Lectures 8, 9, 10, and 11: Homology Searching

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String Comparison

The two "killer apps" of modern string processing algorithms have been computational biology and searching the Internet.
While *exact matching* algorithms are more important in text processing than biology, they are important (1) to illustrate techniques, and (2) as a component of heuristics for approximate matching.
Three primary classes of string matching problems arise, determining whether preprocessing techniques are appropriate:

- *Fixed texts, variable patterns* – e.g. search the human genome or the Bible.

  Suffix trees/arrays are data structures to efficiently support repeated queries on fixed strings.

- *Variable texts*, *fixed patterns* – e.g. search a news feed for dirty words, or the latest Genbank entries for specific motifs.

  Such applications justify preprocessing the set of patterns so as to speed search.

- *Variable texts*, *variable patterns* – e.g. the naive $O(nm)$ brute force search algorithm.

  Suffix trees solve this problem in linear time, but with excessive complexity and overhead.

Efficient Exact String Matching

We have seen the naive brute force algorithm searching for pattern p in text t in $O(mn)$ time, where $m = |p|$ and $n = |t|$. The brute force algorithm can be viewed as sliding the pattern across from left to right by *one position* when we detect a mismatch between the pattern and the text.

But in certain circumstances we can slide the pattern to the right by more than one position:

```
pattern:        ABCDABCE
text:       ....ABCDABCD....
```

Since we know the last seven characters of the text must be $ABCDABC$, we can shift the pattern four positions without missing any matches.

The Knuth-Morris-Pratt Algorithm

Whenever a character match fails, we can shift the pattern forward according to the *failure function* $fail(q)$, which is the length of the longest prefix of P which is a *proper* suffix of P_q

```
i:          1 2 3 4 5 6 7 8 9 10
P[i]:       a b a b a b a b c a
fail[i]:    0 0 1 2 3 4 5 6 0 1
```

Given this prefix function we can match efficiently – on a character match we increment the pointers, on a mismatch we slide the pattern one step plus the failure function.

Example

```
a b b b a b a b a b a c a b a b a b a
a b *
    *
      *
          a b a b a b a *
                        *
                          a b a b a b a
```

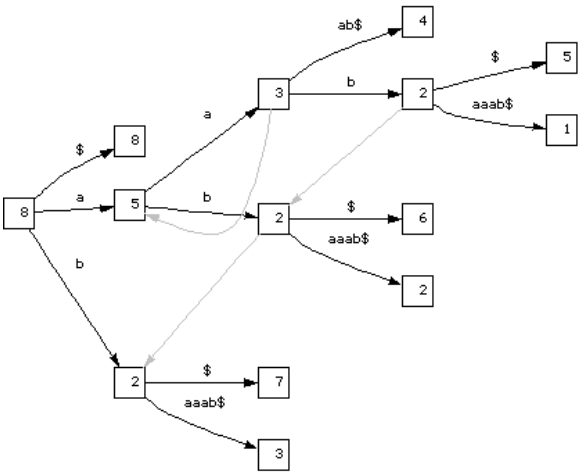
We match the pattern according to where we are in the text – we never look backwards in the text.

The failure function for the pattern can be constructed in $O(m)$ preprocessing, and hence does not change the asymptotic complexity.

Computing the Failure Function

The failure function can be constructed in linear time using suffix trees, which is more instructive than the standard simpler-to-program algorithm.

The failure function is defined by the match length between each leaf and the entire string (suffix 1).



string	a	a	b	a	a	a	b
match length	0	1	0	1	2	1	3
failure function	0	1	0	1	2	2	3

Each match applies over all *prefixes* of the match, so we take the maximum match length at each position.

This can be done in linear time by scanning the failure array backwards and update the values as $f[n] = max(f[n], f[n+1] - 1)$.

Time Complexity of KMP

If the pattern rejects near the beginning, we did not waste much time in the search.

If the pattern rejects near the end (e.g. EEEEEEEH in E^m, there is an opportunity to slide it back many positions.

Note that we never move backwards through the text to compare a character again – we slide the *pattern* forward accordingly.

There are "improved" failure functions which do even cleverer preprocessing to reduce the number of pattern shifts. However, such improvements have little effect in practice and are tricky to program correctly.

Amortized Analysis

The linearity of KMP follows from an *amortized* analysis. Each time we move forward in the text we add c steps to our account, while each mismatch costs us one step.

Provided the account never goes negative, we only did $O(n)$ work total since there were a total of $\leq cn$ steps put in the account.

Thus if many consecutive mismatches requires the pattern to shift repeatedly, it is only because we have enough previous forward moves to compensate.

The Boyer-Moore Algorithm

An alternate linear algorithm, *Boyer-Moore*, starts matching from right side of the pattern instead of the left side:

```
pattern:    ABCDABCD
text:       ABCDABCE....
```

In this example, the last character in the window does not occur anywhere in the pattern. Thus we can shift the pattern m positions after *one* comparison.

Thus the best case time for Boyer-Moore is *sub-linear*, i.e. $O(n/m)$. The worst-case is $O(n+rm)$, where r is the number of times the pattern occurs in the text.

