Lecture Notes for INF281 Basics of Bioinformatics Sequence Analysis

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Contents

Part I

1 Introduction

1.1 Introduction to Molecular Biology

Molecular biology is the study of biology focusing on organisms and cells at the molecular level.

Five essential facts about cells

1. Two primary types of cells - eukaryotes and prokaryotes

- Eukaryote: animals & plants
- Prokaryote: bacteria & archaea

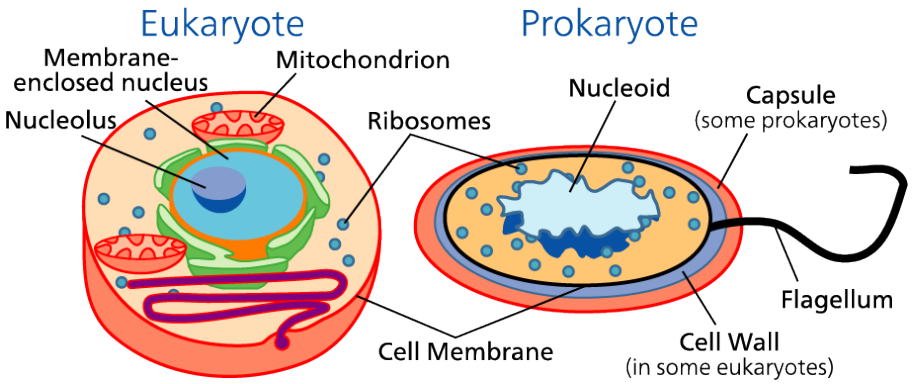


Figure 1.1: Eukaryotic and prokaryotic cells (source: Science Primer, Wikimedia Commons)

2. Cell size - around 1 to 100 micrometers

- Cell Size and Scale: `http://learn.genetics.utah.edu/content/cells/scale`

3. The number of cells

- Prokaryotes: 1 cell
- Human: Estimate of 15 trillion cells

4. An animal cell and cell organelles

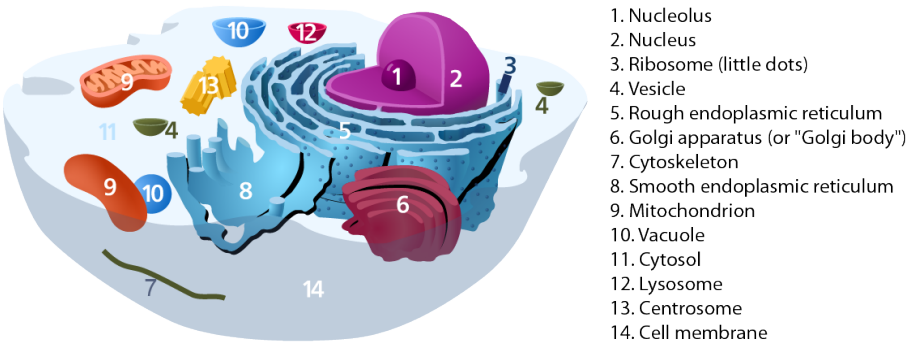


Figure 1.2: An animal cell and organelles (source: Kelvinsong, Wikimedia Commons)

5. Cellular processes

- Cell growth, cell development, cell signaling,
- Example: `http://www.nature.com/nrg/multimedia/rnai`

Central dogma of molecular biology

It describes the information flow within a cell.

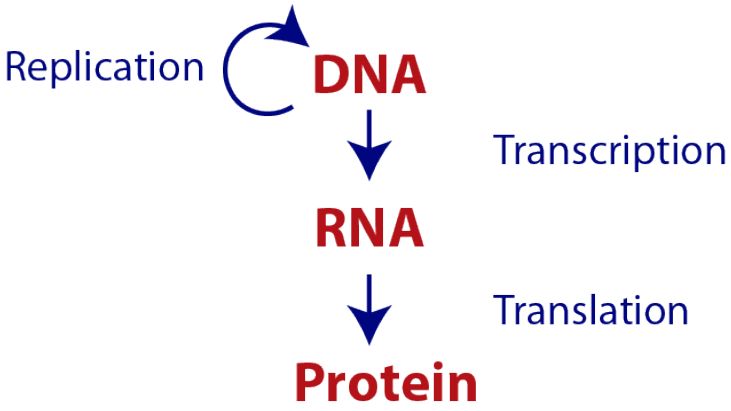


Figure 1.3: Central dogma of molecular biology

DNA (deoxyribonucleic acid)

DNA stores genetic information. It has four different bases: cytosine (C), guanine (G), adenine (A), and thymine (T).

