Alignment of Long Sequences

BMI/CS 776

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Goals for Lecture

the key concepts to understand are the following

- how large-scale alignment differs from the simple case
- the canonical three step approach of large-scale aligners
- using suffix trees to find MUMs (alignment seeds)
- using tries and threaded tries to find alignment seeds
- constrained dynamic programming to align between/ around anchors
- using sparse DP to find a chain of local alignments

Pairwise Large-Scale Alignment: Task Definition

Given

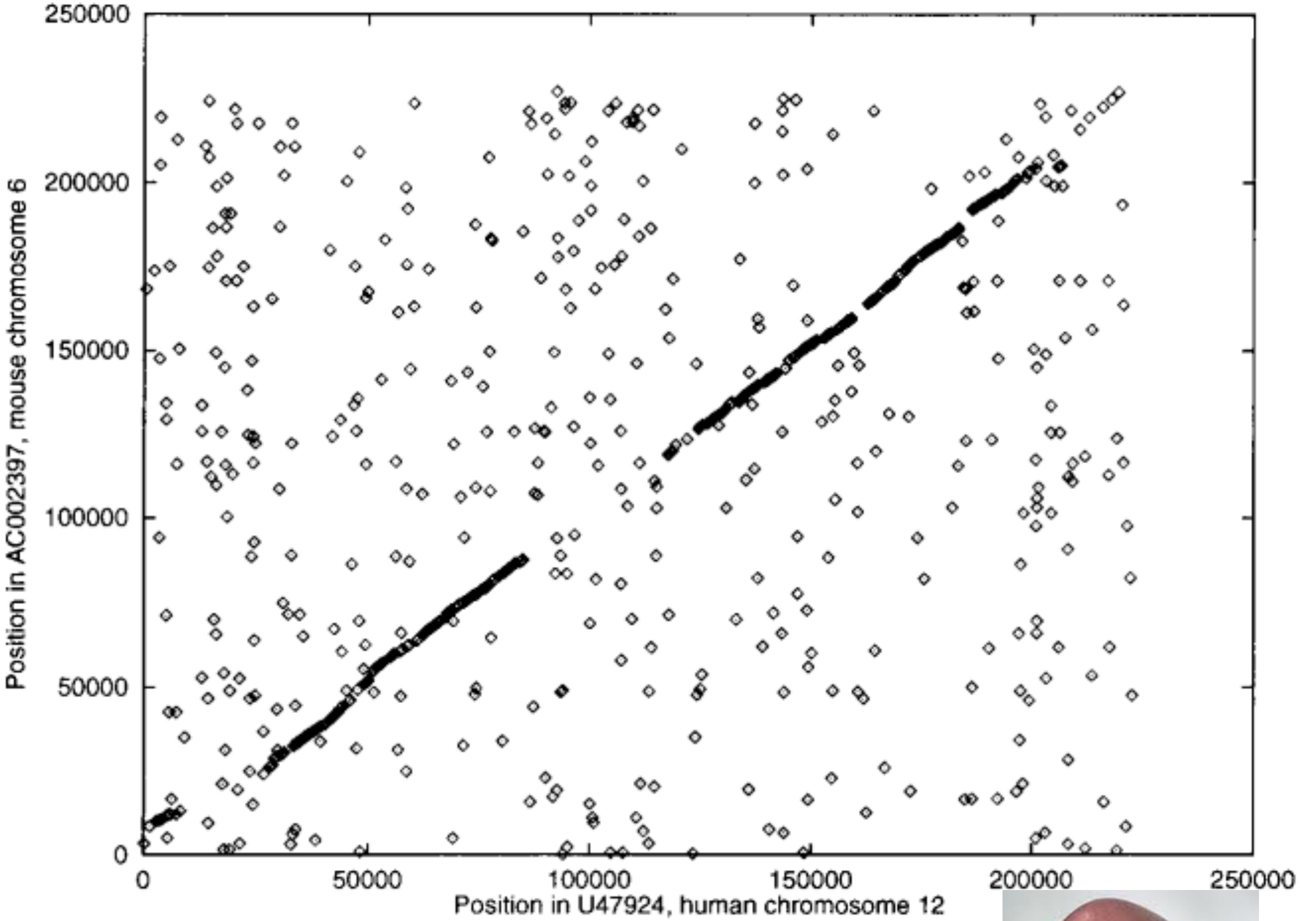
- a pair of large-scale sequences (e.g. chromosomes)
- a method for scoring the alignment (e.g. substitution matrices, insertion/deletion parameters)

Do

- construct global alignment: identify all matching positions between the two sequences

Large Scale Alignment Example:

Mouse Chr6 vs. Human Chr12



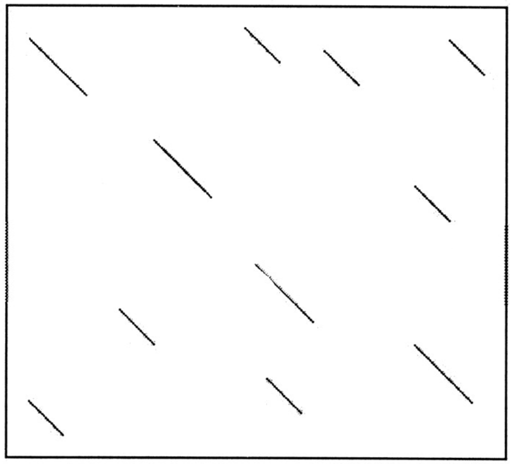
Why the Problem is Challenging

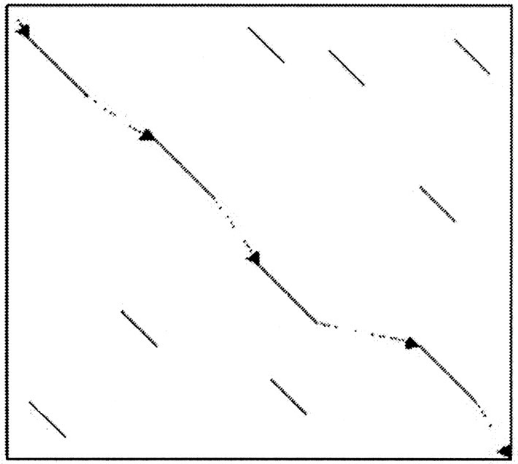
- sequences too big to make $O(n^2)$ dynamic-programming methods practical

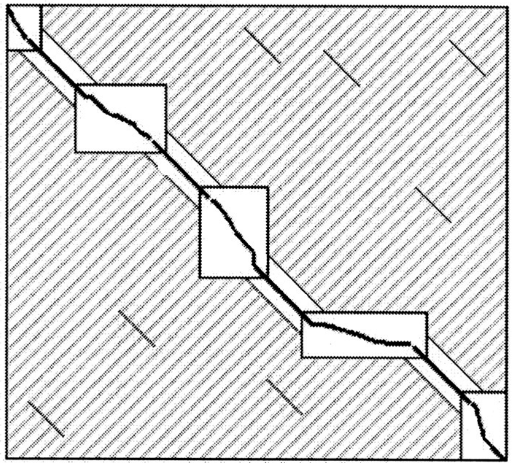
- long sequences are less likely to be colinear because of *rearrangements*
  - initially we'll assume colinearity
  - we'll consider rearrangements in next lecture

General Strategy

Figure from: Brudno et al. *Genome Research*, 2003







1. perform pattern matching to find seeds for global alignment
2. find a good chain of anchors
3. fill in remainder with standard but constrained alignment method

Comparison of Large-Scale Alignment Methods

Method	Pattern matching	Chaining
MUMmer	suffix tree - MUMs	LIS variant
AVID	suffix tree - exact & wobble matches	Smith-Waterman variant
LAGAN	*k*-mer trie, inexact matches	sparse DP

The MUMmer System

Delcher et al., *Nucleic Acids Research*, 1999

Given: genomes A and B

1. find all maximal, unique, matching subsequences (MUMs)
2. extract the longest possible set of matches that occur in the same order in both genomes
3. close the gaps

Step 1: Finding Seeds in MUMmer

- *maximal unique match* (MUM):
  - occurs exactly once in both genomes A and B
  - not contained in any longer MUM

```
Genome A:   tcgatcGACGATCGCGGCCGTAGATCGAATAACGAGAGAGCATAAcgactta
Genome B:   gcattaGACGATCGCGGCCGTAGATCGAATAACGAGAGAGCATAAtccagag
```

mismatches

- key insight: a significantly long MUM is certain to be part of the global alignment

Suffix Trees

- substring problem:
  - given text S of length m
  - preprocess S in $O(m)$ time
  - such that, given query string Q of length n, find occurrence (if any) of Q in S in $O(n)$ time
- suffix trees solve this problem, and others

Suffix Tree Definition

- a suffix tree T for a string S of length m is a tree with the following properties:
  - *rooted* and *directed*
  - m leaves, labeled 1 to m
  - each edge labeled by a substring of S
  - concatenation of edge labels on path from root to leaf i is suffix i of S (we will denote this by $S_{i \ldots m}$)
  - each internal non-root node has at least two children
  - edges out of a node must begin with different characters

key property

Suffixes

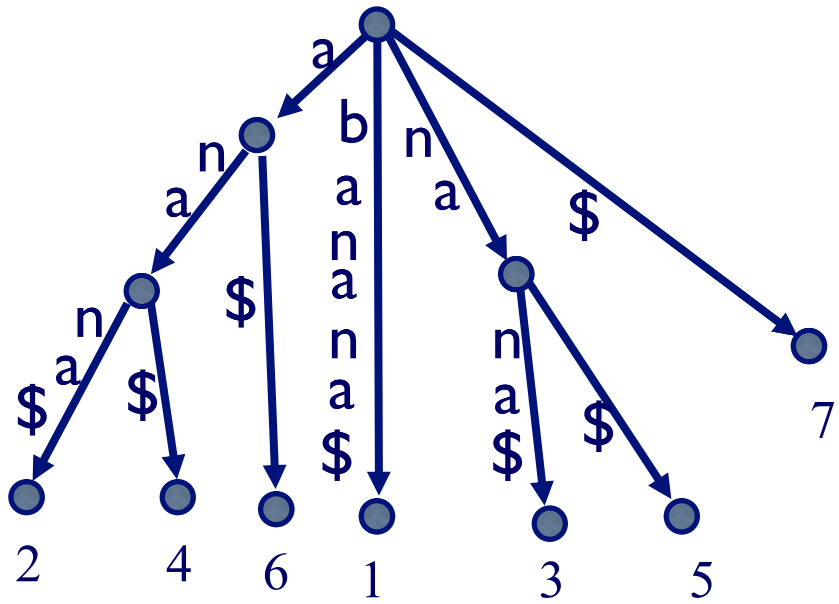
S = "banana$"

suffixes of S

- $
- a$
- na$
- ana$
- nana$
- anana$
- banana$

Suffix Tree Example

- S = "banana$"
- add '$' to end so that suffix tree exists (no suffix is a prefix of another suffix)



