Lecture 08
sequence alignment

Hibiscus lasiocarpus
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Missouri Botanical Garden

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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

TCCAAGCGTTATC

same length, same content → TCCAAGCGTTATC

AATCAGTGGTATC ← same length, diff. content

diff. length, diff. content → TAGTGGTATC

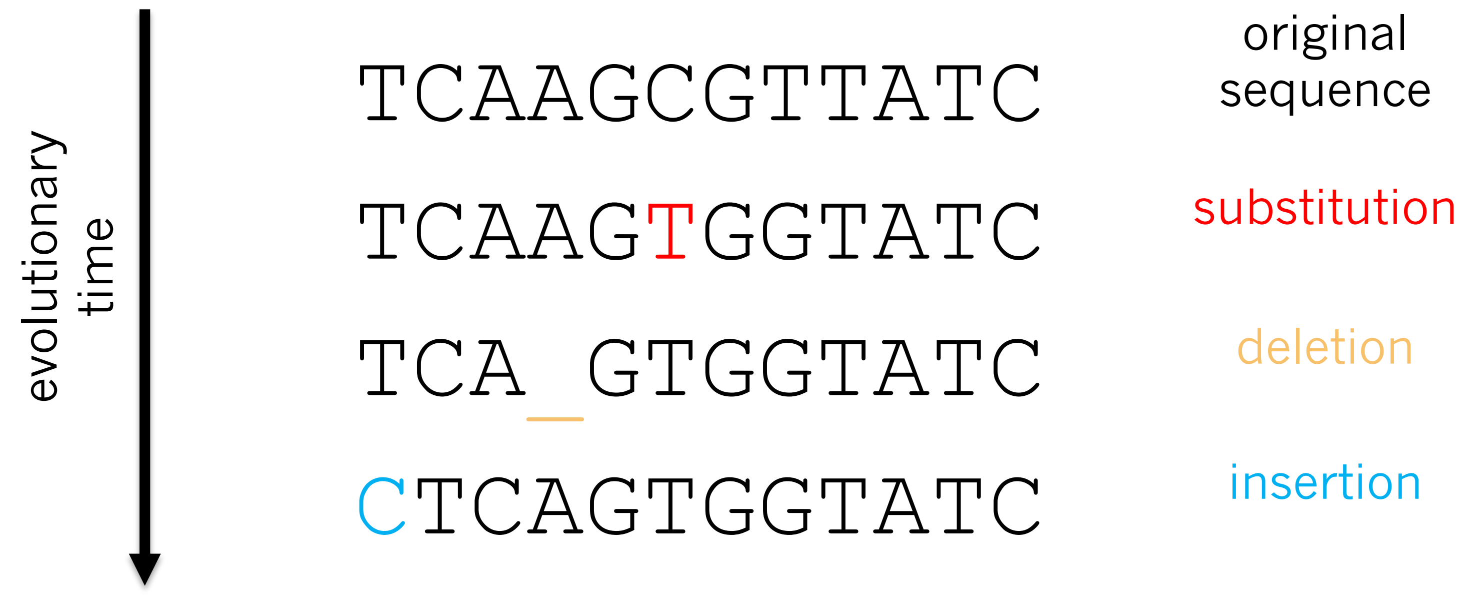
Sequence alignment

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?



evolutionary time

TCAAGCGTTATC — original sequence

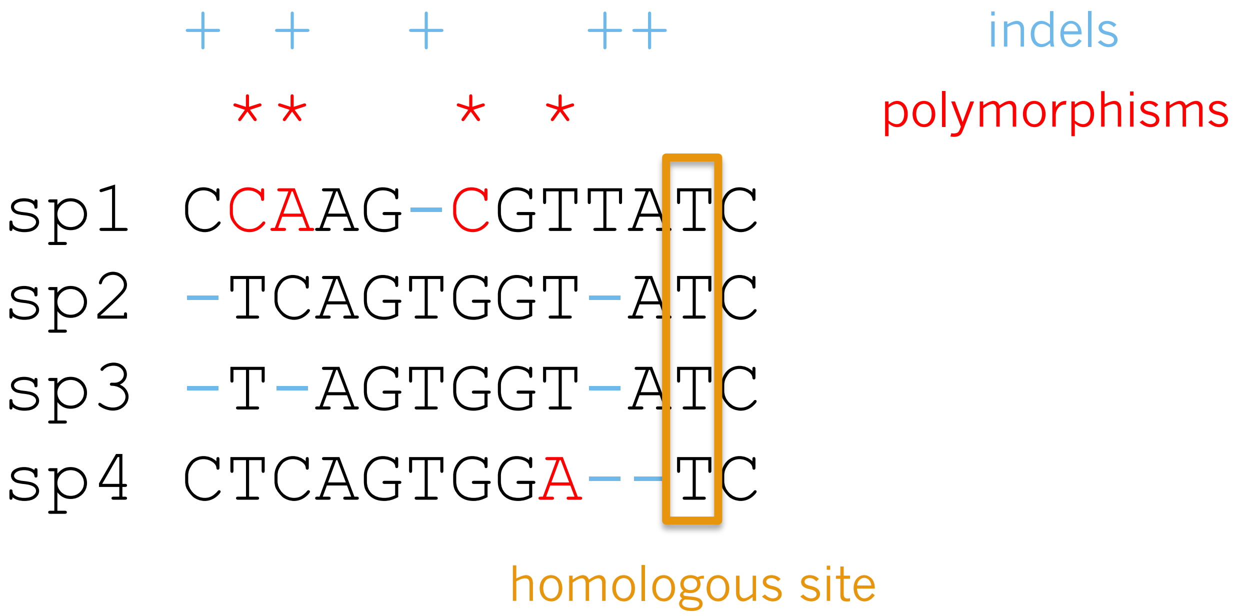
TCAAGTGGTATC — substitution

TCA_GTGGTATC — deletion

CTCAGTGGTATC — insertion

Sequence alignment

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



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

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: maximize alignment score

```
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: maximize alignment score

```
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

S2 \ S1	S1	T	G	A	C
S2	0	← -2			
T	↑ -2				
C					
A					
T					
C					

Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: ?
S2: ?

