Module 1
Bioinformatics Basics

Taking a look behind the curtain

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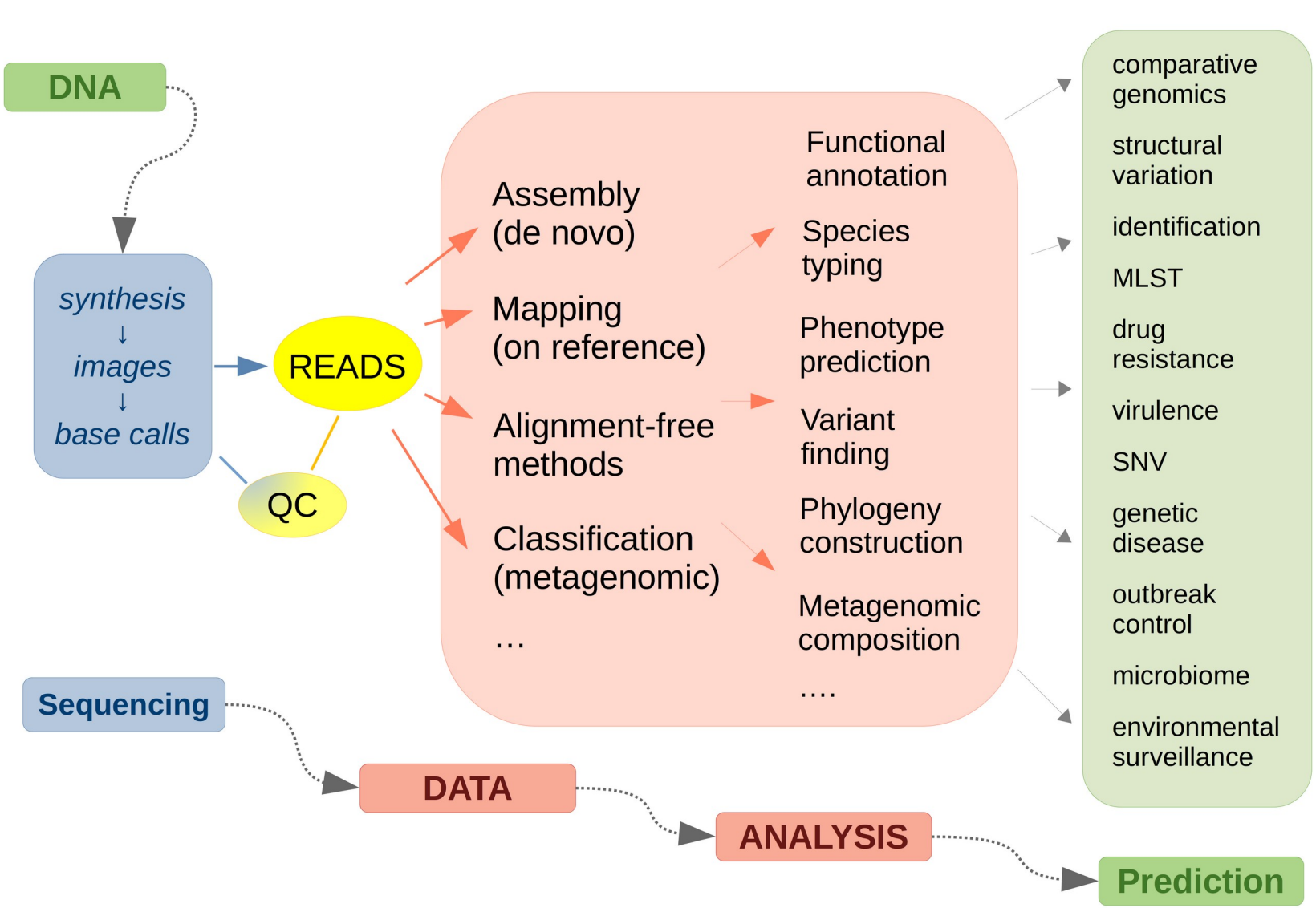
Topics

- What do all these technical terms mean?
  - What are reads, assembly, FASTQ, FASTA?
  - More terminology: alignment, quality scores, coverage, depth
- How is sequencer output turned into a genome?
- How does species identification and typing work?
- How do we find AMR genes and mutations?
- What is happening “behind the curtain” in the tools we use?



