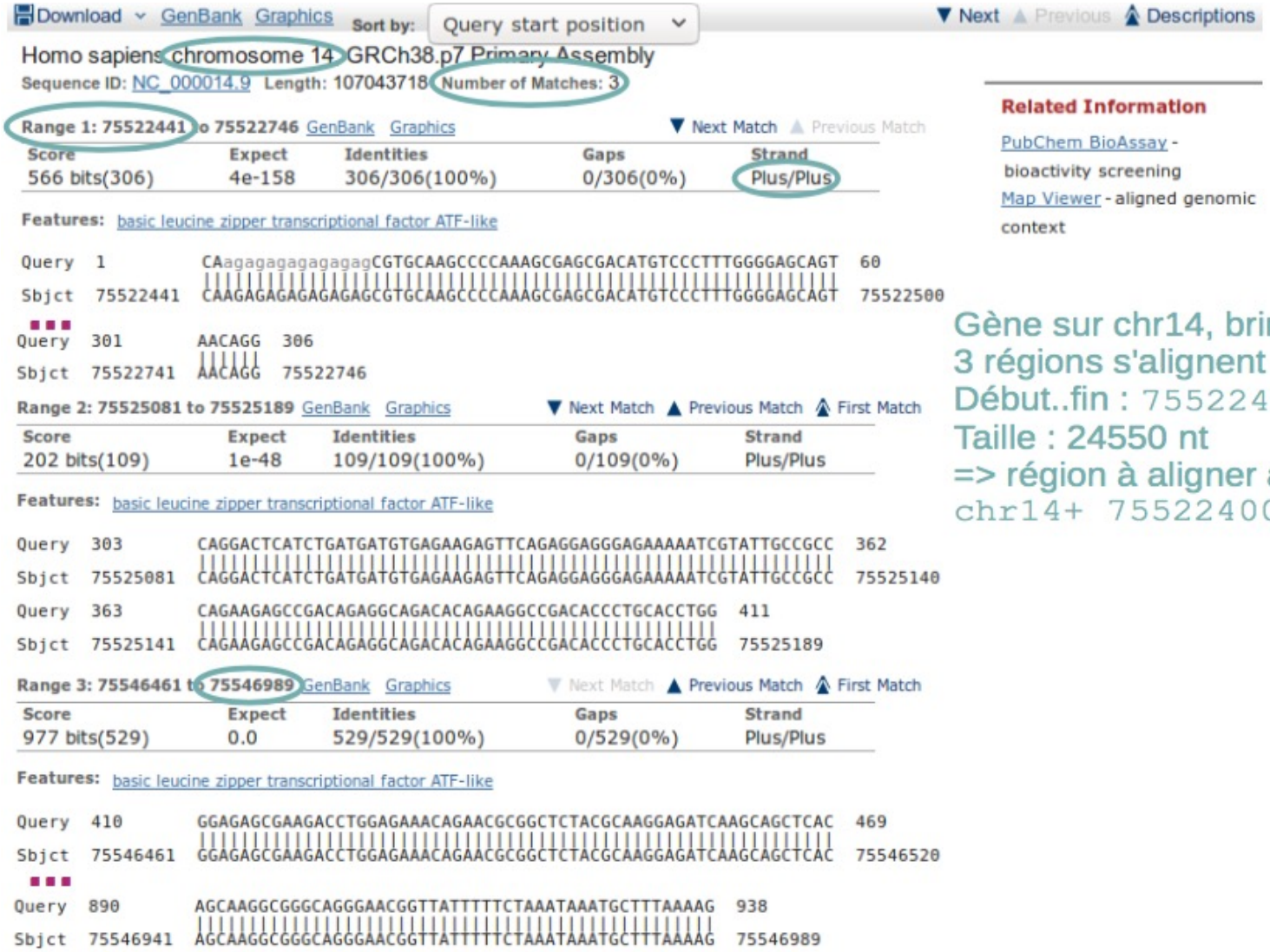
Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

