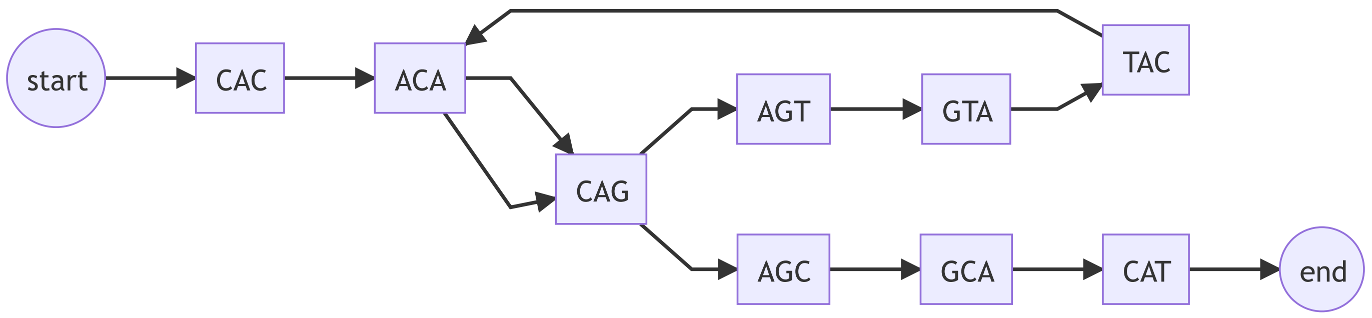
BIOL8706: Dividing and conquering sequence alignment using De Bruijn Graphs



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

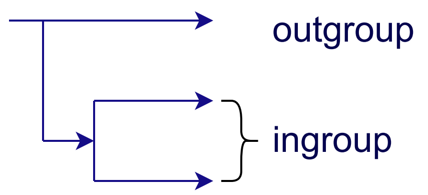
Australian National University

Sequence alignment

Sequences $\longrightarrow$ **Alignment** $\longrightarrow$ **Phylogenetic relationship**

Sequence A	[ACAGTACTGACAT]
Sequence B	[ACAGCTGCAT]
Sequence C	[ACAGCTACAT]

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



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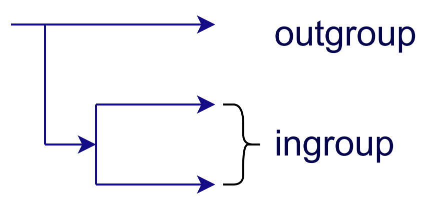

We start with a set of DNA sequences (Note all different lengths)

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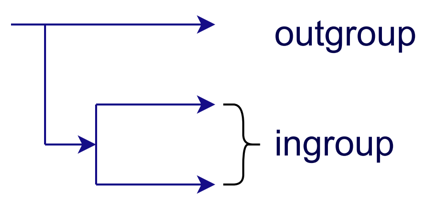
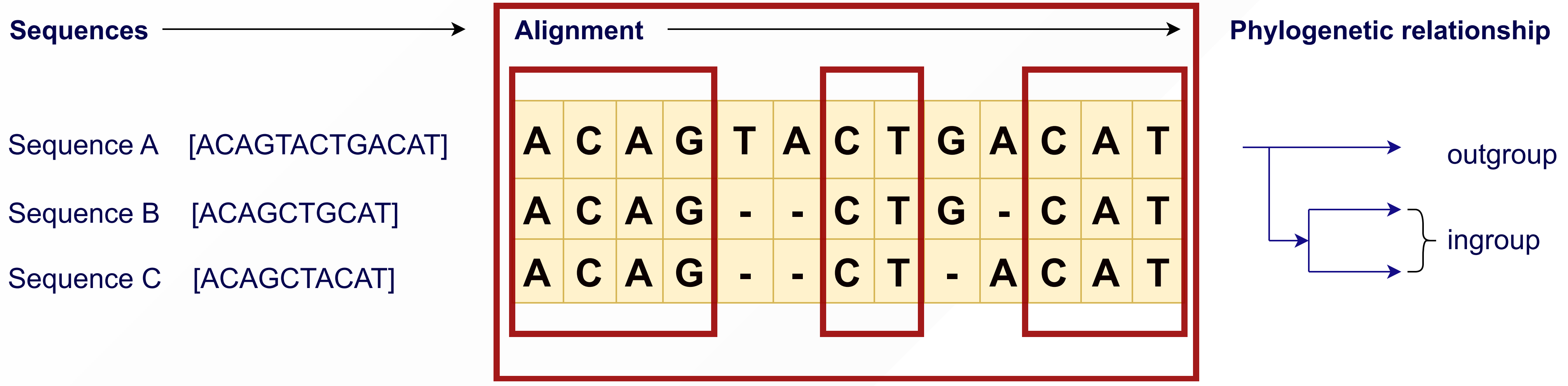


We align those sequences

Sequence alignment


Sequences
Alignment
Phylogenetic relationship
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A C A G T A C T G A C A T
A C A G - - C T G - C A T
A C A G - - C T - A C A T
outgroup
ingroup

By lining up regions that are similar

Sequence alignment

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

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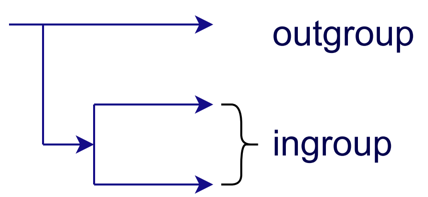

Noting those that are different

Sequence alignment

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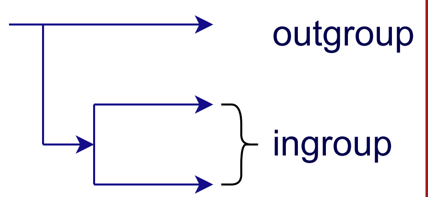

And we can infer evolutionary relationships between those sequences

- ingroup (1 letter different)
- outgroup (3 letters different)

Sequence alignment

Sequences → **Alignment** →

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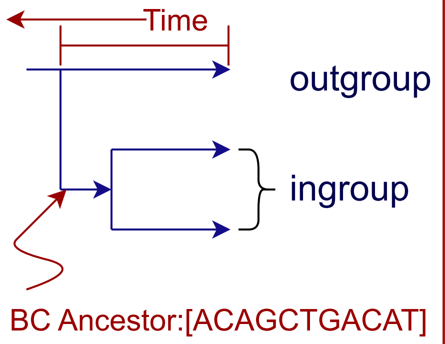
Phylogenetic relationship



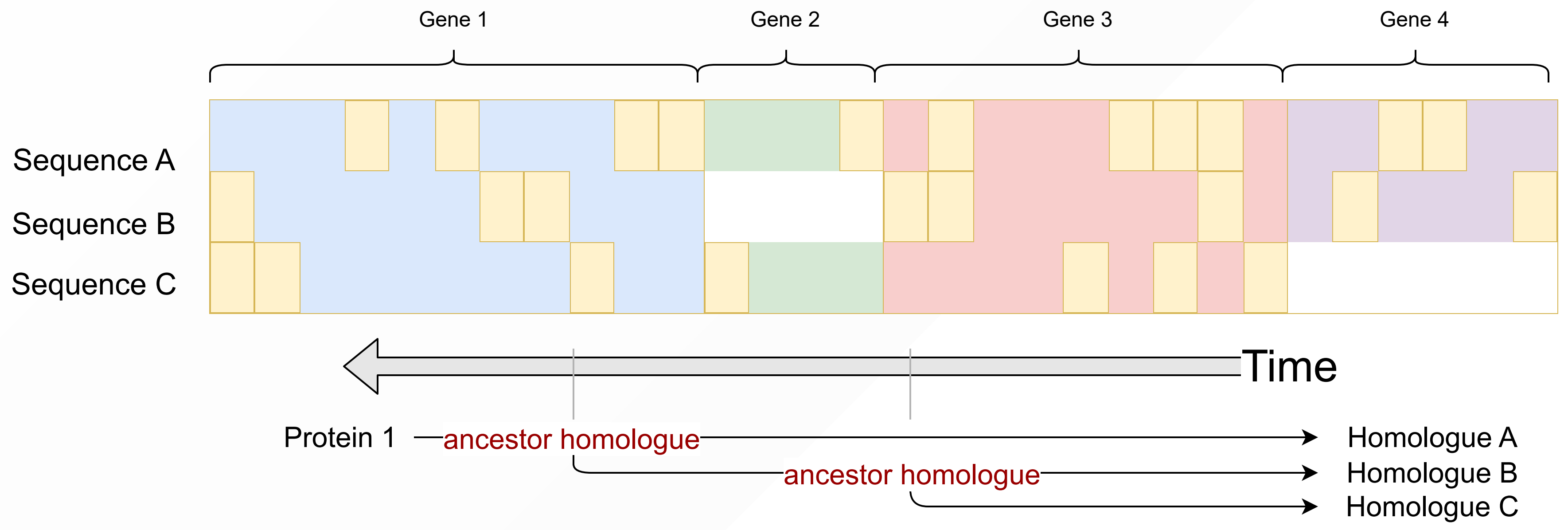
And we can infer evolutionary relationships between those sequences

- ingroup (1 letter different)
- outgroup (3 letters different)
- extinct common ancestor sequence
- how long ago sequences diverged

The central dogma of biology

$$\text{DNA} \xrightarrow{\text{transcribe}} \text{RNA} \xrightarrow{\text{translate}} \text{Protein}$$

