BI694

Bioinformatics & Phylogenetics

Winter Semester 2017

WEEK 4

Overview

- BLAST: **B**asic **L**ocal **A**lignment **T**ool
  - Local alignment!
  - Used to compare sequences to database
  - Described by Altschul et al. 1990

Overview

- BLAST: **B**asic **L**ocal **A**lignment **T**ool
  - Identify highest scoring pair (HSP) between sequences
  - Produces ungapped alignments
  - Newer versions allow for gaps in alignment

BLAST algorithm

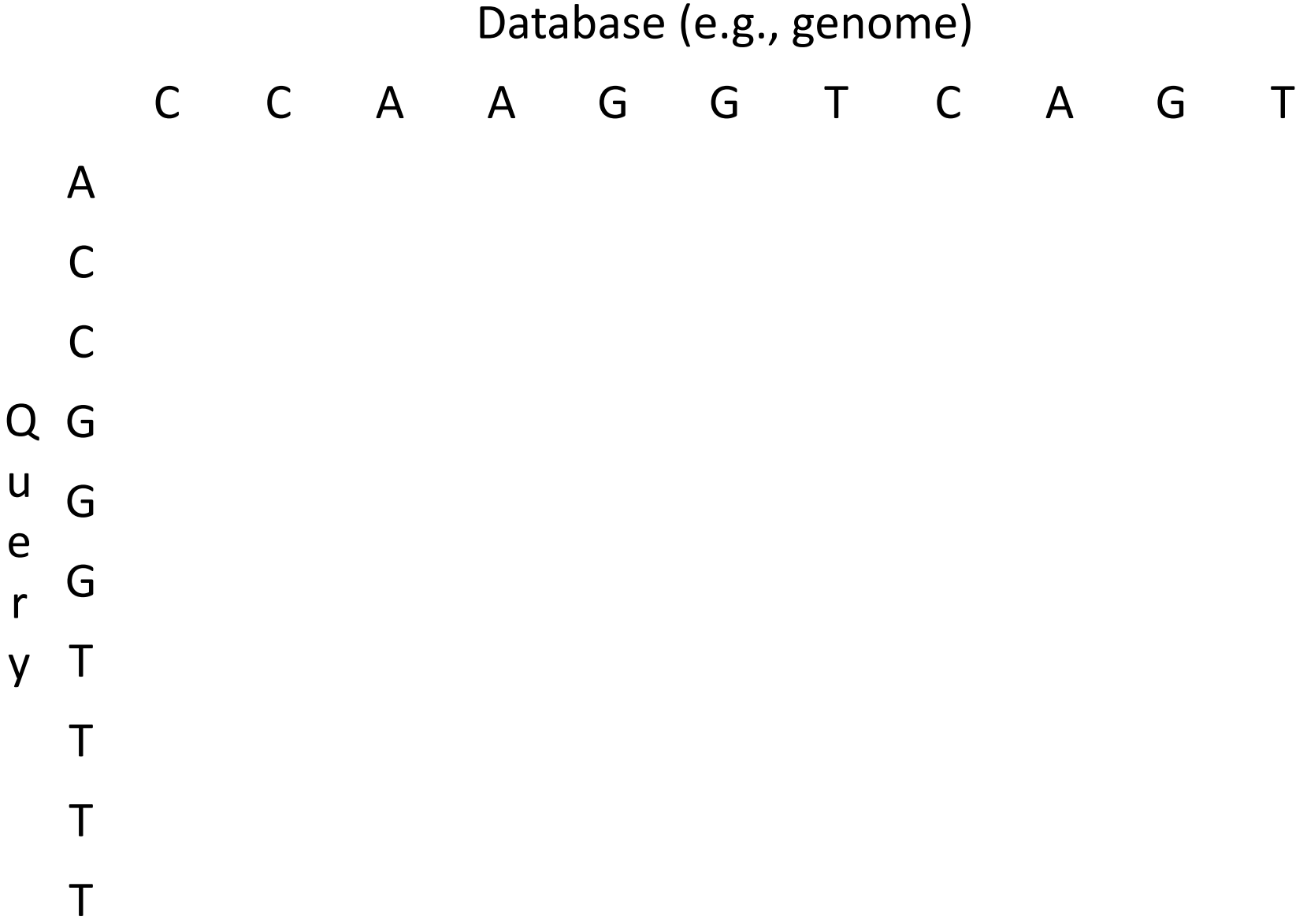
Database (e.g., genome)

C C A A G G T C A G T

BLAST algorithm

How do we find the best subset of sequences that match between query and database?

