Lecture 08
sequence alignment

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Lecture 08 outline

Last time: sequence data

This time: sequence alignment

- sources of sequence variation
- pairwise alignment
- progressive alignment
- generative alignment

Sequence variation

Many questions in genome biology are fundamentally ***comparative***

- where is this gene located in the genome?
- what amino acid differences cause two proteins to differ in function?
- how are two genes evolutionarily related?

Sequence variation

Any two sequences can differ in length and/or content

Sequence alignment

An ***alignment*** defines which parts of the sequence are evolutionary or functionally comparable (***homologous***)

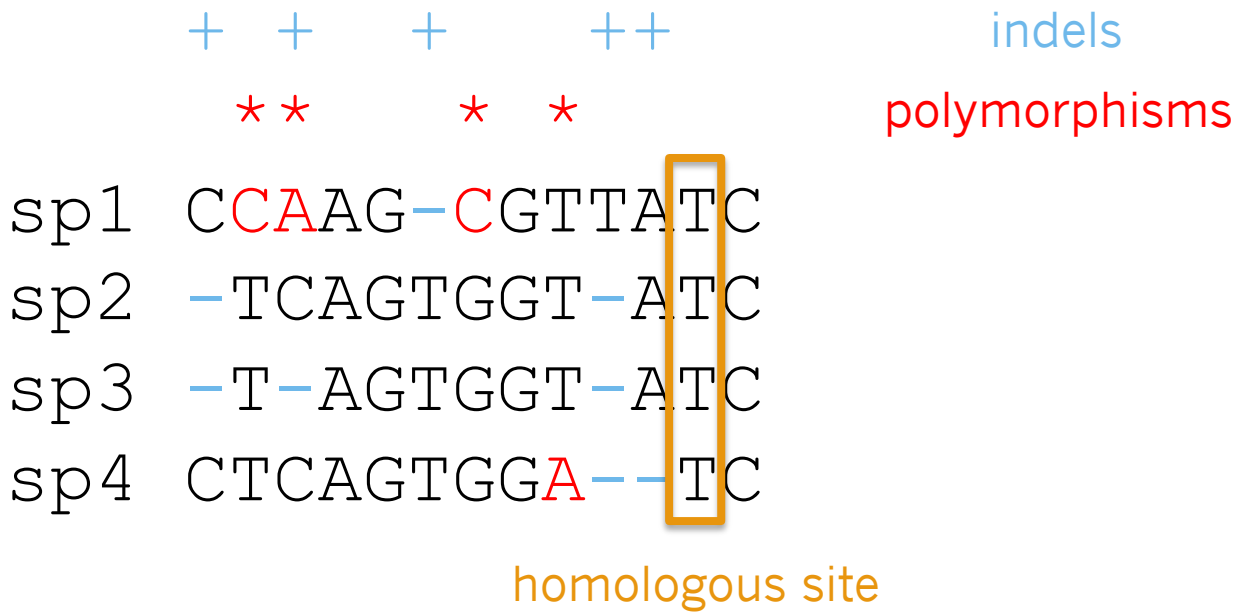
(unaligned sequences)

```
sp1 CCAAGCGTTATC
sp2 TCAGTGGTATC
sp3 TAGTGGTATC
sp4 CTCAGTGGATC
```

What creates sequence variation?

Sequence alignment

An ***alignment*** defines which parts of the sequence are evolutionary or functionally comparable (***homologous***)



substitutions are common
indels are rare

```
  + +   +    ++
   **    *  *
sp1 CCAAG-CGTTATC
sp2 -TCAGTGGT-ATC
sp3 -T-AGTGGT-ATC
sp4 CTCAGTGGA--TC
```

8 indels
4 mismatches

substitutions are rare
indels are common

```
   +++  +     ++
          *
sp1 C-CAAGCGGTTATC
sp2 -TCA-GTGGT-ATC
sp3 -T-A-GTGGT-ATC
sp4 CTCA-GTGG--ATC
```

11 indels
1 mismatches

Alignment methods

Alignment algorithms find the matrix for which:

- rows are different sequences/genes
- columns are homologous characters
- some optimization criterion is maximized

Two dominant method families:

- ***heuristic methods*** optimize "match scores" for alignment matrix
- ***generative methods*** reconstruct the most probable history that generated the alignment matrix

Heuristic alignment

Example: minimize cost of alignment

```
TCAA---GTATCGACCT
TCATGCGGTATT-ACCT
```

+1	Match (11)
-1	Mismatch (2)
-2	Gap open (2)
-1	Gap extension (2)

+1x11 + -1x2 + -2x2 + -1x2 = +3

Heuristic alignment

Example: minimize cost of alignment

```
TCAA----GTAT-CGACCT
TCA-TGCGGTATT--ACCT
```

+1 Match (11)
-1 Mismatch (0)
-2 Gap open (4)
-1 Gap extension (4)

+1x11 + -1x0 + -2x4 + -1x4 = -1

Needleman-Wunsch example

Recursive algorithm

- compute *local cost* to add a match, mismatch, or gap to the pairwise alignment for each cell
- construct path based on best cost
- continue until you reach the final site
- traverse path in reverse order to obtain alignment

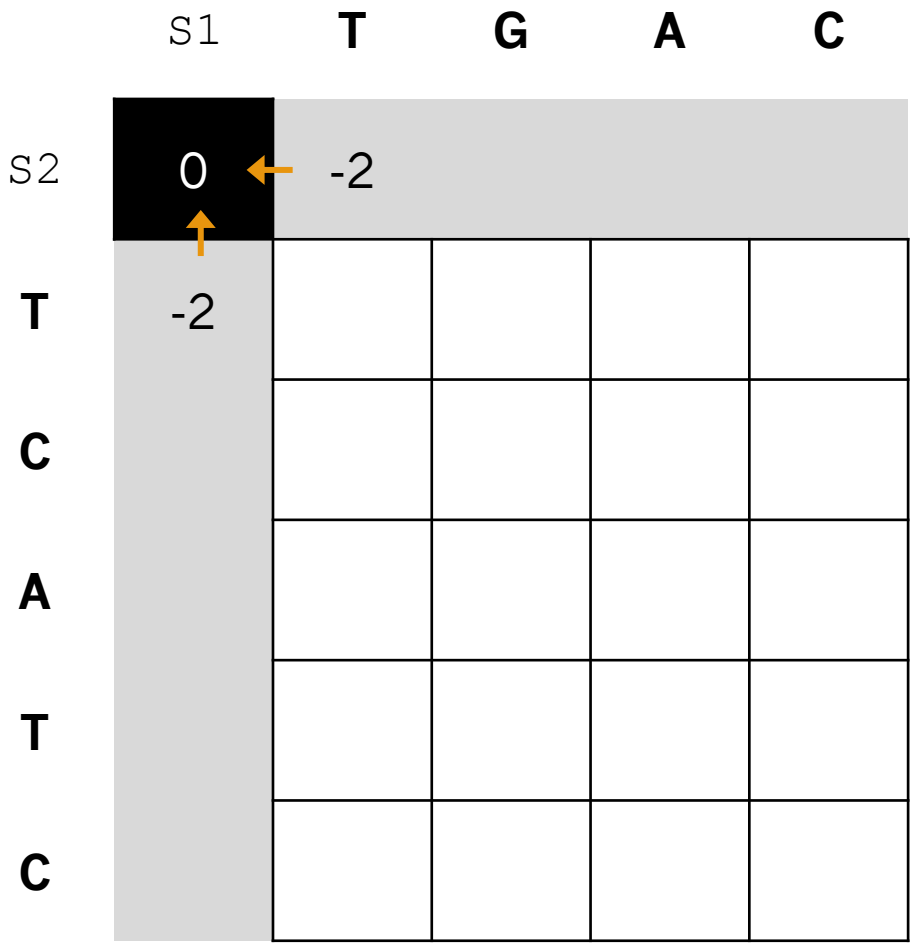
Needleman-Wunsch example

S1		T	G	A	C
S2	0				
T					
C					
A					
T					
C					

Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: ?
S2: ?

