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## Local Alignment

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## Local Alignment

- I. Insert Outline Here
  - A. The way you regularly would....
  - B.

**Instructor's Notes for**  
**Local Alignment**

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# A More Natural Treatment of Gaps

It is a biological reality that mutations that add or delete nucleotides to the genome are less likely than simple mutations that change the nucleotide. This is why gaps are penalized more than point mutations.

|            |
|------------|
| ACAGCAGTAT |
| A--G--G-A- |

Furthermore, it is a biological reality that the presence of a gap is more significant than the actual length of the gap.

|             |
|-------------|
| ACAGCAGTAT  |
| --AGGA----- |

To reflect this, we charge a lot to "start" a gap and less to "continue" the gap.

# Affine Gap Penalties

So consider this gap penalty function:

The first gap in a contiguous string of gaps costs -3, and each additional gap in that string costs -1.

What is the score of each of these alignments:

|                          |                           |
|--------------------------|---------------------------|
| ACAGCAGTAT<br>A--G--G-A- | ACAGCAGTAT<br>--AGGA----- |
|--------------------------|---------------------------|

Notice how this notion gives preference to the more natural alignment on the right, similar to the way our semi-global alignment (no charge for end gaps) preferred more natural alignments.

# Optimal Affine Gap Penalty Alignment

Let's align ACAGCAGTAT and AGGA under the system:

Match: +1  
Mismatch: -1  
Start gap: -3  
Continue gap: -1

How did we do this with the regular gap penalty?  
What could our last column look like?

Either  $\begin{bmatrix} T \\ A \end{bmatrix}$ ,  $\begin{bmatrix} T \\ - \end{bmatrix}$  or  $\begin{bmatrix} - \\ A \end{bmatrix}$ .

What optimal alignments would you like to know?

|           |           |            |
|-----------|-----------|------------|
| ACAGCAGTA | ACAGCAGTA | ACAGCAGTAT |
| AGG       | AGGA      | AGG        |
| score: -6 | score: -4 | score: -7  |

What is the optimal cost?

# We Need to keep More Information

Align ACAGCAGTAT and AGGA

Match: +1

Mismatch: -1

Start gap: -3

Continue gap: -1

The last column is either  $\begin{bmatrix} T \\ A \end{bmatrix}$ ,  $\begin{bmatrix} T \\ - \end{bmatrix}$  or  $\begin{bmatrix} - \\ A \end{bmatrix}$ .

Here are the optimal alignments of some pairs of prefixes with each of *their* possible last columns:

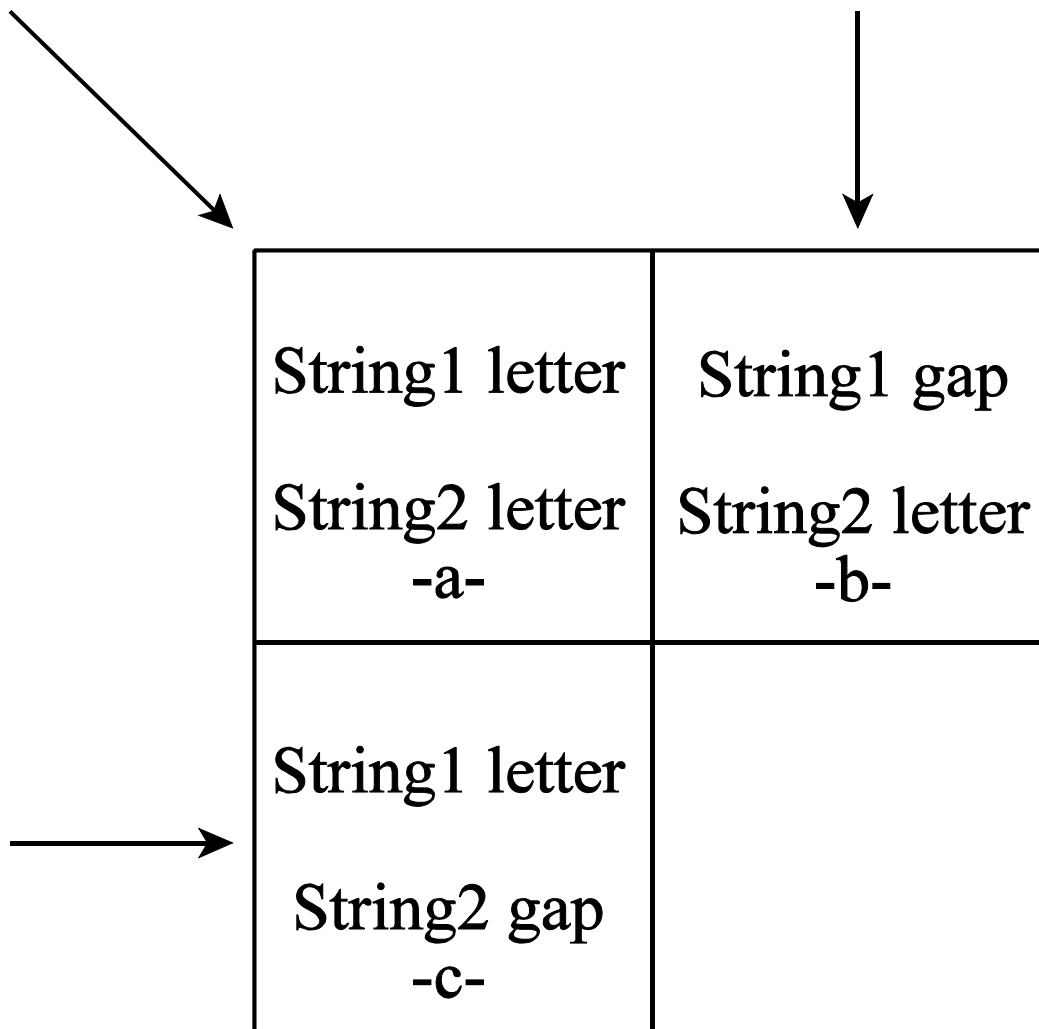
| ACAGCAGTA<br>AGG |    |    | ACAGCAGTA<br>AGGA |    |    | ACAGCAGTAT<br>AGG |    |     |
|------------------|----|----|-------------------|----|----|-------------------|----|-----|
| A                | A  | -  | A                 | A  | -  | T                 | T  | -   |
| G                | -  | G  | A                 | -  | A  | G                 | -  | G   |
| -7               | -6 | -9 | -4                | -5 | -8 | -8                | -7 | -10 |

Now can you tell me the optimal alignment?

# A More Interesting Matrix

For the optimal alignment of each pair of prefixes, we need to keep track of its optimal alignment for each possible last column.

Each entry of the matrix becomes three entries, one for each direction into that cell.