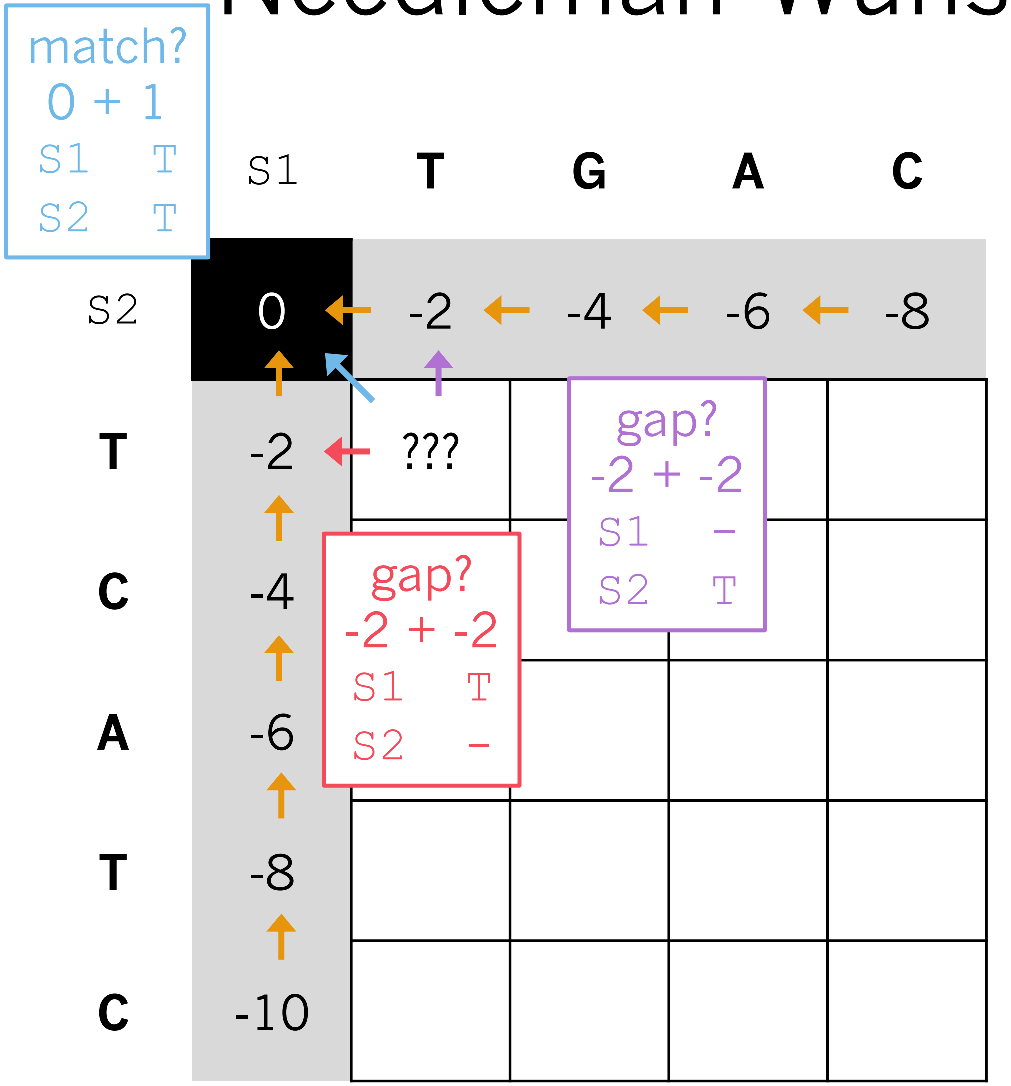
Needleman-Wunsch example

	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



match?
0 + 1
S1 T
S2 T

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

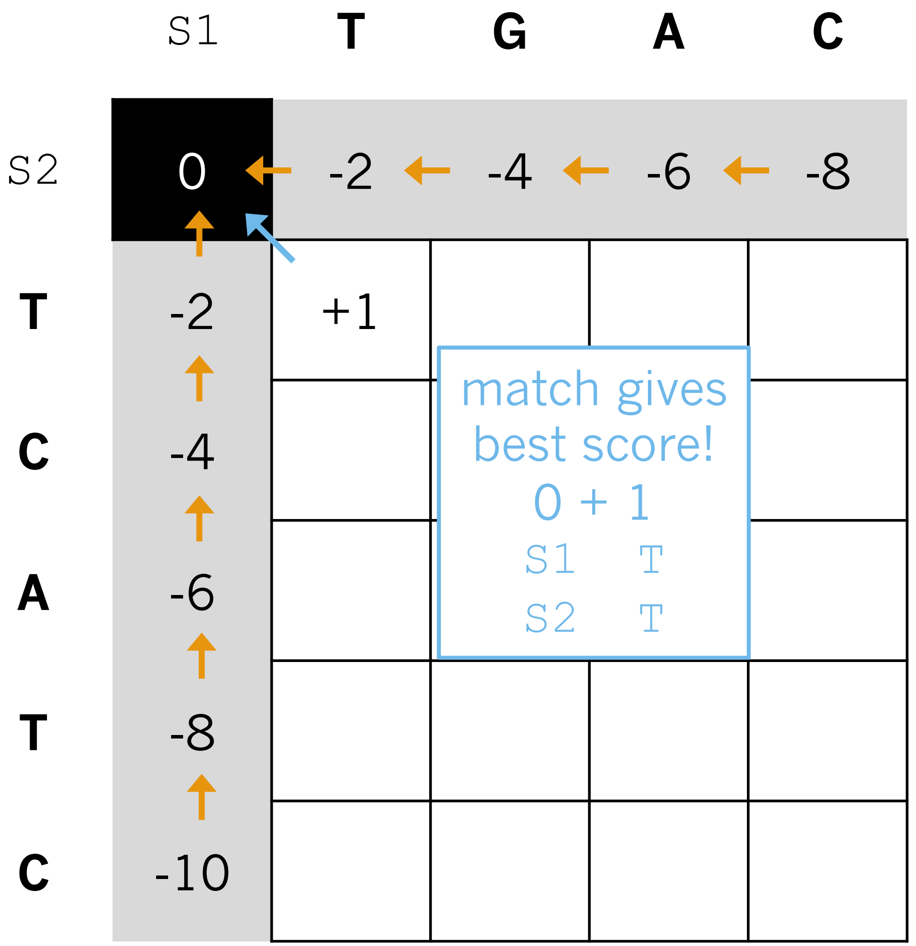
gap?
-2 + -2
S1 –
S2 T

gap?
-2 + -2
S1 T
S2 –

Type	Score
Match	+1
Mismatch	-1
Gap	-2

```
S1: ?
S2: ?
```

Needleman-Wunsch example



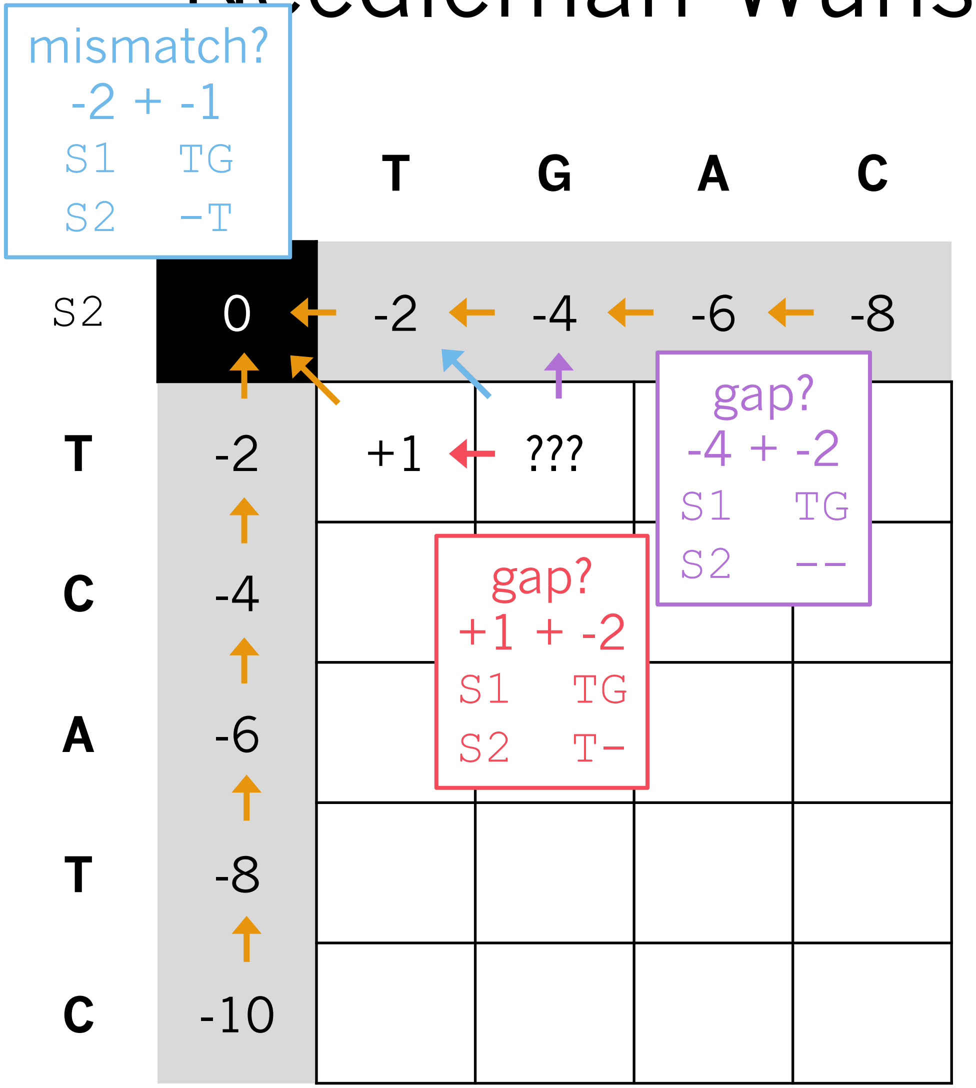
	S1	T	G	A	C
S2	0	-2	-4	-6	-8
T	-2	+1			
C	-4				
A	-6				
T	-8				
C	-10				