Needleman-Wunsch example



Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: ?
S2: ?

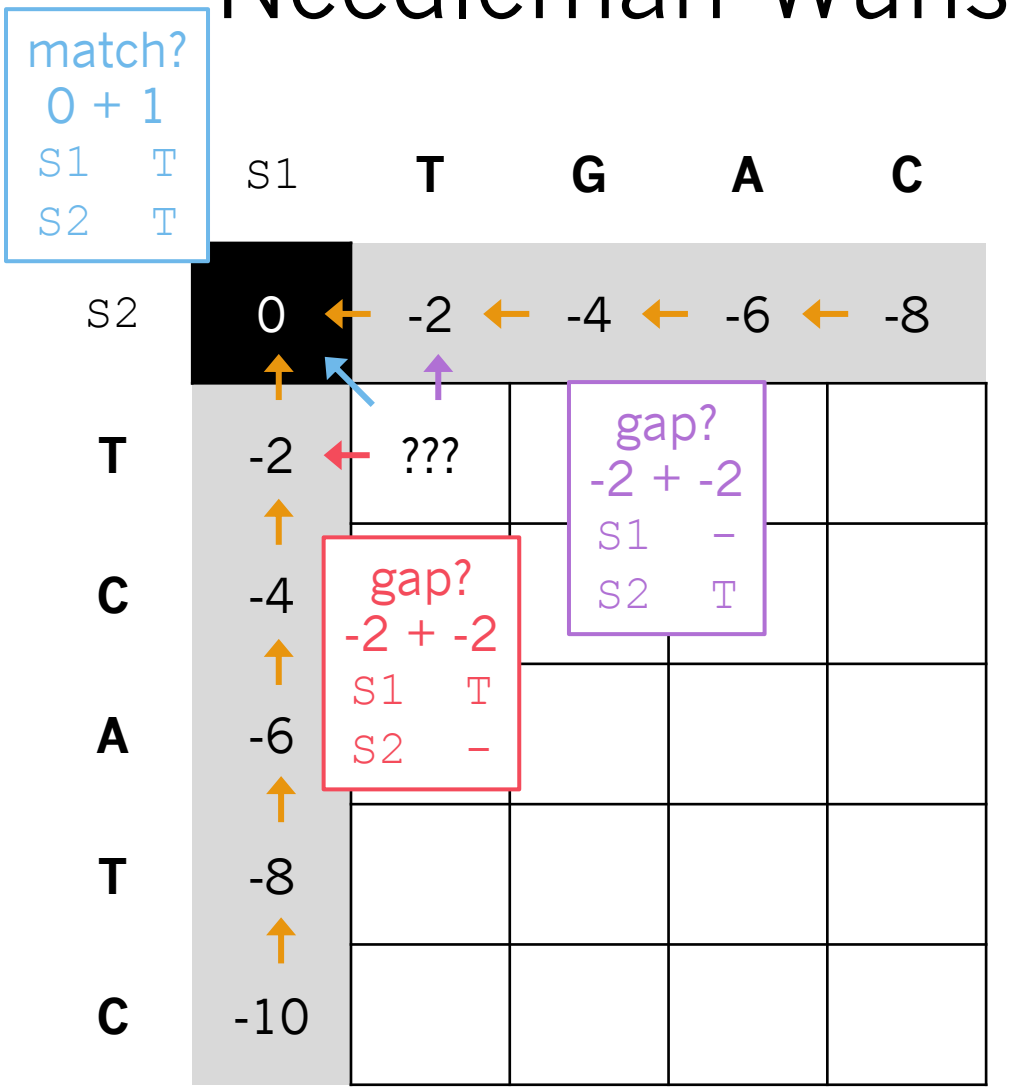
Needleman-Wunsch example

S1		T	G	A	C
S2	0 ←	-2 ←	-4 ←	-6 ←	-8
	↑				
T	-2				
	↑				
C	-4				
	↑				
A	-6				
	↑				
T	-8				
	↑				
C	-10				

Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: ?
S2: ?

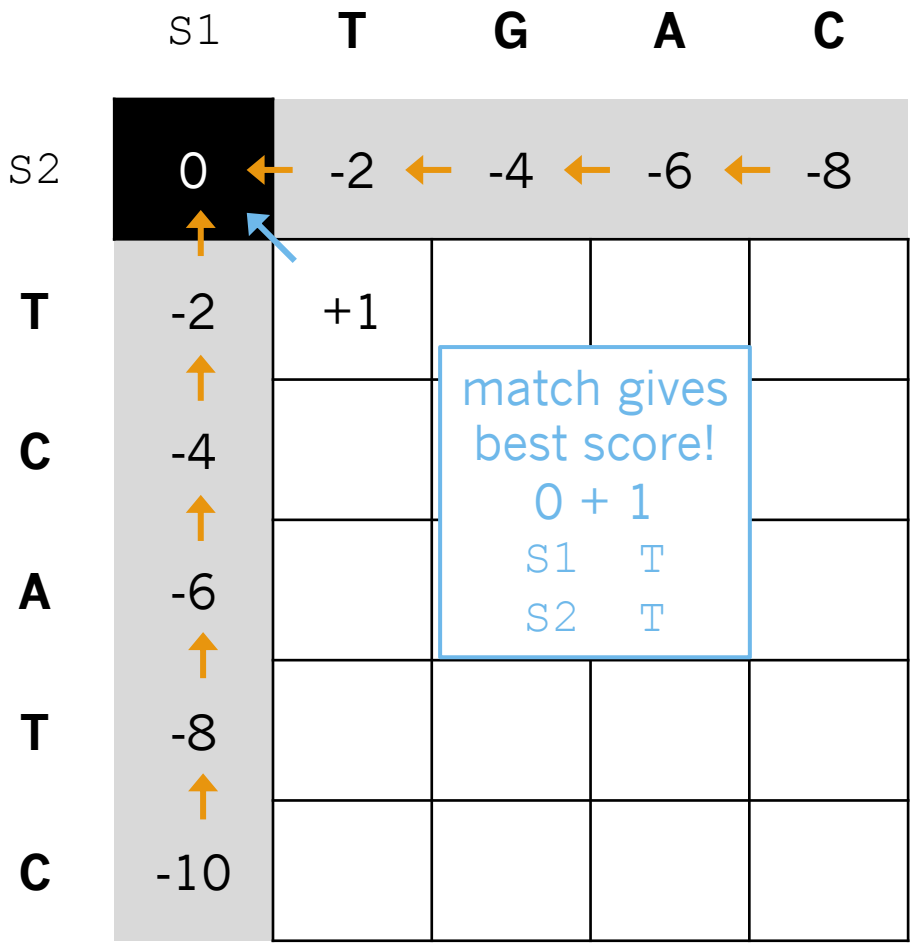
Needleman-Wunsch example



Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: ?
S2: ?