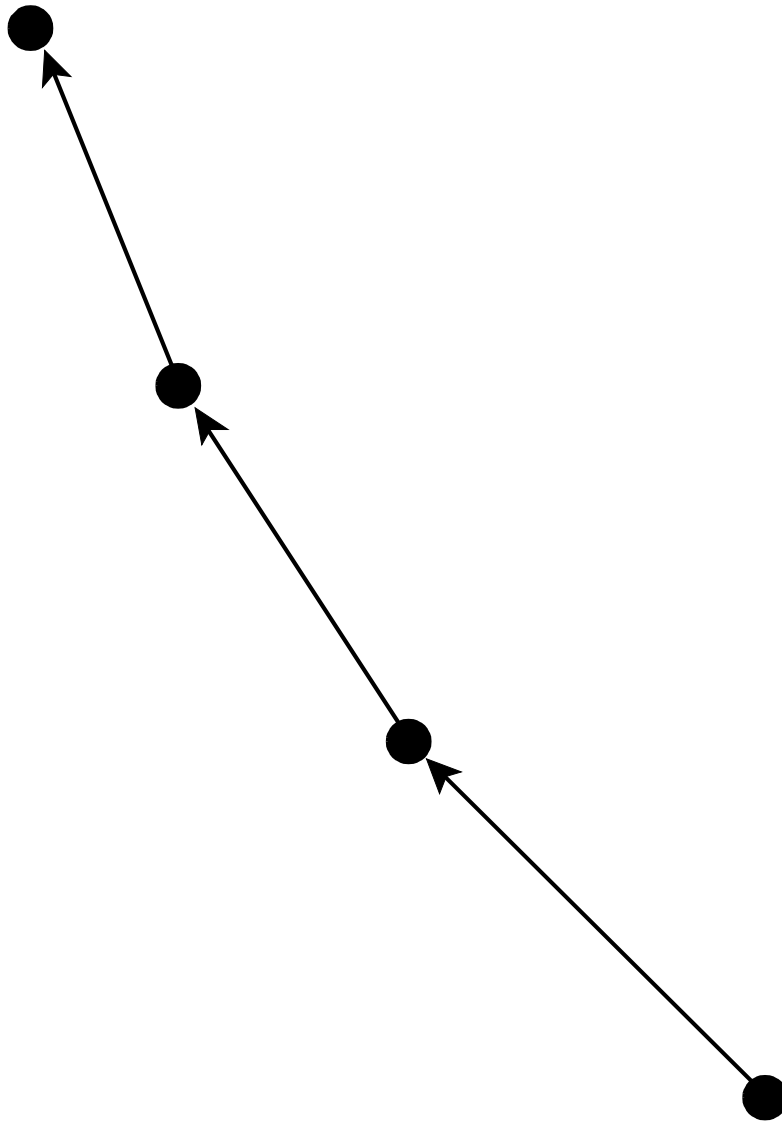
# A Simple Affine Gap Alignment

|   |  | A | C |
|---|--|---|---|
|   |  |   |   |
| G |  |   |   |
| A |  |   |   |
| T |  |   |   |

# A Simple Affine Gap Alignment

|     |  |     | A   |     | C   |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
|-----|--|-----|---|-----|-----|-----|--|-----|--|-----|-----|-----|--|-----|--|-----|-----|----|--|
|     |  |     | <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>0</td><td>-99</td></tr><tr><td>-99</td><td></td></tr></table> | 0   | -99 | -99 |  | a-- | <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-99</td><td>-99</td></tr><tr><td>-3</td><td></td></tr></table> | -99 | -99 | -3  |  | --c | <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-99</td><td>-99</td></tr><tr><td>-4</td><td></td></tr></table> | -99 | -99 | -4 |  |
| 0   | -99  |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| -99 |  |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| -99 | -99  |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| -3  |  |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| -99 | -99  |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| -4  |  |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
|     | a--  | a-- | --c   | --c | --c |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| G   | <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-99</td><td>-3</td></tr><tr><td>-99</td><td></td></tr></table> | -99 | -3  | -99 |     | -b- | <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-1</td><td>-6</td></tr><tr><td>-6</td><td></td></tr></table> | -1  | -6   | -6  |     | a-- | <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-4</td><td>-7</td></tr><tr><td>-4</td><td></td></tr></table> | -4  | -7   | -4  |     |    |  |
| -99 | -3   |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| -99 |  |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| -1  | -6   |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| -6  |  |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| -4  | -7   |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| -4  |  |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
|     | -b-  | -b- | a--   | a-- | a-c |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| A   | <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-99</td><td>-4</td></tr><tr><td>-99</td><td></td></tr></table> | -99 | -4  | -99 |     | -b- | <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-2</td><td>-4</td></tr><tr><td>-7</td><td></td></tr></table> | -2  | -4   | -7  |     | a-- | <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-2</td><td>-7</td></tr><tr><td>-5</td><td></td></tr></table> | -2  | -7   | -5  |     |    |  |
| -99 | -4   |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| -99 |  |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| -2  | -4   |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| -7  |  |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| -2  | -7   |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| -5  |  |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
|     | -b-  | -b- | ab-   | a-- | a-- |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| T   | <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-99</td><td>-5</td></tr><tr><td>-99</td><td></td></tr></table> | -99 | -5  | -99 |     | -b- | <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-5</td><td>-5</td></tr><tr><td>-8</td><td></td></tr></table> | -5  | -5   | -8  |     | ab- | <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-3</td><td>-5</td></tr><tr><td>-8</td><td></td></tr></table> | -3  | -5   | -8  |     |    |  |
| -99 | -5   |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| -99 |  |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| -5  | -5   |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| -8  |  |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| -3  | -5   |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |
| -8  |  |     |   |     |     |     |  |     |  |     |     |     |  |     |  |     |     |    |  |

# Tracing Back



# Slightly More Interesting

|     |   | A   | T   | G   |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
|-----|---|---|-----|-----|-----|--|---|-----|-----|---|--|---|-----|-----|---|--|---|-----|-----|---|--|
|     |   | <table border="1"><tr><td>0</td><td>-99</td></tr><tr><td>-99</td><td></td></tr></table> | 0   | -99 | -99 |  | <table border="1"><tr><td>-99</td><td>-99</td></tr><tr><td>0</td><td></td></tr></table> | -99 | -99 | 0 |  | <table border="1"><tr><td>-99</td><td>-99</td></tr><tr><td>0</td><td></td></tr></table> | -99 | -99 | 0 |  | <table border="1"><tr><td>-99</td><td>-99</td></tr><tr><td>0</td><td></td></tr></table> | -99 | -99 | 0 |  |
| 0   | -99   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -99 |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -99 | -99   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| 0   |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -99 | -99   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| 0   |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -99 | -99   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| 0   |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
|     | a--   | a--   | --c | --c | --c | --c  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| C   | <table border="1"><tr><td>-99</td><td>0</td></tr><tr><td>-99</td><td></td></tr></table> | -99   | 0   | -99 |     | <table border="1"><tr><td>-1</td><td>-3</td></tr><tr><td>-3</td><td></td></tr></table> | -1  | -3  | -3  |   | <table border="1"><tr><td>-1</td><td>-3</td></tr><tr><td>-4</td><td></td></tr></table> | -1  | -3  | -4  |   | <table border="1"><tr><td>-1</td><td>-3</td></tr><tr><td>-4</td><td></td></tr></table> | -1  | -3  | -4  |   |  |
| -99 | 0   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -99 |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -1  | -3  |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -3  |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -1  | -3  |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -4  |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -1  | -3  |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -4  |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
|     | -b-   | -b-   | ab- | a-- | ab- | a--  | ab-   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| T   | <table border="1"><tr><td>-99</td><td>0</td></tr><tr><td>-99</td><td></td></tr></table> | -99   | 0   | -99 |     | <table border="1"><tr><td>-1</td><td>-4</td></tr><tr><td>-3</td><td></td></tr></table> | -1  | -4  | -3  |   | <table border="1"><tr><td>0</td><td>-4</td></tr><tr><td>-4</td><td></td></tr></table>  | 0   | -4  | -4  |   | <table border="1"><tr><td>-2</td><td>-4</td></tr><tr><td>-3</td><td></td></tr></table> | -2  | -4  | -3  |   |  |
| -99 | 0   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -99 |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -1  | -4  |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -3  |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| 0   | -4  |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -4  |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -2  | -4  |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -3  |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
|     | -b-   | -b-   | a-- | a-- | a-- | a--  | ab-   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| T   | <table border="1"><tr><td>-99</td><td>0</td></tr><tr><td>-99</td><td></td></tr></table> | -99   | 0   | -99 |     | <table border="1"><tr><td>-1</td><td>-4</td></tr><tr><td>-3</td><td></td></tr></table> | -1  | -4  | -3  |   | <table border="1"><tr><td>0</td><td>-3</td></tr><tr><td>-4</td><td></td></tr></table>  | 0   | -3  | -4  |   | <table border="1"><tr><td>-1</td><td>-5</td></tr><tr><td>-3</td><td></td></tr></table> | -1  | -5  | -3  |   |  |
| -99 | 0   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -99 |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -1  | -4  |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -3  |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| 0   | -3  |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -4  |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -1  | -5  |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -3  |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
|     | -b-   | -b-   | a-- | a-- | a-- | a--  | a--   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| A   | <table border="1"><tr><td>-99</td><td>0</td></tr><tr><td>-99</td><td></td></tr></table> | -99   | 0   | -99 |     | <table border="1"><tr><td>1</td><td>-4</td></tr><tr><td>-3</td><td></td></tr></table>  | 1   | -4  | -3  |   | <table border="1"><tr><td>-2</td><td>-3</td></tr><tr><td>-2</td><td></td></tr></table> | -2  | -3  | -2  |   | <table border="1"><tr><td>-1</td><td>-4</td></tr><tr><td>-3</td><td></td></tr></table> | -1  | -4  | -3  |   |  |
| -99 | 0   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -99 |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| 1   | -4  |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -3  |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -2  | -3  |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -2  |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -1  | -4  |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -3  |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
|     | -b-   | -b-   | a-- | a-- | -b- | a-c  | a--   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| G   | <table border="1"><tr><td>-99</td><td>0</td></tr><tr><td>-99</td><td></td></tr></table> | -99   | 0   | -99 |     | <table border="1"><tr><td>-1</td><td>-2</td></tr><tr><td>-3</td><td></td></tr></table> | -1  | -2  | -3  |   | <table border="1"><tr><td>0</td><td>-4</td></tr><tr><td>-4</td><td></td></tr></table>  | 0   | -4  | -4  |   | <table border="1"><tr><td>-1</td><td>-4</td></tr><tr><td>-3</td><td></td></tr></table> | -1  | -4  | -3  |   |  |
| -99 | 0   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -99 |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -1  | -2  |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -3  |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| 0   | -4  |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -4  |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -1  | -4  |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |
| -3  |   |   |     |     |     |  |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |

# Local Alignment

Given two sequences:

```
ATGCTGACACGTA
ACTACGCTCACAC
```

Select a substring from each so that their alignment score is as large as possible.

