Graphs for reference-free analysis of sequencing data

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Colib'read workshop, November 2016, Paris

Motivation

Reference-free sequencing data analysis:
no genome sequence, or choosing not to use it

Some applications:

1. Read error correction
2. DNA/RNA/metaDNA assembly
3. Alternative splicing detection
4. DNA variants detection
5. Transcript quantification

Presentation will be application-agnostic. We'll talk about the overlap graph, string graph, de Bruijn graph, A-Bruijn graph, superstring graph

This talk

.. will be technical!

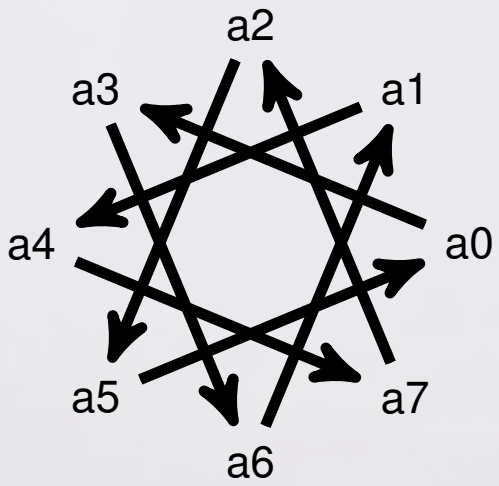
Computer Scientists: sit back and relax, it gets challenging in the second part

(as Pierre would say,) *Biologists*:
the first part will possibly be helpful to understand other talks

Vocabulary

A graph is:

- a set of nodes, and
- a set of edges (directed or not)



k-mers are strings of length *k*

Graphs for sequencing data

Graphs represent overlaps between sequences in reads.

Two widely used families of graphs for sequencing data:

- de Bruijn graphs — generally for Illumina data
- string graphs — generally for Sanger/PacBio/Nanopore data

Overlap graphs

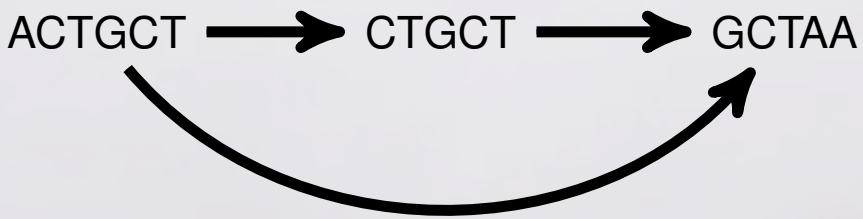
First, agree on some definition of "what is an overlap".

1. **Nodes** = reads.
2. **Edges** = overlap between two reads.

In this example, let's say that an overlap needs to be:

- exact
- and over at least 3 characters.

```
ACTGCT
 CTGCT (overlap of length 5)
   GCTAA (overlap of length 3)
```



String graphs

A **string graph** is obtained from an overlap graph by removing redundancy:

- redundant reads (those fully contained in another read)
- transitively redundant edges (if $a \to c$ and $a \to b \to c$, then remove $a \to c$)

Example:

```
ACTGCT
 CTGCT (overlap length 5)
   GCTAA (overlap length 3)
```

ACTGCT → GCTAA

Let's have inexact overlaps here

```
ACTGCT
 CTACT
   GCTAA
```

ACTGCT → CTACT → GCTAA

String graph / de Bruijn graph (4)

So, which is better?

- String graphs capture whole read information
- de Bruijn graphs are conceptually simpler:
  - single node length
  - single overlap definition

string graphs have been mostly used for long reads and de Bruijn graphs mostly for short reads.

String graphs are also known as the **Overlap Layout Consensus** (OLC) method.

de Bruijn graphs

A **de Bruijn** graph for a fixed integer k:

1. **Nodes** = all *k-mers* (substrings of length k) in the reads.
2. There is an **edge** between x and y if the $(k-1)$-mer prefix of y matches exactly the $(k-1)$-mer suffix of x.

