Bacterial gene and genome annotation

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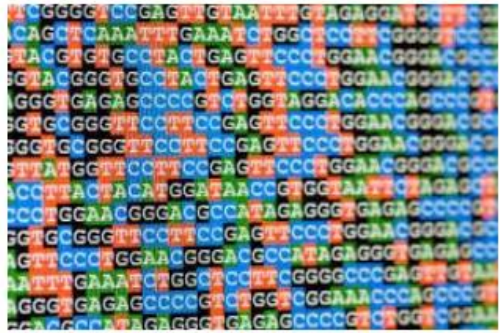
Bacterial genomes: small genomes

6,000,000,000

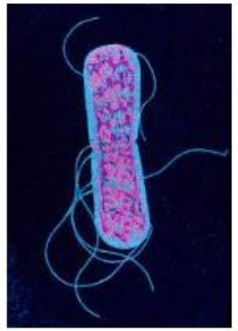
letters

30,000 genes



Genome

A T G C



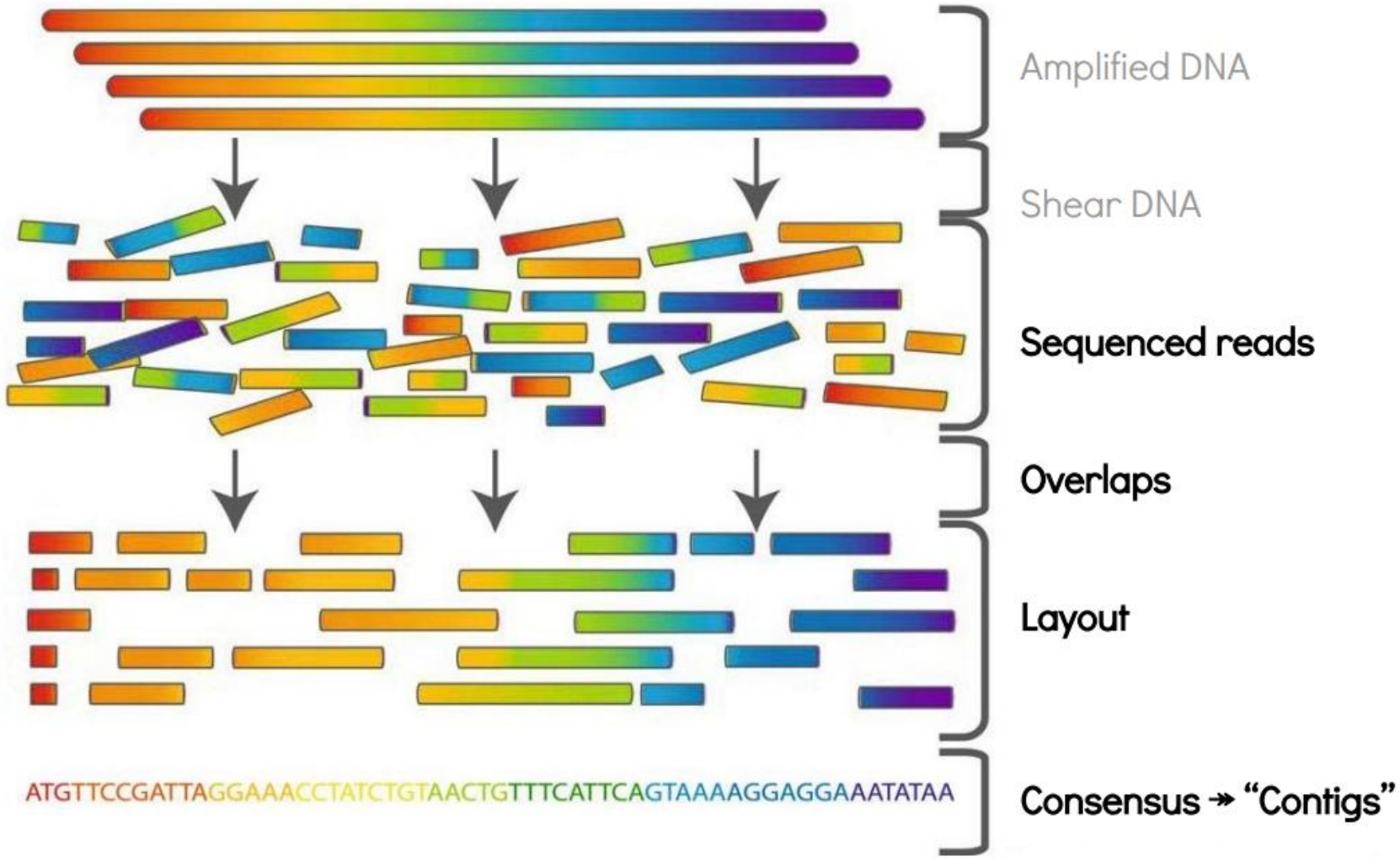
3,000,000

letters

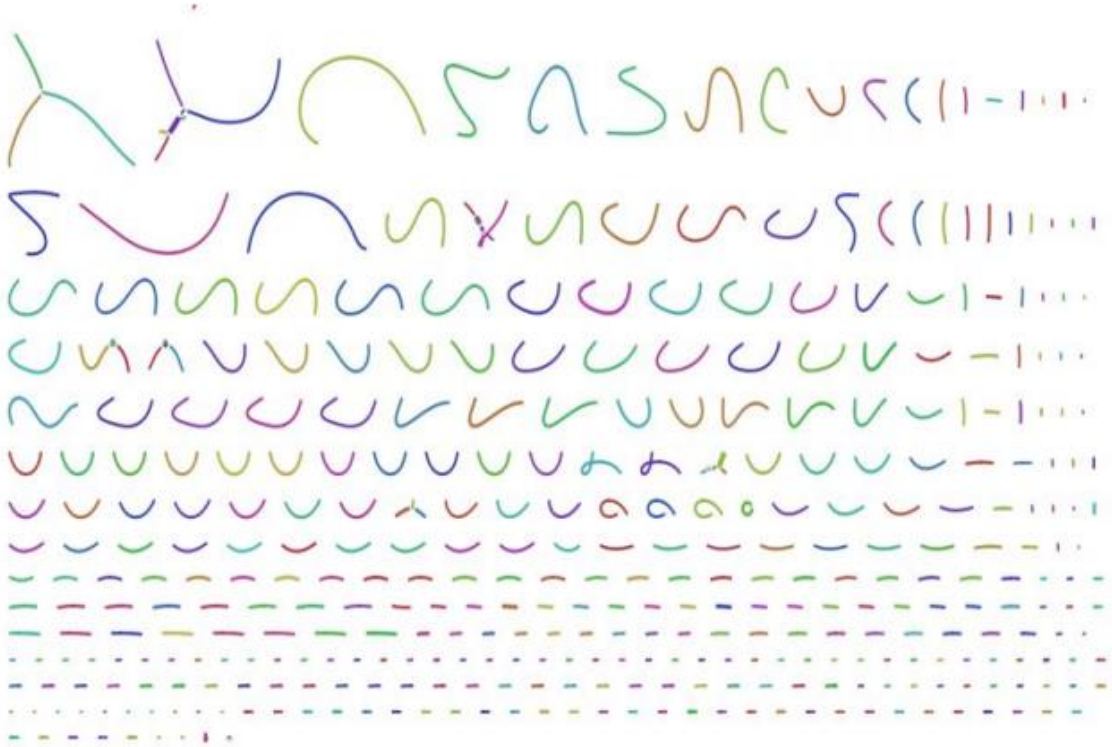
3,000 genes

https://angus.readthedocs.io/en/2016/_static/bacterial_genome_annotation.pdf

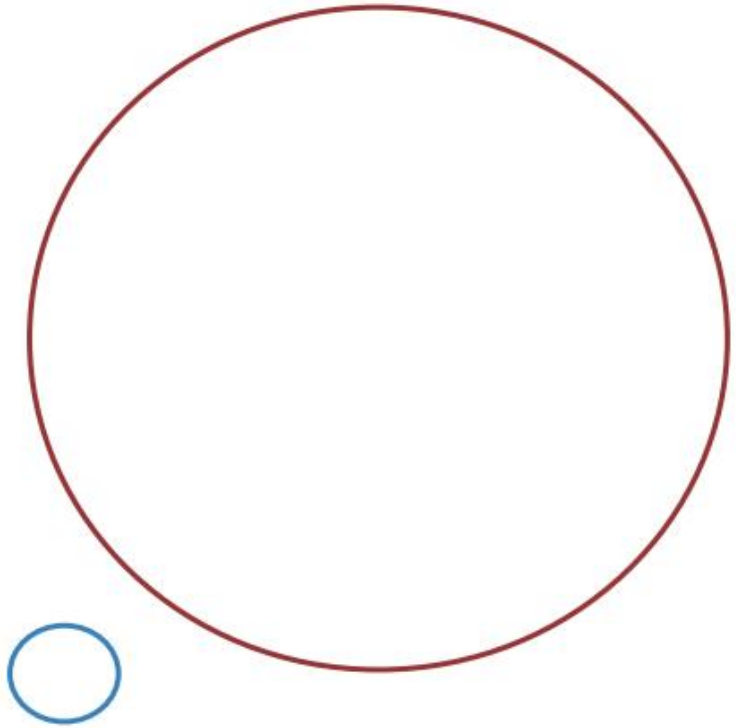
De novo assembly



Draft vs Finished genomes



Lots of contigs



One contig per replicon

```
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48601 agagccgggt tgaaggtgac tgaagtgaaa tgggacagta gaggcggggg gggtggtgag
48661 ttcctggagg tgggggggtgt gggaacctgc tgtgtactga gatgcacccc tgccagttct
48721 gcctgaagat ttgaggcggg gggcaggggg gcggagtgaa gtcattttac tggtaagtaa
48781 ttttaaacct tttaatatta aagcaaacgt ggatatgtaa tgaatgaaat tcattctgga
48841 atgaaaaatt cacgtgatgt tgaaaaataa cacggggctt cagagaggac tttctggctg