This is called the *local alignment* problem.

Can you find a good local alignment:

|                                     |
|-------------------------------------|
| match = +1, mismatch = -1, gap = -2 |
|-------------------------------------|

Did you find:

```
GCTGACAC
GCTCACAC
```

# Local Alignment Algorithm

It's *way* easier than the affine gap matrix.

It's even easier than our first method.

|                                     |
|-------------------------------------|
| match = +1, mismatch = -1, gap = -2 |
|-------------------------------------|

Could this be an optimal local alignment of two long sequences:

```
CGTT-AGGGCTTA-C
CAATGAGGGCTTACC
```

No, for two kinds of reason:

- We can lop off stuff at the beginning of the alignment to obtain a better one, because the stuff has negative score.
- Same with the end

# Local Alignment Algorithm

Here's a close-up of that alignment:

|    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| C  | G  | T  | T  | -  | A  | G  | G  | G  | C  | T  | T  | A  | -  | C  |
| C  | A  | A  | T  | G  | A  | G  | G  | G  | C  | T  | T  | A  | C  | C  |
| +1 | -1 | -1 | +1 | -2 | +1 | +1 | +1 | +1 | +1 | +1 | +1 | +1 | -2 | +1 |
|    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |

Let's put running totals in the bottom row:

Algorithmically, how could we have discovered that initial section to lop off? Whenever a running total becomes negative, just start over. Set the would-be negative cell to "0."

|    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| C  | G  | T  | T  | -  | A  | G  | G  | G  | C  | T  | T  | A  | -  | C  |
| C  | A  | A  | T  | G  | A  | G  | G  | G  | C  | T  | T  | A  | C  | C  |
| +1 | -1 | -1 | +1 | -2 | +1 | +1 | +1 | +1 | +1 | +1 | +1 | +1 | -2 | +1 |
| +1 | 0  | 0  | +1 | 0  | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 6  | 7  |

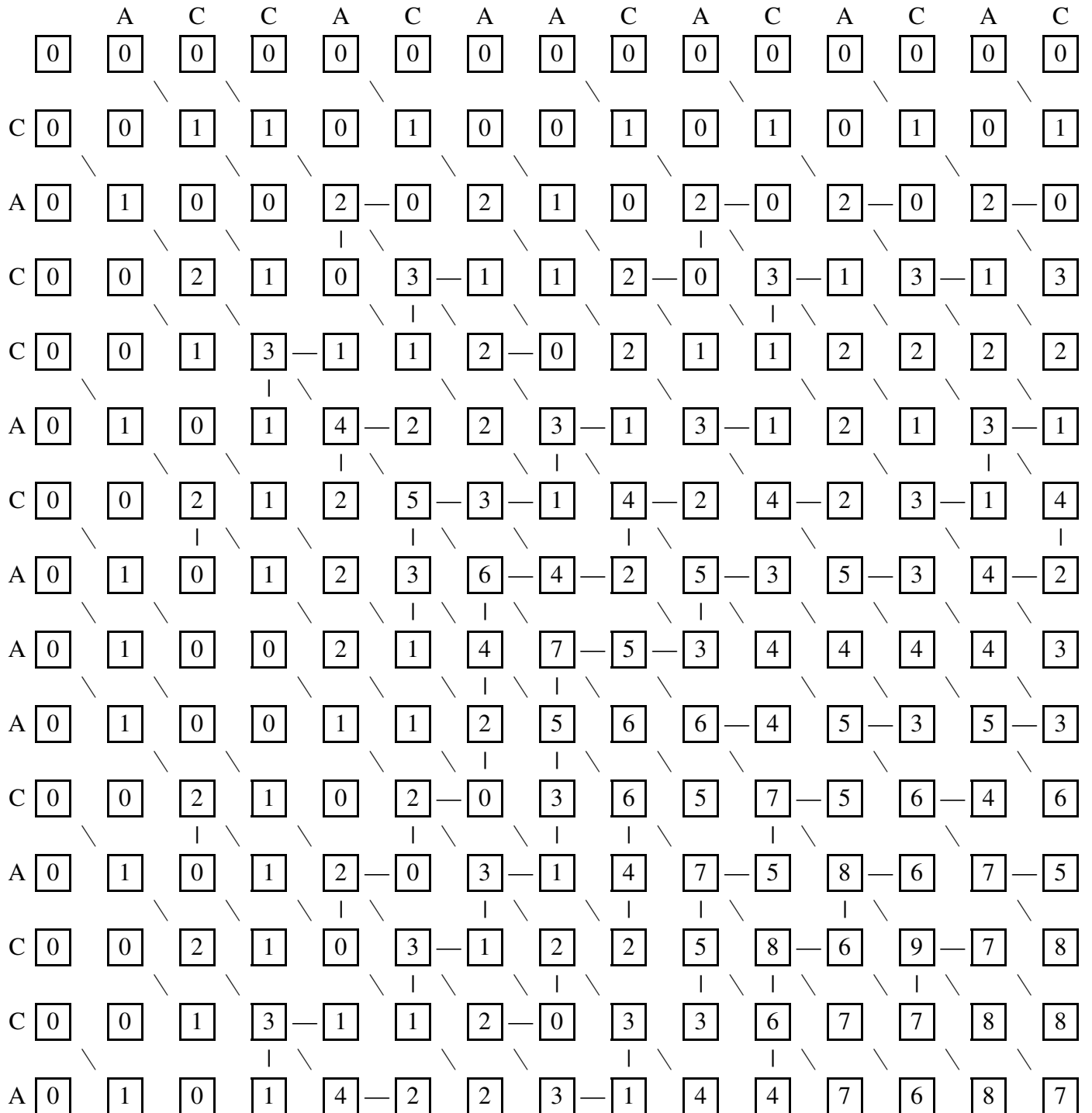
# Local Alignment Algorithm (Smith-Waterman)

|   |  | A                        | C                        | T                        | C                        | A                        |
|---|--|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|   |  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| T |  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| T |  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| C |  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| A |  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| T |  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

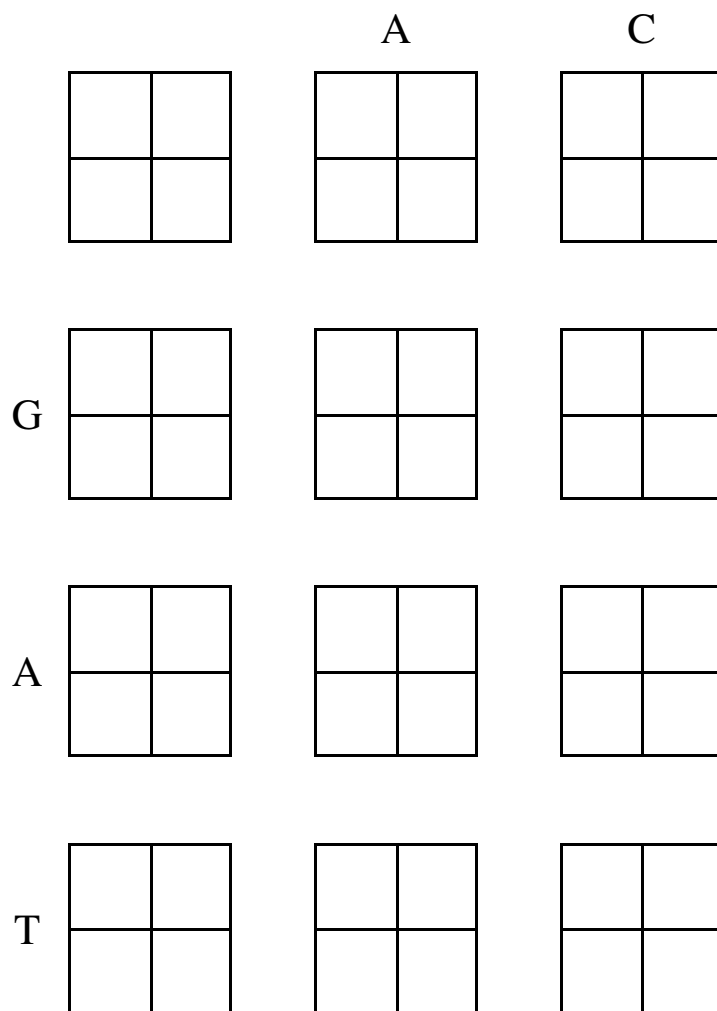
The algorithm is the same as our original alignment algorithm, except that if an entry is about to be negative, we make it “0” instead.



