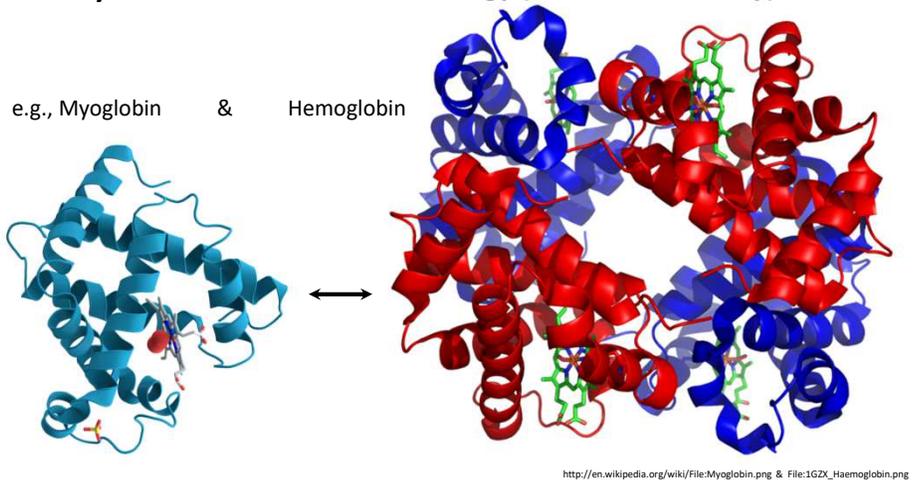


Typically, to be “biologically related” means to share a common ancestor. In biology, we call this *homologous*.

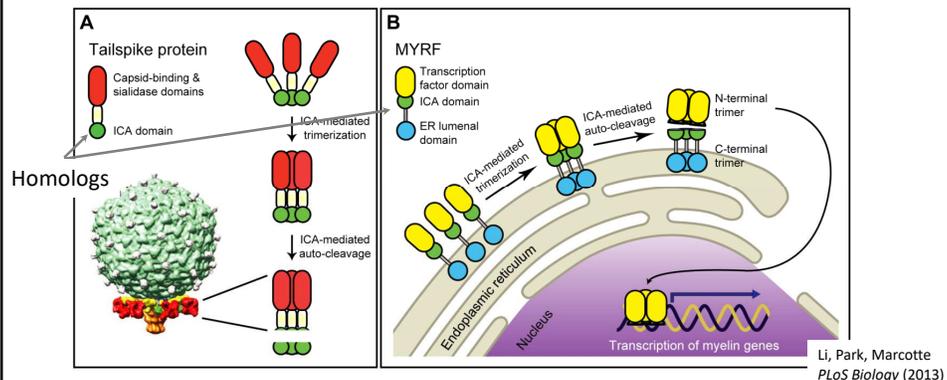
Two proteins sharing a common ancestor are said to be *homologs*. Homology often implies structural similarity & sometimes (not always) sequence similarity. **A statistically significant sequence or structural similarity can be used to infer homology (common ancestry).**



1

**In practice, searching for sequence or structural similarity is one of the most powerful computational approaches to discover a gene’s function. We can often gain insight about a protein from its homologs.**

For example, my lab discovered that myelinating the neurons in your brain reuses the same biochemical mechanism that phage use to make capsids. The key breakthrough was recognizing that the human and phage proteins contained homologous domains.

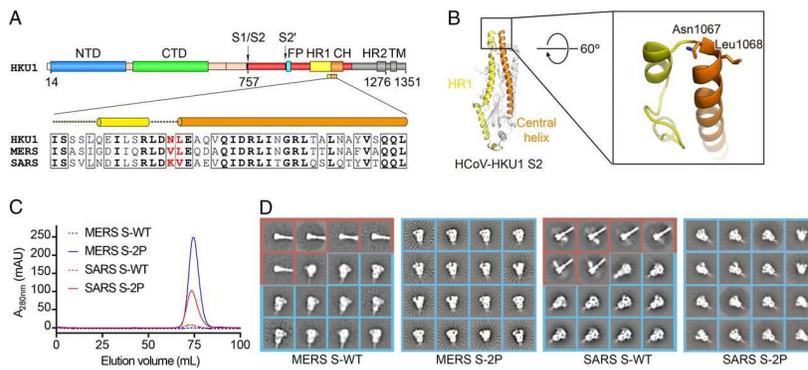


2

**& for a very timely example, here's the "trillion dollar" paper from the McLellan lab that the SARS-CoV-2 vaccines are designed from based on homology to MERS and SARS spike antigens**

**Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen**

Jesper Pallelesen, Nianshuang Wang, Kizmekia S. Corbett, Daniel Wrapp, Robert N. Kirchdoerfer, Hannah L. Turner, Christopher A. Cottrell, Michelle M. Becker, Lingshu Wang, Wei Shi, Wang-Pu Kong, Erica L. Anders, Armita N. Ketenbach, Mark R. Denison, James D. Chappell, Barney S. Graham, Andrew B. Ward, and Jason S. McLellan  
 PNAS August 29, 2017, 114 (35):E7348-E7357; first published August 14, 2017; https://doi.org/10.1073/pnas.1707204114



3

**Sequence alignment algorithms such as BLAST, PSI-BLAST, FASTA, and the Needleman–Wunsch & Smith-Waterman algorithms arguably comprise some of the most important driver technologies of modern biology and underlie the sequencing revolution.**