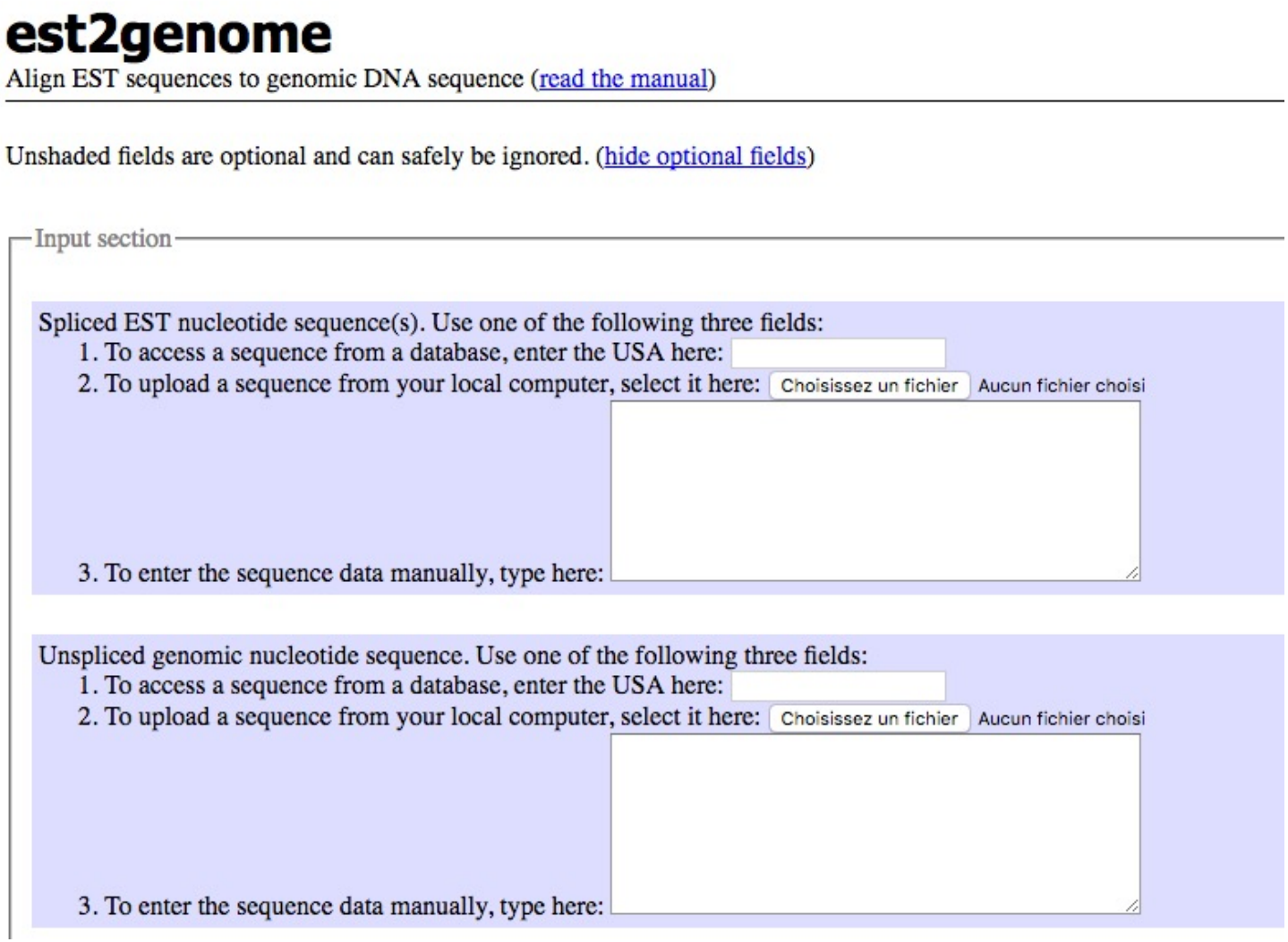
	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Homo sapiens chromosome 14, GRCh38.p7 Primary Assembly	977	1746	100%	0.0	100%	NC_000014.9
☐	Homo sapiens chromosome 14 genomic scaffold, GRCh38.p7 Primary Assembly HSCHR1	977	1746	100%	0.0	100%	NT_026437.13
☐	Homo sapiens chromosome 14, alternate assembly CHM1_1.1, whole genome shotgun se	977	1746	100%	0.0	100%	NC_018925.2
☐	Homo sapiens chromosome 14 genomic scaffold, alternate assembly CHM1_1.1, whole ge	977	1746	100%	0.0	100%	NW_004929393.1

