UK Health Security Agency

De novo assembly

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Overview

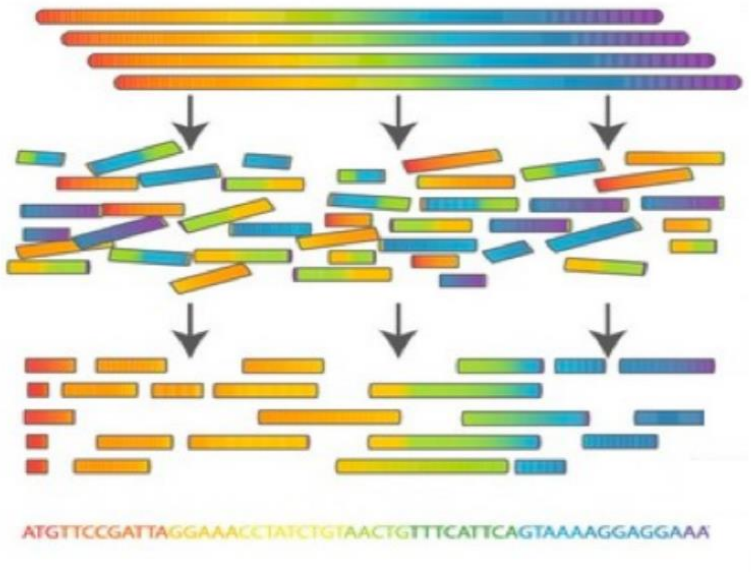
- What is de novo assembly?
  - How does it differ from reference mapping?
- When might it be used?
- How does it work?
- Pitfalls & difficulties with virus data
- Outputs

What is *de novo* assembly?

Basic definition:

“The process of reconstructing sample sequence(s) without any guide reference(s)”

De novo: “from the beginning”



Sample sequences

Sequencing reads

Assembly

Sequence!

Commins *et al.* Bio. Proc. Online (2009) 11(1)

What is *de novo* assembly?

“Trying to solve a huge jigsaw puzzle where you don’t have the picture”

But there are also a number of additional confounding issues:

1. All the pieces are blank
2. Some are missing
3. Some are damaged
4. Many are duplicates
5. None are edges
6. Some belong to other puzzles

When might it be used?

