Bioinformatics 1: lecture 4

Followup of lecture 3?

Molecular evolution

Global, semi-global and local

Affine gap penalty

How sequences evolve

- point mutations (single base changes)
- deletion (loss of residues within the sequence)
- insertion (gain of residue within the sequence)
- truncation (loss of either end)
- extension (gain of residues at either end)

Mechanisms of insertion or extension:

- duplication or whole gene or domain
- polymerase "stutter"
- transposable element
- more??

How alignments are scored

- point mutations ..... substitution matrix
- deletion ..... gap penalty
- insertion ..... gap penalty
- truncation ..... end gap penalty
- extension ..... end gap penalty

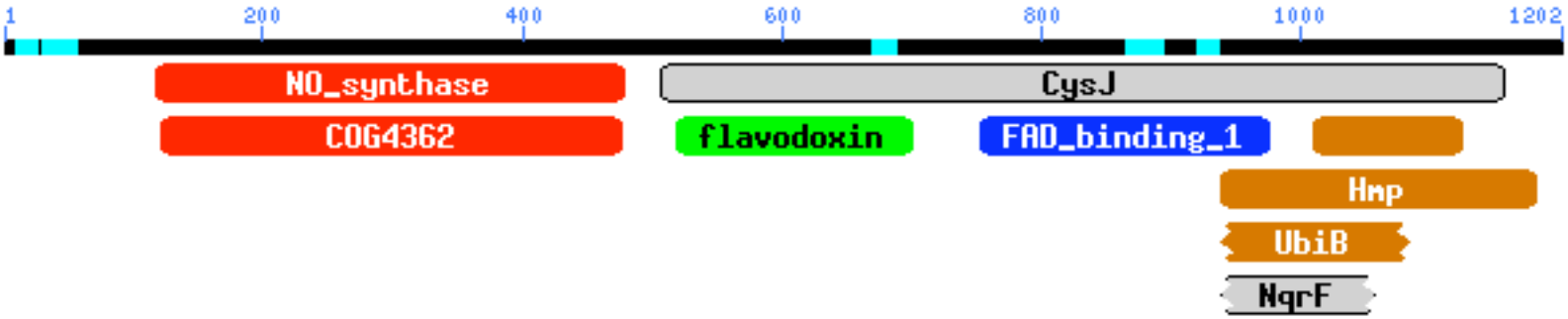
Therefore, an **alignment algorithm** is really

A Model for Sequence Evolution!

> *That means the way we do alignment should be closely aligned to what we know about how things evolve.*

- point mutations ..... relatively frequent, usually bad
- deletion ..... infrequent, always bad, location dependent
- insertion ..... infrequent, always bad, location dependent
- truncation ..... frequent, not so bad
- extension ..... frequent, not so bad

Extension/truncation, domains : end gaps



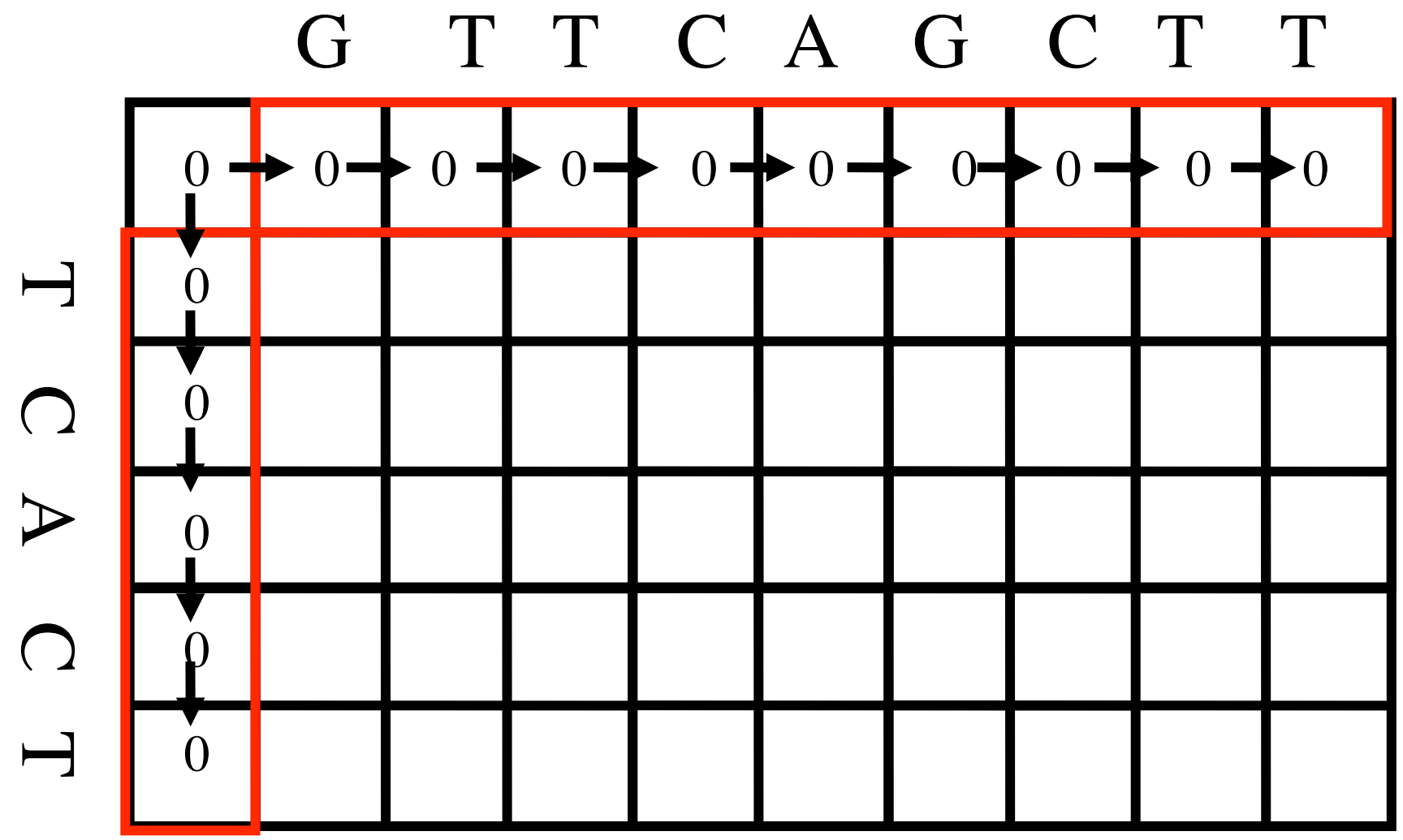
Example: here is an alignment of mouse nitric oxide synthase (thick black line). It has multiple domains which are homologous to several shorter proteins. If we penalize end gaps, what happens to the score of the true alignment? Did "end gaps" evolve the same way as internal gaps? (no!)