48901 gcagcagact ccagattccc agggcccctg caccctcctc tgcccacagg gcaccttaat
48961 ggagaaggtg tgggaggaga gccaggccgg agtcagagca cactggtgac tccacatttg
49021 cagcgtgccc tgcctctctc ctgaggcttg gcaacgtgca atatgctaag caaactcccc
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49201 ggagtgaagc actcgggtga aaaggtacaa ggaagtcagg gacaggagtg tggggacatc
49261 acctagacaa tgacagagaa gaggggcaca gccgagtgag gggagagggg ccggcagtcc
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49381 aagagacctg agttcaaatt ctggctccac cactgaccac ctgtgtaacc ttgaactgct
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49501 gcccctgtga gaacgaaatg gtgcaagcac caaggagcct cagcaaaggt cgggcctgcc
49561 cccgcctggc caaacctttc ctcttcagga ggccacggca accgtagttt gacagaagag
49621 cagcaccttg atttaatgct tcccagcatg tgtccttgag caagtcacct aacctctctg
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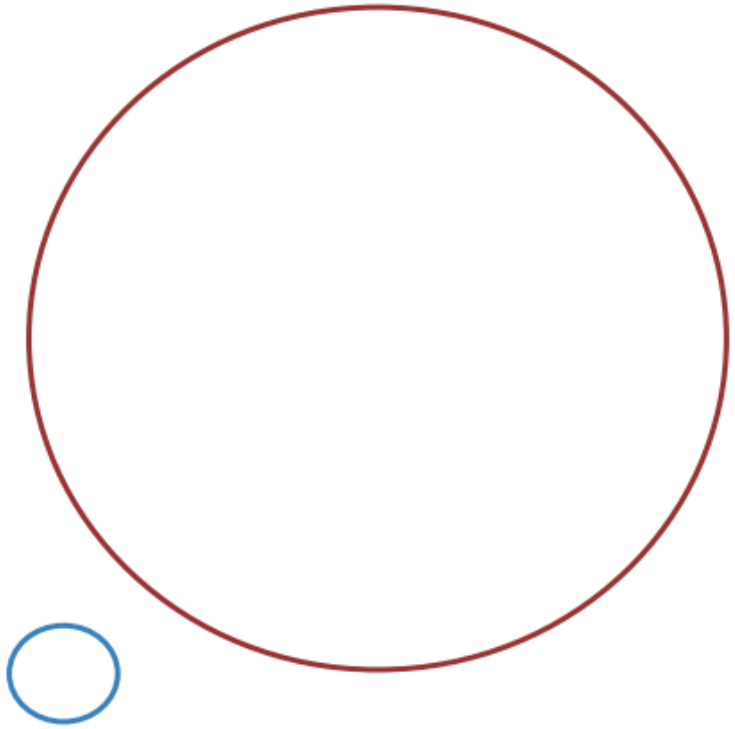

One contig per replicon

https://www.ncbi.nlm.nih.gov/nuccore/806904736

Genome Annotation: Adding biological info to sequences