**So, let's start learning bioinformatics algorithms by learning how to align two protein sequences.**

4

**Live demo:**

[http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&BLAST\\_PROGRAMS=blastp  
&PAGE\\_TYPE=BlastSearch&SHOW\\_DEFAULTS=on&LINK\\_LOC=blasthome](http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&BLAST_PROGRAMS=blastp&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on&LINK_LOC=blasthome)

MVLSPADKTNVKAAWGKVGGAHAGEYGAELERMFLSFPTTKTYFPHFDLSHGSAQVKGHG  
KKVADALTNVAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTP  
AVHASLDKFLASVSTVLTSKYR

Title: All non-redundant GenBank CDS translations+PDB+SwissProt+PIR+PRF excluding environmental samples from WGS projects

Molecule Type: Protein

Update date: 2021/01/12

Number of sequences: 340,795,435

5

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per Ident	Acc Len	Accession
<input checked="" type="checkbox"/> hemoglobin alpha 2 [synthetic construct]	<a href="#">synthetic construct</a>	287	287	100%	9e-98	100.00%	143	<a href="#">AAV29522.1</a>
<input checked="" type="checkbox"/> hemoglobin alpha subunit family protein [Citrobacter freundii]	<a href="#">Citrobacter freundii</a>	286	286	100%	1e-97	100.00%	142	<a href="#">WIP_108961785.1</a>
<input checked="" type="checkbox"/> A cis-proline in alpha-hemoglobin stabilizing Protein directs the structural reorganization of alpha-hemoglobin [Homo sapiens]	<a href="#">Homo sapiens</a>	286	286	100%	2e-97	100.00%	145	<a href="#">3IA3_B</a>
<input checked="" type="checkbox"/> mutant hemoglobin alpha 2 globin chain [Homo sapiens]	<a href="#">Homo sapiens</a>	286	286	100%	2e-97	99.30%	142	<a href="#">AK266543.1</a>
<input checked="" type="checkbox"/> mutant hemoglobin subunit alpha 2 [Homo sapiens]	<a href="#">Homo sapiens</a>	286	286	100%	3e-97	99.30%	142	<a href="#">AXY55002.1</a>
<input checked="" type="checkbox"/> hemoglobin alpha-2 [Homo sapiens]	<a href="#">Homo sapiens</a>	285	285	100%	4e-97	99.30%	142	<a href="#">AN04486.1</a>
<input checked="" type="checkbox"/> alpha-2-globin [Homo sapiens]	<a href="#">Homo sapiens</a>	285	285	100%	5e-97	99.30%	142	<a href="#">AAF72612.1</a>
<input checked="" type="checkbox"/> TPA_globin C1 [Homo sapiens]	<a href="#">Homo sapiens</a>	286	286	100%	6e-97	100.00%	177	<a href="#">SAI82135.1</a>
<input checked="" type="checkbox"/> hemoglobin subunit alpha [Gorilla gorilla gorilla]	<a href="#">Gorilla gorilla gorilla</a>	285	285	100%	7e-97	99.30%	142	<a href="#">XP_030858702.1</a>
<input checked="" type="checkbox"/> hemoglobin alpha 1-2 hybrid [Homo sapiens]	<a href="#">Homo sapiens</a>	284	284	100%	1e-96	99.30%	142	<a href="#">BFF56145.1</a>
<input checked="" type="checkbox"/> mutant hemoglobin subunit alpha 2 [Homo sapiens]	<a href="#">Homo sapiens</a>	284	284	100%	1e-96	99.30%	142	<a href="#">AXY54998.1</a>
<input checked="" type="checkbox"/> mutant hemoglobin subunit alpha 2 [Homo sapiens]	<a href="#">Homo sapiens</a>	284	284	100%	1e-96	99.30%	142	<a href="#">AXY55003.1</a>
<input checked="" type="checkbox"/> mutant hemoglobin subunit alpha 2 [Homo sapiens]	<a href="#">Homo sapiens</a>	284	284	100%	1e-96	99.30%	142	<a href="#">AXY55001.1</a>
<input checked="" type="checkbox"/> Hemoglobin Thionville: An Alpha-Chain Variant With A Substitution Of A Glutamate For Valine At Na-1 And Hae1	<a href="#">Homo sapiens</a>	284	284	100%	1e-96	99.30%	143	<a href="#">1BAB_A</a>
<input checked="" type="checkbox"/> hemoglobin alpha-1 globin chain [Homo sapiens]	<a href="#">Homo sapiens</a>	284	284	100%	1e-96	99.30%	142	<a href="#">AAK37554.1</a>
<input checked="" type="checkbox"/> Hemoglobin (Val Beta1 Met_Tro Beta37 Tyr) Mutant [Homo sapiens]	<a href="#">Homo sapiens</a>	284	284	99%	2e-96	100.00%	141	<a href="#">1A00_A</a>
<input checked="" type="checkbox"/> alpha 2 globin variant [Homo sapiens]	<a href="#">Homo sapiens</a>	284	284	100%	2e-96	99.30%	142	<a href="#">BAD97112.1</a>

6

Descriptions **Graphic Summary** Alignments Taxonomy

hover to see the title click to show alignments  Show Conserved Domains

Alignment Scores  < 40  40 - 50  50 - 80  80 - 200  >= 200

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

Query seq. MVLSPADKTNVKAAGKVGVAHAGEYGAELERMFLSFPTTKTYFPHFDLSHGSA  
 Specific hits Hb-alpha\_like  
 Superfamilies Globin\_like superFamily

