

# CHAPTER 4: PRODUCING AND ANALYZING SEQUENCE ALIGNMENTS

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# Motivation

- You have recently sequenced a gene and its CDS begins with
  - GGCGGAGCCAGGCCGGCCTAGAGTCACTTCTCC
- You have isolated a protein and its amino acid sequence is
  - MGKEIPTDAPWEAQHADKWDKMTMKELIDKICWTKTA
- Questions:
  - What does this protein do?
  - What are the important functional regions?
  - Do other organisms have similar genes or proteins?
- To answer these questions we can find similar sequences, identified through sequence alignments, using tools such as BLAST

# Sequence alignment

- Two sequences should be aligned in such a way that maximizes their *similarity*
  - If they derive from a common ancestor, characters (bases or amino acids) derived from the same ancestral base should be aligned
  - Shared domains in proteins (and important regions in nucleotide sequences) should align, even if the sequences are not similar overall
- Alignment should take into account biological mutations and other events
  - Point mutations
  - Insertions or deletions (indels)
  - Gene duplications and pseudogenes (a gene copy that does not produce a functional protein)
    - The human genome has up to 20,000 pseudogenes!

# Sequence alignment example

- Consider the alignment of two hypothetical protein sequences:

THISSEQUENCE and THATSEQUENCE

<b>T</b>	<b>H</b>	I	S	<b>S</b>	<b>E</b>	<b>Q</b>	<b>U</b>	<b>E</b>	<b>N</b>	<b>C</b>	<b>E</b>
<b>T</b>	<b>H</b>	A	T	<b>S</b>	<b>E</b>	<b>Q</b>	<b>U</b>	<b>E</b>	<b>N</b>	<b>C</b>	<b>E</b>

# Sequence alignment example (different lengths)

- Now consider the alignment of two hypothetical protein sequences:

THATSEQUENCE and THISISASEQUENCE,

where the amino acids I, S, and A were inserted into one of the original sequences

<b>T</b>	<b>H</b>	A	T	S	E	Q	U	<b>E</b>	N	C	<b>E</b>			
<b>T</b>	<b>H</b>	I	S	I	S	A	S	<b>E</b>	Q	U	<b>E</b>	N	C	E

- When aligning both sequences from the beginning
  - similarity which is obvious to us is lost
  - false matches are created because of differences in length

# Sequence alignment example (different lengths)

- The solution is to introduce a **gap**, which corresponds to an insertion or a deletion and is usually indicated by a dash (-) in an alignment

<b>T</b>	<b>H</b>	I	S	I	S	<b>A</b>	-	<b>S</b>	<b>E</b>	<b>Q</b>	<b>U</b>	<b>E</b>	<b>N</b>	<b>C</b>	<b>E</b>
<b>T</b>	<b>H</b>	-	-	-	-	<b>A</b>	T	<b>S</b>	<b>E</b>	<b>Q</b>	<b>U</b>	<b>E</b>	<b>N</b>	<b>C</b>	<b>E</b>

- There are always multiple possible alignments, and the best alignment is not always obvious
- The alignment must be selected using a quantitative scoring measure

# Sequence homology

- **Similarity** is a descriptive term indicating that two or more sequences have a certain degree of identity or likeness
- **Homologous sequences** (or homologues) are sequences that are descended from a common ancestor
- Homologous genes will accumulate different mutations (**divergent evolution**) during the course of evolution and their sequences are often not identical.
- **Convergent evolution** is when sequences with high similarity are not homologous
- Sequences with high similarity are *inferred* to be homologous
  - But homologous sequences may not have high similarity



## Homology is more easily detected from protein sequences

- Number of possible characters in nucleotides vs. proteins?
- Matches in nucleotide sequences are more likely due to chance than matches in protein sequences
- The genetic code is redundant
  - Identical amino acid sequences can be encoded by different nucleotide sequences
  - Nucleotide sequences are more likely to change over time
- Structure and function of a protein is determined by its amino acid sequence (although this is determined by the nucleotide sequence)

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SEMESTER**



# Scoring alignments

- Since multiple alignments are always possible, the best possible alignment is determined based on an alignment **score**
  - The **optimal alignment** is the alignment with the best score (multiple optimal alignments are possible)
  - **Suboptimal alignments** have slightly less scores than the best one
- The **percentage** or **percent identity** of an alignment is equal to the number of identical matches in an alignment divided by the length of the alignment (including gaps)