Blastn result against the genome, alignments



Gène sur chr14, brin +
3 régions s'alignent => 3 exons ?
Début..fin : 75522441..75546989
Taille : 24550 nt
=> région à aligner avec l'ARNm :
chr14+ 75522400..75547000

EST2genome



http://www.bioinformatics.nl/cgi-bin/emboss/est2genome

EST2genome results

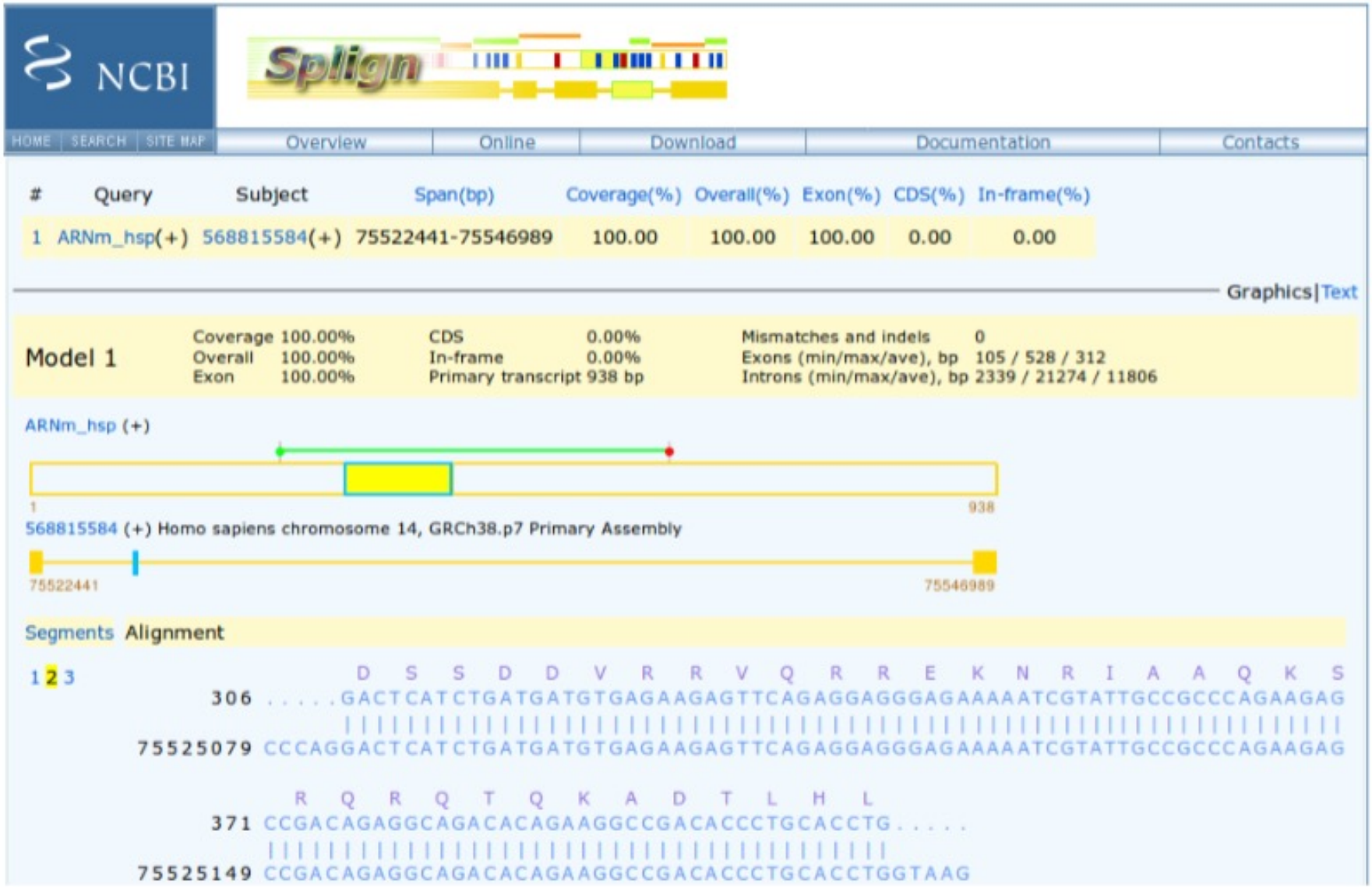
- Determination of **the position of exons** on **chr 14**, **region 75522400..75547000**

```
Exon       305 100.0    42   346  NC_000014     1   305 ARNm_hsp
+Intron    -20   0.0   347  2684  NC_000014
Exon       105 100.0  2685  2789  NC_000014   306   410 ARNm_hsp
+Intron    -20   0.0  2790 24062  NC_000014
Exon       528 100.0 24063 24590  NC_000014   411   938 ARNm_hsp

Span       898 100.0    42 24590  NC_000014     1   938 ARNm_hsp

Segment    305 100.0    42   346  NC_000014     1   305 ARNm_hsp
Segment    105 100.0  2685  2789  NC_000014   306   410 ARNm_hsp
Segment    528 100.0 24063 24590  NC_000014   411   938 ARNm_hsp
```