```
48541 agcccttcaa agaaatgttc tcagcaggca tggagcccag gacttgctcc ctttgggtag
48601 agagccgggt tgaaggtgac tgaagtgaaa tgggacagta gaggcggggg gggtggtgag
48661 ttcctggagg tgggggtgt gggaacctgc tgtgtactga gatgcacccc tgccagttct
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49621 cagcaccttg atttaatgct tcccagcatg tgtccttgag caagtcacct aacctctctg
49681 ggctgcttcc tcattgggaa aatatggctg ccagtaaaac ctgccctgtc cacctcctgg
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50701 agcttccaca gccagcgctg gacagggagg gtccagatat acccactagt gccctcacca
```

ribosome binding site

delta toxin
PubMed: 15353161

ACCGGCCGAGACAGCGAGCATATGCAGGAAGCGGCAGGAATAAGGA
AAAGCAGCCTCCTGACTTTCCTCGCTTGGTGGTTTGAGTGGACCTC
CCAGGCCAGTGCCGGGCCCCTCATAGGAGAGGAAGCTCGGGAGGTG
GCCAGGCGGCAGGAAGGCGCACCCCCCCAGCAATCCGCGCGCCGGG
ACAGAATGCCCTGCAGGAACTTCTTCTAGAAGACCTTCTCCTCCTG
CAAATAAAACCTCACCCATGAATGCTCACGCAAGTTTAATTACAGA
CCTGAAACAAGATGCCATTGTCCCCCGGCCTCCTGCTGCTGCTGCT
CTCCGTCCGTCCGTGGGCCACGGCCACCGCTTTTTTTTTTGCC

transfer RNA
Leu-(UUR)

tandem repeat
CCGT x 3

homopolymer
10 x T

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*

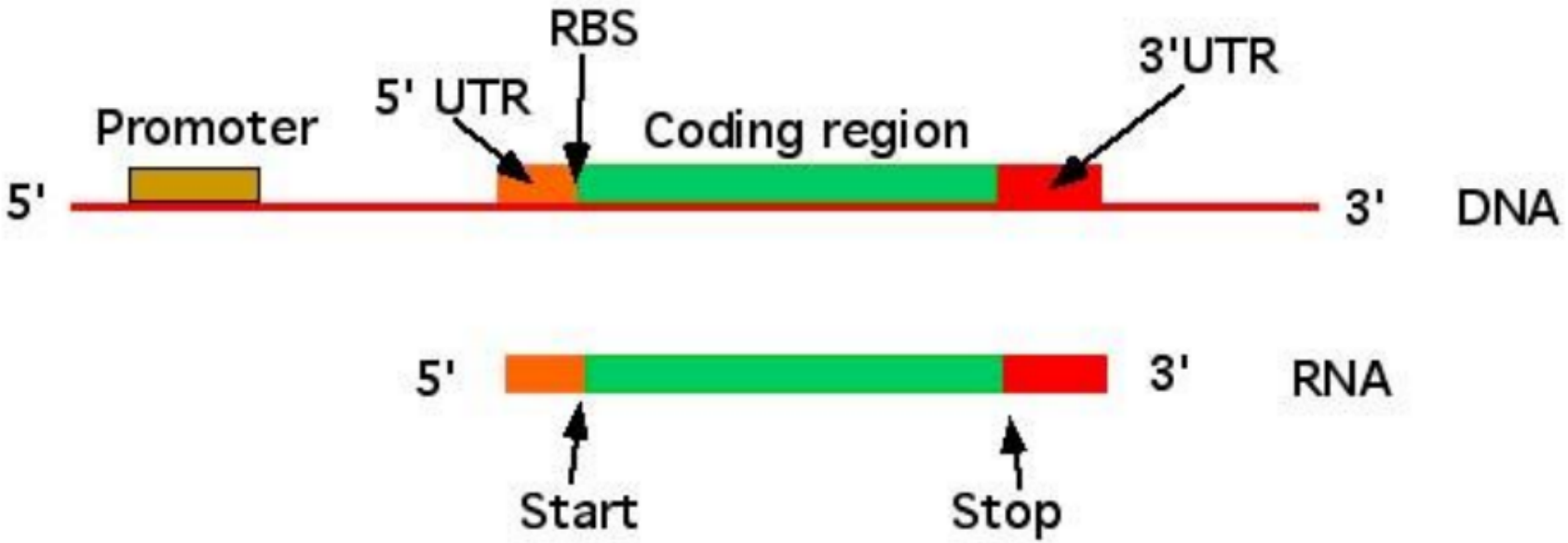
Bacterial feature types

- protein coding genes
  - promoter (-10, -35)
  - ribosome binding site (RBS)
  - coding sequence (CDS)
    - signal peptide, protein domains, structure
  - terminator
- non coding genes
  - transfer RNA (tRNA)
  - ribosomal RNA (rRNA)
  - non-coding RNA (ncRNA)
- other
  - repeat patterns, operons, origin of replication, ...

Key bacterial features

- tRNA
  - easy to find and annotate: anti-codon
- rRNA
  - easy to find and annotate: 5s 16s 23s
- CDS
  - straightforward to find candidates
    - false positives are often small ORFs
    - wrong start codon
  - partial genes, remnants
  - pseudogenes
  - assigning function is the bulk of the workload

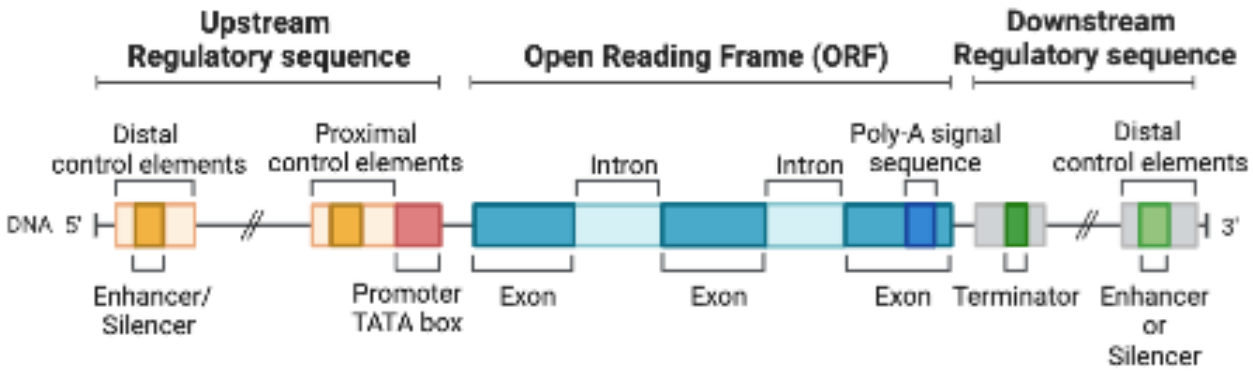
Bacterial genes: no introns!



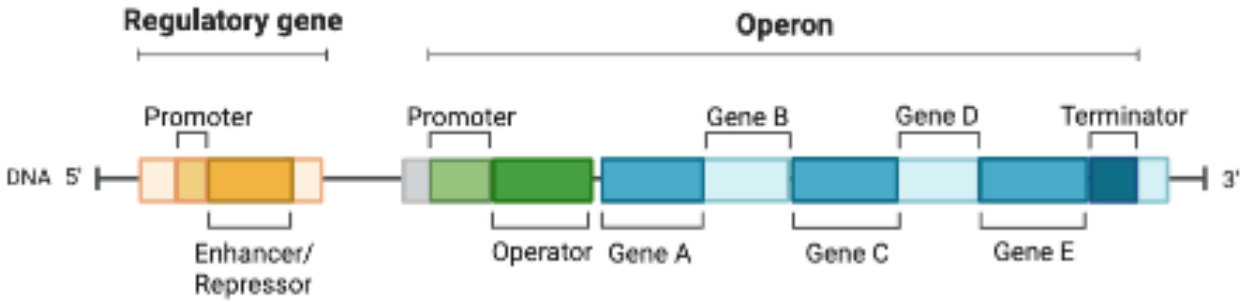