If we can align homologous genes, we can infer homologous proteins



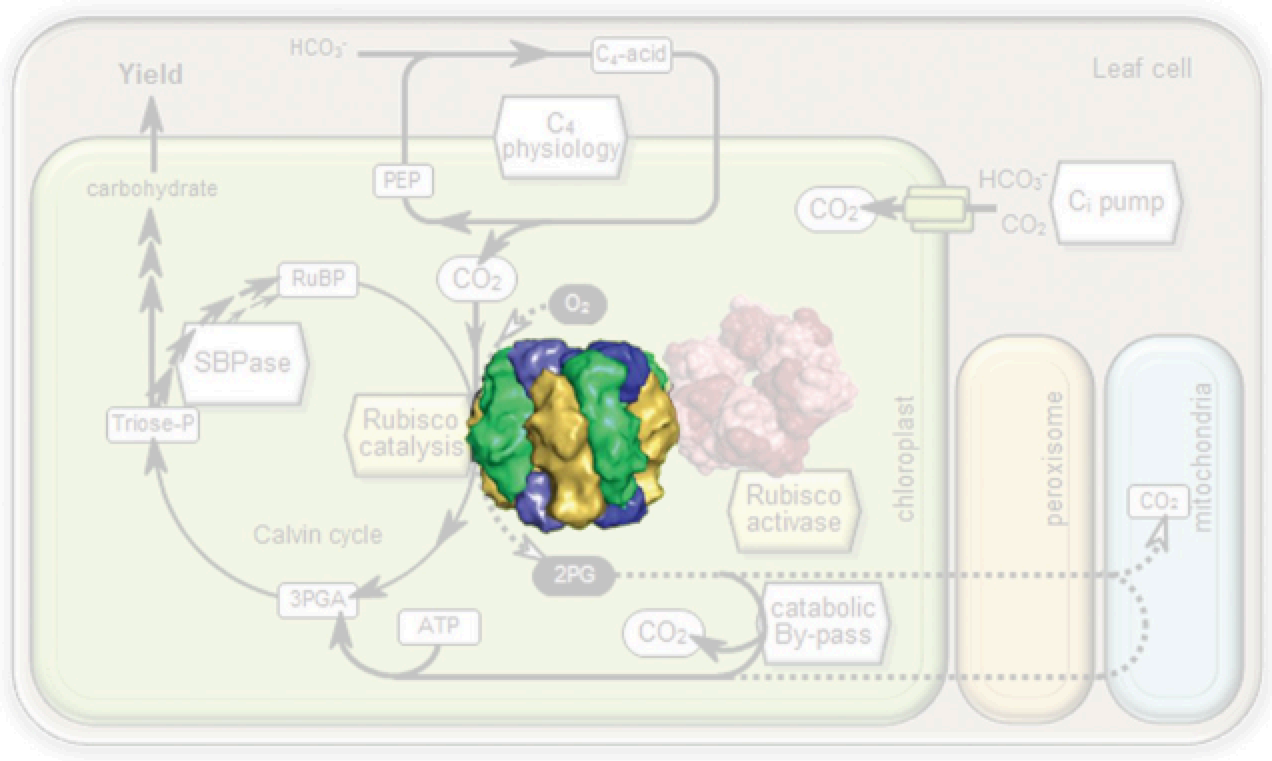
Sequence alignment is a **time machine** for homologous proteins

3 big ideas that require sequence alignment

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

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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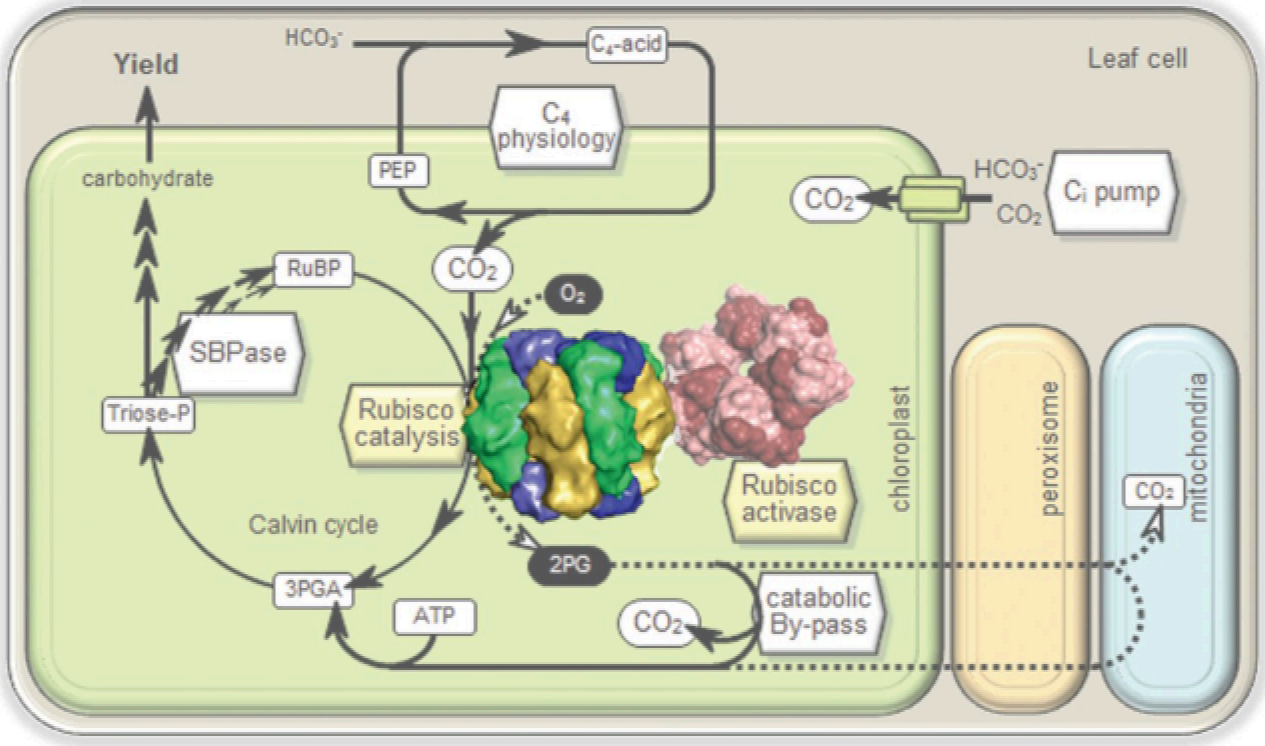

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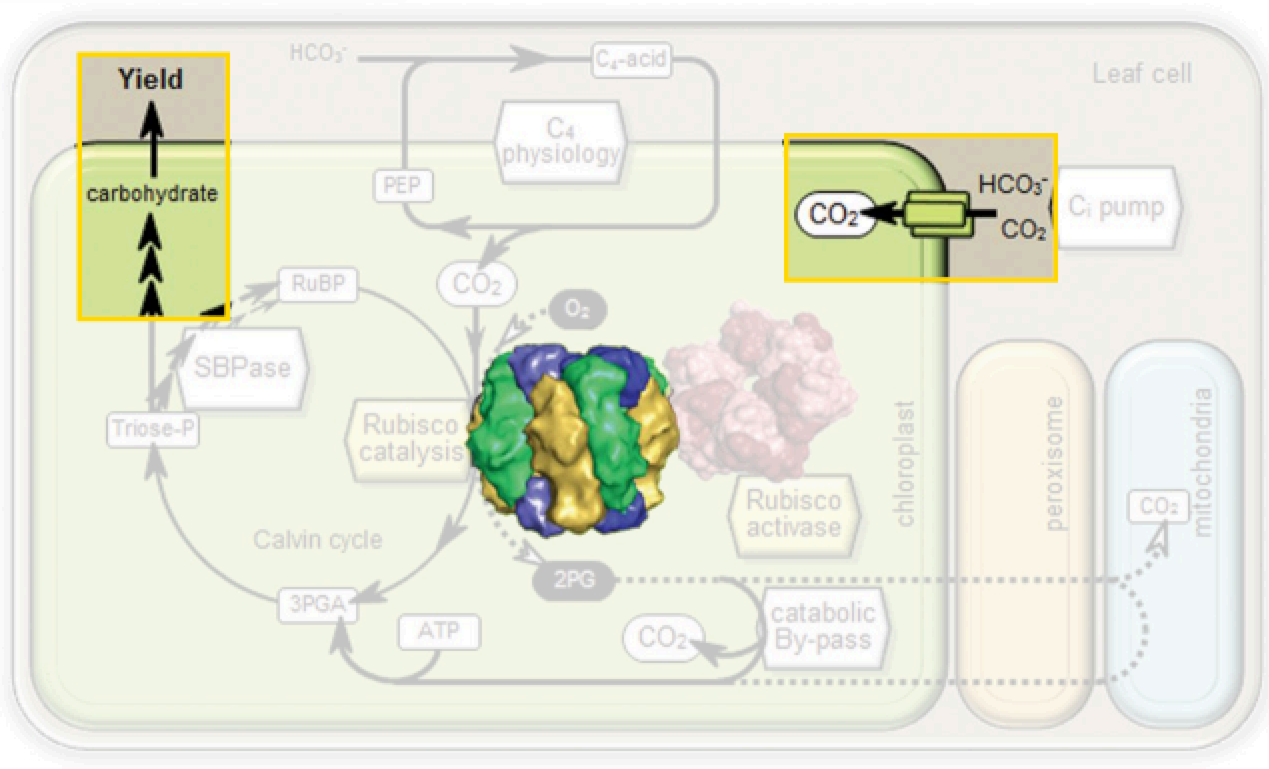
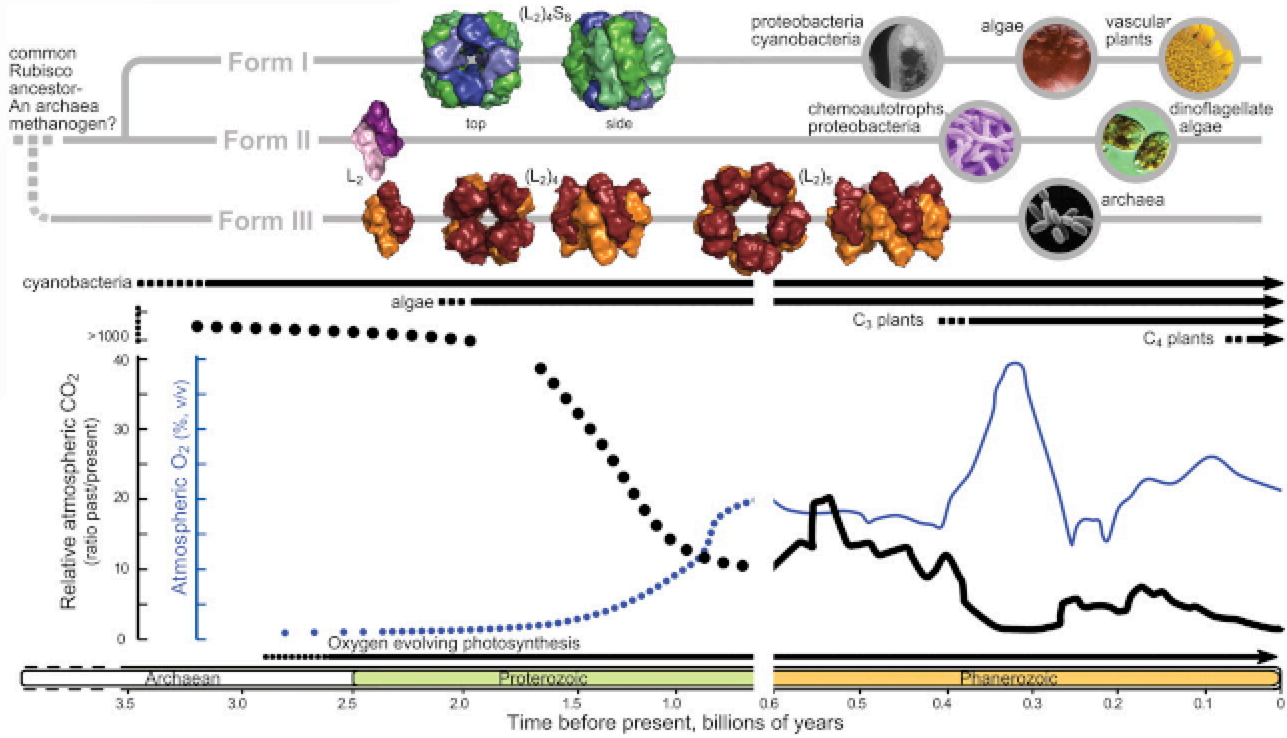


Evolution of RuBisCO

- 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](https://doi.org/10.1104/pp.110.164814)

The value of understanding RuBisCO’s features?

