

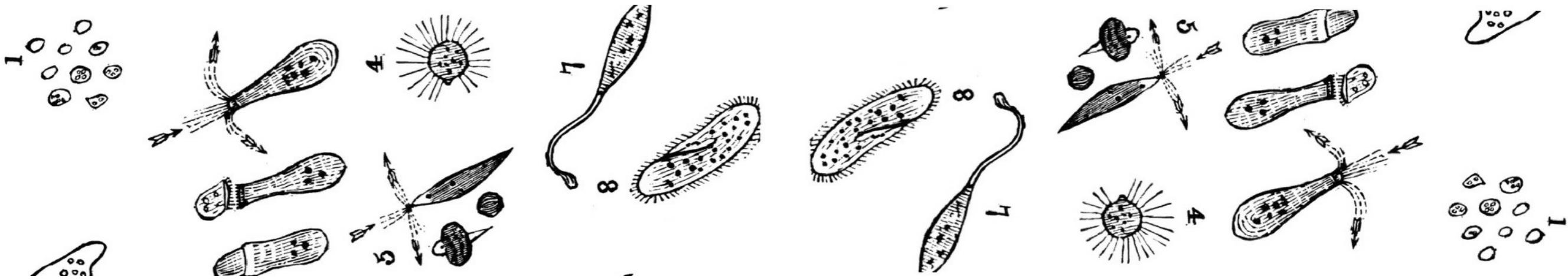


CENTER FOR BIOINFORMATICS
& INTEGRATIVE BIOLOGY

Alineamiento de secuencias

www.castrolab.org
www.cbib.cl

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20 de marzo de 2018



Todo en bioinformática es comparativo

- ...y prácticamente todo comienza con un alineamiento de secuencias
- Alinear secuencias es comúnmente un procedimiento al cual los investigadores no le prestan mucha atención...

Todo en bioinformática es comparativo

- ... sin embargo las consecuencias de los errores de alineamiento son arrastrados durante todo un análisis
- equivalente en biología molecular —> equilibrar mal el pH de una solución

La raíz de los errores en alineamientos

- Confiar ciegamente en un programa —> biología molecular vs. biología computacional
- La matriz de costo de un alineamiento

¿Qué es un alineamiento?

- Un alineamiento es una hipótesis de homología

Sequence alignment of NREGERIRF and RLEGERVRY across 40 homologs. The alignment shows high conservation of the NREGER motif at the top and the RLEGER motif at the bottom. A red box highlights a region between positions 435 and 445 where the NREGER sequence varies significantly among homologs, while the RLEGER sequence remains constant.

¿Qué es una matriz de costo?

- Es una función matemática que determina el puntaje de un alineamiento.
- De entre muchos posibles alineamientos, los programas escogen el que tenga el mejor puntaje

¿Qué es una matriz de costo?

- Están definidas por una penalización sobre los “gaps”, extender “gaps”, y “mismatches”

ATTGACCTGA
| | | | |
AT - - CCTGA

Match = 1 punto
Gap = -1 punto
Total = 6

Match = 1 punto
Abrir gap = -1 punto
Extensión de gap = -1.5
Total = 3

¿Qué es una matriz de costo?

- Están definidas por una penalización sobre los “gaps”, extender “gaps”, y “mismatches”

ACCTGATCCG	ACCTGATCCG
AC-TGATCAG	ACTGA-TCAG
S=8-4-3=1	S=5-4-12=-11

Figure 1.2. Alternate alignments of a pair of sequences illustrating a simple scoring function with matches = +1, mismatches = -3, and gaps = -4. The alignment on the left is better than the alignment on the right because its overall score is larger (1 vs. -11).

¿Es el alineamiento con mejor puntaje el verdadero?

- Usar genes bien caracterizados
- Simular datos (conoce la respuesta correcta)
- BaliBase → <http://www.lbgi.fr/balibase/>

The screenshot shows a journal article page from the website of the journal **Proteins: Structure, Function, and Bioinformatics**. The article is titled **BALiBASE 3.0: Latest developments of the multiple sequence alignment benchmark**, authored by Julie D. Thompson, Patrice Koehl, Raymond Ripp, and Olivier Poch. It was first published on 25 July 2005. The page includes links for PDF download, journal exploration, research article details, and a table of contents for Volume 61, Issue 1, dated 1 October 2005, with pages 127–136.

Proteins: Structure, Function, and Bioinformatics [Explore this journal >](#)

Research Article

BALiBASE 3.0: Latest developments of the multiple sequence alignment benchmark

Julie D. Thompson [✉](#), Patrice Koehl, Raymond Ripp, Olivier Poch

First published: 25 July 2005 [Full publication history](#)

DOI: 10.1002/prot.20527 [View/save citation](#)

PROTEINS

View issue TOC
Volume 61, Issue 1
1 October 2005
Pages 127–136

¿Qué puede pasar si tus genes están mal alineados?

- Búsqueda de motivos conservados
- Modelos de proteínas
- Árboles filogenéticos
- Selección positiva

Alineamiento local
versus global

Diferencias

Alineamiento Local

alinear regiones locales de secuencias

secuencias relacionadas distamente; rearreglos; dominios compartidos

Algoritmo popular = Smith–Waterman

Alineamiento Global

alinear dos secuencias de extremo a extremo

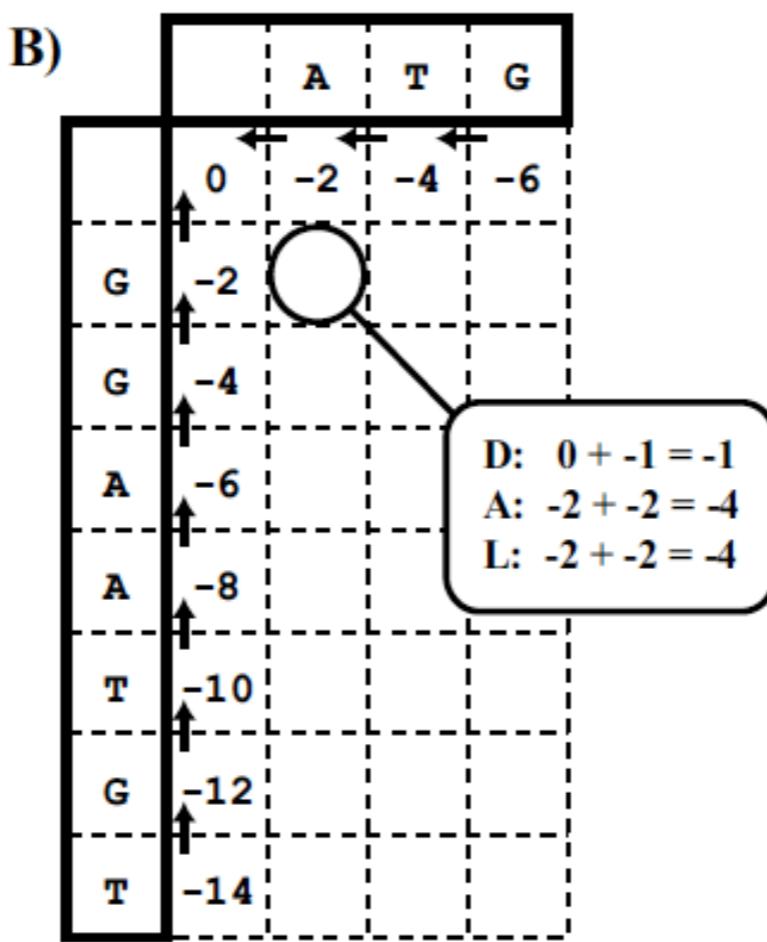
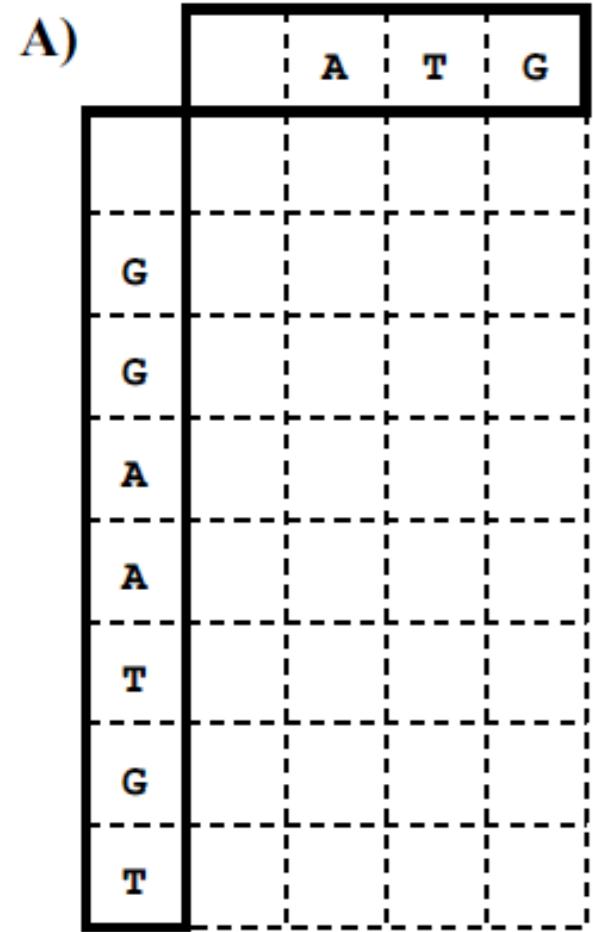
ideal para secuencias “cercanas” evolutivamente

