

Implementação do algoritmo Smith-Waterman

O algoritmo Smith-Waterman é utilizado para alinhamento local de sequências de DNA ou aminoácidos. Esse algoritmo retorna o melhor alinhamento local possível, ou seja, tenta encontrar a maior subsequência, porém, é muito importante a escolha de um sistema de pontuação e/ou matriz de substituição adequados ao problema.

Implementou-se o algoritmo, em Python 3.8, para o alinhamento de duas sequências. O código fonte está disponível no *Github* (<https://github.com/glenjasper/smith-waterman.git>). O algoritmo aceita um arquivo fasta contendo as duas sequências biológicas, para o qual é requerido escolher a matriz de substituição (BLOSUM ou PAM) e o *gap penalty* para sequências proteicas, entretanto para sequências de DNA podem-se configurar o *match* e o *mismatch*. Além do alinhamento feito, o algoritmo retorna a matriz de pontuação em um arquivo TXT. Também, é possível visualizar o alinhamento através do *framework* Dash (<https://dash.plotly.com>), que oferece uma visualização simples e amigável em HTML.

Pré-requisitos

`pip install dash`

`pip install dash-bio`

Uso básico

```
$ python smith_waterman.py --help
```

```
usage: smith_waterman.py [-h] -t {nt,aa} [-sm {BLOSUM45, BLOSUM50, BLOSUM62, BLOSUM80,
BLOSUM90, PAM30, PAM70, PAM250}] -f FILE [-m MATCH] [-mi MISMATCH_PENALTY] [-gap
GAP_PENALTY] [-o FOLDER] [--version]
```

Implementation of the Smith–Waterman algorithm

optional arguments:

`-h, --help` show this help message and exit

`-t {nt,aa}, --type {nt,aa}`

nt: Nucleotide sequence | aa: Amino acid sequence

`-sm {BLOSUM45, BLOSUM50, BLOSUM62, BLOSUM80, BLOSUM90, PAM30, PAM70, PAM250}, --substitution_matrix {BLOSUM45, BLOSUM50, BLOSUM62, BLOSUM80, BLOSUM90, PAM30, PAM70, PAM250}`

Substitution Matrix type (Only for amino acid sequence) [default: BLOSUM62].

`-f FILE, --fasta FILE`

Fasta file

`-m MATCH, --match MATCH`

Match value (Only for nucleotide sequence) [default: 1].

`-mi MISMATCH_PENALTY, --mismatch_penalty MISMATCH_PENALTY`

Mismatch penalty value (Only for nucleotide sequence) [default: 0].

`-gap GAP_PENALTY, --gap_penalty GAP_PENALTY`

Gap penalty value [default: 0].

`-o FOLDER, --output FOLDER`

Output folder

`--version` show program's version number and exit

Parâmetros

Parâmetro	Descrição	Possíveis valores	Default
-t --type	Tipo de sequências a alinhar. Podem ser de aminoácidos ou nucleotídeos.	nt, aa	
-sm --substitution_matrix	Matriz de substituição BLOSUM ou PAM, usado quando as sequencias forem proteicas.	BLOSUM45, BLOSUM50, BLOSUM62, BLOSUM80, BLOSUM90, PAM30, PAM70, PAM250	BLOSUM62
-f --fasta	Arquivo fasta que contem as sequências biológicas a serem alinhadas.		
-m --match	Valor de <i>match</i> . Usado quando as sequencias forem de DNA.		1
-mi --mismatch_penalty	Valor de <i>mismatch</i> . Usado quando as sequencias forem de DNA.		0
-gap --gap_penalty	Valor de <i>gap penalty</i> .		0
-o --output	Pasta de saída.		

Exemplos

1. Realizar os alinhamentos LOCAL e GLOBAL das sequências proteicas CALMLVTAPMLLLVTLLCALCSAVLYDSSSYVYYYQSAFRPPSGWHLQGGAYAVVNISSEFNAG e AAMLLSLLCALLYSDDSSYVYYYQPPGWYGGAG (contidas no arquivo **file1.fa**), com a matriz de substituição BLOSUM80 e *gap penalty* -5.

Alinhamento LOCAL

```
$ python smith_waterman.py -t aa -f file1.fa -sm blosum80 -gap -1 -o out_align1
```

Output:

```
2021-02-05 15:01:12 #####
2021-02-05 15:01:12 ##### Smith-Waterman Algorithm #####
2021-02-05 15:01:12 #####
2021-02-05 15:01:12 Input:
2021-02-05 15:01:12 Fasta file: C:\Users\Glen\Dropbox\UFMG\Disciplinas\Bioinformática\Atividades\TP1\script\smith-waterman\file1.fa
2021-02-05 15:01:12
2021-02-05 15:01:12 Parameters:
2021-02-05 15:01:12 Matrix: BLOSUM80
2021-02-05 15:01:12 Gap penalty: -1
2021-02-05 15:01:12
2021-02-05 15:01:12 Alignment:
2021-02-05 15:01:12 Score: 138
2021-02-05 15:01:12
2021-02-05 15:01:12 sequence1 APMLLLVTLLCALCSAVLY-DSSSYVYYYQSAFRPPSGWHLQGGGA
2021-02-05 15:01:12 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
2021-02-05 15:01:12 sequence2 AAW-LL-SLLCAL----LYSDDSSYVYYYQ---PP-GWY--GGA
2021-02-05 15:01:12
2021-02-05 15:01:12 Matrix file: C:\Users\Glen\Dropbox\UFMG\Disciplinas\Bioinformática\Atividades\TP1\script\smith-waterman\out_align1\alignment_matrix.txt
2021-02-05 15:01:12
2021-02-05 15:01:12 Running Dash's alignment viewer
2021-02-05 15:01:12 Run the address http://127.0.0.1:8050 in your browser
Dash is running on http://127.0.0.1:8050/
```