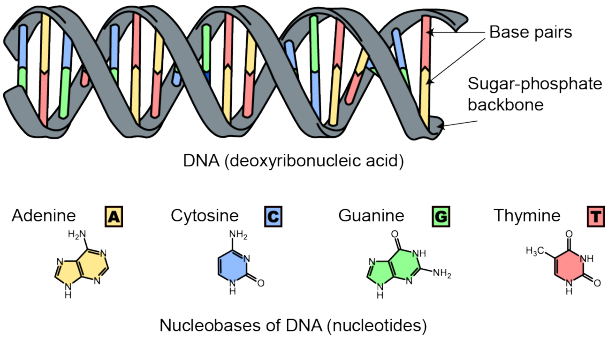


Figure 1.4: DNA double helix and base pairs
(modified from the original version by Sponk, Wikimedia Commons)

Base pair matching (Watson-Crick base pair)

Adenine (A) pairs with thymine (T), whereas cytosine (C) pairs with guanine (G).

```
DNA strand1: ACGT
             ||||
DNA strand2: TGCA
```

RNA (Ribonucleic acid)

RNA has various biological roles and several sub-classes. Messenger RNAs (mRNAs) convey genetic information. It has four different bases: cytosine (C), guanine (G), adenine (A), and uracil (U).

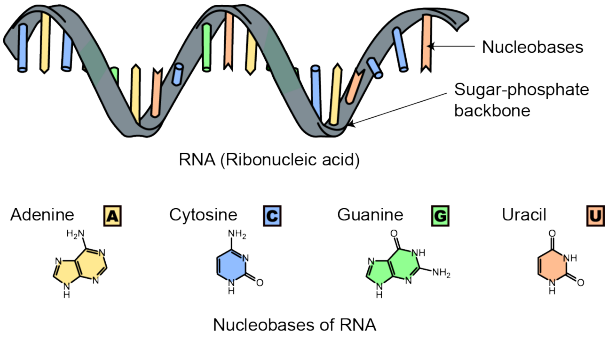


Figure 1.5: Single strand RNA
(modified from the original version by Sponk, Wikimedia Commons)

Transcription: mRNAs are transcribed from DNAs

```
DNA: ACGT -------> RNA: ACGU
        Transcription
```

Protein

Proteins are large molecules consisting of amino acids. There are 20 common amino acids.

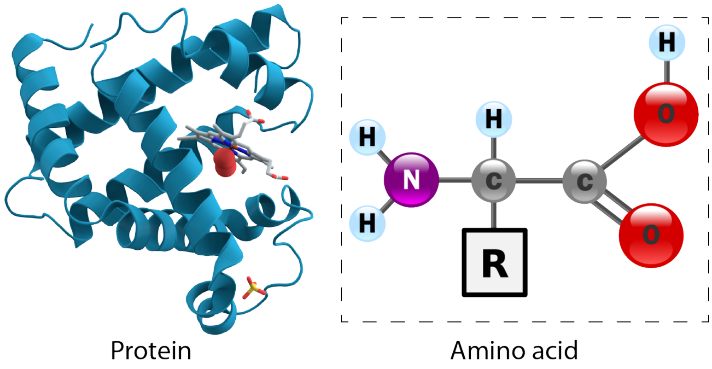


Figure 1.6: Protein 3D structure and amino acids
(sources: AzaToth, Wikimedia Commons, YassineMrabet, Wikimedia Commons)

Translation: Amino-acids are translated from mRNAs

```
mRNA: GUC -------> AA: Valine
        Translation
```

Universal genetic code

A codon consists of three nucleic acids. Single-letter or three-letter names can be used for amino acids.