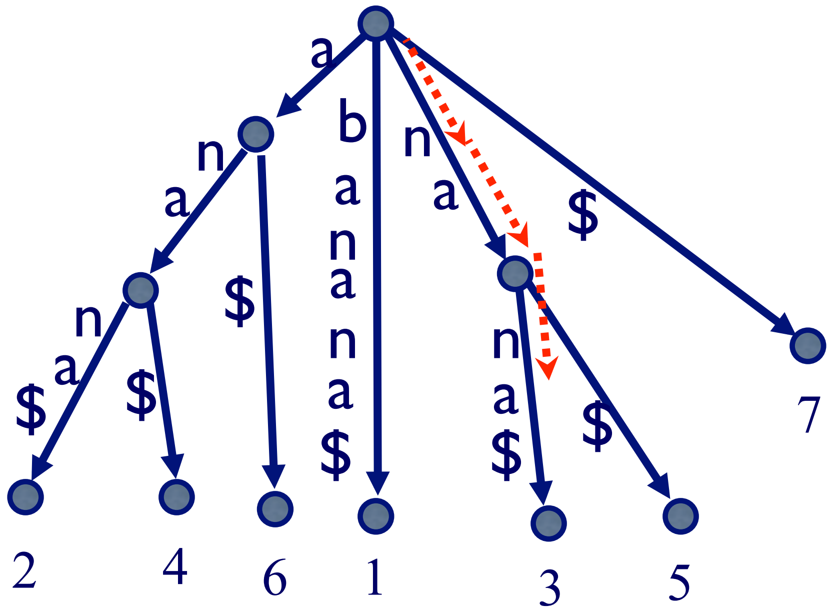
Solving the Substring Problem

- assume we have suffix tree T
- FindMatch(Q, T):
  - follow (unique) path down from root of T according to characters in Q
  - if all of Q is found to be a prefix of such a path

    return label of some leaf below this path
  - else, return no match found

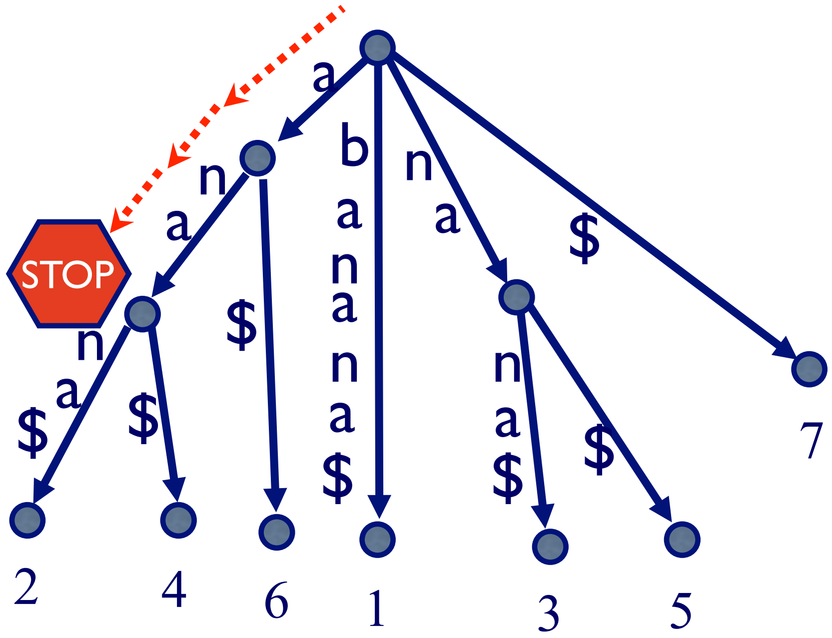
Solving the Substring Problem

Q = nan



return 3

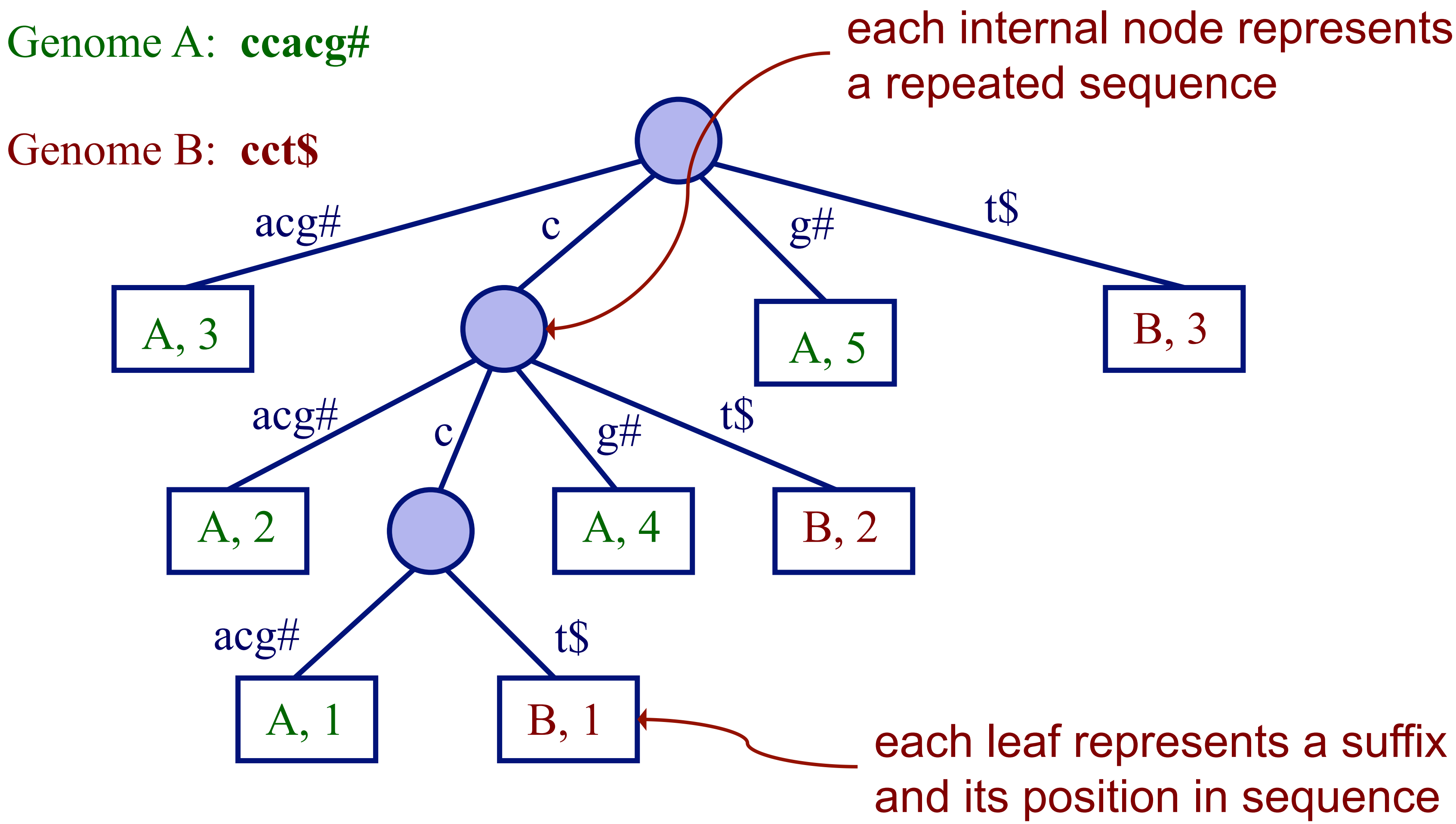
Q = anab



return no match found

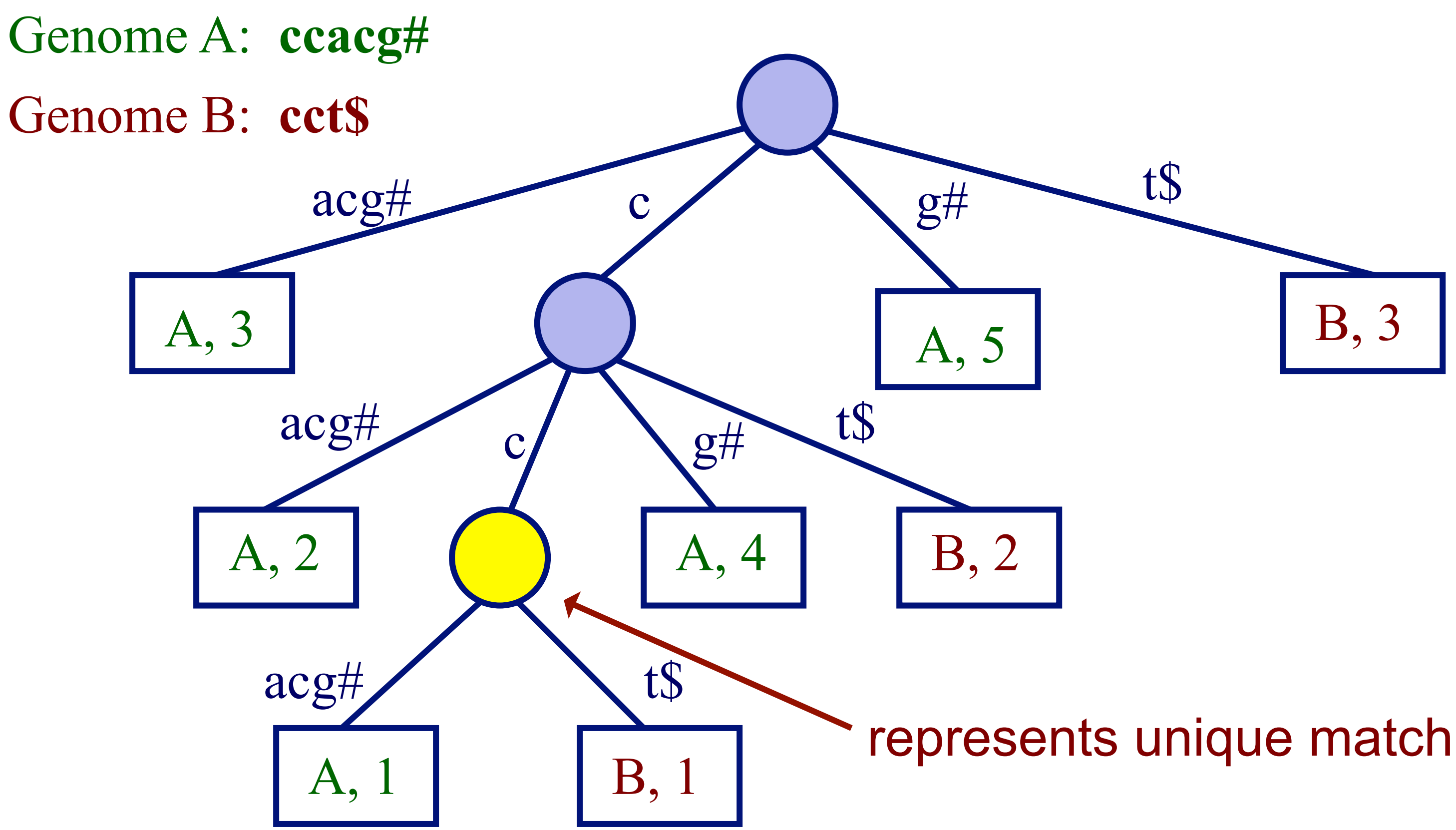
MUMs and *Generalized* Suffix Trees

- build one suffix tree for both genomes *A* and *B*
- label each leaf node with genome it represents