Alinhamento GLOBAL

```
$ python needleman_wunsch.py -t aa -f file1.fa -sm blosum80 -gap -5 -o out_align1
```

Output:

```
2021-02-05 14:57:09 #####
2021-02-05 14:57:09 ##### Needleman-Wunsch Algorithm #####
2021-02-05 14:57:09 #####
2021-02-05 14:57:09 Input:
2021-02-05 14:57:09 Fasta file: C:\Users\Glen\Dropbox\UFMG\Disciplinas\Bioinformática\Atividades\TP1\script\needleman-wunsch\file1.fa
2021-02-05 14:57:09 Parameters:
2021-02-05 14:57:09 Matrix: BLOSUM80
2021-02-05 14:57:09 Gap penalty: -5
2021-02-05 14:57:09 Alignment:
2021-02-05 14:57:09 Score: -6
2021-02-05 14:57:09 sequence1 CALMLVTAPMLLLVTLTLLCALCSAVLY-DSSSYVYVYQSAFRPPSGWHLQGGAYAVVNISS
2021-02-05 14:57:09 sequence2 -A-----A-M-LL-SLLCAL----LYSDSSVYVYQ----PP-GWY--GG-----
2021-02-05 14:57:09 sequence1 EFNWAG
2021-02-05 14:57:09 sequence2 ----AG
2021-02-05 14:57:09 Matrix file: C:\Users\Glen\Dropbox\UFMG\Disciplinas\Bioinformática\Atividades\TP1\script\needleman-wunsch\out_align1\alignment_matrix.txt
2021-02-05 14:57:09
2021-02-05 14:57:09 Running Dash's alignment viewer
2021-02-05 14:57:09 Run the address http://127.0.0.1:8050 in your browser
Dash is running on http://127.0.0.1:8050/
```

Matriz de substituição (ver arquivo **alignment_matrix.txt**):

Para ambos os casos é possível visualizar os alinhamento com o *framework* Dash (HTML+CSS):

Vista do alinhamento com Needleman-Wunsch



Vista do alinhamento com Smith-Waterman



Enquanto com o algoritmo Needleman-Wunsch tenta estender o alinhamento, através de todo o comprimento das sequências, já com o algoritmo Smith-Waterman tenta-se achar o maior comprimento da subsequência em um local.

2. Realizar os alinhamentos LOCAL e GLOBAL das sequências de DNA AATTTACGCGGCATTATAATACAATCGTCTGCATGGCCGGA e ATTGCATTATAGGATACAATCGTGG (contidas no arquivo **file2.fa**), usando os valores de *match* +2, *mismatch* -1 e *gap penalty* -3.

Alinhamento LOCAL

```
$ python smith_waterman.py -t nt -f file2.fa -m 2 -mi -1 -gap -3 -o out_align2
```

Output:

```

2021-02-05 15:38:39 #####
2021-02-05 15:38:39 ##### Smith-Waterman Algorithm #####
2021-02-05 15:38:39 #####
2021-02-05 15:38:39 Input:
2021-02-05 15:38:39 Fasta file: C:\Users\Glen\Dropbox\UFMG\Disciplinas\Bioinformática\Atividades\TP1\script\smith-waterman\file2.fa
2021-02-05 15:38:39 Parameters:
2021-02-05 15:38:39 Match: 2
2021-02-05 15:38:39 Mismatch penalty: -1
2021-02-05 15:38:39 Gap penalty: -3
2021-02-05 15:38:39 Alignment:
2021-02-05 15:38:39 Score: 29
2021-02-05 15:38:39 sequence1 GCATT-TATAATACAATCGT
2021-02-05 15:38:39          |||| |••|
2021-02-05 15:38:39 sequence2 GCATTATAGGATACAATCGT
2021-02-05 15:38:39 Matrix file: C:\Users\Glen\Dropbox\UFMG\Disciplinas\Bioinformática\Atividades\TP1\script\smith-waterman\out_align2\alignment_matrix.txt
2021-02-05 15:38:39 Running Dash's alignment viewer
2021-02-05 15:38:39 Run the address http://127.0.0.1:8050 in your browser
Dash is running on http://127.0.0.1:8050/

```

Alinhamento GLOBAL

\$ python needleman_wunsch.py -t nt -f file2.fa -m 2 -mi -1 -gap -3 -o out_align2

Output:

```

2021-02-05 15:38:43 #####
2021-02-05 15:38:43 ##### Needleman-Wunsch Algorithm #####
2021-02-05 15:38:43 #####
2021-02-05 15:38:43 Input:
2021-02-05 15:38:43 Fasta file: C:\Users\Glen\Dropbox\UFMG\Disciplinas\Bioinformática\Atividades\TP1\script\needleman-wunsch\file2.fa
2021-02-05 15:38:43 Parameters:
2021-02-05 15:38:43 Match: 2
2021-02-05 15:38:43 Mismatch penalty: -1
2021-02-05 15:38:43 Gap penalty: -3
2021-02-05 15:38:43 Alignment:
2021-02-05 15:38:43 Score: -15
2021-02-05 15:38:43 sequence1 AATTTACGCGGCATT-TATAATACAATCGTCTGCATGGCCGGA
2021-02-05 15:38:43          | ||      |||| |••| || | | ||
2021-02-05 15:38:43 sequence2 -A-TT-----GCATTATAGGATACAA---TC-G--T---GG-
2021-02-05 15:38:43 Matrix file: C:\Users\Glen\Dropbox\UFMG\Disciplinas\Bioinformática\Atividades\TP1\script\needleman-wunsch\out_align2\alignment_matrix.txt
2021-02-05 15:38:43 Running Dash's alignment viewer
2021-02-05 15:38:43 Run the address http://127.0.0.1:8050 in your browser
Dash is running on http://127.0.0.1:8050/