Algoritmo popular = Needleman–Wunsch

Alineamiento global

match = +1
mismatch = -1
gap = -2

Figure 1.4. Illustration of Needleman–Wunsch (1970) global alignment algorithm. (A) Setting up the matrix. (B) The first row and column are filled with increasing multiples of the gap cost. The first cell will be given the maximum of three possible values. (C) The value for the first cell is entered along with the path that led to the value. The possible values for the second cell are illustrated. (D) The value for the second cell is entered; multiple paths are recorded since multiple paths led to the maximum score. (E) The completed matrix. (F) The completed matrix with all suboptimal paths removed. Tracing the arrows from the bottom right corner to the upper left leads to four possible paths and (therefore) four equally optimal alignments.



o global

match = +1
mismatch = -1
gap = -2

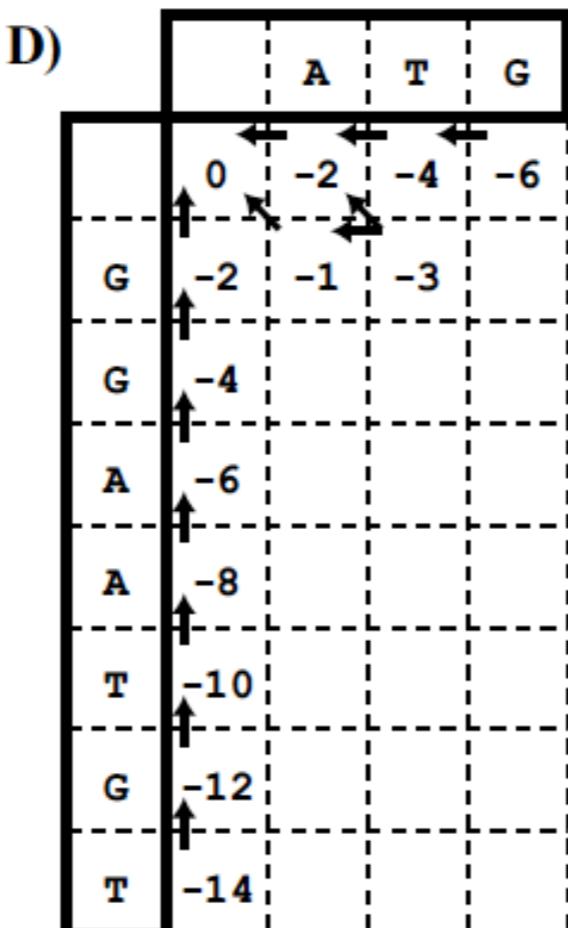
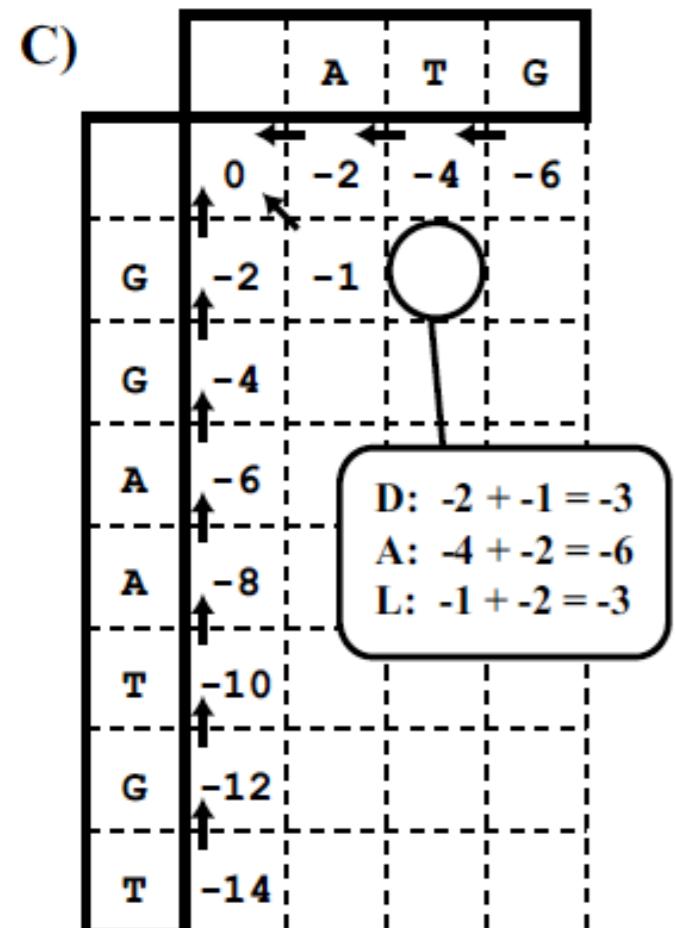


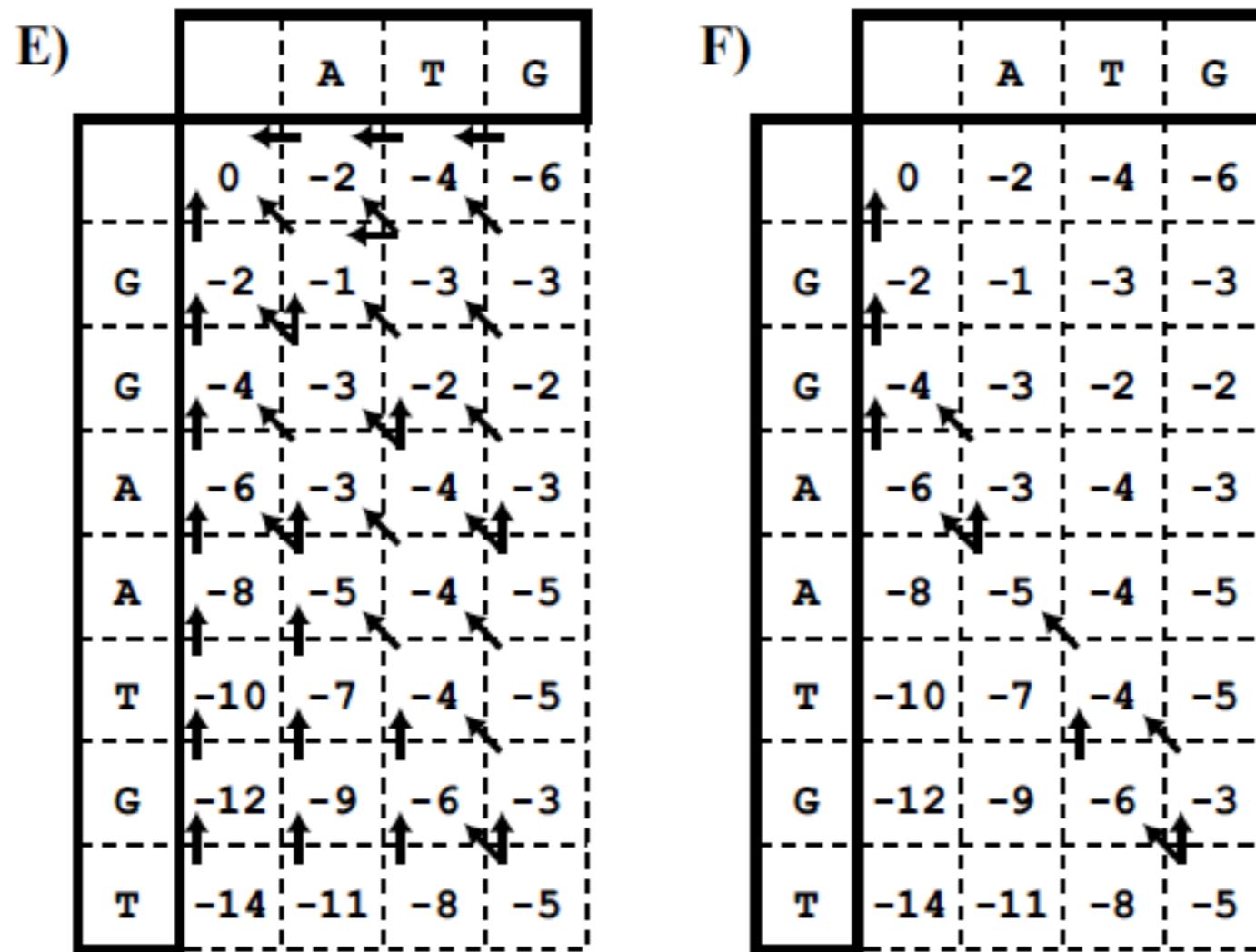
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Alineamiento global

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Alineamiento global



match = +1
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 gap = -2

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Alineamiento global

GGAATGG
---ATG-

GGAATGG
---AT-G

GGAATGG
--A-TG-

GGAATGG
--A-T-G

Figure 1.5. Four equally optimal global alignments of sequences GGAATGG and ATG derived from the alignment matrix shown in Figure 1.2.