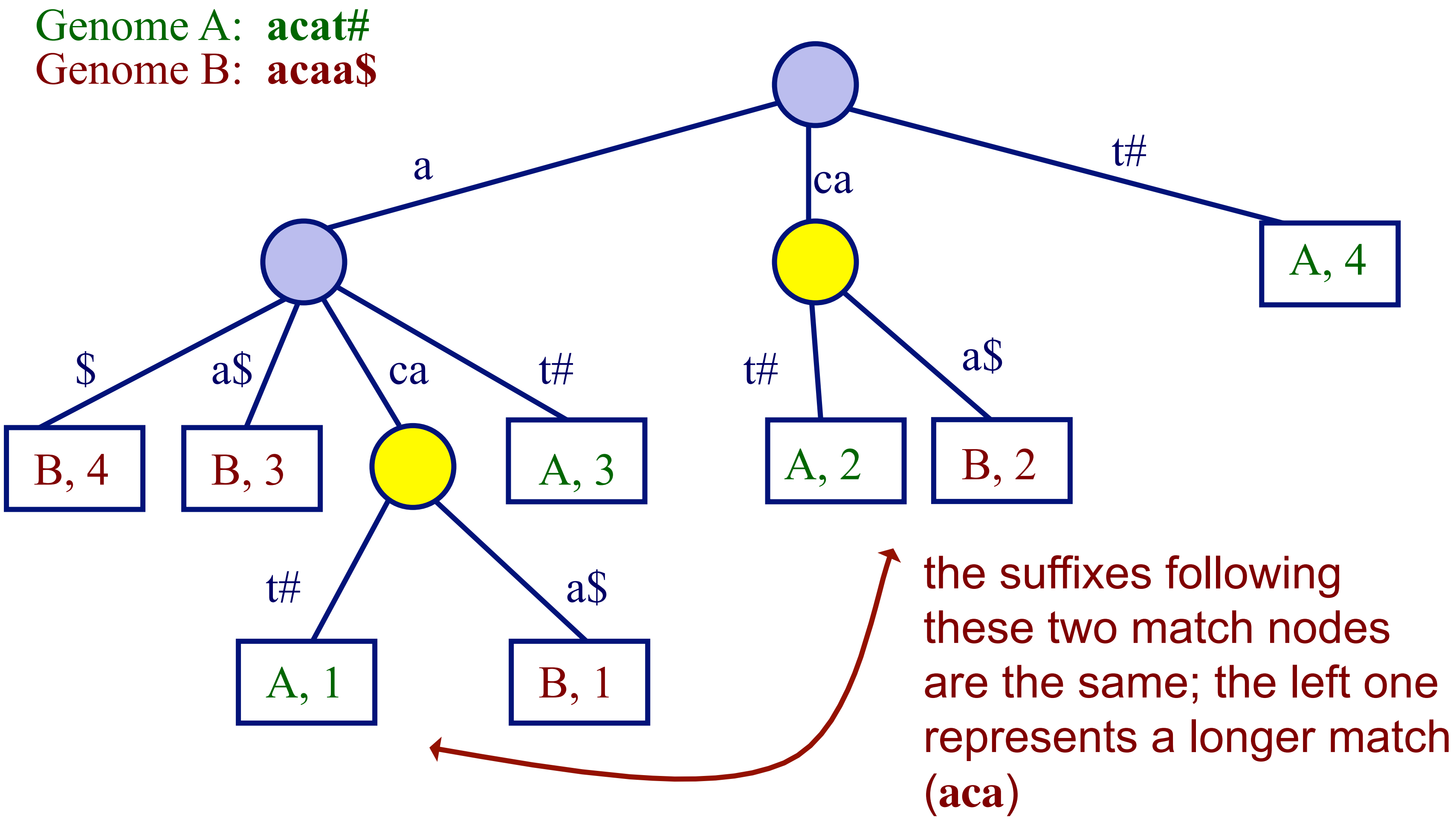
MUMs and Suffix Trees

- unique match: internal node with 2 children, leaf nodes from different genomes
- but these matches are not necessarily maximal



MUMs and Suffix Trees

- to identify <u>maximal</u> matches, can compare suffixes following unique match nodes



Using Suffix Trees to Find MUMs

- $O(n)$ time to construct suffix tree for both sequences (of lengths $\leq n$)
- $O(n)$ time to find MUMs - one scan of the tree (which is $O(n)$ in size)
- $O(n)$ possible MUMs in contrast to $O(n^2)$ possible exact matches

- main parameter of approach: length of shortest MUM that should be identified (20 – 50 bases)

Step 2: Chaining in MUMmer

- sort MUMs according to position in genome A
- solve variation of *Longest Increasing Subsequence* (LIS) problem to find sequences in ascending order in both genomes

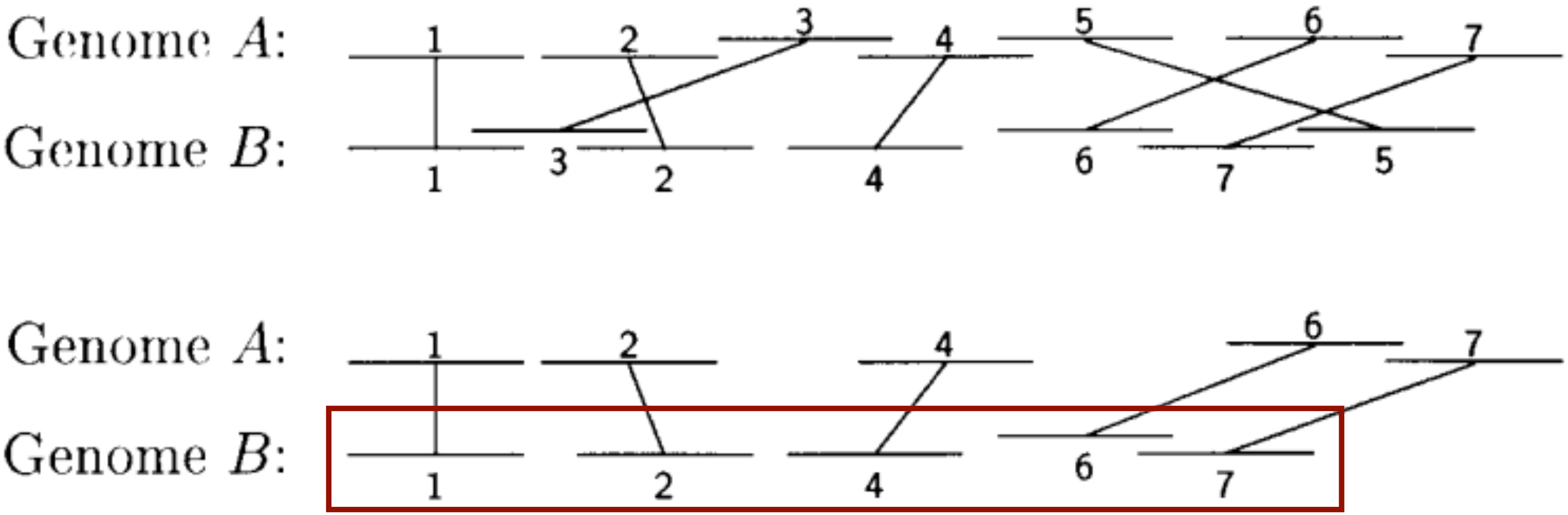


Figure from: Delcher et al., *Nucleic Acids Research* 27, 1999

Finding Longest Subsequence

- unlike ordinary LIS problems, MUMmer takes into account
  - lengths of sequences represented by MUMs
  - overlaps
- requires $O(k \log k)$ time where k is number of MUMs

Types of Gaps in a MUMmer Alignment

1. SNP: exactly one base (indicated by ^) differs between the two sequences. It is surrounded by exact-match sequence.

```
Genome A:   cgtcatgggcgttcgtcgttg
Genome B:   cgtcatgggcattcgtcgttg
                      ^
```

2. Insertion: a sequence that occurs in one genome but not the other.

```
Genome A:   cggggtaaccgc..................cctggtcggg
Genome B:   cggggtaaccgcgttgctcggggtaaccgccctggtcggg
                        ^^^^^^^^^^^^^^^^^^
```

3. Highly polymorphic region: many mutations in a short region.

```
Genome A:   ccgcctcgcctgg.gctggcgcccgctc
Genome B:   ccgcctcgccagttgaccgcgcccgctc
                      ^ ^^ ^^^
```

4. Repeat sequence: the repeat is shown in uppercase. Note that the first copy of the repeat in Genome B is imperfect, containing one mismatch to the other three identical copies.

```
Genome A:   cTGGGTGGGACAACGTaaaaaaaaaTGGGTGGGACAACGTc
Genome B:   aTGGGTGGGGCgACGTgggggggggTGGGTGGGACAACGTa
            ^          ^                            ^
```

Figure from: Delcher et al., *Nucleic Acids Research* 27, 1999

Step 3: Close the Gaps

- SNPs:
  - between MUMs: trivial to detect
  - otherwise: handle like repeats
- inserts
  - transpositions (subsequences that were deleted from one location and inserted elsewhere): look for out-of-sequence MUMs
  - simple insertions: trivial to detect

Step 3: Close the Gaps

- polymorphic regions
  - short ones: align them with dynamic programming method
  - long ones: call MUMmer recursively w/ reduced min MUM length
- repeats
  - detected by overlapping MUMs

