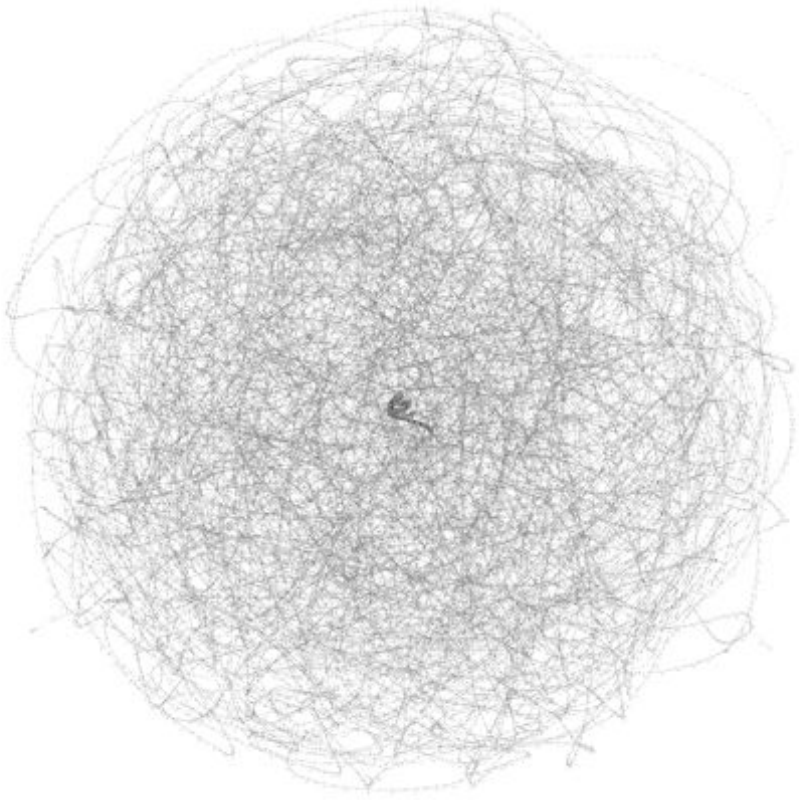
splitMEM: graphical pan-genome analysis with suffix skips

Shoshana Marcus

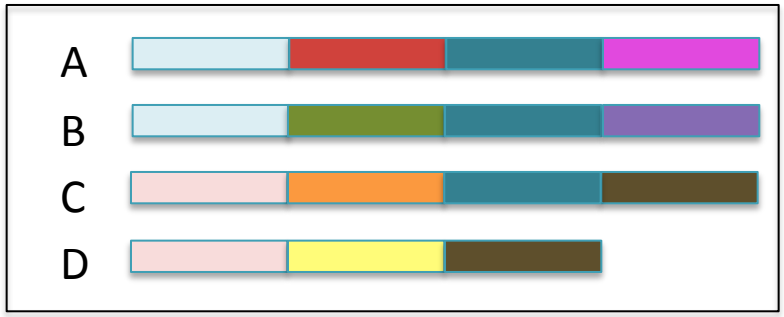
May 7, 2014

Outline



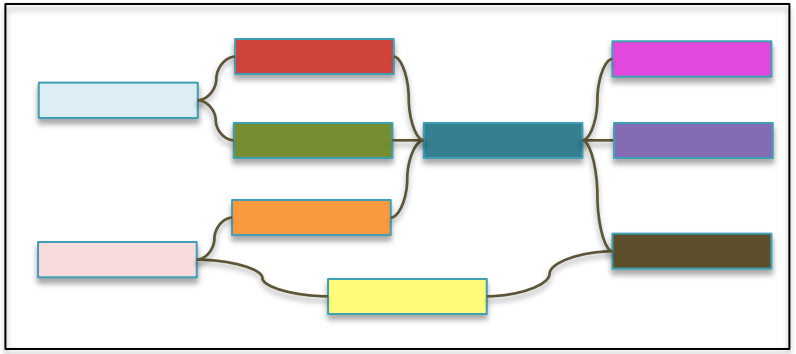
Objective

Input



- Several complete genomes
- Available today for many microbial species, near future for higher eukaryotes
- Pan-genome: analyze multiple genomes of species together

Output



Compressed de Bruijn graph

- Graphical representation depicts how population variants relate to each other, especially where they diverge at branch points
- How well conserved is a sequence?
- What are network properties?

de Bruijn graph

- Node for each distinct kmer
- Directed edge connects consecutive kmers
- Nodes overlap by k-1 bp
- Self-loops, multi-edges

AGAAGTCC
ATAAGTTA

Reconstruct original sequence:

Eulerian path through graph, visit each edge once

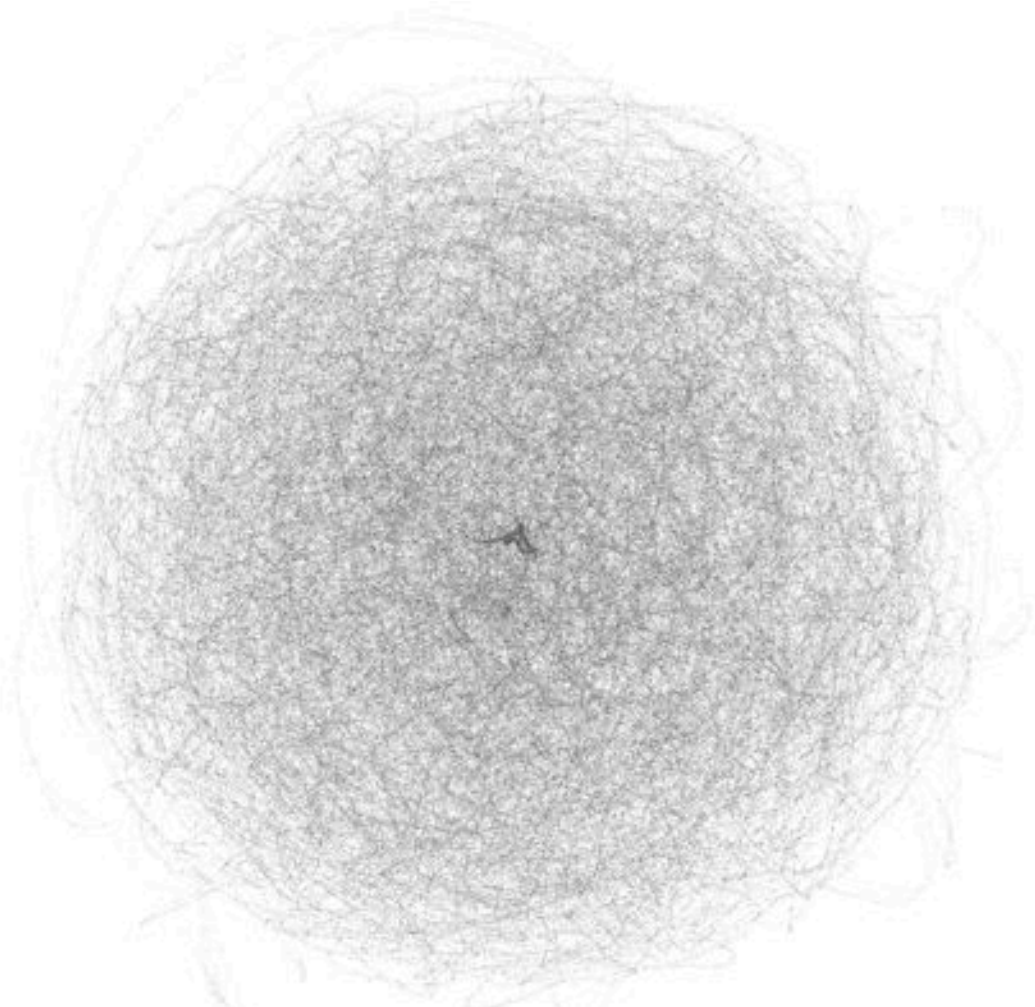
Compressed de Bruijn graph

- Merge non-branching chains of nodes
- Min. number of nodes that preserve path labels

✧Usually built from uncompressed graph

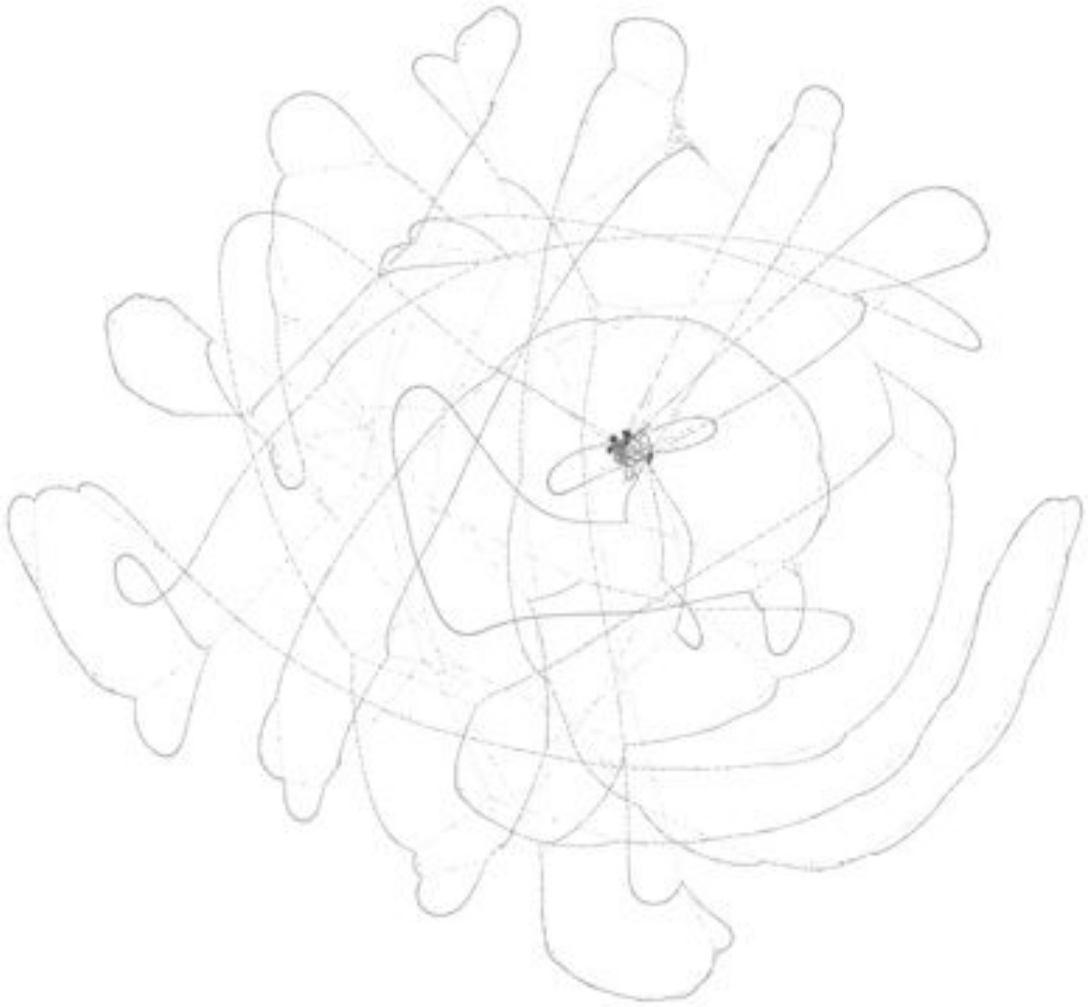
✧We build directly in O(n log n) time and space

Compresssed de Bruijn graph



9 strains of *Bacillus anthracis k=25*

Compresssed de Bruijn graph



9 strains of *Bacillus anthracis k=1000*

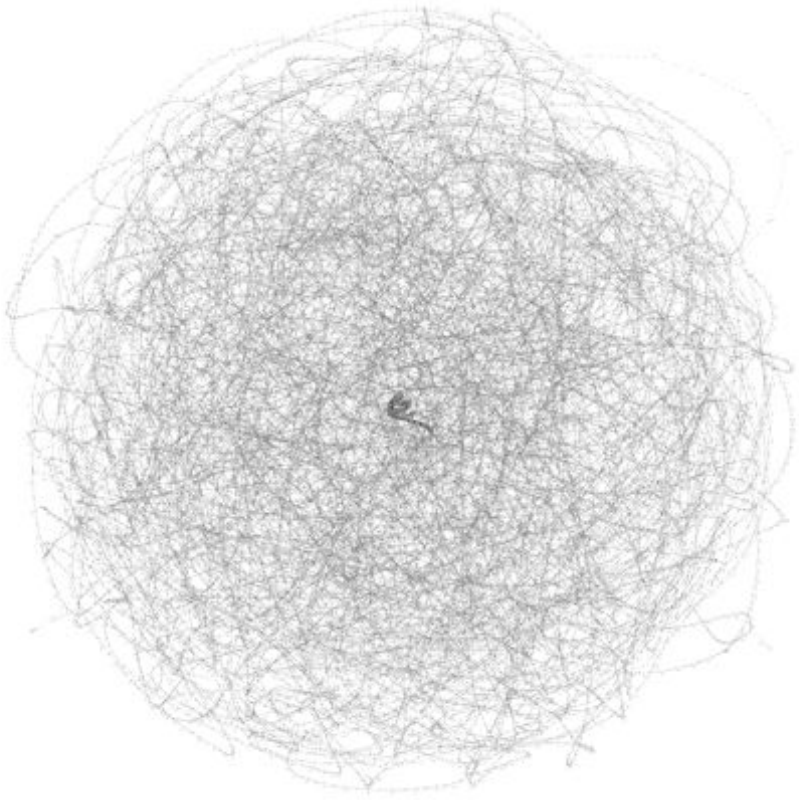
Outline

1 Overview

2 Data Structures

3 splitMEM Algorithm

4 Pan-genome Analysis



Suffix Tree

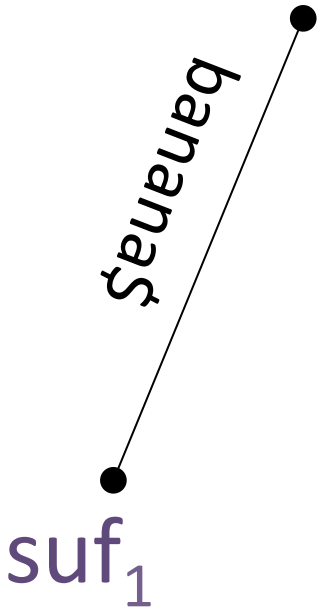
- Rooted, directed tree with leaf for each suffix.
- Each internal node, except the root, has at least two children.

- Each edge is labeled with nonempty substring.
- No two siblings begin with the same character.
- Path from root to leaf i spells suffix S[i . . . n].
- Append special character $ to guarantee each suffix ends at leaf.