Alineamiento global

- Existen secuencias que no pueden ser alineadas de extremo a extremo

AB--CDEF

ABEDC--F

ABCDEF

ABEDCF

ABCDE--F

AB--EDCF

Figure 1.6. Illustration of global alignment problem. Sequences ABCDEF and ABEDCF cannot be properly aligned because the homologous sections of the sequences are not in the same order.

Alineamiento local

- Adaptación de Needleman-Wunsch —> permite un cuarto valor = 0

	A	T	G	
G	0	0	0	0
G	0	0	0	1
G	0	0	0	1
A	0	1	0	0
A	0	1	0	0
T	0	0	2	0
G	0	0	0	3
T	0	0	0	1

Figure 1.7. Completed score and trace-back matrix for local alignment using the Smith and Waterman (1981b) algorithm.

Alineamiento local

- Adaptación de Needleman-Wunsch —> permite un cuarto valor = 0

	A	T	G	
A	0	0	0	0
T	0	0	0	0
G	0	0	0	1
A	0	0	0	1
A	0	1	0	0
A	0	1	0	0
T	0	0	2	0
G	0	0	0	3
T	0	0	0	1

Figure 1.7. Completed score and trace-back matrix for local alignment using the Smith and Waterman (1981b) algorithm.

ATG
ATG

Comparación local/global

CAGCCTCGCTTAG
AATGCCATTGACGG

A)

CA-GCC-TCGCTTAG	CA-GCC-TCGCTTAG	CA-GCC-TCGCTTAG	CA-GCC-TCGCTTAG
AATGCCATTGACG-G	AATGCCATTGAC-GG	AATGCCATTGA-CGG	AATGCCATTG-ACGG

B)

GCC
GCC

+1 match, -1 mismatch, -2 gap

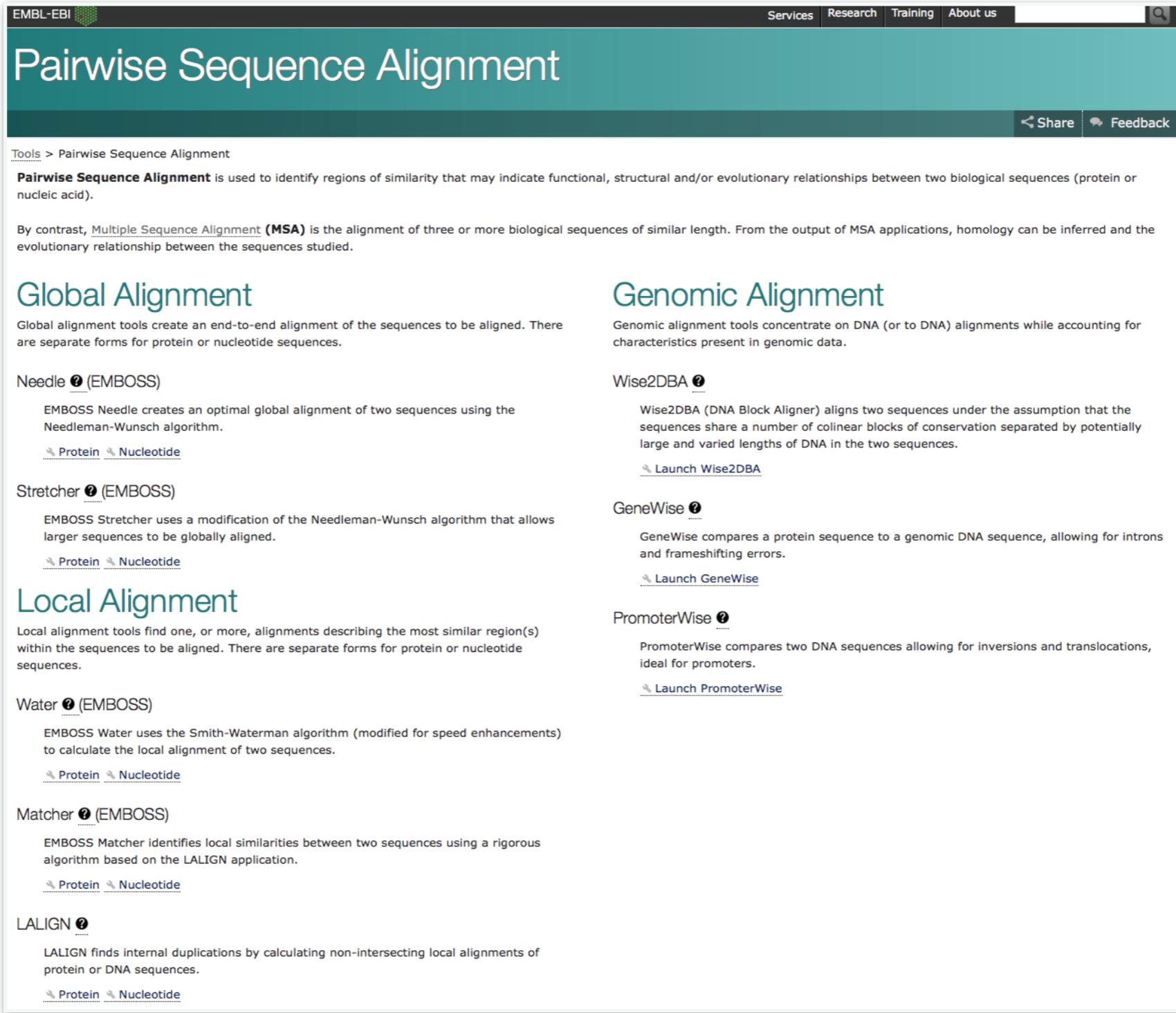
¿Cómo determinar qué valores usar?

- La mayoría de la gente usa los valores “por defecto”, i.e., los que los autores pusieron
- proporción “mismatch”/“gap cost” lo más importante
- sustituciones/indels

GCC - TCG
GCCATTG

Figure 1.10. The optimal local alignment of sequences CAGCCTCGCTTAG and AATGCCATTGACGG with a cost function with matches = +1, mismatches = -0.3, and gaps = -1.3. Contrast with the local alignment in Figure 1.9B.

Buen lugar para empezar = <http://www.ebi.ac.uk/Tools/psa/>



The screenshot shows the homepage of the EMBL-EBI Pairwise Sequence Alignment tool. The header includes the EMBL-EBI logo, navigation links for Services, Research, Training, and About us, and a search bar. Below the header, the page title is "Pairwise Sequence Alignment". A sub-navigation bar shows "Tools > Pairwise Sequence Alignment". The main content area is divided into two main sections: "Global Alignment" on the left and "Genomic Alignment" on the right.

Global Alignment

- Needle** (EMBOSS)
- EMBOSS Needle creates an optimal global alignment of two sequences using the Needleman-Wunsch algorithm.
Protein Nucleotide
- Stretcher** (EMBOSS)
- EMBOSS Stretcher uses a modification of the Needleman-Wunsch algorithm that allows larger sequences to be globally aligned.
Protein Nucleotide

Local Alignment

- Water** (EMBOSS)
- EMBOSS Water uses the Smith-Waterman algorithm (modified for speed enhancements) to calculate the local alignment of two sequences.
Protein Nucleotide
- Matcher** (EMBOSS)
- EMBOSS Matcher identifies local similarities between two sequences using a rigorous algorithm based on the LALIGN application.
Protein Nucleotide
- LALIGN**
- LALIGN finds internal duplications by calculating non-intersecting local alignments of protein or DNA sequences.
Protein Nucleotide

Genomic Alignment

- Wise2DBA**
Wise2DBA (DNA Block Aligner) aligns two sequences under the assumption that the sequences share a number of colinear blocks of conservation separated by potentially large and varied lengths of DNA in the two sequences.
Launch Wise2DBA
- GeneWise**
GeneWise compares a protein sequence to a genomic DNA sequence, allowing for introns and frameshifting errors.
Launch GeneWise
- PromoterWise**
PromoterWise compares two DNA sequences allowing for inversions and translocations, ideal for promoters.
Launch PromoterWise

Alineamiento múltiple

- Diferentes estrategias, e.g., matrices multidimensionales
- Alineamiento progresivo → Feng and Doolittle 1987

Article
Journal of Molecular Evolution
August 1987, Volume 25, Issue 4, pp 351-360

First online:

Progressive sequence alignment as a prerequisite to correct phylogenetic trees

Da-Fei Feng, Russell F. Doolittle



Look Inside >

Alineamiento múltiple: pasos

1. Calcular todos los alineamientos de pares
 1. para n secuencias, $n \times (n-1)/2$ pares
2. Calcular dendrograma usando algoritmo de clustering (UPGMA; Neighbor Joining)
3. Las secuencias más similares son alineadas primero de acuerdo al dendrograma

