Outline: Jan 31

- DBG assembly in practice
- Velvet assembler
- Evaluation of assemblies
- (if time) Start: string alignment

Notes:
• Lab 1 due tonight (fill out README)
• Assembly reading posted: spend 1.5 hours max
• Office hours today 1-3pm
• Fill out partner form for Lab 2 (if you know who you want to work with)
Recap: Creating Eulerian paths
Recap Fleury’s Algorithm

- Start with vertex $u$ where $(\text{outdegree} - \text{indegree}) = 1$
- Until there are no more edges:
  - Choose $e = (u,v)$ where removing $e$ will not disconnect the graph (if possible)
  - Traverse $e$, delete $e$, and proceed with $u = v$
Recap Fleury’s Algorithm

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Avoids going from:
A -> B -> C
And then getting stuck
Recap recursive algorithm

\[
\text{find\_cycle}(u, \text{cycle}):
\]
\[
\text{for each edge } e = (u, v):
\]
\[
\quad \text{remove } e
\]
\[
\quad \text{find\_cycle}(v, \text{cycle})
\]
\[
\text{push } u \text{ onto } \text{cycle}
\]

To use in practice:
- Start with \text{cycle} as an empty stack
- Start at any node \( u \) (or start at a node with (outdegree – indegree) = 1 for a path)
- After completion, pop elements off \text{cycle} to find the correct order
De Bruijn graphs in practice
De Bruijn graph

A procedure for making a De Bruijn graph for a genome

Assume *perfect sequencing* where each length-$k$ substring is sequenced exactly once with no errors

Pick a substring length $k$: 5

Start with an input string: \texttt{a_long_long_long_time}

Take each $k$ mer and split into left and right $k-1$ mers

Add $k-1$ mers as nodes to De Bruijn graph (if not already there), add edge from left $k-1$ mer to right $k-1$ mer
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Building $k$-mer graph with reads

* Pick $k$ (for $L = 100$ bp, $k = 20$-40 is common)
* For each read:
  For each $k$-mer in read:
  - add L&R ($k$-1)-mers to graph as nodes (if not already there)
  - add edge from L -> R

From last time: # $k$-mers is $O(G)$ => # nodes is $O(G)$, and # edges is $O(G)$

But: how do we know if ($k$-1)-mer is already in our graph or not? Do we have to compare with all nodes?

- $n = 6 \times 10^9$ reads
- $L = 100$ bp
- $G = 3 \times 10^9$ bp (humans)
Implementation considerations

1) In practice, k-mers must be hashed so we can easily compare them.

2) When graphs become larger, recursive solutions are no longer practical.
What “messes” up our DBG?

1) Repeats of length \((k-1)\) or longer <- correction!

2) Gaps in coverage

3) Differences in coverage

4) Sequencing errors
Handout 2: work with someone new
Issues with DBGs

Gaps in coverage can lead to *disconnected* graph

Graph for `a_long_long_long_time`, $k = 5$ but *omitting* `ong_t`:

Connected components are individually Eulerian, overall graph is not
Issues with DBGs

De Bruijn graph

*Differences* in coverage also lead to non-Eulerian graph

Graph for *a_long_long_long_time*, $k = 5$ but with *extra copy* of *ong_t*:

Graph has 4 *semi-balanced* nodes, isn’t Eulerian

*Slide: adapted from Ben Langmead, John Hopkins*
Issues with DBGs

De Bruijn graph

Errors and differences between chromosomes also lead to non-Eulerian graphs

Graph for a_long_long_long_time, k = 5 but with error that turns a copy of long_ into 1xng_

Graph is not connected; largest component is not Eulerian
One workaround for coverage issues:

In typical assembly projects, average coverage is ~ 30 - 50

Same edge might appear in dozens of copies; let’s use edge weights instead

Weight = # times k-mer occurs

Using weights, there’s one weighted edge for each distinct k-mer

Before: one edge per k-mer

After: one weighted edge per distinct k-mer
Issues with DBGs

Casting assembly as Eulerian walk is appealing, but not practical

Uneven coverage, sequencing errors, etc make graph non-Eulerian

Even if graph were Eulerian, repeats yield many possible walks


*De Bruijn Superwalk Problem* (DBSP) is an improved formulation where we seek a walk over the De Bruijn graph, where walk contains each read as a *subwalk*

Proven NP-hard!


Slide: adapted from Ben Langmead, John Hopkins
What did we give up by going from OLC to DBG?

Reads are immediately split into shorter $k$-mers; can’t resolve repeats as well as overlap graph.

Only a very specific type of “overlap” is considered, which makes dealing with errors more complicated.

*Read coherence* is lost. Some paths through De Bruijn graph are inconsistent with respect to input reads.
But we still have advantages...

**Building the de Bruijn graph:**
- $O(nL)$ since we go through each read and k-mers along the length of the read
- $O(G)$ space to store since both # edges and # nodes are $O(G)$

**Finding paths through the graph:**
- assuming Eulerian, $O(G)$ to traverse since # edges is $O(G)$
Velvet Assembler
Velvet Assembler (Zerbino & Birney, 2008)

- The first truly practical de Bruijn graph assembler
- Combines several algorithms to simplify the raw k-mer graph

**Resource**

**Velvet: Algorithms for de novo short read assembly using de Bruijn graphs**

Daniel R. Zerbino and Ewan Birney

EMBL-European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, United Kingdom

We have developed a new set of algorithms, collectively called “Velvet,” to manipulate de Bruijn graphs for genomic sequence assembly. A de Bruijn graph is a compact representation based on short words (k-mers) that is ideal for high coverage, very short read (25–50 bp) data sets. Applying Velvet to very short reads and paired-ends information only, one can produce contigs of significant length, up to 50-kb N50 length in simulations of prokaryotic data and 3-kb N50 on simulated mammalian BACs. When applied to real Solexa data sets without read pairs, Velvet generated contigs of ~8 kb in a prokaryote and 2 kb in a mammalian BAC, in close agreement with our simulated results without read-pair information. Velvet represents a new approach to assembly that can leverage very short reads in combination with read pairs to produce useful assemblies.

[Supplemental material is available online at www.genome.org. The code for Velvet is freely available, under the GNU Public License, at http://www.ebi.ac.uk/~zerbino/velvet.]
Velvet paper: Figure 1
Combine nodes into “blocks” when graph is “linear”

Reverse complement
Velvet paper: Figure 1

Each (k-1)-mer adds one nucleotide of marginal information

Combine nodes into “blocks” when graph is “linear”

Reverse complement
Velvet paper: Figure 2 (Tour Bus algorithm)
When we hit D for the second time, compare the sequences of the two ways we got there: BC and B'C'. If they are judged similar, error correct and merge.
Evaluating Assemblies
N50: for a set of contigs, N50 is the greatest length such that at least half the bases of the assembly are in a contig with length N50 or longer.

1a. Contigs, sorted according to their lengths.
Assembly evaluation

- **N50**: for a set of contigs, N50 is the greatest length such that at least half the bases of the assembly are in a contig with length N50 or longer.

![Diagram of contigs, sorted according to their lengths.](Image: The Molecular Ecologist)
Why is N50 a bad evaluation metric?
Why is N50 a bad evaluation metric?

- We could just loop through cycles in our a graph over and over, generating large (incorrect) contigs

- We need a better way to evaluate the quality of assemblies

- Take away: simulated data is every valuable. Take an existing genome, simulate random reads, then try to reconstruct.

- How do we tell how similar our reconstruction is to the original genome we simulated from?
Next topic: sequence alignment

- Goal: given two sequences, what is the best match or “alignment” between them?