```

Além de visualizar os alinhamentos usando o *framework* Dash, também nos arquivos *.log* encontram-se o resumo dos alinhamentos:

Log do alinhamento com o algoritmo Needleman-Wunsch:

```

sequence1      AATTTACGCGGCATT-TATAATACAATCGTCTGCATGGCCGGA
               | ||      |||| |••| || | | ||
sequence2      -A-TT-----GCATTATAGGATACAA---TC-G--T---GG-

```

Log do alinhamento com o algoritmo Smith-Waterman:

```

sequence1      GCATT-TATAATACAATCGT
               |||| |••|
sequence2      GCATTATAGGATACAATCGT

```

Nestes alinhamentos observam-se que enquanto o alinhamento tenta estender o alinhamento com respeito à sequência de maior comprimento, no alinhamento local, o alinhamento fica concentrado em um lugar.

Adicionalmente podem-se apreciar as matrizes de pontuação, para ambos os casos se ressaltaram em amarelo o caminho que segue o *backtracking*:

Matriz de pontuação do alinhamento global:

-	A	A	T	T	T	A	C	G	C	G	G	C	A	T	T	T	A	T	A	A	T	A	C	A	A	T	C	G	T	C	T	G	C	A	T	G	G	C	C	G	G	A				
-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
A	0	2 d	2 d	0 z	0 z	0 z	2 d	0 z	0 z	0 z	0 z	0 z	2 d	0 z	0 z	0 z	0 z	2 d	0 z	2 d	2 d	0 z	2 d	2 d	0 z	2 d	2 d	0 z	0 z	0 z	0 z	0 z	2 d	0 z	0 z	0 z	0 z	0 z	0 z	0 z	0 z	2 d				
T	0	0 z	1 d	4 d	2 d	2 d	0 z	1 d	0 z	0 z	0 z	0 z	0 z	0 z	4 d	2 d	2 d	0 z	4 d	1 l	1 d	1 d	0 z	4 d	1 l	1 d	0 z	4 d	1 l	1 d	0 z	2 d	0 z	2 d	1 d	0 z	0 z	0 z	0 z	0 z	0 z	0 z				
T	0	0 z	0 z	3 d	6 d	4 d	1 d	0 z	0 d	0 z	0 z	0 z	0 z	0 z	2 d	6 d	4 d	1 d	2 d	3 d	0 d	0 z	3 d	0 d	0 z	3 d	0 d	0 z	3 d	0 d	2 d	4 d	4 d	1 d	0 z	0 z	0 z	0 z	0 z	0 z	0 z	0 z				
G	0	0 z	0 z	0 z	0 z	0 u	2 d	4 d	5 d	2 l	4 d	1 l	1 d	4 d	1 l	0 z	0 u	2 d	5 d	2 l	1 d	0 d	4 d	1 l	1 u	6 d	3 l	0 l	0 z	1 d	1 d	3 d	0 d	4 d	1 l	0 z	0 z	4 d	5 d	2 l	0 z	2 d	2 d	0 z		
C	0	2 d	2 d	0 z	0 z	0 z	4 d	3 d	4 d	1 d	3 d	0 d	1 u	6 d	3 l	0 l	0 z	4 d	3 d	4 d	2 d	0 z	3 d	1 u	6 d	3 d	0 l	0 z	1 d	1 d	3 d	0 d	4 d	3 u	8 d	5 l	2 l	0 d	4 u	6 d	3 d	0 d	3 d			
T	0	0 z	1 d	4 d	2 d	2 d	1 u	3 d	2 d	3 d	0 d	2 d	0 z	3 u	8 d	5 d	2 d	1 u	6 d	3 l	3 d	4 d	1 l	2 u	3 u	5 d	5 d	2 l	0 z	3 d	0 d	5 d	2 d	0 u	5 u	10 d	7 l	4 l	1 l	3 d	5 d	2 d	0 u			
T	0	0 z	0 z	3 d	6 d	4 d	1 d	0 d	2 d	1 d	2 d	0 z	0 z	1 d	0 u	5 d	10 d	7 d	4 l	3 d	5 d	2 d	5 d	3 d	0 d	1 d	2 d	7 d	4 d	1 d	2 d	2 d	2 d	4 d	1 d	2 d	7 d	9 d	6 d	3 d	0 d	2 d	4 d	1 d		
A	0	2 d	2 d	0 u	3 u	5 d	6 d	3 l	0 l	0 l	1 d	0 d	1 d	0 z	3 d	2 u	7 u	9 d	9 d	6 l	5 d	7 d	4 l	2 d	3 d	4 u	6 d	3 d	4 u	6 d	3 d	4 u	6 d	8 d	5 d	2 d	0 z	1 d	6 d	6 d						
T	0	0 z	1 d	4 d	2 d	5 d	4 d	5 d	2 d	0 z	0 d	0 z	0 z	0 d	0 u	5 d	4 d	9 d	8 d	11 d	8 l	5 l	9 d	6 l	6 d	3 d	1 d	5 d	3 d	5 d	5 d	2 l	3 d	3 d	3 d	0 d	0 z	2 d	5 d	3 d	5 d	7 d	4 d	1 d	0 z	3 u
