splitMEM: graphical pan-genome analysis with suffix skips

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Outline

1. Overview
2. Data Structures
3. splitMEM Algorithm
4. Pan-genome Analysis
Motivation

- 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?

A
B
C
D

Input

Output
de Bruijn graph

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

通常是构建松散的图

直接构建的时间和空间复杂度为 $O(n \log g)$

在实践中，更接近线性，对于大型集合尤其如此。
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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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 $S[i \ldots n]$.
- Append special character $\$$ to guarantee each suffix ends at leaf.
Constructing Suffix Tree

$O(n)$ time

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.

We are interested in MEMs length $\geq k$
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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Compressed de Bruijn graph

Types of nodes:

i. repeatNodes
ii. uniqueNodes

Input:

AGAAGTCC$ATAAAGTTA
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

TGCA...GGCA
Overlapping MEMs

TGCCATCGCCAAACCAT

TGCCATCGCCAAACCAT
Tandem Repeat

AGGCTTTGGCCTTGGGCTTTGGGCTTA
AGGCTTTGGCTTTGGGCTTTGGGCTTA
AGGCTTTGGGCTTTGGGCTTTGGGCTTA
AGGCTTTGGGCTTTGGGCTTTGGGCTTA
AGGCTTTGGGCTTTGGGCTTTGGGCTTA
repeatNodes

1. **Construct set of repeatNodes**
   
   1. Build suffix tree of genome
   2. Mark internal nodes that are MEMs, length $\geq k$
   3. Preprocess suffix tree for LMA queries

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

Traverse suffix link.
Look for MEM as ancestor.
Split MEM to repeatNodes

Traverse suffix link.
Look for MEM as ancestor.
Split MEM to repeatNodes

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
  o C++
  o open source [http://splitmem.sourceforge.net](http://splitmem.sourceforge.net)

• Input modes:
  o single genome: fasta file
  o 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*

<table>
<thead>
<tr>
<th>Species</th>
<th>K</th>
<th>Nodes</th>
<th>Edges</th>
<th>Avg. Degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. anthracis</td>
<td>25</td>
<td>103926</td>
<td>138468</td>
<td>1.33</td>
</tr>
<tr>
<td>B. anthracis</td>
<td>100</td>
<td>41343</td>
<td>54954</td>
<td>1.32</td>
</tr>
<tr>
<td>B. anthracis</td>
<td>1000</td>
<td>6627</td>
<td>8659</td>
<td>1.30</td>
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<tr>
<td>E. coli</td>
<td>25</td>
<td>494783</td>
<td>662081</td>
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<td>230996</td>
<td>308256</td>
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<tr>
<td>E. coli</td>
<td>1000</td>
<td>11900</td>
<td>15695</td>
<td>1.31</td>
</tr>
</tbody>
</table>
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

Fraction of nodes with each level of genome sharing

B. anthracis

E. coli
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.*
http://biorxiv.org/content/early/2014/04/06/003954
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iPlant Collaborative™
Thank You!
Scaling Experiment:
62 E. Coli Genomes

Practical to run on large datasets
Linear time and space because \( \max(|\text{MEM}|) \) is bounded by the largest single genome size (\(~\text{constant}~\))
Pan-genome analysis

Histogram of Node Lengths

Node Length Histogram for *B. anthracis* (k=100)
mean=382 +/- 4501 max=451679

Node Length Histogram for *E. coli* (k=100)
mean=194 +/- 394 max=40838
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