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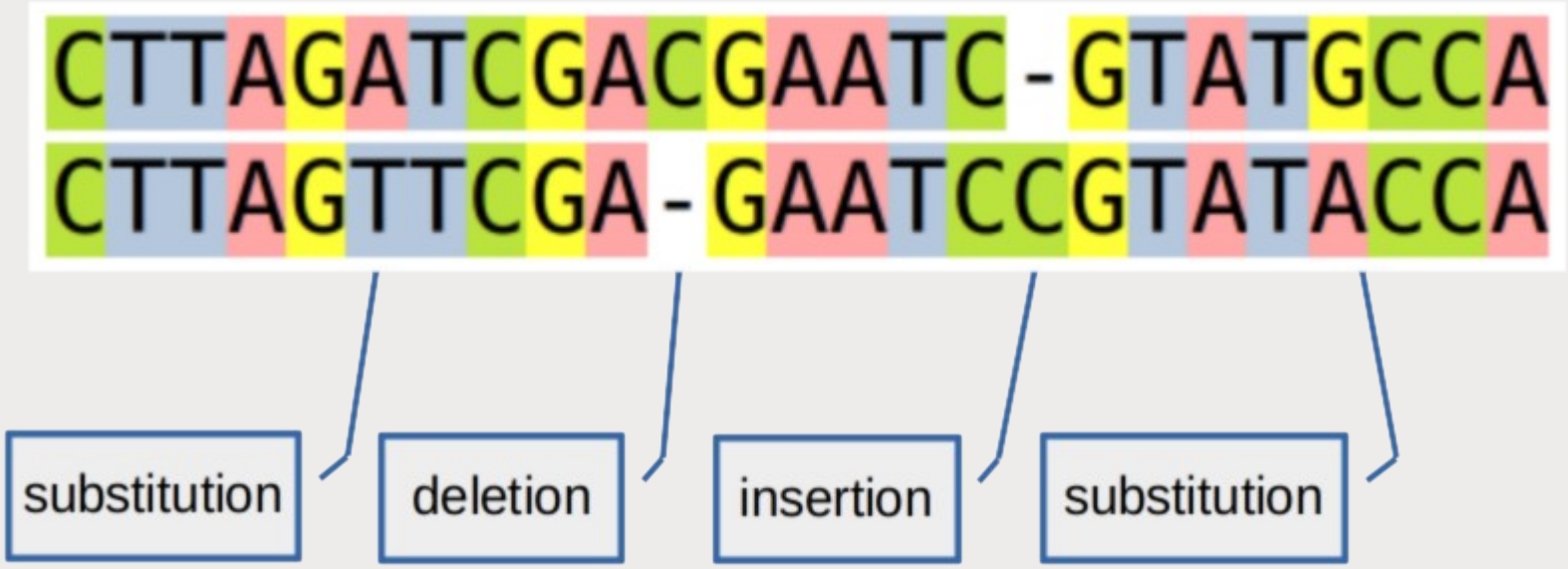
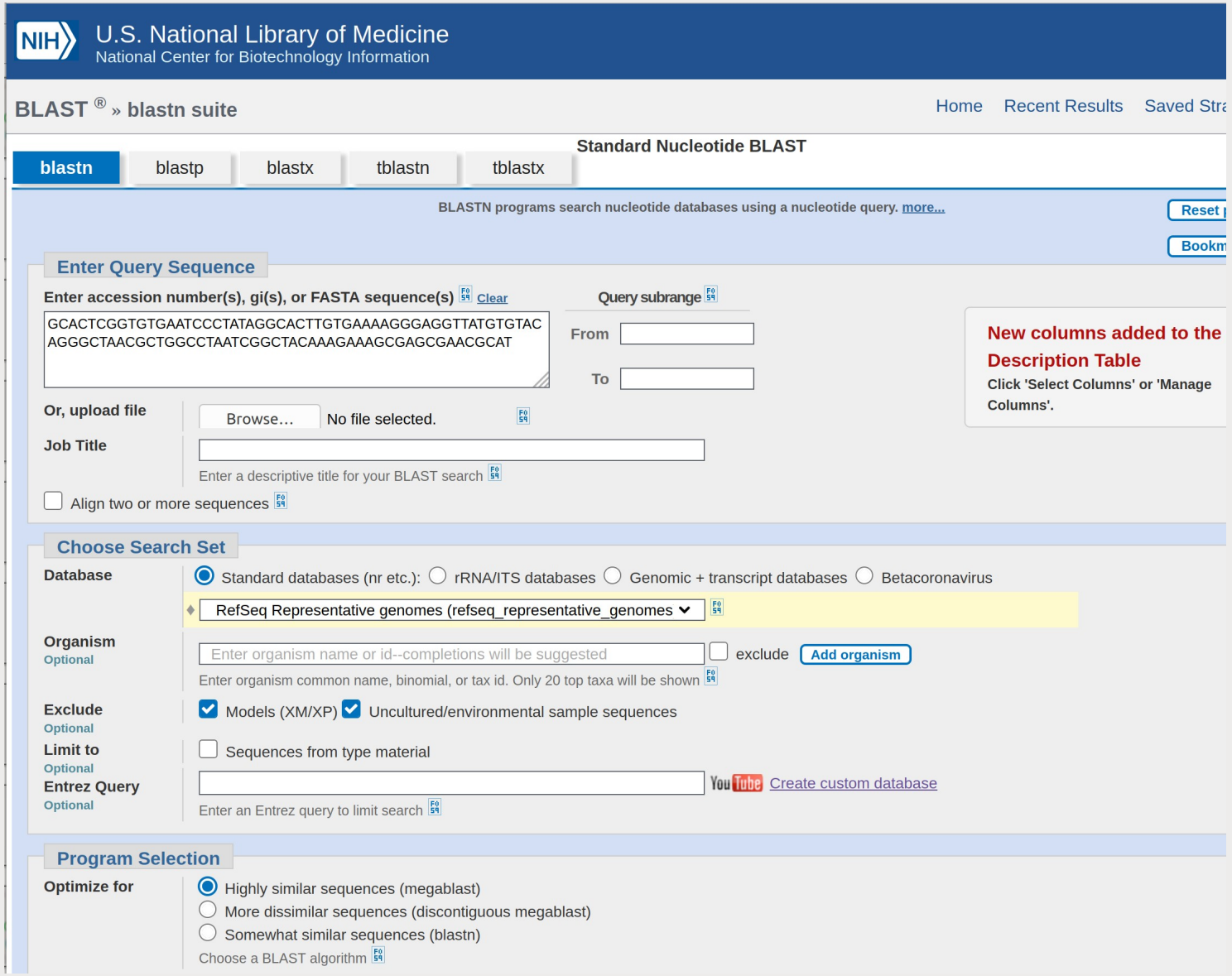
Bioinformatician Bird's Eye View

- Everything starts with **reads** ...
- … and ends at (just before) prediction
- Trend toward end user operable **pipelines** that perform a workflow of analyses
- These analyses are built using a still fast-growing toolset
- Rapid innovation continues – keep abreast of the "data deluge"
- But at the basis are a relatively small number of 'core operations'



Alignment

- At the heart of bioinformatics
- Alignments can be **scored** to give an alignment quality
- Dissimilarity of two sequences: **edit distance.** How many changes turn one into the other?
- Edit penalties can be **weighted**, e.g:
  - Gap vs substitution
  - Transversion vs transition
  - Observed substitution rates
  - ...