match gives best score!
0 + 1
S1 T
S2 T

Type	Score
Match	+1
Mismatch	-1
Gap	-2

```
S1: ?
S2: ?
```

Needleman-Wunsch example



mismatch?
-2 + -1
S1 TG
S2 -T

gap?
-4 + -2
S1 TG
S2 --

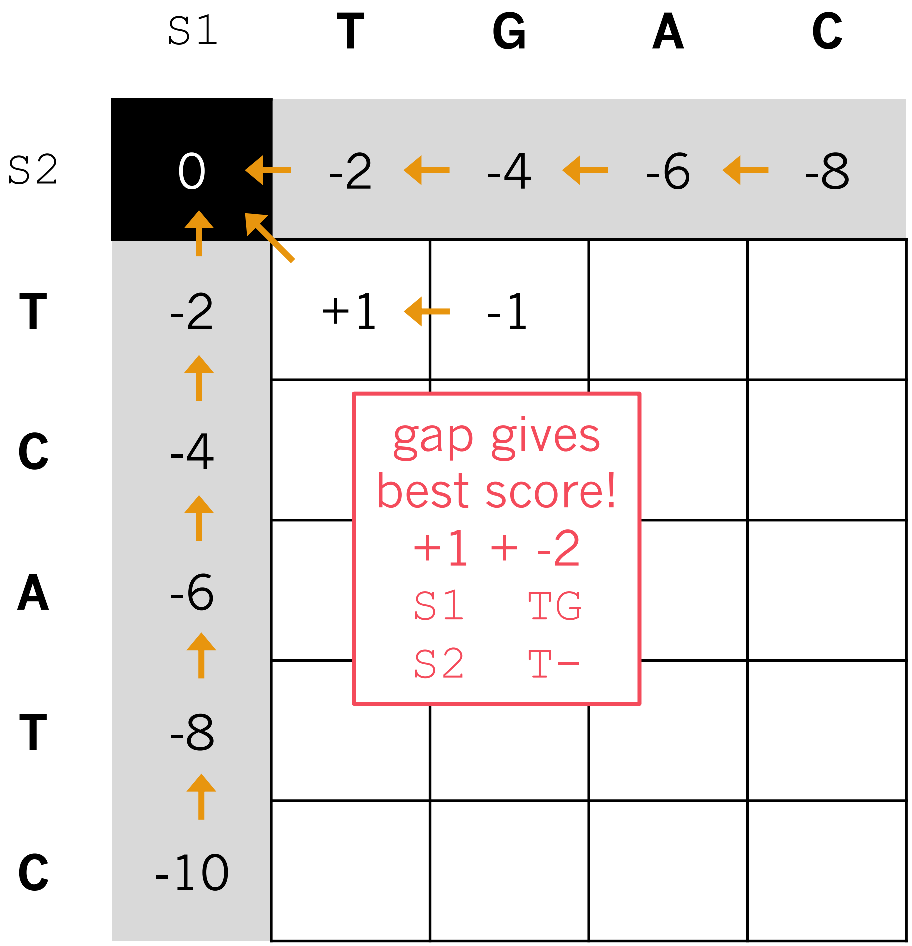
gap?
+1 + -2
S1 TG
S2 T-

		T	G	A	C
S2	0	-2	-4	-6	-8
T	-2	+1	???		
C	-4				
A	-6				
T	-8				
C	-10				

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	+1	-1		
C	-4				
A	-6				
T	-8				
C	-10				

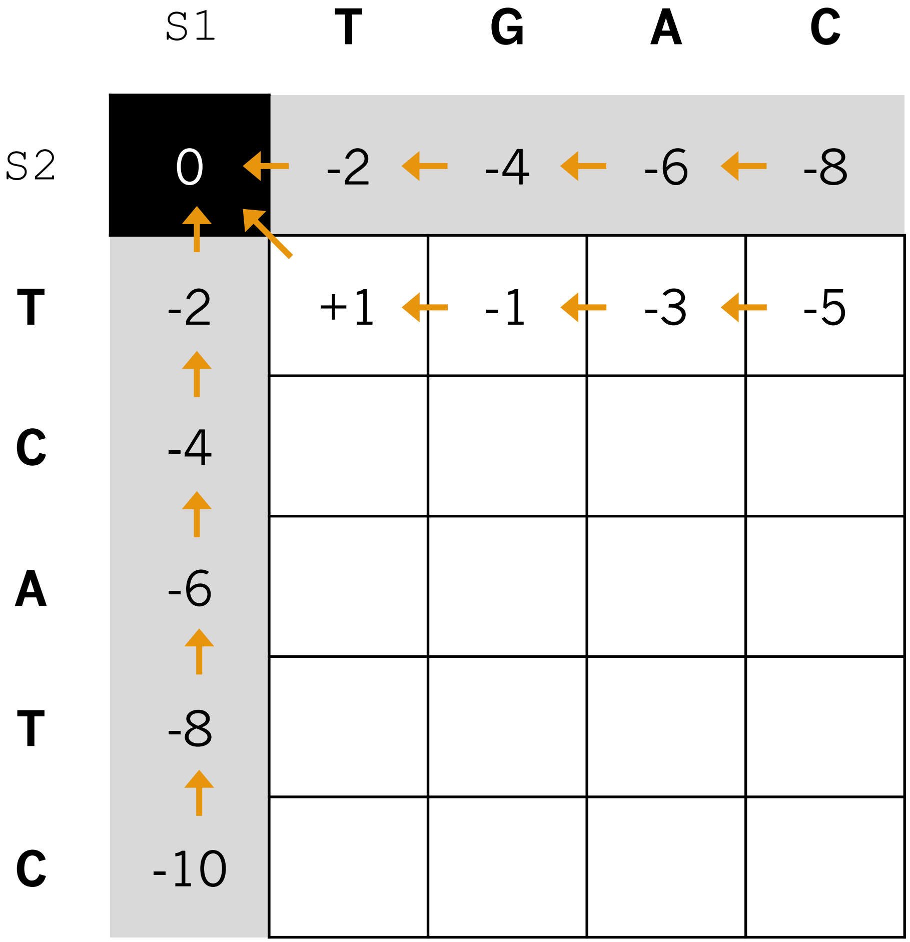
gap gives best score!
+1 + -2

```
S1   TG
S2   T-
```

Type	Score
Match	+1
Mismatch	-1
Gap	-2

```
S1: ?
S2: ?
```

Needleman-Wunsch example

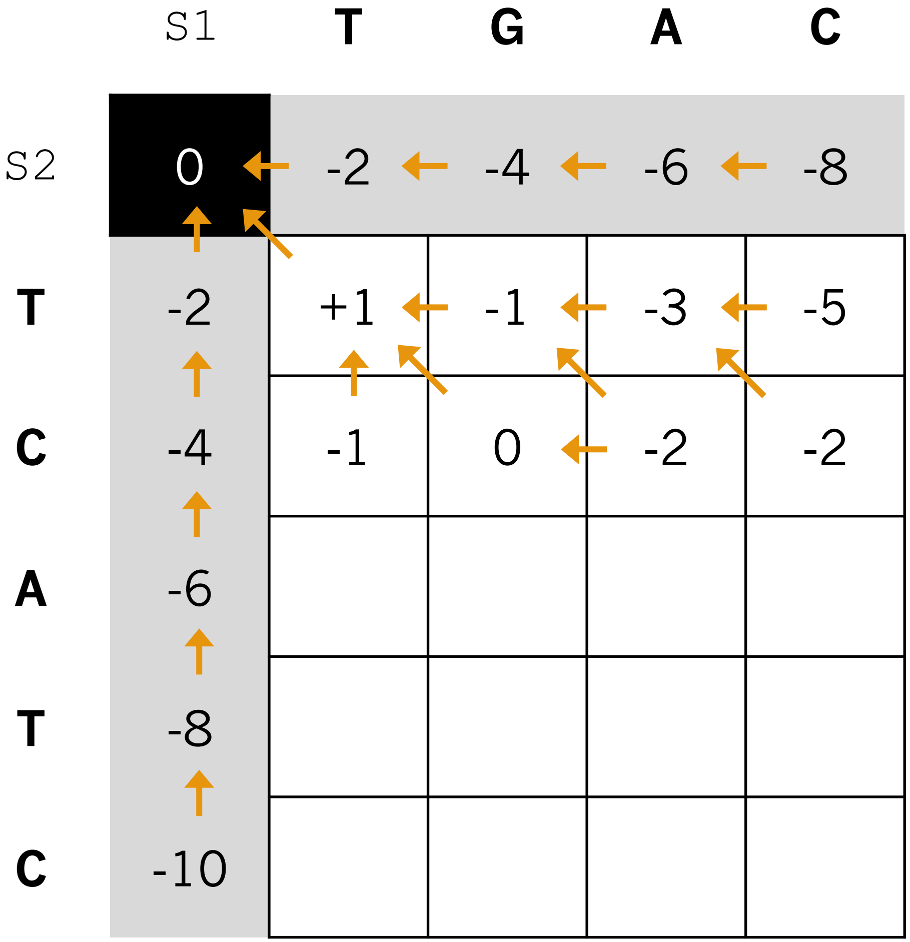


S2	S1	T	G	A	C
S2	0	← -2	← -4	← -6	← -8
T	↑ -2	↖ +1	← -1	← -3	← -5
C	↑ -4				
A	↑ -6				
T	↑ -8				
C	↑ -10				

Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: ?
S2: ?