Alphabet Fail

The algorithm precomputes a table of size $|\Sigma|$ describing how far to shift for a mismatch on each letter of the alphabet, i.e. depending upon the position of the rightmost occurrence of that letter in the pattern.

Further, since we know that the suffix of the pattern matched up to the point of mismatch, we can precompute a table recording the next place where the suffix occurs in the pattern. We can use the *bigger* of the two shifts, since no intermediate position can define a legal match.

Boyer-Moore *may* be the fastest algorithm for alphanumeric string matching in practice when implemented correctly.

Randomized String Matching

The *Rabin-Karp* algorithm computes an appropriate hash function on all m-length strings of the text, and does a brute force comparison only if the hash value is the same for the text window and the pattern.
An appropriate hash function is

$$H(S) = \sum_{i=1}^{m-1} d^i S_i \bmod q$$

which treats each string as an m-digit base-d number, mod q. This hash function can be computed *incrementally* in constant time as we slide the window from left to right since

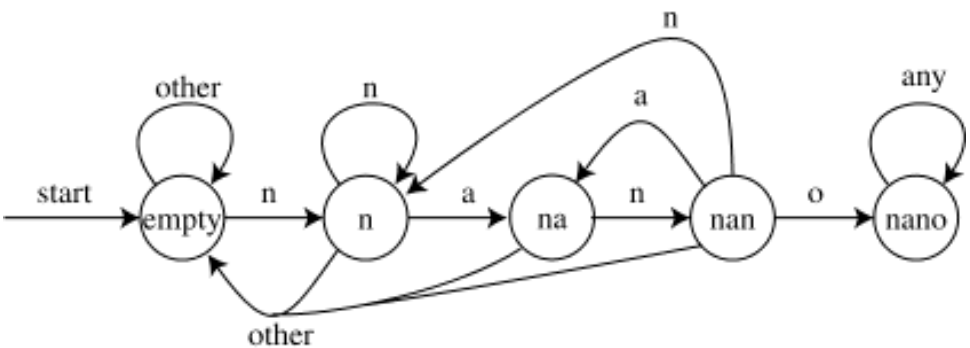
$$H(S_{j+1}) = dH(S_j) + S_{j+1} - d^m S_{j-m}$$

Randomized Analysis

Further, if q is a random prime the expected number of false positives is small enough to yield a *randomized* linear algorithm.

Multiple Exact Patterns

Many applications require searching a text for occurrences of any one of many patterns, e.g. searching text for dirty words or searching a genome for any one of a set of known motifs.
Pattern matching with *wild card* characters ($ACG?T$) is an important special case of multiple patterns.
Techniques from *automata theory* come into play, since any finite set of patterns can be modeled by *regular expressions*, and many interesting infinite sets (e.g. $G(AT) * C$) as well.
The standard UNIX tool *grep* stands for "general regular expression pattern matcher".



The Aho-Corasick algorithm builds a DFA from the set of patterns and then walks through the text in linear time, taking action when reaching any accepting state.

Approximate String Comparison

An important generalization of exact string matching is measuring the *distance* between two or more strings.

These problems arise naturally in biology because DNA / protein sequences tend to be structurally conserved across species over the course of evolution, so functionally similar genes in different organisms can be detected via approximate string matching.

Computer science applications of approximate string matching included spell checking and file difference testing.

A reasonable distance on strings measure minimizes the cost of the *changes* which have to be made to convert one string to another.

Edit Operations

There are three natural types of changes:

- *Substitution* – Change a single character from pattern s to a different character in text t, e.g. 'shot' to 'spot'.
- *Insertion* – Insert a single character into pattern s to help it match text t, e.g. 'ago' to 'agog'.
- *Deletion* – Delete a single character from pattern s to help it match text t, e.g. 'hour' to 'our'.

Edit Distance and Similarity Scores

Computer scientists usually measure *distance* between strings x and y by the minimum number of insertions, deletions, and substitutions to transform x to y.

Certain mathematical properties are expected of any distance measure, or *metric*:

1. $d(x, y) \geq 0$ for all x, y.
2. $d(x, y) = 0$ iff $x = y$.
3. $d(x, y) = d(y, x)$ (symmetry)
4. $d(x, y) \leq d(x, z) + d(z, y)$ for all x, y, and z. (triangle inequality)

Biologists typically instead measure a sequence *similarity score* which gets larger the more similar the sequences are. Similar algorithms can be used to optimize both measures.

Pairwise String Alignment

An elegant algorithm for finding the minimum cost sequence of changes to transform string S to string T is based on the observation that the correct action on the rightmost characters of S and T can be computed knowing the costs of matching various prefixes:

```
#define MATCH           0       /* symbol for match */
#define INSERT          1       /* symbol for insert */
#define DELETE          2       /* symbol for delete */

int string_compare(char *s, char *t, int i, int j)
{
        int k;                  /* counter */
        int opt[3];             /* cost of the three options */
        int lowest_cost;        /* lowest cost */

        if (i == 0) return(j * indel(' '));
        if (j == 0) return(i * indel(' '));

        opt[MATCH] = string_compare(s,t,i-1,j-1) + match(s[i],t[j]);
        opt[INSERT] = string_compare(s,t,i,j-1) + indel(t[j]);
        opt[DELETE] = string_compare(s,t,i-1,j) + indel(s[i]);

        lowest_cost = opt[MATCH];
        for (k=INSERT; k<=DELETE; k++)
                if (opt[k] < lowest_cost) lowest_cost = opt[k];

        return( lowest_cost );
}
```

Exponential!