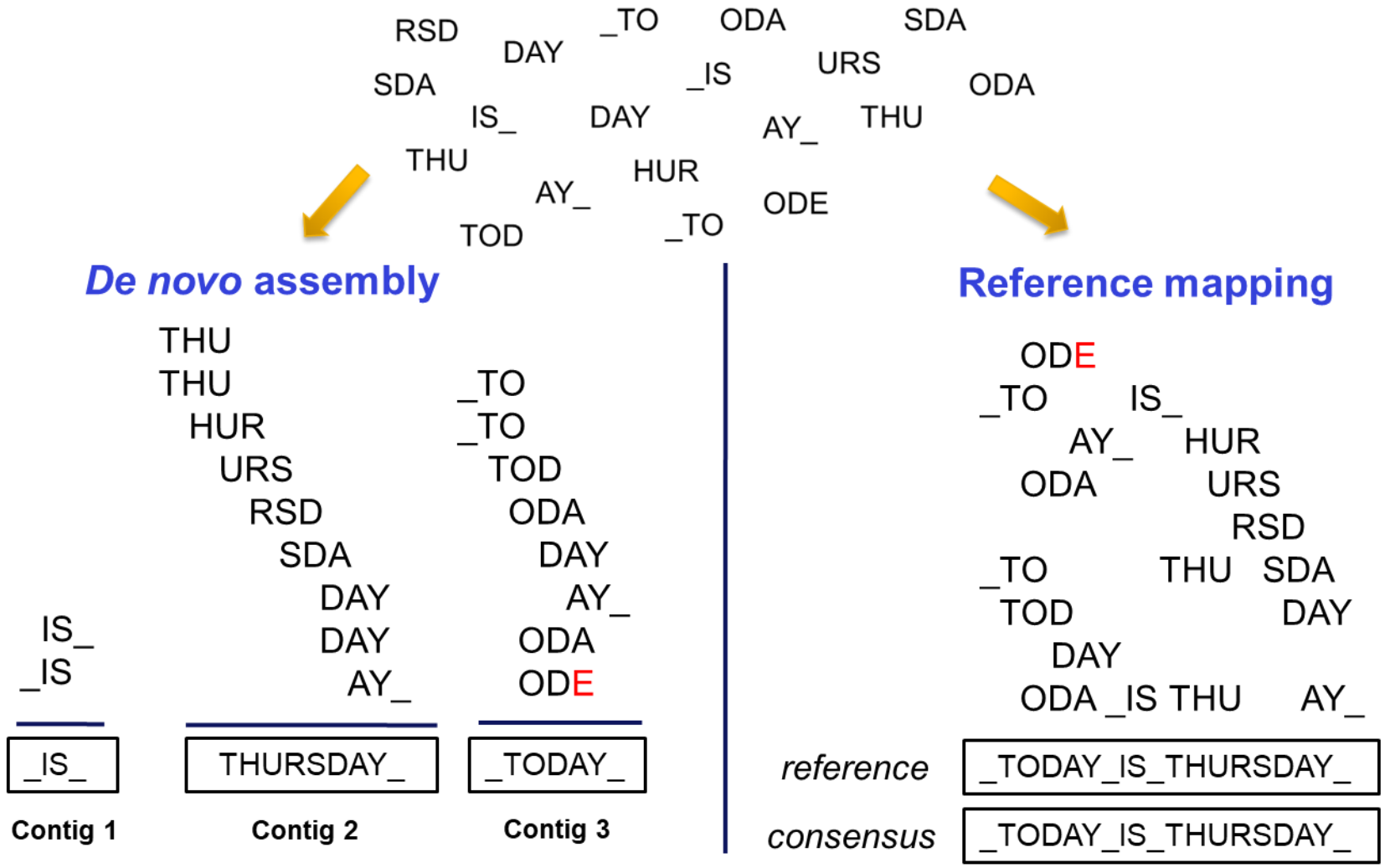
So why not use a reference?

- No suitable references for this virus
- Poorly studied
- Hard to sequence
- Unreliable sequence data
- Highly divergent species (e.g. many RNA viruses)

Target not specified

- Metagenomic analysis
- Syndromic testing
- Unknown aetiological agent of disease

How does it work?



How does it work?

Principles

1. Find overlaps between reads
2. Build a graph of overlaps
3. Correct errors
4. Traverse graph
5. Deliver contigs

Methods

1. Greedy Approach
2. Overlap-Consensus-Layout
3. De Bruijn graphs
4. Other (e.g. VICUNA)

How does it work – Greedy Approach?

1. Iterate through pairs of reads
2. If an overlap exists, merge the two reads into one
   - Overlap detection criteria can be parameterized
3. Repeat until no further overlaps are possible

Slow

Inaccurate

Easily confounded by repeats

How does it work – Overlap-Layout-Consensus?

1. Find all overlaps between reads
   - Again, overlap detection criteria can be parameterized
2. Create a graph of all overlaps
3. Traverse graph to find an unambiguous path
   - “Hamiltonian path” (each *vertex* only once)

