

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

T	H	I	S	S	E	Q	U	E	N	C	E
T	H	A	T	S	E	Q	U	E	N	C	E

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

T	H	A	T	S	E	Q	U	E	N	C	E			
T	H	I	S	I	S	A	S	E	Q	U	E	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

T	H	I	S	I	S	A	-	S	E	Q	U	E	N	C	E
T	H	-	-	-	-	A	T	S	E	Q	U	E	N	C	E

- 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)

Scoring alignments

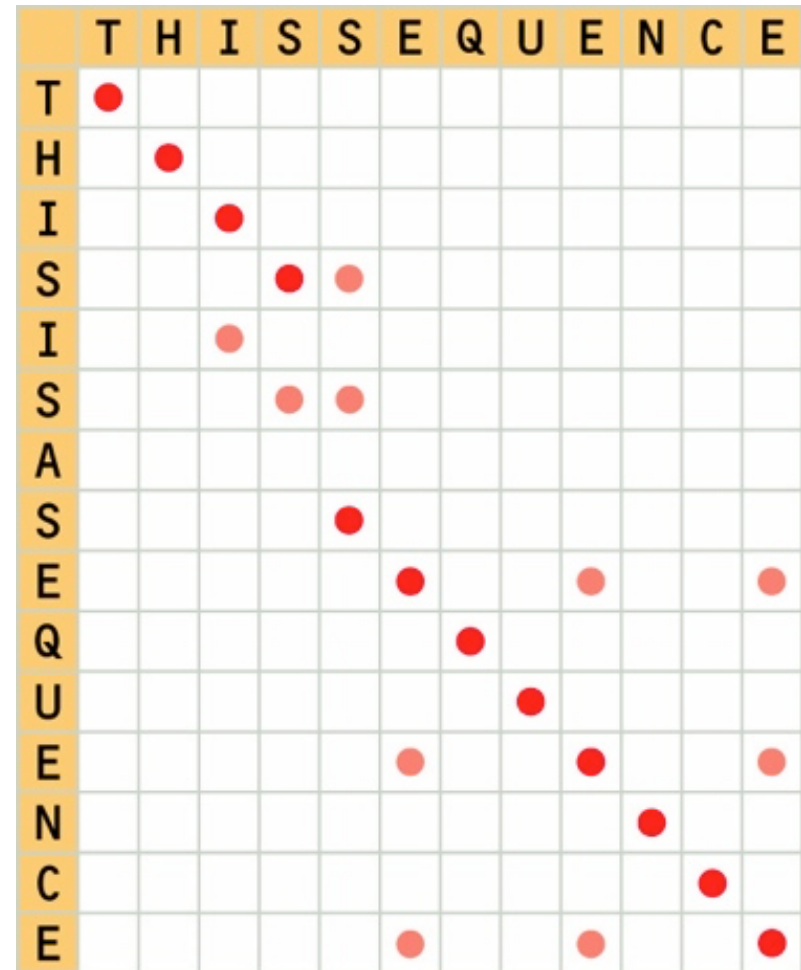
- Since multiple alignments are always possible, the best possible alignment is determined based on an alignment **score**
 - The **optimal alignment** is the alignment with the best score (multiple optimal alignments are possible)
 - **Suboptimal alignments** have slightly less scores than the best 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)