How much time does this program take? Exponential in the length of the strings!

Making this tractible requires realizing that we are repeatedly performing the same computations on each pair of prefixes.

Dynamic Programming

The entire state of the recursive call is governed by the index positions into the strings. Thus there are only $|S| \times |T|$ different calls.

By storing the answers in a table and looking them up instead of recomputing, the algorithm takes quadratic time.

Dynamic programming is the algorithmic technique of efficiently computing recurrence relations by storing partial results. It is very powerful on any *ordered* structures, like character strings, permutations, and rooted trees.

DP Table

The table data structure keeps track of the cost of reaching this position plus the last move which took us to this cell.

```
typedef struct {
        int cost;               /* cost of reaching this cell */
        int parent;             /* parent cell */
} cell;

cell m[MAXLEN][MAXLEN];         /* dynamic programming table */
```

General Edit Distance via Dynamic Programming

Note how we use and update the table of partial results.

```
int string_compare(char *s, char *t)
{
        int i,j,k;              /* counters */
        int opt[3];             /* cost of the three options */

        for (i=0; i<MAXLEN; i++) {
           row_init(i);
           column_init(i);
        }

        for (i=1; i<strlen(s); i++)
           for (j=1; j<strlen(t); j++) {
                opt[MATCH] = m[i-1][j-1].cost + match(s[i],t[j]);
                opt[INSERT] = m[i][j-1].cost + indel(t[j]);
                opt[DELETE] = m[i-1][j].cost + indel(s[i]);

                m[i][j].cost = opt[1];
                m[i][j].parent = 1;
                for (k=2; k<=3; k++)
                        if (opt[k] < m[i][j].cost) {
                                m[i][j].cost = opt[k];
                                m[i][j].parent = k;
                        }
           }

        goal_cell(s,t,&i,&j);
        return( m[i][j].cost );
}
```

To determine the value of cell (i, j), we need the the cells $(i-1, j-1)$, $(i, j-1)$, and $(i-1, j)$. Any evaluation order with this property will do, including the row-major order we used.

Standard String Edit Distance

The function `string_compare` is very general, and must be customized to a particular application.

It uses problem-specific subroutines `match` and `indel` to return the costs of character pair transitions:

```
row_init(int i)
{
  m[0][i].cost = i;
  if (i>0)
    m[0][i].parent=INSERT;
  else
    m[0][i].parent = -1;
}
```

```
column_init(int i)
{
  m[i][0].cost = i;
  if (i>0)
    m[i][0].parent=DELETE;
  else
    m[0][i].parent = -1;
}
```

The functions `row_init` and `column_init` to initialize the boundary conditions.

```
int match(char c, char d)
{
  if (c == d) return(0);
  else return(1);
}
```

```
int indel(char c)
{
  return(1);
}
```

The function `goal_cell` returns the desired final cell of interest in the matrix.

```
goal_cell(char *s, char *t, int *i, int *j)
{
  *i = strlen(s) - 1;
  *j = strlen(t) - 1;
}
```

Changing these functions lets us do substring matching, longest common subsequence, and maximum monotone subsequence as special cases.

String Matching Example: Cost Matrix

The cost matrix in converting *thou shalt not* to *you should not*:

		y	o	u	-	s	h	o	u	l	d	-	n	o	t
:	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
t:	1	1	2	3	4	5	6	7	8	9	10	11	12	13	13
h:	2	2	2	3	4	5	5	6	7	8	9	10	11	12	13
o:	3	3	2	3	4	5	6	5	6	7	8	9	10	11	12
u:	4	4	3	2	3	4	5	6	5	6	7	8	9	10	11
-:	5	5	4	3	2	3	4	5	6	6	7	7	8	9	10
s:	6	6	5	4	3	2	3	4	5	6	7	8	8	9	10
h:	7	7	6	5	4	3	2	3	4	5	6	7	8	9	10
a:	8	8	7	6	5	4	3	3	4	5	6	7	8	9	10
l:	9	9	8	7	6	5	4	4	4	4	5	6	7	8	9
t:	10	10	9	8	7	6	5	5	5	5	5	6	7	8	8
-:	11	11	10	9	8	7	6	6	6	6	6	5	6	7	8
n:	12	12	11	10	9	8	7	7	7	7	7	6	5	6	7
o:	13	13	12	11	10	9	8	7	8	8	8	7	6	5	6
t:	14	14	13	12	11	10	9	8	8	9	9	8	7	6	5