Unless the two proteins are known to be single domains,

it makes more sense NOT to penalize end gaps.

How to NOT penalize end gaps

First: *To ignore **starting** gap penalties*, set gap rows to zero (keep the traceback arrows).



How to NOT penalize end gaps

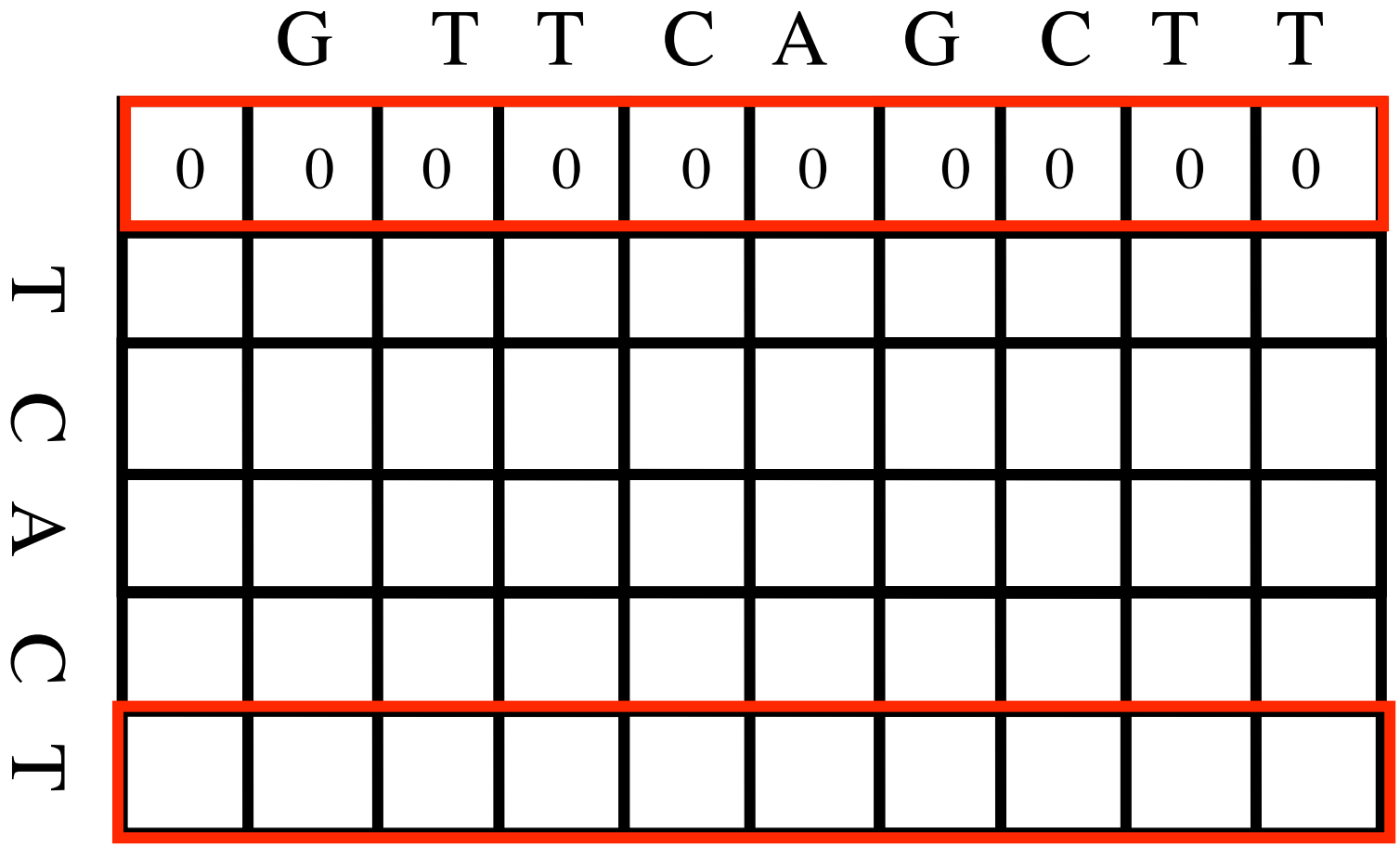
*Second: To ignore **ending** gap penalty, start the traceback with the MAX score at the **end of either sequence**.*