Q: Can we design more efficient RuBisCO?

$\Uparrow$ RuBisCO efficiency would lead to

- Crop yield $\Uparrow$
- Carbon sequestration $\Uparrow$

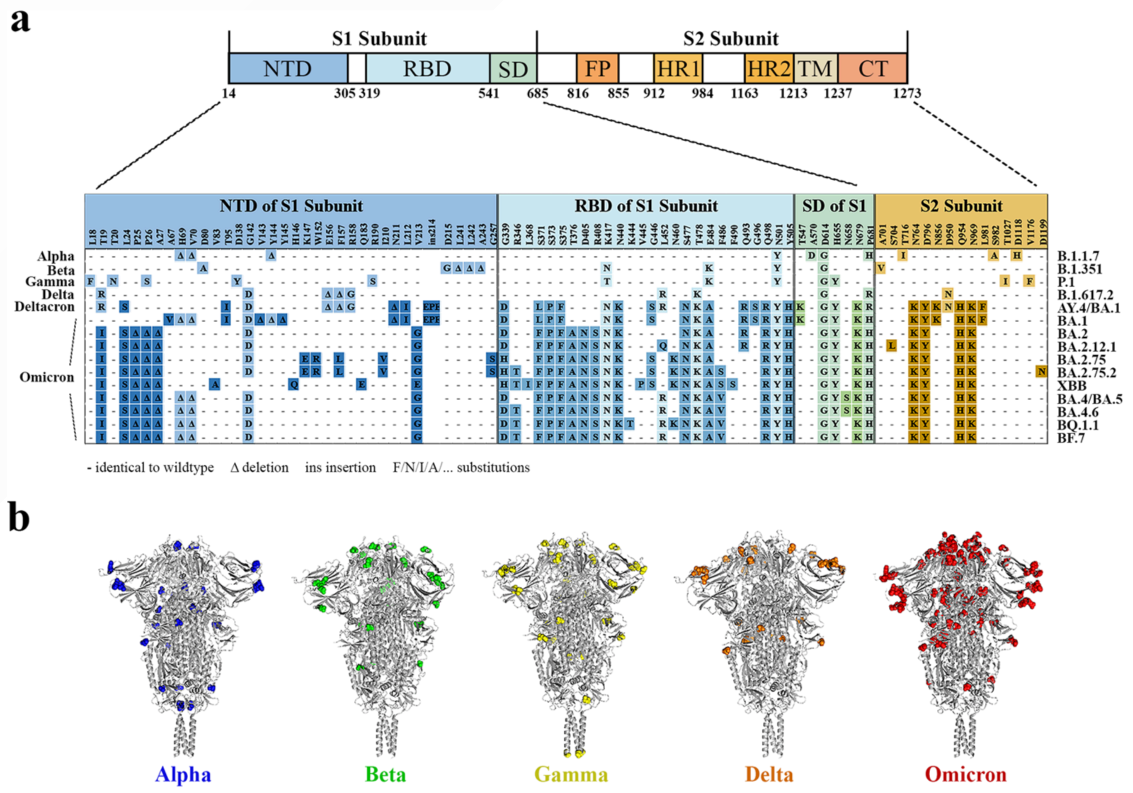
This is a big question that sequence alignment can contribute to answering

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 virus's trajectory

These are big questions that sequence alignment can contribute to answering

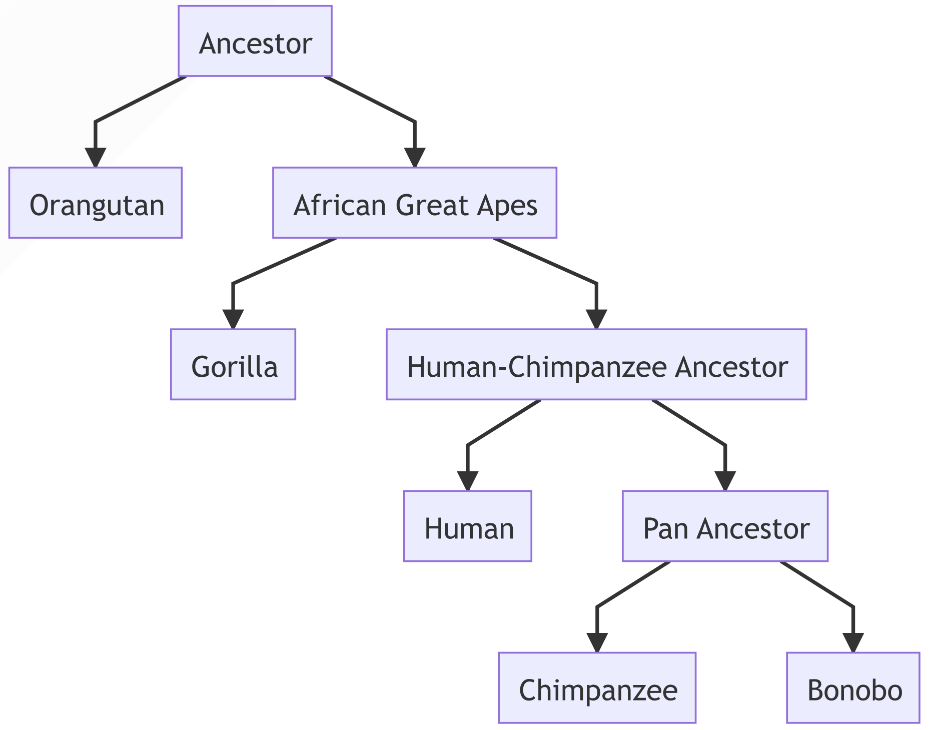


Alignment of S mutation points of SARS-CoV-2 variants

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 evil jigsaw puzzle
- Since 1972 it's become a computational problem
- The task is to compare **each** letter in **each** sequence with **all** the letters of **every** other sequence.

- The terms: **each**, **all** and **every** should tell you 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

$O(L_1 \times L_2 \times \ldots L_n)$ **or** $O(L^n)$

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 the order of $Work(L^n)$

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]	$\text{millions}^{\text{hundreds of thousands}}$
SARS-CoV-2	~29 kbp	>5M[2]	$\text{29 thousand}^{\text{5 million}}$
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 in GISAID and other public repositories as of Oct 2021 - www.nature.com/articles/s41588-022-01033-y

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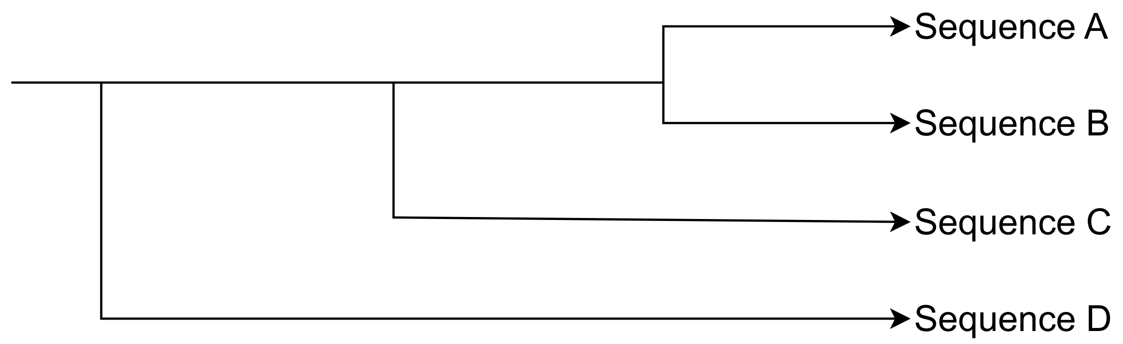
Progressive alignment

Progressive alignment is a strategy that reduces the work required

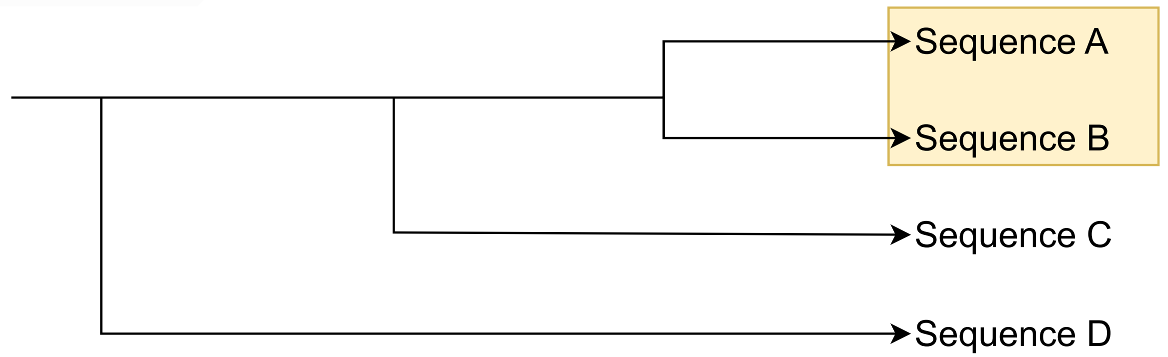


Progressive alignment

Progressive alignment is a strategy that reduces the work required

Strategy:

- align the 2 most closely related sequences into a statistical model called a profile



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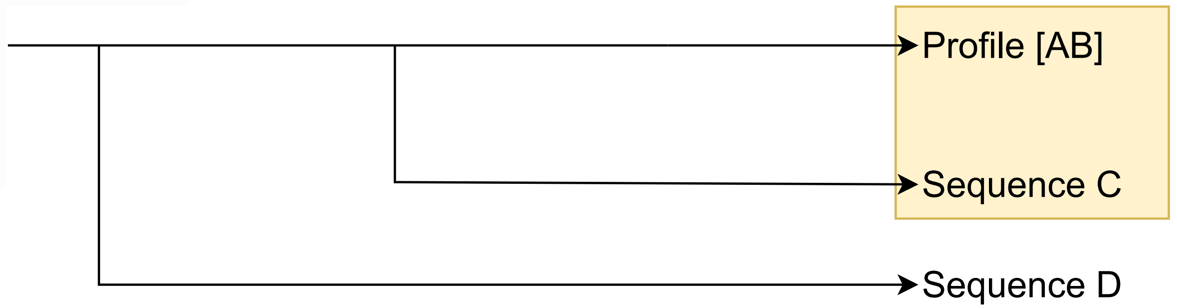
- align that profile with the next most closely related sequence