Alineamiento múltiple: pasos - versión gráfica

calcular alineamientos de a pares para todas las secuencias

S_1 : PPGVKSDCAS
 S_2 : PADGVVKDCAS
 S_3 : PPDGKSDS
 S_4 : GADGKDCCS
 S_5 : GADGKDCAS



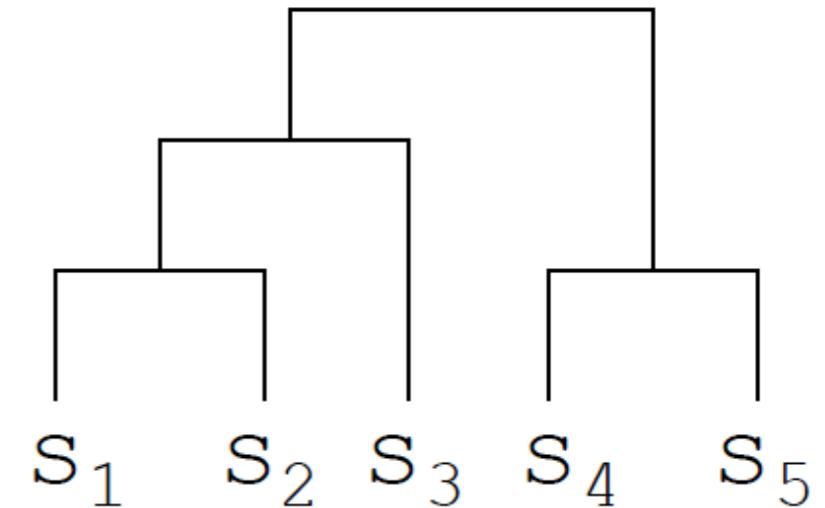
	S_1	S_2	S_3	S_4	S_5
S_1	0	0.111	0.25	0.555	0.444
S_2		0	0.375	0.222	0.111
S_3			0	0.5	0.5
S_4				0	0.111
S_5					0

Alineamiento múltiple: pasos

- versión gráfica

crear un dendrograma guía (árbol guía)

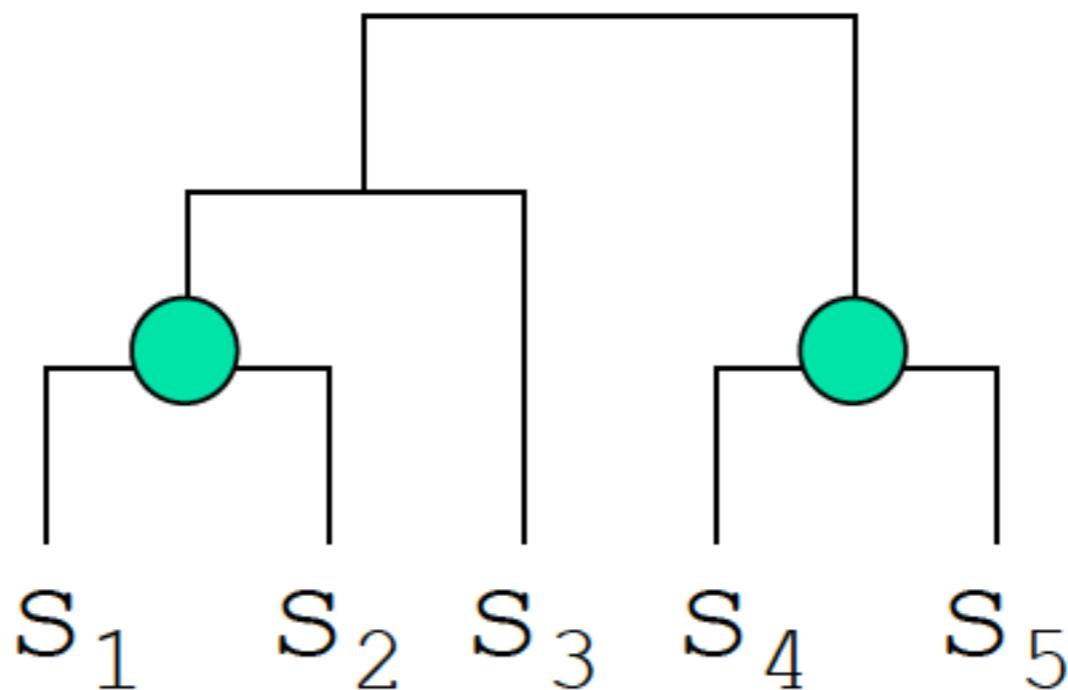
	S ₁	S ₂	S ₃	S ₄	S ₅
S ₁	0	0.111	0.25	0.555	0.444
S ₂		0	0.375	0.222	0.111
S ₃			0	0.5	0.5
S ₄				0	0.111
S ₅					0



Alineamiento múltiple: pasos

- versión gráfica

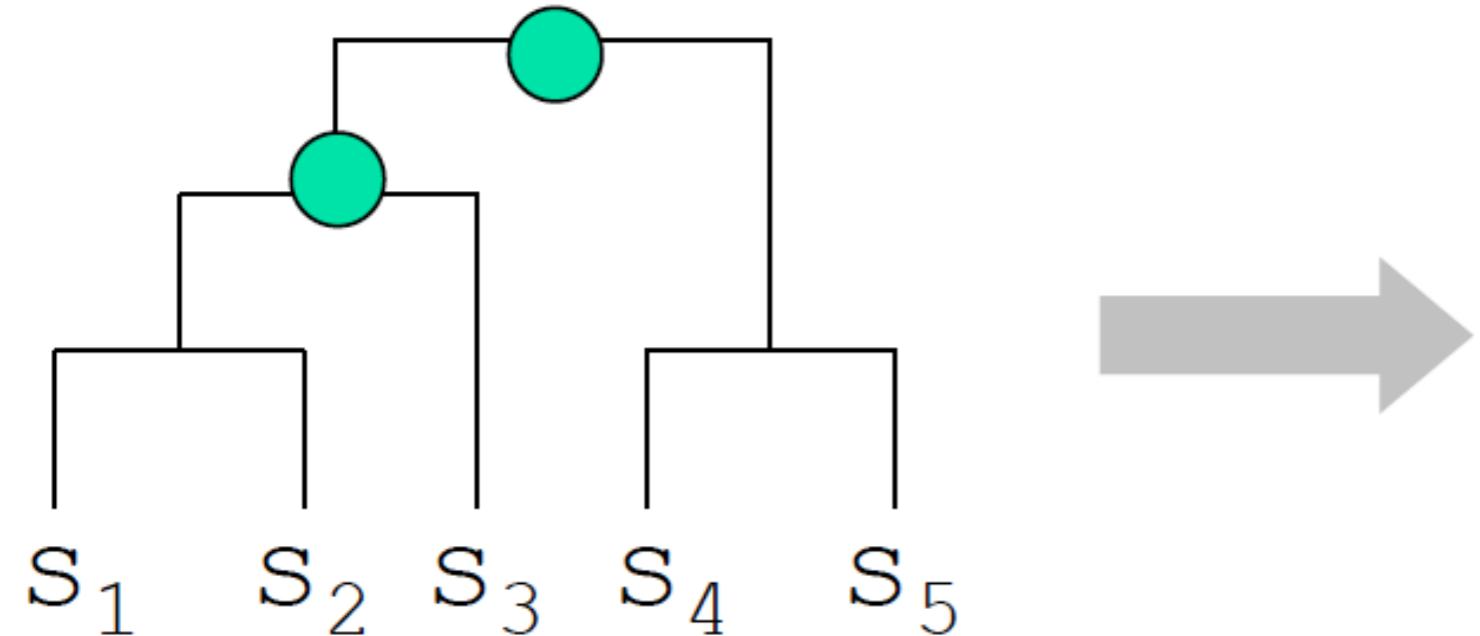
alineamos secuencias más similares primero