- have >= 3 potential start codons (species dependent)
- haploid, but lots of horizontal gene transfer
- methylation used as primitive immune system
  - restriction modification system against phage

Prokaryotic vs eukaryotic Genes

Eukaryotic Gene Structure

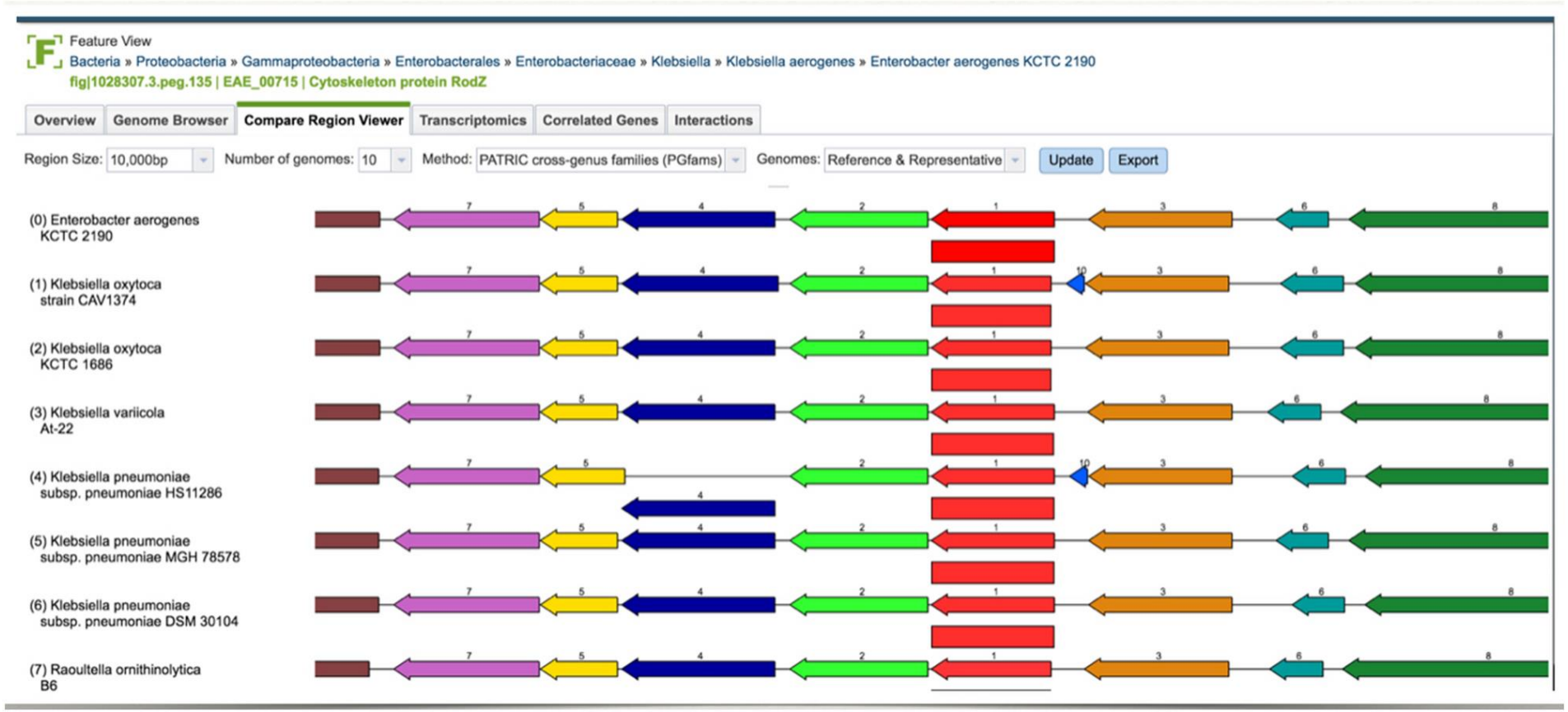


Prokaryotic Gene Structure



https://study.com/skill/practice/contrasting-the-regulation-of-gene-expression-in-prokaryotic-eukaryotic-organisms-questions.html

Detecting operons in bacterial genomes via visual representation learning



Snapshot of the Compare Region Viewer service provided by PATRIC (https://www.patricbrc.org). The image shows a genomic region of the query genome (first row) aligned against a set of other genomes, anchored at the focus gene (represented as a red arrow). The service starts with finding other genes that are of the same family as the focus gene, and then aligns their flanking regions accordingly.

https://www.nature.com/articles/s41598-021-81169-9

Automatic Genome Annotation

Two strategies for identifying coding genes:

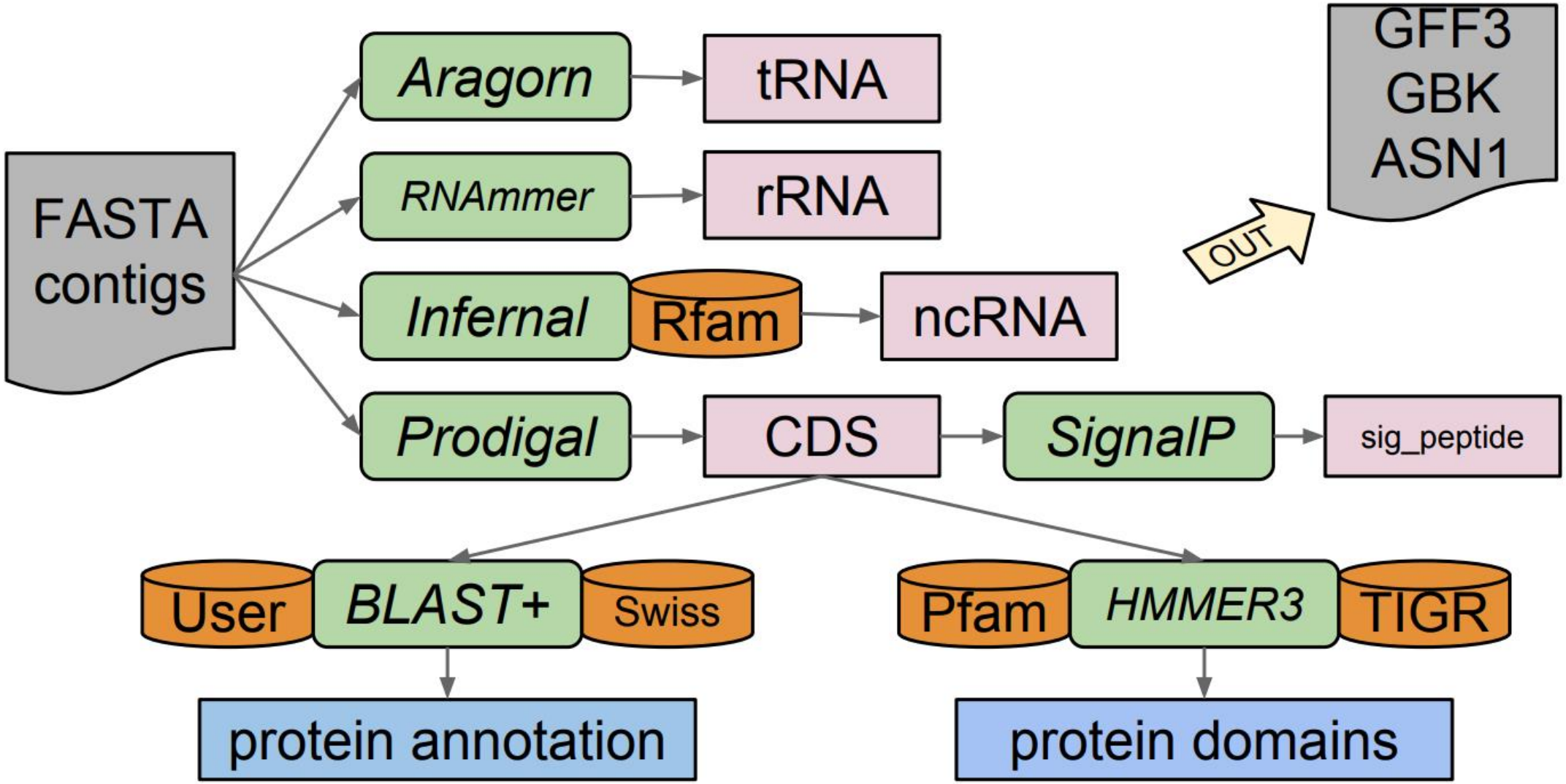
- **sequence alignment**
  - find known protein sequences in the contigs
    - transfer the annotation across
  - will miss proteins not in your database
  - may miss partial proteins

- ***ab initio* gene finding**
  - find candidate open reading frames
    - build model of ribosome binding sites
    - predict coding regions
  - may choose the incorrect start codon
  - may miss atypical genes, overpredict small genes

Some good existing tools

Software	*ab initio*	align-ment	Availability	Speed
RAST	yes	yes	web only	12-24 hours
xBASE	yes	no	web only	>4 hours
BG7	no	yes	standalone	>10 hours
PGAAP (NCBI)	yes	yes	email / we	>1 month

Prokka pipeline (simplified)



Prokka pipeline (simplified)

Bioinformatics Advance Access published March 18, 2014

Genome Analysis

Prokka: rapid prokaryotic genome annotation