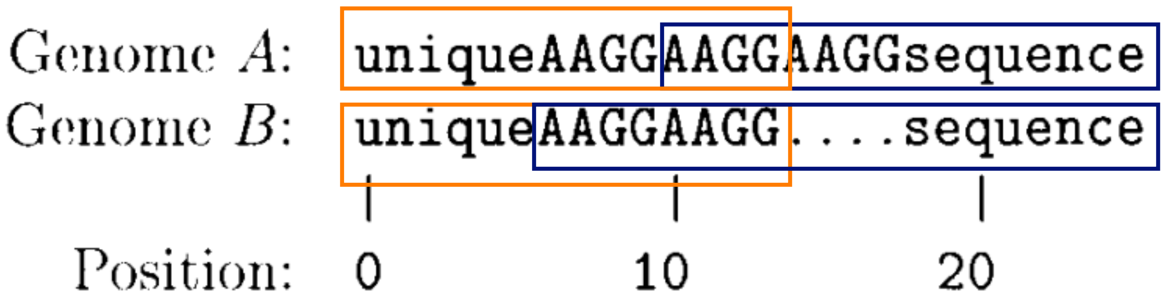


Figure from: Delcher et al. Nucleic Acids Research 27, 1999

The LAGAN Method

Brudno et al., *Genome Research*, 2003

Given: genomes *A* and *B*

anchors = find_anchors(A, B)

step 3: finish global alignment with DP constrained by *anchors*

find_anchors(A, B)

step 1: find local alignments by matching, chaining *k*-mer seeds

step 2: *anchors* = highest-weight sequence of local alignments

for each pair of adjacent anchors a_1, a_2 in *anchors*

if a_1, a_2 are more than *d* bases apart

A', *B*' = sequences between a_1, a_2

sub-anchors = find_anchors(*A', B'*)

insert *sub-anchors* between a_1, a_2 in *anchors*

return *anchors*

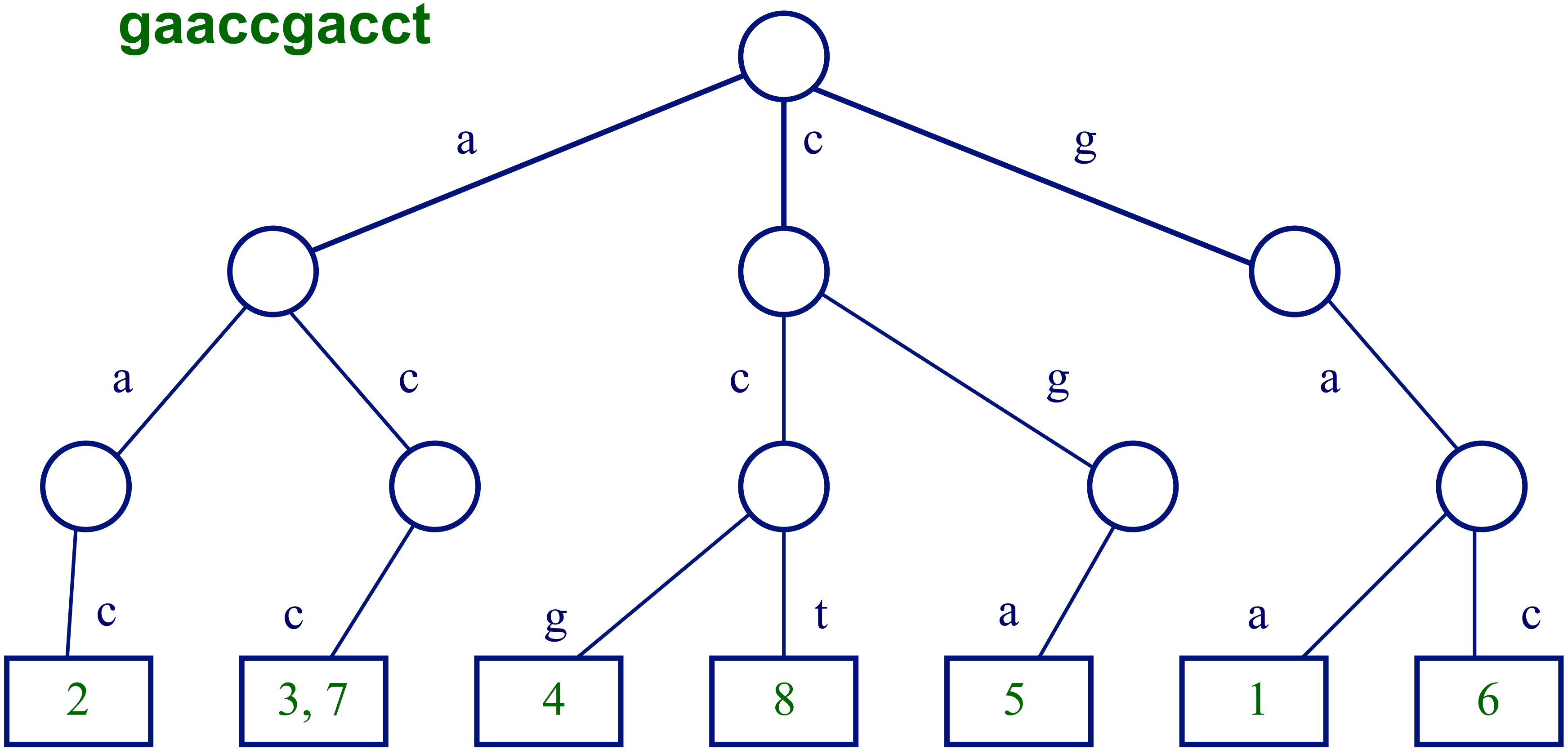
Step 1a: Finding Seeds in LAGAN

- *degenerate k-mers*: matching *k*-long sequences with a small number of mismatches allowed
- by default, LAGAN uses 10-mers and allows 1 mismatch

cacg cgcgctacat acct
acta cgcggtacat cgta

Finding Seeds in LAGAN

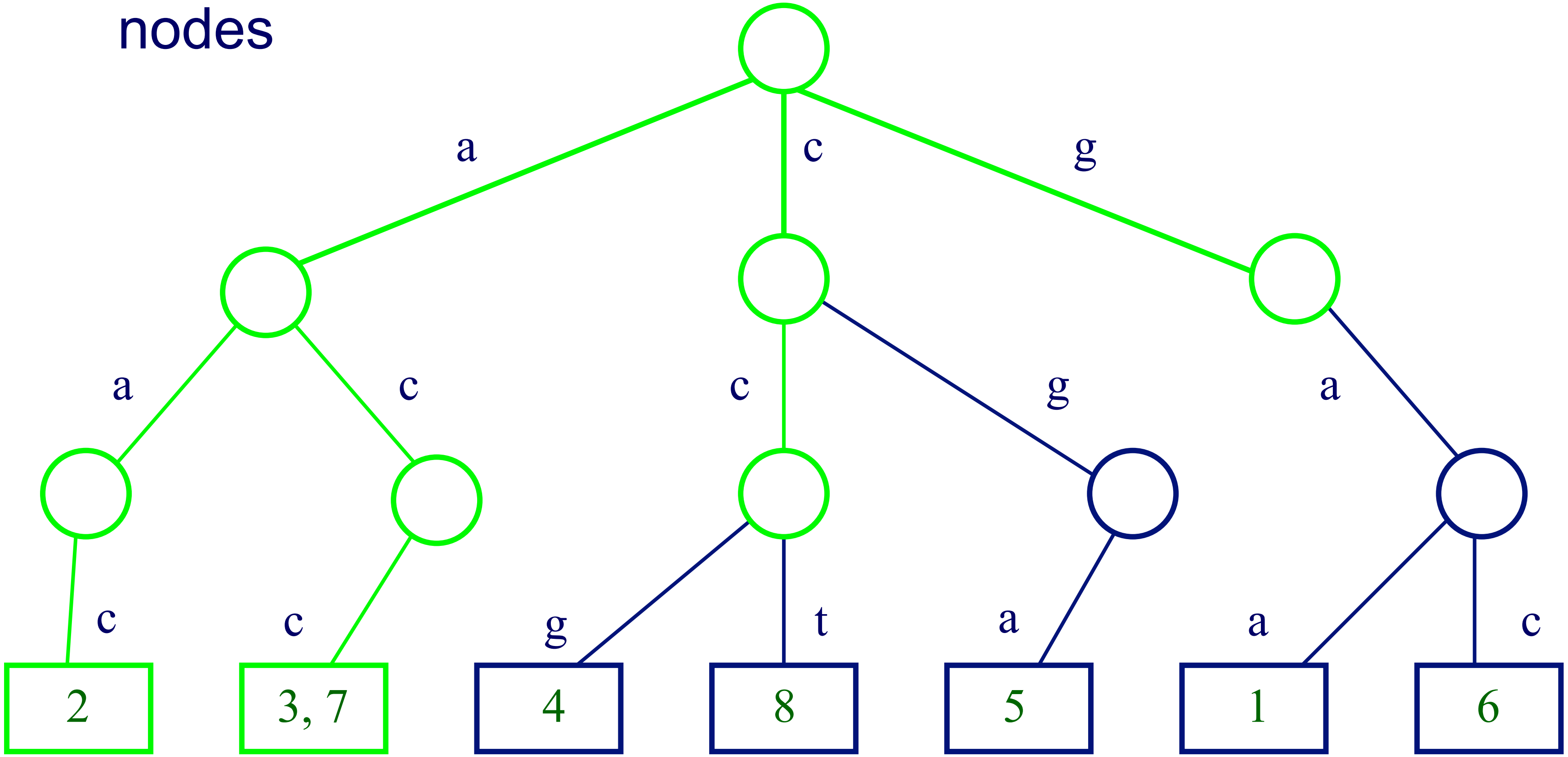
- example: a *trie* to represent all 3-mers of the sequence **gaaccgacct**



- one sequence is used to build the trie
- the other sequence (the query) is "walked" through to find matching *k*-mers

