

Introduction to Bioinformatics (2003)

Scenario 3: Scoring and protein alignments

Outline:

- I. Scoring sequence alignments
- II. Scoring tables for protein
- III. Practical *Blast*

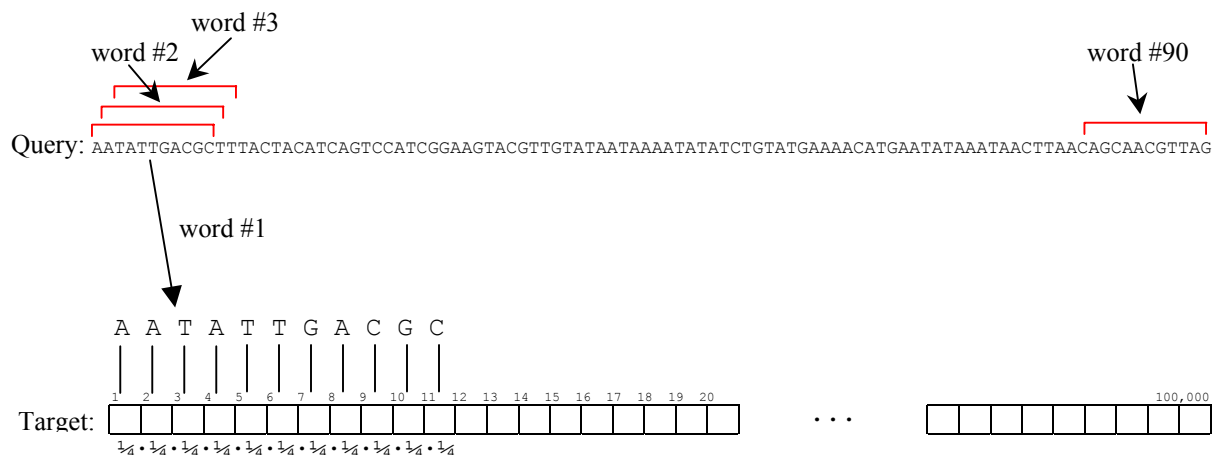
I. Scoring sequence alignments

Our version of *BlastN* prints out every word match it finds, after extending it in both directions as far as it can. Real *BlastN* doesn't do that. Instead, it calculates a score for each match, ranks all matches by their scores, and provides the top x matches that exceed a threshold y (you can specify x and y). The score thus serves to order the matches, so you can look at the best ones first, and also tosses out matches that are worse than you want to consider.

What kind of scoring system would be appropriate for sequence alignments? The first idea that might come to mind would be to use directly the raw score that *BlastN* calculates, i.e. one of the numbers found in scoring tables. This is OK for ranking matches, but suppose that *all* of the matches were no good, according to your tastes. How could you prevent *BlastN* from providing you with pages of garbage?

A better idea is to transform the raw score into a probability. If a match would occur frequently in a random sequence, you don't want to know about it. If you'd have to go through 10^{180} random sequences to find a match as good as the one *BlastN* is considering, then you want the program to pass that one on to you. So, we need to talk about expected frequency.

Suppose you're following Blast as it compares a 100-nucleotide sequence with a 100,000-nucleotide sequence. The first step is to extract a word from the query (let's say that the word size had been set to 11 nucleotides). What's the probability that this particular 11 nucleotides match the first 11 nucleotides of the 100,000-nucleotide sequence? Not much, sure, but we need a number. Let's simplify the situation and say that the probability of a match of one nucleotide is $\frac{1}{4}$. If so, then:



The probability of a match between word #1 and positions 1-11 of the target is $(\frac{1}{4})^{11}$. But there is the same probability of a match between word #1 and positions 2–12 and 3–13, etc., all the way up to positions 99,990 – 100,000. That’s about 99,990 possible matches, each with a probability of $(\frac{1}{4})^{11}$, giving a combined probability of $99,990 \cdot (\frac{1}{4})^{11}$. But that’s not the end: there are also words #2, #3, all the way up to word #90,^a so there are now $90 \cdot 99,990$ ways of getting a match, each with a probability of $(\frac{1}{4})^{11}$. That gives a total expected number of matches of:

$$90 \cdot 99,990 \cdot (\frac{1}{4})^{11}$$

or more generally:

$$(1) \ E = m \cdot n \cdot p^S$$

where **E** is the expected number of matches, **m** is the effective length of the query, **n** is the effective length of the target, **p** is the probability of a single match, and **S** is (for the moment) the total number of matches.