A	0	2 d	2 d	1 u	3 d	2 u	7 d	4 l	4 d	1 d	0 z	0 z	0 z	2 d	2 u	4 d	6 u	11 d	8 l	13 d	10 d	7 l	11 d	8 l	8 d	7 d	7 d	4 d	1 d	6 d	3 d	3 d	1 d	0 z	1 d	4 d	6 d	3 l	3 d	8 d	5 d	2 l				
G	0	0 z	1 d	1 d	0 d	0 d	2 d	4 u	6 d	6 d	3 d	3 d	2 d	0 z	0 z	1 d	1 d	3 d	8 u	10 d	10 u	12 d	9 d	8 u	10 d	7 d	7 d	4 d	1 d	6 d	3 l	3 d	3 d	1 d	0 z	1 d	4 d	6 d	3 l	3 d	8 d	5 d	2 l			
G	0	0 z	0 z	0 d	0 d	0 z	1 d	3 d	8 d	5 d	5 d	5 d	2 l	0 z	0 z	0 z	0 d	5 d	5 d	7 d	9 d	9 d	11 d	8 d	7 d	9 d	6 d	6 d	3 d	3 d	5 d	2 d	2 d	5 d	2 d	0 z	3 d	6 d	5 d	2 d	5 d	10 d	7 l			
A	0	2 d	2 d	0 z	0 z	0 z	2 d	0 d	5 u	7 d	4 d	4 d	4 d	4 d	1 l	0 z	0 z	2 d	4 d	9 d	11 d	8 d	13 d	10 l	9 d	11 d	8 l	5 d	2 d	2 d	4 d	1 d	1 d	2 u	4 d	4 d	1 l	0 u	3 u	5 d	4 d	2 u	7 u	12 d		
T	0	0 z	1 d	4 d	2 d	2 d	0 z	1 d	2 u	4 d	6 d	3 d	3 d	3 d	6 d	3 d	2 d	0 z	4 d	6 u	8 d	13 d	10 l	12 d	9 d	8 d	13 d	10 l	7 d	4 d	1 d	6 d	3 l	1 d	3 d	6 d	3 l	0 l	2 d	5 d	4 d	3 d	4 u	9 u		
A	0	2 d	2 d	1 u	3 d	1 d	4 d	1 l	0 d	1 d	3 d	5 d	2 d	5 d	3 u	5 d	2 d	4 d	1 l	6 d	8 d	10 u	15 d	12 l	14 d	11 d	10 u	12 d	9 d	6 d	3 d	3 u	5 d	2 d	3 d	3 u	5 d	2 d	0 z	1 d	3 d	2 d	6 d			
C	0	0 z	1 d	1 d	0 d	2 d	1 u	6 d	3 l	2 d	0 d	2 d	7 d	4 l	4 l	4 d	2 d	4 d	1 d	3 d	3 u	5 d	7 d	12 u	17 d	14 l	13 d	10 d	12 d	11 d	8 d	8 d	5 l	2 d	7 d	4 l	2 d	2 d	4 d	4 d	2 d	3 u				
A	0	2 d	2 d	0 d	0 d	0 z	4 d	3 u	5 d	2 d	1 d	0 d	0 z	4 u	9 d	6 l	3 d	1 d	6 d	3 l	5 d	5 d	7 d	4 d	9 d	14 u	19 d	16 d	13 l	10 l	11 d	10 d	7 d	7 d	4 d	4 u	9 d	6 l	3 l	1 d	3 d	3 d	4 d			
T	0	0 z	1 d	6 d	3 d	2 d	0 z	1 d	2 d	1 d	3 d	0 d	0 z	3 u	8 d	10 d	7 d	4 l	5 d	4 d	9 d	6 l	8 d	13 u	18 u	23 d	20 l	17 l	14 d	11 l	11 d	8 l	5 d	3 u	8 d	7 d	4 d	1 d	0 z	1 d	1 d	0 z				
C	0	0 z	0 z	3 u	5 d	2 d	1 d	2 d	0 d	4 d	1 l	2 d	2 d	0 d	0 u	5 u	7 d	9 d	6 d	3 d	4 d	3 d	6 u	8 d	8 d	10 u	15 u	20 u	25 d	22 l	19 l	16 d	13 l	10 d	10 d	7 l	5 u	7 d	6 d	6 d	3 d	0 l	0 d	0 d		
G	0	0 z	0 z	0 u	2 d	4 d	1 d	0 d	4 d	1 l	6 d	3 d	2 d	1 d	1 u	2 u	4 d	6 d	8 d	5 d	2 d	3 d	3 u	5 d	7 d	7 d	12 u	17 u	22 u	27 d	24 l	21 l	18 d	15 d	12 l	9 d	6 d	7 d	9 d	6 l	5 d	2 d	0 z			
T	0	0 z	0 z	2 d	2 d	4 d	3 d	0 d	1 u	3 d	3 u	5 d	2 d	0 d	3 d	4 d	6 d	5 d	10 d	7 l	4 l	5 d	2 d	4 d	6 d	9 u	14 d	19 u	24 u	29 d	26 l	23 d	20 l	17 l	14 l	11 d	8 l	6 d	8 d	5 d	4 d	1 d				
G	0	0 z	0 z	0 z	1 d	1 d	3 d	2 d	2 d	0 d	5 d	5 d	4 d	1 d	0 u	2 d	3 d	5 d	7 u	9 d	3 d	5 d	3 d	4 d	1 d	3 d	6 u	11 u	16 u	21 d	26 u	28 d	25 d	22 l	19 l	16 l	13 d	10 d	7 l	7 d	6 d	3 d				
G	0	0 z	0 z	0 z	0 z	0 d	2 d	4 d	1 d	2 d	7 d	4 d	3 d	0 d	0 z	1 d	2 d	4 d	6 d	8 d	5 d	2 d	3 d	0 d	3 u	8 u	13 u	18 d	23 u	25 d	27 d	27 d	24 d	21 d	18 d	18 d	15 d	12 l	9 l	9 d	9 d	6 l				