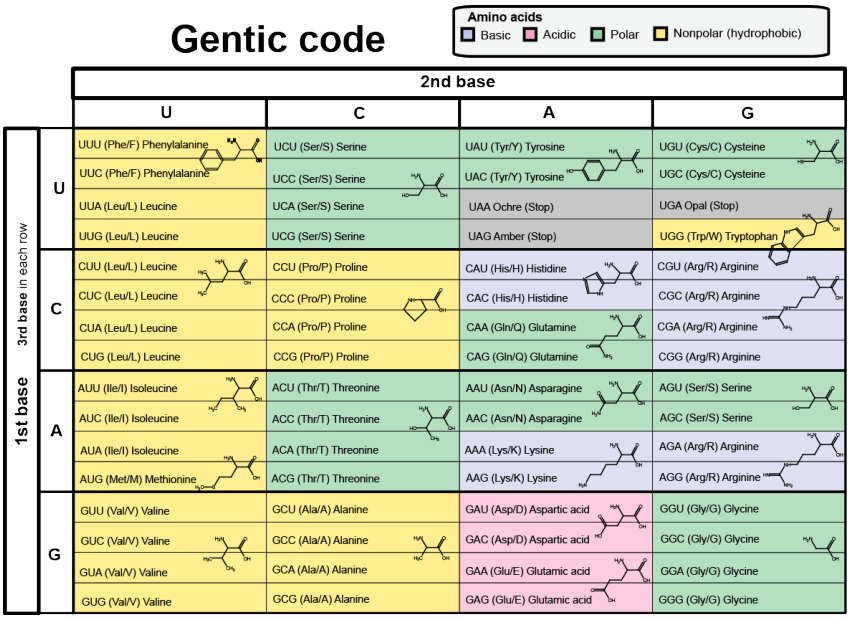


Figure 1.7: Universal genetic code
(modified from the original version by Häggström, Wikimedia Commons)

Cellular functions of proteins

- Enzymes: catalyze chemical reaction
- Cell signaling: hormone (e.g. insulin), antibodies,
- Structural: collagen, cartilage, keratin,

Exercises 1.1

1. Draw a simple diagram of the central dogma of molecular biology and briefly explain the information flow of the molecules.
2. What are the DNA sequences of the opposite strand for the following DNA sequences?

```
Seq1 CCGATT
Seq2 TTACGC
Seq3 ACGCGC
```

3. What are the mRNA sequences transcribed from the following DNA sequences?
4. What are the polypeptide sequences translated from the following mRNA sequences? Answer them with both one-letter and three letter names.

```
Seq1 AUGUUUUAA
Seq2 GCAGCAAAA
```

1.2 Introduction to Biotechnology

Biotechnology is the use of laboratory techniques to study living organism and cells.

Applications of biotechnology

Branches of biotechnology can be explained with different colors.

- Red: medical processes
- Green: agricultural processes
- White: industrial processes
- Blue: marine and aquatic applications

Laboratory tools and equipment

Figure 1.8: Pipette, centrifuge, thermal cycler, and DNA sequencer (sources: Domain, Manske, Rror, RE73 via Wikimedia Commons)

Human genome project

It was a large-scale international research project to determine the whole DNA sequences of human.

- 1990 - 2003
- $2.7 billion

Next generation sequencing

Sequence technologies have been rapidly advanced since the human genome project.
Example: sequence a whole human genome with Illumina HiSeq X Ten.

- One day
- $1000

Protein sequencing

Proteins are generally more studied than DNAs and RNAs, but the whole proteome is generally harder to analyze than the whole genome. MS (mass-spectrometry) based technologies are widely used to sequence proteins.

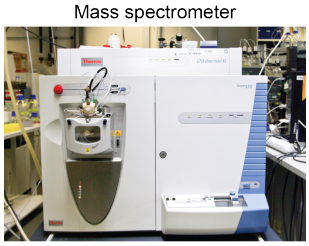


Figure 1.9: Orbitrap mass spectrometer (source: Wiòrkiewicz, Wikimedia Commons)

1.3 Bioinformatics

Bioinformatics uses computational approaches to solve problems in life sciences. It is based on computer science.

Similar or almost equivalent disciplines

- Biostatistics
- Biophysics
- Systems biology
- Computational biology

Not much related with bioinformatics

- Health informatics
- Forensic science

Scope of INF281

We mainly cover the following fields of bioinformatics in this course.

