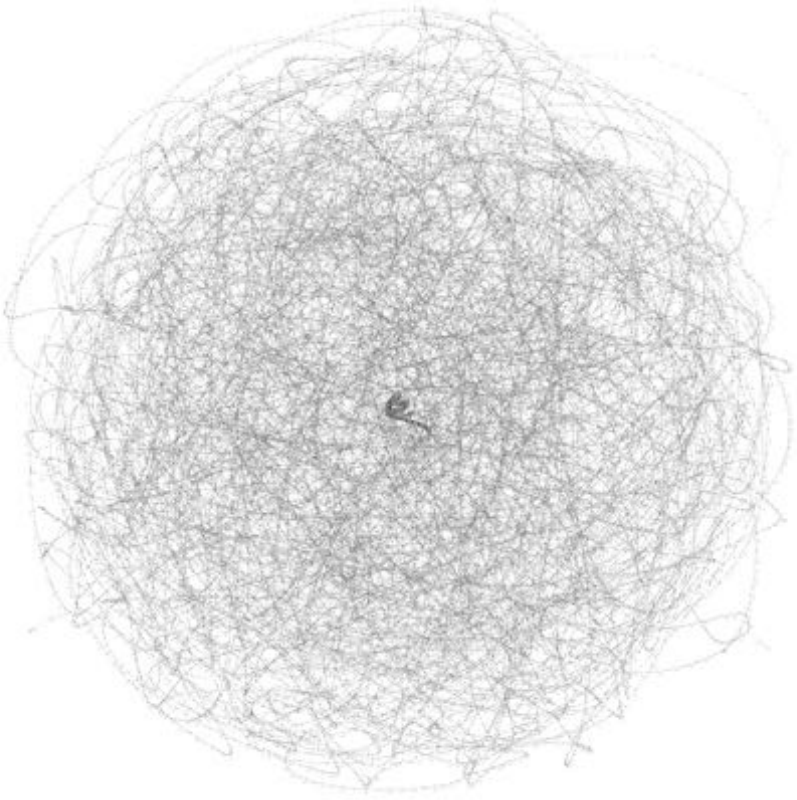
splitMEM: graphical pan-genome analysis with suffix skips

Shoshana Marcus

July 11, 2014

Outline



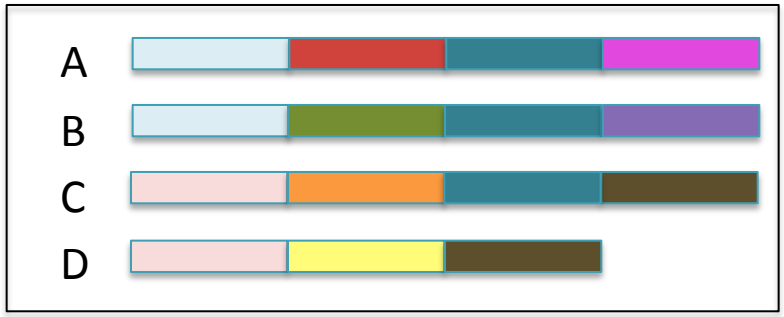
Motivation

GENOME 10K®

- ✧We are sequencing growing collections of genomes.
- ✧Should analyze sets together.

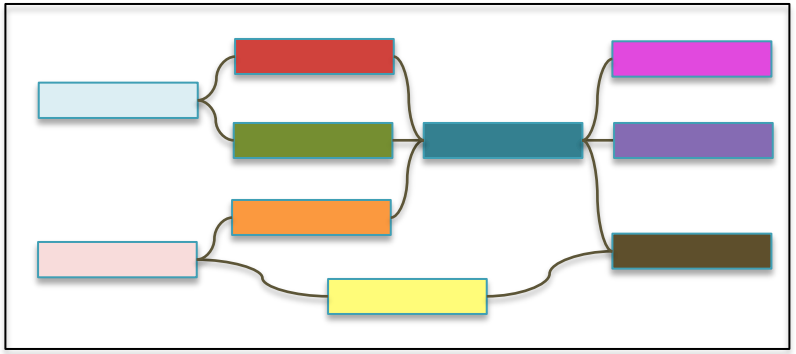
Objective

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

Compressed de Bruijn graph

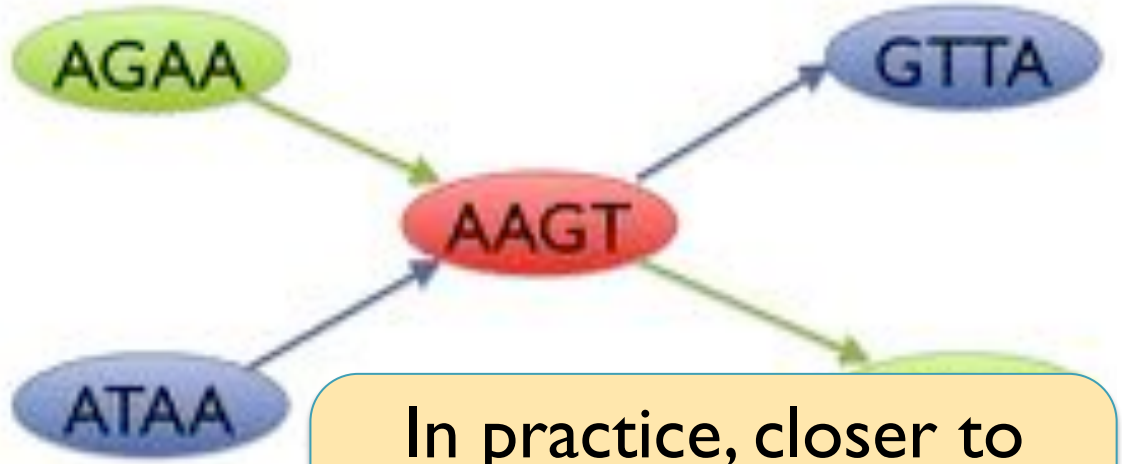
- Merge non-branching chains of nodes
- Min. number of nodes that preserve path labels

✧Usually built from uncompressed graph

✧Build directly O(*n* log *g*) time and space, $g < n$

Compressed de Bruijn graph

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

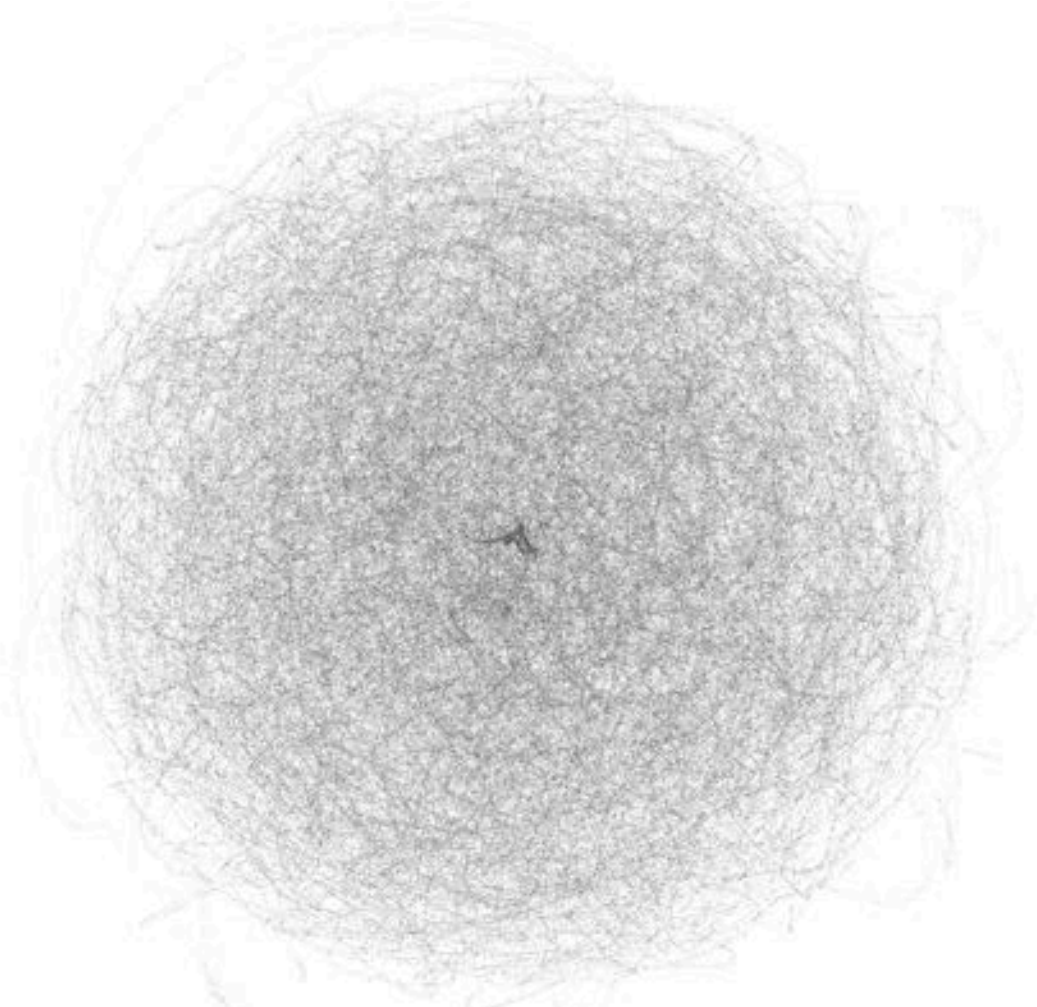


In practice, closer to linear for large collections.

