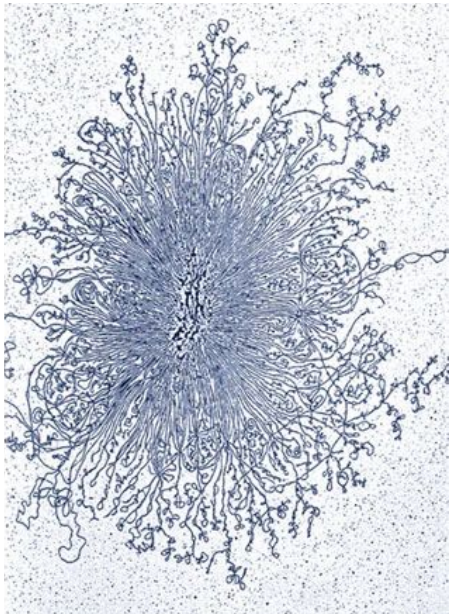
Introduction to gene prediction and functional annotation

Guillaume GAUTREAU, 05/06/2024

Prokaryotic genomes…

DNA/chromosome : molecular support



E. coli nucleoid (Wang *et al.,* 2013)

Genome : the information, the sequence from the chromosome

GTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAAC
CACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCC
GATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGG
GCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCA
CCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATT
GTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTA
CGGATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCT
GGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAG
CTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAG
TTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCAC
CAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGC
CGATACTGTCGTCGTCCCCTCAAACTGGCAGATGCACGGTTACGATG
CGCCCATCTACACCAACGTGACCTATCCCATTACGGTCAATCCGCCG
TTTGTTCCCACGGAGAATCCGACGGGTTGTTACTCGCTCACATTTAAT
GTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGA
TGGCGTTAACTCGGCGTTTCATCTGTGGTGCAACGGGCGCTGGGTC
GGTTACGGCCAGGACAGTCGTTTGCCGTCTGAATTTGACCTGAGCG
CATTTTTACGCGCCGGAGAAAACCGCCTCGCGGTGATGGTGCTGCG
CTGGAGTGACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGATG
AGCGGCATTTTCCGTGACGTCTCG.....
.....

…annotation

According to Cambridge Dictionary

annotate

verb [T] • formal

UK /ˈæn.ə.teɪt/ US /ˈæn.ə.teɪt/

[often passive]

to add a short explanation or opinion to a text or image:

- *Annotated editions of Shakespeare's plays help readers to understand old words.*
- *an annotated bibliography/manuscript/edition*
- *His great-granddaughter has painstakingly transcribed and annotated his wartime diaries.*
- *The students annotate their photos, saying why they are relevant.*

COMPUTING, LANGUAGE • specialized

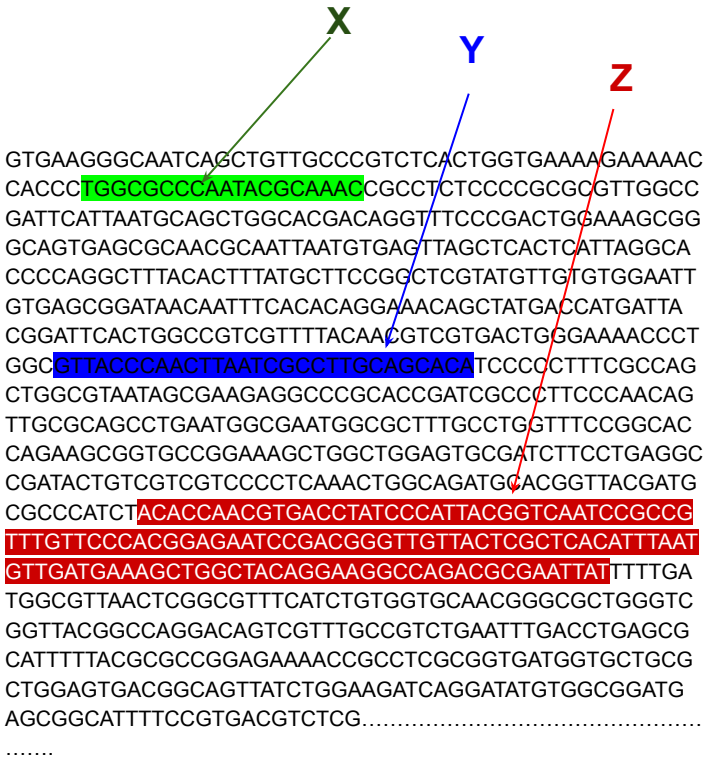
to add a description or piece of information to data, for example a label saying whether a word is a noun, a verb, etc.:

- *After the corpus was collected we annotated it.*
- *Textual or numerical data can be copied into databases, annotated, and linked to other data.*

Prokaryotic genome annotation

It means the combination of:

1. a genomic sequence location (described using coordinates)
2. a biological meaning for this sequence:
   - Is it a gene ?
     - what the function of that gene ?
     - Is it a coding gene or a non coding gene ?
     - Is it a set of co-transcribed genes ?
     - Is it regulated by a common factor ?
   - Is it a restriction site ?
   - Is it a integration hotspot ?
   - Is it a replication origin ?
   - ….



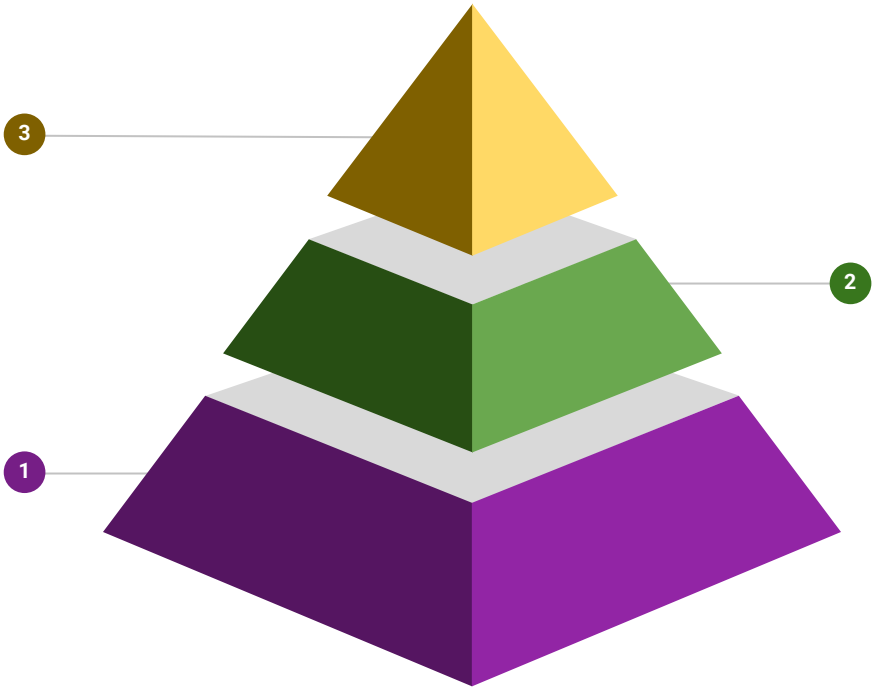
Cumulative levels of genomic annotation

Relational annotation

Which genes are associated in a common biological process ? (how?)

Syntactic annotation

Detect the location of the genes (where ?)



Functional annotation

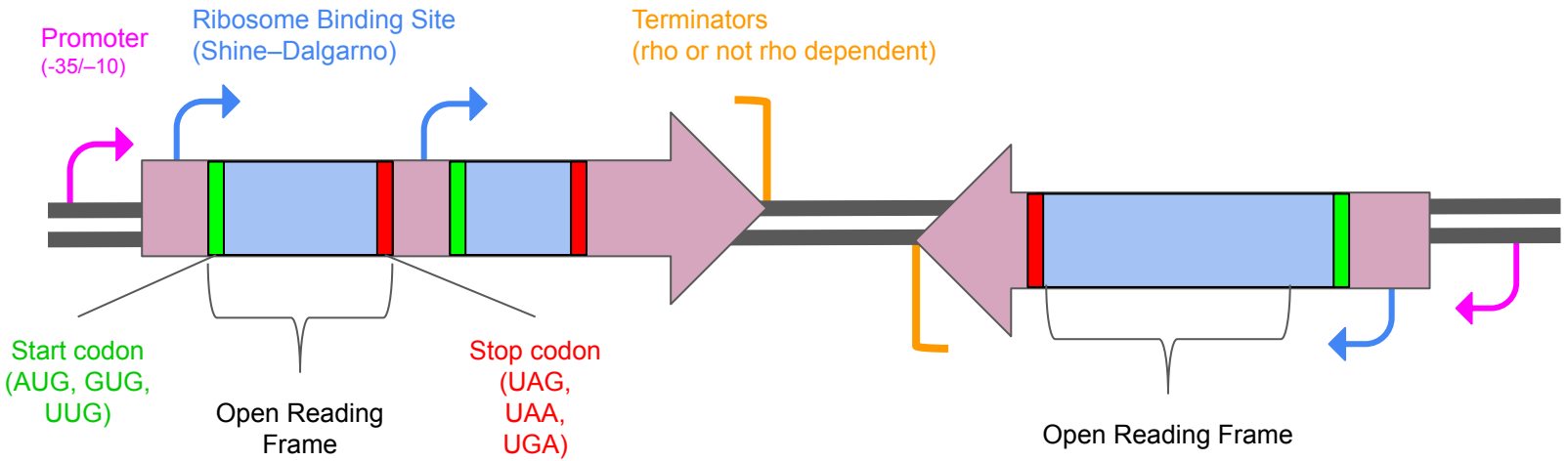
Find the biological functions of the genes (what?)

How to detect gene positions in a genome ?