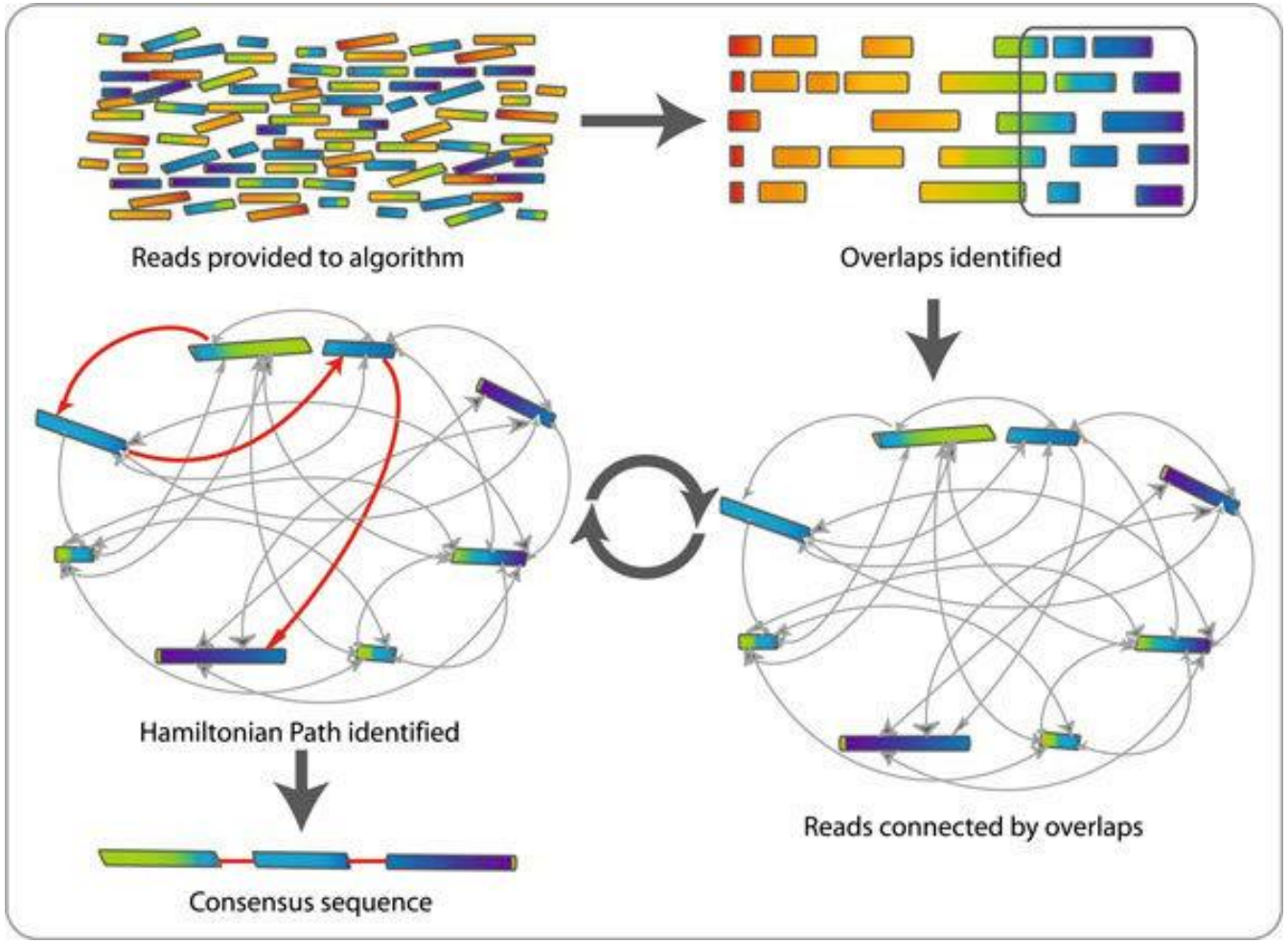
Arachne

PCAP

Newbler

Celera Assembler

How does it work – Overlap-Layout-Consensus?



Commins *et al.* Bio. Proc. Online (2009) 11(1)

How does it work – De Bruijn graphs?