(i.e. use last row or column)

		G	T	T	C	A	G	C	T	T
	0	0	0	0	0	0	0	0	0	0
T	0									
C	0									
A	0									
C	0									
T	0									

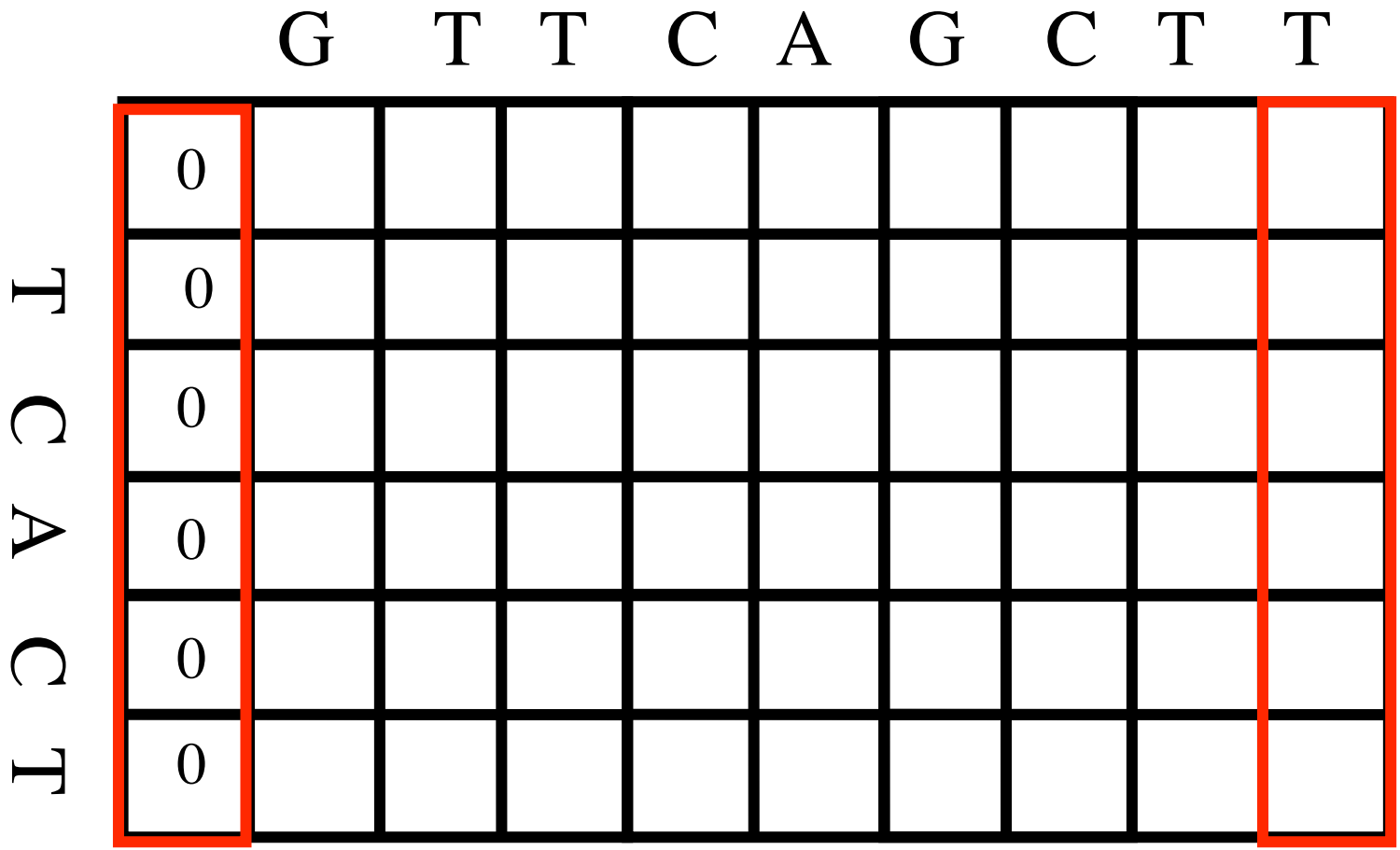
Semi-global: Penalize end gaps on one side

If we penalize end gaps in sequence 2 but not in sequence 1, we are asking for an alignment that *contains all of sequence 2 within sequence 1*.