Constructing Suffix Tree

Naïve Algorithm

S = banana$

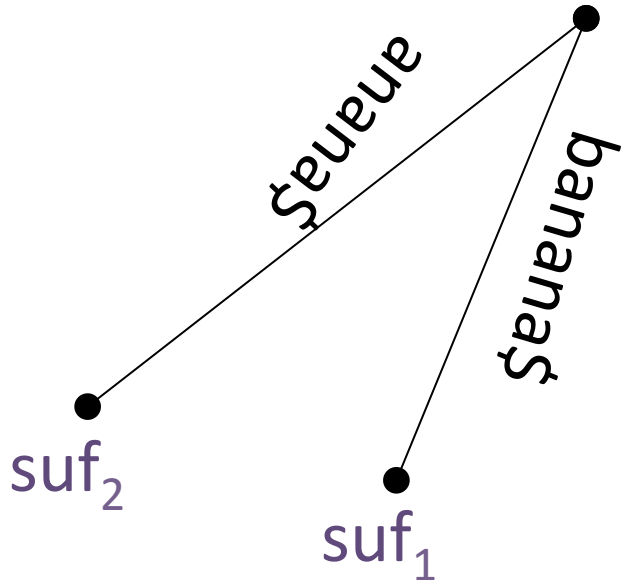


suf_1
banana$

Constructing Suffix Tree

Naïve Algorithm

S = banana$

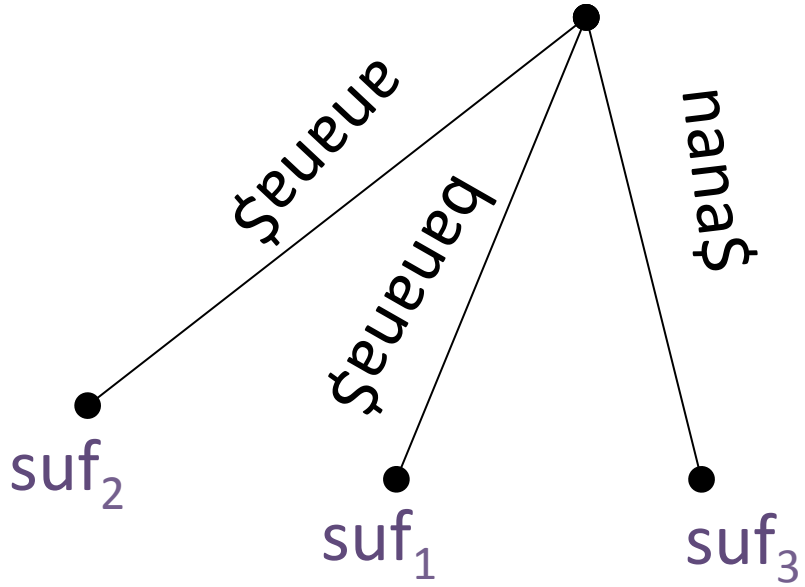


suf_2
anana$

Constructing Suffix Tree

Naïve Algorithm

S = banana$

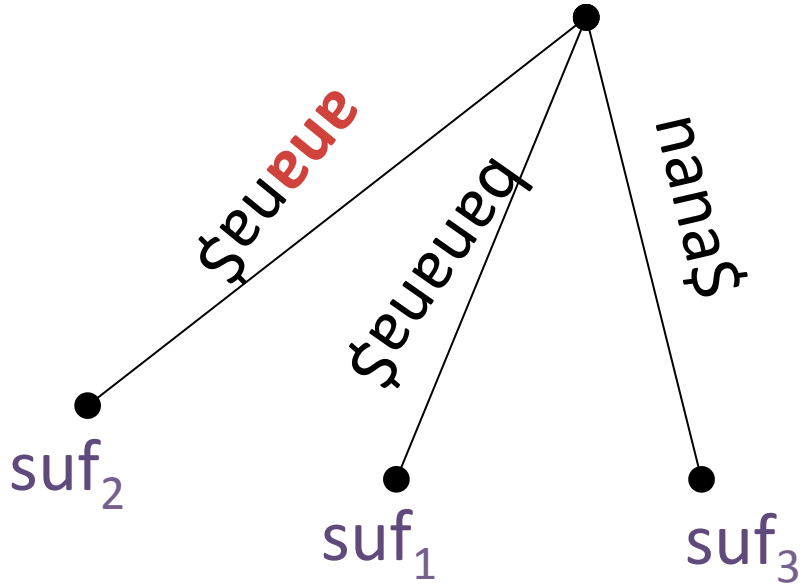


suf_3
nana$

Constructing Suffix Tree

Naïve Algorithm

S = banana$

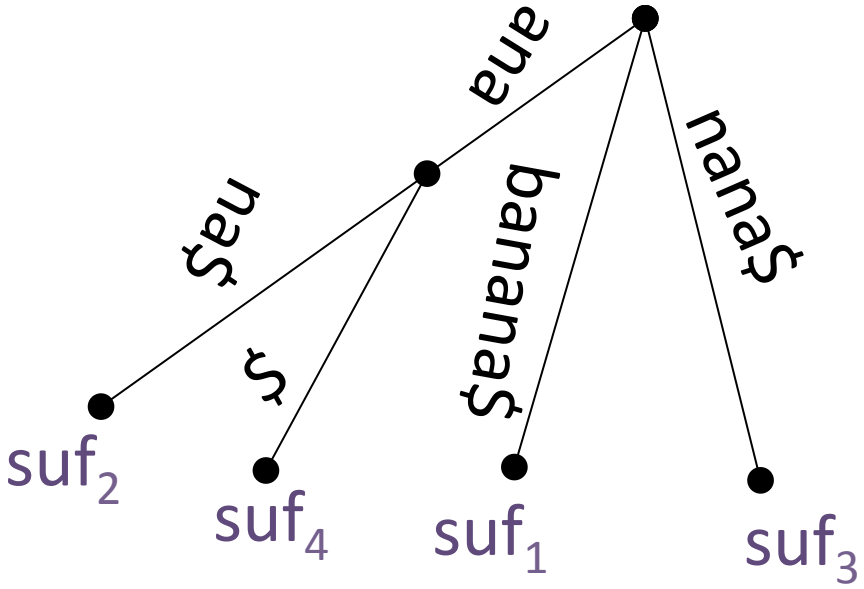


suf_4
ana$

Constructing Suffix Tree

Naïve Algorithm

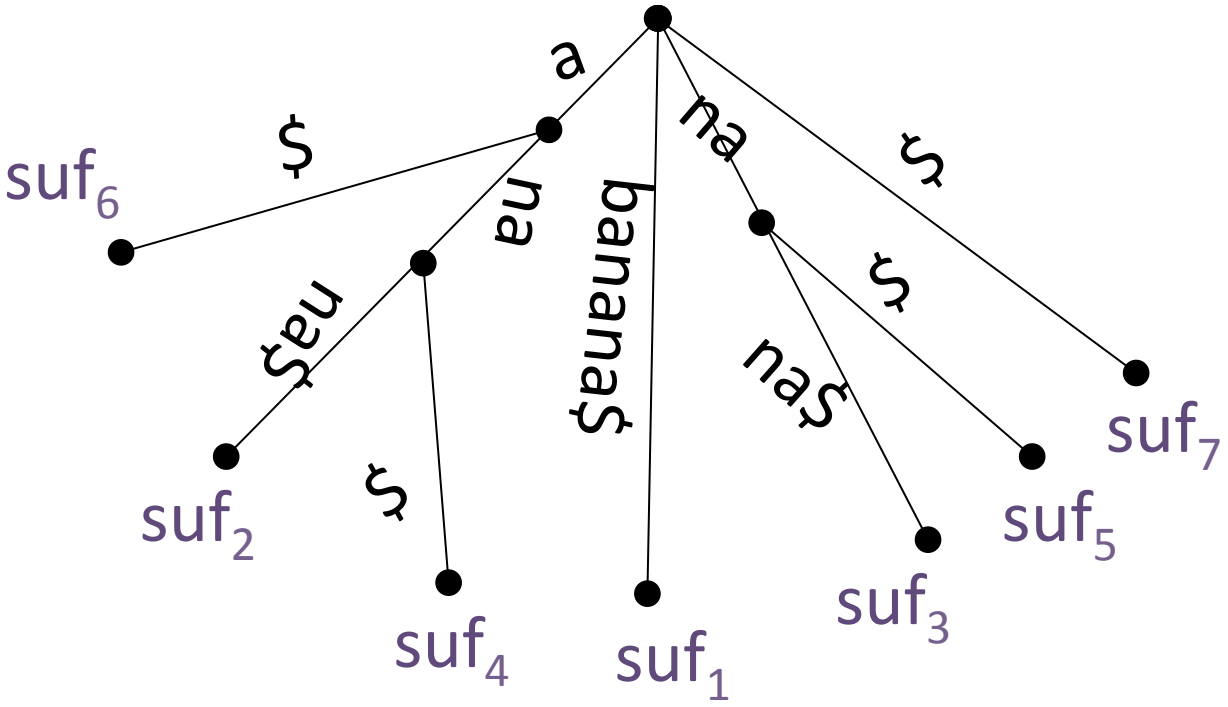
S = banana$



suf_4
ana$

Constructing Suffix Tree

Naïve Algorithm



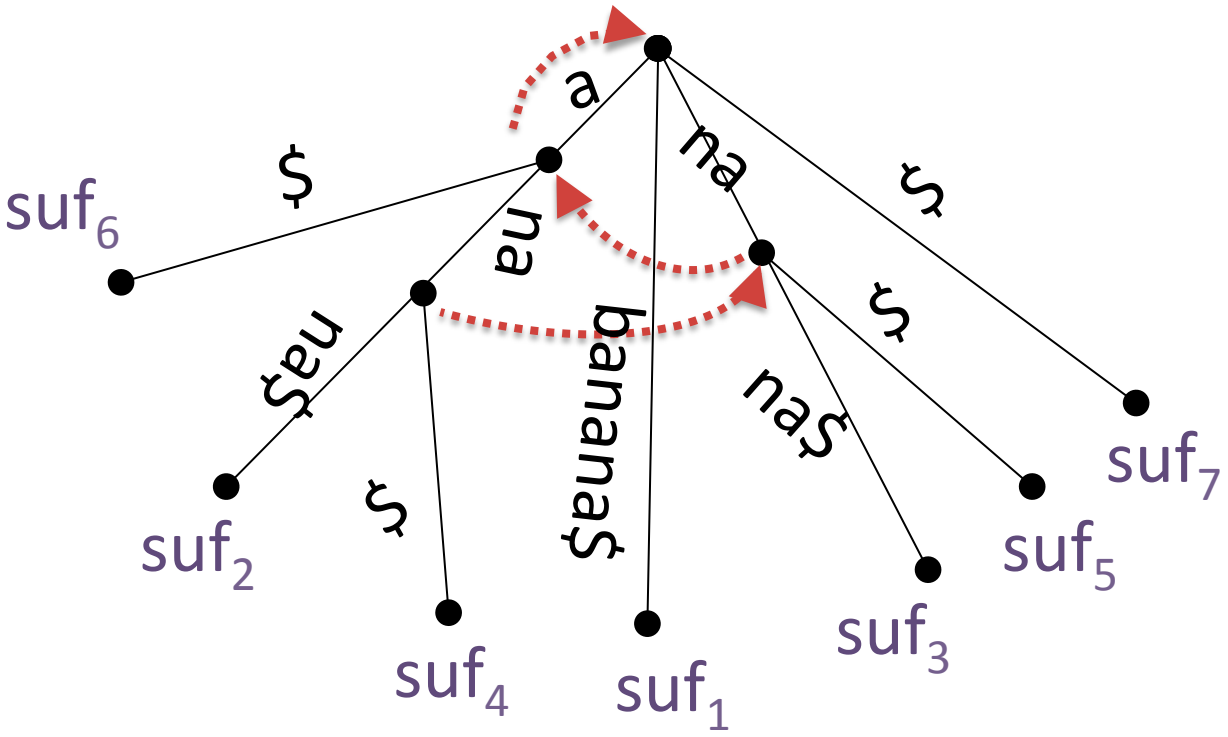
S = banana$

banana$	suf_1
anana$	suf_2
nana$	suf_3
ana$	suf_4
na$	suf_5
a$	suf_6
$	suf_7

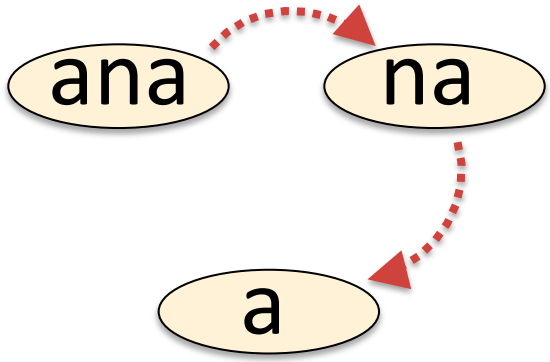
$O(n^2)$ time

Constructing Suffix Tree

O(n) time



Suffix Links

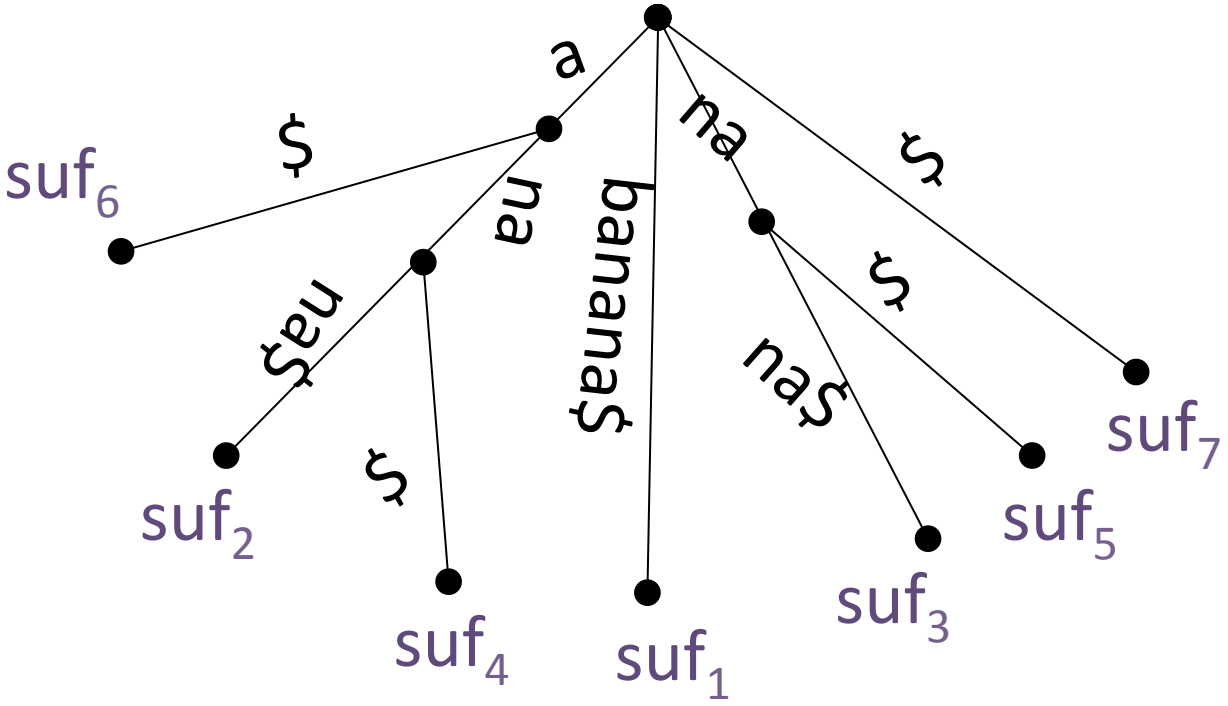


On-line Constructin of Suffix Trees, E. Ukkonen Algorithmica (1995)

Suffix Tree Query

S = banana$

Search for **ban**



Suffix Tree Query

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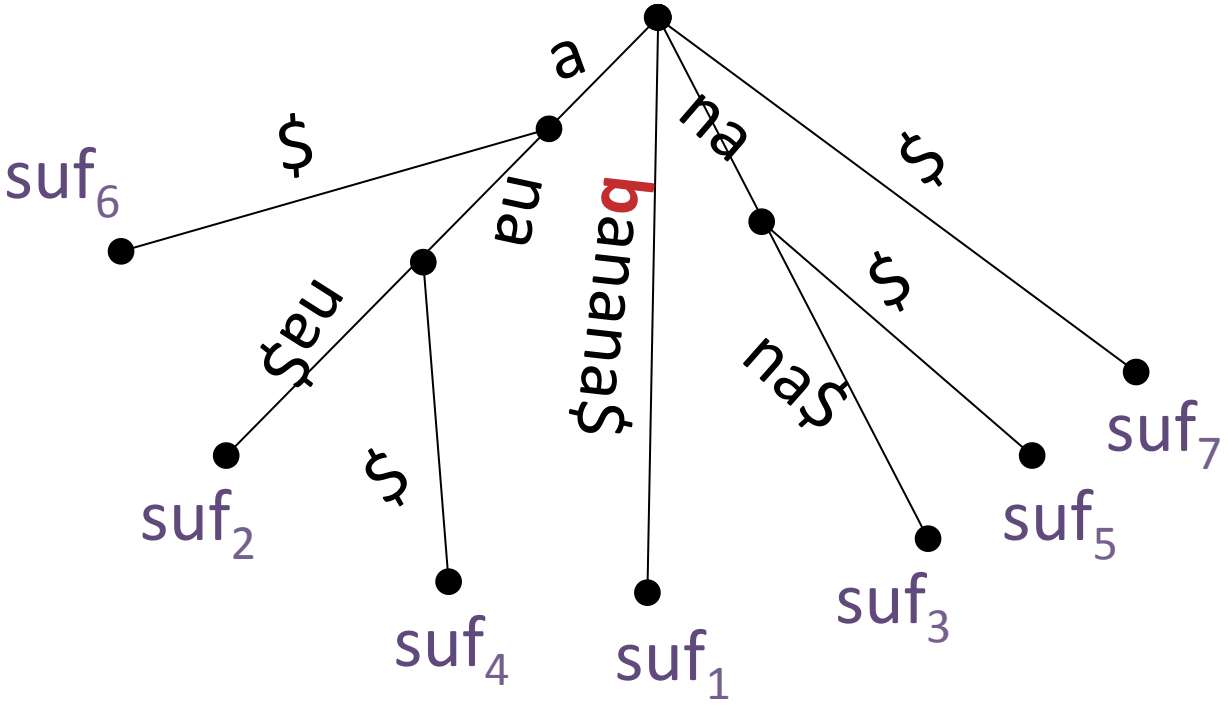