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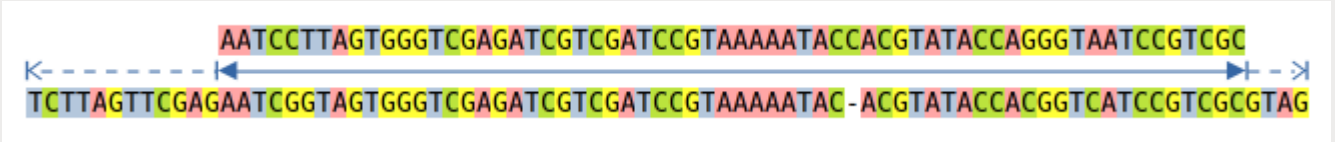
BLAST

- https://blast.ncbi.nlm.nih.gov
- Basic **Local Alignment** Search Tool
- Search for matches of a query sequence in (huge) sequence databases
  - But can be used offline too
- Matches come with metrics that express alignment quality

Metrics you will likely encounter

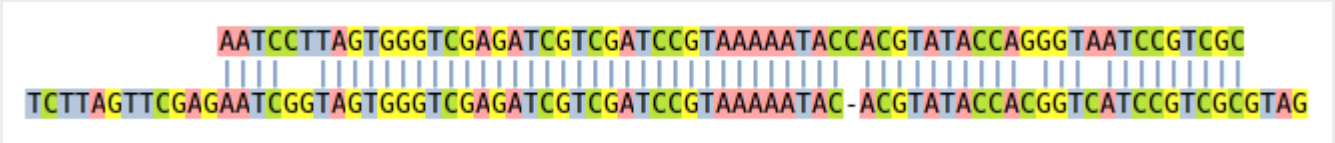
- Coverage
  - Do not confuse with coverage *depth*

Coverage: percentage of target region covered by query (here 80%)

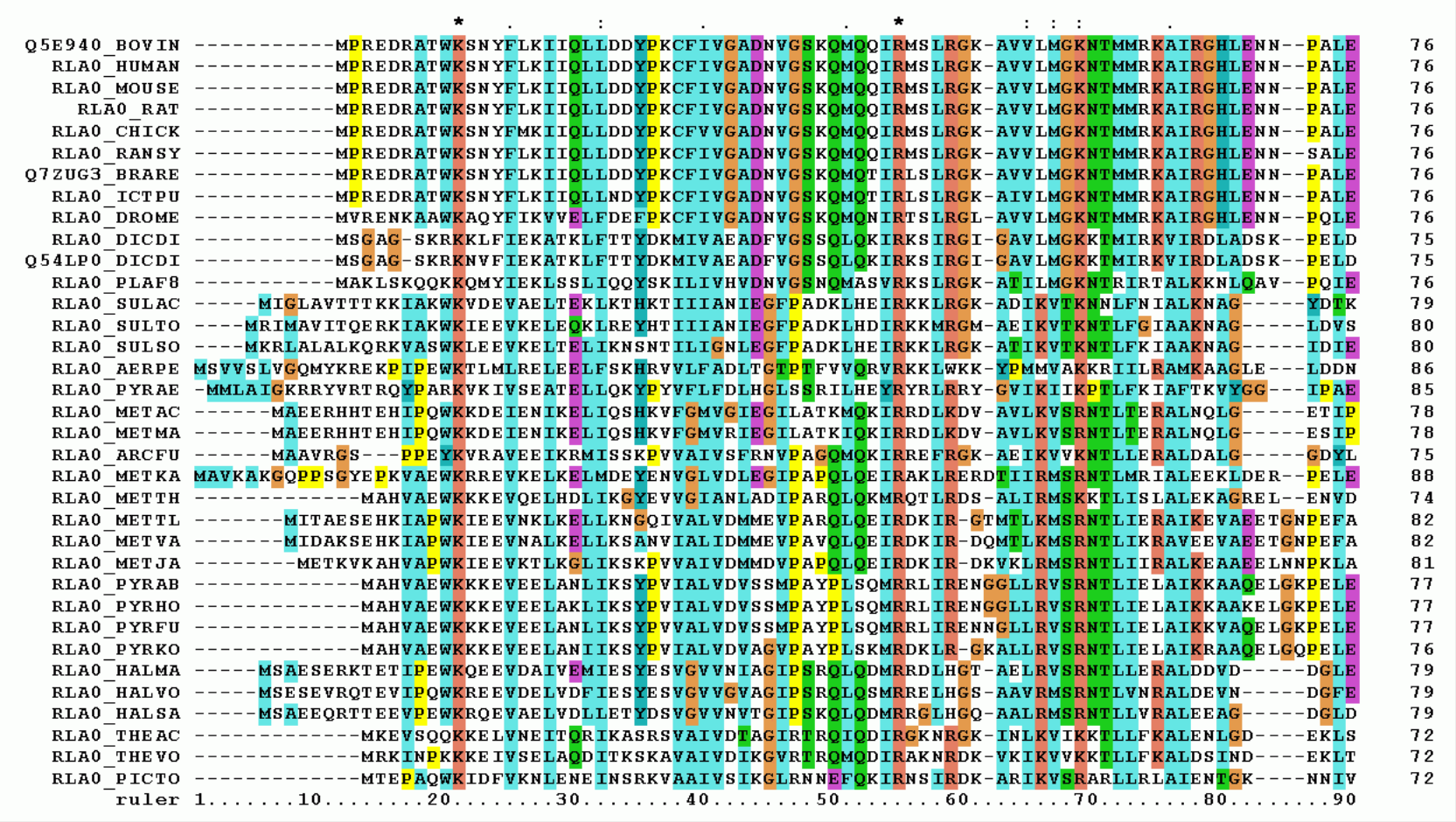


- Percentage identity

Identity: percentage of bases in the alignment that match exactly (here 92%)



- *Bit score, E-value, p-value*: related to the probability of attaining at least the alignment score by chance



https://commons.wikimedia.org/wiki/File:RPLP0_90_ClustalW_aln.gif (CC BY-SA 3.0)

Multiple Alignment

- Same concept, multiple sequences
- Here to illustrate homology of ribosomal protein P0 across species
- Basis for phylogenetic analyses:
  - Multiple-align genomes of a collection of isolates
  - Compute edit distance between every pair
  - Assume edit distance ~ evolutionary distance
  - “Evolutionary distance matrix” is basis for inferring phylogenetic tree

