Lab activity: analysis of transcriptional regulatory networks

1. Introduction

This lab activity consists of four sessions that you will complete during the remaining class sessions of the course. The four sessions will go through the data life cycle, with the implementation of a set of functions defined in the assignments and the analysis of the obtained results.

- Session 1: Data acquisition
- Session 2: Network analysis
- Session 3: Network motifs
- Session 4: Visualization

Our first step will be data acquisition, illustrating where and how we can obtain the data and what obstacles we may face during this process, as well as how to store the data to later use it. Our second step will be to perform basic analyses of the generated network in order to gain insights into the network structure and function. Our third step will be to analyze how these networks are organized internally, identifying recurrent patterns of interconnection known as network motifs. Finally, we will work on generating graphical network representations that adequately convey the results we have obtained through our analyses.

The lab activity will be carried out in **groups of two** and only one submission per group is required (the report must clearly indicate the names and student IDs of each group member). You will turn in the lab activity through the virtual campus, in the specific session for lab submission.

There will be a single deliverable for the lab activity (covering the four sessions). Moreover, before submission of the lab deliverable, an individual oral interview to assess the progress of the work has to be done. Please take into account that you must pass the individual oral interview in order to be able to submit the deliverable for grading.

The deadline for submitting the activity is **June 6th**. The oral interview can be done at class time, in either the May 22nd or May 29th sessions. The calendar for the lab activity is as follows:

Date	15-17	17-19
05/15/2025	Lab session 1: data acquisition	Lab session 2: data acquisition
05/22/2025	Lab session 3: network analysis	Lab session 4: network motifs
05/29/2025	Lab session 5: network visualization	Lab session 6: final tweaks

As you will see from the statement of each session, lab sessions have two components: coding and analyzing results.

Coding needs to be done in Python (3.7 or higher). A template plain python file (.py) will be provided for each session. You need to implement all the code of each part in the given template file[1] (indications of where you need to write your code are included as comments in the template). You can define additional global functions (and import any libraries) if needed, but do not include any other code in the global scope. The algorithm that implements the work for each session (the calls to the functions) needs to be included inside the main scope.

The other component of the project requires you to analyze the information obtained using the code you have developed and reflect on some issues. The answers to these questions should be turned in in a PDF document. At the end of the statement for each session you will find a list detailing the files you need to deliver for that session (and their format).

At the beginning of each class session, we will provide the statements for the session and, in some cases, present some concepts to be able to carry out the proposed work. The rest of the class session will be used to perform the work requested in the lab session description.

2. Session 1: Data acquisition and storage

2.1. Biological background

In this lab activity we will analyze the transcriptional regulatory network of bacterial species. What follows is a short introduction to the molecular biology concepts required to undertake this lab activity.

2.1.1. The master molecule: DNA

Each cell (whether a bacterium or one of the cells in your body) contains all the necessary information to create new copies of itself (and to keep itself alive). We call this type of information *genetic information*. Within the cell, genetic information is stored in a specific type of molecule called DNA. We refer to the totality of the genetic information stored in each cell as the *genome*.

DNA is a polymer made of four different nucleotides (or bases), which we denote as A, C, G and T. For instance, a short DNA sequence could be ACCTGGTTACATC. DNA is set up as a double strand[2], in which each base has a complementary base (A-T, G-C). Hence, our short sequence is in reality a molecule composed of two *strands*:

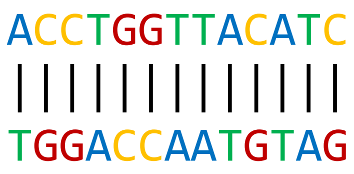


Figure 1 – Double strand representation of the short DNA sequence ACCTGGTTACATC. Since it is a double helix, with two strands, the length of a DNA sequence is conventionally measured in base *pairs* (bp). By convention,

[1] Do not convert the file to a Jupyter Notebook. Because of the length of this activity, plain Python files, which can be easily included from other files and debugged with IDEs, need to be used.

[2] This is used to detect and correct errors during the lifetime of a cell, and especially when DNA needs to be replicated (i.e. when the cell divides). Errors happen very frequently, and cells constantly repair their DNA (just for reference, each day, in each of the cells of your body, about 5,000 bases fall off the backbone of your DNA due to thermal noise).

the top strand is referred to as the *forward* strand, and the bottom one as the *reverse* strand. One is read left-to-right, and the other one right-to-left. Converting one (ACCTGGTTACATC) into the other (GATGTAACCAGGT) is known as *reverse complementing*, since it involves flipping the sequence as well as complementing it (A→T, T→A, C→G, G→C).

2.1.2. Genes and genomes: transcription and translation

Information is only useful insofar as it can be read, and DNA is read all the time. It is read in chunks, which we call *genes*. Genes code for *proteins*, which perform tasks in the cell. In humans, for instance, hemoglobin is a protein used to transport oxygen (and other stuff) in the blood, and the lactase enzyme is a protein used to digest lactose. Hence, you can think of genes as cell microinstructions (e.g. *transport oxygen*, *digest lactose*).