- So exon 1 start: 75522400+42-1

- CDS location on chromosome 14 region: join(284..346,2685..2789,24063..24272)

Splign results



Fgenesh results

G	Str		Feature	Start	End	Score	ORF	Len
1	+	1	CDSf	284	346	9.43	284 - 346	63
1	+	2	CDSi	1573	1644	0.66	1573 - 1644	72
1	+	3	CDSi	2685	2789	18.51	2685 - 2789	105
1	+	4	CDSl	24063	24272	19.16	24063 - 24272	210
1	+		PolA	24574		1.12		

1 exon supplémentaire
épissage alternatif ?

3 exons identiques aux prédictions par comparaison de séquences

CDSf = CDS first (commence par un codon d'initiation)
CDSi = CDS internal (ni codon d'initiation, ni codon de terminaison)
CDSl = CDS last conding segment (se termine par un codon de terminaison)
PolA = signal pour la queue polyA

Augustus results

NC_000014	AUGUSTUS	gene	284	24272	0.89	+	.	Gene g1
NC_000014	AUGUSTUS	mRNA	284	24272	0.89	+	.	mRNA g1.t1
NC_000014	AUGUSTUS	start_codon	284	286	.	+	0	mRNA g1.t1
NC_000014	AUGUSTUS	initial	284	346	1	+	0	mRNA g1.t1
NC_000014	AUGUSTUS	internal	2685	2789	0.99	+	0	mRNA g1.t1
NC_000014	AUGUSTUS	terminal	24063	24272	1	+	0	mRNA g1.t1
NC_000014	AUGUSTUS	CDS	284	346	1	+	0	mRNA g1.t1
NC_000014	AUGUSTUS	CDS	2685	2789	0.99	+	0	mRNA g1.t1
NC_000014	AUGUSTUS	CDS	24063	24272	1	+	0	mRNA g1.t1
NC_000014	AUGUSTUS	stop_codon	24270	24272	.	+	0	mRNA g1.t1