Suffix Tree Query

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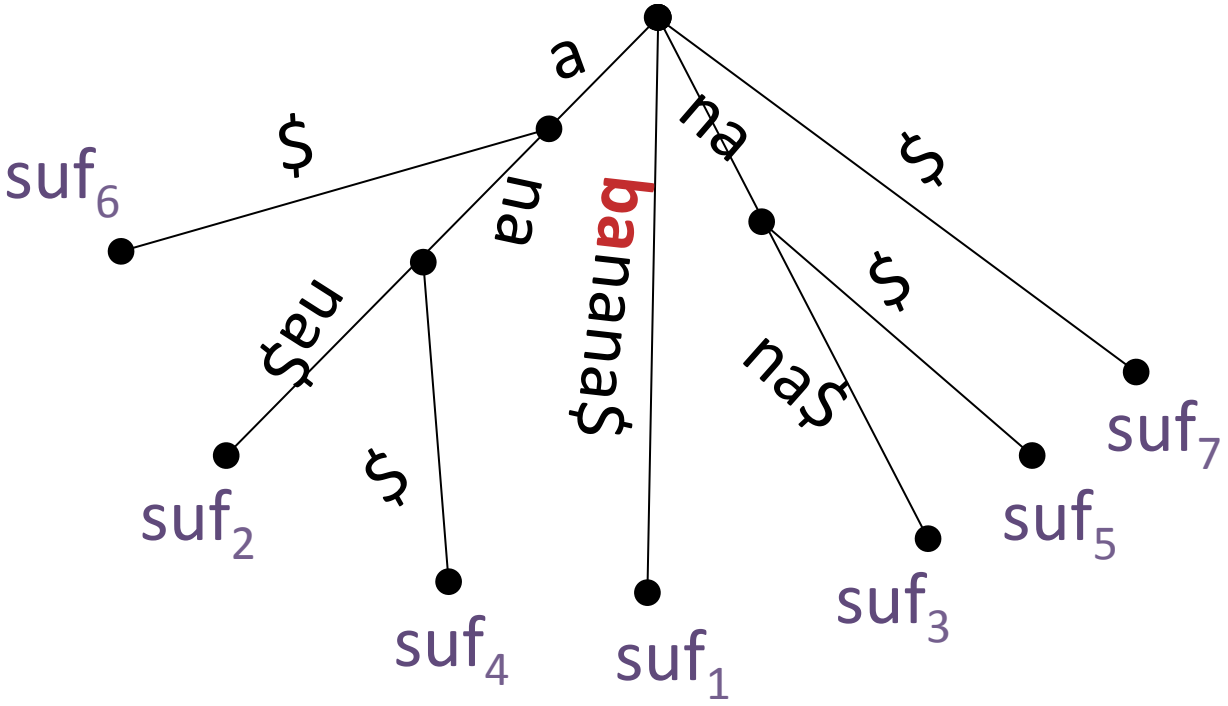
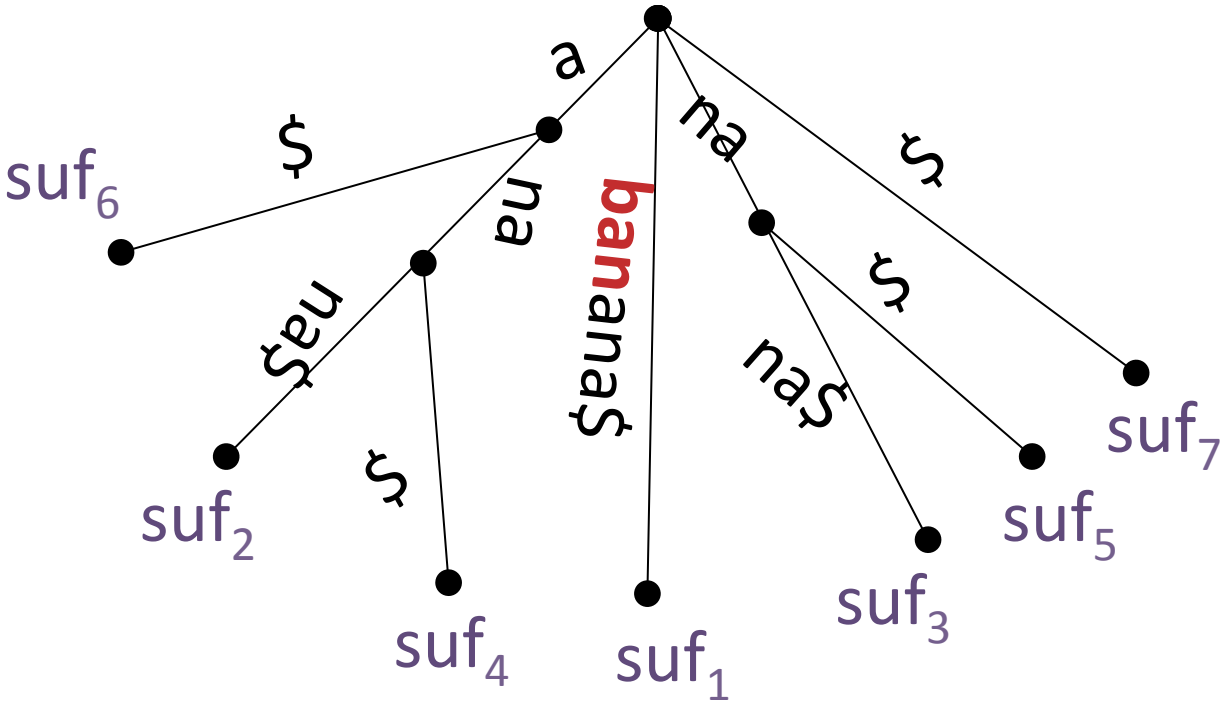
Search for **ban**



Suffix Tree Query

S = banana$

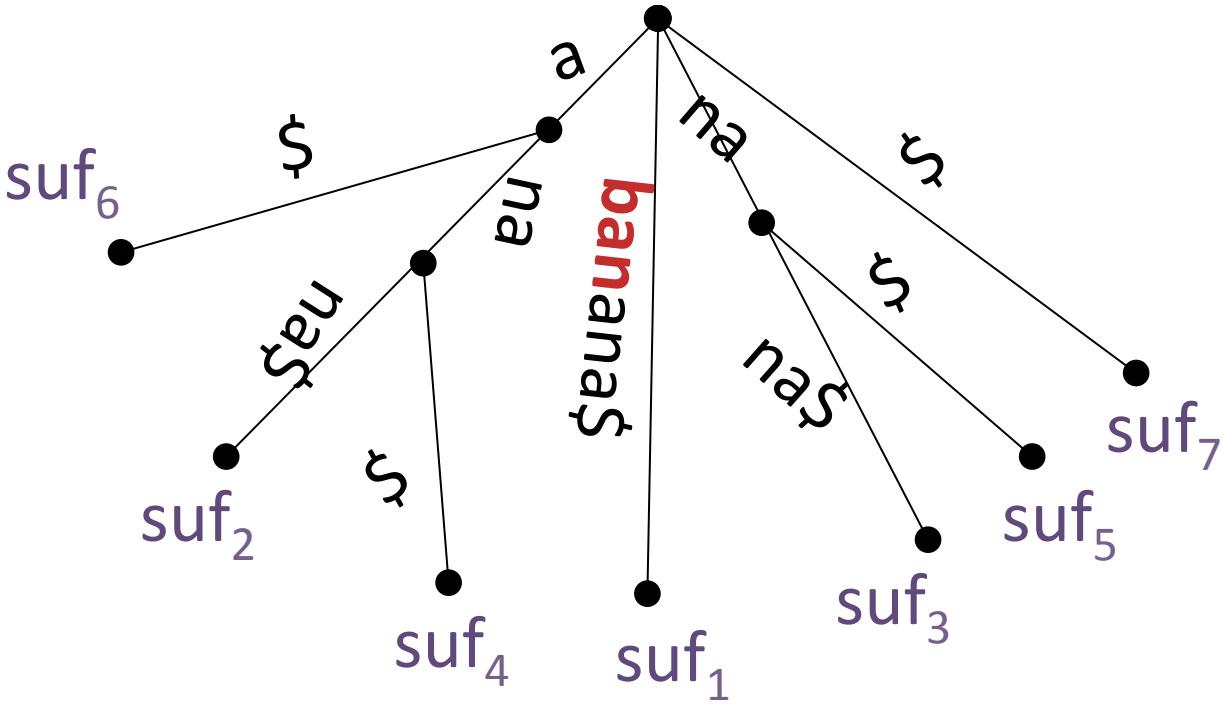


Search for **ban**

Found 1 occurrence

Suffix Tree Query

S = banana$

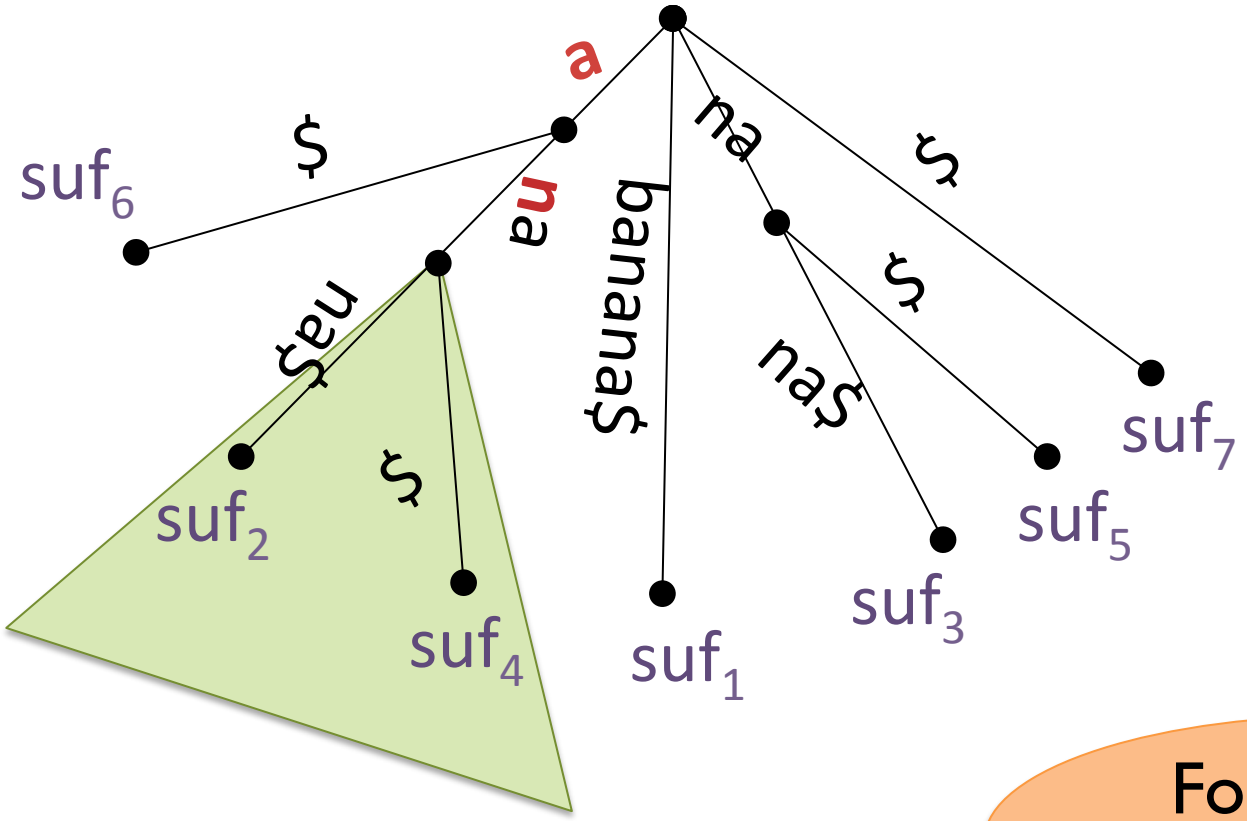


Search for **band**

Not found

Suffix Tree Query

S = banana$



Search for **an**

Found 2 occurrences

Suffix Tree

✧Many applications in computational biology

✧Linear time construction algorithms

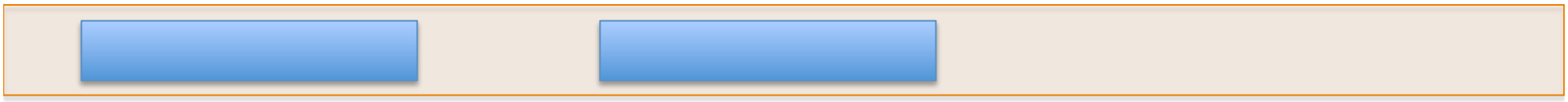
Linear time solutions to

- Genome alignment
- Finding longest common substring
- All-pairs suffix-prefix matching
- **Locating all maximal repetitions**
- And many more…

MEMs

Maximal Exact Match (MEM)

Exact match within sequence that cannot be extended left or right without introducing mismatch.



T G C A C G C A A

We are interested in MEMs length ≥ k

MEMs

Maximal Exact Match (MEM)

Exact match within sequence that cannot be extended left or right without introducing mismatch.

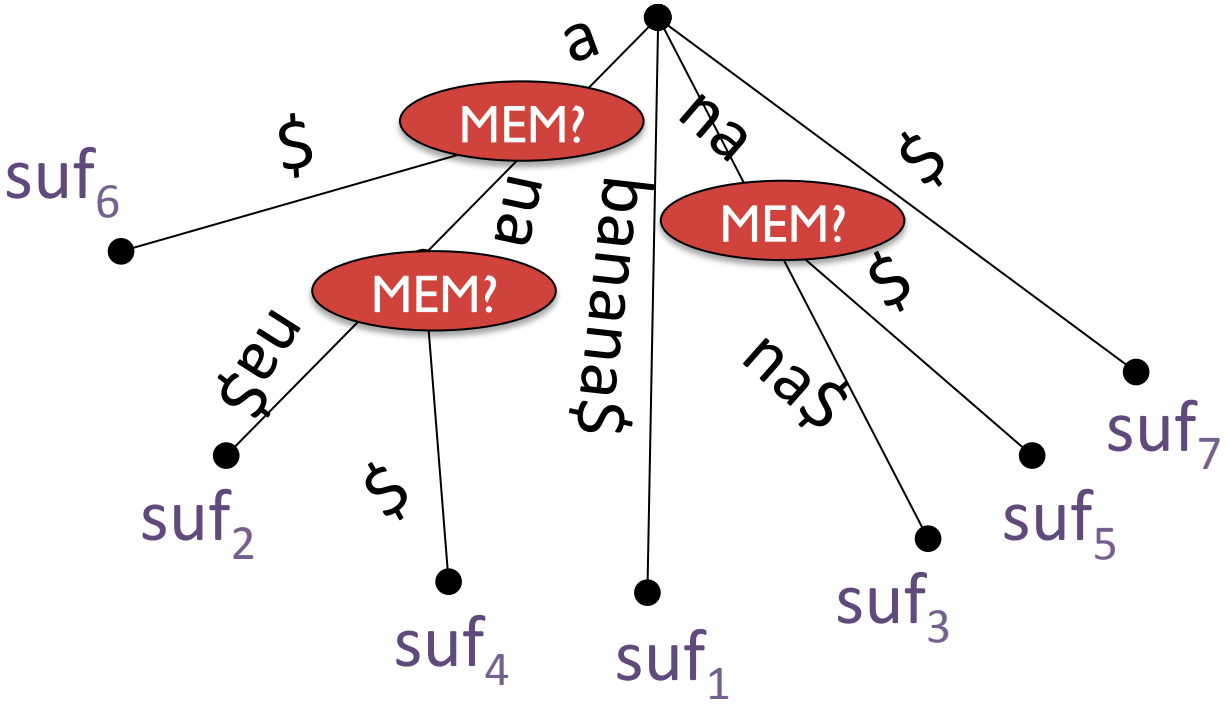
MEMs are internal nodes in the suffix tree that have left-diverse descendants.

(have descendant leaves that represent suffixes with different characters preceding them)

✧Linear-time suffix tree traversal to locate MEMs.