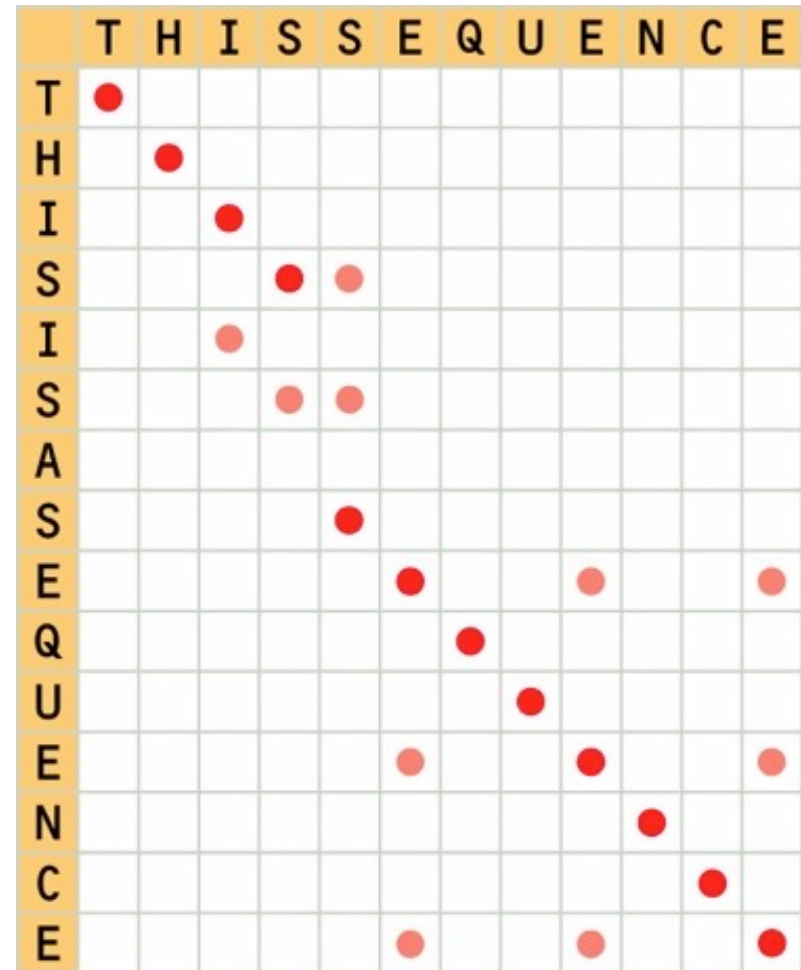
<b>T</b>	<b>H</b>	I	S	I	S	<b>A</b>	-	<b>S</b>	<b>E</b>	<b>Q</b>	<b>U</b>	<b>E</b>	<b>N</b>	<b>C</b>	<b>E</b>
<b>T</b>	<b>H</b>	-	-	-	-	<b>A</b>	T	<b>S</b>	<b>E</b>	<b>Q</b>	<b>U</b>	<b>E</b>	<b>N</b>	<b>C</b>	<b>E</b>

- The above alignment is optimal and has a percent identity of  $11/16 = 68.75\%$