We want to find the best ungapped local alignment…



BLAST algorithm

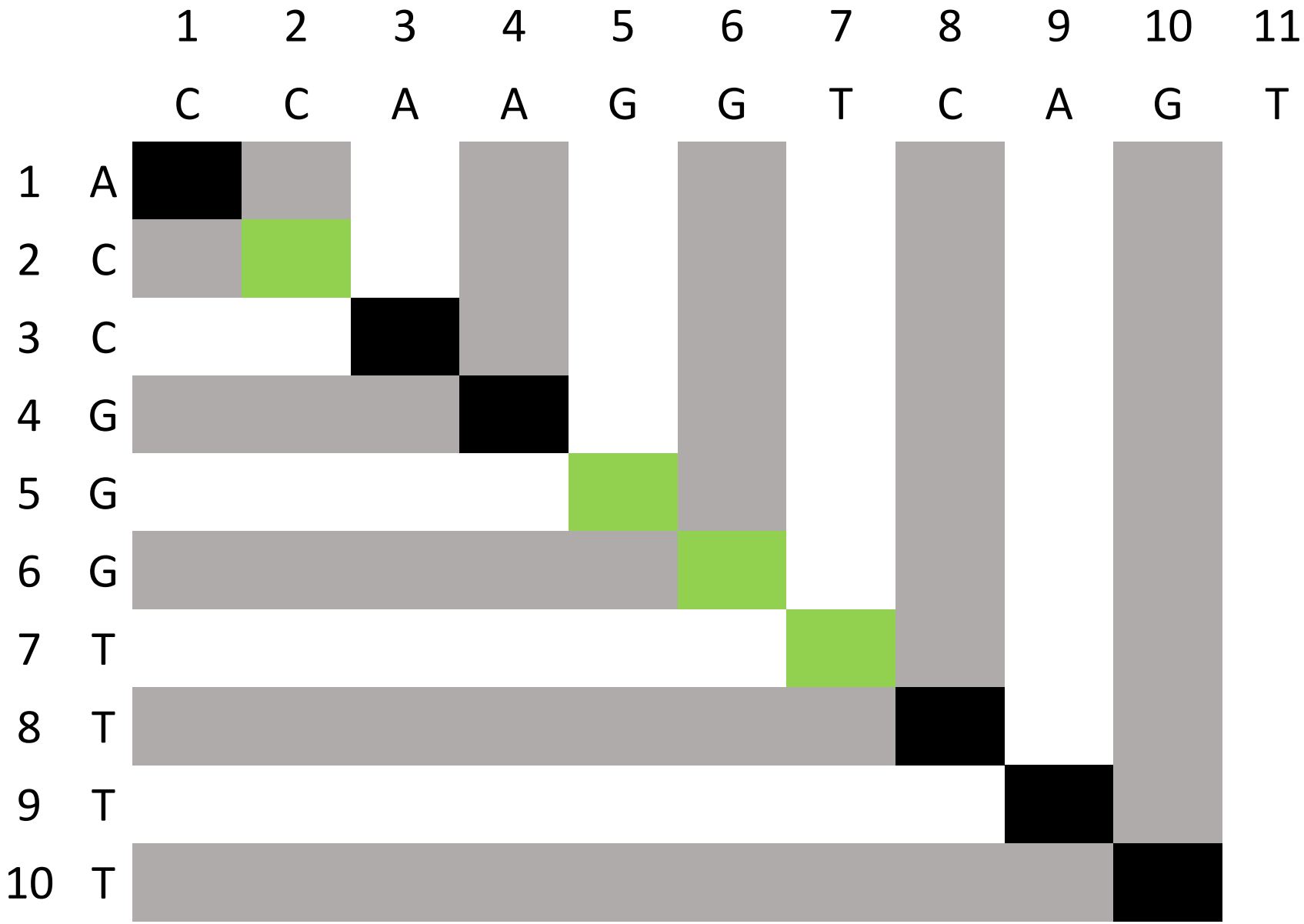
1) Scoring matrix with:

   Sii = +1

   Sij = -1

   $i \neq j$

How do we find the best matching sub-sequences?



BLAST algorithm

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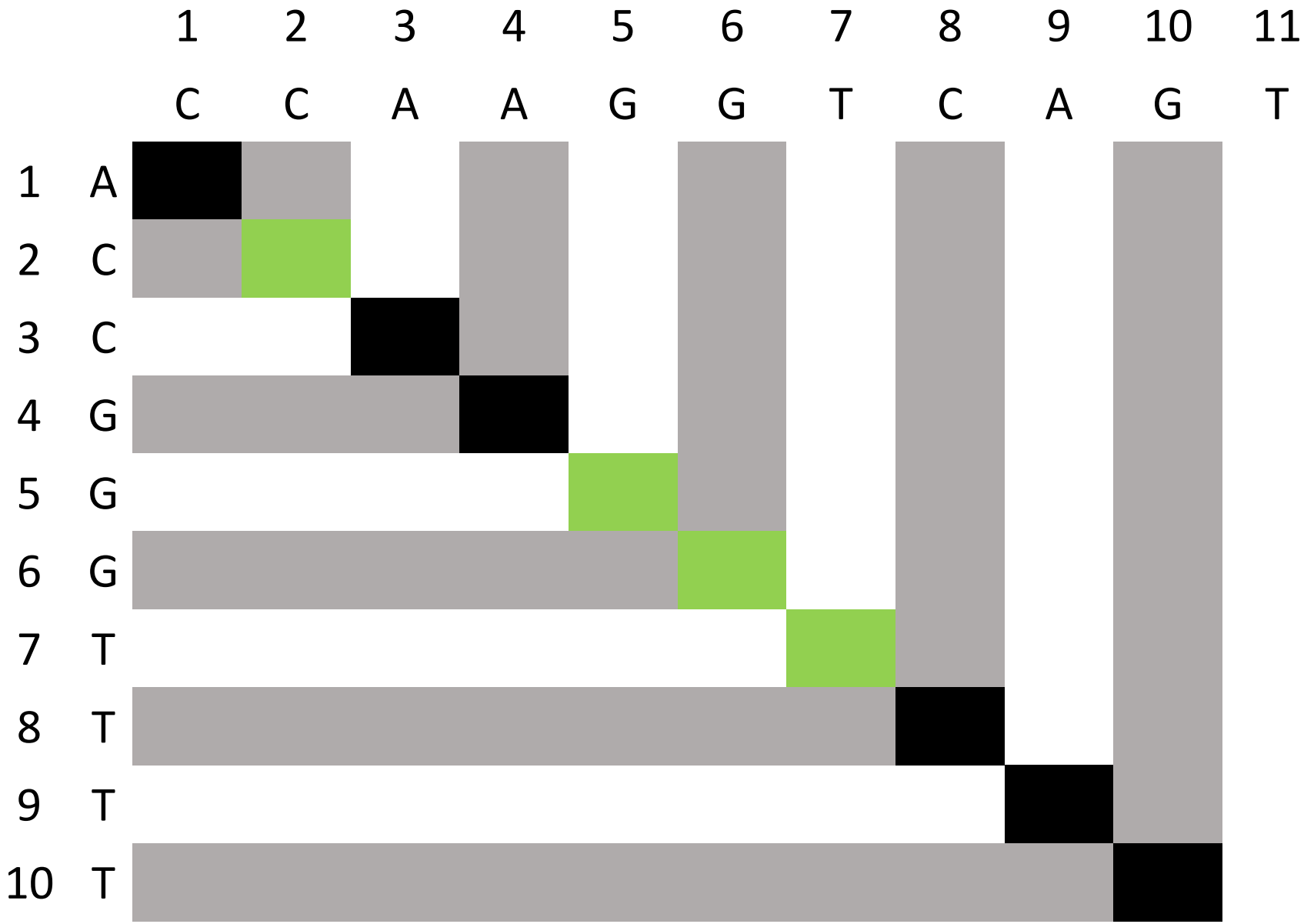
2) Determine highest segment score → Highest Scoring Pair (HSP)