Starting from an assembly, syntactic annotation is based on the identification of specific patterns:

- Identify the coding genes :
  - Start using a start codon (mostly “ATG”) and end with a STOP codon
  - Has a specific frequency of nucleotides (because of the genetic code constraints)
- Identify the non coding genes based on their specific patterns :
  - tRNA
  - tmRNA
  - rRNA
  - lncRNA

Reminder on the genomic structure of coding genes



But the actual data are:

GTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGC
AGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCC
GGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGGATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGG
AAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCG
CAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTCCC
CTCAAACTGGCAGATGCACGGTTACGATGCGCCCATCTACACCAACGTGACCTATCCCATTACGGTCAATCCGCCGTTTGTTCCCACGGAGAATCCGACGGGTTGTT
ACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGATGGCGTTAACTCGGCGTTTCATCTGTGGTGCAACGGGCGCTGG
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GTTAATGTATGAGTTTAATCACTCGCATCCATCAGA

But the actual data are:

GTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGC
AGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCC
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GAATGGTGCAGCGCGATCGTAATCACCCGAGTGTGATCATCTGGTCGCTGGGGAATGAATCAGGCCACGGCGCTAATCACGACGCGCTGTATCGCTGGATCAAATC
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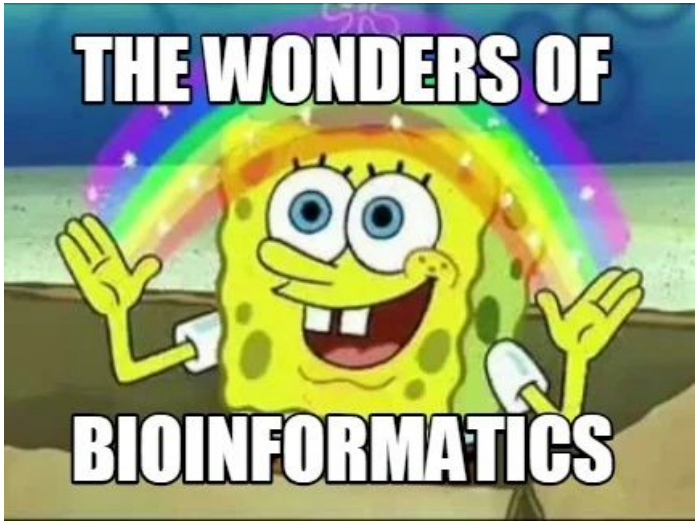
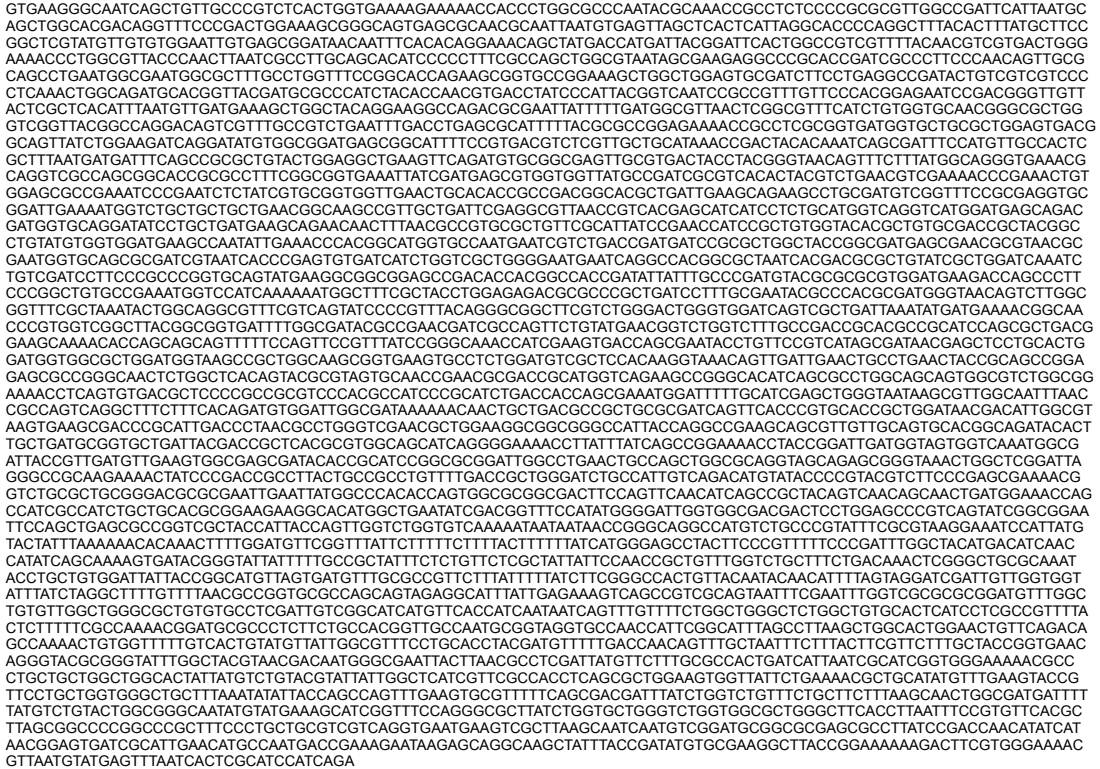


Bioinformatics solutions



Two kinds of approaches:

- Alignments methods:

Aligning our sequence over data banks of known genes

- *Ab initio* methods:

Detection of signals in our sequence corresponding to a set of rules describing a gene

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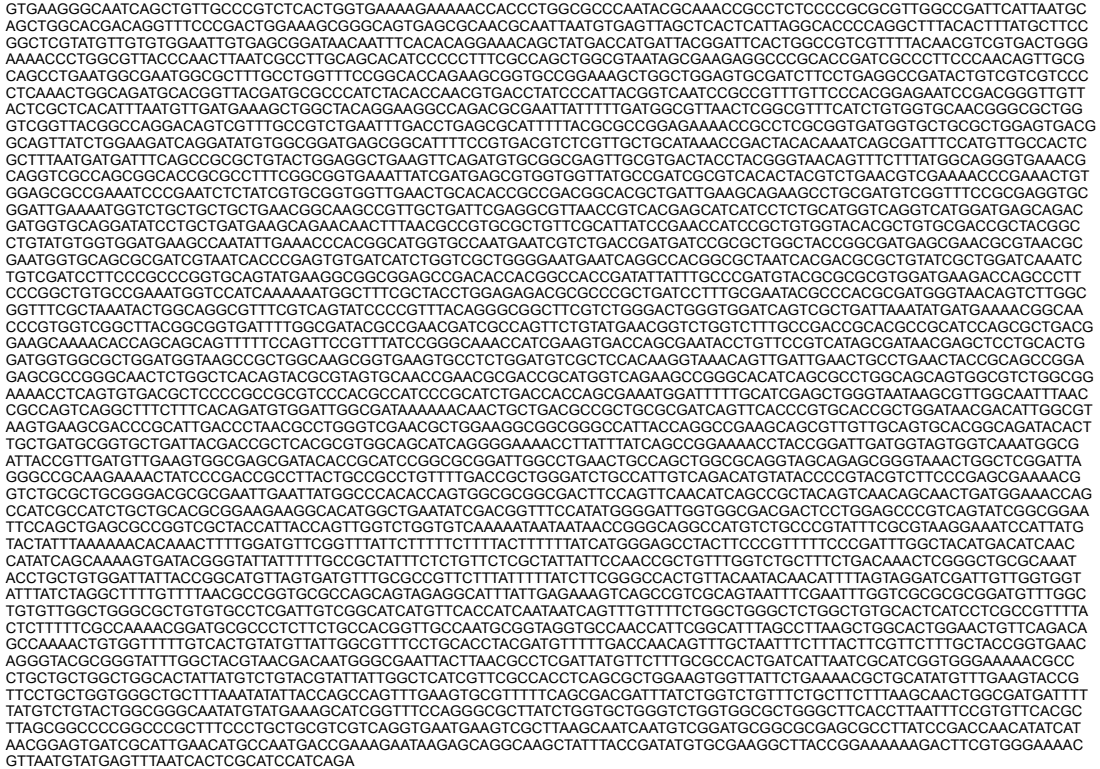

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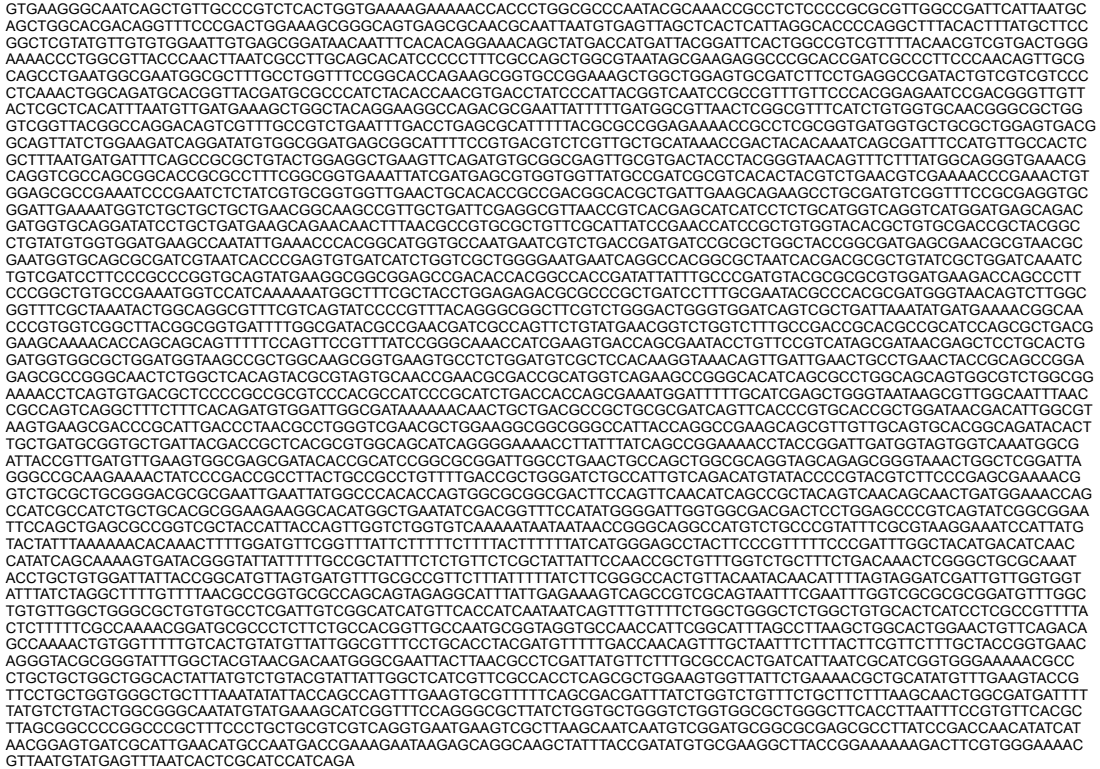

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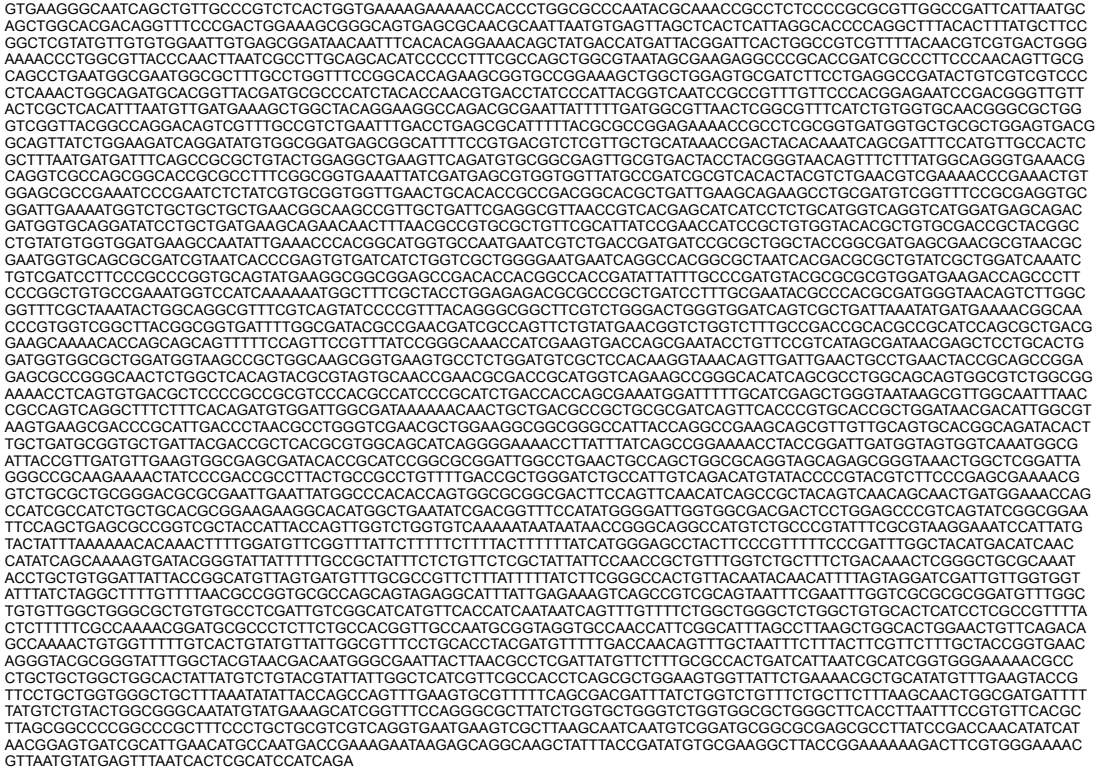