Matriz de pontuação do alinhamento global:

	-	A	A	T	T	T	A	C	G	C	G	G	C	A	T	T	T	A	T	A	A	T	A	C	A	A	T	C	T	G	C	A	-T	G	G	C	C	G	G	A			
-	0	-3	-6	-9	-12	-15	-18	-21	-24	-27	-30	-33	-36	-39	-42	-45	-48	-51	-54	-57	-60	-63	-66	-69	-72	-75	-78	-81	-84	-87	-90	-93	-96	-99	-102	-105	-108	-111	-114	-117	-120	-123	-126
A	-3	2 d	-1 d	-4 l	-7 l	-10 l	-13 d	-16 l	-19 l	-22 l	-25 l	-28 l	-31 l	-34 d	-37 l	-40 l	-43 l	-46 d	-49 l	-52 d	-55 d	-58 l	-61 d	-64 l	-67 d	-70 d	-73 l	-76 l	-79 l	-82 l	-85 l	-88 l	-91 l	-94 l	-97 d	-100 l	-103 l	-106 l	-109 l	-112 l	-115 l	-118 l	-121 d
T	-6	-1 u	1 d	1 d	-2 d	-5 d	-8 l	-11 l	-14 l	-17 l	-20 l	-23 l	-26 l	-29 l	-32 d	-35 d	-38 d	-41 l	-44 d	-47 l	-50 l	-53 d	-56 l	-59 l	-62 l	-65 l	-68 d	-71 l	-74 l	-77 d	-80 l	-83 d	-86 l	-89 l	-92 l	-95 d	-98 l	-101 l	-104 l	-107 l	-110 l	-113 l	-116 l
T	-9	-4 u	2 d	3 d	3 d	0 d	-3 l	-6 l	-9 l	-12 l	-15 l	-18 l	-21 l	-24 l	-27 d	-30 d	-33 d	-36 l	-39 d	-42 l	-45 l	-48 d	-51 l	-54 l	-57 l	-60 d	-63 d	-66 l	-69 l	-72 d	-75 l	-78 d	-81 l	-84 l	-87 l	-90 d	-93 l	-96 l	-99 l	-102 l	-105 l	-108 l	-111 l
G	-12	-7 u	-5 d	0 u	2 d	2 d	-1 d	-4 d	-4 d	-7 l	-10 d	-13 d	-16 l	-19 l	-22 l	-25 l	-28 l	-31 l	-34 l	-37 l	-40 l	-43 l	-46 l	-49 l	-52 l	-55 l	-58 l	-61 l	-64 d	-67 l	-70 l	-73 l	-76 d	-79 l	-82 l	-85 l	-88 d	-91 d	-94 l	-97 l	-100 d	-103 d	-106 l
C	-15	-10 u	-8 d	-3 u	-1 d	1 d	1 d	1 d	-2 l	-2 d	-5 l	-8 l	-11 d	-14 l	-17 l	-20 l	-23 l	-26 l	-29 l	-32 l	-35 l	-38 l	-41 l	-44 d	-47 l	-50 l	-53 l	-56 d	-59 l	-62 l	-65 d	-68 l	-71 l	-74 d	-77 l	-80 l	-83 l	-86 l	-89 d	-92 d	-95 l	-98 l	-101 l
A	-18	-13 d	-8 d	-6 u	-4 d	-2 d	3 d	0 d	0 d	-3 d	-3 d	-6 d	-9 d	-12 l	-15 l	-18 l	-21 d	-24 l	-27 d	-30 d	-33 l	-36 d	-39 l	-42 d	-45 d	-48 l	-51 l	-54 l	-57 l	-60 l	-63 l	-66 l	-69 l	-72 d	-75 l	-78 l	-81 l	-84 l	-87 l	-90 l	-93 l	-96 d	
T	-21	-16 u	-11 u	-6 u	-4 d	-2 d	0 u	2 d	-1 d	-1 d	-4 d	-4 d	-7 d	-10 d	-13 d	-16 l	-19 l	-22 l	-25 l	-28 l	-31 l	-34 l	-37 l	-40 l	-43 d	-46 l	-49 l	-52 l	-55 l	-58 l	-61 d	-64 l	-67 l	-70 d	-73 l	-76 l	-79 l	-82 l	-85 l	-88 l	-91 l		
T	-24	-19 u	-14 u	-9 u	-6 u	-4 d	-2 d	-1 d	-1 d	-2 d	-2 d	-5 d	-8 d	-11 d	-14 d	-17 d	-20 d	-23 d	-26 l	-29 l	-32 l	-35 l	-38 l	-41 l	-44 l	-47 l	-50 l	-53 d	-56 l	-59 l	-62 l	-65 d	-68 l	-71 l	-74 d	-77 l	-80 l	-83 l	-86 l	-89 d			
G	-27	-22 u	-17 u	-12 u	-9 u	-6 u	-4 d	-2 d	-1 d	-1 d	-2 d	-2 d	-5 d	-8 d	-11 d	-14 d	-17 d	-20 d	-23 d	-26 l	-29 l	-32 l	-35 l	-38 l	-41 l	-44 l	-47 l	-50 l	-53 d	-56 l	-59 l	-62 l	-65 d	-68 l	-71 l	-74 d	-77 l	-80 l	-83 l	-86 l			
C	-30	-25 u	-20 u	-15 u	-10 u	-6 u	-4 d	-2 d	-1 d	-1 d	-2 d	-2 d	-5 d	-8 d	-11 d	-14 d	-17 d	-20 d	-23 d	-26 l	-29 l	-32 l	-35 l	-38 l	-41 l	-44 l	-47 l	-50 l	-53 d	-56 l	-59 l	-62 l	-65 d	-68 l	-71 l	-74 d	-77 l	-80 l	-83 l	-86 l			
A	-33	-28 u	-23 u	-18 u	-13 u	-8 u	-3 u	-1 d	-1 d	-2 d	-2 d	-5 d	-8 d	-11 d	-14 d	-17 d	-20 d	-23 d	-26 l	-29 l	-32 l	-35 l	-38 l	-41 l	-44 l	-47 l	-50 l	-53 d	-56 l	-59 l	-62 l	-65 d	-68 l	-71 l	-74 d	-77 l	-80 l	-83 l	-86 l				