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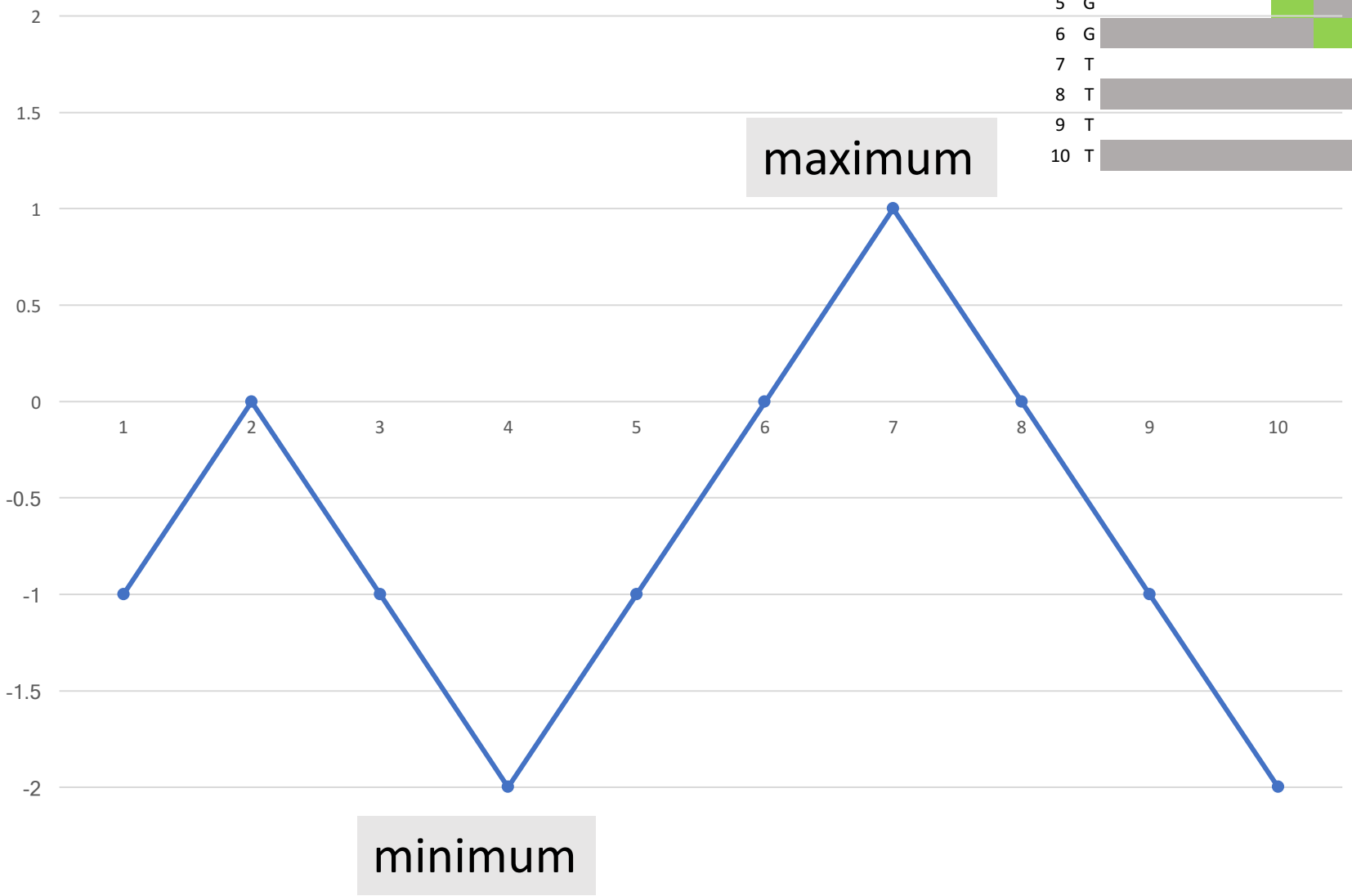

