

Computational Biology

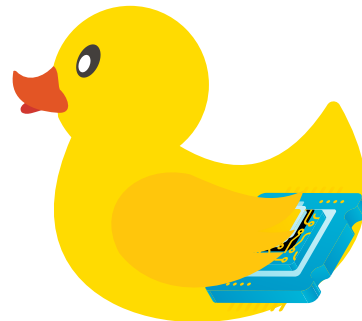
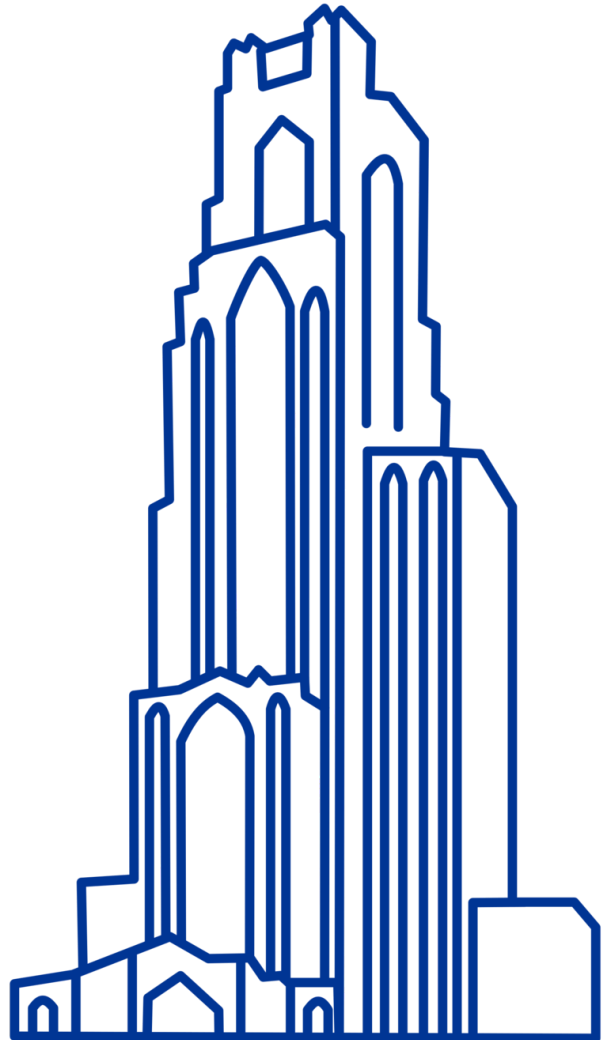
(BIOSC 1540)

Lecture 04A

Gene prediction

Foundations

Jan 28, 2025



Announcements

Assignments

- Assignment [P01C](#) is due Saturday (Feb 1)
- Assignment [P01D](#) will be released on Saturday (Feb 1)

Quizzes

- [Quiz 01](#) is today and will cover lectures [02A](#) to [03B](#)
- [Quiz 02](#) is on Feb 18 and will cover lectures [04A](#) to [06B](#)

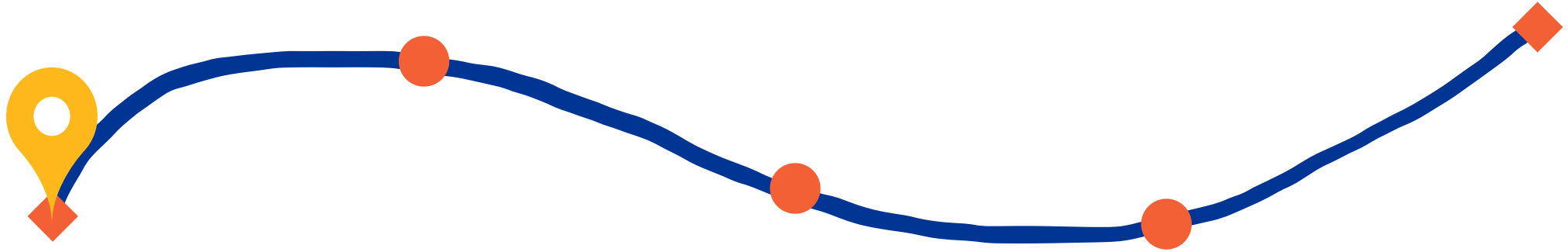
CBytes

- [CByte 01](#) is live and will expire on Feb 1
- [CByte 02](#) is live and will expire on Feb 7
- [CByte 03](#) will be released on Feb 8

Next reward: [Checkpoint Submission Feedback](#)

ATP until the next reward: 1,783

After today, you should have a better understanding of



Quiz 01

**Please put away all materials
as we distribute the quiz**

**Sit with an empty seat between you and
your neighbors for the quiz**

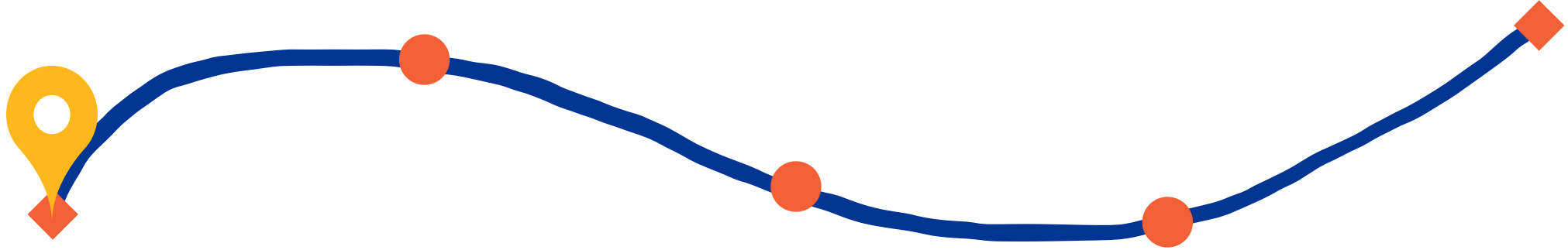
Fill out the cover page, and do not start yet

Quiz end at 9:50 am

<https://www.clockfaceonline.co.uk/clocks/digital/>

When you are finished, please hold on to your quiz and feel free to doodle, write anything, tell me a joke, etc. on the last page

After today, you should have a better understanding of



The biological importance of gene
prediction and genome annotation

???

Genome assembly provides the sequence, but gene prediction and annotation assign meaning

In previous lectures, we explored the process of creating contiguous sequences with genome assembly

TACGATCGGATTACGCGTAGGCTAGCTTACGGACTCGATGTACGATCGGATTACG

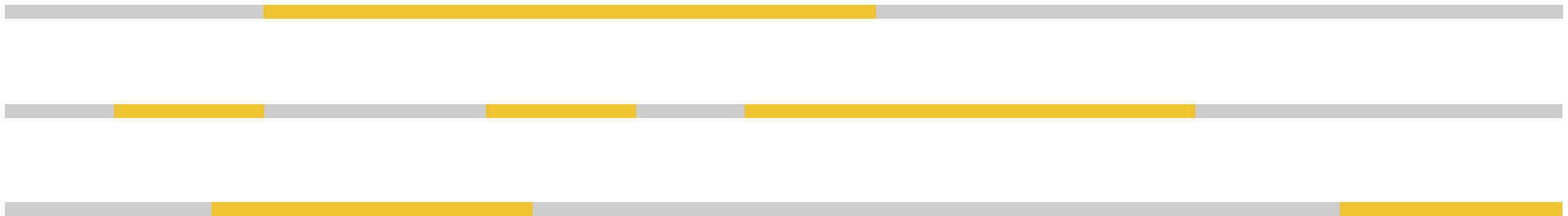
DNA sequence (i.e., contig)

Gene prediction and genome annotation transform **raw sequence data into actionable biological insights**, identifying functional elements like genes, regulatory regions, etc.

Gene prediction locates gene-containing regions and functional elements within a genome

Genes encode proteins, enzymes, and non-coding RNAs essential for cellular function

Predicted genes



We often use **Hidden Markov Models (HMMs)** to statistically predict gene locations

This is also called
"structural annotation"

(Topic for [L04B](#))

Genome annotation links gene sequences to biological functions and processes

Annotation assigns putative functions to genes through experimental evidence, similarity to known genes, or *ab initio* predictions.

Functional annotation helps classify genes into pathways (e.g., KEGG), ontologies (e.g., GO terms), and systems (e.g., metabolic networks).

Job Title NM_001275:Homo sapiens chromogranin A (CHGA),...

