Lecture 18: Approximate Pattern Matching

Study Chapter 9.6 – 9.8

Approximate vs. Exact Pattern Matching

- Previously we have discussed exact pattern matching algorithms
- Usually, because of mutations, it makes much more biological sense to find approximate pattern matches
- Biologists often use fast heuristic approaches to find approximate matches

Heuristic Similarity Searches

- Why heuristics?
  - Genomes are huge: Smith-Waterman quadratic alignment algorithms are too slow
- Observation: Good alignments of two sequences usually have short identical or highly similar subsequences
- Many heuristic methods (i.e., BLAST, FASTA) are based on the idea of *filtration*
  - Find short exact matches, and use them as "seeds" for potential match extension
  - "Filter" out positions with no extendable matches

Dot Plot

- A dot matrix or dot plot shows similarities between two sequences
- FASTA makes an implicit dot matrix of length l matches,
  - tries to find long diagonals (allowing for some mismatches)
- Nucleotide matches

	G	A	T	T	C	G	C	T	T	A	G	T
C					*		*					
T			*	*				*	*			*
G						*					*	
A		*								*		
T			*	*				*	*			*
T			*	*				*	*			*
C					*		*					
C					*		*					
T			*	*				*	*			*
T			*	*				*	*			*
A		*								*		
G						*					*	
T			*	*				*	*			*
C					*		*					
A		*								*		
G	*					*					*	

$l = 1$

Dot Plot

- A dot matrix or dot plot shows similarities between two sequences
- FASTA makes an implicit dot matrix of length *l* matches,
  - tries to find long diagonals (allowing for some mismatches)
- Dinucleotide matches

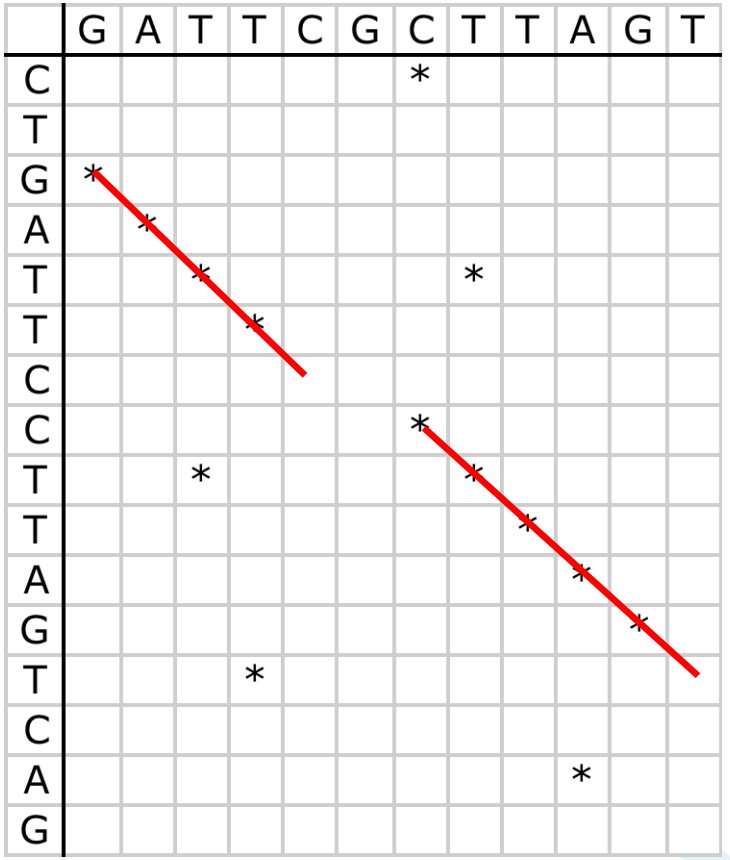
	G	A	T	T	C	G	C	T	T	A	G	T
C							*					
T												
G	*											
A		*										
T			*					*				
T				*								
C												
C							*					
T			*					*				
T									*			
A										*		
G											*	
T				*								
C												
A										*		
G												