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## Dot-plots

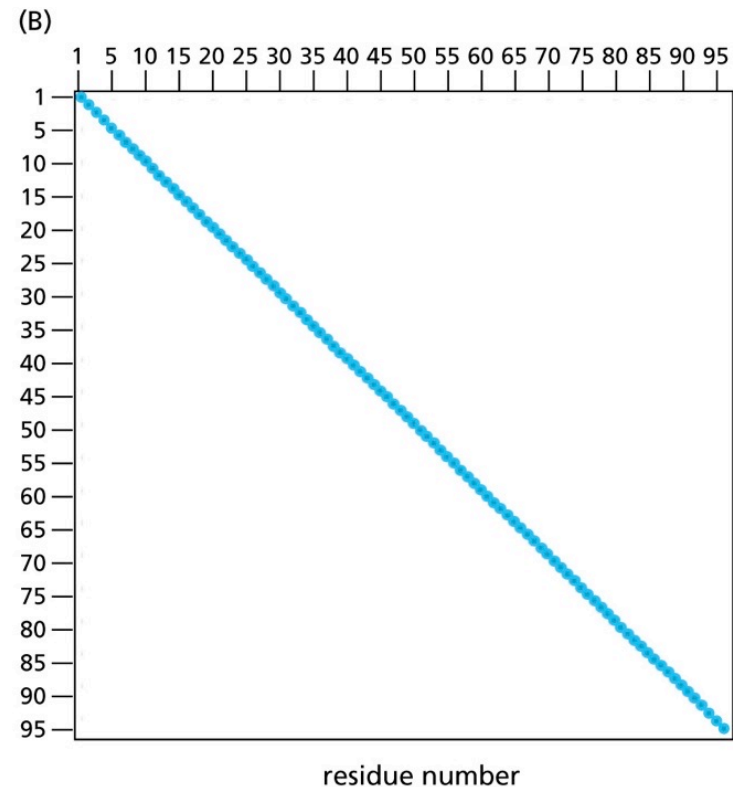
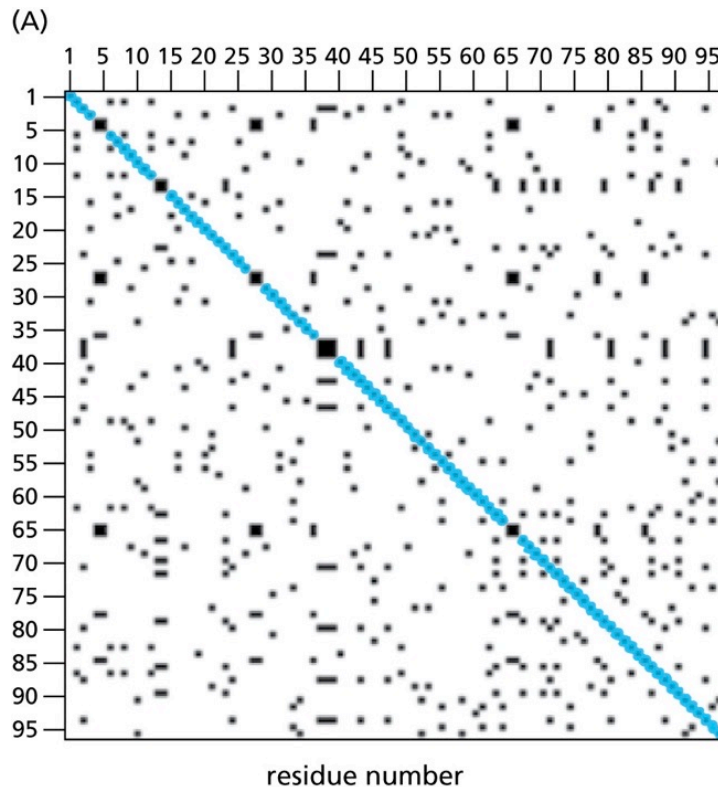
- A **dot-plot** displays the alignment of two sequences and visualizes sequence similarity graphically
- A dot indicates identity between characters of each sequence
- Interruptions along the diagonal indicate a gap
- In addition to visualizing overall similarity, dot-plots can indicate intrasequence repeats