The process by which genes are read in order to become proteins (which will carry out those instructions) is known as the Central Dogma of molecular biology, and it is a two-step process:

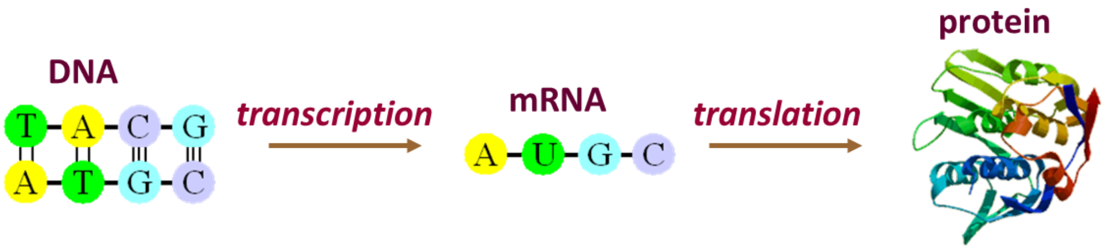
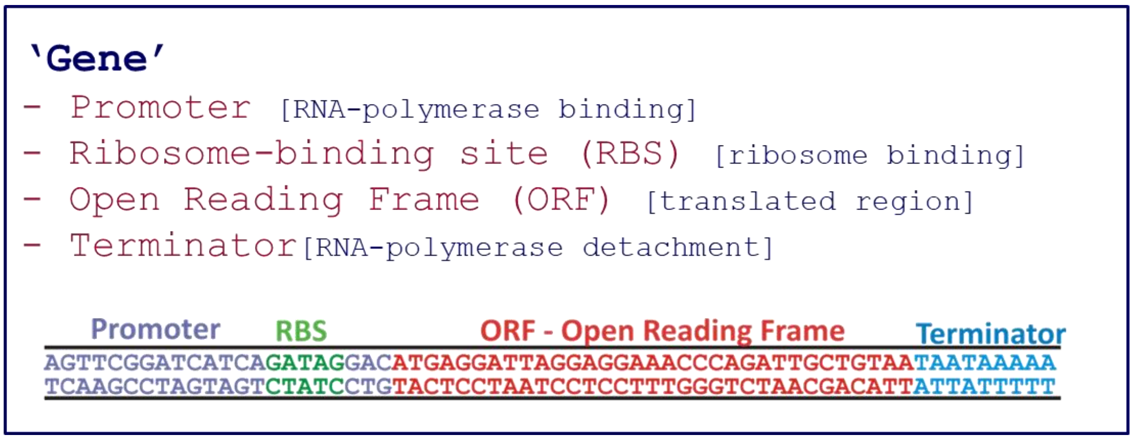


Figure 2 – Schematic representation of the Central Dogma of molecular biology.

Genes are first read (*transcribed*) from DNA into a similar molecule (RNA), which is then read (*translated*) into a protein (this is done using the Genetic Code, because proteins use an alphabet of 20 amino acids, instead of 4 nucleotides as DNA and RNA).

In order to be read, a gene must have a set of signals that enable its *reading* (transcription and then translation). For this lab activity, we will only consider one of them, the *promoter*, which is where a specialized enzyme, RNA-polymerase, binds to initiate transcription.



```
'Gene'
- Promoter [RNA-polymerase binding]
- Ribosome-binding site (RBS) [ribosome binding]
- Open Reading Frame (ORF) [translated region]
- Terminator[RNA-polymerase detachment]
```

Promoter
RBS
ORF - Open Reading Frame
Terminator
AGTTCGGATCATCAGATAGGACATGAGGATTAGGAGGAAACCCAGATTGCTGTAATAATAAAAA
TCAAGCCTAGTAGTCTATCCTGTACTCCTAATCCTCCTTTGGGTCTAACGACATTATTATTTTT

Figure 3 – Schematic representation of a *gene* with its different components: promoter and terminator are used to initiate and terminate *transcription* by the RNA-polymerase enzyme. The RBS is the location where the ribosome binds to the transcribed RNA to initiate *translation*. The ORF is the actual "message" that gets translated into a protein, defined by start (ATG) and stop (TAA, TGA or TAG) signals.

We can model the genome as a long DNA sequence, which contains genes. Because the *promoter* and other signals on the DNA have directionality, genes can be read on one or the other strand.

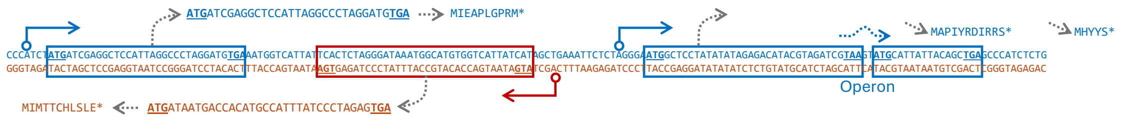

ATGATCGAGGCTCCATTAGGCCCTAGGATGTGA
MIEAPLGPRM*
MAPIYRDIRRS*
MHYYS*
CCCATCTATGATCGAGGCTCCATTAGGCCCTAGGATGTGAAATGGTCATTATTCACTCTAGGGATAAATGGCATGTGGTCATTATCATAGCTGAAATTCTCTAGGGAATGGCTCCTATATATAGAGACATACGTAGATCGTAAGTATGCATTATTACAGCTGAGCCCATCTCTG
GGGTAGATACTAGCTCCGAGGTAATCCGGGATCCTACACTTTACCAGTAATAAGTGAGATCCCTATTTACCGTACACCAGTAATAGTATCGACTTTAAGAGATCCCTTACCGAGGATATATATCTCTGTATGCATCTAGCATTCATACGTAATAATGTCGACTCGGGTAGAGAC
Operon
MIMTTCHLSLE*
ATGATAATGACCACATGCCATTTATCCCTAGAGTGA