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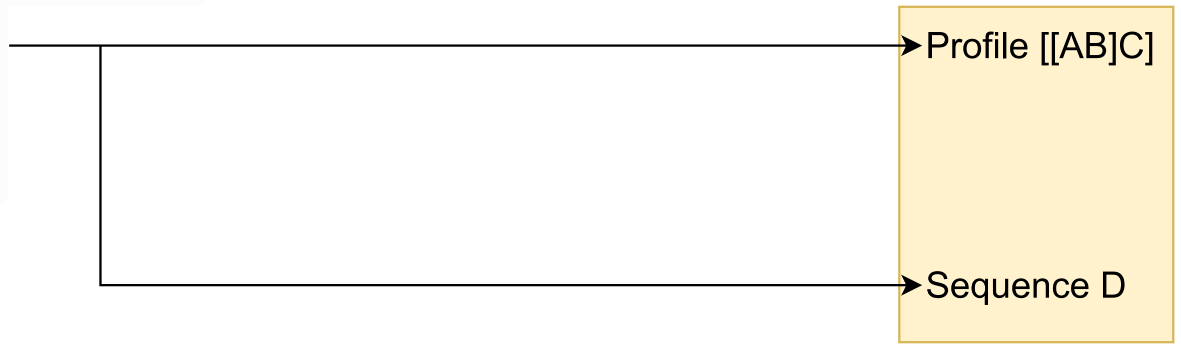

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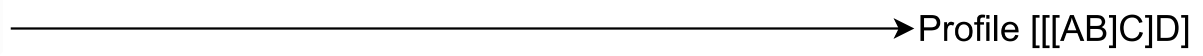
This reduces the order of $Work(L^n) \rightarrow Work(n^2.L^2)$
... which is a lot less $Work$



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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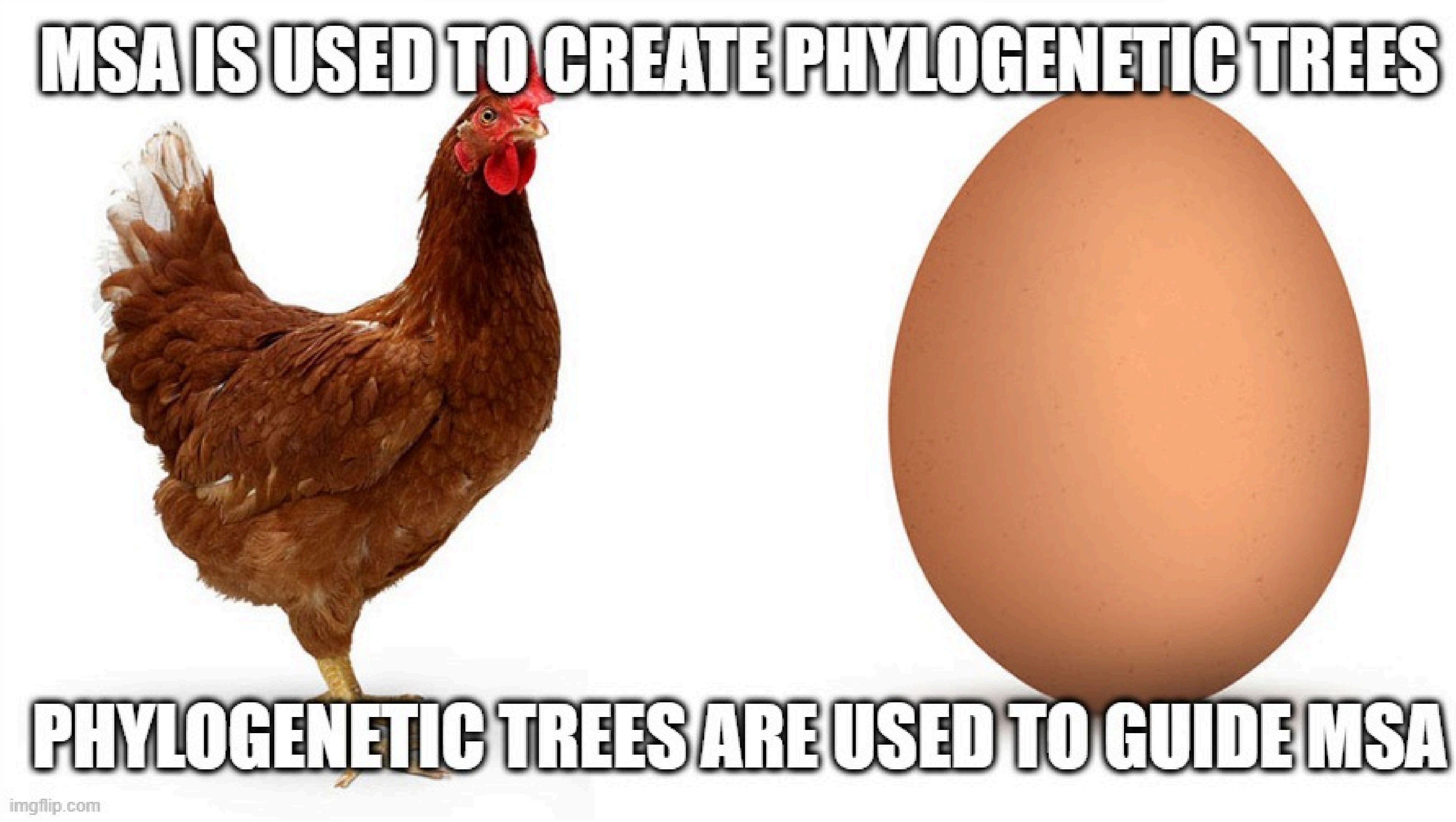
Do you see the problem?


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

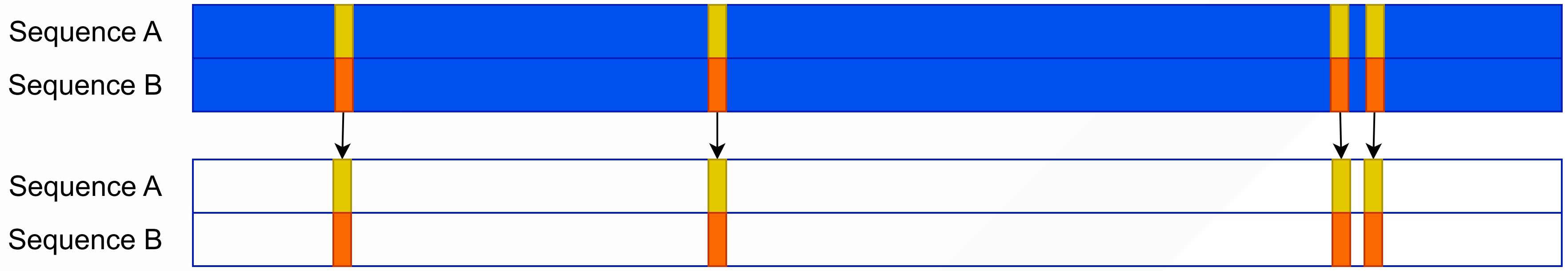
Sequence alignment is sensitive to

- 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?

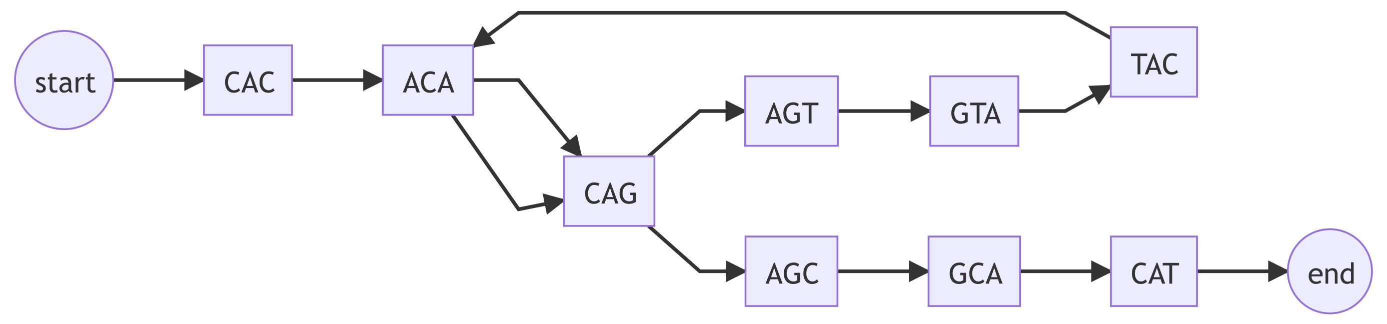


We'd could focus resources on just the regions that differ

Sequence alignment using De Bruijn Graphs

My work builds upon 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



You might have heard of de Bruijn graphs for sequence assembly from reads.

De Bruijn graphs can also be used for sequence alignment.

De Bruijn graphs

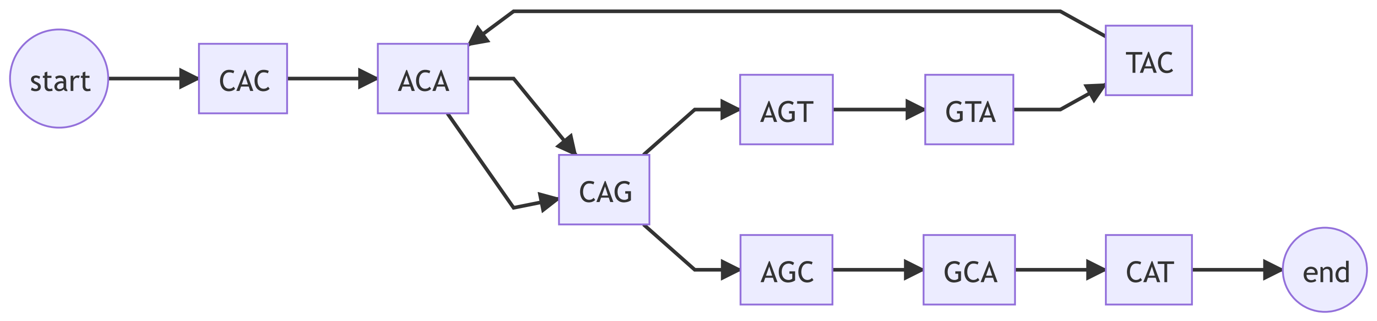
A De Bruijn graph is a directed graph that represents unique overlapping subsequences

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

This "Work" scales linearly not exponentially.