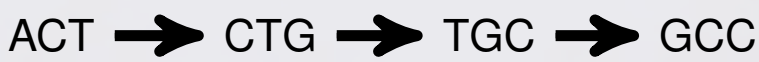
Example for $k = 3$ and a single read:

`ACTG`

de Bruijn graphs

Example for many reads and still $k = 3$.

```
ACTG
 CTGC
  TGCC
```



de Bruijn graphs: redundancy

What happens if we add redundancy?

```
ACTG
ACTG
 CTGC
 CTGC
 CTGC
  TGCC
  TGCC
```

dBG, $k = 3$:

de Bruijn graphs: errors

How is a sequencing error (at the end of a read) impacting the de Bruijn graph?

```
ACTG
 CTGC
 CTGA
  TGCC
```

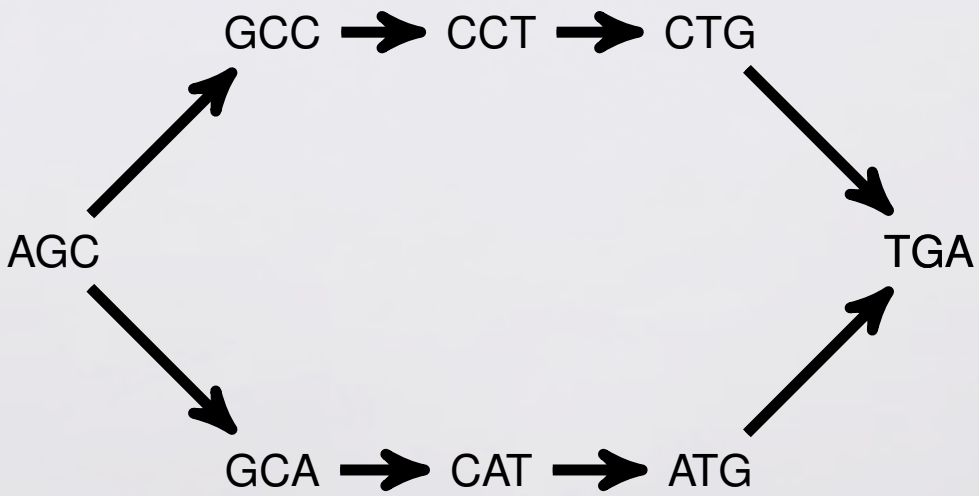
dBG, $k = 3$:

de Bruijn graphs: SNPs

What is the effect of a SNP (or a sequencing error inside a read) on the graph?

AGCCTGA
AGCATGA

dBG, $k = 3$:

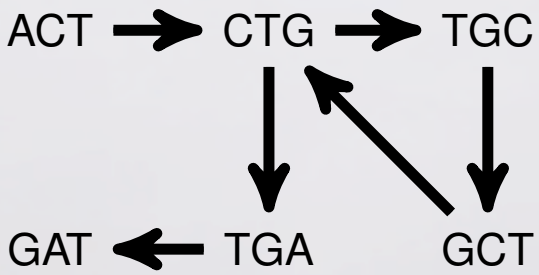


de Bruijn graphs: repeats

What is the effect of a small repeat on the graph?

```
ACTG
 CTGC
  TGCT
   GCTG
    CTGA
     TGAT
```

dBG, $k = 3$:

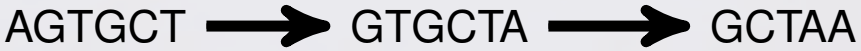


Comparison string graph / de Bruijn graph

On the same example, compare the de Bruijn graph with the string graph:

```
AGTGCT
 GTGCTA
   GCTAA
```

String graph, overlap threshold of 3:



de Bruijn graph, $k = 3$:

Short note on reverse complements

Because sequencing is generally not strand-specific:

> *We always consider that reads (and k-mers) are equal to their reverse complements.*

E.g:
AAA = TTT
ATG = CAT

Short note on dBG definitions

Node-centric dBG:

what we saw.
nodes = k-mers
edges = $(k-1)$-overlaps

Edge-centric dBG:

nodes = k-mers
edges = $(k+1)$-mers

Also called dBG^- and dBG^+ in the south of France.

They're related but not strictly equivalent.

Space needed to represent the dBG

With a hash table: $\geq 2k$ bits / node

Memory-efficient dBG data structures:

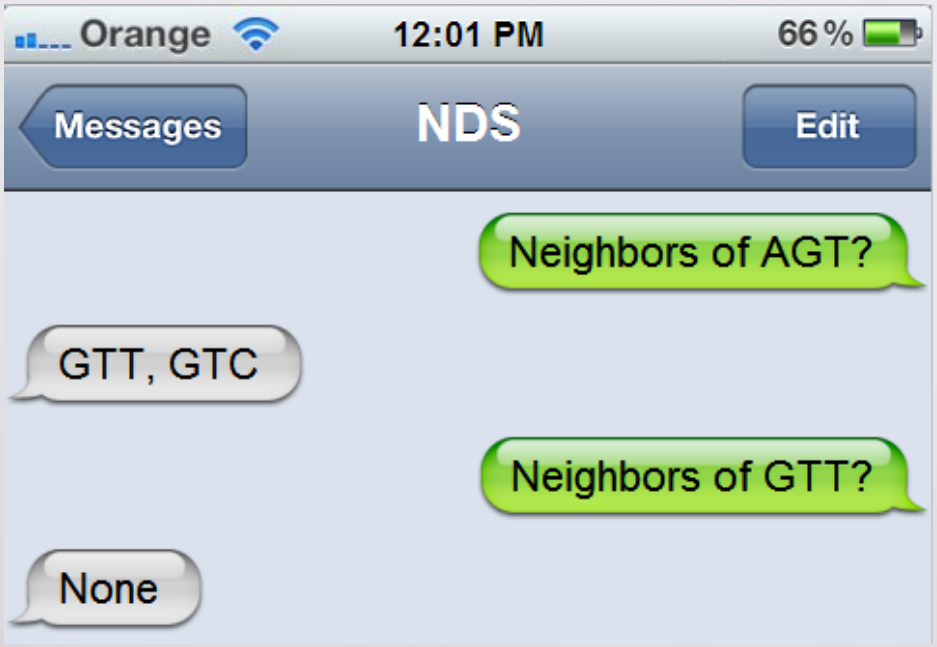
khmer	Bloom filter	[Pell et al. 11]
Minia	BF \ false positives	[Chikhi, Rizk 12], [Salikhov et al. 13]
SDBG	XBW + rank/select	[Bowe et al. 12]

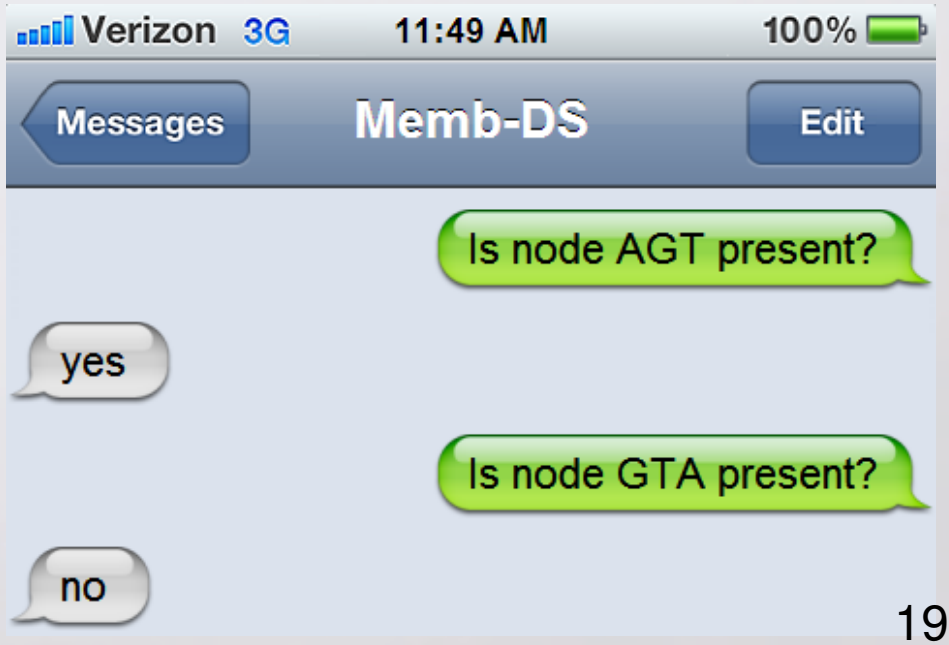
Why are they so efficient? $\rightarrow$ not all operations are supported

Navigational data structures

	NDS	Membership (e.g. hash table)
Traverse dBG from known nodes	✓	✓
Query **membership** of arbitrary nodes	*x*	✓
Enumerate nodes	*x*	✓

NDS has undefined behavior if query node not present.





Software

To just construct the graph:

String graph

Illumina: SGA[1] or SlideSort[2]
PacBio: Minimap + Miniasm[3]

de Bruijn graph

GATB-Core[4] (for developers, navigational index),
BCALM 2[5] (stand-alone, graph on disk),
ABySS[6]

BCALM 2: 3 GB RAM / 2 wall-clock hours for mammalian genome, outputs unitigs.