Needleman-Wunsch example

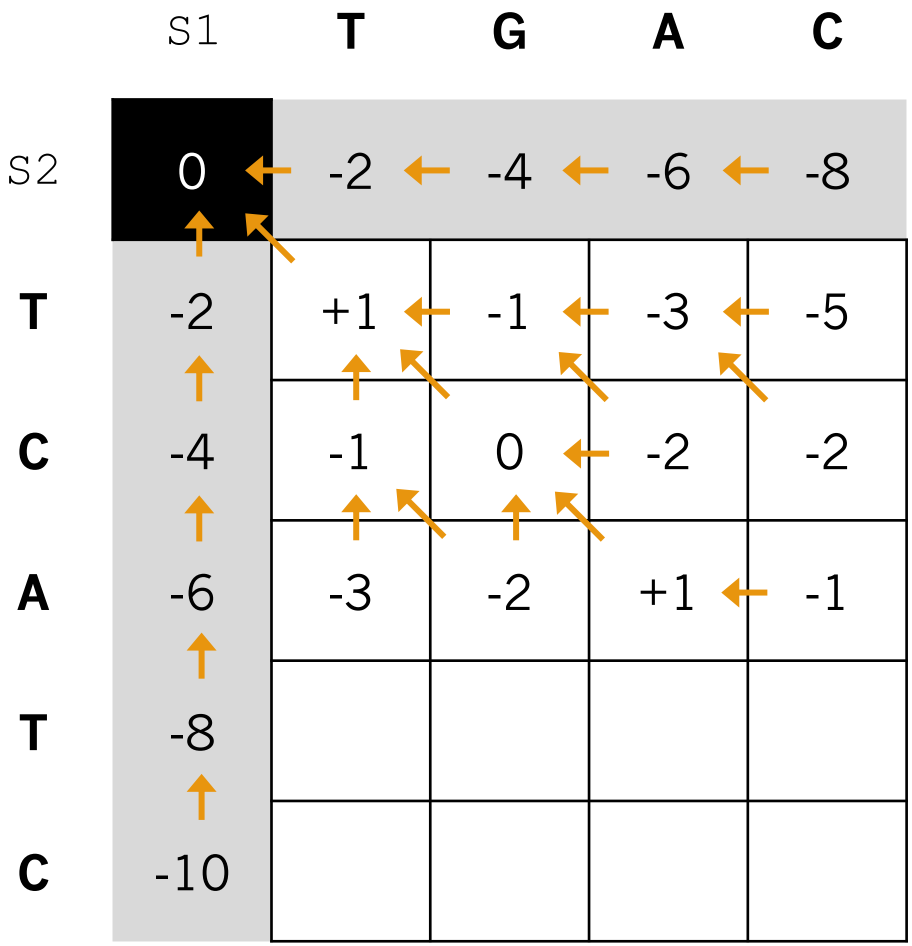


	S1	T	G	A	C
S2	0	-2	-4	-6	-8
T	-2	+1	-1	-3	-5
C	-4	-1	0	-2	-2
A	-6				
T	-8				
C	-10				

Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: ?
S2: ?

Needleman-Wunsch example

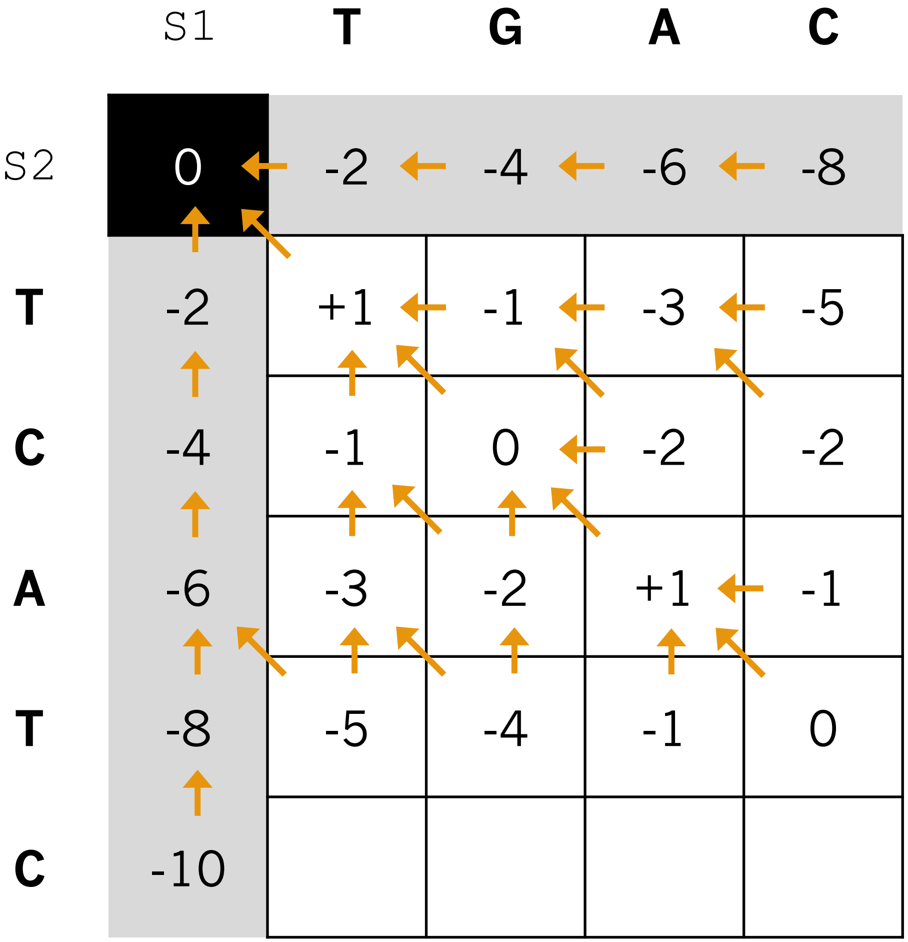


	S1	T	G	A	C
S2	0	-2	-4	-6	-8
T	-2	+1	-1	-3	-5
C	-4	-1	0	-2	-2
A	-6	-3	-2	+1	-1
T	-8				
C	-10				

Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: ?
S2: ?

Needleman-Wunsch example

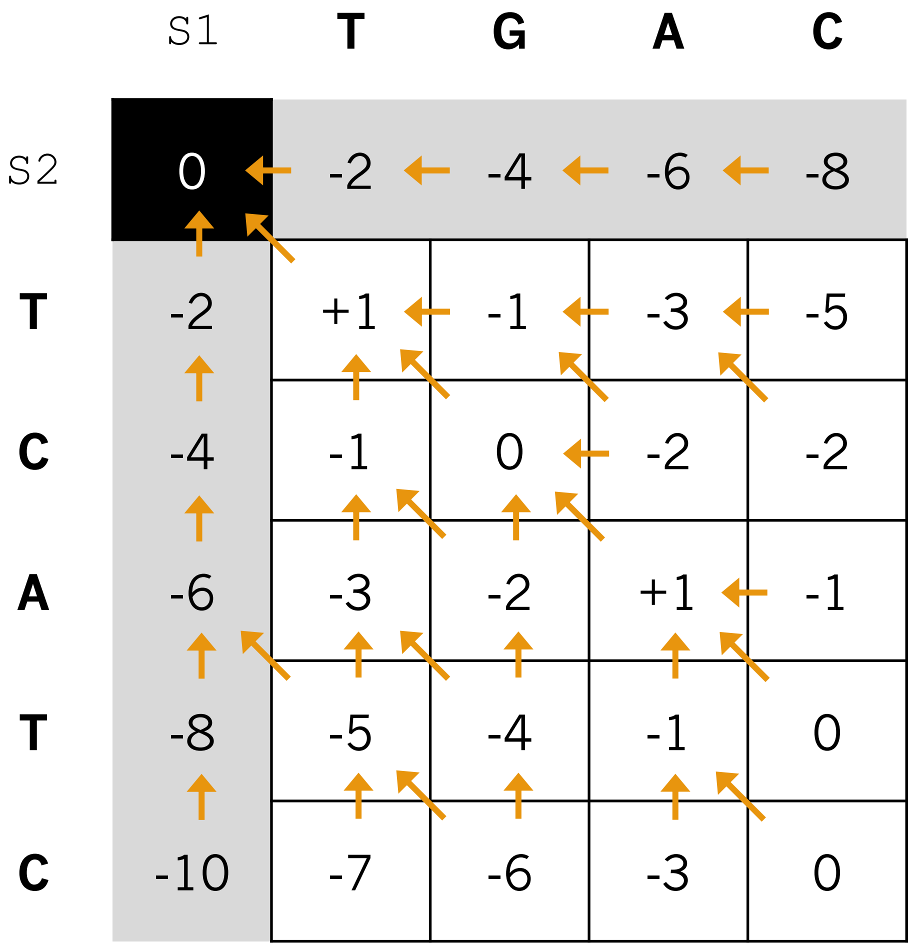


	S1	T	G	A	C
S2	0	-2	-4	-6	-8
T	-2	+1	-1	-3	-5
C	-4	-1	0	-2	-2
A	-6	-3	-2	+1	-1
T	-8	-5	-4	-1	0
C	-10				

Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: ?
S2: ?