- Pairwise alignment
- Database search
- Statistical evaluation
- Multiple alignment
- Phylogenetic tree
- Scoring scheme
- Sequence patterns

Popular bioinformatics programs

BLAST and ClustalW are popular tools for sequence analysis.

- BLAST: a program for database search

  URL: `http://blast.ncbi.nlm.nih.gov`

- ClustalW: a program for multiple alignments

  URL: `http://www.ch.embnet.org/software/ClustalW.html`

Rank	Title	Times cited
1	Protein measurement with the folin phenol reagent	305148
2	Cleavage of structural proteins during the assembly of the head of bacteriophage T4	213005
3	A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding	155530
4	DNA sequencing with chain-terminating inhibitors	65335
5	Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction	60397
6	Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications	53349
7	Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density	46702
8	Density-functional thermochemistry. III. The role of exact exchange	46145
9	A simple method for the isolation and purification of total lipides from animal tissues	45131
10	**Clustal W**: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice	40289
11	Nonparametric estimation from incomplete observations	38600
12	**Basic local alignment search tool**	38380
13	A short history of SHELX	37978
14	**Gapped BLAST and PSI-BLAST**: A new generation of protein database search programs	36410
15	A revised medium for rapid growth and bio assays with tobacco tissue cultures	36132

Table 1.1: The 15 most cited papers of all time
(The top 100 papers, Van Noorden, Maher, and Nuzzo, *Nature*, 2014)

Part II

2 Global pairwise alignment

2.1 Pairwise alignment

A pairwise alignments is a basic sequence structure that consits of two sequences. A global alignment stretches to the whole part of two sequences, whereas a local alignment usually contains only part of the sequences.

Components of pairwise alignment

We name two sequences as database or d and query or q through this course. They may represent sequences from two different species or organisms.

Identical sequences.

```
q: ACGT
d: ACGT
```

One mismatch.

```
q: ACGT
d: ACGA
```

The '-' symbol represents a blank. A single or a set of multile blanks further reprents a gap, which is an indication of insertion or deletion in the course of evoluation between two organisms.

```
q: ACGT
d: A-GT
```

N.B. A gap cannot be aligned with another gap.

Example of a simple scoring scheme

- Match: 1
- Mismatch: 0
- Gap penalty: 1 (use -1 for the actual calculation)

We may use the following notation.

- $R_{ab} = 1$ for a = b
- $R_{ab} = 0$ for a $\neq$ b
- g = 1

Exercise 2.1

Use the simple scoring scheme above and calculate the scores of the following two alignments.

```
Alignment 1
    q: GCA-GCA
    d: GA-TG-A
```

```
Alignment 2
    q: GCA-GCA
    d: GA-TG-A
```

2.2 Alignment by brute–force

A brute–force approach finds the alignment with the highest score by simply considering all possible alignments and calculates the score for each of them.

An example of brute–force approach

We find the optimal alignment for the follwoing sequences by using the scoring scheme below.

Sequences:

```
q: AG
d: ACG
```

Scoring scheme:
$R_{ab} = 1$ for a = b
$R_{ab} = 0$ for a $\neq$ b
g = 1

1. The length of alignment

- Maximum length: length(q) + length(d)
- Minimum length: max(length(q), length(d))

2. All possible alignments when length = 5

```
---AG    A---G    A--G-    AG---    --A-G
ACG--    -ACG-    -AC-G    --ACG    AC-G-

--AG-    -AG--    -A--G    -A-G-    A-G--
AC--G    A--CG    A-CG-    A-C-G    -A-CG
```

3. All possible alignments when length = 4

```
A--G    A-G-    AG--    A--G    -A-G    -AG-
ACG-    AC-G    A-CG    -ACG    ACG-    AC-G

-AG-    A-G-    --AG    --AG    -A-G    AG--
A-CG    -ACG    ACG-    AC-G    A-CG    -ACG
```

4. All possible alignments when length = 3

```
-AG    A-G    AG-
ACG    ACG    ACG
```

5. Alignment with the best score

```
ACG
A-G
```

Score: 1

Search space size of the brute-force approach

The search space size is the number of all possible alignments. It is 25 (10 + 12 + 3) for the example above.