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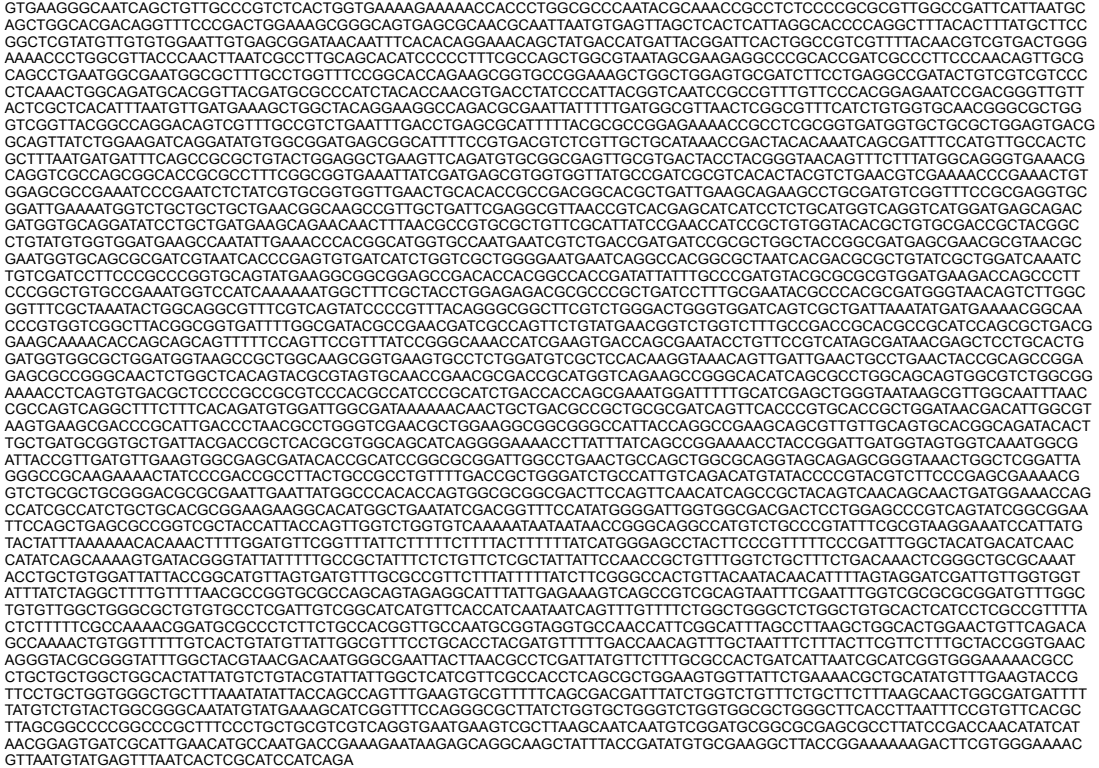
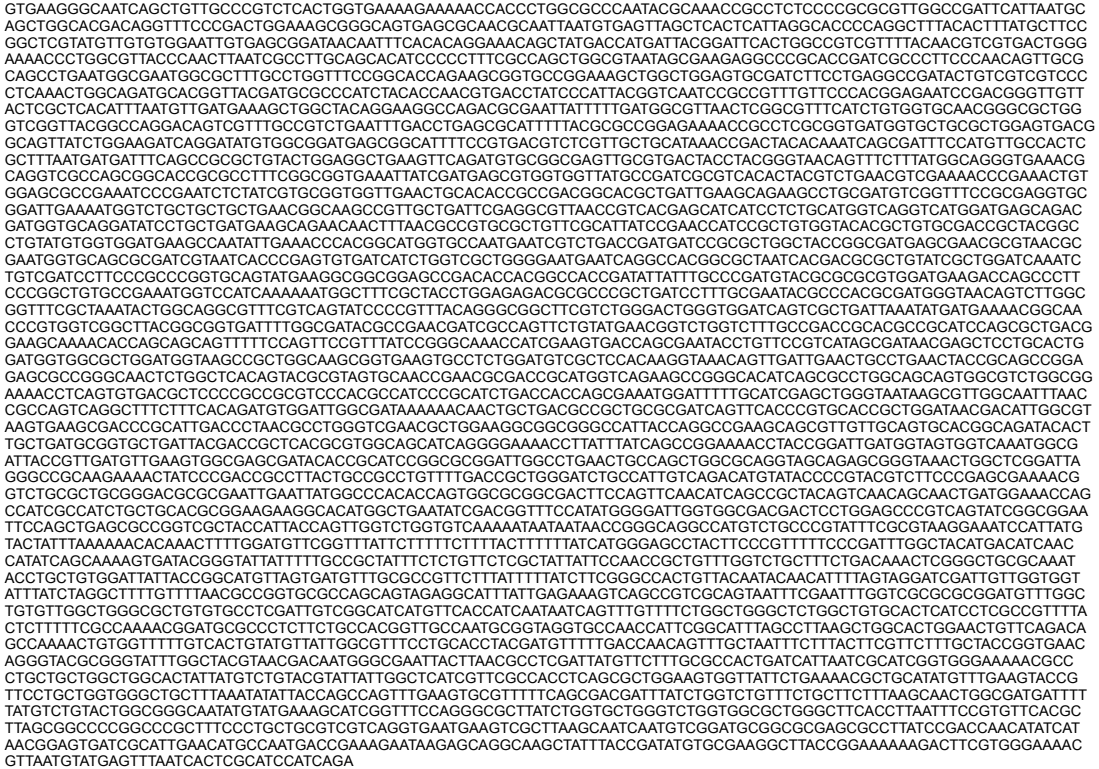


2 main steps :

1) List Open Reading Frames (OFRs) **over the 6 reading frames** :
   - starting with a start codon (mostly ATG)
   - ending with a stop codon (TAA,TAG,TGA)
2) Select valid ORFs

Ab initio methods



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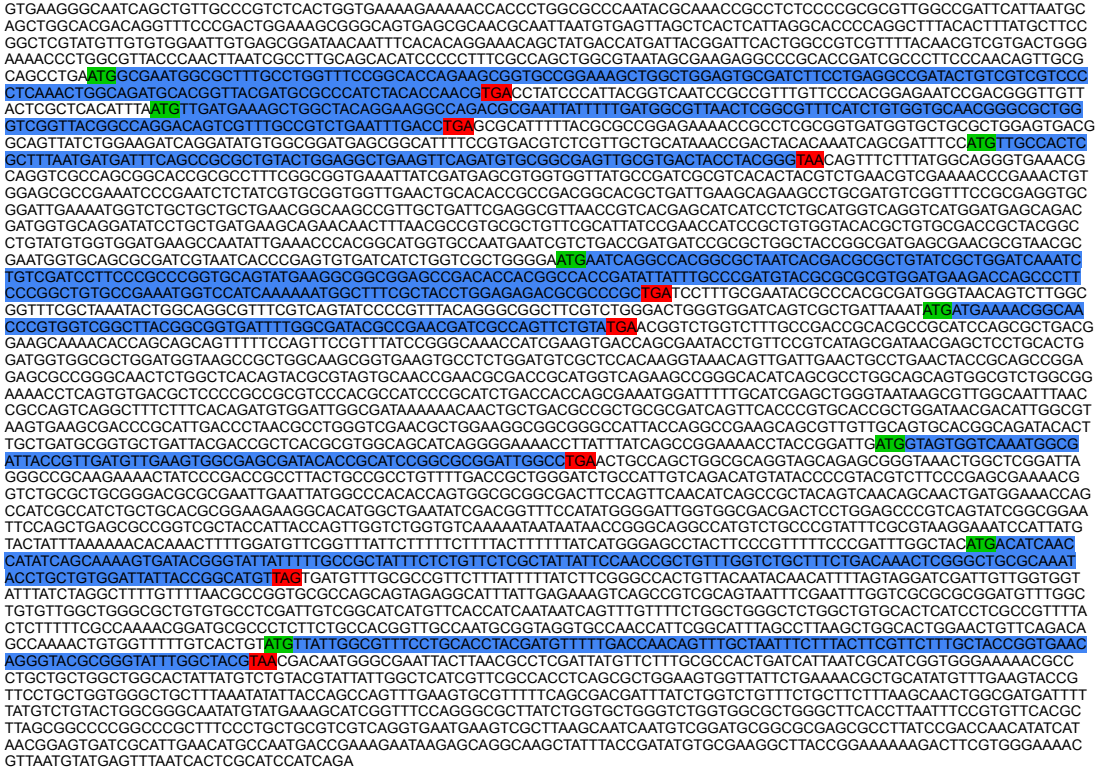
Example of ORF finding* : phase +1



- When several start codons are available, the longest ORF is reported
- Here : 8 ORFs found