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[2] Life Sciences Computation Centre, Victorian Life Sciences Computation Initiative, Melbourne, Australia.

Associate Editor: Prof. Alfonso Valencia

ABSTRACT

Summary: The multiplex capability and high yield of current day DNA sequencing instruments has made bacterial whole genome sequencing a routine affair. The subsequent *de novo* assembly of reads into contigs has been well addressed. The final step of annotating all relevant genomic features on those contig can be achieved slowly using existing web and email-based systems, but these are not applicable for sensitive data or integrating into computational pipelines. Here we introduce Prokka, a command line software tool to fully annotate a draft bacterial genome in about ten minutes on a typical desktop computer. It produces standards-compliant output files for further analysis or viewing in genome browsers.

Availability and Implementation: Prokka is implemented in Perl and is freely available under an open source GPLv2 license from http://vicbioinformatics.com/.

Contact: torsten.seemann@monash.edu

2 DESCRIPTION

2.1 Input

Prokka expects pre-assembled genomic DNA sequences in FASTA format. Finished sequences without gaps are the ideal input, but it is expected that the typical input will be a set of scaffold sequences produced by *de novo* assembly software. This sequence file is the only mandatory parameter to the software.

2.2 Annotation

Prokka relies on external feature prediction tools to identify the coordinates of genomic features within contigs. These tools are listed in Table 1, and all of them, except for Prodigal, provide co-

Example from Prokka

Feature Type:

tRNA

Location:

contig000341 @ 655..730 +

Attributes:

/gene="tRNA-Leu(UUR)"

/anticodon=(pos:678..680,aa:Leu)

/product="transfer RNA-Leu(UUR)"

/inference="profile:Aragorn:1.2"

Provenance

Recording where an annotation came from

Prokka uses Genbank "evidence qualifier" tags:

Wet lab