Alineamiento múltiple: pasos

- versión gráfica

Alineamiento múltiple



S_1 : P-PGVKSDCAS
 S_2 : PADGVK-DCAS
 S_3 : PPDG-KSD--S
 S_4 : GADG-K-DCCS
 S_5 : GADG-K-DCAS

Alineamiento múltiple: otros métodos

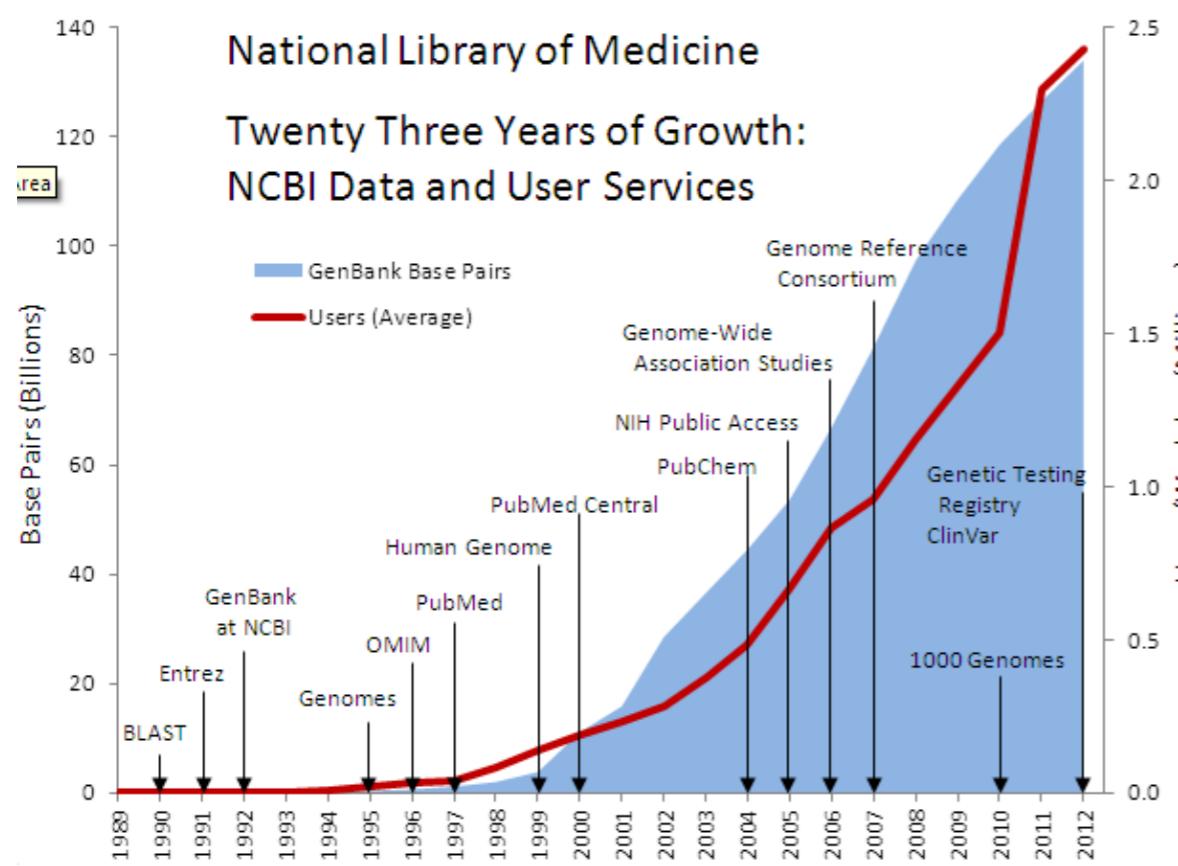
- métodos iterativos —> Muscle [http://www.ebi.ac.uk/
Tools/msa/muscle/](http://www.ebi.ac.uk/Tools/msa/muscle/)
- métodos de consenso —> M-Coffee [http://
www.tcoffee.org/Projects/mcoffee/](http://www.tcoffee.org/Projects/mcoffee/)
- modelos ocultos de Markov —> HMMER [http://
hmmer.org](http://hmmer.org)
- algoritmos genéticos —> SAGA [http://
www.tcoffee.org/Projects/saga/](http://www.tcoffee.org/Projects/saga/)

BLAST

Basic Local Alignment Search Tool

BLAST

- Tipo de alineamiento local especializado para búsqueda en bases de datos
- En segundos entrega resultados



Genetic Sequence Data Bank
February 15 2016
190250235 loci, 207018196067 bases,
from 190250235 reported sequences

BLAST

- Entrega resultados que no se deben a chance
- La premisa es que si dos secuencias se parecen no por chance, entonces son homólogas
- Homología: Desciende de un ancestro común, no necesariamente la misma función

Program	Description
blastp	Compares an amino acid query sequence against a protein sequence database.
blastn	Compares a nucleotide query sequence against a nucleotide sequence database.
blastx	Compares a nucleotide query sequence translated in all reading frames against a protein sequence database. You could use this option to find potential translation products of an unknown nucleotide sequence.
tblastn	Compares a protein query sequence against a nucleotide sequence database dynamically translated in all reading frames.
tblastx	Compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database.

Program	Notes
Megablast	<p>Contiguous</p> <p>Discontiguous</p>
Position Specific	<p>PSI-BLAST</p> <p>RPS-BLAST</p>



nucleotide only



protein only

¿Cómo funciona?

- Matriz de costo
- Corta tu secuencia en fragmentos de 3 nucleótidos
- Esos fragmentos (semillas) son extendidos hasta encontrar un resultado óptimo

Las estadísticas de BLAST son importantes

- Te permiten distinguir entre resultados significativos y por chance
- Los principales son el Score (puntaje), Query Coverage (cobertura de la secuencia de consulta) y el e-value

Las estadísticas de BLAST son importantes*

- Score = La suma de los puntajes para cada posición en la secuencia de consulta y su resultado → **Representa la calidad del alineamiento**
- e-value = No es una probabilidad, es una expectativa. **Representa el número de alineamientos diferentes con puntajes equivalentes o mejores que los que se esperan ocurrían por chance**

*pregunta de prueba

e-value

- **Representa el número de alineamientos diferentes con puntajes equivalentes o mejores que los que se esperan ocurrían por chance**
- e-value bajo (10^{-5}) puede ser indicativo de homología
- Excepción: regiones de baja complejidad como repeticiones, pueden tener e-value bajo pero no ser homólogas

BLAST® Basic Local Alignment Search Tool My NCBI Welcome pevsner. [Sign Out]

NCBI/ BLAST/ blastp suite Standard Protein BLAST

blastn blastp blastx tblastn tblastx

BLASTP programs search protein databases using a protein query. [more...](#) Reset page Bookmark

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) [?](#)

1 >gi|4504349|ref|NP_000509.1| hemoglobin subunit beta [Homo sapiens]
MVKLTPEEKSAVTALWKGKVNVDEVGGEALGRLLVVVYPWTQRFESFGDLSTPDAVMGNPKVKAH
GKKVLGAFSDGLAHLDNLKGTFTALSELHCDKLHVDPENFRLLGNVLVCVLAAHHFGKEFTPVQ
RAYQKUVAGVANALAHKTH

Clear Query subrange [?](#)

From _____ To _____

Or, upload file [Browse...](#) [?](#)

Job Title Enter a descriptive title for your BLAST search [?](#)

Align two or more sequences [?](#)

Choose Search Set

2 Database Reference proteins (refseq_protein) [?](#)

Organism Optional Enter organism name or id—completions will be suggested Exclude [+](#)
Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown. [?](#)

Exclude Optional Models (XM/XP) Uncultured/environmental sample sequences

3 Entrez Query Optional perutz mf[Author]
Enter an Entrez query to limit search [?](#)

Program Selection

4 Algorithm blastp (protein-protein BLAST)
 PSI-BLAST (Position-Specific Iterated BLAST)
 PHI-BLAST (Pattern Hit Initiated BLAST)
 DELTA-BLAST (Domain Enhanced Lookup Time Accelerated BLAST)
Choose a BLAST algorithm [?](#)

BLAST Search database Reference proteins (refseq_protein) using Blastp (protein-protein BLAST)
 Show results in a new window

5 [Algorithm parameters](#) Note: Parameter values that differ from the default are highlighted

Sub-unidad beta de la hemoglobina

FIGURE 4.1 Main page for a BLASTP search at NCBI. The sequence can be input as an accession number, GI identifier, or FASTA-formatted sequence as shown here (arrow 1). The database must be selected (arrow 2) if the default setting is not selected (as here, in which the database is set to RefSeq proteins); the choice is highlighted in yellow. The search can be restricted to a particular organism or taxonomic group, and Entrez queries can be used to further focus the search (arrow 3); here we limit the search to entries including the author Max Perutz. We discuss the BLASTP algorithm in this chapter (arrow 4), and PSI-BLAST, PHI-BLAST, and DELTA-BLAST in Chapter 5. Many of the search parameters can be modified (arrow 5).

