Practical Insights into Bacterial Genomic Annotation

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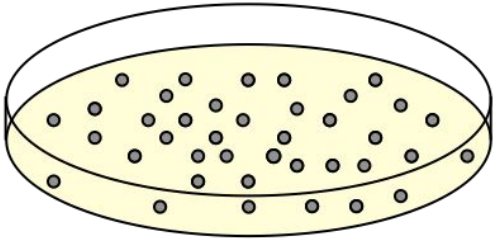
Genome annotation is the process of **identifying genes and other functional elements** within a DNA sequence and attaching information about their possible functions. It transforms a **sequence of nucleotides into a functional map of genetic capabilities**.

Why Annotate Bacterial Genomes?

- Better describe the functional capability of a bacteria
- Identify risk (AMR, virulence, etc) / beneficial (biofuels, etc) factors
- Typing bacteria to find similar strains (Surveillance)
- Identify potential adaptation
- Comparative Genomics
- Microbiome studies

From Bacterial Samples to Genetic Content



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> chromosome
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CGTGACACCTAAGACTGGAACAAGCTCAGAAG
AGTAGTAGGCGAGCATTTTTTGACCGAGTCCG
CTCCTTTCTAACTCACTGACTTCTCGCGGGCCG
TATCCTGCACGCTCAACAGCCAGCGGTGTCCC
GTTACCCTTCAAGCTCATCTTCCTCGAGGTCTG
TTGTAGTACCACACGCCTCTCCCGGCATTAGC
CCGCACTCCTCGACGGGACATTATGTGCCTTC
AGTTCCCGATCTCGGTCGCGCCCAGCCGGAAT
CCCTTAGACACCAGGGCCGCGTGAGCGAGAA
GCGGGGGGAGAACTTTATAGGGCTGTGGCTCA
TACAATAGGGTAAGGTTTCACCACATTTCTTCA
CTTCAGAAGCGACGCCTCCATTTTGCCCTCACC
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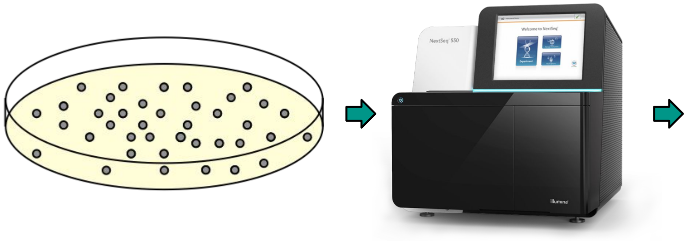
```

From Bacterial Samples to Genetic Content



```
> chromosome
TAACCGGTAAAACACTTTGCTCGTGCACATAGG
CGTGACACCTAAGACTGGAACAAGCTCAGAAG
AGTAGTAGGCGAGCATTTTTTGACCGAGTCCG
CTCCTTTCTAACTCACTGACTTCTCGCGGGCCG
TATCCTGCACGCTCAACAGCCAGCGGTGTCCC
GTTACCCTTCAAGCTCATCTTCCTCGAGGTCTG
TTGTAGTACCACACGCCTCTCCCGGCATTAGC
CCGCACTCCTCGACGGGACATTATGTGCCTTC
AGTTCCCGATCTCGGTCGCGCCCAGCCGGAAT
CCCTTAGACACCAGGGCCGCGTGAGCGAGAA
GCGGGGGGAGAACTTTATAGGGCTGTGGCTCA
TACAATAGGGTAAGGTTTCACCACATTTCTTCA
CTTCAGAAGCGACGCCTCCATTTTGCCCTCACC
CACGGTATAAGACGAAAGCCTAAGGCAACCCC
GGGGTTAGACGCGGTCCTTCTCTCTACT
```

GENOME ANNOTATION

Genetic Potential

CGPS

What is Genome Annotation?

```
> chromosome
TAACCGGTAAAACACTTTGCTCGTGCACATAGG
CGTGACACCTAAGACTGGAACAAGCTCAGAAG
AGTAGTAGGCGAGCATTTTTTGACCGAGTCCG
CTCCTTTCTAACTCACTGACTTCTCGCGGGCCG
TATCCTGCACGCTCAACAGCCAGCGGTGTCCC
GTTACCCTTCAAGCTCATCTTCCTCGAGGTCTG
TTGTAGTACCACACGCCTCTCCCGGCATTAGC
CCGCACTCCTCGACGGGACATTATGTGCCTTC
AGTTCCCGATCTCGGTCGCGCCCAGCCGGAAT
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GCGGGGGGAGAACTTTATAGGGCTGTGGCTCA
TACAATAGGGTAAGGTTTCACCACATTTCTTCA
CTTCAGAAGCGACGCCTCCATTTTGCCCTCACC
CACGGTATAAGACGAAAGCCTAAGGCAACCCC
GGGGTTAGACGCGGTCCTTCTCTCTACT
```

Genetic Potential:

- Gene prediction
- Regulatory genes
- Structural motifs
- Mobile Genetic Elements

All we have are As, Ts, Gs, and Cs mixed up