RID [M3JFFUAU010](#) Search expires on 08-02 01:03 am [Download All](#) ▼

Program BLASTN [Citation](#) ▼

Database refseq_rna [See details](#) ▼

Query ID [NM_001275.4](#)

Description Homo sapiens chromogranin A (CHGA), transcript variant 1, mRNA

Molecule type nucleic acid

Query Length 1985

Other reports [Distance tree of results](#) [MSA viewer](#) [?](#)

Filter Results

Organism only top 20 will appear ☐ exclude

Type common name, binomial, taxid or group name

[+ Add organism](#)

Percent Identity to **E value** to

[Filter](#) [Reset](#)

Descriptions Graphic Summary Alignments Taxonomy

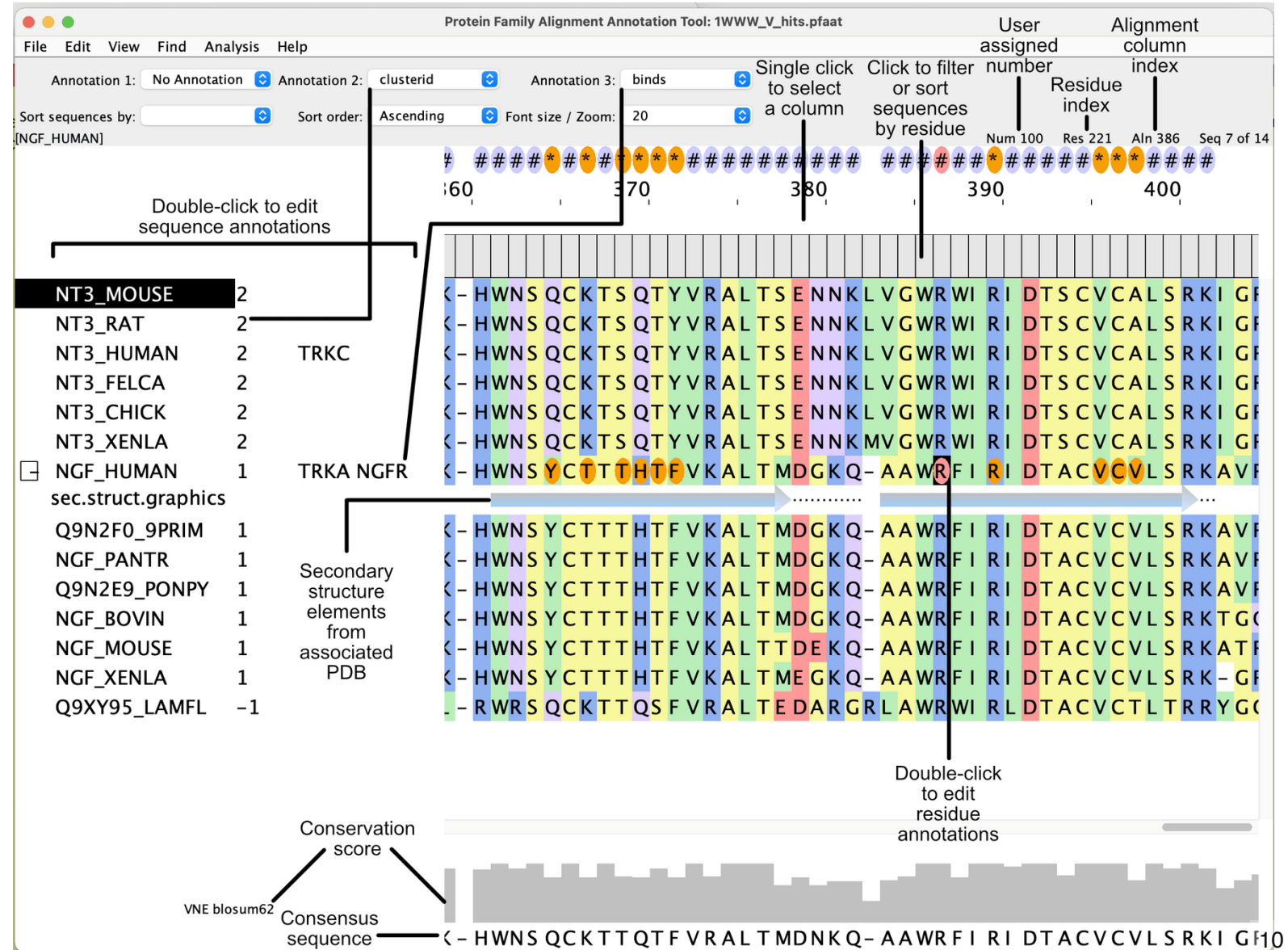
Sequences producing significant alignments [Download](#) ▼ [Manage Columns](#) ▼ Show 100 ▼ [?](#)

☒ select all 7 sequences selected [GenBank](#) [Graphics](#) [Distance tree of results](#)

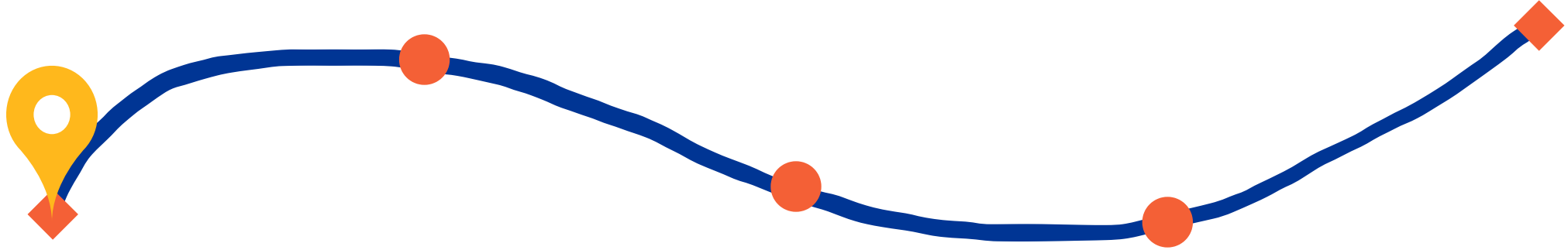
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/>	Macaca mulatta chromogranin A (CHGA), mRNA	3081	3081	99%	0.0	94.77%	NM_001278450.1
<input checked="" type="checkbox"/>	Macaca fascicularis chromogranin A (CHGA), mRNA	3075	3075	99%	0.0	94.72%	NM_001319389.1
<input checked="" type="checkbox"/>	Equus caballus chromogranin A (CHGA), mRNA	1628	1628	93%	0.0	83.08%	NM_001081814.2
<input checked="" type="checkbox"/>	Bos taurus chromogranin A (CHGA), mRNA	1548	1548	95%	0.0	81.91%	NM_181005.2
<input checked="" type="checkbox"/>	Sus scrofa chromogranin A (CHGA), mRNA	1415	1415	80%	0.0	83.08%	NM_001164005.2
<input checked="" type="checkbox"/>	Rattus norvegicus chromogranin A (Chga), mRNA	278	278	20%	4e-72	80.34%	NM_021655.2
<input checked="" type="checkbox"/>	Mus musculus chromogranin A (Chga), mRNA	176	176	11%	2e-41	81.61%	NM_007693.2

Gene prediction and annotation provides the starting point for downstream analyses and discoveries

All downstream (i.e., after) analyses are often gene-specific, so any errors here will propagate



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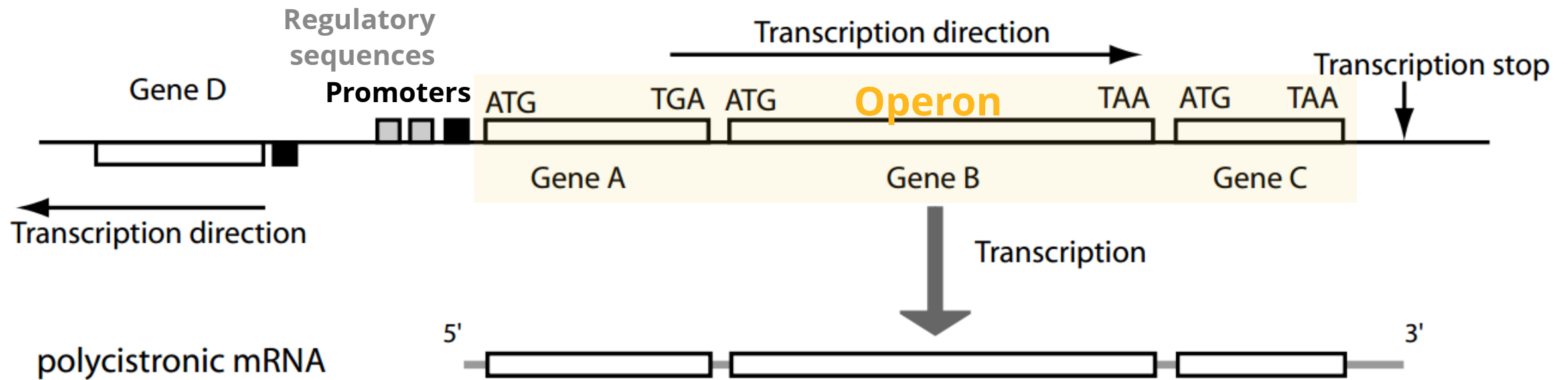


Key differences and challenges of
prokaryotic and eukaryotic gene prediction

Prokaryotes

Prokaryotic genomes are relatively straightforward due to their compact structure

Most genes are organized in **operons**—clusters of co-transcribed genes under a single promoter.



