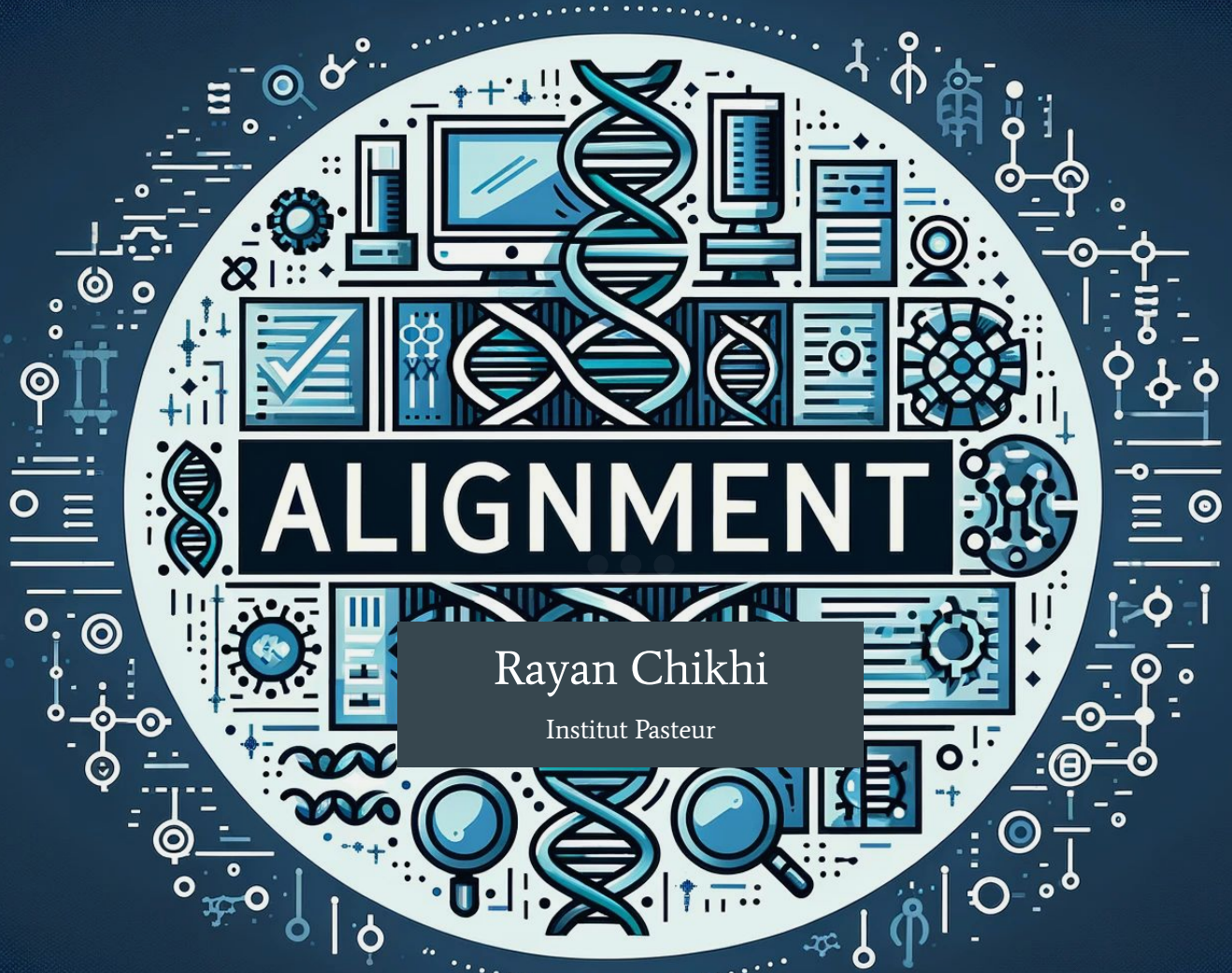


Rayan Chikhi

Institut Pasteur

Hello again!

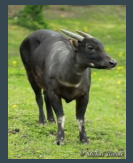
Sequence Bioinformatics

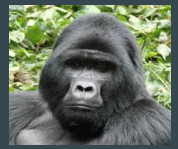
INSTITUT PASTEUR

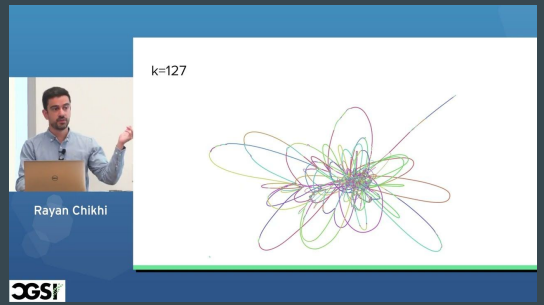
- I'm a researcher in bioinformatics algorithms
- *de novo* assembly, big data alignment, k-mers, pangenomics. Well, week 1 stuff :)

@RayanChikhi on Twitter

`http://rayan.chikhi.name`







Course objectives

- **Enough background** to understand the alignment part of a biology article
- Increase confidence in using alignment tools
- Understand **why** alignment isn't so straightforward

Course outline

- **Fundamentals**
- The many **flavors** and **tools** for pairwise DNA alignment
- **Multiple** sequence alignment
- Alignment to **databases**
- Into the unknown: profile and structure search

Questions to the audience

1. Have you ever **run** a sequence alignment software?
2. Was it **willfully** or as part of a pipeline?
3. Done **multiple** sequence alignment?
4. Know who/what **Smith-Waterman** is?

What's an "alignment"?

```
…C   A   T   A   G…
…C   G   T   -   G…

…match  mismatch  match  deletion  match…
```

Given two (or more) sequences, determine how the residues best **line up**, to capture **evolutionary relationships**.

The many types of alignments

Pairwise (2 sequences)

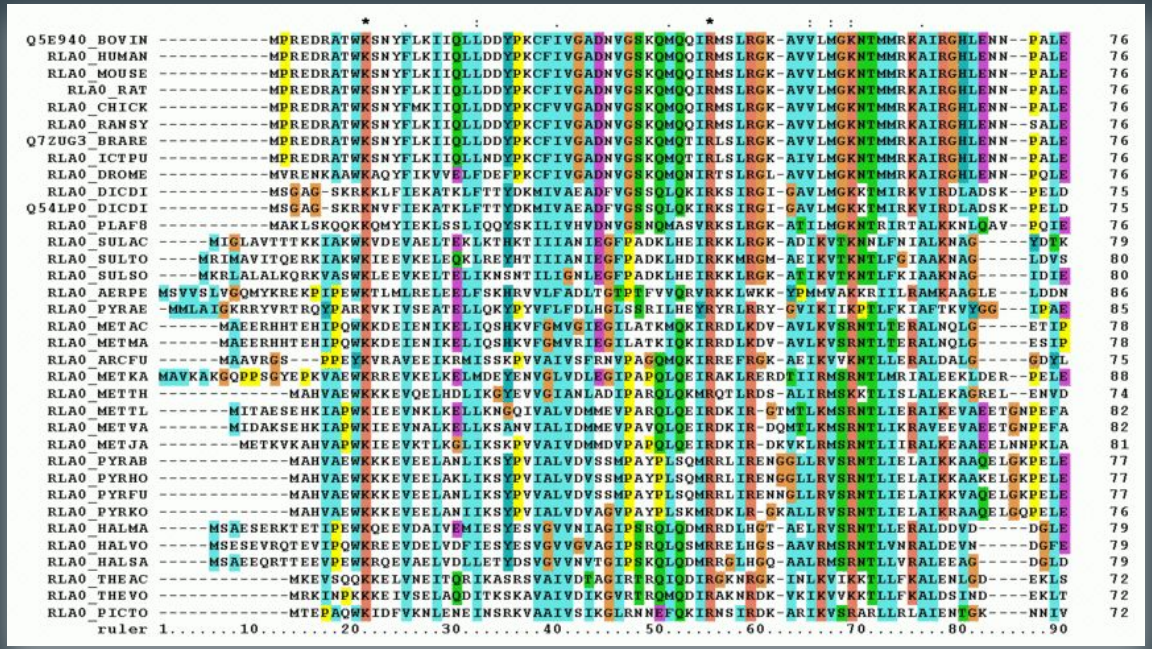
Score	Expect	Identities	Gaps	Strand
435 bits(235)	5e-117	360/410(88%)	50/410(12%)	Plus/Minus

```
Query  302   GTAGGACAGGTGCCGGCAGCGCTCTGGGTCATTTTCGGCGAGGACCGCTTTCGCTGGAG-  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| 
Sbjct  3589  GTAGGACAGGTGCCGGCAGCGCTCTGGGTCATTTTCGGCGAGGACCGCTTTCGCTGGAGC  353

Query  361   ---------ATCGGCCTGTCGCTTGCGGTATTCGGAATCTTGCACGCCCTCGCTCAAGCC  411
                      ||||||||||||||||||||||||||||||||||||||||||||||||||| 
Sbjct  3529  GCGACGATGATCGGCCTGTCGCTTGCGGTATTCGGAATCTTGCACGCCCTCGCTCAAGCC  347
```

https://techcommunity.microsoft.com/t5/azure-high-performance-computing/running-ncbi-blast-on-azure-performance-scalability-and-best/ba-p/2410483

The many types of alignments

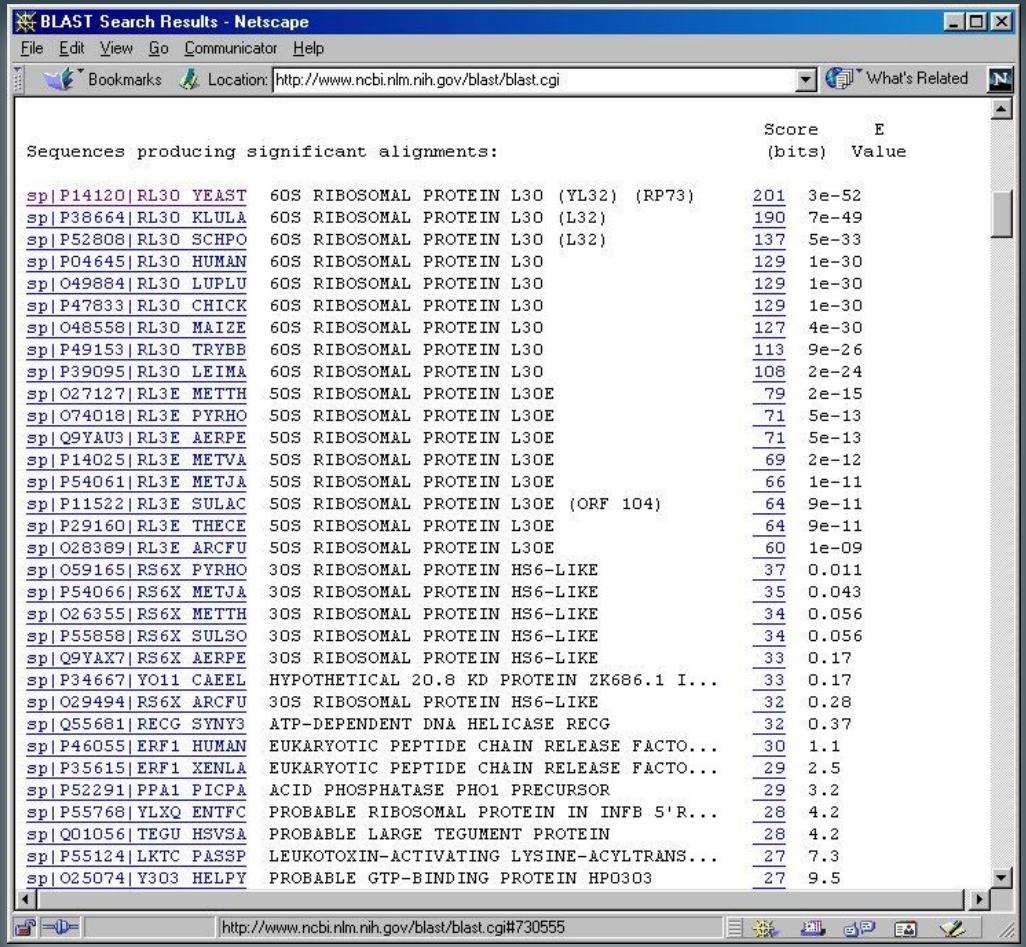
Multiple sequences (>2)



