Alineamiento

Bioinformática para biotecnología BIT120
31 marzo 2016
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www.castrolab.org

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

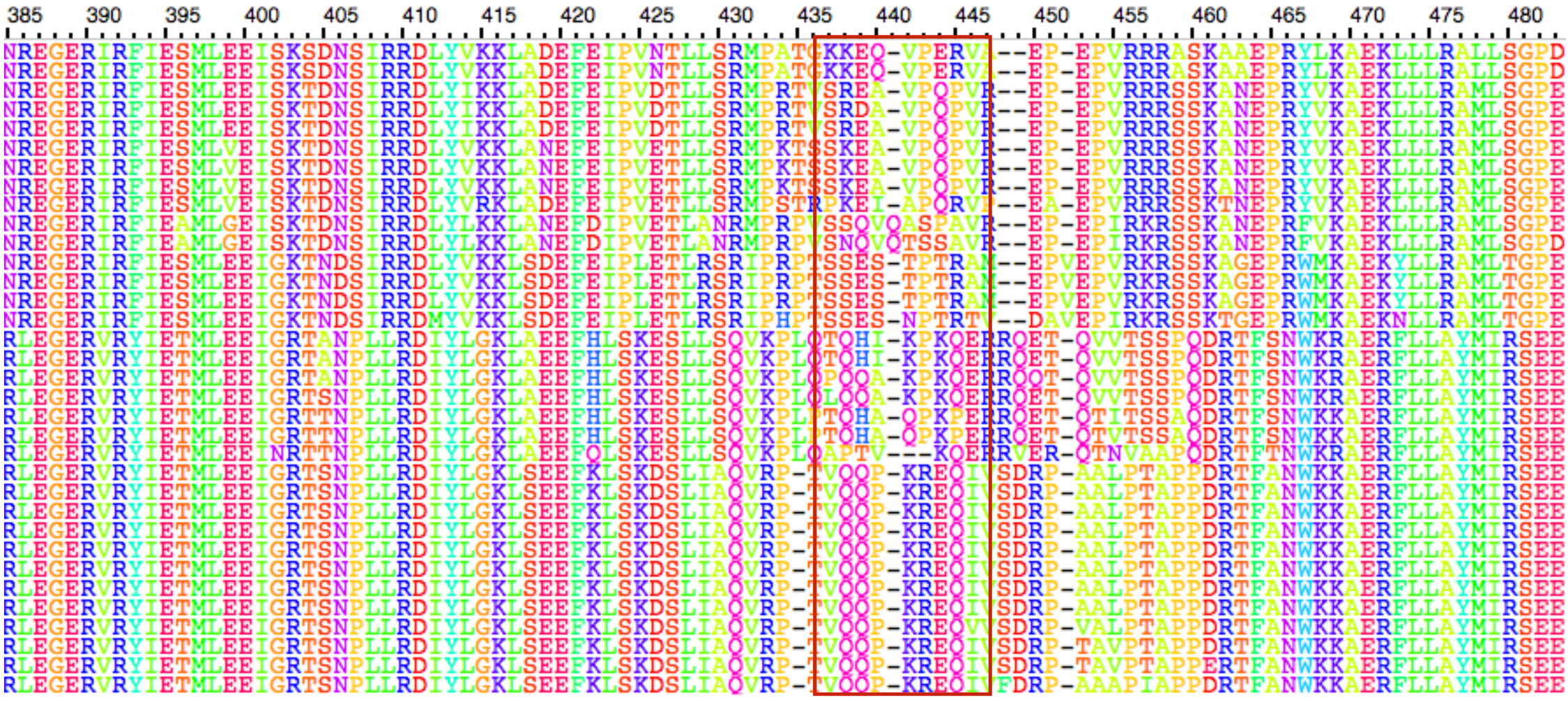
La raíz de los errores en alineamientos

- 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



¿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”

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”

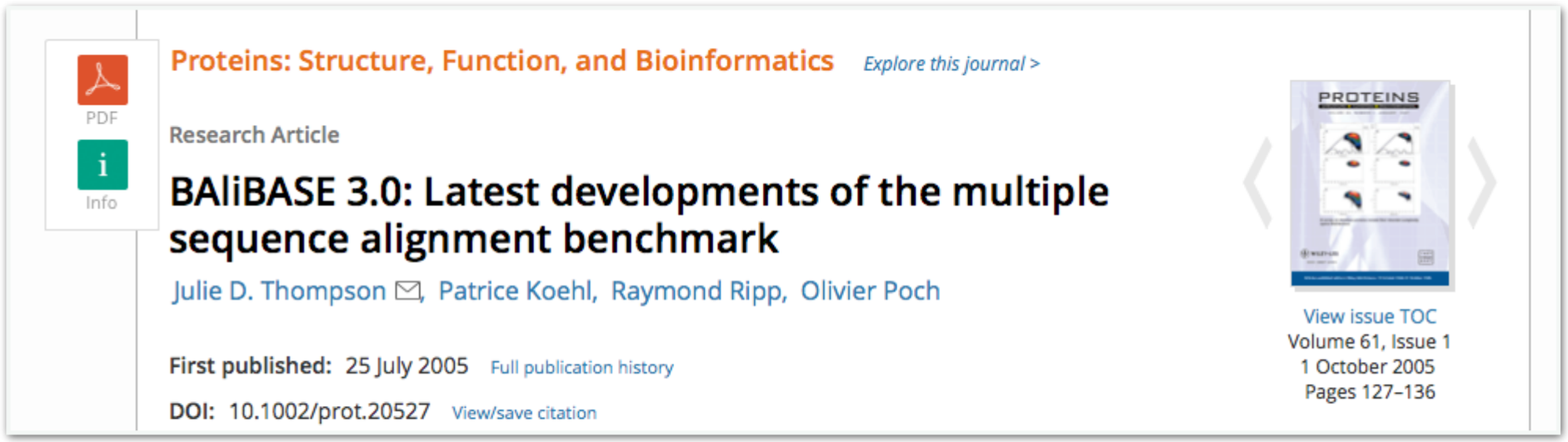
```
ACCTGATCCG        ACCTGATCCG
||  |||||  |      ||     ||  |
AC-TGATCAG        ACTGA-TCAG
S=8-4-3=1         S=5-4-12=-11
```

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).

Sequence Alignment Methods, Models, Concepts, and Strategies Edited by Michael S. Rosenberg

¿Es el alineamiento con mejor puntaje el verdadero?

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



¿Qué puede pasar si tus genes están mal alineados?