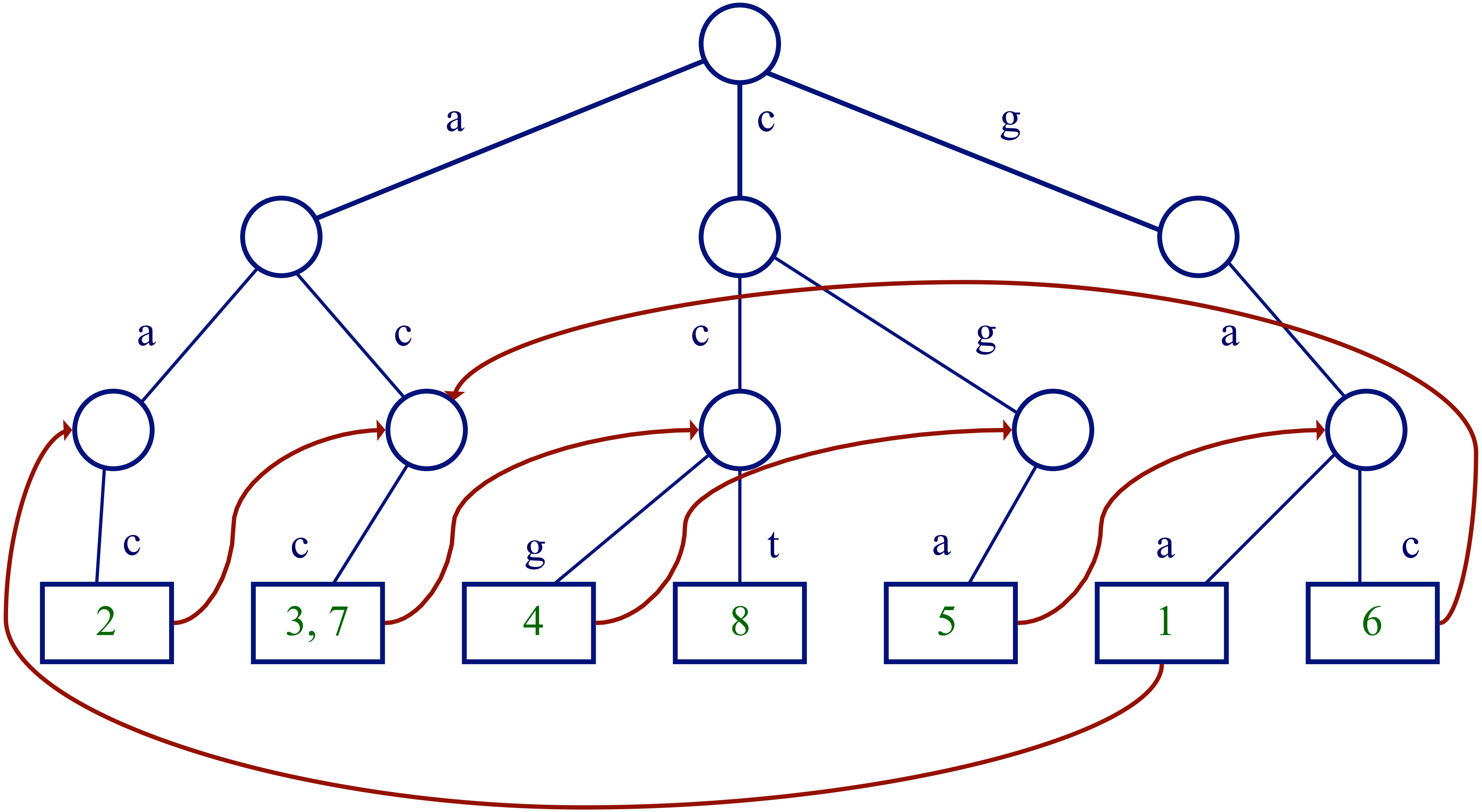
Allowing Degenerate Matches

- suppose we're allowing 1 base to mismatch in looking for matches to the 3-mer **acc**; need to explore green nodes



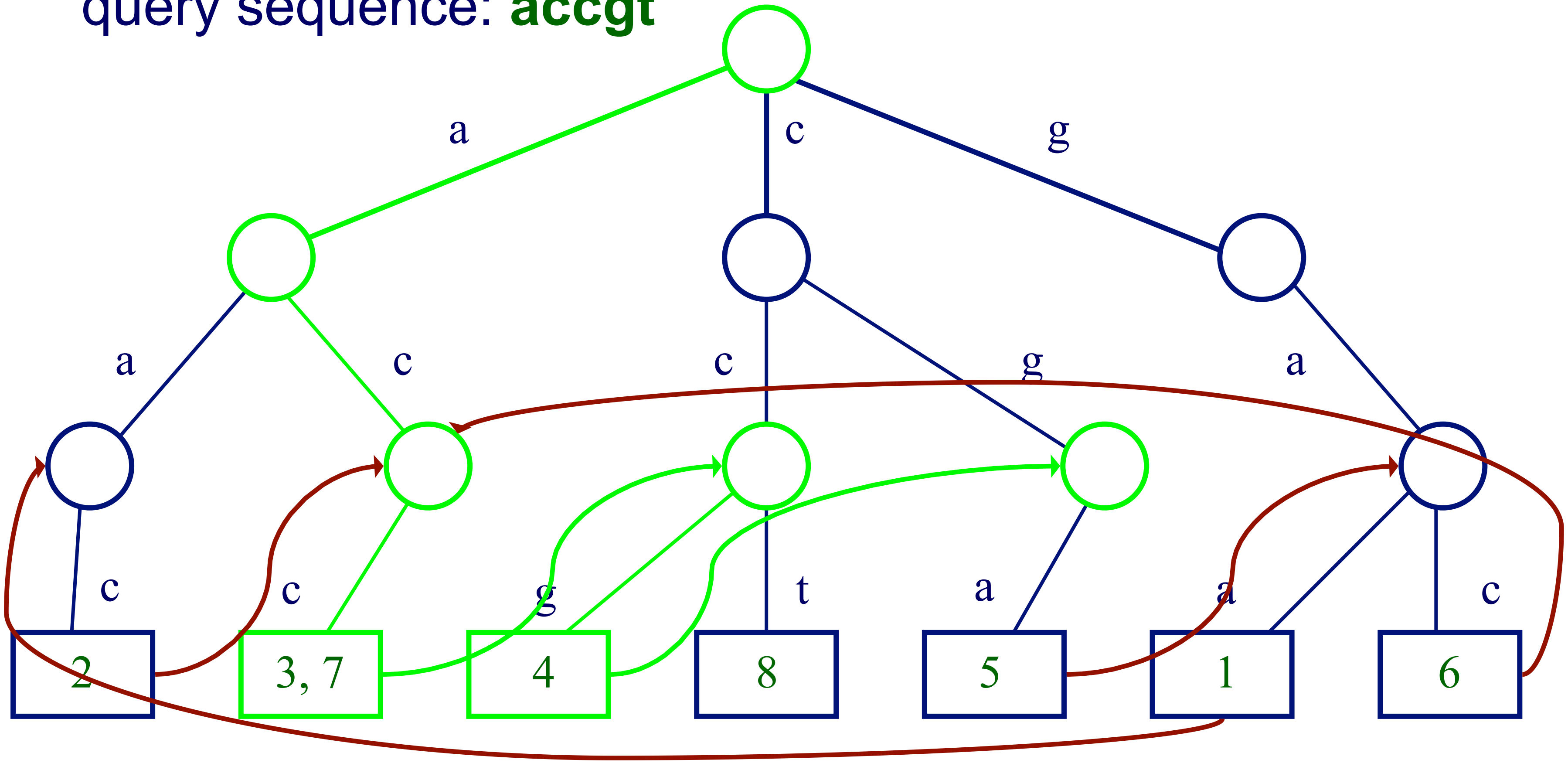
LAGAN Uses Threaded Tries

- in a *threaded trie,* each leaf for word $w_1 \ldots w_p$ has a back pointer to the node for $w_2 \ldots w_p$



Traversing a Threaded Trie

- consider traversing the trie to find 3-mer matches for the query sequence: **accgt**



- usually requires following only two pointers to match against the next *k*-mer, instead of traversing tree from root for each

Step 1b: Chaining Seeds in LAGAN

- can chain seeds s_1 and s_2 if
  - the indices of s_1 > indices of s_2 (for both sequences)
  - s_1 and s_2 are near each other
- keep track of seeds in the "search box" as the query sequence is processed

