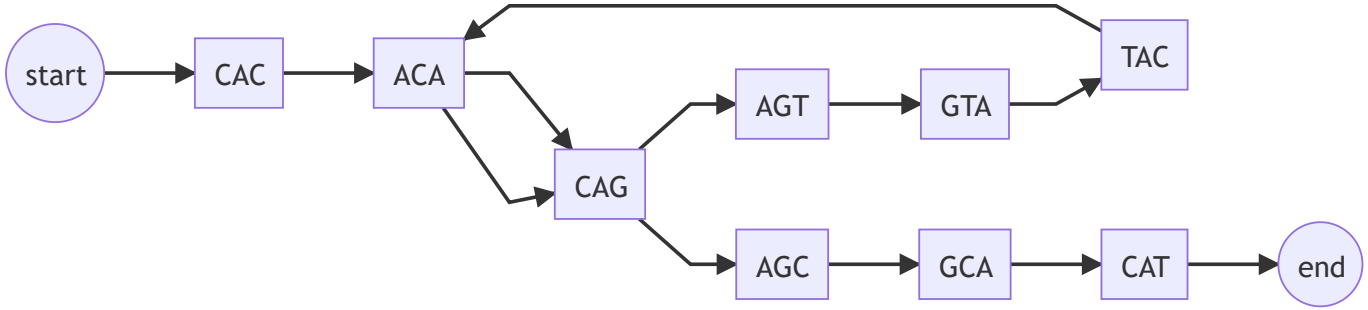
BIOL8706: Dividing and conquering sequence alignment using De Bruijn Graphs



- Student: Richard Morris
- Huttley lab, Australian National University
- Supervisors: Gavin Huttley, Vijini Mallawaarachchi

Australian National University

Sequence alignment

Sequences → **Alignment** → **Phylogenetic relationship**

Sequence A [ACAGTACTGACAT]

Sequence B [ACAGCTGCAT]

Sequence C [ACAGCTACAT]

A	C	A	G	T	A	C	T	G	A	C	A	T
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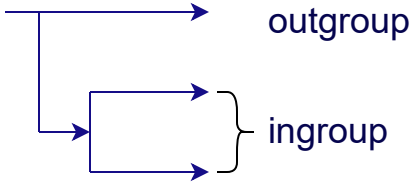

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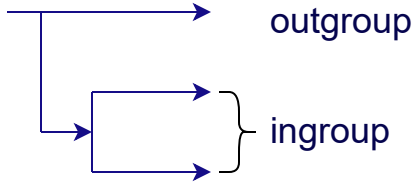

We start with a set of DNA sequences

Sequence alignment

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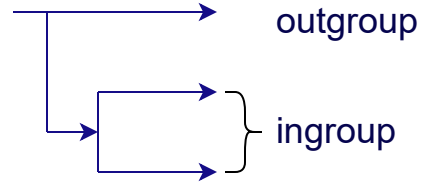
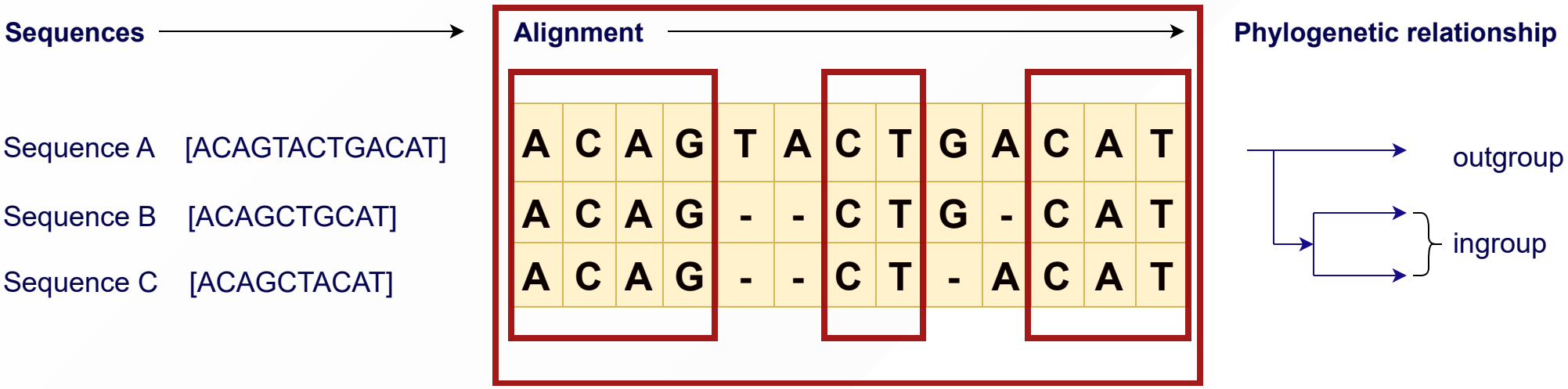


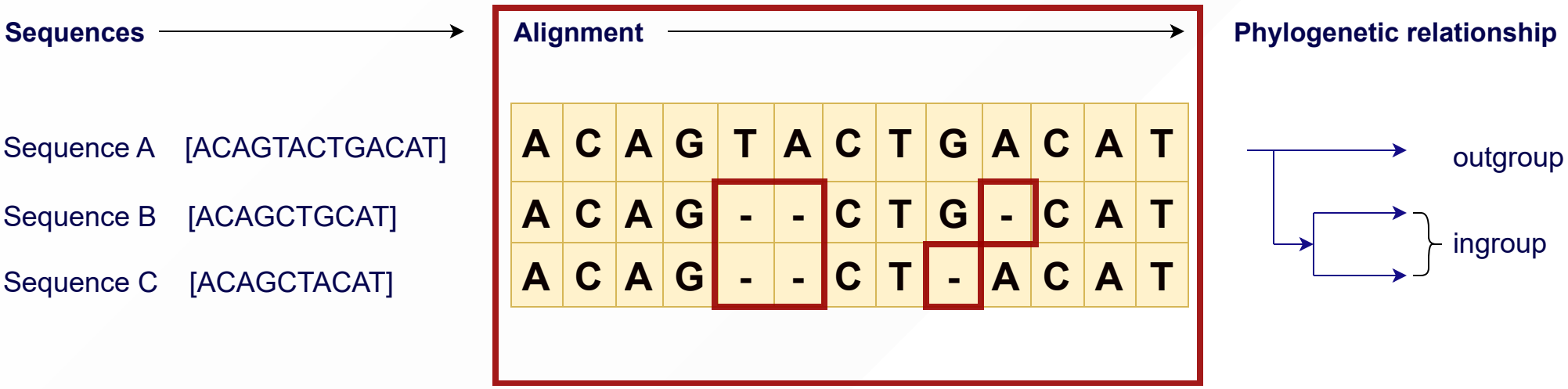
We align those sequences

Sequence alignment



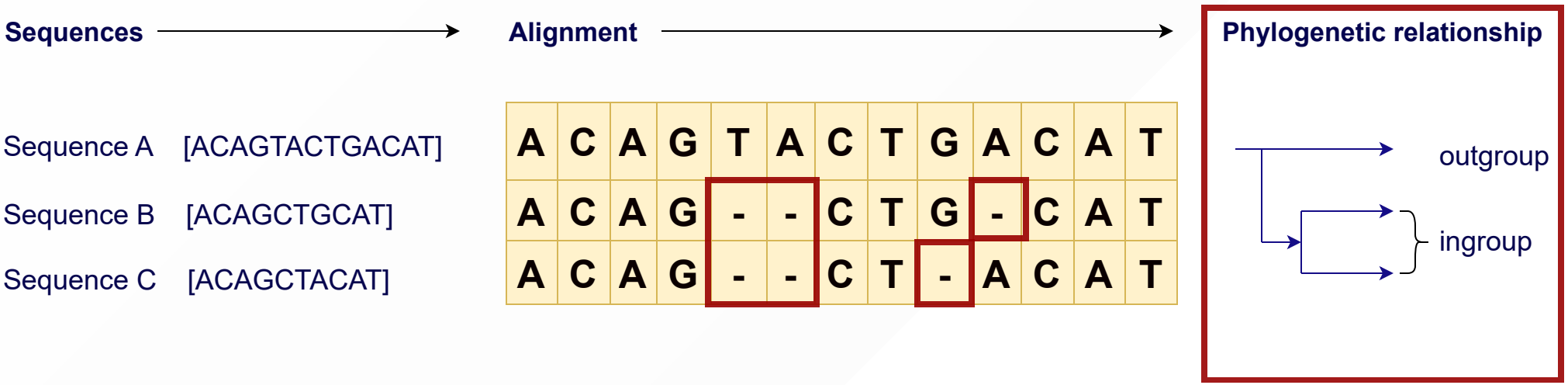
By lining up regions that are similar

Sequence alignment



Noting those that are different

Sequence alignment



And we can infer evolutionary relationships between those sequences

- ingroup
- outgroup

Sequence alignment

Sequences → **Alignment** →

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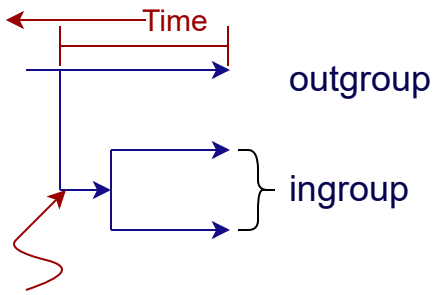
Phylogenetic relationship