Mathematically, it is more convenient to transform this expression into an exponential based on *e*, forcing the expression to look like:

$$(2) \ E = m \cdot n \cdot e^{-\lambda S}$$

To fit this form, it works out that the constant λ has to equal $-\ln(p)$.^b

We now have almost what we want: a score that tells us how frequent we should expect a match to be. Unfortunately for this expression, *BlastN* finds matches that are more complicated than a string of matching nucleotides. This means that the expression has to be modified. I don’t understand half the math necessary to tell you how the ultimate expression arises, but you can see that this expression used by *BlastN* to calculate expected frequency is pretty close to the expression we derived intuitively from first principles. In the actual expression:

$$(3) \ E = K \cdot m \cdot n \cdot e^{-\lambda S}$$

where **K** and λ are empirically derived constants, the expected frequency, **E**, depends on the effective lengths of the query and the target, and it is related exponentially to the score **S**. Furthermore, λ is generally close to $\ln(\frac{1}{4})$, and **K** (a constant that depends on the base composition of the target) is not far from 1.

SQ5. Let’s see these symbols in action. Go to the NCBI web site and do a *BlastN* search of M29081 against all available nucleotide sequences. Look at the very end of the results you obtain to find values for K, λ , m, and n. Then go to any match in the results (you’ll have to scroll down from the top past the summary list) that has an E-value greater than zero. You’ll find the E-value and the raw score, S, in the line that begins:

Score = nnn bits ([raw score]), Expect = [E-value]

Determine from these values whether the given E-value can be calculated by equation (3).

^a Why not 100 words? Because the last word ends at position 100, so must begin at position 90. The number of words = length – wordsize + 1.

^b $m \cdot n \cdot p^S = E = m \cdot n \cdot e^{-\lambda S}$, leads to $p^S = e^{-\lambda S}$, and (taking the log of both sides) $\ln(p) = -\lambda$.

You'll notice in the same line that gave you raw score and E-value, there's a quantity called *bits*. This is yet another way of representing the score. One problem with the raw score is that its significance depends on other parameters (**K** and λ) that can change depending on the base composition of the sequence being examined. One way to transform the raw score into a score whose significance is independent of these quantities is to force the equation for E-value into the form:

$$(4) E = K \cdot m \cdot n \cdot e^{-\lambda S}$$

$$(5) = m \cdot n \cdot 2^{-S'}$$

leading to:

$$(6) S' = [\lambda \cdot S - \ln(K)] / \ln(2)$$

where S' represents the normalized score. Since the E-value depends in the latter expression only on S' and on readily determined quantities (i.e., the effective length of the query sequence, m , and the effective length of the target sequence, n), it is more transportable. Secondly, since S' is present as an exponent of 2, it relates the number of binary digits, or bits, necessary to represent a number, just as the exponent in 10^n tells you how many digits are in the decimal representation of a number. Bits is also the currency of information theory, making it a theoretically attractive unit, one that is interconvertible with the E-value. You might also think of bits as the number of binary digits it takes to represent the probability of a specific match as good as the one reported. Multiply this probability by the sizes of the two sequences considered and you get, as in equation (1), the expected number of matches.

SQ6. Using the same *BlastN* result from SQ1, calculate the E-value from the bit score (S') and equation (5), and determine whether this value corresponds well with the given E-value.