✧Usually built fron

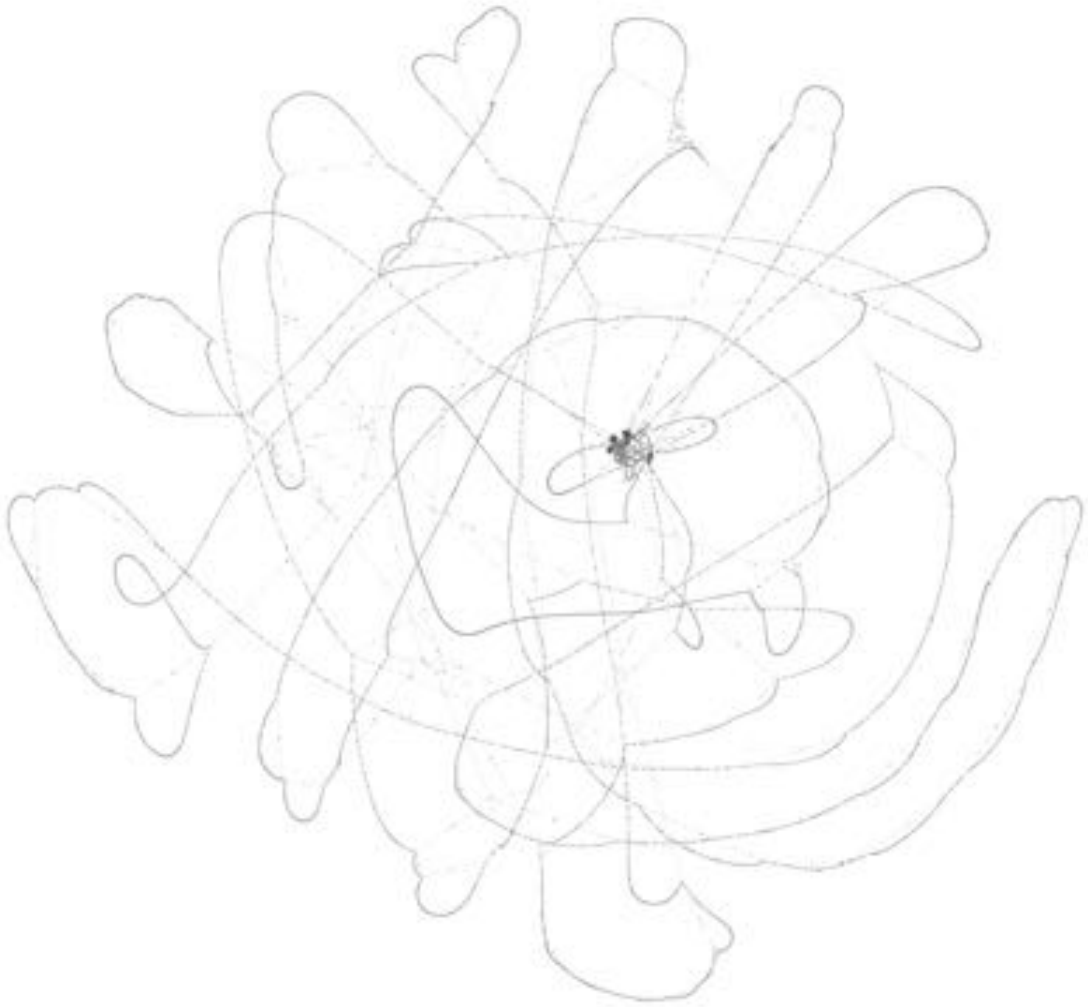
✧Build directly O(*n* log *g*) time and space

Compressed de Bruijn graph



9 strains of *Bacillus anthracis k=25*

Compressed de Bruijn graph



9 strains of *Bacillus anthracis k=1000*

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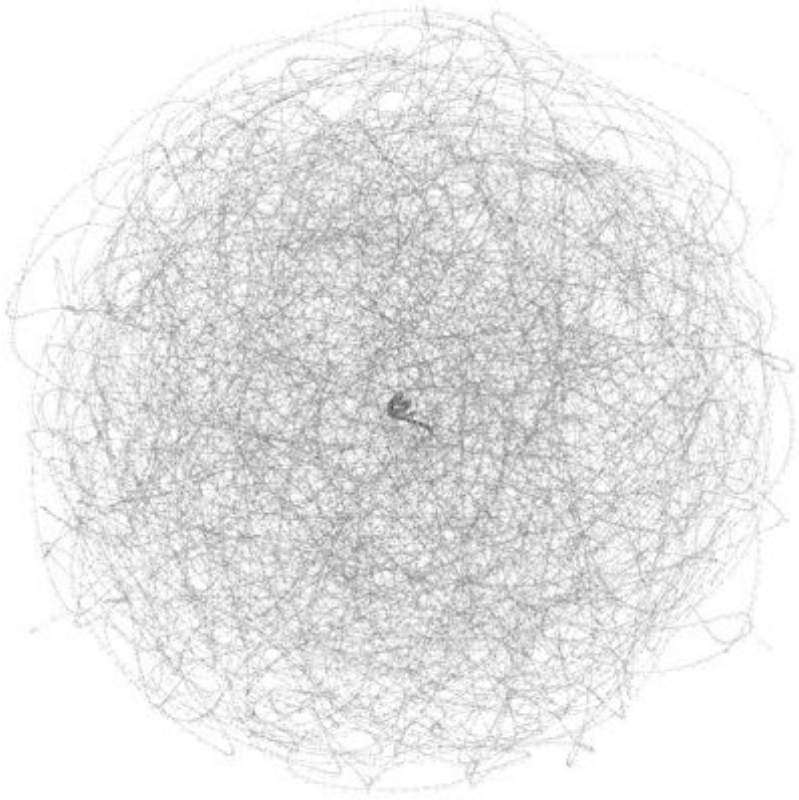
1 Overview

2 Data Structures

3 splitMEM Algorithm

4 Pan-genome Analysis



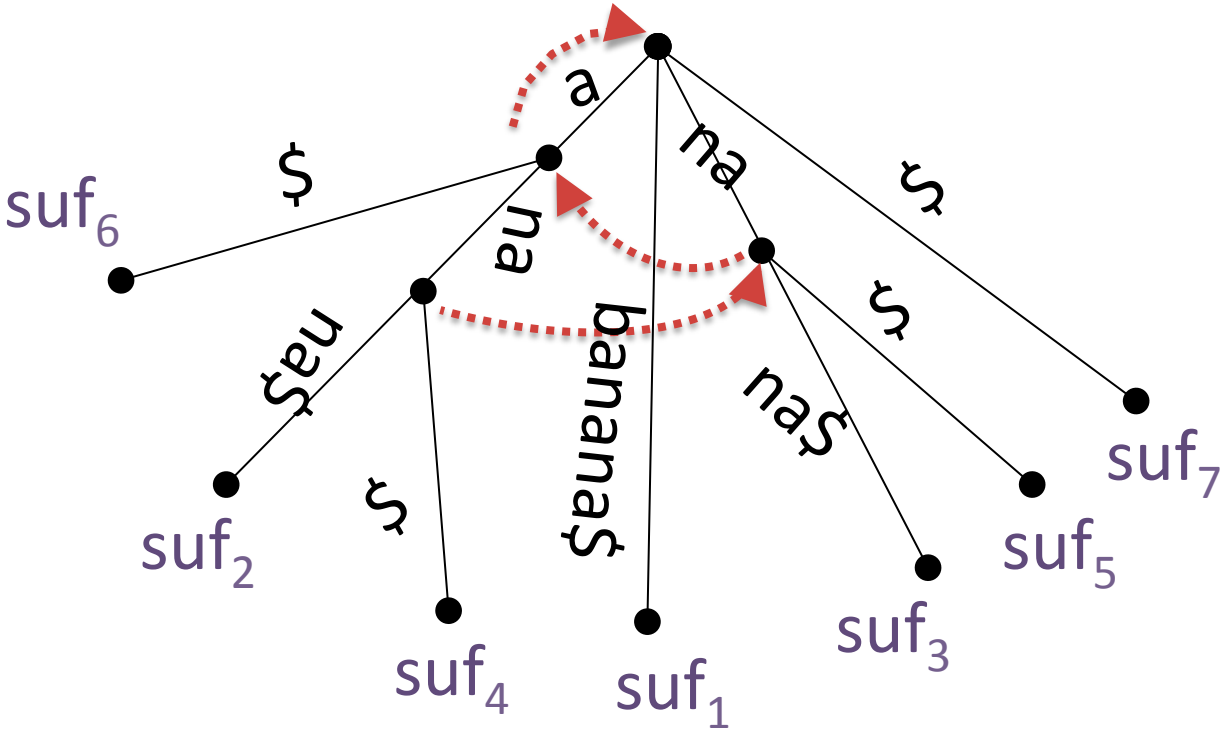
Suffix Tree

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

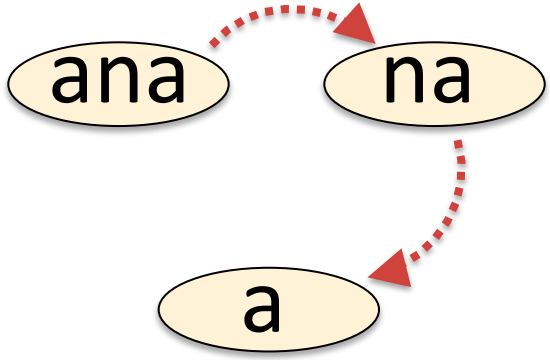
- Each edge is labeled with nonempty substring.
- No two siblings begin with the same character.
- Path from root to leaf i spells suffix S[i . . . n].
- Append special character $ to guarantee each suffix ends at leaf.

Constructing Suffix Tree

O(n) time



Suffix Links



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

Suffix Tree

✧Many applications in computational biology

✧Linear time construction algorithms

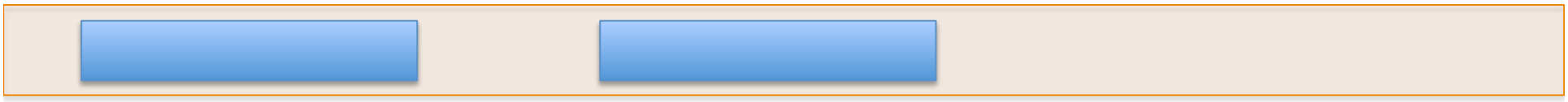
Linear time solutions to

- 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.