1. Derive *k*mers from sequence reads
   - Subsequences of length *k*
2. Create overlap graph using [*k*-1]mers
3. Traverse graph to reconstruct likely sequence
   - "Eulerian path" (each *edge* only once)
4. More memory-efficient than OLC

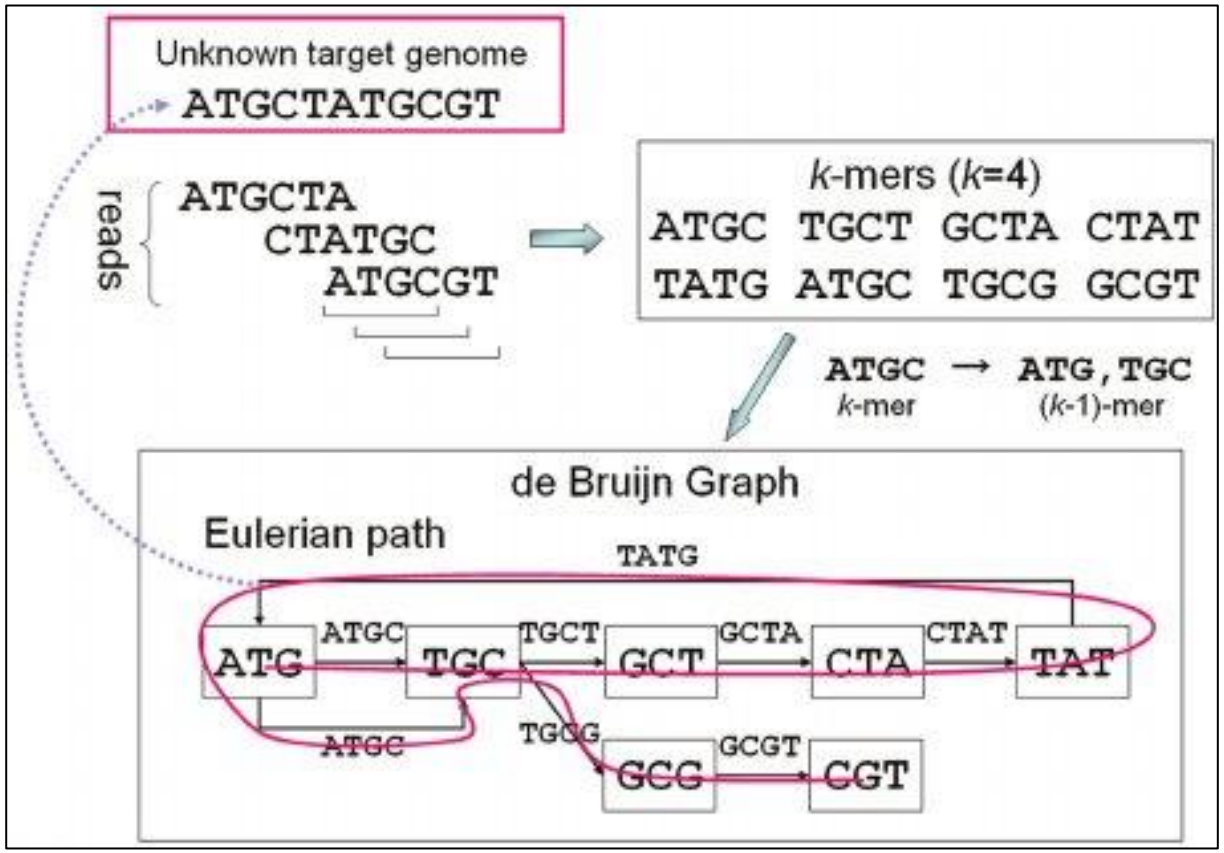
ABySS

SOAPdenovo

Trinity

Ben Langmead, Johns Hopkins
"De Bruijn Graph assembly" pdf

How does it work – De Bruijn graphs?