Consider the following sequence as a de Bruijn graph of order 3 (nodes overlap by 2 characters):

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



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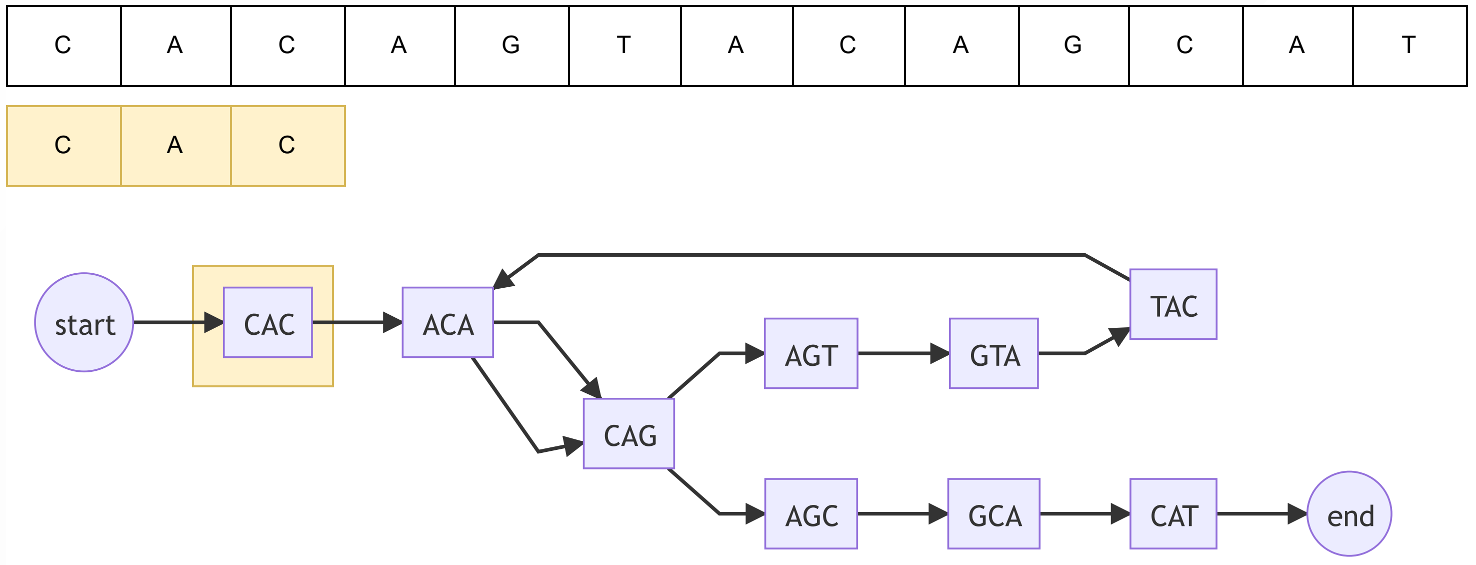

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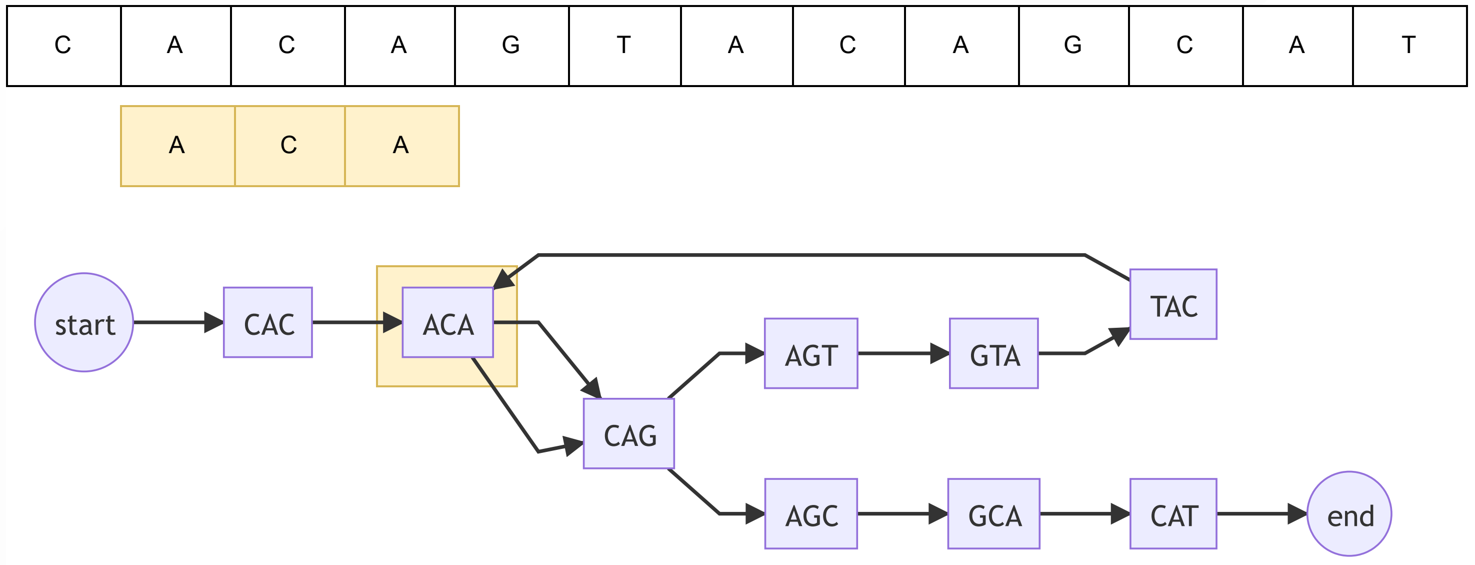

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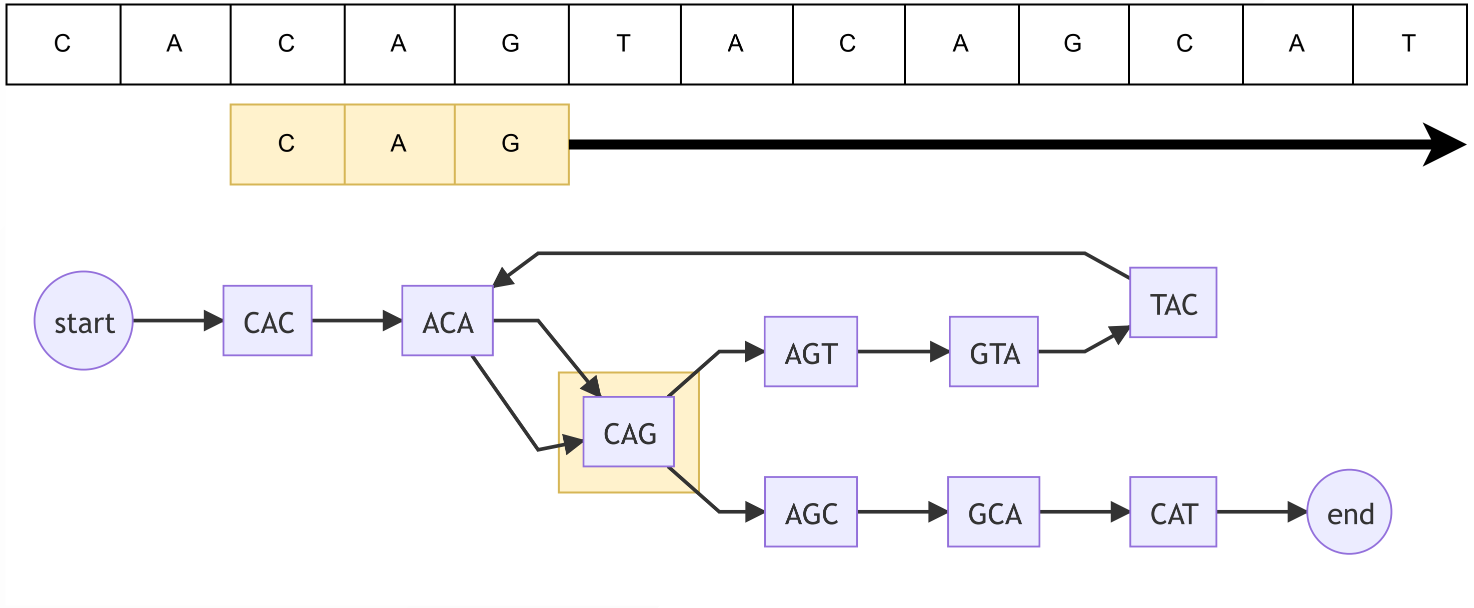
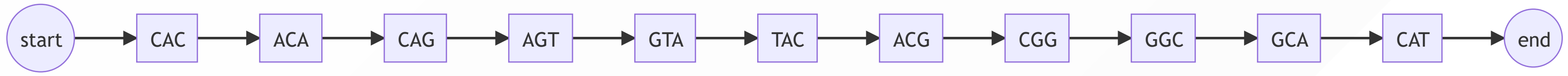


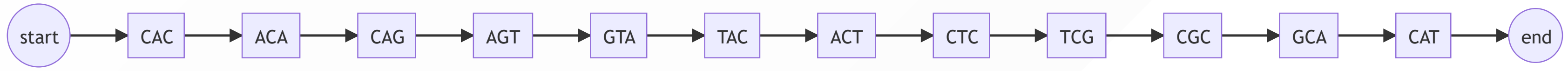
And we keep going until we have created the graph

Reducing the length of sequence to be aligned

Sequence A: `CACAGTACGGCAT`



Sequence B: `CACAGTACTGCAT`



These differ in just one nucleotide in the middle of the sequence

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

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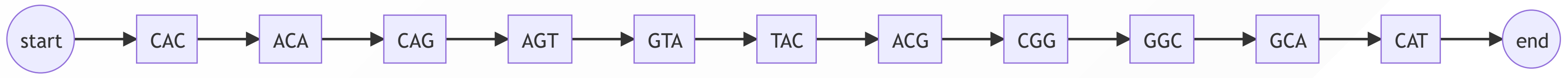
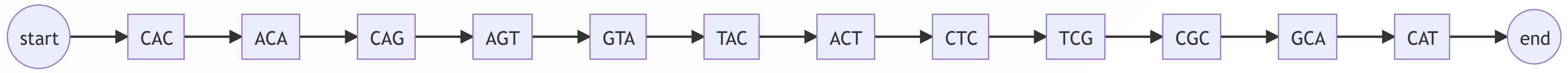


Sequence B: `CACAGTACTGCAT`



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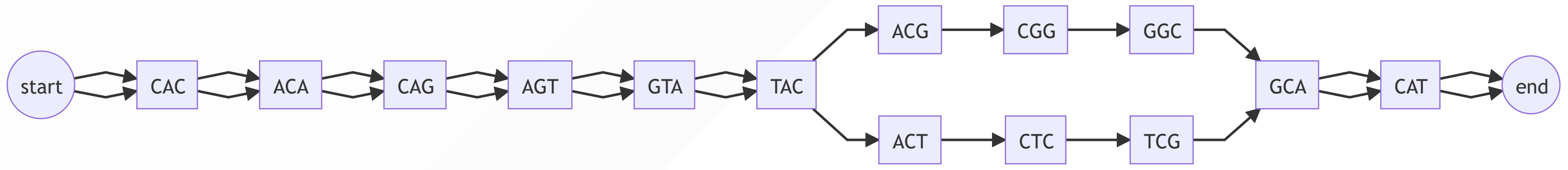
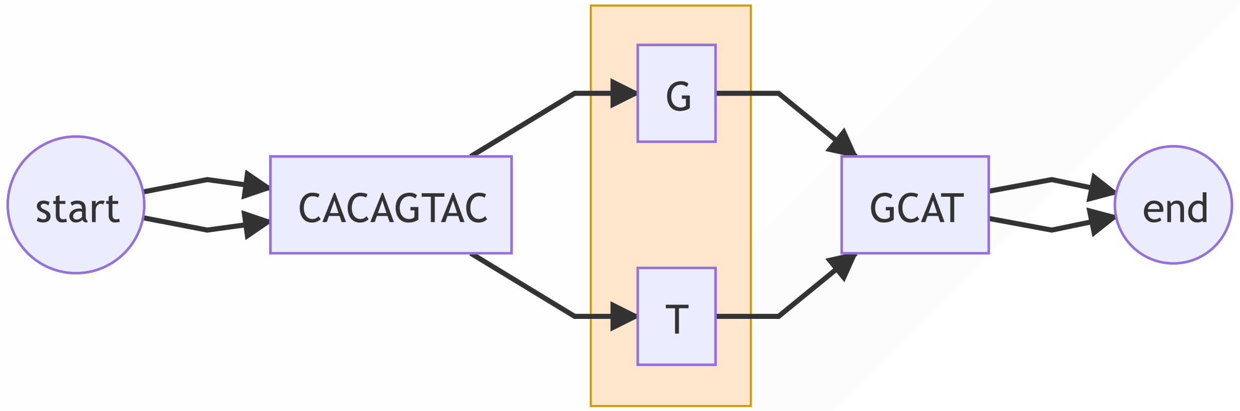