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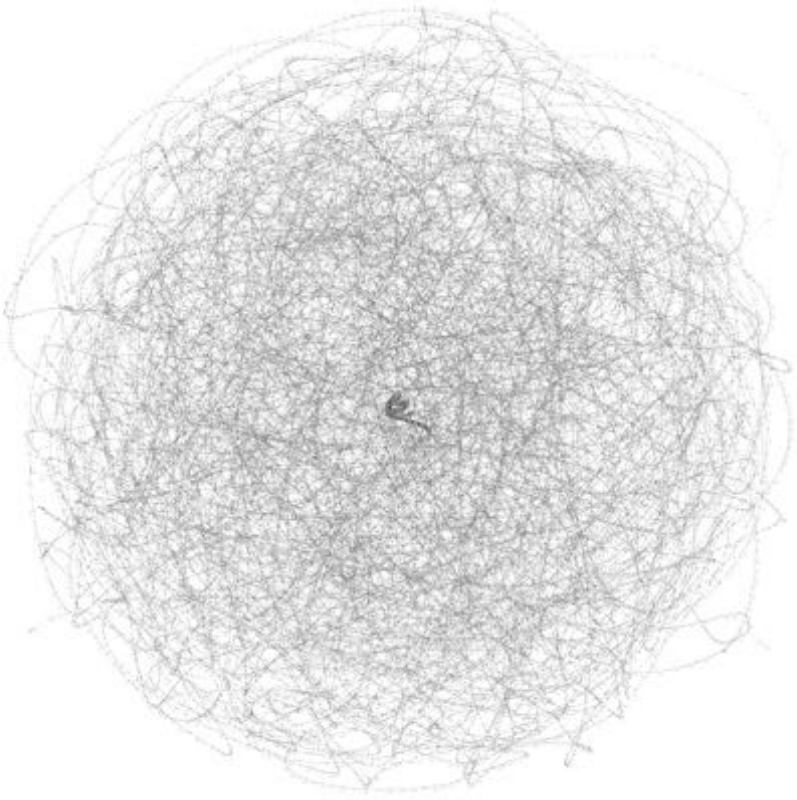


Compresssed de Bruijn graph

Types of nodes:

i. repeatNodes

ii. uniqueNodes

Input:

AGAAGTCC$ATAAGTTA

splitMEM

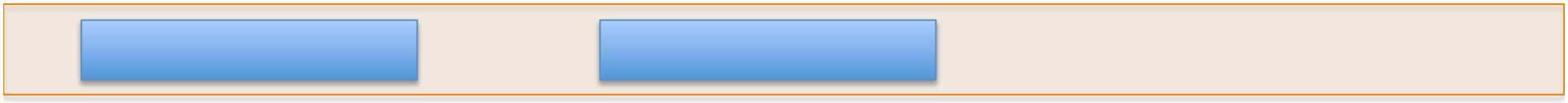
Nodes in compressed de Bruijn graph classified as

i. repeatNodes
ii. uniqueNodes

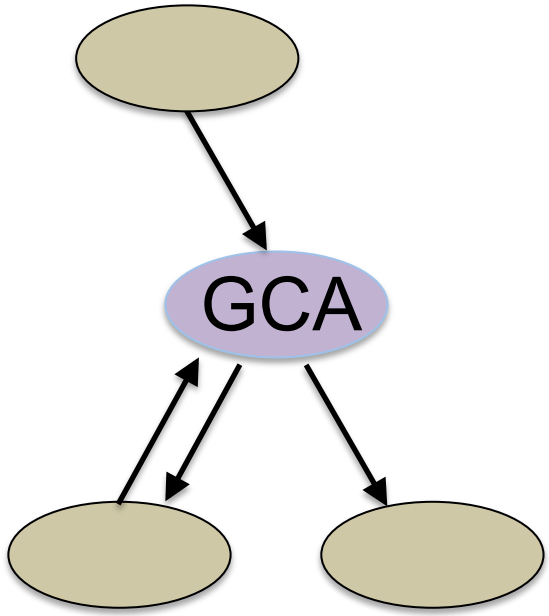
Algorithm:

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

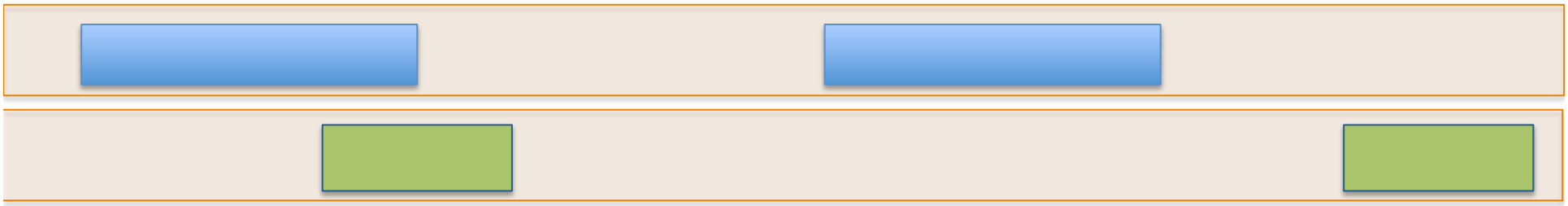
1 MEM occurs twice



TGCAC…GGCAA

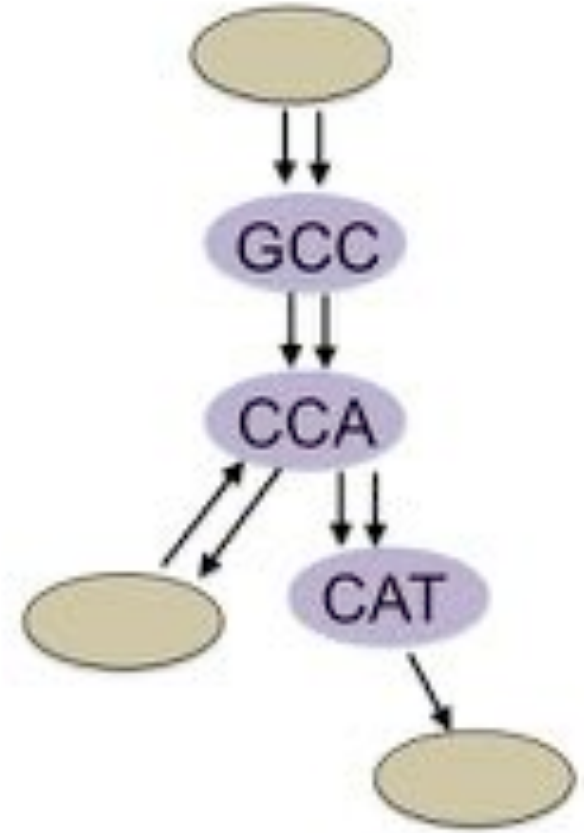


Overlapping MEMs

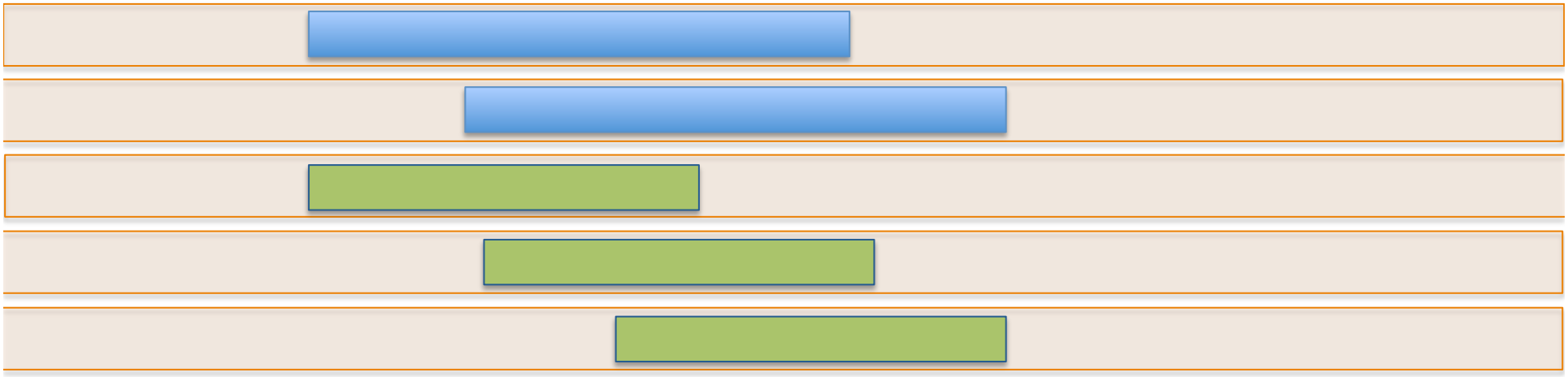


T G C C A T C G C C A A C C A T

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



Tandem Repeat



AGGCTTGGCTTGGCTTGGCTA
AGGCTTGGCTTGGCTTGGCTA
AGGCTTGGCTTGGCTTGGCTA
AGGCTTGGCTTGGCTTGGCTA
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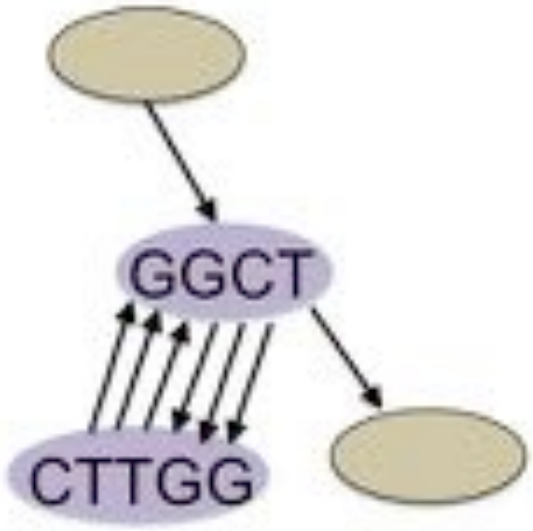