II. Aligning protein sequences

Aligning pairs of protein sequences introduces additional complications, although the search algorithm is only a bit different from that used in aligning nucleic acid sequences. The root of the problem is the more complex functional roles played by amino acids relative to nucleotides. With nucleotides, scoring is simple: there's a gap, a match, or a mismatch. With amino acids, there are various levels of mismatch, as illustrated in Figure 1. The region of the protein shown in the figure is relatively well conserved over evolution, but there is variation in amino acids at

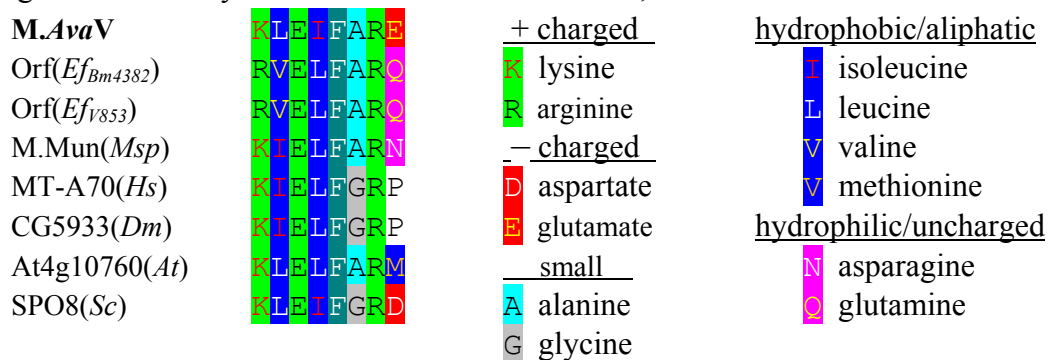


Figure 1: Alignment of a region from eight DNA-modifying enzymes (some putative). Note that the alignment isn't perfect, but most of the imperfections fall into certain families of amino acids.

each position, and there is often order to that variation. For example, the first position is occupied by either lysine or arginine, both positively charged amino acids. The second position shows either isoleucine, leucine, or valine, all aliphatic^c hydrophobic amino acids. The last position is a different matter, however, with a mixed bag of amino acids. One would imagine that in many cases, mutation from one amino acid to a similar amino acid would retain function of the protein, and we would like to score that mismatch differently from a mutation that leads to a more drastic change.

II.A. Percent Accepted Mutation (PAM) tables

We would like to be able to look at a protein and say that this amino acid change is minimally significant, hence a low mismatch penalty, while another is worthy of a high mismatch penalty, but we don't yet know enough about protein structure to do this. Instead, we've asked the proteins to tell us to score their own mutations. This has been done in two ways. Margaret Dayhoff collected pairs of very similar protein sequences that differed from each other in about one amino acid per one hundred residues and analyzed which amino acid were replaced by which others. All of the proteins analyzed were functional, so the amino acid changes did not abolish function and were termed "acceptable". The resulting table, called PAM1 for *Percent Accepted Mutation at 1% change*, provides a guide as to what amino acid substitutions might be considered conservative (more likely to retain function of the protein) and a basis for the construction of a rational scoring table for protein sequence alignment.

However, a table constructed from the comparison of closely related protein is not as applicable to the comparison of proteins that have a much greater degree of divergence. For this reason other PAM tables were calculated. Multiplying the PAM1 matrix by itself gives a PAM2 matrix, representing predicted mutation frequencies amongst proteins with a 2% degree change. By this procedure, matrices as high as PAM400 have been calculated. It may seem surprising that there exist a table that predicts the frequencies of amino acid substitutions in protein with 400% degree of change, but you have to remember that over millions of years, the same position in a protein may change multiple times. A 400% degree of change means that each amino acid position has