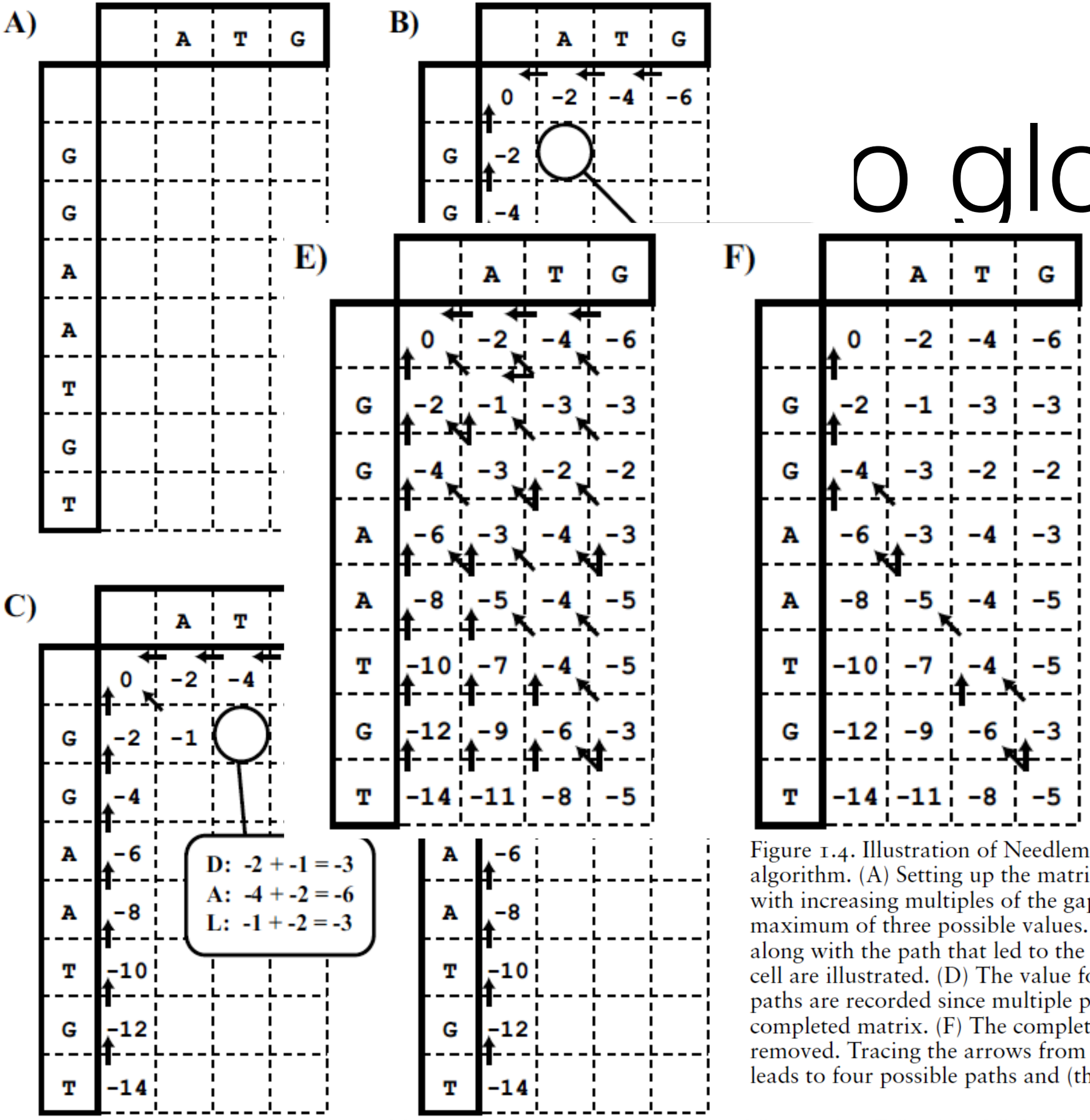
- Búsqueda de motivos conservados
- Modelos de proteínas
- Árboles filogenéticos
- Selección positiva

Alineamiento local versus global

Diferencias

Alineamiento Local	Alineamiento Global
alinear regiones locales de secuencias	alinear dos secuencias de extremo a extremo
secuencias relacionadas distantemente; rearreglos; dominios compartidos	ideal para secuencias “cercanas” evolutivamente
Algoritmo popular = Smith–Waterman	Algoritmo popular = Needleman–Wunsch

o 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.

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Alineamiento global

```
GGAATGG    GGAATGG    GGAATGG    GGAATGG
---ATG-    ---AT-G    --A-TG-    --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.

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Alineamiento global

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

```
AB--CDEF      ABCDEF      ABCDE--F
ABEDC--F      ABEDCF      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.

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Alineamiento local

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

		A	T	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.

ATG

ATG

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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

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¿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.

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

EMBL-EBI Services Research Training About us

Pairwise Sequence Alignment

Share Feedback

Tools > Pairwise Sequence Alignment

Pairwise Sequence Alignment is used to identify regions of similarity that may indicate functional, structural and/or evolutionary relationships between two biological sequences (protein or nucleic acid).

By contrast, Multiple Sequence Alignment **(MSA)** is the alignment of three or more biological sequences of similar length. From the output of MSA applications, homology can be inferred and the evolutionary relationship between the sequences studied.

Global Alignment

Global alignment tools create an end-to-end alignment of the sequences to be aligned. There are separate forms for protein or nucleotide sequences.

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

Local alignment tools find one, or more, alignments describing the most similar region(s) within the sequences to be aligned. There are separate forms for protein or nucleotide sequences.

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

Genomic alignment tools concentrate on DNA (or to DNA) alignments while accounting for characteristics present in genomic data.

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

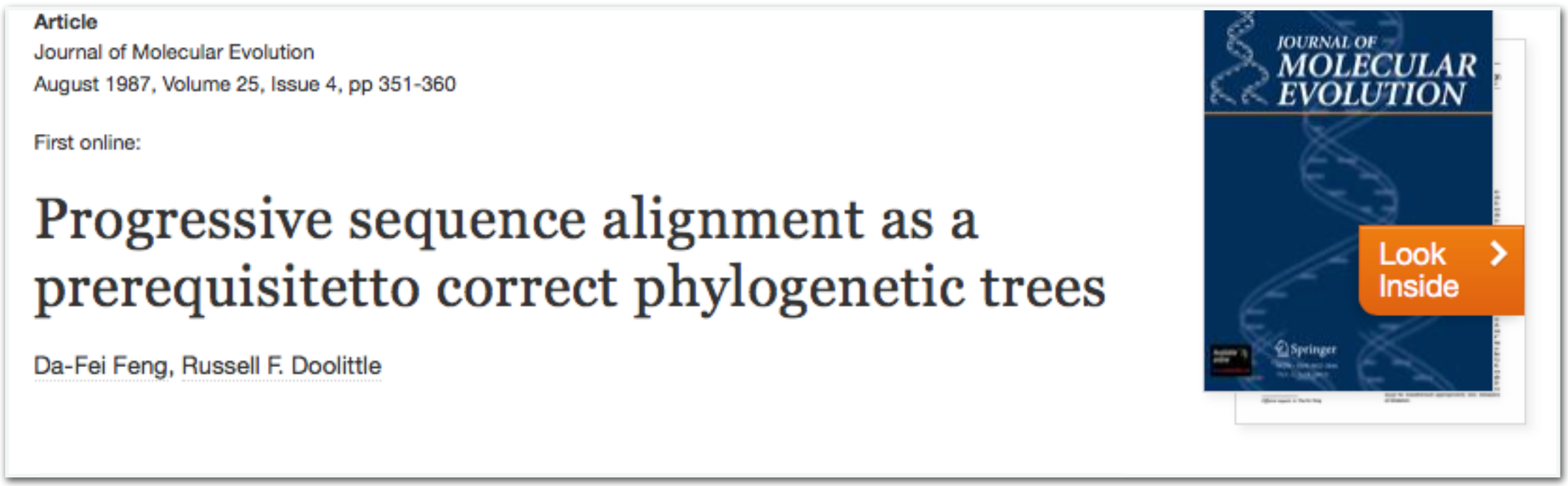
PromoterWise compares two DNA sequences allowing for inversions and translocations, ideal for promoters.