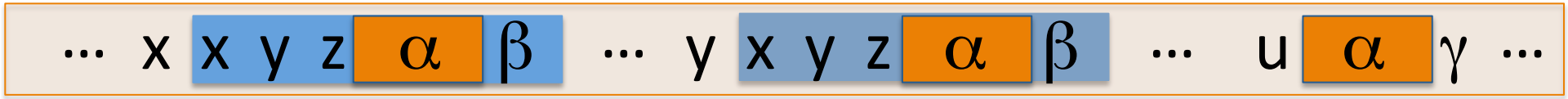
repeatNodes

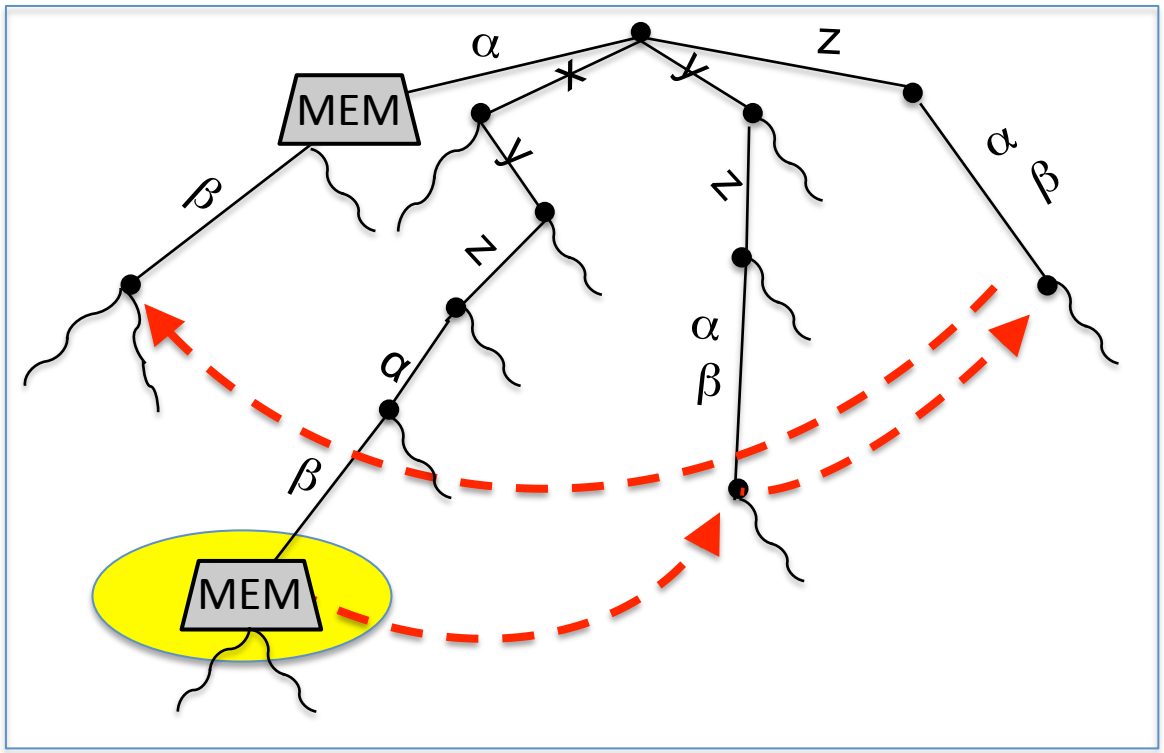
1 Construct set of repeatNodes

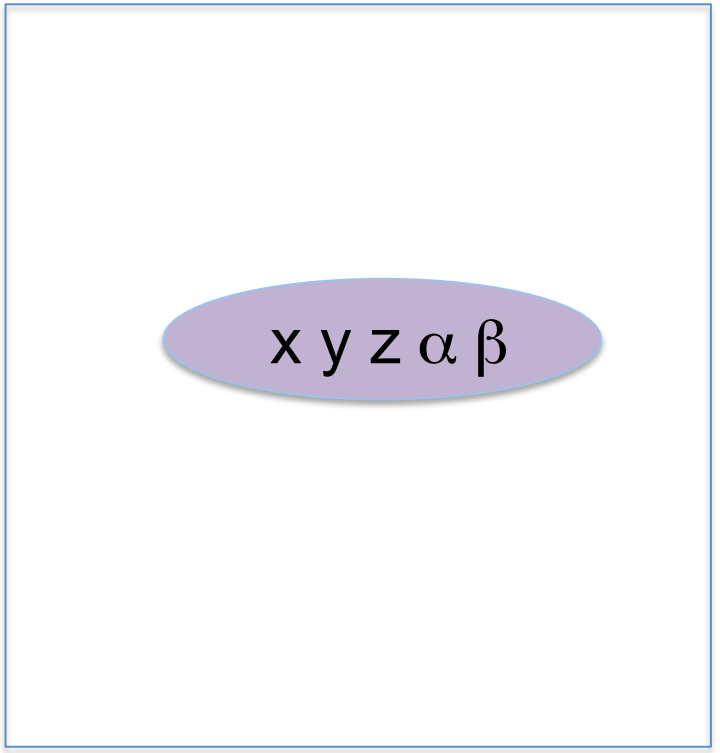
1. Build suffix tree of genome
2. Mark internal nodes that are MEMs, length ≥ k
3. Preprocess suffix tree for LMA queries

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

Split MEM to repeatNodes

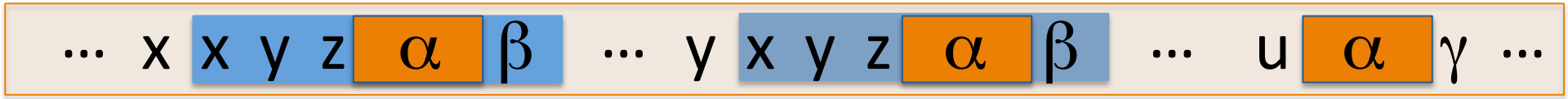


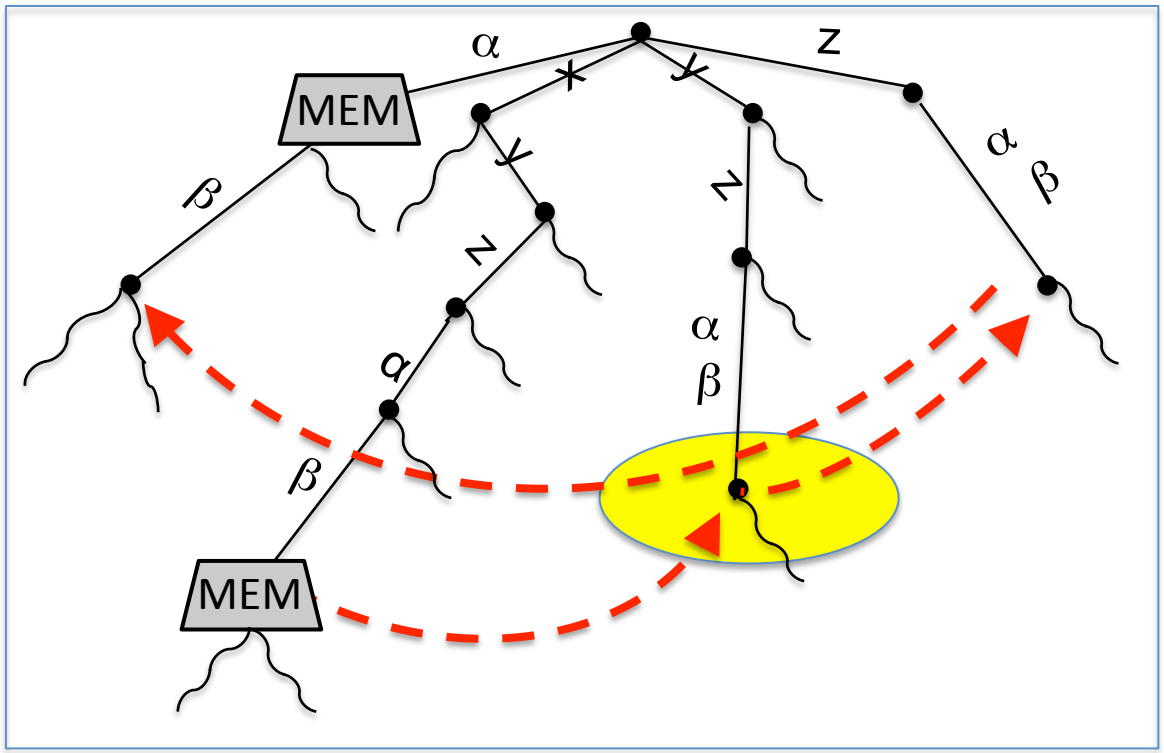


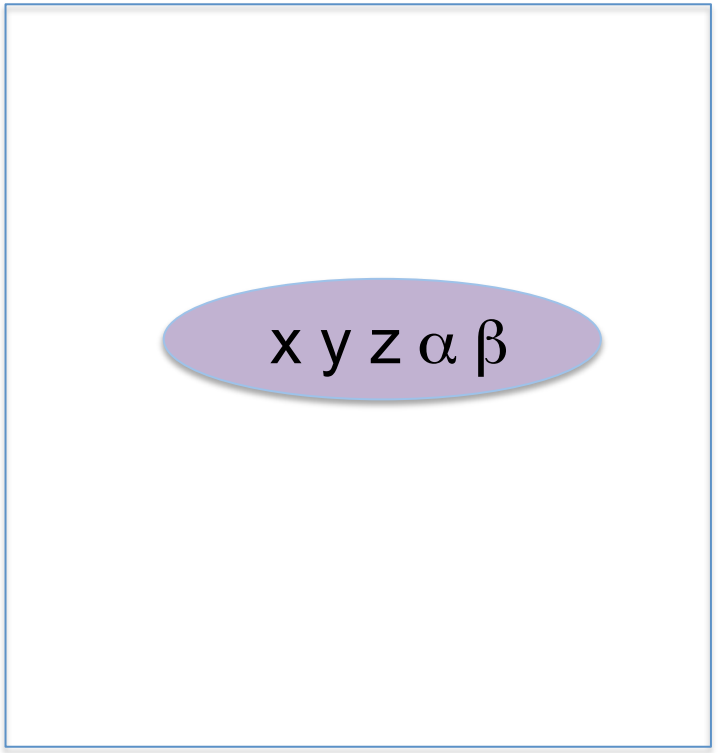


Find MEM in suffix tree.