$l = 2$

Dot Plot

- Identify diagonals above a threshold length

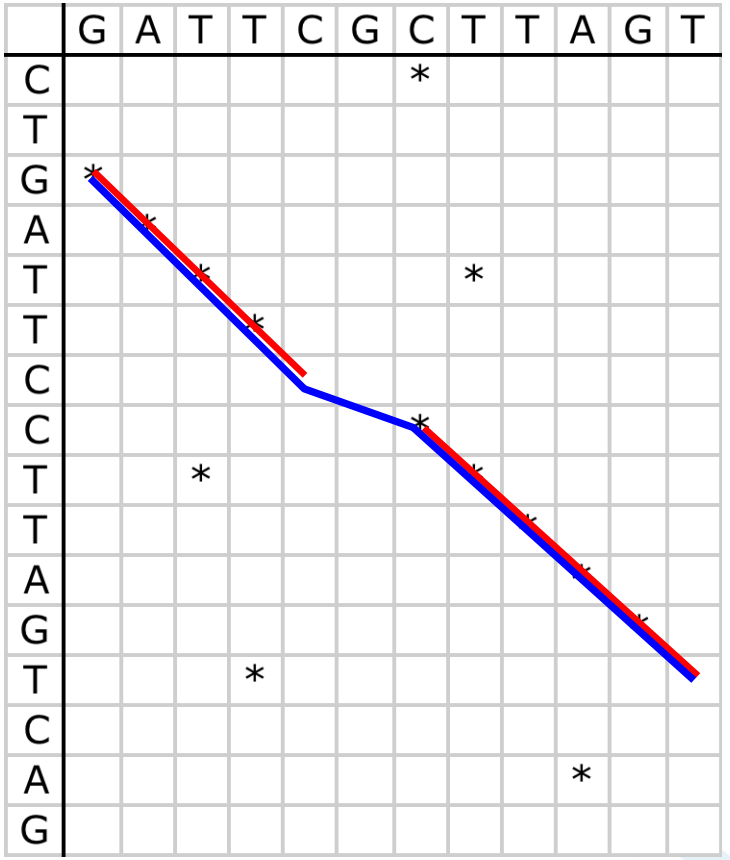
- Diagonals in the dot matrix indicate exact substring matching



$l = 2$

Diagonals in Dot Plots

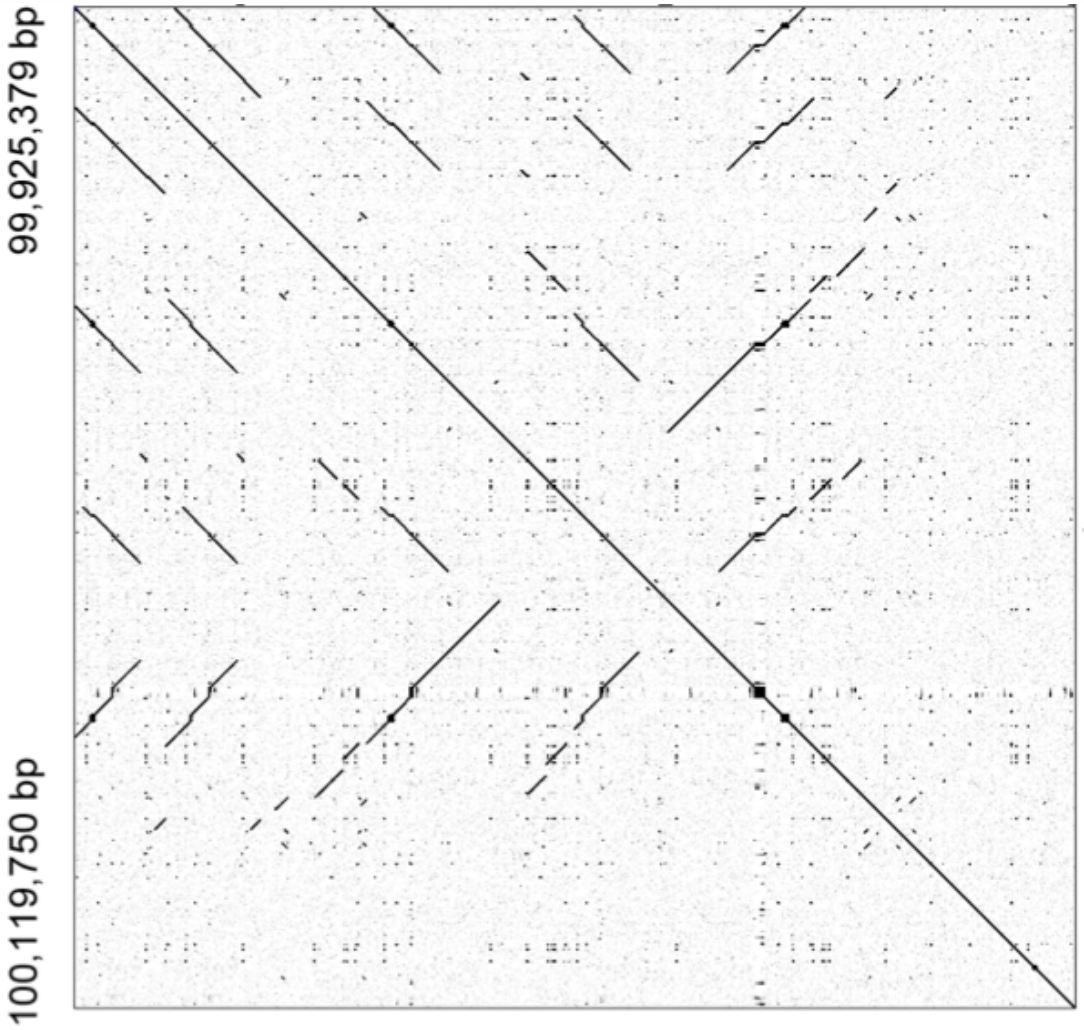
- Extend diagonals and try to link them together, allowing for minimal mismatches/indels
- Linking diagonals reveals approximate matches over longer substrings