**Distribution of the top 106 Blast Hits on 100 subject sequences**

hemoglobin subunit alpha

7

Descriptions Graphic Summary **Alignments** Taxonomy

Alignment view Pairwise

100 sequences selected

[GenPept](#) [Graphics](#) Next Previous Descriptions

**hemoglobin alpha 2, partial [synthetic construct]**  
 Sequence ID: [AAX29522.1](#) Length: 143 Number of Matches: 1

Range 1: 1 to 142 [GenPept](#) [Graphics](#) Next Match Previous Match

Score	Expect	Method	Identities	Positives	Gaps
287 bits(734)	7e-98	Compositional matrix adjust.	142/142(100%)	142/142(100%)	0/142(0%)
Query 1		MVLSPADKTNVKAAGKVGVAHAGEYGAELERMFLSFPTTKTYFPHFDLSHGSA			
Sbjct 1		MVLSPADKTNVKAAGKVGVAHAGEYGAELERMFLSFPTTKTYFPHFDLSHGSA			
Query 61		KKVADALTNVAHVDDMPNALSALSDLHAHKLKRVDPVNFKLLSHCLLVTLAAHL			
Sbjct 61		KKVADALTNVAHVDDMPNALSALSDLHAHKLKRVDPVNFKLLSHCLLVTLAAHL			
Query 121		AVHASLDKFLASVSTVLTSKYR 142			
Sbjct 121		AVHASLDKFLASVSTVLTSKYR 142			

8

## Protein sequence alignment

### Two biologically related proteins with similar sequences:

**FlgA1** EAGNVKLRGRDLTLPRTVLDINQLVDAISLRDLSPDQPIQLTQFRQAWRVKAGQQRVNVIASGD  
++K+K+GRLDTLPP +L+ N A+SLR ++ QP+ R+ W +KAGQ V V+A G+  
**FlgA2** TLQDIKMKQGRDLTLPGALLEPNFAQGAVSLRQINAGQPLTRNMLRRLWIIKAGQDVQVLALGE

### Also biologically related (& fold up into the same 3D protein structure):

**FlgA1** EAGNVKLRGRDLTLPRTVLDINQLVDAISLRDLSPDQPIQLTQFRQAWRVKAGQQRVNVIASGD  
A + P +L I+ R L P + I R+AW V+ G V V  
**FlgA3** LAALKQVTLLIAGKHKPDAMATHABELQGKIAKRTLLPGRYIPTAAIREAWLVEQGAAVQVFFIAG

### But these are biologically unrelated (& fold up into unrelated structures):

**FlgA1** AGNVKLRGRDLTLPRTVLDINQLVDAISLRDLSPDQPIQLTQFRQA-WRVKAGQQRVNVIASGD  
AG+V K G + + PRT ++ I+ P PI +++A WRV A + V V+ GD  
**HvcPP** AGHV--KNGTMRIVGPRTCSNVWNGTFPINATTTGPSIPI PAPNYKKALWRVSATEYVEVVRVGD

(FYI, we'll draw examples from Durbin *et al.*, *Biological Sequence Analysis*, Ch. 1 & 2).

9

To align two sequences, we need to perform 3 steps:

1. We need some way to decide which alignments are better than others.  
For this, we'll invent a way to give the alignments a "score" indicating their quality.
2. Align the two proteins so that they get the best possible score.
3. Decide if the score is "good enough" for us to believe the alignment is biologically significant.

10

**To align two sequences, we need to perform 3 steps:**

1. We need some way to decide which alignments are better than others.  
**For this, we'll invent a way to give the alignments a "score" indicating their quality.**
2. Align the two proteins so that they get the best possible score.
3. Decide if the score is "good enough" for us to believe the alignment is biologically significant.

11

We'll treat mutations as independent events.

This allows us to create an ***additive scoring scheme***.

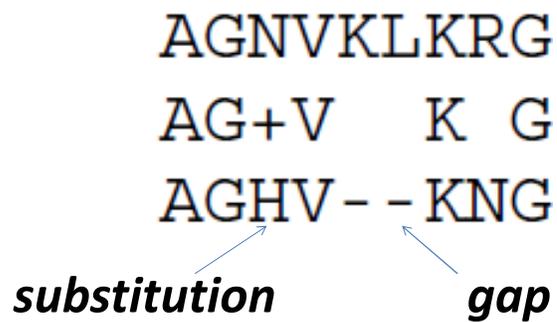
The score for a sequence alignment will be the sum of the scores for aligning each of the individual positions in two sequences.

**What kind of mutations should we expect?**

12

***Substitutions, insertions and deletions.***

Insertions and deletions can be treated as equivalent events by considering one or the other sequence as the reference, and are usually called ***gaps***.



13

Let's consider two models:

First, a **random** model, where amino acids in the sequences occur independently at some given frequencies.

The probability of observing an alignment between  $x$  and  $y$  is just the product of the frequencies ( $q$ ) with which we find each amino acid.

We can write this as:

What does the capital pi mean?

$$P(x, y | R) = \prod_i q_{x_i} \prod_j q_{y_j}$$

What's this mean?                      What's this mean?

14

Second, a **match** model, where amino acids at a given position in the alignment arise from some common ancestor with a probability given by the joint probability  $p_{ab}$ .