Launch PromoterWise

Alineamiento múltiple

- Diferentes estrategias, e.g., matrices múlti-dimensionales

- 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 prerequisitetto correct phylogenetic trees

Da-Fei Feng, Russell F. Doolittle

Alineamiento múltiple: pasos

1. Calcular todos los alineamientos de pares

    1. para n secuencias, n x (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: PADGVKDCAS
S_3: PPDGKSDS
S_4: GADGKDCCS
S_5: GADGKDCAS

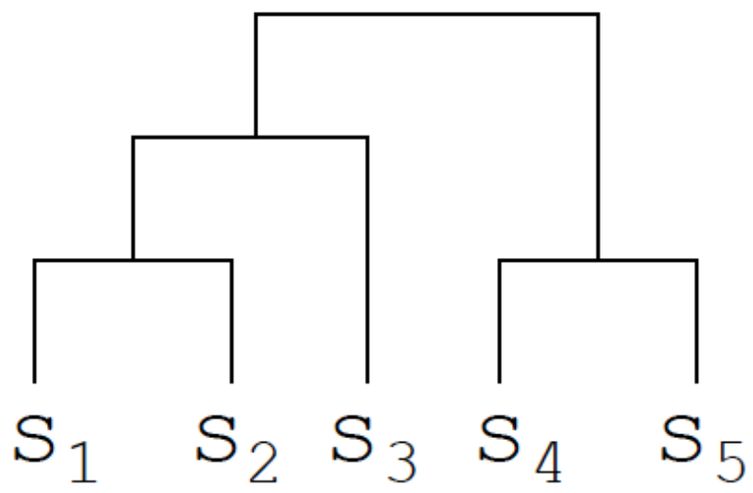
	S_1	S_2	S_3	S_4	S_5
S_1	0	0.111	0.25	0.555	0.444
S_2		0	0.375	0.222	0.111
S_3			0	0.5	0.5
S_4				0	0.111
S_5					0

http://www.comp.nus.edu.sg/~ksung/algo_in_bioinfo/slides/

Alineamiento múltiple: pasos - versión gráfica

crear un dendrograma guía (árbol guía)

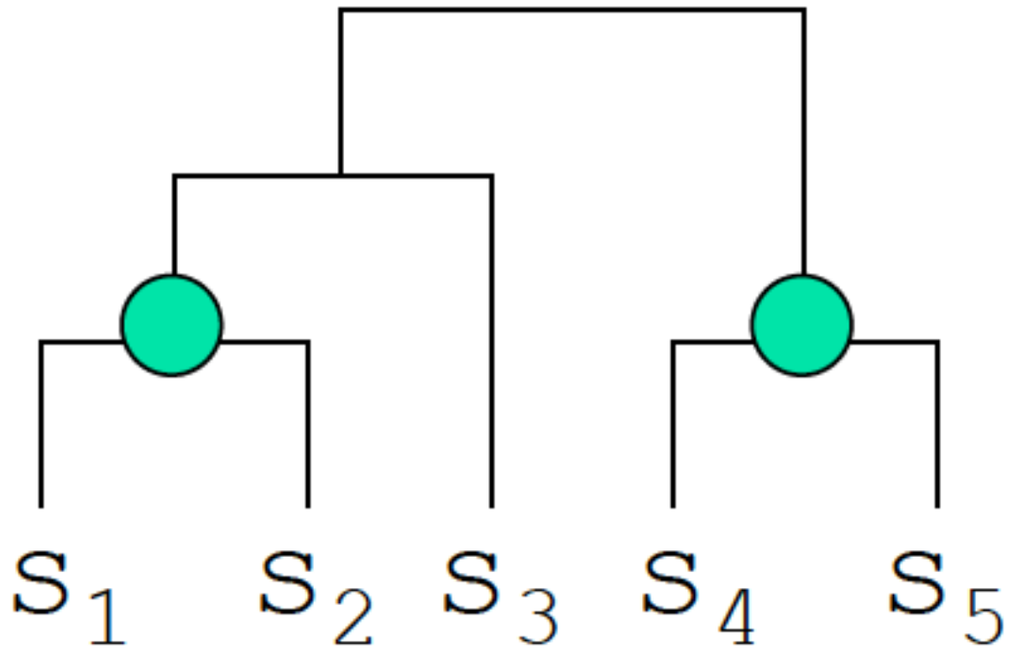
	S_1	S_2	S_3	S_4	S_5
S_1	0	0.111	0.25	0.555	0.444
S_2		0	0.375	0.222	0.111
S_3			0	0.5	0.5
S_4				0	0.111
S_5					0



http://www.comp.nus.edu.sg/~ksung/algo_in_bioinfo/slides/

Alineamiento múltiple: pasos - versión gráfica

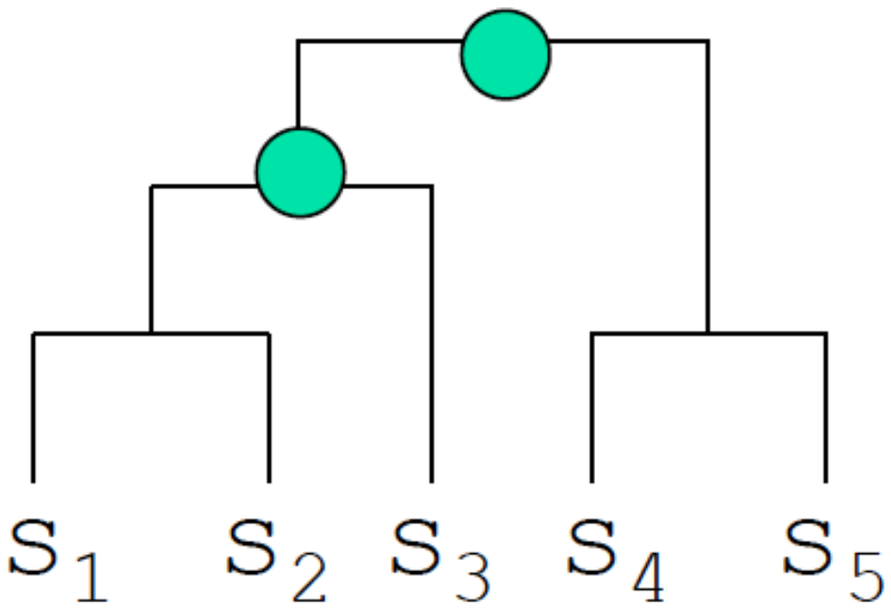
alineamos secuencias más similares primero



http://www.comp.nus.edu.sg/~ksung/algo_in_bioinfo/slides/

Alineamiento múltiple: pasos - versión gráfica

Alineamiento múltiple



```
S1: P-PGVKSDCAS
S2: PADGVK-DCAS
S3: PPDG-KSD--S
S4: GADG-K-DCCS
S5: GADG-K-DCAS
```