- **3 exons**: identical to results obtained by sequence comparison approach
  - Only coding regions (the term mRNA is abused)

GenScan results

Gn.Ex	Type	S	Begin	..End	Len	Fr	Ph	I/Ac	Do/T	CodRg	P....	Tscr..
1.01	Init	+	284	346	63	1	0	83	80	45	0.914	4.35
1.02	Intr	+	2685	2789	105	2	0	114	119	114	0.996	17.51
1.03	Term	+	24063	24272	210	2	0	83	49	404	0.985	33.29
1.04	PlyA	+	24574	24579	6							1.05

- **3 exons**: identical to results obtained by sequence comparison approach
  - Only coding parts
- **PolyA tail** predicted at the same location as FGENESH

Summary of the analysis

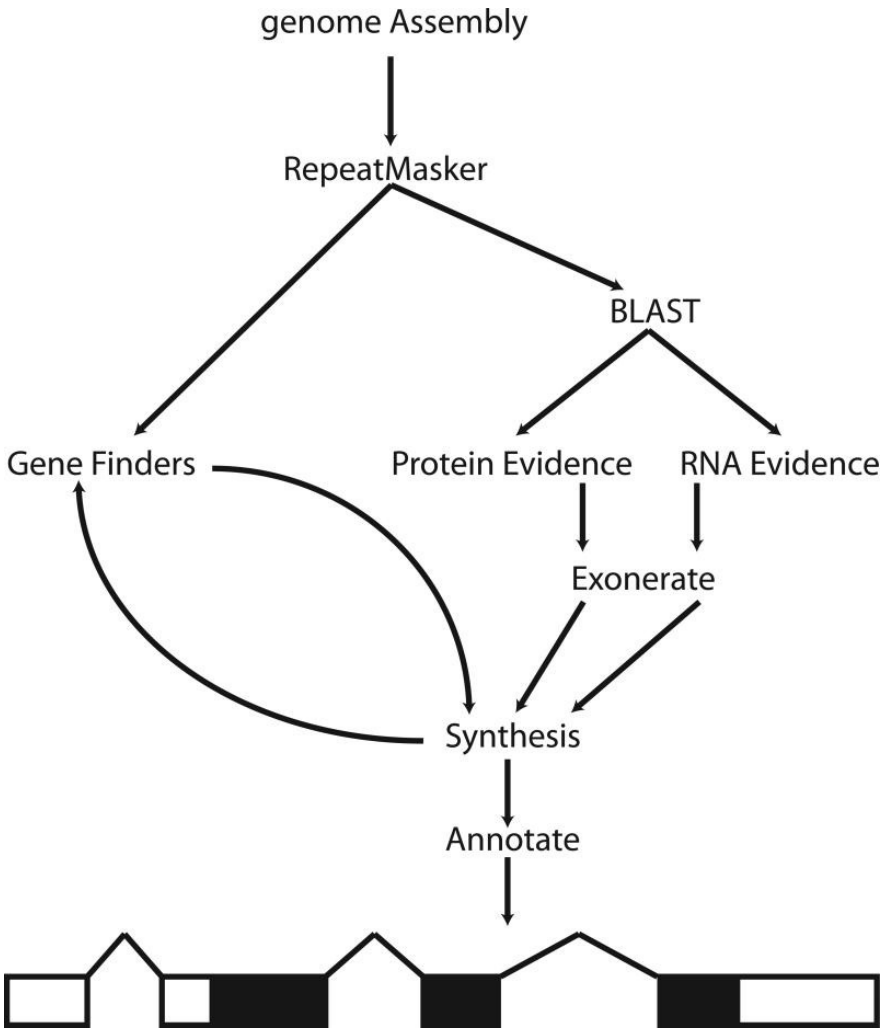
- All predictions agree on **3 exons**
- **One additional coding exon** predicted by FGENESH
  Alternative splicing ?
- The encoded protein is probably a **B-zip transcription factor**

Automatic annotation of a whole genome

- Use an annotation pipeline: Maker, PASA, Gnomon …

- Example: Maker

Cantarel BL, Korf I, Robb SM, Parra G, Ross E, Moore B, Holt C, Sánchez Alvarado A, Yandell M. MAKER: an easy-to-use annotation pipeline designed for emerging model organism genomes. Genome Res. 2008 Jan;18(1):188-96.