```
> chromosome
TAACCGGTAAAACACTTTGCTCGTGCACATAGGCGTGACACCTAAGACTGGAACAAGCTCAGAAGAGTAGTAGGCGAGCATTTT
TTGACCGAGTCCGCTCCTTTCTAACTCACTGACTTCTCGCGGGCCGTATCCTGCACGCTCAACAGCCAGCGGTGTCCCGTTACC
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TCTGTTGTAGTACCACACGCCTCTCCCGGCATTAGCCCGCACTCCTCGACGGGACATTATGTGCCTTCAGTTCCCGATCTCGGT
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TATCCTGCACGCTCAACAGCCAGCGGTGTCCCGTTACCCTTCAAGCTCATCTTCCTCGAGGTCTGTTGTAGTACCACACGCCTC
TCCCGGCATTAGCCCGCACTCCTCGACGGGACATTATGTGCCTTCAGTTCCCGATCTCGGTCGCGCCCAGCCG
```

All we have are As, Ts, Gs, and Cs mixed up

```
> chromosome
TAACCGGTAAAACACTTTGCTCGTGCACATAGGCGTGACACCTAAGACTGGAACAAGCTCAGAAGAGTAGTAGGCGAGCATTTT
TTGACCGAGTCCGCTCCTTTCTAACTCACTGACTTCTCGCGGGCCGTATCCTGCACGCTCAACAGCCAGCGGTGTCCCGTTACC
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TGCCTTCAGTTCCCGATCTCGGTCGCGCCCAGCCGGAATCCCTTAGACACCAGGGCCGCGTGAGCGAGAAGCGGGGGGAGAA
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CCACGGTATAAGACGAAAGCCTAAGGCAACCCCGGGGTTAGACGCGGTCCTTCTCTCTACTTAACCGGTAAAACACTTTGCTCG
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TCTGTTGTAGTACCACACGCCTCTCCCGGCATTAGCCCGCACTCCTCGACGGGACATTATGTGCCTTCAGTTCCCGATCTCGGT
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AGGCAACCCCGGGGTTAGACGCGGTCCTTCTCTCTACTTAACCGGTAAAACACTTTGCTCGTGCACATAGGCGTGACACCTAAG
ACTGGAACAAGCTCAGAAGAGTAGTAGGCGAGCATTTTTTGACCGAGTCCGCTCCTTTCTAACTCACTGACTTCTCGCGGGCCG
TATCCTGCACGCTCAACAGCCAGCGGTGTCCCGTTACCCTTCAAGCTCATCTTCCTCGAGGTCTGTTGTAGTACCACACGCCTC
TCCCGGCATTAGCCCGCACTCCTCGACGGGACATTATGTGCCTTCAGTTCCCGATCTCGGTCGCGCCCAGCCG
```

How do we annotate a genome?

The Annotation Process

- Step 1: Input and Quality Check
- Step 2: Gene Prediction
- Step 3: Functional Prediction
- Step 4: Structural RNA Prediction
- Step 5: Validation and Refinement

The Annotation Process

Step 1: Input and Quality Check

➔ Assembled Genome in FASTA format

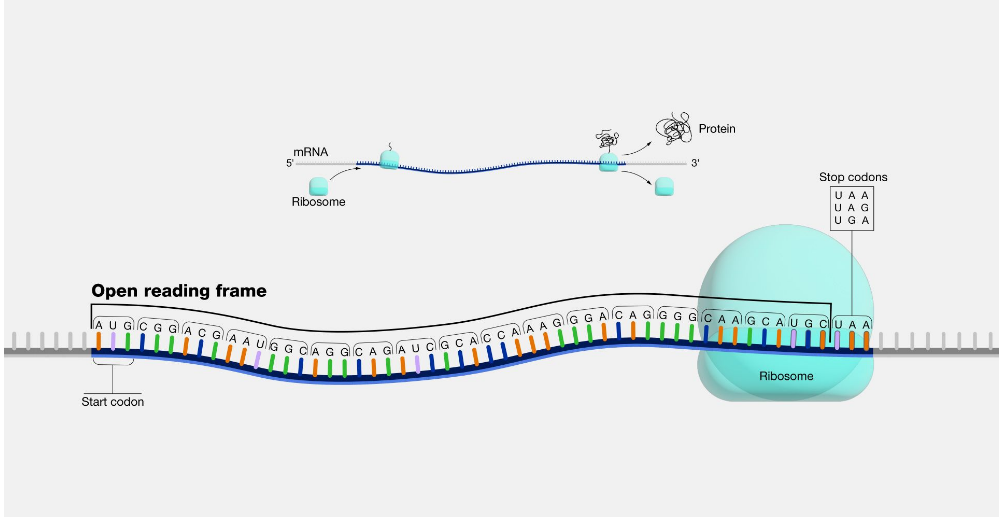
➔ The more contiguous the better

➔ Long read assemblies may resolve certain annotations better

The Annotation Process

Step 2: Gene Prediction

Open reading frames (ORF)


mRNA
5'
3'
Ribosome
Protein
Stop codons
U A A
U A G
U G A
Open reading frame
A U G C G G A C G A A U G G C A G G C A G A U C G C A C C A A A G G G A C A G G G G C A A G C A U G C U A A
Start codon
Ribosome

https://www.genome.gov/genetics-glossary/Open-Reading-Frame

The Annotation Process

Step 2: Gene Prediction

Ab-initio prediction