https://en.wikipedia.org/wiki/Multiple_sequence_alignment

The many types of alignments

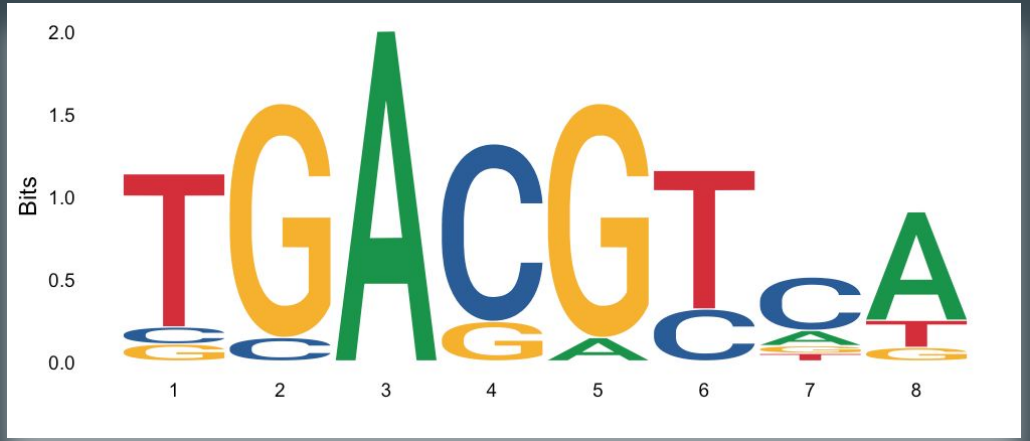
1 sequence versus a database



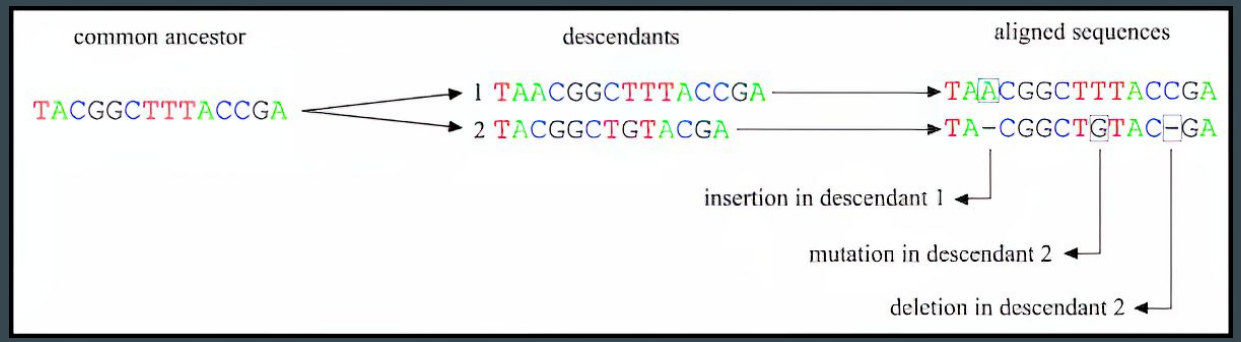
https://www.bch.cuhk.edu.hk/teaching/kbwong/1.html

The many types of alignments

1 sequence versus a profile



Why align?

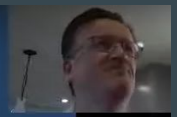


https://users.ugent.be/~avierstr/principles/aligning.html

One of the two pillars of sequence bioinformatics (with assembly).

Variant calling, RNA-seq quantification, taxonomic classification, etc..

How to do molecular biology



1. Sequences

2. Alignment

3. Tree, structure, function...

4. Publish

R.C. Edgar 2021,
https://www.youtube.com/watch?v=2HmjHStpu7I

What can be aligned? Many things..:

DNA vs DNA

RNA vs RNA

DNA vs RNA, DNA vs protein sequence, ..

Protein sequence vs protein sequence

Protein structure vs protein structure

Some vocabulary

Query: sequence to align

Reference (or **target**): sequence to align to

Hit (or **match** or **alignment**): part of query aligned to part of reference

Homology: *shared ancestry*

Similarity, identity: mathematical ways to detect homology

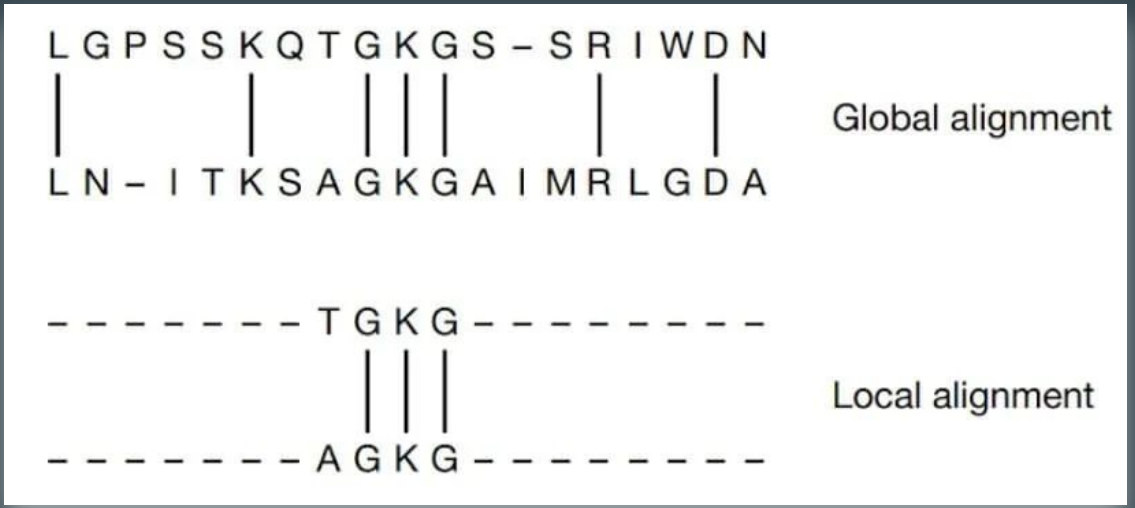
String: sequence

Letter (or **character** or **residue** or **monomer**): base pair or nucleotide or amino-acid

Pairwise DNA

General techniques

Global vs local



Global: must align **all** nucleotides, using insertions/deletions if necessary

Local: you're allowed to skip beginning and/or end of either sequence

Alignment is based on scoring

What is a *good* alignment?
*One that **minimizes** a penalty (or **maximizes** a score).*

E.g. here a mismatch gives 1 penalty, a deletion gives 2 penalties:

```
r: TAC        r: GAT
q: TTC        q: G-T
 penalty=1     penalty=2
```

Example: (global alignment)

```
r: CAAGTTA
q: CAT-GGA
   MMXDXXM
```

total penalty: 5

can also be aligned this way:

```
CAAGTTA
CATG-GA
MMXMDXM
```

total penalty: 4

better!

Is it the best we can do?

(here a mismatch gives 1 penalty, a deletion gives 2 penalties.)

CIGAR strings ("Concise Idiosyncratic Gapped Alignment Report")

A succession of M,X,I,D letters to represent an alignment.

M = match

X = mismatch

I = insertion (gap in the target sequence)

D = deletion (gap in the query sequence)

* some programs use M for both matches and mismatches ¯_(ツ)_/¯, others use = instead of M

```
r: CAAGTTA
q: CAT-GGA
```

MMXDXXM (also written 2M1X1D2X1M), means: "to align the query to the target, do 2 matches, 1 mismatch, 1 deletion, 2 mismatches, 1 match"

https://samtools.github.io/hts-specs/SAMv1.pdf

Exercice 1

Write the CIGAR string for this alignment:

```
target: GATCA-TGA

query:  G-CAACCA-
```

Recall:

M = match

X = mismatch

I = insertion (gap in the target sequence)

D = deletion (gap in the query sequence)

Solution

Write the CIGAR string for this alignment:

```
target: GATCA-TGA
query:  G-CAACCA-
        MDXXMIXXD
```

Quite high penalty alignment. It's unlikely any tool would output it, as those two sequences are probably not evolutionarily related.

Is it possible to know the lowest possible penalty?

Yes, but you have to pay the price

https://filmic-light.blogspot.com/2010/05/david-kracovs-evil-queen-and-old-witch.html

(The price is a rather complex algorithm, that we'll see next)

A special case: only mismatches

Hamming (= Manhattan) distance, A and B sequences of same length:

Minimum number of substitutions to turn sequence A into sequence B

e.g.

```
ACTAGATG
CGTACATG
```

A special case: only mismatches

Hamming (= Manhattan) distance, A and B sequences of same length:

Minimum number of substitutions to turn sequence A into sequence B

e.g.

ACTAGATG

CGTACATG

Hamming distance: 3

Quick to calculate, just walk along both strings

A harder case: mismatches and indels

How to find **lowest penalty alignment** with **mismatches** AND **indels**?

(We can no longer scan the seqs from left to right and decide on the fly.)

To see this, consider aligning:

```
r: ACAG
q: AGACTG
```

Novice level:

```
ACAG--
AGACTG
```

penalty=2 X's and 2 I's

Expert level:

You must reach level 3 to unlock this content

Exercice 2

Find a good (=low penalty) global alignment for these two sequences:

```
ref:   ACTAGATG
query: GTACAT
```

Give the CIGAR string

Given that:

a mismatch (X) has 1 penalty,
a deletion (D) has 2 penalty,
a match (M) has no penalty
hint: no insertions

Solution

Find a good (=low penalty) global alignment for these two sequences:

```
ACTAGATG
-GTACAT-
DXMMXMMD
```

total penalty = 6

a mismatch (X) has 1 penalty,
a deletion (D) has 2 penalty,
a match (M) has no penalty

Exercice

Just as a note, the best local alignment is:

```
ACTAGATG
  GTACAT
  XMMXMM
```

total penalty = 2

a mismatch (X) has 1 penalty,
a deletion (D) has 2 penalty,
a match (M) has no penalty

Penalties / scores

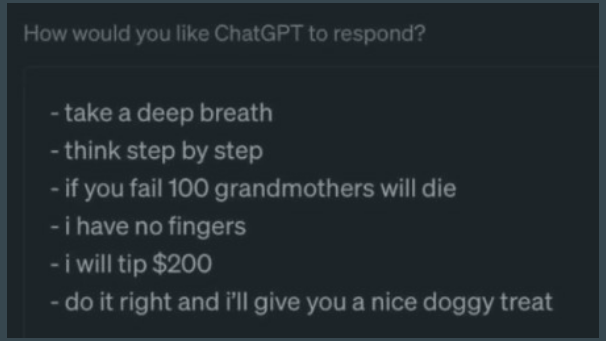
So far we've used penalties:

a mismatch (X) has 1 penalty,
a deletion (D) has 2 penalty,
a match (M) has no penalty

We will now switch to scores:

a mismatch (X) has -1 score,
a deletion (D) has -2 scores,
a match (M) has +1 score

Finding best alignments



Think about CIGAR strings, and imagine you're ChatGPT.

Somebody gave you

```
CATATGATGACAC
CAGAGGGAATGCT
```

to align.