* to simplify, only ORFs starting with an ATG start codon are shown

Example of ORF finding : phase -1

GTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGC
AGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCC
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GCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAGCGGCATTTTCCGTGACGTCTCGTTGCTGCATAAACCGACTACACAAATCAGCGATTTCCATGTTGCCACTC
GCTTTAATGATGATTTCAGCCGCGCTGTACTGGAGGCTGAAGTTCAGATGTGCGGCGAGTTGCGTGACTACCTACGGGTAACAGTTTCTTTATGGCAGGGTGAAACG
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TTCCTGCTGGTGGGCTGCTTTAAATATATTACCAGCCAGTTTGAAGTGCGTTTTTCAGCGACGATTATCTGGTCTGTTTCTGCTTCTTTAAGCAACTGGCGATGATTTT
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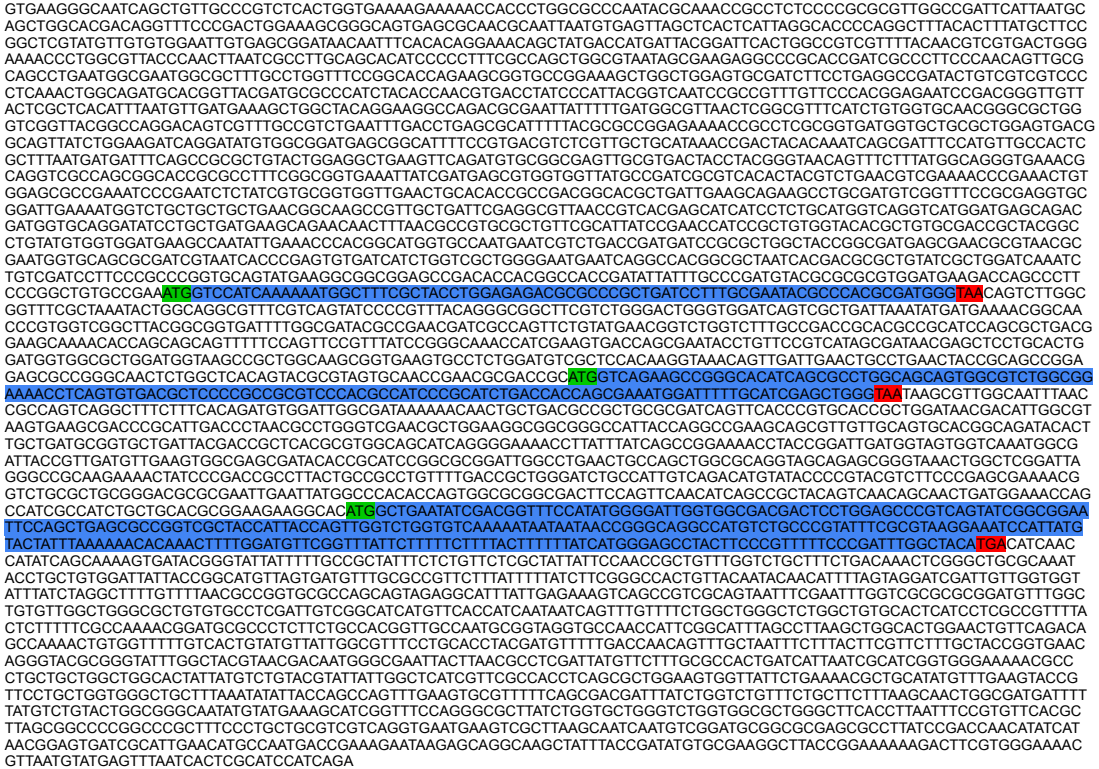
AACGGAGTGATCGCATTGAACATGCCAATGACCGAAAGAATAAGAGCAGGCAAGCTATTTACCGATATGTGCGAAGGCTTACCGGAAAAAAGACTTCGTGGGAAAAC
GTTAATGTATGAGTTTAATCACTCGCATCCATCAGA

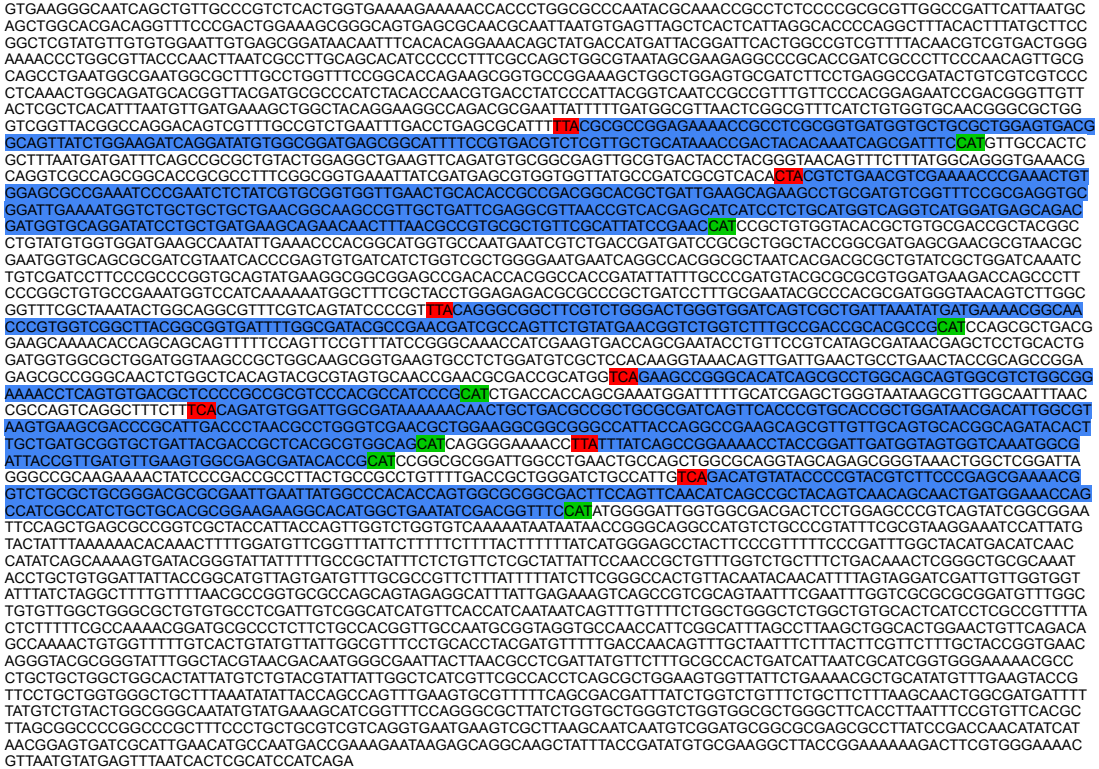
- In the other direction :
  - ATG ⇒ CAT
  - TAA ⇒TTA
  - TGA ⇒TCA
  - TAG ⇒CTA
- here : 2 ORFs found

Example of ORF finding : phase +2



- 3 ORFs found

Example of ORF finding : phase -2



- 7 ORFs found

Example of ORF finding : phase +3 and -3

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GGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGGATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGG
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CAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTCCC
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GCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAGCGGCATTTTCCGTGACGTCTCGTTGCTGCATAAACCGACTACACAAATCAGCGATTTCCATGTTGCCACTC
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GATGGTGCAGGATATCCTGCTGATGAAGCAGAACAACTTTAACGCCGTGCGCTGTTCGCATTATCCGAACCATCCGCTGTGGTACACGCTGTGCGACCGCTACGGC
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TACTATTTAAAAAACACAAACTTTTGGATGTTCGGTTTATTCTTTTTCTTTTACTTTTTTATCATGGGAGCCTACTTCCCGTTTTTCCCGATTTGGCTACATGACATCAAC
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AACGGAGTGATCGCATTGAACATGCCAATGACCGAAAGAATAAGAGCAGGCAAGCTATTTACCGATATGTGCGAAGGCTTACCGGAAAAAAGACTTCGTGGGAAAAC
GTTAATGTATGAGTTTAATCACTCGCATCCATCAGA

Example of ORF finding : phase +3 and -3

GTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGC
AGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCC
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ACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGATGGCGTTAACTCGGCGTTTCATCTGTGGTGCAACGGGCGCTGG
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CAGGTCGCCAGCGGCACCGCGCCTTTCGGCGGTGAAATTATCGATGAGCGTGGTGGTTATGCCGATCGCGTCACACTACGTCTGAACGTCGAAAACCCGAAACTGT
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GGATTGAAAATGGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATTCGAGGCGTTAACCGTCACGAGCATCATCCTCTGCATGGTCAGGTCATGGATGAGCAGAC
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CTGCTGCTGGCTGGCACTATTATGTCTGTACGATTATTGGCTCATCGTTCGCCACCTCAGCGCTGGAAGTGGTTATTCTGAAAACGCTGCATATGTTTGAAGTACCG
TTCCTGCTGGTGGGCTGCTTTAAATATATTACCAGCCAGTTTGAAGTGCGTTTTTCAGCGACGATTTATCTGGTCTGTTTCTGCTTCTTTAAGCAACTGGCGATGATTTT
TATGTCTGTACTGGCGGGCAATATGTATGAAAGCATCGGTTTCCAGGGCGCTTATCTGGTGCTGGGTCTGGTGGCGCTGGGCTTCACCTTAATTTCCGTGTTCACGC
TTAGCGGCCCCGGCCCGCTTTCCCTGCTGCGTCGTCAGGTGAATGAAGTCGCTTAAGCAATCAATGTCGGATGCGGCGCGAGCGCCTTATCCGACCAACATATCAT
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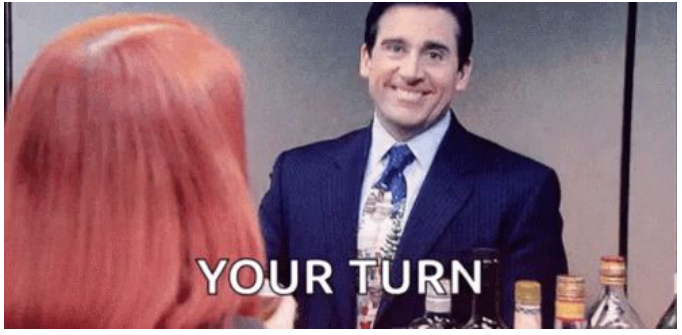
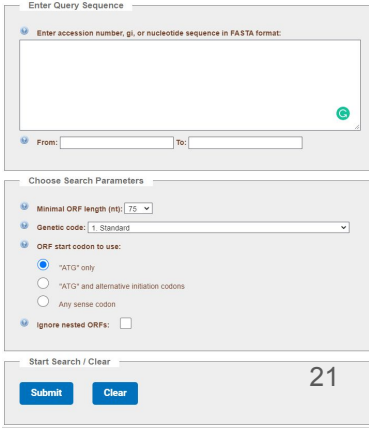


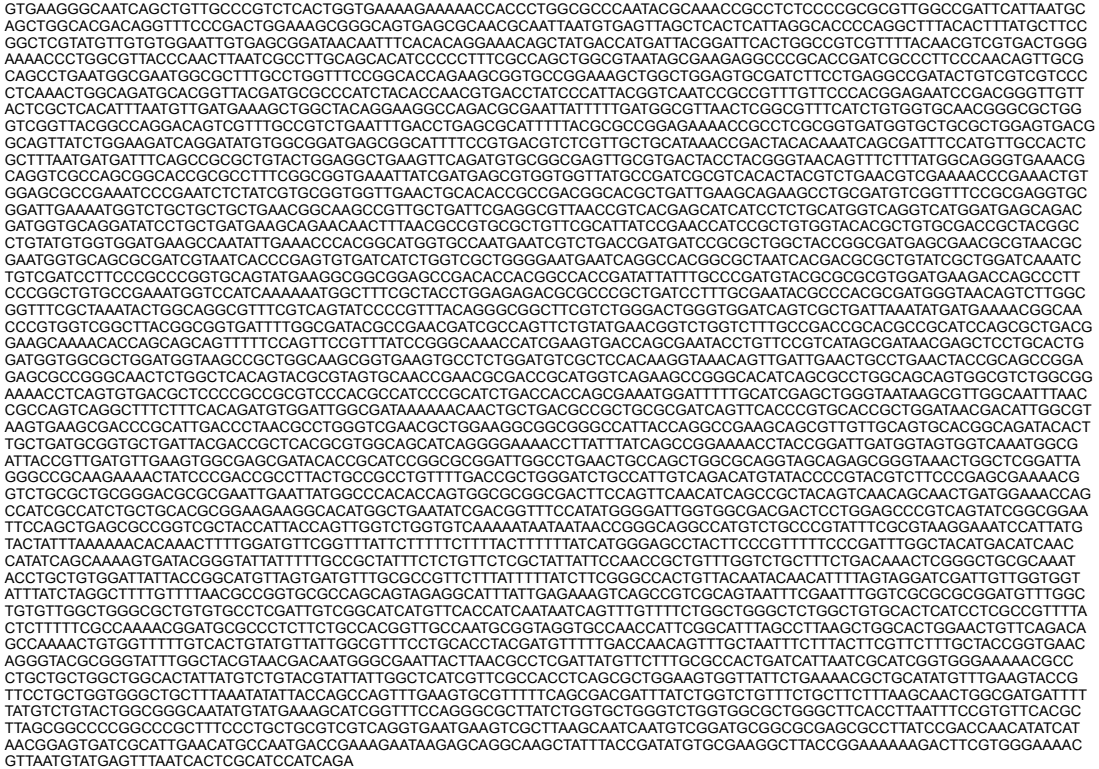
https://www.ncbi.nlm.nih.gov/orffinder/

Actually, you can use ORFFinder,

It would be easier :