Figure 4 – Schematic representation of a segment of a genome, containing several *genes*, illustrating their transcription and then translation. The directionality of the promoter (arrow) determines the strand the gene is read (transcribed) on. Some genes don't have a promoter; they simply are very close to the previous gene and are transcribed together with it. These genes are said to be in an *operon*.

2.1.3. Genetic programming: transcriptional regulation

In a cell, genes are akin to the microinstructions of a processor, and they can be read (i.e. executed). But having microinstructions is of little use if we cannot program with them. Programming, in a cell, is performed by a process known as *transcriptional regulation*.

Like conditional jumps in computer code (e.g. JZ, JNE), some of the microinstructions (proteins) in a cell have *programming* functions. These proteins, known as *transcription factors*, usually have two components: one that detects some signal, and another one that binds to the DNA molecule. By binding to the DNA, transcription factors facilitate or hinder the binding of RNA-polymerase to the promoter of some genes, essentially turning them ON or OFF.

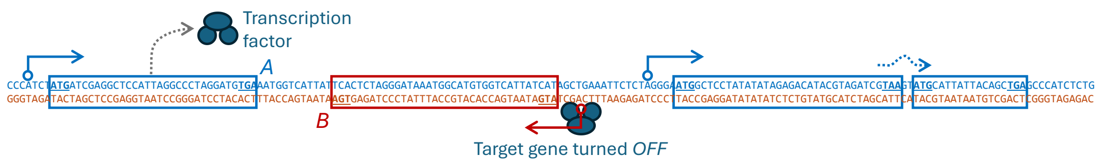

Transcription factor
A
CCCATCTATGATCGAGGCTCCATTAGGCCCTAGGATGTGAAATGGTCATTATTCACTCTAGGGATAAATGGCATGTGGTCATTATCATAGCTGAAATTCTCTAGGGAATGGCTCCTATATATAGAGACATACGTAGATCGTAAGTATGCATTATTACAGCTGAGCCCATCTCTG
GGGTAGATACTAGCTCCGAGGTAATCCGGGATCCTACACTTTACCAGTAATAAGTGAGATCCCTATTTACCGTACACCAGTAATAGTATCGACTTTAAGAGATCCCTTACCGAGGATATATATCTCTGTATGCATCTAGCATTCATACGTAATAATGTCGACTCGGGTAGAGAC
B
Target gene turned *OFF*

Figure 5 – Schematic representation of transcriptional regulation. Gene *A* codes for a transcription factor. Once "read" into a protein, the transcription factor binds to the promoter region of target gene *B*, shutting off its transcription.

2.2. Analyzing transcriptional regulatory networks (TRNs)

Bacterial species have between 80 and 300 transcription factors, and each transcription factor can regulate from very few to hundreds of genes. Some of these genes may code for other transcription factors, and may be regulated by other transcription factors as well. This leads to a complex network of regulatory interactions that we call the transcriptional regulatory network of the cell, and which we will analyze in this lab activity using graph theory approaches.

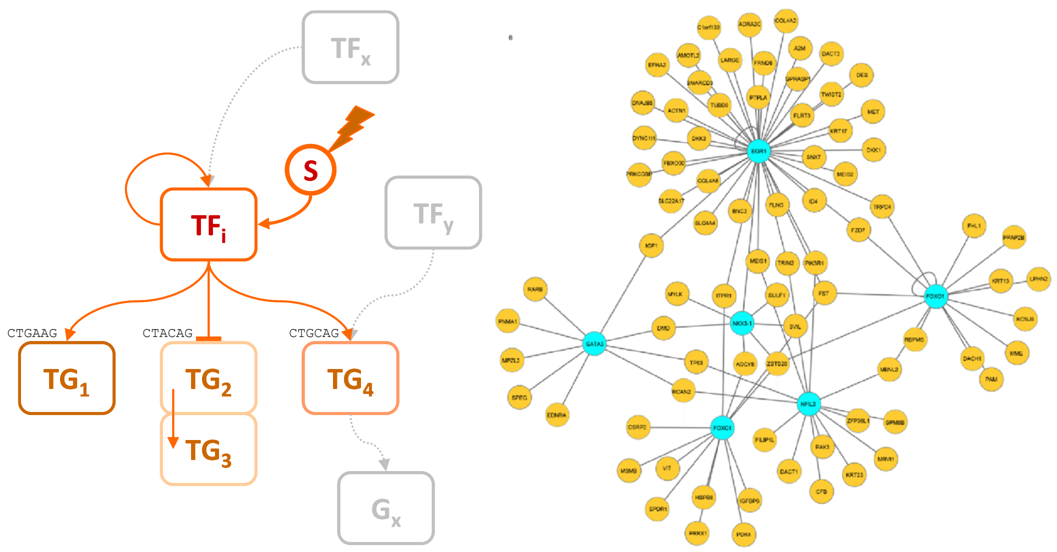
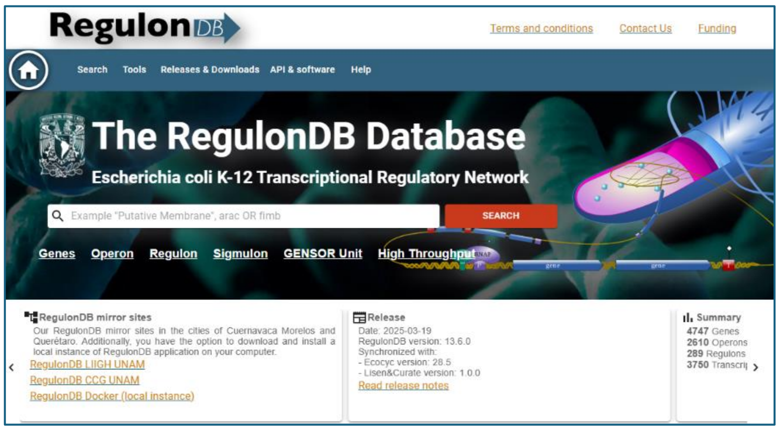