Split MEM to repeatNodes



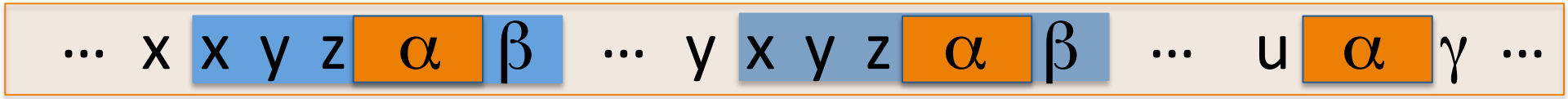


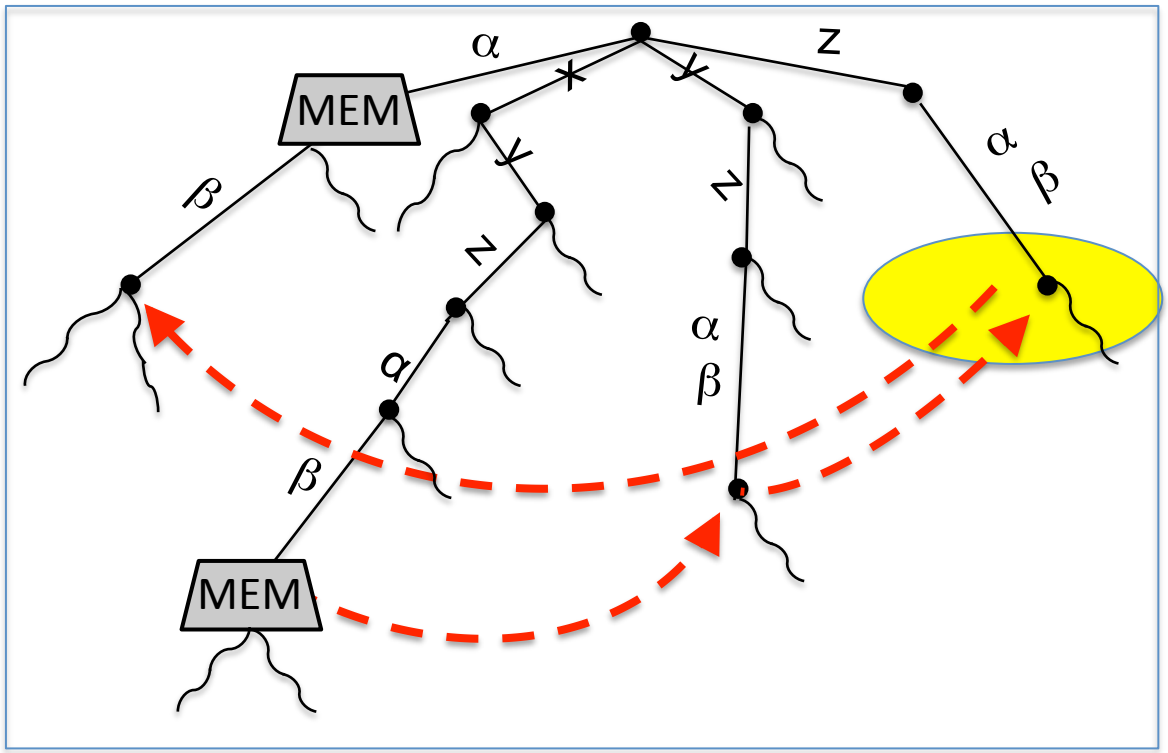


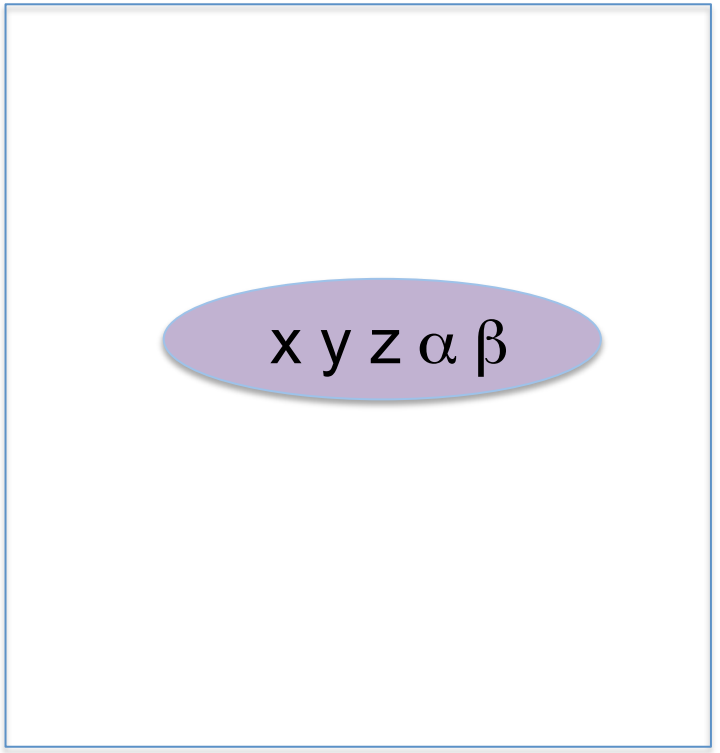
Traverse suffix link.

Look for MEM as ancestor.

Split MEM to repeatNodes







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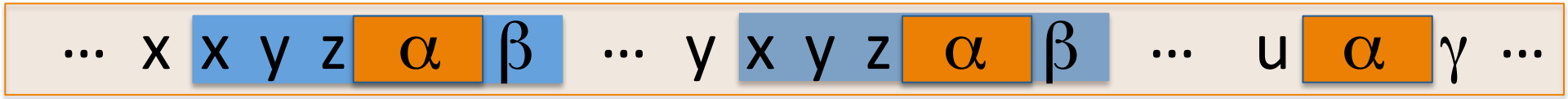