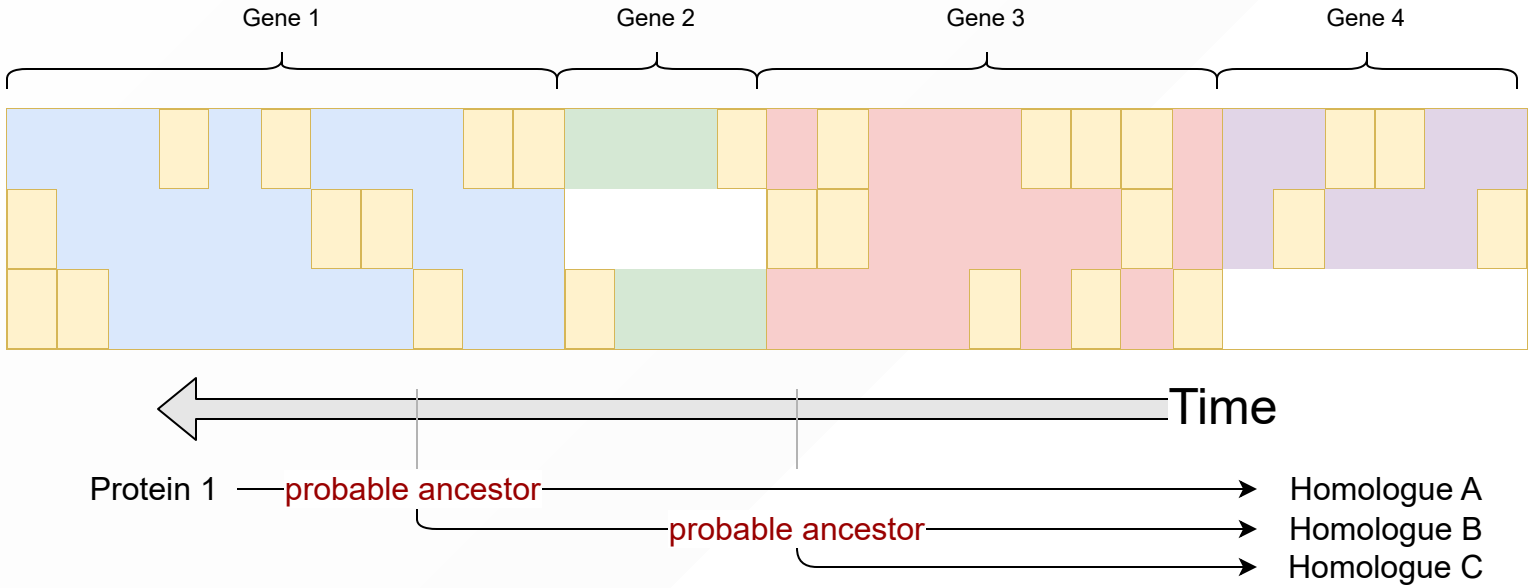
And we can infer evolutionary relationships between those sequences

- ingroup
- outgroup
- extinct common ancestors
- how long ago sequences diverged

Recall the central dogma of biology

$$\text{DNA} \xrightarrow{\text{transcription}} \text{RNA} \xrightarrow{\text{translation}} \text{Protein}$$

If we can infer coding DNA of these ancient ancestors, we can infer the proteins they made, and passed to their descendants



Sequence alignment is a time machine for homologous proteins

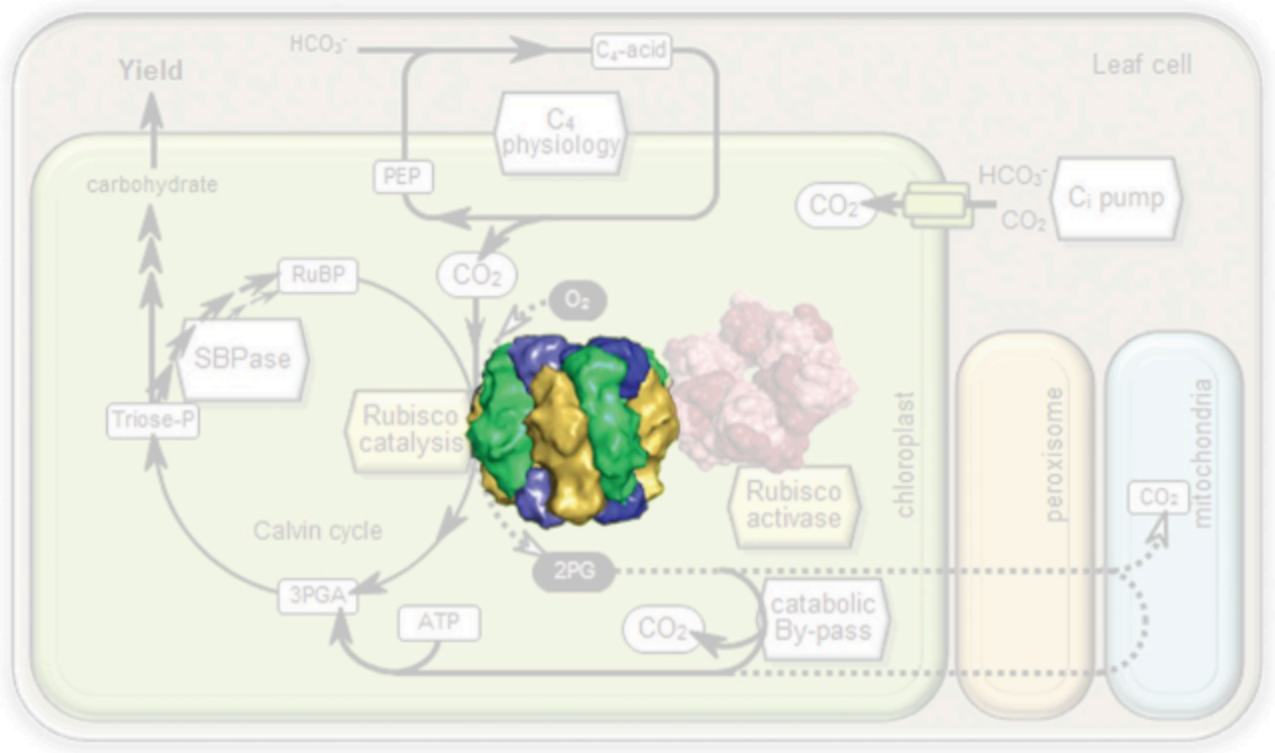
3 big ideas

- design crops for better yield
- predict the trajectory of a virus
- understand our own evolution

These all require sequence alignment

Consider RuBisCO

- The most abundant protein on Earth
- essential component of photosynthesis
- primary role is to convert CO_2 to organic carbon



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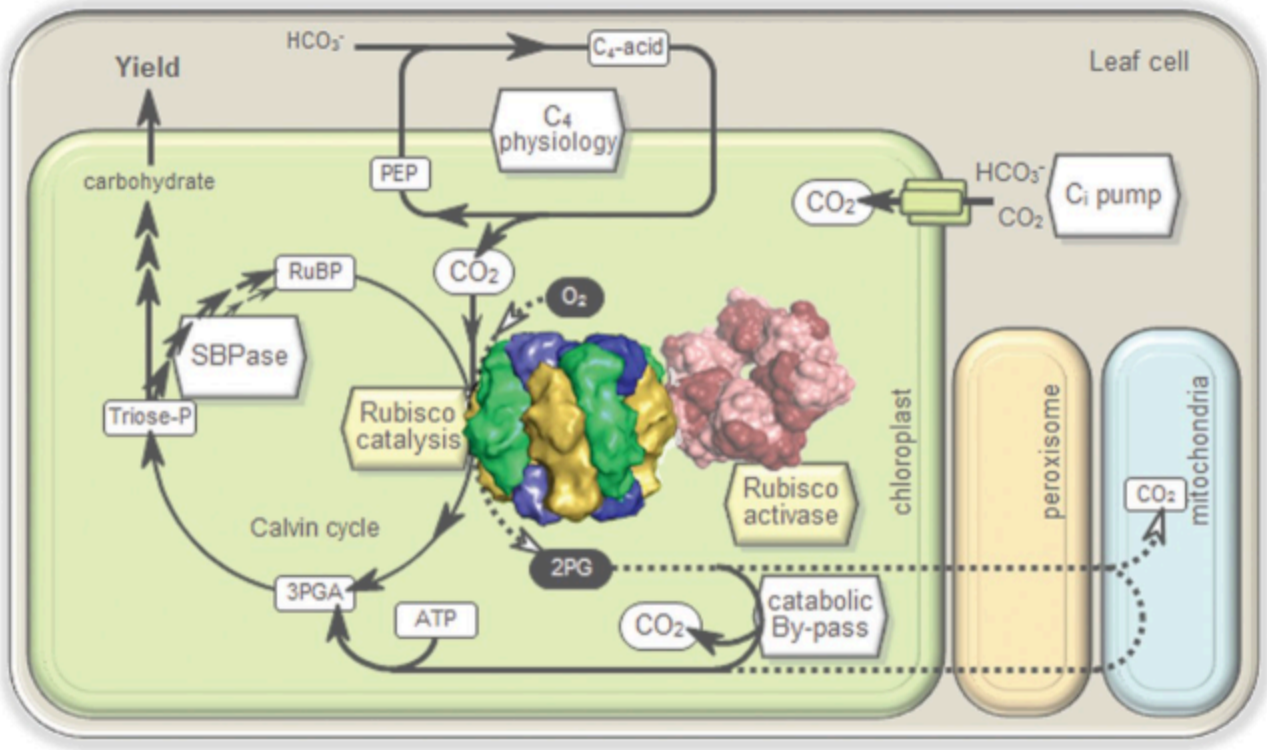