Namiki *et al.* Nuc. Acids. Res. (2012) 40:e155

Viral data - challenges

High levels of variation in the data set

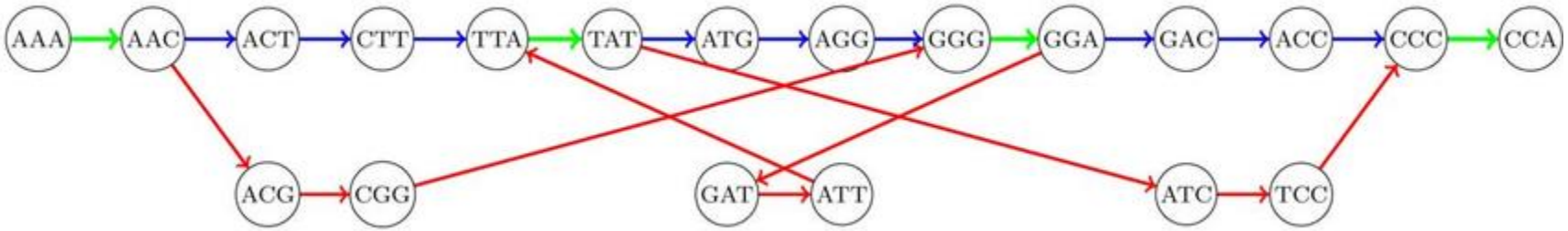
- Errors from sequencing
- Errors from RT-PCR
- Coverage variation from library prep
- Variable quantities of off-target sequence data

High levels of variation in the source virus sample

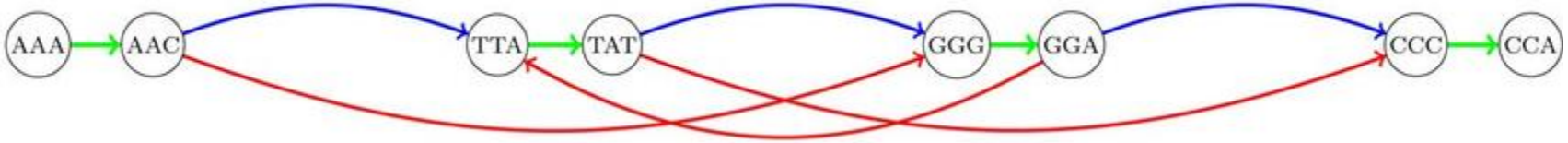
- Continuous quasispecies variation at the nucleotide level
- Length polymorphisms
- “High polyploidy”

Viral data - challenges

OLC & De Bruijn graphs have many “bubbles” & “branches”



Whilst these can be simplified, they can be impossible to resolve



Viral data - challenges

- Traditional assemblers often generate multiple contigs across a single region
- Virus-specific assemblers try to allow for low-level nucleotide variation

Velvet

IVA

SPAdes

VICUNA

- Some tools use the variation to reconstruct haplotypes
  - Linking reads into longer structures
  - HaploClique, Savage

Viral data - challenges

Ways to improve assemblies

1. Trim reads – QC
   - The termini comprise the overlaps; hence critical for good assembly
   - Often of poor quality
   - Errors may be statistically distinguishable from true variation
2. Reduce volume of duplicate information
   - Many near-identical reads can confound even the best assemblers
   - Reduce redundant reads through normalisation (see practical)
3. Eliminate off-target reads
   - Contaminating host/bacterial reads etc.
   - Can be mis-incorporated into graphs and hence contigs
   - Run a mapping against e.g. human/mouse and retain unmapped reads

