# Local Alignment

## Master Document

**Local Alignment**

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

**Instructor's Notes for**

**Local Alignment**

# 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`<br>`AGG`<br>score: –6 | `ACAGCAGTA`<br>`AGGA`<br>score: –4 | `ACAGCAGTAT`<br>`AGG`<br>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:

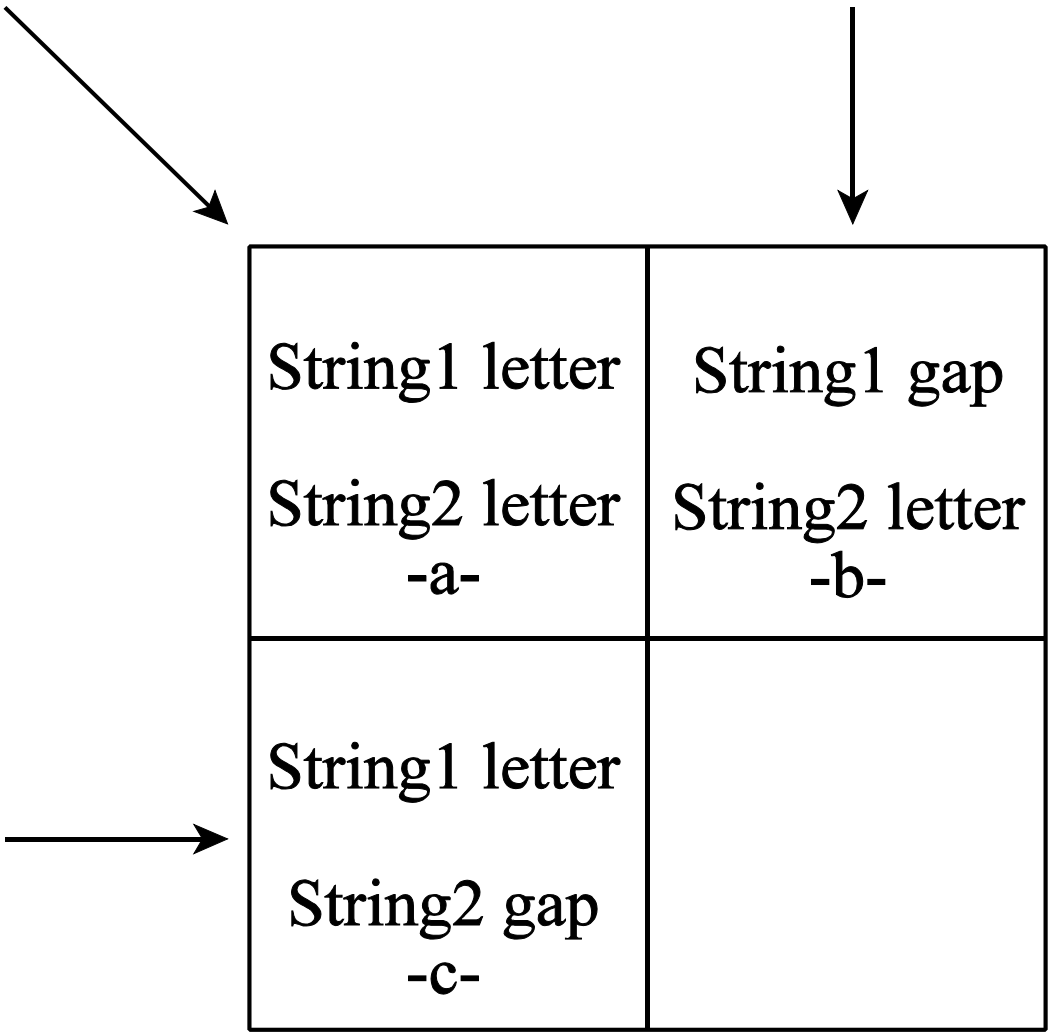
| ACAGCAGTA<br>AGG | | | ACAGCAGTA<br>AGGA | | | ACAGCAGTAT<br>AGG | | |
|---|---|---|---|---|---|---|---|---|
| A<br>G | A<br>– | –<br>G | A<br>A | A<br>– | –<br>A | T<br>G | T<br>– | –<br>G |
| –7 | –6 | –9 | –4 | –5 | –8 | –8 | –7 | –10 |

Now can you tell me the optimal alignment?