Needleman-Wunsch example

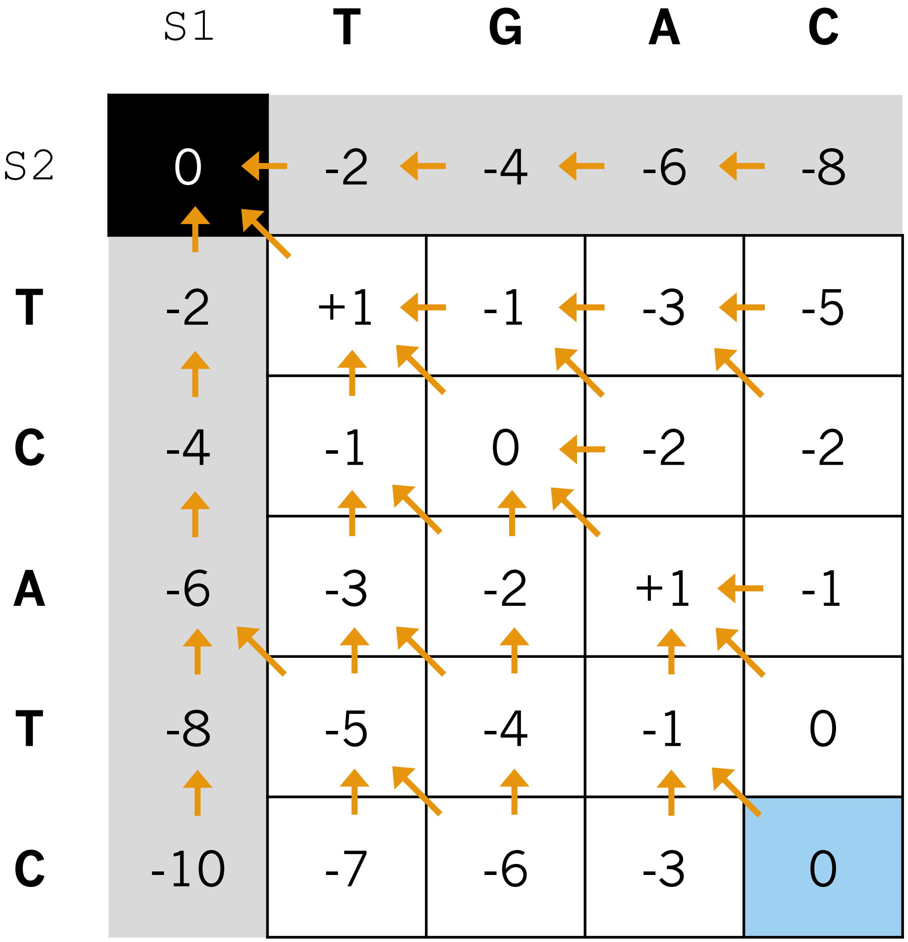


	S1	T	G	A	C
S2	0	-2	-4	-6	-8
T	-2	+1	-1	-3	-5
C	-4	-1	0	-2	-2
A	-6	-3	-2	+1	-1
T	-8	-5	-4	-1	0
C	-10	-7	-6	-3	0

Type	Score
Match	+1
Mismatch	-1
Gap	-2

S1: ?
S2: ?