# Dot-plots and background noise

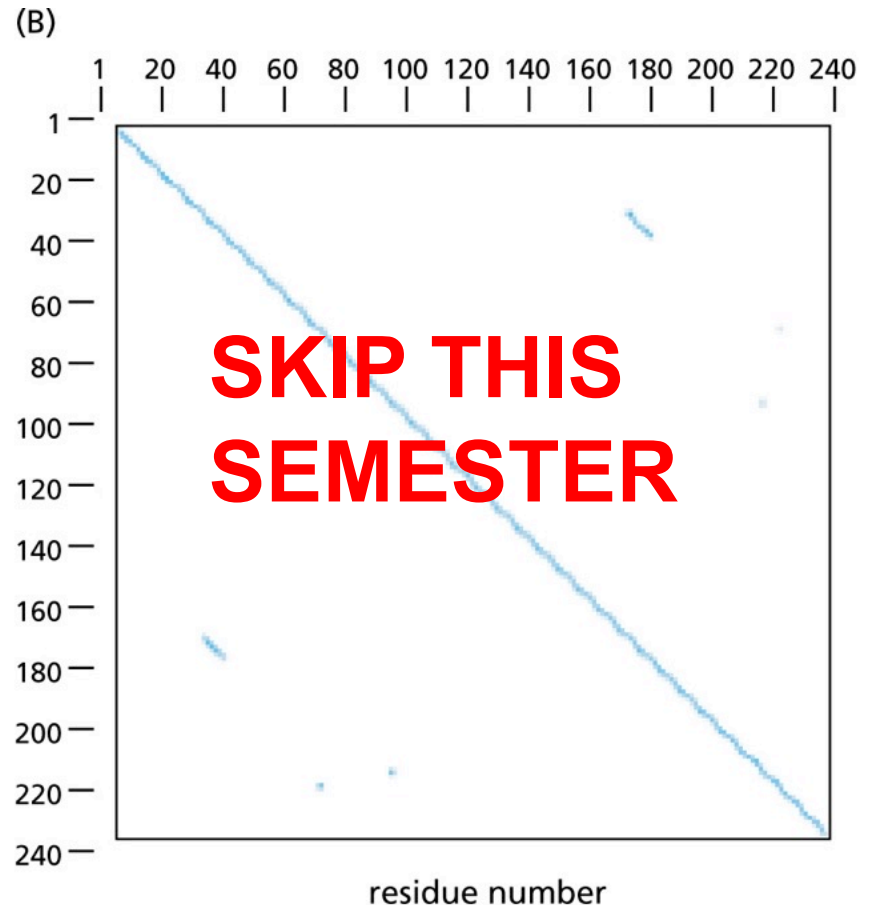
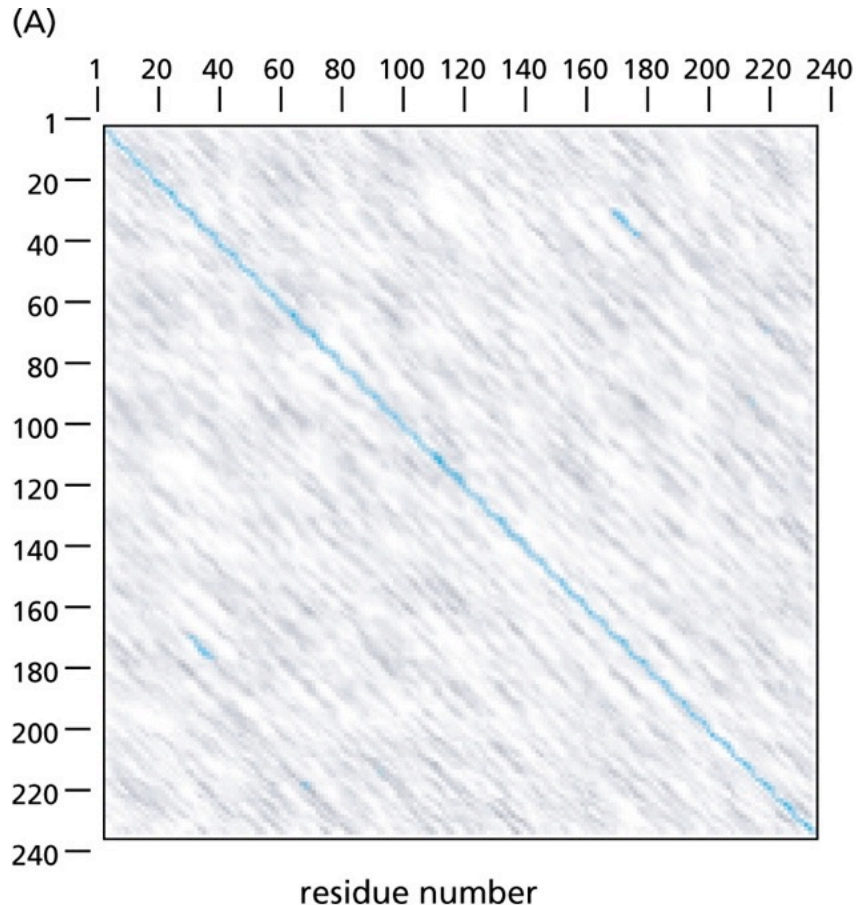
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- A. Dot-plot of an SH2 domain with itself
- B. The same dot-plot but with background noise removed, based on a window of 10 residues and a minimum identity score within each window of 3