String Matching Example: Parent Matrix

		y	o	u	-	s	h	o	u	l	d	-	n	o	t
:	-1	2	2	2	2	2	2	2	2	2	2	2	2	2	2
t:	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1
h:	3	1	1	1	1	1	1	2	2	2	2	2	2	2	2
o:	3	1	1	1	1	1	1	1	2	2	2	2	2	1	2
u:	3	1	3	1	2	2	2	2	1	2	2	2	2	2	2
-:	3	1	3	3	1	2	2	2	2	1	1	1	2	2	2
s:	3	1	3	3	3	1	2	2	2	2	1	1	1	1	1
h:	3	1	3	3	3	3	1	2	2	2	2	2	2	1	1
a:	3	1	3	3	3	3	3	1	1	1	1	1	1	1	1
l:	3	1	3	3	3	3	3	1	1	1	2	2	2	2	2
t:	3	1	3	3	3	3	3	1	1	1	1	1	1	1	1
-:	3	1	3	3	1	3	3	1	1	1	1	1	2	2	2
n:	3	1	3	3	3	3	3	1	1	1	1	3	1	2	2
o:	3	1	1	3	3	3	3	1	1	1	1	3	3	1	2
t:	3	1	3	3	3	3	3	3	1	1	1	3	3	3	1

Reconstructing the Alignment

Once we have the dynamic programming matrix, we have to walk backwards through it to reconstruct the alignment.

Either explicit back pointers can be kept, or we can remake decisions of how we got to the critical cells starting from the back.

```
reconstruct_path(char *s, char *t, int i, int j)
{
        if (m[i][j].parent == -1) return;

        if (m[i][j].parent == MATCH) {
                reconstruct_path(s,t,i-1,j-1);
                match_out(s, t, i, j);
                return;
        }
        if (m[i][j].parent == INSERT) {
                reconstruct_path(s,t,i,j-1);
                insert_out(t,j);
                return;
        }
        if (m[i][j].parent == DELETE) {
                reconstruct_path(s,t,i-1,j);
                delete_out(s,i);
                return;
        }
}
```

The actions we take on traceback are governed by `match_out`, `insert_out`, and `delete_out`:

```
insert_out(char *t, int j)
{
        printf("I");
}

delete_out(char *s, int i)
{
        printf("D");
}
```

```
match_out(char *s,char *t,int i,int j)
{
        if (s[i] == t[j]) printf("M");
        else printf("S");
}
```

The edit sequence from “thou-shalt-not” to “you-should-not” is `DSMMMMMISMSMMMM` – meaning delete the first ‘t’, replace the ‘h’ with ‘y’, match the next five characters before inserting an ‘o’, replace ‘a’ with ’u’, replace the ‘t’ with a ‘d’.

Other Applications: Longest Common Subsequence

By changing the relative costs for matching and substitution we can get different alignments.

If we make the cost of substitution so high as to be greater than the cost of inserting and deleting characters, the returned alignment will seek only to match the largest number of possible characters, returning the *longest common subsequence*.

```
int match(char c, char d)
{
        if (c == d) return(0);
        else return(MAXLEN);
}
```

This shows us that the exact replacement costs can have a big impact on the optimal alignment.

By changing our traceback functions we get different behavior:

```
insert_out(char *t,int j)
{
}

delete_out(char *s,int i)
{
}
```

```
match_out(char *s,char *t,
          int i,int j)
{
   if (s[i]==t[j])
       printf("%c",s[i]);
}
```

```
abracadabra
abbababa
length of longest common subsequence = 6
abaaba
```

Substring Matching

Suppose we want to search for a short pattern (say ‘Skiena’ allowing mispellings) in a long text.

Using the approximate string matching function will achieve little sensitivity, since most of the edit cost will be deleting the body of the full text.

We can use the same basic edit distance function for this task, but must adjust the initialization so that the substring matches in the middle of the text are not discouraged:

```
row_init(int i)        /* what is m[0][i]? */
{
       m[0][i].cost = 0;              /* NOTE CHANGE */
       m[0][i].parent = -1;           /* NOTE CHANGE */
}
```

Now the goal cell is the cheapest cell matching the entire pattern:

```
goal_cell(char *s, char *t, int *i, int *j)
{
     int k;                  /* counter */

     *i = strlen(s) - 1;
     *j = 0;

     for (k=1; k<strlen(t); k++)
        if (m[*i][k].cost < m[*i][*j].cost) *j = k;
}
```

Biological Sequence Comparison

Constructing a meaningful alignment of two sequences requires using a appropriate function to measure the cost of changing between each possible pair symbols.

In correcting text entered by a fast typist, we might penalize pairs of symbols near each other on the keyboard less than those on different sides, for example.

For genomic sequences, the weights/scores governing the cost of changing between bases are given by *PAM matrices* for “point accepted mutations”

DNA Matricies

DNA PAM matrices include *Blast similarity* and *transition / transversion* matrices.

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

	A	T	C	G
A	0	5	5	1
T	5	0	1	5
C	5	1	0	5
G	1	5	5	0

The four nucleotide bases are classified as either *purines* (Adenine and Guanine) or *pyrimidines* (Cytosine and Thymine).

Transitions are mutations which stay within the class (e.g. A→G or C→T), while *transversions* cross classes (e.g. A→C, A→T etc.).

Transitions are more common than transversions.

Genetic Code Matrix

The following *genetic code matrix* calculates the minimum number of DNA base changes to go from a codon for i to a codon for j.

Met→Tyr requires all 3 positions to change.