MEMs in Suffix Tree

Possible MEMs: a, ana, na



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anana$	suf_2
nana$	suf_3
ana$	suf_4
na$	suf_5
a$	suf_6
$	suf_7

MEMs are internal nodes in suffix tree with left-diverse descendants

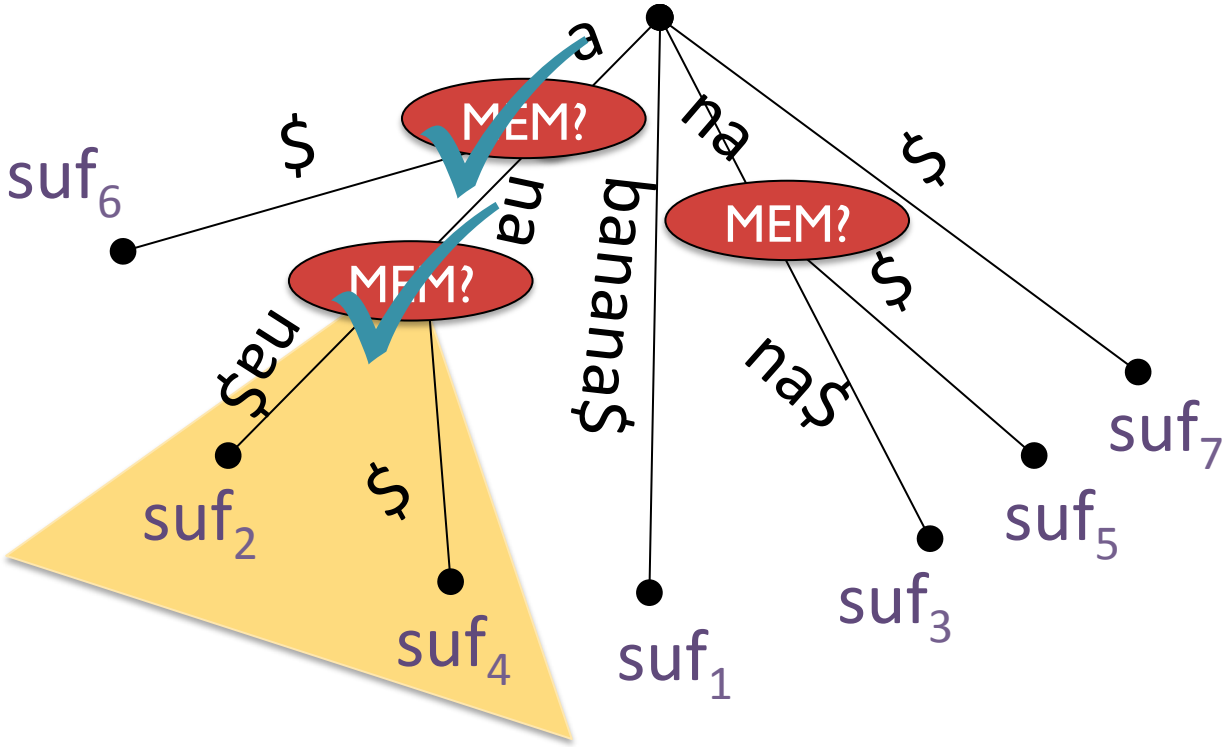
MEMs in Suffix Tree

MEMs: a, ana

S = banana$

banana$ suf_2

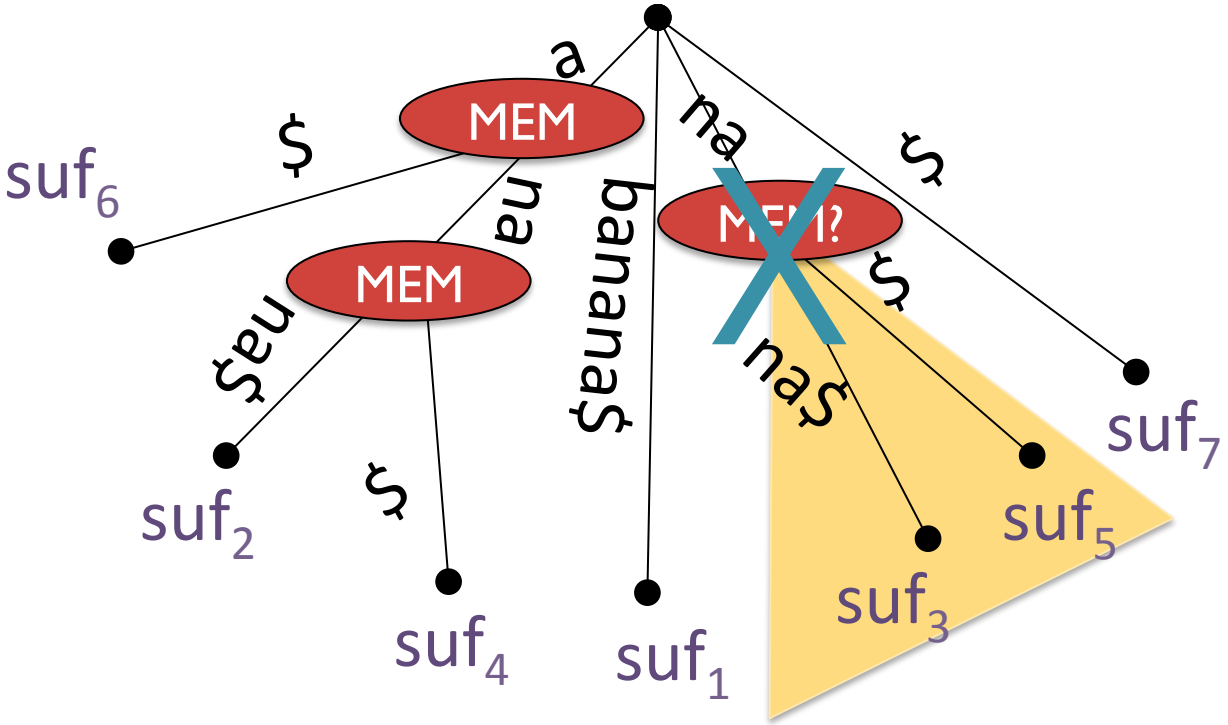
nana$ suf_4



MEMs are internal nodes in suffix tree with left-diverse descendants

MEMs in Suffix Tree

MEMs: a, ana



S = banana$

anana$ suf_3

ana$ suf_5

MEMs are internal nodes in suffix tree with left-diverse descendants

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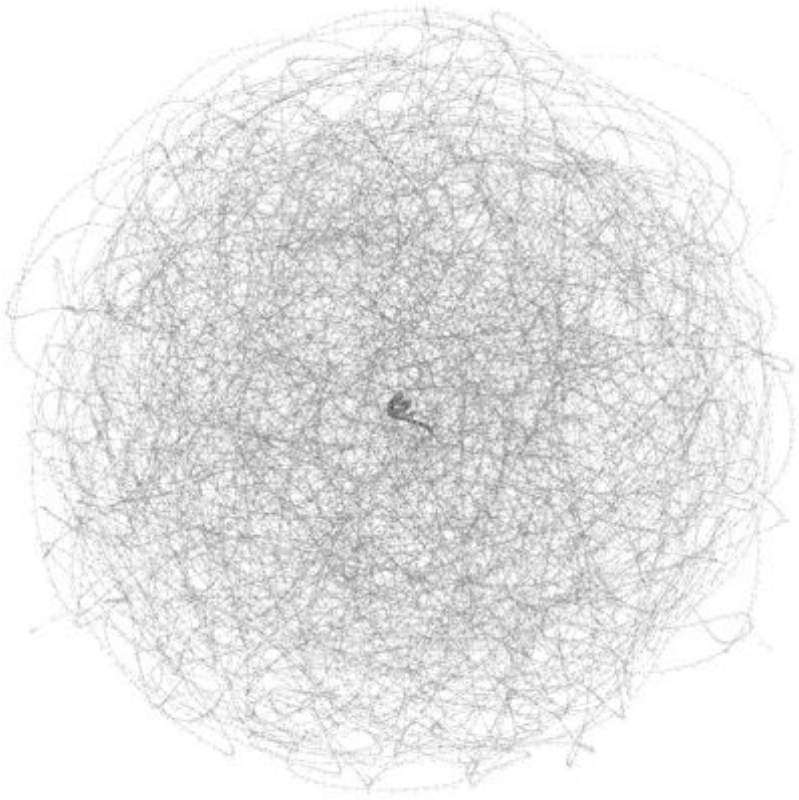


Compresssed de Bruijn graph

Types of nodes:

i. repeatNodes

ii. uniqueNodes

Input:

AGAAGTCC$ATAAGTTA

splitMEM

Nodes in compressed de Bruijn graph classified as

i. repeatNodes
ii. uniqueNodes

Algorithm:

1 Construct set of repeatNodes
2 Sort start positions of repeatNodes
3 Create edges and uniqueNodes to link non-contiguous repeatNodes

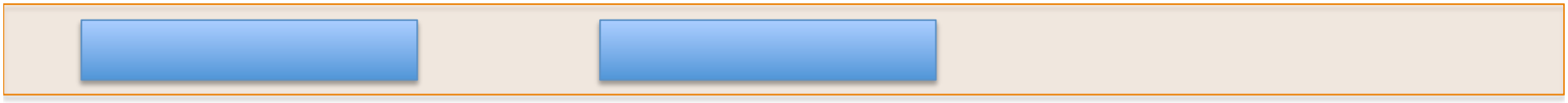
repeatNodes

1 Construct set of repeatNodes

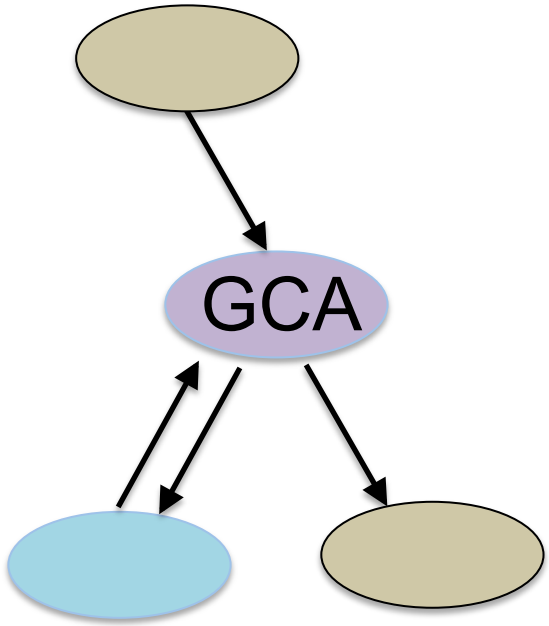
1. Build suffix tree of genome
2. Mark internal nodes that are MEMs, length ≥ k
3. Preprocess suffix tree for LMA queries

4. Compute repeatNodes in compressed de Bruijn graph by decomposing MEMs and extracting overlapping components, length ≥ k