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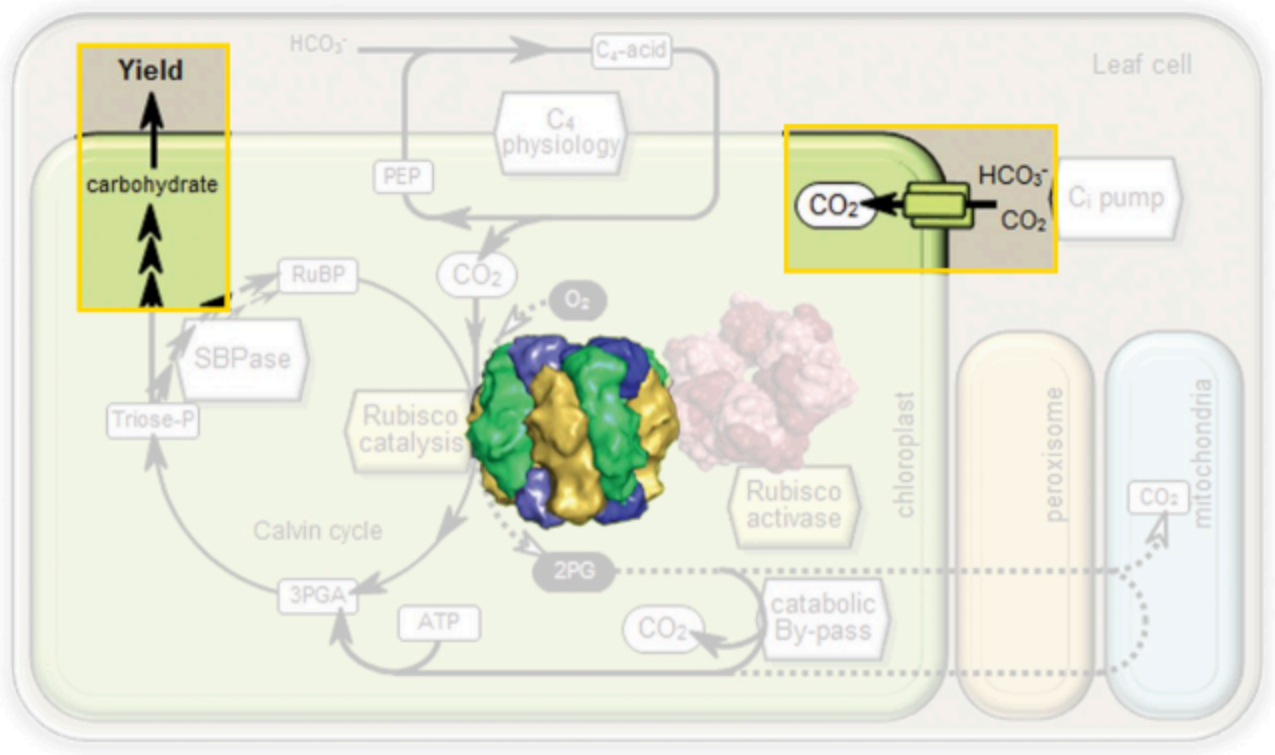
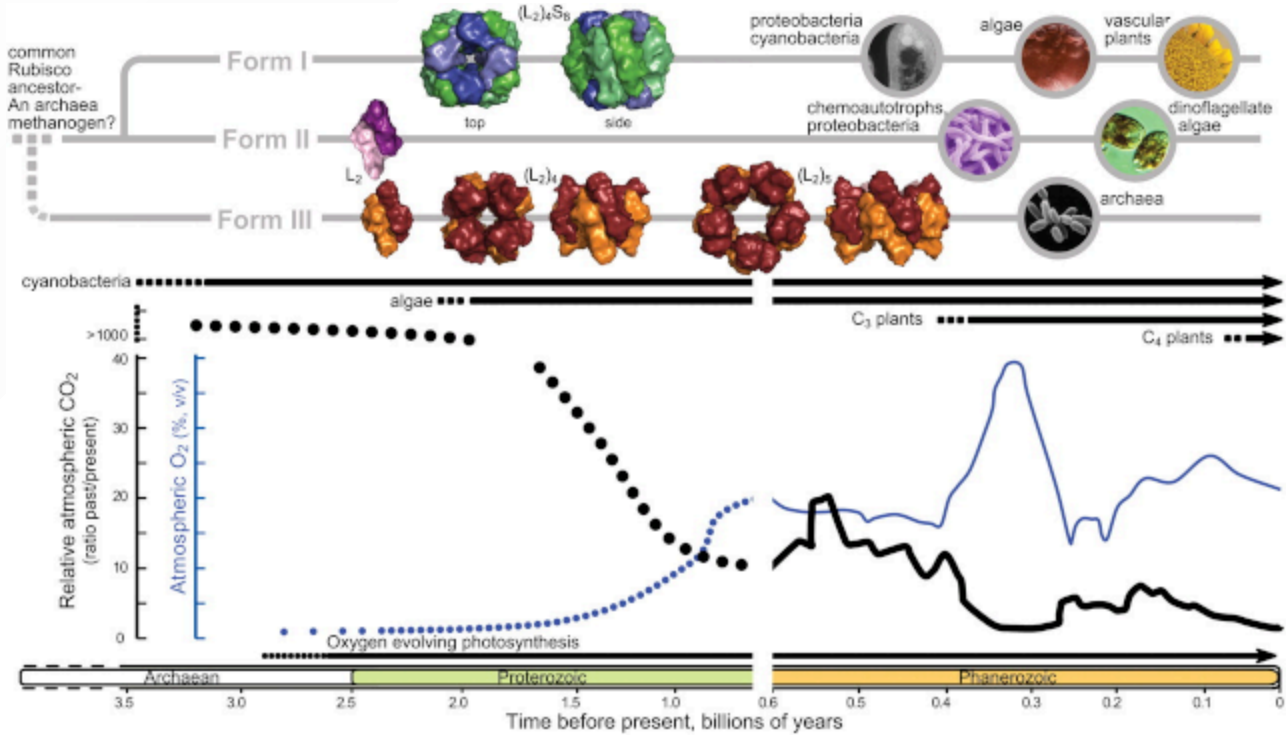


Parry et al 2012 doi.org/10.1093/jxb/ers336

Evolution of RuBisCO

- Sequence alignment can infer the evolutionary history of RuBisCO
- when novel features appeared
- And compare that with a geological understanding of the atmosphere at that time

ie: We can associate **features** in the protein with the **environment** in which it evolved?



Whitney et al 2012 doi.org/10.1104/pp.110.164814

The value of understanding RuBisCO features?

Q: Can we design more efficient RuBisCO?

⇑ RuBisCO efficiency would lead to

- Crop yield ⇑
- Carbon sequestration ⇑

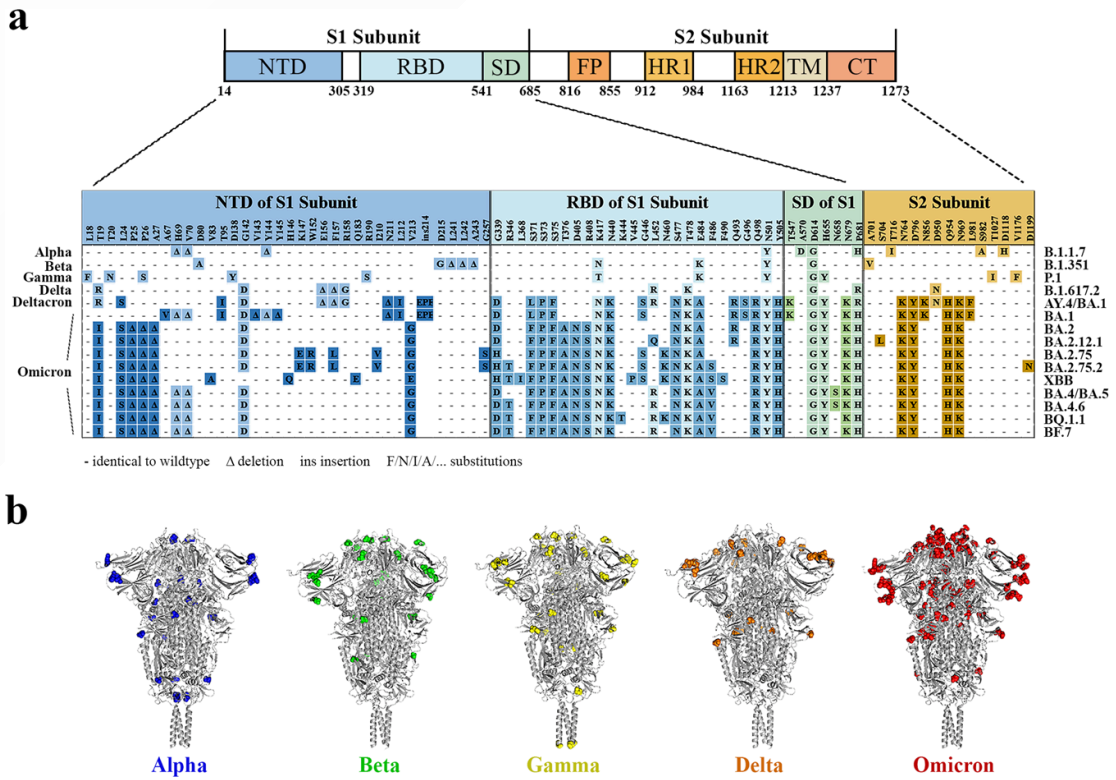
This is a big question that sequence alignment can contribute to answering

Consider the spike protein of SARS-CoV-2

Sequence alignment

- allows us to identify conserved regions for vaccine/drug development
- can help us predict the trajectory

These are big questions that sequence alignment can contribute to answering

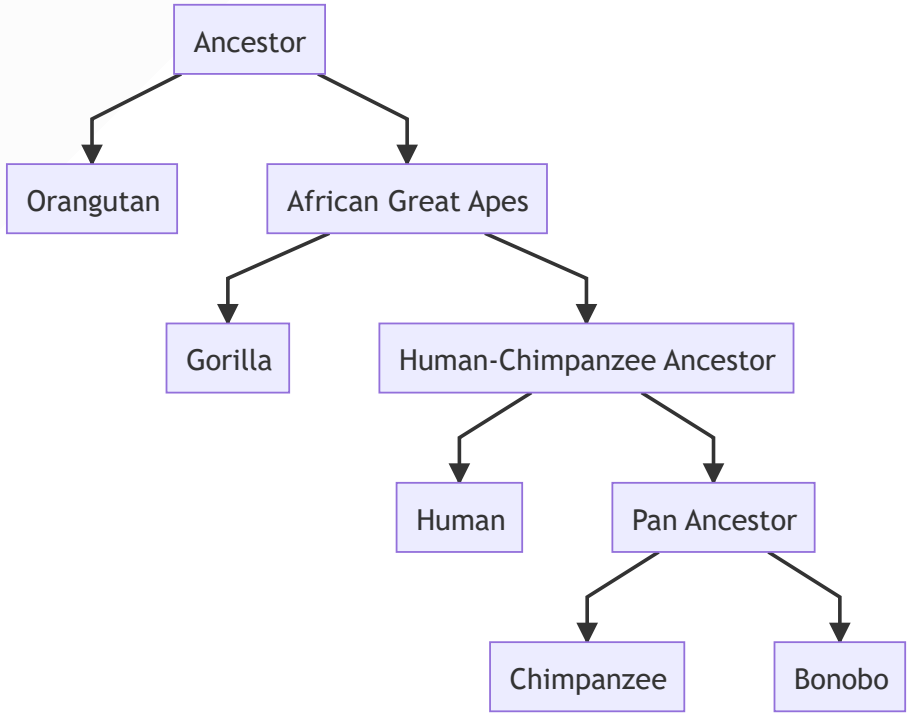


Alignment of S mutation points of SARS-CoV-2 variants

Consider our immediate family

- What can we learn about our own evolution from our closest relatives?
- Can that knowledge inform biomedical science

This is a big question that sequence alignment can contribute to answering



The family tree of great apes

Sequence alignment is a big job

- Historically sequence alignment was done manually, like a really big jigsaw puzzle
- Since 1972 it's become a computational problem
- to compare **each** letter in **each** sequence with **all** the letters of **every** other sequence.