Semi-global: Penalize end gaps on one side

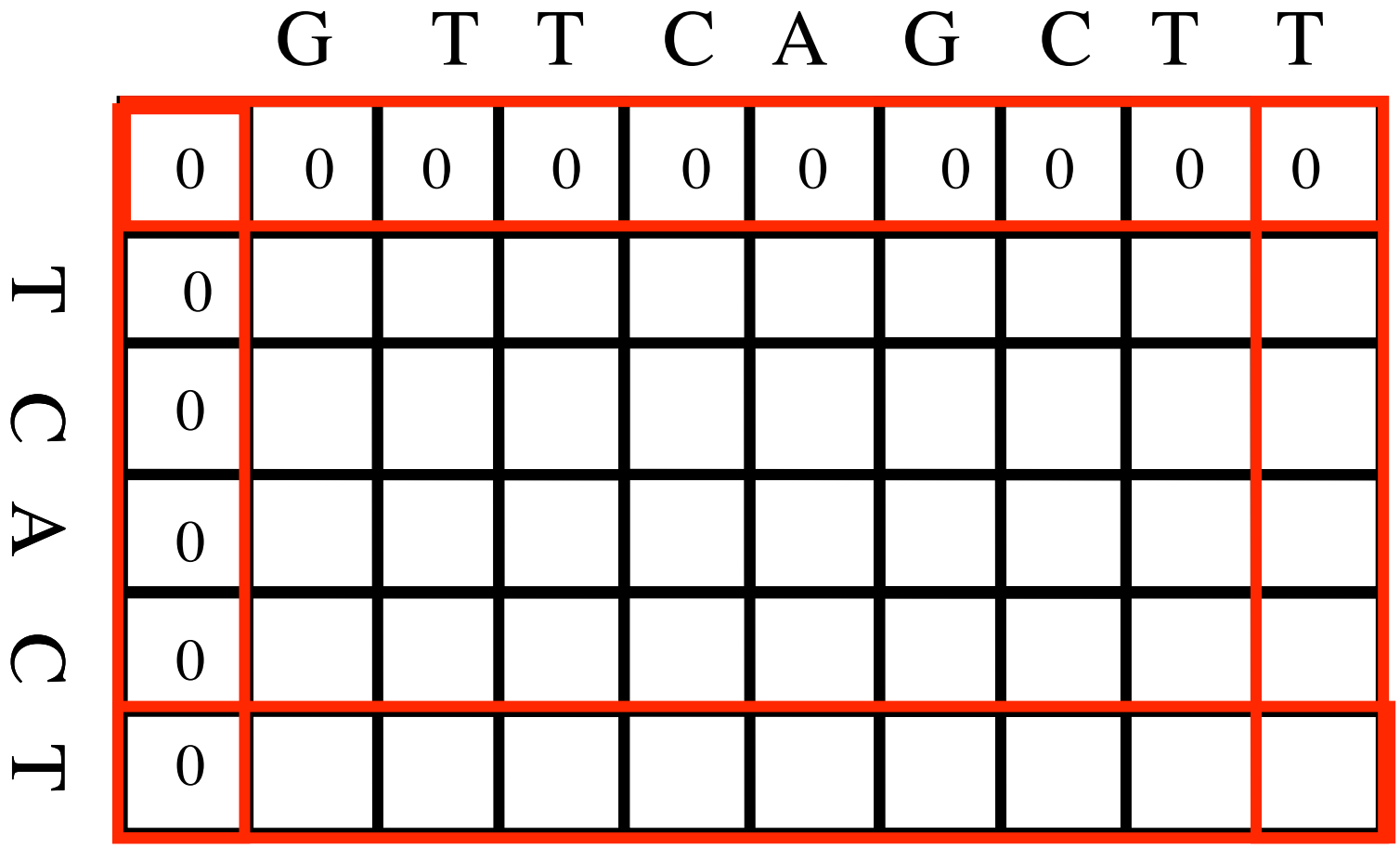
If we penalize end gaps in sequence 1 but not in sequence 2, we are asking for an alignment that *contains all of sequence 1 within sequence 2*.



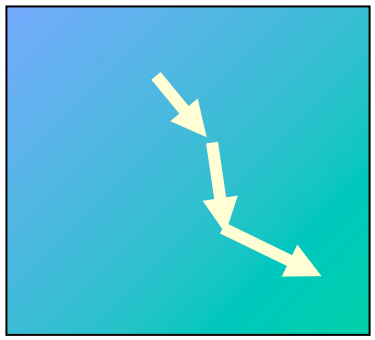
Semi-global: no end gaps

If we penalize end gaps in neither sequence, we are asking for the best alignment that contains at least two of the 4 termini.

Good for identifying terminal domains in two multi-domain proteins.