Reads and Assemblies

- … and FASTQ & FASTA
- *De novo* assembly
- Hybrid assembly

```
@M02836:3:000000000-AD058:1:1101:19894:6963 1:N:0:8
ACCGCAAGATGCGACACCACTTACATTAGGTCAAGAAATCTCCGGTTGGGCGGCGATGCTTGAGCATAATATCAAACAT...
+
3A1>A11>11@DA1EEGG0F1EBF12F1111BBFDEGHGDHHFG/AAAEE@?!!!###?2BBBG>FEG2>F22>E02B/...
@M02836:3:000000000-AD058:1:1101:11069:5821 1:N:0:8
CGGTGTAATGTTGAGATAGTGACTAAGAGAAACAGACGAATGAAAGAAAATTATTATTATTTGGAGCAAACTTCAGAAA...
+
9!!!>FD&&FFGGGFGFGGGFHHGCHHHGHHHGFHHGGGHGGCHHHHFHHHFHFGH>>9.9.7777$$$$$########...
@M02836:3:000000000-AD058:1:1101:18069:7032 1:N:0:8
ACATTACCGCAGCAGCCTCGACAAGTACAAATCTCCTGTCAATTGCGTATTACTGAACGTTTATAATTAGGCACACCAG...
+
&&&&&*.,3***+(%(""%%&$'$$')&)06*'/53)*%,'+(%%%%(&0)5.373:0')-/101..)*+14;9:96/-...

... and so on ...
```

One read is 4 lines

Sequence

Q-scores

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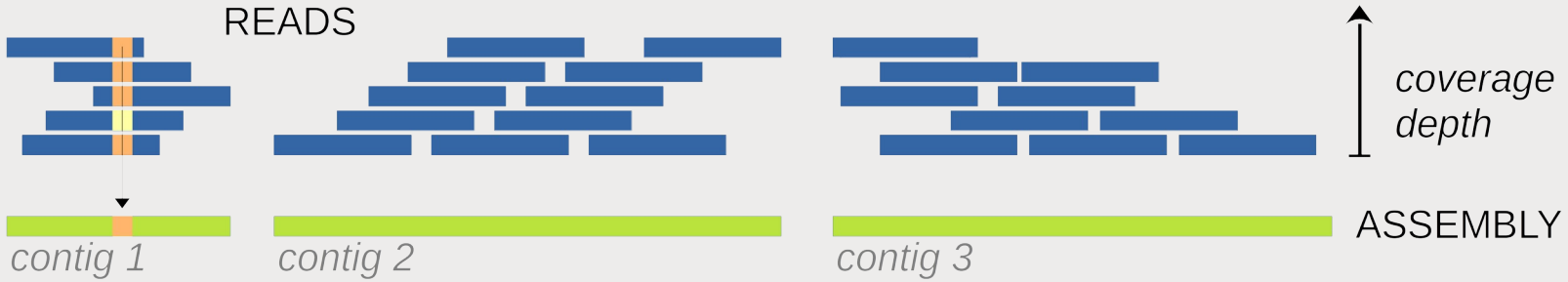
FASTQ

- Produced by the sequencer, one or two files per sample
- Contains (often millions) of **reads**: the nucleotide sequences of the fragments in your library
- … for *each* base an estimate of its **accuracy**, its Q-score:

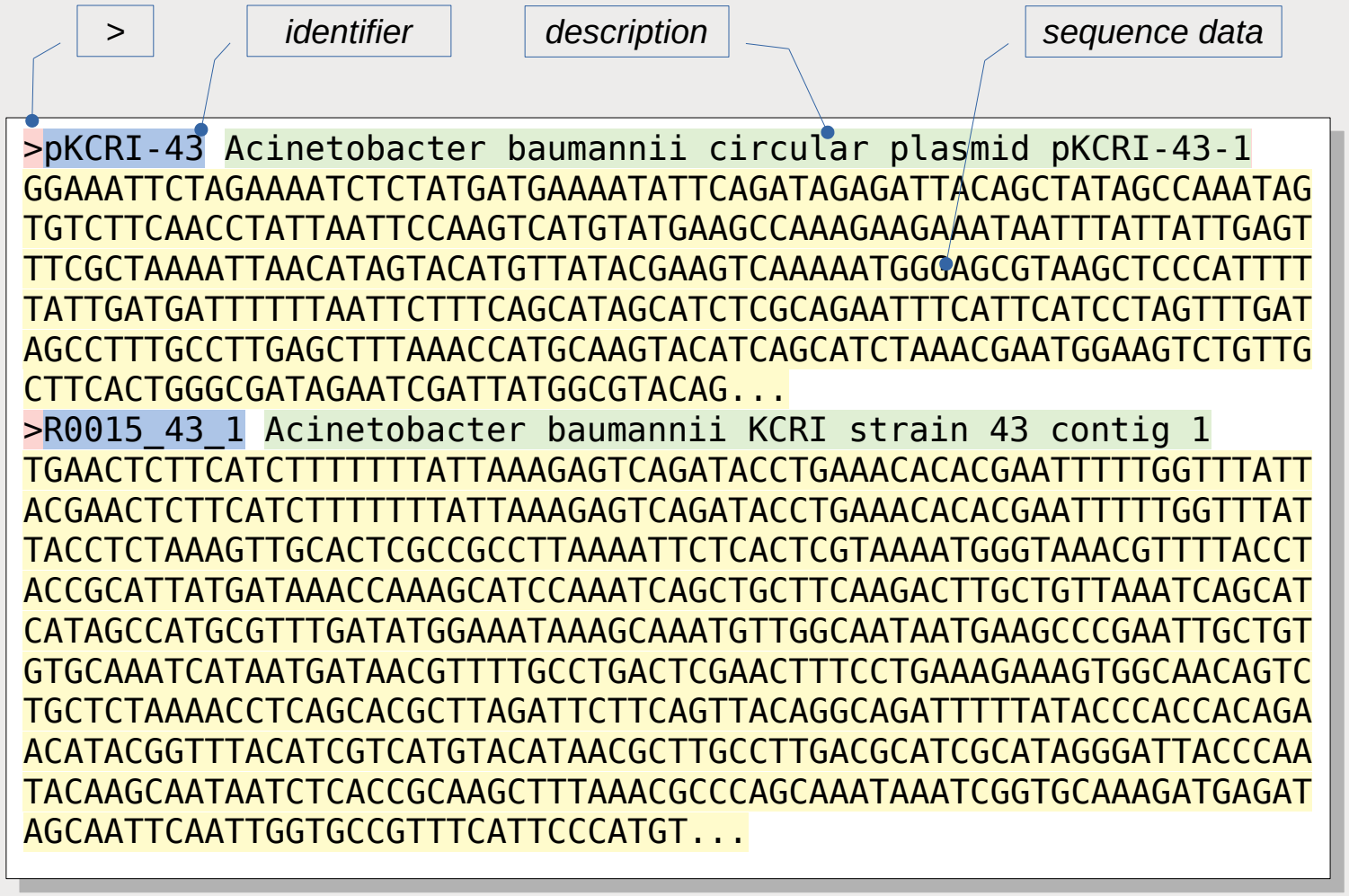
  Q30 (on the Phred scale) means 1:1000 probability of being incorrect.