```
/experiment="EXISTENCE:Northern blot"
```

Dry lab

```
/inference="similar to DNA sequence:INSD:AACN010222672.1"
/inference="profile:tRNAscan:2.1"
/inference="protein motif:InterPro:IPR001900"
/inference="ab initio prediction:Glimmer:3.0"
```

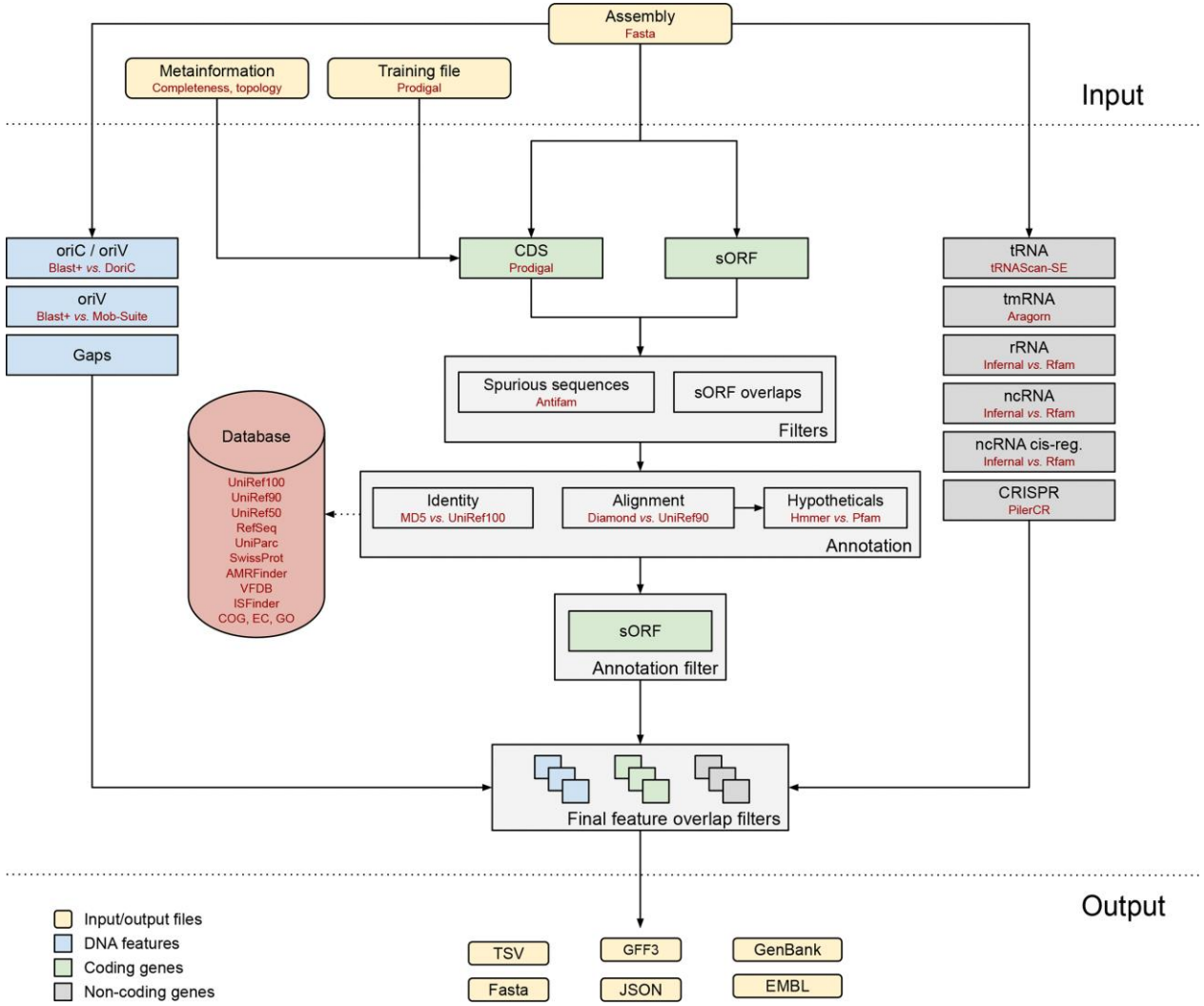
Curating genomes: Improving annotations

- Some annotations are wrong
  - False annotation
  - Missing annotation
  - Partially wrong annotation
- Curation
  - Manual effort to improve annotations
  - Community curation

Web Apollo: a web-based genomic annotation editing platform

https://genomebiology.biomedcentral.com/articles/10.1186/gb-2013-14-8-r93