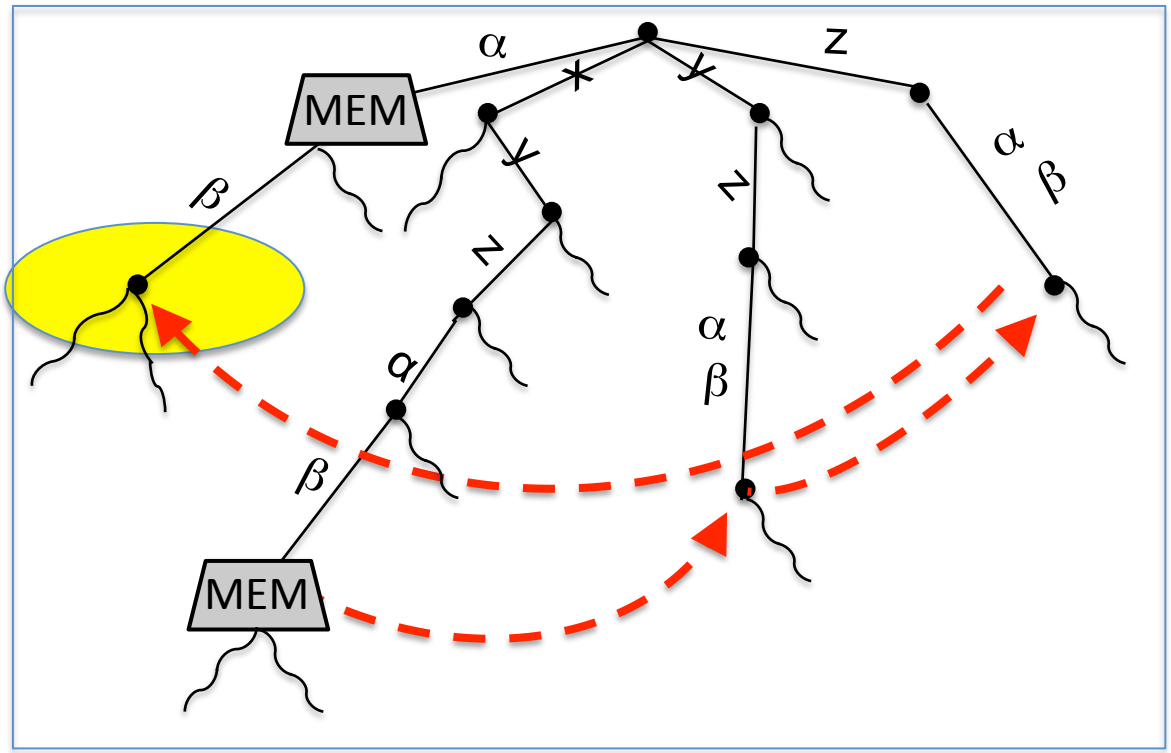

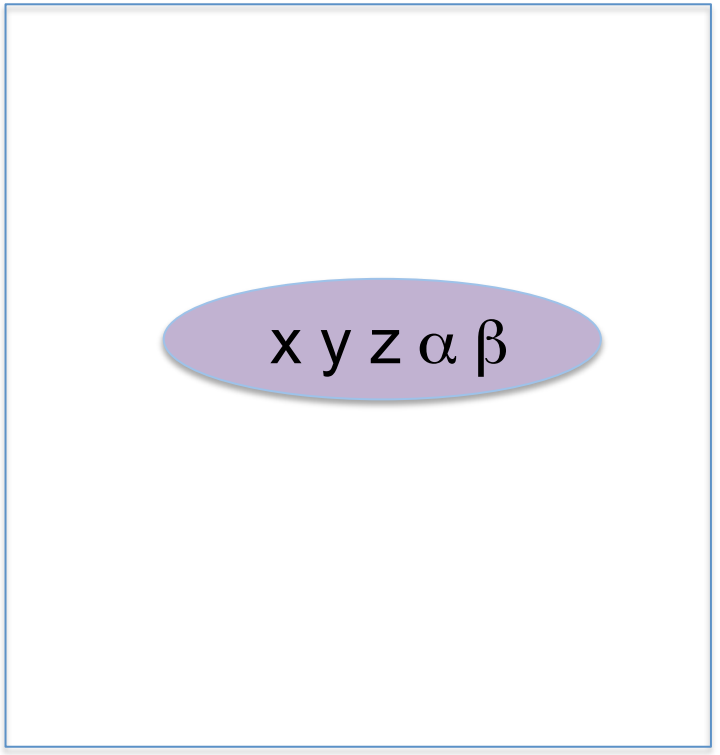

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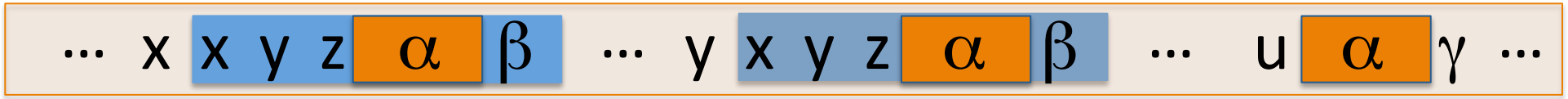
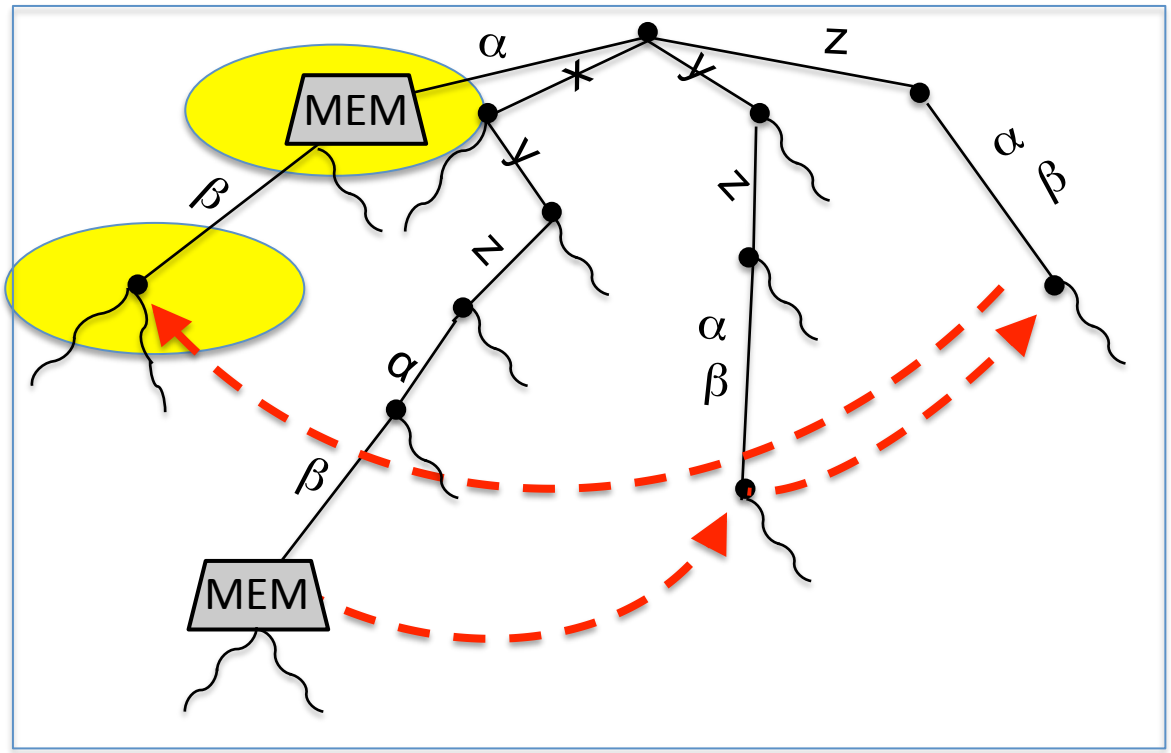
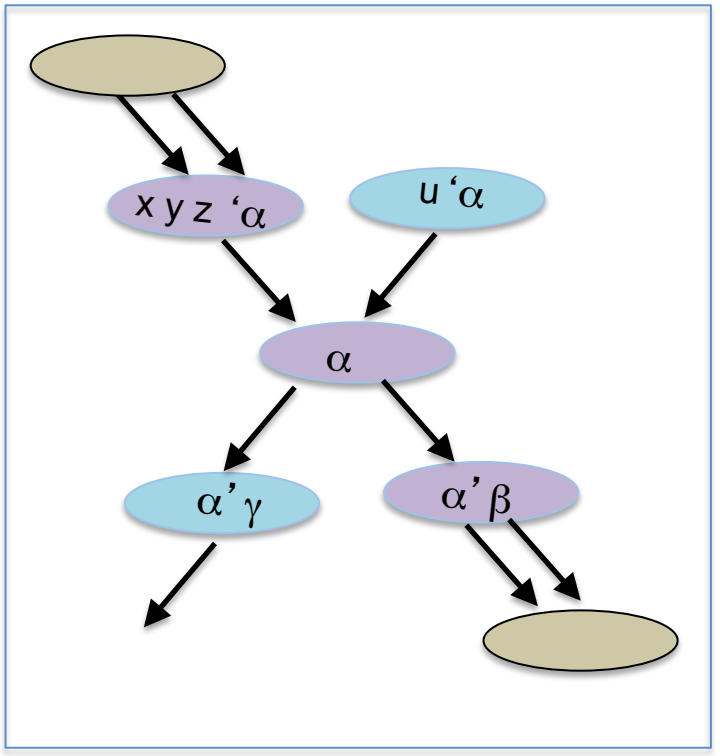






Found MEM as ancestor. Decompose.

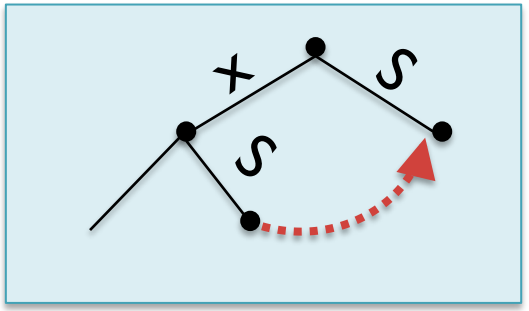
Remove embedded MEM (suffix links). Find next embedded MEM.

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

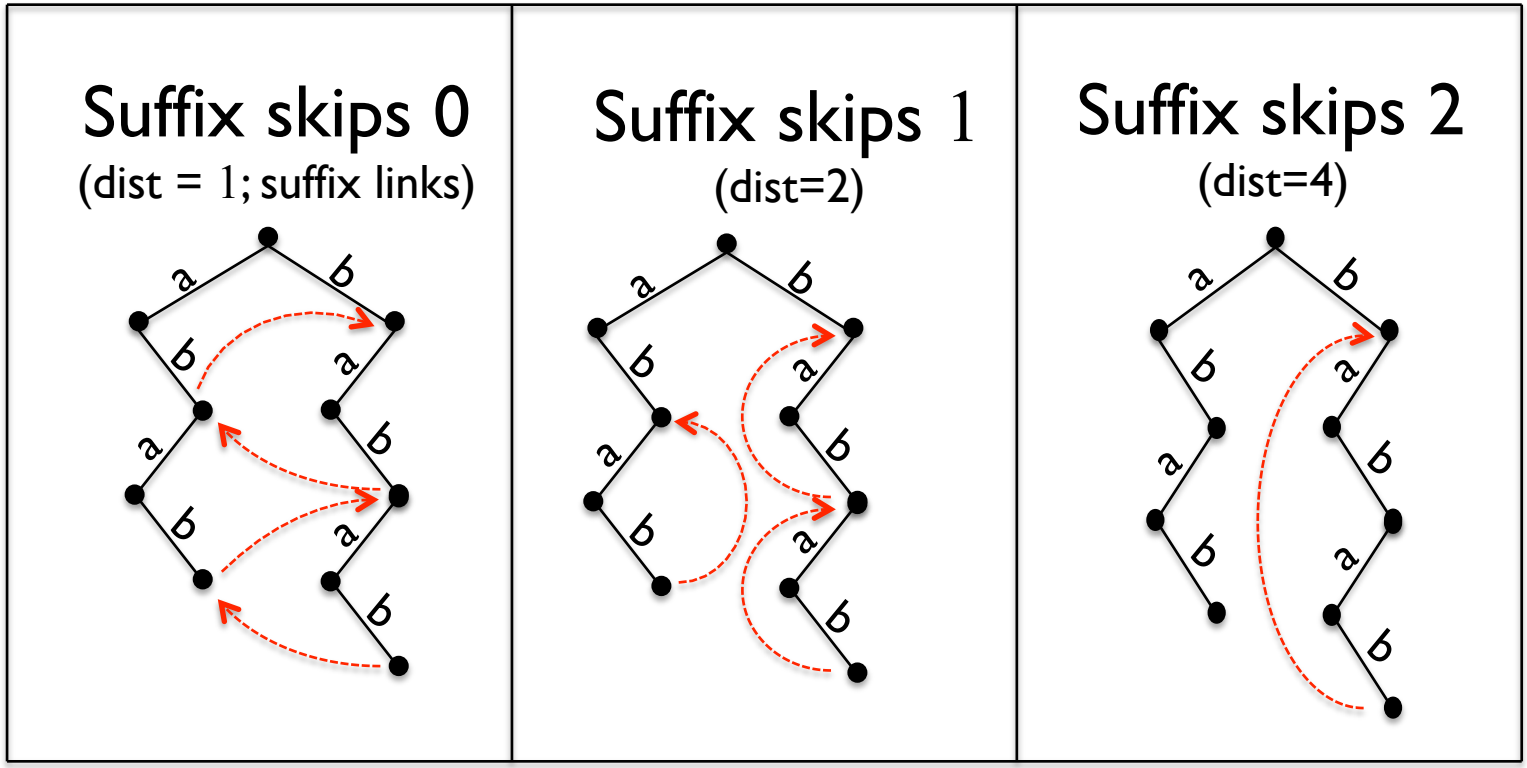


Suffix skip:

- 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

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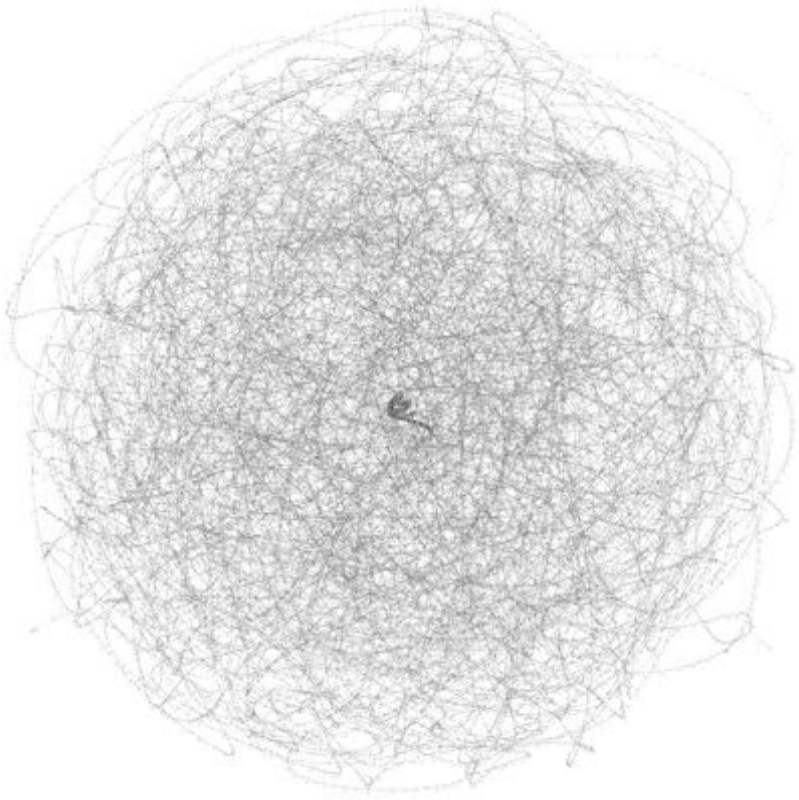
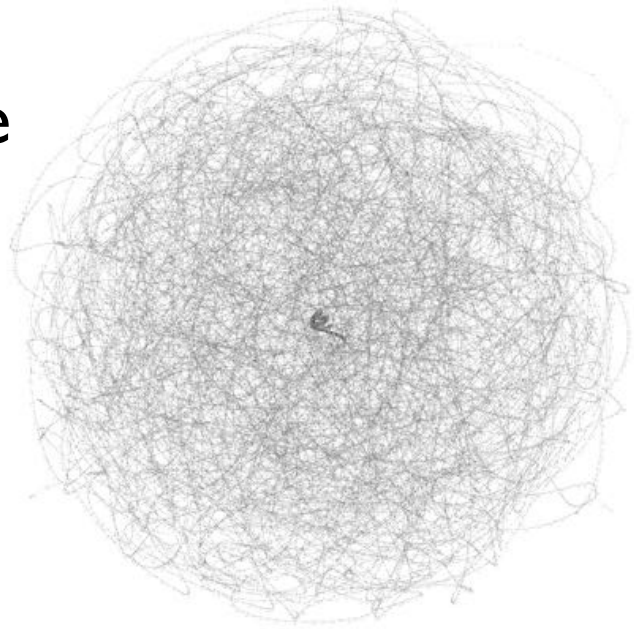


Pan-genome analysis

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

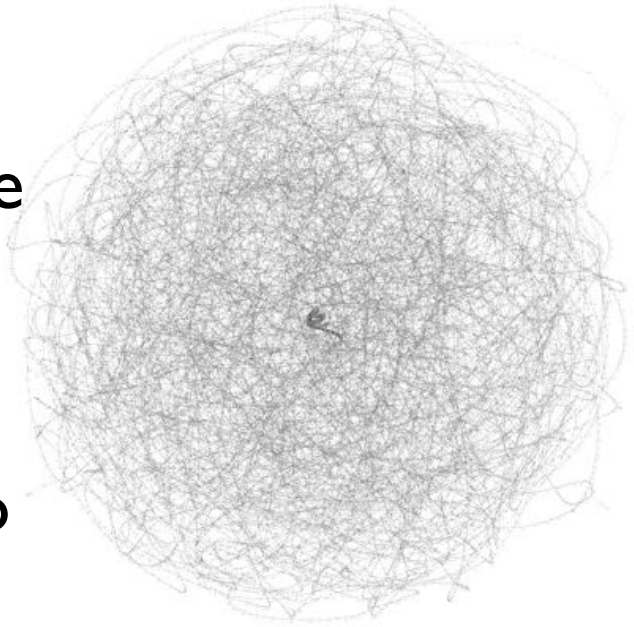
Graphs of main chromosomes

- 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*



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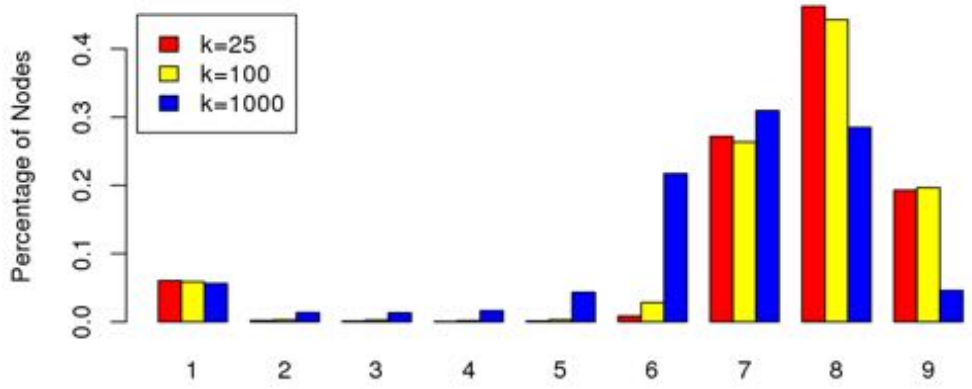
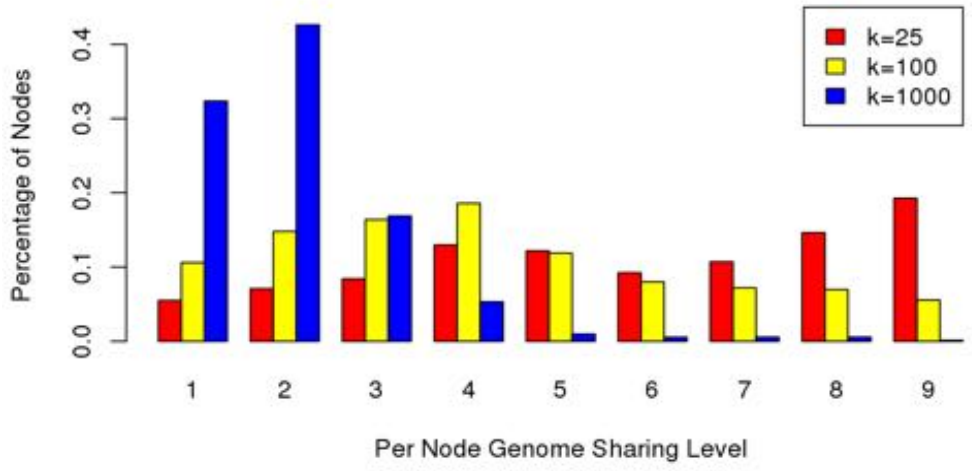
Pan-genome analysis



B. anthracis



E. coli

Fraction of nodes with each level of genome sharing

Contributions

- 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

Acknowledgments

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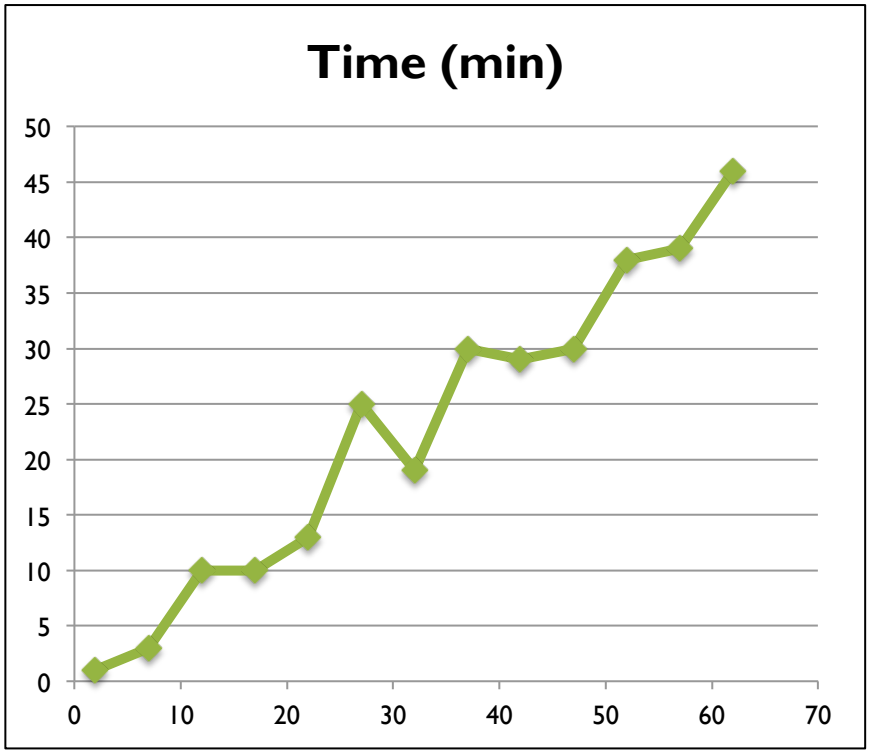
James Gurtowski
Schatz Lab