T	-36	-31 u	-26 u	-21 u	-16 u	-11 u	-6 u	-3 u	-1 d	-1 d	-2 d	-2 d	-5 d	-8 d	-11 d	-14 d	-17 d	-20 d	-23 d	-26 l	-29 l	-32 l	-35 l	-38 l	-41 l	-44 l	-47 l	-50 l	-53 d	-56 l	-59 l	-62 l	-65 d	-68 l	-71 l	-74 d	-77 l	-80 l	-83 l	-86 l			
C	-39	-34 u	-29 u	-24 u	-19 u	-14 u	-9 u	-7 d	-2 d	-2 d	-3 d	-1 d	-1 d	-3 d	-4 d	-6 d	-4 d	-6 d	-4 d	-7 d	-10 d	-13 d	-16 l	-19 l	-22 l	-25 l	-28 l	-31 l	-34 l	-37 d	-40 l	-43 d	-46 l	-49 l	-52 l	-55 d	-58 l	-61 d	-64 l				
G	-42	-37 d	-32 d	-27 u	-22 u	-17 u	-12 u	-10 d	-5 u	-3 d	-4 d	-1 d	-1 d	-3 d	-4 d	-6 d	-4 d	-7 d	-10 d	-13 d	-16 l	-19 l	-22 l	-25 l	-28 l	-31 l	-34 l	-37 d	-40 l	-43 d	-46 l	-49 l	-52 l	-55 d	-58 l	-61 d	-64 l	-67 l	-70 d				
C	-45	-40 u	-35 u	-30 u	-25 u	-20 u	-15 u	-13 d	-8 u	-6 d	-4 d	-1 d	-1 d	-3 d	-4 d	-6 d	-4 d	-7 d	-10 d	-13 d	-16 l	-19 l	-22 l	-25 l	-28 l	-31 l	-34 l	-37 d	-40 l	-43 d	-46 l	-49 l	-52 l	-55 d	-58 l	-61 d	-64 l	-67 l	-70 d	-73 l			
A	-48	-43 u	-38 u	-33 u	-28 u	-23 u	-18 u	-16 u	-10 u	-8 d	-6 d	-3 d	-3 d	-5 d	-6 d	-8 d	-6 d	-9 d	-12 d	-15 d	-18 d	-21 d	-24 d	-27 d	-30 d	-33 d	-36 d	-39 d	-42 d	-45 d	-48 d	-51 d	-54 d	-57 d	-60 d	-63 d	-66 d	-69 d	-72 d	-75 d			
T	-51	-46 u	-41 u	-36 u	-31 u	-26 u	-21 u	-19 u	-11 u	-9 d	-7 d	-4 d	-4 d	-6 d	-7 d	-9 d	-7 d	-10 d	-13 d	-16 l	-19 l	-22 l	-25 l	-28 l	-31 l	-34 l	-37 d	-40 l	-43 d	-46 l	-49 l	-52 l	-55 d	-58 l	-61 d	-64 l	-67 l	-70 d	-73 l				
C	-54	-49 u	-44 u	-39 u	-34 u	-29 u	-24 u	-22 u	-13 u	-11 d	-9 d	-6 d	-6 d	-8 d	-9 d	-11 d	-9 d	-12 d	-15 d	-18 d	-21 d	-24 d	-27 d	-30 d	-33 d	-36 d	-39 d	-42 d	-45 d	-48 d	-51 d	-54 d	-57 d	-60 d	-63 d	-66 d	-69 d	-72 d	-75 d				
G	-57	-52 u	-47 u	-42 u	-37 u	-32 u	-27 u	-25 u	-16 u	-14 d	-12 d	-9 d	-9 d	-11 d	-12 d	-14 d	-12 d	-15 d	-18 d	-21 d	-24 d	-27 d	-30 d	-33 d	-36 d	-39 d	-42 d	-45 d	-48 d	-51 d	-54 d	-57 d	-60 d	-63 d	-66 d	-69 d	-72 d	-75 d					
C	-60	-55 u	-50 u	-45 u	-40 u	-35 u	-30 u	-28 u	-19 u	-17 d	-15 d	-12 d	-12 d	-14 d	-15 d	-17 d	-15 d	-18 d	-21 d	-24 d	-27 d	-30 d	-33 d	-36 d	-39 d	-42 d	-45 d	-48 d	-51 d	-54 d	-57 d	-60 d	-63 d	-66 d	-69 d	-72 d	-75 d	-78 d					
A	-63	-58 u <td>-53 u<td>-48 u<td>-43 u<td>-38 u<td>-33 u<td>-31 u<td>-22 u</td><td>-20 d</td><td>-18 d</td><td>-15 d</td><td>-15 d</td><td>-17 d</td><td>-18 d</td><td>-20 d</td><td>-18 d</td><td>-21 d</td><td>-24 d</td><td>-27 d</td><td>-30 d</td><td>-33 d</td><td>-36 d</td><td>-39 d</td><td>-42 d</td><td>-45 d</td><td>-48 d</td><td>-51 d</td><td>-54 d</td><td>-57 d</td><td>-60 d</td><td>-63 d</td><td>-66 d</td><td>-69 d</td><td>-72 d</td><td>-75 d</td><td>-78 d</td><td>-81 d</td></td></td></td></td></td></td>	-53 u <td>-48 u<td>-43 u<td>-38 u<td>-33 u<td>-31 u<td>-22 u</td><td>-20 d</td><td>-18 d</td><td>-15 d</td><td>-15 d</td><td>-17 d</td><td>-18 