$l = 2$

A Realistic Dot-Plot

- On the right is a dot-plot of approximately ~200 KB of genomic sequence compared to itself.
- L = 20 with >= 90% concordance
- What do the off diagonal traces represent?



Approximate Pattern Matching (APM)

- Goal: *Find all approximate occurrences of a pattern in a text*
- Input:
  - pattern **p** = $p_1 \ldots p_n$
  - text **t** = $t_1 \ldots t_m$
  - the maximum number of mismatches k
- Output: All positions $1 \leq i \leq (m - n + 1)$ such that $t_i \ldots t_{i+n-1}$ and $p_1 \ldots p_n$ have at most k mismatches
  - i.e., Hamming distance between $t_i \ldots t_{i+n-1}$ and **p** $\leq k$

APM: A Brute-Force Algorithm

ApproximatePatternMatching(p, t, *k*)

```
1    n ← length of pattern p
2    m ← length of text t
3    for i ← 1 to m – n + 1
4       dist ← 0
5       for j ← 1 to n
6          if t_{i+j-1} != p_j
7             dist ← dist + 1
8       if dist ≤ k
9          output i
```

APM: Running Time

- That algorithm runs in O(*nm*).

- Extend "Approximate Pattern Matching" to a more general "Query Matching Problem":
  - Match *n-length substring of the query* (not the full pattern) to a substring in a text with at most ***k*** mismatches
  - **Motivation**: we may seek similarities to some gene, but not know which parts of the gene to consider