Needleman-Wunsch example

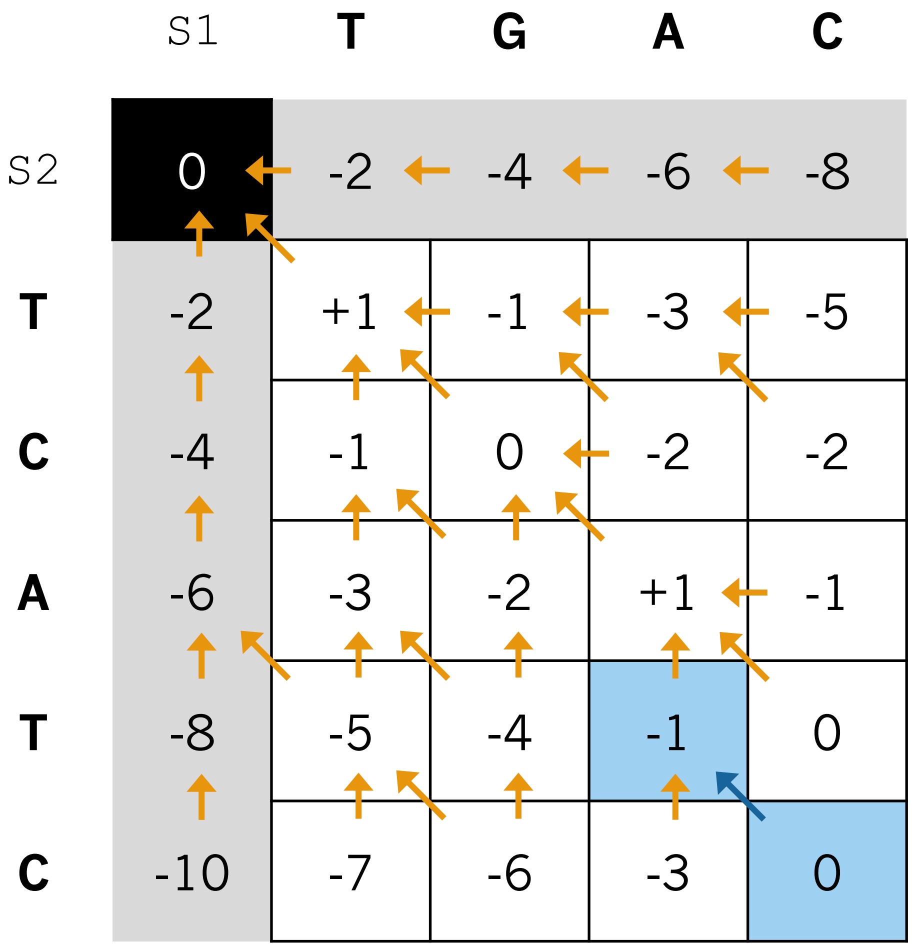


	S1	T	G	A	C
S2	0	-2	-4	-6	-8
T	-2	+1	-1	-3	-5
C	-4	-1	0	-2	-2
A	-6	-3	-2	+1	-1
T	-8	-5	-4	-1	0
C	-10	-7	-6	-3	0

Type	Score
Match	+1
Mismatch	-1
Gap	-2

```
S1: C
S2: C
```

Needleman-Wunsch example

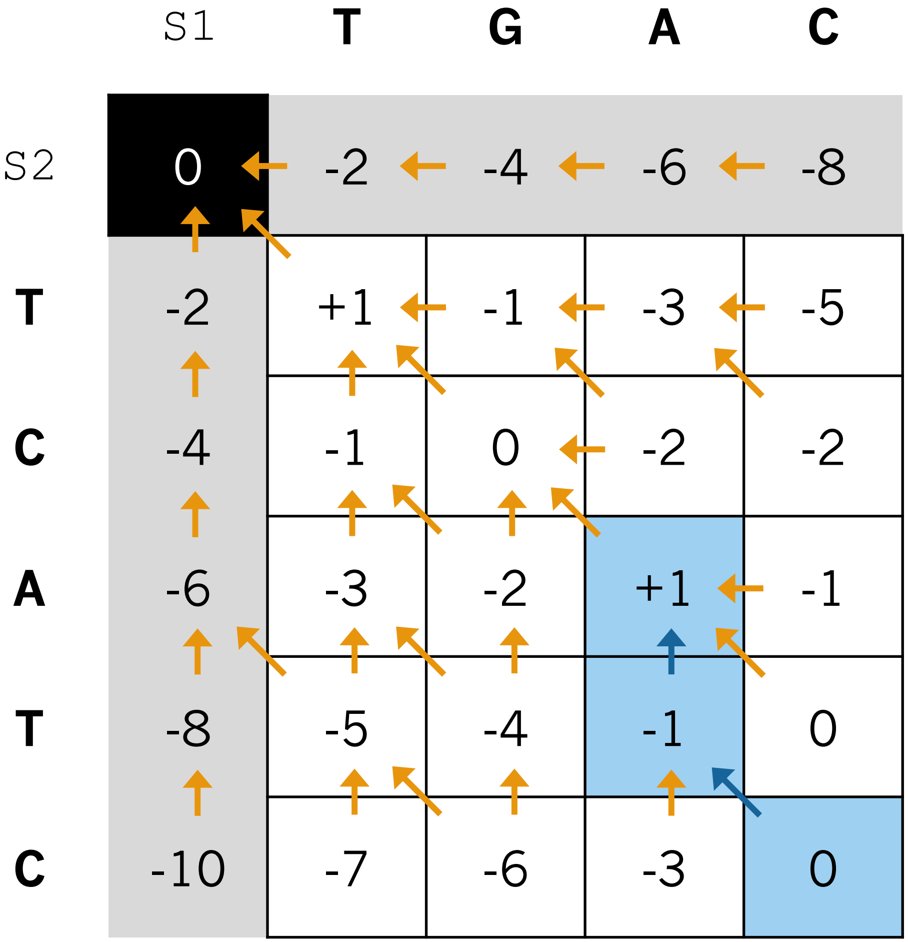


	S1	T	G	A	C
S2	0	-2	-4	-6	-8
T	-2	+1	-1	-3	-5
C	-4	-1	0	-2	-2
A	-6	-3	-2	+1	-1
T	-8	-5	-4	-1	0
C	-10	-7	-6	-3	0

Type	Score
Match	+1
Mismatch	-1
Gap	-2

```
S1: AC
S2: TC
```

Needleman-Wunsch example

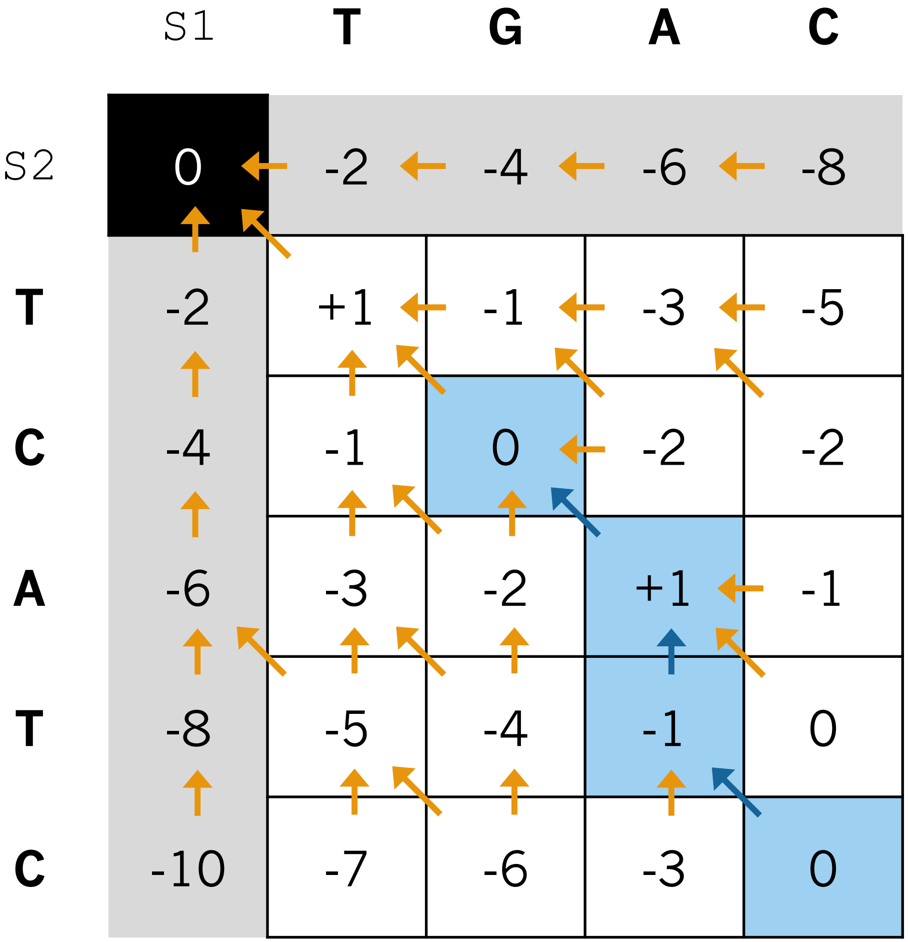


	S1	T	G	A	C
S2	0	-2	-4	-6	-8
T	-2	+1	-1	-3	-5
C	-4	-1	0	-2	-2
A	-6	-3	-2	+1	-1
T	-8	-5	-4	-1	0
C	-10	-7	-6	-3	0