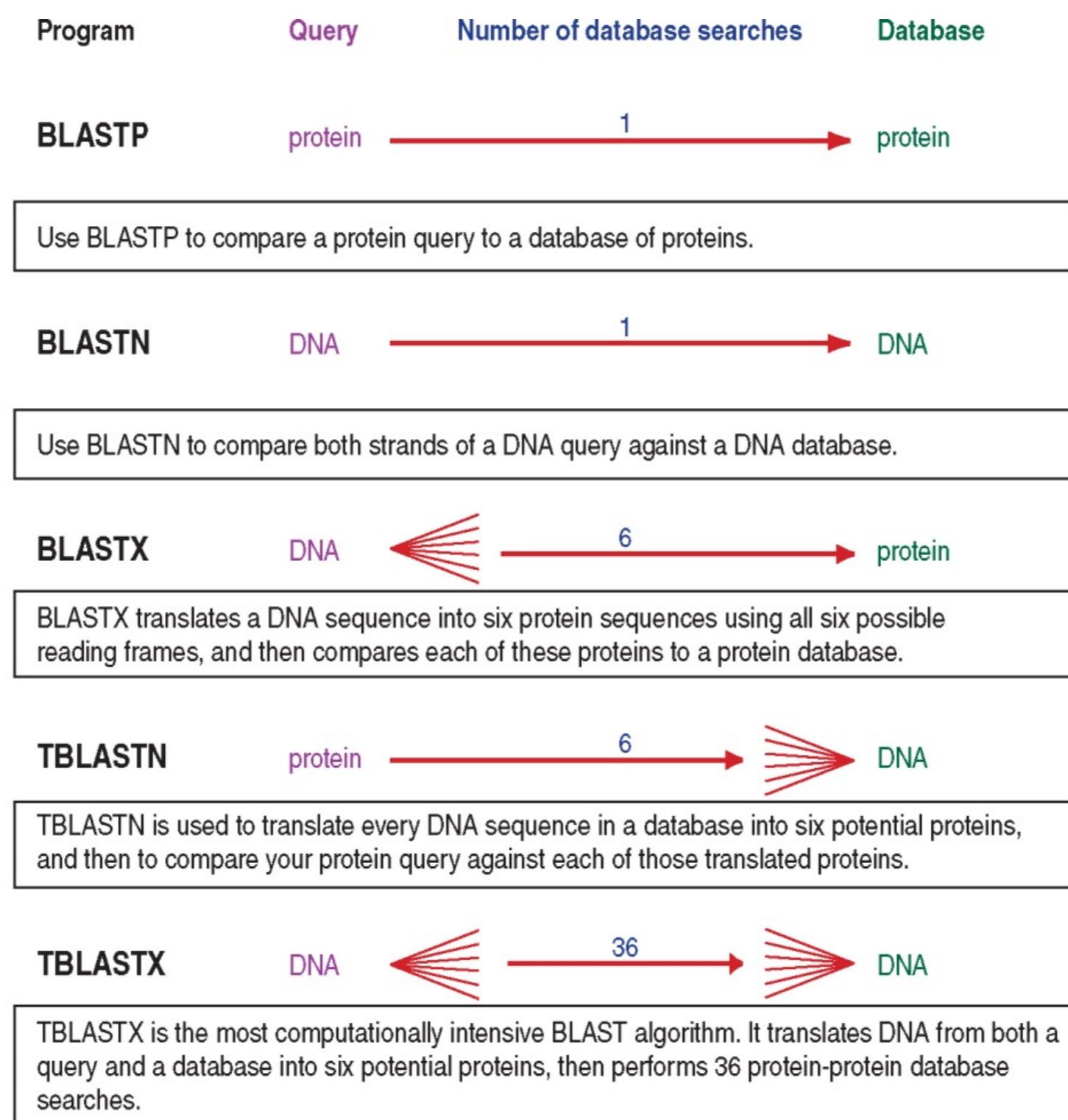


FIGURE 4.2 Overview of the five main BLAST algorithms. Note that the suffix P refers to protein (as in BLASTP), N refers to nucleotide, and X refers to a DNA query that is dynamically translated into six protein sequences. The prefix T refers to “translating,” in which a DNA database is dynamically translated into six proteins.

Homo sapiens hemoglobin, beta (HBB), mRNA

NCBI Reference Sequence: NM_000518.4

[GenBank](#) [FASTA](#)



¿Cuántos marcos de lectura existen?

FIGURE 4.3 DNA can potentially encode six different proteins. To demonstrate this, we view the NCBI Nucleotide entry for HBB and select the “graphics” view; The two strands of DNA sequence are shown (arrow 1). In this zoomed view, only a portion of the HBB sequence is displayed. From the top strand, three potential proteins are encoded (frames +1, +2, +3) with the corresponding amino acids indicated in gray using the single-letter amino acid abbreviations. In this case, frame +3 corresponds to the frame used for translation (arrow 2). Note that frames +1 and +2 as well as frame -3 include stop codons (asterisks shaded red). The lower portion of the display includes the amino acid sequence of the corresponding protein (arrow 3) as well as the corresponding nucleotides (matching frame +3); features indicated with black shading represent a site that may be acetylated or glycosylated and a globin domain.

Algorithm parameters

General Parameters

1 → Max target sequences 100 Select the maximum number of aligned sequences to display ?

2 → Short queries Automatically adjust parameters for short input sequences ?

3 → Expect threshold 10 ?

4 → Word size 3 ?

5 → Max matches in a query range 0 ?

Scoring Parameters

6 → Matrix BLOSUM62 ?

7 → Gap Costs Existence: 11 Extension: 1 ?

8 → Compositional adjustments Conditional compositional score matrix adjustment ?

Filters and Masking

9 → Filter Low complexity regions ?

10 → Mask Mask for lookup table only ?
 Mask lower case letters ?

BLAST Search database Non-redundant protein sequences (nr) using Blastp (protein-protein BLAST)
 Show results in a new window

FIGURE 4.4 Optional BLASTP parameters. Numbered arrows refer to discussion in the text.

Bioinformatics and Functional Genomics, Third Edition, Jonathan Pevsner.

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Companion Website: www.wiley.com/go/pevsnerbioinformatics

Graphic Summary

Show Conserved Domains

Putative conserved domains have been detected, click on the image below for detailed results.



Distribution of 27 Blast Hits on the Query Sequence

Mouse-over to show define and scores, click to show alignments

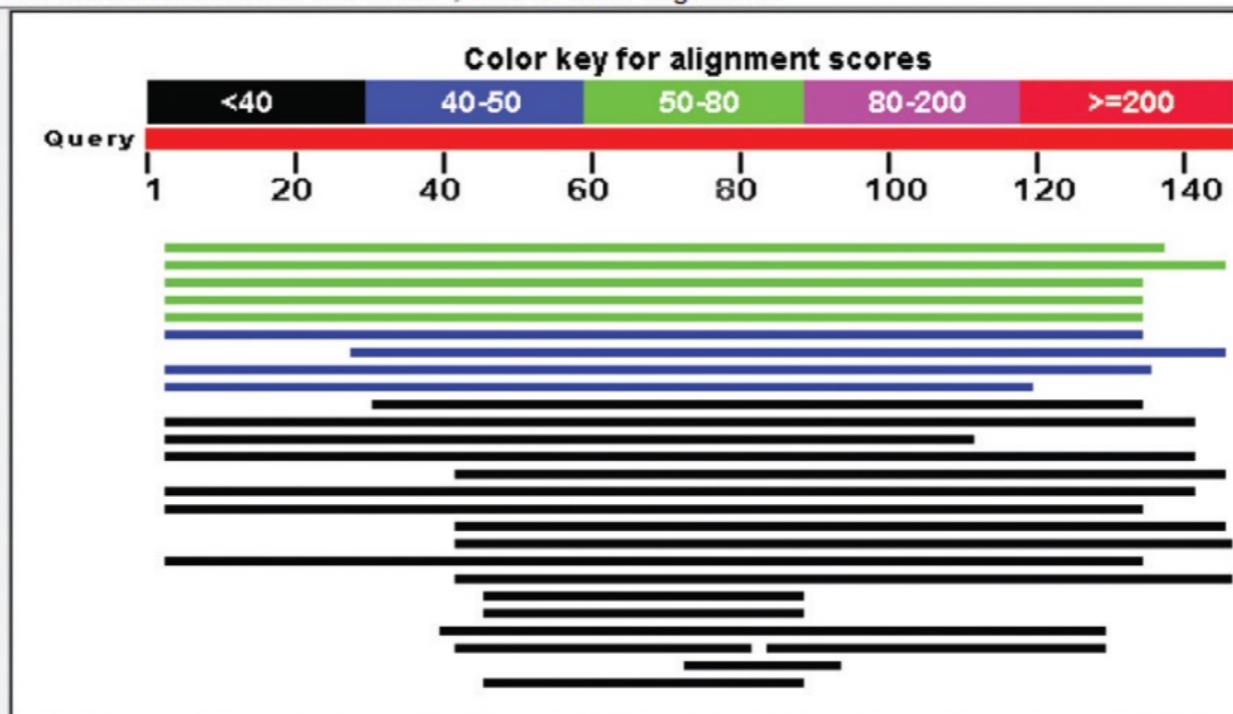


FIGURE 4.8 The graphic summary of BLAST results includes a display of conserved domains (here showing a match to the globin protein family), then a color-coded distribution of hits. Here the *x* axis corresponds to the length of the query (147 amino acid residues for beta globin), with each database match characterized by a color-coded score (e.g., five matches shaded green have scores of 50–80) and lengths (one of the five green database hits includes an aligned region that extends fully to the carboxy-terminus of the HBB query, while the other four do not). This graphic can be useful to summarize the regions in which database matches align to the query.