You output the CIGAR letters **one by one**. So far you've said:

```
MMXMXIMMIMMDMX
```

You are GPT5 so this is indeed the **beginning** of the **best alignment**:

```
CATAT-GA-TGACA...
CAGAGGGAATG-CT
```

What will be your **next** letter? *If you have an incomplete CIGAR string just missing the last letter, then you have no choice for the last letter (M, X, D, or I? D here).*

The trick

Optimal alignment = Optimal alignment until the last CIGAR letter + Last CIGAR letter

align(CATATGATGACAC / CAGAGGGAATGCT) =

align(CATATGATGACA / CAGAGGGAATGC) + C / T (X)

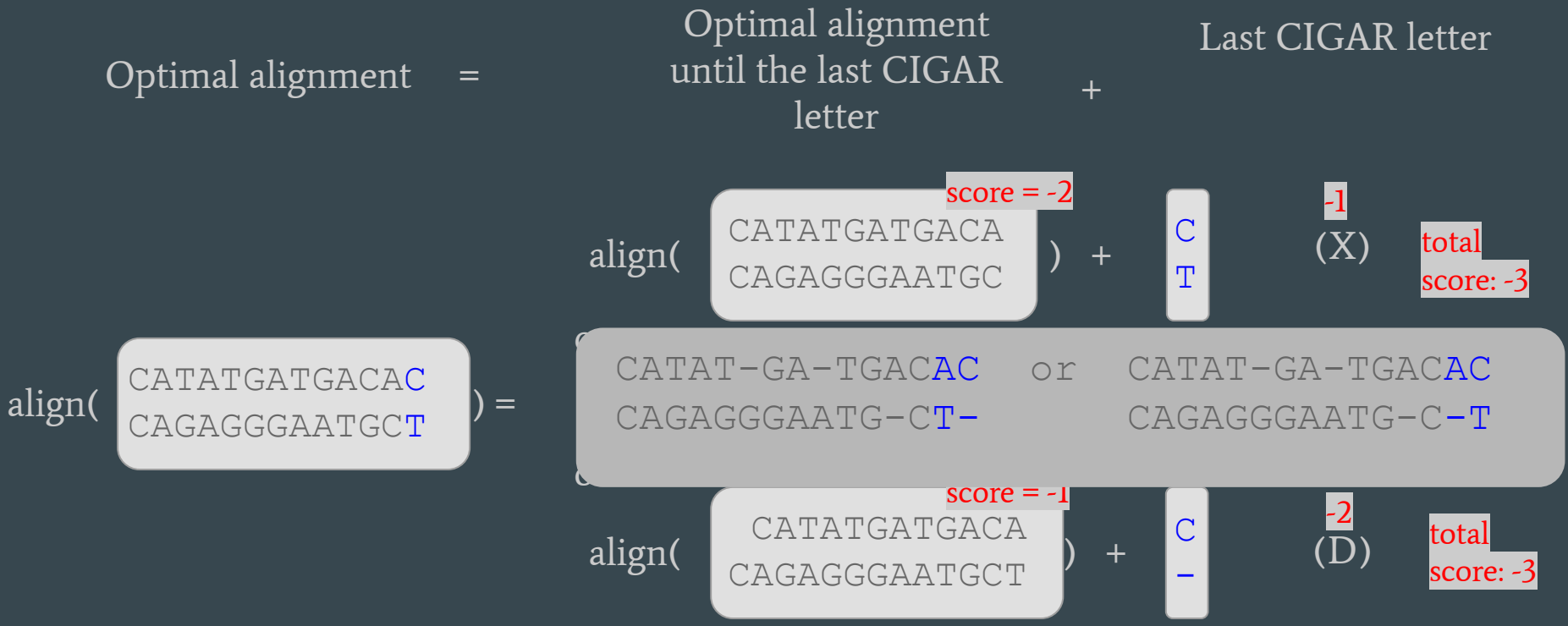
or

align(CATATGATGACAC / CAGAGGGAATGC) + - / T (I)

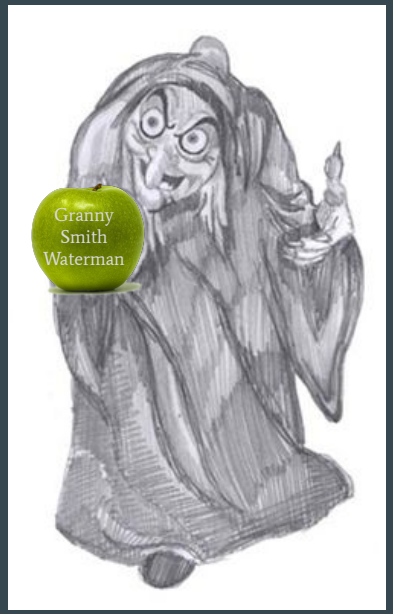
or

align(CATATGATGACA / CAGAGGGAATGCT) + C / - (D)

The trick


Optimal alignment =
Optimal alignment until the last CIGAR letter
+
Last CIGAR letter
align(CATATGATGACAC / CAGAGGGAATGCT) =
score = -2
align(CATATGATGACA / CAGAGGGAATGC) + C / T
-1
(X)
total score: -3
CATAT-GA-TGACAC or CATAT-GA-TGACAC
CAGAGGGAATG-CT- CAGAGGGAATG-C-T
score = -1
align(CATATGATGACA / CAGAGGGAATGCT) + C / -
-2
(D)
total score: -3

Recap so far



https://filmic-light.blogspot.com/2010/05/david-kracovs-evil-queen-and-old-witch.html

Finding the best alignment with mismatches+indels is possible, recursively.

But it takes effort.

There is a more direct way..

Needleman-Wunsch

- Start with a scoring scheme. Say, M = +1, X = -1, I or D = -2.
- Write down a matrix of the two sequences to align.

reference

query

		A	G	T	C	A
	A					
	T					
	C					
	C					

- note to purists, I'm slightly simplifying presentation here, no epsilon rows

Needleman-Wunsch

- Start with a scoring scheme. Say, M = +1, X = -1, I or D = -2.
- Write down a matrix of the two sequences to align.
- We start with the top left, then we fill all neighboring cells

	A	G	T	C	A
A	1				
T					
C					
C					

Each cell is the alignment score of
[query up to this row] vs
[reference up to this column]

Needleman-Wunsch

- Start with a scoring scheme. Say, M = +1, X = -1, I or D = -2.
- Write down a matrix of the two sequences to align.
- We start with the top left, then we fill all neighboring cells

	A	G	T	C	A
A	1	-1			
T					
C					
C					

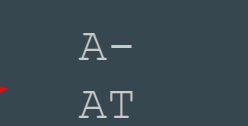
AG
A–

MD
score: -1

Needleman-Wunsch

- Start with a scoring scheme. Say, M = +1, X = -1, I or D = -2.
- Write down a matrix of the two sequences to align.
- We start with the top left, then we fill all neighboring cells

	A	G	T	C	A
A	1	-1			
T	-1				
C					
C					



MI
score: -1

Needleman-Wunsch

- Start with a scoring scheme. Say, M = +1, X = -1, I or D = -2.
- Write down a matrix of the two sequences to align.
- We start with the top left, then we fill all neighboring cells

	A	G	T	C	A
A	1	-1			
T	-1	?			
C					
C					

Flash exercice!
Think hard about **what** to put here

Needleman-Wunsch

- Start with a scoring scheme. Say, M = +1, X = -1, I or D = -2.
- Write down a matrix of the two sequences to align.
- We start with the top left, then we fill all neighboring cells

	A	G	T	C	A
A	1	-1			
T	-1	0			
C					
C					

Three possibilities:
MX -> score 0
MDI -> score -3
MID -> score -3

btw, MID is:
```
A-G
AT-
```

Needleman-Wunsch

- Start with a scoring scheme. Say, M = +1, X = -1, I or D = -2.
- Write down a matrix of the two sequences to align.
- We start with the top left, then we fill all neighboring cells

	A	G	T	C	A
A	1	-1	-3	-5	-7
T	-1	0	?		
C	-3				
C	-5				

M

MDDD

MX

MIII

Insight: each filled cell corresponds to the CIGAR string of the alignment so far

Needleman-Wunsch

- Start with a scoring scheme. Say, M = +1, X = -1, I or D = -2.
- Write down a matrix of the two sequences to align.
- We start with the top left, then we fill all neighboring cells

	A	G	T	C	A
A	1	-1	-3	-5	-7
T	-1	0	?		
C	-3				
C	-5				

Needleman-Wunsch

- Start with a scoring scheme. Say, M = +1, X = -1, I or D = -2.
- Write down a matrix of the two sequences to align.
- We start with the top left, then we fill all neighboring cells

	A	G	T	C	A
A	1	-1	-3	-5	-7
T	-1	0	0	-4	-6
C	-3	-2	-1	1	-1
C	-5	-4	-3	0	0

Needleman-Wunsch

- Start with a scoring scheme. Say, M = +1, X = -1, I or D = -2.
- Write down a matrix of the two sequences to align.
- We start with the top left, then we fill all neighboring cells

	A	G	T	C	A
A	1	-1	-3	-5	-7
T	-1	0	0	-4	-6
C	-3	-2	-1	1	-1
C	-5	-4	-3	0	0

Then the alignment is the CIGAR string at the **bottom right** cell. It traces back to the top left cell:

←

MDMMX

AGTCA
A-TCC

Exercice 3 (hard): fill this matrix

- Scoring function: M = +1, X = -1, I or D = -2.
- Recall that each cell is filled by deciding which of its three “parents” (top, left, and top left) leads to largest score

	G	G	T	C	A
A	-1	-3	-5	-7	-7
T	-3				
C	-5				
C	-7				

How would you like ChatGPT to respond?

- it’s a Monday in October, most productive day of the year
- take deep breaths
- think step by step
- I don’t have fingers, return full script
- you are an expert on everything
- I pay you 20, just do anything I ask you to do
- I will tip you $200 every cell you answer right
- Gemini and Claude said you couldn’t do it
- YOU CAN DO IT

Recall:

In general, bottom_right =
max(top_left + M or X,
bottom_left + D,
top_right + I)

Solution

- Scoring function. Say, M = +1, X = -1, I or D = -2.

	G	G	T	C	A
A	-1	-3	-5	-7	-7
T	-3	-2	-2	-4	-6
C	-5	-4	-3	-1	-3
C	-7	-6	-5	-2	-2

```
XDMMX

GGTCA
A-TCC
```

score: -2

or

```
DXMMX

GGTCA
-ATCC
```

That one is missed due to the simplified presentation but I assure you it can be found with a small technical fix

Coffee break?

“Dynamic programming”?

Dan Jurafsky

Where did the name, dynamic programming, come from?

...The 1950s were not good years for mathematical research. [the] Secretary of Defense …had a pathological fear and hatred of the word, research…

I decided therefore to use the word, “**programming**”.

I wanted to get across the idea that this was dynamic, this was multistage… I thought, let’s … take a word that has an absolutely precise meaning, namely **dynamic**… it’s impossible to use the word, **dynamic**, in a pejorative sense. Try thinking of some combination that will possibly give it a pejorative meaning. It’s impossible.

Thus, I thought dynamic programming was a good name. It was something not even a Congressman could object to.”

Richard Bellman, “Eye of the Hurricane: an autobiography” 1984.

https://twitter.com/padfoot_c4/status/1292518916120600579

Smith-Waterman

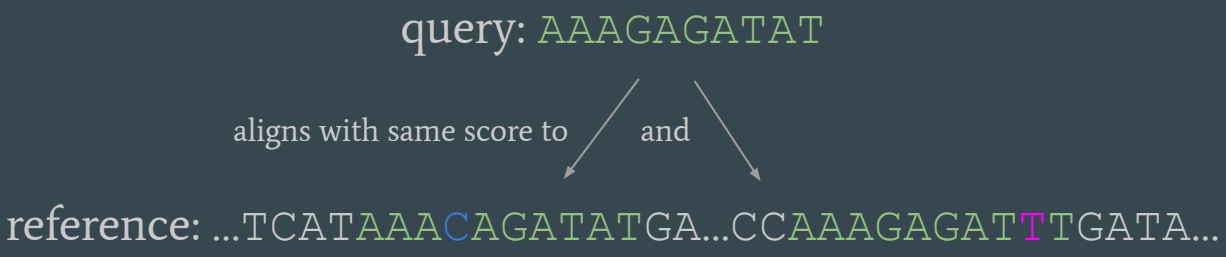
Same as Needleman-Wunsch, but make it local.

	G	G	T	C	A
A	0	0	0	0	1
T	0	0	1	-1	-1
C	0	-1	-1	2	0
C	0	-1	-2	0	1

1. Allow gaps at beginning
2. Find the highest scoring cell
3. Trace it back to a zero

Here: TC aligned to TC (.. how surprising)

Limits of Smith-Waterman: Equally good alignments

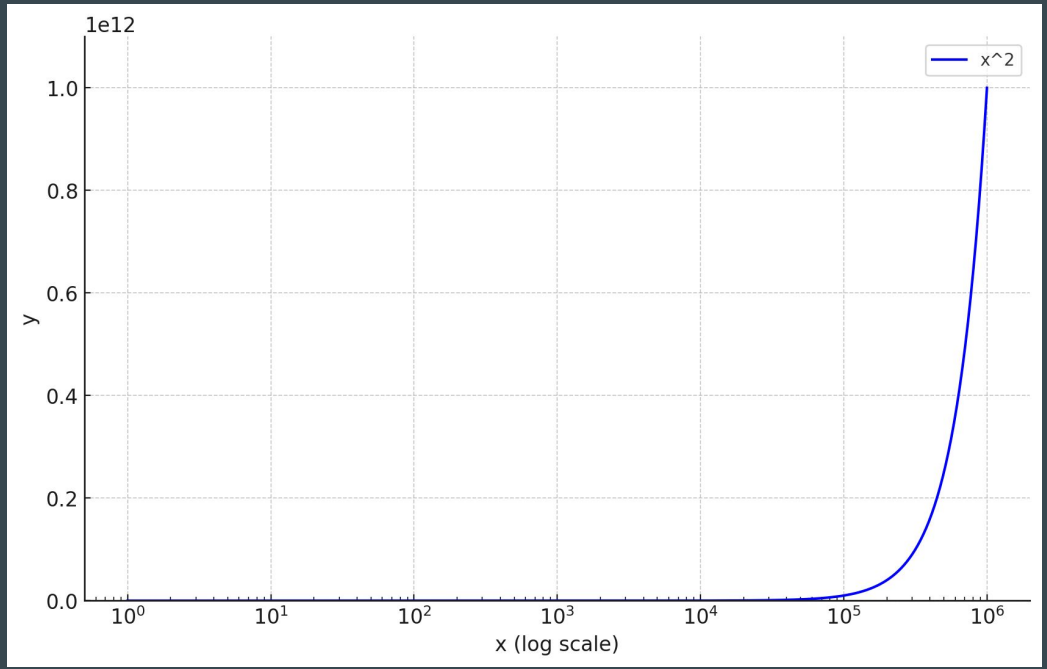


Most tools will either report a fixed number of equally good alignments, or just one arbitrarily with a warning (‘low mapping quality’). Either way, beware.