Table 2: PAM1 amino acid change frequencies*

| | | Ala | Arg | Asn | Asp | Cys | Gln | Glu | Gly | His | Ile | Leu | Lys | Met | Phe | Pro | Ser | Thr | Trp | Tyr | Val | |
|-----|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | A | A | R | N | D | C | Q | E | G | H | I | L | K | M | F | P | S | T | W | Y | V | |
| Ala | A | 9867 | 2 | 9 | 10 | 3 | 8 | 17 | 21 | 2 | 6 | 4 | 2 | 6 | 2 | 22 | 35 | 32 | 0 | 2 | 18 | |
| Arg | R | 1 | 9913 | 1 | 0 | 1 | 10 | 0 | 10 | 3 | 1 | 19 | 4 | 1 | 4 | 6 | 1 | 8 | 0 | 1 | | |
| Asn | N | 4 | 1 | 9822 | 36 | 0 | 4 | 6 | 6 | 21 | 3 | 1 | 13 | 0 | 1 | 2 | 20 | 9 | 1 | 4 | 1 | |
| Asp | D | 6 | 0 | 42 | 9859 | 0 | 6 | 53 | 6 | 4 | 1 | 0 | 3 | 0 | 0 | 1 | 5 | 3 | 0 | 0 | 1 | |
| Cys | C | 1 | 1 | 0 | 0 | 9973 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 5 | 1 | 0 | 3 | 2 | |
| Gln | Q | 3 | 9 | 4 | 5 | 0 | 9876 | 27 | 1 | 23 | 1 | 3 | 6 | 4 | 0 | 6 | 2 | 2 | 0 | 0 | 1 | |
| Glu | E | 10 | 0 | 7 | 56 | 0 | 35 | 9865 | 4 | 2 | 3 | 1 | 4 | 1 | 0 | 3 | 4 | 2 | 0 | 1 | 2 | |
| Gly | G | 21 | 1 | 12 | 11 | 1 | 3 | 7 | 9935 | 1 | 0 | 1 | 2 | 1 | 1 | 3 | 21 | 3 | 0 | 0 | 5 | |
| His | H | 1 | 8 | 18 | 3 | 1 | 20 | 1 | 0 | 9912 | 0 | 1 | 1 | 0 | 2 | 3 | 1 | 1 | 1 | 4 | 1 | |
| Ile | I | 2 | 2 | 3 | 1 | 2 | 1 | 2 | 0 | 0 | 9872 | 9 | 2 | 12 | 7 | 0 | 1 | 7 | 0 | 1 | 33 | |
| Leu | L | 3 | 1 | 3 | 0 | 0 | 6 | 1 | 1 | 4 | 22 | 9947 | 2 | 45 | 13 | 3 | 1 | 3 | 4 | 2 | 15 | |
| Lys | K | 2 | 37 | 25 | 6 | 0 | 12 | 7 | 2 | 2 | 4 | 1 | 9926 | 20 | 0 | 3 | 8 | 11 | 0 | 1 | 1 | |
| Met | M | 1 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 5 | 8 | 4 | 9874 | 1 | 0 | 1 | 2 | 0 | 0 | 4 | |
| Phe | F | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 2 | 8 | 6 | 0 | 4 | 9946 | 0 | 2 | 1 | 3 | 28 | 0 | |
| Pro | P | 13 | 5 | 2 | 1 | 1 | 8 | 3 | 2 | 5 | 1 | 2 | 2 | 1 | 1 | 9926 | 12 | 4 | 0 | 0 | 2 | |
| Ser | S | 28 | 11 | 34 | 7 | 11 | 4 | 6 | 16 | 2 | 2 | 1 | 7 | 4 | 3 | 17 | 9840 | 38 | 5 | 2 | 2 | |
| Thr | T | 22 | 2 | 13 | 4 | 1 | 3 | 2 | 2 | 1 | 11 | 2 | 8 | 6 | 1 | 5 | 32 | 9871 | 0 | 2 | 9 | |
| Trp | W | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 9976 | 1 | 0 | |
| Tyr | Y | 1 | 0 | 3 | 0 | 3 | 0 | 1 | 0 | 4 | 1 | 1 | 0 | 0 | 21 | 0 | 1 | 1 | 2 | 9945 | 1 | |
| Val | V | 13 | 2 | 1 | 1 | 3 | 2 | 2 | 3 | 3 | 57 | 11 | 1 | 17 | 1 | 3 | 2 | 10 | 0 | 2 | 9901 | |
| | | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 | 10000 |

* Each cell gives the number of times the amino acid listed horizontally changed to the amino acid listed vertically, per 10,000 changes of the first amino acid.

^c An "aliphatic" amino acid is one means that has a side chain consisting solely of a hydrocarbon chain.

changed, on average, 4 times. Figure 2 provides a theoretical correlation between the degree of change and the percent similarity between protein. PAM tables higher than PAM250 are of limited value, because with such a low degree of similarity, matches between amino acids may have arisen by chance rather than by common descent. PAM250 is commonly used in Blast searches.