Sequences producing significant alignments:

Select: All None Selected:2

	Description	Max score	Total score	Query cover	E value	Max ident	Accession
<input checked="" type="checkbox"/>	PREDICTED: cytoglobin-2-like isoform 1 [Bombus terrestris] >ref XP_003396833.1 PREDI	59.7	59.7	91%	1e-10	29%	XP_003396832.1
<input checked="" type="checkbox"/>	PREDICTED: cytoglobin-2-like isoform 1 [Bombus impatiens] >ref XP_003494220.1 PREDI	58.5	58.5	97%	3e-10	28%	XP_003494219.1
<input type="checkbox"/>	PREDICTED: globin-like [Megachile rotundata]	57.8	57.8	89%	6e-10	29%	XP_003707185.1
<input type="checkbox"/>	PREDICTED: globin-like [Apis florea]	53.9	53.9	89%	1e-08	30%	XP_003690810.1
<input type="checkbox"/>	globin 1 [Apis mellifera]	52.8	52.8	89%	4e-08	30%	NP_001071291.1
<input type="checkbox"/>	PREDICTED: cytoglobin-2-like isoform 1 [Bombus terrestris] >ref XP_003396831.1 PREDI	45.1	45.1	89%	2e-05	26%	XP_003396830.1
<input type="checkbox"/>	PREDICTED: neuroglobin-like, partial [Acyrthosiphon pisum]	42.4	42.4	80%	2e-04	23%	XP_001946608.2
<input type="checkbox"/>	globin, putative [Ixodes scapularis]	42.7	42.7	90%	2e-04	25%	XP_002414906.1

FIGURE 4.9 A typical BLASTP output includes a list of database sequences that match the query. Links are provided to that database entry (e.g., an NCBI Protein entry) and to the pairwise alignment to the query. The bit score and *E* value for each alignment are also provided. Note that the best matches at the top of the list have large bit scores and small *E* values.

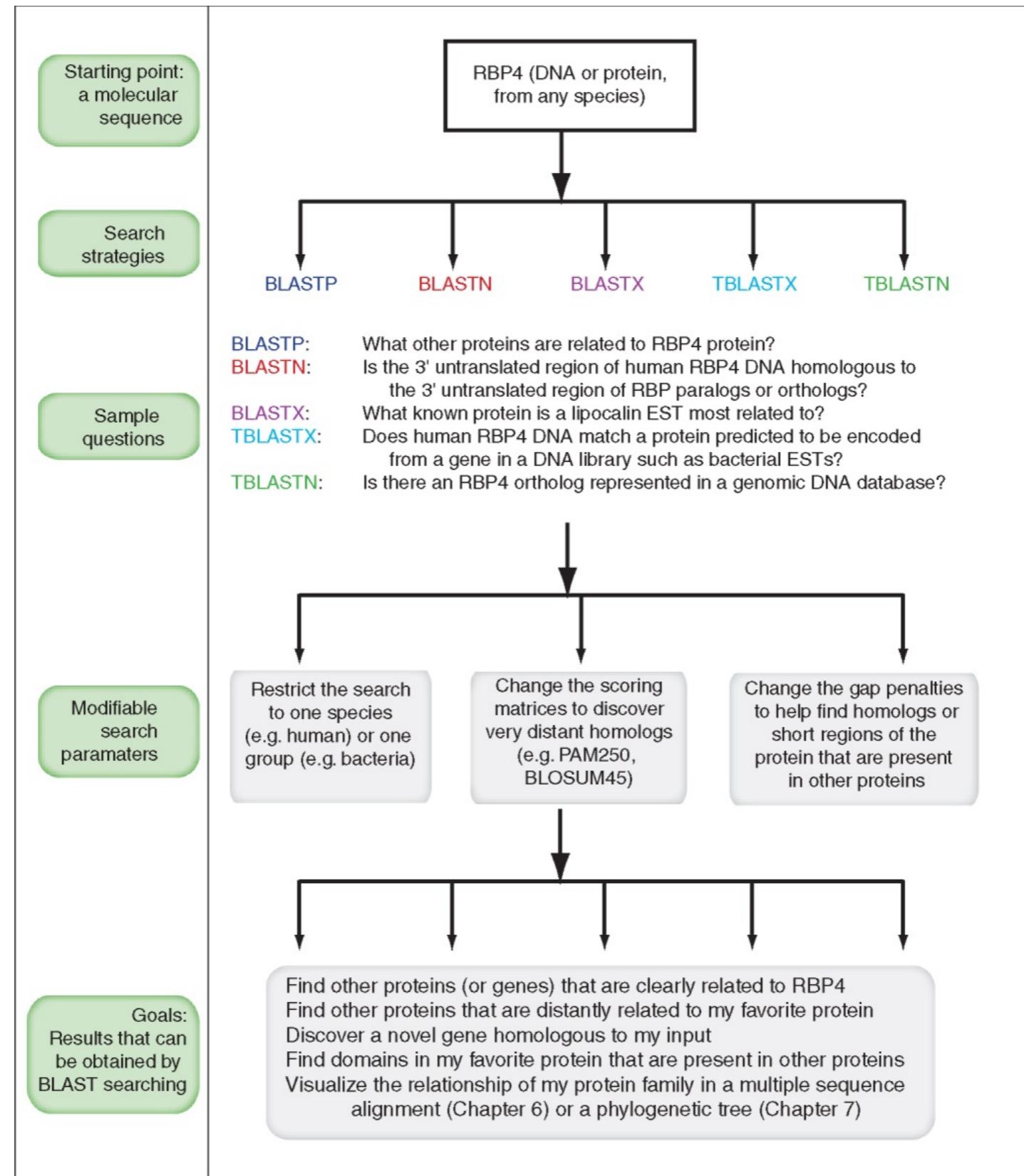


FIGURE 4.15 Overview of BLAST searching strategies. There are many hundreds of questions that can be addressed with BLAST searching, from characterizing the genome of an organism to evaluating the sequence variation in a single gene.