http://www.comp.nus.edu.sg/~ksung/algo_in_bioinfo/slides/

Alineamiento múltiple: otros métodos

- métodos iterativos —> Muscle http://www.ebi.ac.uk/Tools/msa/muscle/

- métodos de consenso —> M-Coffee http://www.tcoffee.org/Projects/mcoffee/

- modelos ocultos de Markov —> HMMER http://hmmer.org

- algoritmos genéticos —> SAGA http://www.tcoffee.org/Projects/saga/

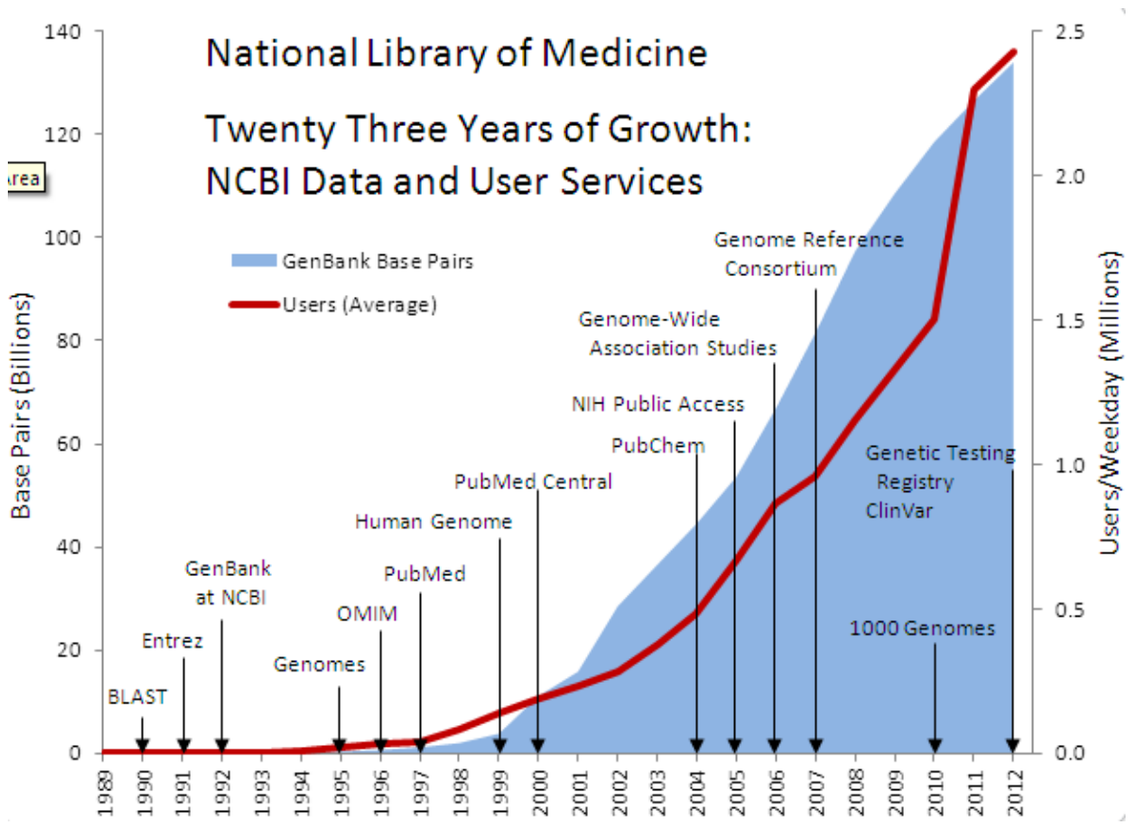
BLAST

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	Contiguous	Nearly identical sequences
	Discontiguous	Cross-species comparison
Position Specific	PSI-BLAST	Automatically generates a position specific score matrix (PSSM)
	RPS-BLAST	Searches a database of PSI-BLAST PSSMs

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 ocurran por chance**

*pregunta de prueba

e-value

- **Representa el número de alineamientos diferentes con puntajes equivalentes o mejores que los que se esperan ocurran 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

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).

Bioinformatics and Functional Genomics, Third Edition, Jonathan Pevsner.

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

Sub-unidad beta de la hemoglobina

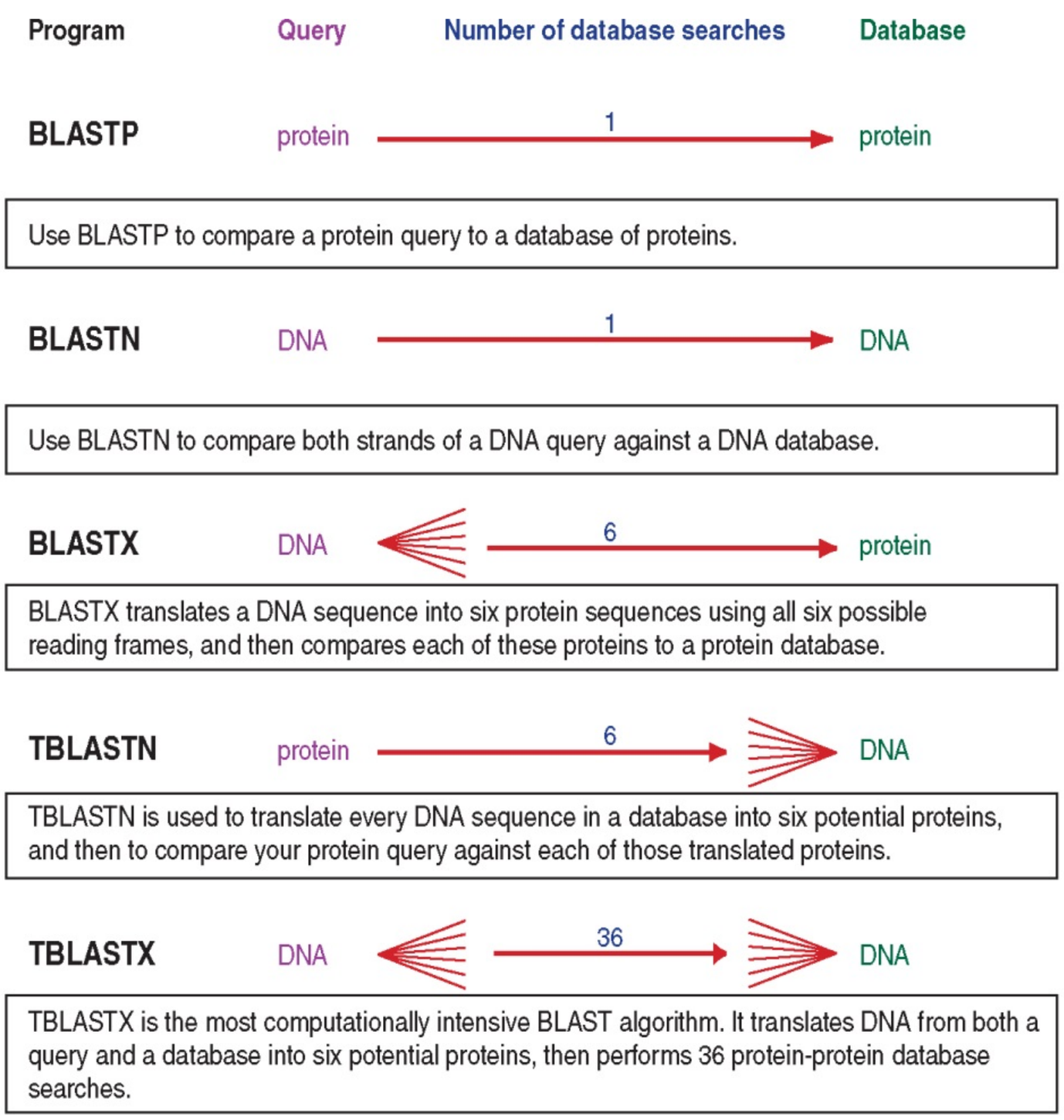


FIGURE 4.2 Overview of the five main BLAST algorithms. Note that the suffix P refers to protein (as in BLASTP), N refers to nucleotide, and X refers to a DNA query that is dynamically translated into six protein sequences. The prefix T refers to "translating," in which a DNA database is dynamically translated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