So, under this model, the probability of the alignment is the product of the probabilities of seeing the individual amino acids aligned.

We can write that as:

What does the capital pi mean again?

$$P(x, y | M) = \prod_i p_{x_i, y_i}$$

What's this mean?

What's this mean?

15

To decide which model better describes an alignment, we'll take the ratio:

$$\frac{P(x, y | M)}{P(x, y | R)} = \frac{\prod_i p_{x_i, y_i}}{\prod_i q_{x_i} \prod_j q_{y_j}} = \prod_i \frac{p_{x_i, y_i}}{q_{x_i} q_{y_i}}$$

What did these mean again?

Such a ratio of probabilities under 2 different models is called an **odds ratio**.

Where else have you heard odds ratios used?

Basically: if the ratio  $> 1$ , model  $M$  is more probable  
if  $< 1$ , model  $R$  is more probable.

16

Now, to convert this to an additive score  $S$ , we can simply take the logarithm of the odds ratio (called the **log odds ratio**):

$$S = \sum_i s(x_i, y_i)$$

This is just the score for aligning one amino acid with another amino acid:

$$s(a, b) = \log\left(\frac{P_{ab}}{P_a P_b}\right)$$

Here written  $a$  and  $b$  rather than  $x_i$  and  $y_i$  to emphasize that this score reflects the inherent preference of the two amino acids ( $a$  and  $b$ ) to be aligned.

**Almost done with step 1...**

17

The last trick:

Take a big set of pre-aligned protein sequence alignments (that are correct!) and measure all of the pairwise amino acid substitution scores (the  $s(a,b)$ 's). Put them in a 20x20 **amino acid substitution matrix** :

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	5	-2	-1	-2	-1	-1	-1	0	-2	-1	-2	-1	-1	-3	-1	1	0	-3	-2	0
R	-2	7	-1	-2	-4	1	0	-3	0	-4	-3	3	-2	-3	-3	-1	-1	-3	-1	-3
N	-1	-1	7	2	-2	0	0	0	1	-3	-4	0	-2	-4	-2	1	0	-4	-2	-3
D	-2	-2	2	8	-4	0	2	-1	-1	-4	-4	-1	-4	-5	-1	0	-1	-5	-3	-4
C	-1	-4	-2	-4	13	-3	-3	-3	-3	-2	-2	-3	-2	-2	-4	-1	-1	-5	-3	-1
Q	-1	1	0	0	-3	7	2	-2	1	-3	-2	2	0	-4	-1	0	-1	-1	-1	-3
E	-1	0	0	2	-3	2	6	-3	0	-4	-3	1	-2	-3	-1	-1	-1	-3	-2	-3
G	0	-3	0	-1	-3	-2	-3	8	-2	-4	-4	-2	-3	-4	-2	0	-2	-3	-3	-4
H	-2	0	1	-1	-3	1	0	-2	10	-4	-3	0	-1	-1	-2	-1	-2	-3	2	-4
I	-1	-4	-3	-4	-2	-3	-4	-4	-4	5	2	-3	2	0	-3	-3	-1	-3	-1	4
L	-2	-3	-4	-4	-2	-2	-3	-4	-3	2	5	-3	3	1	-4	-3	-1	-2	-1	1
K	-1	3	0	-1	-3	2	1	-2	0	-3	-3	6	-2	-4	-1	0	-1	-3	-2	-3
M	-1	-2	-2	-4	-2	0	-2	-3	-1	2	3	-2	7	0	-3	-2	-1	-1	0	1
F	-3	-3	-4	-5	-2	-4	-3	-4	-1	0	1	-4	0	8	-4	-3	-2	1	4	-1
P	-1	-3	-2	-1	-4	-1	-1	-2	-2	-3	-4	-1	-3	-4	10	-1	-1	-4	-3	-3
S	1	-1	1	0	-1	0	-1	0	-1	-3	-3	0	-2	-3	-1	5	2	-4	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-2	-1	2	5	-3	-2	0	3
W	-3	-3	-4	-5	-5	-1	-3	-3	-3	-3	-2	-3	-1	1	-4	-4	-3	15	2	-3
Y	-2	-1	-2	-3	-3	-1	-2	-3	2	-1	-1	-2	0	4	-3	-2	-2	2	8	-1
V	0	-3	-3	-4	-1	-3	-3	-4	-4	4	1	-3	1	-1	-3	-2	0	-3	-1	5

18

This is the **BLOSUM50** matrix.

(The numbers are scaled & rounded off to the nearest integer):

What's the score for aspartate (D) aligning with itself?

How about aspartate with phenylalanine (F)? Why?

