

Lecture 2.1: Genome assembly algorithms

Professors: Jacques Rougemont, Anne-Florence Bitbol, Raphaëlle Luisier



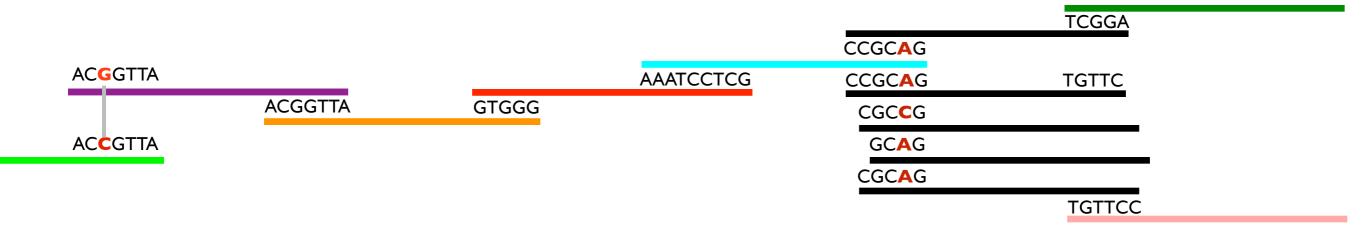
Fragments assembly

General Procedure:

Overlap → Layout → Consensus

Difficulties:

• Computing overlap with sequencing errors (1-3%) and unknown orientation



Contiguous sequence)

Assembling a genome

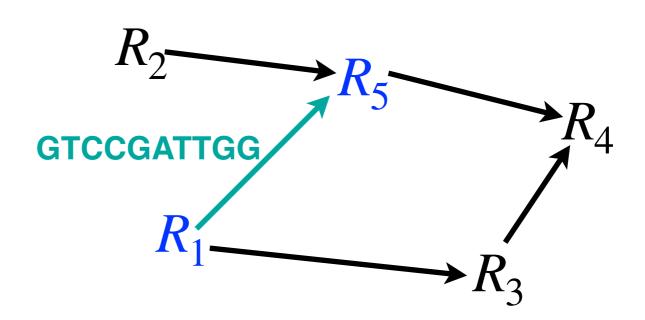
We start with a simpler problem:

sequencing provides N reads $R_1, ..., R_N$ all of length L, when 2 reads overlap, it is always by ℓ nucleotides

$$R_1$$
 = ACGTGTCCGATTGG
 R_5 = GTCCGATTGGTGTA

$$L = 14$$
, $\ell = 10$

overlap graph: vertices = reads edges = overlaps



Overlap graph

Contig is a Hamiltonian path

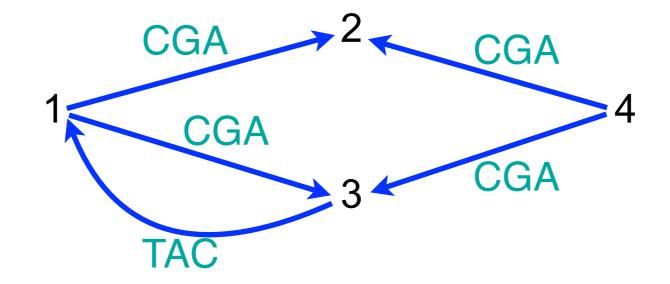
1.TACCGA

2.CGATCG

3.CGATAC

4.ATTCGA

$$N = 4$$
, $L = 6$, $\ell = 3$



contig:

$$4 \rightarrow 3 \rightarrow 1 \rightarrow 2$$

ATTCGA CGATAC TACCGA CGATCG

ATTCGATACCGATCG

Overlap graph

Hamiltonian paths are hard to find

Definition: A **Hamiltonian path** in a graph is a path visiting every vertex once and only once

Finding a Hamiltonian path is a NP-complete problem: there is no good algorithm to solve this problem

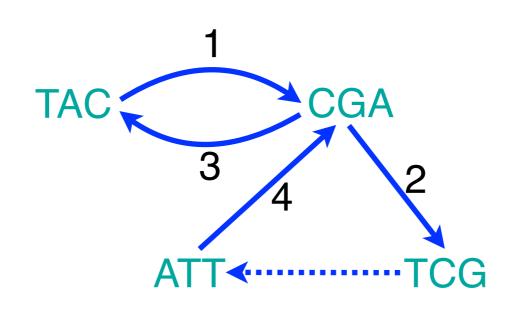
Definition: An **Eulerian path** in a graph is a path visiting every edge once and only once. If the path closes on itself it is called an **Eulerian cycle**.