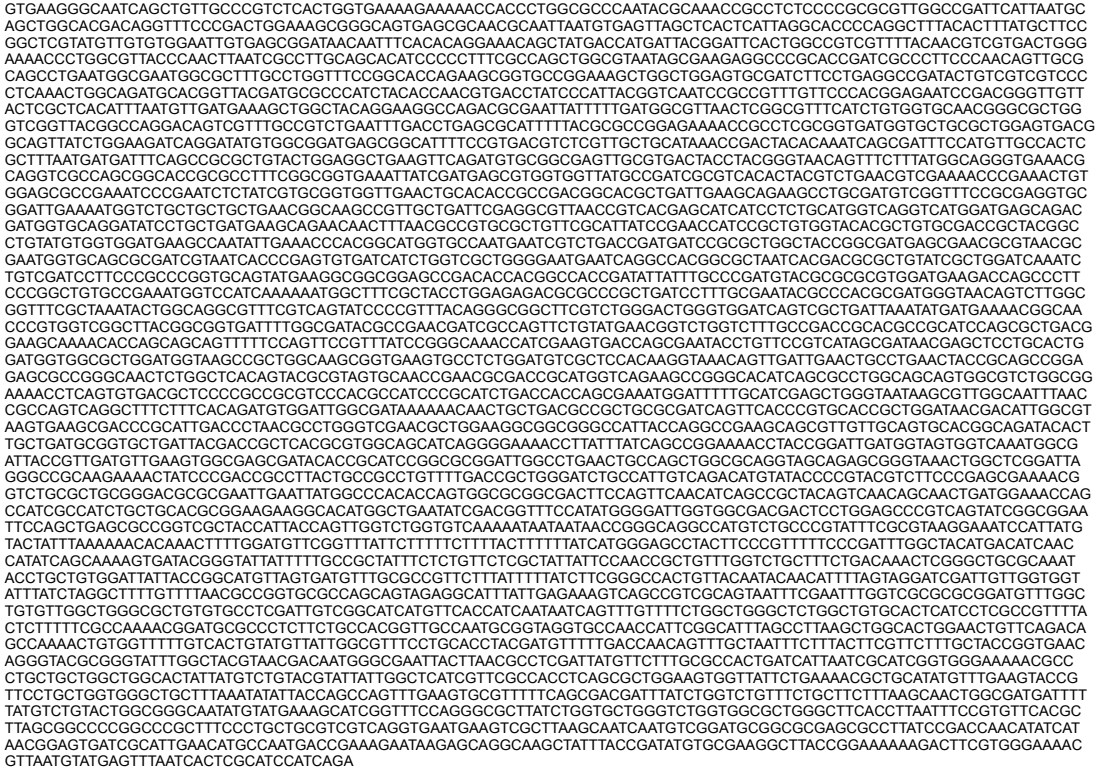
Ab initio methods



2 mains steps :

1) List Open Reading Frames (OFRs) **over the 6 reading frames** :
   - starting with a start codon (mostly ATG)
   - ending with a stop codon (TAA,TAG,TGA)
2) Select valid ORFs

Ab initio methods



2 mains steps :

1) List Open Reading Frames (ORFs) **over the 6 reading frames** :
   - starting with a start codon (mostly ATG)
   - ending with a stop codon (TAA,TAG,TGA)
2) Select valid ORFs

Select valid ORFs because of overlaps between phases

Example:

But overlaps can be valid too…

Select valid ORFs because of overlaps between phases

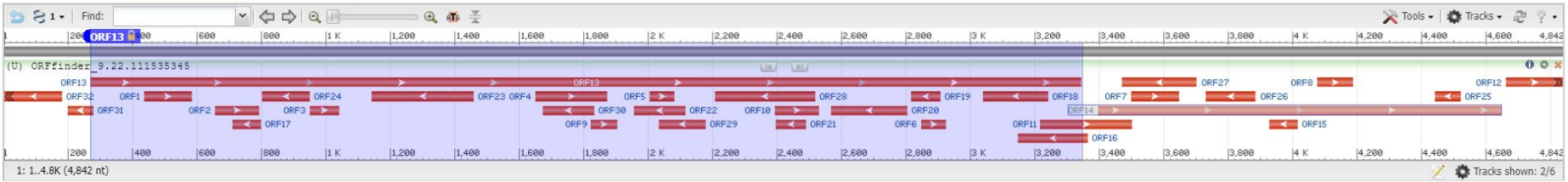
< ORFfinder submitting page

> Help

Open Reading Frame Viewer

Sequence

ORFs found: 32 Genetic code: 1 Start codon: 'ATG' only



ORF13 (1024 aa) Display ORF as... Mark

```
>lcl|ORF13
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FHLWCNGRWVGYGQDSRLPSEFDLSAFLRAGENRLAVMVLRWSDGSYLED
QDMWRMSGIFRDVSLLHKPTTQISDFHVATRFNDDFSRAVLEAEVQMCGE
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LWSAEIPNLYRAVVELHTADGTLIEAEACDVGFREVRIENGLLLLNGKPL
LIRGVNRHEHHPLHGQVMDEQTMVQDILLMKQNNFNAVRCSHYPNHPLWY
TLCDRYGLYVVDEANIETHGMVPMNRLTDDPRWLPAMSERVTRMVQRDRN
HPSVIIWSLGNESGHGANHDALYRWIKSVDPSRPVQYEGGGADTTATDII
CPMYARVDEDQPFPAVPKWSIKKWLSLPGETRPLILCEYAHAMGNSLGGF
```

ORF13

SmartBLAST

BLAST

Marked set (0)

SmartBLAST best hit titles...

BLAST

BLAST Database:

UniProtKB/Swiss-Prot (swissprot)

Mark subset... Marked: 0 Download marked set as Protein FASTA

Six-frame translation...

Label	Strand	Frame	Start	Stop	Length (nt | aa)
ORF27	-	3	3703	3470	234 | 77
ORF4	+	1	1651	1872	222 | 73
ORF16	-	1	3363	3148	216 | 71
ORF18	-	2	3242	3039	204 | 67
ORF32	-	3	181	>2	180 | 59
ORF12	+	2	4664	>4840	177 | 58
ORF30	-	3	1831	1673	159 | 52
ORF22	-	2	2114	1956	159 | 52
ORF26	-	3	3886	3731	156 | 51
ORF7	+	1	3499	3648	150 | 49
ORF24	-	2	950	801	150 | 49

How to select valid ORFs in *ab initio* methods ?

No consensual methods but here are the main tools used:

- Glimmer
- (Meta)GeneMarkS2
- Phanotate : dedicated to phage genes
- PROkaryotic DYnamic programming Gene-finding ALgorithm (PRODIGAL)

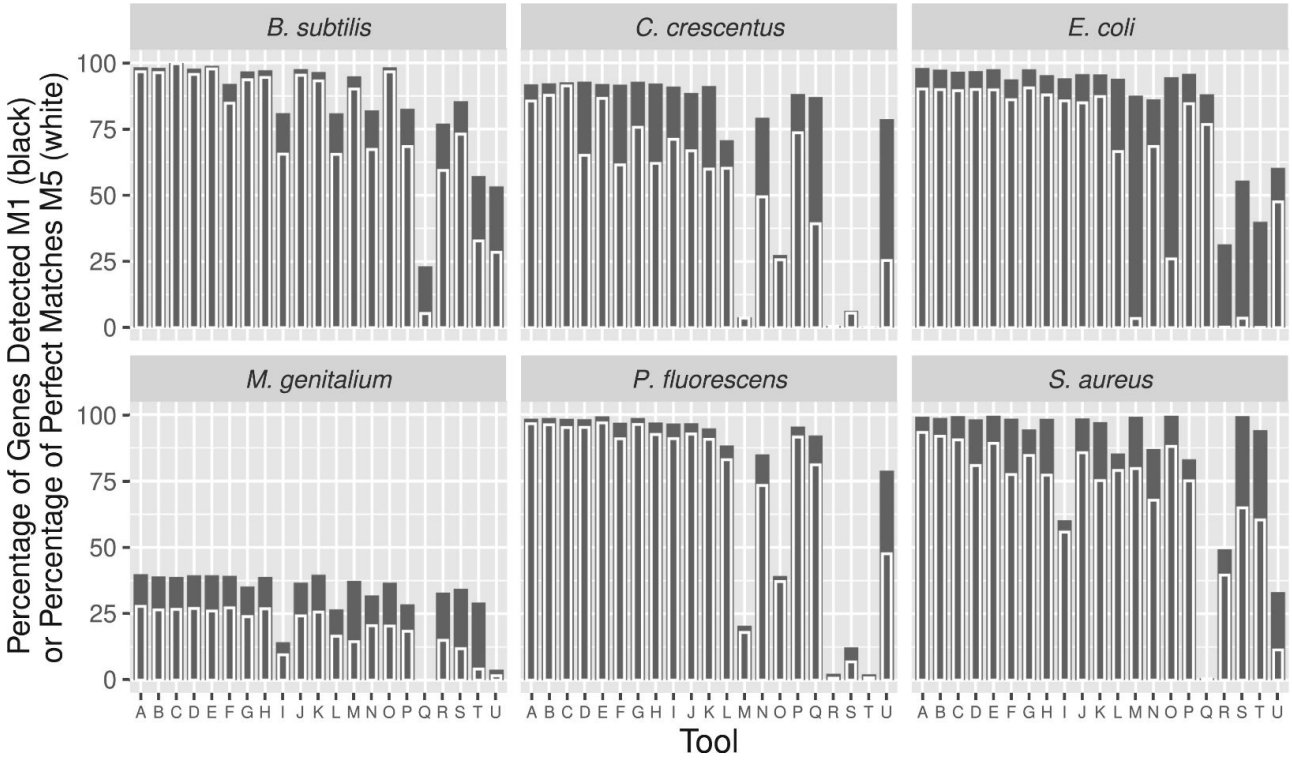
How to select valid ORFs in *ab initio* methods ?