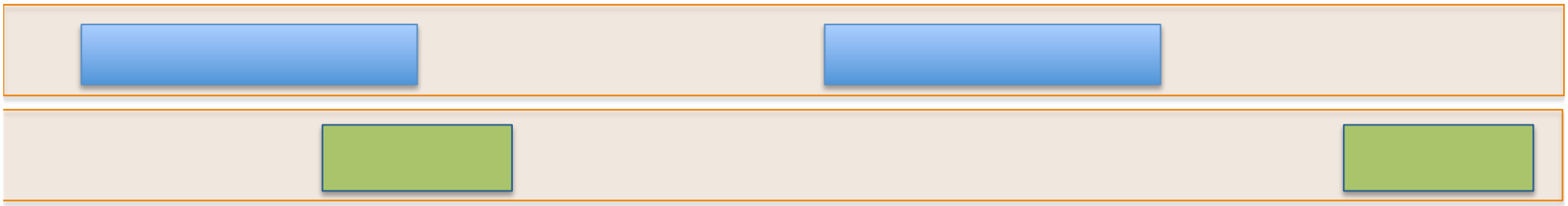
1 MEM occurs twice



TGCAC…GGCAA

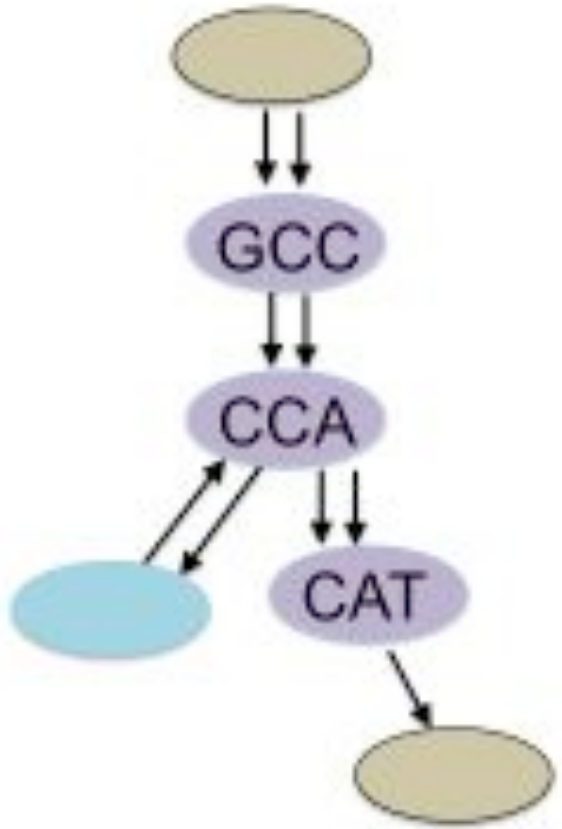


Overlapping MEMs

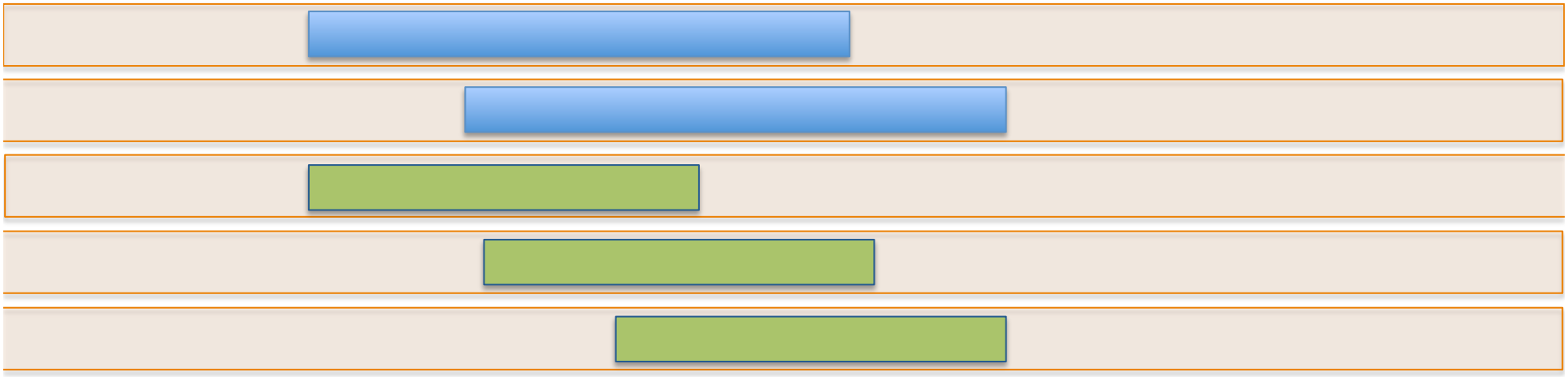


T G C C A T C G C C A A C C A T

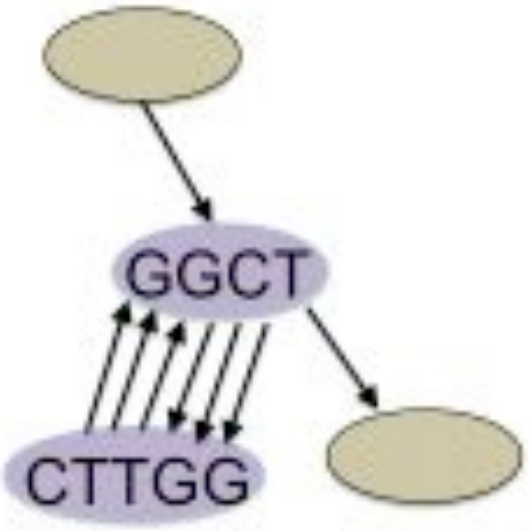
T G C C A T C G C C A A C C A T



Tandem Repeat



AGGCTTGGCTTGGCTTGGCTA
AGGCTTGGCTTGGCTTGGCTA
AGGCTTGGCTTGGCTTGGCTA
AGGCTTGGCTTGGCTTGGCTA
AGGCTTGGCTTGGCTTGGCTA



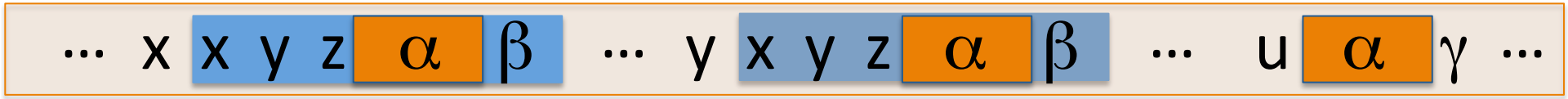
repeatNodes

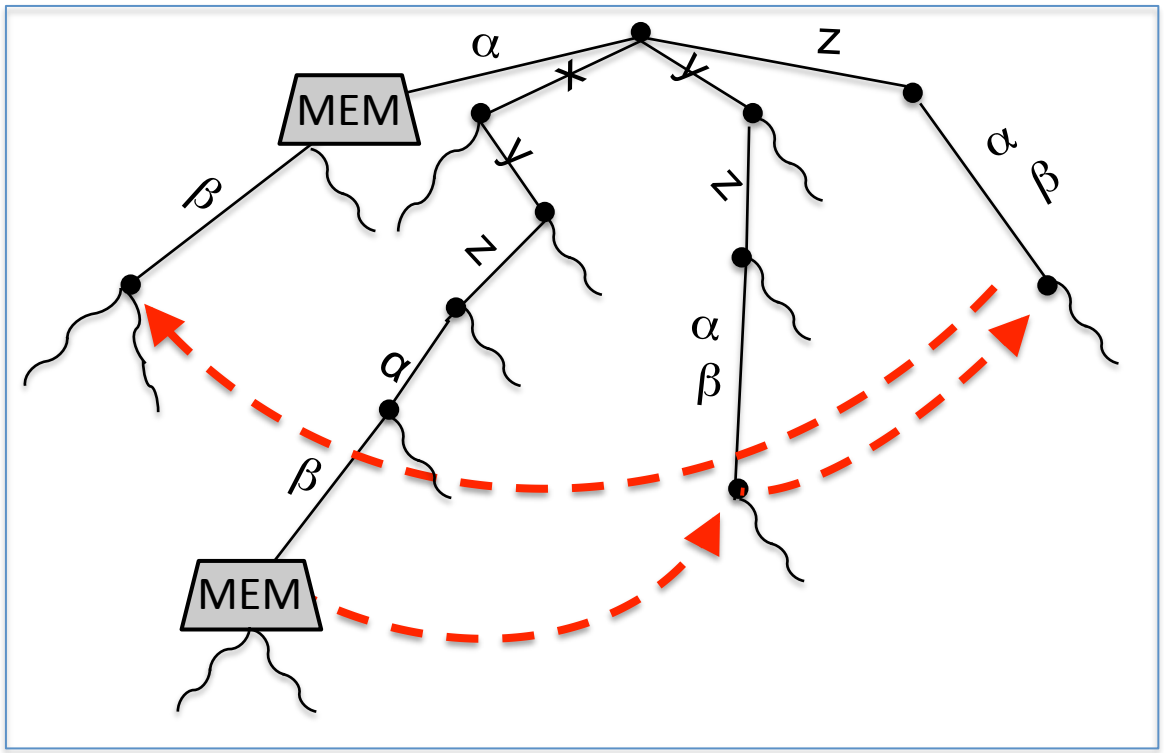
1 Construct set of repeatNodes

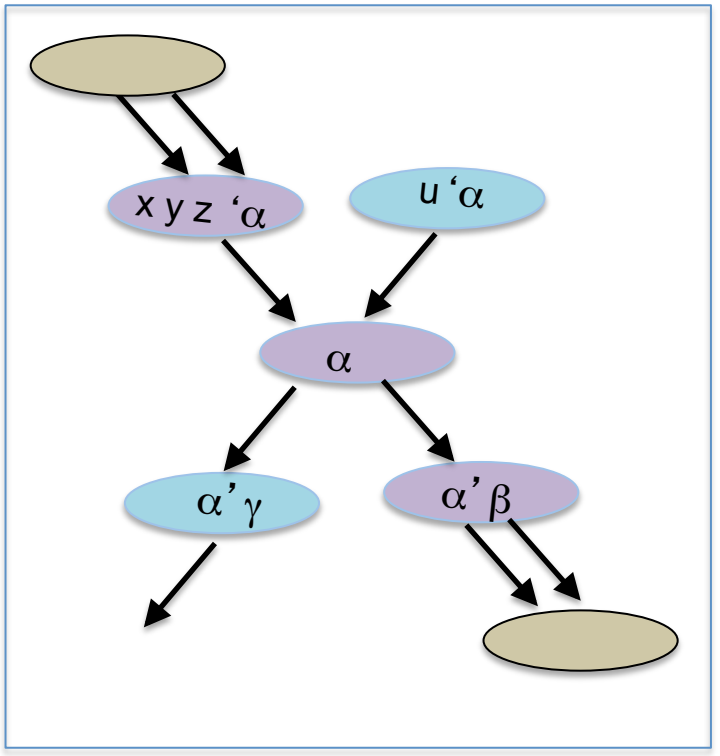
1. Build suffix tree of genome
2. Mark internal nodes that are MEMs, length ≥ k
3. Preprocess suffix tree for LMA queries

4. Compute repeatNodes in compressed de Bruijn graph by decomposing MEMs and extracting overlapping components, length ≥ k

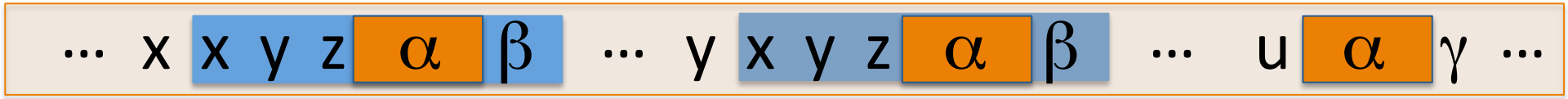
Split MEM to repeatNodes

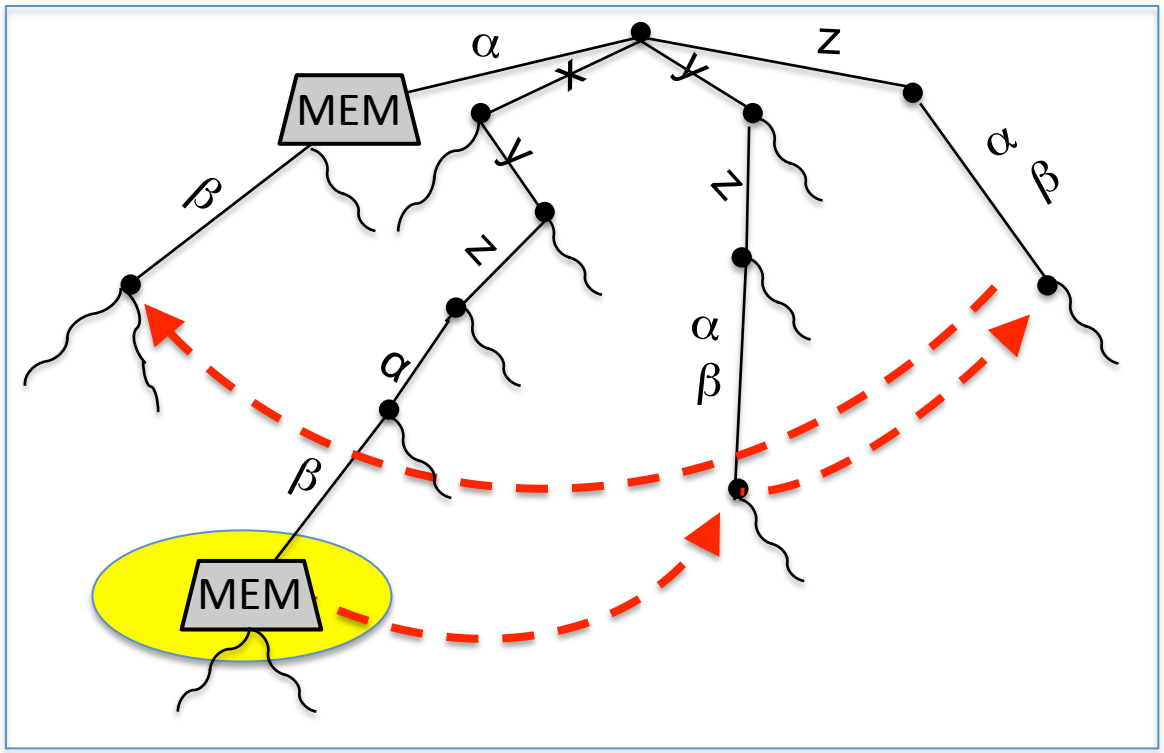


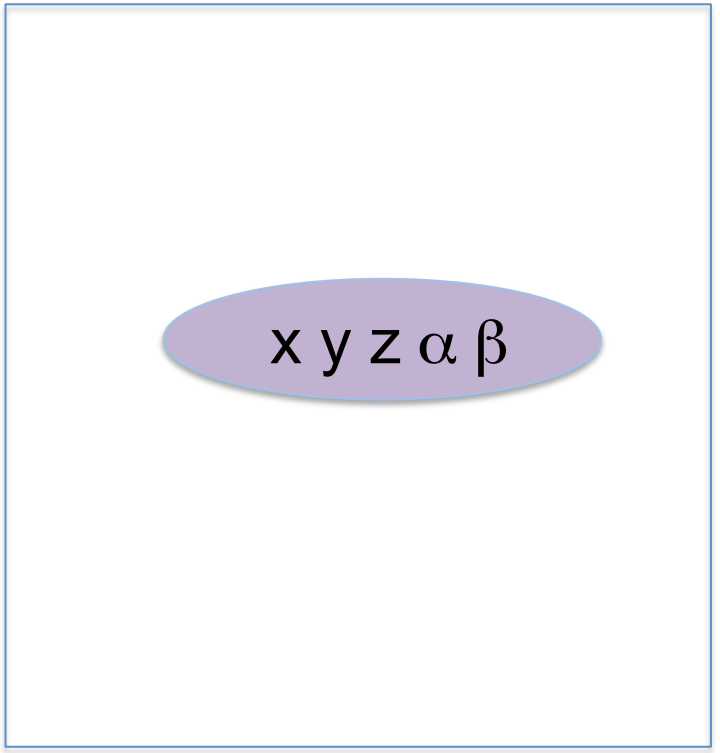




Split MEM to repeatNodes

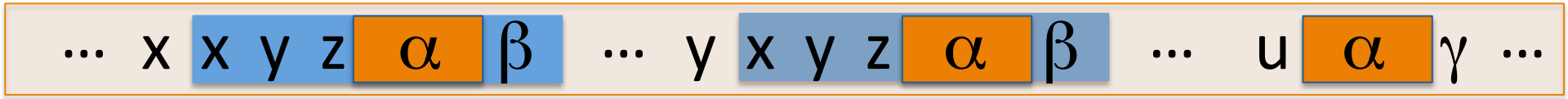


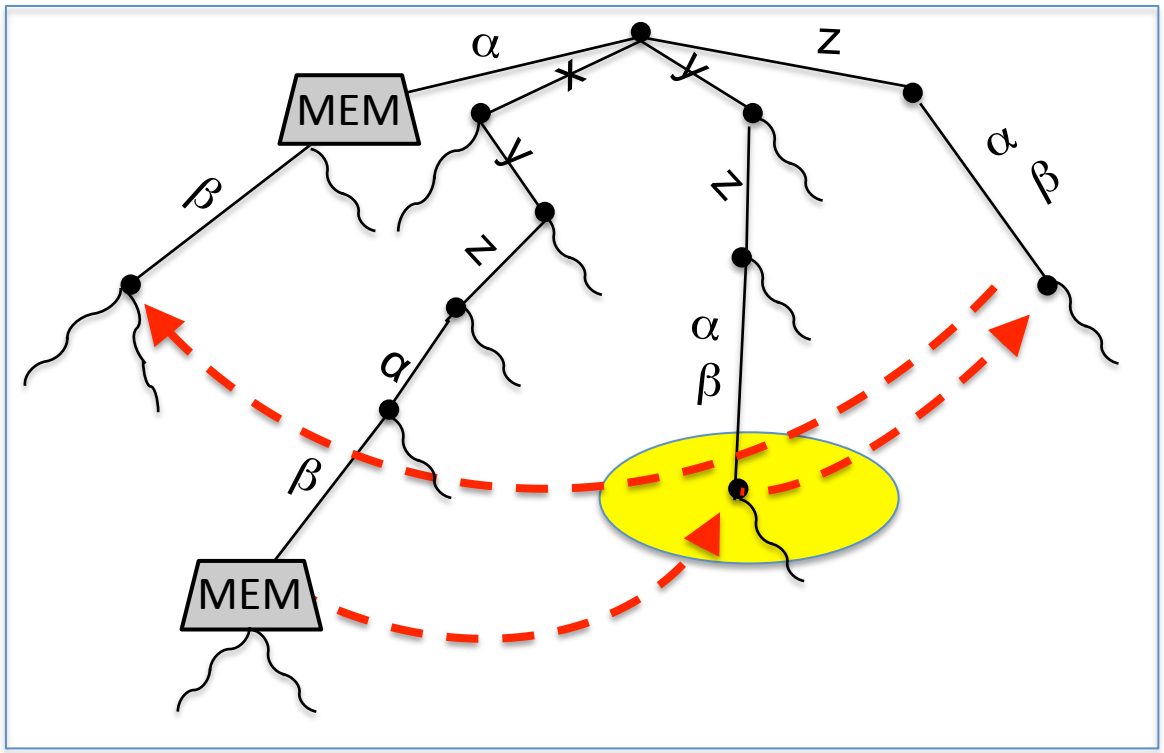


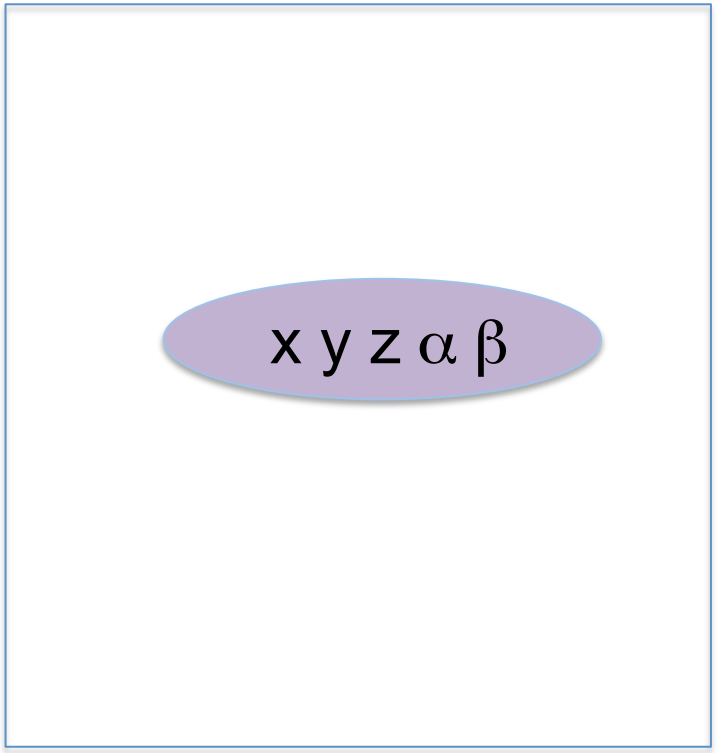


Find MEM in suffix tree.