Theorem: There exists an Eulerian cycle in a graph if and only if the graph is balanced: for each vertex *v*:

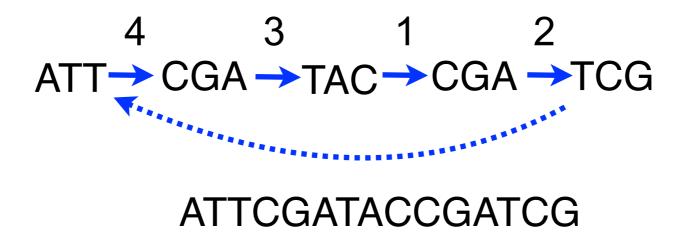
indegree(v) = outdegree(v)

Dual graph

1.TACCGA 2.CGATCG 3.CGATAC 4.ATTCGA vertices are overlaps: TAC, CGA, TCG, ATT edges are reads



contig is an Eulerian path:



Euler assembler

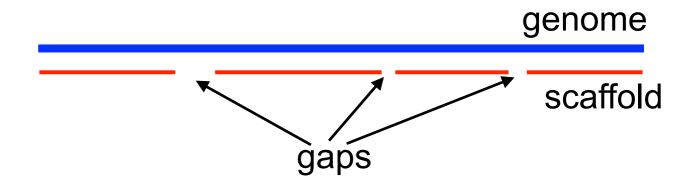
Problem:

- Reads have variable length (sometimes)
- Reads have sequencing errors
- Reads have random orientation
- Overlap size is variable and unknown
- Graph is not balanced and is highly redundant

Strategy:

- Construct a de Bruijn graph
- Heuristically simplify graph
- Extract many quasi-eulerian paths

⇒ many disjoint contigs



de Bruijn graph

Reads:

overlap parameter: 1. ATTCGAT

 $\ell = 4$

all ℓ -mers:

ATTC, TTCG, TCGA, CGAT,

CGAT, GATC, ATCG,

CGAT, GATA, ATAC, TACC, ACCG, CCGA

2. CGATCG

3. CGATACCGA

unique $(\ell-1)$ -mers: ATT, TTC, TCG, CGA, GAT,

ATC, ATA, TAC, ACC, CCG

Dual graph:

quasi-Eulerian cycle:

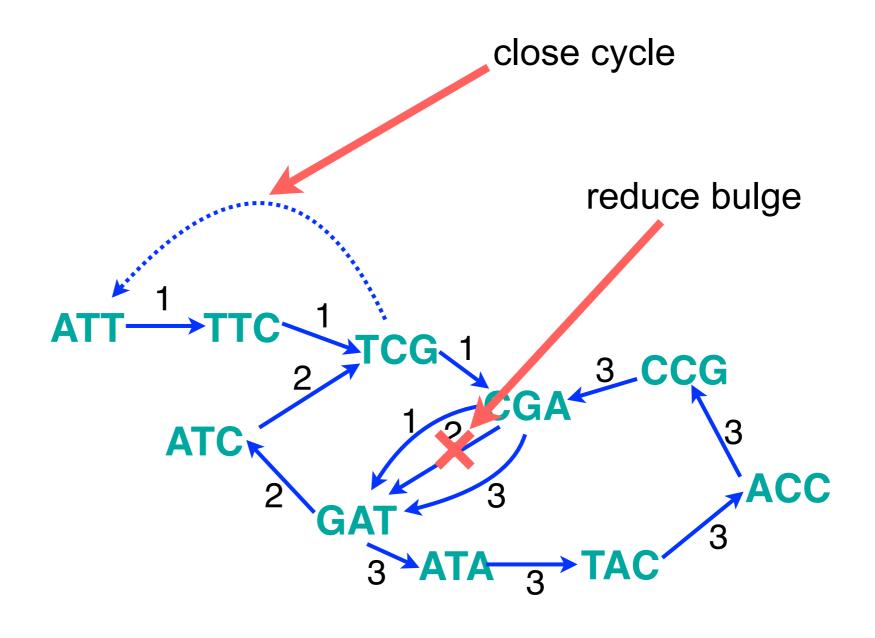
CGA GA

ATC

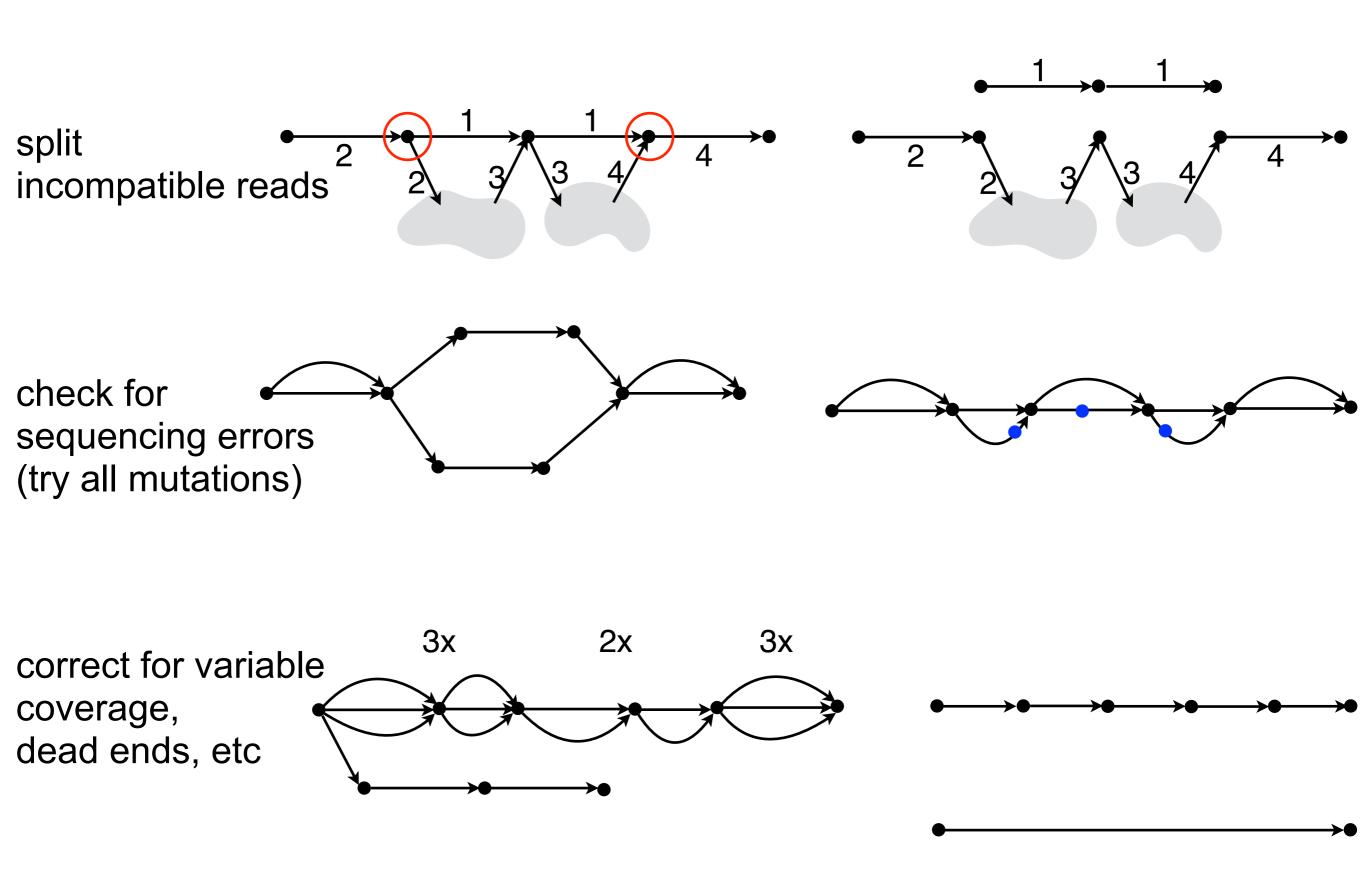
ATTCGATACCGATCG

de Bruijn graph





Heuristics: reduce graph inbalance



Paired-end sequencing

2 reads from same DNA fragment, from both ends

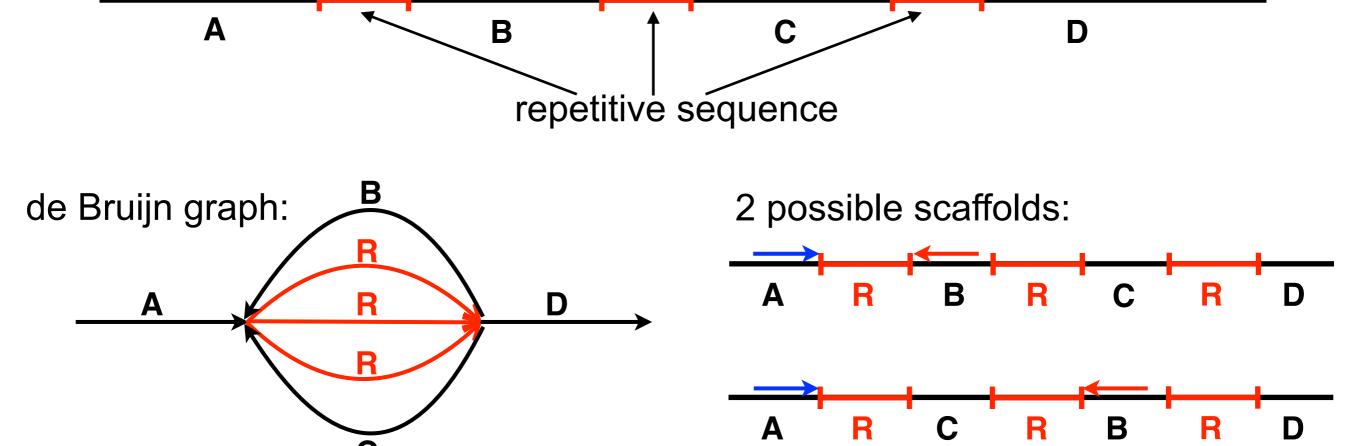
Genome:

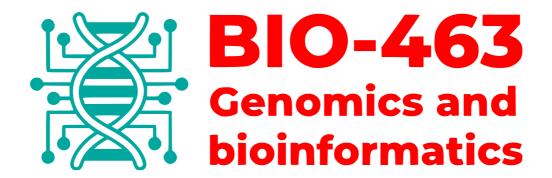


Fragment size known: ~ 10kb

Read length: 1kb

≫10kb



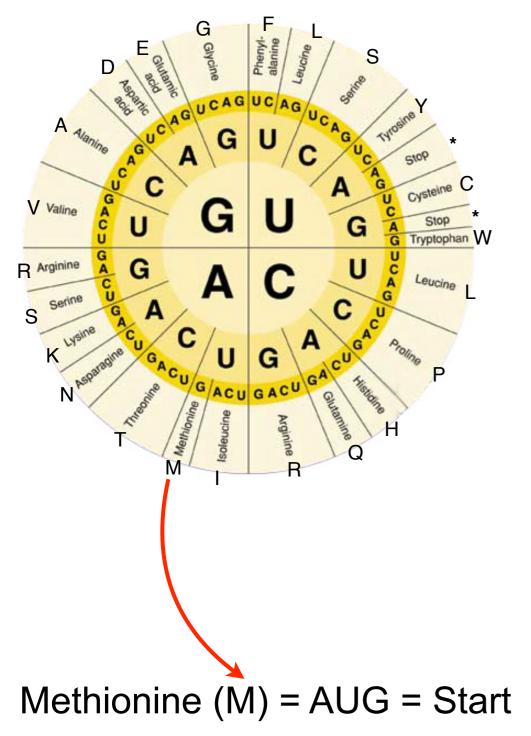


Lecture 2.2: Sequence alignments

Professors: Jacques Rougemont, Anne-Florence Bitbol, Raphaëlle Luisier



Open Reading Frames (ORFs)



6-frame translation

atgatcgacgcctcctcagcaagctga

M I D A S S A S *

* S T P P Q Q A

D R R L L S K L

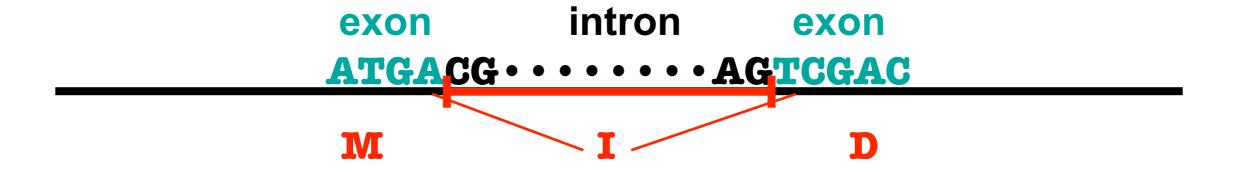
tcagcttgctgaggaggcgtcgatcat
S A C * G G V D H

Q L A E E A S I

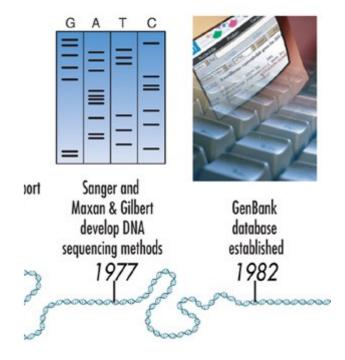
S L L R R R S

The Genetic Code

- On a bacterial genome, practically all proteins can be identified by direct translation (maybe ignoring short ORFs)
- In eukaryotes, genes have introns and alternative splicing



- We will use comparison to known transcripts to identify gene structures in the genome
- There are large databases of RNA sequences (full transcripts or fragments)



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Sequence alignments

Definition: An alignment of the two sequences X (length n) and Y (length m) is a sequence of operations:

match M, **delete** D, and **insert** I such that

$$#M + #D = n$$
 $#M + #I = m$

- •Is one alignment a better choice than the other?
- Are these alignments significant or not?

Sequence alignments

CACCGCATC-TG DDMMMMMMMIDM --CCGCAGGA-G CACCGCATC-TG MDMDMMMDMIMM C-C-GCA-GGAG

... (167960 possibilities)

How many different alignments exist? $\binom{n+m}{m}$

$\underline{\hspace{1cm}}$ m	10	100	200
1	11	101	201
50	$8 \cdot 10^{10}$	10^{40}	10^{53}
100	$5 \cdot 10^{13}$	10 ⁵⁹	10 ⁸¹

Scoring an alignment

We calculate a quality score for each alignment based on a scoring matrix

$$M = \begin{pmatrix} M & A & C & G & T & -D \\ A & 2 & -1 & -1 & -1 & -\gamma \\ C & -1 & 2 & -1 & -1 & -\gamma \\ -1 & -1 & -1 & 2 & -1 & -\gamma \\ T & -1 & -1 & -1 & 2 & -\gamma \\ \hline I & & & & & \\ mismatch & & & & \\ gap penalty & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & \\ & &$$

sequence+gaps character no
$$k$$

$$S(X',Y'|M) = \sum_{k=1}^{L} M(X_k',Y_k')$$

$$X'$$
 CACCGCATC-TG DDMMMMMMMIDM Y' --CCGCAGGA-G

$$-\gamma - \gamma + 2 + 2 + 2 + 2 + 2 + 2 - 1 - 1 - \gamma - \gamma + 2 = 10 - 4\gamma$$

Scoring matrix must be:

- symmetric
- •diagonal > 0, off-diagonal < 0
- M(-,-) impossible: $-\infty$

We always use:

- All diagonal element equal
- All gaps equal

Scoring an alignment

We calculate a quality score for each alignment based on a scoring matrix

$$M = \begin{matrix} A & C & G & T & - \\ A & C & G & T & - \\ C & -1 & -1 & -1 & -\gamma \\ -1 & 2 & -1 & -1 & -\gamma \\ -1 & -1 & 2 & -1 & -\gamma \\ -1 & -1 & -1 & 2 & -\gamma \\ -\gamma & -\gamma & -\gamma & -\gamma & -\infty \end{matrix} \end{matrix} \qquad S_{\text{affine}}(X', Y'|M, \delta) = \sum_{k=1}^{L} M(X'_k, Y'_k) - G\delta$$

number of gap opening

$$S_{\text{affine}}(X', Y'|M, \delta) = \sum_{k=1}^{L} M(X'_k, Y'_k) - G\delta$$

affine gap penalty

$$-\gamma - \gamma + 2 + 2 + 2 + 2 + 2 + 2 - 1 - 1 - \gamma - \gamma + 2 - \frac{3\delta}{\delta} = 10 - 4\gamma - \frac{3\delta}{\delta}$$

Examples

Which of these alignments is better depends on choice of scoring matrix

$$\gamma = 2$$
: $-4-3+12=5$ > $-16+12=-4$

$$-16+12=-4$$

$$\gamma = 0$$
: $-3 + 12 = 9$

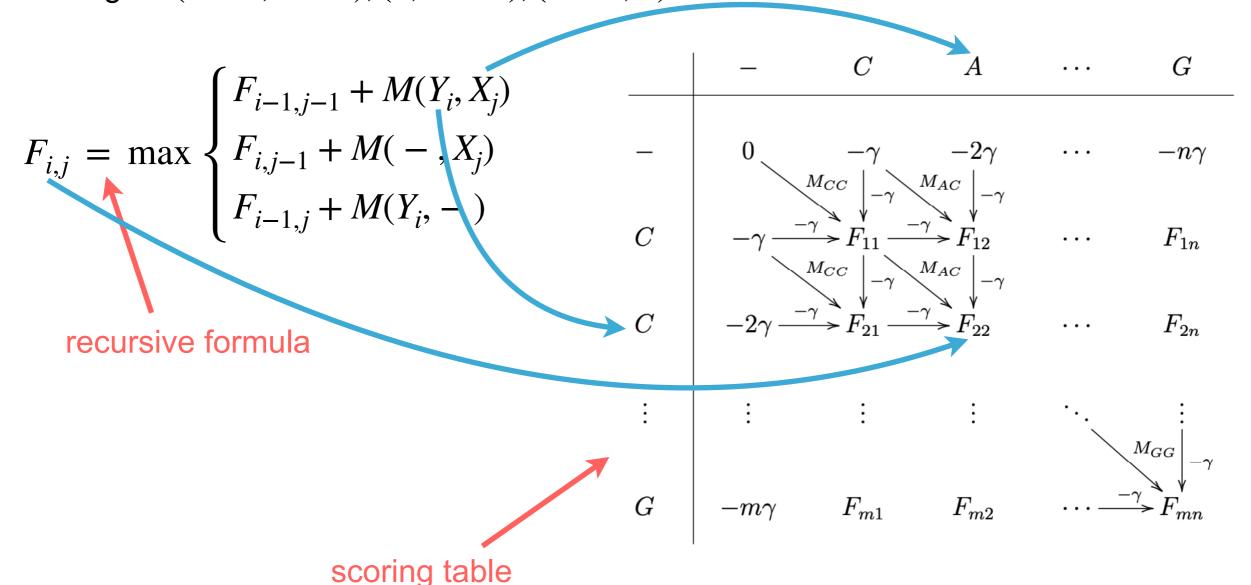
$$\gamma = 0, \delta = 2 : -3 + 12 - 2 = 7 > 12 - 6 = 6$$

$$12 - 6 = 6$$

$$M = \begin{pmatrix} A & C & G & T & - \\ A & C & G & T & -1 \\ C & -1 & -1 & -1 & -\gamma \\ -1 & 2 & -1 & -1 & -\gamma \\ -1 & -1 & 2 & -1 & -\gamma \\ -1 & -1 & -1 & 2 & -\gamma \\ -\gamma & -\gamma & -\gamma & -\gamma & -\infty \end{pmatrix}$$