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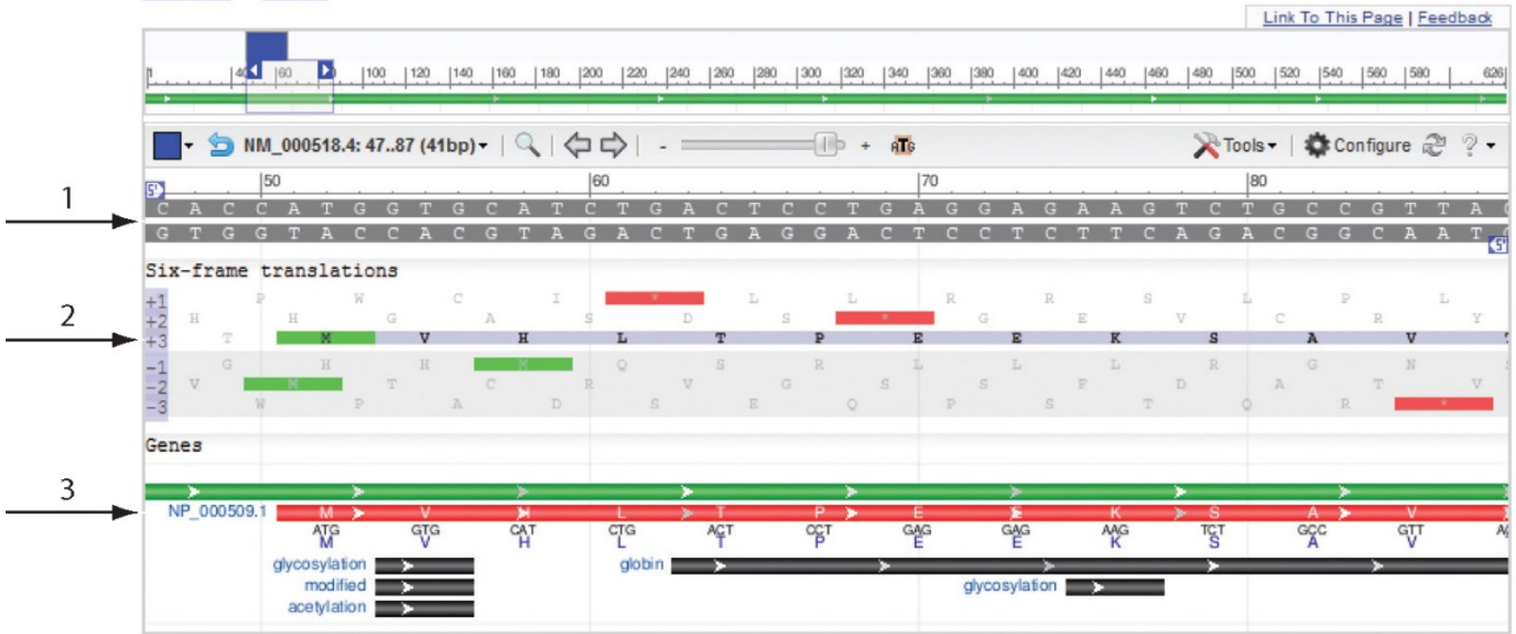

¿Cuántos marcos de lectura existen?

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.

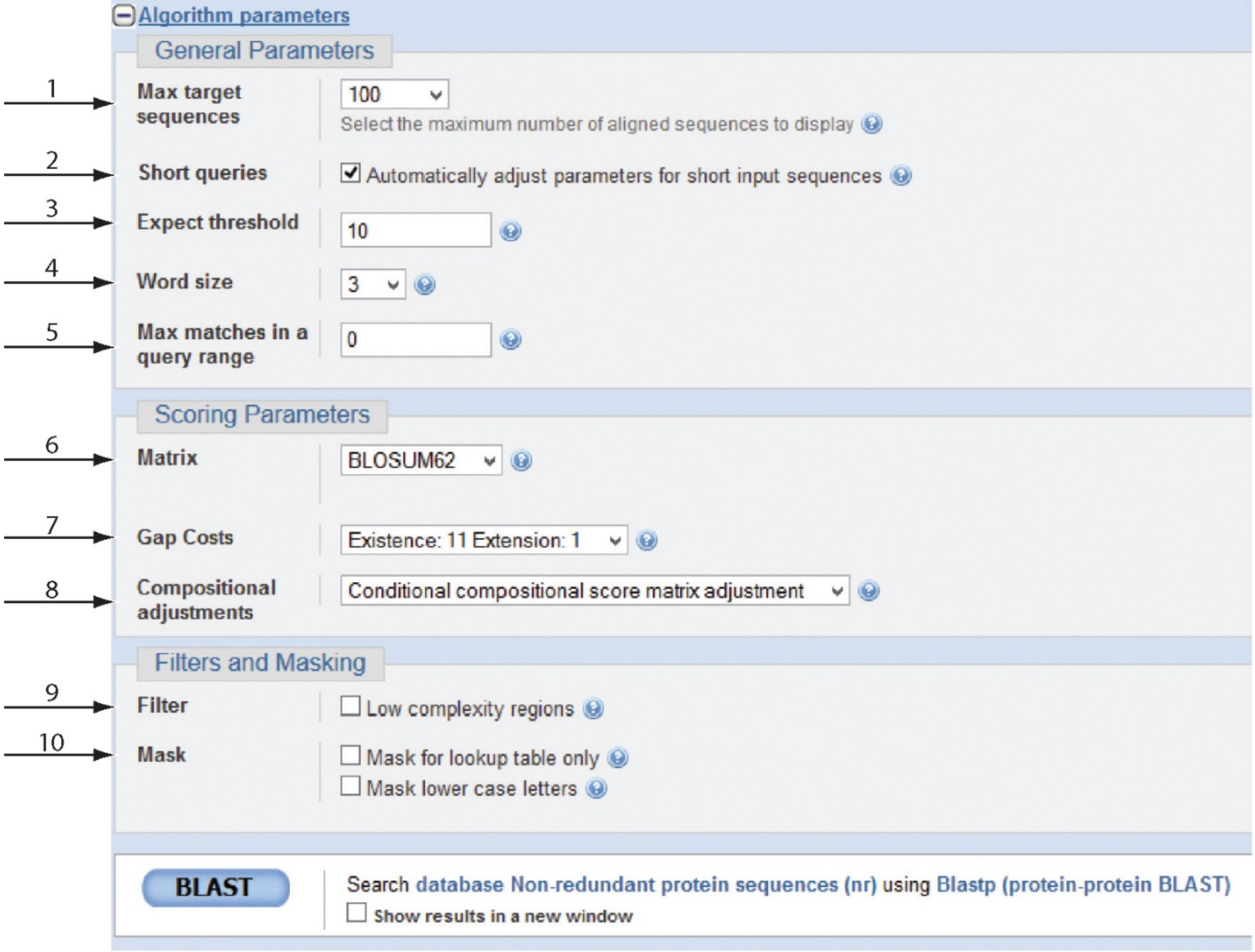

FIGURE 4.4 Optional BLASTP parameters. Numbered arrows refer to discussion in the text.

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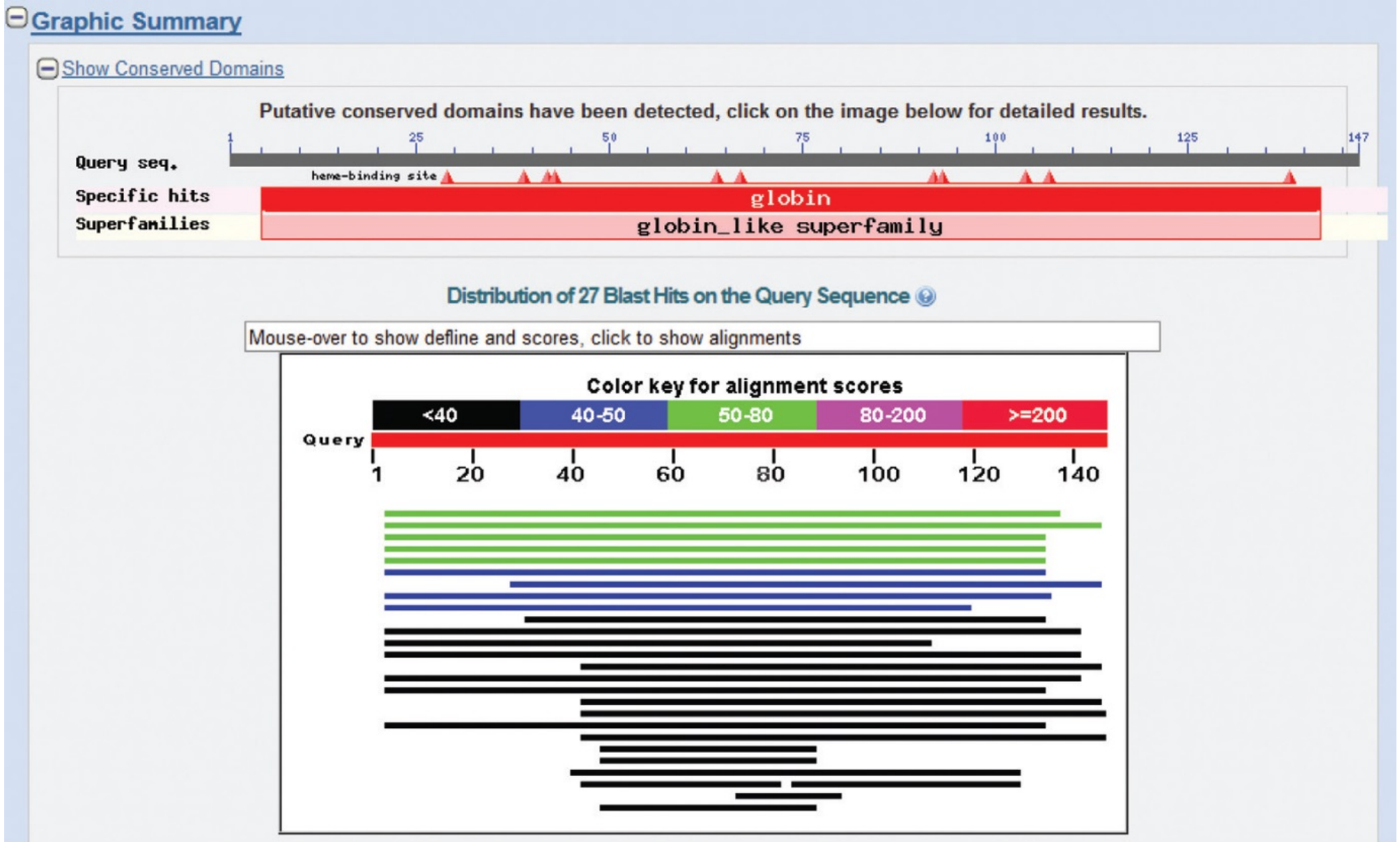