# Dot-plots showing BRCA2 repeat domain

Background is removed using a window of 30 and a minimum score of 5



# Similarity versus identity

- Genuine matches do not have to be identical
- Certain non-identical amino acids may have
  - Similar physical and chemical properties
  - May be more likely to be present at the same region than others in related sequences
- Percent similarity is calculated in the same way as percent identity but both identical and similar matches are considered

<b>T</b>	<b>H</b>	<b>I</b>	<b>S</b>	I	S	A	<b>S</b>	<b>E</b>	<b>Q</b>	<b>U</b>	<b>E</b>	<b>N</b>	<b>C</b>	<b>E</b>
		.	.											
<b>T</b>	<b>H</b>	<b>A</b>	<b>T</b>	—	—	—	<b>S</b>	<b>E</b>	<b>Q</b>	<b>U</b>	<b>E</b>	<b>N</b>	<b>C</b>	<b>E</b>

- Isoleucine (I) and alanine (A) are hydrophobic; serine (S) and threonine (T) are polar
- Percent similarity is  $12/15 = 80\%$

# Substitution matrices

- For protein sequences, the score for each aligned pair of amino acids is determined by a **substitution matrix**, which has values for all possible pairs of residues.
- Example using BLOSUM-62 matrix:

Seq1: T H I S S E Q U E N C E

Seq2: T H A T S E Q U E N C E

Score: 5 8 -1 1 4 5 5 0 5 6 9 5

***This alignment has an overall score (S) of 52***

# BLOSUM-62 matrix

C	9	small and polar residues																		
S	-1	4																		
T	-1	1	5																	
P	-3	-1	-1	7																
A	0	1	0	-1	4	small and nonpolar														
G	-3	0	-2	-2	0	6														
N	-3	1	0	-2	-2	0	6	polar or acidic residues												
D	-3	0	-1	-1	-2	-1	1	6												
E	-4	0	-1	-1	-1	-2	0	2	5											
Q	-3	0	-1	-1	-1	-2	0	0	2	5										
H	-3	-1	-2	-2	-2	-2	1	-1	0	0	8	basic								
R	-3	-1	-1	-2	-1	-2	0	-2	0	1	0	5								
K	-3	0	-1	-1	-1	-2	0	-1	1	1	-1	2	5							
M	-1	-1	-1	-2	-1	-3	-2	-3	-2	0	-2	-1	-1	5	large and hydrophobic					
I	-1	-2	-1	-3	-1	-4	-3	-3	-3	-3	-3	-3	-3	1	4					
L	-1	-2	-1	-3	-1	-4	-3	-4	-3	-2	-3	-2	-2	2	2	4				
V	-1	-2	0	-2	0	-3	-3	-3	-2	-2	-3	3	2	1	3	1	4			
F	-2	-2	-2	-4	-2	-3	-3	-3	-3	-3	-1	-3	-3	0	0	0	-1	6	aromatic	
Y	-2	-2	-2	-3	-2	-3	-2	-3	-2	-1	2	-2	-2	-1	-1	-1	-1	3	7	
W	-2	-3	-2	-4	-3	-2	-4	-4	-3	-2	-2	-3	-3	-1	-3	-2	-3	1	2	
	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W



# Substitution matrices

- BLOSUM matrices
  - BLOck SUBstitution Matrix
  - Based on local alignments to detect conserved short regions
  - Sequences grouped based on percent identity, where the percent identity threshold for grouping determines the specific BLOSUM matrix
    - BLOSUM-62 is based on grouping aligned sequences with no more than 62% identity
  - Substitution frequencies are then calculated
  - Positive scores indicate conservative (more likely) substitutions
  - Negative scores indicate non-conservative (less likely) substitutions
  - All BLOSUM matrices are based on observed alignments



# Substitution matrices

- Point Accepted Mutation (PAM) matrices
  - Based on amino acid frequencies in alignment of similar (>85% identical) and homologous protein sequences
  - Probabilities were calculated for whether a given amino acid mutates to any other over a given period of time
  - The logarithm of this probability gives the substitution score
  - Based on number of changes from each amino acid and total number of occurrences
  - There are multiple PAM matrices and the PAM # corresponds to the number of accepted point mutations per 100 residues.
  - All PAM matrices are based on PAM1; others are inferred.
  - For example, the PAM250 contains scores based on an expected evolutionary distance corresponding to 250 point accepted mutations for every 100 amino acid residues