```
> chromosome
TAACCGATGTAACACTTTGCTCGTGCACATAGGCGTGACACCTAAGACTGGAACAAGCTCAGAAGAGTAGTAGGCGAGCATTTTTTGA

CCGAGTCCGCTCCTTTCTAACTCACTGACTTCTCGCGGGCCATGTCCTGCACGCTCAACAGCCAGCGGTGTCCCGTTACCCTTCAAG

CTCATCTTCCTCGAGGTCTGTTGTAGTACCACACGCCTCTCCCGGCATATGCCCGCACTCCTCGACGGGACATTATGTGCCTTCAGTT

CCCGATCTCGGTCGCGCCCAGCCGGAATCCCTATGGCACCAGGGCCGCGTGAGCGAGAAGCGGGGGAGAACTTTATAGGGCTGT

GGCTCATACAATAGGGTAAGGTTTCACCACATTTCTTCACTTCAGAAGCGACGCCTCCATTTTGCCCTCACCCACGGTATAAGACGAA

AGCCTAAGGCAACCCCGGGGTATGGCGCGGTCCTTCTCTCTACTTAACCGGTAAAACACTTTGCTCGTGCACATAGGCGTGACACCT
```

The Annotation Process

Start Codon	**ATG**

Step 2: Gene Prediction

Ab-initio prediction of open reading frames (ORF)

> chromosome

TAACCG**ATG**TAACACTTTGCTCGTGCACATAGGCGTGACACCTAAGACTGGAACAAGCTCAGAAGAGTAGTAGGCGAGCATTTTTTGA

CCGAGTCCGCTCCTTTCTAACTCACTGACTTCTCGCGGGCC**ATG**TCCTGCACGCTCAACAGCCAGCGGTGTCCCGTTACCCTTCAAG

CTCATCTTCCTCGAGGTCTGTTGTAGTACCACACGCCTCTCCCGGCAT**ATG**CCCGCACTCCTCGACGGGACATT**ATG**TGCCTTCAGTT

CCCGATCTCGGTCGCGCCCAGCCGGAATCCCT**ATG**GCACCAGGGCCGCGTGAGCGAGAAGCGGGGGGAGAACTTTATAGGGCTGT

GGCTCATACAATAGGGTAAGGTTTCACCACATTTCTTCACTTCAGAAGCGACGCCTCCATTTTGCCCTCACCCACGGTATAAGACGAA

AGCCTAAGGCAACCCCGGGGTATGGCGCGGTCCTTCTCTCTACTTAACCGGTAAAACACTTTGCTCGTGCACATAGGCGTGACACCT

The Annotation Process

Step 2: Gene Prediction

E.g. Ab-initio prediction of open reading frames (ORF)

Start Codon	**ATG**
Stop Codon	**TAG** **TGA** **TAA**

> chromosome

TAACCG**ATG**TAACACTTTGCTCGTGCACA**TAG**GCG**TGA**CACC**TAA**GACTGGAACAAGCTCAGAAGAG**TAGTAG**GCGAGCATTTTT**TGA**

CCGAGTCCGCTCCTTTC**TAA**CTCAC**TGA**CTTCTCGCGGGCC**ATG**TCCTGCACGCTCAACAGCCAGCGGTGTCCCGTTACCCTTCAAG

CTCATCTTCCTCGAGGTCTGTTG**TAG**TACCACACGCCTCTCCCGGCAT**ATG**CCCGCACTCCTCGACGGGACATT**ATG**TGCCTTCAGTT