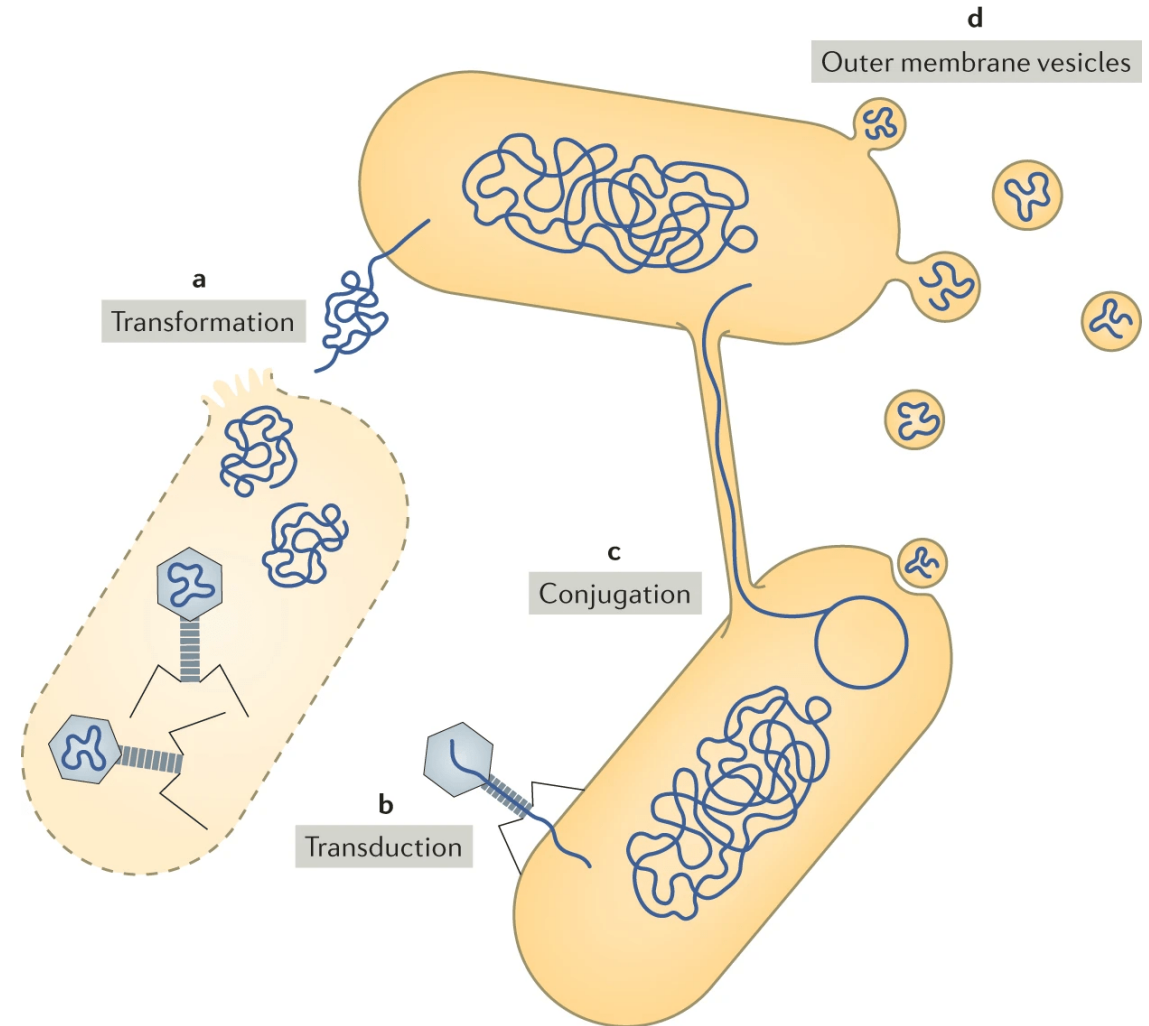
Polycistronic: coding sequences for two or more polypeptide chains that are transcribed in succession from the same promoter

Most genes are readily identifiable by open reading frame (ORF) detection.

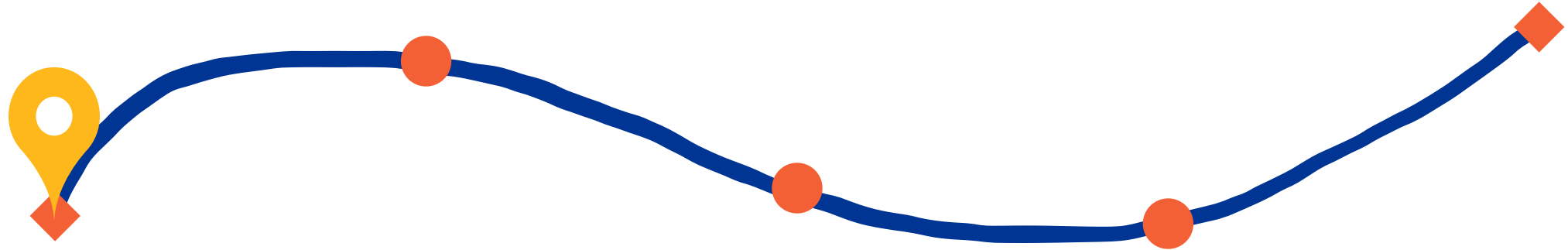
While prokaryotic genomes are simpler, challenges still exist

Horizontal gene transfer: Foreign genes may lack organism-specific sequence patterns, complicating detection.

Short genes: Genes shorter than 150 bp are harder to distinguish from random ORFs.