Rapid growth of search space size

```
Example 1
    q: ACGACG
    d: AGAG
```

Search space size: 1289

```
Example 2
    q: ACGACGACGACG
    d: AGAGAGAG
```

Search space size: 4,673,345

Exercise 2.2

Find the alignment with the best score for the sequences. Use the simple scoring scheme below.

Sequences:

```
q: A
d: AC
```

Scoring scheme:
$R_{ab} = 1$ for a = b
$R_{ab} = 0$ for a $\neq$ b
g = 1

1. What are the maximum and minimum lengths of the alignment?
2. What is the search space size when the brute-force approach is used?
3. Identify all possible alignments.
4. What is the best score?

2.3 Table representation of alignment

Several data strcutres can be used to repesent an alignment. The table representation is frequently used and also makes the process clear when we combine it with dynamic programming (DP) later.

Data structures and algorithms

It is important to consider the following aspects before solving computational problems.

1. Identify and analyze the problem you want to solve
2. Pick up an algorithm that can efficiently solve the problem
3. Decide a data structure that works with the algorithm of your choice

We use a table format (2D array) to solve global alignments by dynamic programming.

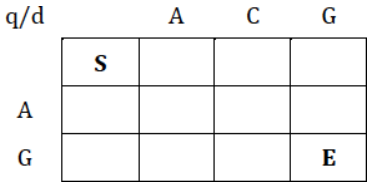
Example of table format

Alignment:

```
q: -AG-
d: A-CG
```

1. Initial setup

1. Make a table with the size of (1 + length(q)) by (1 + length(b))
2. Add the database sequence as column labels
3. And the query sequence as row labels

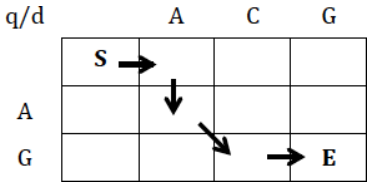


2. Add arrows

We use three types of arrows to form an alignment.

- Move diagonally: add the letters from q and d to the alignment
- Move vertically: add - and the letter from d to the alignment
- Move horizontally: add the letter from q and - to the alignment

It shoud start from S and stops at E.



Exercise 2.3

Find the corresponding alignments for Table 1, 2 and 3.

Table 1

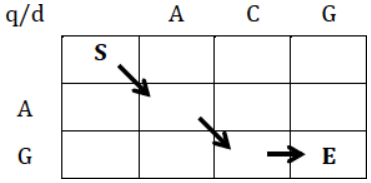


Table 2

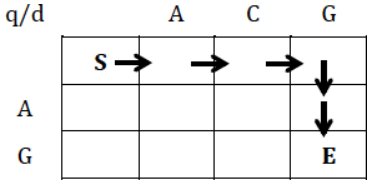
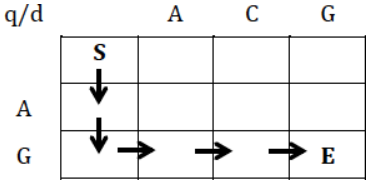


Table 3



2.4 Global alignment with DP

Dynamic programming (DP) provides a solution for a multi-stage decision process, in which larger decisions recursively nest smaller decisions.

Memorize the best score in a table cell

The most basic step of DP procedures is updatig a cell with the hightet score from the three different scores calculated from its adjacent cells. DP ends when all the table cells are updated.

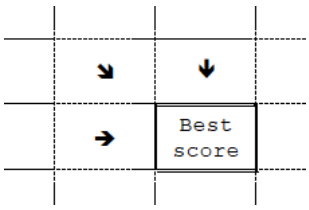
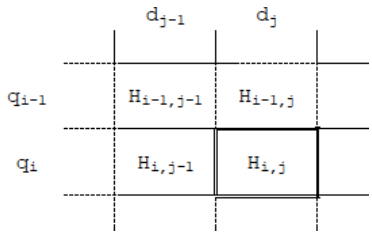


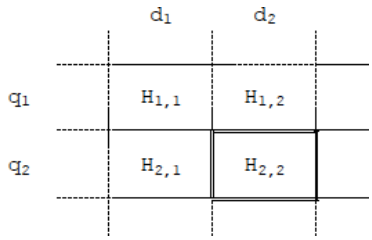
Table notation and indices

$H_{i,j}$ represents the score of the cell for the current update. $H_{i-1,j}$, $H_{i,j-1}$,and $H_{i-1,j-1}$ are the scores of the adjacent cells.