Type	Score
Match	+1
Mismatch	-1
Gap	-2

```
S1: A-C
S2: ATC
```

Needleman-Wunsch example

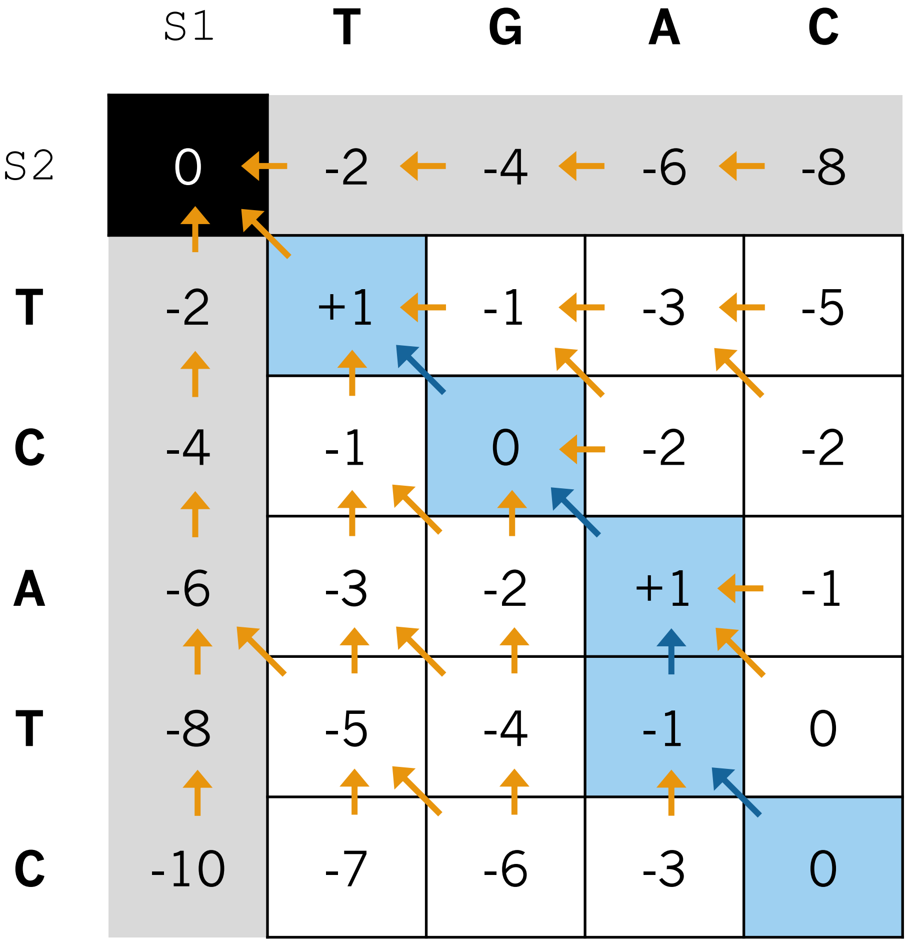


	S1	T	G	A	C
S2	0	-2	-4	-6	-8
T	-2	+1	-1	-3	-5
C	-4	-1	0	-2	-2
A	-6	-3	-2	+1	-1
T	-8	-5	-4	-1	0
C	-10	-7	-6	-3	0

Type	Score
Match	+1
Mismatch	-1
Gap	-2

```
S1: GA-C
S2: CATC
```

Needleman-Wunsch example

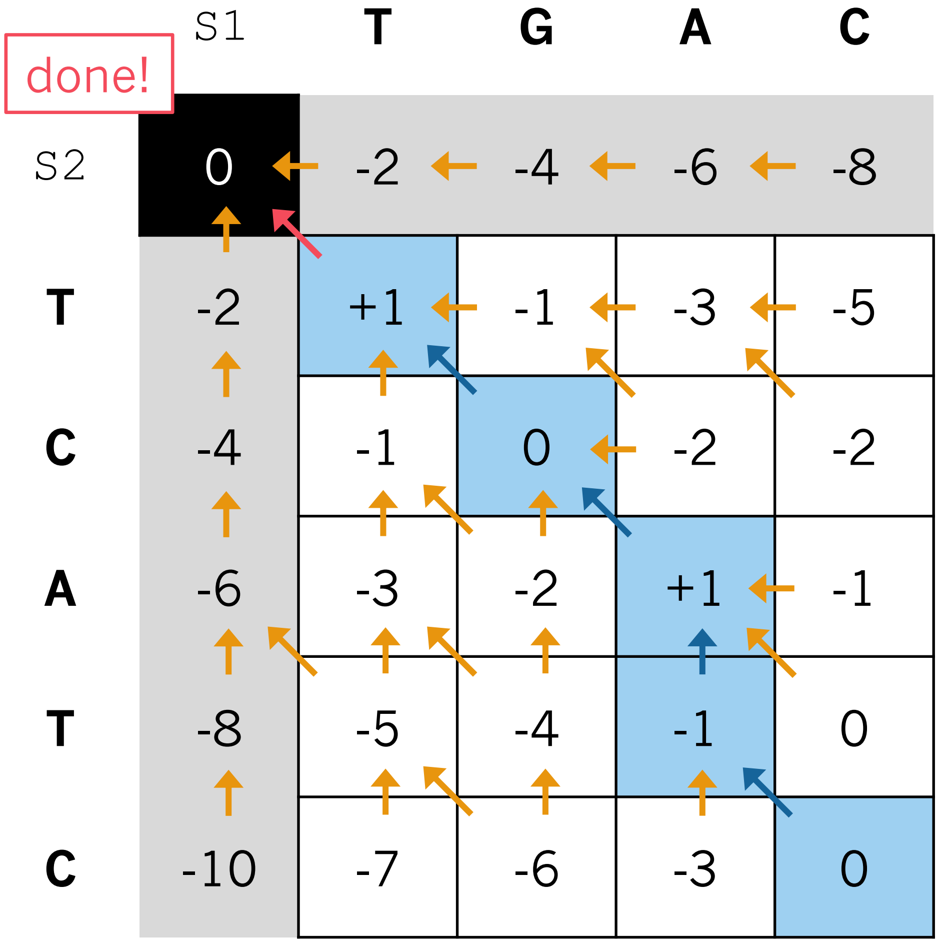


	S1	T	G	A	C
S2	0	-2	-4	-6	-8
T	-2	+1	-1	-3	-5
C	-4	-1	0	-2	-2
A	-6	-3	-2	+1	-1
T	-8	-5	-4	-1	0
C	-10	-7	-6	-3	0

Type	Score
Match	+1
Mismatch	-1
Gap	-2

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

Needleman-Wunsch example



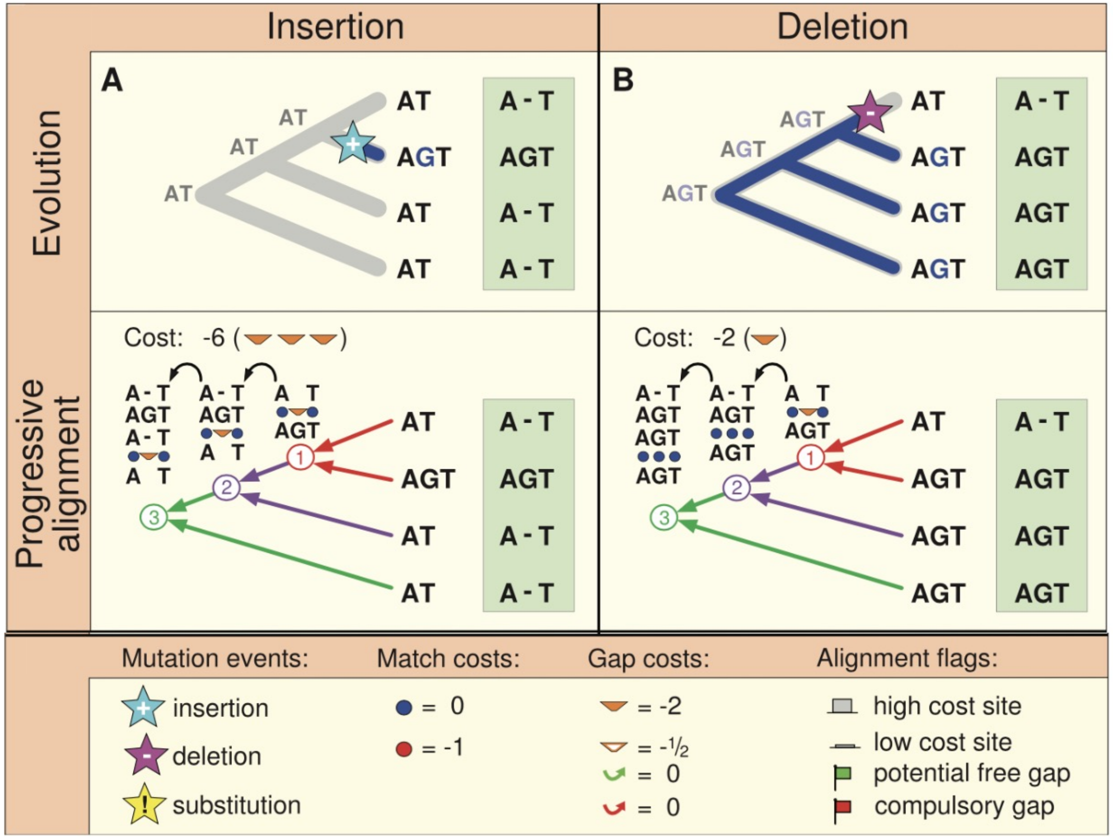
done!