After today, you should have a better understanding of

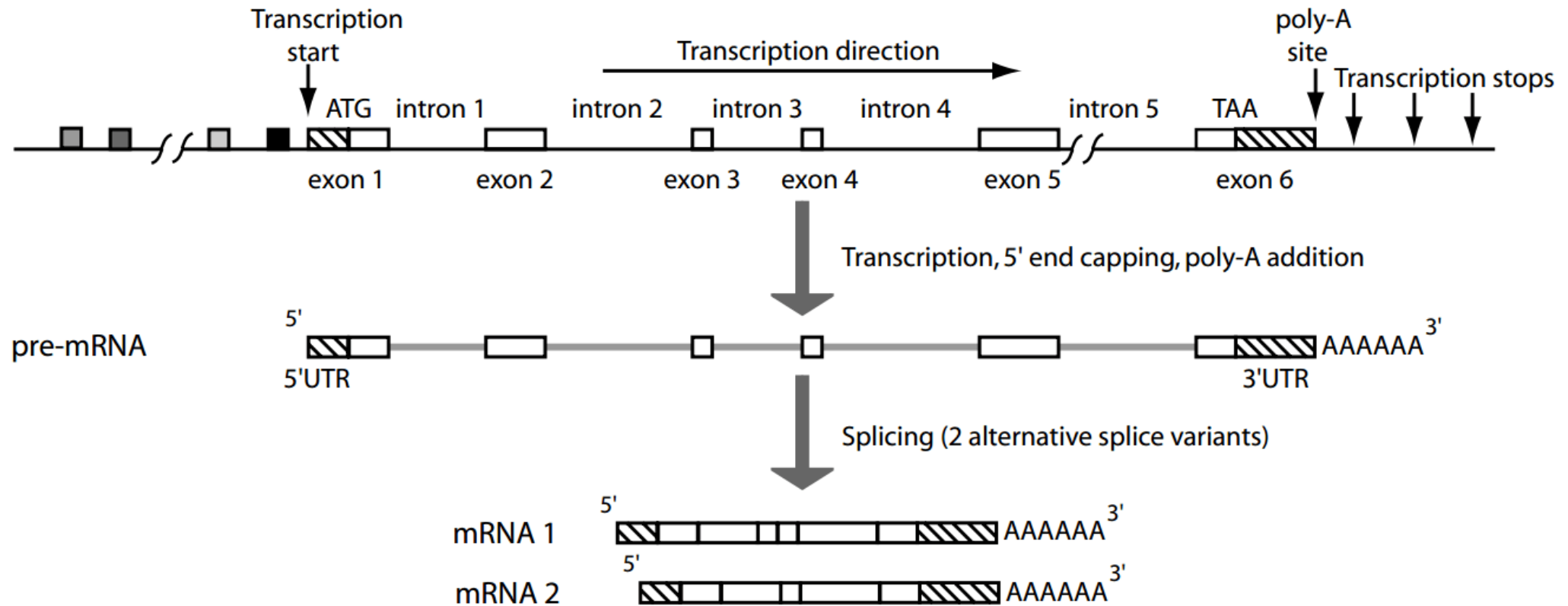


Key differences and challenges of
prokaryotic and eukaryotic gene prediction

Eukaryotes

Eukaryotic genomes are more complex due to non-coding regions and regulatory sequences

Genes contain **introns** (non-coding regions) and **exons** (coding regions), requiring splicing for expression



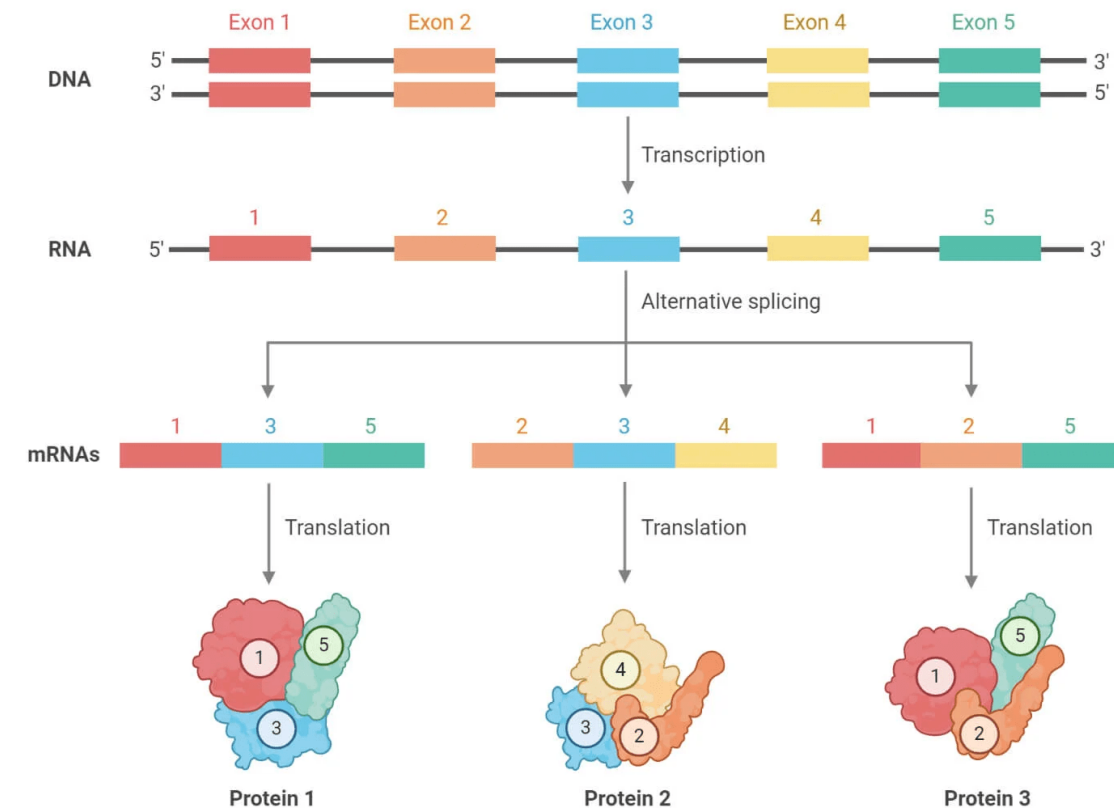
Intergenic (i.e., between genes) regions are large and often contain regulatory elements (e.g., enhancers, silencers).

Eukaryotic gene prediction faces additional challenges due to complexity

Eukaryotic genes undergo splicing which will remove introns and then join exons to form mature mRNA

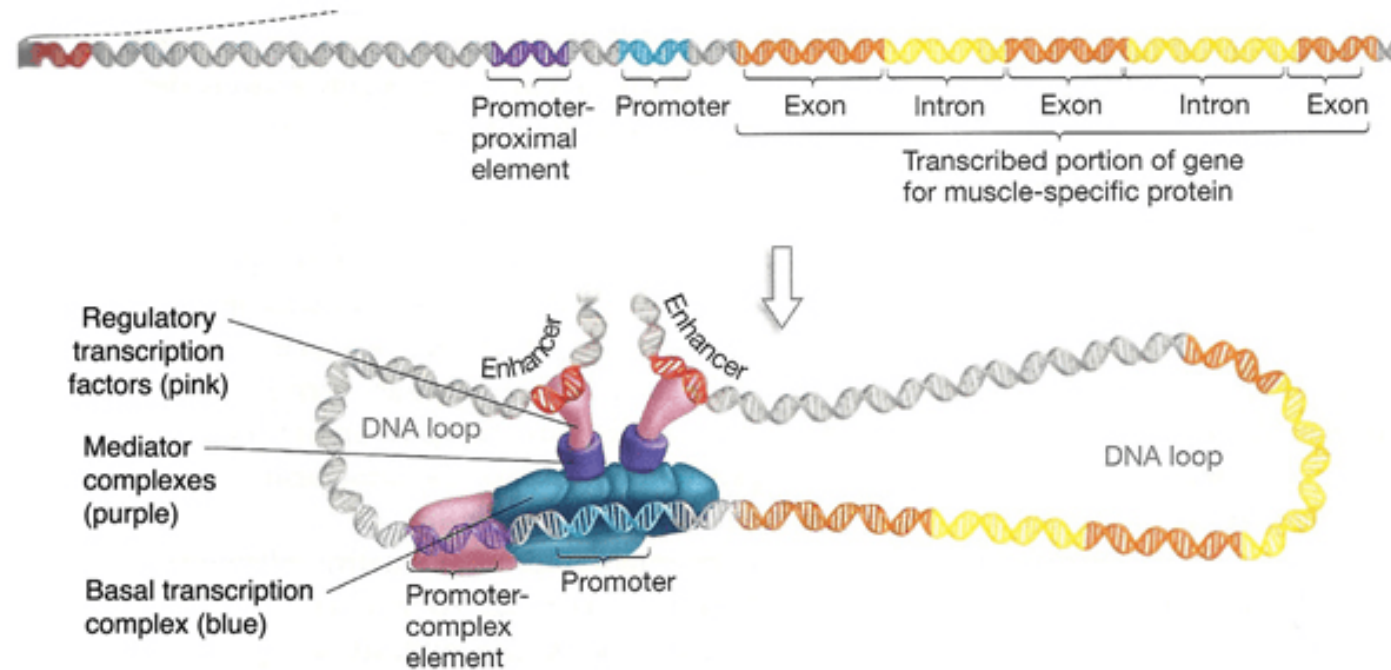
Gene prediction has to predict intron boundaries, which are often much longer than exons and not always consistent

Furthermore, eukaryotes use alternative splicing to join different exons of the same gene to form multiple different proteins



Regulatory elements are critical for expression but are hard to predict

Promoters, enhancers, and silencers regulate transcription but are often far from the gene they control.