Reducing the length of sequence to be aligned

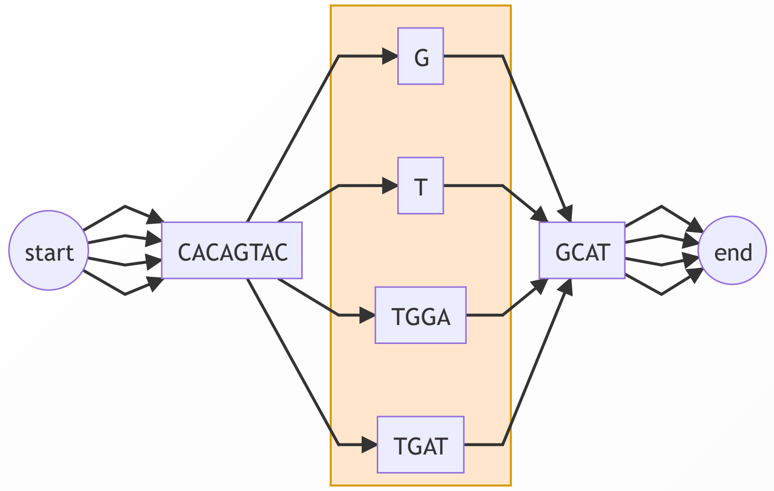
If we transform the graph into a partial order graph, we can see the regions that are similar which we don't need to align, and the regions that are different (in the gold box) which we do.



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

De Bruijn multiple sequence alignment

And we can extend this trivially to multiple sequences. Consider aligning 4 sequences



Now we've reduced Work(13x13x16x16) down to Work(1x1x5x4)

= **2,163x** less “work”

Taking the de Bruijn graph to the next level

- recall exact alignment has a Work order of $Work(L^n)$ or $Work(L_1 \times L_2 \times \cdots \times L_n)$
- if we reduce the length of the sequences we need to align then we reduce L

Taking the de Bruijn graph to the next level

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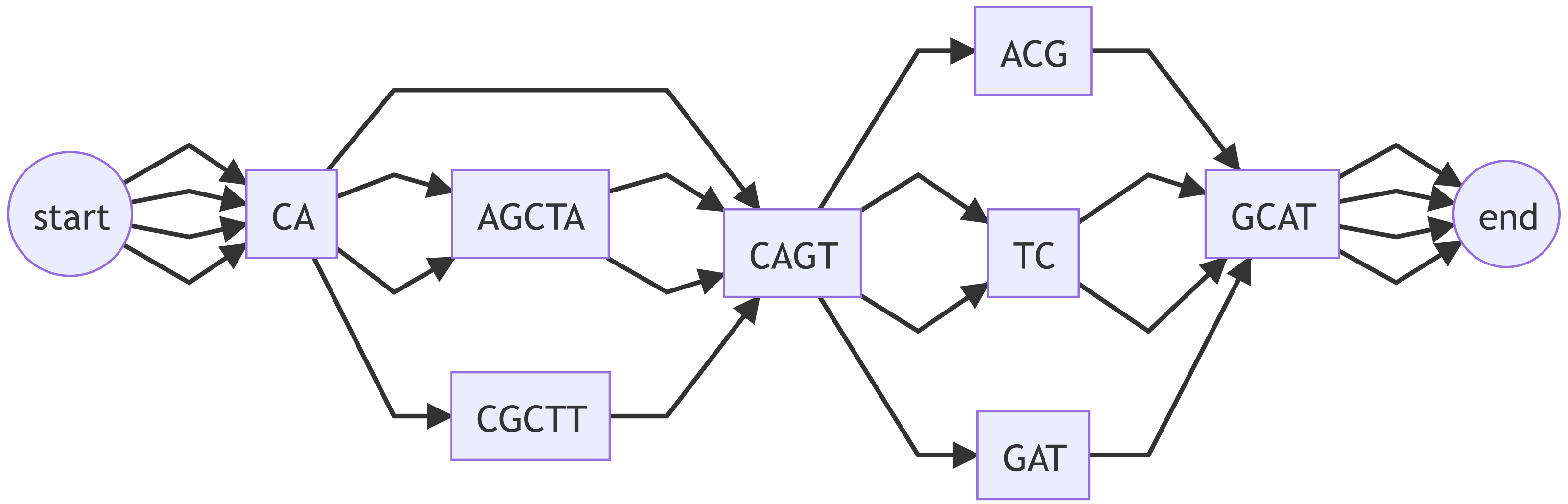
Can we change the number of sequences to align?

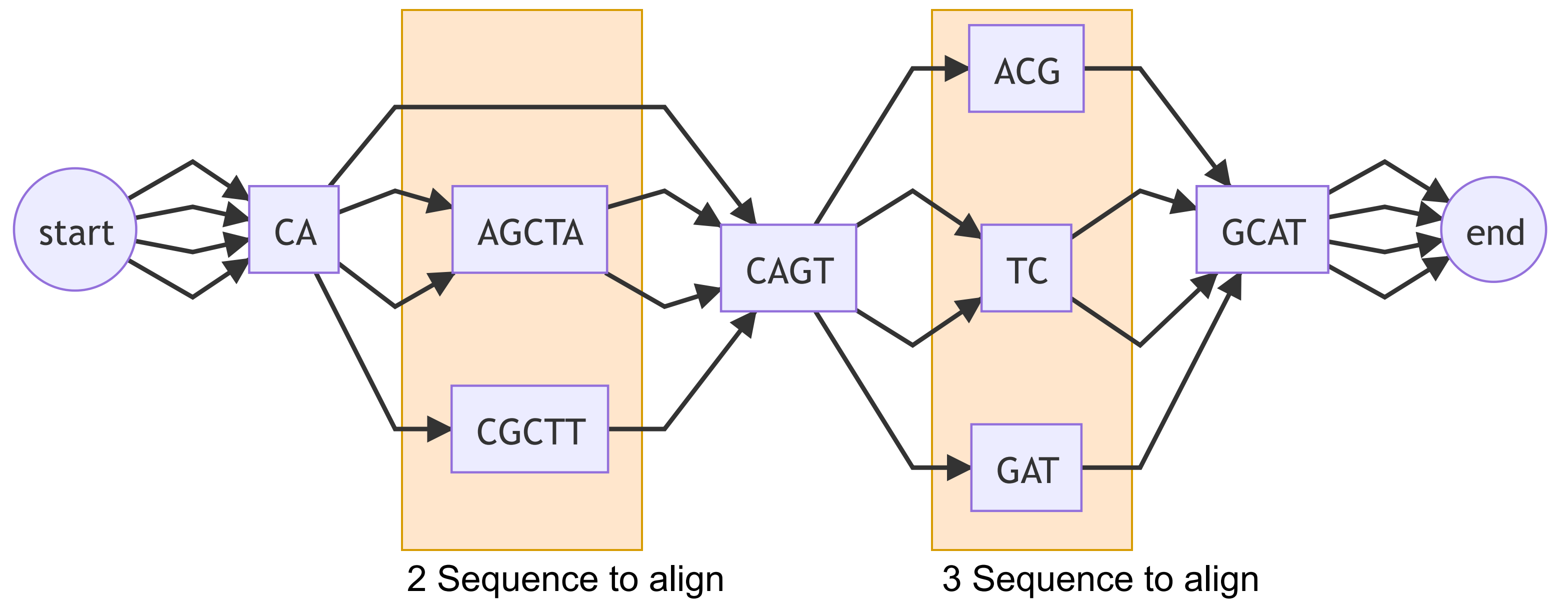
Reducing the **number** of sequences to be aligned

Consider this partial order graph containing 4 sequences with overlaps removed



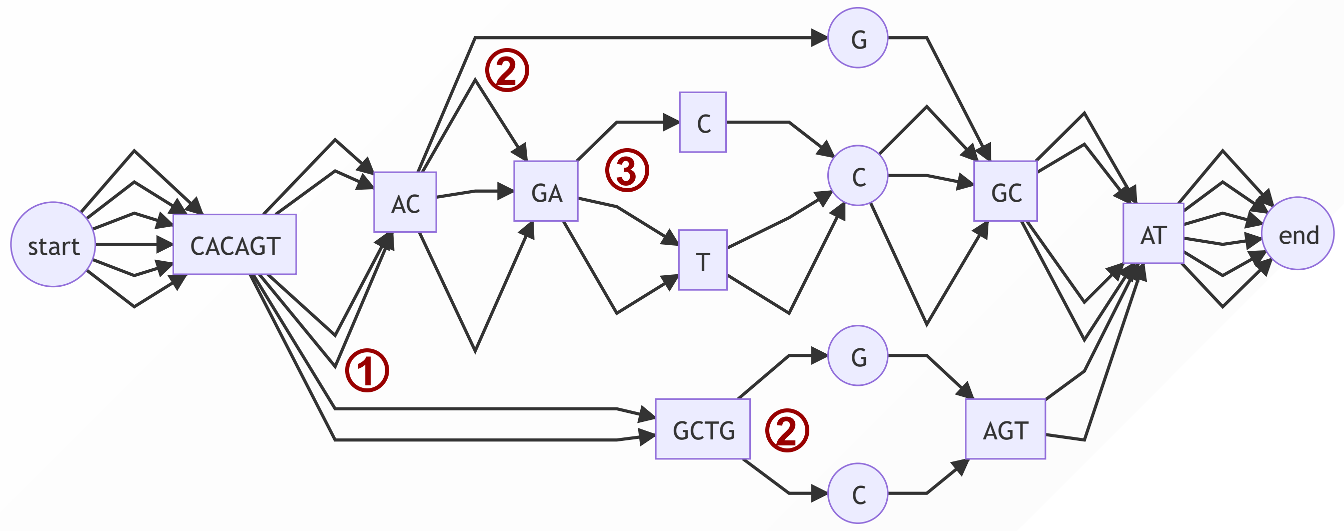
Reducing the **number** of sequences to be aligned