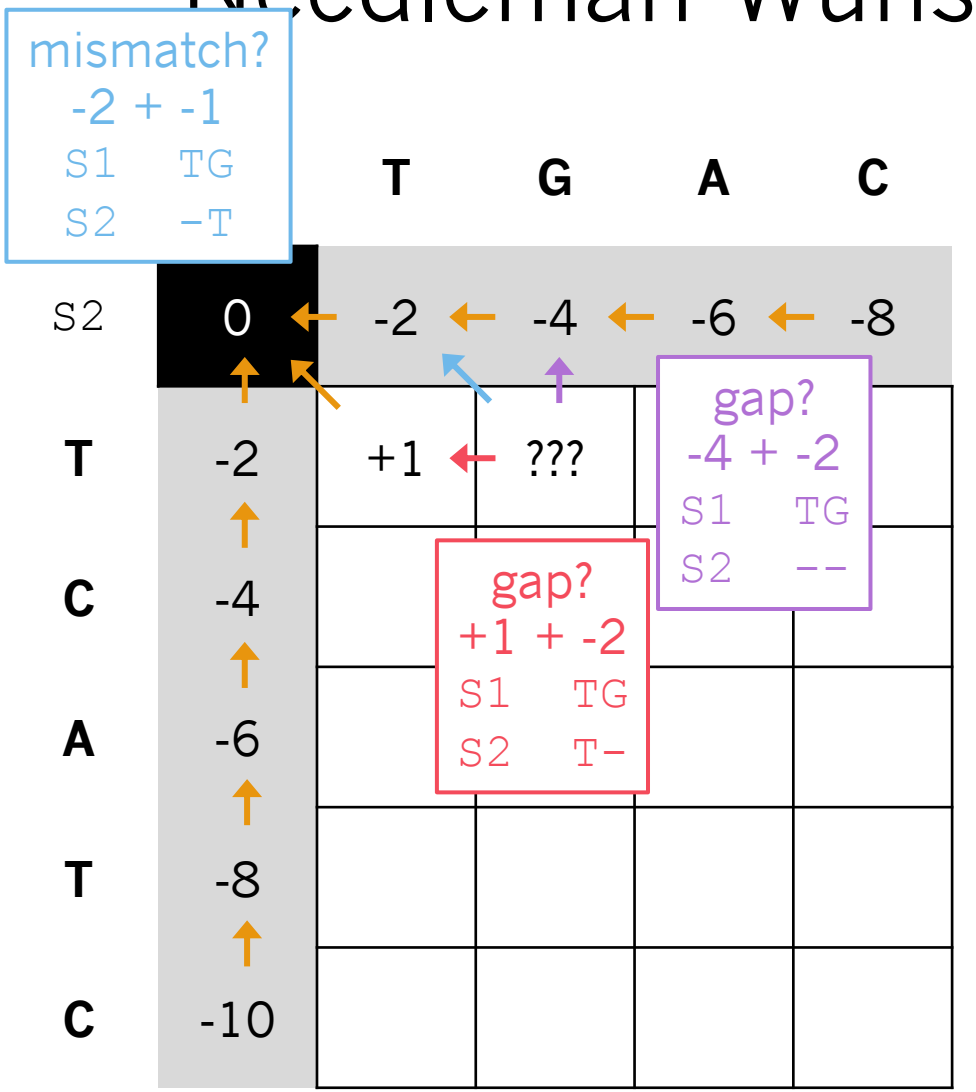
Needleman-Wunsch example



Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: ?
S2: ?

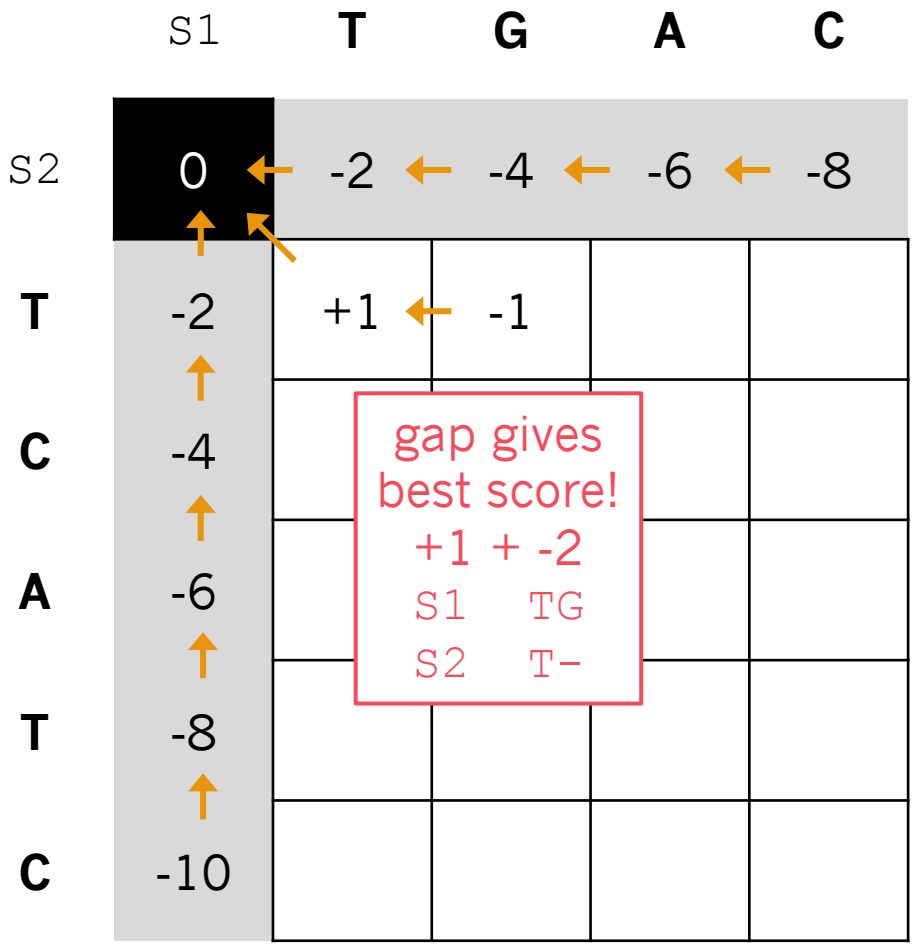
Needleman-Wunsch example



Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: ?
S2: ?

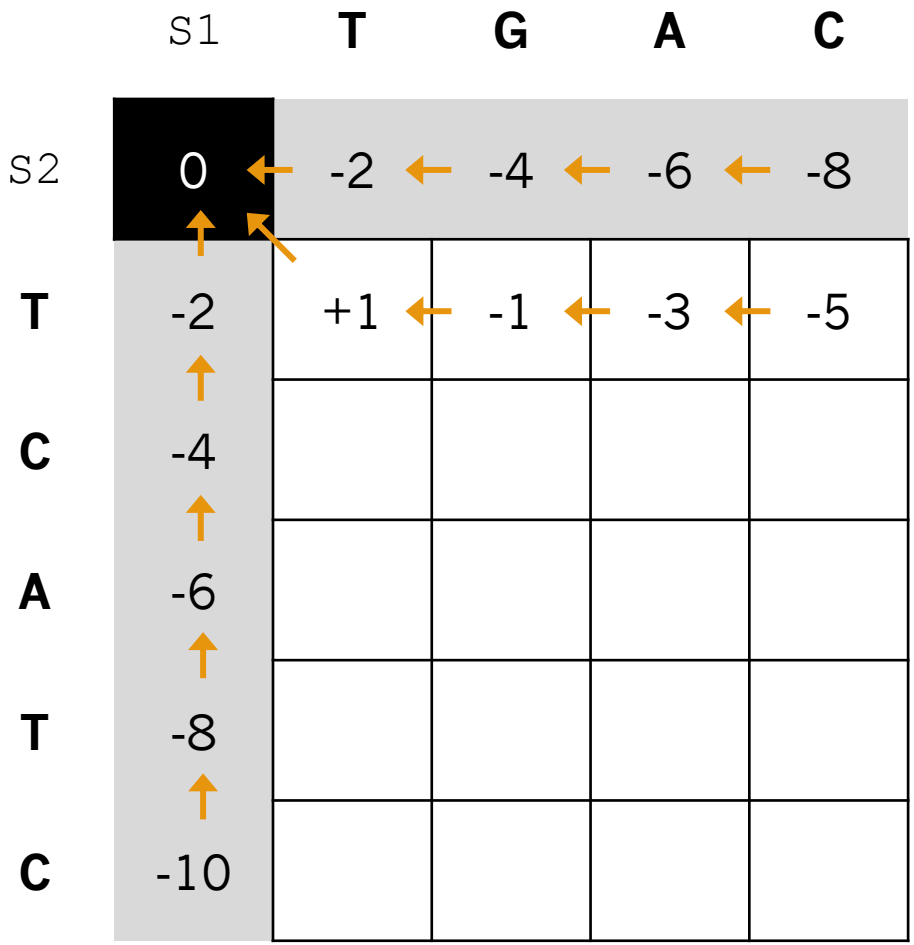
Needleman-Wunsch example



Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: ?
S2: ?