De novo outputs

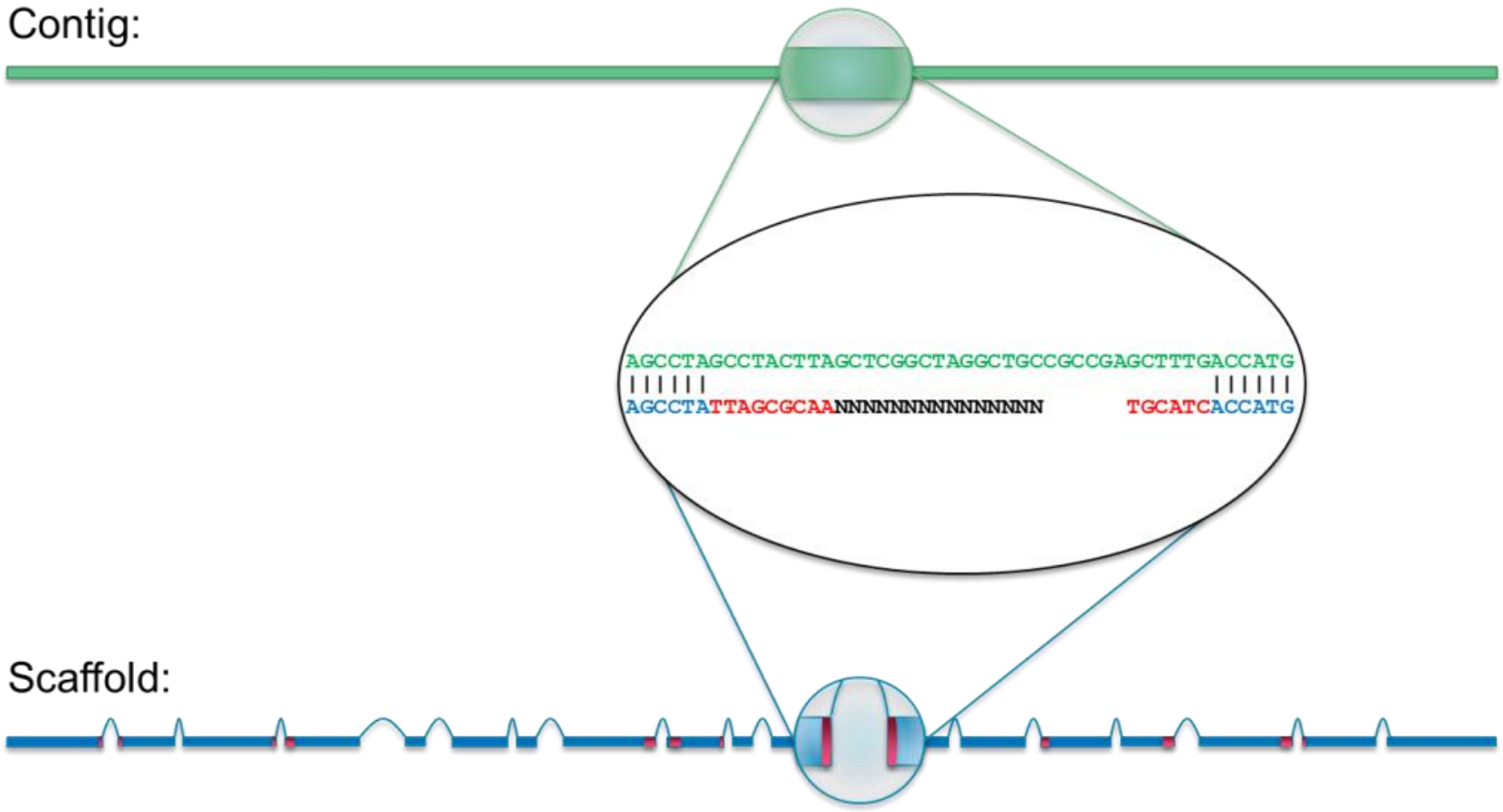
Contigs

- Continuous stretches of sequence containing only A, C, G, or T without gaps.
- These can be interrogated for similarity to known targets or used as reference sequences for mapping

Scaffolds

- Created by joining contigs together using additional information regarding the relative position and orientation of the contigs with reference to a genome
- The contigs within a scaffold are separated by gaps, which are denoted by a variable number of ‘N’ letters.
- In viral genomes, these can represent repeat regions, or unresolved areas of structural uncertainty

De novo outputs



PacBio: ow.ly/fAUJ304re2V

Practical

Three parts

1. SPAdes, QUAST, and BLAST
2. Comparing ‘raw’ and ‘clean’ data
3. Normalising data

Command prompt

- Written `GCV2025:~$` in the documentation, lines following this are to be typed

Results files

- If time is running short, type

```
GCV2025:~$ unzip results.zip
```

and the output files will magically appear for you to look at