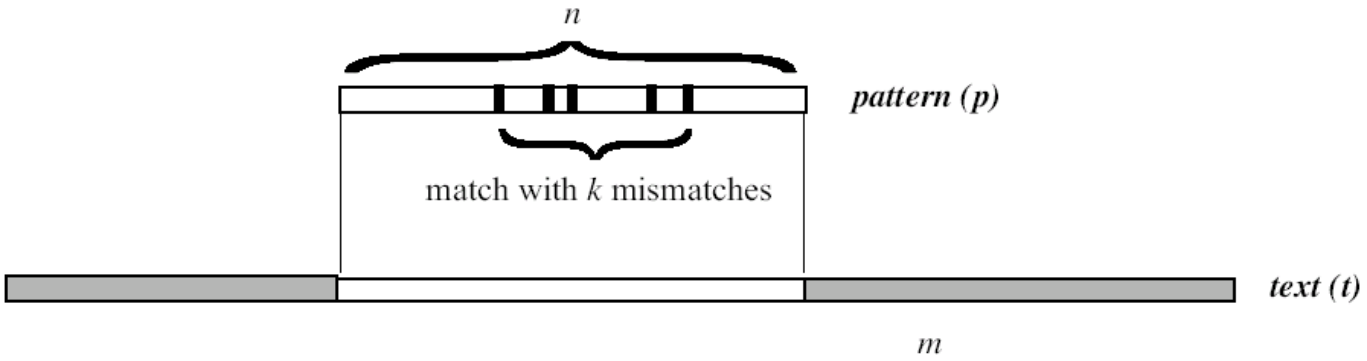
Query Matching Problem

- Goal: *Find all substrings of the query that approximately match the text*

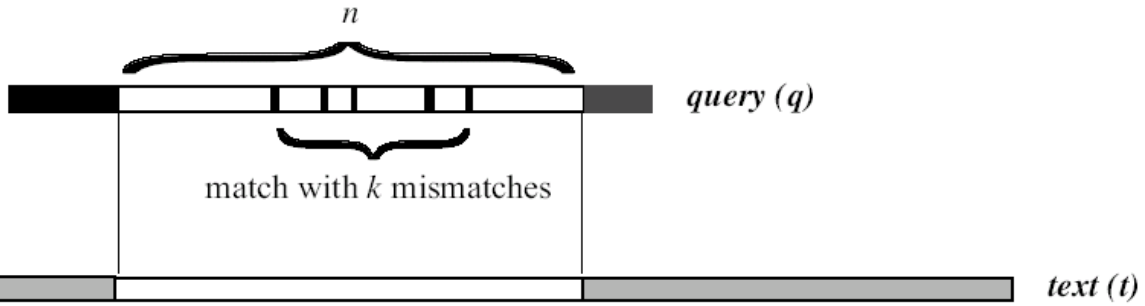
- Input: Query **q** = $q_1 \ldots q_w$,
  text **t** = $t_1 \ldots t_m$,
  n (length of matching substrings $n \leq w \leq m$),
  k (maximum number of mismatches)

- Output: All pairs of positions (i, j) such that the
  n-letter substring of **q** starting at i
  approximately matches the
  n-letter substring of **t** starting at j,
  with at most k mismatches

Approximate Pattern Matching vs Query Matching



(a) Approximate Pattern Matching



(b) Query Matching

Query Matching: Main Idea

- Approximately matching strings share some perfectly matching substrings.

- Instead of searching for approximately matching strings (difficult) search for perfectly matching substrings first (easy).

Filtration in Query Matching

- We want all n-matches between a query and a text with up to k mismatches
- "Filter" out positions that do not match between text and query

- **Potential match detection**: find all matches of ℓ-tuples in query and text for some small ℓ

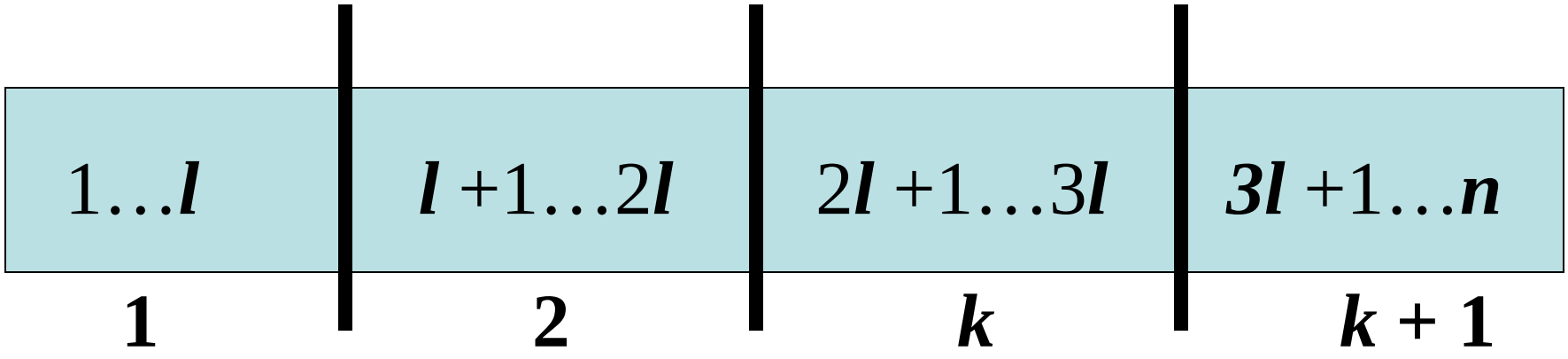
- **Potential match verification**: Verify each potential match by extending it to the left and right, until $(k + 1)$ mismatches are found

Filtration: Match Detection

- If $x_1 \ldots x_n$ and $y_1 \ldots y_n$ match with at most $k << n$ mismatches they must share ℓ–mers that are perfect matches, with $\ell = \lfloor n/(k+1) \rfloor$
- Break string of length n into k+1 parts, each of length $\lfloor n/(k+1) \rfloor$
  - k mismatches can affect at most k of these k+1 parts
  - At least one of these k+1 parts is perfectly matched

Filtration: Match Detection (cont'd)