FIGURE 4.8 The graphic summary of BLAST results includes a display of conserved domains (here showing a match to the globin protein family), then a color-coded distribution of hits. Here the *x* axis corresponds to the length of the query (147 amino acid residues for beta globin), with each database 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.

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Sequences producing significant alignments:

Select: All None Selected:2

Alignments Download GenPept Graphics Distance tree of results Multiple alignment

	Description	Max score	Total score	Query cover	E value	Max ident	Accession
☑	PREDICTED: cytoglobin-2-like isoform 1 [Bombus terrestris] >ref|XP_003396833.1| PREDIC	59.7	59.7	91%	1e-10	29%	XP_003396832.1
☑	PREDICTED: cytoglobin-2-like isoform 1 [Bombus impatiens] >ref|XP_003494220.1| PREDI	58.5	58.5	97%	3e-10	28%	XP_003494219.1
☐	PREDICTED: globin-like [Megachile rotundata]	57.8	57.8	89%	6e-10	29%	XP_003707185.1
☐	PREDICTED: globin-like [Apis florea]	53.9	53.9	89%	1e-08	30%	XP_003690810.1
☐	globin 1 [Apis mellifera]	52.8	52.8	89%	4e-08	30%	NP_001071291.1
☐	PREDICTED: cytoglobin-2-like isoform 1 [Bombus terrestris] >ref|XP_003396831.1| PREDIC	45.1	45.1	89%	2e-05	26%	XP_003396830.1
☐	PREDICTED: neuroglobin-like, partial [Acyrthosiphon pisum]	42.4	42.4	80%	2e-04	23%	XP_001946608.2
☐	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.

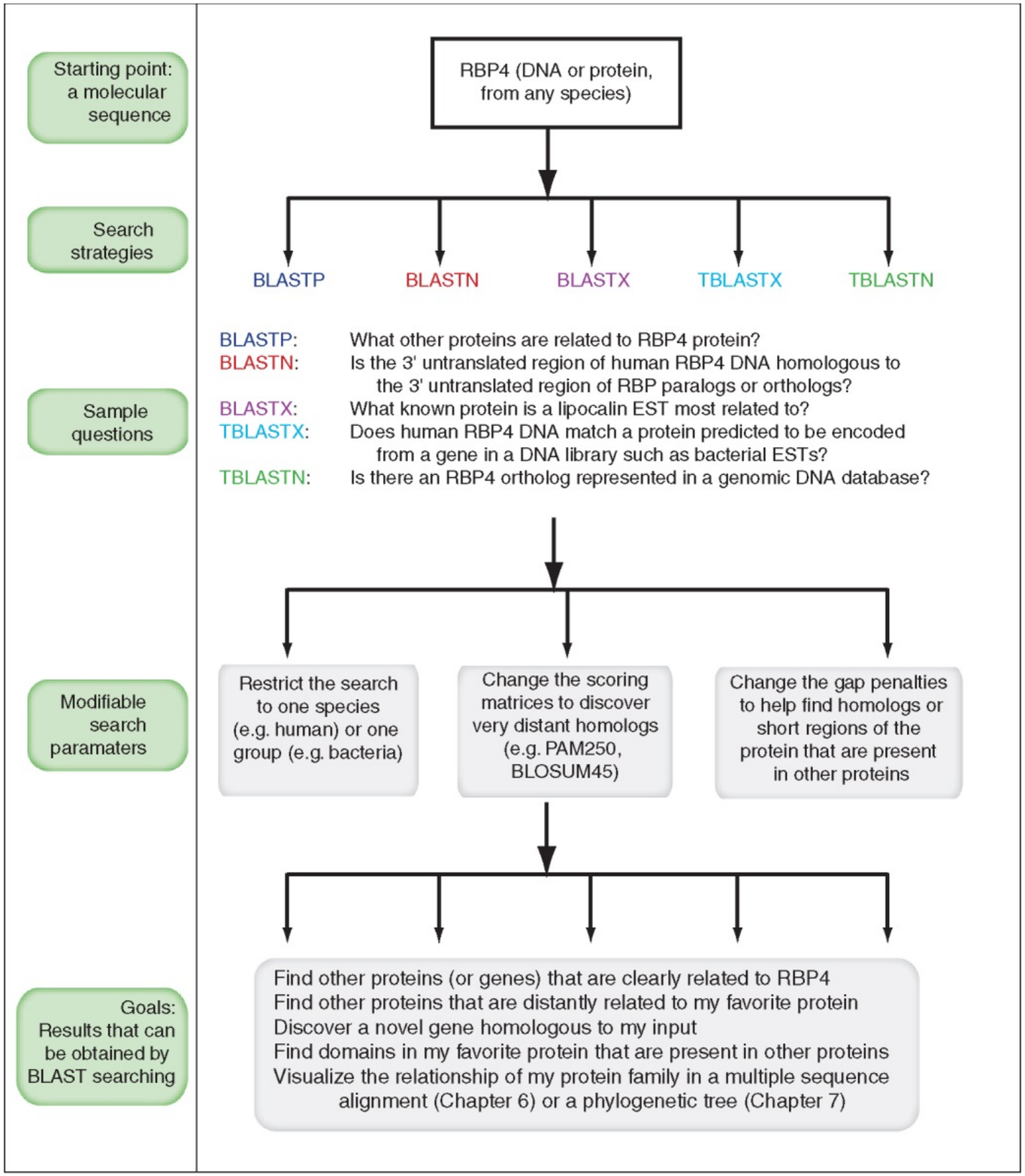


FIGURE 4.15 Overview of BLAST searching strategies. There are many hundreds of questions that can be addressed with BLAST searching, from characterizing the genome of an organism to evaluating the sequence variation in a single gene.