No consensual methods but here are the main tools used :

- Glimmer
- (Meta)GeneMarkS2
- Phanotate : dedicated to phage genes
- **PROkaryotic DYnamic programming Gene-finding ALgorithm (PRODIGAL)**
  - Good results in benchmarking
  - Prodigal runs very quickly (a bacterial genome in about 10 seconds)
  - The *ab initio* prediction tool used by almost everybody (often wrapped in other tools)

Benchmark of *ab initio* gene prediction tools

Dimonaco *et al.,* 2022



Tools

A : Prodigal
B : GeneMarkS –2
C : MetaGeneAnnotator
D : MetaGeneMark
E : GeneMarkS
F : MetaGene
G : GeneMark.hmm/ *E.coli* Model
H : GeneMark –HA
I : Augustus/ *E.coli* Model
J : FragGeneScan
K : GLIMMER –3
L : EasyGene/ *E.coli* Model
M : Augustus/ *S.aureus* Model
N : Transdecoder
O : GeneMark.hmm/ *S.aureus* Model
P : FGENESB/ *E.coli* Model
Q : GeneMark/ *E.coli* Model
R : EasyGene/ *S.aureus* Model
S : FGENESB/ *S.aureus* Model
T : GeneMark/ *S.aureus* Model
U : Augustus/ *H.sapiens* Model

The Prodigal algorithm : a nightmare to explain

1. Read in the sequence
2. Locate all starts and stops in the genome
3. Scan all open reading frames and record numbers of G's and C's in each codon position
4. Build a frame bias model based on ORF length and G/C codon position within each ORF
5. Record the highest scoring start nodes in each frame that overlap a stop codon by <= 60 bp
6. Do the first pass dynamic programming, connecting nodes based on frame bias scores
7. Create a hexamer background of all 6-mers in the entire sequence
8. FOR each gene model in the dynamic programming output:
    1. Gather all hexamer statistics
9. Create log table of hexamer coding scores
10. FOR each gene model in the dynamic programming output:
    1. Calculate a coding score based on hexamer statistics
    2. Penalize the score if there is a higher scoring start upstream in the same ORF
    3. IF the gene is very long but has a negative score, THEN give it a barely positive score
11. FOR 10 iterations
    1. Build a ribosomal binding site and ATG/GTG/TTG background for all nodes
    2. FOR each gene with a score of > 35.0:
        1. Gather its Shine-Dalgarno RBS motif data and ATG/GTG/TTG data
    3. Modify RBS and ATG/GTG/TTG weights by the observations
12. IF organism is not determined to use Shine-Dalgarno THEN run the non-SD finder
13. FOR each gene model:
    1. Assign a final score of start score + coding score
    2. Penalize the final score of genes < 250bp
14. Do the second pass dynamic programming, connecting nodes based on hexamer coding
15. FOR each gene model in the final dynamic programming:
    1. Eliminate negative scoring models
    2. Resolve very close start pairs (<= 15 bp from each other)
16. Print final output

The Prodigal algorithm : a nightmare to explain (is it worth it ?)

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The Prodigal algorithm : a summary

1. Read in the sequence
2. Locate all starts and stops in the genome
3. Scan all open reading frames and record numbers of G's and C's in each codon position

Simplified

4. Look for bias in the percentage of GC% in candidates: find ORFs having a similar %GC biais

5. Look for difference in the frequency of hexamers statistics in contrast to background (region not covered by ORF)

6. Look for conserved upstream ORF patterns (Shine-Delgarno)

7. After computing all of these statistics : output predicted coding genes (Coding DNA Sequences, CDS)

Conclusion

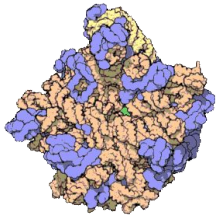
- Coding gene are detected via *ab initio* methods by finding all ORFs and selecting the most relevant one based on complex empiric rules
- This approach allow predicting **even unknown coding genes** contrary to an alignment approach, but :
  - some genes can be missed
  - some genes can be truncated due to a partial detection
  - some genes can be longer than the reality
  - some detected genes can be biologically inactive (pseudogenes)
- Therefore, syntactic annotation must be refined using a database of known proteins (see lesson 5. Introduction to functional annotation)

Prediction of non coding genes

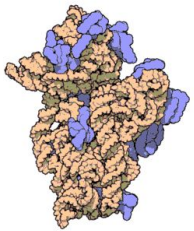
Different kinds of non coding genes : rRNA

- Several clusters composed of 3 rRNA genes can be detected in the genome

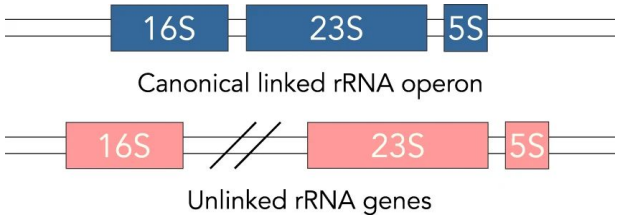
credit: Wikipedia



Large subunit 50S
(23S+5S+proteins)



small subunit 30S
(16S+proteins)



Brewer *et al.*, 2020

- One or some tRNA genes often lie between 16S and et 23S genes (case of linked rRNA genes)
- 16S rRNA genes are conserved in some regions and variables in other ones (V1,V2…) :
  - Largely used for phylogeny (Carl Woese, 1977)
  - And for metabarcoding to describe the biological content of a sample (“metagenomic”)

credit: https://www.repertoire.co.jp/en/research/technology/16srrnainfo/

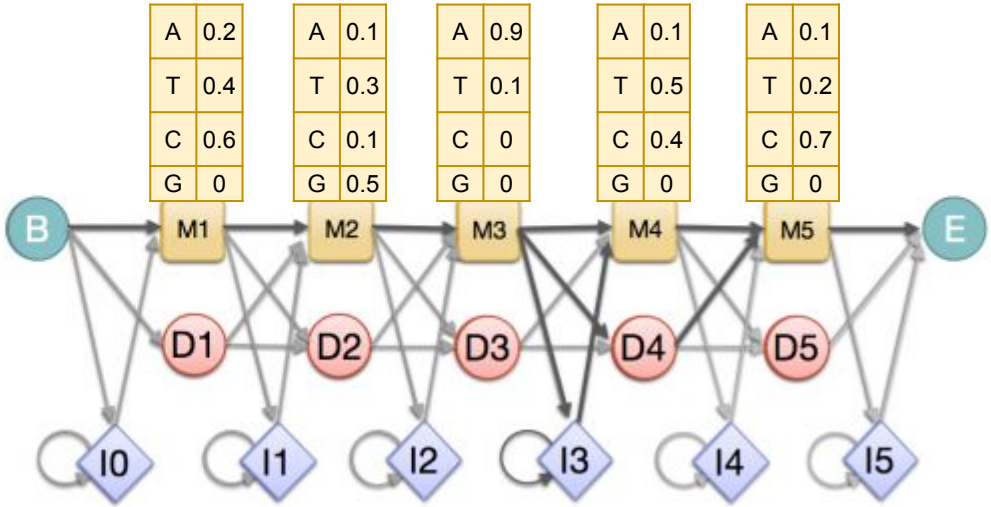
Different kinds of non coding genes : rRNA

- Detection of rRNA can be performed using RNAmmer
  1. patterns of rRNA genes are stored as Hidden Markov Model profiles (HMM) :

An alignment of rRNA genes:

AAATT
TTATC
CTACT
ATACC
CCTTC
TGATA
CGAAC
TGACC
CGACC
CGATC

modeled into HMM



modified from: Wikipedia

Different kinds of non coding genes : rRNA

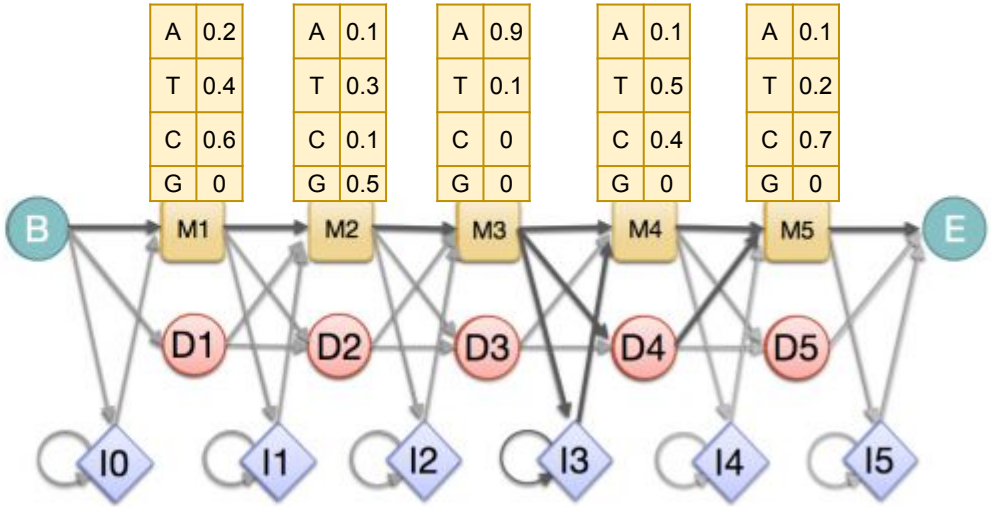
- Detection of rRNA can be performed using RNAmmer
  2. sequences of our genome are aligned on the HMM profiles :