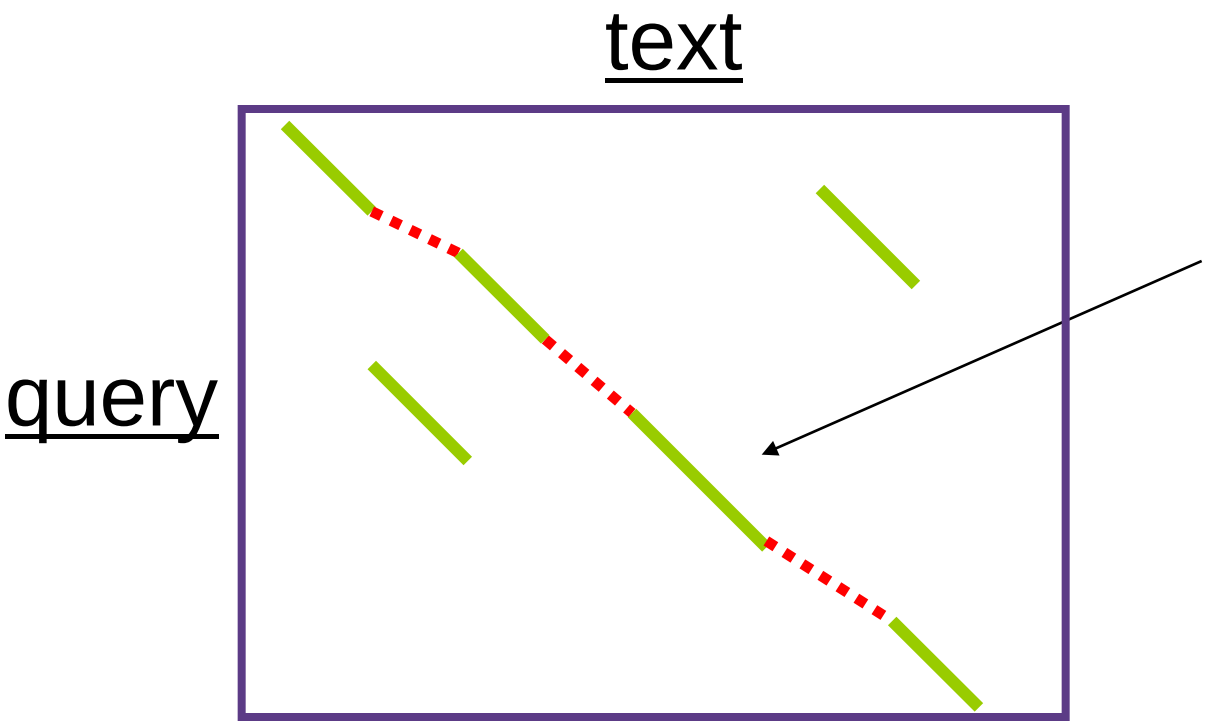
- Suppose $k = 3$. We would then have $l=n/(k+1)=n/4$:



- There are at most k mismatches in n, so at the very least there must be one out of the $k+1$ ℓ–tuples without a mismatch

Filtration: Match Verification

- For each ℓ-match we find, try to extend the match further to see if it is substantial



Extend perfect match of length ℓ until we find an approximate match of length n with no more than k mismatches

Filtration: Example

	$k = 0$	$k = 1$	$k = 2$	$k = 3$	$k = 4$	$k = 5$
ℓ-tuple length	n	$n/2$	$n/3$	$n/4$	$n/5$	$n/6$

Shorter **perfect matches** required

Performance **decreases**

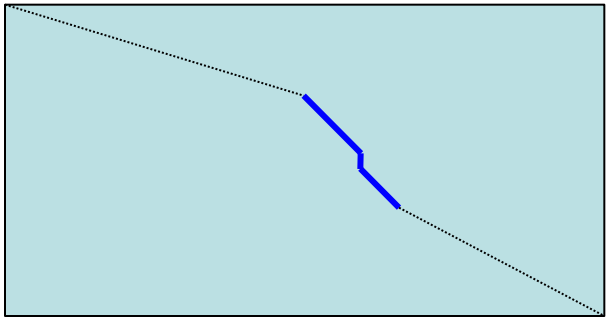
Local alignment is too slow…

- Quadratic local alignment is too slow when looking for similarities between long strings (e.g. the entire GenBank database)
- Guaranteed to find the optimal local alignment
- Sets the standard for sensitivity
- **B**asic **L**ocal **A**lignment **S**earch **T**ool
  - Altschul, S., Gish, W., Miller, W., Myers, E. & Lipman, D.J. Journal of Mol. Biol., 1990
- Search sequence databases for local alignments to a query