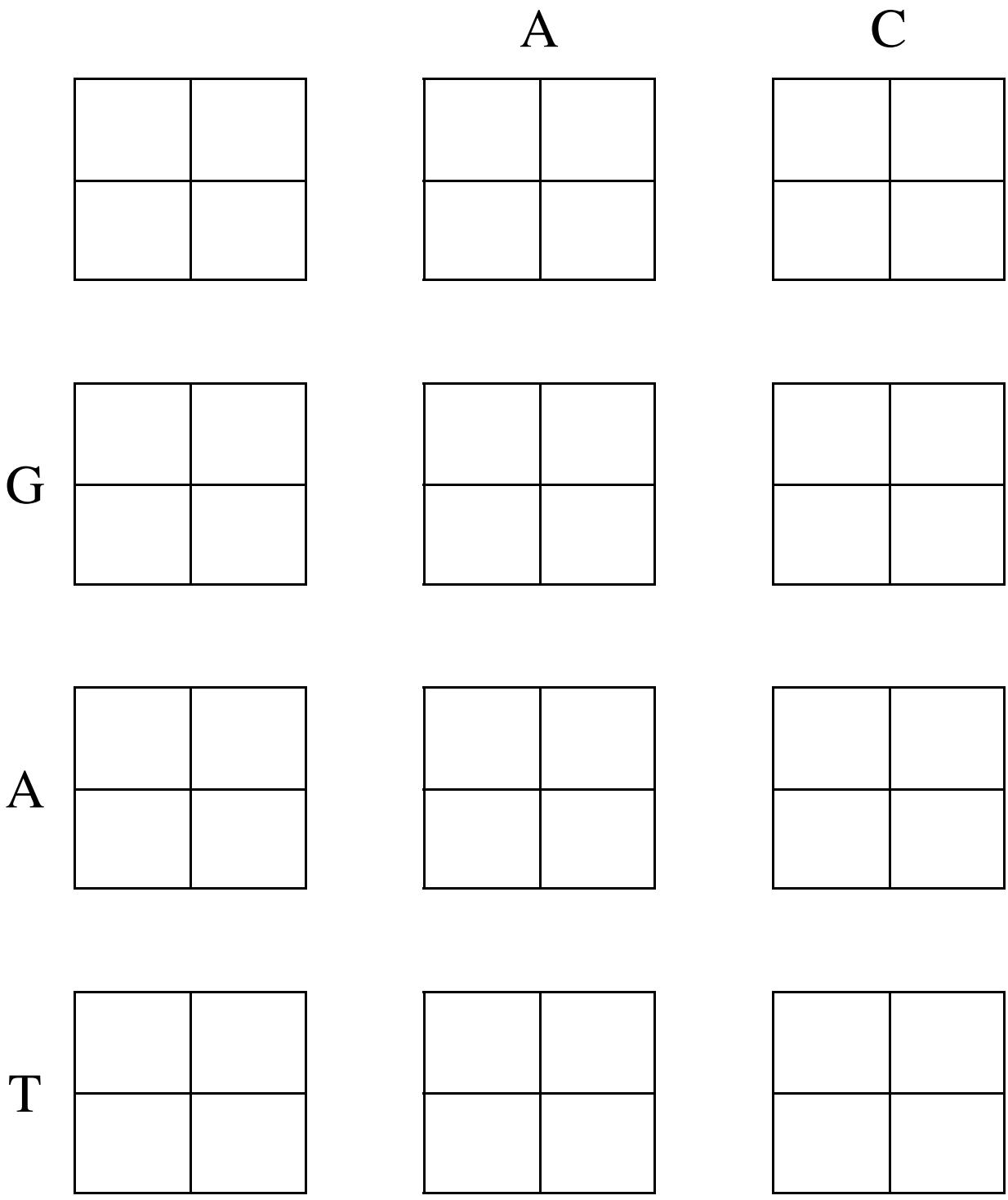
# A More Interesting Matrix

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

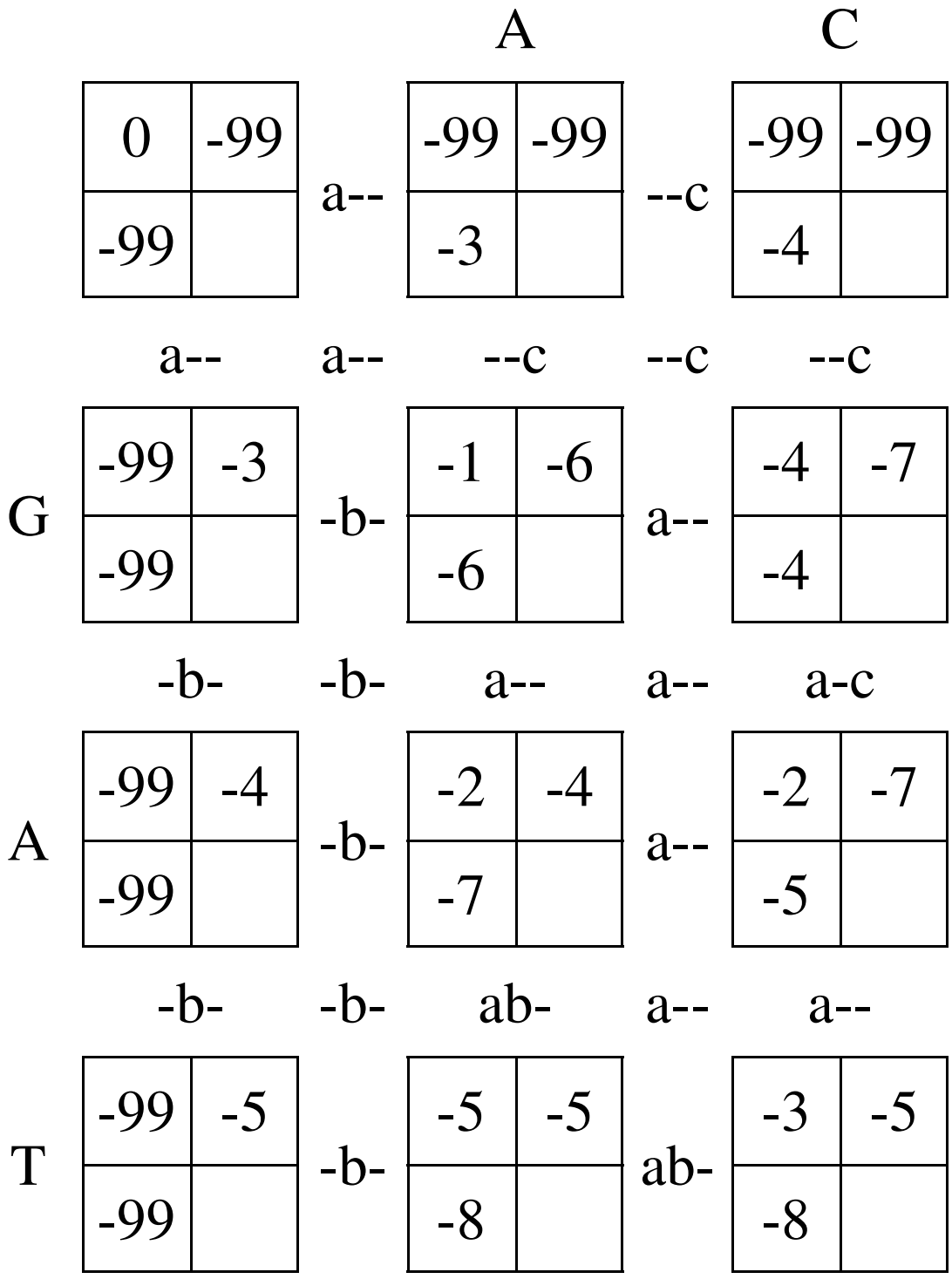
Each entry of the matrix becomes three entries, one for each direction into that cell.