Figure 6 – [Left] Local structure of the cell's TRN, with a TF regulating several genes (TG$_2$ and TG$_3$ are in an operon), including itself. Other TFs (TF$_Y$) can regulate the same target genes, or (TF$_X$) regulate the TF itself, leading to [Right] a complex network structure (TFs shown in blue, TGs in yellow).

2.3. Primary data sources

2.3.1. The RegulonDB database

The RegulonDB database is a database of experimentally-validated TF-binding sites (TFBS) of the bacterium *Escherichia coli*, a human commensal. The database is maintained by Julio Collado-Vides' team at the *Centro de Ciencias Genómicas* of the *Universidad Nacional Autónoma de México* (UNAM), and contains information on the binding sites and regulatory activity of 184 *E. coli* transcription factors.



The RegulonDB Database

Escherichia coli K-12 Transcriptional Regulatory Network

Example "Putative Membrane", arac OR fimb

RegulonDB mirror sites

Our RegulonDB mirror sites in the cities of Cuernavaca Morelos and Querétaro. Additionally, you have the option to download and install a local instance of RegulonDB application on your computer.

RegulonDB LIIGH UNAM

RegulonDB CCG UNAM

RegulonDB Docker (local instance)

Release

Date: 2025-03-19

RegulonDB version: 13.6.0

Synchronized with:

- Ecocyc version: 28.5
- Lisen&Curate version: 1.0.0

Read release notes

Summary

4747 Genes

2610 Operons

289 Regulons

3750 Transcri

Figure 7 – The RegulonDB landing page at https://regulondb.ccg.unam.mx/.

2.3.2. The NCBI GenBank database

The National Center for Biotechnology Information (NCBI) of the National Library of Medicine (NLM) is one of the largest genetic data repositories in the world. Among others, the NCBI hosts the GenBank database, which contains genetic sequences for thousands of species and is used by biomedical researchers across the world.

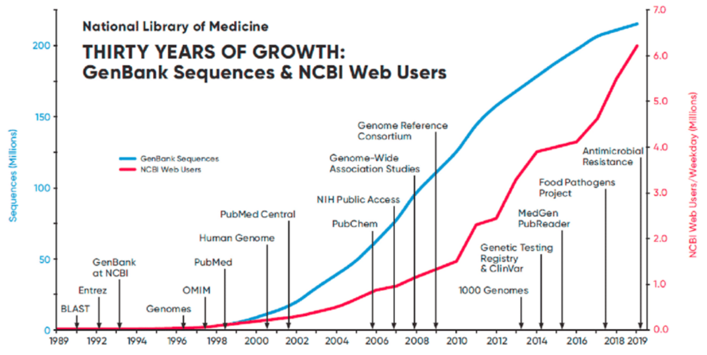


Figure 8 – Growth in sequence number and web user activity of the NCBI and its GenBank database (source: NLM 2021 Congressional Justification; Gaffney *et al.* *Global Food Security*, 26, 2020, doi: `10.1016/j.gfs.2020.100411`).

2.4. Dataset acquisition and storage

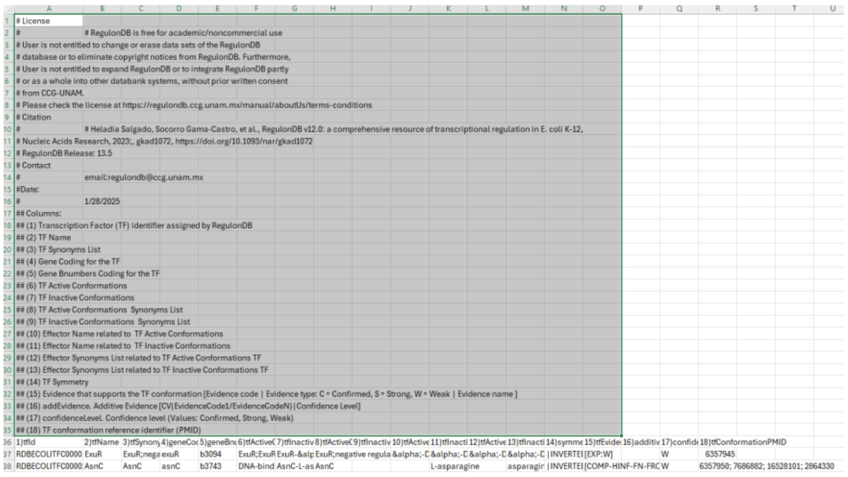
In this first lab activity we will acquire data from RegulonDB and from the NCBI GenBank database to generate TF-binding motif models. Together with the statement of this first session, a skeleton file where the functions to be implemented in each activity are explicitly stated is attached (`Lab AGX 202425 P1 skeleton.py`).