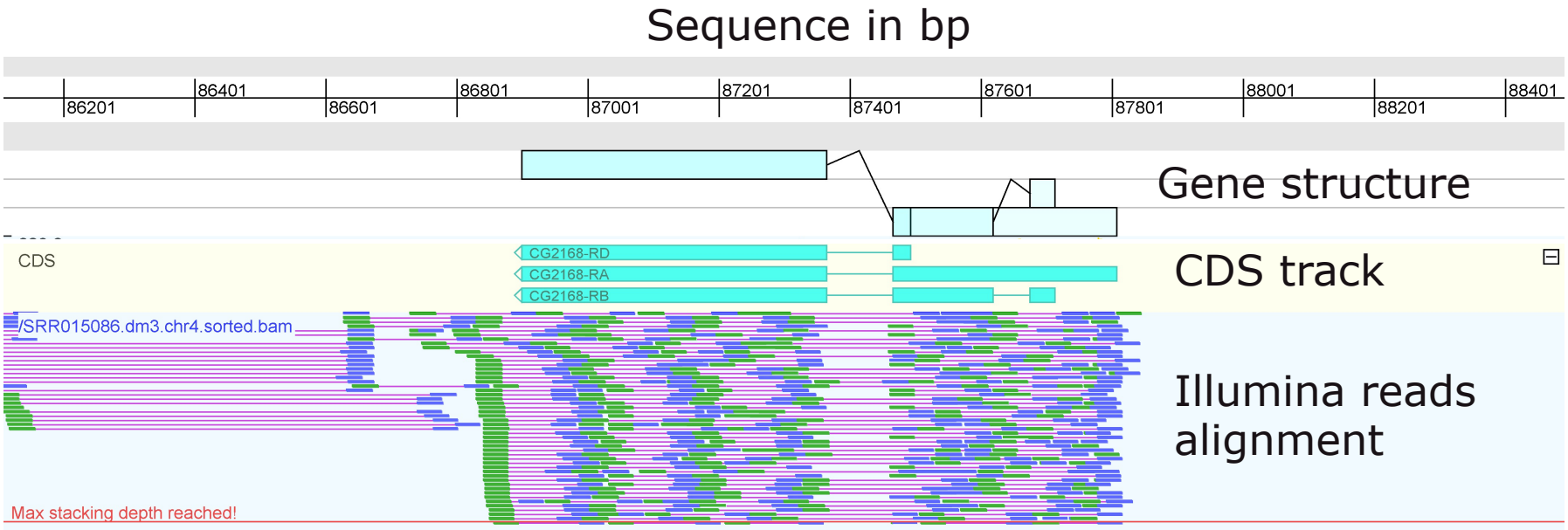


Campbell MS, Holt C, Moore B, Yandell M. Genome Annotation and Curation Using MAKER and MAKER-P. Curr Protoc Bioinformatics. 2014 Dec 12;48:4.11.1-39.

Viewing annotation data

- Five commonly used formats for annotations: **GFF3**, GenBank, BED, GTF and EMBL
  GFF3→ https://github.com/The-Sequence-Ontology/Specifications/blob/master/gff3.md

- Use of a genome visualisation tool (IGV, Jbrows, GenomeView...)