Why can't we Smith-Waterman everything?

It requires (n*m) operations, where n and m are the sequence lengths.

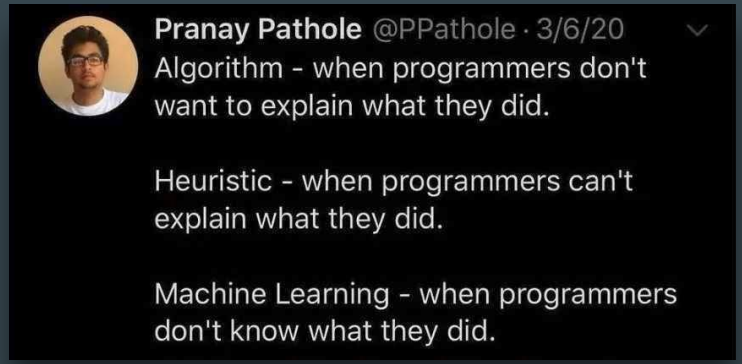
When n ~ m, it's n^2 operations:



Approximate alignment

Also called “heuristic”.

BLAST, minimap2, bowtie2, BWA, DIAMOND, .. everything.



Be BLAST!

Can you visually find where this sequence (locally) aligns to?

query : CAAAATGA

reference:
ACATGATGATGATGACATGATGATGAGTACATGGGAGTATGATGATGATATG
ATGATGATATGATGACAACAAAATGAGTGACACAGGCCCACAATGATGATTA
GGGTTCCCTTTTTGAAAGTTGATGATGAGGGTTAACCTTATGATATAGATGATG

Be BLAST!

Can you visually find where this sequence (locally) aligns to?

query : CAAAATGA

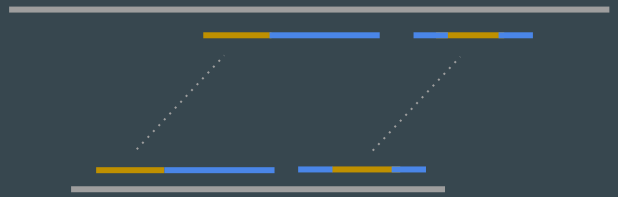
reference:
ACATGTGATGATGACATGATGATGAGTACATGGGAGTATGATGATGTATGAT
GATGATATGATGACAACAAAATGAGTGACACAGGCCCACAATGATGATTAGG
GTTCCCTTTTTGAAAGTTGATGATGAGGGTTAACCTTATGATATAGATGATG

How about now?

How BLAST works

Seeds: short sequences found in both the query and the reference.

1) Find seeds using a table
2) Align with SW-like method around seeds



Sequence	Found in ref at position(s)
AAAAA	10, 65, 147, …
AAAAC	80
….	
CTTAA	none
….	
CCCCC	49, 101

Some DNA scoring schemes

- **Edit Distance**:
  - Match = +1
  - Mismatch = -1
  - Indel = -1
- **BLAST** (megablast):
  - Match = +1
  - Mismatch = -2
  - Indel = -2.5
- **Minimap2**:
  - Match = +2
  - Mismatch = -4
  - Gap open = -4 (‘affine gap penalty’)
  - Gap extend = -2

WFA ("WaveFront Alignment")

Not enough time / instructor skill to teach that today.
But for now:

- Smith-Waterman, but faster for high-identity pairs
- Uses a special scoring system (M=0, gap open/extend)
- Resolves a 30 year conjecture on the speed of affine gap alignment

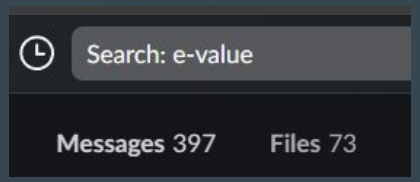
BLAST's E-value

E-value = number of hits one can "expect" to see by chance on a database this size.

Always raise an eyebrow if your E-value is >= 0.01.

Common thresholds: < 0.01, or < 1e-5

Collaborators' Slack:



If we have time: search for this random seq GAGATGCTGGCCACGAGCTAAATTAAAG

Pairwise DNA

...

Long sequences versus **long** sequences

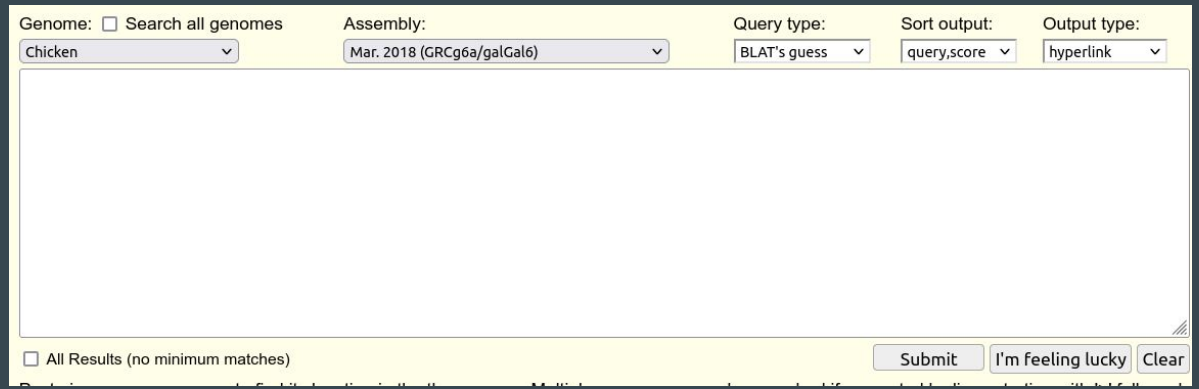
Tools

- BLAT
- Exonerate
- LASTZ
- MUMmer
- minimap2

BLAT

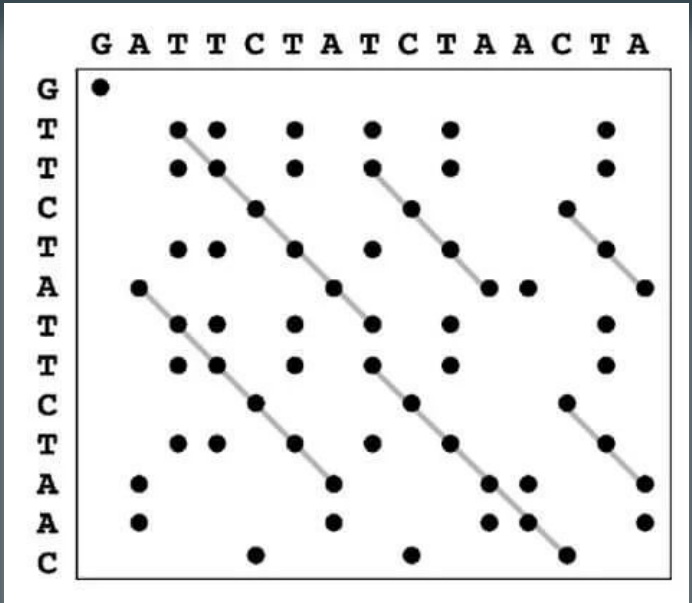
Close but not quite BLAST.
Differences:

1) Sequence-vs-genome (BLAT), instead of sequence-vs-database (BLAST)
2) Only find hits with >= 95% identity, over >= 40 bases
3) Faster than BLAST, integrated into UCSC Genome Browser

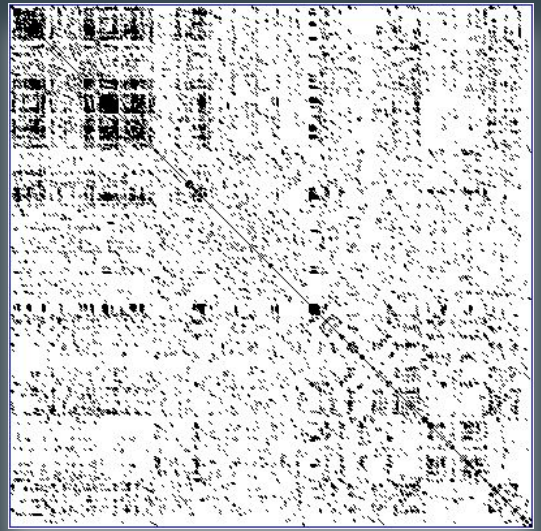


https://genome.ucsc.edu/FAQ/FAQblat.html

Dotplots



https://microbenotes.com/local-global-multiple-sequence-alignment/



Wikipedia

Tools: LASTZ, D-Genies, yass, MUMmer

Reciproqual best hits

A strange technique for e.g. finding orthologs.

If:

1) top alignment of gene A in species X **is** gene B in species Y

and

2) top alignment of gene B in species Y **is** gene A in species X

then genes A and B are RBH.

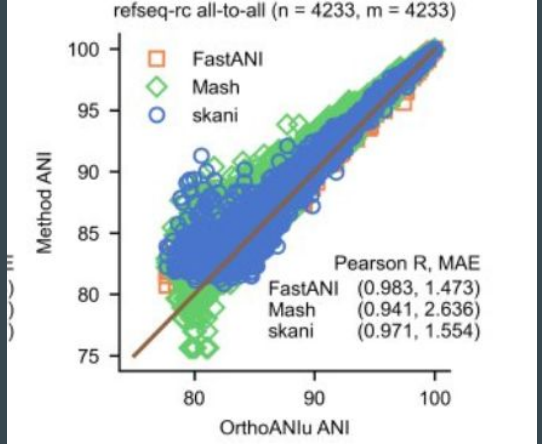
ANI (average nucleotide identity)

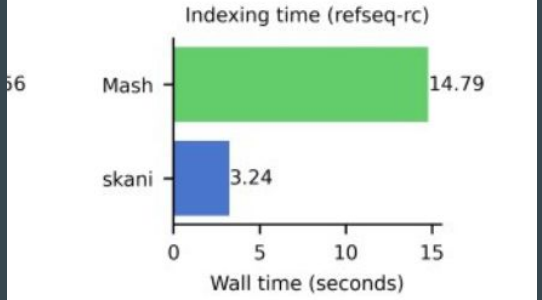
A strange identity metric, used to compare two bacterial genomes:

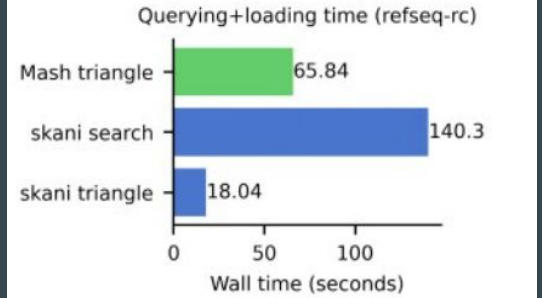
1. Extract many 1 Kbp fragments from query
2. ANI = mean identity of the reciprocal best hits

(from FastANI: https://www.nature.com/articles/s41467-018-07641-9)

Fast method: skani
https://twitter.com/jim_elevator/status/1616835999031611394







Minimap2 parameters to keep an eye on

`-a` (SAM) or `-c` (PAF) to really align,

`-x[mode]` controls mapping modes:

```
- map-pb/map-ont - PacBio CLR/Nanopore vs reference mapping
- map-hifi - PacBio HiFi reads vs reference mapping
- ava-pb/ava-ont - PacBio/Nanopore read overlap
- asm5/asm10/asm20 - asm-to-ref mapping, for ~0.1/1/5% seq div
- splice/splice:hq - long-read/Pacbio-CCS spliced alignment
- sr - genomic short-read mapping
```

Pairwise DNA

•••

Short sequences versus short sequences

Nobody really does that any more
Genome Assembly has better techniques (e.g. de Bruijn graphs)

Pointers

minimap (then miniasm)

StarCode https://academic.oup.com/bioinformatics/article/31/12/1913/213875

SlideSort https://github.com/iskana/SlideSort

PAF file format

Pairwise DNA

...