	A	S	G	L	K	V	T	P	E	D	N	I	Q	R	F	Y	C	H	M	W	Z	B	X
A	0	1	1	2	2	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2
S	1	0	1	1	2	2	1	1	2	2	1	1	2	1	1	1	1	2	2	1	2	2	2
G	1	1	0	2	2	1	2	2	1	1	2	2	2	1	2	2	1	2	2	1	2	2	2
L	2	1	2	0	2	1	2	1	2	2	2	1	1	1	1	2	2	1	1	1	2	2	2
K	2	2	2	2	0	2	1	2	1	2	1	1	1	1	2	2	2	2	1	2	1	2	2
V	1	2	1	1	2	0	2	2	1	1	2	1	2	2	1	2	2	2	1	2	2	2	2
T	1	1	2	2	1	2	0	1	2	2	1	1	2	1	2	2	2	2	1	2	2	2	2
P	1	1	2	1	2	2	1	0	2	2	2	2	1	1	2	2	2	1	2	2	2	2	2
E	1	2	1	2	1	1	2	2	0	1	2	2	1	2	2	2	2	2	2	2	1	2	2
D	1	2	1	2	2	1	2	2	1	0	1	2	2	2	2	1	2	1	2	2	2	1	2
N	2	1	2	2	1	2	1	2	2	1	0	1	2	2	2	1	2	1	2	2	2	1	2
I	2	1	2	1	1	1	1	2	2	2	1	0	2	1	1	2	2	2	1	2	2	2	2
Q	2	2	2	1	1	2	2	1	1	2	2	2	0	1	2	2	2	1	2	2	1	2	2
R	2	1	1	1	1	2	1	1	2	2	2	1	1	0	2	2	1	1	1	1	2	2	2
F	2	1	2	1	2	1	2	2	2	2	2	1	2	2	0	1	1	2	2	2	2	2	2
Y	2	1	2	2	2	2	2	2	2	1	1	2	2	2	1	0	1	1	3	2	2	1	2
C	2	1	1	2	2	2	2	2	2	2	2	2	2	1	1	1	0	2	2	1	2	2	2
H	2	2	2	1	2	2	2	1	2	1	1	2	1	1	2	1	2	0	2	2	2	1	2
M	2	2	2	1	1	1	1	2	2	2	2	1	2	1	2	3	2	2	0	2	2	2	2
W	2	1	1	1	2	2	2	2	2	2	2	2	2	1	2	2	1	2	2	0	2	2	2
Z	2	2	2	2	1	2	2	2	1	2	2	2	1	2	2	2	2	2	2	2	1	2	2
B	2	2	2	2	2	2	2	2	2	1	1	2	2	2	2	1	2	1	2	2	2	1	2
X	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2

Hydrophobicity Matrix

Amino acids differ to the extent that they like (*hydrophilic*) or don't like (*hydrophobic*) water.

Hydrophobic residues do not want to be on the surface of a protein.

	R	K	D	E	B	Z	S	N	Q	G	X	T	H	A	C	M	P	V	L	I	Y	F	W
R	10	10	9	9	8	8	6	6	6	5	5	5	5	5	4	3	3	3	3	3	2	1	0
K	10	10	9	9	8	8	6	6	6	5	5	5	5	5	4	3	3	3	3	3	2	1	0
D	9	9	10	10	8	8	7	6	6	6	5	5	5	5	5	4	4	4	3	3	3	2	1
E	9	9	10	10	8	8	7	6	6	6	5	5	5	5	5	4	4	4	3	3	3	2	1
B	8	8	8	8	10	10	8	8	8	8	7	7	7	7	6	6	6	5	5	5	4	4	3
Z	8	8	8	8	10	10	8	8	8	8	7	7	7	7	6	6	6	5	5	5	4	4	3
S	6	6	7	7	8	8	10	10	10	10	9	9	9	9	8	8	7	7	7	7	6	6	4
N	6	6	6	6	8	8	10	10	10	10	9	9	9	9	8	8	8	7	7	7	6	6	4
Q	6	6	6	6	8	8	10	10	10	10	9	9	9	9	8	8	8	7	7	7	6	6	4
G	5	5	6	6	8	8	10	10	10	10	9	9	9	9	8	8	8	8	7	7	6	6	5
X	5	5	5	5	7	7	9	9	9	9	10	10	10	10	9	9	8	8	8	8	7	7	5
T	5	5	5	5	7	7	9	9	9	9	10	10	10	10	9	9	8	8	8	8	7	7	5
H	5	5	5	5	7	7	9	9	9	9	10	10	10	10	9	9	9	8	8	8	7	7	5
A	5	5	5	5	7	7	9	9	9	9	10	10	10	10	9	9	9	8	8	8	7	7	5
C	4	4	5	5	6	6	8	8	8	8	9	9	9	9	10	10	9	9	9	9	8	8	5
M	3	3	4	4	6	6	8	8	8	8	9	9	9	9	10	10	10	10	9	9	8	8	7
P	3	3	4	4	6	6	7	8	8	8	8	8	9	9	9	10	10	10	9	9	9	8	7
V	3	3	4	4	5	5	7	7	7	8	8	8	8	8	9	10	10	10	10	10	9	8	7
L	3	3	3	3	5	5	7	7	7	7	8	8	8	8	9	9	9	10	10	10	9	9	8
I	3	3	3	3	5	5	7	7	7	7	8	8	8	8	9	9	9	10	10	10	9	9	8
Y	2	2	3	3	4	4	6	6	6	6	7	7	7	7	8	8	9	9	9	9	10	10	8
F	1	1	2	2	4	4	6	6	6	6	7	7	7	7	8	8	8	8	9	9	10	10	9
W	0	0	1	1	3	3	4	4	4	5	5	5	5	5	6	7	7	7	8	8	8	9	10