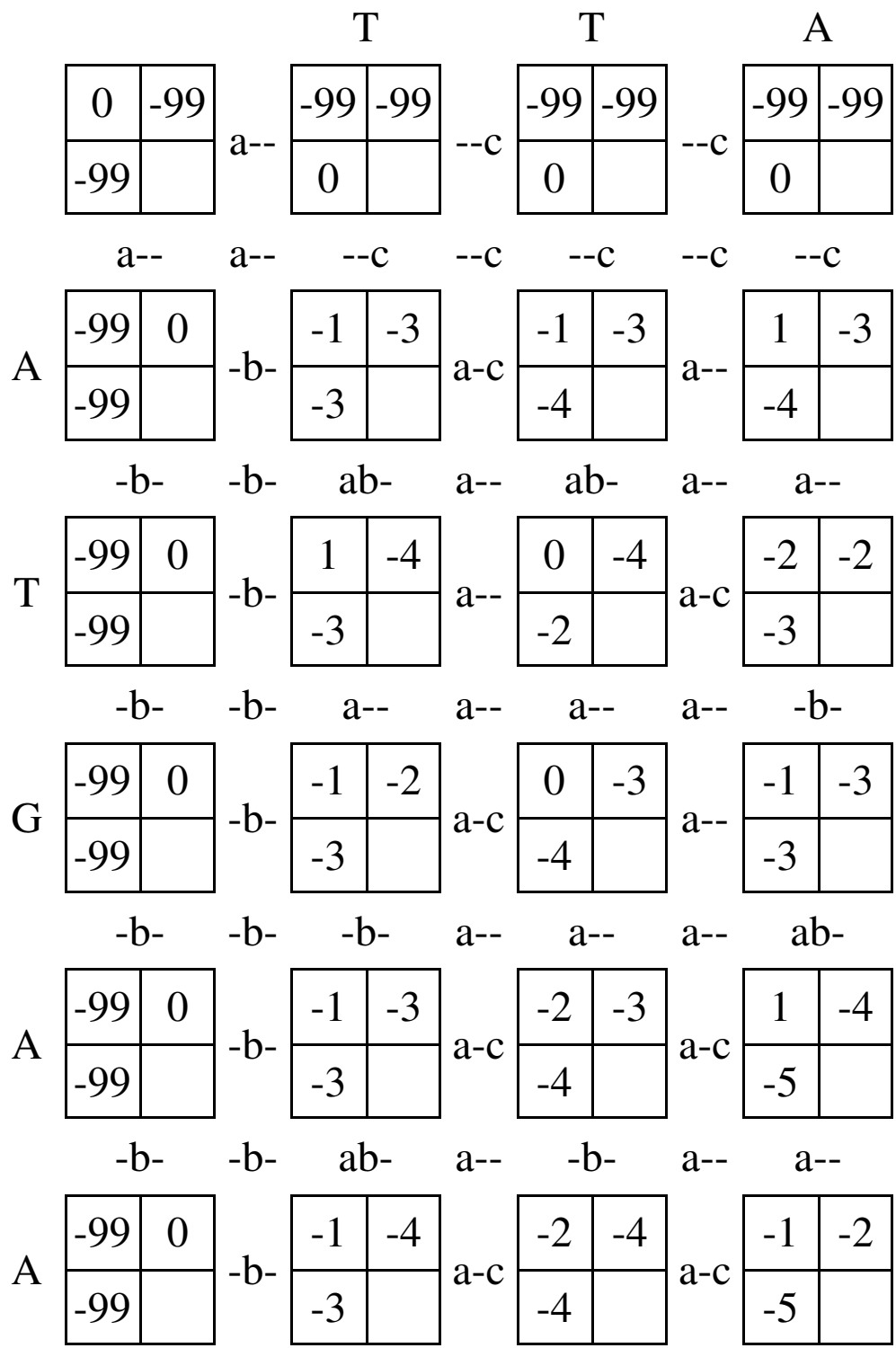
# Tracing Back in Smith-Waterman



# Handout #1 — A Simple Affine Gap Alignment



# Handout #2 — Tracing Back in an Affine Gap Matrix



## Handout #3 — Introduction to Local Alignment

Given two strings, a local alignment is an alignment of two substrings, one taken from each string.

For example, given the two strings

```
ATGCTGACACGTA
ACTACGCTCACAC
```

the following would be a local alignment of score 0:

```
TGCTG
TAC-G
```

What is the best local alignment you can find in those two strings?

## Handout #4 — Local Alignment Algorithm

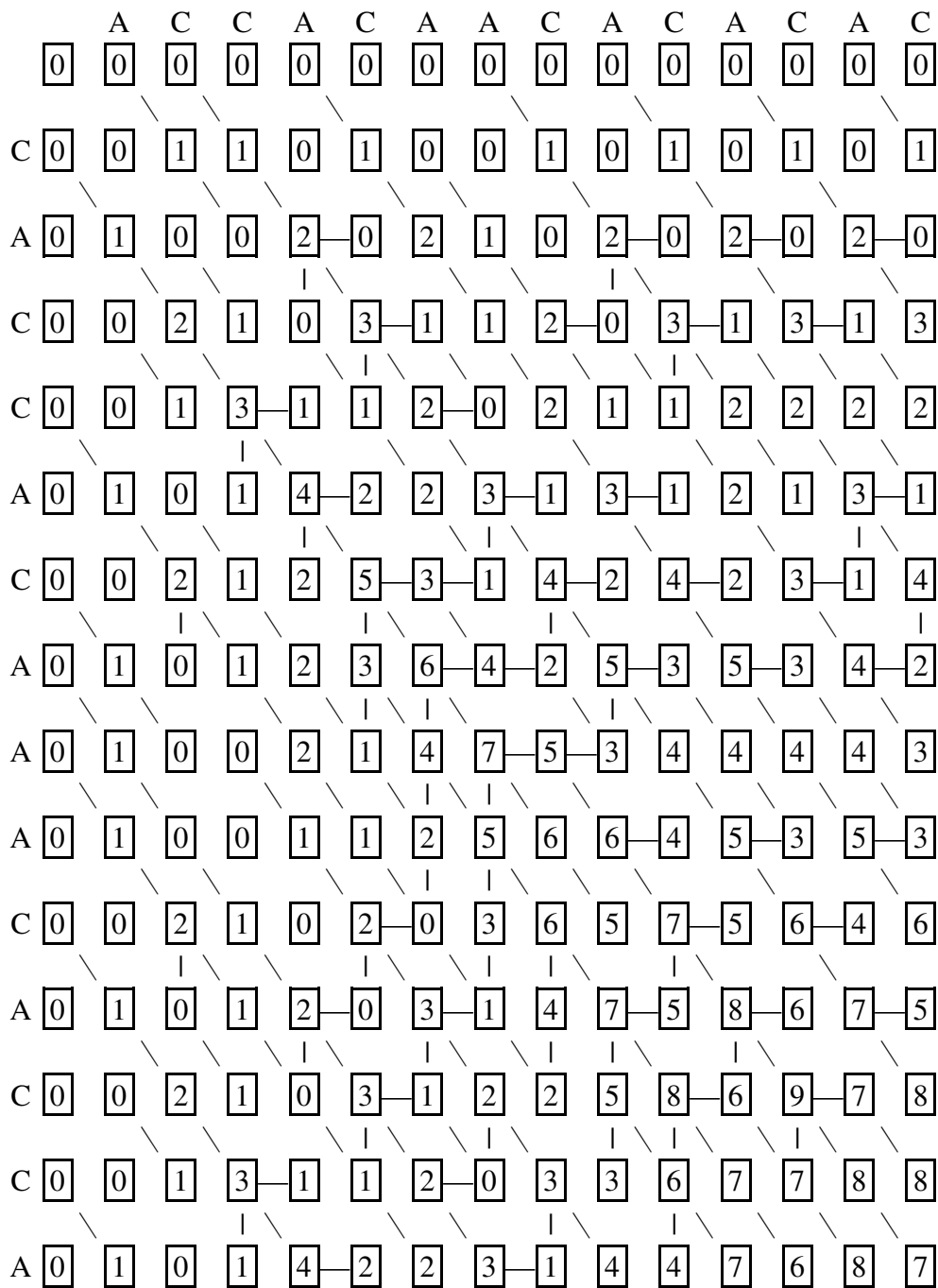
The algorithm is the same as our original alignment algorithm, except that if an entry is about to be negative, we make it “0” instead.