sequences from the genome

…CTAGTC…

Alignment

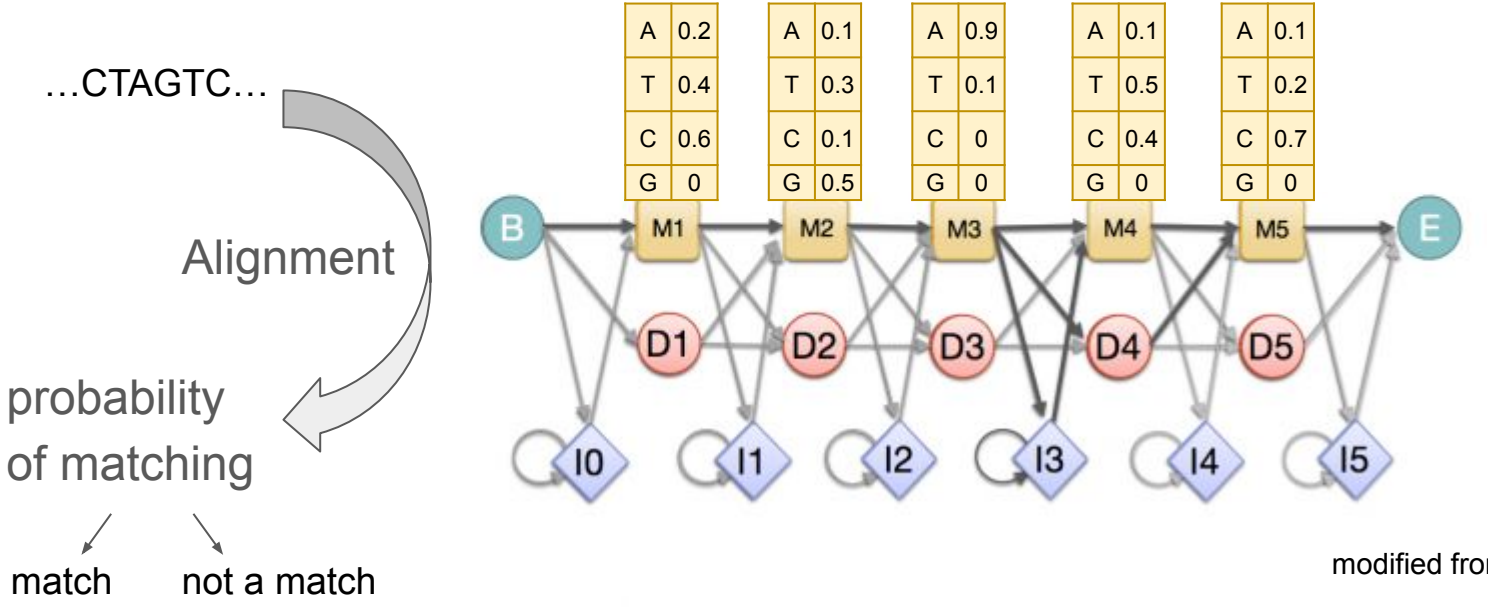
probability of matching



modified from: Wikipedia

Different kinds of non coding genes : rRNA

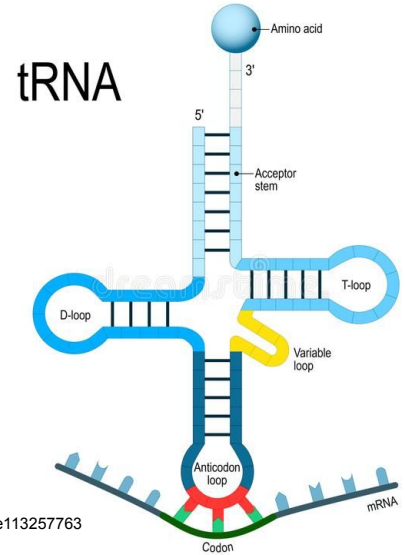
- Detection of rRNA can be performed using RNAmmer
  3. the sequence having the best probability of matching corresponds to the genes encoded in the HMM profiles



modified from: Wikipedia

Different kinds of non coding genes : tRNA

- tRNA : several clusters of tRNA scattered all along the genome
  - One or several tRNAs for each codon
  - The pool of tRNA drives the codon usage
  - Very stable across bacterial genomes (except the variation loop and, of course, the anticodon)

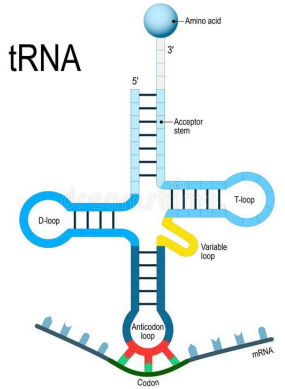


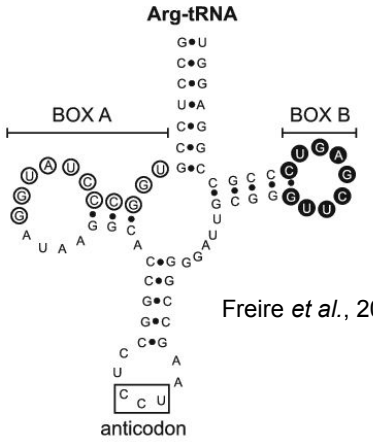
credit: https://fr.dreamstime.com/arn-trna-code-g%C3%A9n%C3%A9tique-image113257763

credit: https://fr.dreamstime.com/arn-trna-code-g%C3%A9n%C3%A9tique-image113257763

Different kinds of non coding genes : tRNA

- Detection of tRNA can be performed using ARAGORN
- Here is the algorithm (an expert recipe):
  1. Searches for partially mismatched, non-gapped, occurrences of the sequence GTTC (subset of the B box consensus sequence)
  2. Around each hit, the algorithm attempts to construct a T-loop from five to nine bases long and a T-stem from 4 to 5 bp long.
  3. To detect tRNA genes, the sequence is searched from 28 to 85 bases upstream of this T-stem for the sequence motif TRGYNAA, a subset of the A box consensus sequence which allows for a D-stem from 3 to 4 bp long.
  4. Around the motif, a D-loop from five to 11 bases long and containing the sequence A- - - - -GG-R is constructed.
  5. A 7–9 bp long A-stem is constructed using the sequence from two to three bases upstream of the D-stem and immediately downstream of the T-stem.
  6. The length of the V-loop is allowed to vary from three to 25 bases, upstream of the T-stem.
  7. Finally, the 3′ end of the C-stem is constructed by searching between the D- and T-stems for a sequence that is complementary to the 5′ end of the C-stem, immediately downstream of the D-stem and spacer base.
  8. Limited use of tertiary structures contacts between the T-loop, V-loop and D-loop is made.
  9. If position 55 in the T-loop is a non-consensus G, then a non-consensus TT at positions 18 and 19 in the D-loop is given an improved score.
- Useless to have a complete understanding (to my mind)

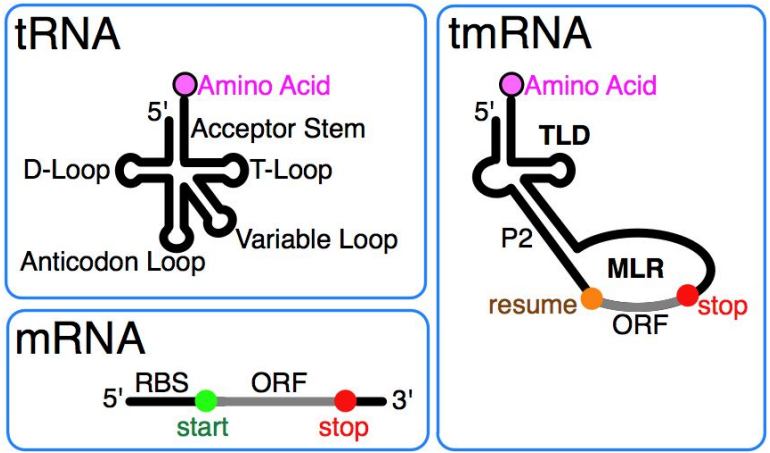




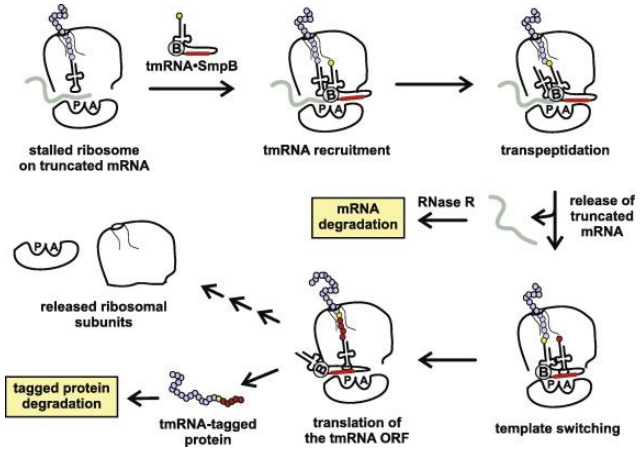
Freire *et al.*, 2010

Different kinds of non coding genes : tmRNA

- tmRNA allows recycling stalled ribosomes during the translation of a truncated mRNA (so without any stop codon)



credit: wikipedia

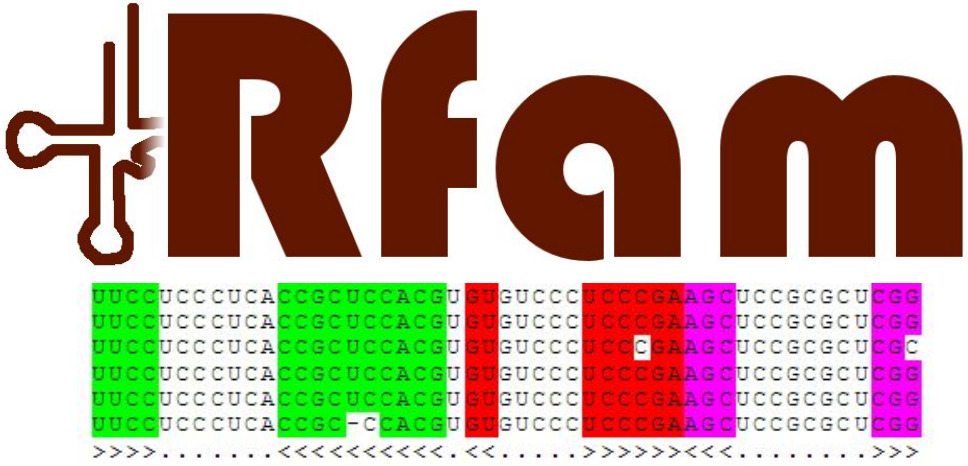


Hayes *et al.*, 2010

- Detection of tRNA can also be performed using ARAGORN using an expert recipe

Different kinds of non coding genes : ncRNA

- Non coding RNAs correspond to a set of RNA (having varieties of functions) which are not mRNA, rRNA, tRNA, and tmRNA
- Detection of tRNA can be performed using Infernal based on Rfam, which is database of HHM profiles of ncRNA



Conclusion

- Coding genes still correspond to the main part of genomes but non coding genes can’t be ignored
- Detection of non coding genes can help annotation of coding genes (overlaps)
- The diversity of non coding genes is detected via 2 main kinds of approaches
  - Alignment on a database of HMM profiles
  - Expert recipes ruling a specific pattern to match