# PAM vs. BLOSUM Substitution matrices

- Choice depends on evolutionary distance
- For closely related sequences
  - Use higher BLOSUM number and lower PAM number
- For distantly related sequences
  - Use lower BLOSUM number or higher PAM number

# Inserting Gaps

- A **gap** in a sequence alignment indicates an insertion or deletion in the sequence
- When a gap is introduced, a **gap opening penalty** is added to the score
  - Insertions and deletions are not likely to occur in regions of structural importance
- Insertions tend to be several residues long
  - A smaller **gap extension** penalty is added each time a gap is extended
- Gaps cannot be aligned with each other

# Gap Penalties

- A "gap" (composed of a sequence of gap characters in the alignment, e.g., - - - ) has a penalty composed of a **gap opening penalty** for the initial character of each gap and a **gap extension penalty** for each subsequent character. Typically gaps are not penalized if they occur at the beginning or end of the alignment (this is known as a semi-global alignment)
- Here we use a gap opening penalty of 10 and a gap extension penalty of 1

Seq1:	S	E	Q	U	E	N	-	E
Seq2:	-	-	Q	-	-	N	C	E
Score:	0	0	5	-10	-1	6	-10	5

Diagram illustrating a semi-global alignment between Seq1 and Seq2. The alignment is shown with gaps (hyphens) and scores. Red boxes highlight specific gap regions, and red arrows point to them with labels:

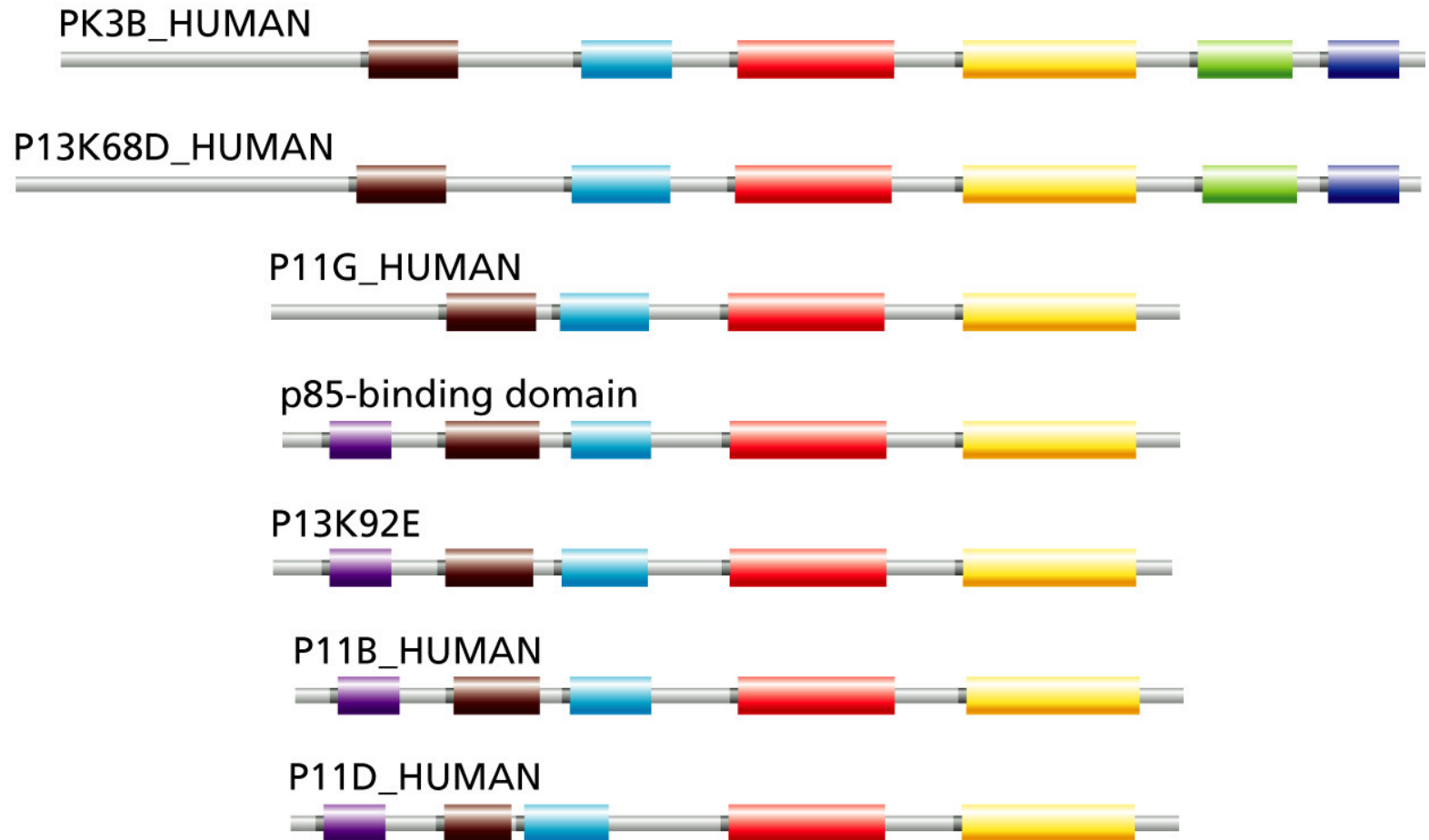
- Not penalized in semi-global alignment (points to the gap at the beginning of Seq2)
- Gap opening penalty (points to the first gap character in the third gap region)
- Gap extension penalty (points to the second gap character in the third gap region)
- Gap opening penalty (points to the first gap character in the fourth gap region)