We then obtain our good alignments by finding large entries in the resulting matrix, and tracing them back, up to but not including, a “0” entry.

|   | A                        | C                        | T                        | C                        | A                        |
|---|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|   | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| T | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| T | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| C | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| A | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| T | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

# Handout #5 — Tracing Back in the Smith Waterman Matrix

Find all optimal local alignments indicated by this scoring matrix.



# Handout #6 — Smith-Waterman with an Amino Acid Substitution Matrix

Here is the BLOSUM62 scoring matrix:

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

Find an optimal local alignment using the Smith-Waterman algorithm with the scores given above.

|   |  | K                    | A                    | T                    | H                    | Y                    |
|---|--|----------------------|----------------------|----------------------|----------------------|----------------------|
|   |  | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| C |  | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| H |  | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| C |  | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |
| K |  | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> |

## Handout #0 — Workshop Session — Supplemental Note Page

|                          |                         |
|--------------------------|-------------------------|
| ACAGCAGTAT<br>A--G--G-A- | ACAGCAGTAT<br>--AGGA--- |
|--------------------------|-------------------------|

Let's align ACAGCAGTAT and AGGA under the system:  
Match = +1, Mismatch = -1, Start gap = -3, Continue gap = -1:

Could this be an optimal local alignment of two long sequences:

```
CGTT-AGGGCTTA-C  
CAATGAGGGCTTACC
```

|   |  | A   | G   | G   | A   |
|---|--|---|---|---|---|
|   | $\begin{matrix} 0 & -99 \\ -99 & \end{matrix}$   | a-- $\begin{matrix} -99 & -99 \\ -2 & \end{matrix}$ | --c $\begin{matrix} -99 & -99 \\ -3 & \end{matrix}$ | --c $\begin{matrix} -99 & -99 \\ -4 & \end{matrix}$ | --c $\begin{matrix} -99 & -99 \\ -5 & \end{matrix}$ |
|   | a--  | a--   | --c   | --c   | --c   |
| A | $\begin{matrix} -99 & -2 \\ -99 & \end{matrix}$  | -b- $\begin{matrix} 1 & -4 \\ -4 & \end{matrix}$    | a-- $\begin{matrix} -3 & -5 \\ -1 & \end{matrix}$   | --c $\begin{matrix} -4 & -6 \\ -2 & \end{matrix}$   | --c $\begin{matrix} -3 & -7 \\ -3 & \end{matrix}$   |
|   | -b-  | -b-   | a--   | a--   | --c   |
| C | $\begin{matrix} -99 & -3 \\ -99 & \end{matrix}$  | -b- $\begin{matrix} -3 & -1 \\ -5 & \end{matrix}$   | -b- $\begin{matrix} 0 & -3 \\ -3 & \end{matrix}$    | a-- $\begin{matrix} -2 & -4 \\ -2 & \end{matrix}$   | --c $\begin{matrix} -3 & -5 \\ -3 & \end{matrix}$   |
|   | -b-  | -b-   | -b-   | -b-   | a--   |
| A | $\begin{matrix} -99 & -4 \\ -99 & \end{matrix}$  | -b- $\begin{matrix} -2 & -2 \\ -6 & \end{matrix}$   | - ab $\begin{matrix} -2 & -2 \\ -4 & \end{matrix}$  | - ab $\begin{matrix} -1 & -4 \\ -4 & \end{matrix}$  | a-- $\begin{matrix} -1 & -5 \\ -3 & \end{matrix}$   |
|   | -b-  | -b-   | -b-   | ab  | ab  |
| G | $\begin{matrix} -99 & -5 \\ -99 & \end{matrix}$  | -b- $\begin{matrix} -5 & -3 \\ -7 & \end{matrix}$   | -b- $\begin{matrix} -1 & -3 \\ -5 & \end{matrix}$   | a-- $\begin{matrix} -1 & -3 \\ -3 & \end{matrix}$   | a-- $\begin{matrix} -2 & -3 \\ -3 & \end{matrix}$   |
|   | -b-  | -b-   | -b-   | -b-   | a--   |
| C | $\begin{matrix} -99 & -6 \\ -99 & \end{matrix}$  | -b- $\begin{matrix} -6 & -4 \\ -8 & \end{matrix}$   | -b- $\begin{matrix} -4 & -3 \\ -6 & \end{matrix}$   | -b- $\begin{matrix} -2 & -3 \\ -5 & \end{matrix}$   | a-- $\begin{matrix} -2 & -4 \\ -4 & \end{matrix}$   |
|   | -b-  | -b-   | -b-   | -b-   | ab-   |
| A | $\begin{matrix} -99 & -7 \\ -99 & \end{matrix}$  | -b- $\begin{matrix} -5 & -5 \\ -9 & \end{matrix}$   | - ab $\begin{matrix} -5 & -4 \\ -7 & \end{matrix}$  | -b- $\begin{matrix} -4 & -4 \\ -6 & \end{matrix}$   | ab $\begin{matrix} -1 & -4 \\ -6 & \end{matrix}$    |
|   | -b-  | -b-   | -b-   | ab  | ab  |
| G | $\begin{matrix} -99 & -8 \\ -99 & \end{matrix}$  | -b- $\begin{matrix} -8 & -6 \\ -10 & \end{matrix}$  | -b- $\begin{matrix} -4 & -5 \\ -8 & \end{matrix}$   | a-- $\begin{matrix} -3 & -5 \\ -6 & \end{matrix}$   | a-- $\begin{matrix} -5 & -3 \\ -5 & \end{matrix}$   |
|   | -b-  | -b-   | -b-   | -b-   | ab-   |
| T | $\begin{matrix} -99 & -9 \\ -99 & \end{matrix}$  | -b- $\begin{matrix} -9 & -7 \\ -11 & \end{matrix}$  | -b- $\begin{matrix} -7 & -6 \\ -9 & \end{matrix}$   | -b- $\begin{matrix} -5 & -5 \\ -8 & \end{matrix}$   | ab $\begin{matrix} -4 & -4 \\ -7 & \end{matrix}$    |
|   | -b-  | -b-   | -b-   | -b-   | ab  |
| A | $\begin{matrix} -99 & -10 \\ -99 & \end{matrix}$ | -b- $\begin{matrix} -8 & -8 \\ -12 & \end{matrix}$  | - ab $\begin{matrix} -8 & -7 \\ -10 & \end{matrix}$ | -b- $\begin{matrix} -7 & -6 \\ -9 & \end{matrix}$   | -b- $\begin{matrix} -4 & -5 \\ -8 & \end{matrix}$   |
|   | -b-  | -b-   | -b-   | ab  | ab  |
| T | $\begin{matrix} -99 & -11 \\ -99 & \end{matrix}$ | -b- $\begin{matrix} -11 & -9 \\ -13 & \end{matrix}$ | -b- $\begin{matrix} -9 & -8 \\ -11 & \end{matrix}$  | -b- $\begin{matrix} -8 & -7 \\ -10 & \end{matrix}$  | -b- $\begin{matrix} -7 & -6 \\ -9 & \end{matrix}$   |