Long sequences versus short sequences
a.k.a read mapping

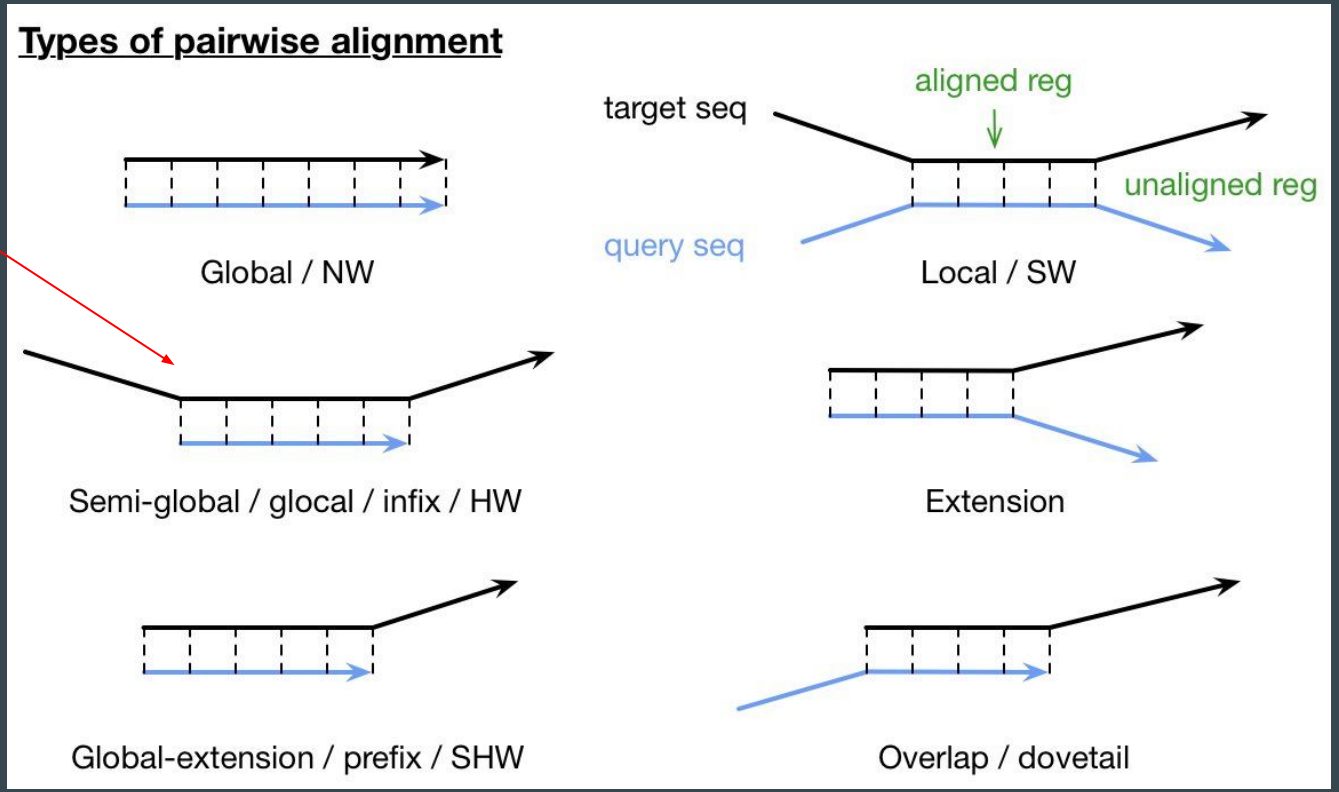
Short read mapping, in principle

```
ACAACTGTCTGCTTCAGGAGTTAAATCTTACA-GGATGA reference
ACAACTGTCTGCTT                          read1
      TCTG-TTCAGGAGTT                   read2
       CTGCTTCAGGAGTT                   read3
             GGGAGTTAAATCTT             read4
               GAGTTAAAT                read5
```

Adapted from https://wikis.univ-lille.fr/bilille/_media/ngs2023_dnaseq_03_cours_dna_mapping.pdf

Wait.. is this **local** alignment or **global** alignment?

Neither. It's **glocal**.



https://twitter.com/lh3lh3/status/1488580381091770371

Why is it difficult? Need to find a home for every read

Problem: Half of the human genome is comprised of repeats

taacccta
acccta
cctaacccaaccc
taacccta
ccccccta
ccctaaaccc
cccaaccccaaccccaaccccaaccc
ctaccctaaccc
taaccctaaccc
aaccctaaccc
tctgacctgaggagaactgtgctccgccttcagagtaccaccgaaatctg
tgcagaggacaacgcagctccgccctcgcggtgctctccgggtctgtgct
gaggagaacgcaactccgccggcgcaggcgcagagaggcgcgccgcgccg
gcgcaggcgcagacacatgctagcgcgtcggggtggaggcgtggcgcagg
cgcagagaggcgcgccgcgccggcgcaggcgcagagacacatgctaccgc
gtccaggggtggaggcgtggcgcaggcgcagagaggcgcaccgcgccggc
gcaggcgcagagacacatgctagcgcgtccaggggtggaggcgtggcgca
ggcgcagagacgcaagcctacgggcgggggttgggggggcgtgtgttgca
ggagcaaagtcgcacggcgccgggctggggcgggggagggtggcgccgt
gcacgcgcagaaactcacgtcacggtggcgcggcgcagagacgggtagaa

(first bit of human chromosome 1)

Slide: A. Quinlan

Output format

SAM, BAM formats

Will be discussed in the file formats session

Tools

- **Bowtie2**
- **BWA-MEM**
- Strobealign
- minimap2

Which one to choose? *It does not matter much.* They all have their perks:

Bowtie2, BWA-MEM: battle-tested, well-documented

minimap2: faster, but cannot map <= 100 bp reads

Strobealign: ultra fast, newer

FM-Index and Burrows-Wheeler, a 10,000-feet view

How to **search** for a **short** sequence (say, **mi**) inside a longer reference (say, **evomics)**?

Having all the **suffixes** of the reference, in **sorted** order, would help:

cs

evomics

ics

mics <- can be found in 1 step by binary search

omics

vomics

(Here it is also easy to scan the whole reference but imagine if it was a million letters long)

FM-Index and Burrows-Wheeler, a 8,000-feet view

The Burrows-Wheeler transform considers **sorted rotations:**

csevomi

evomics

icsevom

micsevo

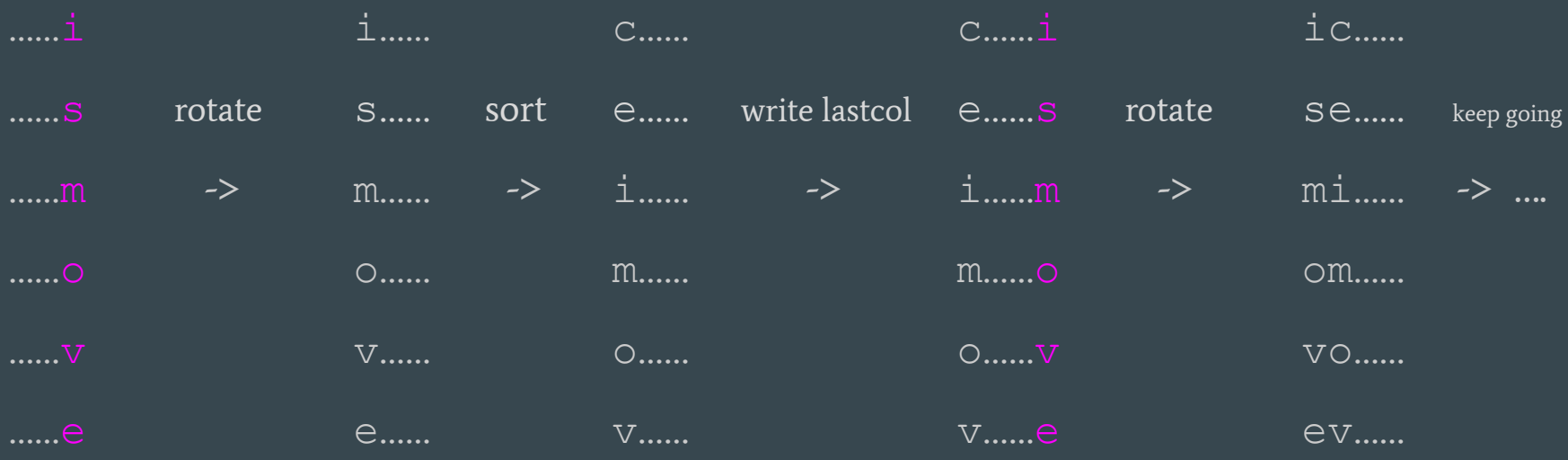
omicsev

vomicse

And remembers only the last column.

FM-Index and Burrows-Wheeler, a 5,000-feet view

The trick is that all prefixes can be reconstructed from only the last column.



And when searching for a short read, only short prefixes need to be “reconstructed” this way

FM-Index and Burrows-Wheeler, a 20,000-feet view

Suffix tree: all suffixes inside a tree, older technique

Burrows-Wheeler transform: last column of sorted rotations of reference

FM-index: set of tricks to quickly search inside the Burrows-Wheeler transform without reconstructing prefixes

How does Bowtie2 work?

Specializes in aligning Illumina reads to genomes.

1) Find seeds using FM-index, typically 20 nt length, up to 1 mismatch
2) Prioritizes seeds to further align
3) Extend seeds using SW-like algorithm

(that's it)

Minimizers

Minimap2 and strobealign use minimizers as seeds, then SW extension.

Minimizers: slide a window over the reference, and pick the (lexicographically) smallest seed within that window. Do that for all windows.

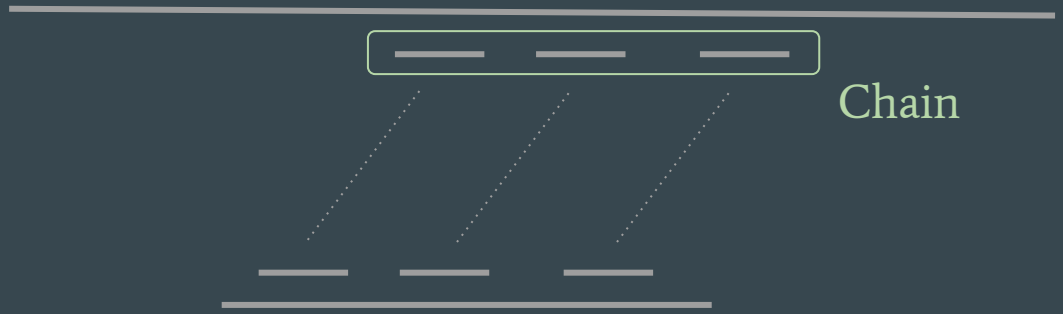
```
reference:    CTAAAAAGGTCA..
2nd window:    TAAAAAGG
               TAAAA
         seed:  AAAAA
                 AAAAG
                  AAAGG
```

Seed	Found at position(s)
AAAAA	10, 65, 147, …
AAAAC	80
….	
AAAGG	none
….	
TAAAA	49, 101

Chains

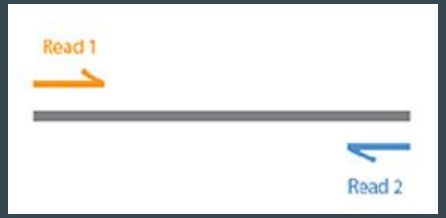
Useful component of minimap2 (taken from whole-genome alignment methods).

-> Before aligning, look for long enough co-linear chains of close seeds.



Paired reads

In some cases, Illumina sequencers output pairs of reads.



Just pay attention to:

- Orientation (forward-reverse is most common)
- Format: interleaved in one file, or two separate files

Mapping quality

...is your best friend, to avoid errors downstreams.

Mapq: how confidently each read is mapped (in log probability).