d</td><td>-20 d</td><td>-18 d</td><td>-21 d</td><td>-24 d</td><td>-27 d</td><td>-30 d</td><td>-33 d</td><td>-36 d</td><td>-39 d</td><td>-42 d</td><td>-45 d</td><td>-48 d</td><td>-51 d</td><td>-54 d</td><td>-57 d</td><td>-60 d</td><td>-63 d</td><td>-66 d</td><td>-69 d</td><td>-72 d</td><td>-75 d</td><td>-78 d</td><td>-81 d</td></td></td></td></td></td>	-48 u <td>-43 u<td>-38 u<td>-33 u<td>-31 u<td>-22 u</td><td>-20 d</td><td>-18 d</td><td>-15 d</td><td>-15 d</td><td>-17 d</td><td>-18 d</td><td>-20 d</td><td>-18 d</td><td>-21 d</td><td>-24 d</td><td>-27 d</td><td>-30 d</td><td>-33 d</td><td>-36 d</td><td>-39 d</td><td>-42 d</td><td>-45 d</td><td>-48 d</td><td>-51 d</td><td>-54 d</td><td>-57 d</td><td>-60 d</td><td>-63 d</td><td>-66 d</td><td>-69 d</td><td>-72 d</td><td>-75 d</td><td>-78 d</td><td>-81 d</td></td></td></td></td>	-43 u <td>-38 u<td>-33 u<td>-31 u<td>-22 u</td><td>-20 d</td><td>-18 d</td><td>-15 d</td><td>-15 d</td><td>-17 d</td><td>-18 d</td><td>-20 d</td><td>-18 d</td><td>-21 d</td><td>-24 d</td><td>-27 d</td><td>-30 d</td><td>-33 d</td><td>-36 d</td><td>-39 d</td><td>-42 d</td><td>-45 d</td><td>-48 d</td><td>-51 d</td><td>-54 d</td><td>-57 d</td><td>-60 d</td><td>-63 d</td><td>-66 d</td><td>-69 d</td><td>-72 d</td><td>-75 d</td><td>-78 d</td><td>-81 d</td></td></td></td>	-38 u <td>-33 u<td>-31 u<td>-22 u</td><td>-20 d</td><td>-18 d</td><td>-15 d</td><td>-15 d</td><td>-17 d</td><td>-18 d</td><td>-20 d</td><td>-18 d</td><td>-21 d</td><td>-24 d</td><td>-27 d</td><td>-30 d</td><td>-33 d</td><td>-36 d</td><td>-39 d</td><td>-42 d</td><td>-45 d</td><td>-48 d</td><td>-51 d</td><td>-54 d</td><td>-57 d</td><td>-60 d</td><td>-63 d</td><td>-66 d</td><td>-69 d</td><td>-72 d</td><td>-75 d</td><td>-78 d</td><td>-81 d</td></td></td>	-33 u <td>-31 u<td>-22 u</td><td>-20 d</td><td>-18 d</td><td>-15 d</td><td>-15 d</td><td>-17 d</td><td>-18 d</td><td>-20 d</td><td>-18 d</td><td>-21 d</td><td>-24 d</td><td>-27 d</td><td>-30 d</td><td>-33 d</td><td>-36 d</td><td>-39 d</td><td>-42 d</td><td>-45 d</td><td>-48 d</td><td>-51 d</td><td>-54 d</td><td>-57 d</td><td>-60 d</td><td>-63 d</td><td>-66 d</td><td>-69 d</td><td>-72 d</td><td>-75 d</td><td>-78 d</td><td>-81 d</td></td>	-31 u <td>-22 u</td> <td>-20 d</td> <td>-18 d</td> <td>-15 d</td> <td>-15 d</td> <td>-17 d</td> <td>-18 d</td> <td>-20 d</td> <td>-18 d</td> <td>-21 d</td> <td>-24 d</td> <td>-27 d</td> <td>-30 d</td> <td>-33 d</td> <td>-36 d</td> <td>-39 d</td> <td>-42 d</td> <td>-45 d</td> <td>-48 d</td> <td>-51 d</td> <td>-54 d</td> <td>-57 d</td> <td>-60 d</td> <td>-63 d</td> <td>-66 d</td> <td>-69 d</td> <td>-72 d</td> <td>-75 d</td> <td>-78 d</td> <td>-81 d</td>	-22 u	-20 d	-18 d	-15 d	-15 d	-17 d	-18 d	-20 d	-18 d	-21 d	-24 d	-27 d	-30 d	-33 d	-36 d	-39 d	-42 d	-45 d	-48 d	-51 d	-54 d	-57 d	-60 d	-63 d	-66 d	-69 d	-72 d	-75 d	-78 d	-81 d					