T	H	I	S	I	S	A	-	S	E	Q	U	E	N	C	E
T	H	-	-	-	-	A	T	S	E	Q	U	E	N	C	E

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

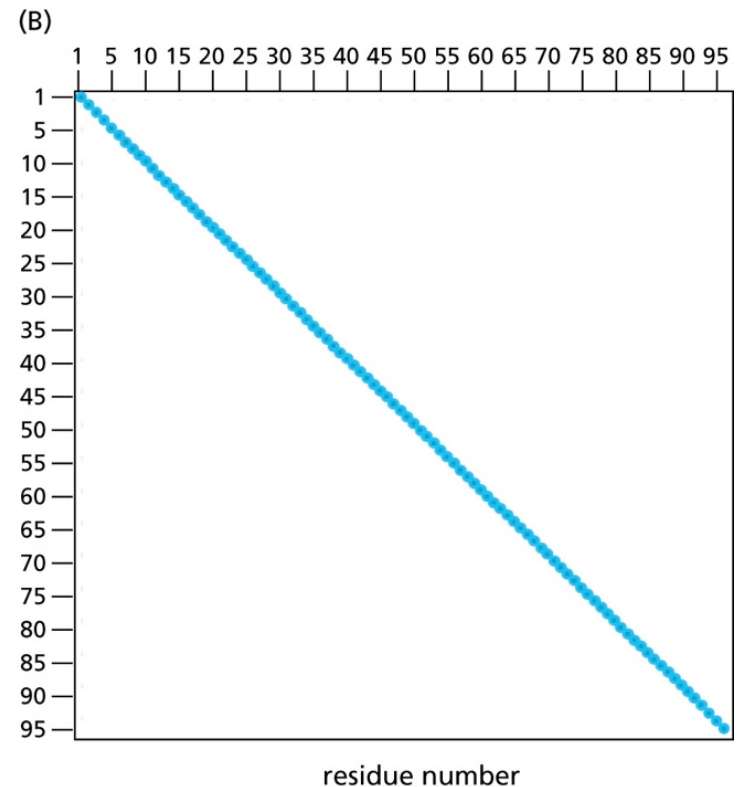
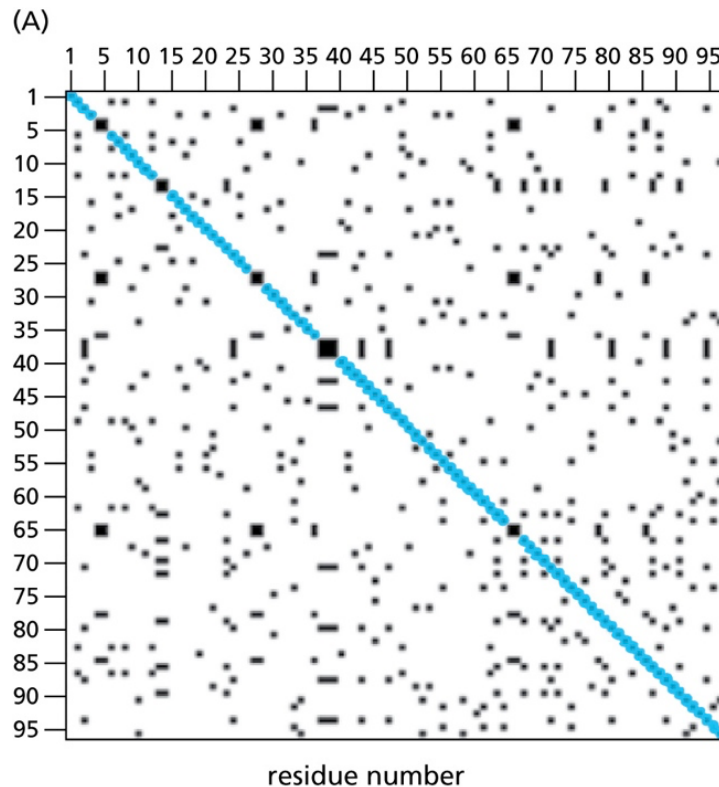
Dot-plots