- The read headers have technical metadata, relevant for QA
- File extension usually `.fastq.gz` or `.fq.gz`

Assembly *(de novo)*



- Goal: reconstruct the genome of the organism from the reads
- The “exploding newspapers” analogy (Pevzner & Compeau)
- Puzzle together increasingly longer contigs by joining ones with overlapping edges
- Result: a set of contigs (unitigs) that can’t be joined further as
  - no other contig overlaps, or
  - multiple overlap (so which to choose)?



identifier

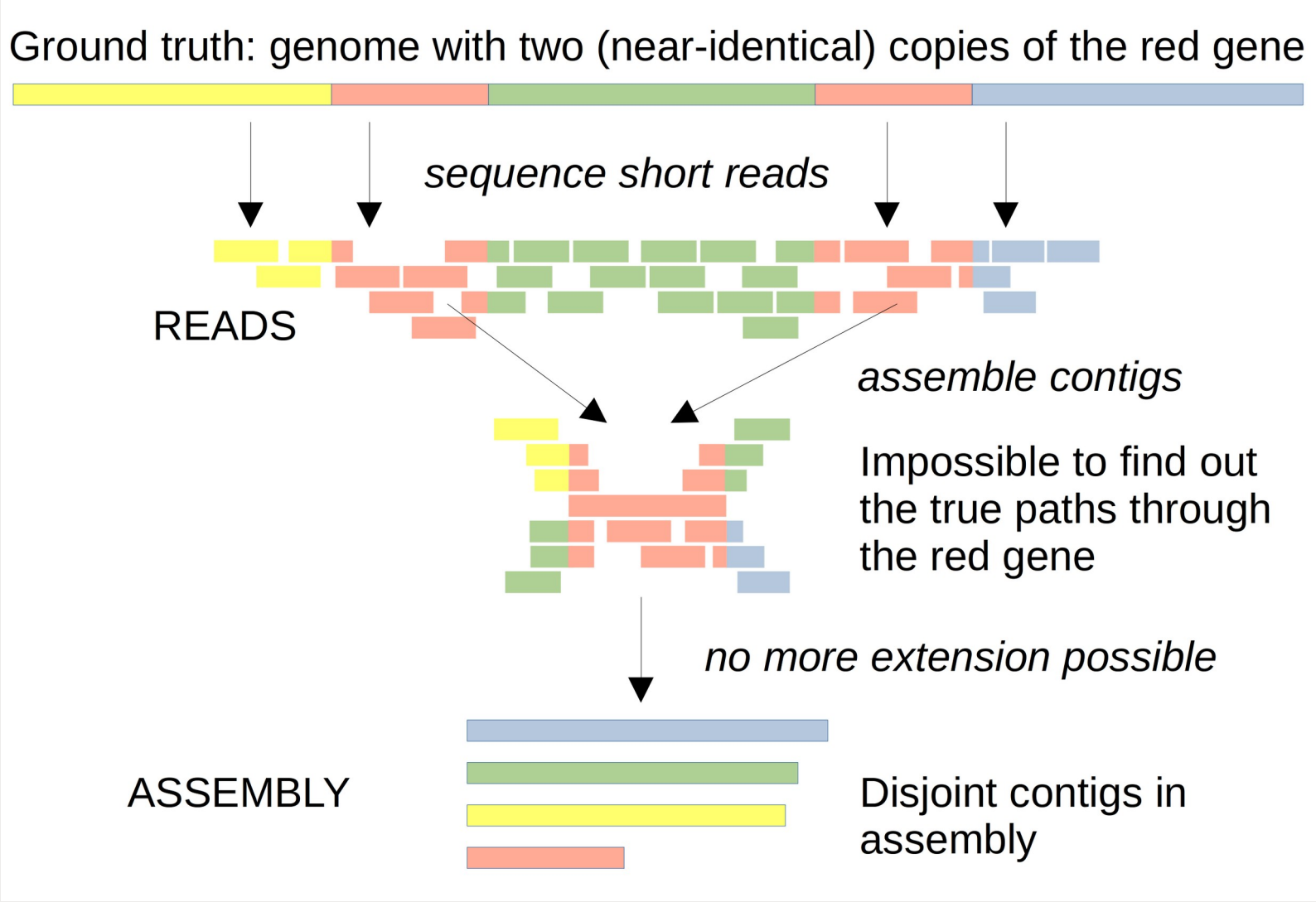
description

sequence data

```
>pKCRI-43 Acinetobacter baumannii circular plasmid pKCRI-43-1
GGAAATTCTAGAAAATCTCTATGATGAAAATATTCAGATAGAGATTACAGCTATAGCCAAATAG
TGTCTTCAACCTATTAATTCCAAGTCATGTATGAAGCCAAAGAAGAAATAATTTATTATTGAGT
TTCGCTAAAATTAACATAGTACATGTTATACGAAGTCAAAAATGGCAGCGTAAGCTCCCATTTT
TATTGATGATTTTTTAATTCTTTCAGCATAGCATCTCGCAGAATTTCATTCATCCTAGTTTGAT
AGCCTTTGCCTTGAGCTTTAAACCATGCAAGTACATCAGCATCTAAACGAATGGAAGTCTGTTG
CTTCACTGGGCGATAGAATCGATTATGGCGTACAG...
>R0015_43_1 Acinetobacter baumannii KCRI strain 43 contig 1
TGAACTCTTCATCTTTTTTTATTAAAGAGTCAGATACCTGAAACACACGAATTTTTGGTTTATT
ACGAACTCTTCATCTTTTTTTATTAAAGAGTCAGATACCTGAAACACACGAATTTTTGGTTTAT
TACCTCTAAAGTTGCACTCGCCGCCTTAAAATTCTCACTCGTAAAATGGGTAAACGTTTTACCT
ACCGCATTATGATAAACCAAAGCATCCAAATCAGCTGCTTCAAGACTTGCTGTTAAATCAGCAT
CATAGCCATGCGTTTGATATGGAAATAAAGCAAATGTTGGCAATAATGAAGCCCGAATTGCTGT
GTGCAAATCATAATGATAACGTTTTGCCTGACTCGAACTTTCCTGAAAGAAAGTGGCAACAGTC