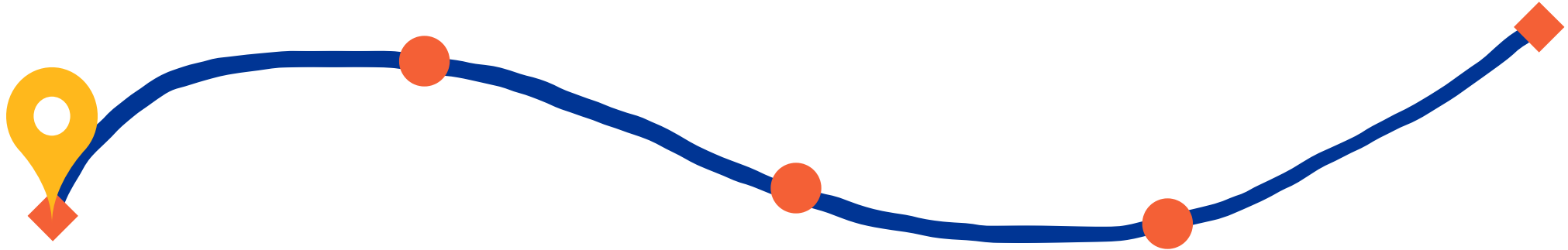
These elements lack a universal sequence pattern, making them difficult to identify.

Eukaryotic gene prediction faces additional challenges due to complexity

Repetitive sequences: Large portions of eukaryotic genomes are repetitive, often confusing prediction algorithms.

AGCTGATC CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT
CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT
CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT
CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT
CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT
CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT CGAT TTAGCCGA

After today, you should have a better understanding of



The principles behind *ab initio* and homology-based
gene prediction approaches

Ab initio

Ab initio gene prediction identifies genes based on intrinsic sequence features

Detects genes without requiring prior knowledge or reference sequences.

Relies on patterns like:

- Start and stop codons.
- Coding sequence biases (e.g., codon usage, GC content).
- Splice sites and promoter regions (in eukaryotes).

We use HMMs to detect these

```
10      20      30      40      50
1 ACCAGGCTTTATAATGTGGGAGAGCCTCCGGGGGGGTTCATAATGCGATG
2 AGATACGGTCATCGGTATTAATGGAGGAGGGGTGGTCACTGCTCATCGT
3 ATATAAGCCAGGGAGGCTGAAAGAGATCCGTTACTATACTCTTCTTTAT
4 AAGGGATGATGTCTCTCTGCGGTGATTCGGCGACTGGTTTAACCACAATA
5 ATAATGACGCAAACGTACAAACGGATCCTCCGGTCACTTAAGGCGAGAT
6 AAAAGGGCACTGCTGGAGTACGTCACGTAGTTCCCGATAAGATTAAGCCA
7 CAGTCCCTGGGCTGATAATGGTCATCGCATACCGGGTCCAGATATTAGC
8 ACGGCTGCTCAGGAGCAGGTGGGAGCCACTGCTGCCATGATTCGCAAAAA
9 ATAACCTATGAACGGACTCCACTTCTAATGGCCCTGAGCATCTGGAGCCG
10 GAGCTAATGCGCAATAGTATGATAATGCGGTGGTCTACCCTAGAAGTCTGA
11 TGAGCGGTCAAATCGCTTTTCGAGGAAATGTGGCAAATTAGGGCTGGCTTG
12 GGAGCTGGTACTGTCAGTCCCATATAAGTCGAGCTGTGGTAATGCGCG
13 CTCATCGAGCAGGTTAGGAAGGAACGCAAGATGATGGGGCTATCTAGCAT
14 CAAATAAGGCGCTTCTGATCCCAACGCGTGGTGACCGTTAAGTAATAATG
15 AGGATCAAAAACAGCAAATGGTAGTGACCGAGCGTCGACCGAACATCGAC
16 TGATAATGCTGACGGAGGGGCGGTGTCACATAAAAGTAGCGATGTATCT
17 TAAGGCGCGCCGAGGTTGATGATGGAGAGGTGGATCTGATGAGGCATTTG
18 ACTCCCTCGTGATGATGCTGATCTCTCAAATTGCTTCATTGAATTATATA
19 AGAGCCTGGTCAGGTAGTGGGATACTAGGGACGTTCAATAAATGATAATG
20 AAAAGCACGTACTGGCAAGAGTCAAGTAGTACGTGGATAAAAGGAGTCGG
21 CGGCTGCTGGGTCCAGCTCTGCTGCCATGATTCGGCCGCGGCCACTAAAA
22 ATCCAAATAATGCCATTCGAGGTCAAATCTGTAAGGACAGTTAAAAAAT
23 ATAAGAGGGCTACGATTGCCGTCACTTCGTTGCATACACCCCTTAAAGT
24 TTGCAACGCTGTACCTGACGACGTCATCAAGGAGGTCTTAATGAGCATGC
25 AAACGCGACAGATACTCTGCTATGATTATGGTCCTGACGAAATACTGATG
26 AGCCGCTGTGATTGTCTGCTGTATTGCACTGACGATGCCATACTTATCAA
27 TTCGTCACTGATATAAGCACTCGCATCTAGGCGACGGTACACGGCAGGTT
28 AATGCGCAGTGTCAATTAATAATGTGGGAGAAAGATTAGTGCCTGACCTT
29 ATGATTTCTATGCAAAGTTCTCATGATCGACATTACTGGAGGTAGGGCG
```

Ab initio methods are powerful but limited by genome complexity

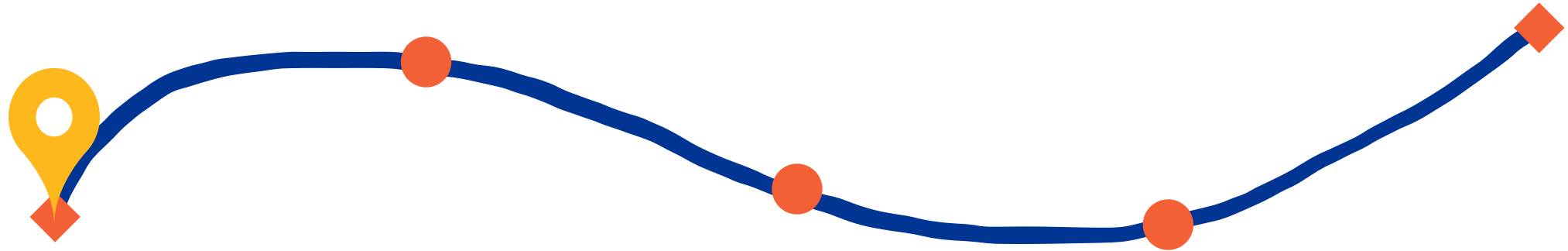
Prokaryotes: Compact genomes make ORF detection easier, but short genes and overlapping genes can still pose challenges.

Eukaryotes:

- Accurate prediction requires identifying introns, exons, and splice sites.
- Alternative splicing and non-coding regions can confound predictions.

False positives and false negatives are common, especially in large, complex genomes.