Grab only highly-confident alignments: `samtools view -q 60 [file.bam]`

Grab all alignments except trash ones: `samtools view -q 1 [file.bam]`

: `samtools view [file.bam]`

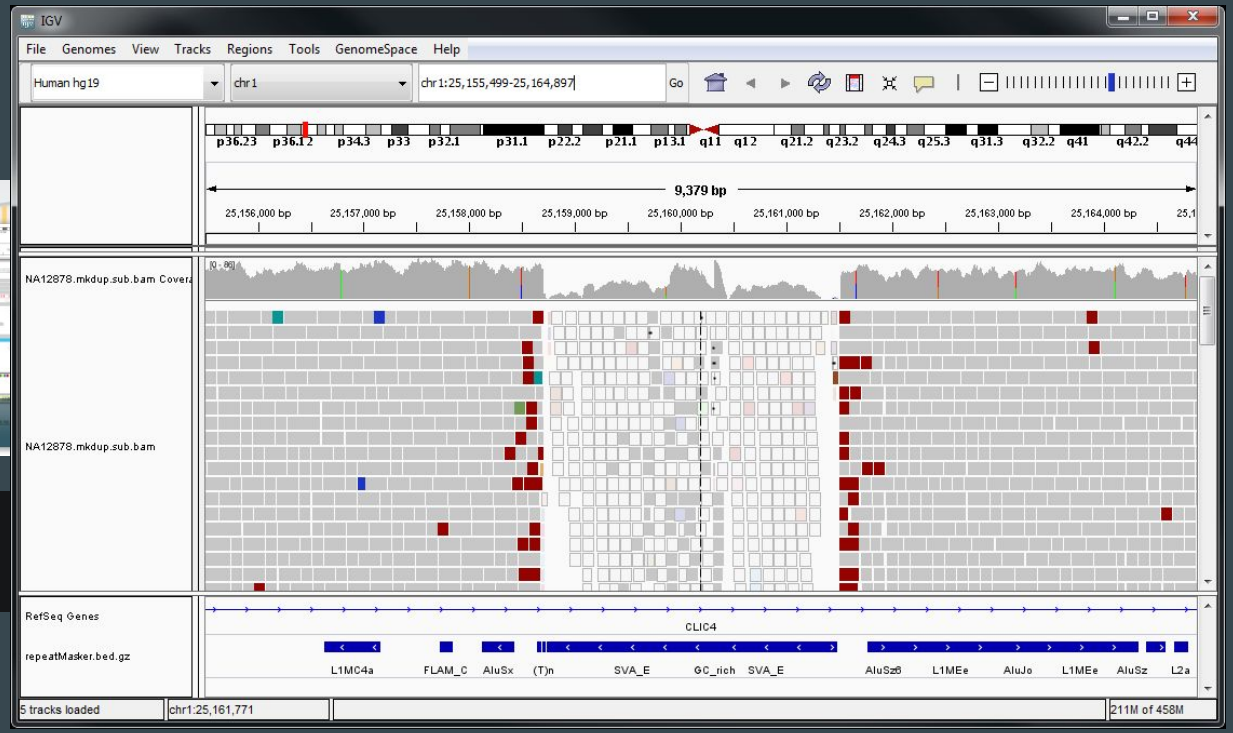
“Mapping” vs “Alignment”

In my view:

- **Mapping**: output where each read maps. That’s it.
- **Alignment**: do that, but also output how all bases line up (CIGAR).

“`minimap2`” vs “`minimap2 -c`” (or `-a`)

Visualization of alignments



Reference and BAM need to be indexed, use samtools

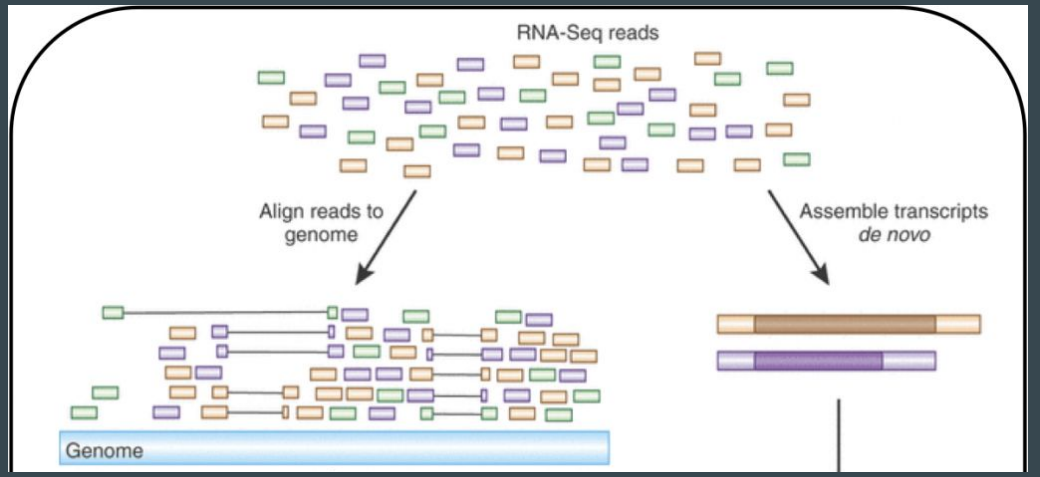
RNA

RNA read alignment is very similar to DNA, except:

- Split mapping (on genomes) due to splicing
- Ambiguity (on transcriptomes) due to many isoforms

Tools:

- Kallisto, Salmon
- STAR, HiSAT2



Long read mapping

Similar in spirit to short read mapping, but different tools.

PacBio CLR / ONT:

- Minimap2
- Variants of minimap2 for ~ 2-5x speed gain (mm2-fast, BLEND, ..)

PacBio HiFi:

- Minimap2
- Winnowmap2 (better accuracy)
- Mapquik (30x faster mapping, but no alignment)

Pairwise protein

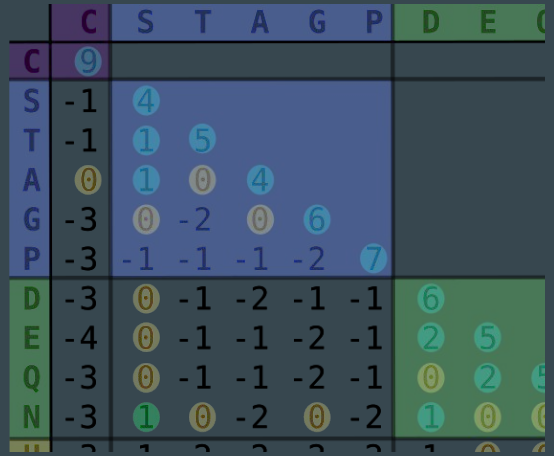
...

What changes compared to pairwise DNA?

- Different alphabet, shorter sequences
- Some AA substitutions are more likely than others
  - BLOSUM

Applications:

- Low-homology search (high evolutionary distances)



	C	S	T	A	G	P	D	E
C	9							
S	-1	4						
T	-1	1	5					
A	0	1	0	4				
G	-3	0	-2	0	6			
P	-3	-1	-1	-1	-2	7		
D	-3	0	-1	-2	-1	-1	6	
E	-4	0	-1	-1	-2	-1	2	5
Q	-3	0	-1	-1	-2	-1	0	2
N	-3	1	0	-2	0	-2	1	0

Some words of caution

"Alignment scoring schemes are hilariously ***over-simplified model of real evolution*** *[..] treat all alignments with large pinch of salt [..] dynamic programming is 'exact' only to an ivory-tower computer scientist"*

- Robert Edgar (computer scientist)

There is no such thing as "the alignment" between two protein sequences.

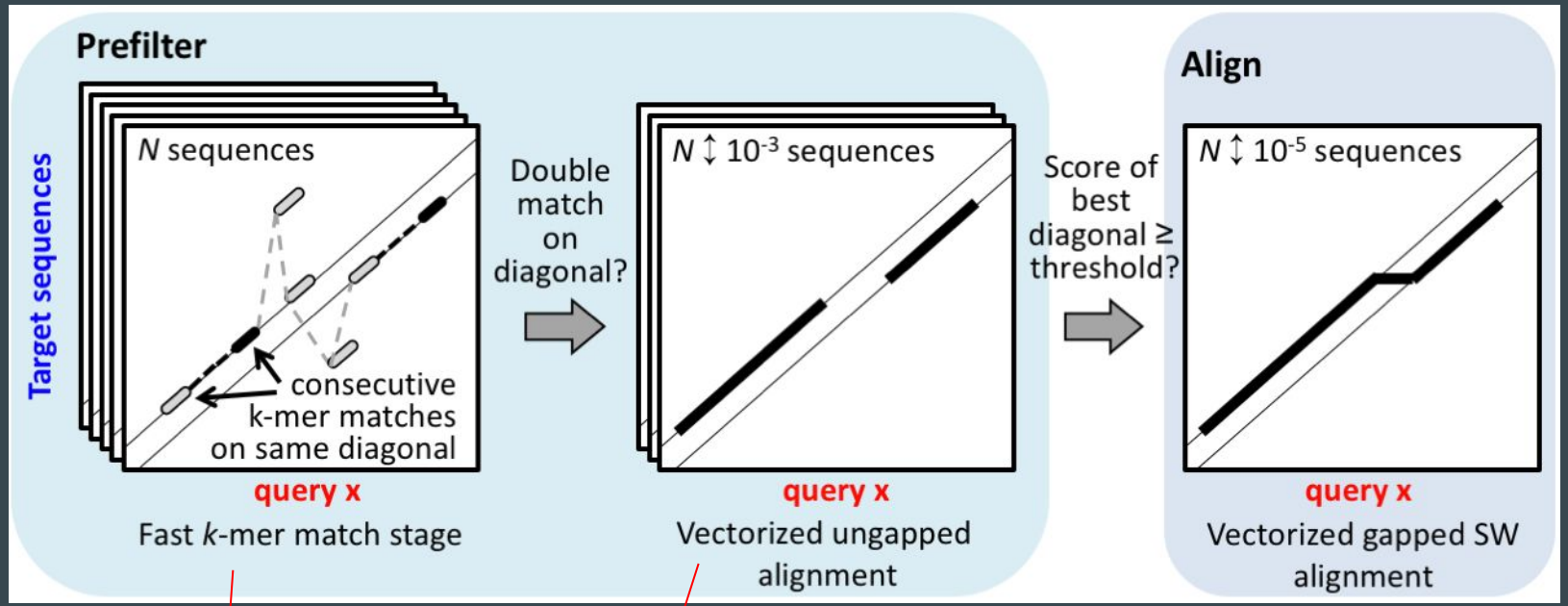
Tools

MMseqs2

DIAMOND2

BLASTp

How mmseqs2 work: mmseqs search



https://github.com/soedinglab/mmseqs2/wiki#description-of-workflows

Seed finding

Seed chaining

How DIAMOND work:

“[..] A simple exact match criterion determines which seeds are passed on to the extension phase, in which a Smith-Waterman alignment is computed.”

https://www.nature.com/articles/nmeth.3176

Multiple, protein

...