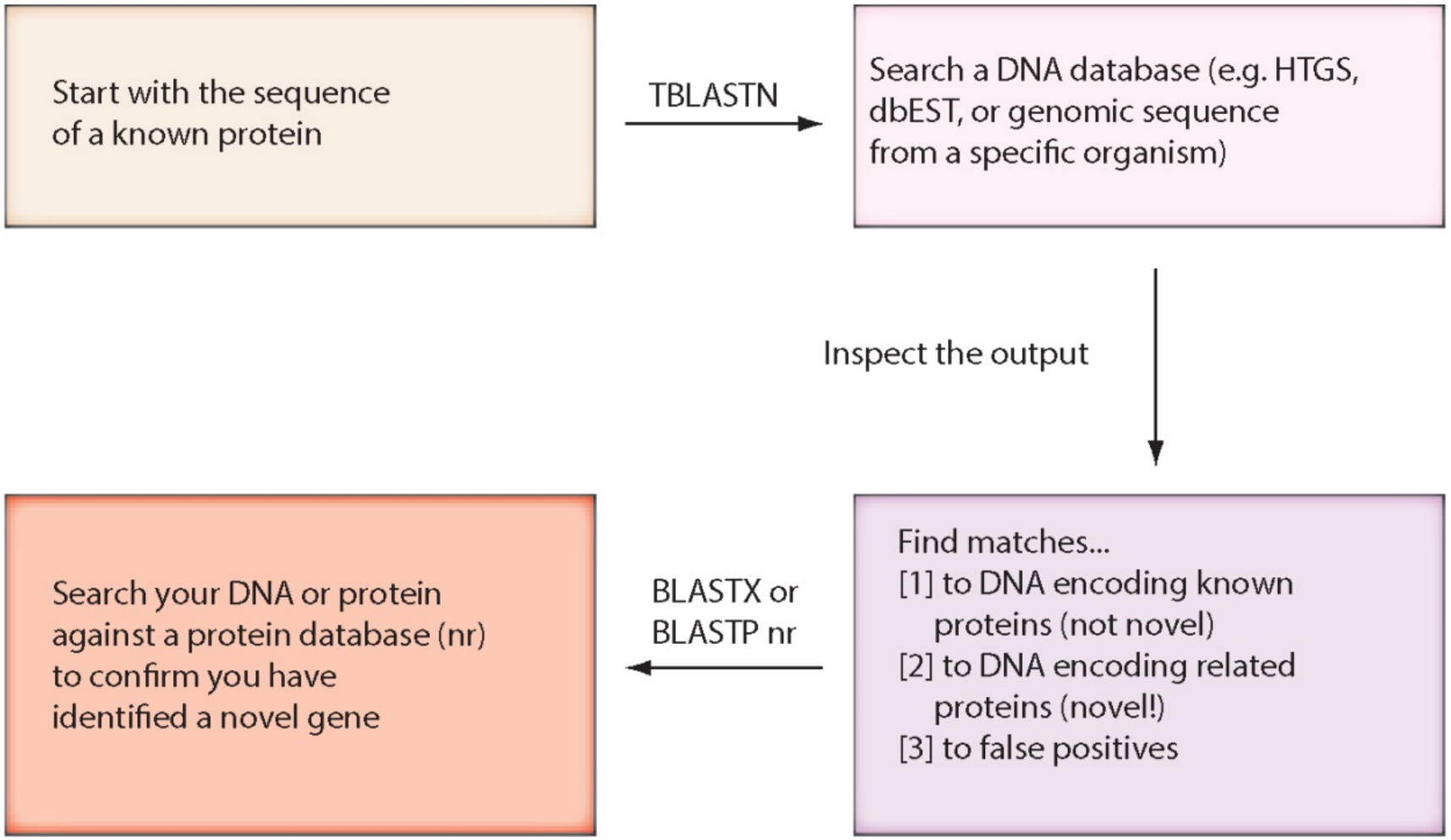


FIGURE 4.23 How to discover a novel gene by BLAST searching. Begin with the sequence of a known protein such as human beta globin. Perform a TBLASTN search of a DNA database. It is unlikely that there are many "novel" genes in the well-characterized genomes of organisms such as human, yeast, or *E. coli*. 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.

Bioinformatics and Functional Genomics, Third Edition, Jonathan Pevsner.

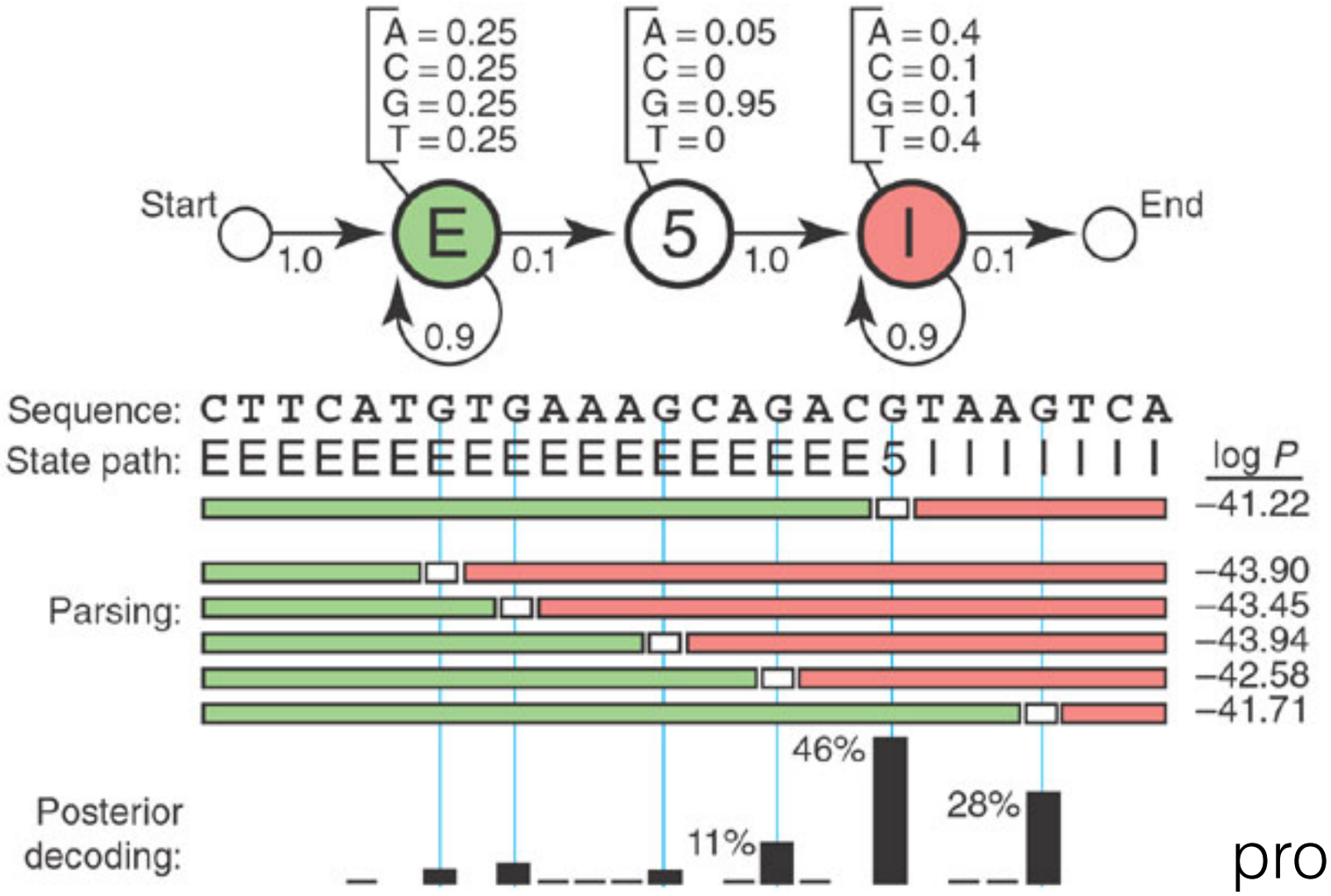
Companion Website: www.wiley.com/go/pevsnerbioinformatics

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

http://www.nature.com/nbt/journal/v22/n10/full/nbt1004-1315.html

Modelos de Markov Ocultos (HMMs)