TGCTCTAAAACCTCAGCACGCTTAGATTCTTCAGTTACAGGCAGATTTTTATACCCACCACAGA
ACATACGGTTTACATCGTCATGTACATAACGCTTGCCTTGACGCATCGCATAGGGATTACCCAA
TACAAGCAATAATCTCACCGCAAGCTTTAAACGCCCAGCAAATAAATCGGTGCAAAGATGAGAT
AGCAATTCAATTGGTGCCGTTTCATTCCCATGT...
```

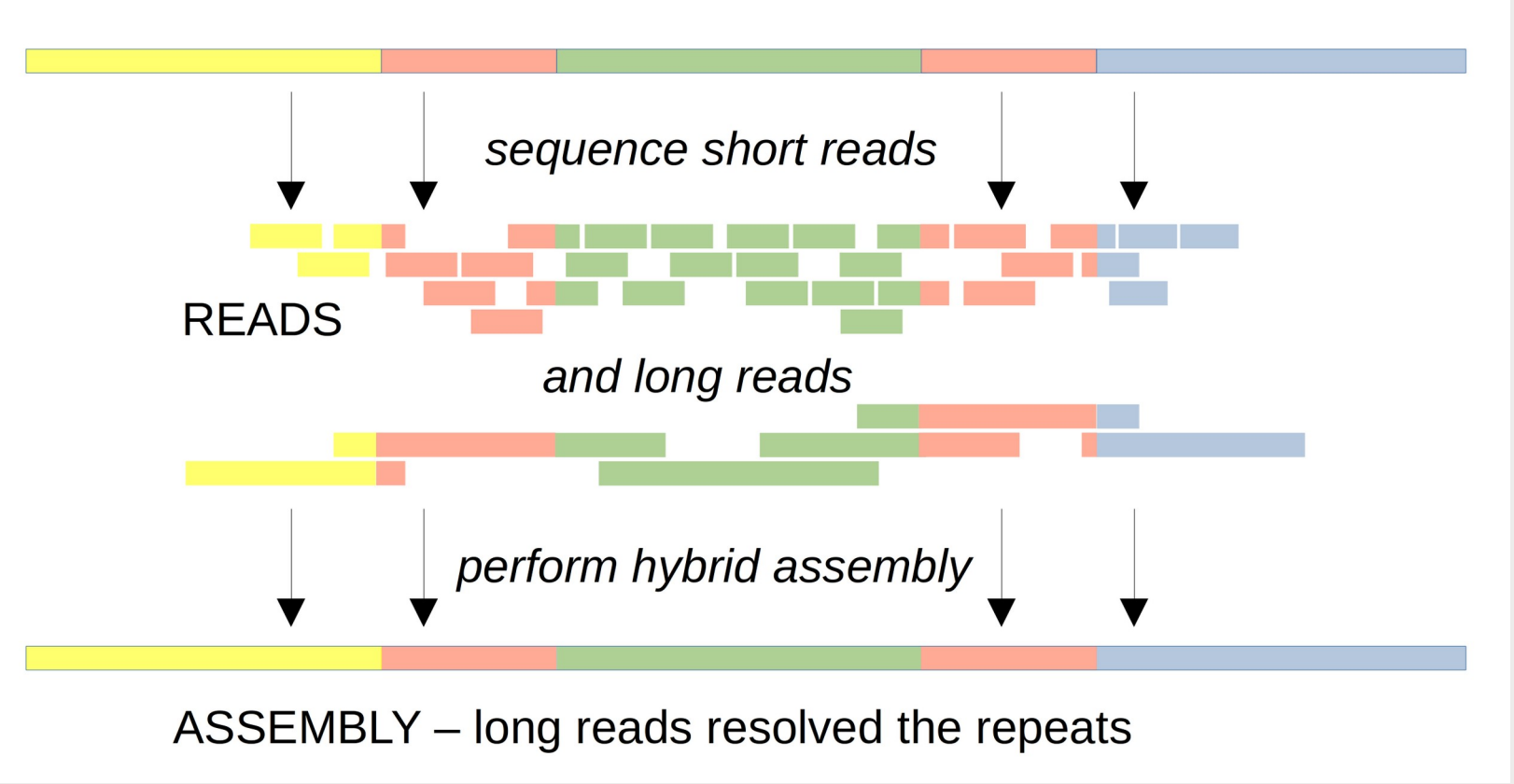
FASTA

- Contains one or more sequences
  - For instance the contigs produced by an assembler
  - But can be any nucleotide or amino acid sequence
- Each sequence preceded by an identifying header
- Common file name extensions: `.fas`, `.fasta`, `fsa`, `.fna`, `.faa`; compressed `.gz`
-



The problem with repeats

- When there are near-identical repeats of a region that is larger than the read length …
- … then the assembler cannot tell from which of the copies the reads were read
- … so it produces a single contig covering either repeat region
- … and it gets in trouble at the edges of the contig, where there are two possible continuations
- So it must split the contigs there, and we can't know their order



Hybrid assembly

- Combine short and long reads
- “Best of both worlds”:
  - Short reads provide accuracy
  - Long reads for structure

Brief Recap

- FASTQ contains reads
  - Nucleotide sequences of your library fragments
  - With a quality score for every base read
- FASTA files contain sequences
  - Typically an assembled genome (broken into contigs)
  - But can be any collection of sequences: AMR genes, alleles of some gene, protein products, etc.