Global alignment: The Needleman-Wunsch algorithm

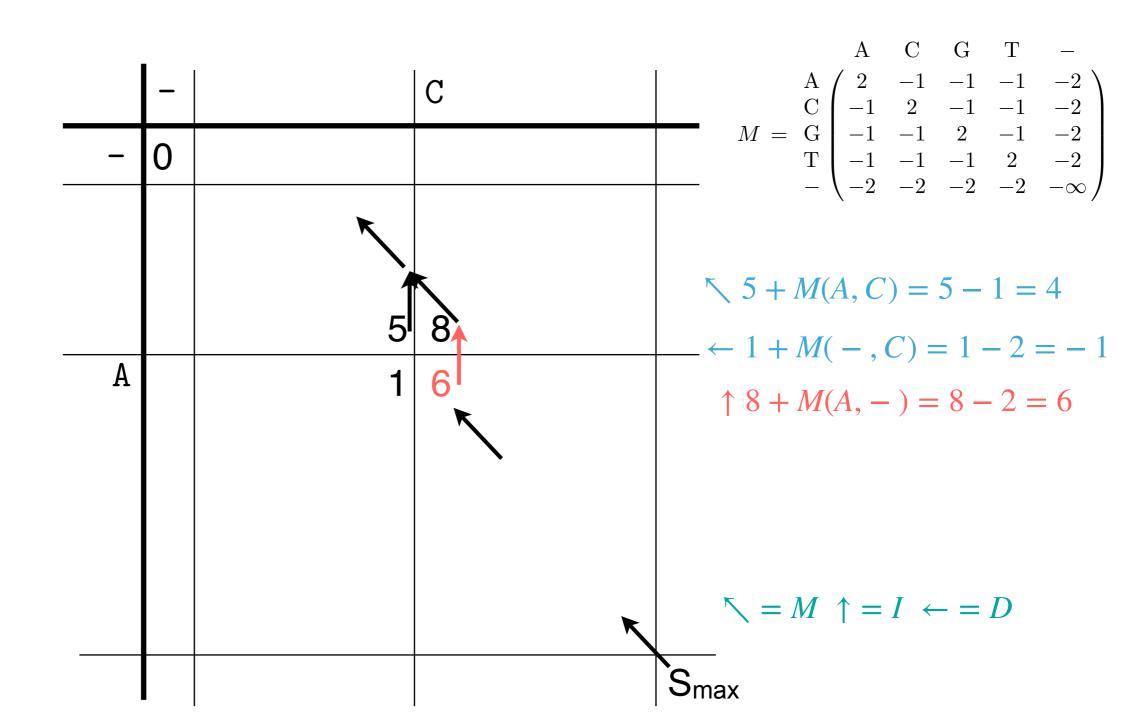
- \bullet Can we find the best scoring alignment (given M) without searching through all possibilities?
- Dynamic programming: a class of algorithms that work by recursively extending the solution of a sub-problem.
- We find the alignment of sequences of length (n, m) by extending the alignments of lengths (n 1, m 1), (n, m 1), (n 1, m)



Global alignment: The Needleman-Wunsch algorithm

$$F_{i,j} = \max \begin{cases} F_{i-1,j-1} + M(Y_i, X_j) \\ F_{i,j-1} + M(-, X_j) \\ F_{i-1,j} + M(Y_i, -) \end{cases}$$

- Optimal score is at bottom-right
- Backtracking follows optimal alignment



Global alignment: The Needleman-Wunsch algorithm

$$M = \begin{pmatrix} A & C & G & T & - \\ A & C & -1 & -1 & -1 & -2 \\ -1 & 2 & -1 & -1 & -2 \\ -1 & -1 & 2 & -1 & -2 \\ T & -1 & -1 & 2 & -2 \\ -2 & -2 & -2 & -2 & -\infty \end{pmatrix}$$

$$M = \begin{pmatrix} A & C & G & T & - \\ A & C & G & T & - \\ C & -1 & 2 & -1 & -1 & -2 \\ -1 & -1 & 2 & -1 & -2 \\ -1 & -1 & -1 & 2 & -2 \\ -1 & -2 & -2 & -2 & -\infty \end{pmatrix}$$

$$C = \begin{pmatrix} -2 & 2 & -4 & -6 & -8 & -10 \\ -2 & 2 & -4 & -6 & -8 & -10 \\ -2 & 2 & -4 & -6 & -8 & -10 \\ -2 & 2 & -4 & -6 & -10 \\ -2 & 2 & -2 & -2 & -2 & -2 \end{pmatrix}$$

$$C = \begin{pmatrix} -2 & 2 & 0 & -2 & -4 & -6 \\ -2 & -1 & 3 & 4 & -2 \\ -2 & -1$$

Cost of the algorithm (in time and memory):

$$\mathcal{O}((m+1)(n+1)) = 6 \times 5 = 30$$

compare to number of possible alignments:

$$\binom{m+n}{m} = \binom{9}{4} = 126$$

CACCG MDMMM C-CCG

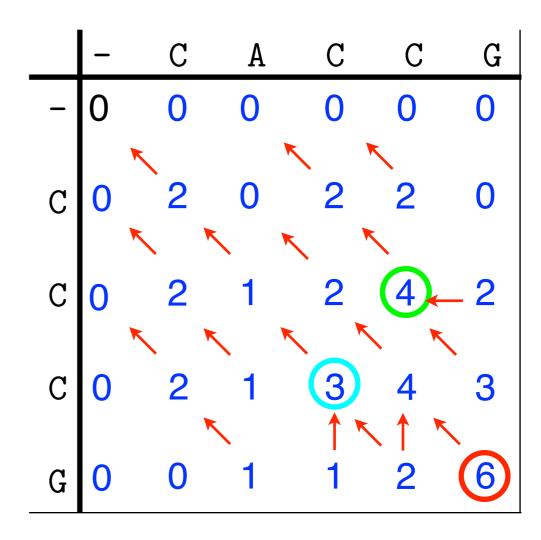
$$abla = M \uparrow = I \leftarrow = D$$