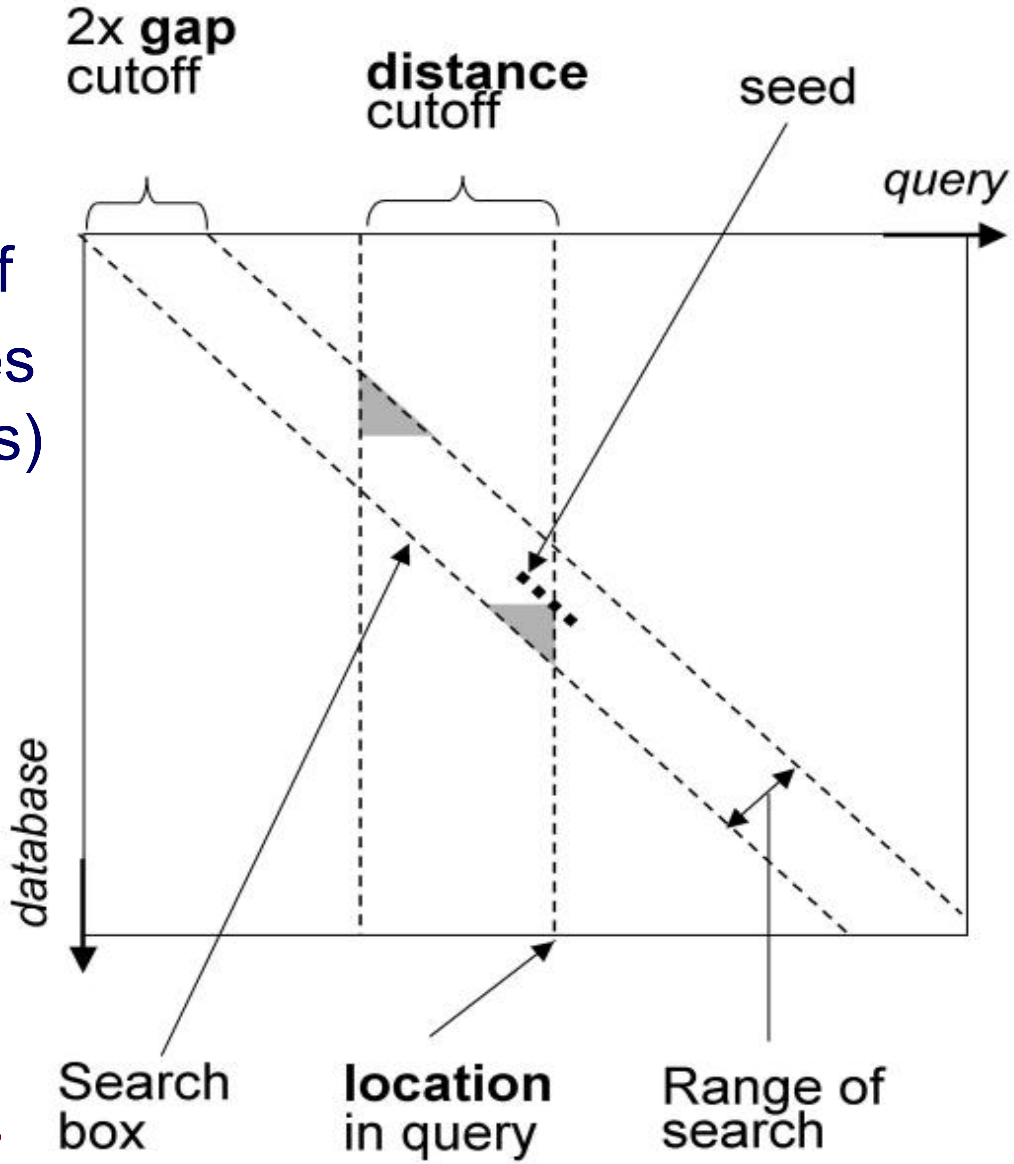
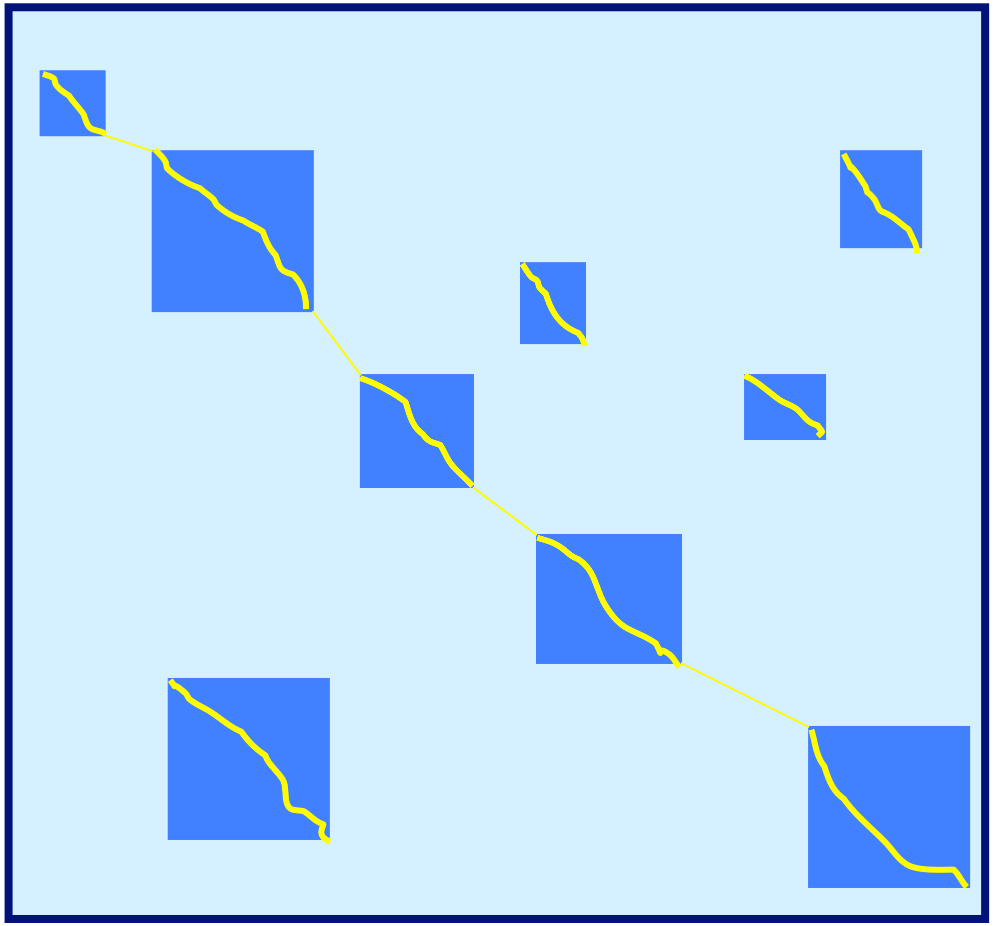


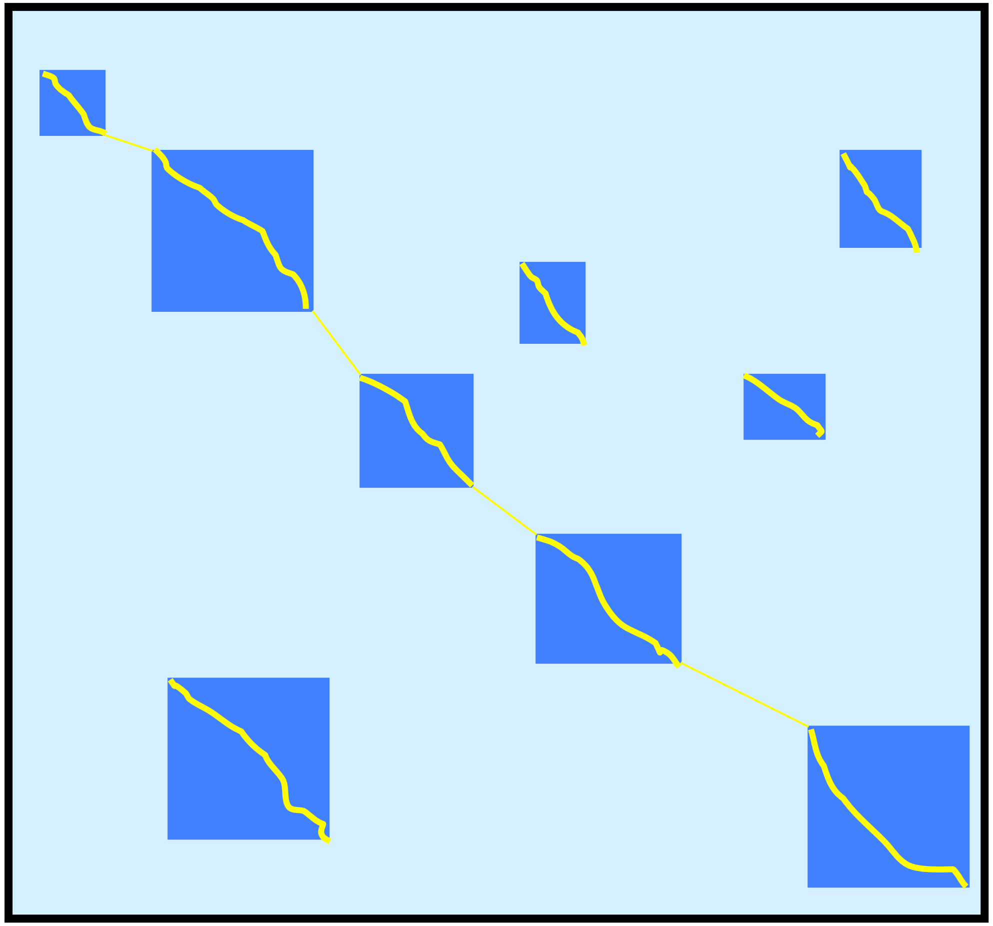
Figure from: Brudno et al. *BMC Bioinformatics*, 2003

Step 2: Chaining in LAGAN

- use *sparse dynamic programming* to chain local alignments



The Problem: Find a Chain of Local Alignments



(x,y) → (x',y')

requires

x < x'
y < y'

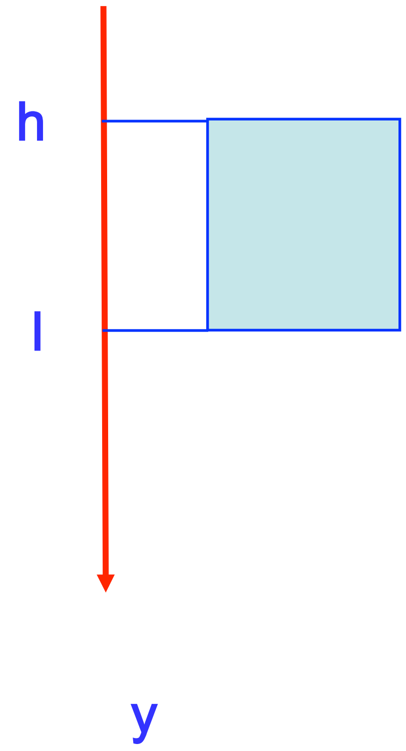
Each local alignment has a weight

FIND the chain with highest total weight

Slide from Serafim Batzoglou, Stanford University

Sparse DP for rectangle chaining

- 1,…, N: rectangles
- (h_j, l_j): y-coordinates of rectangle j
- w(j): weight of rectangle j
- V(j): optimal score of chain ending in j
- L: list of triplets $(l_j, V(j), j)$
  - L is sorted by l_j: smallest (North) to largest (South) value
  - L is implemented as a balanced binary tree



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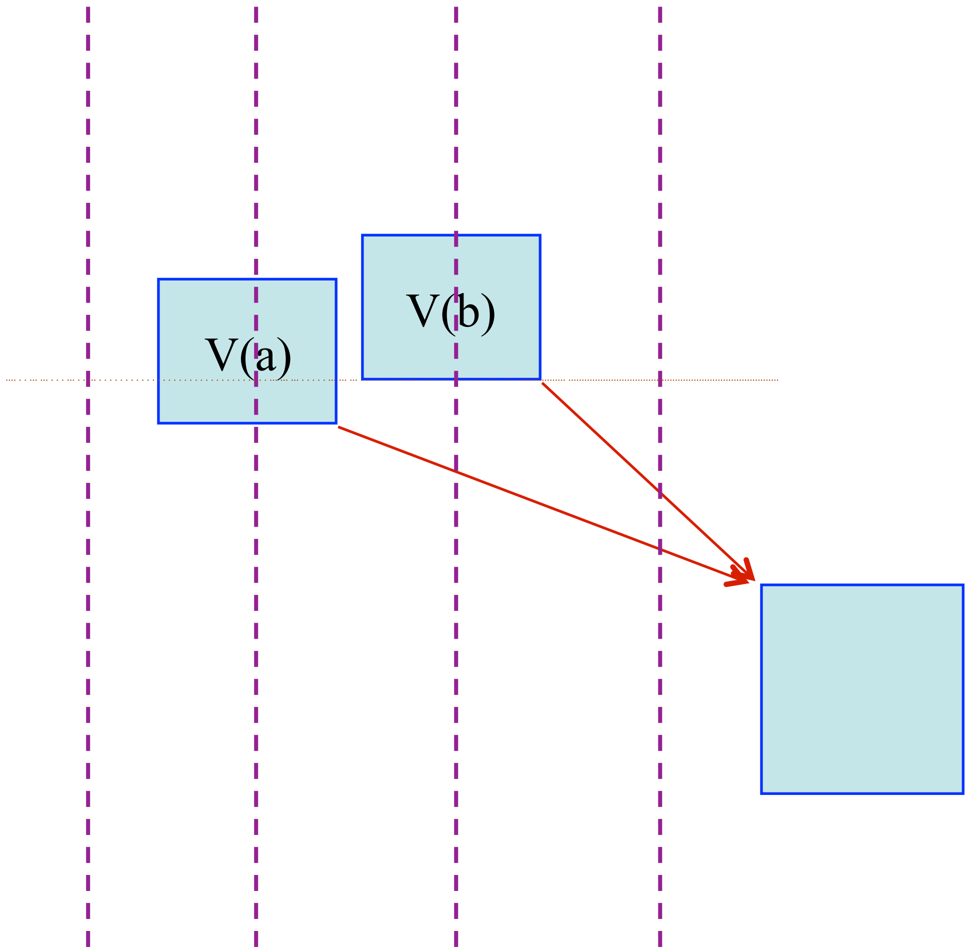
Sparse DP for rectangle chaining