Consider this partial order graph containing 4 sequences



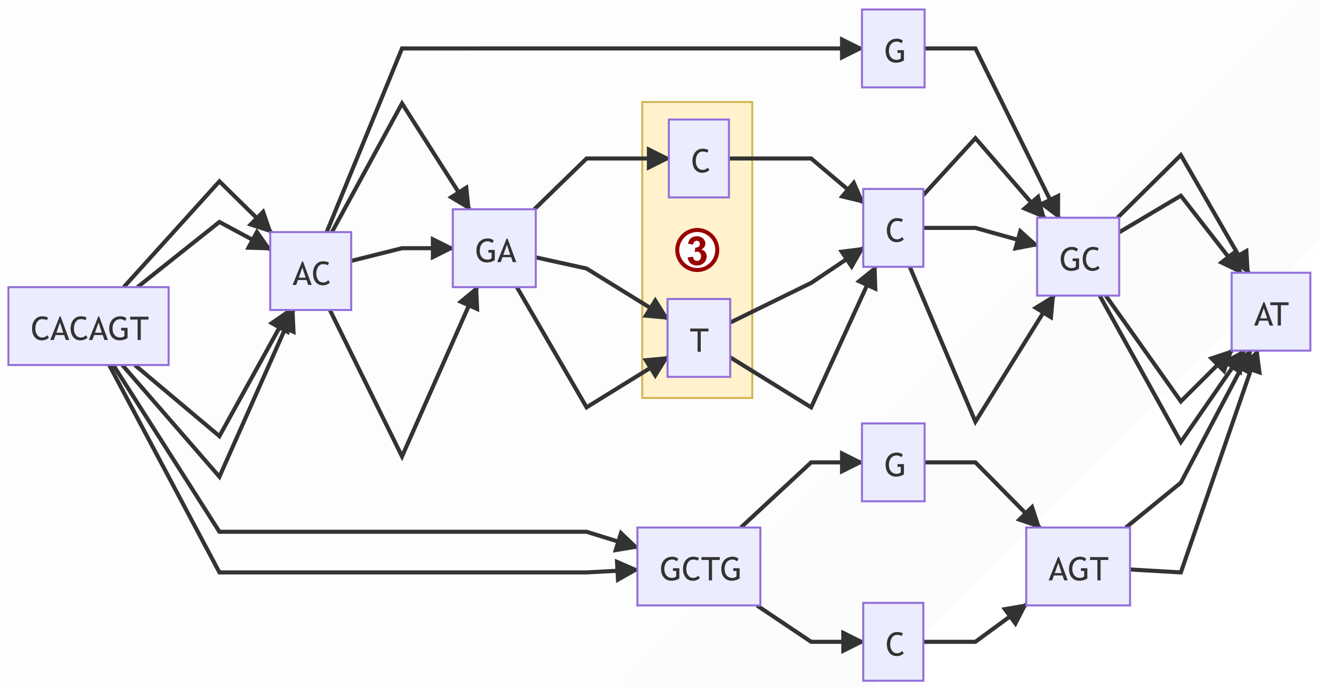
exhaustive alignment	reduce length	reduce length & number
$Work(13 \times 14 \times 17 \times 17)$	$Work(5^4 + 3 \times 2 \times 2 \times 3)$	$Work(5^2 + 3 \times 2 \times 3)$
52,598	661 (**79x** vs exhaustive)	43 (**1223x** vs exhaustive)

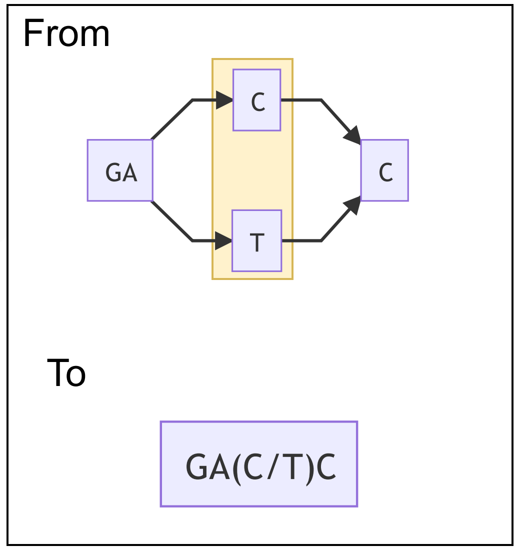
Reduce the dependence on the phylogeny



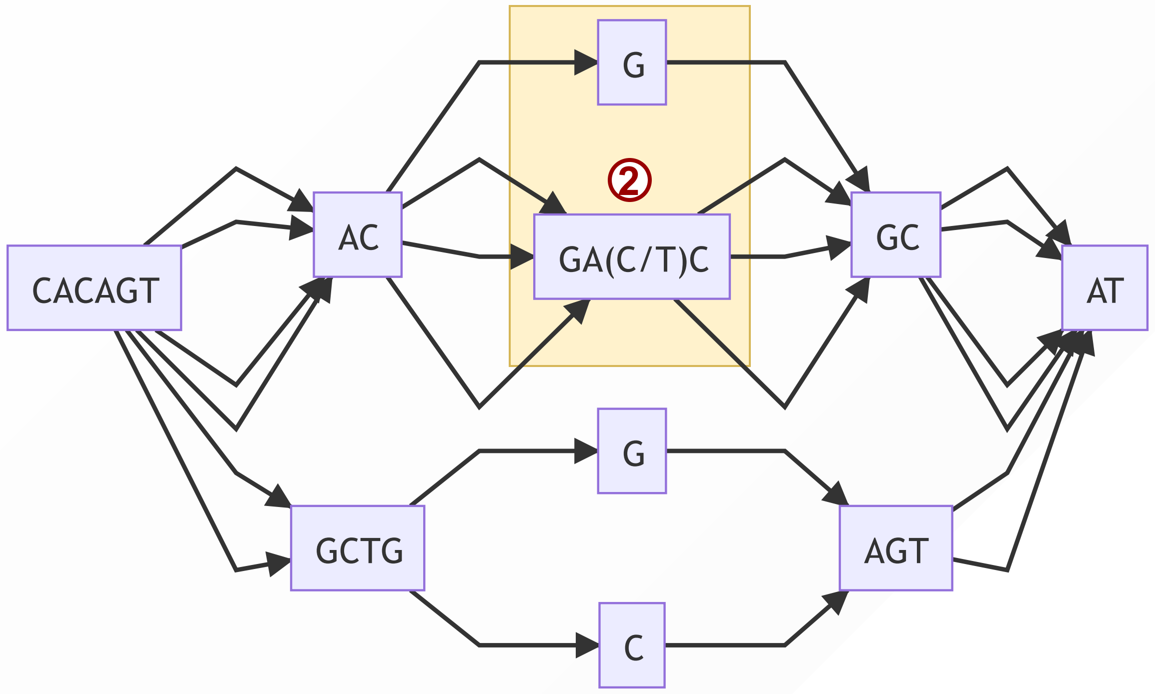
By ordering progressive alignment by descending “bubble” depth, we can progressively align without needing to know in advance the phylogenetic relation between sequences.