Bakta is a tool for the rapid & standardized annotation of bacterial genomes and plasmids from both isolates and metagenome-assembled genomes (MAGs)



Overview of the Bakta annotation workflow (Schwengers *et al.* 2021)

Further structural annotation

- **PlasmidFinder** (Carattoli and Hasman 2020), a tool for the identification and typing of plasmid sequences in Whole-Genome Sequencing.

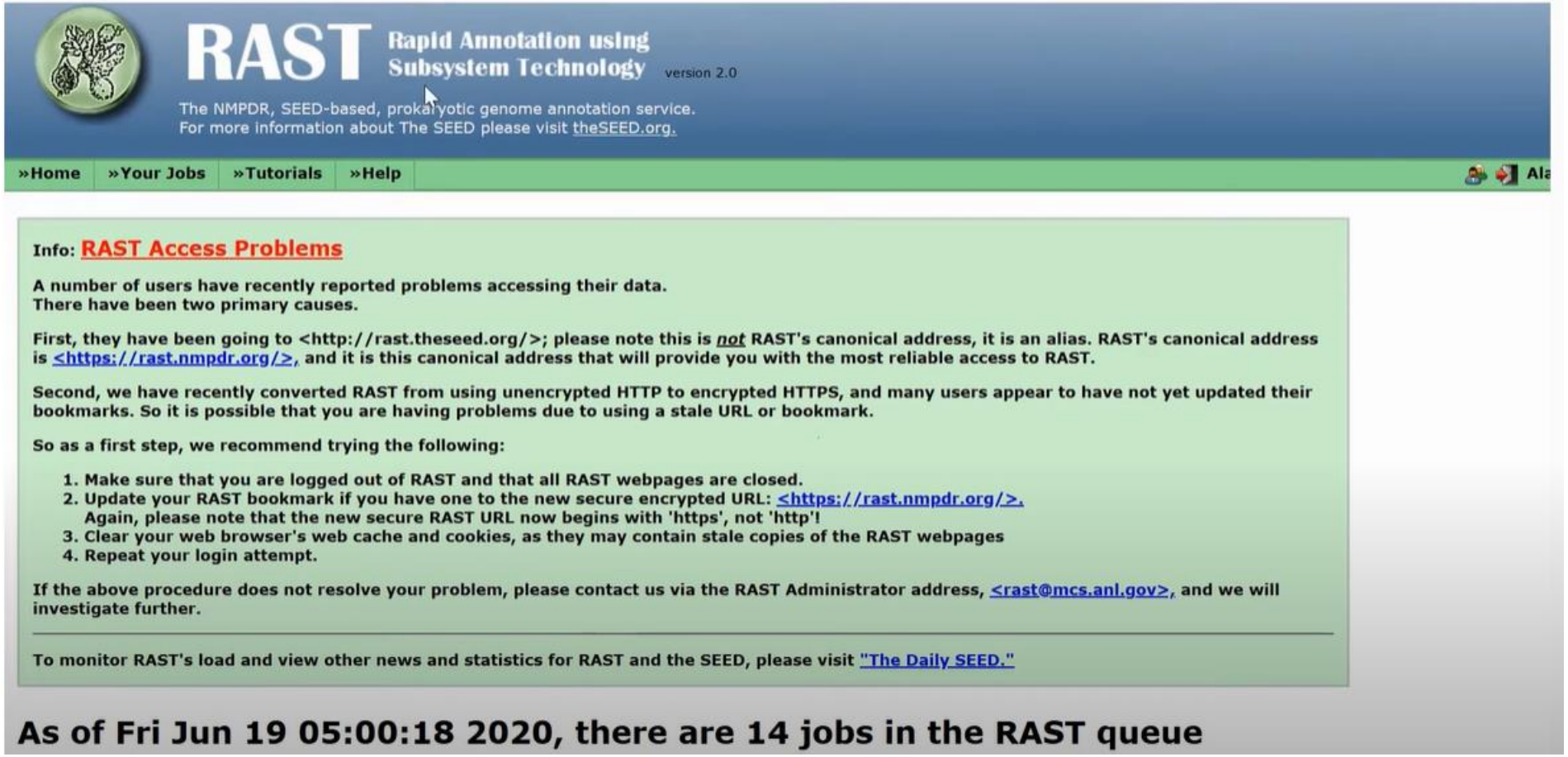
- **IntegronFinder** (Néron *et al.* 2022), a tool for detecting integrons

- An **integron** is minimally composed of:
  - ❑ a gene encoding for a site-specific recombinase (intI)