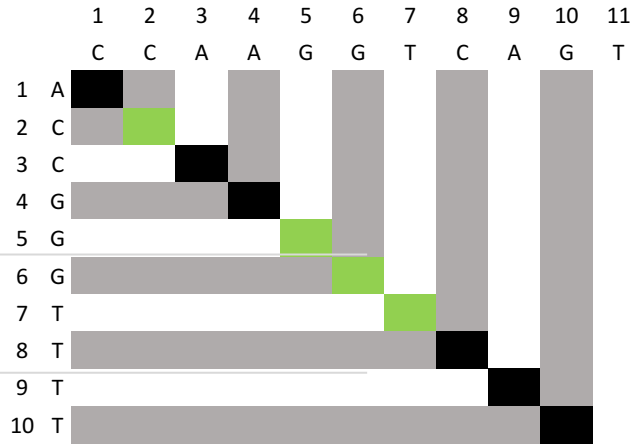

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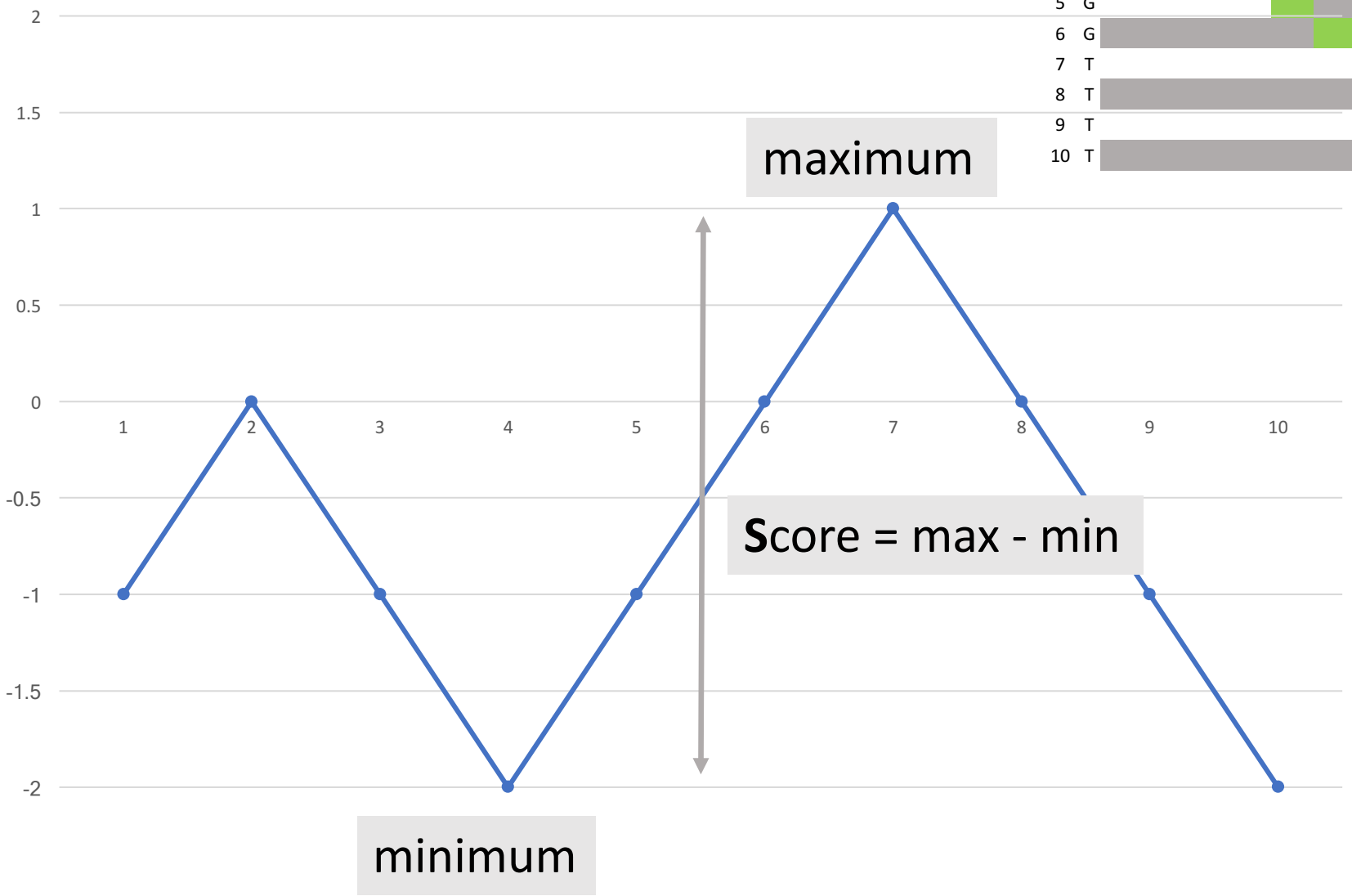

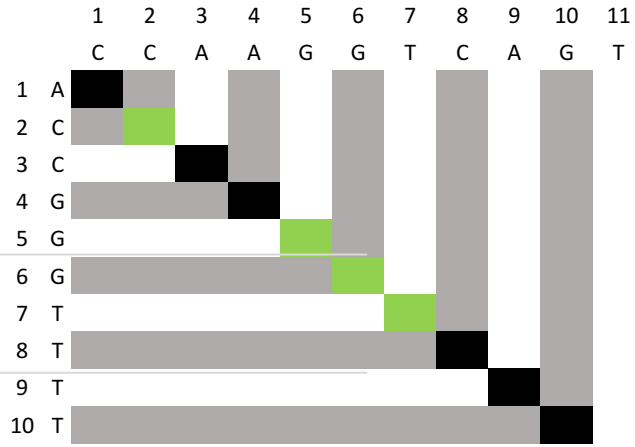

BLAST algorithm

1) Scoring matrix with:
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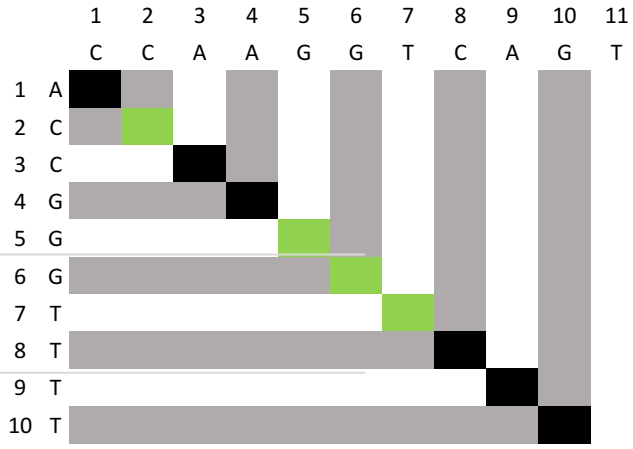
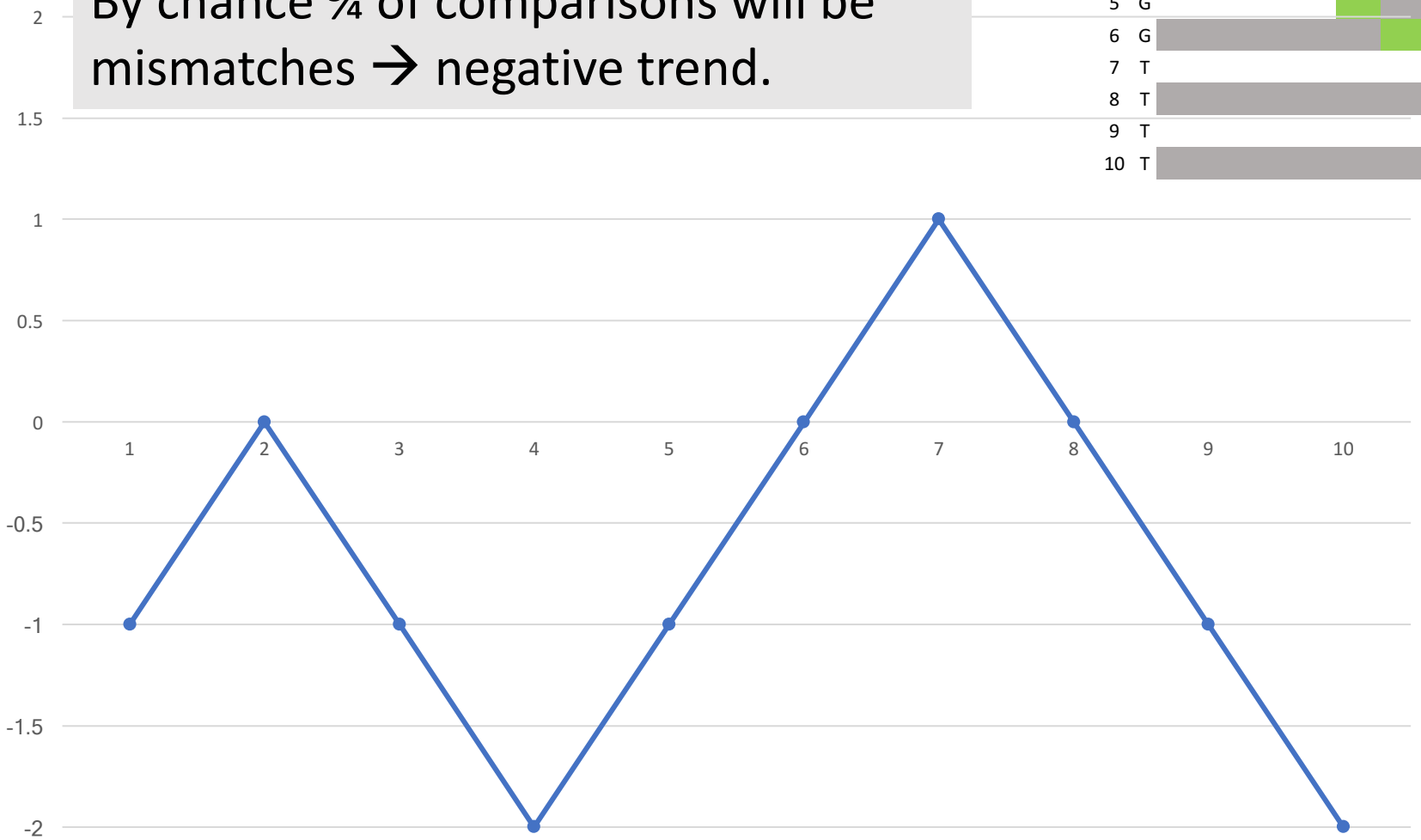
By chance ¾ of comparisons will be mismatches → negative trend.