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	5	-2	-1	-2	-1	-1	-1	0	-2	-1	-2	-1	-1	-3	-1	1	0	-3	-2	0
R	-2	7	-1	-2	-4	1	0	-3	0	-4	-3	3	-2	-3	-3	-1	-1	-3	-1	-3
N	-1	-1	7	2	-2	0	0	0	1	-3	-4	0	-2	-4	-2	1	0	-4	-2	-3
D	-2	-2	2	8	-4	0	2	-1	-1	-4	-4	-1	-4	-5	-1	0	-1	-5	-3	-4
C	-1	-4	-2	-4	13	-3	-3	-3	-3	-2	-2	-3	-2	-2	-4	-1	-1	-5	-3	-1
Q	-1	1	0	0	-3	7	2	-2	1	-3	-2	2	0	-4	-1	0	-1	-1	-1	-3
E	-1	0	0	2	-3	2	6	-3	0	-4	-3	1	-2	-3	-1	-1	-1	-3	-2	-3
G	0	-3	0	-1	-3	-2	-3	8	-2	-4	-4	-2	-3	-4	-2	0	-2	-3	-3	-4
H	-2	0	1	-1	-3	1	0	-2	10	-4	-3	0	-1	-1	-2	-1	-2	-3	2	-4
I	-1	-4	-3	-4	-2	-3	-4	-4	-4	5	2	-3	2	0	-3	-3	-1	-3	-1	4
L	-2	-3	-4	-4	-2	-2	-3	-4	-3	2	5	-3	3	1	-4	-3	-1	-2	-1	1
K	-1	3	0	-1	-3	2	1	-2	0	-3	-3	6	-2	-4	-1	0	-1	-3	-2	-3
M	-1	-2	-2	-4	-2	0	-2	-3	-1	2	3	-2	7	0	-3	-2	-1	-1	0	1
F	-3	-3	-4	-5	-2	-4	-3	-4	-1	0	1	-4	0	8	-4	-3	-2	1	4	-1
P	-1	-3	-2	-1	-4	-1	-1	-2	-2	-3	-4	-1	-3	-4	10	-1	-1	-4	-3	-3
S	1	-1	1	0	-1	0	-1	0	-1	-3	-3	0	-2	-3	-1	5	2	-4	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-2	-1	2	5	-3	-2	0	0
W	-3	-3	-4	-5	-5	-1	-3	-3	-3	-3	-2	-3	-1	1	-4	-4	-3	15	2	-3
Y	-2	-1	-2	-3	-3	-1	-2	-3	2	-1	-1	-2	0	4	-3	-2	-2	2	8	-1
V	0	-3	-3	-4	-1	-3	-3	-4	-4	4	1	-3	1	-1	-3	-2	0	-3	-1	5

19

Using this matrix, we can score any alignment as the sum of scores of individual pairs of amino acids.

For example, the top alignment in our earlier example:

```

FlgA1 EAGNVKLRGRLDTLPPRTVLDINQLVDAISLRDLSPDQPIQLTQFRQAWRVKAGQQRVNVIASGD
      ++K+K+GRDLTLPP  +L+ N   A+SLR ++  QP+      R+ W +KAGQ V V+A G+
FlgA2 TLQDIKMKQGRDLTLPPGALLEPNFAQGAVSLRQINAGQPLTRNMLRRLWIKAGQDVQVLALGE
  
```

gets the score:

$$S(\text{FlgA1}, \text{FlgA2}) = -1 - 2 - 2 + 2 + 4 + 6 + \dots = 186$$

We also need to penalize **gaps**. For now, let's just use a constant penalty **d** for each amino acid gap in an alignment, *i. e.*:

$$\text{the penalty for a gap of length } g = -g*d$$

20

PAM	vs.	BLOSUM
		
<p>Margaret Dayhoff (1925-1983) Developed point accepted mutation matrices (PAM matrices)</p>		<p>Steve and Jorja Henikoff Developed BLOSUM matrices</p>
<p><u>Calibrated for different evolutionary times</u> PAM-<math>n</math> = <math>n</math> substitutions per 100 residues e.g. matrices from PAM1 to PAM250 measure PAM1, calculate higher PAMs from that</p>		<p><u>Calibrated for different % identity sequences</u> BLOSUM-<math>n</math> = for sequences of about <math>n</math> % identity averages substitution probabilities over sequence clusters, gives better estimates for highly divergent cases</p>
<p><u>Explicit model of evolution</u> (calculated using a phylogenetic tree)</p>		<p><u>Implicit model of evolution</u> (calculated from blocks of aligned sequences)</p>

21

**To align two sequences, we need to perform 3 steps:**

1. We need some way to decide which alignments are better than others.  
For this, we'll invent a way to give the alignments a "score" indicating their quality.
2. **Align the two proteins so that they get the best possible score.**
3. Decide if the score is "good enough" for us to believe the alignment is biologically significant.

22

**A sense of scale:**

There are  $\binom{2n}{n} \approx \frac{2^{2n}}{\sqrt{\pi n}}$  possible global alignments between two sequences of length  $n$  if we use gaps

So, with 2 sequences of length 100, that's  $> 10^{60}$  possible alignments

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We'll use something called *dynamic programming*.

This is **mathematically guaranteed** to find the best scoring alignment, and uses *recursion*. This means problems are broken into sub-problems, which are in turn broken into sub-problems, etc, until the simplest sub-problems can be solved.

We're going to find the best *local* alignment—the best matching internal alignment—without forcing all of the amino acids to align (i.e. to match *globally*).

i.e., this  $\longrightarrow$       ATGCAT  
   ATGCAT

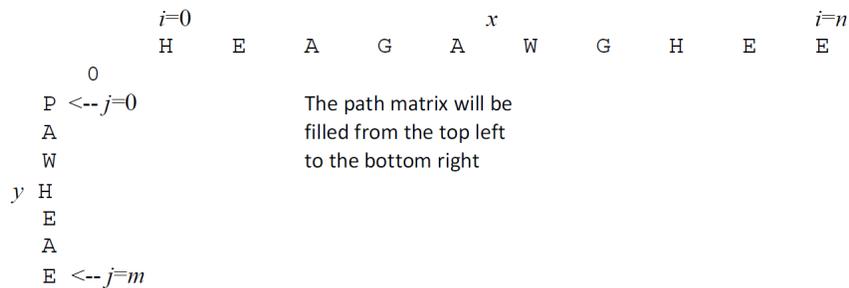
Not this  $\longrightarrow$       ACGTTATGCATGACGTA  
                                 -C---ATGCAT-----T-

24

**Here's the main idea:**

We'll make a **path matrix**, showing the possible alignments and their scores. There are simple rules for how to fill in the matrix.