Main idea:

- Sweep through x-coordinates
- To the right of **b**, anything chainable to **a** is chainable to b
- Therefore, if **V(b) > V(a)**, rectangle a is "useless" for subsequent chaining
- In L, keep rectangles j sorted with increasing l_j-coordinates ⇒
  sorted with increasing V(j) score



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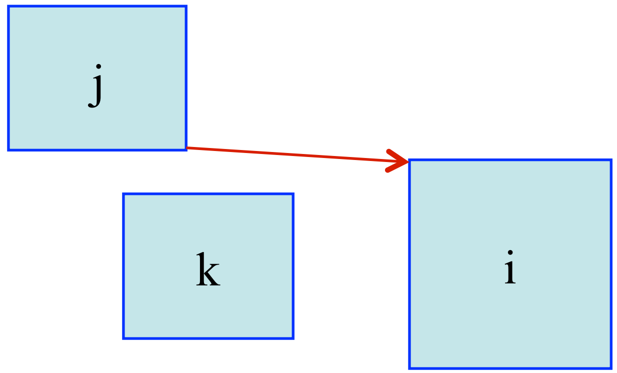
Sparse DP for rectangle chaining

Go through rectangle x-coordinates, from lowest to highest:



1. When on the leftmost end of rectangle i:
   a. j: rectangle in L, with largest $l_j < h_i$
   b. $V(i) = w(i) + V(j)$
2. When on the rightmost end of i:
   a. k: rectangle in L, with largest $l_k \le l_i$
   b. If $V(i) > V(k)$:
      i. **INSERT** $(l_i, V(i), i)$ in L
      ii. **REMOVE** all $(l_j, V(j), j)$ with $V(j) \le V(i)$ & $l_j \ge l_i$

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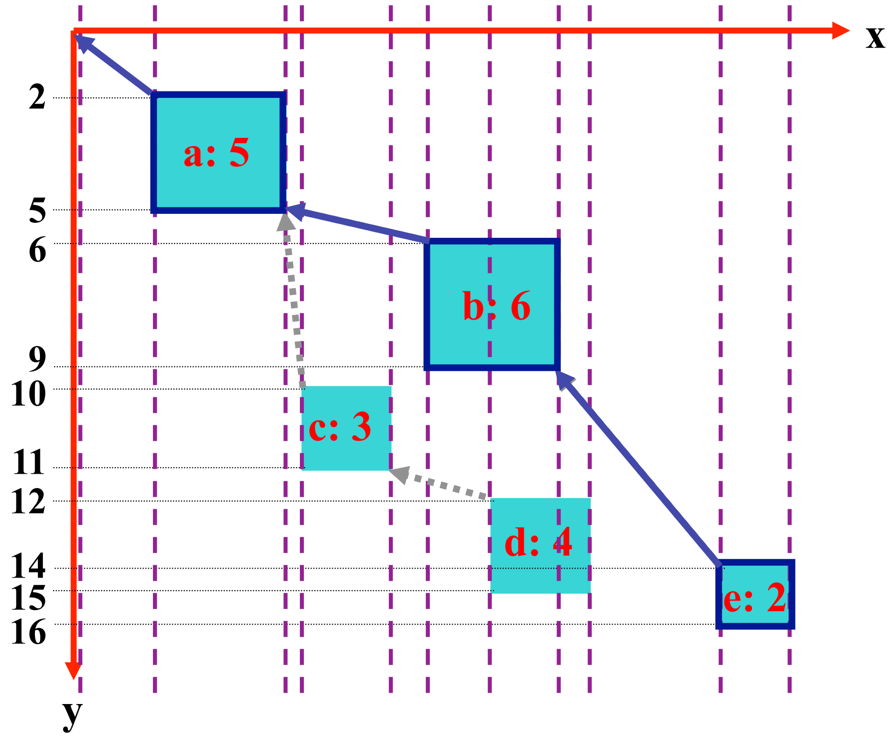
Example



V

a	b	c	d	e
5	11	8	12	13

L

l_i	5	9	15	16
V(i)	5	11	12	13
i	a	b	d	e

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Slide from Serafim Batzoglou, Stanford University

Time Analysis

1. Sorting the x-coords takes O(N log N)

2. Going through x-coords: N steps

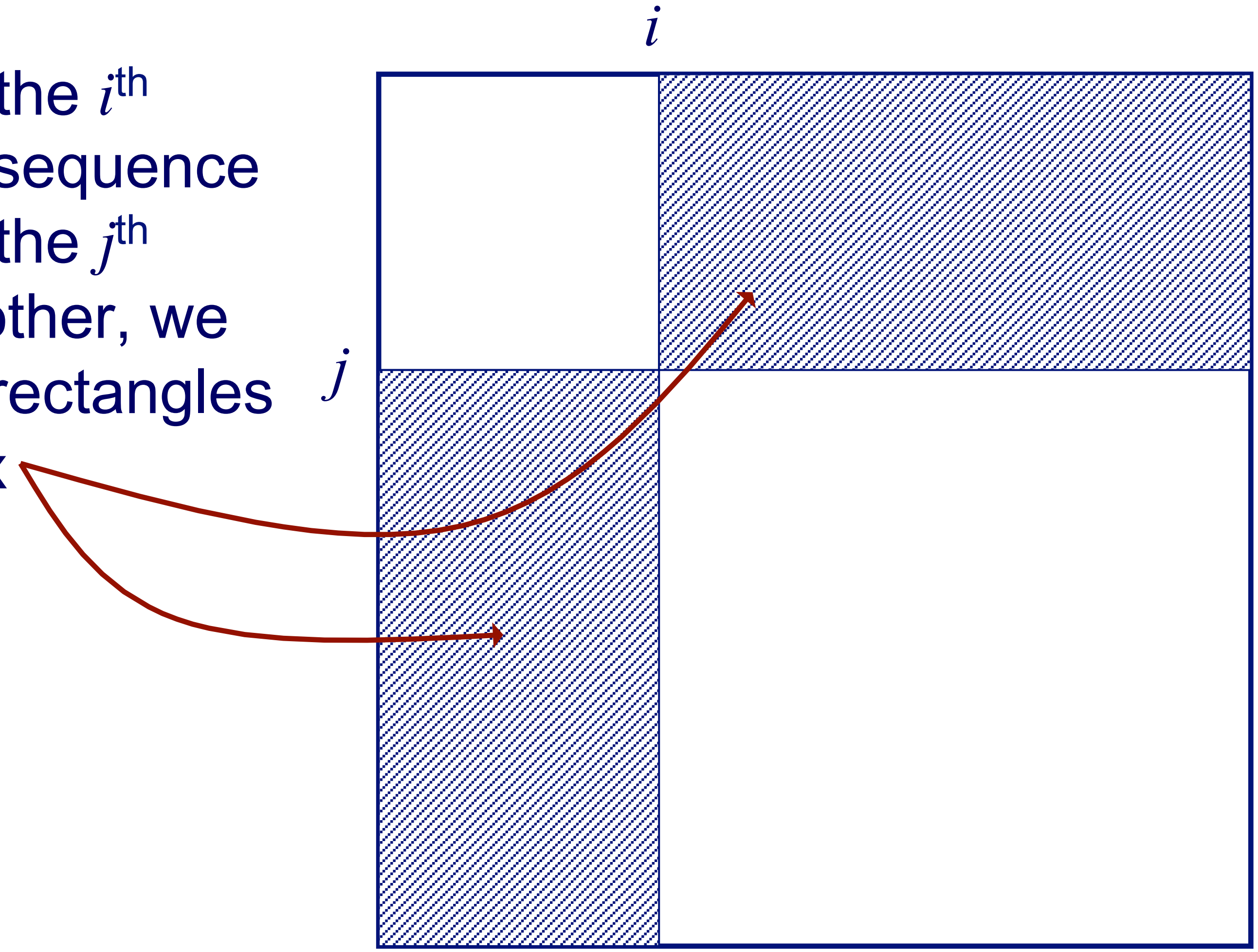
3. Each of N steps requires O(log N) time:

   - Searching L takes log N
   - Inserting to L takes log N
   - All deletions are consecutive, so log N per deletion
   - Each element is deleted at most once: N log N for all deletions

     - Recall that INSERT, DELETE, SUCCESSOR, take O(log N) time in a balanced binary search tree

Constrained Dynamic Programming

- if we know that the i^{th} element in one sequence must align with the j^{th} element in the other, we can ignore two rectangles in the DP matrix



Step 3: Computing the Global Alignment in LAGAN

- given an anchor that starts at (i, j) and ends at (i', j'), LAGAN limits the DP to the unshaded regions
- thus anchors are somewhat flexible