After today, you should have a better understanding of



The principles behind *ab initio* and homology-based
gene prediction approaches

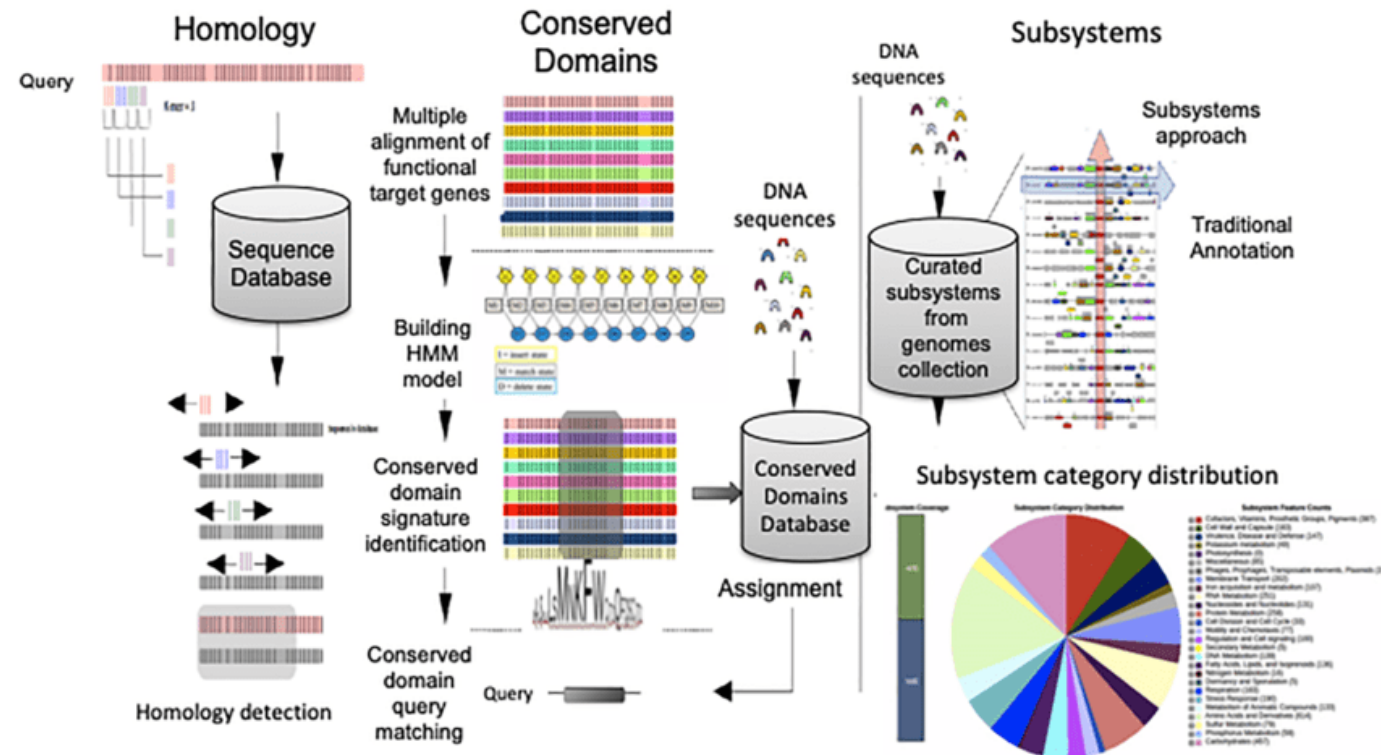
Homology

Homology-based gene prediction identifies genes by comparing sequences to known databases

Searches for regions of similarity between the query genome and annotated sequences in databases.

Assumes genes are evolutionarily conserved across species.

Tools often use sequence alignment methods (e.g., BLAST, HMMER) to detect homologous genes.



Homology-based methods depend on accurate and complete reference data

Advantages: High accuracy for conserved genes with reliable reference sequences.

Limitations:

- Cannot predict novel genes or those without significant similarity to database entries.
- Errors in reference annotations propagate into predictions.
- Divergence and mutation can obscure homology signals.

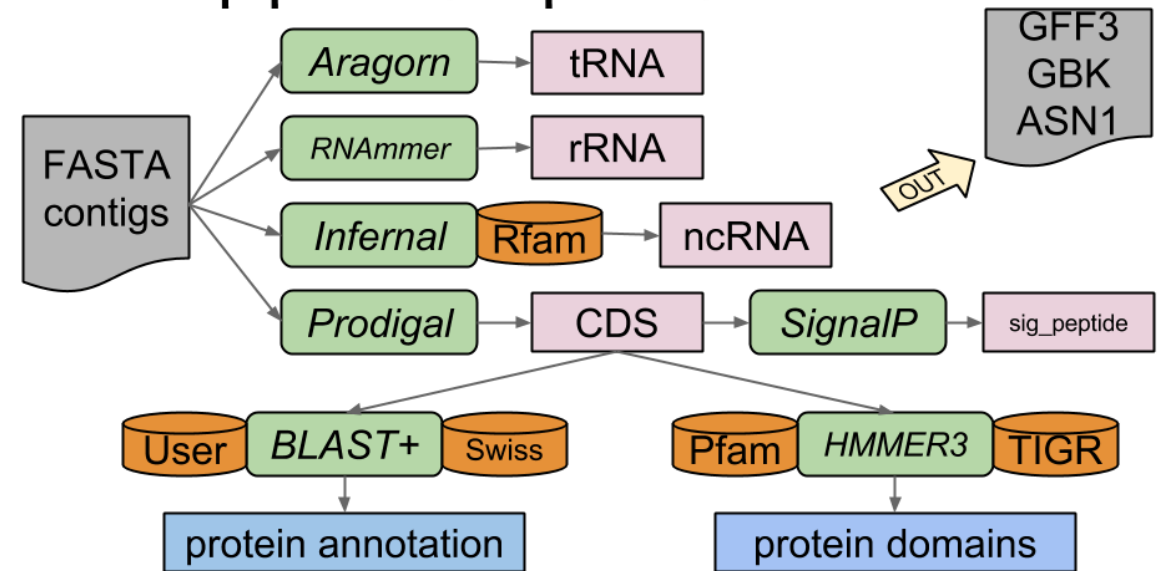
Combining *ab initio* and homology-based methods improves gene prediction accuracy

Ab initio methods can detect novel genes, filling gaps left by homology-based methods.

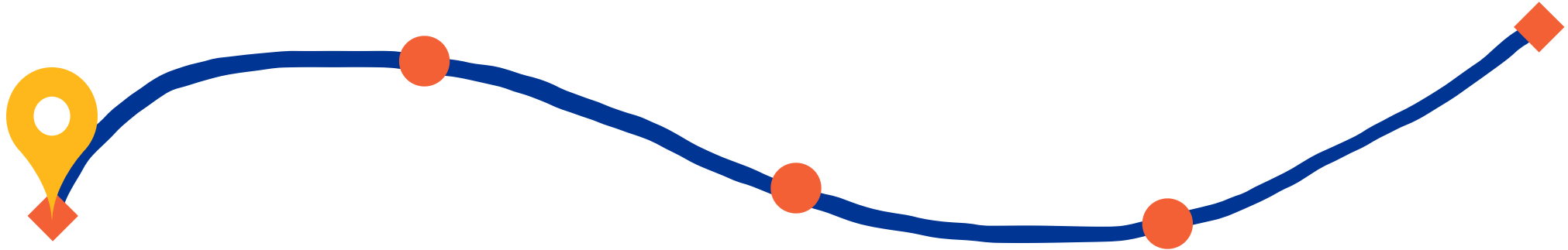
Homology-based methods provide functional validation for predictions from *ab initio*.

Integrated pipelines (e.g., Prokka, AUGUSTUS) use both approaches to produce more reliable results.

Prokka pipeline (simplified)



After today, you should have a better understanding of



Practical examples of gene prediction tools
and how to interpret their outputs

???

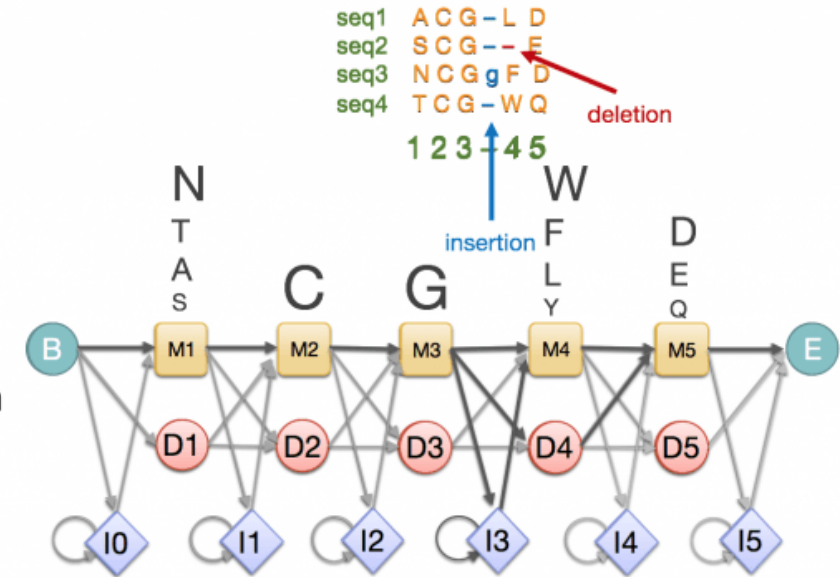
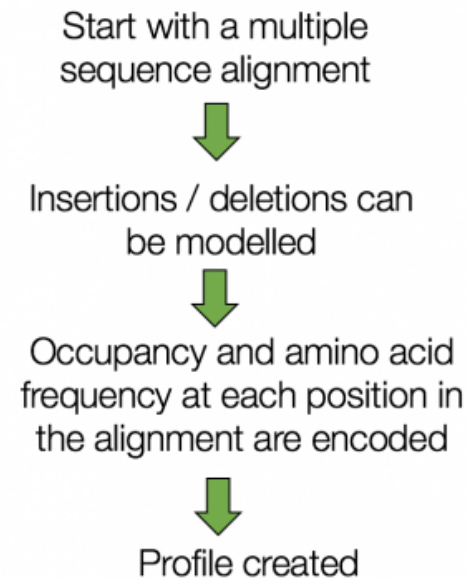
HMMER uses Hidden Markov Models (HMMs) for detecting homologous genes

Aligns query sequences to profiles of known genes/proteins in curated databases like [Pfam](#).