**This will test all possible alignments & give us the top-scoring alignment between the two sequences.**



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**Here are the rules:**

For a given square in the matrix  $F(i,j)$ , we look at the squares to its left  $F(i-1,j)$ , top  $F(i,j-1)$ , and top-left  $F(i-1,j-1)$ . Each should have a score.

We consider **3 possible events & choose the one scoring the highest:**

- |                               |   |  |   |
|-------------------------------|---|--|---|
|                               |  |  |  |
| (1) $x_i$ is aligned to $y_j$ |   |  | $F(i-1,j-1) + s(x_i,y_j)$   |
| (2) $x_i$ is aligned to a gap |   |  | $F(i-1,j) - d$  |
| (3) $y_j$ is aligned to a gap |   |  | $F(i,j-1) - d$  |

For this example, we'll use  $d = 8$ . We also set the left-most & top-most entries to zero.

26

**Just two more rules:**

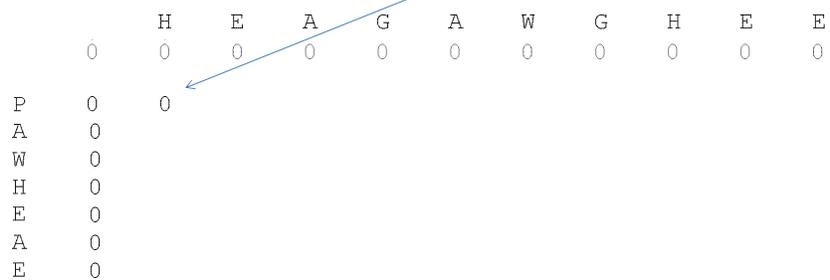
**If the score is negative, set it equal to zero.**

At each step, we also keep track of which event was chosen by **drawing an arrow from the cell we just filled back to the cell which contributed its score to this one.**

**That's it! Just repeat this to fill the entire matrix.**

27

Here we go! Start with the borders & the first entry.



A BLOSUM matrix is shown with a blue arrow pointing from the top-right cell to the first cell. The matrix is as follows:

		H	E	A	G	A	W	G	H	E	E
	0	0	0	0	0	0	0	0	0	0	0
P	0	0									
A	0										
W	0										
H	0										
E	0										
A	0										
E	0										

Why is this zero?

What's the score from our BLOSUM matrix for substituting H for P?

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Next round!

		H	E	A	G	A	W	G	H	E	E
	0	0	0	0	0	0	0	0	0	0	0
P	0	0	0								
A	0	0	0								
W	0										
H	0										
E	0										
A	0										
E	0										

Terrible! Again, none of the possible give positive scores.  
We have to go a bit further in before we find a positive score...

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A few more rounds, and a positive score at last!

		H	E	A	G	A	W	G	H	E	E
	0	0	0	0	0	0	0	0	0	0	0
P	0	0	0	0							
A	0	0	0	5							
W	0	0	0								
H	0										
E	0										
A	0										
E	0										

How did we get this one?

30

& a few more rounds...

	0	H 0	E 0	A 0	G 0	A 0	W 0	G 0	H 0	E 0	E 0
P	0	0	0	0	0						
A	0	0	0	5	0						
W	0	0	0	0	2						
H	0	10	2	0	0						
E	0										
A	0										
E	0										

What does this mean?

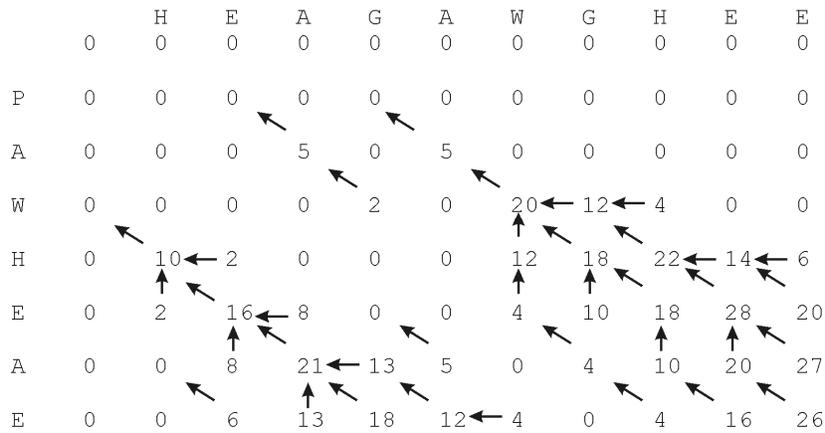
31

The whole thing filled in!