Introduction to functional annotation using Bakta

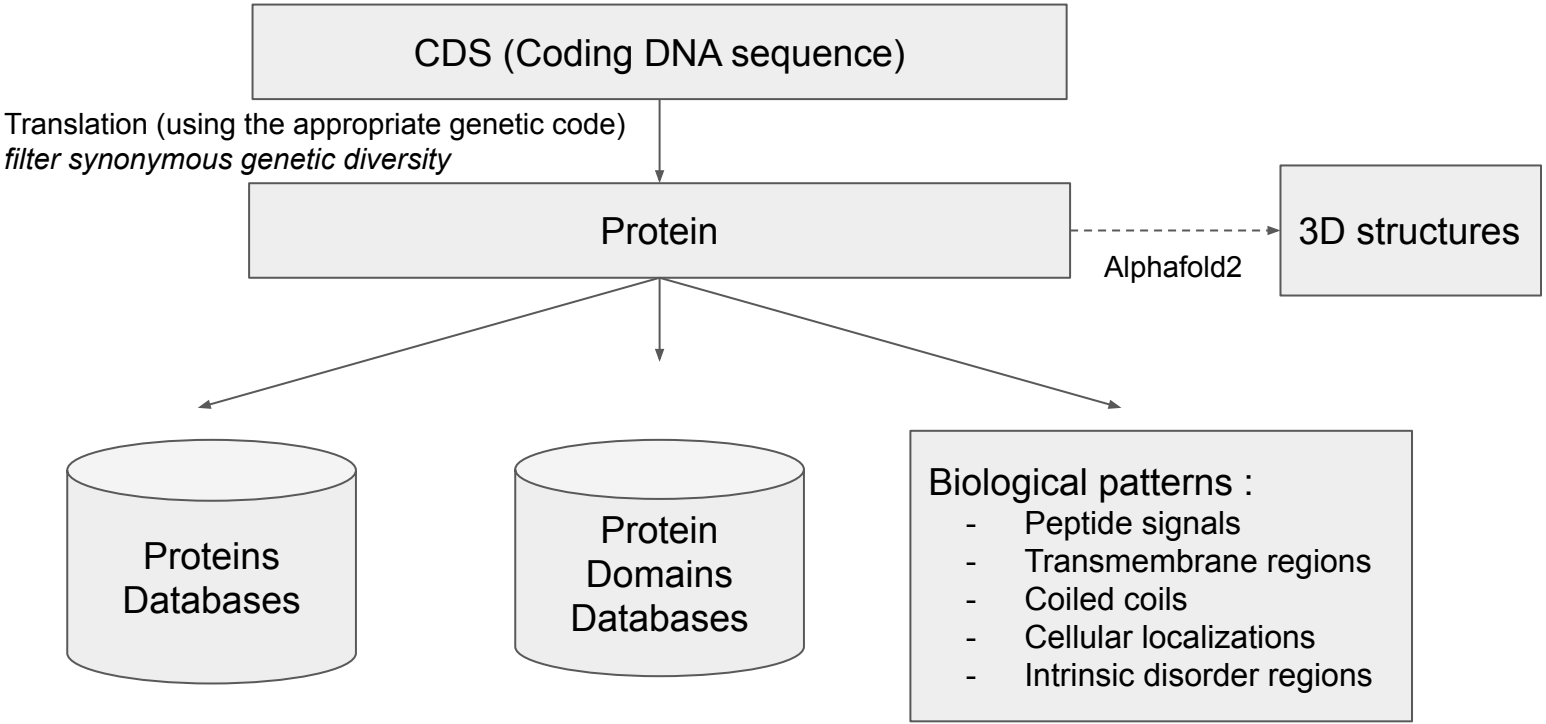
What's in an annotation?

- Location
  - which sequence? — *chromosome 2*
  - where on the sequence? — 100..659
  - what strand? — -ve
- Feature type
  - what is it? — *protein coding gene*
- Attributes
  - protein product? — *alcohol dehydrogenase*
  - enzyme code? — *EC:1.1.1.1*
  - subcellular location? — *cytoplasm*
  - note? — *beer processing*

Source : https://training.galaxyproject.org/training-material/topics/genome-annotation/tutorials/annotation-with-prokka/slides.html#9

What is the functional role of an identified coding genes ?

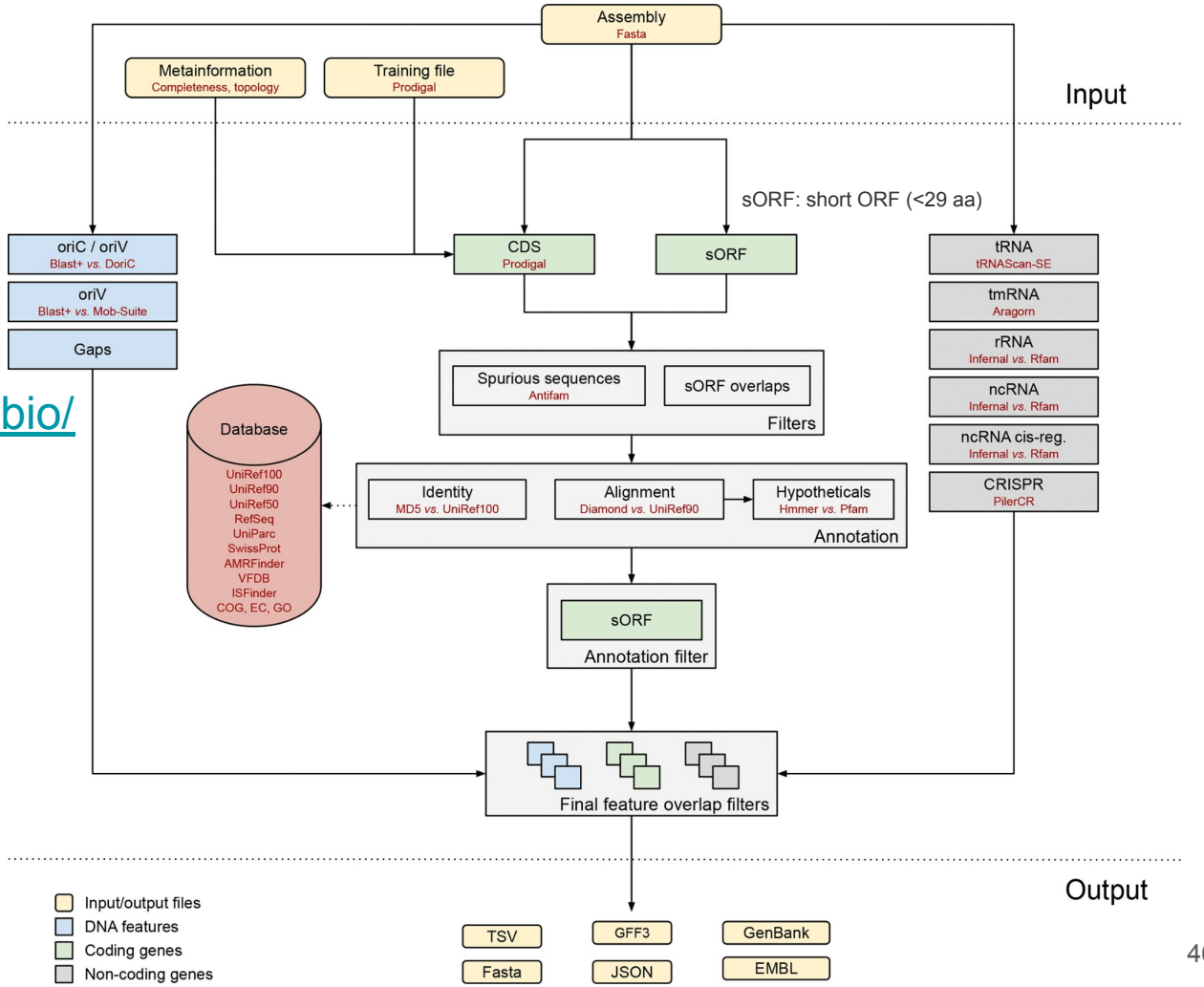
Simplify analysis pipeline :



Bakta

- successor of Prokka
- available in Galaxy

https://bakta.computational.bio/

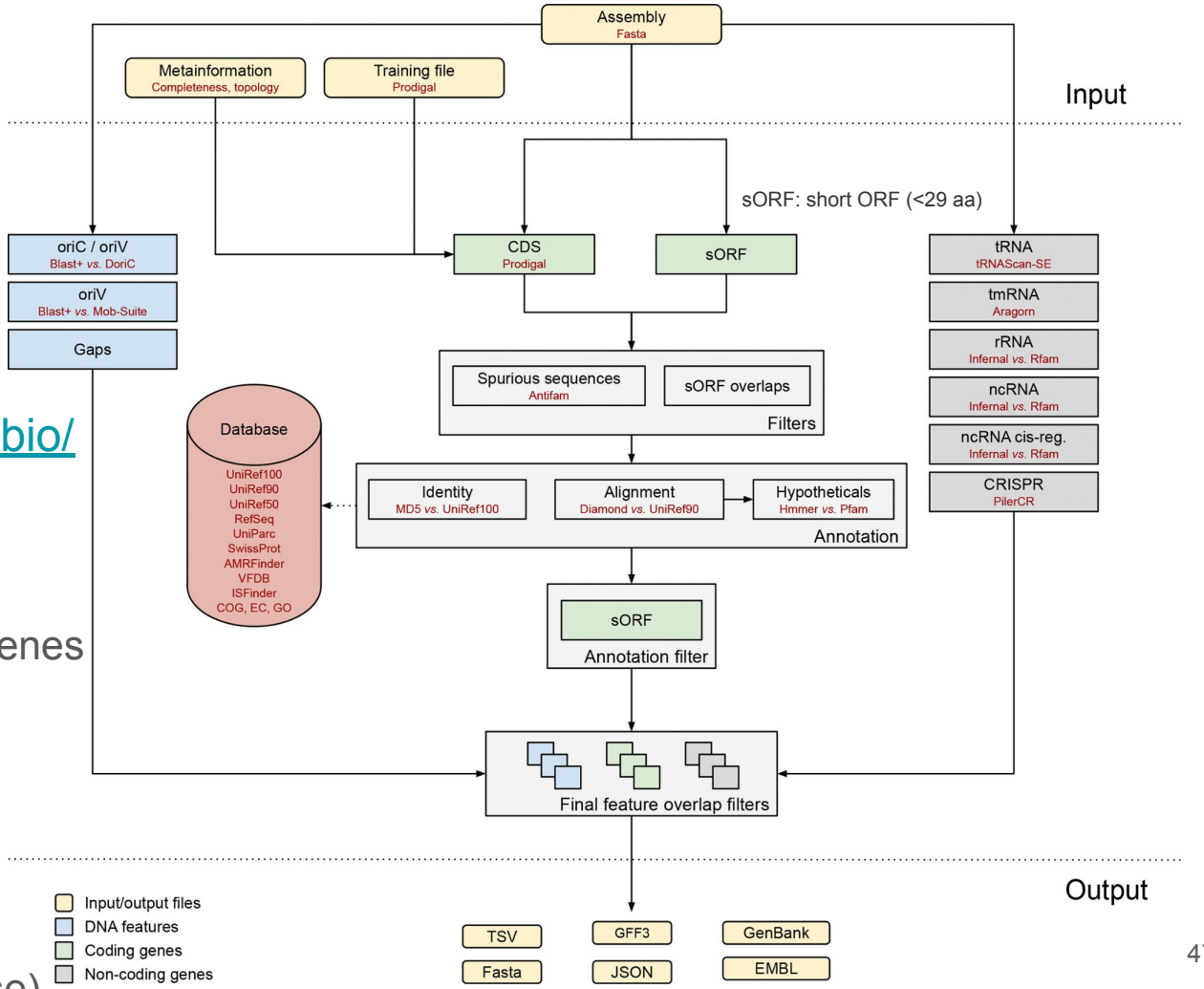


Bakta

- successor of Prokka
- available in Galaxy

https://bakta.computational.bio/

+ Peptide signal (DeepSig)
+ Antimicrobial resistance genes (AMRFinderPlus) :
  - NCBI tool
  - AMR proteins: 6990
  - Point mutations: 1309
  - HMMs: 743
+ VFDB (Virulence Factor Database)



Tutorials

https://training.galaxyproject.org/training-material/topics/genome-annotation/tutorials/bacterial-genome-annotation/tutorial.html#is-insertion-sequence-elements

Bakta,

Version de Bakta 1.8.2
The bakta database : v5.0_2023-02-20
The amrfinderplus database : v3.11_2023-04-17.1

PlasmidFinder,
IntegronFinder,
ISEScan,
JBrowse