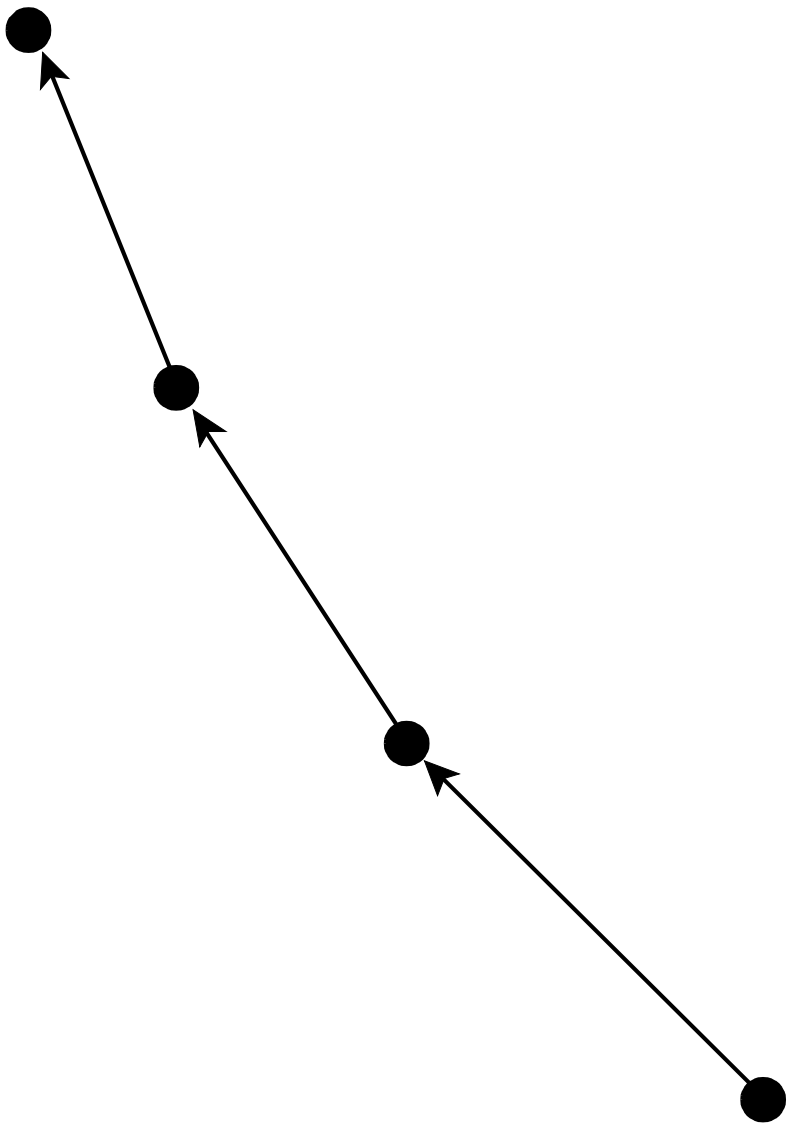
# A Simple Affine Gap Alignment



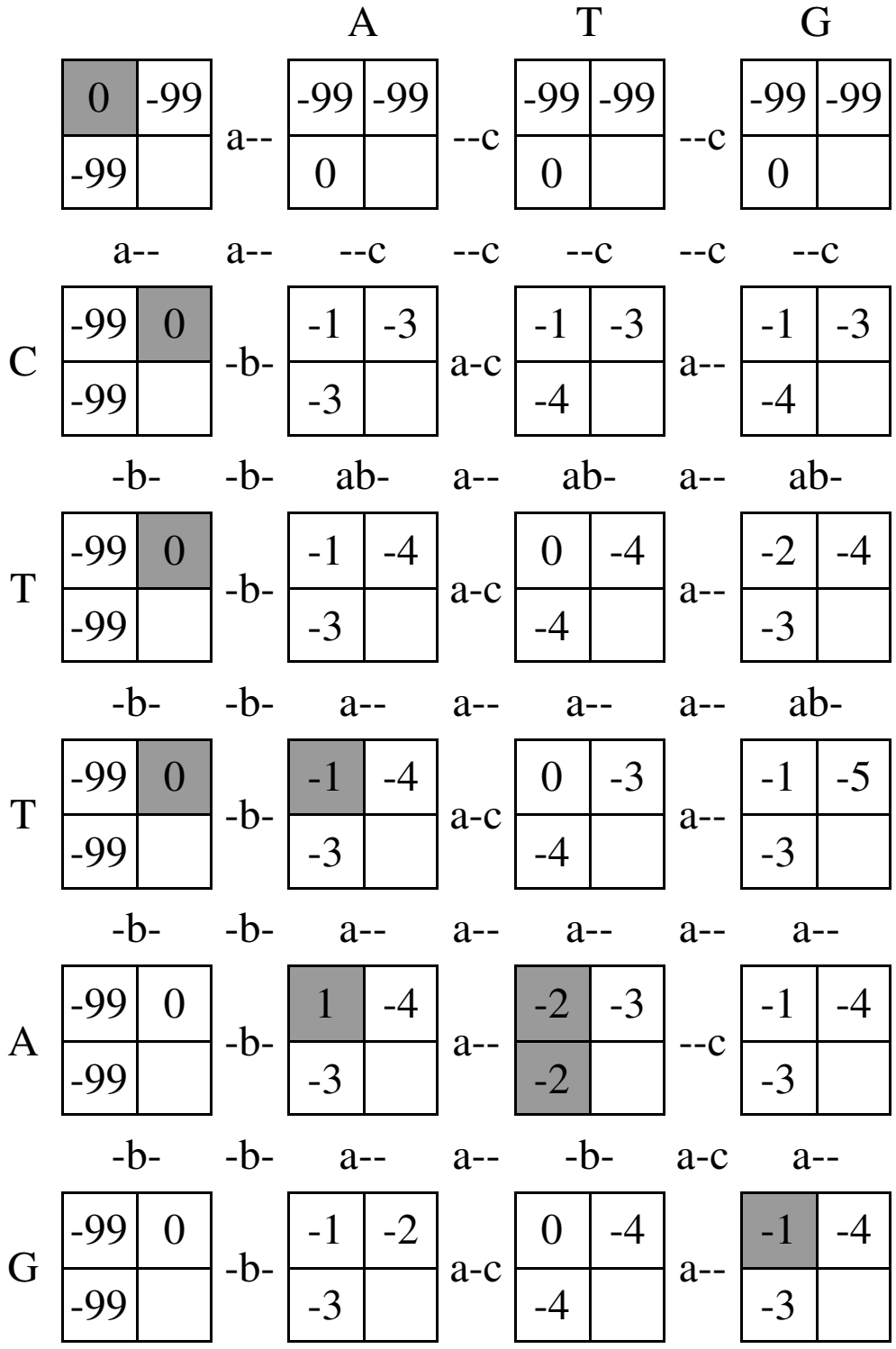
# A Simple Affine Gap Alignment



# Tracing Back



# Slightly More Interesting



# 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 |
| | | | | | | | | | | | | | | |

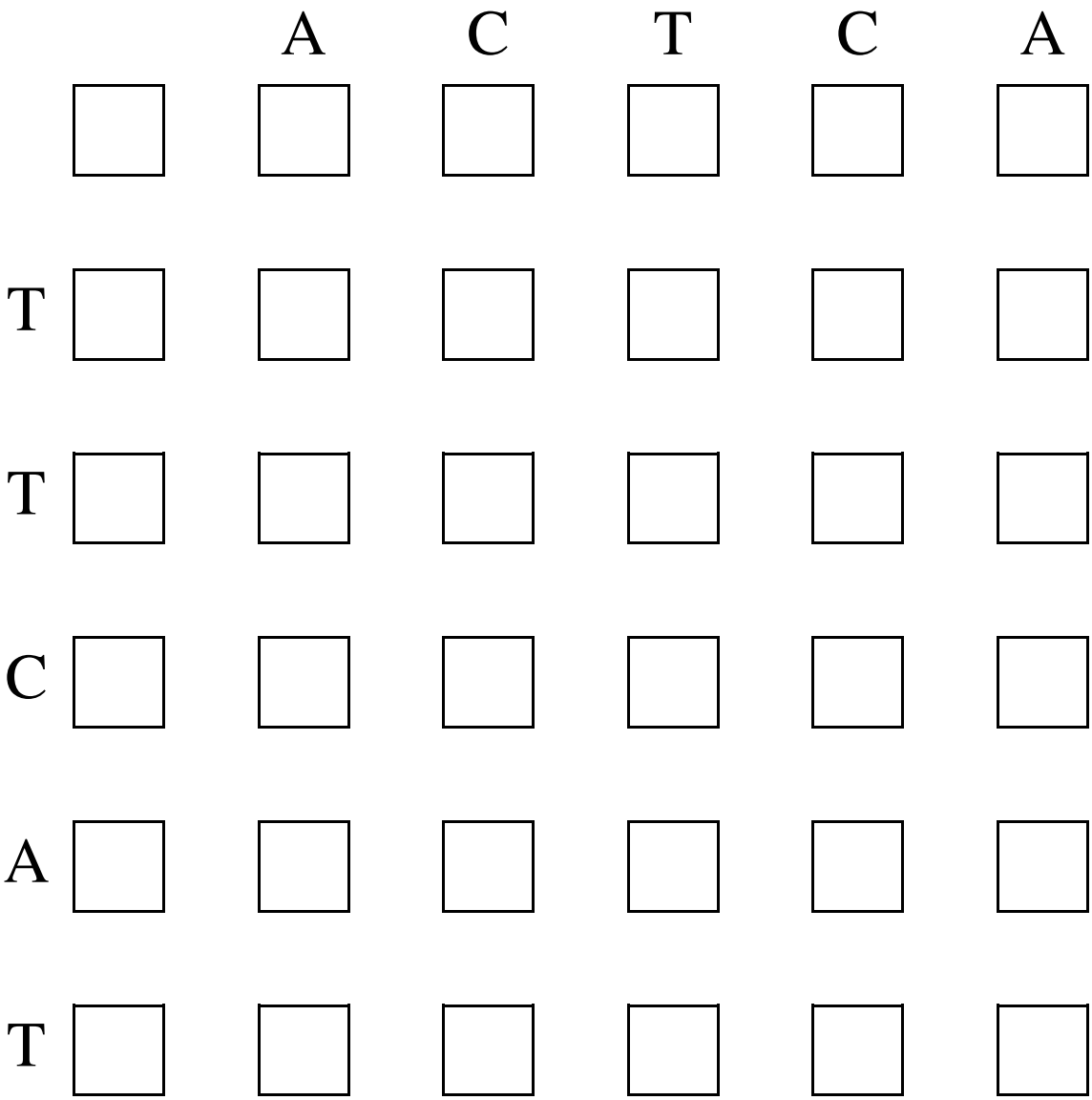
Let's put running totals in the bottom row:

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 |

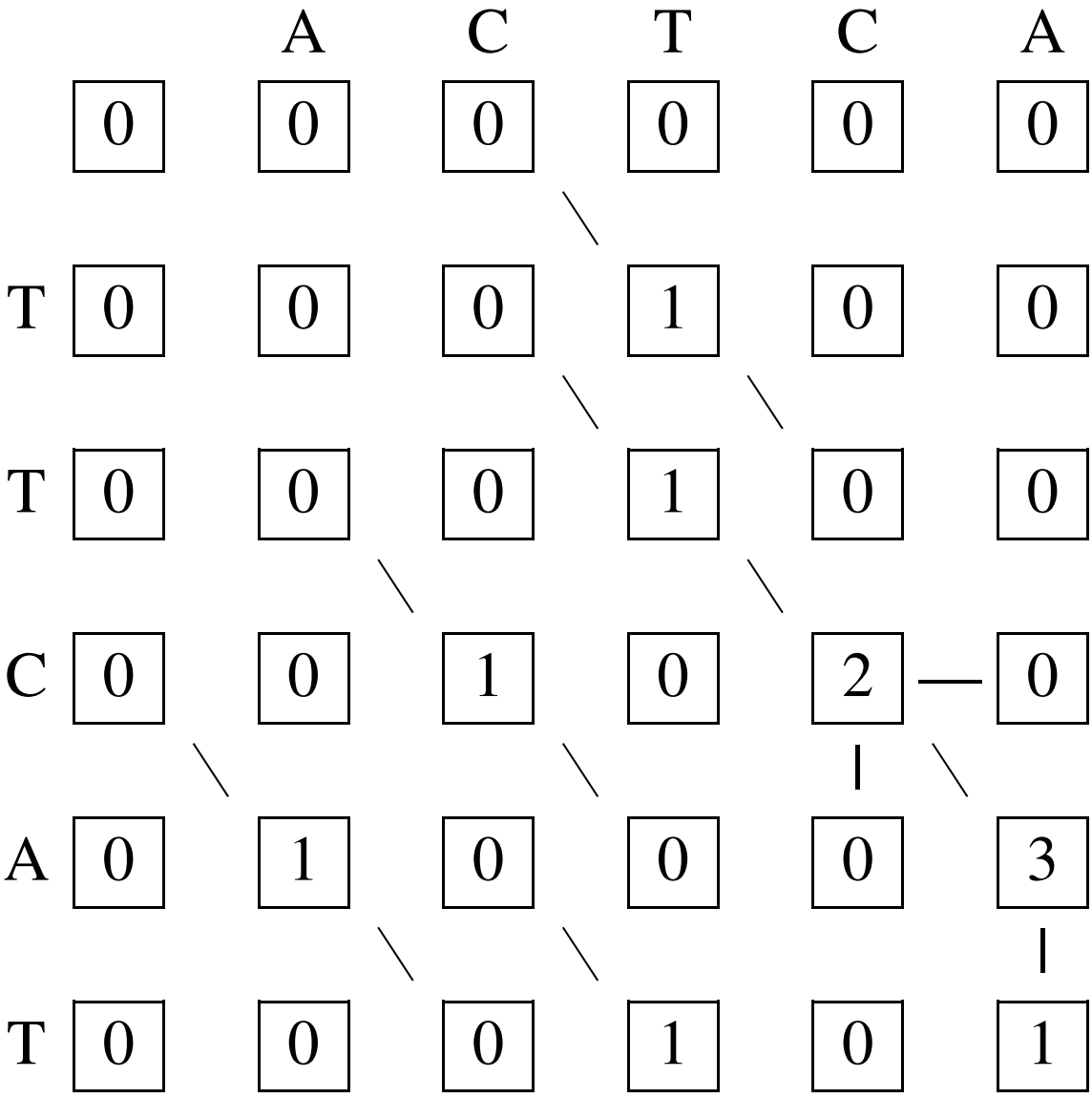
# Local Alignment Algorithm

(Smith-Waterman)