Split MEM to repeatNodes



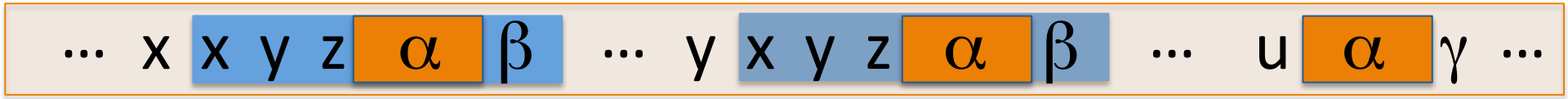


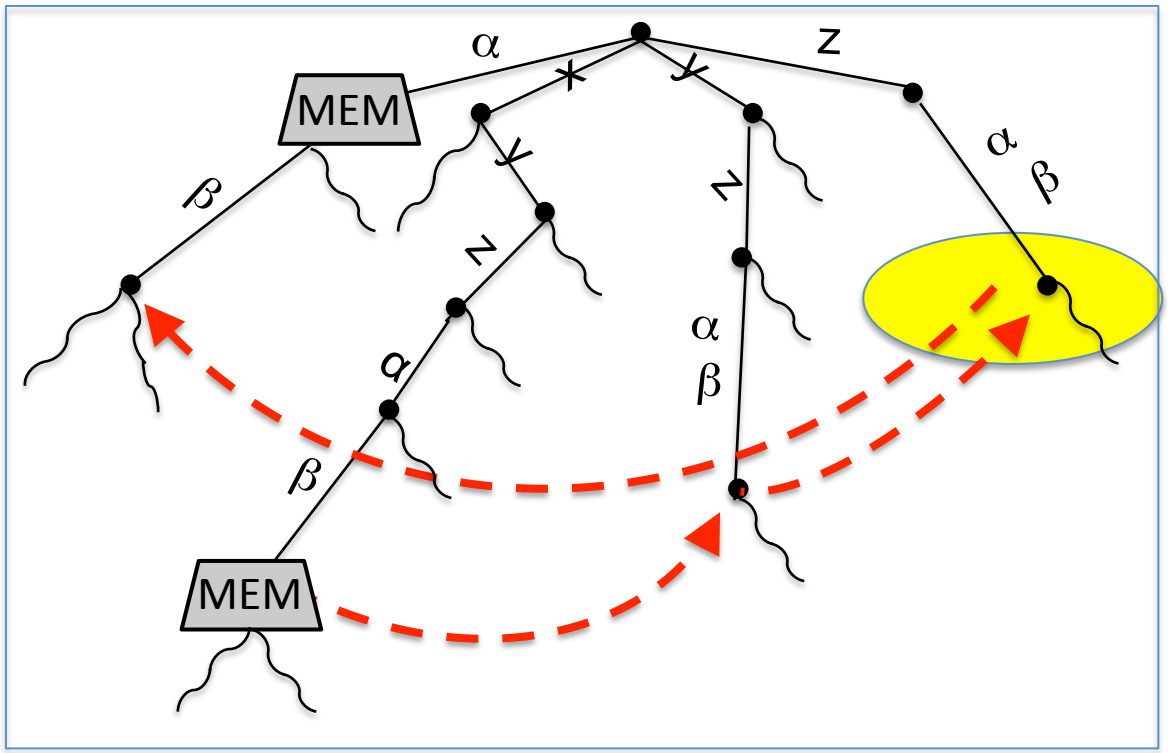


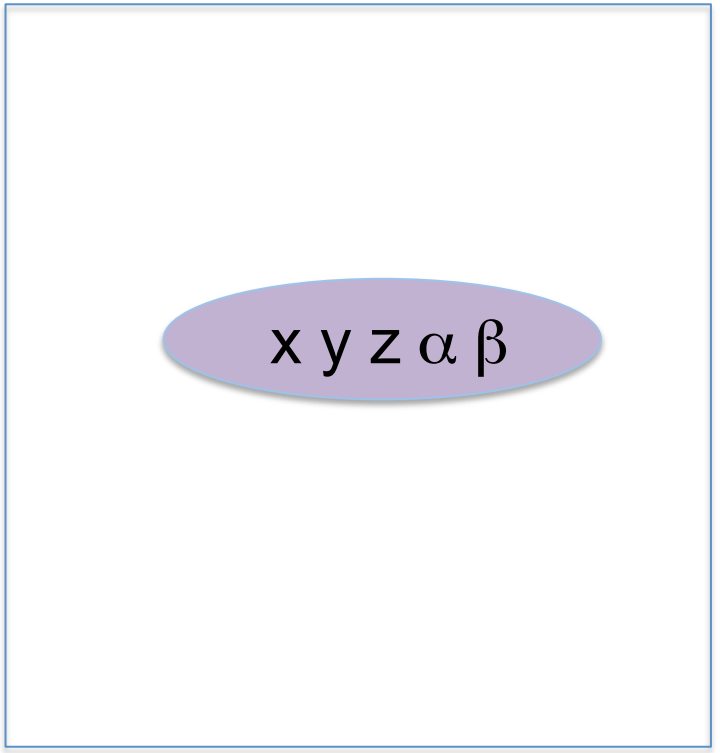
Traverse suffix link.

Look for MEM as ancestor.

Split MEM to repeatNodes







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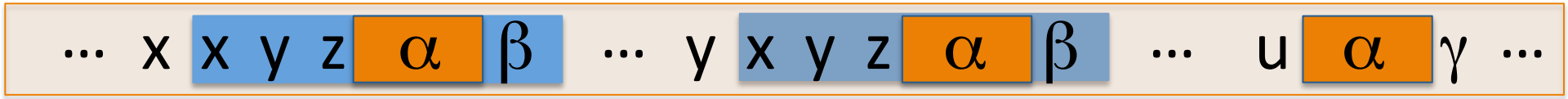

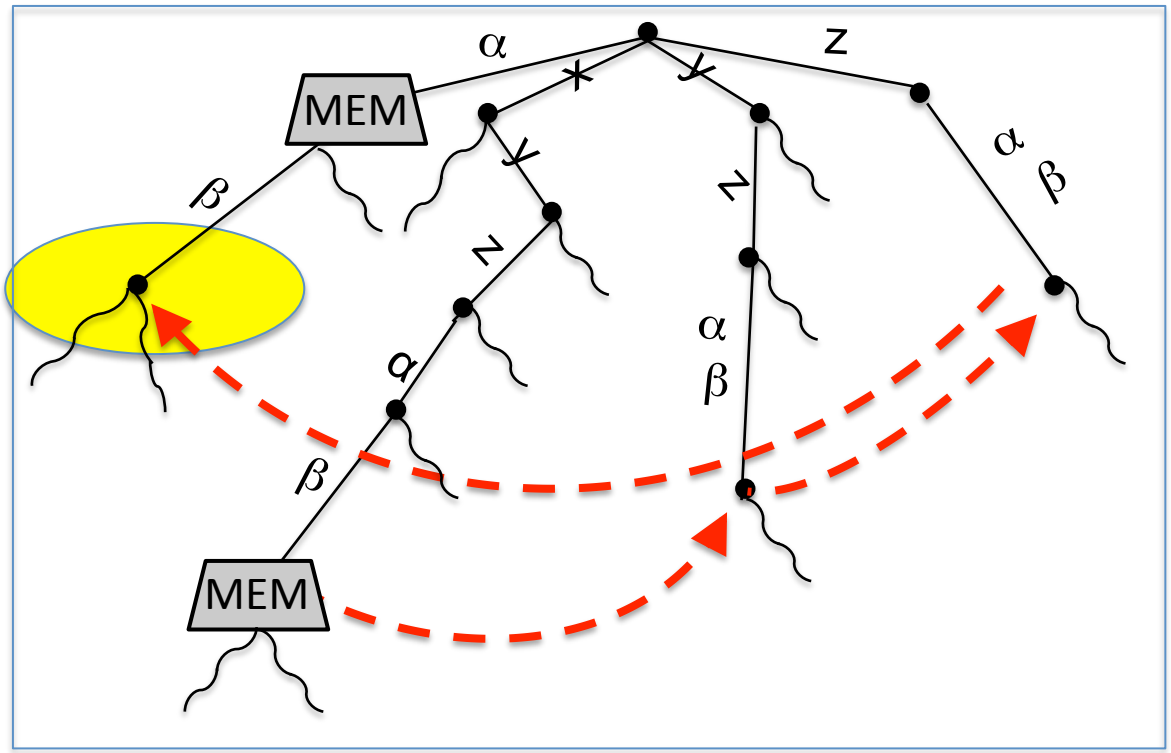

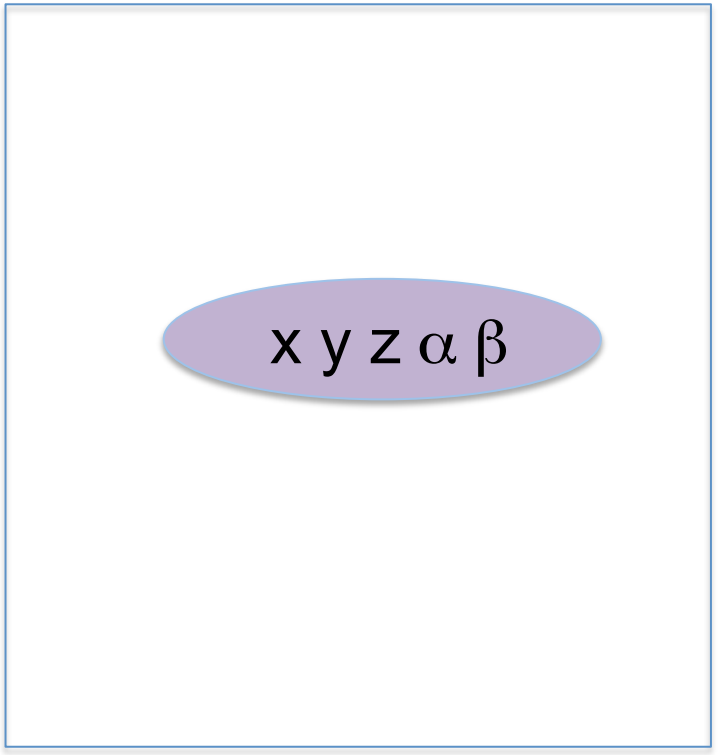

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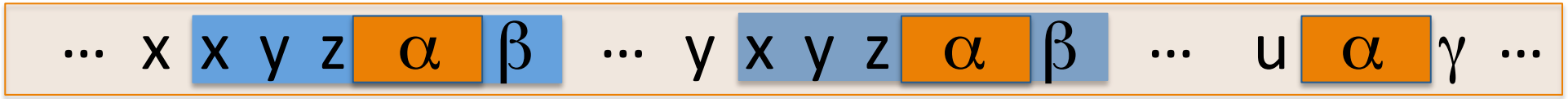
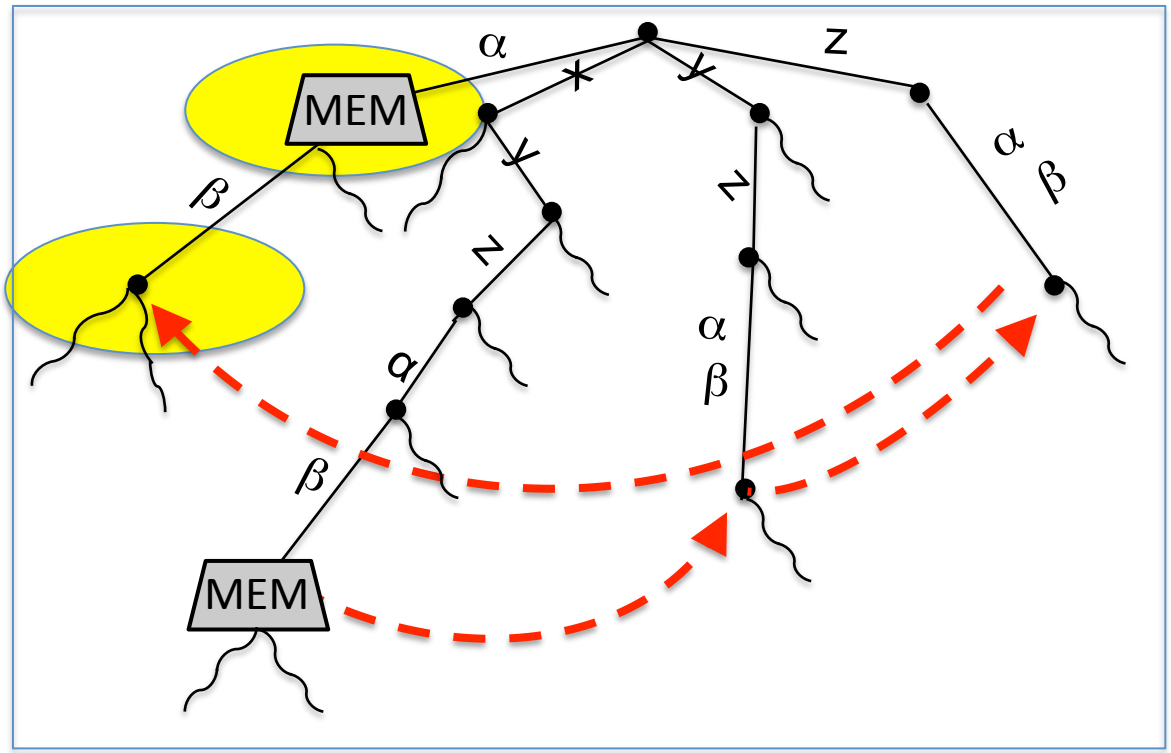
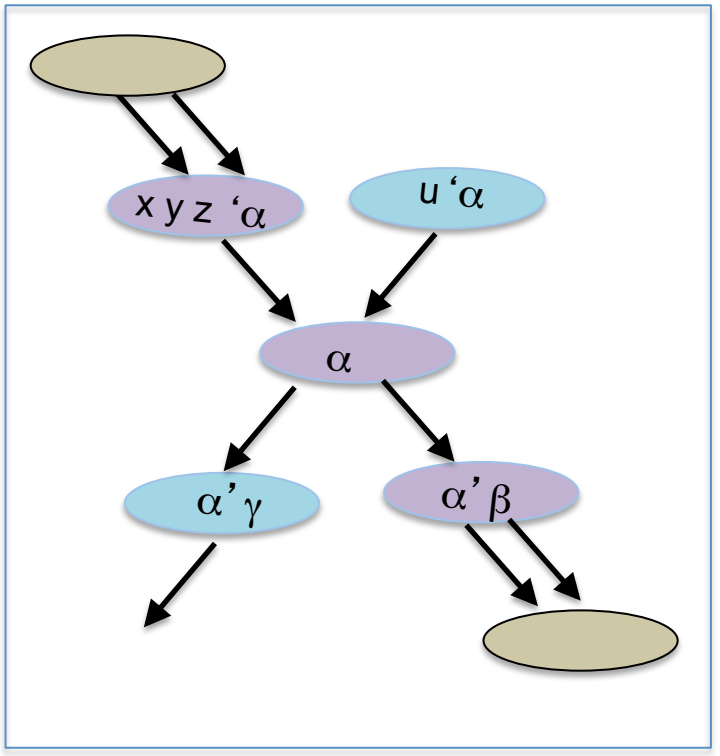






Found MEM as ancestor. Decompose.

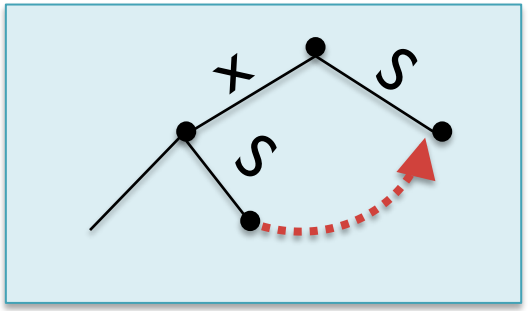
Remove embedded MEM (suffix links). Find next embedded MEM.