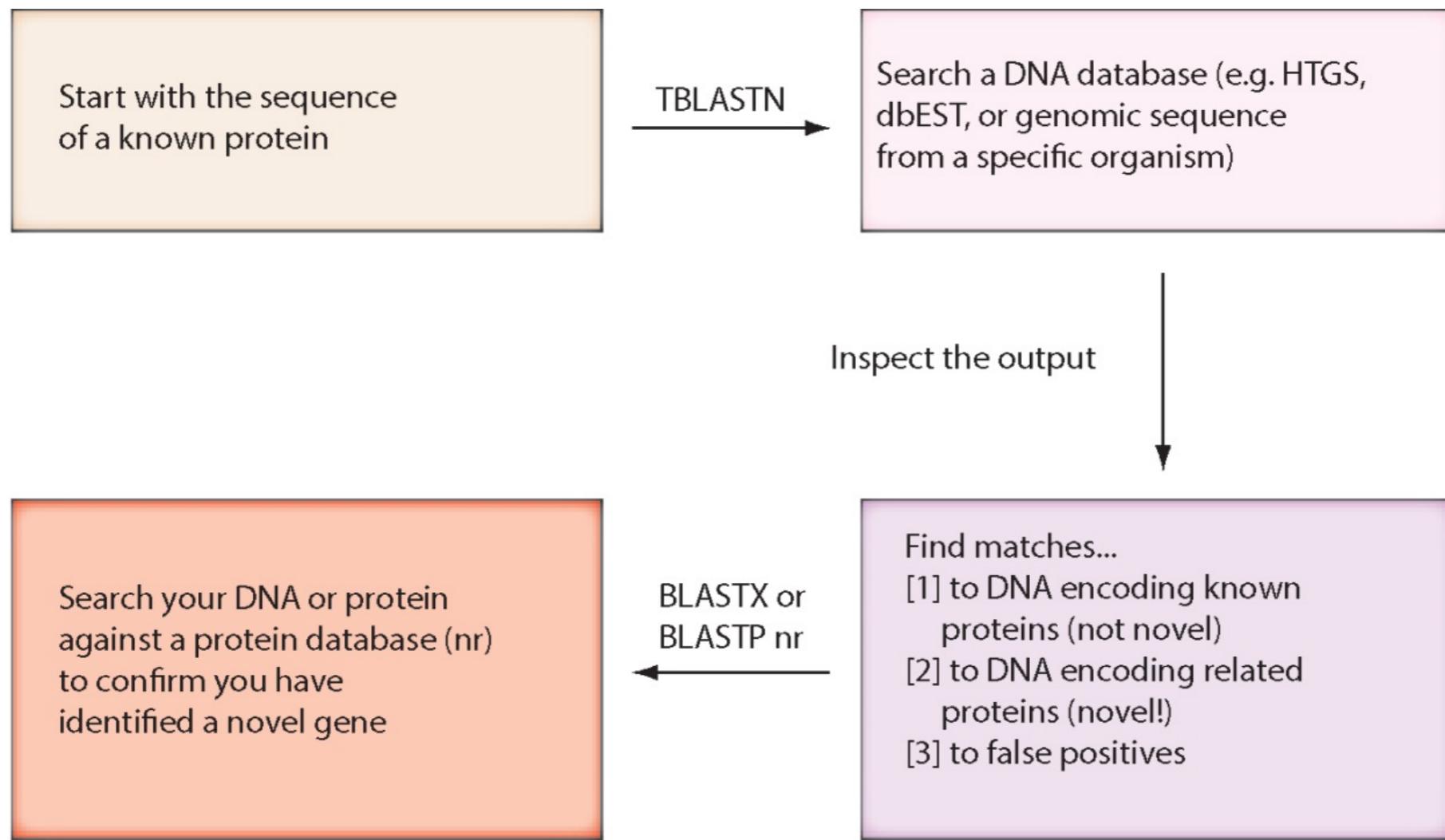
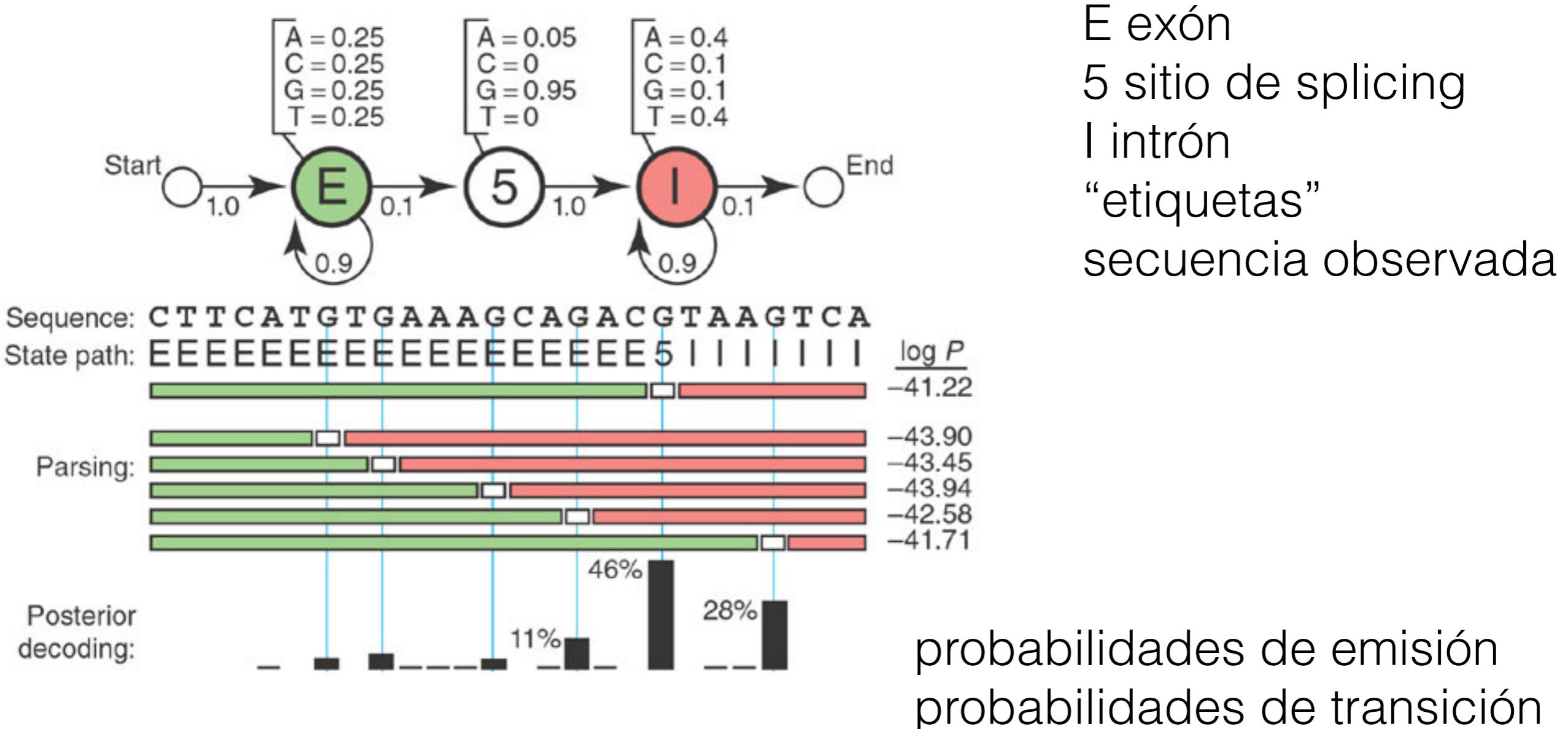


FIGURE 4.23 How to discover a novel gene by BLAST searching. Begin with the sequence of a known protein such as human beta globin. Perform a TBLASTN search of a DNA database. It is unlikely that there are many “novel” genes in the well-characterized genomes of organisms such as human, yeast, or *E. coli*. It may therefore be helpful to search databases of organisms that are poorly characterized or not fully annotated. The TBLASTN search may result in two types of significant matches: (1) matches of your query to known proteins that are already annotated; and (2) homologous proteins that have not yet been annotated (“novel” genes and corresponding novel proteins). (3) The DNA sequence corresponding to the putative novel gene may be searched using the BLASTX algorithm against the nonredundant (nr) database. This may confirm that the DNA does indeed encode a protein that has no perfect match to any described protein.

Modelos de Markov Ocultos (HMMs)

- En inglés “Hidden Markov Models”
- Son la caja de herramientas conceptual para generar un modelo probabilístico
- En vez de preguntarse cuáles son los valores de la matriz de costo para mi alineamiento, HMMs evaluar la probabilidad de que un número de combinaciones sea cierta

Modelos de Markov Ocultos (HMMs)



Ejemplo para encontrar la mejor transición de exón a intrón

Estrategias para mejorar alineamientos

- Si tienes secuencias codificantes → usa un alineamiento traducido (translated alignment)

Direct nucleotide alignment

A color-coded alignment of nine DNA sequences. Nucleotides are represented by four colors: blue for A, green for C, red for G, and orange for T. The alignment shows high conservation of sequence across the samples.

Average % of identity: 78%
(ranging from 57 to 88%)

Amino acid alignment

A color-coded alignment of nine protein sequences. Amino acids are represented by four colors: blue for K, green for F, red for M, and orange for L. The alignment shows high conservation of sequence across the samples.

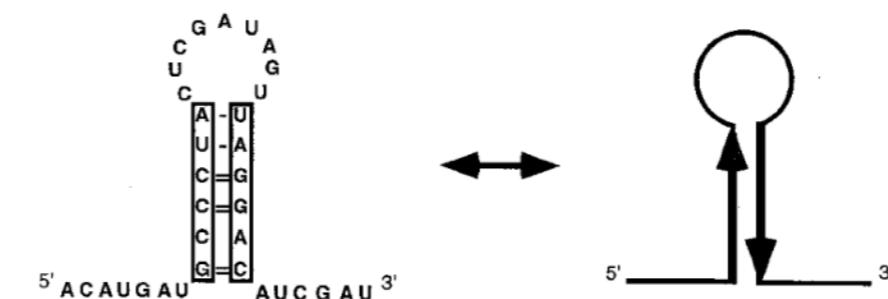
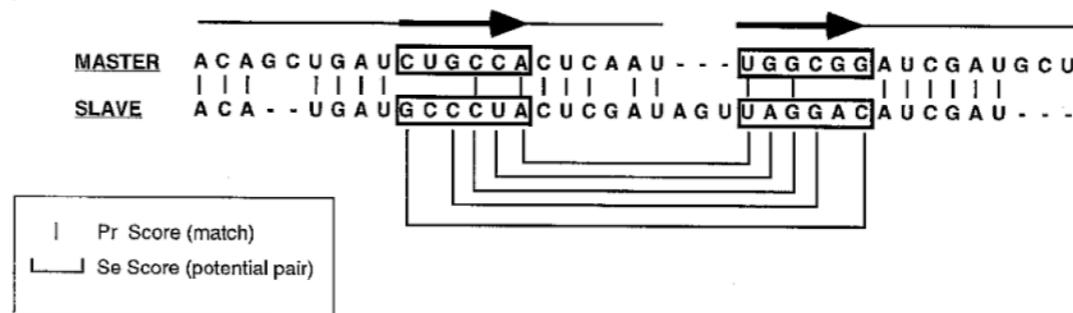
Back-translation

Average % of identity: 73%
(ranging from 33 to 88%)

A color-coded alignment of nine DNA sequences, representing the back-translation of the amino acid alignment. The sequences are identical to the original direct nucleotide alignment shown above.

Estrategias para mejorar alineamientos

- Si tienes secuencias que forman estructuras secundarias —> usa un alineamiento ad hoc (structure-aware)



En resumen

- Alineamiento de secuencias es central en bioinformática —> métodos comparativos
- Errores en el alineamiento son arrastrados
- Matriz de costos determinan el alineamiento final y su puntaje
- Alineamientos locales y globales
- Alineamiento múltiple es una extensión del global
- BLAST, alineamiento local para búsqueda en bases de datos
- HMMs —> modelos probabilísticos
- Translated alignments / structure-aware alignments