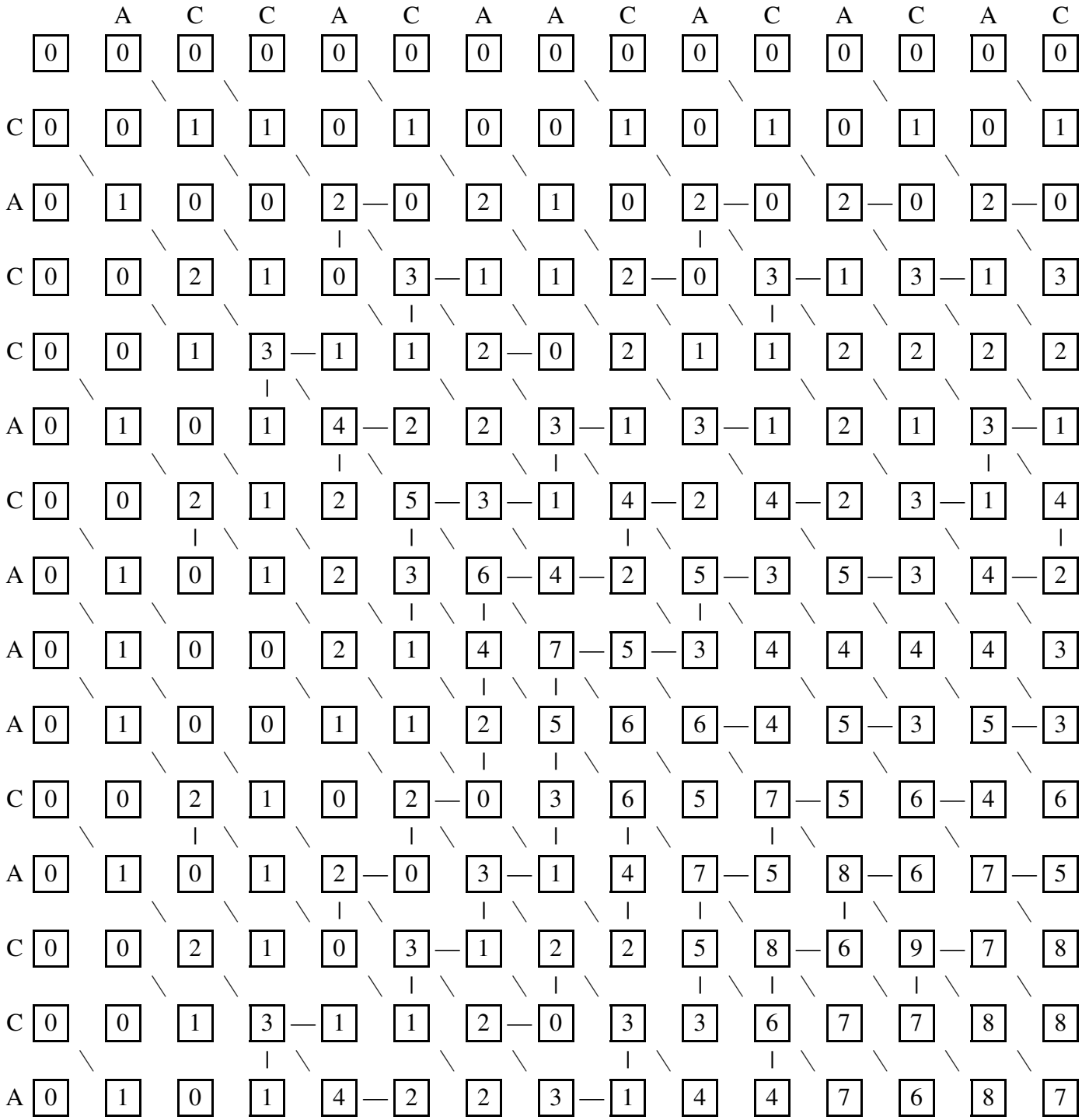

The algorithm is the same as our original alignment algorithm, except that if an entry is about to be negative, we make it “0" instead.

# Tracing Back in Smith-Waterman

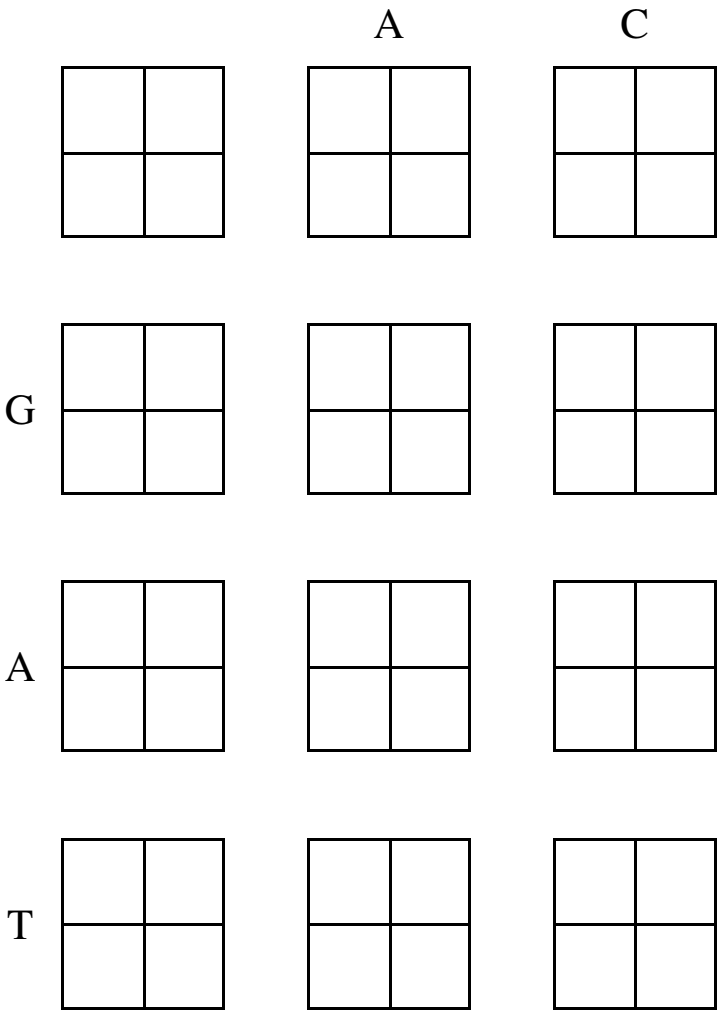



To find an optimal alignmnet, find a largest entry anywhere in the matrix and trace it back, up to but not including a “0.”