- The terms: **each**, **all** and **every** suggests that it will be a big job for computers too.

Exhaustive sequence alignment takes time

A computational scientist might say that the asymptotic complexity of an exhaustive alignment is given by the big-O notation

$$O(L^n)$$

Where:

- L is the average length of the sequence
- n is the number of sequences

"Big-O tells you how code **slows** as data **grows**" *Ned Batchelder*

Too much math?

Let’s rephrase this big-O notation as “Work”

So we can reframe this as “Work” **slows** as data **grows**

Sequence length	number of sequences	“Work” required (comparisons)
1,000	2	1 Million
1,000	3	1 Billion
1,000	4	1 Trillion
1,000	5	1 Quadrillion

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Sequence length	number of sequences	“Work” required (comparisons)
1,000	3	1 Billion
2,000	3	8 Billion
3,000	3	27 Billion
4,000	3	64 Billion

The scale of our 3 big problems

Genomes	Length (bp)	Number	"Work" required
RuBisCO producers	1.5-500 mbp	350K[1]	millions[hundreds of thousands]
SARS-CoV-2	~29 kbp	>5M[2]	29 thousand[5 million]
Great apes	~30mbp	5	30 million[5]

[1] back of the napkin math = 300K species of **plants** + 10's of thousands of species of **algae** + thousands of species of **cyanobacter**

[2] 5.1M as of Oct 2021 - www.nature.com/articles/s41588-022-01033-y


WE'RE GONNA NEED
MORE HAMSTERS
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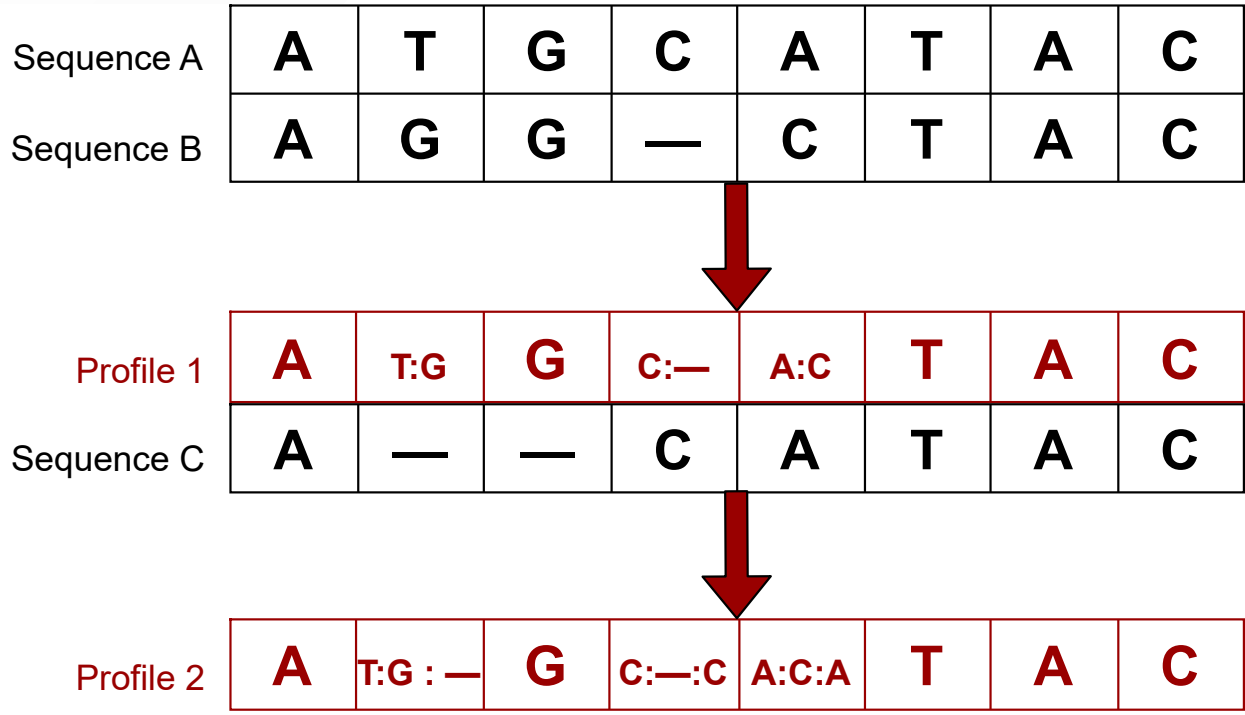
Progressive alignment

- align the 2 most closely related sequences into a statistical model called a profile
- align that profile with the next most closely related sequence
- Do that $\binom{n}{2}$ times

This reduces the work required from $O(L^n) \rightarrow O(n^2.L^2)$

... which is a lot less “Work”



Progressive multiple sequence alignment (MSA)

- To align multiple sequences first reconstruct a phylogeny to order by distance
- To reconstruct a phylogeny first align all sequences

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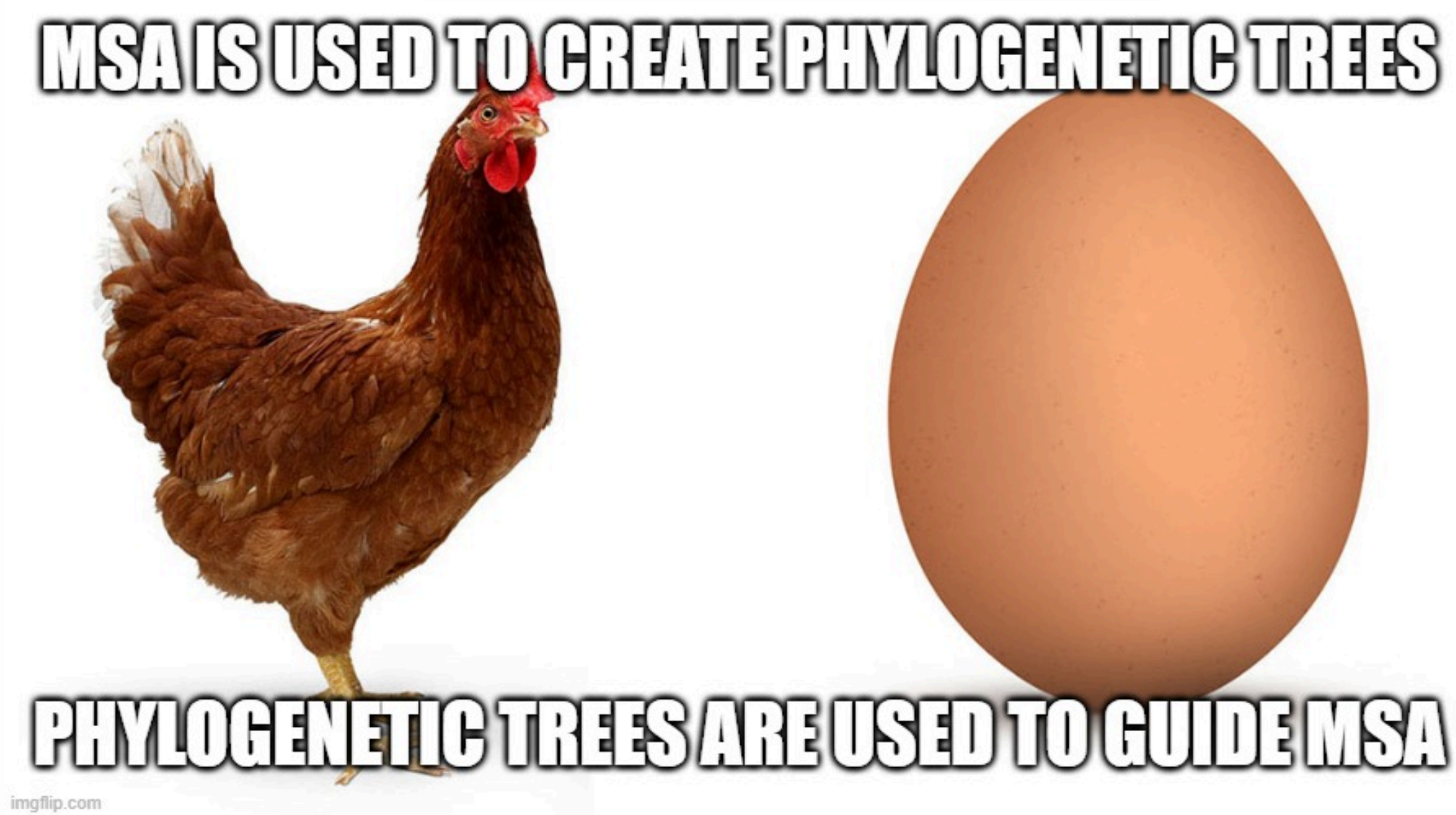
Do you see the problem?


MSA IS USED TO CREATE PHYLOGENETIC TREES
PHYLOGENETIC TREES ARE USED TO GUIDE MSA
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The problem space

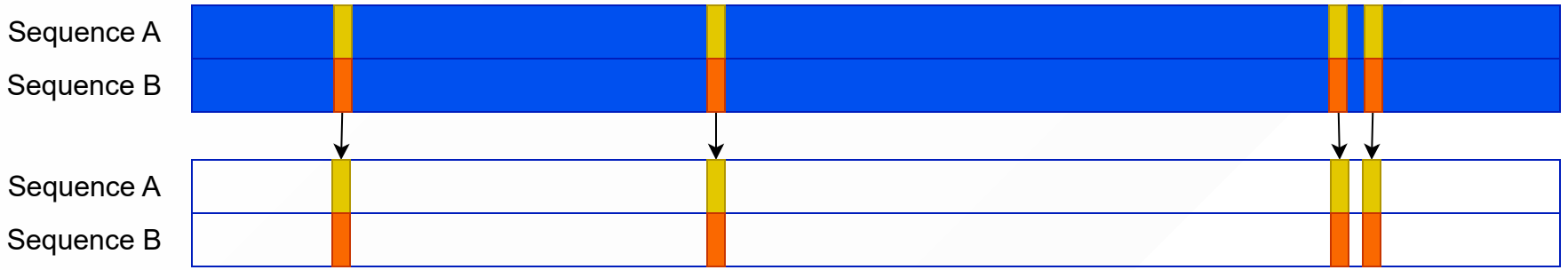
Recall: Sequence alignment is sensitive to

- The **length** of sequences to be aligned
- The **number** of sequences to be aligned
- the “ Chicken and Egg ” problem

An ideal strategy would reduce

- The **length** of sequences to be aligned
- The **number** of sequences to be aligned
- Dependence on knowing the phylogeny in advance

What if we could quickly remove similar regions?