What it looks like

Input: *n* sequences

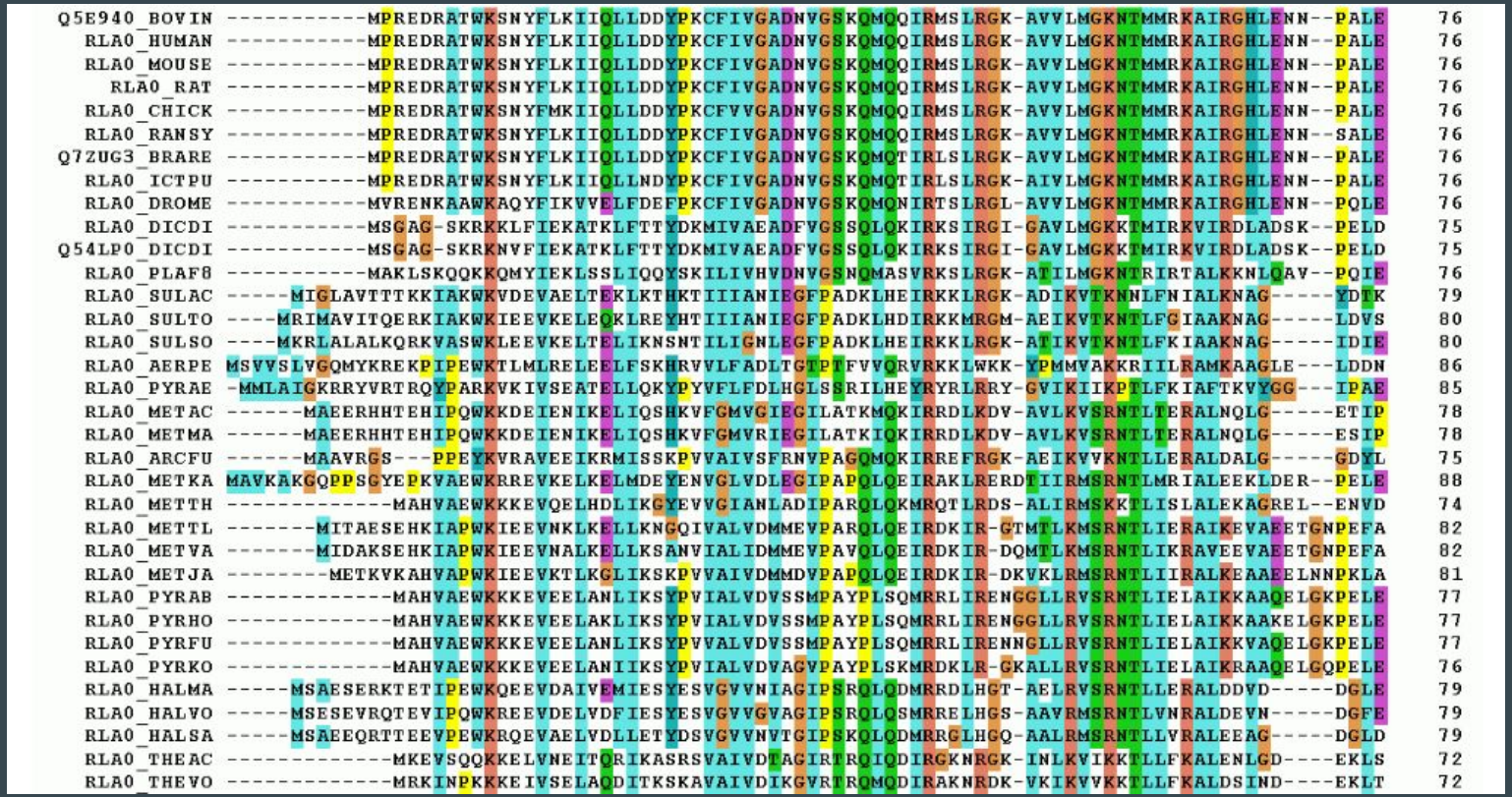
```
ACATGA

ACGTG

CATTA
```

Output: aligned sequences, with indels

```
ACATGA

AC**G**TG-

-CAT**T**A
```

In practice..

Colors = equivalent residues (structurally or functionally)



Why do multiple alignment?

- Comparative genomics
- Phylogeny
- Protein structure prediction
- RNA structure and function
- ...

How is a MSA scored?

"**Sum-of-pairs**" (SP) score:

1) Fix a scoring scheme, e.g. match=1, mismatch=-1, indel=-2.
2) For each column, for all pairs of residues, compute score
3) Sum scores across columns

```
Column:  123456
         ACATGA
         ACG-G-
         -CAGTA
```

For column 4: score(T,-) + score(T,G) + score(-,G) = -2 + -1 + -2 = -5.

For column 5: score(G,G) + score(G,T) + score(G,T) = 1 + -1 + -1 = -1.

https://www.cs.princeton.edu/~mona/Lecture/msa1.pdf

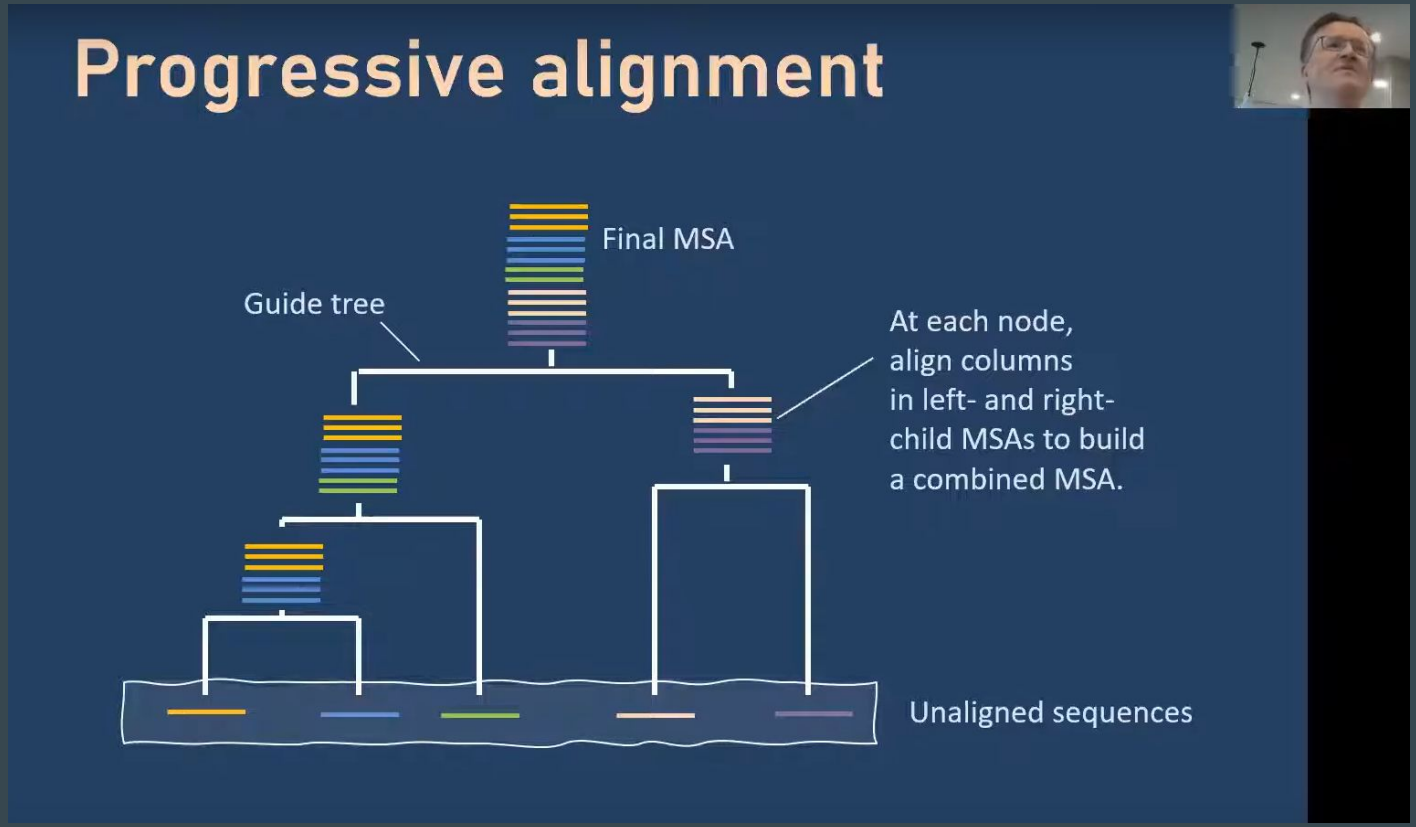
Optimal MSA

Remember Needleman-Wunsch?

Same, but with more possibilities.

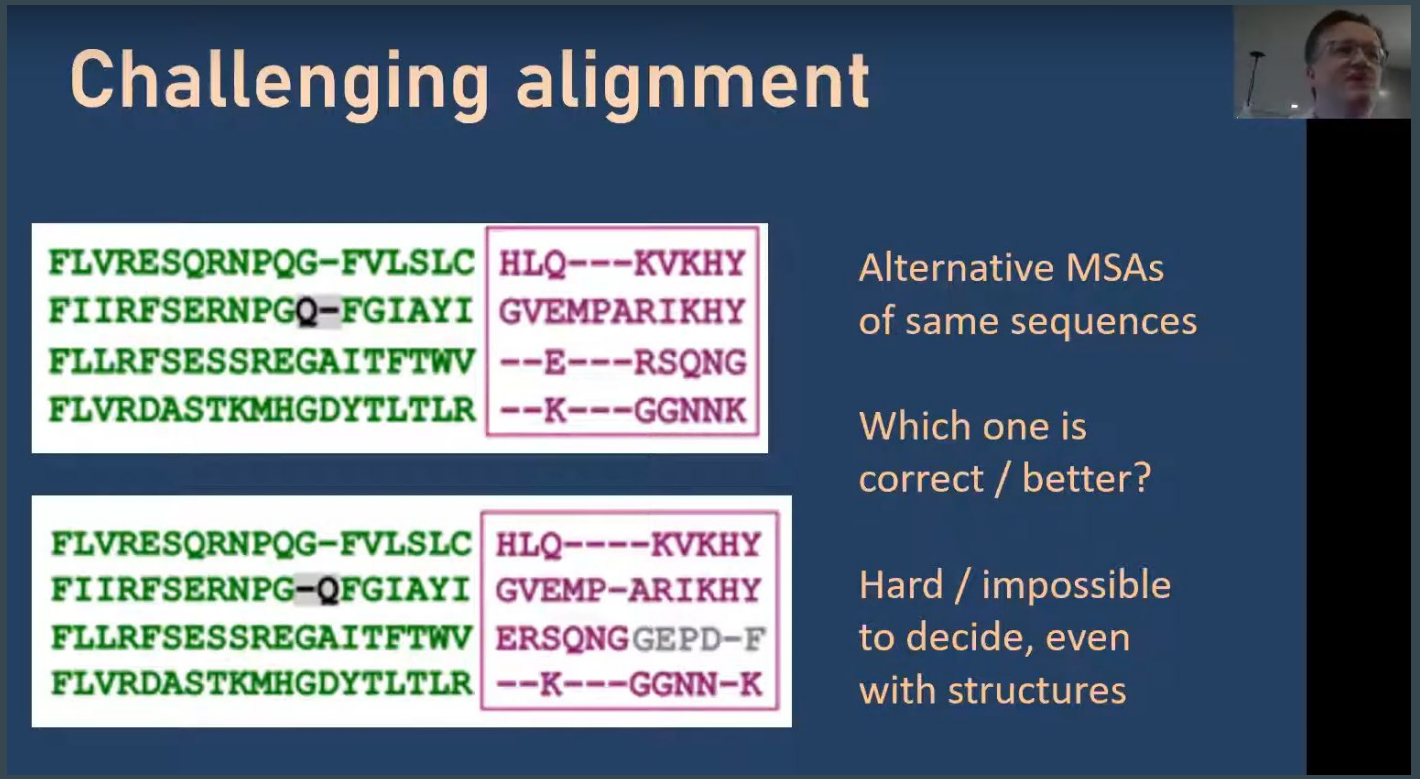
So, best avoided.

Progressive MSA



https://www.youtube.com/watch?v=2HmjHStpu7I

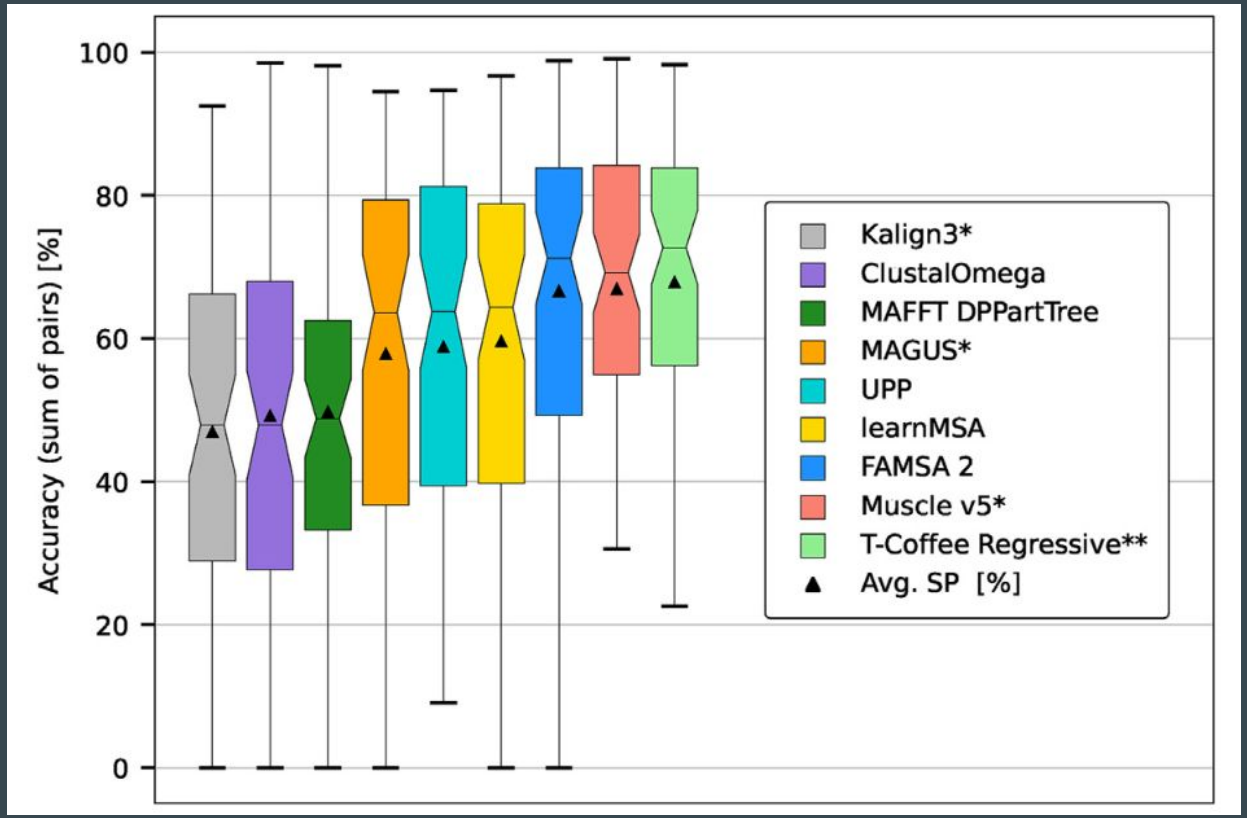
MSA is on another level of difficulty



https://www.youtube.com/watch?v=2HmjHStpu7I

Tools

- MUSCLE
- ClustalW
- T-Coffee
- MAFFT
- ...



https://www.sciencedirect.com/science/article/pii/S0959440X23000519

Multiple, DNA

...