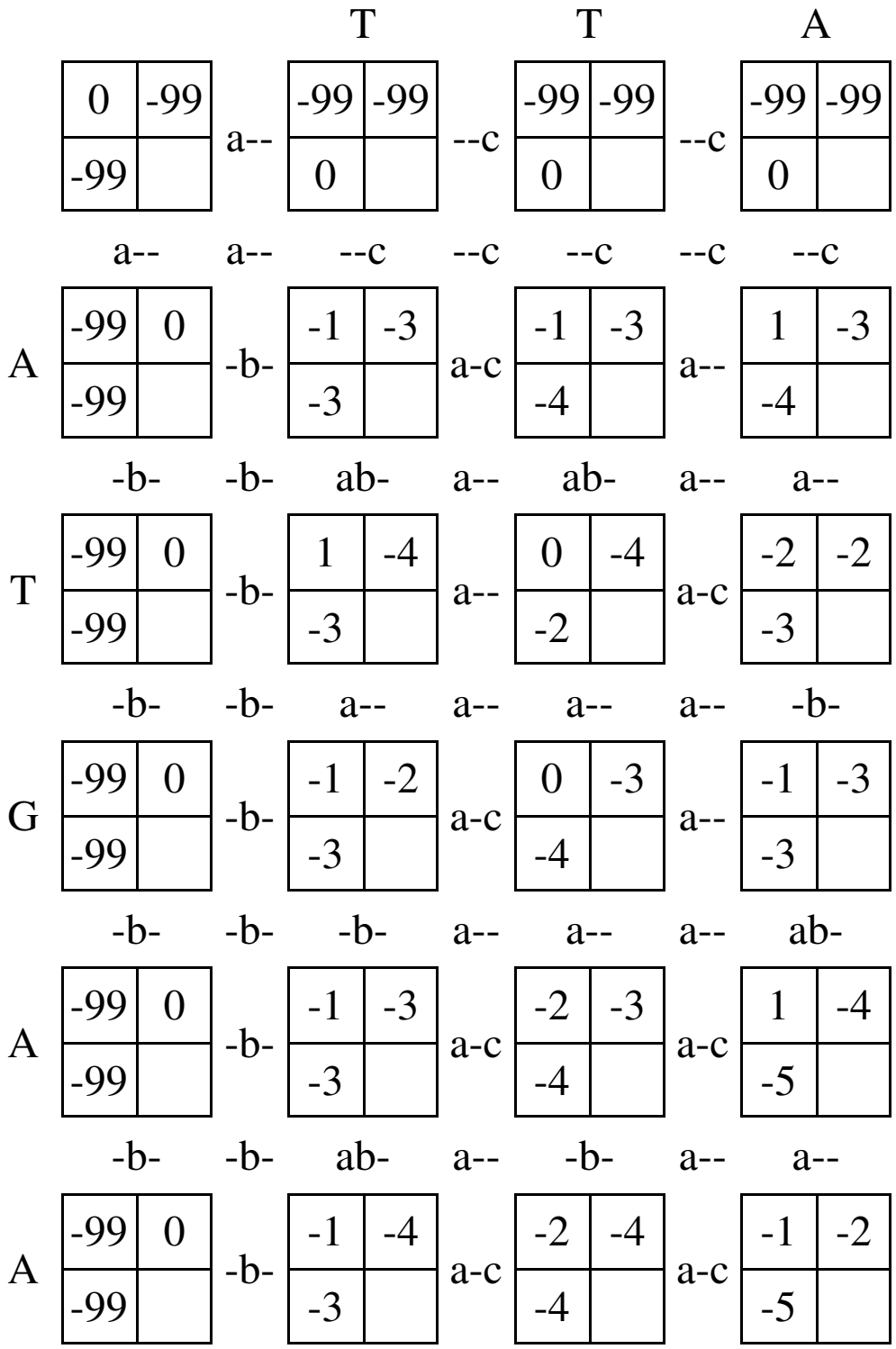
# Tracing Back in Smith-Waterman



# 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:

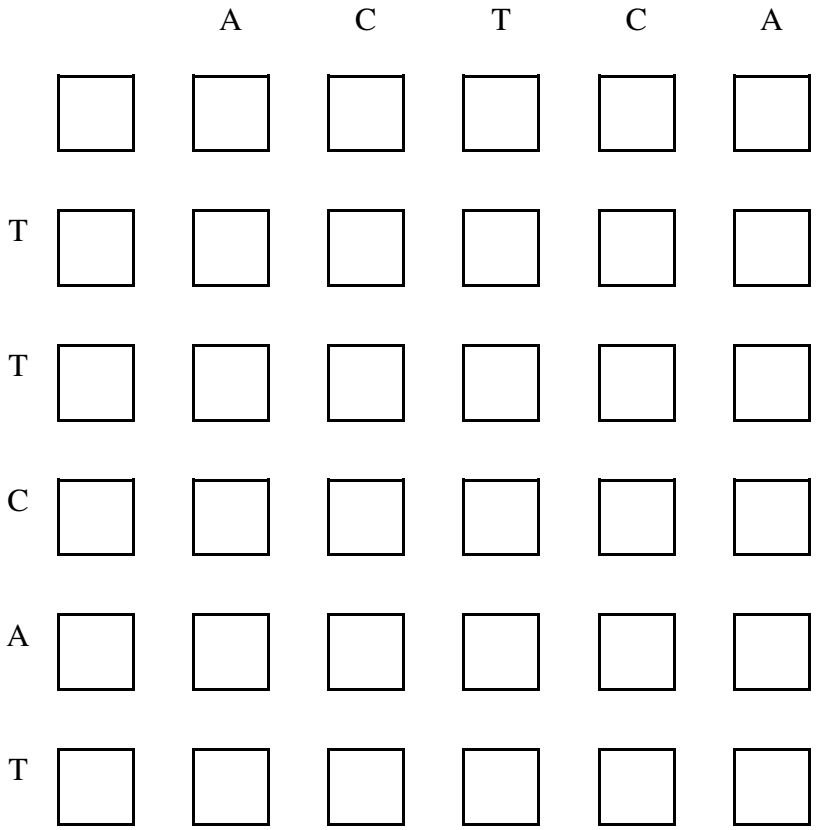
```
TGCTG
TAC-G
```

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

# Handout #4 — Local Alignment Algorithm

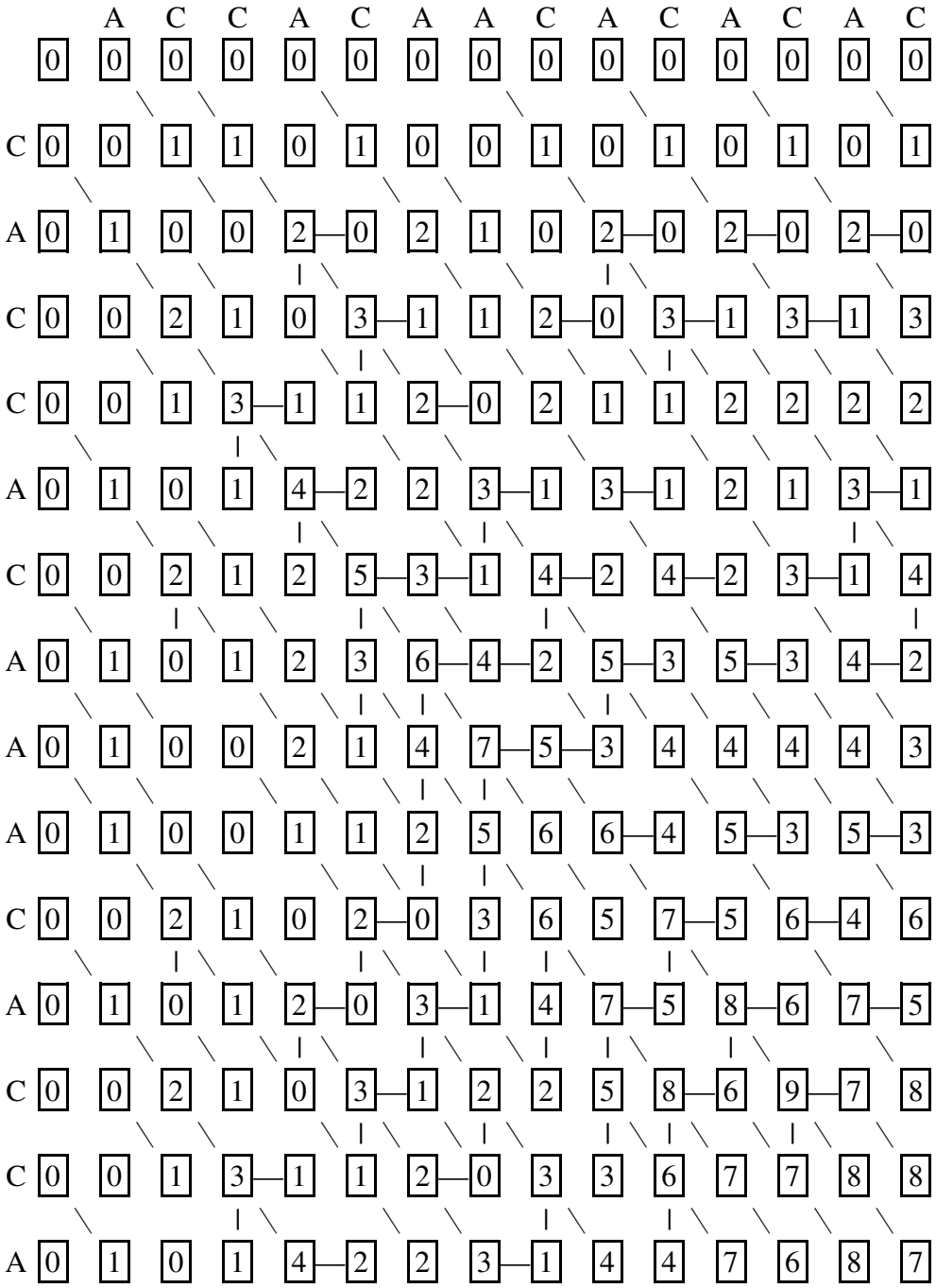
The algorithm is the same as our original alignment algorithm, except that if an entry is about to be negative, we make it “0" instead.