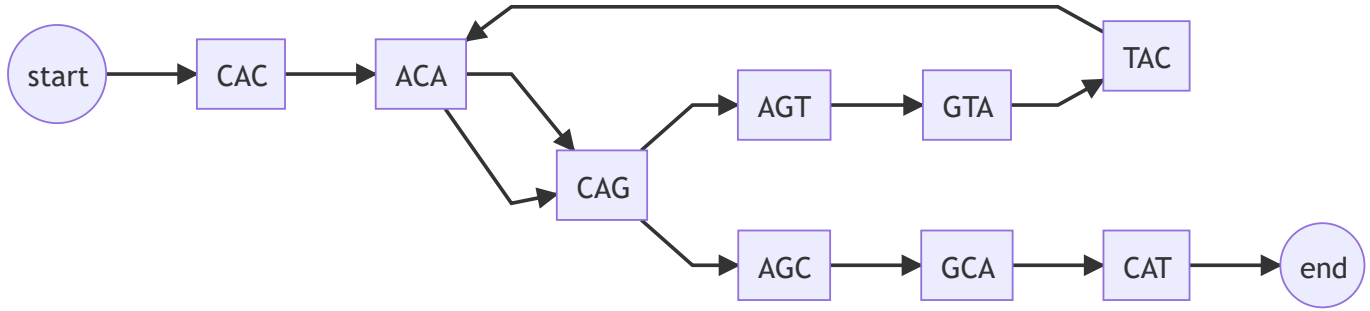


We'd could focus resources on just the regions that differ

Sequence alignment using De Bruijn Graphs

This work builds on the work by Xingjian Leng in 2022, under the supervision of Dr. Yu Lin and Prof. Gavin Huttley.

Xingjian tackled the length problem using de Bruijn graphs



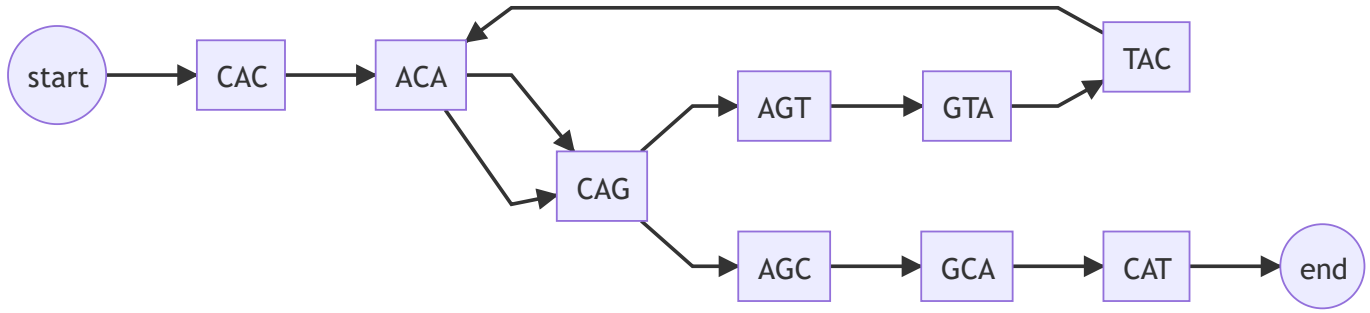
De Bruijn graphs

A De Bruijn graph is a directed graph that represents unique overlapping subsequences

Building a De Bruijn graph is $O(nL)$

This "Work" scales linearly not exponentially.

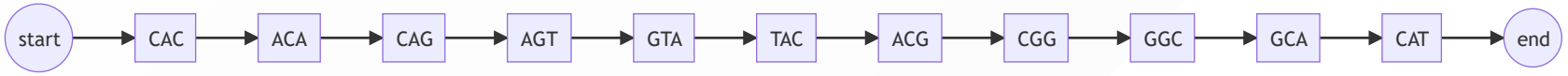
The sequence CACAGTACAGCAT as a de Bruijn graph of order 3 (nodes overlap by 2 characters) looks like:



Reducing the length of sequence to be aligned

Consider the DNA sequence $CACAGTACGGCAT$

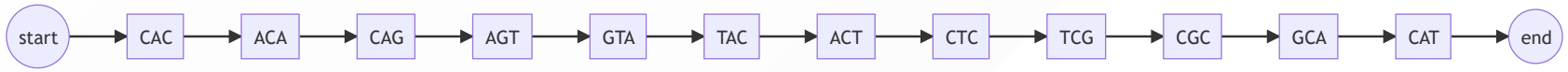
When we represent that as a de Bruijn graph it looks like this:



Reducing the length of sequence to be aligned

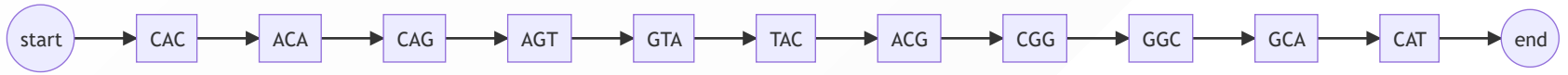
Consider we want to align that sequence $CACAGTAC\boxed{G}GCAT$ to the very similar sequence $CACAGTAC\boxed{T}CGCAT$

Which as a De Bruijn graph looks like this:

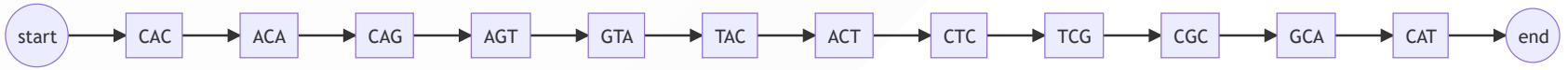


Reducing the length of sequence to be aligned

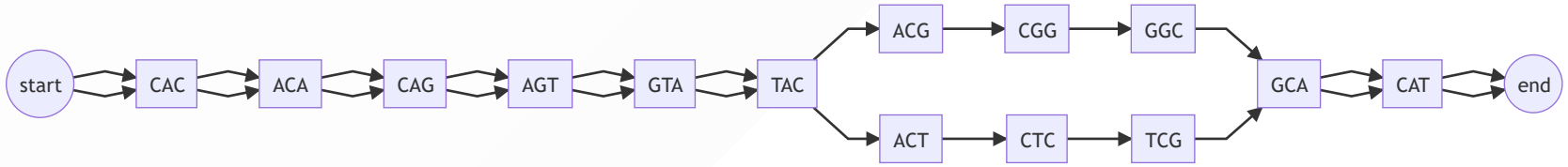
Sequence A:



Sequence B:

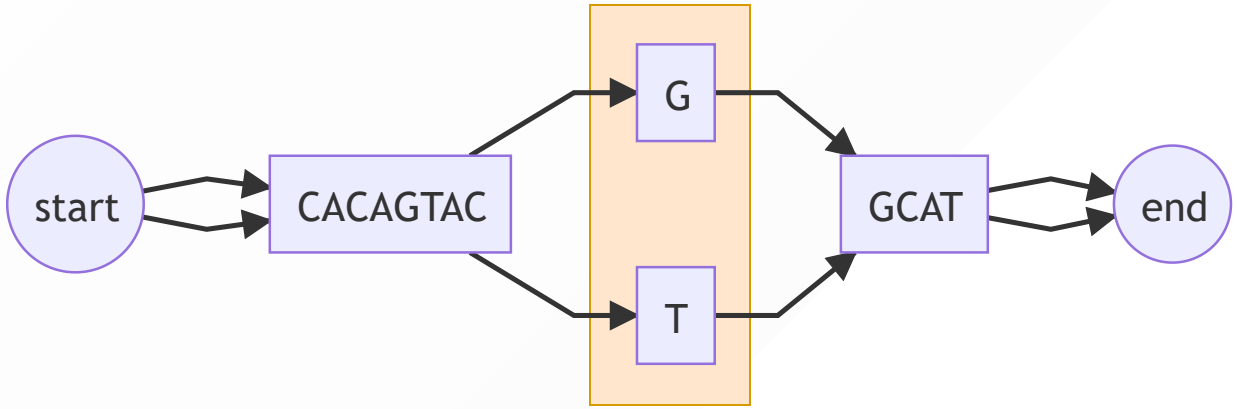


If we combine both sequences into a single de Bruijn graph, it will develop **“bubbles”** where regions are different.



Reducing the length of sequence to be aligned

If we can collect nodes into runs of characters, with overlaps removed, we can see the regions that are similar which we don't need to align, and the regions that are different (in the gold box) which we do.

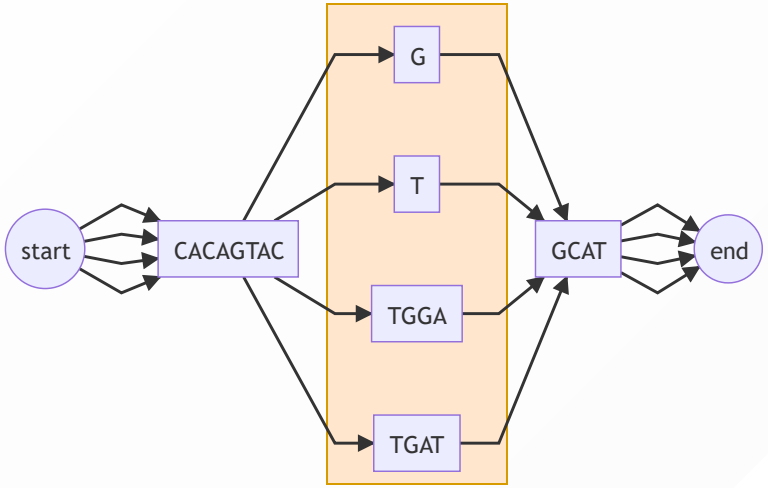


Now we can use a traditional algorithm to align the regions $\boxed{G}$ and $\boxed{T}$, and we've reduced our "Work" function from $O(14 \times 14)$ down to $O(1 \times 1)$ = **196x** less "work".