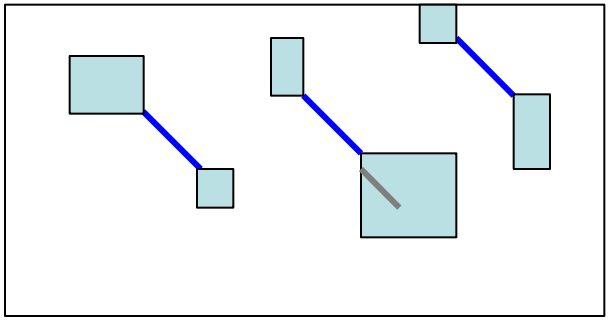
$$s_{i,j} = \max \begin{cases} 0 \\ s_{i-1,j} + \delta(v_i, -) \\ s_{i,j-1} + \delta(-, w_j) \\ s_{i-1,j-1} + \delta(v_i, w_j) \end{cases}$$

BLAST

- Great improvement in speed, with only a modest decrease in sensitivity
- Opts to minimizes search space instead of exploring entire search space between two sequences
- Finds short exact matches ("seeds"), explore locally around these "hits"



Search space of Local Alignment



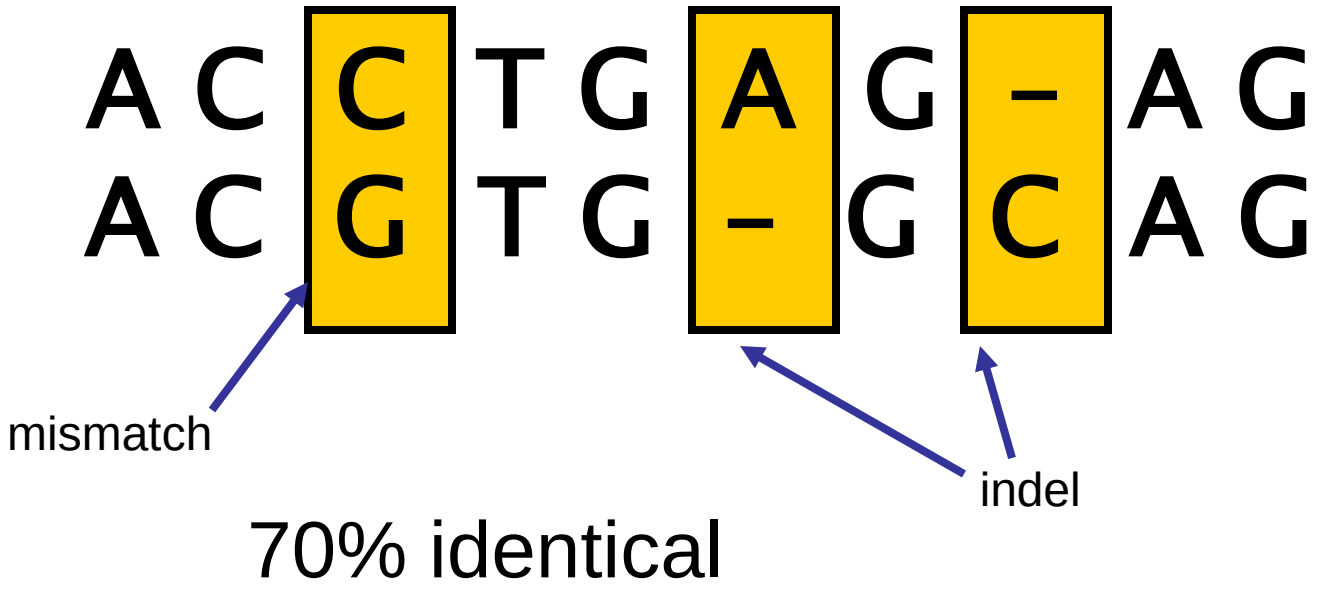
Search space of BLAST

Similarity

- BLAST only continues it's search as long as regions are sufficiently *similar*
- Measuring the extent of similarity between two sequences
  - Based on percent sequence identity
  - Based on conservation

Percent Sequence Identity

- The extent to which two nucleotide or amino acid sequences are invariant



Conservation

- Amino acid changes that preserve the physico-chemical properties of the original residue
  - Polar to polar
    - aspartate → glutamate
  - Nonpolar to nonpolar
    - alanine → valine
  - Similarly behaving residues
    - leucine to isoleucine
- Nucleotide changes that preserve molecular shape
  - Transitions (A-G, C-T) are more similar than Transversions (A-C, A-T, C-G, G-T)

Assessing Sequence Similarity

- How good of a local alignment score can be expected from chance alone
- "Chance" relates to comparison of sequences that are generated randomly based upon a certain sequence model
- Sequence models may take into account:
  - nucleotide frequency
  - dinucelotide frequency (e.g. C+G content in mammals)
  - common repeats
  - etc.