Local alignment: The Smith-Waterman algorithm

$$F_{i,j} = \max \begin{cases} 0 \\ F_{i-1,j-1} + M(Y_i, X_j) \\ F_{i,j-1} + M(-, X_j) \\ F_{i-1,j} + M(Y_i, -) \end{cases}$$
 •Optimal score is highest anywhere in table •Backtrack from a high score until you reach a 0

Possible local alignments:

CCG	CC	CAC
MMM	MM	MMM
CCG	CC	CCC
score 6	score: 4	score:3



How to make a scoring matrix

- The scoring matrix contains "prior information" about what we consider a relevant alignment
- A standard interpretation of alignment scores is as log-likelihood ratios
- You can estimate them empirically

Negative set: random sequences

Frequency of nucleotide pair $= q_{\rm C} \cdot q_{\rm A}$ = product of individual frequencies

$$L_{\mathsf{random}}(X,Y) = \prod_i q_{x_i} q_{y_i}$$

$$L_{\text{model}}(X,Y) = \prod q_{x}q_{y}$$

$$L_{\text{model}}(X,Y) = \prod p_{x}q_{y}$$

$$\mathcal{L}(X,Y) = \log\left(\frac{L_{\text{model}}(X,Y)}{L_{\text{random}}(X,Y)}\right) = \sum_{i} (\log p_{x_iy_i} - \log q_{x_i} - \log q_{y_i}) = \sum_{i} M(x_i,y_i)$$

Positive set: curated pairs of homologous sequences

Count frequency of each nucleotide pair $= p_{xy}$

$$L_{\mathsf{model}}(X,Y) = \prod_{i} p_{x_i y_i}$$

Empirical chemical similarity of amino-acids: wikipedia:Substitution matrix

BLAST

Basic Local Alignment Software Tool search local alignements of query ("gene") in a large database ("genome")

- 1.Remove low-complexity (repeat-like) regions from query
- 2.Cut query in small words (DNA: 11 bases, AA: 3 residues), look for exact matches in the database (pre-computed table)
- 3.Perform a Smith-Waterman alignment in the neighborhood of each hit to produce a high-scoring segment pair (HSP)

Ranking of HSP is performed by **E-value**, assuming an extreme value distribution:

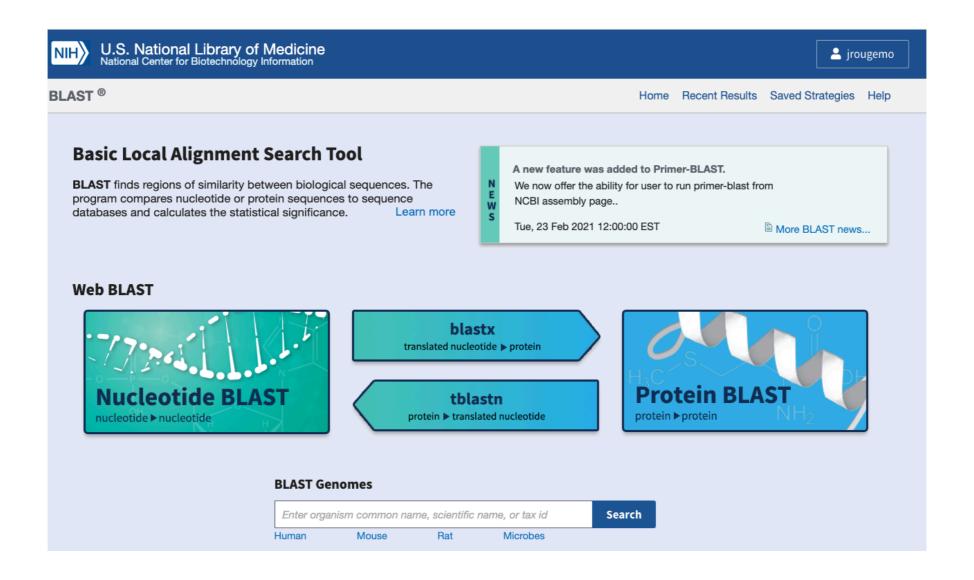
$$E = Kmne^{-\lambda S}$$
 S-W score sizes of query and database

Parameters K, λ have been empirically tuned.

Ranking will not change if you rescale all scores as $S' = \frac{\lambda S - \log K}{\log 2}$ $E = mn2^{-S'}$ "bit-score"

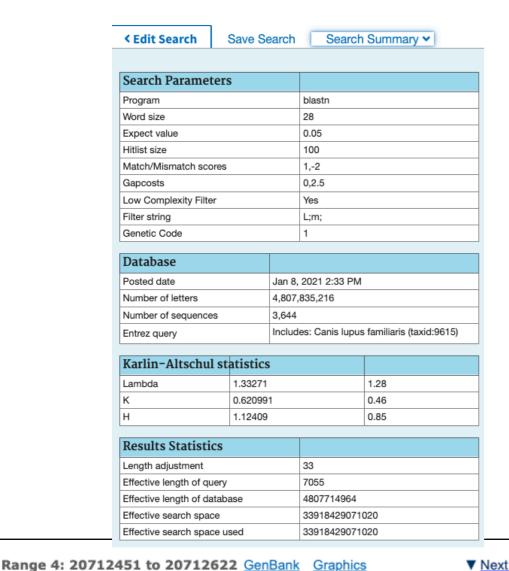
BLAST

Basic Local Alignment Software Tool search local alignements of query ("gene") in a large database ("genome")



blast.ncbi.nlm.nih.gov

- Database: dog genome
- Query: human BRCA1



149/172(87%)