Local Alignment



A local alignment can start and end anywhere in the alignment matrix.

start

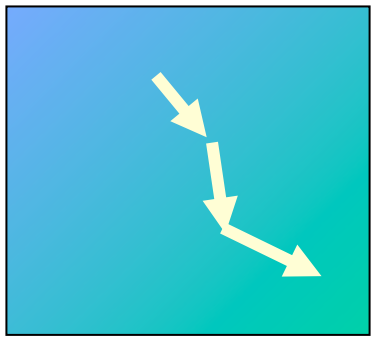
		A	T	S	F	M
P						
G						
T						
S						
F						**end**
E						
P						

$$A(i,j) = \mathrm{MAX} \begin{cases} A(i-1,j-1) + S(i,j) & \searrow \\ A(i,j-1) + \text{gap} & \downarrow \\ A(i-1,j) + \text{gap} & \rightarrow \\ 0 + \text{match score} & \textbf{start} \end{cases}$$

arrow

```
TSF
TSF
```

end is the maximum score anywhere in the *matrix*.



Local Alignment

- Asks for largest domain, sub-domain, or set of contiguous domains that are in common between two sequences.
- Worst score is always zero (0) for “no alignment”
- More appropriate than global or semi-global when there are no assumptions about the sequence relationship.
- Used for database searches.
- Required to obtain e-values

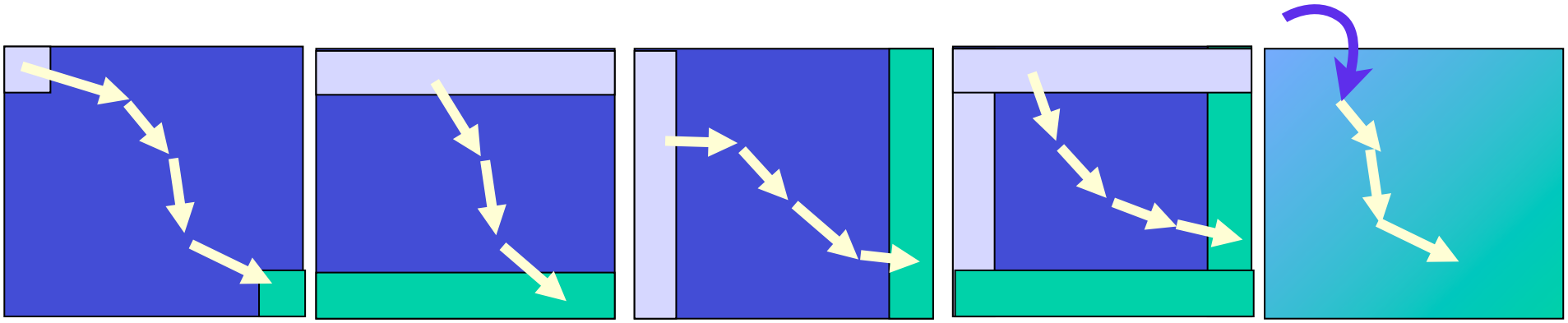
Global, semi-global, and local alignment

The choice of alignment method makes a statement about how the sequences are related. Was one sequence inserted into the other?

- **Global alignment** (end gaps) requires that all 4 termini are counted. In general, the two sequences are about the same length.

- **Semi-global** (no end gaps in 1 or both seqs) requires that one of the two sequences be completely contained in the other or that 2 or the 4 the termini be included.

- **Local alignment** finds subsequences in both. Does not require that the termini be included in the alignment.



The **optimal** alignment may be **no alignment**

If the maximum score in the alignment matrix is < 0.,
then the optimal local alignment has score = 0 and
looks like this:

```
ATSFM~~~~~~~