	S1	T	G	A	C
S2	0	-2	-4	-6	-8
T	-2	+1	-1	-3	-5
C	-4	-1	0	-2	-2
A	-6	-3	-2	+1	-1
T	-8	-5	-4	-1	0
C	-10	-7	-6	-3	0

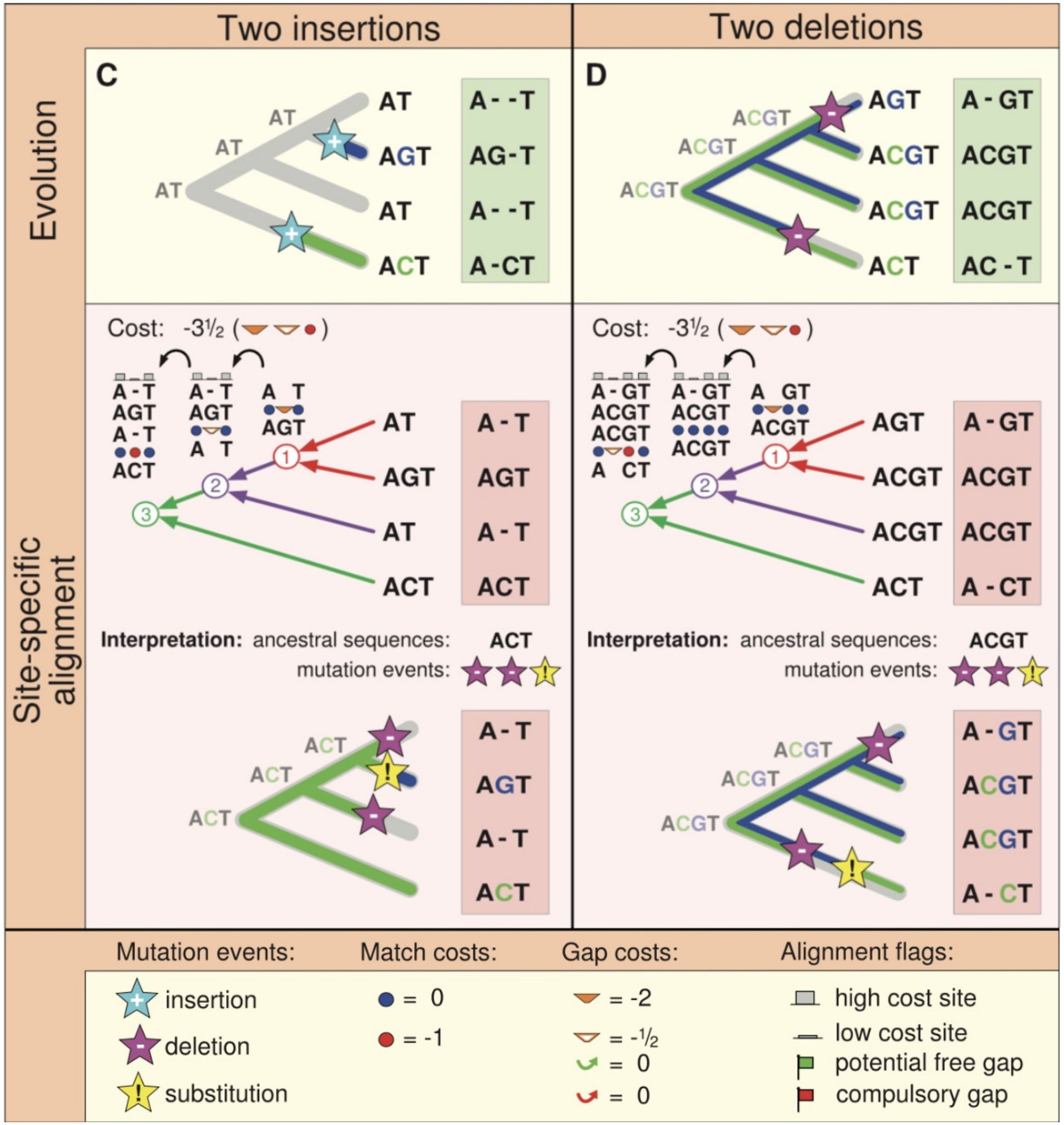
Type	Score
Match	+1
Mismatch	-1
Gap	-2

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

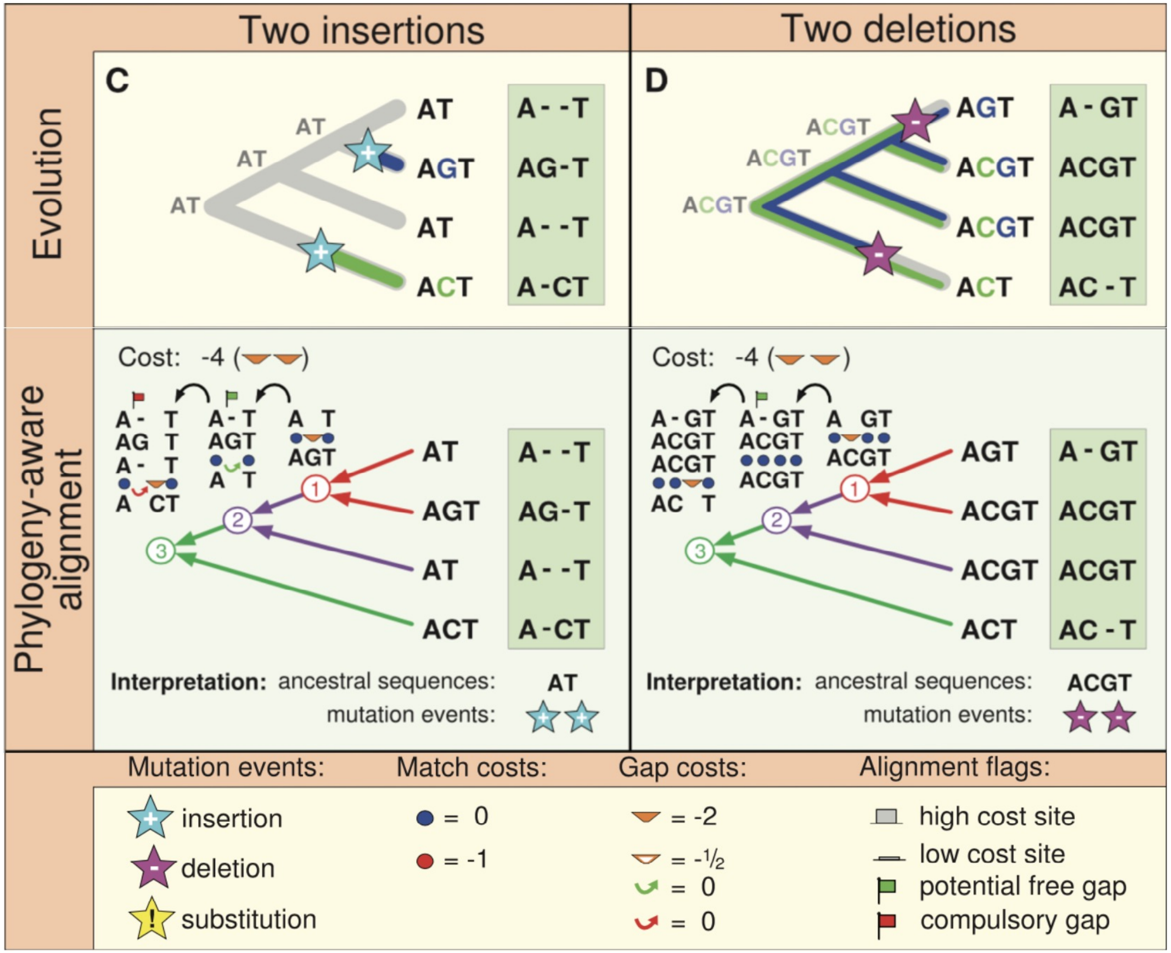
See the two-insertion scenario (left)

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

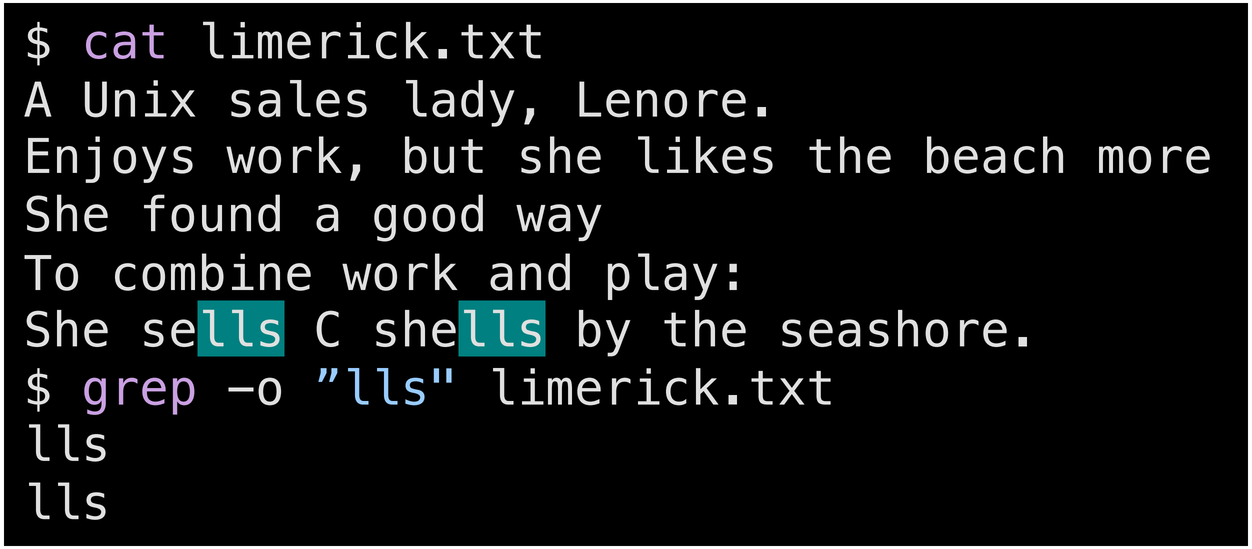


This software (PRANK; left) “flags” events as scored so they’re not double-counted

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