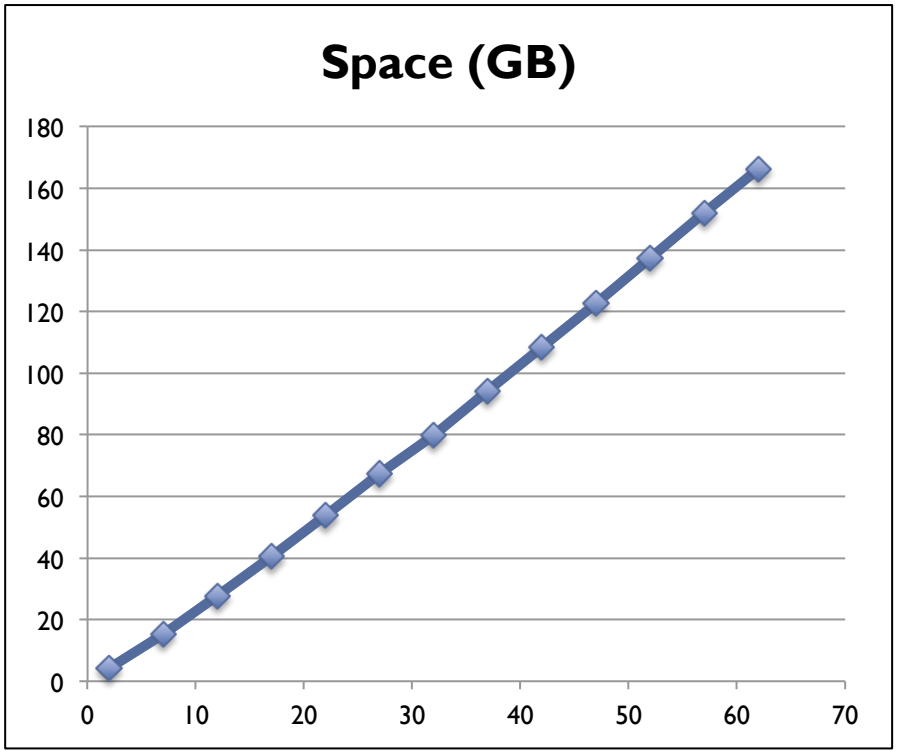
IT department
Todd Heywood

iPlant Collaborative™

Thank You!

Scaling Experiment:
62 *E. Coli* Genomes

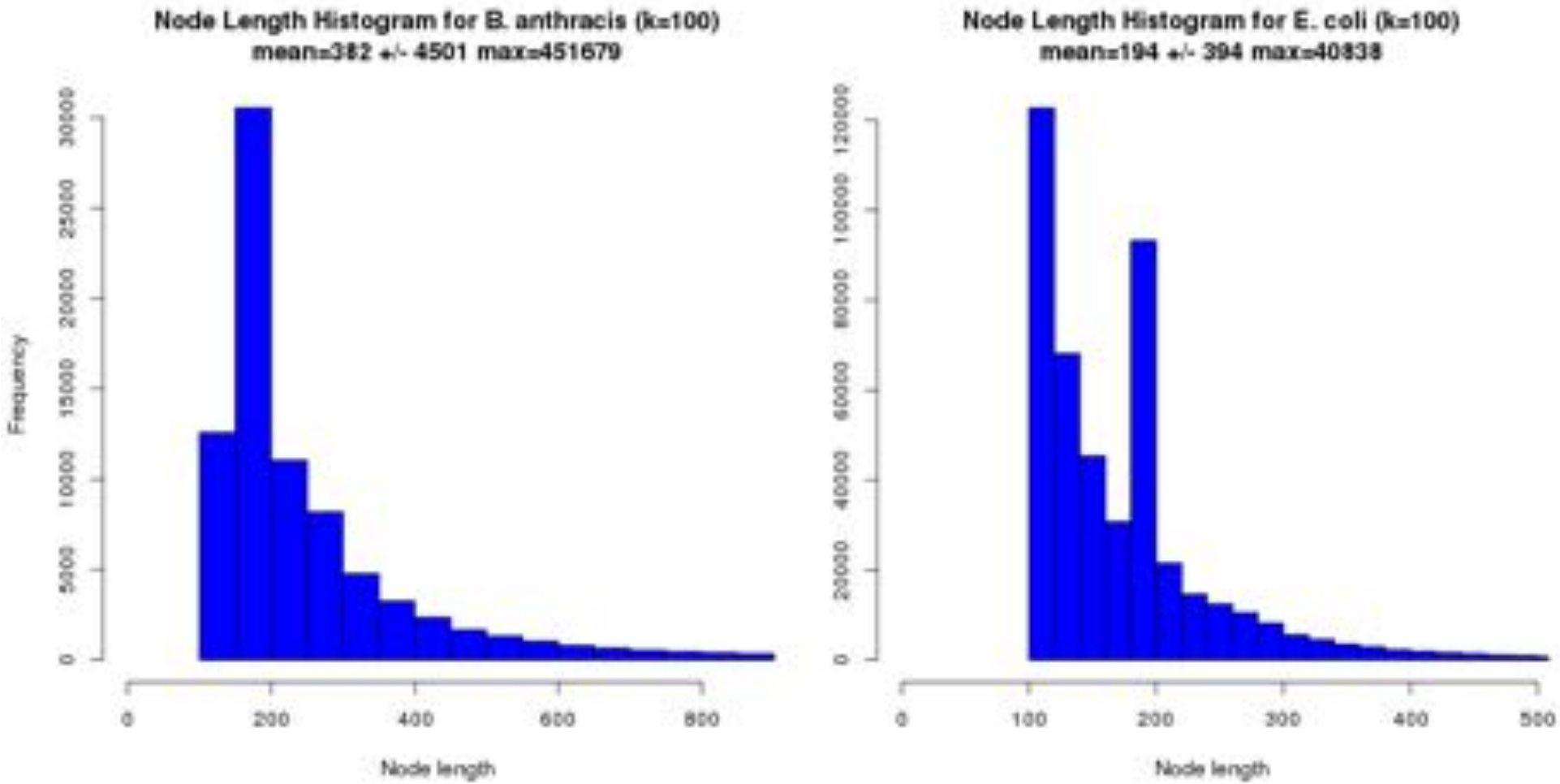




Practical to run on large datasets

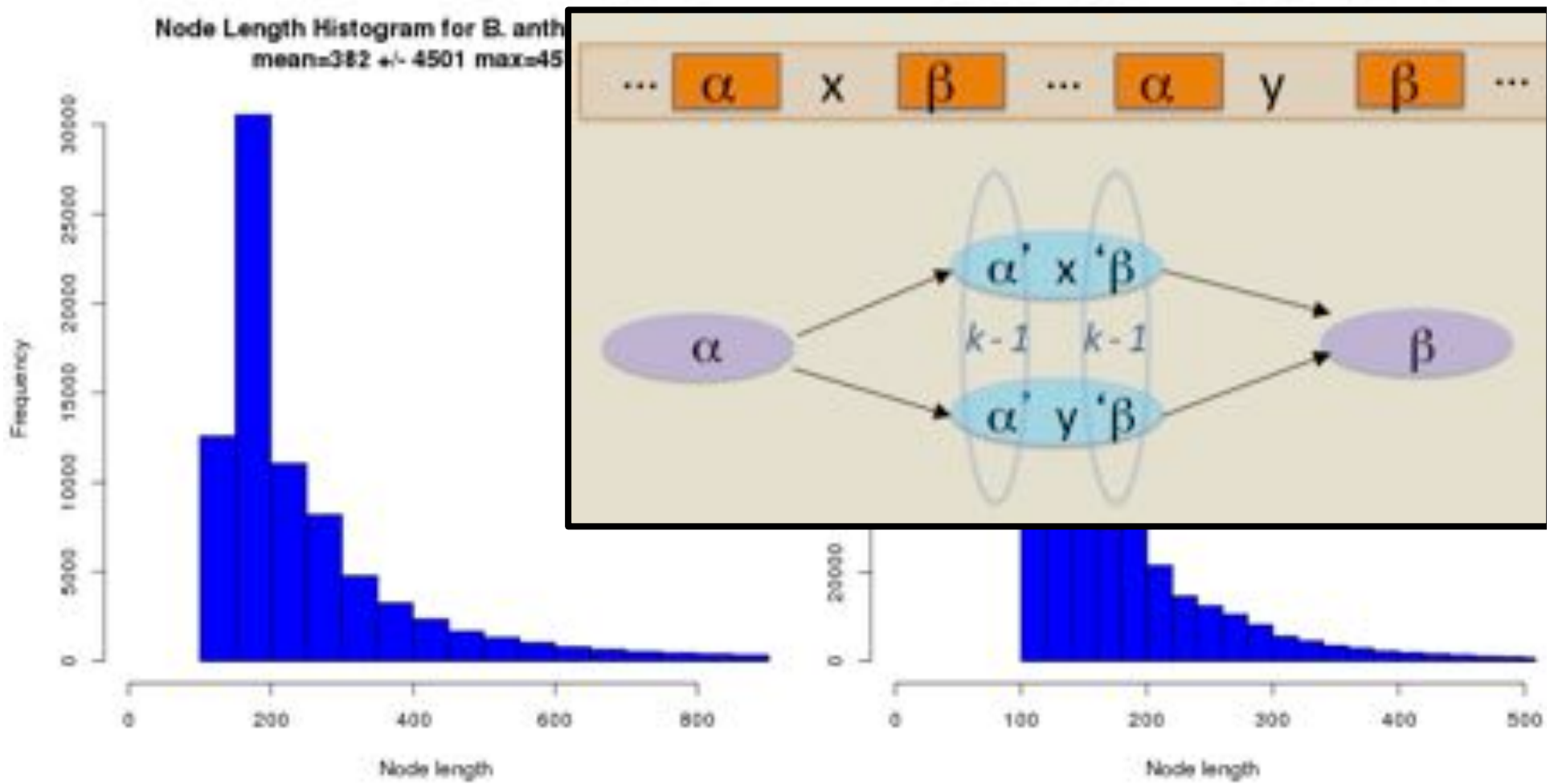
Linear time and space because max(|MEM|) is bounded by the largest single genome size (~constant)

Pan-genome analysis



Histogram of Node Lengths

Pan-genome analysis



Histogram of Node Lengths

Spike at 2k: SNPs

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