~~~~~PGTSFEP
```

In class exercise: gaps

- In Genious: Select NCBI-->Protein
- Search for **1DRF**. Drag the first result to your folder "inclass" (or whatever you called it)
- Search for **2DRC**. Do the same.
- Select, shift-select the 2 sequences. Select Alignment. Use Geneious Align, BLOSUM62, Gap open=12, Gap extend=3, Global with free end gaps.
- Count the number of gaps (initiations, not characters)
- Select statistics, record pairwise % identity.
- Re-align by selecting the alignment, and choosing Alignment. Do the experiments on the next page.

Geneious align worksheet

gap extension penalty

gap opening penalty	0	1	3	10
0	86, 28.8			>50, 24.7
1				
3				
12			6 , 26.2	
20				
50	0, 0.0			0, 0.0

Record: # of gaps , % Identity

Affine gap penalty-- theory

- Each gap represents an evolutionary event (duplication, polymerase stutter, deletion/ligation, etc.)
- If the alignment has "evolutionary distance" meaning, then the gap penalty score should be proportional to the number of gaps.

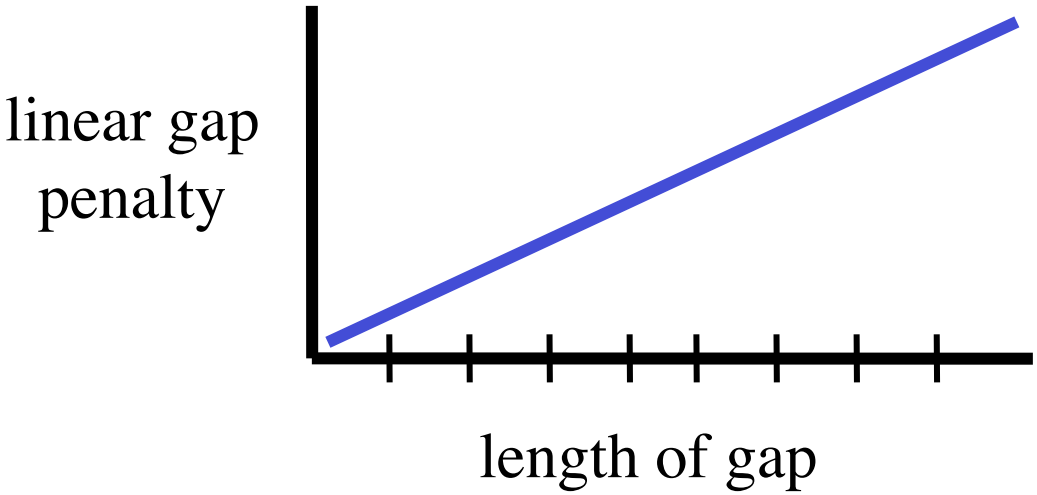
Are long gaps proportionally less likely?

Which alignment is intuitively better?

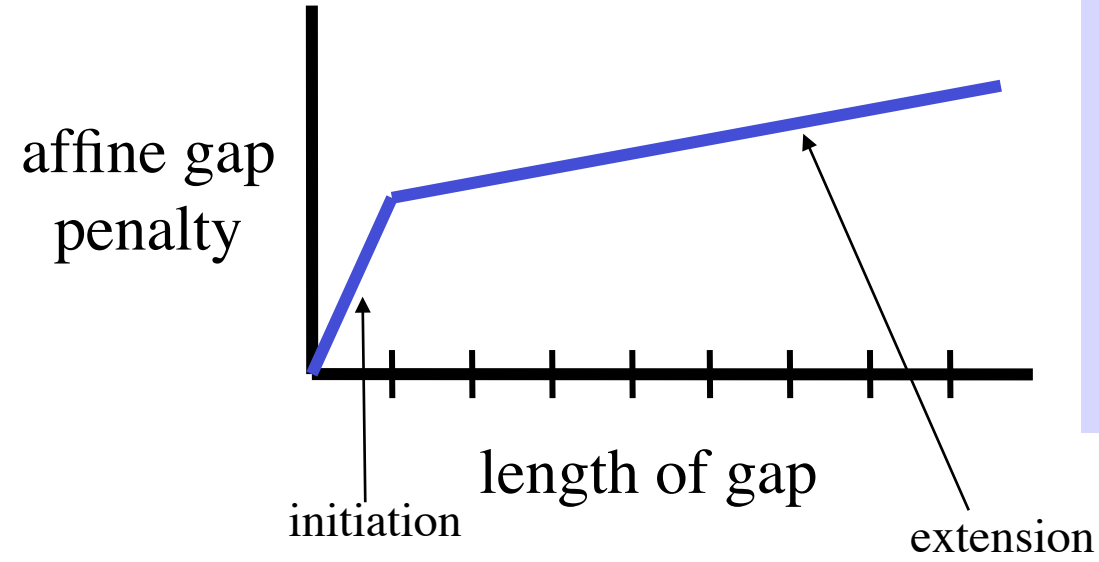
```
AGGCTACT~T~TCA
 GGCTACTATATCA
```

```
AGGCTACTTT~~CA
 GGCTACTATATCA
```

Linear versus Affine gap penalty



Gap penalty for the whole sequence is just the total number of gap characters times a constant.



Gap penalty for the whole sequence is the function.
N*(gap initiation penalty) + E*(gap extension penalty)

where N is the number of gap initiation characters, E is the number of gap extension characters

Example: affine gap

gap *initiation* = -5 gap *extension* = -1

-5 -5