CCCGATCTCGGTCGCGCCCAGCCGGAATCCCT**ATG**GCACCAGGGCCGCG**TGA**GCGAGAAGCGGGGGGAGAACTTTA**TAG**GGCTGT

GGCTCATACAA**TAG**GG**TAA**GGTTTCACCACATTTCTTCACTTCAGAAGCGACGCCTCCATTTTGCCCTCACCCACGGTA**TAA**GACGAA

AGCC**TAA**GGCAACCCCGGGGTATGGCGCGGTCCTTCTCTCTACT**TAA**CCGG**TAA**AACACTTTGCTCGTGCACA**TAG**GCG**TGA**CACCT

The Annotation Process

Start Codon	**ATG**
Stop Codon	**TAG** **TGA** **TAA**

Step 2: Gene Prediction

Ab-initio prediction of open reading frames (ORF)

> chromosome

TAACCG**ATG**TAACACTTTGCTCGTGCACA**TAG**GCG**TGA**CACC**TAA**GACTGGAACAAGCTCAGAAGAG**TAGTAG**GCGAGCATTTTT**TG**
A

ORF1

CCGAGTCCGCTCCTTTC**TAA**CTCAC**TGA**CTTCTCGCGGGCC**ATG**TCCTGCACGCTCAACAGCCAGCGGTGTCCCGTTACCCTTCAAG

ORF2

CTCATCTTCCTCGAGGTCTGTTG**TAG**TACCACACGCCTCTCCCGGCAT**ATG**CCCGCACTCCTCGACGGGACATT**ATG**TGCCTTCAGTT

CCCGATCTCGGTCGCGCCCAGCCGGAATCCCT**ATG**GCACCAGGGCCGCG**TGA**GCGAGAAGCGGGGGAGAACTTTA**TAG**GGCTGT

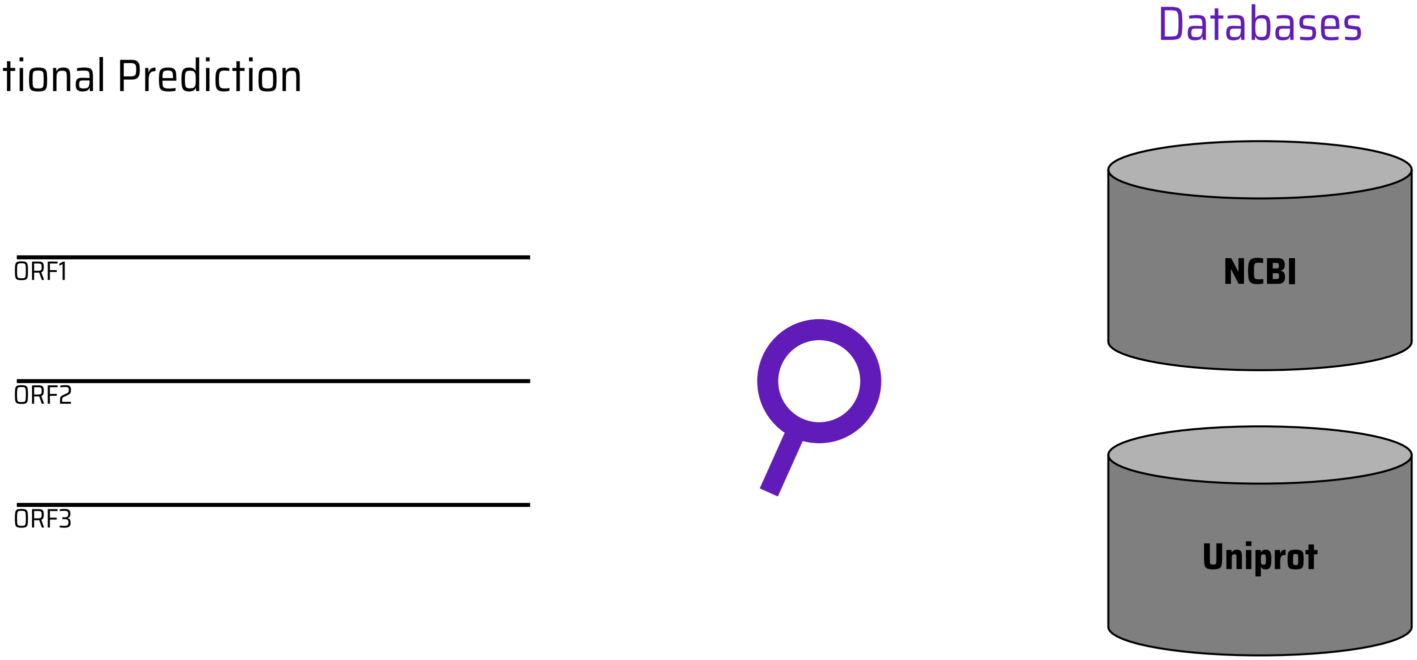
ORF3

GGCTCATACAA**TAG**GG**TAA**GGTTTCACCACATTTCTTCACTTCAGAAGCGACGCCTCCATTTTGCCCTCACCCACGGTA**TAA**GACGAA

AGCC**TAA**GGCAACCCCGGGGTATGGCGCGGTCCTTCTCTCTACT**TAA**CCGG**TAA**AACACTTTGCTCGTGCACA**TAG**GCG**TGA**CACCT

The Annotation Process

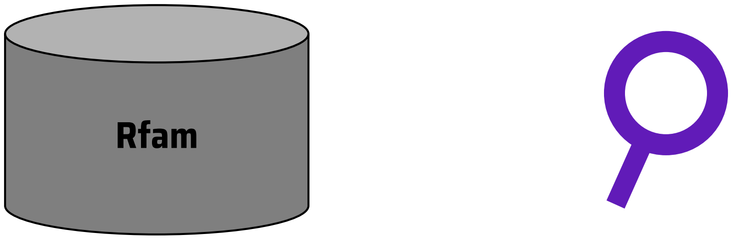
Step 3: Functional Prediction



The Annotation Process

Step 4: Structural RNA Prediction
(tRNAs, rRNAs, other non-coding RNA)

Databases



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> chromosome
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CUCAUCUUCCUCGAGGUCUGUUGUAGUACCACACGCCUCUCCCGGCAUAUGCCCGCACUACAAGC

CCCGAUCUCGGUCGCGCCCAGCCGGAAUCCCUAUGGCACCAGGGCCGCGUGAGCGAGAACAAGC

GGCUCAUACAAUAGGGUAAGGUUUCACCACAUUUCUUCACUUCAGAAGCGACGCCUCCAACAAGC

AGCCUAAGGCAACCCCGGGGUAUGGCGCGGUCCUUCUCUCUACUUAACCGGUAAAACACACAAGC
```