You know enough to do species detection

- Assemble the genome of your isolate
- Download the *Microbial 16S rRNA* database from NCBI
- BLAST your assembled genome against this database
- Pick the highest scoring alignment
- Check that its coverage and identity percentages are good
- Voilà, your own species finder!

And you can do AMR detection too!

- Assemble the genome of your isolate
- Obtain FASTA files with the sequences of known AMR genes
  - Freely downloadable, e.g. DTU CGE (genomicepidemiology.org)
- BLAST the genes against your assembled genome
- List all high scoring alignments with sufficient coverage and identity
- Voilà, your own AMR Finder!

- But … what about point mutations?

Mapping

- Core bioinformatics procedure
- Mapping has a **target**, e.g. **reference** gene or genome
- Map all **reads** for an isolate on the target – dropping unmapped ones
- Alternative for *de novo* assembly when we know the organism: map on a **reference genome**
- Particularly appropriate when the goal is finding **SNP**s (e.g. in phylogeny)
- The basis for **variant calling** and obtaining **consensus sequence**

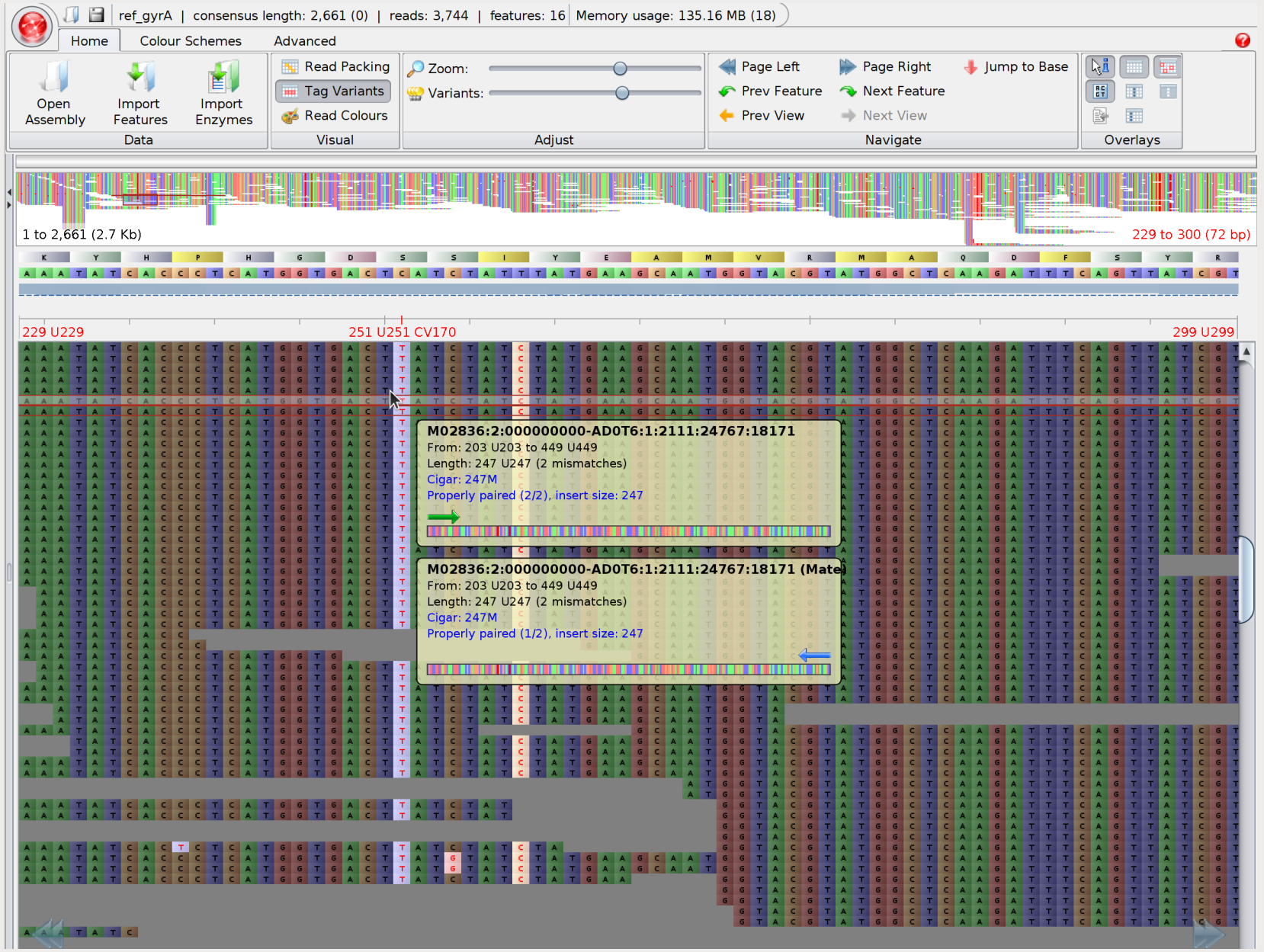


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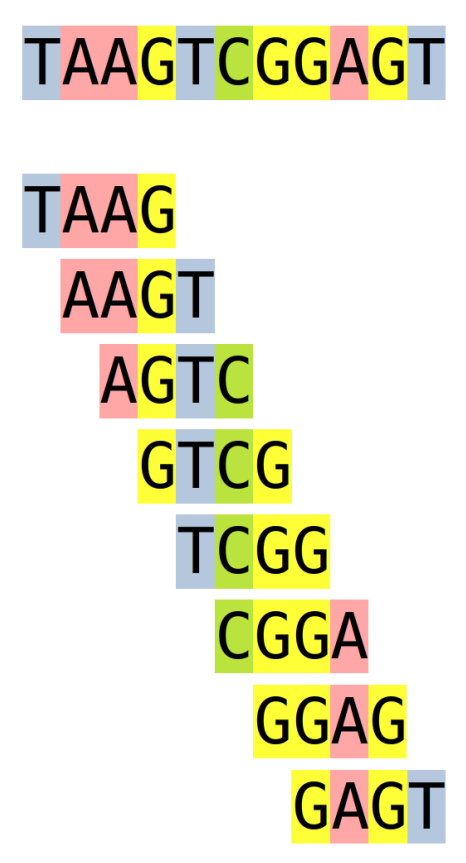
Illustration (tool: Tablet)