De Bruijn multiple sequence alignment

And we can extend this trivially to multiple sequences. Consider aligning the following sequences

`CACAGTACGGCAT` `CACAGTACTGCAT` `CACAGTACTGGAGCAT` & `CACAGTACTGATGCAT`



Now we've reduced O(13x13x16x16) down to O(1x1x5x4) = **2,163x** less “work”

Taking the de Bruijn graph to the next level

- recall an exact alignment has an order complexity of $O(L^n)$ or $O(L_1 \times L_2 \times \dots)$ for pairwise alignment
- if we reduce the length of the sequences we need to align then we reduce L

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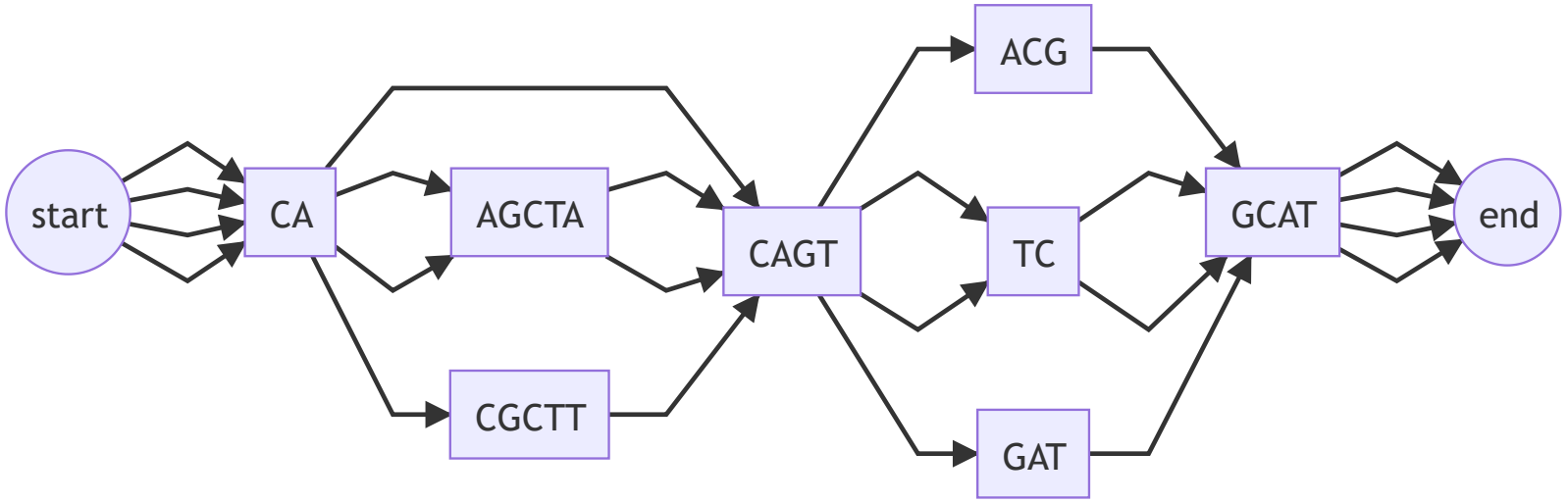
How about n?

Reducing the number of sequences to be aligned

Consider this partial order graph containing 4 sequences with overlaps removed



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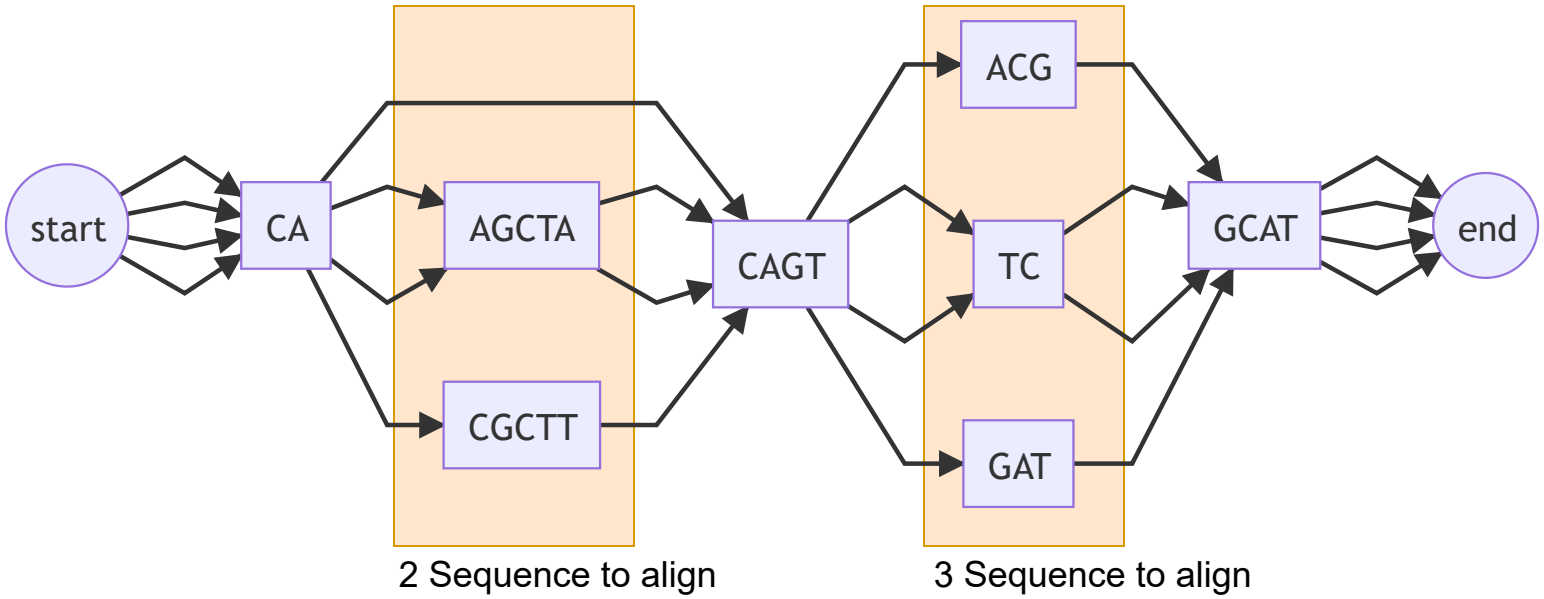
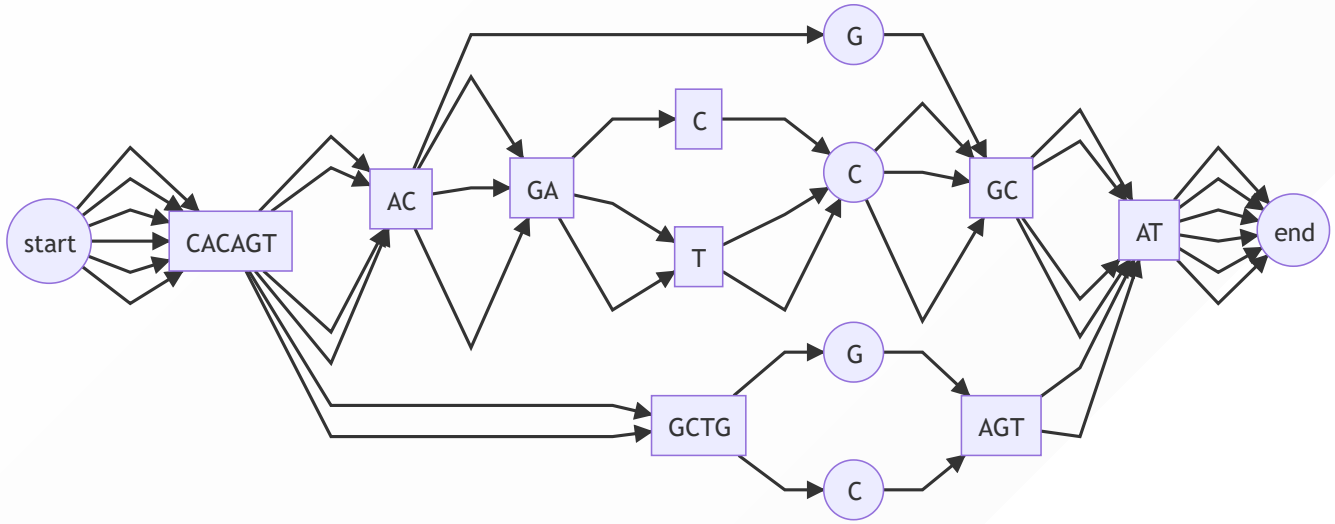


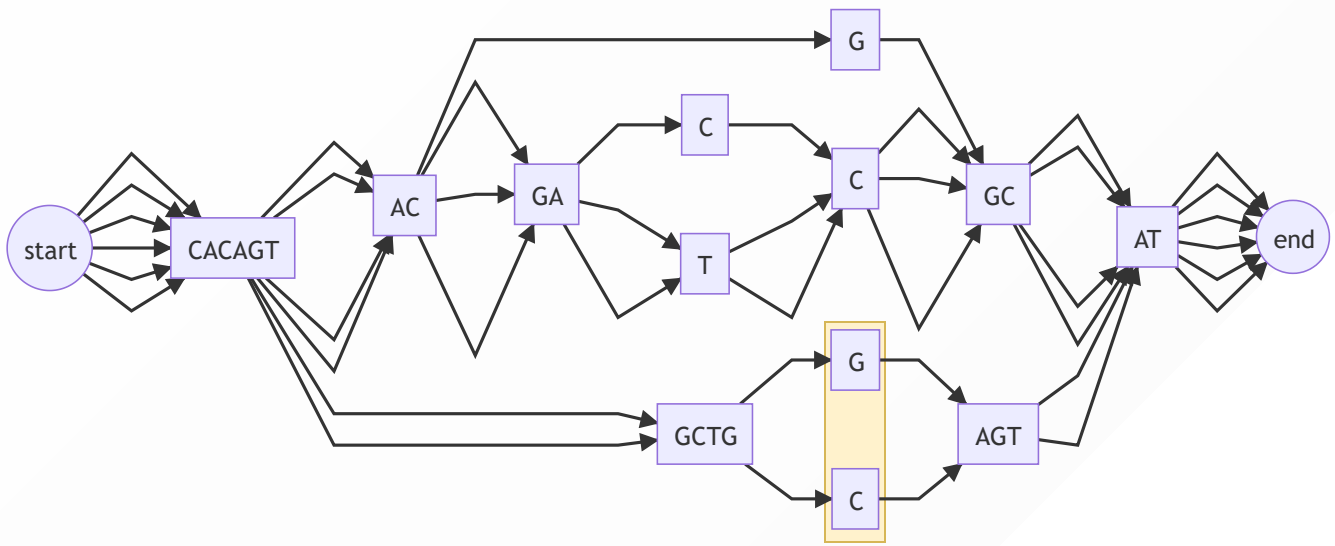
exhaustive alignment	reduce length	reduce length & number
$O(13 \times 14 \times 17 \times 17)$	$O(5^4 + 3 \times 2 \times 2 \times 3)$	$O(5^2 + 3 \times 2 \times 3)$
52,598	661 (**79x** vs exhaustive)	43 (**1223x** vs exhaustive)