Exemple de visualisation par GenomeView

Viewing annotation data

Homo sapiens chromosome 14, GRCh38.p7 Primary Assembly

NCBI Reference Sequence: NC_000014.9

GenBank Graphics

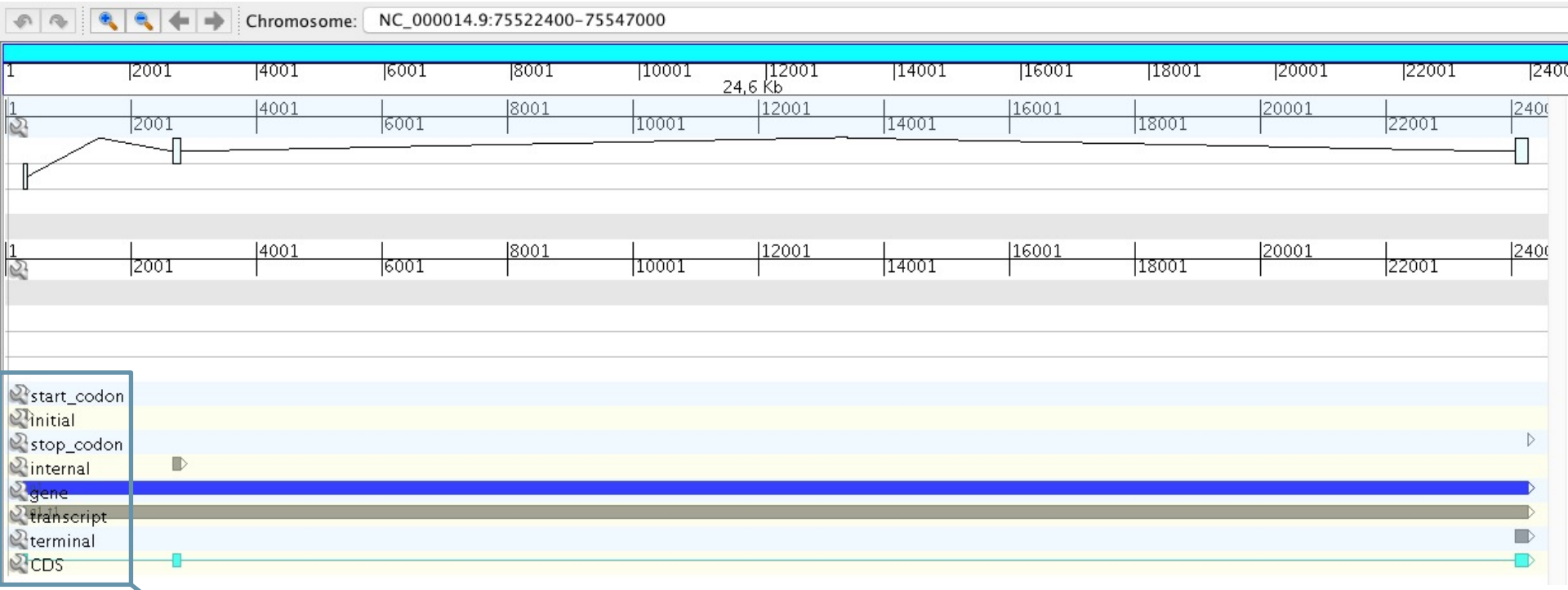
```
>NC_000014.9:75522400-75547000 Homo sapiens chromosome 14, GRCh38.p7 Primary
Assembly
TTTCCGCCCATGTGACTTCCAGCGTGAGTTACCAGAAACCACAAGAGAGAGAGAGAGCGTGCAAGCCCCA
AAGCGAGCGACATGTCCCTTTGGGGAGCAGTCCCTCTGCACCCCAGAGTGAGGAGGACGCAGGGGTCAGA
GGTGGCTACAGGGCAGGCAGAGGAGGCACCTGTAGGGGGTGGTGGGCTGGTGGCCCAGGAGAAGTCAGGA
AGGGAGCCCAGCTGGTGACAAGAGAGCCCAGAGGTGCCTGGGGCTGAGTGTGAGAGCCCGGAAGATTTCA
GCCATGCCTCACAGCTCCGACAGCAGTGACTCCAGCTTCAGCCGCTCTCCTCCCCCTGGCAAACAGGTAG
AGTCCTCCTTTTTCTCTCTCCTACCTTCTGATTCTCCTGGGGGATGGAAAGAGAGCCAGGCTTCCTTGTC
CTGCCCAGGGAGCTGAGGATGGAGGAAGTGGCTCGTTGCACGGGCACTCTGTTAGACTTAGGACATGGAA
TTTGCTACTAAGCTGTGCATATTGGCAGAGATCCTCATCCTTCCACCCATTCTGCCAAAGCCCCTTTTTC
TCTCCATTTTCCAAGGCTGCCTATCACCTCTGCCTCACTGGGGTTGCCACCCTAAAAAGCTTTCTAGGAA
GCAAAGAGGAGGATGAACATCAAAGAATGCAGAGAAAAGAGTCTACTGTTCTCCAAGGCTGTAGAAAAGT
```