|     |   | A   | G   | G   | A   |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
|-----|---|-----|-----|-----|-----|---|-----|-----|-----|--|---|-----|-----|-----|--|---|-----|-----|-----|--|--|-----|-----|-----|--|
|     | <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td style="background-color: #cccccc;">0</td><td>-99</td></tr><tr><td>-99</td><td></td></tr></table>  | 0   | -99 | -99 |     | a-- <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-99</td><td>-99</td></tr><tr><td>-2</td><td></td></tr></table>  | -99 | -99 | -2  |  | --c <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-99</td><td>-99</td></tr><tr><td>-3</td><td></td></tr></table>  | -99 | -99 | -3  |  | --c <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-99</td><td>-99</td></tr><tr><td>-4</td><td></td></tr></table>  | -99 | -99 | -4  |  | --c <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-99</td><td>-99</td></tr><tr><td>-5</td><td></td></tr></table>   | -99 | -99 | -5  |  |
| 0   | -99   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -99 |   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -99 | -99   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -2  |   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -99 | -99   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -3  |   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -99 | -99   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -4  |   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -99 | -99   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -5  |   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
|     | a--   | a-- | --c | --c | --c |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| A   | <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-99</td><td style="background-color: #cccccc;">-2</td></tr><tr><td>-99</td><td></td></tr></table> | -99 | -2  | -99 |     | -b- <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>1</td><td>-5</td></tr><tr><td>-5</td><td></td></tr></table>   | 1   | -5  | -5  |  | a-- <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-3</td><td>-6</td></tr><tr><td>-2</td><td></td></tr></table>  | -3  | -6  | -2  |  | --c <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-4</td><td>-7</td></tr><tr><td>-3</td><td></td></tr></table>  | -4  | -7  | -3  |  | --c <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-3</td><td>-8</td></tr><tr><td>-4</td><td></td></tr></table>   | -3  | -8  | -4  |  |
| -99 | -2  |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -99 |   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| 1   | -5  |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -5  |   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -3  | -6  |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -2  |   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -4  | -7  |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -3  |   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -3  | -8  |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -4  |   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
|     | -b-   | -b- | a-- | a-- | --c |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| C   | <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-99</td><td style="background-color: #cccccc;">-3</td></tr><tr><td>-99</td><td></td></tr></table> | -99 | -3  | -99 |     | -b- <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-3</td><td>-2</td></tr><tr><td>-6</td><td></td></tr></table>  | -3  | -2  | -6  |  | -b- <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>0</td><td>-5</td></tr><tr><td>-5</td><td></td></tr></table>   | 0   | -5  | -5  |  | a-- <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-3</td><td>-6</td></tr><tr><td>-3</td><td></td></tr></table>  | -3  | -6  | -3  |  | --c <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td>-4</td><td>-6</td></tr><tr><td>-4</td><td></td></tr></table>   | -4  | -6  | -4  |  |
| -99 | -3  |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -99 |   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -3  | -2  |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -6  |   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| 0   | -5  |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -5  |   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -3  | -6  |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -3  |   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -4  | -6  |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -4  |   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
|     | -b-   | -b- | -b- | -b- | a-- |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
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| -12 |   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -9  | -7  |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |
| -12 |   |     |     |     |     |   |     |     |     |  |   |     |     |     |  |   |     |     |     |  |  |     |     |     |  |

## Exercises — Local Alignment

### Quick Concepts:

1. What optimal global alignment is suggested by the affine gap penalty grid below?

|     |  | A   | C   | T   |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
|-----|--|---|-----|-----|-----|--|--|-----|-----|----|---|--|-----|-----|----|---|--|-----|-----|----|--|
|     |  | <table border="1"><tr><td>0</td><td>-99</td></tr><tr><td>-99</td><td></td></tr></table> | 0   | -99 | -99 |  | <table border="1"><tr><td>-99</td><td>-99</td></tr><tr><td>-3</td><td></td></tr></table> | -99 | -99 | -3 |   | <table border="1"><tr><td>-99</td><td>-99</td></tr><tr><td>-4</td><td></td></tr></table> | -99 | -99 | -4 |   | <table border="1"><tr><td>-99</td><td>-99</td></tr><tr><td>-5</td><td></td></tr></table> | -99 | -99 | -5 |  |
| 0   | -99  |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -99 |  |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -99 | -99  |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -3  |  |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -99 | -99  |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -4  |  |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -99 | -99  |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -5  |  |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
|     | a--  | --c   | --c | --c |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
|     | a--  | --c   | --c | --c |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| G   | <table border="1"><tr><td>-99</td><td>-3</td></tr><tr><td>-99</td><td></td></tr></table> | -99   | -3  | -99 |     | <table border="1"><tr><td>-5</td><td>-5</td></tr><tr><td>-5</td><td></td></tr></table> | -5   | -5  | -5  |    | <table border="1"><tr><td>-8</td><td>-6</td></tr><tr><td>-6</td><td></td></tr></table>  | -8   | -6  | -6  |    | <table border="1"><tr><td>-9</td><td>-7</td></tr><tr><td>-7</td><td></td></tr></table>  | -9   | -7  | -7  |    |  |
| -99 | -3   |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -99 |  |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -5  | -5   |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -5  |  |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -8  | -6   |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -6  |  |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -9  | -7   |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -7  |  |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
|     | -b-  | -b-   | -b- | -b- |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
|     | -b-  | -b-   | -b- | -b- |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| A   | <table border="1"><tr><td>-99</td><td>-4</td></tr><tr><td>-99</td><td></td></tr></table> | -99   | -4  | -99 |     | <table border="1"><tr><td>-2</td><td>-6</td></tr><tr><td>-6</td><td></td></tr></table> | -2   | -6  | -6  |    | <table border="1"><tr><td>-10</td><td>-7</td></tr><tr><td>-4</td><td></td></tr></table> | -10  | -7  | -4  |    | <table border="1"><tr><td>-11</td><td>-8</td></tr><tr><td>-5</td><td></td></tr></table> | -11  | -8  | -5  |    |  |
| -99 | -4   |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -99 |  |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -2  | -6   |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -6  |  |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -10 | -7   |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -4  |  |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -11 | -8   |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -5  |  |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
|     | -b-  | -b-   | -b- | -b- |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
|     | -b-  | -b-   | -b- | -b- |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| T   | <table border="1"><tr><td>-99</td><td>-5</td></tr><tr><td>-99</td><td></td></tr></table> | -99   | -5  | -99 |     | <table border="1"><tr><td>-9</td><td>-4</td></tr><tr><td>-7</td><td></td></tr></table> | -9   | -4  | -7  |    | <table border="1"><tr><td>-7</td><td>-6</td></tr><tr><td>-6</td><td></td></tr></table>  | -7   | -6  | -6  |    | <table border="1"><tr><td>-3</td><td>-7</td></tr><tr><td>-7</td><td></td></tr></table>  | -3   | -7  | -7  |    |  |
| -99 | -5   |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -99 |  |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -9  | -4   |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -7  |  |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -7  | -6   |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -6  |  |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -3  | -7   |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
| -7  |  |   |     |     |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
|     | -b-  | -b-   | -b- | -b- |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |
|     | -b-  | -b-   | -b- | -b- |     |  |  |     |     |    |   |  |     |     |    |   |  |     |     |    |  |

2. What are the four gap penalties (start an initial gap, continue an initial gap, start another gap, continue another gap) used in the alignment above? Also, what are the match and mismatch scores?
3. What optimal local alignment is suggested by the scoring matrix below?

|   | S | C | A | T | T | E | R |
|---|---|---|---|---|---|---|---|
|   | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| T | 0 | 0 | 0 | 4 | 2 | 0 | 0 |
| H | 0 | 0 | 0 | 2 | 3 | 1 | 0 |
| E | 0 | 0 | 0 | 0 | 1 | 5 | 3 |
| R | 0 | 0 | 0 | 0 | 0 | 3 | 7 |

**Presentation Problems:**

4. The strings "GATTACA" and "TAGCAT" have been aligned using an affine gap penalty function in which matches score +1, mismatches cost -1, gaps cost -2 to start and -1 to continue. Complete the alignment begun in the table below.