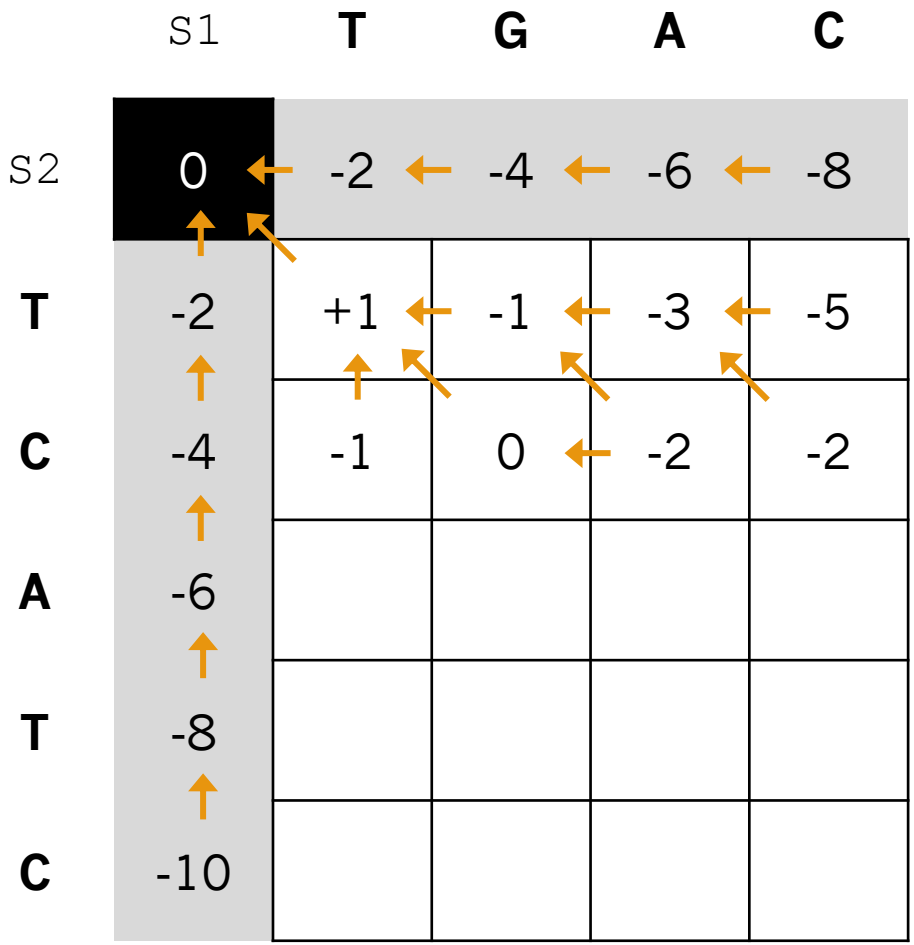
Needleman-Wunsch example



Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: ?
S2: ?

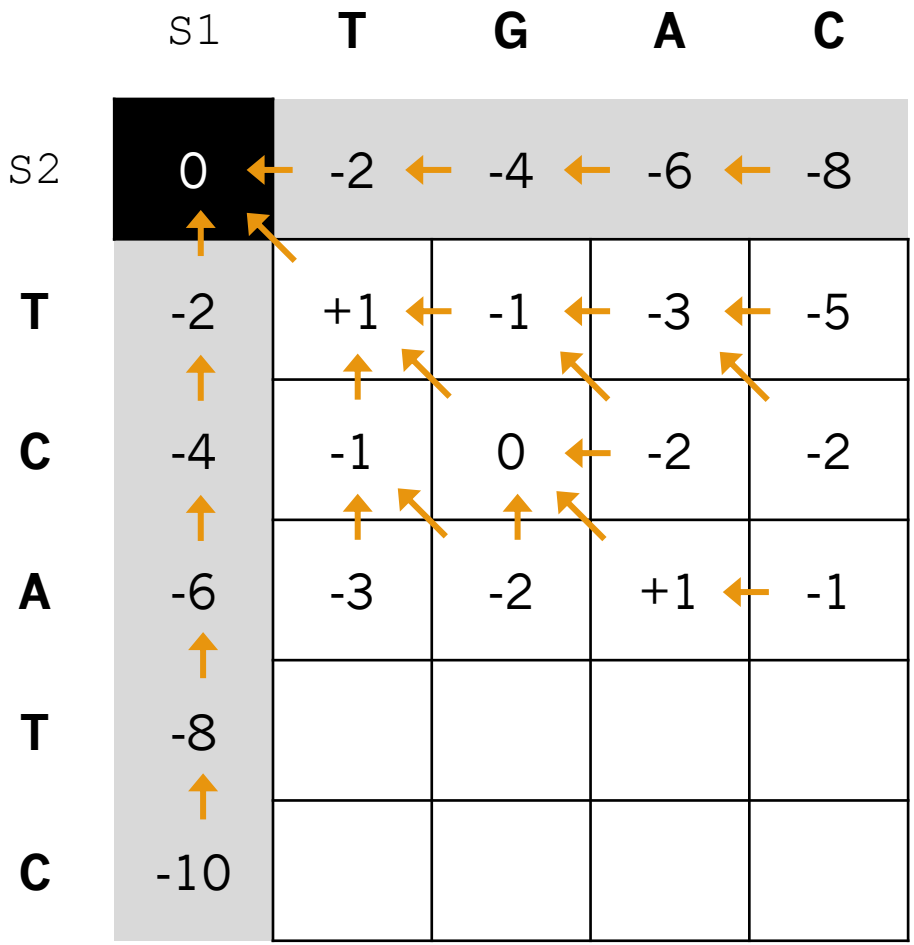
Needleman-Wunsch example



Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: ?
S2: ?

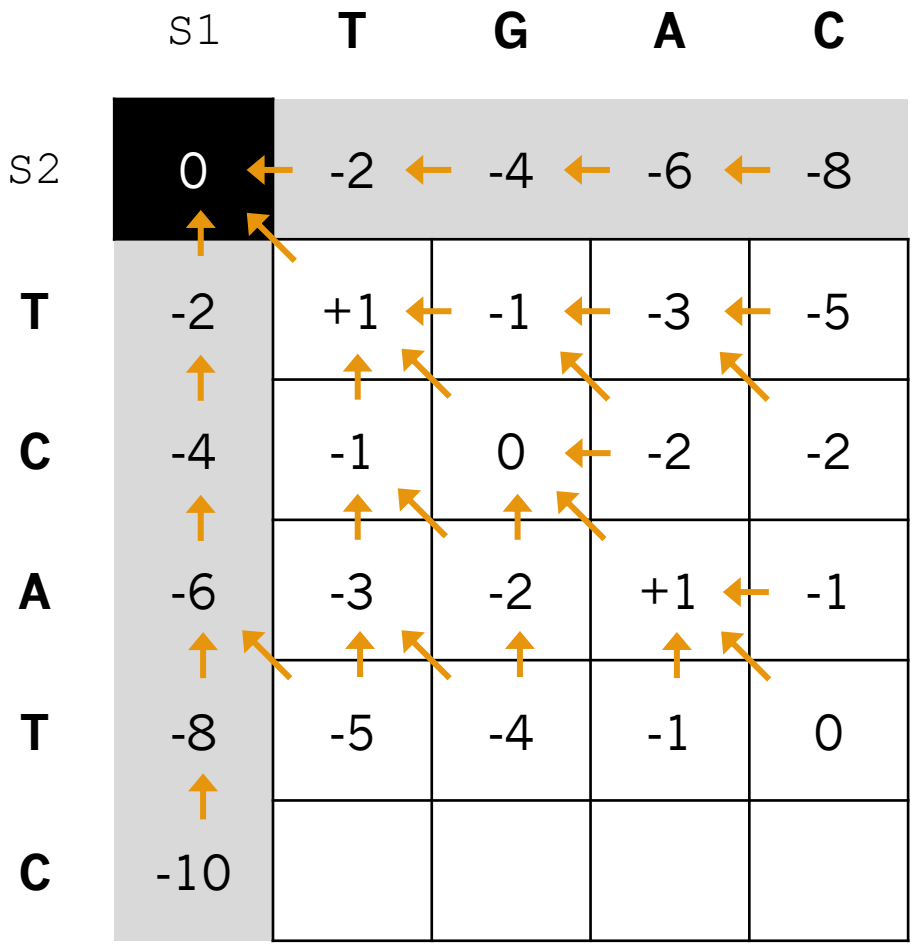
Needleman-Wunsch example



Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: ?
S2: ?