2.4.1. File download

We will first navigate to the Releases & Download section of RegulonDB and we will download two files: `TF-RISet` and `TF-Set`.

- `TF-RISet` contains information on the experimentally-validated regulatory interactions between transcription factors and their target genes. Among other nuggets of information, for each regulatory interaction we have the TFBS sequence, its genomic location, the experimental evidence for the regulatory activity and the scientific article where it was reported.
- `TF-Set` contains information on *E. coli* transcription factors, including the identifier (*locus_tag*) of the gene that codes for the TF.

These are tab-separated (TSV) files that, inconveniently, contain an explanation header. We will edit out this header, so that the files start on the column headers row, in order to facilitate their automated processing.



```
# License
#	# RegulonDB is free for academic/noncommercial use
# User is not entitled to change or erase data sets of the RegulonDB
# database or to eliminate copyright notices from RegulonDB. Furthermore,
# User is not entitled to expand RegulonDB or to integrate RegulonDB partly
# or as a whole into other databank systems, without prior written consent
# from CCG-UNAM.
# Please check the license at https://regulondb.ccg.unam.mx/manual/aboutUs/terms-conditions
# Citation
#	# Heladia Salgado, Socorro Gama-Castro, et al., RegulonDB v12.0: a comprehensive resource of transcriptional regulation in E. coli K-12,
# Nucleic Acids Research, 2023;, gkad1072, https://doi.org/10.1093/nar/gkad1072
# RegulonDB Release: 13.5
# Contact
#	email:regulondb@ccg.unam.mx
#Date:
#	1/28/2025
## Columns:
## (1) Transcription Factor (TF) identifier assigned by RegulonDB
## (2) TF Name
## (3) TF Synonyms List
## (4) Gene Coding for the TF
## (5) Gene Bnumbers Coding for the TF
## (6) TF Active Conformations
## (7) TF Inactive Conformations
## (8) TF Active Conformations  Synonyms List
## (9) TF Inactive Conformations  Synonyms List
## (10) Effector Name related to  TF Active Conformations
## (11) Effector Name related to  TF Inactive Conformations
## (12) Effector Synonyms List related to TF Active Conformations TF
## (13) Effector Synonyms List related to TF Inactive Conformations TF
## (14) TF Symmetry
## (15) Evidence that supports the TF conformation [Evidence code | Evidence type: C = Confirmed, S = Strong, W = Weak | Evidence name ]
## (16) addEvidence. Additive Evidence [CV(EvidenceCode1/EvidenceCodeN)|Confidence Level]
## (17) confidenceLevel. Confidence level (Values: Confirmed, Strong, Weak)
## (18) TF conformation reference identifier (PMID)
```

1)tfId	2)tfName	3)tfSynony	4)geneCoc	5)geneBnu	6)tfActive(	7)tfInactiv	8)tfActive(	9)tfInactiv	10)tfActive	11)tfInacti	12)tfActive	13)tfInacti	14)symme	15)tfEvide	16)additiv	17)confide	18)tfConformationPMID
RDBECOLITFC0000	ExuR	ExuR;nega	exuR	b3094	ExuR;ExuR	ExuR-&alp	ExuR;negative regula		&alpha;-D	&alpha;-D	&alpha;-D	&alpha;-D	|INVERTEI	[EXP:W]		W	6357945
RDBECOLITFC0000	AsnC	AsnC	asnC	b3743	DNA-bind	AsnC-L-as	AsnC			L-asparagine		asparagir	|INVERTEI	[COMP-HINF-FN-FRC		W	6357950; 7686882; 16528101; 2864330

Figure 9 – Rows to be removed from the TF-Set file.

The complete genome of the *E. coli* reference strain (*Escherichia coli* str. K-12 substr. MG1655) is available on the NCBI GenBank Nucleotide database (https://www.ncbi.nlm.nih.gov/nuccore/) under accession number `U00096.3`. We will search for this accession number and download the nucleotide sequence in *GenBank (full)* format through the Send to → File link. We will save the file as "`U00096.3.gb`".