  - ❑ a proximal recombination site (attI), which is recognized by the integrase and at which gene cassettes may be inserted
  - ❑ a promoter (Pc) which directs transcription of cassette-encoded genes

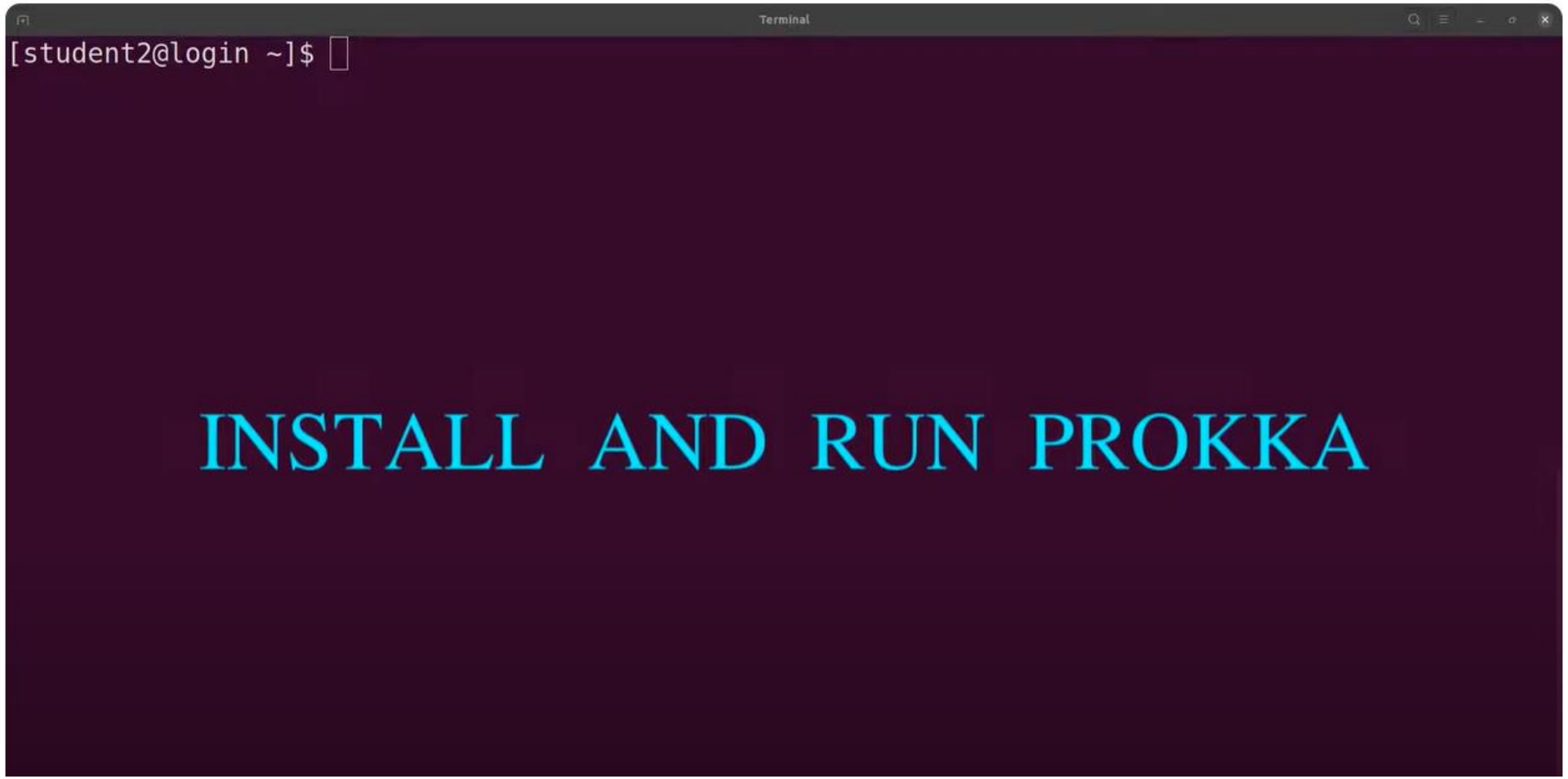
- **IS (Insertion Sequence)** elements: a short DNA sequence that acts as a simple transposable element

- **ISEScan** (Xie and Tang 2017) for detection of IS

Exercise 1: Using RAST to annotate bacterial genome sequence as following https://www.youtube.com/watch?v=pICJ4Gmqq1U



Exercise 2: Using PROKKA to annotate bacterial genome sequence as following
https://www.youtube.com/watch?v=NYbYxGf-8NM&list=PLe1-kjuYBZ04YmORKfXBCOqGDDYJtudVA&index=8



Thank you for your attention!