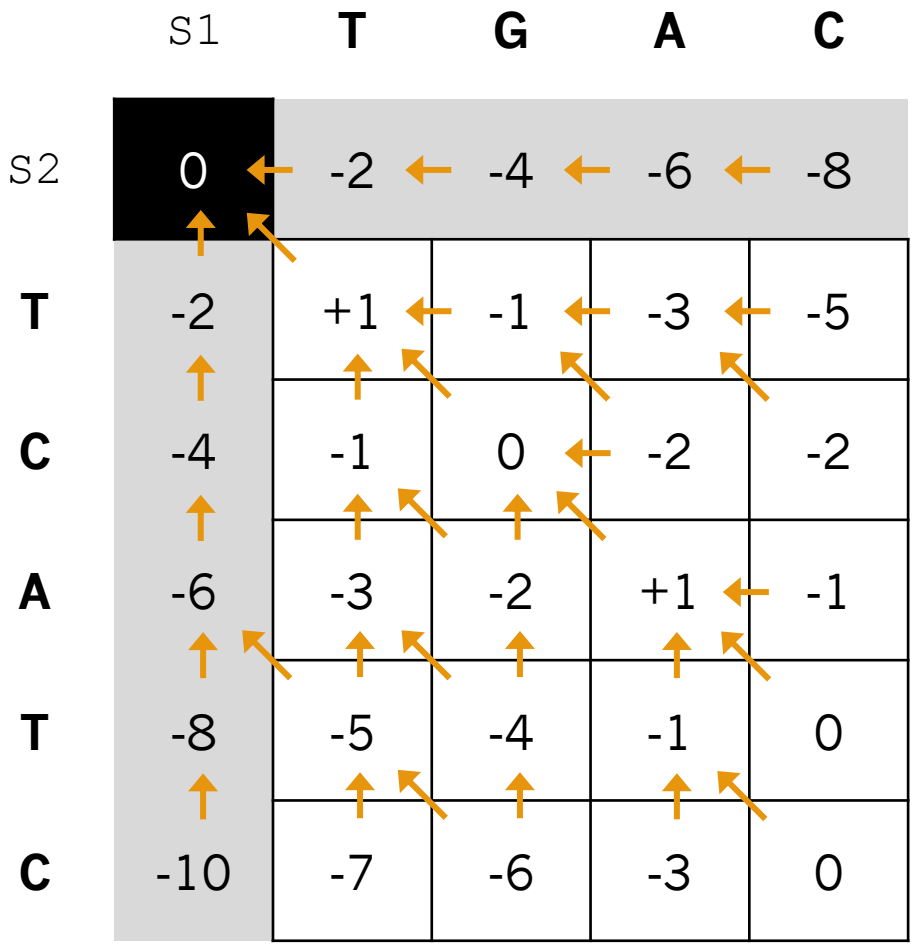
Needleman-Wunsch example



Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: ?
S2: ?

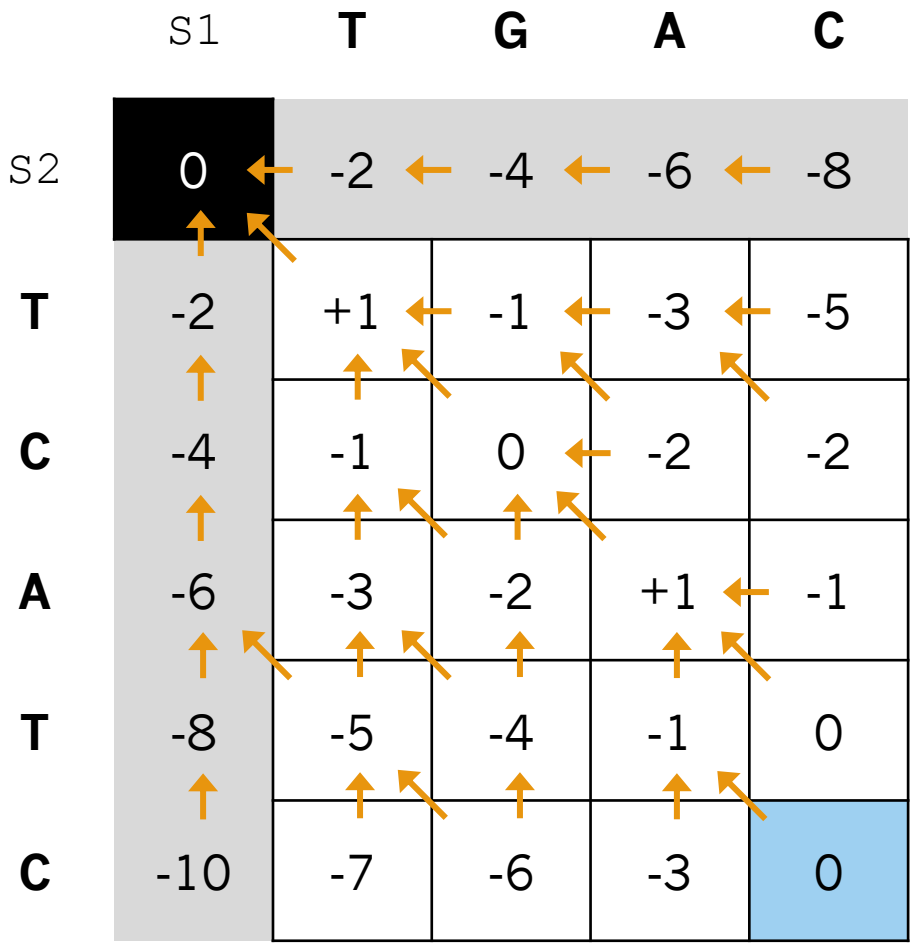
Needleman-Wunsch example



Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: ?
S2: ?