- Mapping of the reads of a *Staph aureus* isolate on reference *gyrA* gene
- Mutation S84L on *gyrA* is known to contribute to Quinolone resistance
- We spot C>T in nearly all 170 reads covering nt pos 251, thus call variant T with confidence
- Meaning that codon T**C**A (S) on reference is T**T**A (L) on isolate

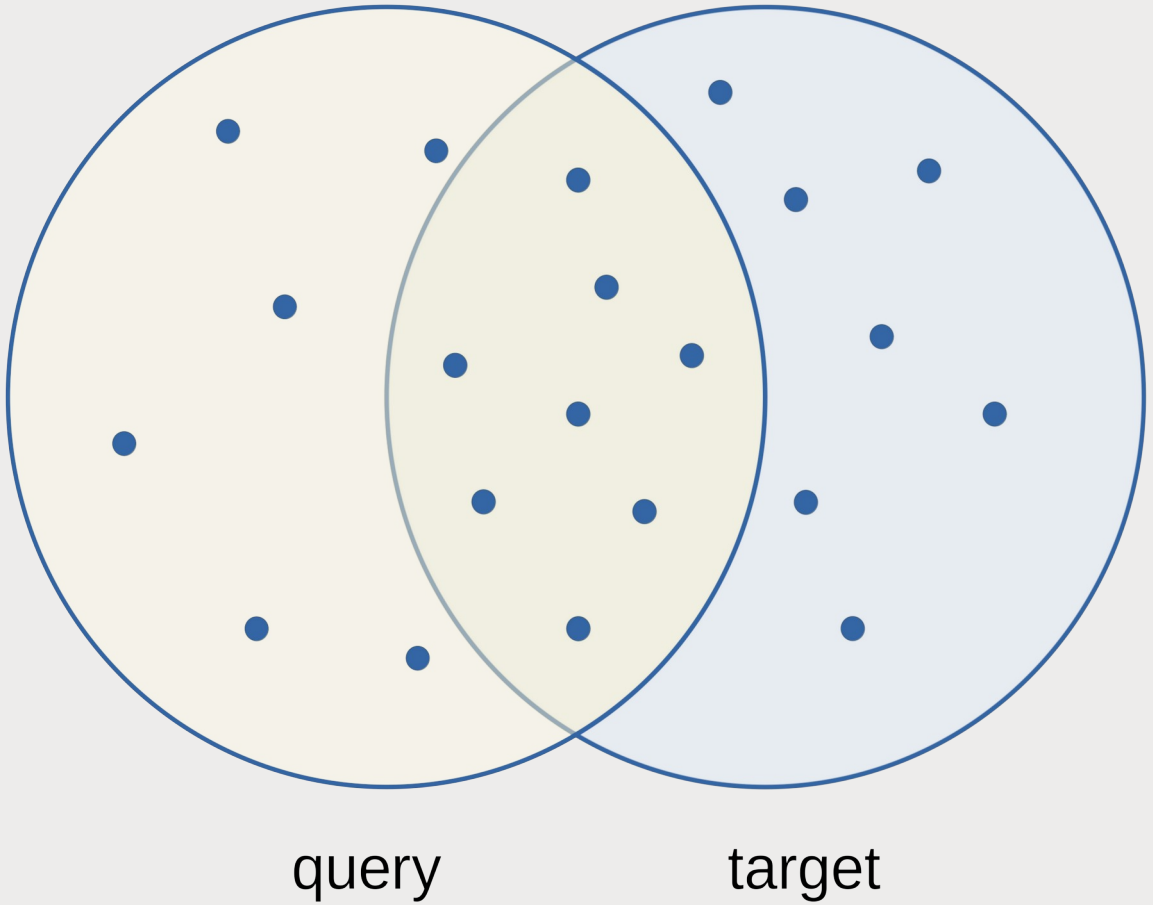
What can your do with the extended tool box?

- Detect AMR caused by point mutations
- MLST by mapping reads on profile alleles
- Discover and submit novel MLST alleles
- Accurate SNP detection for phylogenetic analysis
- Analyse outbreaks by assessing relatedness of isolates
- Identify virus strains, detect novel variants



What is a k-mer?

- A *k*-mer is a subsequence of length *k*
- The *k*-mers of a sequence are *all* its subsequences of length *k*
- The *k*-mer composition of a sequence is like a “spectrum”
  - Can be used to identify a sequence
- Computers can deal with k-mers very efficiently
  - Assemblers, mappers, binners all make use of k-mers
  - Can scale to extremely large databases



Alignment-free methods

- Alignment-free: no assembly, no mapping, no reference – just count *k*-mers
- For instance:
  - Tally every distinct *k*-mer in the query
  - Compare with the *k*-mer composition of the targets
  - Pick the target that shares the most *k*-mers with the query
- Applicable to reads and contigs
- Fast even with huge databases

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Summarising ...

- FASTQ files contain reads and a quality score for every base
- FASTA files contain plain sequences (genes, genomes, ...)
- Assembly reconstructs the genome from reads
- Mapping piles up aligning reads on a reference sequence
- Alignment and mapping underlie many genomic analyses
- K-mers enable rapid search through large datasets
- Alignment-free methods combine speed and huge data sets

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Thank you

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