PAM Matrices

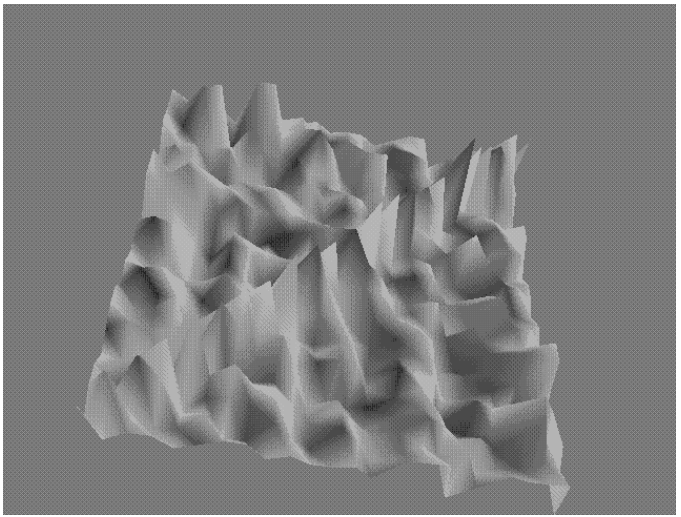
This variety of possible matrix criteria make judging the significance of an alignment tricky.

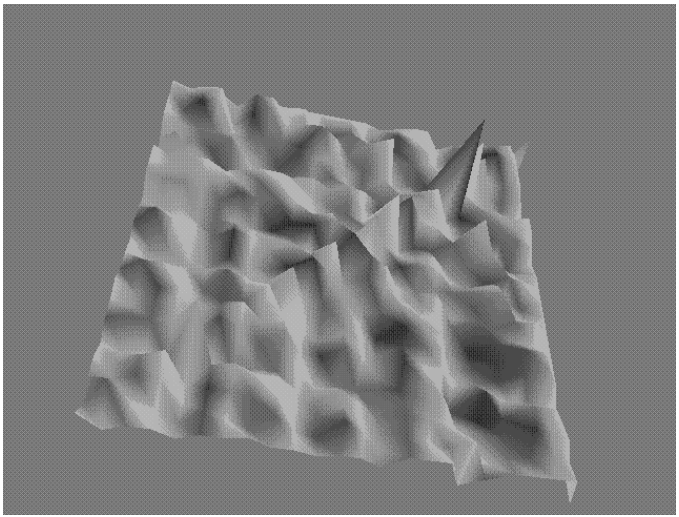
Most widely used are *PAM matrices*, for “point accepted mutations”.

These were constructed by aligning very similar proteins, and tabulating how often each substitution occurred.

The PAM1 matrix scores the transitions when the proteins differ in 1% of the residues.

Raising this to higher powers gives us PAM matrices suited for comparing more distantly related proteins.





Note the main diagonal on these plots of the PAM50 and PAM250 matrices.

More modern than the PAM matrices are the *Blosum* matrices, reflecting additional sequence alignment data.

Local Alignment

The critical problem of biological interest is in comparing two long sequences and finding *local* areas of similarity.

Typical applications include (1) what regions have been conserved between mouse and human, and (2) recognizing coding regions in 'split genes'.

Here using similarity scores is more meaningful than edit distance. We want $d(i, j)$ to be the highest-scoring local alignment ending at $S[i]$ and $T[j]$.

This way to compute this is typically called the *Smith-Waterman* algorithm.

Edit Distance vs. Smith-Waterman

It is the same basic algorithm as for edit distance, except:

- We maximize instead of minimize.
- We have the option of starting our local alignment fresh at each cell in the matrix, i.e. 0 is always an allowable cost.
- We scan all mn cells at the end to see which gives us the best score, not just those along the last row or column.

Smith-Waterman Program

```
int smith_waterman(char *s, char *t)
{
    int i,j,k;              /* counters */
    int opt[3];             /* cost of the three options */
    int match(); int indel();

    for (i=0; i<=strlen(s); i++)
        for (j=0; j<=strlen(t); j++)
            cell_init(i,j);

    for (i=1; i<strlen(s); i++)
      for (j=1; j<strlen(t); j++) {
         opt[MATCH] = m[i-1][j-1].cost + match(s[i],t[j]);
         opt[INSERT] = m[i][j-1].cost + indel(t[j]);
         opt[DELETE] = m[i-1][j].cost + indel(s[i]);

         m[i][j].cost = 0;
         m[i][j].parent = -1;
         for (k=MATCH; k<=DELETE; k++)
            if (opt[k] > m[i][j].cost) {
                   m[i][j].cost = opt[k];
                   m[i][j].parent = k;
            }
      }

    goal_cell(s,t,&i,&j);
    return( m[i][j].cost );
}
```