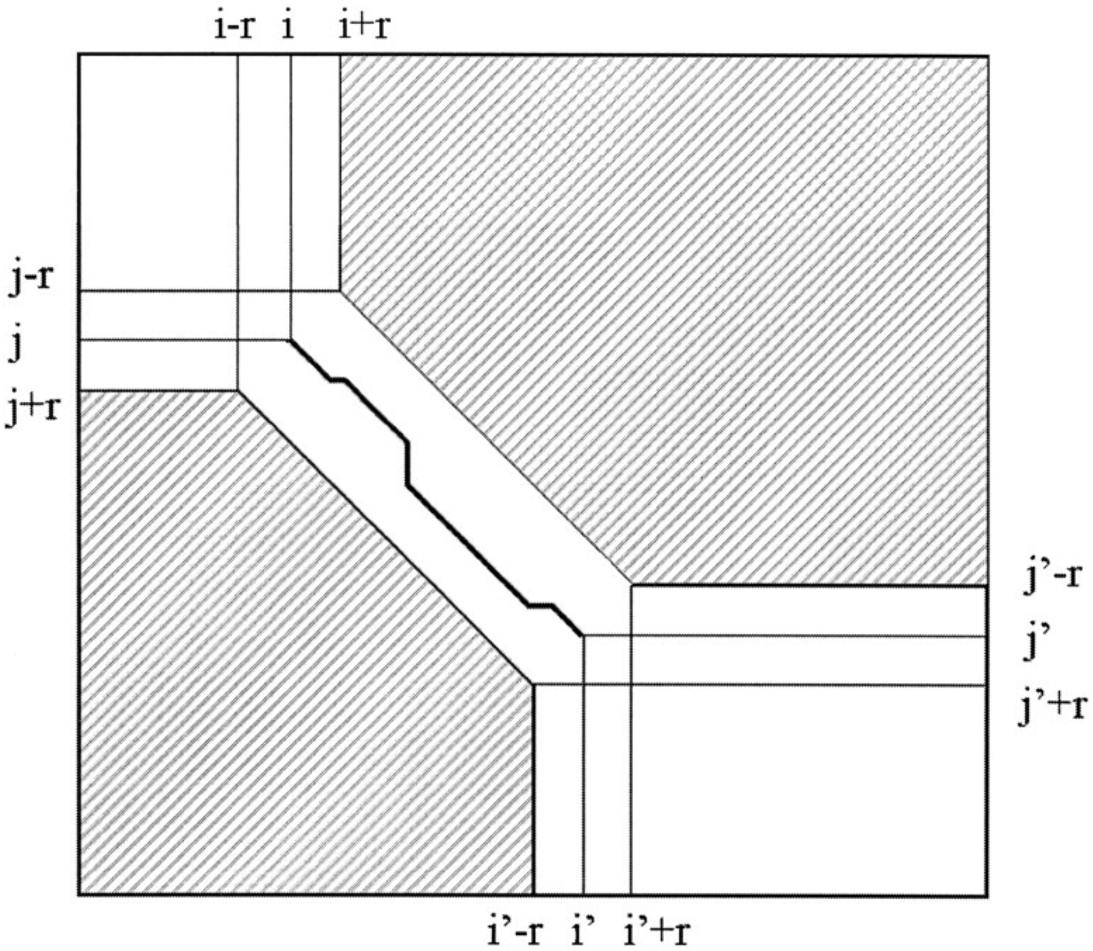
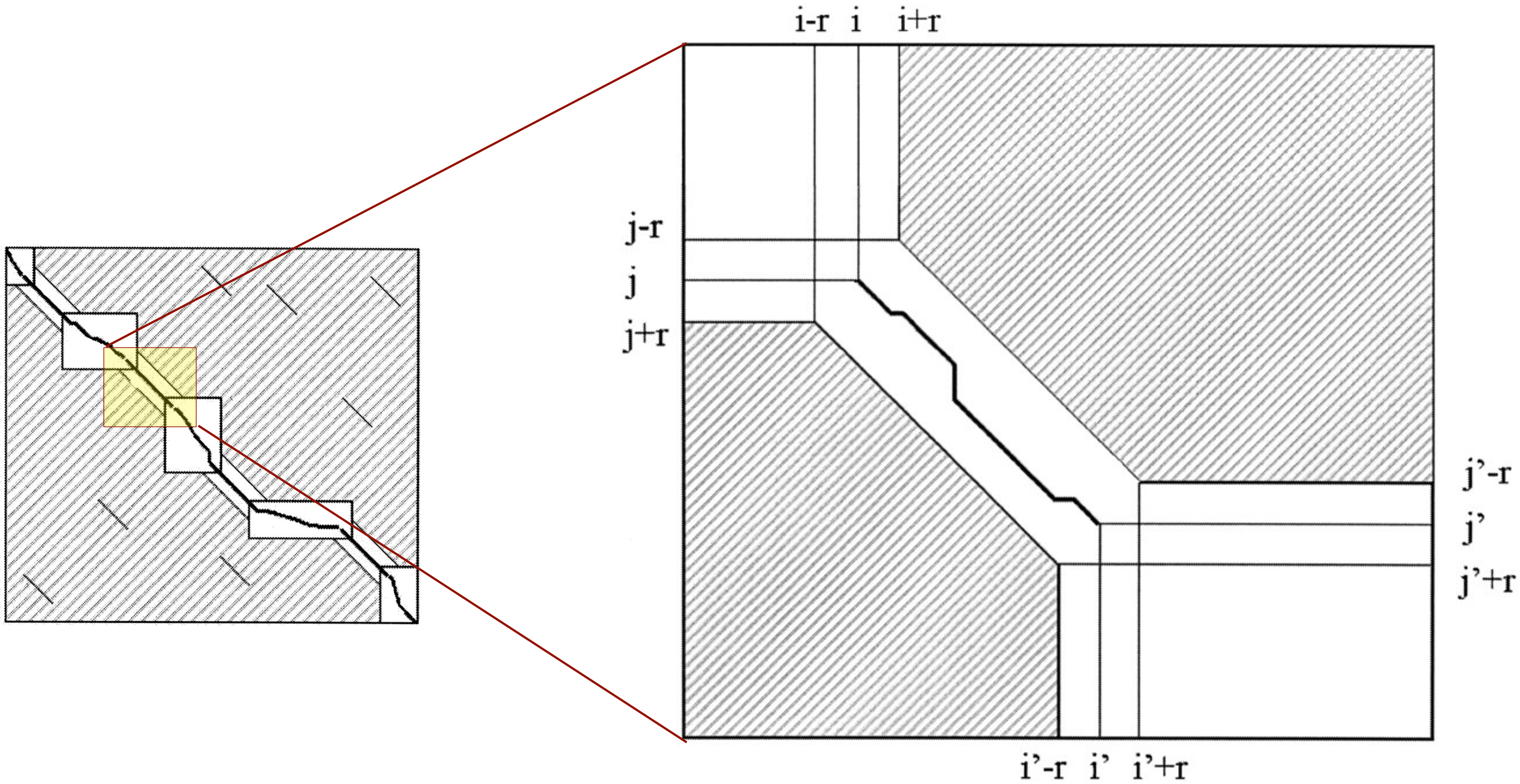


Figure from: Brudno et al. *Genome Research*, 2003

Step 3: Computing the Global Alignment in LAGAN



Figures from: Brudno et al. *Genome Research*, 2003

Example Alignment:
E. Coli O157:H7 vs. E. coli K-12

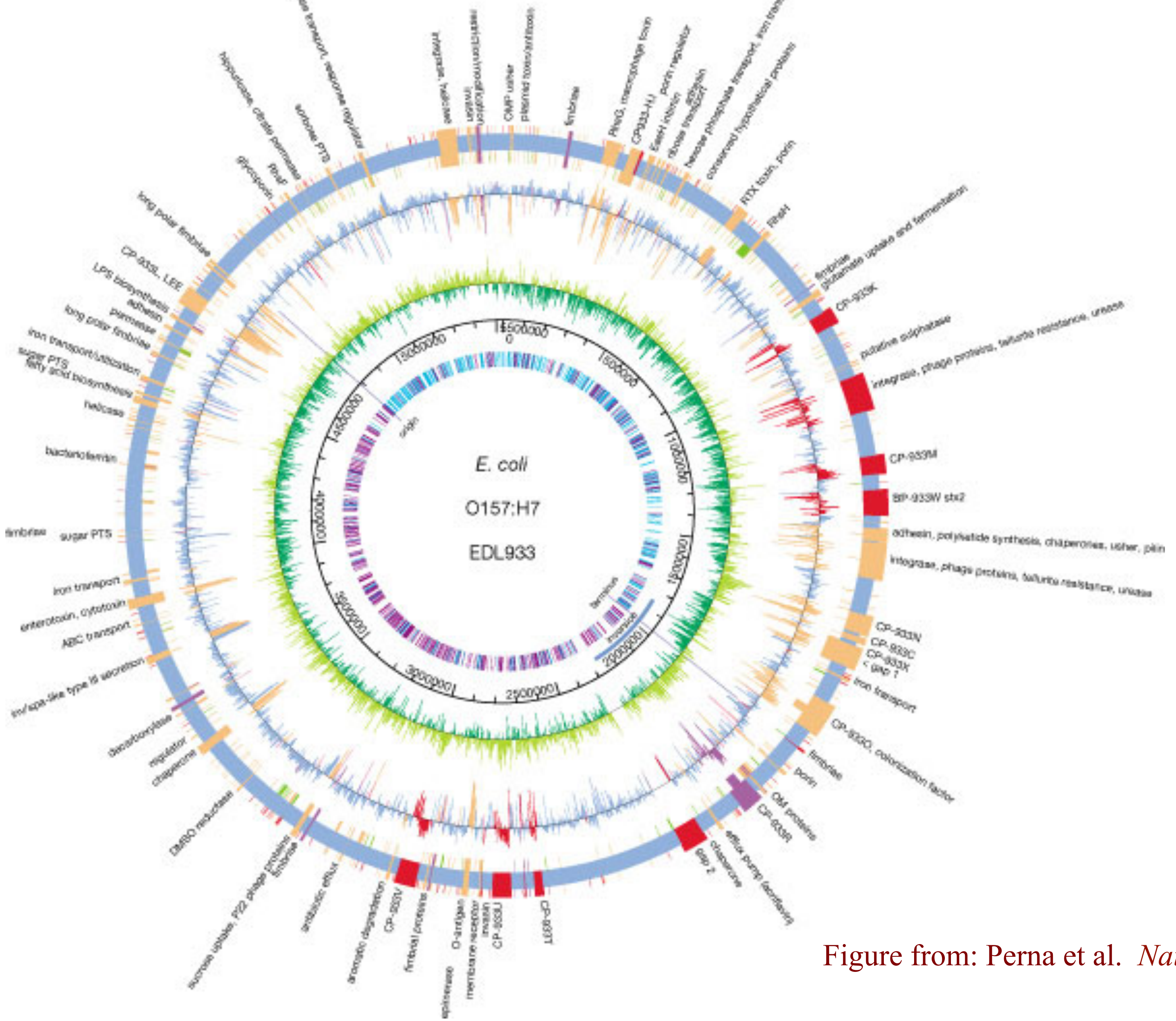


Figure from: Perna et al. *Nature*, 2001