- 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



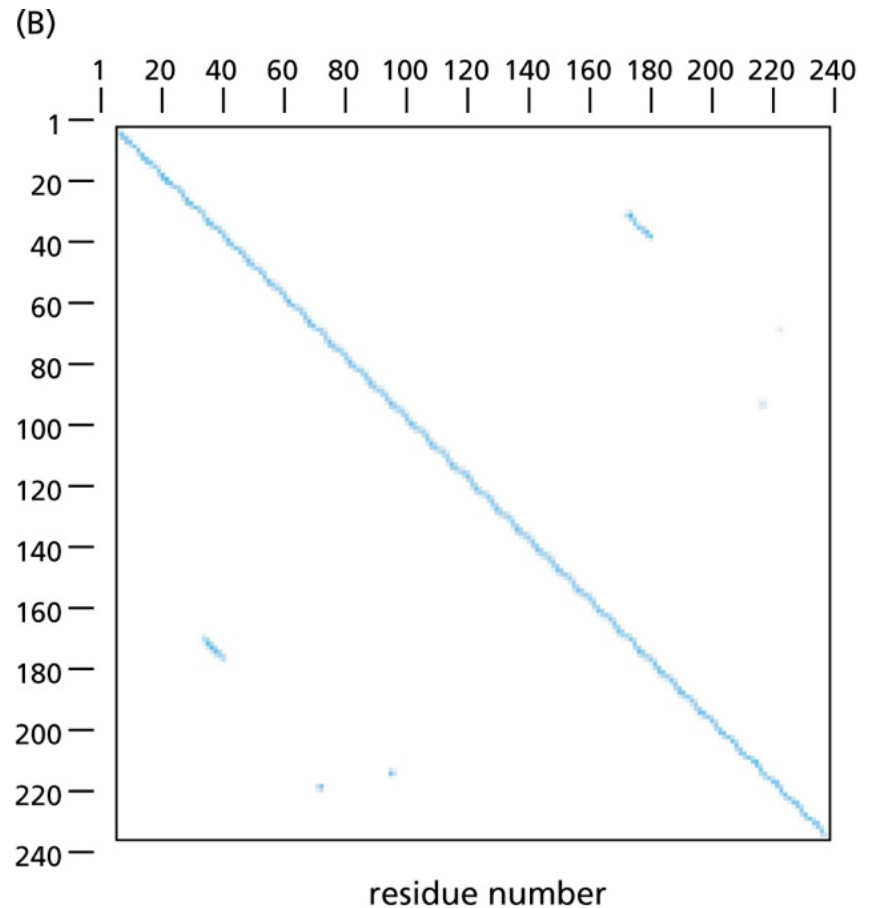
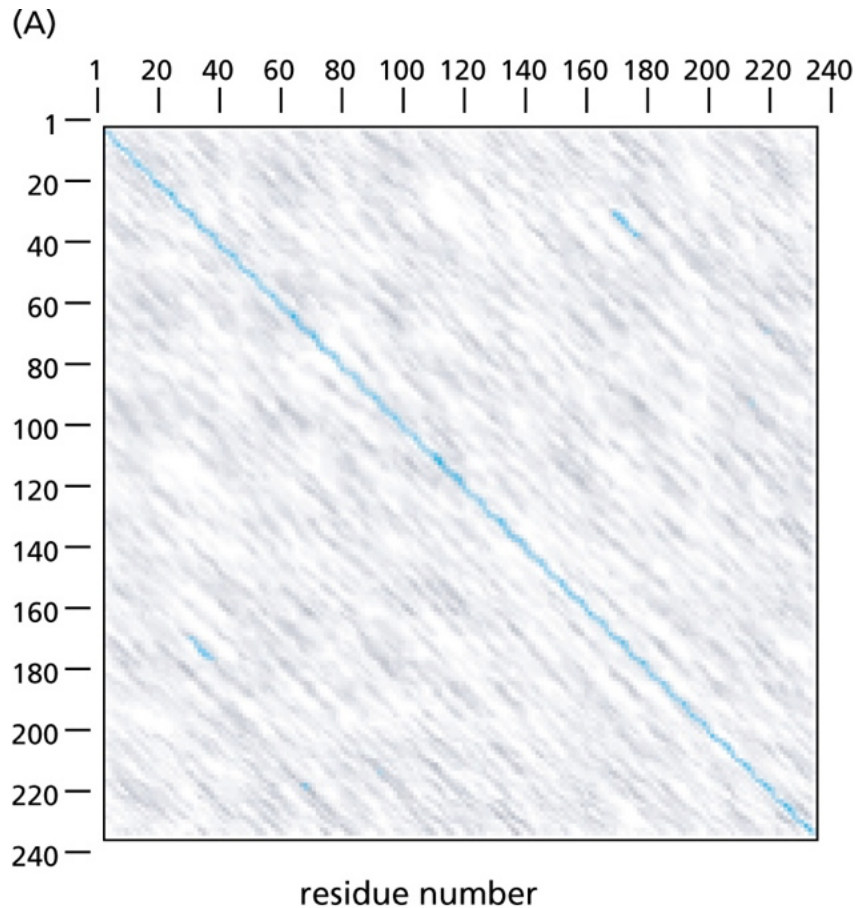
Dot-plots and background noise