	0	H 0	E 0	A 0	G 0	A 0	W 0	G 0	H 0	E 0	E 0
P	0	0	0	0	0	0	0	0	0	0	0
A	0	0	0	5	0	5	0	0	0	0	0
W	0	0	0	0	2	0	20	12	4	0	0
H	0	10	2	0	0	0	12	18	22	14	6
E	0	2	16	8	0	0	4	10	18	28	20
A	0	0	8	21	13	5	0	4	10	20	27
E	0	0	6	13	18	12	4	0	4	16	26

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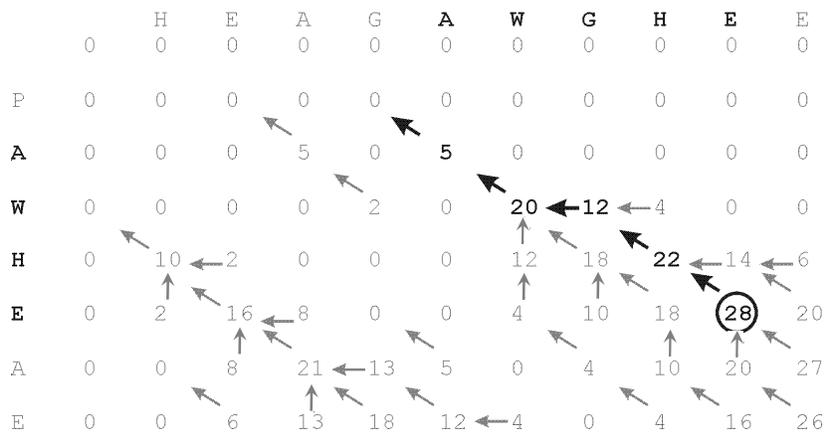
Now, find the optimal alignment using a *traceback* process:  
**Look for the highest score, then follow the arrows back.**  
**The alignment “grows” from right to left**



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This gives the following alignment:    A W G H E  
   A W - H E

(Note: for gaps, the arrow points to the sequence that gets the gap)



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**To align two sequences, we need to perform 3 steps:**

1. We need some way to decide which alignments are better than others.  
For this, we'll invent a way to give the alignments a "score" indicating their quality.
2. Align the two proteins so that they get the best possible score.
3. **Decide if the score is "good enough" for us to believe the alignment is biologically significant.**

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This algorithm always gives the best alignment.

Every pair of sequences can be aligned in some fashion.

**So, when is a score "good enough"?**

How can we figure this out?

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Here's one approach:

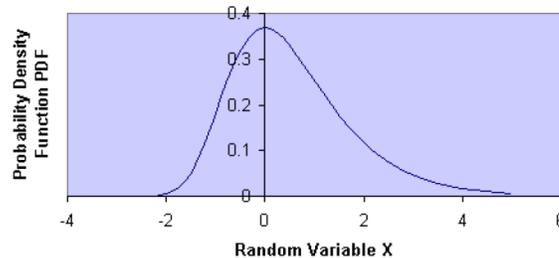
**Shuffle one sequence. Calculate the best alignment & its score.  
Repeat 1000 times.**

If we never see a score as high as the real one, we say the real score has <1 in a 1000 chance of happening just by luck.

But if we want something that only occurs < 1 in a million, we'd have to shuffle 1,000,000 times...

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Luckily, alignment scores follow a well-behaved distribution, the **extreme value distribution**, so we can do a few trials & fit to this.



# random trials & their average score

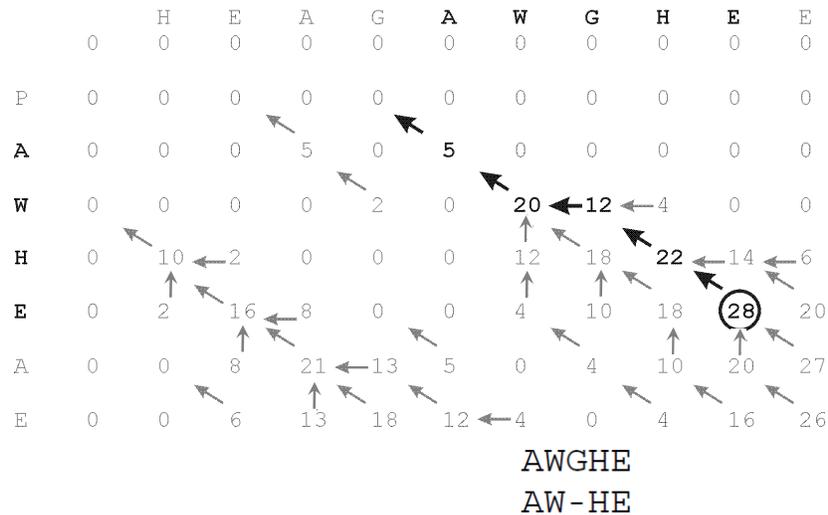
$$p(\text{max score} \leq X) \approx e^{-kNe^{\lambda(X-\mu)}}$$

This p-value gives the significance of your alignment.  
But, if we search a database and perform many alignments, we still need something more (next time).

Describe the shape & can be fit from a few trials

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Some extensions: Local vs. global alignments  
 How might you force the full sequences to align?



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Some extensions: Local vs. global alignments  
 How might you force the full sequences to align?

A few tiny changes:

Initialize only the top left cell of the path matrix to zero  
 (not all top and left cells).