2.4.2. Biology in Python: the `biopython` package

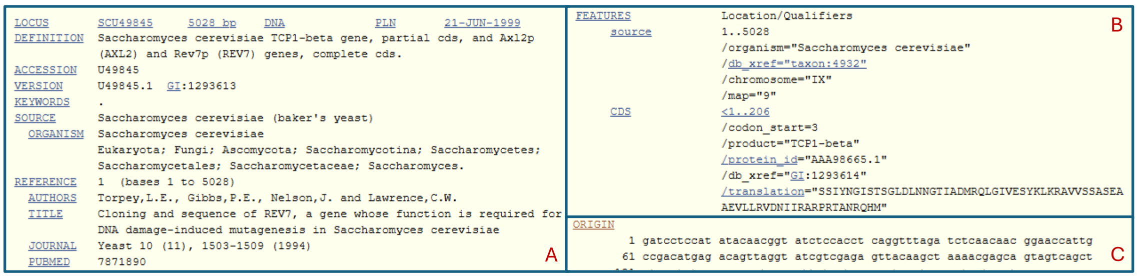
Bioinformatics is a scientific discipline focused on the analysis of large amounts of biological data to infer new biological knowledge. Although many programming languages are used in bioinformatics, the discipline has gradually adopted Python as a standard, propelled in large part by the development of the `biopython` package (https://biopython.org/). This package contains libraries defining classes for most biological data elements (e.g. genetic sequences) as well as parsers to read and write from and to common bioinformatics tools and pipelines.

The `biopython` package, as well as other packages needed for this lab can be installed by creating a conda environment with the provided `agx_lab` YAML file.

```
conda env create -f agx_lab.yml
```

2.4.3. Genome processing

A central object in bioinformatics is the genome. Genome files are most often distributed using the GenBank format, which contains the DNA sequence of the genome, annotations (e.g. the species name) and features (e.g. from position *X* to position *Y*, in the forward strand, there is gene *Z*).



```
LOCUS       SCU49845     5028 bp    DNA             PLN       21-JUN-1999
DEFINITION  Saccharomyces cerevisiae TCP1-beta gene, partial cds, and Axl2p
            (AXL2) and Rev7p (REV7) genes, complete cds.
ACCESSION   U49845
VERSION     U49845.1  GI:1293613
KEYWORDS    .
SOURCE      Saccharomyces cerevisiae (baker's yeast)
  ORGANISM  Saccharomyces cerevisiae
            Eukaryota; Fungi; Ascomycota; Saccharomycotina; Saccharomycetes;
            Saccharomycetales; Saccharomycetaceae; Saccharomyces.
REFERENCE   1  (bases 1 to 5028)
  AUTHORS   Torpey,L.E., Gibbs,P.E., Nelson,J. and Lawrence,C.W.
  TITLE     Cloning and sequence of REV7, a gene whose function is required for
            DNA damage-induced mutagenesis in Saccharomyces cerevisiae
  JOURNAL   Yeast 10 (11), 1503-1509 (1994)
  PUBMED    7871890
```

```
FEATURES             Location/Qualifiers
     source          1..5028
                     /organism="Saccharomyces cerevisiae"
                     /db_xref="taxon:4932"
                     /chromosome="IX"
                     /map="9"
     CDS             <1..206
                     /codon_start=3
                     /product="TCP1-beta"
                     /protein_id="AAA98665.1"
                     /db_xref="GI:1293614"
                     /translation="SSIYNGISTSGLDLNNGTIADMRQLGIVESYKLKRAVVSSASEA
                     AEVLLRVDNIIRARPRTANRQHM"
```

```
ORIGIN
        1 gatcctccat atacaacggt atctccacct caggtttaga tctcaacaac ggaaccattg
       61 ccgacatgag acagttaggt atcgtcgaga gttacaagct aaaacgagca gtagtcagct
```

Figure 10 – The three main elements of a GenBank-formatted file. [A] Annotations. [B] Features. [C] Sequence. See https://www.ncbi.nlm.nih.gov/genbank/samplerecord/ for details.

When you read in a genome file with `biopython` (using the `SeqIO` `read` command and specifying that you are reading "genbank" format) the three central elements of the GenBank file become attributes of the SeqRecord object that is generated. Hence, `record.features` is a list with all the features in the genome.

Each feature in a genome has a `location` (start, end and strand) and a `type`. The most common feature types are *genes* and *CDS* (which stands for 'coding sequence'; the part of the gene that is translated into protein). A feature will also have a number of `qualifiers`, organized as a dictionary. Hence, `record.features[N].qualifiers` is a dictionary with keys such as `'gene'` or `'product'`.

We will be working primarily with the following qualifiers:

- `gene`: the *name* of the gene the feature corresponds to (e.g. *dapB*)
- `locus_tag`: a unique *identifier* for that feature in the genome (e.g. *b0031*)
- `protein_id`: a unique *identifier* for the protein coded by the CDS feature (e.g. AAC73142.1)
- `product`: the *function* (if known) of the protein coded by the CDS feature (e.g. 4-hydroxy-tetrahydrodipicolinate reductase)

1. **(0.5 points)** Implement a function that will return a list of tuples (`locus_tag`, `protein_id`) with all the CDS features in a genome sequence record object whose `product` qualifier contains the query string.

```python
def feature_list(genome : SeqRecord, query : str) -> list:
    '''
    Extract CDS features with specific feature description.
    - param genome : SeqRecord
        genome SeqRecord object to be analyzed.
    - param query : str
        feature descriptor.
    - return list
        list of tuples (locus_tag, protein_id) matching descriptor.
    '''
    # ------- IMPLEMENT HERE THE BODY OF THE FUNCTION ------- #
    pass
    # ----------------- END OF FUNCTION --------------------- #
```