Identifies genes based on conserved domains or motifs.

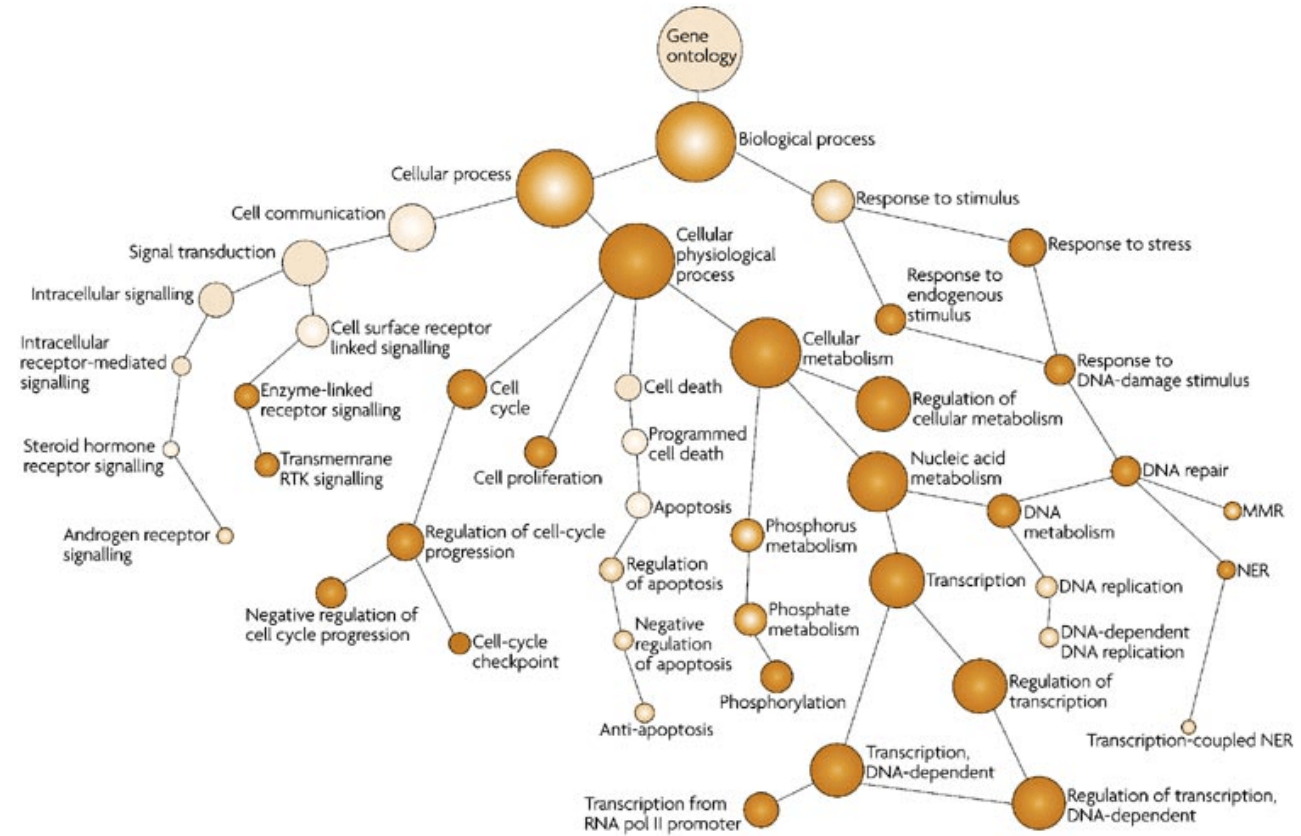
Outputs:

- Alignment scores for detected homologs.
- Functional annotations from database hits.



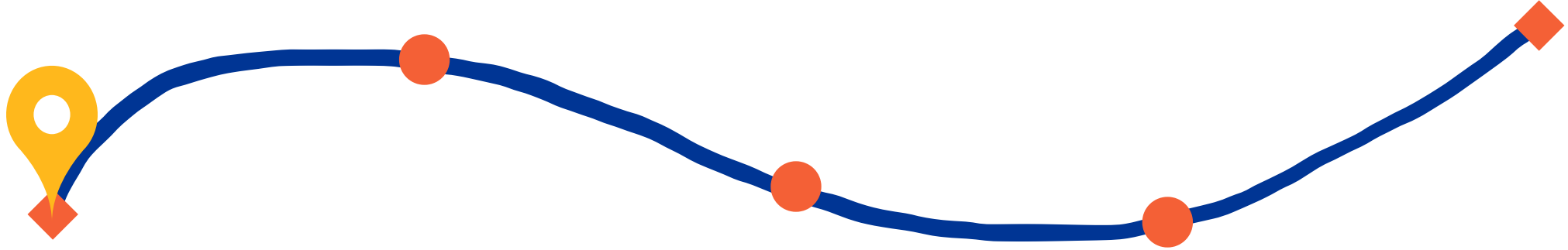
Interpreting outputs requires understanding key metrics and visualizations

- **Gene locations:** Coordinates of start and stop codons or exon-intron boundaries.
- **Scores:** Confidence values for predictions, such as e-values in HMMER or reliability scores in AUGUSTUS.
- **Functional annotations:** Gene ontology (GO) terms, protein domains, or pathway mappings.



Nature Reviews | Cancer

After today, you should have a better understanding of



Practical examples of gene prediction tools
and how to interpret their outputs

Prokka

Gene prediction tools apply computational principles to real-world problems

Selecting the right tool depends on the organism, genome complexity, and research goals.

Prokka is a popular tool for prokaryotic genome annotation

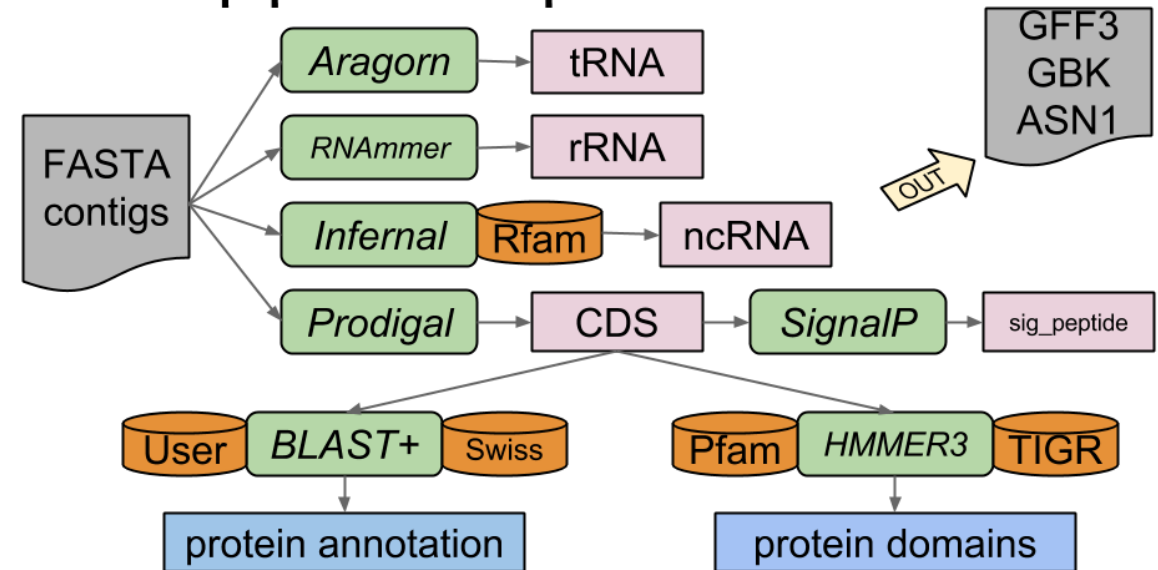
Combines *ab initio* and homology-based methods for prokaryotic genomes.

Annotates coding sequences, tRNAs, rRNAs, and regulatory regions.

Outputs:

- GenBank files for visualization.
- FASTA files of predicted genes/proteins.
- Summary statistics of genome features.