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.



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

Find all optimal local alignments indicated by this scoring matrix.

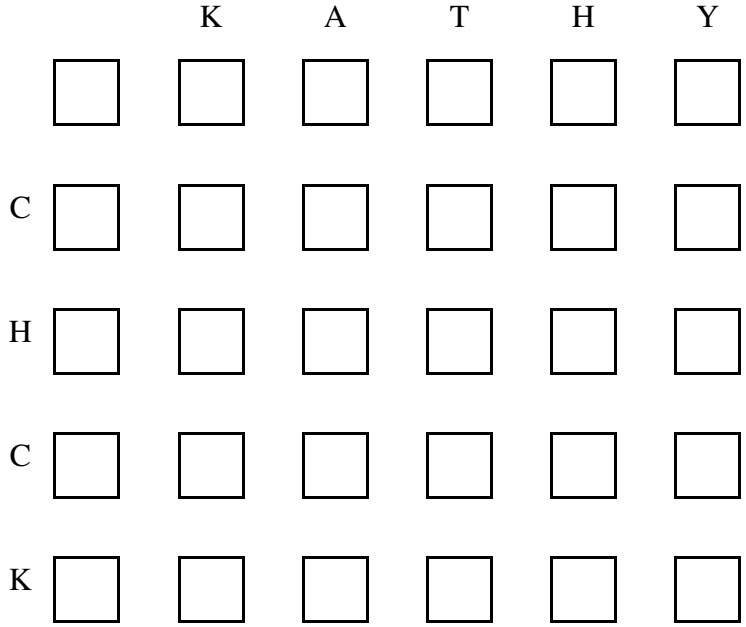


# Handout #6 — Smith-Waterman with an Amino Acid Substitution Matrix

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.



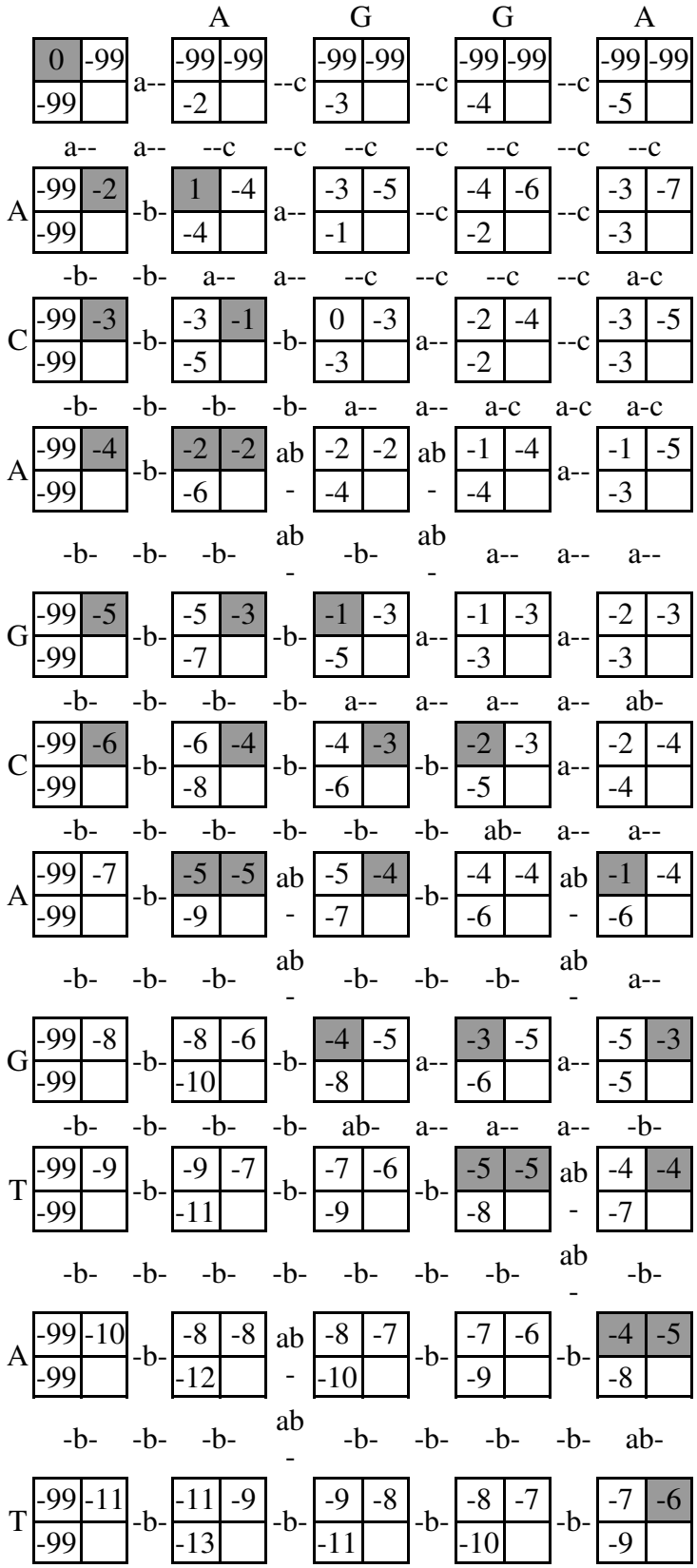
## Handout #0 — Workshop Session — Supplemental Note Page

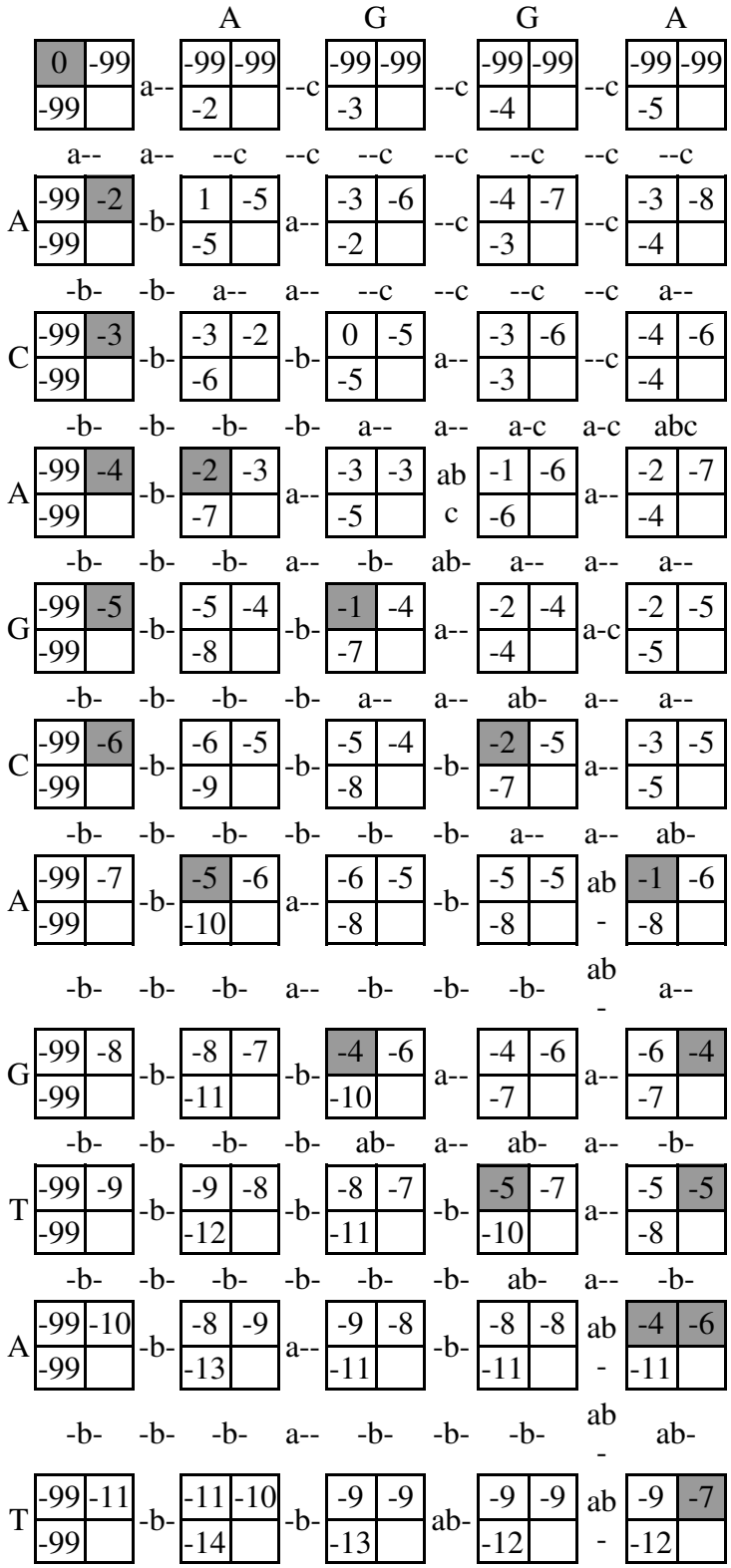
| | |
|---|---|
| ACAGCAGTAT<br>A--G--G-A- | ACAGCAGTAT<br>--AGGA--- |