|   |        |           |           |           |           |           |           |           |
|---|--------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
|   |        | G         | A         | T         | T         | A         | C         | A         |
|   | 0 -99  | -99 -99   | -99 -99   | -99 -99   | -99 -99   | -99 -99   |           |           |
|   | -99    | a-- -2    | --c -3    | --c -4    | --c -5    | --c -6    |           |           |
| T | -99 -2 | -b- -1 -4 | a-- -3 -5 | --c -2 -6 | a-- -3 -7 | a-c -6 -8 |           |           |
|   | -99    | -b- -4    | a-- -3    | --c -4    | a-- -4    | a-c -5    |           |           |
| A | -99 -3 | -b- -3 -3 | ab- 0 -5  | a-- -4 -4 | --c -3 -5 | --c -2 -7 |           |           |
|   | -99    | -b- -5    | ab- -5    | a-- -2    | --c -3    | --c -4    |           |           |
| G | -99 -4 | -b- -2 -4 | a-- -4 -2 |           |           |           |           |           |
|   | -99    | -b- -6    | a-- -4    |           |           |           |           |           |
| C | -99 -5 | -b- -5 -4 | -b- -3 -3 |           |           |           |           |           |
|   | -99    | -b- -7    | -b- -6    |           |           |           |           |           |
| A | -99 -6 | -b- -6 -5 | -b- -3 -4 |           |           |           |           |           |
|   | -99    | -b- -8    | -b- -7    |           |           |           |           |           |
| T | -99 -7 | -b- -7 -6 | -b- -6 -5 |           |           |           |           |           |
|   | -99    | -b- -9    | -b- -8    |           |           |           |           |           |
|   |        |           |           |           |           |           | --c -4 -4 | a-- -4 -4 |

5. Give all optimal global alignments of the strings in the previous problem.
6. Suppose that in the alignment of "GATTACA" and "TAGCAT" above we did not charge for gaps inserted at the end of the alignment, but we do still charge for gaps at the beginning. List all the optimal alignments in this case.

7. Suppose two sequences are aligned twice, once globally and once locally, under the same match, mismatch and gap scoring. Is it the case that the set of edges in the Smith-Waterman matrix (on the right) must be a subset of the edges in the Needleman-Wunsch matrix (on the left)?

|   |    |    |    |    |   |   |   |   |
|---|----|----|----|----|---|---|---|---|
|   |    | A  | C  | T  |   | A | C | T |
|   | 0  | -2 | -4 | -6 |   | 0 | 0 | 0 |
| G | -2 | -1 | -3 | -5 | G | 0 | 0 | 0 |
| A | -4 | -1 | -2 | -4 | A | 0 | 1 | 0 |
| C | -6 | -3 | 0  | -2 | C | 0 | 0 | 2 |
|   |    |    |    |    |   |   |   |   |

8. Here is the BLOSUM62 scoring matrix. What is the score of the following amino acid alignment if the start gap penalty is -11 and the gap continuation penalty is -1?

AMINO---ACID  
GLIDERBRACES

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

9. Find an optimal local alignment using the above scoring matrix with gap penalty -4.

|   |                                |                                |                                |                                |                                |                                |
|---|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
|   |                                | W                              | R                              | A                              | P                              | S                              |
|   | <input type="text" value="0"/> | <input type="text" value="0"/> | <input type="text" value="0"/> | <input type="text" value="0"/> | <input type="text" value="0"/> | <input type="text" value="0"/> |
| S | <input type="text" value="0"/> | <input type="text"/>           | <input type="text"/>           | <input type="text"/>           | <input type="text"/>           | <input type="text"/>           |
| W | <input type="text" value="0"/> | <input type="text"/>           | <input type="text"/>           | <input type="text"/>           | <input type="text"/>           | <input type="text"/>           |
| I | <input type="text" value="0"/> | <input type="text"/>           | <input type="text"/>           | <input type="text"/>           | <input type="text"/>           | <input type="text"/>           |
| P | <input type="text" value="0"/> | <input type="text"/>           | <input type="text"/>           | <input type="text"/>           | <input type="text"/>           | <input type="text"/>           |
| E | <input type="text" value="0"/> | <input type="text"/>           | <input type="text"/>           | <input type="text"/>           | <input type="text"/>           | <input type="text"/>           |

10. Find all optimal local alignments for the Local Alignment matrix shown below. What is the optimal score?

|   | C | G | T | C | G | T | A | A | A | C | A | T | G | A | C |   |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|   | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |   |
| G | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| G | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| T | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| A | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 3 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 |
| C | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
| G | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 |
| G | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| T | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| T | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| G | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| T | 0 | 0 | 0 | 2 | 0 | 0 | 3 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| T | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| C | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| C | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| G | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| T | 0 | 0 | 0 | 3 | 1 | 0 | 3 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| T | 0 | 0 | 0 | 1 | 2 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| A | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 3 | 1 | 0 | 1 | 0 | 0 | 1 | 0 |
| G | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 1 | 0 | 0 |
| C | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 0 | 0 | 0 | 1 |
| C | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 1 |

11. We can combine the Smith-Waterman algorithm with our affine gap penalty trick to obtain optimal local alignments under an affine gap penalty function. I began this below, charging -2 to start a gap, -1 to continue a gap, +5 for a match and -1 for a mismatch.

a. Unfortunately, I didn't have time to finish it.