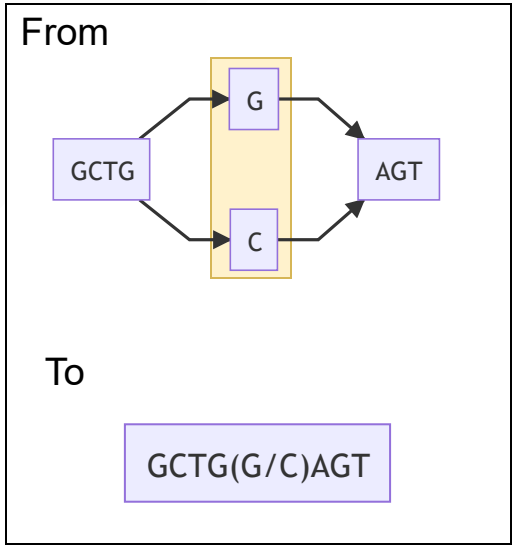
Reduce the dependence on the phylogeny



By ordering progressive alignment by descending “bubble” depth, we can progressively align without needing to know in advance the phylogenetic relation between sequences.

Reduce the dependence on the phylogeny





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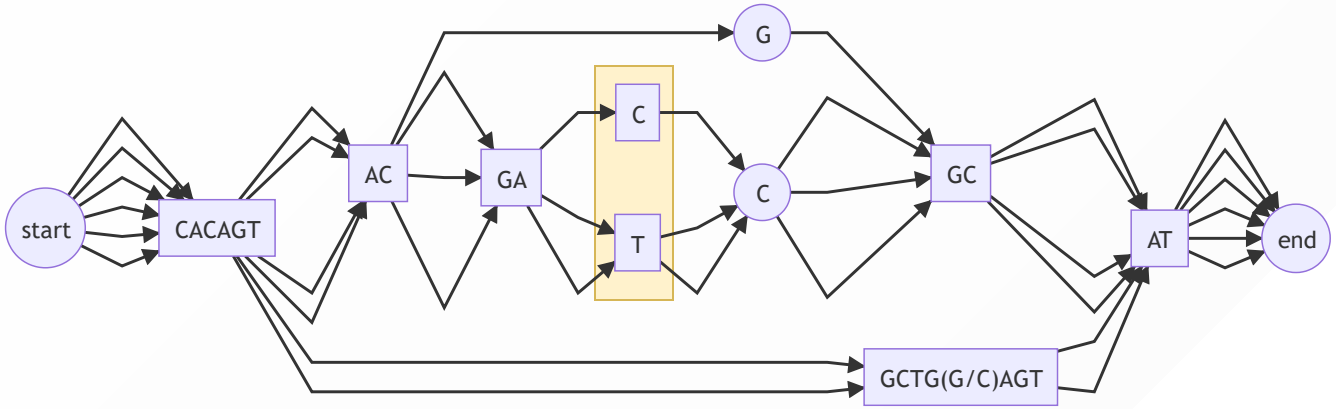
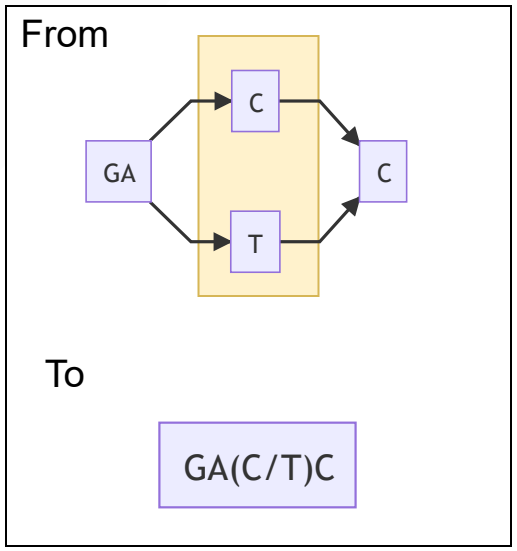
Reduce the dependence on the phylogeny





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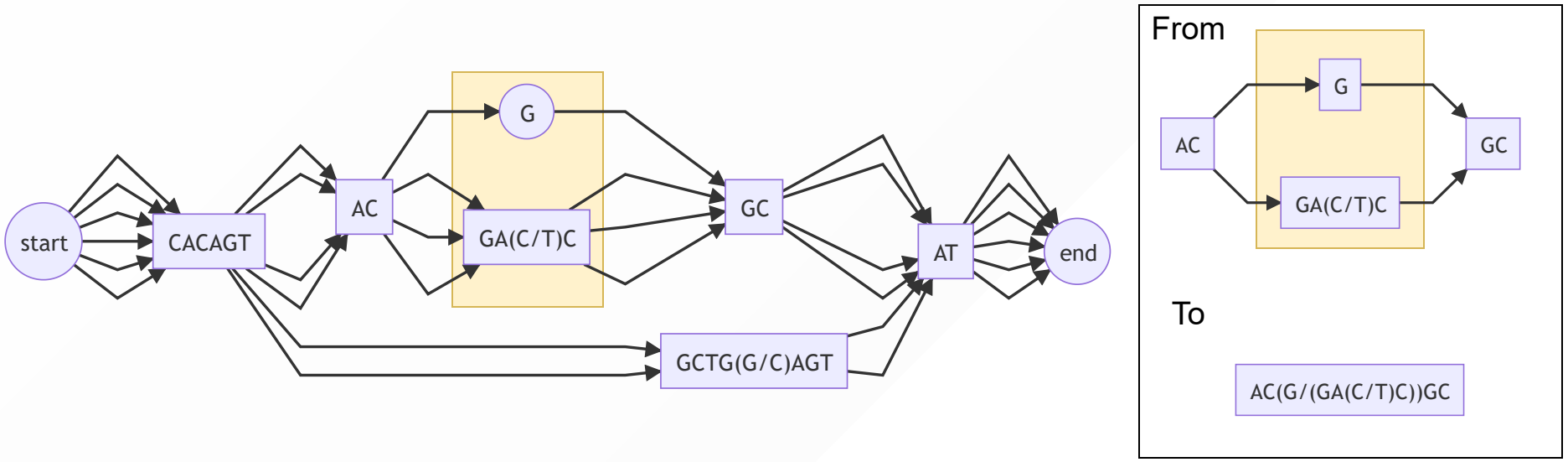

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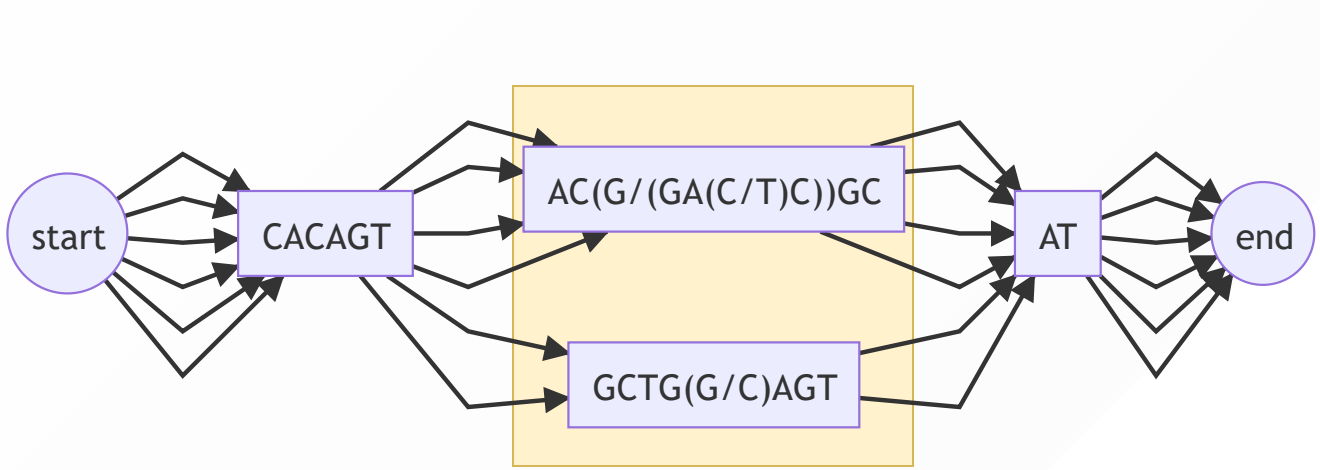
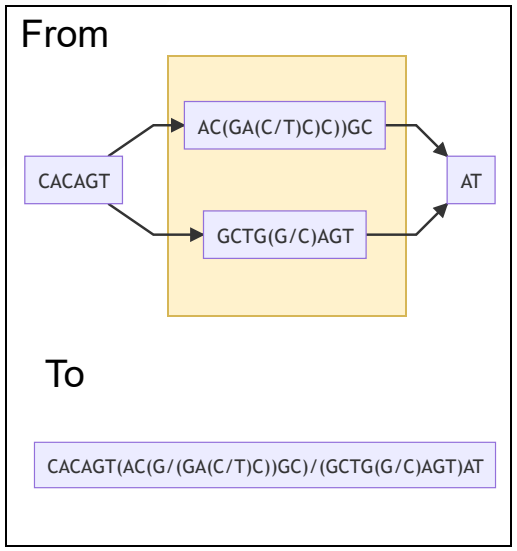




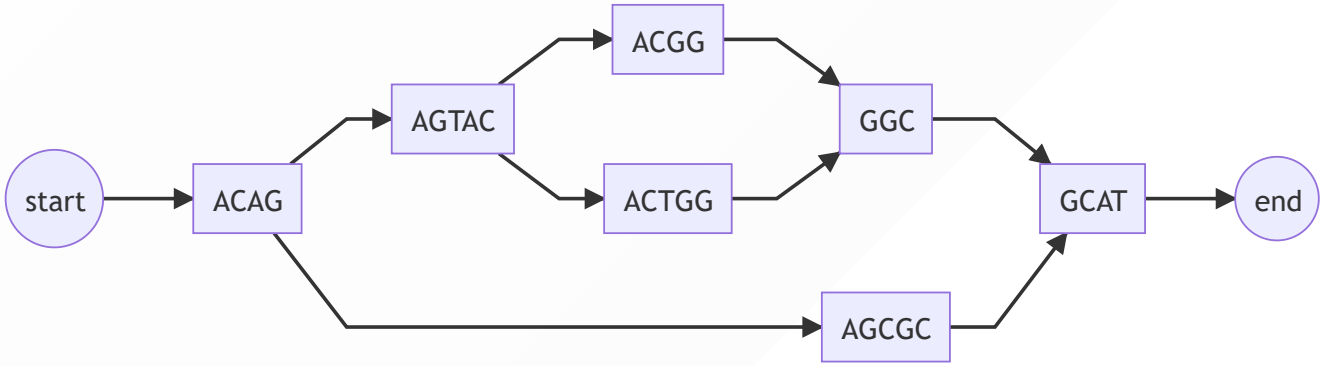
By ordering progressive alignment by descending “bubble” depth, we can progressively align without needing to know in advance the phylogenetic relation between sequences.