The Annotation Process

Step 4: Structural RNA Prediction
(tRNAs, rRNAs, other non-coding RNA)

> chromosome

UAACCGAUGUAACACUUUGCUCGUGCACAUAGGCGUGACACCUAAGACUGGAACAAGCUCAGAAGAGUAGUAGGCGAGCAUUUUUUGA

ORF1

CCGAGUCCGCUCCUUUCUAACUCACUGACUUCUCGCGGGCCAUGUCCUGCACGCUCAACAGCCAGCGGUGUCCCGUUACCCUUCAAG

ORF2

CUCAUCUUCCUCGAGGUCUGUUGUAGUACCACACGCCUCUCCCGGCAUAUGCCCGCACUCCUCGACGGGACAUUAUGUGCCUUCAGUU

CCCGAUCUCGGUCGCGCCCAGCCGGAAUCCCUAUGGCACCAGGGCCGCGUGAGCGAGAAGCGGGGGGAGAACUUUAUAGGGCUGU

ORF3

GGCUCAUACAAUAGGGUAAGGUUUCACCACAUUUCUUCACUUCAGAAGCGACGCCUCCAUUUUGCCCUCACCCACGGUAUAAGACGAA

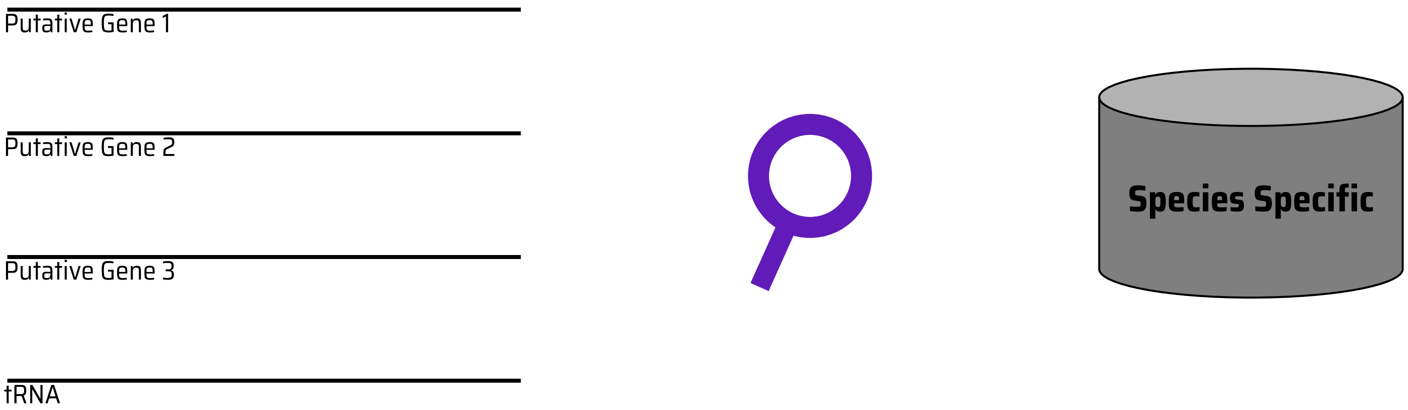
AGCCUAAGGCAACCCCGGGGUAUGGCGCGGUCCUUCUCUCUACUUAACCGGUAAAACACUUUGCUCGUGCACAUAGGCGUGACACCU