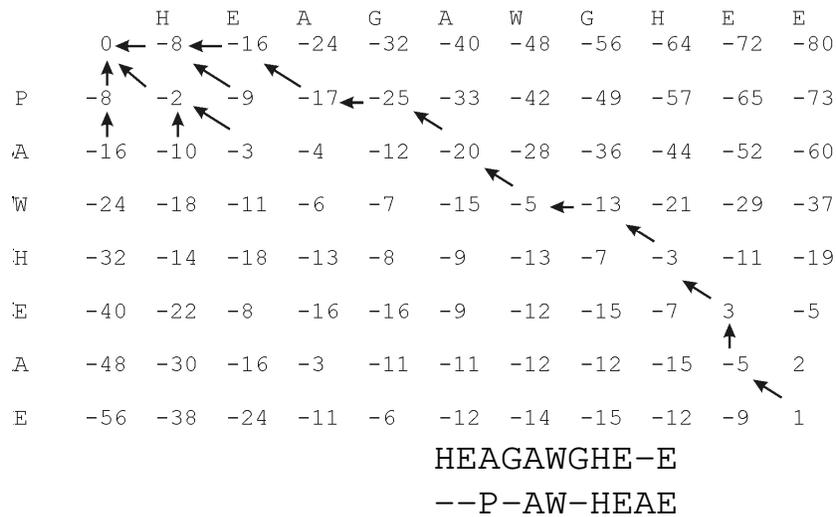
Leave the negative values (don't set them to zero).

The optimal alignment should start at the top left cell and  
 finish at the bottom right cell of the path matrix.

Start the trace-back at the bottom right cell

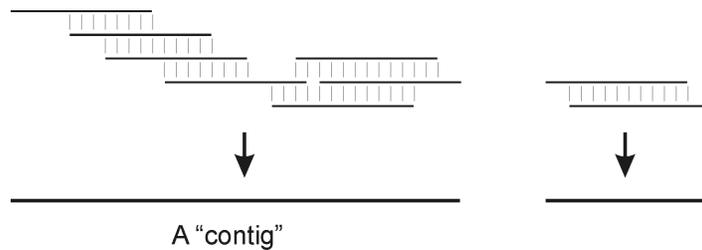
40

Some extensions: Local vs. global alignments  
 How might you force the full sequences to align?



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Some extensions:  
 What about overlapping sequences?  
 e.g. as in 'shotgun sequencing' genomes where  
 'contigs' are built up from overlapping sequences



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Some extensions:

What about overlapping sequences?

Modify global alignment to not penalize overhangs:

The optimal alignment should start at the top or left edge and finish at the bottom or right edge of the path matrix.

Set these boundary conditions :

$$F(i,0) = 0 \text{ for } i=1 \text{ to } n$$

$$F(0,j) = 0 \text{ for } j=1 \text{ to } m$$

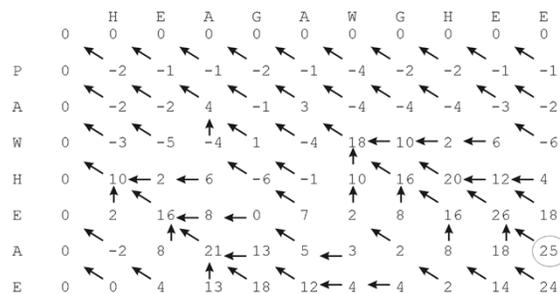
Start the traceback at the cell with the highest score on the right or bottom border

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Some extensions:

What about overlapping sequences?

e.g. as in 'shotgun sequencing' genomes where 'contigs' are built up from overlapping sequences



(overhang = HEA)

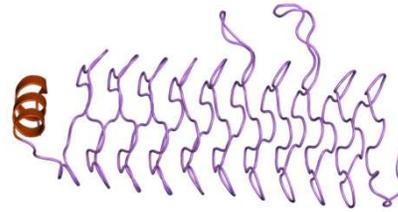
**GAWGHEE**

**PAW-HEA**

(overhang = E)

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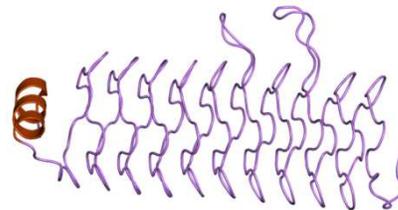
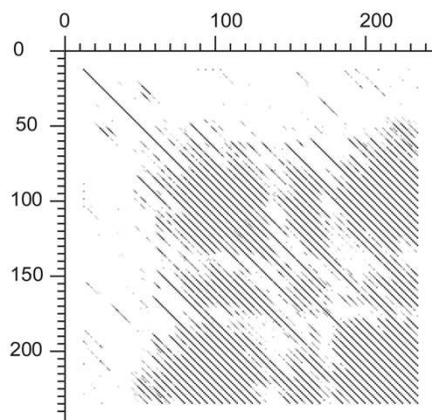
Some extensions:  
How might you find repetitive sequences?



Structure of the pentapeptide  
repeat protein HetL  
(from wiki, PMID18952182)

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Align the sequence to itself and ignore the diagonal (optimal) alignment  
→ High-scoring off-diagonal alignments will be repeats



Structure of the pentapeptide  
repeat protein HetL  
(from wiki, PMID18952182)

Dot plot (quick visualization of  
sequence similarity)  
of the pentapeptide repeat  
protein HgIK protein vs. itself  
([http://en.wikipedia.org/wiki/Pentapeptide\\_repeat](http://en.wikipedia.org/wiki/Pentapeptide_repeat))

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