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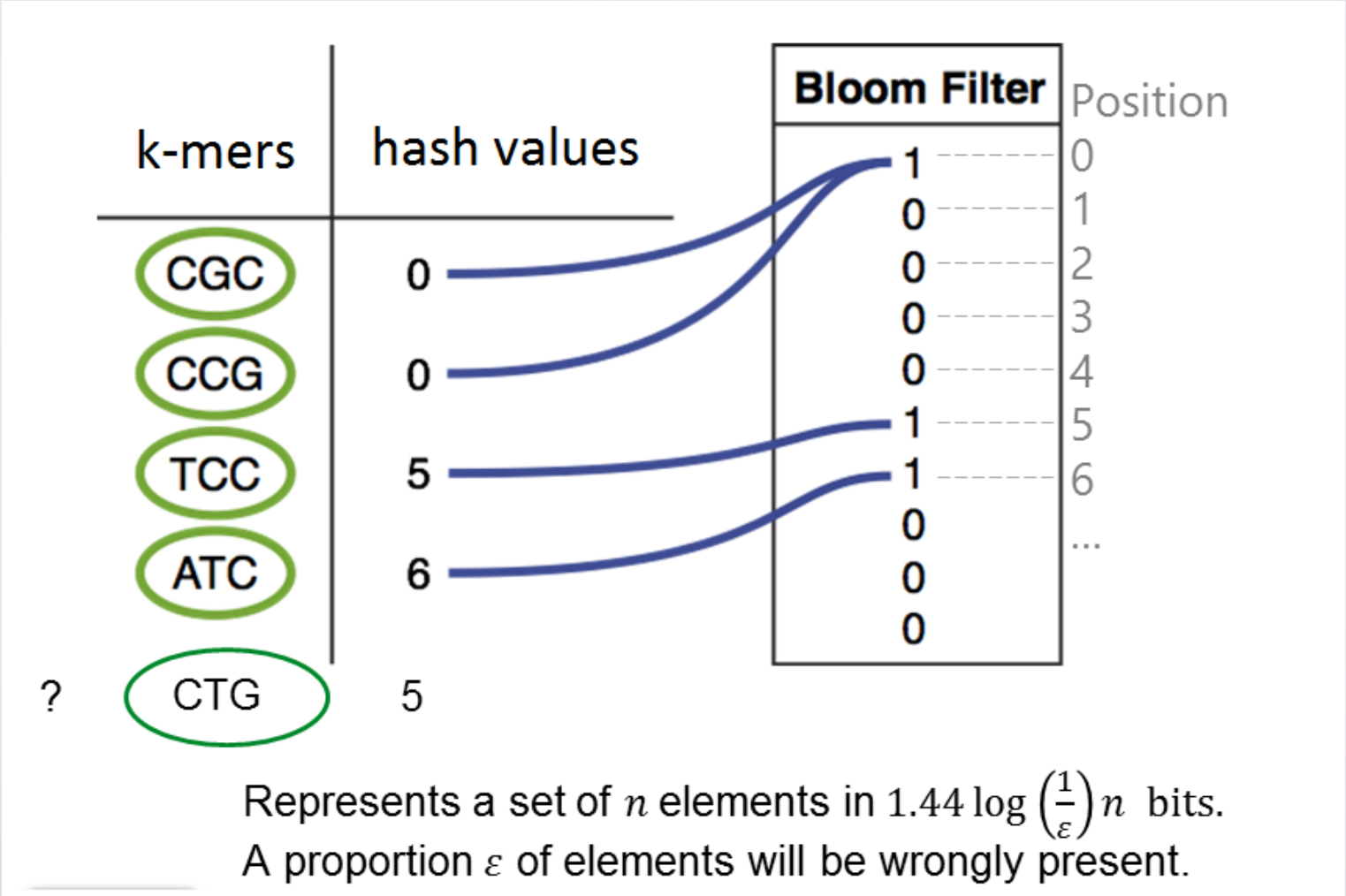
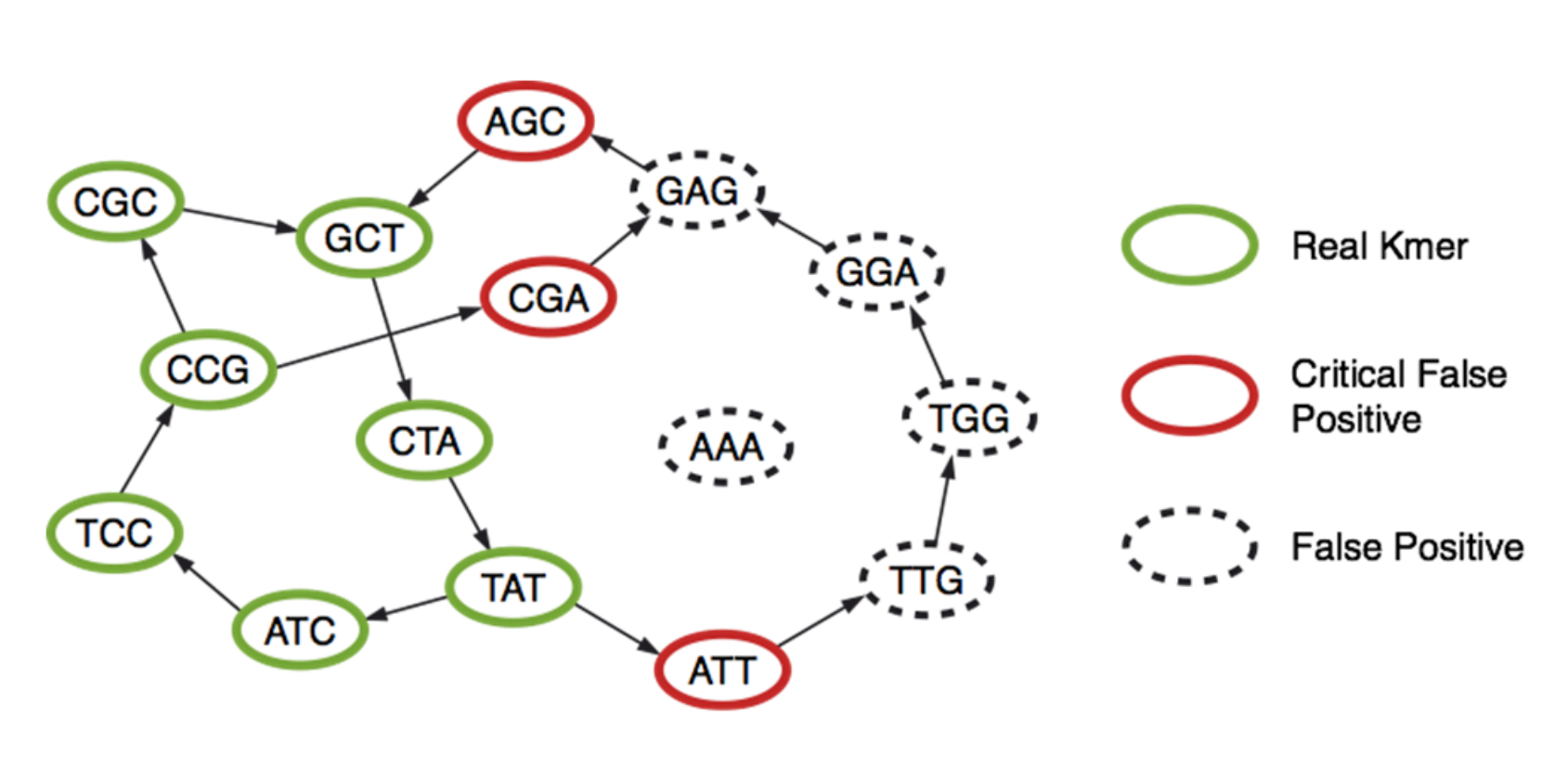
[1] github.com/jts/sga
[2] github.com/iskana/SlideSort
[3] github.com/lh3/miniasm
[4] github.com/GATB/gatb-core
[5] github.com/GATB/bcalm
[6] github.com/bcgsc/abyss