tRNA

The Annotation Process

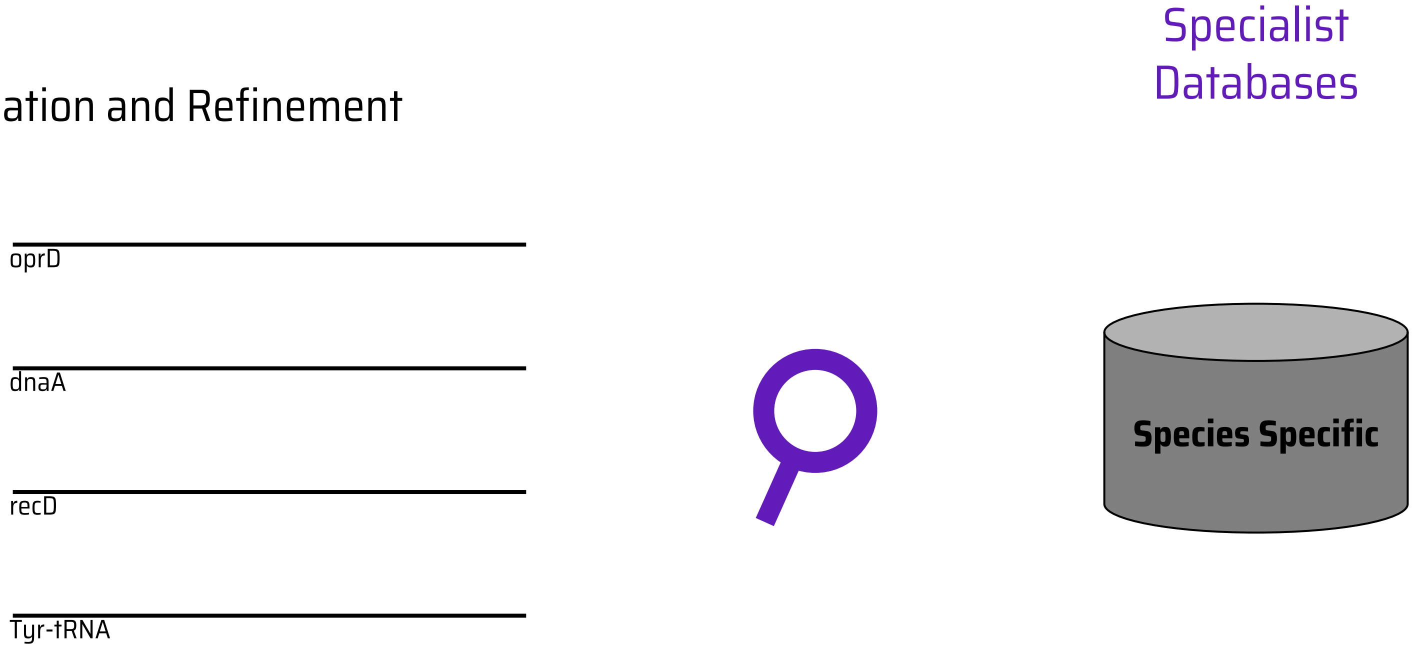
Step 5: Validation and Refinement

Specialist Databases

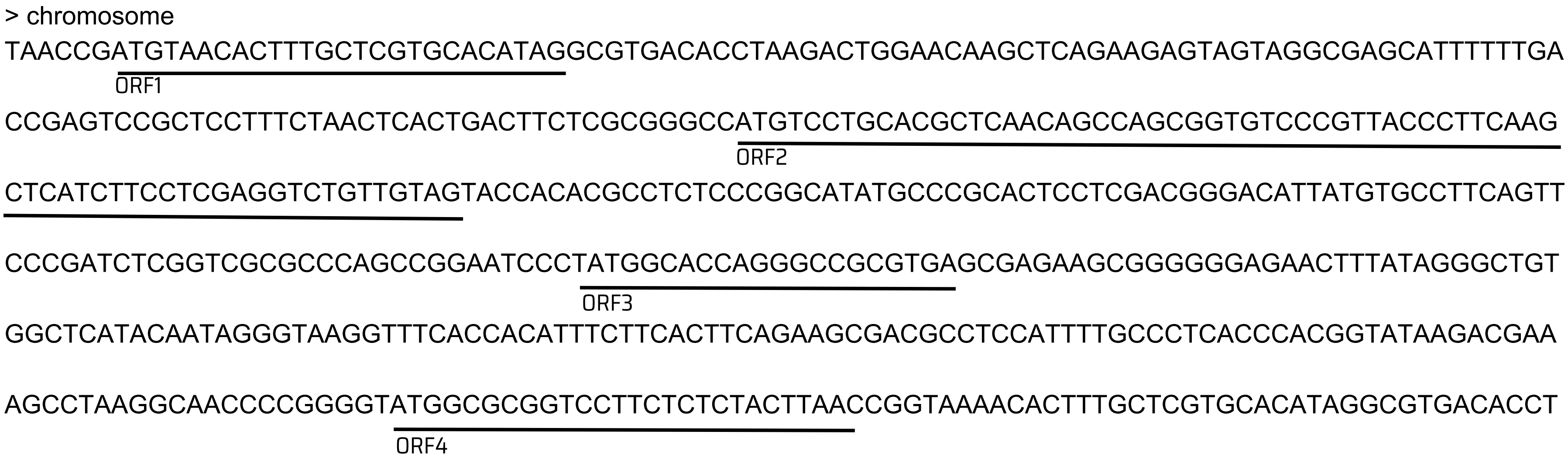


The Annotation Process

Step 5: Validation and Refinement



Annotated Genome



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> chromosome
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CTCATCTTCCTCGAGGTCTGTTGTAGTACCACACGCCTCTCCCGGCATATGCCCGCACTCCTCGACGGGACATTATGTGCCTTCAGTT
CCCGATCTCGGTCGCGCCCAGCCGGAATCCCTATGGCACCAGGGCCGCGTGAGCGAGAAGCGGGGGGAGAACTTTATAGGGCTGT