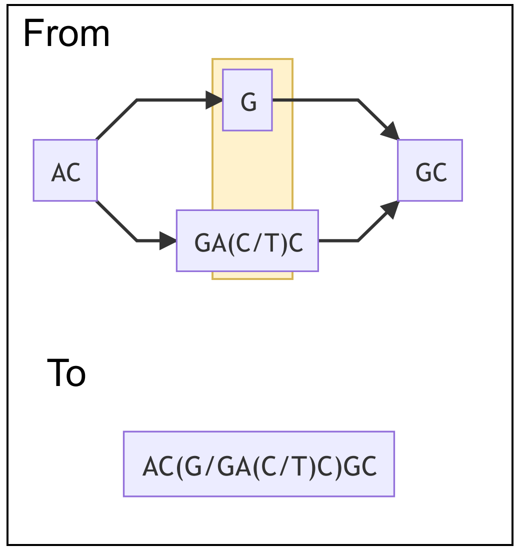
Reduce the dependence on the phylogeny



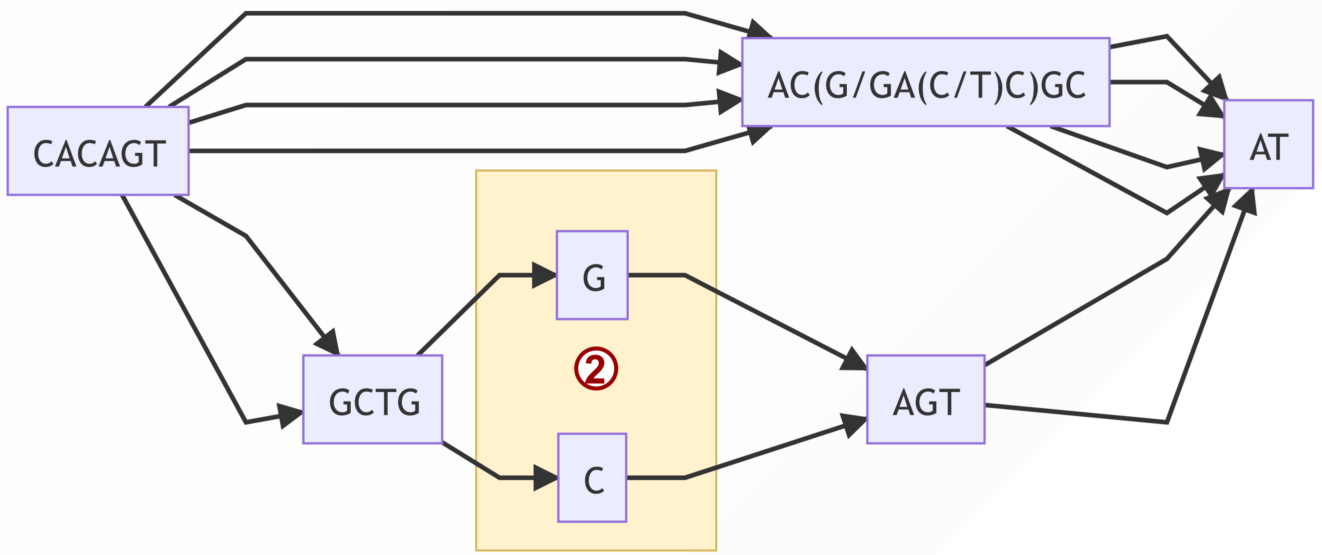


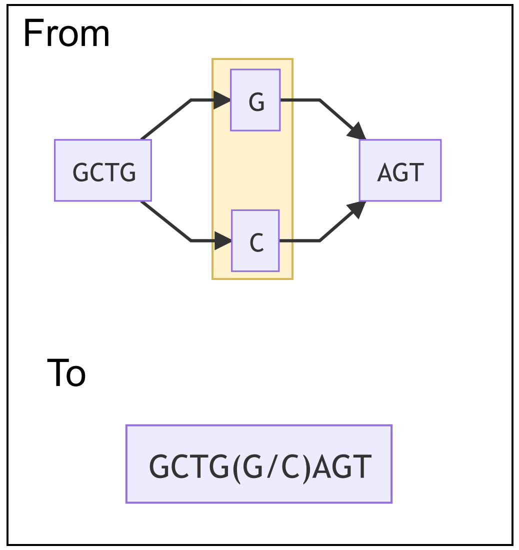
Reduce the dependence on the phylogeny



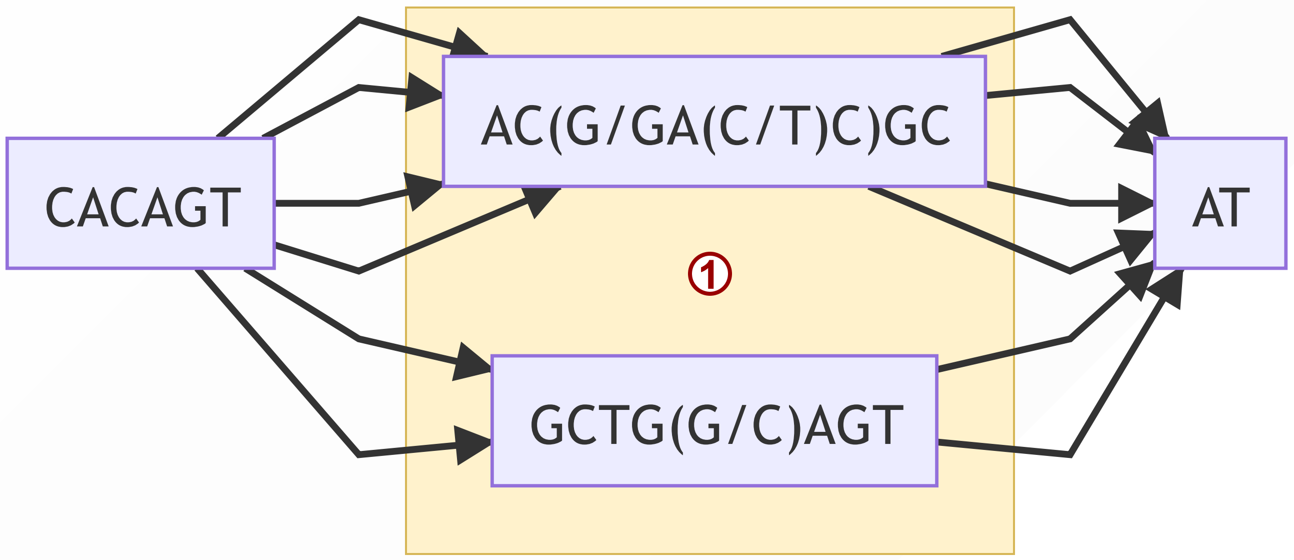


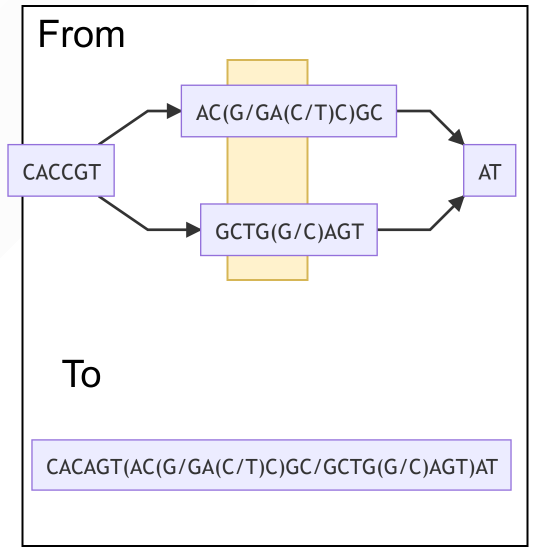
Reduce the dependence on the phylogeny





Reduce the dependence on the phylogeny





Reduce the dependence on the phylogeny

CACAGT(AC(G/GA(C/T)C)GC/GCTG(G/C)AGT)AT

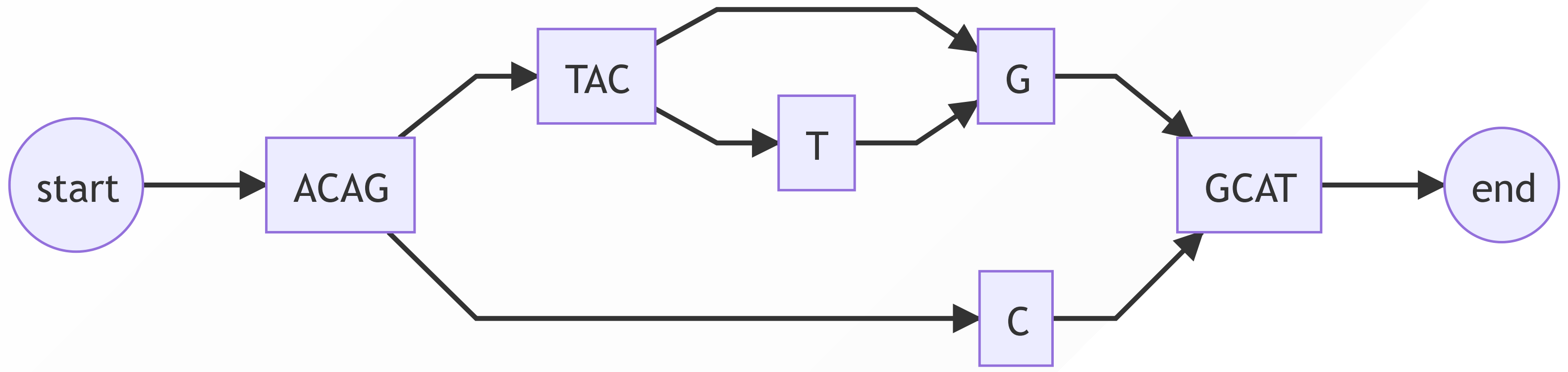
Alignment completed without requiring a phylogenetic guide tree

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 of segments to align
  - identify “bubbles”
  - Develop unit tests to verify correctness
- Develop statistics for de Bruijn graphs to predict efficiency

Results: Work statistic

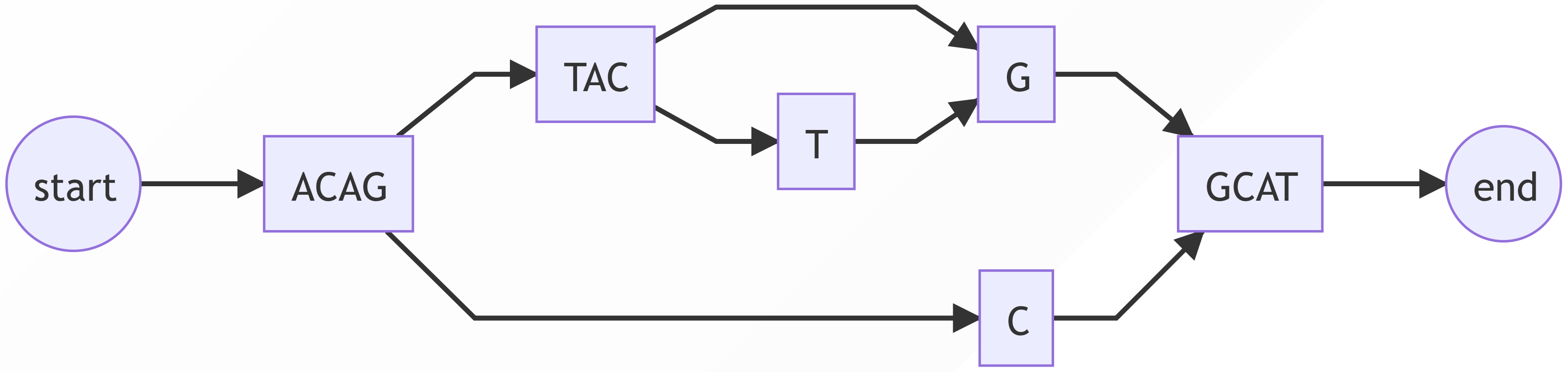
Consider the same order 3 de Bruijn graph



- Work calculates the order of alignment work using 4 strategies
  - Exact = $13 \times 12 \times 9 = 1404$
  - Progressive = $13 \times 12 + 13 \times 9 = 285$
  - DBG_L = $4 \times 5 + 5 \times 1 = 25$ (simplification of sequence length)
  - DBG_LN = $0 \times 1 + 5 \times 1 = 5$ (simplification of sequence length and count)

Results: Quickwork statistic

Consider this order 3 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 ACAG and GCAT (already aligned sections) removed TAC+T+G+C

Quickwork = $\sum \text{non aligned node length}$ = 6
Quickwork has an order of $Work(node\_count)$

Results: Calculated order complexity

Sample Sequence sets

Genomes	Average Length (bp)	Number	Description
BRCA1_divergent	~3k	56	BRCA1 gene divergent sample of 7 chosen from among 56 mammal species
BRCA1_hominae	~3k	4	BRCA1 gene from 4 hominae
SARS-CoV-2	30k	22	SARS-CoV-2 genomes (citation needed)
IBD_phage	40k	60	IBD phage components (https://doi.org/10.1016/j.cell.2015.01.002)
Ocean_phage	40k	130	Tara oceans phage components (https://doi.org/10.1126/science.1261605)

Work metric	Description
Exact	Exhaustive alignment
Progressive	Progressive alignment
dBG(3)_L	De Bruijn graph of order 3 with length simplification
dBG(3)_LN	De Bruijn graph of order 3 with length and number simplification
dBG(k)_LN	De Bruijn graph of order k with length and number simplification

Results: Calculated order of Work

Genomes	Exact	Progressive	dBG(3)_L	dBG(3)_LN	dBG(4)_LN	dBG(5)_LN	dBG(6)_LN	dBG(7)_LN	dBG(8)_LN
BRCA1_divergent									
BRCA1_hominae									
SARS-CoV-2									
IBD_phage									
Ocean_phage									

Results: Quickwork

Genomes	dBG(3)	dBG(4)	dBG(5)	dBG(6)	dBG(7)	dBG(8)	dBG(9)
BRCA1_divergent							
BRCA1_hominae							
SARS-CoV-2							
IBD_phage							
Ocean_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

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

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
- Identify strategies for chosing the ideal kmer length for a given set of sequences

Thanks

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

… and the Huttleylab

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… and the Huttleylab

Q&A

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

<<Back to Errata

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: $O(mn)$

<<Back to Errata

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

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

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

Multiple sequence alignment (MAS) strategies

<<Back to Errata

- 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

<<Back to Errata

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