2. **(1 point)** Implement a function that, given a gene qualifier value (e.g. *insL1*) and its type (e.g. `gene`), will return the index and the specified qualifier (e.g. `locus_tag`) for the corresponding `gene` (type) feature in the genome.

```python
def gene_qualifier(query : str, query_field : str,
                   target_field : str, genome : SeqRecord) -> tuple:
```

```
    '''
    Obtain the specified qualifier identifier for a given gene qualifier
    - param: query: str
        gene name/locus_tag to map to corresponding locus_tag/name
    - param: query_field: str
        query type indicator (gene/locus_tag/protein_id/product)
    - param: target_field: str
        target type indicator (gene/locus_tag/protein_id/product)
    - param: genome : SeqRecord
        genome SeqRecord object containing features
    - return: tuple
        int : feature index
        str : specified qualifier for gene (empty string if no match)
    '''
    # ------- IMPLEMENT HERE THE BODY OF THE FUNCTION ------- #
    pass
    # ----------------- END OF FUNCTION --------------------- #
```

2.4.4. Operon detection

Bacterial genomes have evolved to be as efficient as possible. If several genes share a common, specific function (e.g. degrade lactose), they will need to be "read" (i.e. transcribed) at the same time, and this can be done most efficiently if they are transcribed in block from a single promoter (you can think of this as a programming *function*, encompassing several instructions, that we call in response to an IF-ELSE condition). We call sets of genes transcribed from a single promoter "operons".

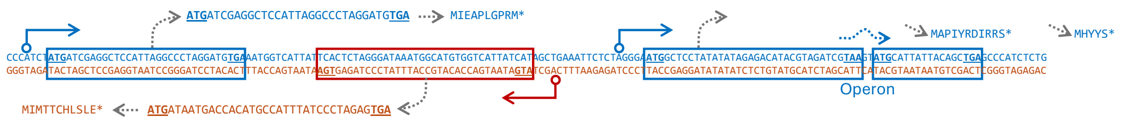
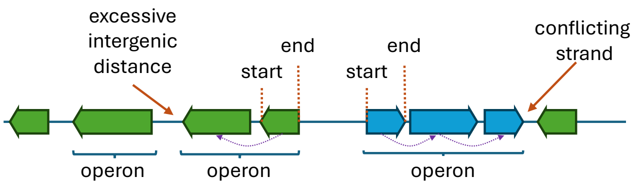

ATGATCGAGGCTCCATTAGGCCCTAGGATGTGA
MIEAPLGPRM*
MAPIYRDIRRS*
MHYYS*
CCCATCTATGATCGAGGCTCCATTAGGCCCTAGGATGTGAAATGGTCATTATTCACTCTAGGGATAAATGGCATGTGGTCATTATCATAGCTGAAATTCTCTAGGGAATGGCTCCTATATATAGAGACATACGTAGATCGTAAGTATGCATTATTACAGCTGAGCCCATCTCTG
GGGTAGATACTAGCTCCGAGGTAATCCGGGATCCTACACTTTACCAGTAATAAGTGAGATCCCTATTTACCGTACACCAGTAATAGTATCGACTTTAAGAGATCCCTTACCGAGGATATATATCTCTGTATGCATCTAGCATTCATACGTAATAATGTCGACTCGGGTAGAGAC
Operon
MIMTTCHLSLE*
ATGATAATGACCACATGCCATTTATCCCTAGAGTGA

Figure 11 – Schematic representation of a segment of a genome, containing several *genes*, illustrating their transcription and then translation. The directionality of the promoter (arrow) determines the strand the gene is read (transcribed) on. Some genes don't have a promoter; they simply are very close to the previous gene and are transcribed together with it. These genes are said to be in an *operon*.

Genes in an operon are always oriented in the same direction (i.e. read on the same strand), and have small intergenic regions between them (i.e. the space between one gene and the next is short). We will use these two features to identify the set of genes that conform an operon, given the first gene.

3. **(2.5 points)** Implement a function that, given a gene `locus_tag` identifier (e.g. *b0016*) and a maximum intergenic distance (e.g. 100 bp), will return a list containing all the genes in the operon.



Bear in mind that you will need to process genes in different strands differently, moving from one gene to the next and checking that both conditions (strand and maximum intergenic distance) are met to include the next gene in the operon.

```python
def operon(locus_tag : str, max_intergenic_dist : int, genome : SeqRecord) -> list:
    '''
    Obtain the locus_tag identifier for a given gene name
    - param: locus_tag : str
        locus_tag of lead operon gene
    - param: max_intergenic_dist : int
        maximum distance between consecutive, same strand genes
    - param: genome : SeqRecord
        genome SeqRecord object for operon inference
    - return: list
        list of locus_tags conforming operon (including query)
    '''
    # ------- IMPLEMENT HERE THE BODY OF THE FUNCTION ------- #
    #pass
    # ----------------- END OF FUNCTION --------------------- #
```

2.4.5. Network generation

The `TF-RISet` and `TFSet` files we downloaded from RegulonDB contain the data we need to recreate the transcriptional regulatory network of *E. coli*. The resulting network will be a directed graph.