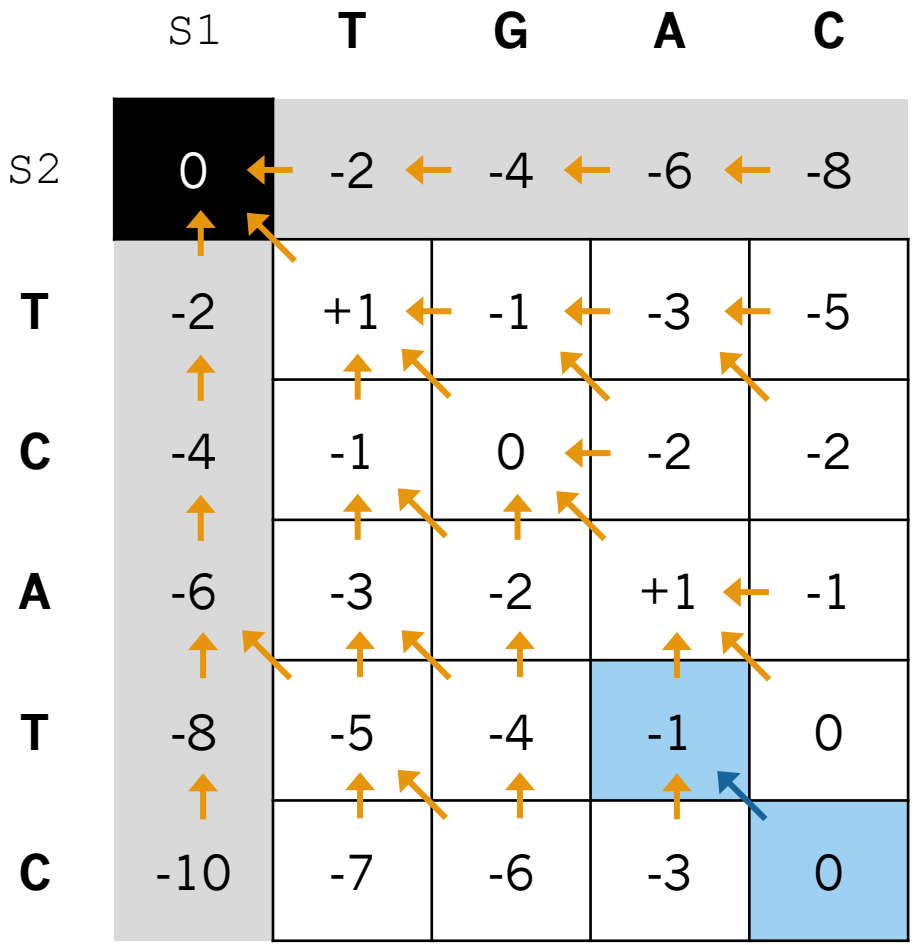
Needleman-Wunsch example



Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: C
S2: C

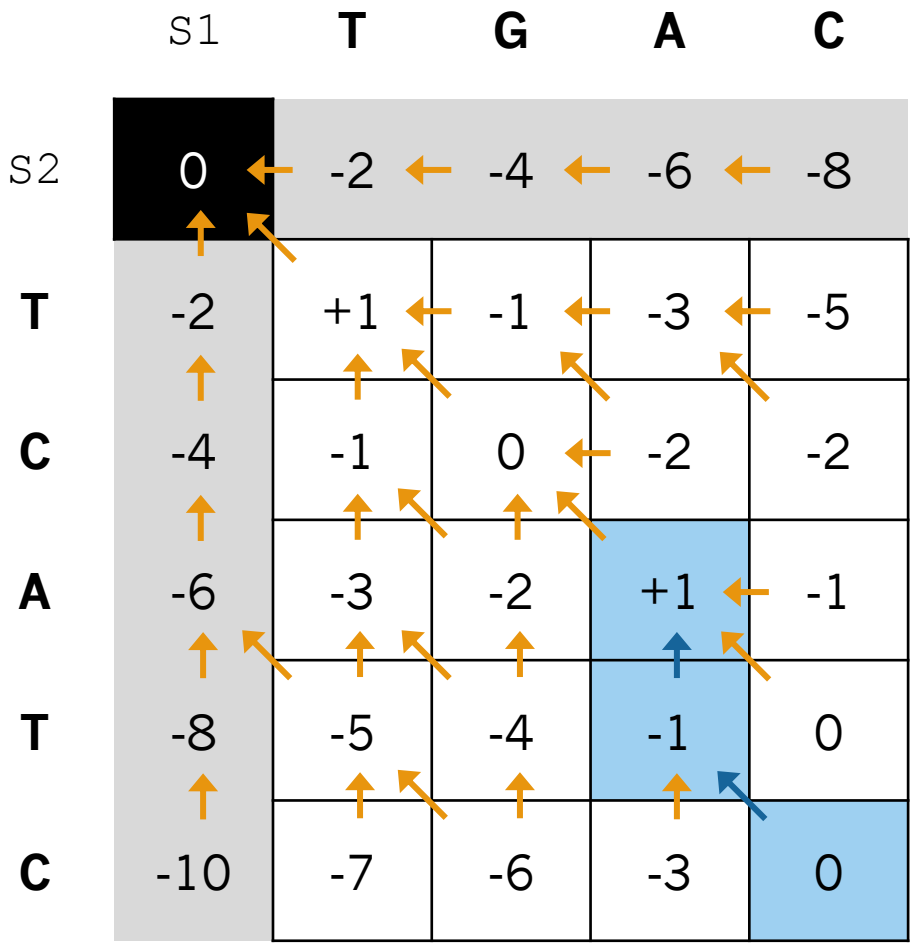
Needleman-Wunsch example



Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: AC
S2: TC

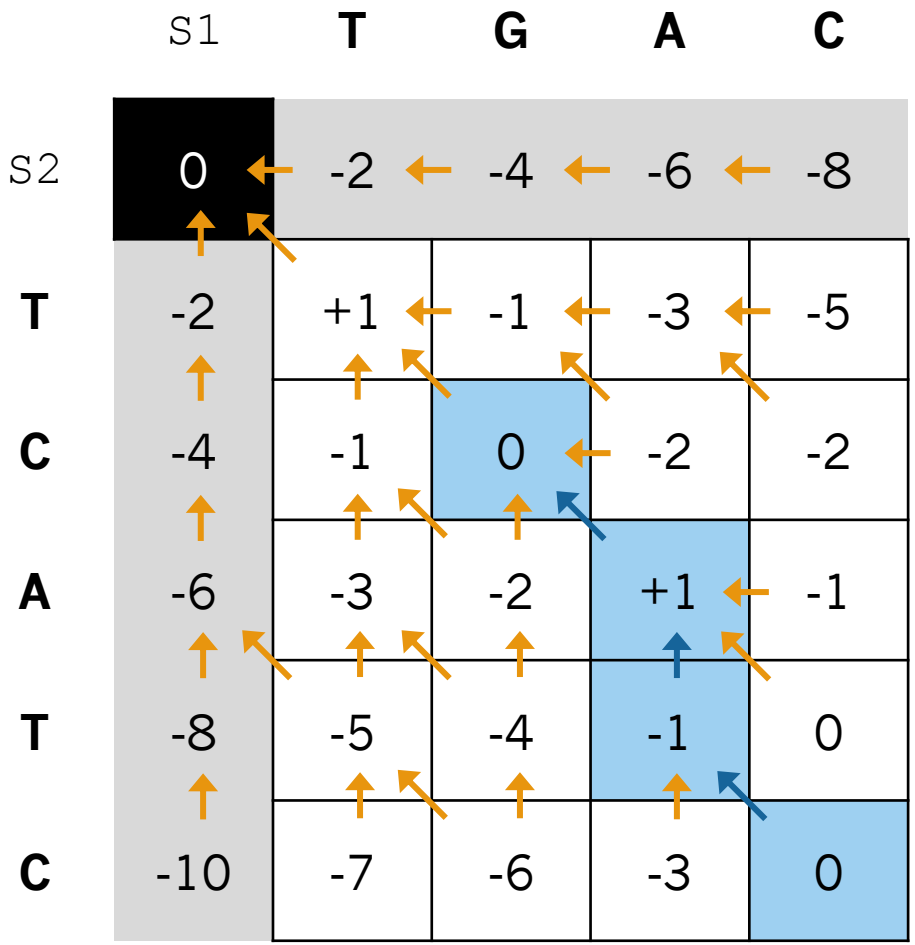
Needleman-Wunsch example



Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: A–C
S2: ATC

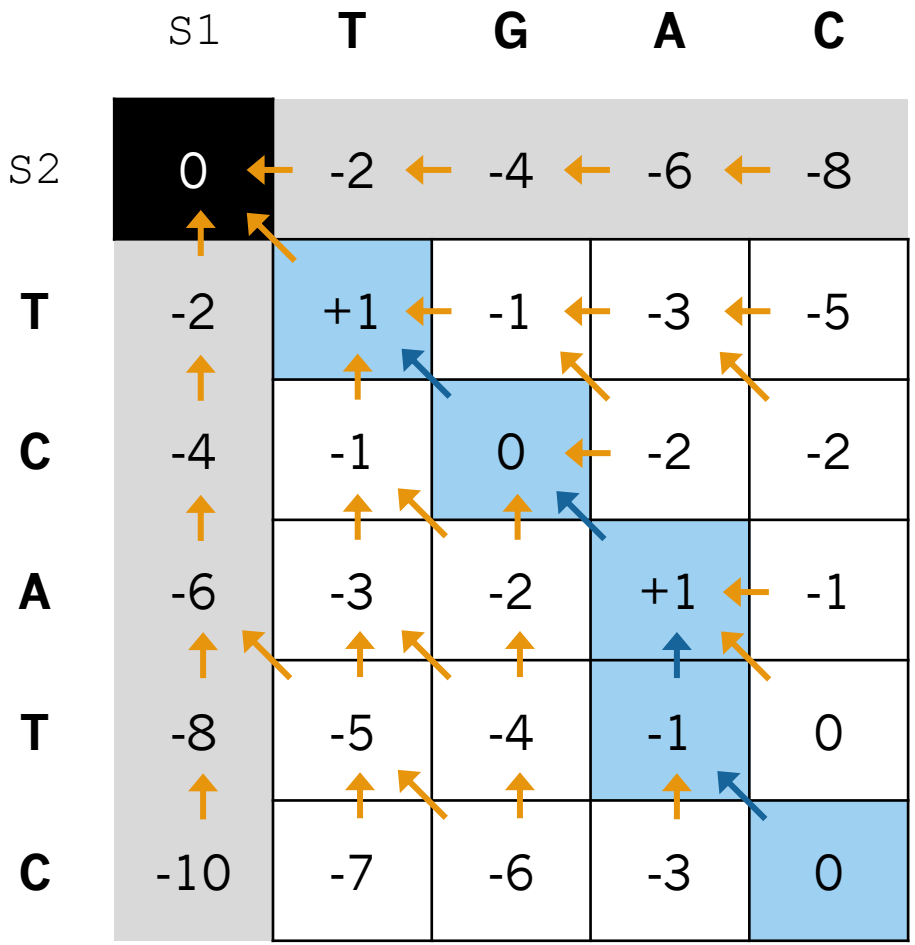
Needleman-Wunsch example



Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: GA–C
S2: CATC

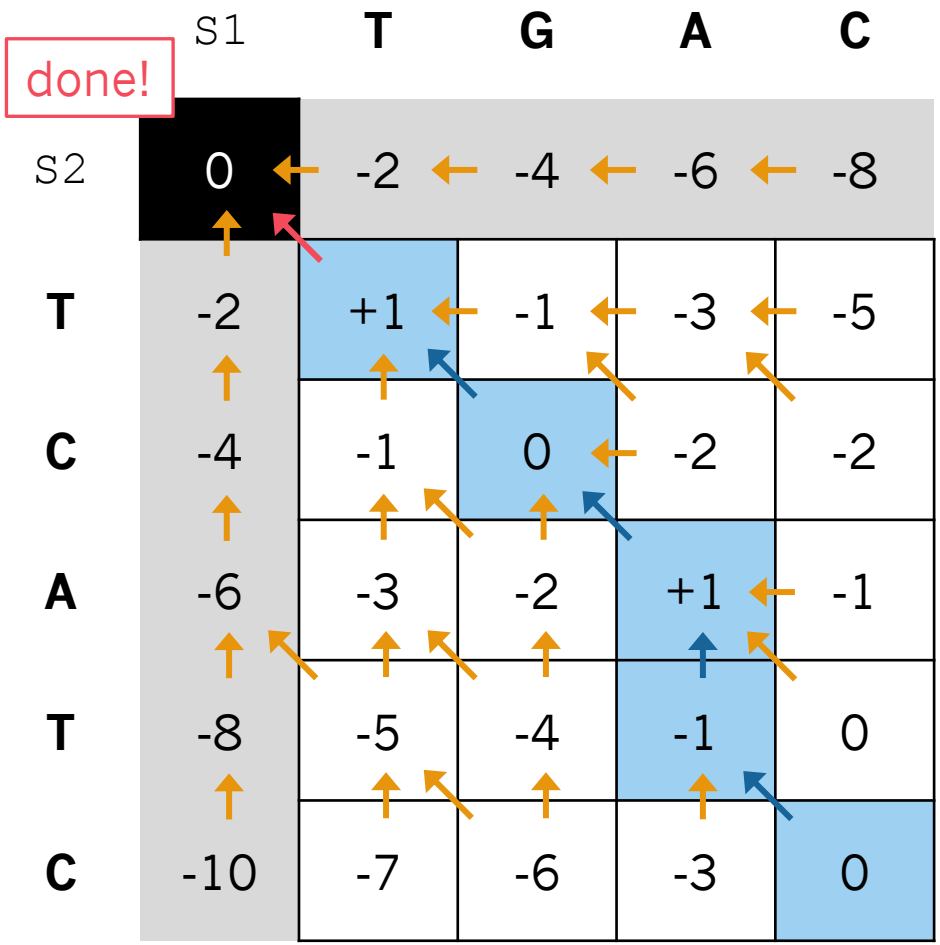
Needleman-Wunsch example



Type	Score
Match	+1
Mismatch	-1