```
AGGCTACT~T~TCA
 GGCTACTATATCA
```

-10

-5 -1

```
AGGCTACTTT~~CA
 GGCTACTATATCA
```

-6

Affine Gap DP

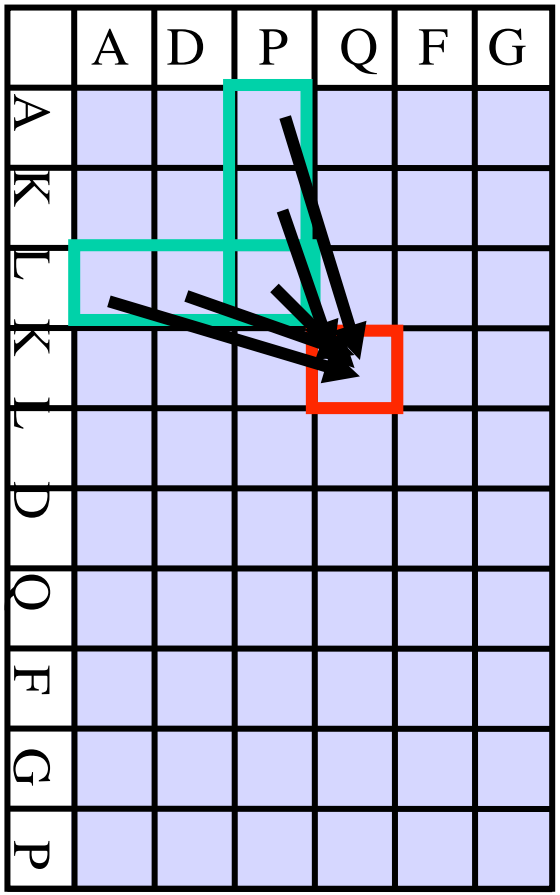
- You can have **5 types of arrows**, instead of just three.

(1) Match
(2) Open a gap in first sequence.
(3) Open a gap in second sequence.
(4) Extend a gap in first sequence.
(5) Extend a gap in second sequence.

---or---

- You can have variable length arrows.

Affine gap DP algorithm using variable length arrows



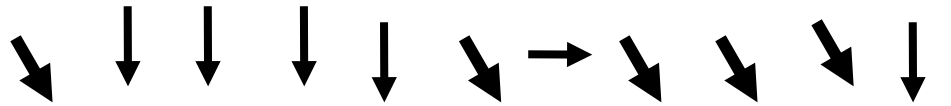
$$S_{i,j} = \max_{n} \{ S_{i-1,j-1} + s(i,j),$$
$$S_{i-1-n,j-1} + s(i,j) - g_{init} - (n-1)\, g_{ext},$$
$$S_{i-1,j-1-n} + s(i,j) - g_{init} - (n-1)\, g_{ext} \}$$

...where s(i,j) is the substiution score, *n* is the length of the gap, g_{init} is the gap initiation penalty, and g_{ext} is the gap extension penalty.

Notes: All arrows end in match. Gap-to-gap not possible. Local or semi-global only. End-gaps not scored. Arrows still translate to an alignment. Still optimal.

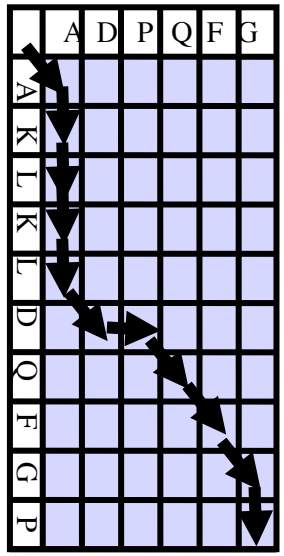
Traceback for linear gap penalty

Each arrow advances either one sequence or both, by 1. Each column has one arrow.



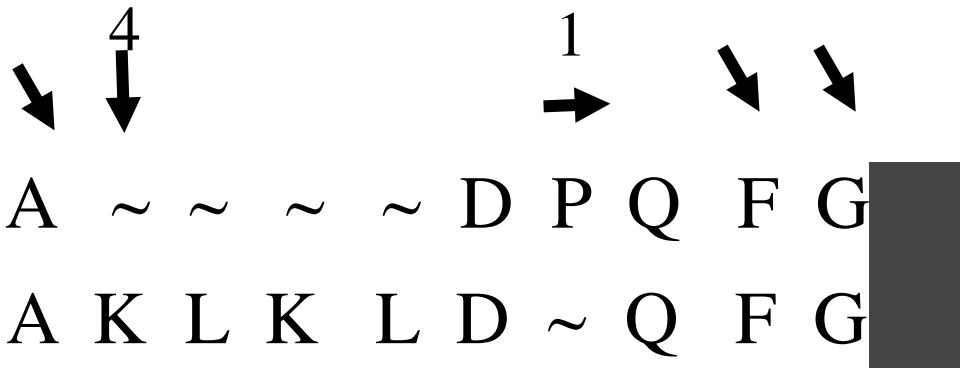
A ~ ~ ~ ~ D P Q F G ~

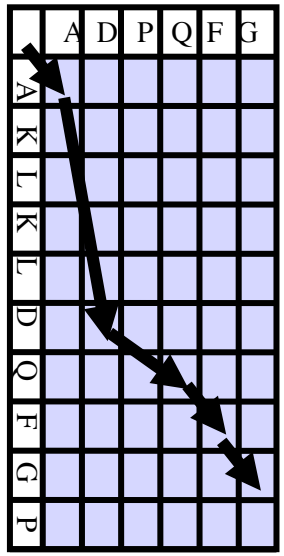
A K L K L D ~ Q F G P



Traceback for affine gap DP

Each arrow advances one sequence by 1, the other sequence by *n*. Output of one arrow is *n* columns. Last of *n* columns is a match. Number of arrows is ≤ number of columns.





Structure-based alignments are the "gold standard"

A structure-based alignment is a sequence alignment that comes from a protein structure superposition.

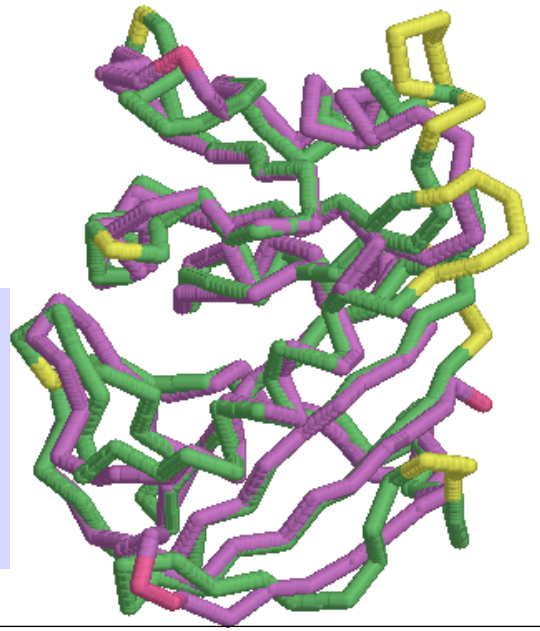
```
2DRC:A     1/2      MISLIAALAVDRVIGMENAM-PFNLPADLAWFKRNTL-------DKPVIMGRHTWESIG-
1DRF:_     3/4      SLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQRMTTTSSVEGKQNLVIMGKKTWFSIPE