- 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

T	H	I	S	I	S	A	S	E	Q	U	E	N	C	E
		.	.											
T	H	A	T	—	—	—	S	E	Q	U	E	N	C	E

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

Seq2: - - Q - - N C E

Score: 0 0 5 -10 -1 6 9 5

Not penalized in semi-global alignment

Gap opening penalty

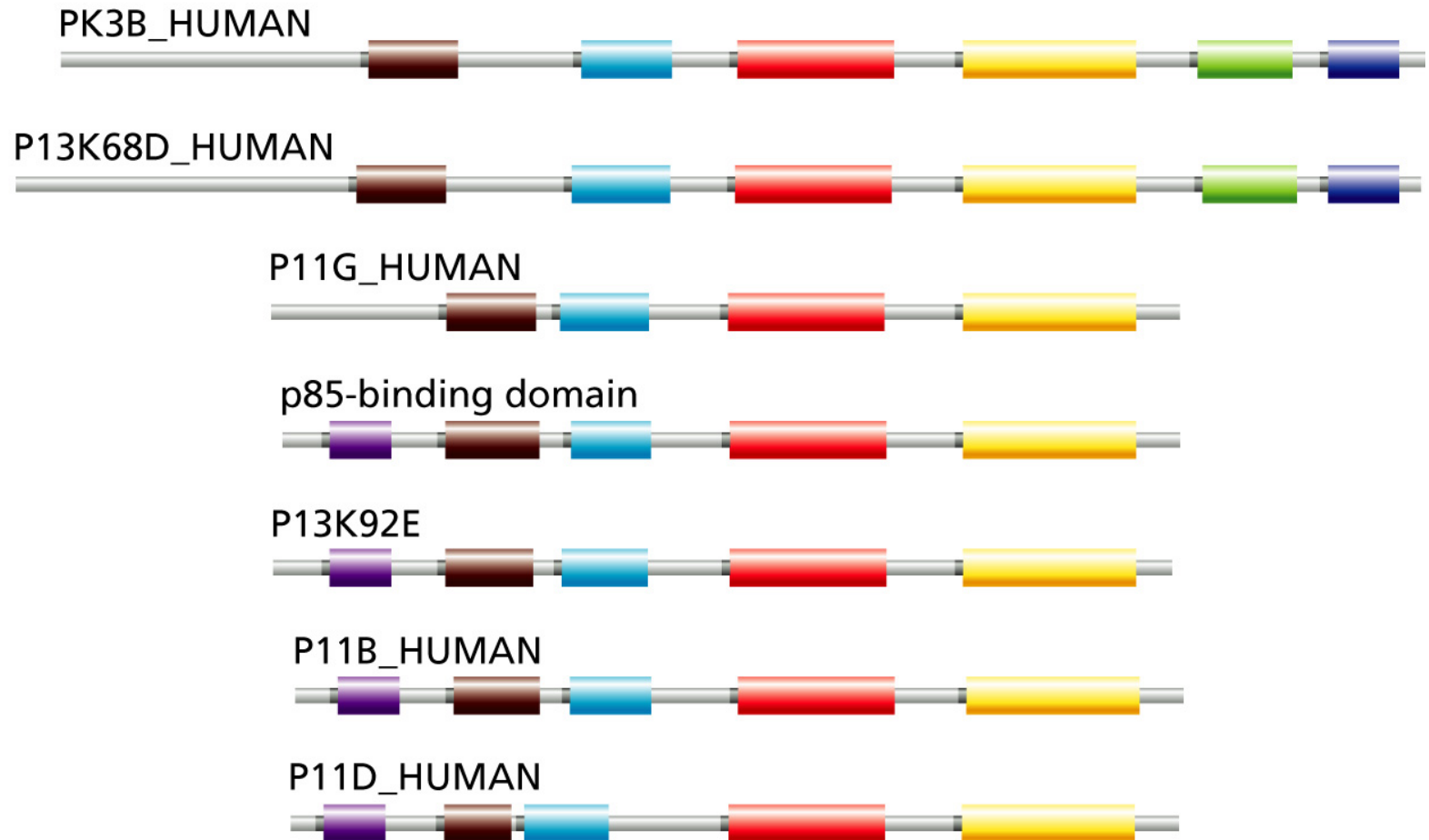
Gap extension penalty

This semi-global alignment has an overall score (S) of 14

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

	10	20	30	40	50
PI3-kinase	HQLGNLR--LEE	CRI--MSSAKRPL	LWLNWENPDIM	SELLFQNN	EIIFKNGDDLRQDMLT
cAMP PK	GNAAAAKKGX	EQESVKEFLAKAKED	FLKKWENPAQNT	AHL	DQFERIKTLGTGSFGRVML-
	10	20	30	40	50

	60	70	80	90	100	110
PI3-kinase	LQIIRIME--NI	WQNQGLDLRML	PYGCLSIGDCV	GLIEVVRNSHT	IMQ-IQCKGGLK	GAL
cAMP PK	---VKHMETGN	HYAMKILDKQK	VVK-----L	KQIEHTLNEKR	ILQAVNFPFLV	KLEF
	60	70	80	90	100	

	120	130	140	150	160	
PI3-kinase	QFNSHT-LHQWLK	DNKGGEIYDAA--	IDLFTRSCAGY	CVATFILGIG	DRHNSNIMVKD-D	
cAMP PK	SFKDNSNLYM	VMEYVPGGEMF	SHLRRIGRFSE	PHARFYAAQIV	LTFEYLSL	DLIYRDLK
	110	120	130	140	150	160

	170	180	190	200	210	220
PI3-kinase	GQLFHIIDFGH	FLDHKKKKFGY	KRERP-----	FVLTQDFL---	IVISKGAQECT	TKTREFE
cAMP PK	PENLLIDQQGYI	--QVTDFGFAK-	RVKGRTWXLCG	TPEYLAPEIIL	SKGYNKAVD	WWALG
	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} for two sequences of length 1000, but 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