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1) Scoring matrix with:

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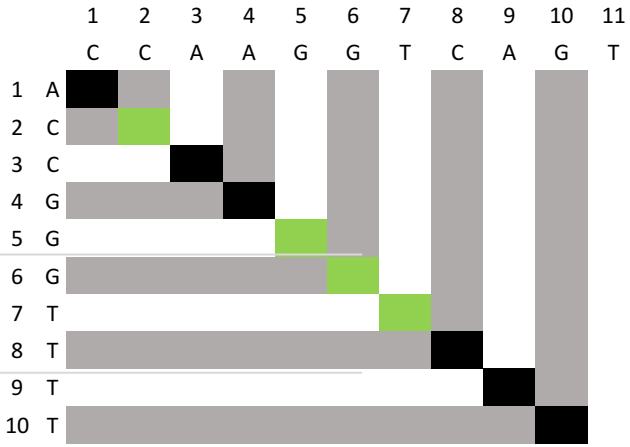
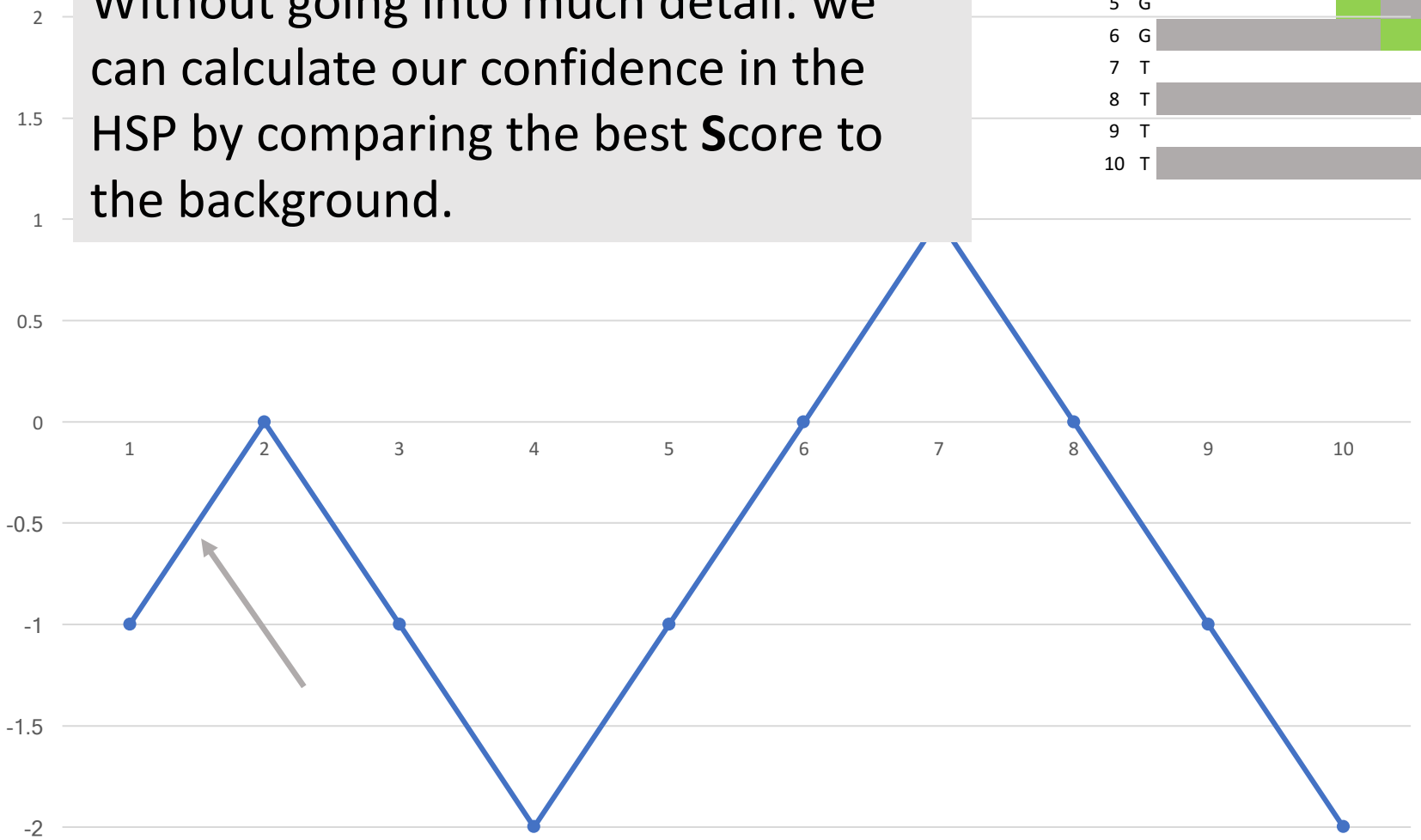
Without going into much detail: we can calculate our confidence in the HSP by comparing the best **S**core to the background.