|     |   | G   | A   | T   | T   | A   | C   | A   |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
|-----|---|---|-----|-----|-----|---|---|-----|-----|---|--|---|-----|-----|---|--|---|-----|-----|---|--|---|-----|--|----|--|---|-----|-----|---|---|---|-----|-----|---|---|---|-----|-----|---|--|
|     |   | <table border="1"><tr><td>0</td><td>-99</td></tr><tr><td>-99</td><td></td></tr></table> | 0   | -99 | -99 |   | <table border="1"><tr><td>-99</td><td>-99</td></tr><tr><td>0</td><td></td></tr></table> | -99 | -99 | 0 |  | <table border="1"><tr><td>-99</td><td>-99</td></tr><tr><td>0</td><td></td></tr></table> | -99 | -99 | 0 |  | <table border="1"><tr><td>-99</td><td>-99</td></tr><tr><td>0</td><td></td></tr></table> | -99 | -99 | 0 |  | <table border="1"><tr><td>-99</td><td>-99</td></tr><tr><td>0</td><td></td></tr></table> | -99 | -99  | 0  |  | <table border="1"><tr><td>-99</td><td>-99</td></tr><tr><td>0</td><td></td></tr></table> | -99 | -99 | 0 |   | <table border="1"><tr><td>-99</td><td>-99</td></tr><tr><td>0</td><td></td></tr></table> | -99 | -99 | 0 |   | <table border="1"><tr><td>-99</td><td>-99</td></tr><tr><td>0</td><td></td></tr></table> | -99 | -99 | 0 |  |
| 0   | -99   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 | -99   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 | -99   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 | -99   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 | -99   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 | -99   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 | -99   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 | -99   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
|     | a--   | ---   | --- | --- | --- | ---   | ---   | --- |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| C   | <table border="1"><tr><td>-99</td><td>0</td></tr><tr><td>-99</td><td></td></tr></table> | -99   | 0   | -99 |     | <table border="1"><tr><td>0</td><td>0</td></tr><tr><td>0</td><td></td></tr></table> | 0   | 0   | 0   |   | <table border="1"><tr><td>0</td><td>0</td></tr><tr><td>0</td><td></td></tr></table>  | 0   | 0   | 0   |   | <table border="1"><tr><td>0</td><td>0</td></tr><tr><td>0</td><td></td></tr></table>  | 0   | 0   | 0   |   | <table border="1"><tr><td>0</td><td>0</td></tr><tr><td>0</td><td></td></tr></table>  | 0   | 0   | 0  |    | <table border="1"><tr><td>0</td><td>0</td></tr><tr><td>0</td><td></td></tr></table>    | 0   | 0   | 0   |   | <table border="1"><tr><td>5</td><td>0</td></tr><tr><td>0</td><td></td></tr></table> | 5   | 0   | 0   |   | <table border="1"><tr><td>0</td><td>0</td></tr><tr><td>3</td><td></td></tr></table> | 0   | 0   | 3   |   |  |
| -99 | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 5   | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 3   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
|     | -b-   | ---   | --- | abc | abc | ---   | ---   | a-- |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| T   | <table border="1"><tr><td>-99</td><td>0</td></tr><tr><td>-99</td><td></td></tr></table> | -99   | 0   | -99 |     | <table border="1"><tr><td>0</td><td>0</td></tr><tr><td>0</td><td></td></tr></table> | 0   | 0   | 0   |   | <table border="1"><tr><td>0</td><td>0</td></tr><tr><td>0</td><td></td></tr></table>  | 0   | 0   | 0   |   | <table border="1"><tr><td>5</td><td>0</td></tr><tr><td>0</td><td></td></tr></table>  | 5   | 0   | 0   |   | <table border="1"><tr><td>5</td><td>0</td></tr><tr><td>3</td><td></td></tr></table>  | 5   | 0   | 3  |    | <table border="1"><tr><td>0</td><td>0</td></tr><tr><td>3</td><td></td></tr></table>    | 0   | 0   | 3   |   | <table border="1"><tr><td>0</td><td>3</td></tr><tr><td>2</td><td></td></tr></table> | 0   | 3   | 2   |   | <table border="1"><tr><td>4</td><td>1</td></tr><tr><td>1</td><td></td></tr></table> | 4   | 1   | 1   |   |  |
| -99 | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 5   | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 5   | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 3   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 3   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   | 3   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 2   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 4   | 1   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 1   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
|     | -b-   | ---   | abc | --- | a-- | a--   | a--   | a-- |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| A   | <table border="1"><tr><td>-99</td><td>0</td></tr><tr><td>-99</td><td></td></tr></table> | -99   | 0   | -99 |     | <table border="1"><tr><td>0</td><td>0</td></tr><tr><td>0</td><td></td></tr></table> | 0   | 0   | 0   |   | <table border="1"><tr><td>5</td><td>0</td></tr><tr><td>0</td><td></td></tr></table>  | 5   | 0   | 0   |   | <table border="1"><tr><td>0</td><td>3</td></tr><tr><td>3</td><td></td></tr></table>  | 0   | 3   | 3   |   | <table border="1"><tr><td>4</td><td>3</td></tr><tr><td>2</td><td></td></tr></table>  | 4   | 3   | 2  |    | <table border="1"><tr><td>10</td><td>1</td></tr><tr><td>2</td><td></td></tr></table>   | 10  | 1   | 2   |   | <table border="1"><tr><td>2</td><td>2</td></tr><tr><td>8</td><td></td></tr></table> | 2   | 2   | 8   |   | <table border="1"><tr><td>8</td><td>2</td></tr><tr><td>7</td><td></td></tr></table> | 8   | 2   | 7   |   |  |
| -99 | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 5   | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   | 3   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 3   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 4   | 3   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 2   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 10  | 1   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 2   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 2   | 2   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 8   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 8   | 2   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 7   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
|     | -b-   | ---   | a-- | a-- | -b- | -bc   | ab-   | a-- |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| T   | <table border="1"><tr><td>-99</td><td>0</td></tr><tr><td>-99</td><td></td></tr></table> | -99   | 0   | -99 |     | <table border="1"><tr><td>0</td><td>0</td></tr><tr><td>0</td><td></td></tr></table> | 0   | 0   | 0   |   | <table border="1"><tr><td>0</td><td>3</td></tr><tr><td>0</td><td></td></tr></table>  | 0   | 3   | 0   |   | <table border="1"><tr><td>10</td><td>2</td></tr><tr><td>1</td><td></td></tr></table> | 10  | 2   | 1   |   | <table border="1"><tr><td>8</td><td>2</td></tr><tr><td>8</td><td></td></tr></table>  | 8   | 2   | 8  |    | <table border="1"><tr><td>3</td><td>8</td></tr><tr><td>7</td><td></td></tr></table>    | 3   | 8   | 7   |   | <table border="1"><tr><td>9</td><td>6</td></tr><tr><td>6</td><td></td></tr></table> | 9   | 6   | 6   |   | <table border="1"><tr><td>7</td><td>6</td></tr><tr><td>7</td><td></td></tr></table> | 7   | 6   | 7   |   |  |
| -99 | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   | 3   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 10  | 2   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 1   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 8   | 2   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 8   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 3   | 8   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 7   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 9   | 6   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 6   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 7   | 6   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 7   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
|     | -b-   | ---   | -b- | -b- | a-- | a--   | a-c   | a-c |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| T   | <table border="1"><tr><td>-99</td><td>0</td></tr><tr><td>-99</td><td></td></tr></table> | -99   | 0   | -99 |     | <table border="1"><tr><td>0</td><td>0</td></tr><tr><td>0</td><td></td></tr></table> | 0   | 0   | 0   |   | <table border="1"><tr><td>0</td><td>2</td></tr><tr><td>0</td><td></td></tr></table>  | 0   | 2   | 0   |   | <table border="1"><tr><td>8</td><td>8</td></tr><tr><td>0</td><td></td></tr></table>  | 8   | 8   | 0   |   | <table border="1"><tr><td>15</td><td>6</td></tr><tr><td>6</td><td></td></tr></table> | 15  | 6   | 6  |    | <table border="1"><tr><td>7</td><td>7</td></tr><tr><td>13</td><td></td></tr></table>   | 7   | 7   | 13  |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   | 2   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 8   | 8   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 15  | 6   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 6   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 7   | 7   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 13  |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
|     | -b-   | -b-   | --- | -b- | -b- | -b-   | ab-   | a-- |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| G   | <table border="1"><tr><td>-99</td><td>0</td></tr><tr><td>-99</td><td></td></tr></table> | -99   | 0   | -99 |     | <table border="1"><tr><td>5</td><td>0</td></tr><tr><td>0</td><td></td></tr></table> | 5   | 0   | 0   |   | <table border="1"><tr><td>0</td><td>1</td></tr><tr><td>3</td><td></td></tr></table>  | 0   | 1   | 3   |   | <table border="1"><tr><td>1</td><td>7</td></tr><tr><td>2</td><td></td></tr></table>  | 1   | 7   | 2   |   | <table border="1"><tr><td>7</td><td>13</td></tr><tr><td>5</td><td></td></tr></table> | 7   | 13  | 5  |    | <table border="1"><tr><td>14</td><td>11</td></tr><tr><td>11</td><td></td></tr></table> | 14  | 11  | 11  |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 5   | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   | 1   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 3   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 1   | 7   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 2   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 7   | 13  |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 5   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 14  | 11  |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 11  |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
|     | -b-   | -b-   | a-- | a-- | --c | --c   | -b-   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| G   | <table border="1"><tr><td>-99</td><td>0</td></tr><tr><td>-99</td><td></td></tr></table> | -99   | 0   | -99 |     | <table border="1"><tr><td>5</td><td>3</td></tr><tr><td>0</td><td></td></tr></table> | 5   | 3   | 0   |   | <table border="1"><tr><td>4</td><td>1</td></tr><tr><td>3</td><td></td></tr></table>  | 4   | 1   | 3   |   | <table border="1"><tr><td>2</td><td>6</td></tr><tr><td>2</td><td></td></tr></table>  | 2   | 6   | 2   |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 5   | 3   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 4   | 1   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 3   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 2   | 6   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 2   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
|     | -b-   | ---   | a-- | a-- | a-- | a--   | -b-   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| A   | <table border="1"><tr><td>-99</td><td>0</td></tr><tr><td>-99</td><td></td></tr></table> | -99   | 0   | -99 |     | <table border="1"><tr><td>0</td><td>3</td></tr><tr><td>0</td><td></td></tr></table> | 0   | 3   | 0   |   | <table border="1"><tr><td>10</td><td>2</td></tr><tr><td>1</td><td></td></tr></table> | 10  | 2   | 1   |   | <table border="1"><tr><td>3</td><td>5</td></tr><tr><td>8</td><td></td></tr></table>  | 3   | 5   | 8   |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   | 3   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 10  | 2   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 1   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 3   | 5   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 8   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
|     | -b-   | ---   | -b- | -b- | a-- | a--   | --c   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| C   | <table border="1"><tr><td>-99</td><td>0</td></tr><tr><td>-99</td><td></td></tr></table> | -99   | 0   | -99 |     | <table border="1"><tr><td>0</td><td>2</td></tr><tr><td>0</td><td></td></tr></table> | 0   | 2   | 0   |   | <table border="1"><tr><td>2</td><td>8</td></tr><tr><td>0</td><td></td></tr></table>  | 2   | 8   | 0   |   | <table border="1"><tr><td>9</td><td>6</td></tr><tr><td>6</td><td></td></tr></table>  | 9   | 6   | 6   |   |  |   |     | <table border="1"><tr><td>14</td><td>16</td></tr><tr><td>20</td><td></td></tr></table> | 14 | 16   | 20  |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 | 0   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| -99 |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   | 2   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 2   | 8   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 0   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 9   | 6   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 6   |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 14  | 16  |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |
| 20  |   |   |     |     |     |   |   |     |     |   |  |   |     |     |   |  |   |     |     |   |  |   |     |  |    |  |   |     |     |   |   |   |     |     |   |   |   |     |     |   |  |

b. Give all optimal local alignments indicated by the (completed) table above?