Prokka pipeline (simplified)

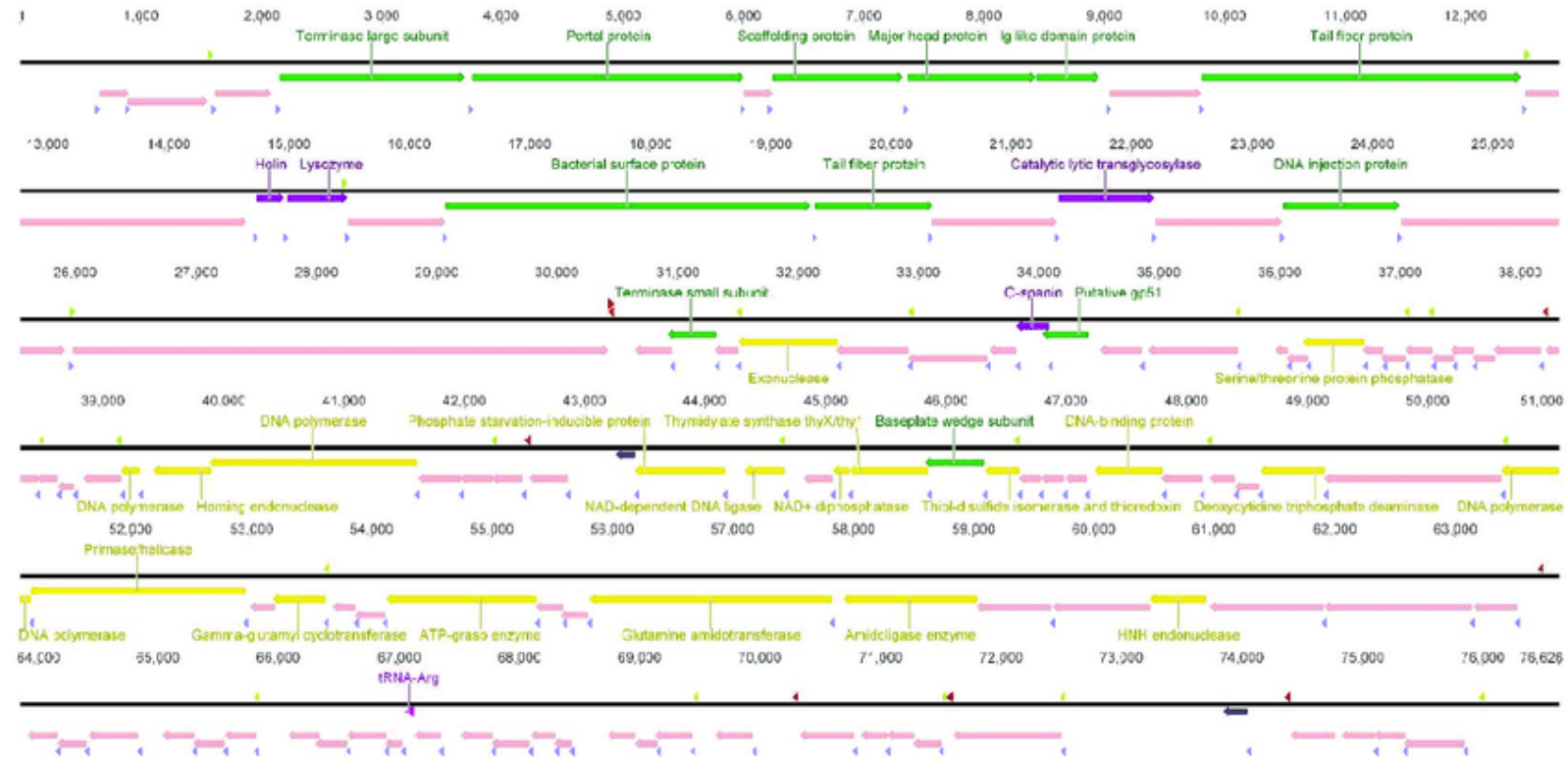


Prokka provides an efficient workflow for bacterial genome annotation

Inputs: Assembled genome in FASTA format.

Outputs:

- A list of coding sequences (CDSs) with predicted functions.
- Identification of antibiotic resistance genes (e.g., beta-lactamases).

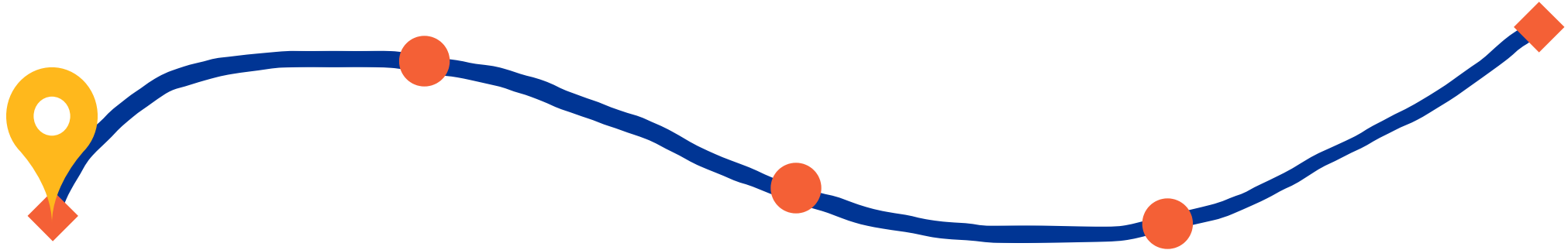


Prokka output files example

```
>ECNNONJI_02637 Dihydrofolate reductase
MTLSILVAHDLQRVIGFENQLPWHLPNDLKHVKKLSTGHTLVMGRKTFESIGKPLPNRRN
VVLTSDTSFNVEGVDVIHSIEDIYQLPGHVFIFFGGQTLFEEMIDKVDDMYITVIEGKFRG
DTFFPPYTFEDWEVASSVEGKLDEKNTIPHTFLHLIRKK
```

Extension	Description
.gff	This is the master annotation in GFF3 format, containing both sequences and annotations. It can be viewed directly in Artemis or IGV.
.gbk	This is a standard Genbank file derived from the master .gff. If the input to prokka was a multi-FASTA, then this will be a multi-Genbank, with one record for each sequence.
.fna	Nucleotide FASTA file of the input contig sequences.
.faa	Protein FASTA file of the translated CDS sequences.
.ffn	Nucleotide FASTA file of all the prediction transcripts (CDS, rRNA, tRNA, tmRNA, misc_RNA)
.sqn	An ASN1 format "Sequin" file for submission to Genbank. It needs to be edited to set the correct taxonomy, authors, related publication etc.
.fsa	Nucleotide FASTA file of the input contig sequences, used by "tbl2asn" to create the .sqn file. It is mostly the same as the .fna file, but with extra Sequin tags in the sequence description lines.
.tbl	Feature Table file, used by "tbl2asn" to create the .sqn file.
.err	Unacceptable annotations - the NCBI discrepancy report.
.log	Contains all the output that Prokka produced during its run. This is a record of what settings you used, even if the --quiet option was enabled.
.txt	Statistics relating to the annotated features found.
.tsv	Tab-separated file of all features: locus_tag,ftype,len_bp,gene,EC_number,COG,product

After today, you should have a better understanding of



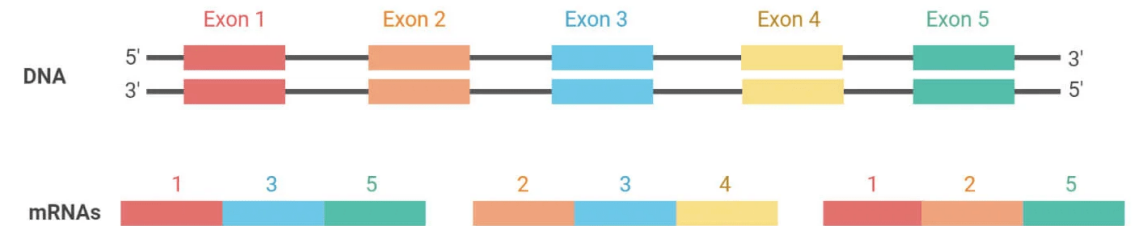
Practical examples of gene prediction tools
and how to interpret their outputs

AGUSTUS

AUGUSTUS excels at predicting genes in eukaryotic genomes

Focuses on *ab initio* gene prediction but integrates hints like RNA-seq data for improved accuracy

Suitable for genomes with limited or no reference annotations



Outputs:

- Predicted gene structures, including exons, introns, and UTRs.
- GFF3 files for integration with genome browsers.

Before the next class, you should

Lecture 04A:

Gene prediction -
Foundations

Lecture 04B:

Gene prediction -
Methodology



Today



Thursday

- Start [P01C](#) (due Jan 31)
- Work on [CByte 01](#) and [CByte 02](#)