Suffix Skips

✧ Reduce $O(n^2)$ time to $O(n \log n)$ time

Suffix link: quickly navigate to distant part of tree

- Pointer from internal node labeled *xS* to node *S*
- Trim 1 character in $O(1)$ time
- Trim c characters in $O(c)$ time

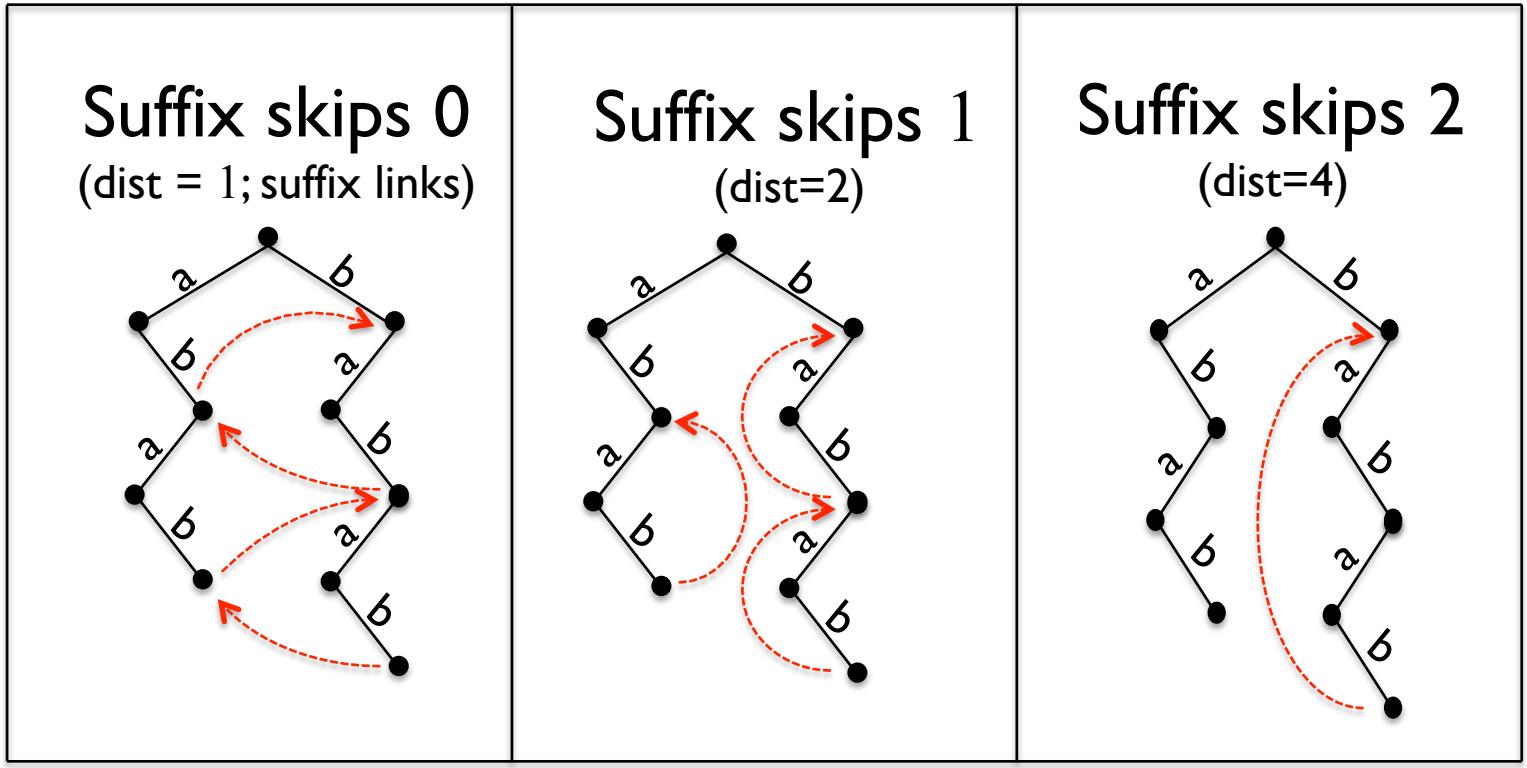


Suffix skip:

- Trim c characters in $O(\log c)$ time

Suffix Skips

Genome: babab



Additional Preprocessing:

pointer jumping to rapidly add additional links

splitMEM

- splitMEM software
  - C++
  - open source http://splitmem.sourceforge.net
- Input modes:
  - single genome: fasta file
  - pan-genome: multi-fasta file
- Multi *k*-mer

  construct several compressed de Bruijn graphs without rebuilding suffix tree

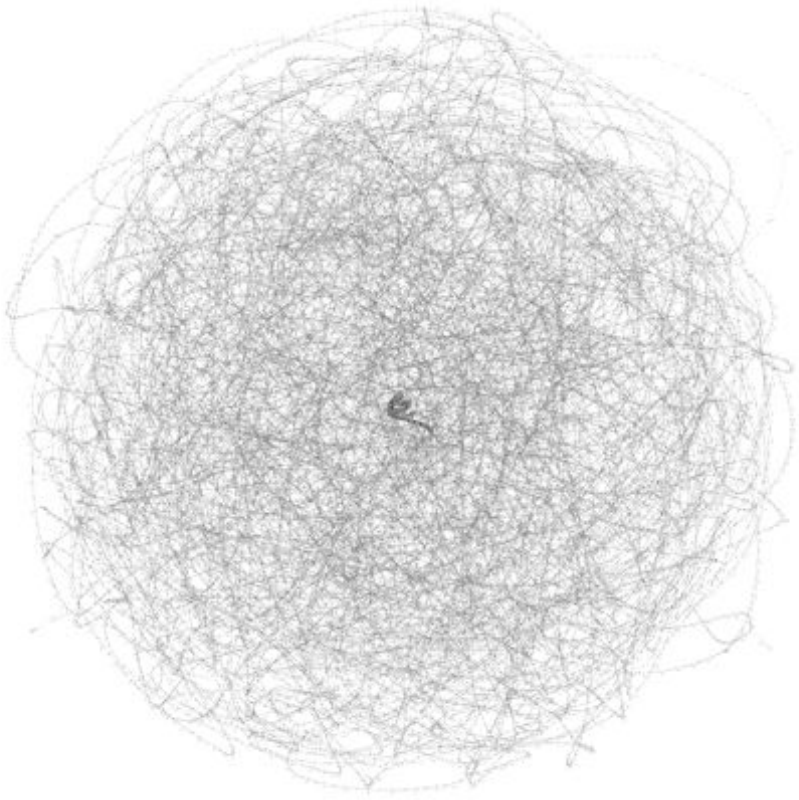
Outline

1 Overview

2 Data Structures

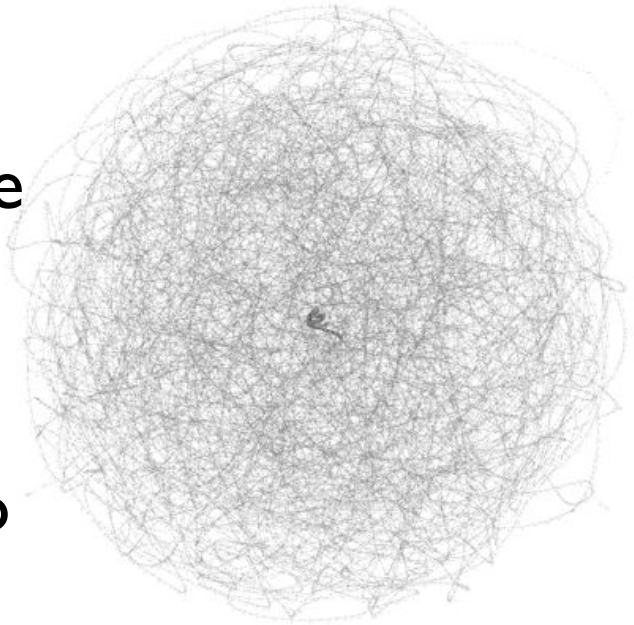
3 splitMEM Algorithm

4 Pan-genome Analysis



Pan-genome analysis

B. Anthracis and *E. coli*



Examine graph properties:

- Number nodes, edges, avg. degree
- Node length distribution
- Genome sharing among nodes
- Distribution of node distances to core genome

Other properties that can be studied:

- Girth, Diameter, Modularity, Network Motifs, etc.
- Functional enrichment of highly conserved or genome specific genes.

Pan-genome analysis

Strain	Size	Accession
B. anthracis A0248 uid33543	5178 KB	CP001598
B. anthracis A16R uid40353	5179 KB	CP001974
B. anthracis A16 uid40303	5179 KB	CP001970
B. anthracis Ames 0581 uid10784	5178 KB	AE017334
B. anthracis Ames uid309	5178 KB	AE016879
B. anthracis CDC 684 uid31329	5181 KB	CP001215
B. anthracis CI uid36309	5147 KB	CP001746
B. anthracis H9401 uid49361	5170 KB	CP000091
B. anthracis str Sterne uid10878	5180 KB	AE017225
E. coli 0127 H6 E2348 69 uid32571	4919 KB	FM180568
E. coli 042 uid40647	5193 KB	FN554766
E. coli 536 uid16235	4893 KB	CP000247
E. coli 55989 uid33413	5107 KB	CU928145
E. coli ABU 83972 uid38725	5083 KB	CP001671
E. coli APEC O1 uid16718	5034 KB	CP000468
E. coli APEC O78 uid184588	4753 KB	CP004009
E. coli BL21 DE3 uid20713	4516 KB	CP001509
E. coli BL21 DE3 uid28965	4516 KB	AM946981

Pan-genome analysis

Graphs of main chromosomes

- 9 strains of *Bacillus anthracis*
- Selection of 9 strains of *Escherichia coli*

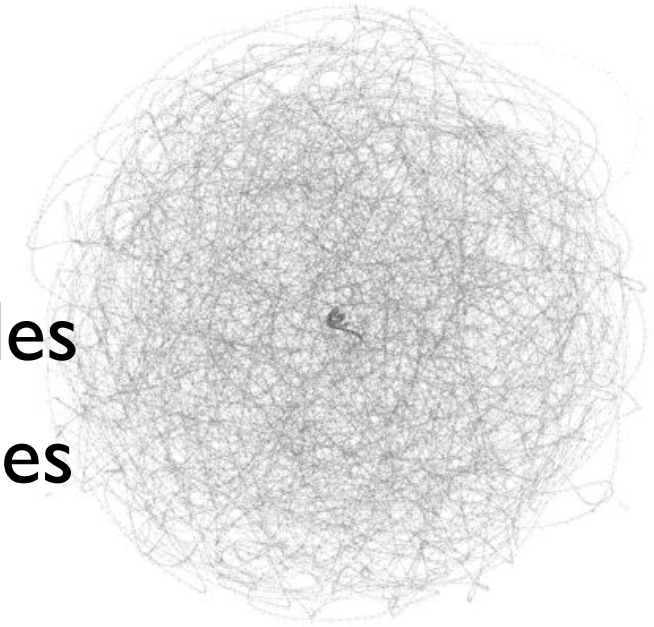
Species	K	Nodes	Edges	Avg. Degree
B. anthracis	25	103926	138468	1.33
B. anthracis	100	41343	54954	1.32
B. anthracis	1000	6627	8659	1.30
E. coli	25	494783	662081	1.33
E. coli	100	230996	308256	1.33
E. coli	1000	11900	15695	1.31

Pan-genome analysis

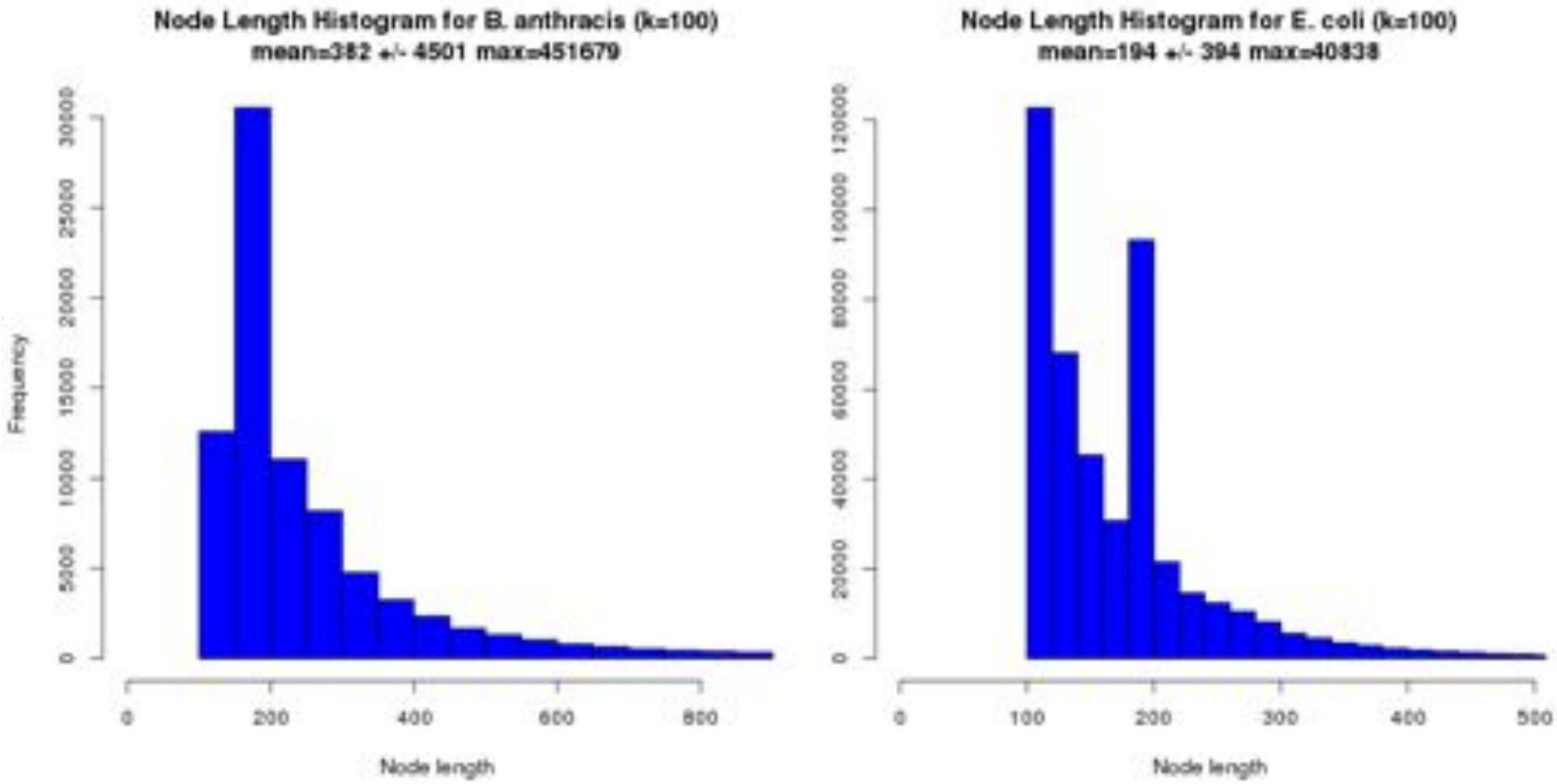
B. Anthracis and *E. coli*

Examine graph properties

- Node length distribution
- Genome sharing among nodes
- Distribution of node distances to core genome