Sequence in FASTA forma

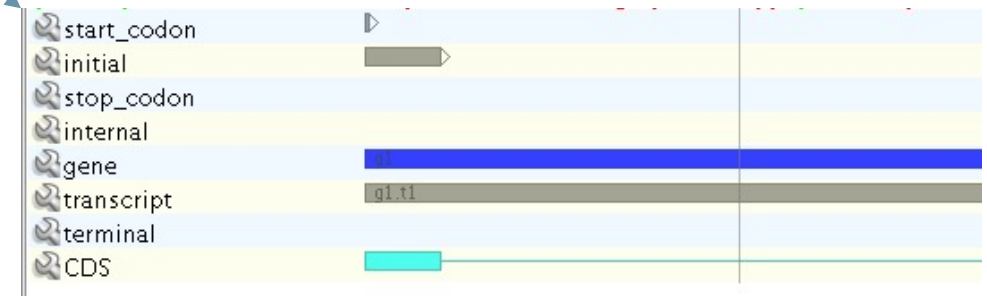
NC_000014	AUGUSTUS	gene	284	24272	0.89	+	.	Gene g1
NC_000014	AUGUSTUS	mRNA	284	24272	0.89	+	.	mRNA g1.t1
NC_000014	AUGUSTUS	start_codon	284	286	.	+	0	mRNA g1.t1
NC_000014	AUGUSTUS	initial	284	346	1	+	0	mRNA g1.t1
NC_000014	AUGUSTUS	internal	2685	2789	0.99	+	0	mRNA g1.t1
NC_000014	AUGUSTUS	terminal	24063	24272	1	+	0	mRNA g1.t1
NC_000014	AUGUSTUS	CDS	284	346	1	+	0	mRNA g1.t1
NC_000014	AUGUSTUS	CDS	2685	2789	0.99	+	0	mRNA g1.t1
NC_000014	AUGUSTUS	CDS	24063	24272	1	+	0	mRNA g1.t1
NC_000014	AUGUSTUS	stop_codon	24270	24272	.	+	0	mRNA g1.t1

Gene prediction by AUGUSTUS in GFF3 format

Visualising annotation data in GenomeView



Different tracks



References

- Annotation of **prokaryotic** genomes
  - Richardson EJ, Watson M. The automatic annotation of bacterial genomes. Brief Bioinform. 2013 Jan;14(1):1-12.

- Annotation of **eukaryotic** genomes
  - Stein L. Genome annotation: from sequence to biology. Nat Rev Genet. 2001 Jul;2(7):493-503.
  - Yandell M, Ence D. A beginner's guide to eukaryotic genome annotation. Nat Rev Genet. 2012 Apr 18;13(5):329-42.
  - Mudge JM, Harrow J. The state of play in higher eukaryote gene annotation. Nat Rev Genet. 2016 Dec;17(12):758-772.

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