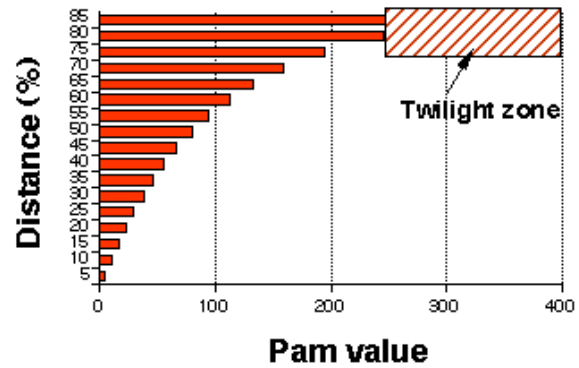


Figure 2: Relationship between degree of change (Pam value) and dissimilarity between two proteins. The hatched box indicates the degree of dissimilarity sufficient to cast doubt on a conclusion of common lineage between two proteins. (from Doolittle, 1987)

SQ7. Amongst protein pairs that are 99% similar to each other, what fraction of arginines in one protein correspond to lysines in the other (at equivalent positions)? What fraction of arginines in one correspond to leucines in the other?

SQ8. What PAM table would be appropriate to search for proteins about 50% identical to a query sequence?

II.B. Block Substitution Matrices (BLOSUM)

PAM tables suffer from several deficiencies. Since they're all calculated from PAM1, any error in that table is propagated throughout the rest. Since the original data consisted of only 1572 amino acid changes, many possible changes were not observed (e.g. aspartate to arginine). Even though such changes must be possible at some frequency, no PAM table will not admit this. Secondly, the procedure of multiplying PAM matrices to get tables for less similar proteins presumes the debatable evolutionary model in which similar and dissimilar protein pairs arose from the same kind of amino acid substitutions. Third, PAM tables are built from a consideration of amino acid substitutions at all positions in similar proteins. It may well be that the pattern of amino acid substitutions would differ in different regions.

Henikoff and Henikoff (1992)^d proposed an alternative strategy to address the deficiencies in PAM tables. They compiled a list of conserved blocks of amino acid sequences (regions within proteins that are relatively conserved across many organisms) and calculated the frequency of each amino acid change. Owing to the size of their database, even the least common amino acid transitions is represented 2369 times. The table derived from blocks in which there is 62% identity of amino acids is called BLOSUM62 (Block substitution matrix, 62%). Two other tables, BLOSUM45 and BLOSUM80 are also available, derived from blocks with 45% and 80% identity, respectively. Note that BLOSUM's numbering system is the opposite in direction from PAM's. Per BLOSUM: low numbers mean low identity. Per PAM: low numbers mean low change (so high identity).

II.C. Constructing scoring matrices from PAM and BLOSUM tables

Blast uses PAM and BLOSUM tables to calculate a probability that a series of matches and mismatches would occur given the presumed model. To facilitate this calculation, the tables are modified in the following way.

^d Henikoff S & Henikoff J (1992). Proc Natl Acad Sci USA 89:10915-10919.

- The frequency of each amino acid-amino acid transition is rendered as an odds ratio. For example, for the transition from asp-glu (in either direction):

$$\frac{\text{Total number of asp-glu transitions observed}}{2 \cdot (\text{frequency of asp residues}) \cdot (\text{frequency of glu residues})} = \frac{\text{relative frequency}}{\text{expected frequency}}$$

The expected frequency is the fraction of transitions you'd expect just from the frequency of the component amino acids. So the odds ratio is the degree to which the frequency of an amino acid transition deviates from expectation.

- From the odds ratio, the log odds ratio (**lod**) is derived:

$$\text{lod} = \log_{10}(\text{relative frequency}/\text{expected frequency})$$

Adding the **lod** scores is equivalent to multiplying the odds ratios, and since addition is faster than multiplication, the program becomes more efficient.

- The **lod** score is multiplied by 10 and only the integer portion is shown. This makes the table easier to read, though making it more difficult to comprehend how the number relates to the odds ratio.