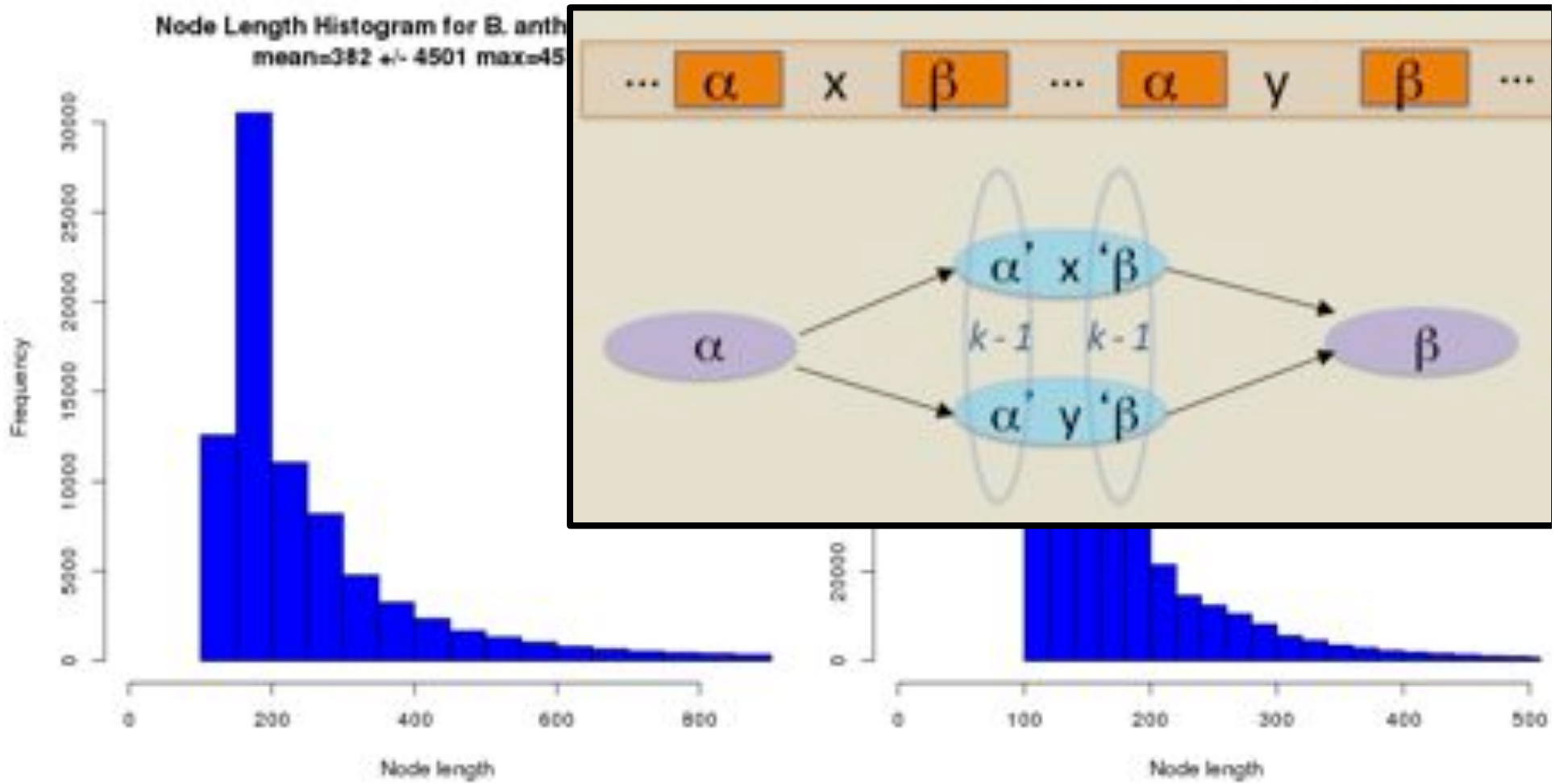


Pan-genome analysis



Histogram of Node Lengths

Pan-genome analysis



Histogram of Node Lengths

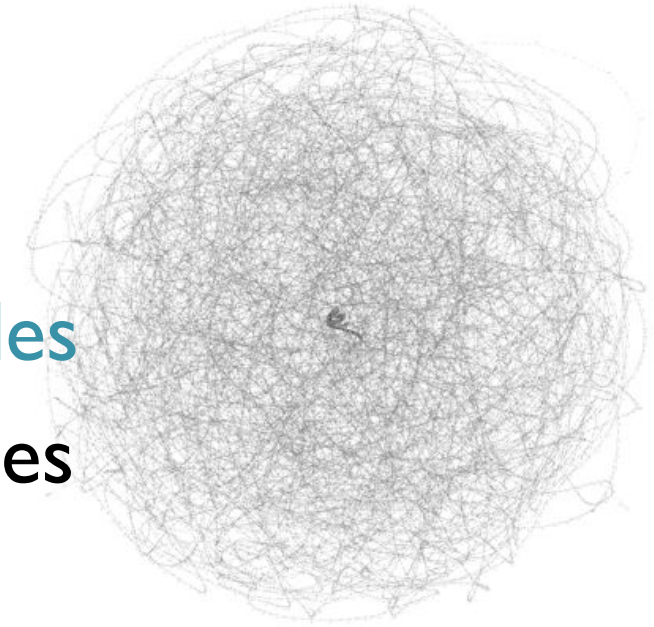
Spike at 2k: SNPs

Pan-genome analysis

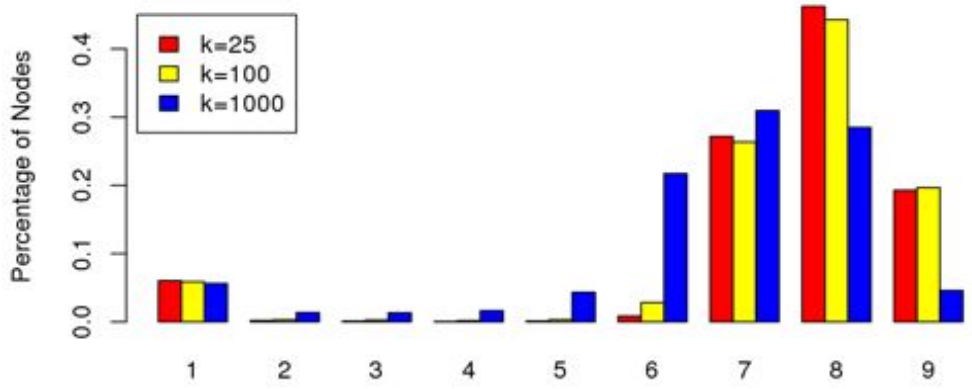
B. Anthracis and *E. coli*

Examine graph properties

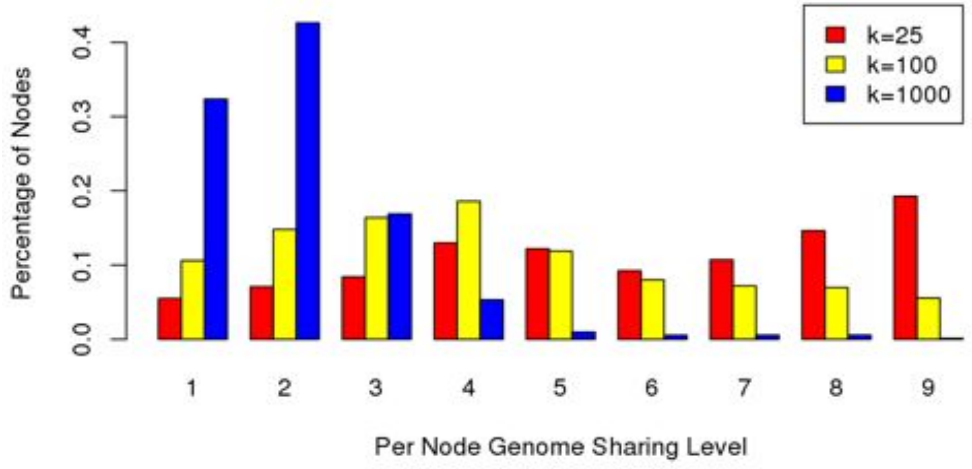
- Node length distribution
- Genome sharing among nodes
- Distribution of node distances to core genome



Pan-genome analysis



B. anthracis



E. coli

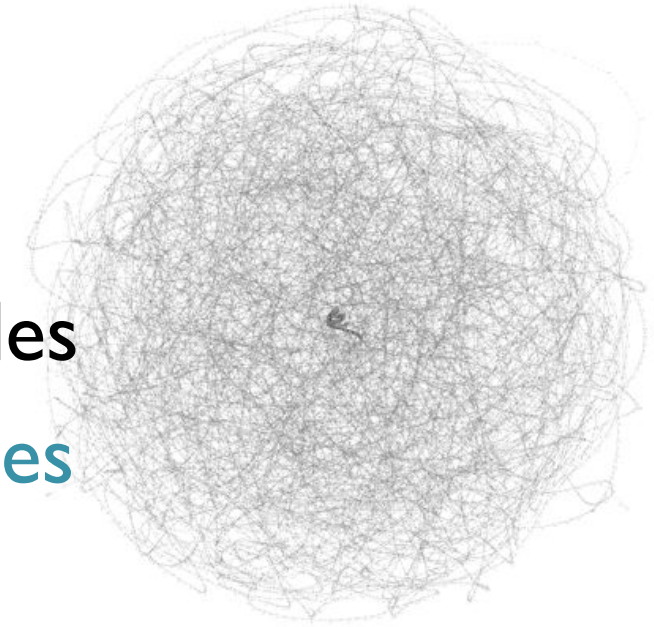
Fraction of nodes with each level of genome sharing

Pan-genome analysis

B. Anthracis and *E. coli*

Examine graph properties

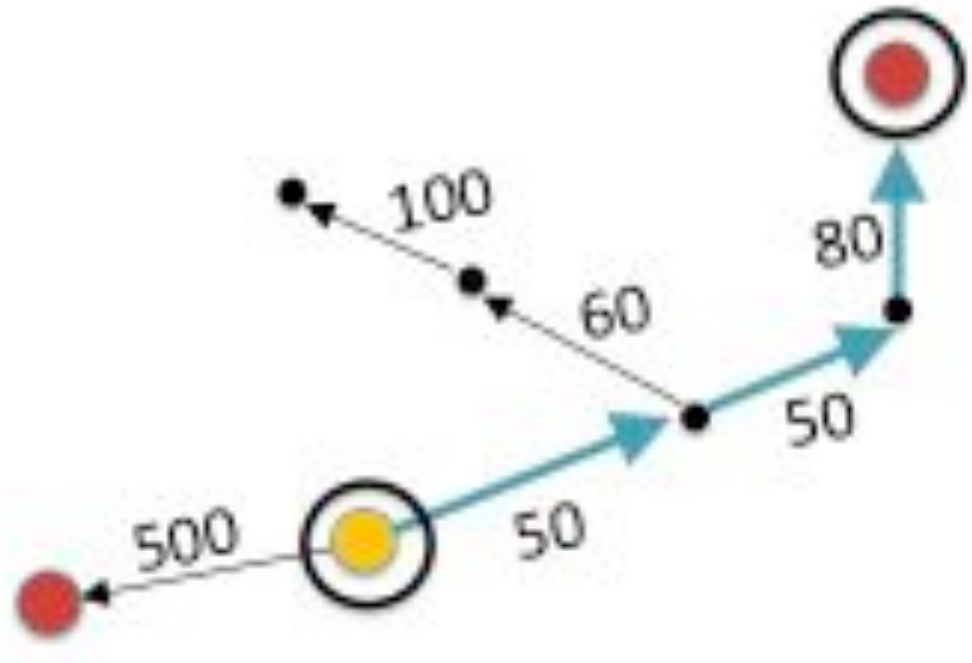
- Node length distribution
- Genome sharing among nodes
- Distribution of node distances to core genome



Pan-genome analysis

Graph encodes sequence context of segments.

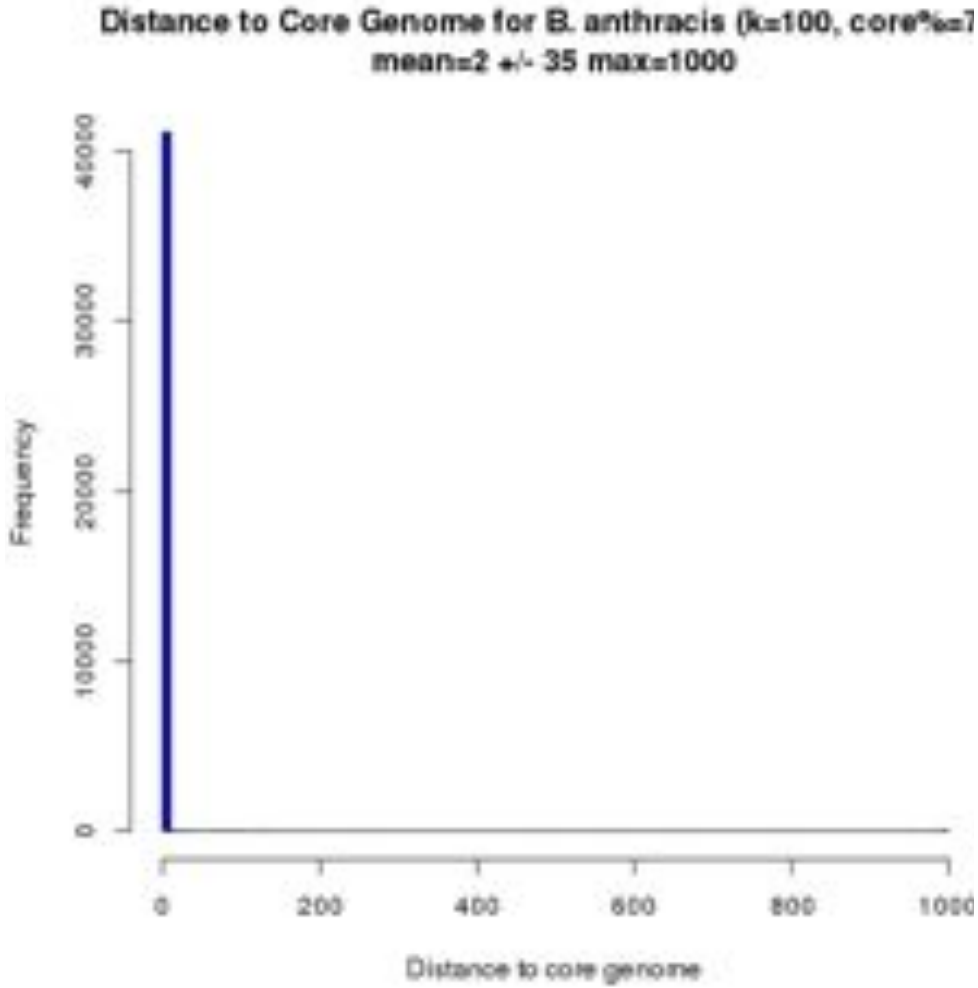
Core genome: subsequences that occur in at least 70% of underlying genomes.

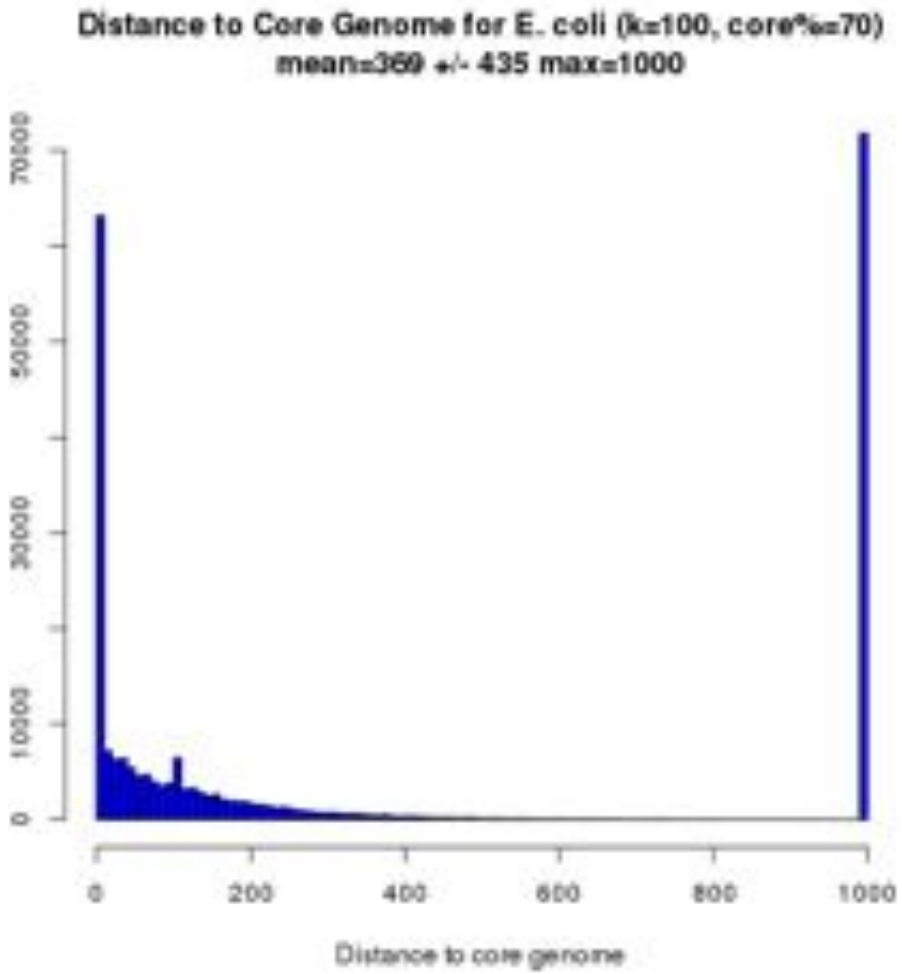


Branch and Bound Search

Nodes can be further in terms of hops while closer by base pairs.

Pan-genome analysis





Node distances to core genome

Searched 1000-hop radius

Summary

- Identify pan-genome relationships graphically.
- Topological relationship between suffix tree and compressed de Bruijn graph.
- Direct construction of compressed de Bruijn graph for single or pan-genome.
- Introduce suffix skips.
- Explore pan-genome graphs of *B. anthracis*, *E. coli*.

SplitMEM: Graphical pan-genome analysis with suffix skips.

Marcus, S, Lee, H, Schatz, MC (2014) *BioRxiv*

http://biorxiv.org/content/early/2014/04/06/003954

Future work

Improve splitMEM software:

- Reduce space using compressed full-text index instead of suffix tree
- Approximate indexing of strains to form a pan-genome graph
- Alignment of reads to pan-genome

Biological applications:

- Functional enrichment of core-genome and genome specific segments
- Expand study to larger collection of microbes and larger genomes

Acknowledgments

Michael Schatz
Hayan Lee
Giuseppe Narzisi
James Gurtowski
Schatz Lab

IT department
Todd Heywood

iPlant Collaborative™

Thank You!

Pan-genome analysis

Branch and bound search (like Dijkstra's shortest path algorithm) to compute bp distance from each non-core node to core genome:

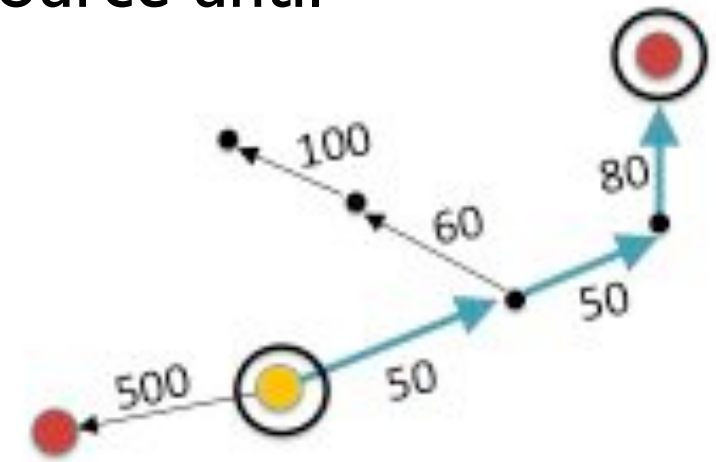
Traverse all distinct paths from source until

- a core node is reached

OR

- current node was visited

  by a shorter path



Bounded search

once a core node is found, its distance bounds maximum search distance along other paths