2DRC:A    52/53     --RPLPGRKNIILSSQP--GTDDRVTWVKSVDEAIAACG------DVPEIMVIGGGRVYE
1DRF:_    63/64     KNRPLKGRINLVLSRELKEPPQGAHFLSRSLDDALKLTEQPELANKVDMVWIVGGSSVYK

2DRC:A   102/103    QFLPK--AQKLYLTHIDAEVEGDTHFPDYEPDDWESVF------SEFHDADAQNSHSYCF
1DRF:_   123/124    EAMNHPGHLKLFVTRIMQDFESDTFFPEIDLEKYKLLPEYPGVLSDVQEE---KGIKYKF

2DRC:A   154/155    EILERR
1DRF:_   180/181    EVYEKN
```

Look carefully. What do you see? Lots of mismatches (id=38%), few gaps (8), gaps are long (1-7).



Two similar structures may be superimposed. The parts that overlay well are the matches (purple and green), and the parts that do not overlay well are the insertions (yellow and red).

Aligned positions have similar chemical 3D environment

BAliBase

- A database of curated multiple sequence alignments.

- http://www-bio3d-igbmc.u-strasbg.fr/balibase/

Does gap to gap make sense???

Special rules may apply for going from *I* to *D* and *D* to *I*.

```
AGGCTACT~TATCA
 GGCTACTA~ATCA
```

If you think this alignment does not make sense, then D to I and I to D can simply be **disallowed** in the DP algorithm. Most programs do this.

[Exception: For a global alignment, D-to-I or I-to-D arrows are allowed at the ends of alignments because there is no other way to complete the matrix.]

Machine learning the gap penalty

- Create a database of sequence alignments (BAliBase)
  - Training set.
  - must be non-redundant
  - must be representative
- Define an objective function
  - function of all alignments
  - converges on a maximum as alignments converge on Training set
- Explore parameter space
  - may be exhaustive search, or something smarter
- Cross-validate.
  - Report the accuracy on a Test set (non-redundent, representative, no overlap with Training set)

Improved pairwise alignments of proteins in the Twilight Zone using local structure predictions

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Simple objective function: count number of matches that are also found in the Training set.

Fig. 6-a

```
                1R69 01 SISSRVKSKRIQLGLNQAELAQKVGTTQQSIE-Q-LENGKTKRPRFLPELASALGVSVDWLLNGT
BLOSUM40        1NEQ 17 ----------------------------------------GLKKRKLSLSALSRQFGYAPTTLANA-
SDM             1NEQ 31 -----------QFGYAPTTLANALERHWPKGE-QIIANALETKPEVI-------------------
HMMSUM-D3       1NEQ 13 DVIAGLKKRKLSL----SALSRQFGYAPTTLANA-LERHWPKGEQII---ANALETKPEVIWPSR
HMMSUM-D3+NS    1NEQ 13 DVIAGLKKRKLSLSALSRQFGYAPTTLANALE-R-------HWPKGEQIIANALETKPEVIWPSR
HMMSUM-D        1NEQ 14 -----VIAGLKKRKLSLSALSRQFGYAPTTLA-N-ALERHWPKGEQIIANALETKPEVIWPSR--
HMMSUM-DNS      1NEQ 11 --RADVIAGLKKRKLSLSALSRQFGYAPTTLA-N-ALERHWPKGEQI--IANALETKPEVIWPSR
BAliBASE        1NEQ 09 WHRADVIAGLKKRKLSLSALSRQFGYAPTTLA-N-ALER--HWPKGEQIIANALETKPEVIWPSR
```

Review

- Scoring should reflect true evolutionary distance
- Semi-global alignment is good for finding domain boundaries
- Local alignment is used for database searches
- Affine gap penalty is better than linear
- Structure-based alignments are the gold standard