BLAST: Segment Score

- BLAST uses scoring matrices (δ) to improve on efficiency of match detection (we did this earlier for pairwise alignments)
  - Some proteins may have very different amino acid sequences, but are still similar (PAM, Blosum)
- For any two ℓ-mers $x_1 \ldots x_l$ and $y_1 \ldots y_l$:
  - Segment pair: pair of ℓ-mers, one from each sequence
  - Segment score: $\Sigma_{i=1}^{\ell} \delta(x_i, y_i)$

BLAST: Locally Maximal Segment Pairs

- A segment pair is maximal if it has the best score over all segment pairs
- A segment pair is locally maximal if its score can't be improved by extending or shortening
- Statistically significant *locally maximal* segment pairs are of biological interest
- BLAST finds all locally maximal segment pairs (MSPs) with scores above some threshold
  - A significantly high threshold will filter out some statistically insignificant matches

BLAST: Statistics

- Threshold: Altschul-Dembo-Karlin statistics
  - Identifies smallest segment score that is unlikely to happen by chance

- # matches with score > θ is approximately Poisson-distributed with mean:

$$E(\theta) = Kmne^{-\lambda\theta}$$

K is a constant, m and n are the lengths of the two compared sequences, λ is a positive root of:

$$\Sigma_{x,y\ in\ A}(p_x p_y e^{\lambda\delta(x,y)}) = 1$$

where p_x and p_y are frequencies of amino acids x and y, δ is the scoring matrix, and A is the twenty letter amino acid alphabet

P-values

- The probability of finding exactly k MSPs with a score ≥ θ is given by:

$$(E(\theta)^k e^{-E(\theta)})/k!$$

- For $k = 0$, that chance is:

$$e^{-E(\theta)}$$

- Thus the probability of finding at least one MSP with a score ≥ θ is:

$$p(MSP > 0) = 1 - e^{-E(\theta)}$$

BLAST algorithm

- Keyword search of all substrings of length w from the query of length n, in database of length m with score above threshold
  - w = 11 for DNA queries, w =3 for proteins

- Local alignment extension for each found keyword
  - Extend result until longest match above threshold is achieved

- Running time O(nm)

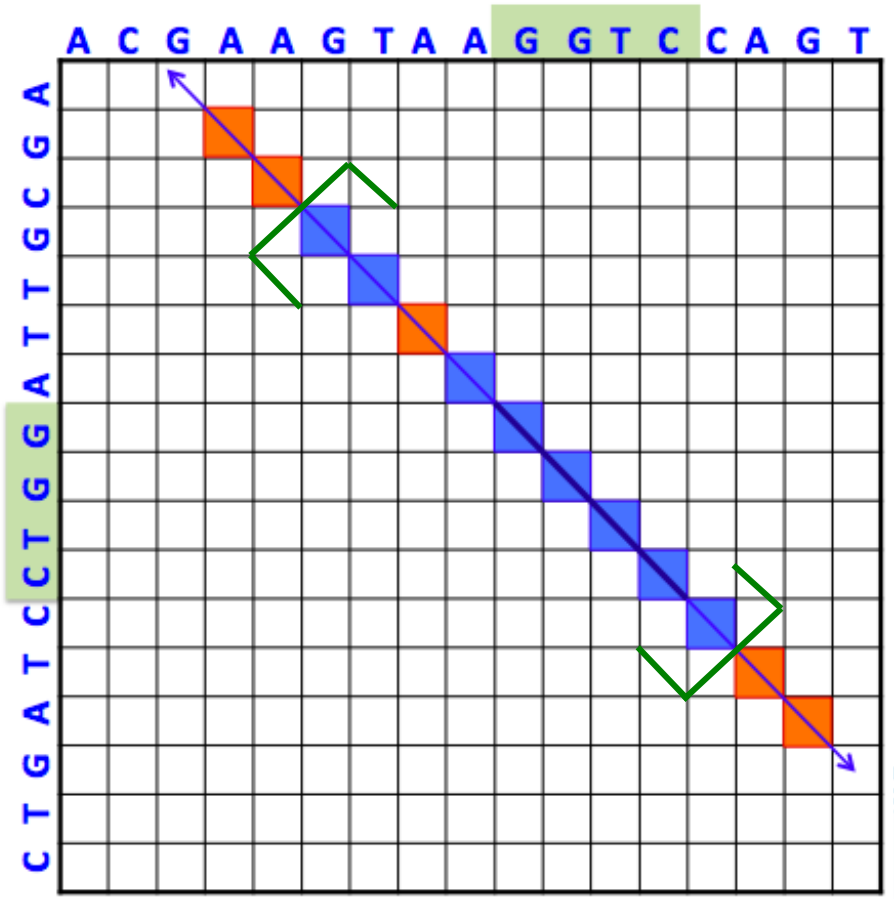
Original BLAST

- **Dictionary**
  - All words of length w
- **Alignment**
  - *Ungapped* extensions until score falls below some statistical threshold
- **Output**
  - All local alignments with score > threshold