Supporting Routines

```
goal_cell(char *s, char *t, int *x, int *y)
{
    int i,j;                        /* counters */

    *x = *y = 0;

    for (i=0; i<strlen(s); i++)
            for (j=0; j<strlen(t); j++)
                if (m[i][j].cost > m[*x][*y].cost) {
                        *x = i;
                        *y = j;
                }
}

int match(char c, char d)
{
        if (c == d) return(+5);
        else return(-4);
}

int indel(char c)
{
        return(-4);
}

cell_init(int i, int j)
{
        m[i][j].cost = 0;
        m[i][j].parent = -1;
}
```

Smith-Waterman Example

Note how the maximum score (31) is not achieved with the exact match `public` but includes the preceeding ‘e’.
This is because we score a match more than we penalize a mismatch/indel.

		b	e	a	t	_	r	e	p	u	b	l	i	c	a	n	s
:	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
f:	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
o:	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
r:	0	0	0	0	0	0	5	1	0	0	0	0	0	0	0	0	0
_:	0	0	0	0	0	5	1	1	0	0	0	0	0	0	0	0	0
t:	0	0	0	0	5	1	1	0	0	0	0	0	0	0	0	0	0
h:	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
e:	0	0	5	1	0	0	0	5	1	0	0	0	0	0	0	0	0
_:	0	0	1	1	0	5	1	1	1	0	0	0	0	0	0	0	0
p:	0	0	0	0	0	1	1	0	6	2	0	0	0	0	0	0	0
u:	0	0	0	0	0	0	0	0	2	11	7	3	0	0	0	0	0
b:	0	5	1	0	0	0	0	0	0	7	16	12	8	4	0	0	0
l:	0	1	1	0	0	0	0	0	0	3	12	21	17	13	9	5	1
i:	0	0	0	0	0	0	0	0	0	0	8	17	26	22	18	14	10
c:	0	0	0	0	0	0	0	0	0	0	4	13	22	31	27	23	19
_:	0	0	0	0	0	5	1	0	0	0	0	9	18	27	27	23	19
g:	0	0	0	0	0	1	1	0	0	0	0	5	14	23	23	23	19
o:	0	0	0	0	0	0	0	0	0	0	0	1	10	19	19	19	19
o:	0	0	0	0	0	0	0	0	0	0	0	0	6	15	15	15	15
d:	0	0	0	0	0	0	0	0	0	0	0	0	2	11	11	11	11

Gap Penalties

Gaps in sequence alignments occur for several reasons, including (1) the deletion of introns from split genes, (2) the insertion of mobile sequence elements such as transposons, (3) because conserved parts of a protein may include several relatively small docking sites.

Gap in alignments can be modeled as repeated base deletions, where the penalty for a gap is just a linear function of its length.

However, in many applications (e.g. exons/introns, extracting good quotes for the back of a book jacket) the length of the gap is relatively unimportant.

Affine Gap Costs

Thus a more meaningful model charges a fixed penalty for the existence of each gap, plus another penalty depending upon the length of the gap.

Affine gap penalties are $A + Bt$ for gaps of length t, while *logarithmic* gap penalties of the form $A + B \lg t$ are suggested by empirical data.

Arbitrary Gap Weights

Suppose the gap cost is a completely general function of length for which we cannot assume monotonicity or any other property.

Then we must explicitly try every possible length deletion/insertion at every possible position, i.e. $V(i,j)$ takes the best of the following options:

$$G(i,j) = V(i-1,j-1) + match(i,j)$$

$$E(i,j) = \max_{k=0}^{j-1} V(i,k) + indel(j-k)$$

$$F(i,j) = \max_{k=0}^{i-1} V(k,j) + indel(i-k)$$

Because we are doing a linear amount of work for each cell, the time complexity goes to $O(n^2m + nm^2)$ or $O(n^3)$ if $n < m$.
This algorithm is often called Needleman-Wunsch.

Affine Gap Weights

By being clever we can avoid the extra linear cost of looking for the start of the gap for *affine* gap penalties, i.e. penalties of the form $A + Bt$ for gaps of length t

We will use the insertion and deletion recurrences E and F to encode the cost of being in gap mode, meaning we have already paid the cost of initiating the gap.

$$V(i,j) = \max(E(i,j), F(i,j), G(i,j))$$

$$G(i,j) = V(i-1, j-1) + match(i,j)$$

$$E(i,j) = \max(E(i,j-1), V(i,j-1) - A) - B$$

$$F(i,j) = \max(F(i-1,j), V(i-1,j) - A) - B$$

With constant amount of work per cell, this algorithm takes $O(mn)$ time, same as without gap costs.

The default in FASTA sets $A = 10$ and $B = 2$, so starting a gap costs the equivalent of five deletions.

The special case of *convex* penalty functions (including logarithmic costs) can be solved in $O(nm \log mn)$ time with a more complicated algorithm.

Space Efficient Dynamic Programming

Quadratic space will kill you faster than quadratic time. $(30,000)^2$ bytes equals one gigabyte, while $(30,000)^2$ operations takes 1000 seconds on a machine doing 1 million steps per second.

The dynamic programming algorithms we have seen only look at neighboring rows/columns to make their decision.

Computing the *highest cell score* in the matrix does not require keeping more than then last column and the best value to date, for a total of $O(n)$ space, where $n \leq m$.