GGCTCATACAATAGGGTAAGGTTTCACCACATTTCTTCACTTCAGAAGCGACGCCTCCATTTTGCCCTCACCCACGGTATAAGACGAA
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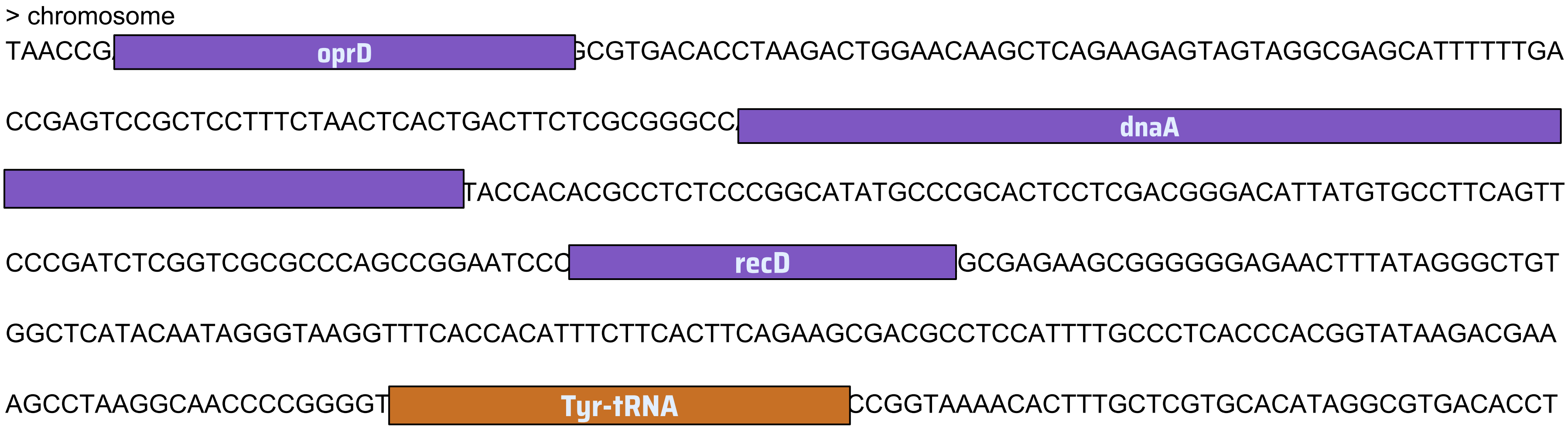
Annotated Genome



> chromosome
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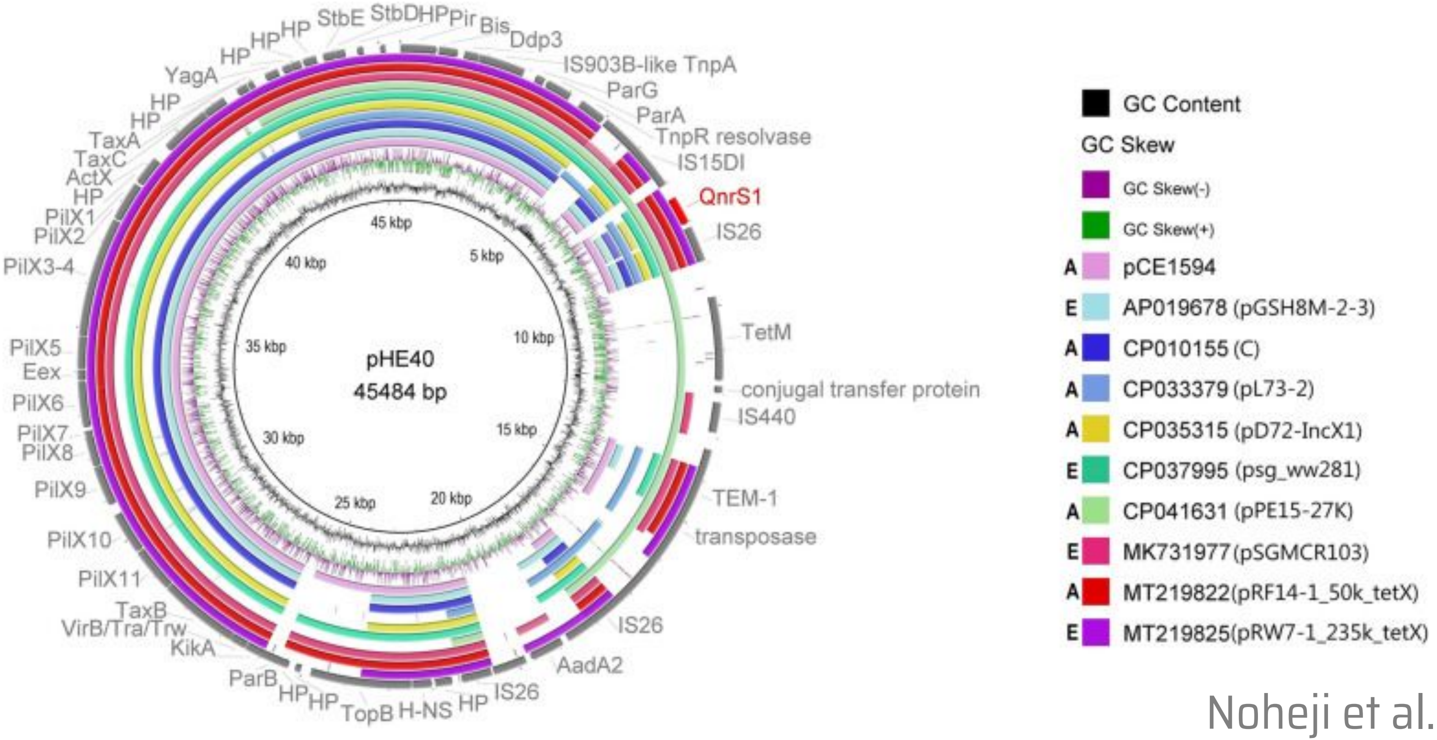
CCGAGTCCGCTCCTTTCTAACTCACTGACTTCTCGCGGGCCA
TACCACACGCCTCTCCCGGCATATGCCCGCACTCCTCGACGGGACATTATGTGCCTTCAGTT
CCCGATCTCGGTCGCGCCCAGCCGGAATCCC GCGAGAAGCGGGGGGAGAACTTTATAGGGCTGT
GGCTCATACAATAGGGTAAGGTTTCACCACATTTCTTCACTTCAGAAGCGACGCCTCCATTTTGCCCTCACCCACGGTATAAGACGAA
AGCCTAAGGCAACCCCGGGGT CCGGTAAAACACTTTGCTCGTGCACATAGGCGTGACACCT