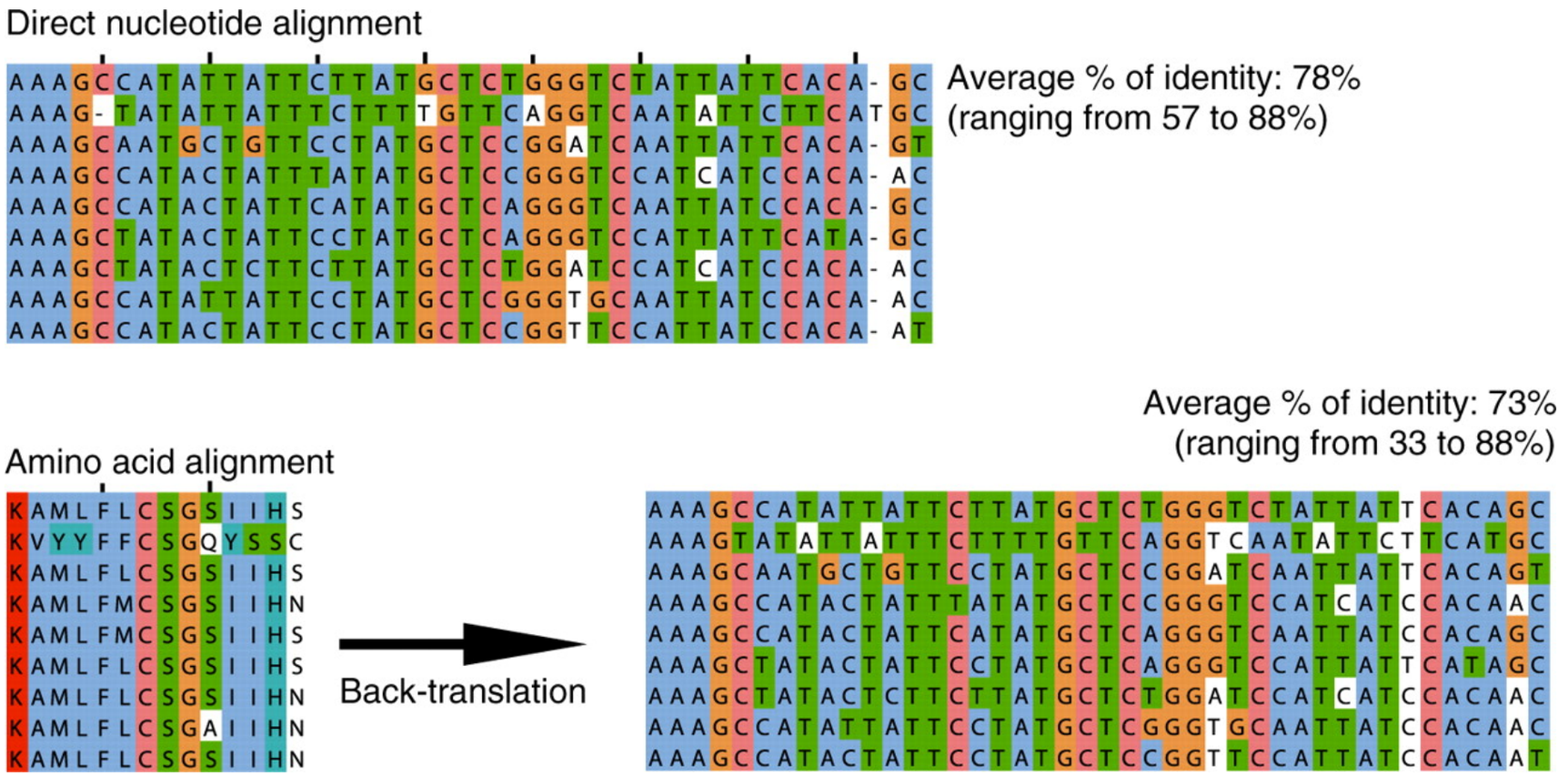
E exón
5 sitio de splicing
I intrón
“etiquetas”
secuencia observada

probabilidades de emisión
probabilidades de transición

Ejemplo para encontrar la mejor transición de expón a intrón

Estrategias para mejorar alineamientos

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

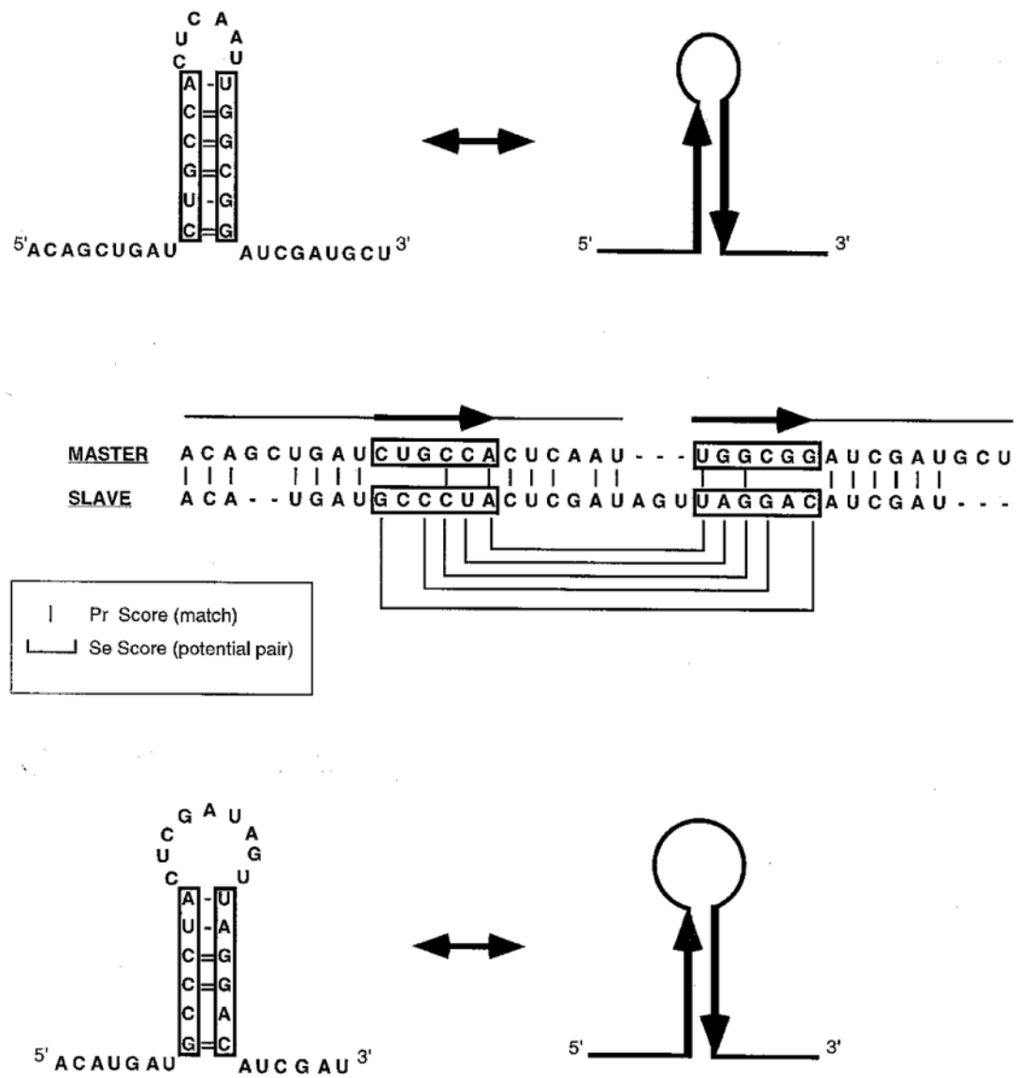


Federico Abascal et al. Nucl. Acids Res. 2010;38:W7-W13

TranslatorX; MACSE

Estrategias para mejorar alineamientos

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



http://tcoffee.org/Projects/raga/

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