Project aims

- Investigate De Bruijn graphs for multi-sequence alignment (MSA)
- Build a python library to
  - Resolve the De Bruijn graph to a partial order graph
  - identify “bubbles”
  - Develop unit tests to verify correctness of the algorithm
- Develop statistics for de Bruijn graphs to predict efficiency

Results: Quickwork statistic

Consider this de Bruijn graph containing 3 sequences [`ACAGTACGGCAT` , `ACAGTACTGGCAT` , `ACAGCGCAT`] of length 12, 13 and 9
When Transformed into a partial order graph

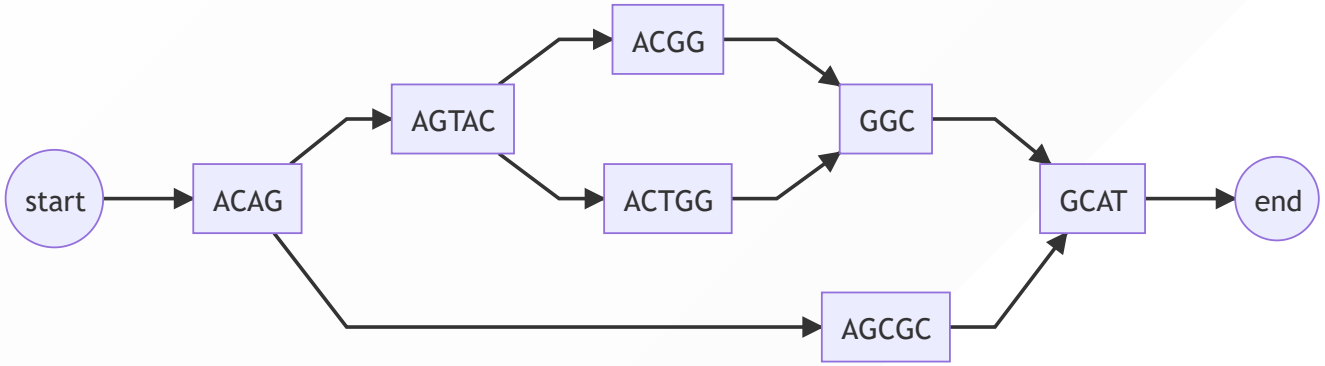


Contains the following nodes (left to right) with overlaped sections removed AC+T+G+C+AT

Quickwork = $\sum \text{node length - overlap} = 7$
Quickwork is an estimate of alignment required in the de Bruijn graph
Quickwork has a " Work " function of $O(node\_count)$

Results: Work statistic

Consider the same de Bruijn graph



- Work calculates the order complexity of alignment using 4 strategies
  - Exact = $13 \times 12 \times 9 = 1404$
  - Progressive = $13 \times 12 + 13 \times 9 = 285$
  - DBG_L = $7 \times 8 + 8 \times 1 = 64$ (simplification of sequence length)
  - DBG_LN = $0 \times 1 + 5 \times 1 = 5$ (simplification of sequence length and count)

Results: Calculated from alignable sequences

- BRCA1 genes in 56 species (citation needed)
- BRCA1 genes in primates (citation needed)
- SARS-CoV-2 genomes (citation needed)
- IBD phage components (https://doi.org/10.1016/j.cell.2015.01.002)
- Tara oceans phage components (https://doi.org/10.1126/science.1261605)

Results: Calculated order complexity from alignable sequences

kmer	Genomes	Exact	Progressive	dBG_L	dBG_LN
3	BRCA1 56 species				
3	BRCA1 primates				
3	SARS-CoV-2				
3	IBD phage				
3	Tara oceans phage				

Results: Calculated order complexity from alignable sequences

kmer	Genomes	Exact	Progressive	dBG_L	dBG_LN
6	BRCA1 56 species				
6	BRCA1 primates				
6	SARS-CoV-2				
6	IBD phage				
6	Tara oceans phage				

Results: Calculated order complexity from alignable sequences

kmer	Genomes	Exact	Progressive	dBG_L	dBG_LN
9	BRCA1 56 species				
9	BRCA1 primates				
9	SARS-CoV-2				
9	IBD phage				
9	Tara oceans phage				

Results: Calculated Quickwork from alignable sequences

Genomes	dBG(3)	dBG(4)	dBG(5)	dBG(6)	dBG(7)	dBG(8)	dBG(9)
BRCA1 56 species							
BRCA1 primates							
SARS-CoV-2							
IBD phage							
Tara oceans phage							

Sample unit tests: cyclic sequences

```
def test_pog_cycle(output_dir: Path):
    dbg = dbg_align.DeBrujinGraph(3,cogent3.DNA)
    dbg.add_sequence({
        "seq1": "ACAGTACGGCAT",
        "seq2": "ACAGTACTGGCAT",
        "seq3":"ACAGCGCGCAT" # contains cycle
        })
    with open(output_dir / "cycle.md", "w") as f:
        f.write("```mermaid\n")
        f.write(dbg.to_mermaid())
        f.write("```")
    assert dbg.has_cycles()
    assert len(dbg) == 3
    assert dbg.names() == ["seq1", "seq2", "seq3"]
    assert dbg["seq3"] == "ACAGCGCGCAT" # contains cycle

    dbg.to_pog()
    # write mermaid out to testout folder
    with open(output_dir / "cycle_compressed.md", "w") as f:
        f.write("```mermaid\n")
        f.write(dbg.to_mermaid())
        f.write("```")
```

Discussion

de Bruijn graphs offer an interesting method to

- Break the tautology at the heart of both Sequence alignment, and Phylogenetic reconstruction
- Reduce the impact of both sequence length and sequence number over traditional alignment approaches

This method may make some very big questions tractable

Future directions

Investigate the potential of using de Bruijn Graphs to;

- Identify reverse complimented regions from a dBG
- Identify genetic distance and infer phylogeny from a dBG
- Process sequences in databases storing dBG structures back to the database, reducing active memory limits for large numbers of large sequences
- Investigate advantage wrt species subject to lateral gene flow
  - eg: Bacteria, Archaea
  - identifying multi-rooted phylogenies
- Investigate using dBG's for targeted sequence extraction using pattern recognition templates (start and stop fragments similar to PCR primers)

Thanks

- Gavin Huttley
- Yu Lin
- Vijini Mallawaarachchi
- Xinjian Leng

... and the Huttleylab

Questions

Errata

> Abandon all hope ye who pass this point
>
> *Tolkein ... probably*

- Sequence alignment order complexity
- Pairwise sequence alignment methods
- Multiple sequence alignment strategies
- unit tests

Sequence alignment order complexity

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Pairwise sequence alignment

- Compare every letter in one sequence to every letter in the other
- order complexity of $O(mn)$
    - where **m** and **n** are lengths of the sequences

Multiple sequence alignment (MSA)

- Perform a pairwise alignment of every sequence to every other sequence
- order complexity of $O(L^n)$
    - where **L** is the length of the sequences
    - **n** is the number of sequences

Pairwise sequence alignment methods: $O(mn)$

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- Needlemann-Wunsch algorithm: global alignment for highly similar sequences
  - scoring system that penalises gaps and mismatches
- Smith-Waterman algorithm: better for local alignment to find conserved domains
  - allows for alignment to reset when the score falls to 0

Compare each nucleotide in one sequence to each nucleotide in the other sequence

Given a simple scoring system +1 match, -1 mismatch, -2 gap (δ)

Where $F(i,j) = \max \text{ of the following}$

$$\begin{aligned}
&\nwarrow F(i-1,j-1) + s(A_i, B_j), \quad (\text{match/mismatch}) \\
&\Uparrow F(i-1,j) + \delta, \quad (\text{deletion}) \\
&\Leftarrow F(i,j-1) + \delta, \quad (\text{insertion})
\end{aligned}$$

	gap	A	G	C	A	A
gap	***0***	⇐-2	⇐-4	⇐-6	⇐-8	⇐-10
A	⇑-2	⇖ ***1***	⇐ **-1**	⇐-3	⇐-5	⇐⇖-7
C	⇑-4	⇑-1	⇖0	⇖ ***0***	⇐-2	⇐-4

Multiple sequence alignment (MAS) strategies

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- Pairwise alignment of each possible pair
  - $\binom{n}{2} \times O(L^2) = \frac{n(n-1)}{2} \times O(L^2) = O(n^2.L^2)$
- Progressive alignment eg: ClustalW
  - create a guide tree
  - Progressively align pairs most closely related to profiles, and then align profiles
- Iterative methods eg: MUSCLE, T-Coffee, MAAFT
  - create an preliminary fast less accurate alignment
  - iteratively improve alignment using some scoring function
  - Complete when some convergence criterion is met
- Hidden markov models $O(nL) + O(LM)$ (M is the number of states in the model)
  - eg: HMMER
  - create a statical model of the transition between states
  - Determine likely alignment based on the model

Unit tests

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library against edge case sequence alignments
* long sequences
* numerous sequences
* cyclic sequences
* bubbles within bubbles
* sequential bubbles