Note that reconstructing the optimal *alignment* does seem to require keeping the entire matrix, however.

But Hirshberg found a clever way to reconstruct the align-

ment in $O(nm)$ time using only $O(n)$ space, by recomputing the appropriate portions of the matrix.

For each cell, we drag along the row number where the optimal path to in crossed the middle ($m/2$nd) column.

This requires only $O(n)$ extra memory, one cell per row.

This works because the crossing point k of the $(m/2)$nd column means that the optimal alignment lies in the sub matrices A from (1,1) to $(m/2, k)$, and B from $(m/2, k)$ to (m, n).

Note that the number of cells in A and B totals only half of the the original mn cells.

Further, these dynamic programming algorithms are linear in the number of cells they compute.

Thus the total amount of recomputation done is

$$\sum_{i=0}^{\lg m} mn/2^i = 2mn$$

so the total work remains $O(mn)$

Heuristic String Comparison

Dynamic programming methods for sequence alignment give the highest quality results.

However, quadratic $O(nm)$ algorithms are only feasible for comparing two modest sized sequences.

Comparing the human genome against mouse with a quadratic algorithm ($3,000,000,000^2$ operations) at a billion operations per second equals 285 years!

Comparing your target sequence against the entire database is hopeless, even with special purpose hardware.

Heuristic algorithms (BLAST/FASTA) are used for an initial scan of the database to find a small number of hits, and then Smith-Waterman finds the optimal alignment.

FASTA

FASTA is a heuristic string alignment program which is based upon finding short exact matches (k-mers) between the query sequence and the database.

The trick is to choose k large enough that there are relatively few hits, but small enough that we are likely to have an exact k-mer match between related sequences.

Recommended values of k are 2 for protein sequences and 6 for DNA sequences.

Note that there are at most $n - k + 1$ distinct k-mers in a sequence of length n.

A *hash table* of all k-mers in the database can be built once and used repeatedly for efficiently looking up the k-mers in query strings.
A dynamic programming-like algorithm is used to align the k-mer hits between query and database, but the problem is much smaller since there are far fewer hits than bases.

BLAST

BLAST stands for “Basic Local Alignment Search Tool”.
BLAST also breaks the query into k-mers in order to search a hash table, but for input k-mer q constructs *all* other k-mers which lie within a distance t of q.
By processing all these patterns into an automata, BLAST can then make one linear-time pass through the database to find all exact matches and group them in an alignment.
The window size k is typically 3-5 for protein sequences and 12 for DNA sequences.
Conventional wisdom has BLAST as faster than FASTA, but perhaps a little less accurate.

Using Blast

Several variants of the *Basic Local Alignment Search Tool* are available at www.ncbi.nlm.nih.gov/BLAST/

- *blastp* – amino acid query sequence to protein sequence database
- *blastn* – nucleotide query sequence to nucleotide sequence database
- *blastx* – nucleotide query sequence translated in all reading frames against a protein sequence database
- *tblastn* – protein query sequence against nucleotide sequence database translated in all reading frames

- *tblastx* – most intensive computationally; compares 6 frame translations of nucleotides query sequence against 6 frame translation of nucleotide sequence database

You can download your own local copy of the code and databases, or use web resources.

Databases

Database choices include:

- *nr* – all non-redundant sequences (from particular databases)
- *est* – expressed-sequence tags (RNA from expressed genes) in human, mouse, etc.
- *month* – new releases from the past 30 days
- *genomes* – from Drosophila, yeast, E.coli, human, etc.

Your query sequence can be (1) an amino acid or nucleotide sequence you type or paste in, or (2) the accession or GI number of Genbank entry.
There are a wide range of output formats.

Advanced Search Parameters

You can select the organism you are interested in to limit your search.

You can change the *expect value* E, the threshold for reporting matches against a database sequence. The default threshold of 10 means that 10 matches are expected to be found merely by chance (Karlin and Altschul). Lower expect thresholds are more stringent, leading to fewer chance matches being reported.

The expect value decreases exponentially with the alignment score S.

You can use *filter* to masks sequences of low compositional complexity, i.e. eliminate statistically significant but biologi-

cally uninteresting reports.

You can select the cost comparison matrix. The default is BLOSUM62, but with this matrix fairly long alignments are requires to rise above background. PAM matrices are recommends if search for short alignments.

Significance Scores

To assess whether a given alignment constitutes evidence for homology, it helps to know how strong an alignment can be expected from chance alone.

A model for expected number of *high-scoring segment pairs* (HSPs) with score at least S is

$$E = Kmne^{-\lambda S}$$

where m and n are the sequence lengths and K and λ are scaling parameters.

Doubling length of either sequence doubles E, as it should.

Doubling the score for an HSP to $2x$ requires it to attain the score x twice in a row, so E should decrease exponentially with score.

The number of random HSPs with score $\geq S$ is described by a Poisson distribution, so the probability of finding exactly a HSPs with score $\geq S$ is given by $e^{-E}E^a/a!$.

Hence the probability of finding 0 HSPs is e^{-E}, and the probability of finding at least one is $p = 1 - e^{-E}$. This is the *p-value* of the score.

A statistical method to measure significance is to generate many random sequence pairs of the appropriate length and composition, and calculate the optimal alignment score for each.