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
```

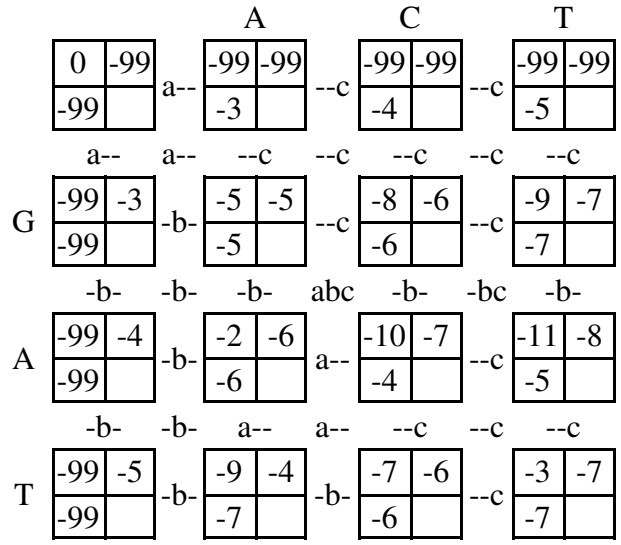

A G G A
0 -99 -99 -99 -99 -99 -99 -99 -99 -99 -99
-99 -2 -3 -4 -5
a-- a-- --c --c --c --c --c --c --c
A -99 -2 -b- 1 -4 a-- -3 -5 --c -4 -6 --c -3 -7
-99 -4 -1 -2 -3
-b- -b- a-- a-- --c --c --c --c a-c
C -99 -3 -b- -3 -1 -b- 0 -3 a-- -2 -4 --c -3 -5
-99 -5 -3 -2 -3
-b- -b- -b- -b- a-- a-- a-c a-c a-c
A -99 -4 -b- -2 -2 ab- -2 -2 ab- -1 -4 a-- -1 -5
-99 -6 -4 -4 -3
-b- -b- -b- ab- -b- ab- a-- a-- a--
G -99 -5 -b- -5 -3 -b- -1 -3 a-- -1 -3 a-- -2 -3
-99 -7 -5 -3 -3
-b- -b- -b- -b- a-- a-- a-- a-- ab-
C -99 -6 -b- -6 -4 -b- -4 -3 -b- -2 -3 a-- -2 -4
-99 -8 -6 -5 -4
-b- -b- -b- -b- -b- -b- ab- a-- a--
A -99 -7 -b- -5 -5 ab- -5 -4 -b- -4 -4 ab- -1 -4
-99 -9 -7 -6 -6
-b- -b- -b- ab- -b- -b- -b- ab- a--
G -99 -8 -b- -8 -6 -b- -4 -5 a-- -3 -5 a-- -5 -3
-99 -10 -8 -6 -5
-b- -b- -b- -b- ab- a-- a-- a-- -b-
T -99 -9 -b- -9 -7 -b- -7 -6 -b- -5 -5 ab- -4 -4
-99 -11 -9 -8 -7
-b- -b- -b- -b- -b- -b- -b- ab- -b-
A -99 -10 -b- -8 -8 ab- -8 -7 -b- -7 -6 -b- -4 -5
-99 -12 -10 -9 -8
-b- -b- -b- ab- -b- -b- -b- -b- ab-
T -99 -11 -b- -11 -9 -b- -9 -8 -b- -8 -7 -b- -7 -6
-99 -13 -11 -10 -9


A G G A
0 -99 -99 -99 -99 -99 -99 -99 -99 -99 -99
-99 -2 -3 -4 -5
a-- --c --c --c
A -99 -2 1 -5 -3 -6 -4 -7 -3 -8
-99 -5 -2 -3 -4
-b- a-- --c --c --c
C -99 -3 -3 -2 0 -5 -3 -6 -4 -6
-99 -6 -5 -3 -4
-b- -b- a-- a-c abc
A -99 -4 -2 -3 -3 -3 -1 -6 -2 -7
-99 -7 -5 -6 -4
ab c
-b- -b- -b- a-- -b- ab- a-- a--
G -99 -5 -5 -4 -1 -4 -2 -4 -2 -5
-99 -8 -7 -4 -5
-b- -b- -b- -b- a-- a-- ab- a--
C -99 -6 -6 -5 -5 -4 -2 -5 -3 -5
-99 -9 -8 -7 -5
-b- -b- -b- -b- -b- -b- a-- a-- ab-
A -99 -7 -5 -6 -6 -5 -5 -5 ab -1 -6
-99 -10 -8 -8 - -8
-b- -b- -b- a-- -b- -b- -b- ab- a--
G -99 -8 -8 -7 -4 -6 -4 -6 -6 -4
-99 -11 -10 -7 -7
-b- -b- -b- -b- ab- a-- ab- a-- -b-
T -99 -9 -9 -8 -8 -7 -5 -7 -5 -5
-99 -12 -11 -10 -8
-b- -b- -b- -b- -b- -b- ab- a-- -b-
A -99 -10 -8 -9 -9 -8 -8 -8 ab -4 -6
-99 -13 -11 -11 - -11
-b- -b- -b- a-- -b- -b- -b- ab- ab-
T -99 -11 -11 -10 -9 -9 -9 -9 ab -9 -7
-99 -14 -13 -12 - -12

## Exercises — Local Alignment

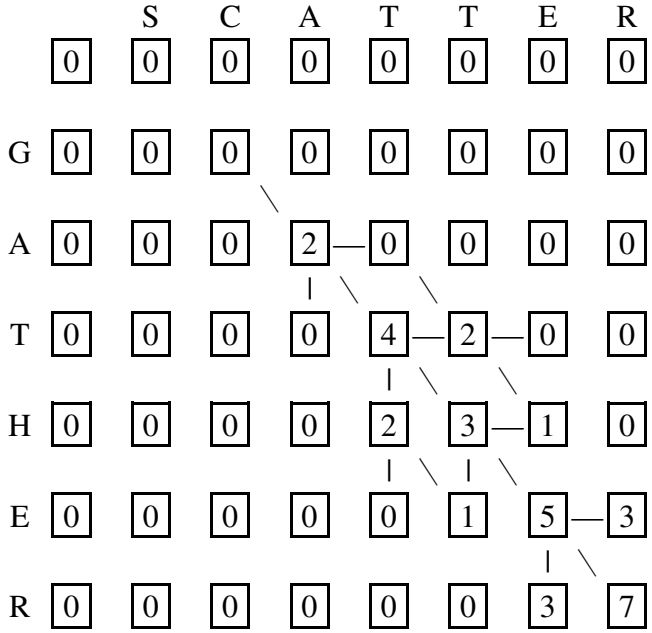
## Quick Concepts:

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




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?



## Presentation Problems:

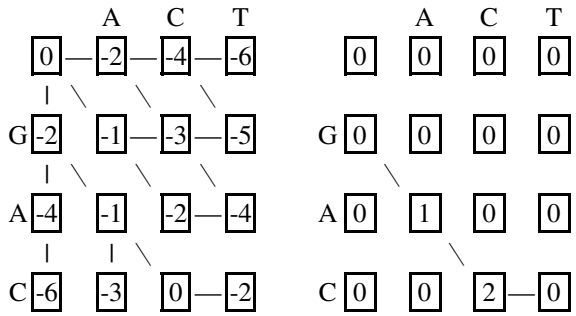
4. The strings "GATTACA" and "TAGCAT" have been aligned using an affine gap penalty function in which matches score +1, mismatches cost -1, gaps cost -2 to start and -1 to continue. Complete the alignment begun in the table below.

| | | G | A | T | T | A | C | A |
|---|---|---|---|---|---|---|---|---|
| | 0 / -99 / -99 | -99 / -99 / -2 | -99 / -99 / -3 | -99 / -99 / -4 | -99 / -99 / -5 | -99 / -99 / -6 | | |
| T | -99 / -2 / -99 | -1 / -4 / -4 | -3 / -5 / -3 | -2 / -6 / -4 | -3 / -7 / -4 | -6 / -8 / -5 | | |