The `TFSet` file contains information on the different *E. coli* transcription factors, including the name and locus tag of the genes that code for these proteins. The `TF-RISet` file contains information on the regulatory interaction between a TF and its target gene (TG), which can be the first gene in an operon. This information includes the name of the TF, the start, end, strand and length of the TF-binding site, and the name and other information of the target gene.

Using the data in the `TFSet` file we will build a TF dictionary, with the TF name as key, and the gene and `locus_tag` information for the TF gene as values. We will use this dictionary to get the TF gene information as we process the `TF-RISet` file.

For the `TF-RISet` file, we will process each row (one regulatory interaction), adding the TF and target genes as nodes to the network, and establishing a link (directed edge) between them. We will assess whether the target gene is the first gene of an operon and, if so, we will also add links from the TF to all the operon genes. We will add a `ntype` field to designate transcription factor ('TF') and target gene ('TG') nodes. A TF can be a target gene for another TF. We should make sure that we do not overwrite the `ntype` attribute of an existing TF node with a 'TG' qualifier.

Hence, we will store the following information for nodes:

- locus tag
    - ntype ('TF' or 'TG')
    - name (gene name)

4. **(2 points)** Implement a function that, given two file names (`TF-RISet` and `TFSet`) and a genome record, returns a directed graph with the reconstructed transcriptional regulatory network.

```python
def TF_RISet_parse(tf_riset_filename : str, tf_set_filename : str, \
                   detect_operons : bool, max_intergenic_dist : int, \
                   genome : SeqRecord) -> nx.DiGraph:
```

```
    """
    Parse TF-RISet file to obtain a TRN graph.
    The TFSet file will be used to extract information on the gene coding for
    each transcription factor [4)geneCodingForTF, 5)geneBnumberCodingForTF]
    The TF-RISet file will be used to extract genes regulated by each TF.
    We will create nodes using their locus_tag identifier, and save the gene
    name as a 'name' attribute.
    If selected, operons will be predicted for each of these genes to determine
    the entire set of genes regulated by the TF.

    - param: tf_riset_filename : str
        name/path of the TF_RISet file
    - param: tf_set_filename : str
        name/path of the TFSet file
    - param: detect_operons : bool
        whether we run operon detection
    - param: max_intergenic_dist : int
        maximum distance between consecutive, same strand genes
    - param: genome : SeqRecord
        genome SeqRecord object to extract information from
    - return: TF dictionary
    """
    # ------- IMPLEMENT HERE THE BODY OF THE FUNCTION ------- #
    pass
    # ----------------- END OF FUNCTION --------------------- #
```

5. **(1 points)** Using the previous functions:

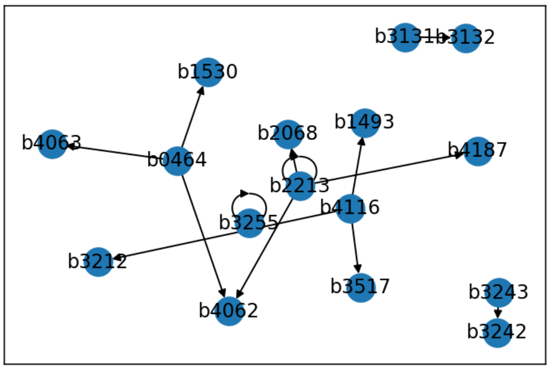
    a. Load the *E. coli* genome from the `U00096.3.gb` file into a biopython genome object.

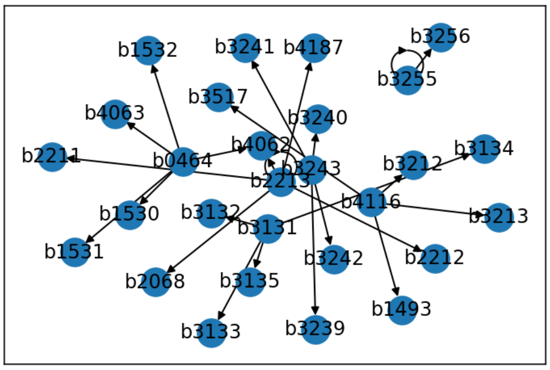
    b. Generate a graph with the reconstructed transcriptional regulatory network of *E. coli*, with (max intergenic distance = 100) and without using operon detection.

    c. Save the networks as GraphML files (`Ecoli_TRN.graphml`, (`Ecoli_operon_TRN.graphml`).

    d. Implement any required computations to answer the report questions.

You can use the provided `miniTF-RISet.tsv` file to check that your code is working as expected. You should obtain networks like the ones shown below.





2.5. Questions to answer in the report

1. **(0.5 points)** Report on the order and size of both networks. Explain why these numbers may be different from the number of rows in the `TF-RISet` file.
2. **(1.5 points)** Report the number of TFs and TGs in both networks, and the fraction of genes in the *E. coli* network that are captured as being involved in transcriptional regulatory interactions in the RegulonDB dataset. What does this tell us about the genomic organization of the *E. coli* transcriptional regulatory network?
3. **(1 points)** Report on the nodes with maximum in- and out-degree (and their respective in- and out-degrees). Are they the same on both networks? Why?