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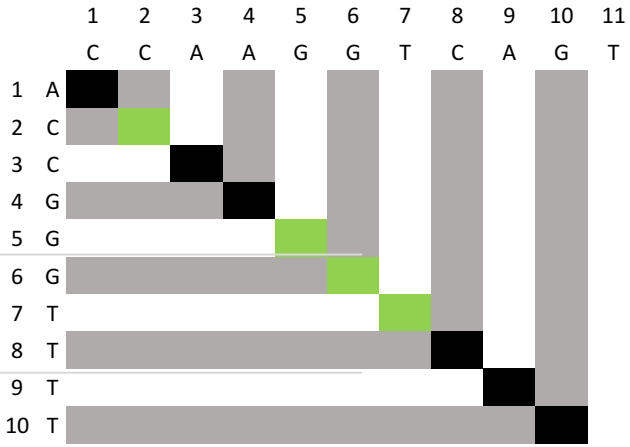

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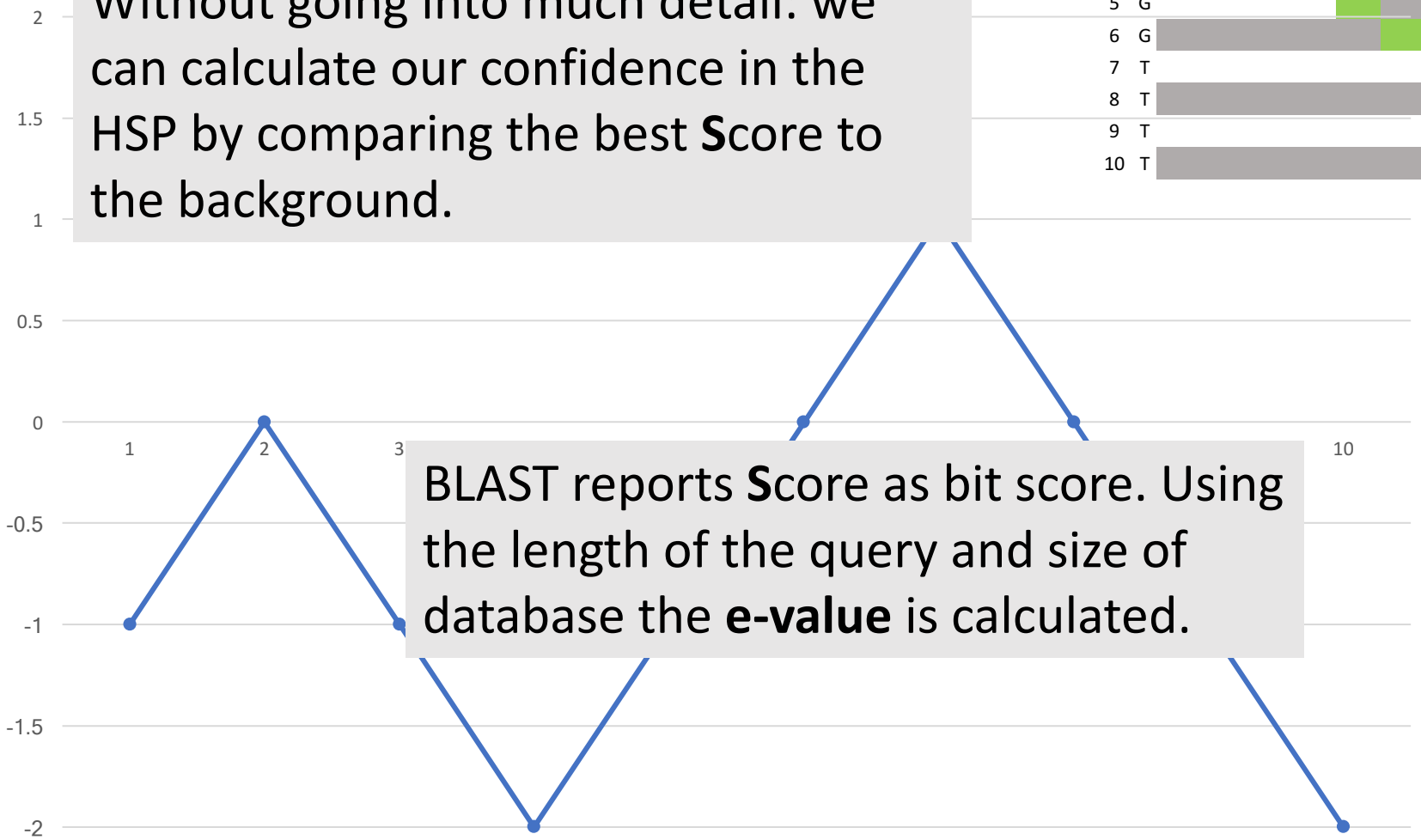