Original BLAST: Example

- $w = 4$
- Exact keyword match of **GGTC**
- Extend diagonals with mismatches until score is under some threshold (65%)
- Trim to until all mismatches are interior
- Output result:

```
GTAAGGTCC
|| ||||||
GTTAGGTCC
```

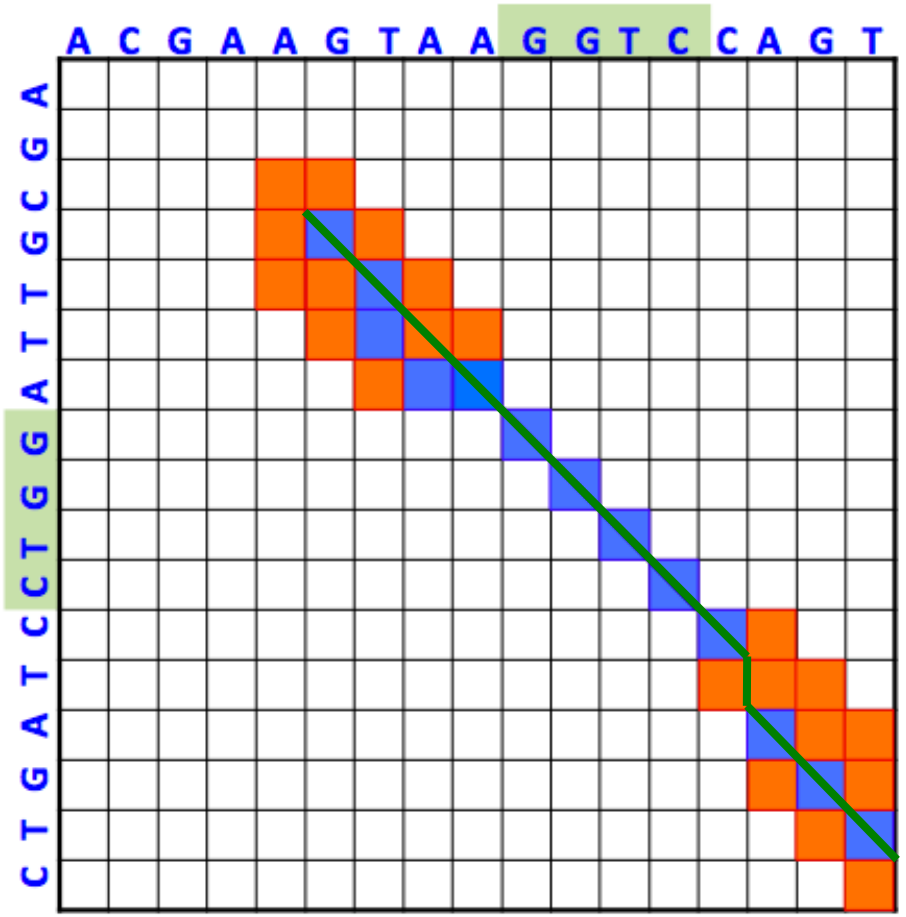


From lectures by Serafim Batzoglou (Stanford)

Gapped BLAST : Example

- Original BLAST exact keyword search, then:
- Extend with gaps around ends of exact match until score < *threshold*
- Output result:

```
GTAAGGTCCAGT
||  |||||  |||
GTTAGGTC-AGT
```



From lectures by Serafim Batzoglou (Stanford)

Incarnations of BLAST

- blastn: Nucleotide-nucleotide
- blastp: Protein-protein
- blastx: Translated query vs. protein database
- tblastn: Protein query vs. translated database
- tblastx: Translated query vs. translated database (6 frames each)

Incarnations of BLAST (cont'd)

- PSI-BLAST
  - Find members of a protein family or build a custom position-specific score matrix
- Megablast:
  - Search longer sequences with fewer differences
- WU-BLAST: (Wash U BLAST)
  - Optimized, added features

Timeline

- 1970: Needleman-Wunsch global alignment algorithm
- 1981: Smith-Waterman local alignment algorithm
- 1985: FASTA
- 1990: BLAST (basic local alignment search tool)
- 2000s: BLAST has become too slow in "genome vs. genome" comparisons - new faster algorithms evolve!
  - Pattern Hunter
  - BLAT