Cell $H_{i,j}$ and its adjcent cells



Example



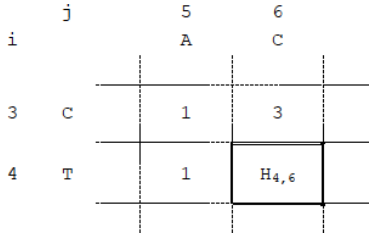
Calculation of three candidate scores

$H_{i,j}^{(0)}$, $H_{i,j}^{(1)}$, and $H_{i,j}^{(2)}$ represent the three candidate scores of $H_{i,j}$. They are respectively calculated as:

$$H_{i,j}^{(0)} = H_{i-1,j} - g \qquad (vertical)$$
$$H_{i,j}^{(1)} = H_{i,j-1} - g \qquad (horizontal)$$
$$H_{i,j}^{(2)} = H_{i-1,j-1} + R_{a,b} \qquad (diagonal)$$

Exercise 2.4

Calculate the scores of $H_{4,6}^{(0)}$, $H_{4,6}^{(1)}$, and $H_{4,6}^{(2)}$ first and then update $H_{4,6}$.



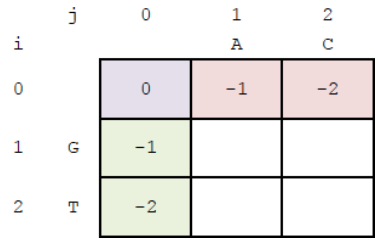
Scoring scheme:
$R_{ab} = 1$ for a = b
$R_{ab} = 0$ for a $\neq$ b
g = 1

Initialization

The first row and the first column can be calcuated independently from the adjcent cells.

$$H_{0,j} : j * -1 * g$$
$$H_{i,0} : i * -1 * g$$

Example



Exercise 2.5

Update all cells of Table 1 and 2. Use the scoring scheme in Exercise 2.4.

Table 1

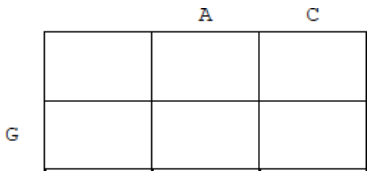
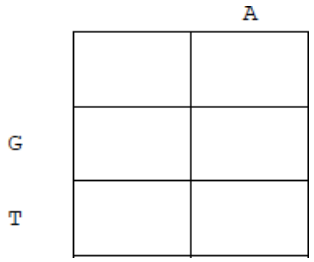


Table 2



Sub-solutions

In DP, larger decisions recursively nest smaller decisions. For instance, Table S is included in Table L.

Table S

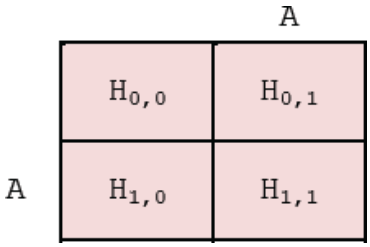
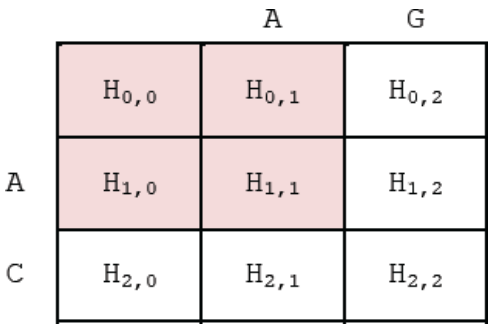


Table L



Psedo-code of updating DP table for global alignment

Algorithm 2.1: Update dynamic programming table for global alignment

$H_{i,j}$: Dyanamic programming table
$R_{a,b}$: Match/mismatch scores
g : Gap penalty

```
// Initialization
for i ← 0 to m do
    H_{i,0} ← i * −1 * g;
end
for j ← 1 to n do
    H_{0,j} ← j * −1 * g;
end

// Main loop for table update
for i ← 1 to m do
    for j ← 1 to n do
        H_{i,j} ← max(H_{i−1,j} − g, H_{i,j−1} − g, H_{i−1,j−1} + R_{a,b});
    end
end
```