Gap	-2

```
S1: TGA-C
S2: TCATC
```

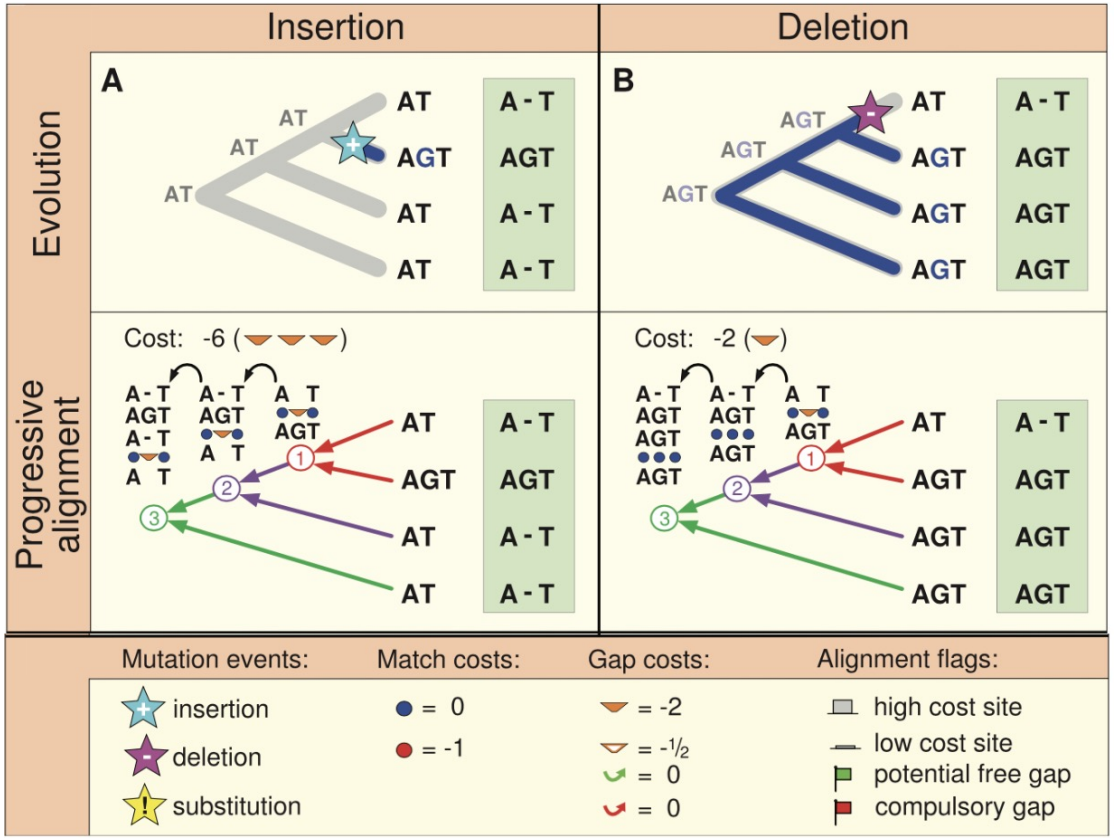
Needleman-Wunsch example



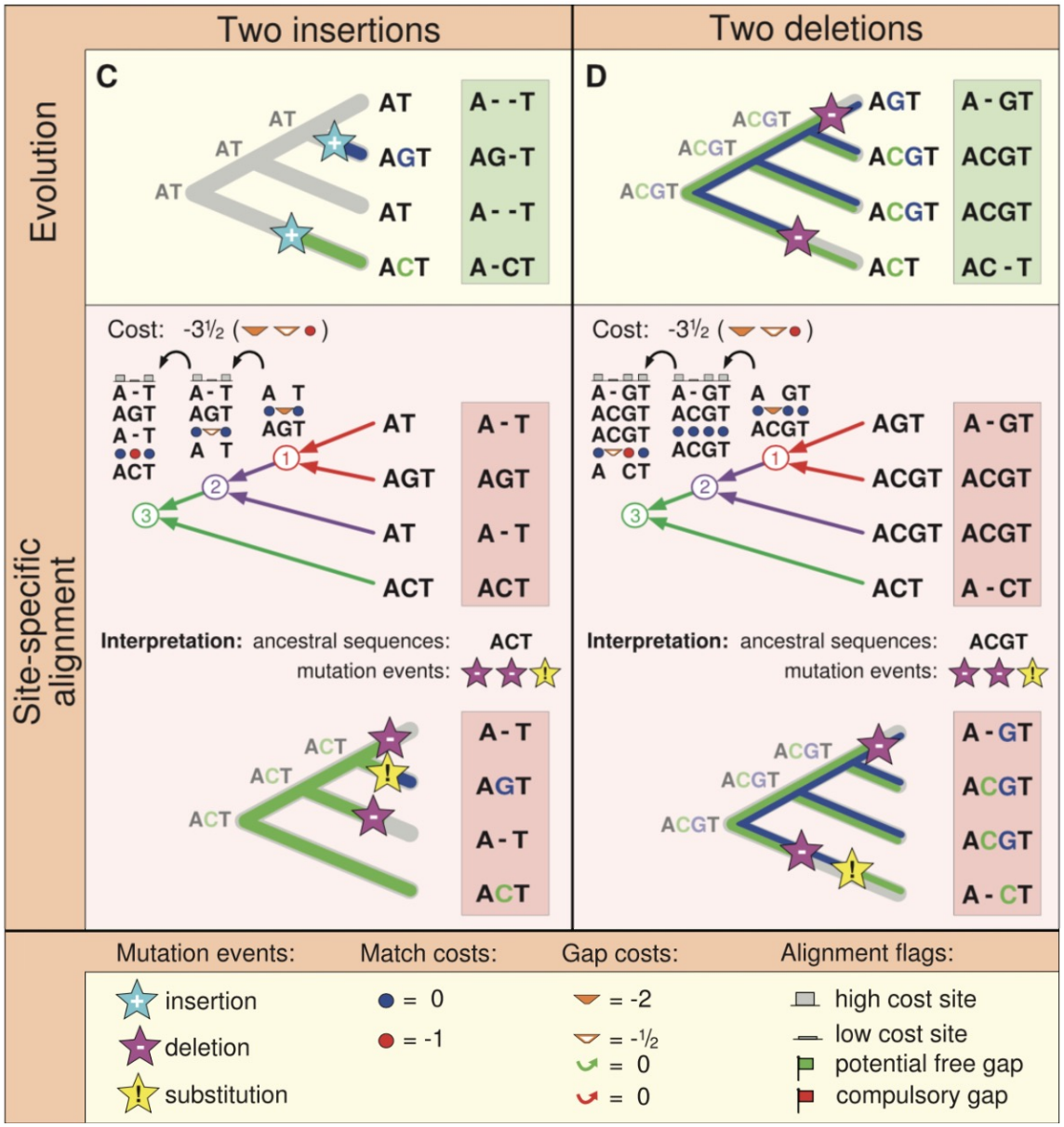
Type	Score
Match	+1
Mismatch	-1
Gap	-2

```
S1: TGA-C
S2: TCATC
```

Progressive alignment



Aligns multiple sequences by *progressively* adding new sequences to alignment based on a ***guide tree*** (*phylogeny*)



Even mildly complex evolutionary scenarios can cause progressive alignments to produce inaccurate homology statements

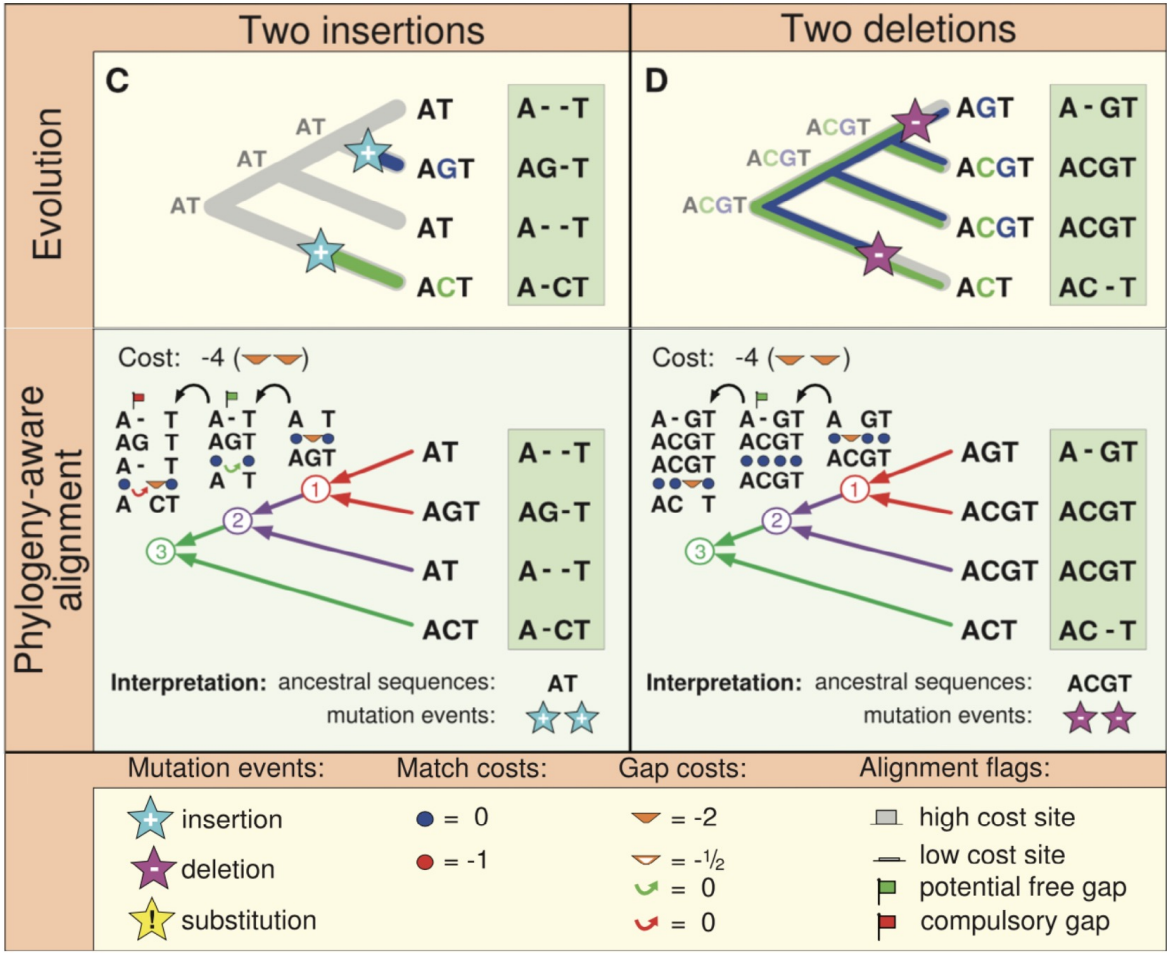
See the two-insertion scenario (left)

Generative alignment

Generative methods uses guide tree to reconstruct the series of substitution, insertion, and deletion events that most likely generated a sequence alignment

Alignment score depends on rates of:

- substitution
- deletion
- insertion



This software (PRANK; left) "flags" events as scored so they're not double-counted

Generative alignments tend to be "gappier"

More evolutionarily accurate statements of homology

only print matching text, *grep -o*

```
$ cat limerick.txt
A Unix sales lady, Lenore.
Enjoys work, but she likes the beach more
She found a good way
To combine work and play:
She sells C shells by the seashore.
$ grep -o ”lls" limerick.txt
lls
lls
```

Overview for Lab 08