| A | -99 / -3 / -99 | -3 / -3 / -5 | 0 / -5 / -5 | -4 / -4 / -2 | -3 / -5 / -3 | -2 / -7 / -4 | | |
| G | -99 / -4 / -99 | -2 / -4 / -6 | -4 / -2 / -4 | | | | | |
| C | -99 / -5 / -99 | -5 / -4 / -7 | -3 / -3 / -6 | | | | | |
| A | -99 / -6 / -99 | -6 / -5 / -8 | -3 / -4 / -7 | | | | | |
| T | -99 / -7 / -99 | -7 / -6 / -9 | -6 / -5 / -8 | | | | | -4 / -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)?



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?

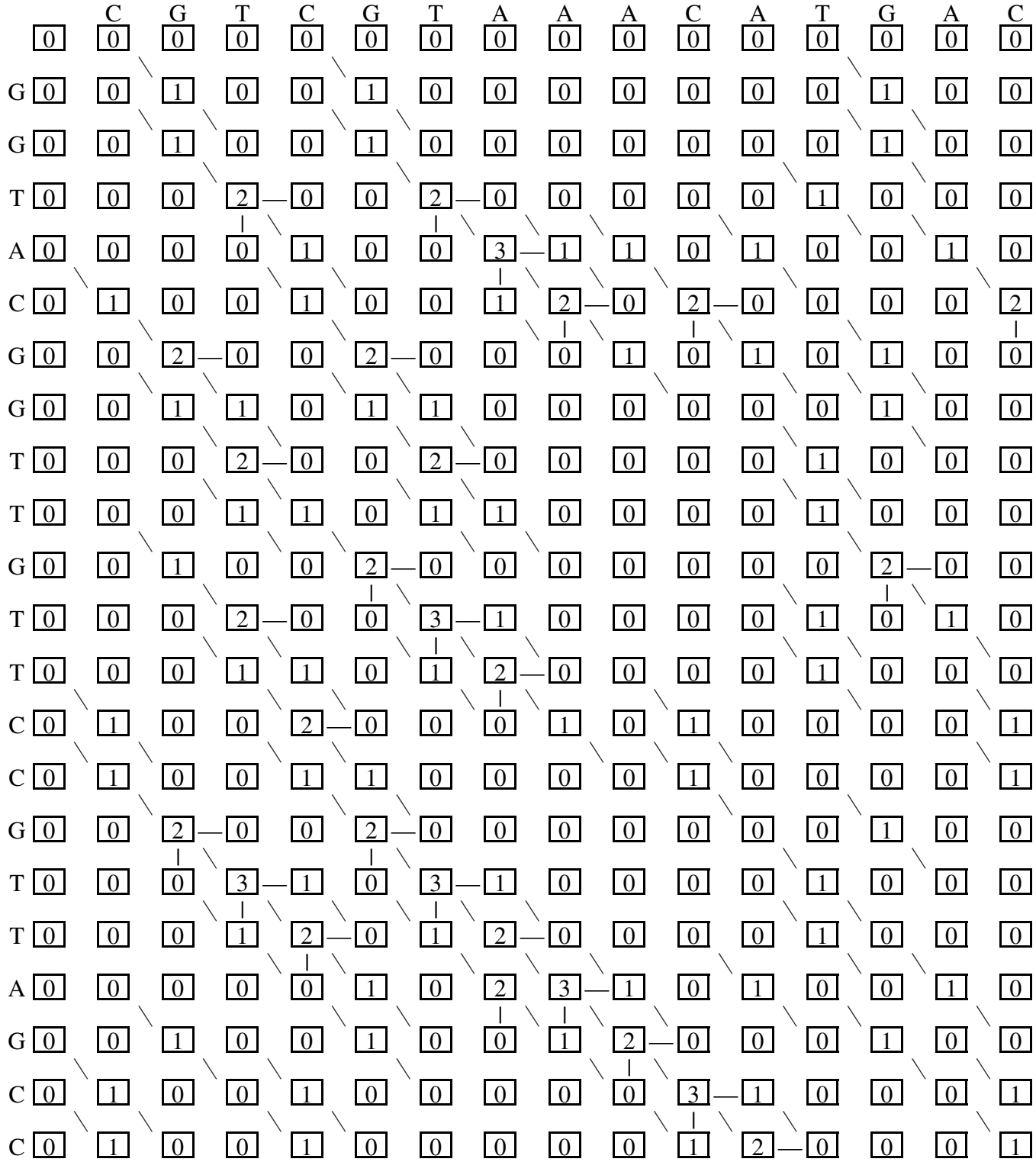
```
AMINO---ACID
GLIDERBRACES
```

| | 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 |
|---|---|---|---|---|---|---|
| | 0 | 0 | 0 | 0 | 0 | 0 |
| S | 0 | | | | | |
| W | 0 | | | | | |
| I | 0 | | | | | |
| P | 0 | | | | | |
| E | 0 | | | | | |

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



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 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 -99 / -99 | a-- | -99 -99 / 0 | --c | -99 -99 / 0 | --c | -99 -99 / 0 | --c | -99 -99 / 0 | --c | -99 -99 / 0 | --c | -99 -99 / 0 | --c | -99 -99 / 0 |
| | a-- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --c | --- | --- | --- |
| C | -99 0 / -99 | --- | 0 0 / 0 | --- | 0 0 / 0 | --- | 0 0 / 0 | --- | 0 0 / 0 | --- | 0 0 / 0 | --- | 5 0 / 0 | a-- | 0 0 / 3 |
| | -b- | --- | --- | --- | --- | abc | --- | abc | --- | --- | --- | --- | a-- | a-- | --c |
| T | -99 0 / -99 | --- | 0 0 / 0 | --- | 0 0 / 0 | --- | 5 0 / 0 | a-- | 5 0 / 3 | a-- | 0 0 / 3 | --c | 0 3 / 2 | -bc | 4 1 / 1 |
| | -b- | --- | --- | abc | --- | --- | a-- | a-- | a-- | a-- | --c | --c | -b- | -b- | a-- |
| A | -99 0 / -99 | --- | 0 0 / 0 | --- | 5 0 / 0 | a-- | 0 3 / 3 | --c | 4 3 / 2 | a-- | 10 1 / 2 | a-- | 2 2 / 8 | --c | 8 2 / 7 |
| | -b- | --- | --- | --- | a-- | a-- | -b- | -bc | ab- | a-- | a-- | a-- | --c | --c | a-- |
| T | -99 0 / -99 | --- | 0 0 / 0 | --- | 0 3 / 0 | -b- | 10 2 / 1 | a-- | 8 2 / 8 | --c | 3 8 / 7 | -bc | 9 6 / 6 | a-- | 7 6 / 7 |
| | -b- | --- | --- | --- | -b- | -b- | a-- | a-- | a-c | a-c | -b- | | | | |
| T | -99 0 / -99 | --- | 0 0 / 0 | --- | 0 2 / 0 | -b- | 8 8 / 0 | ab- | 15 6 / 6 | a-- | 7 7 / 13 | | | | |
| | -b- | -b- | --- | --- | -b- | -b- | -b- | ab- | a-- | a-- | --c | | | | |
| G | -99 0 / -99 | --- | 5 0 / 0 | a-- | 0 1 / 3 | --c | 1 7 / 2 | -b- | 7 13 / 5 | -b- | 14 11 / 11 | | | | |
| | -b- | -b- | a-- | a-- | --c | --c | -b- | | | | | | | | |
| G | -99 0 / -99 | --- | 5 3 / 0 | a-- | 4 1 / 3 | a-c | 2 6 / 2 | | | | | | | | |
| | -b- | --- | a-- | a-- | a-- | a-- | -b- | | | | | | | | |
| A | -99 0 / -99 | --- | 0 3 / 0 | -b- | 10 2 / 1 | a-- | 3 5 / 8 | | | | | | | | |
| | -b- | --- | -b- | -b- | a-- | a-- | --c | | | | | | | --c | a-- |
| C | -99 0 / -99 | --- | 0 2 / 0 | -b- | 2 8 / 0 | -b- | 9 6 / 6 | | | | | | | a-- | 14 16 / 20 |

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