What it may look like?



Noheji et al. 2022

Format of Annotation output

GFF3

```
                            start   end       strand         attributes
NC_002945.4 feature     gene  1524  2345  .     -     .     ID=gene:BQ2027_MB0001;Name=dnaA;biotype=protein_coding;
  seqid      source     type            score       phase
```

Format of Annotation output

GFF3

```
     CDS             complement(1524..2345)
                     /codon_start=1
                     /gene="BQ2027_MB0001"
                     /product="chromosomal replication initiator protein dnaA"
                     /label=dnaA
                     /note="activates initiation of DNA replication in bacteria."
                     /translation="MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGYI
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                     LPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGAS
                     LIKHW"
```

Format of Annotation output

GBK

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                       /note="activates initiation of DNA replication in bacteria."
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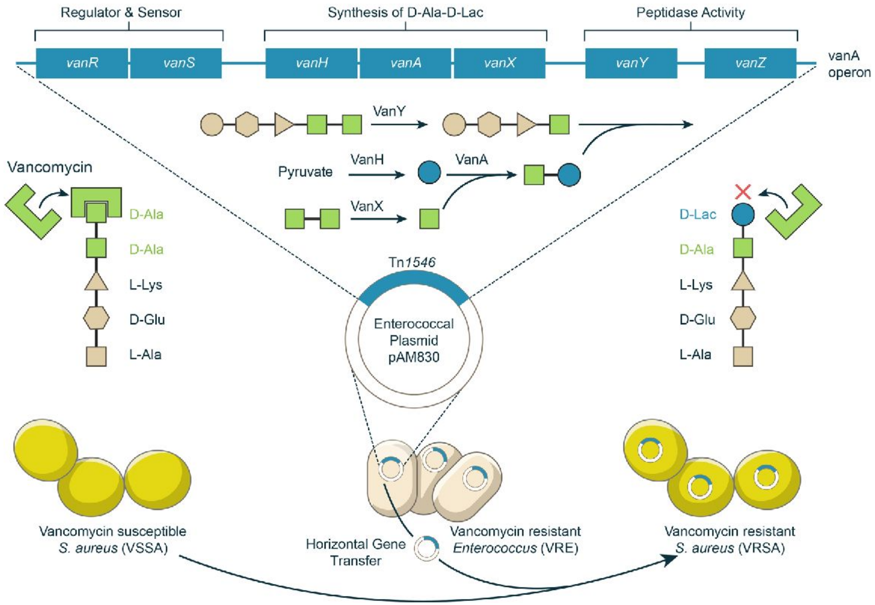
                       LIKHW"
```

Challenges and Innovations

- Complexity of Microbial Genomes
  - Hard to assemble genomes
  - Repeat Regions
- Accuracy of Prediction Algorithms
  - Outdates / Incomplete databases
  - Atypical gene structures
  - Homology based errors
- New methods
  - Data Integration
  - Regular database updates
  - Unsupervised Machine Learning Models

Annotation Exercise

- Annotate three E. faecium genomes with Prokka and Proksee
- Explore annotation results
- Compare AMR potential



Thank you!

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