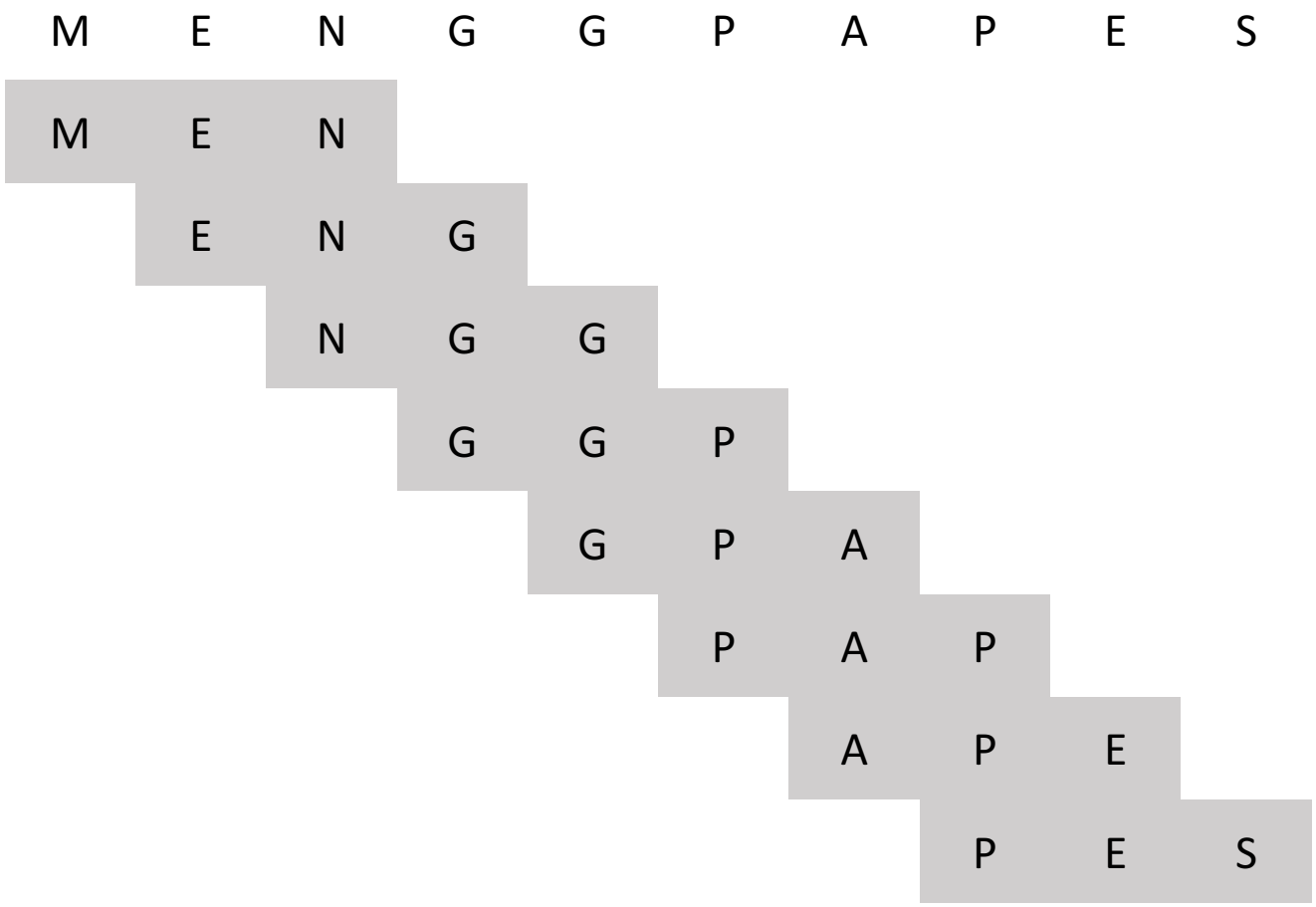
BLAST reports **S**core as bit score. Using the length of the query and size of database the **e-value** is calculated.

BLAST algorithm

M E N G G P A P E S

BLAST algorithm

1) Create all **W**ords of length 3 (kmers)



BLAST algorithm

1) Create all **W**ords of length 3 (kmers)
2) Find exact matches for all **W**

I	P	A	G	G	P	A	P	E	S

M	E	N	G	G	P	A	P	E	S
M	E	N							
	E	N	G						
		N	G	G					
			G	G	P				
				G	P	A			
					P	A	P		
						A	P	E	
							P	E	S

BLAST algorithm

1) Create all **W**ords of length 3 (kmers)
2) Find exact matches for all **W**
3) Score all hits

	I	P	A	G	G	P	A	P	E	S
	1	-2	-2	6	6	7	4	7	5	4
	M	E	N	G	G	P	A	P	E	S
	M	E	N							
		E	N	G						
			N	G	G					
19				G	G	P				
17					G	P	A			
18						P	A	P		
18							A	P	E	
16								P	E	S