Figure 3 shows the BLOSUM62 table in its log odds ratio form. A scoring matrix would use this table to replace match rewards and mismatch penalties. The alignment of the first sequences given in Figure 1:

would give a score (using BLOSUM62) of:

$$2+1+5+2+6+4+5+2 = 27$$

Gaps are still dealt with using gap open and gap extension penalties as in *BlastN*.

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

Figure 3: BLOSUM62. Each number represents the \log_{10} of the odds that a given amino acid transition occurs. Since the table is symmetrical (the direction of change is irrelevant), only half of it is shown.

SQ9. What score do you calculate for the alignment between the last two sequences given in Figure 1?

II.D. One additional difference between *BlastP* and *BlastN*: The initial match

BlastN begins by looking for an exact match in the target sequence of a word extracted from the query sequence. Unfortunately, exact matches in proteins are too rare for this procedure to be useful. Instead, *BlastP* (analogous to *BlastN*, but acting on protein sequences) searches for exact and *almost* exact matches to words. How exact the match has to be is determined by what is known as the neighborhood word threshold value (**T**). For example, if **T** is set to 13, then the word **FIM** will find the following sequences: **FIM** (score=15), **FIM** (score=13), and **FLM**

(score=14). Any of these three sequences in the target will instigate a search for extensions in both directions.

SQ10. What sequences would be found by VLI using a T value of 13? Considering your answer, do you think that this system or the T value used has a defect?

III. Practical *Blast*

You now know how to score DNA and protein alignments, you have an idea how *BlastN* and *BlastP* work... how does this change your life? If you do alignments (and there's probably nothing more common in bioinformatics than that), you should now be able to talk to *Blast* better, to make it do what you want.

III.A. Which scoring matrix to use? (protein searches only)

From the considerations discussed above, you shouldn't be surprised to learn that BLOSUM matrices outperform their PAM counterparts, so if you are searching for protein that figure to be around 45%, 62%, or 80% identical, then choose BLOSUM45, BLOSUM62, or BLOSUM80, respectively (BLOSUM62 is *BlastP*'s default). If you're using a short query, then you want to choose a matrix that demands a higher degree of identity (otherwise true matches will get mixed in with the background). In that case, PAM30 (length <35 amino acids) or PAM70 (length between 35 and 50 amino acids) is preferable to any available BLOSUM table.

III.B. What scoring parameters to use?

If you want to find the most significant matches, leave the Blast default parameters alone. You'll often get a patchwork of partial matches between your query and the target, as shown in

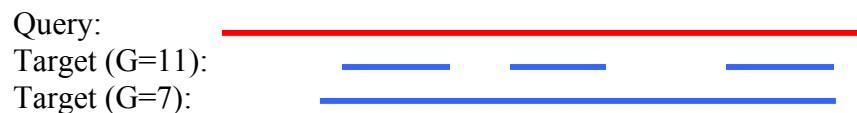


Figure 4: Effect of changing gap open penalty (G). Hypothetical example of results of a Blast search of a query sequence (red line) against a target sequence. Regions found by the search are shown in blue.

Figure 4. Often it is possible to connect the strong matches with less significant matches by reducing the gap penalties or reducing the mismatch penalty (in the case of nucleotides).

If you're searching for short matches, you may want to reduce the word size and increase the E-value threshold (since even good short matches will get worse scores than the long matches Blast is set up for). If you're looking for significant matches and you're getting a lot of them, you can reduce their number by setting the E-value from 10 (default) to .001. While this will almost always get rid of many spurious matches, you will need to decide if anything valuable is also being tossed out.

The most common mistake people make with Blast may be to search for DNA similarity when they could instead look for protein similarity. Evolution operates at the level of protein function, not DNA sequence, so DNA searches invariably miss distant matches that protein searches find.

The second common mistake people make with Blast is to equate similarity of sequence with similarity of function. This is a large topic that takes us out of the scope of this research simulation, but you might heed the warning given by Persemlidis and Fondon.^e

^e <http://genomebiology.com/2001/2/10/reviews/2002>