Gaps

0/172(0%)

Sbjct 20703912

Score

Query

Query

Query

191 bits(103)

4296

4356

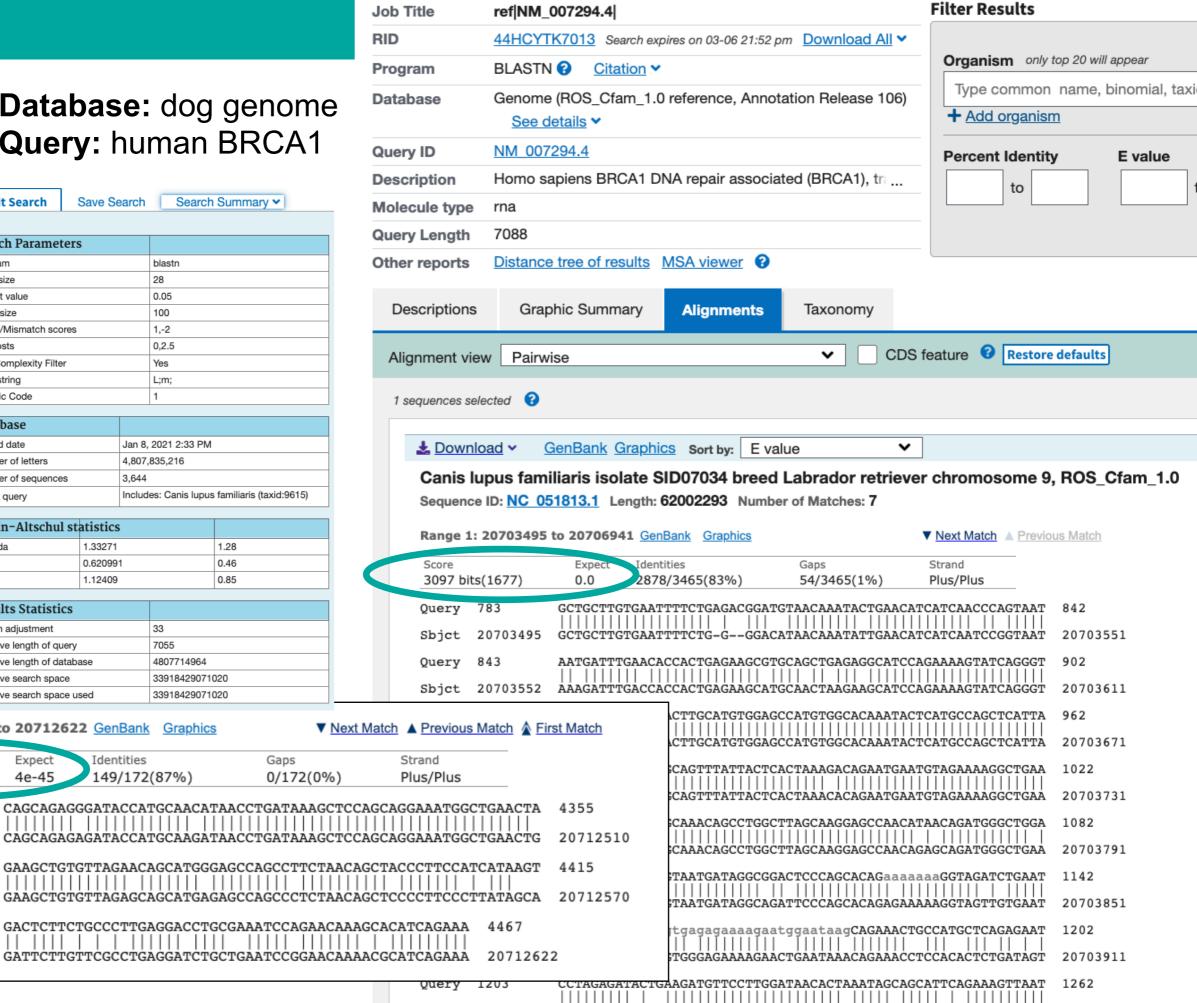
4416

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20712451

Expect

4e-45



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UCSC BLAT = "BLAST-like alignment tool"

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D. melanogaster BLAT Search			Blat								
			In-Silic	o PCR							
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Genome: Search all				orter					Query type:		
D. melanogaster				Variant Annotation Integrator		Release 6 + ISO1 MT/dm6) V			BLAT's guess N		
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			Genom	e Graphs							
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			Other 1	Tools							
☐ All Re	sults (no minin	num matches)									
Paste in a query sequence to find its location in the the genome. Multiple sequences may be searched if separated by line sequence name.											
File Upload: Rather than pasting a sequence, you can choose to upload a text file containing the sequence. Upload sequence: Choose file No file chosen submit file											
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genome.ucsc.edu