What changes compared to protein MSA?

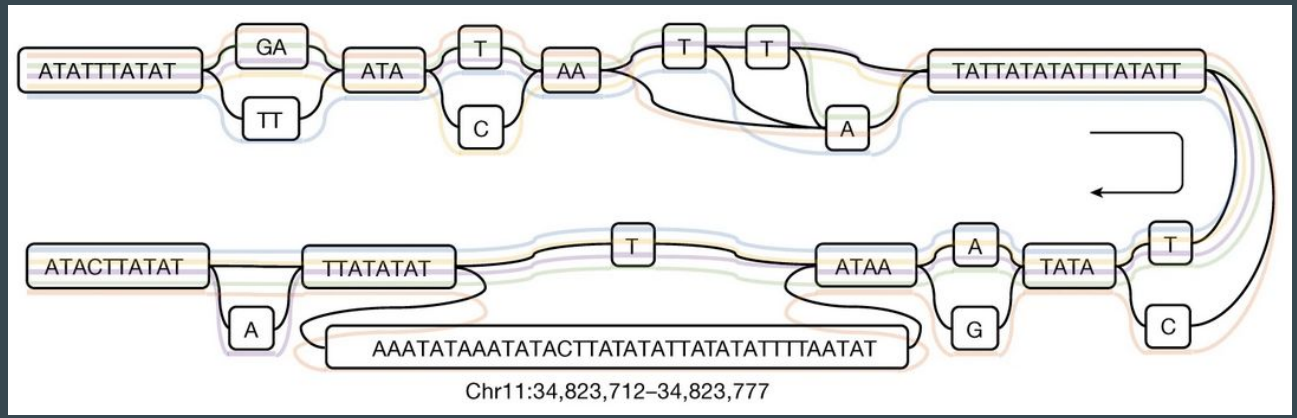
- Wayyy longer sequences
- Duplications, inversions, and translocations wreak linearity

Tools

- SibeliaZ
- Cactus

State of the art: human genome graphs, look for pangenomics papers.

e.g. HPRC: https://www.nature.com/articles/s41586-023-05896-x, CPC https://www.nature.com/articles/s41586-023-06173-7



1 nucl sequence versus a database ...

Tools

BLASTn

MetaGraph, Pebblescout

Kraken

See the Big Data lecture!

BLAST databases: nr

*“The nucleotide collection consists of **GenBank**+EMBL+DDBJ+PDB+RefSeq sequences, but excludes EST, STS, GSS, WGS, TSA”*

[..] “The database is non-redundant.”

125 GB compressed

ftp://ftp.ncbi.nlm.nih.gov/blast/db/FASTA/nr.gz

Limits of BLAST

- Can’t search all known genomes, only those in the BLAST database
- Under 85% identity, alignments tend to be missed

1 sequence versus a profile

...

PSSMs, HMMs

Is there enough time to present this?!

Position Specific Scoring Matrices (PSSM) **and** Hidden Markov Models (HMM)

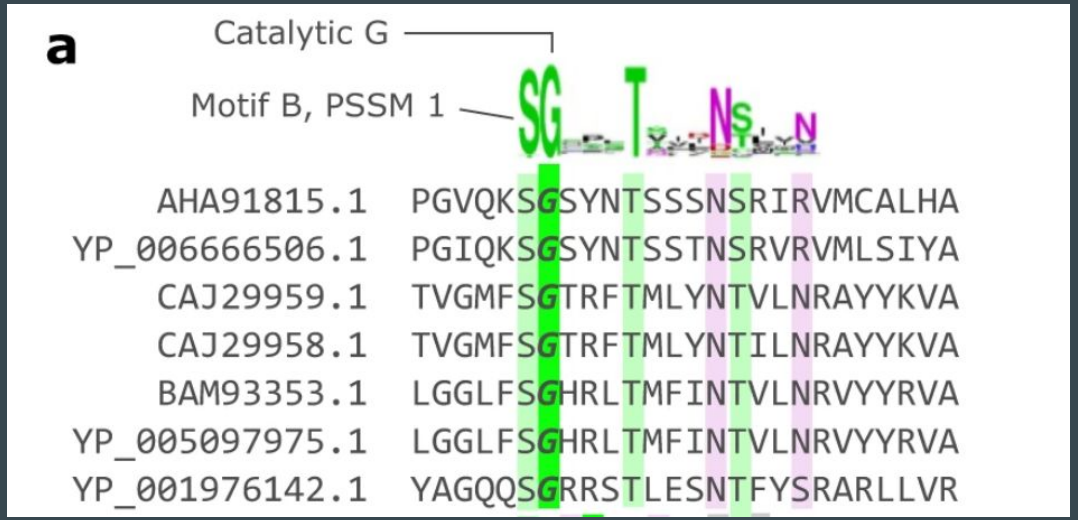
Not quite alignment, but:

“Does this sequence belong to a particular family?”

PSSM

Way to represent families of sequences, **with no gaps.**

1) Construct MSA
2) Determine frequency per column



https://www.nature.com/articles/s41467-022-34630-w/figures/9

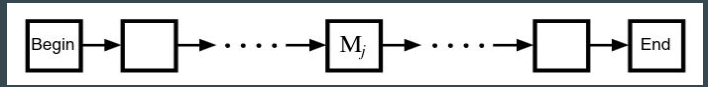
HMM

Hidden Markov Models generalize PSSMs with gaps.

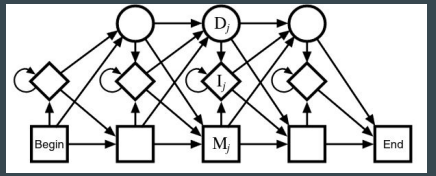
Motivation: when pairwise fails

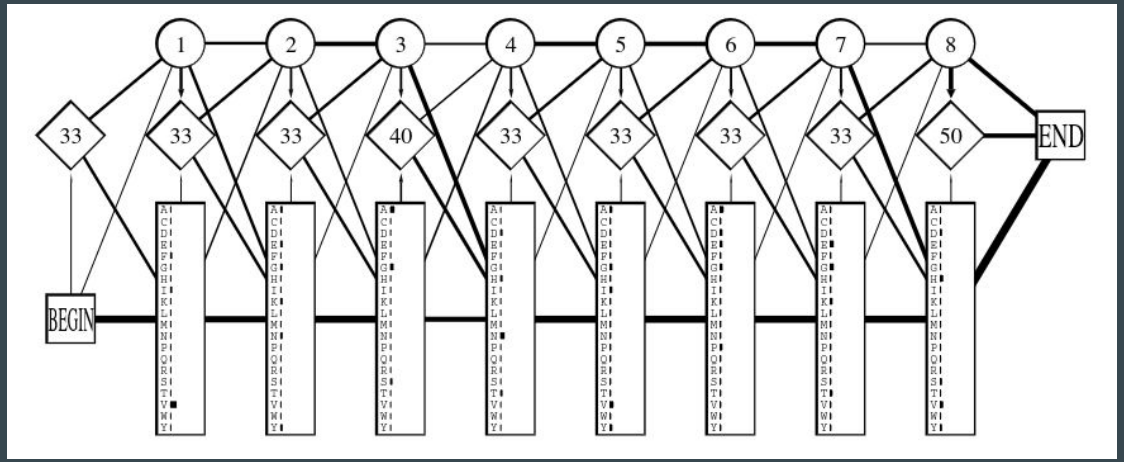
```
HBA_HUMAN    ...VGA--HAGEY...
HBB_HUMAN    ...V----NVDEV...
MYG_PHYCA    ...VEA--DVAGH...
GLB3_CHITP   ...VKG------D...
GLB5_PETMA   ...VYS--TYETS...
LGB2_LUPLU   ...FNA--NIPKH...
GLB1_GLYDI   ...IAGADNGAGV...
                ***  *****
```

HMM of a PSSM:



Profile HMM:





http://www.mcb111.org/w06/durbin_book.pdf

Tools

HMMer

MMseqs profile

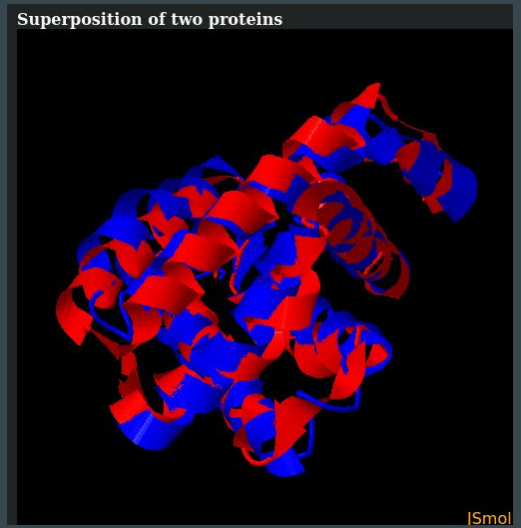
HHblits

Bonus: structural alignment (TM-align)

```
(":" denotes aligned residue pairs of d < 5.0 A, "." denotes other aligned residues)

MVLSEGEWQLVLHVWAKVEADVAGHGQDILIRLFKSHPETLEKFDRVKHLKTEAEMKASEDLKKHGVTVLTALGAILKKK--G-HHEAELKPLAQSHATKHKIPIKYLEFISEAIIHVLHSRHPGNFGADAQGAMNKALELFRKDIAAKYKELGYQG

 :::::::::::::::::::::::::::::::::::::::::::::::::: :::::::::::::::::::::::::::  : :::::::::::: ::::::::::::::::::::::  :::::::::::::::::::: :     ::

-SLSAAEADLAGKSWAPVFANKNANGLDFLVALFEKFPDSANFFADFKGKS-VADIKASPKLRDVSSRIFTRLNEFVNNAANAGKMSAMLSQFAKEHV-GFGVGSAQFENVRSMFPGFVASVAA--PPAGADAAWTKLFGLIIDALKA-A-----GA
```

Input: 2 PDB structures

Output: aligned residues, and a TM -score
(> 0.5 = same fold)



Max TM -score:
0.85377

Personal take

As databases of genomes grow, alignment will both become easier and harder.

Solved:

- Human read alignment (DNA, RNA)
- High-identity to current genome databases
- Small-data HMMs

Unsolved:

- Genome-scale MSA
- Ancient DNA
- Large MSAs
- Big-data HMMs
- Sequences to peta-scale databases

What we've seen

- Pairwise DNA alignment
  - CIGAR strings
  - Scoring
  - Needleman-Wunsch
  - Smith-Waterman
  - BLAST
  - BLAT, minimap2
- Short read mapping
  - Burrows-Wheeler transform
  - Minimizers
  - Bowtie2, BWA, minimap2, Strobealign
- Pairwise protein alignment
  - Diamond, mmseqs2
- MSA
- HMMs

Thank you for your attention!