***This semi-global alignment has an overall score (S) of -5***

# Types of alignments

- A **(semi) global alignment** aligns two sequences across their entire lengths
  - Appropriate for homologous sequences
- A **local alignment** detects shared regions (e.g., domains) which may be missed in global alignments
- A **pairwise alignment** is the alignment of two sequences
- A **multiple alignment** is the simultaneous alignment of more than two sequences

# Many proteins have multiple domains



## (A) local

PI3-kinase DRHNSNIMVKDDGQLFHI DFG

cAMP PK DLKPENLLIDQQGYIQVT DFG

# Local and global alignments

## (B) global

PI3-kinase HQLGNLR--LEEERI--MSSAKRPLWLNWENPDIMSELLFQNNIEIFKNGDDLRRQDMLT  
cAMP PK GNAAAACKKGXEQESVKEFLAKAKEDFLKKWENPAQNTAHL DQFERIKTLGTGSFGRVML--  
10 20 30 40 50

PI3-kinase LQIIRIME--NIWQNQG LDRMLPYGCLSIGDCVGLIEVVRNSHTIMQ-IQCKGGLK GAL  
cAMP PK ---VKHMETGNHYAMKILDKQKVVK-----L KQIEHTLNEKRILQAVNFPFLVKLEF  
60 70 80 90 100

PI3-kinase QFNSHT-LHQWLKDNKG EIIYDAA--IDL FTRSCAGYCVATFILGIG DRHNSNIMVKD-D  
cAMP PK SFKDNSNLYMVMEYVPG GEMFSHLRRIGRFSEPHARFYAAQIVLTFEY LHS LDIYRDLK  
110 120 130 140 150 160

PI3-kinase GQLFHI DFGHFLDHKKKKFGYKRERVP-----FVLTQDFL---IVISKGAQECTKTREFE  
cAMP PK PENLLIDQQGYI--QVT DFGFAK-RVKGRTWXLCGTPEYLAPEIILSKGYNKAVDWWALG  
170 180 190 200 210 220

# Alignment algorithms (preview)

- Needleman-Wunsch (1970) and variations:
  - for aligning two sequences
  - uses dynamic programming to "consider" all possible alignments (there are  $10^{600}$  possible alignments for two sequences of length 1000; there are only  $10^{80}$  atoms in the known universe)
- FASTA: uses a heuristic method for efficient searches (though not guaranteed to find the optimal solution)
  - Creates dictionary of  $k$ -tuples for the query sequence which is checked against sequences in the database
  - A local alignment algorithm is used to complete the alignment
- BLAST (Basic Local Alignment Search Tool): also fast and uses a heuristic
  - Finds short matches (which do not have to be exact)
  - Then uses local alignment to complete the alignment