T	-66	-61 u	-56 u	-51 u	-46 u	-41 u	-36 u	-34 u	-25 u	-23 d	-21 d	-18 d	-18 d	-20 d	-21 d	-23 d	-21 d	-24 d	-27 d	-30 d	-33 d	-36 d	-39 d	-42 d	-45 d	-48 d	-51 d	-54 d	-57 d	-60 d	-63 d	-66 d	-69 d	-72 d	-75 d	-78 d	-81 d	-84 d					
C	-69	-64 u	-59 u	-54 u	-49 u	-44 u	-39 u	-37 u	-28 u	-26 d	-24 d	-21 d	-21 d	-23 d	-24 d	-26 d	-24 d	-27 d	-30 d	-33 d	-36 d	-39 d	-42 d	-45 d	-48 d	-51 d	-54 d	-57 d	-60 d	-63 d	-66 d	-69 d	-72 d	-75 d	-78 d	-81 d	-84 d	-87 d					
G	-72	-67 u	-62 u	-57 u	-52 u	-47 u	-42 u	-40 u	-31 u	-29 d	-27 d	-24 d	-24 d	-26 d	-27 d	-29 d	-27 d	-30 d	-33 d	-36 d	-39 d	-42 d	-45 d	-48 d	-51 d	-54 d	-57 d	-60 d	-63 d	-66 d	-69 d	-72 d	-75 d	-78 d	-81 d	-84 d	-87 d	-90 d					
C	-75	-70 u	-65 u	-60 u	-55 u	-50 u	-45 u	-43 u	-34 u	-32 d	-30 d	-27 d	-27 d	-29 d	-30 d	-32 d	-30 d	-33 d	-36 d	-39 d	-42 d	-45 d	-48 d	-51 d	-54 d	-57 d	-60 d	-63 d	-66 d	-69 d	-72 d	-75 d	-78 d	-81 d	-84 d	-87 d	-90 d	-93 d					
A	-78	-73 u	-68 u	-63 u	-58 u	-53 u	-48 u	-46 u	-37 u	-35 d	-33 d	-30 d	-30 d	-32 d	-33 d	-35 d	-33 d	-36 d	-39 d	-42 d	-45 d	-48 d	-51 d	-54 d	-57 d	-60 d	-63 d	-66 d	-69 d	-72 d	-75 d	-78 d	-81 d	-84 d	-87 d	-90 d	-93 d	-96 d					
T	-81	-76 u	-71 u	-66 u	-61 u	-56 u	-51 u	-49 u	-40 u	-38 d	-36 d	-33 d	-33 d	-35 d	-36 d	-38 d	-36 d	-39 d	-42 d	-45 d	-48 d	-51 d	-54 d	-57 d	-60 d	-63 d	-66 d	-69 d	-72 d	-75 d	-78 d	-81 d	-84 d	-87 d	-90 d	-93 d	-96 d	-99 d					
C	-84	-79 u	-74 u	-69 u	-64 u	-59 u	-54 u	-52 u	-43 u	-41 d	-39 d	-36 d	-36 d	-38 d	-39 d	-41 d	-39 d	-42 d	-45 d	-48 d	-51 d	-54 d	-57 d	-60 d	-63 d	-66 d	-69 d	-72 d	-75 d	-78 d	-81 d	-84 d	-87 d	-90 d	-93 d	-96 d	-99 d	-102 d					
G	-87	-82 u	-77 u	-72 u	-67 u	-62 u	-57 u	-55 u	-46 u	-44 d	-42 d	-39 d	-39 d	-41 d	-42 d	-44 d	-42 d	-45 d	-48 d	-51 d	-54 d	-57 d	-60 d	-63 d	-66 d	-69 d	-72 d	-75 d	-78 d	-81 d	-84 d	-87 d	-90 d	-93 d	-96 d	-99 d	-102 d	-105 d					
C	-90	-85 u	-80 u	-75 u	-70 u	-65 u	-60 u	-58 u	-49 u	-47 d	-45 d	-42 d	-42 d	-44 d	-45 d	-47 d	-45 d	-48 d	-51 d	-54 d	-57 d	-60 d	-63 d	-66 d	-69 d	-72 d	-75 d	-78 d	-81 d	-84 d	-87 d	-90 d	-93 d	-96 d	-99 d	-102 d	-105 d	-108 d					
A	-93	-88 u	-83 u	-78 u	-73 u	-68 u	-63 u	-61 u	-52 u	-50 d	-48 d	-45 d	-45 d	-47 d	-48 d	-50 d	-48 d	-51 d	-54 d	-57 d	-60 d	-63 d	-66 d	-69 d	-72 d	-75 d	-78 d	-81 d	-84 d	-87 d	-90 d	-93 d	-96 d	-99 d	-102 d	-105 d	-108 d	-111 d					
T	-96	-91 u	-86 u	-81 u	-76 u	-71 u	-66 u	-64 u	-55 u	-53 d	-51 d	-48 d	-48 d	-50 d	-51 d	-53 d	-51 d	-54 d	-57 d	-60 d	-63 d	-66 d	-69 d	-72 d	-75 d	-78 d	-81 d	-84 d	-87 d	-90 d	-93 d	-96 d	-99 d	-102 d	-105 d	-108 d	-111 d	-114 d					
C	-99	-94 u	-89 u	-84 u	-79 u	-74 u	-69 u	-67 u	-58 u	-56 d	-54 d	-51 d	-51 d	-53 d	-54 d	-56 d	-54 d	-57 d	-60 d	-63 d	-66 d	-69 d	-72 d	-75 d	-78 d	-81 d	-84 d	-87 d	-90 d	-93 d	-96 d	-99 d	-102 d	-105 d	-108 d	-111 d	-114 d	-117 d					
G	-102	-97 u</																																									