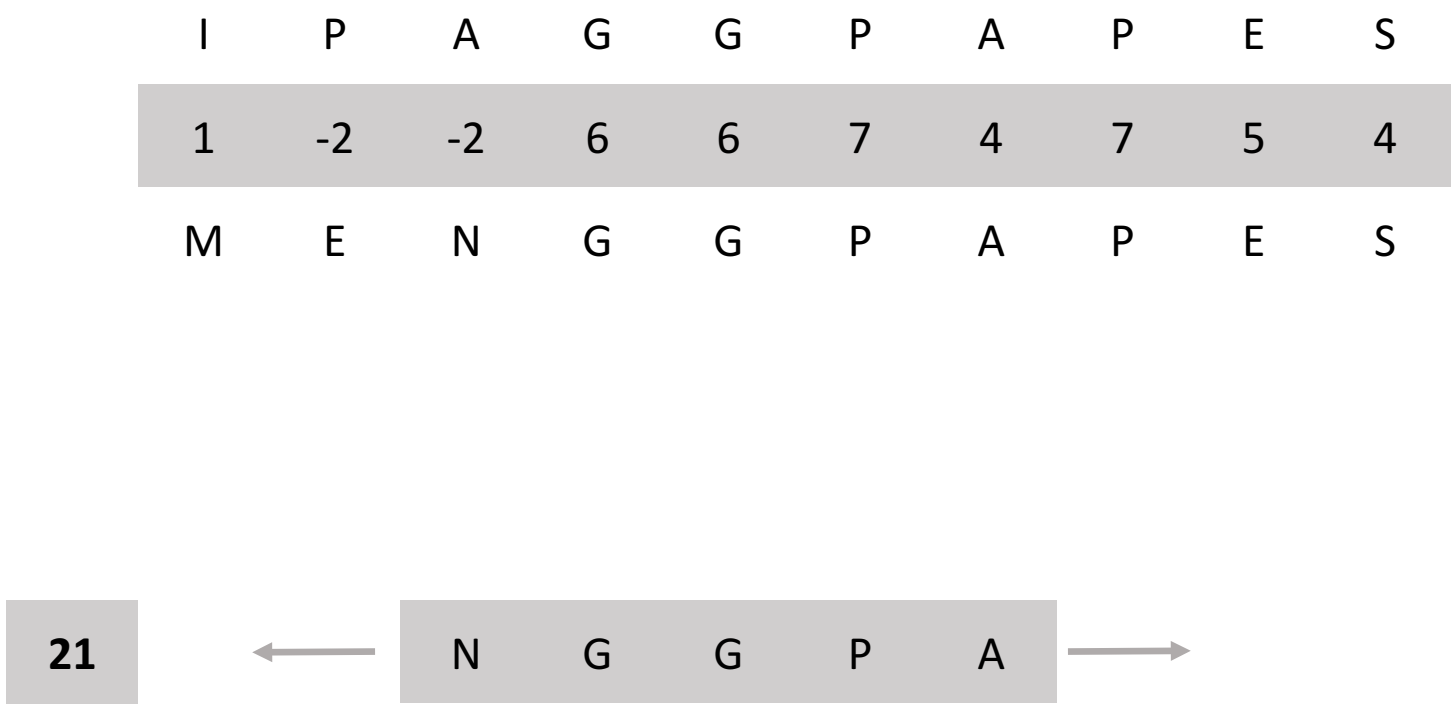
BLAST algorithm

1) Create all **W**ords of length 3 (kmers)
2) Find exact matches for all **W**
3) Score all hits
4) Keep hits above **T**hreshold (19)

	I	P	A	G	G	P	A	P	E	S
	1	-2	-2	6	6	7	4	7	5	4
	M	E	N	G	G	P	A	P	E	S
	M	E	N							
		E	N	G						
			N	G	G					
19				G	G	P				
17					G	P	A			
18						P	A	P		
18							A	P	E	
16								P	E	S

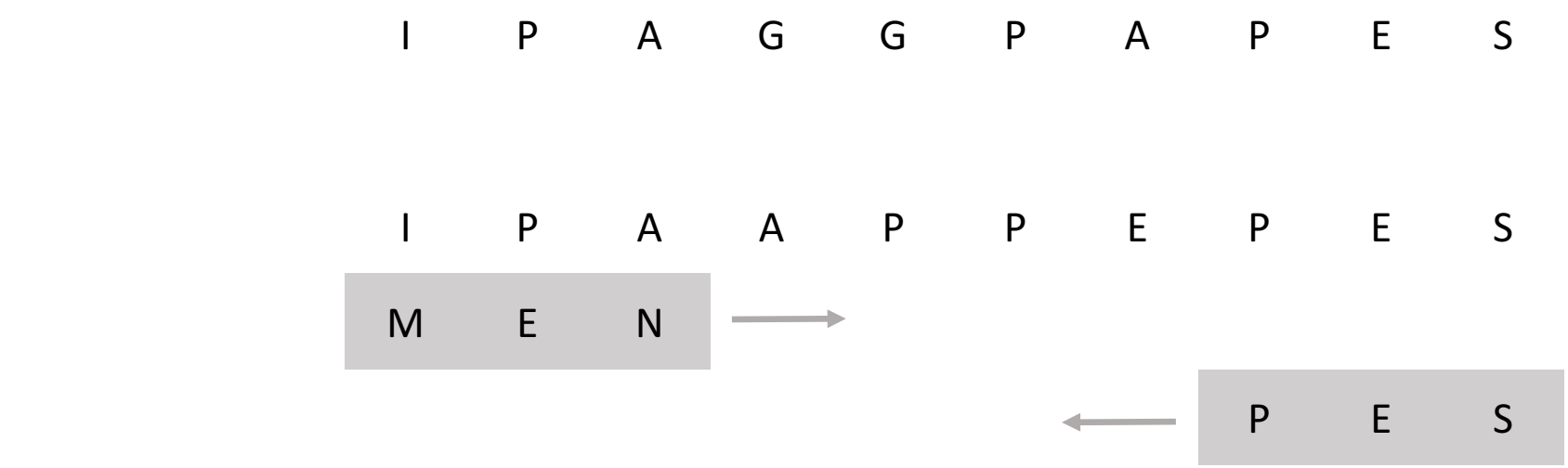
BLAST algorithm

1) Create all **W**ords of length 3 (kmers)
2) Find exact matches for all **W**
3) Score all hits
4) Keep hits above **T**hreshold (19)
5) Extend alignment until **T** drops



BLAST algorithm

- Current versions produce gapped alignments
- Multiple seeds of short kmer matches are kept during initial search
- These matches are extended
- Final alignment reported is a Smith-Waterman alignment



BLAST

BLOSUM62 Scoring Matrix

- **BLO**ck **SU**bstitution **M**atrix
- By Henikoff and Henikoff (1992)
- Default scoring matrix for pairwise alignment of sequences using BLAST
- Based on empirical observations of distantly-related proteins

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4																			
R	-1	5																		
N	-2	0	6																	
D	-2	-2	1	6																
C	0	-3	-3	-3	9															
Q	-1	1	0	0	-3	5														
E	-1	0	0	2	-4	2	5													
G	0	-2	0	-1	-3	-2	-2	6												
H	-2	0	1	-1	-3	0	0	-2	8											
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
K	-1	2	0	-1	-1	1	1	-2	-1	-3	-2	5								
M	-1	-2	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

Pevsner, Bioinformatics and Functional Genomics, 2009

BLAST

```
Query          NLYENFVQATFNALTAEKV
               NY ENF+Q+  + L   +
Subject        NYAENTIQSIISTVEPAQR
```

Centerline provides the following information:

- Letter designates identity of high similarity
- Plus indicates similarity but not very similar
- No symbol equals low similarity

BLAST

Nucleotide scoring matrices for ungapped (left) and gapped alignments (right)

	A	T	C	G
A	5			
T	-4	5		
C	-4	-4	5	
G	-4	-4	-4	5

	A	T	C	G
A	1			
T	-3	1		
C	-3	-3	1	
G	-3	-3	-3	1

Gap opening score: -11
Gap extension: -1

BLAST statistics

- Score (Bits)
  - a conversion of of summed substitution scores
- Expect(e) value
  - Function of the score and database size
  - 1 alignment using a query of this size will by chance produce a score of this value in a database of this size
  - e-value is specific to a database of a certain size

BLAST statistics

- Rules of thumb
  - e value of =< e-5: often used for annotating genes
  - E value of =< e-30: strong evidence of homolgy
  - Length of hit is important when evaluating e values

BLAST programs

Search	Query	Database
blastn	nucleotide	nucleotide
blastx	translated nucleotide in all 6 frames	protein
tblastx	translated nucleotide in all 6 frames	translated nucelotide in all 6 frames
blastp	protein	protein

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Search	Query	Database
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