Focus on the Bloom Filter dBG representation (prelude to GATB)



Adapted from slides by G. Collet

Focus on the Bloom Filter dBG representation (prelude to GATB)



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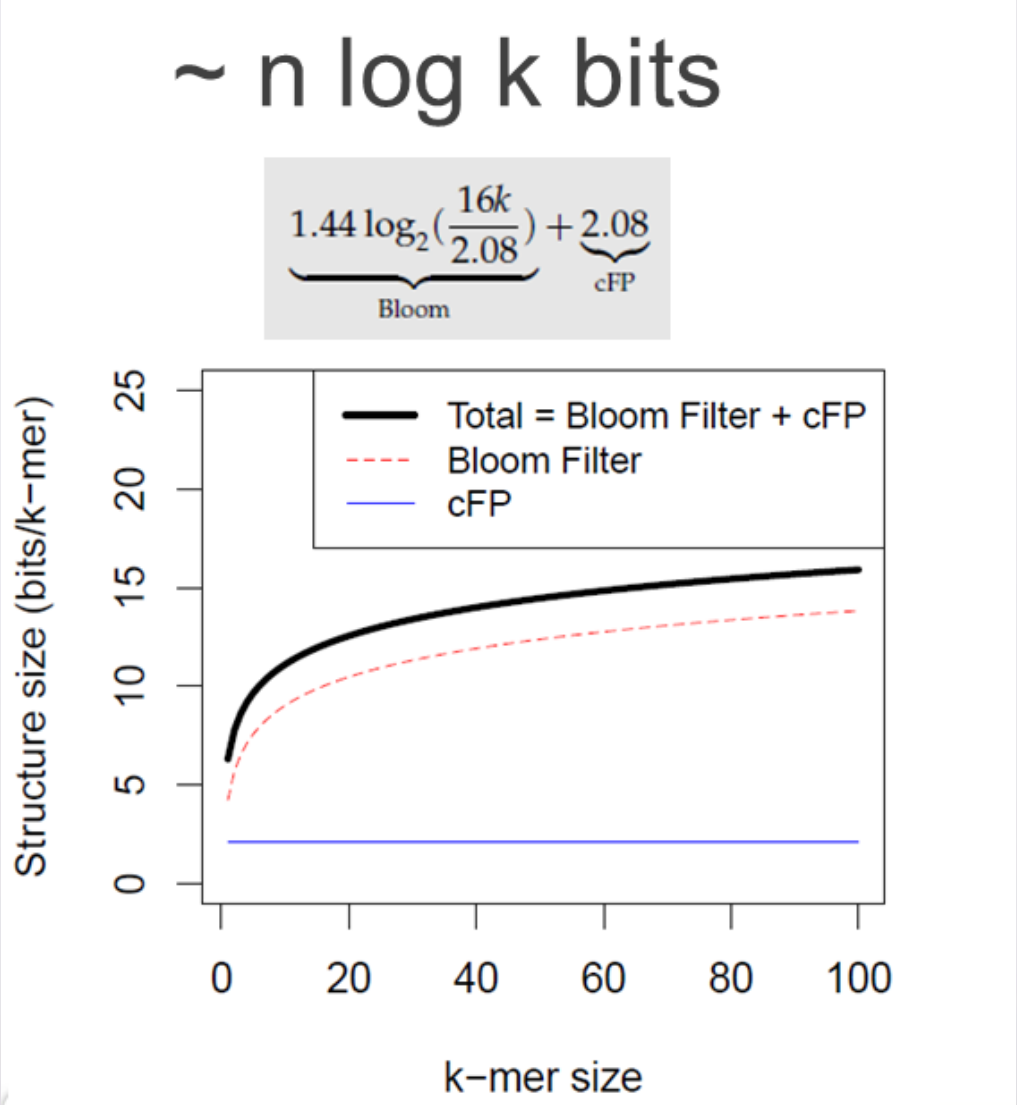
Focus on the Bloom Filter dBG representation (prelude to GATB)



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Compacted de Bruijn graph

Compacted de Bruijn graph:

Each non-branching path becomes a single node (*unitig*).

- no loss of information
- less space

Can be constructed using BCALM.
Some assemblers use it as intermediate representation.

A-Bruijn graphs

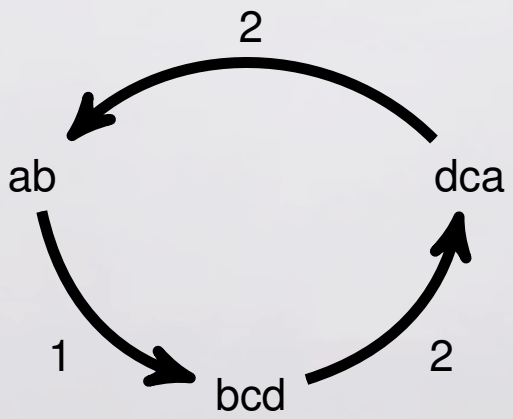
Introduced by [Pevzner 2004]. Generalize de Bruijn graphs.
Recently used in ABruijn assembler (PacBio) [Lin 2016].

Principle: **nodes** = an arbitrary set of words V.
To construct **edges**, given a set of strings (the reads):

- For each string r, find **substrings** that are elements of V.
- Add $v_1 \rightarrow v_2$ when v_1 is *just before* v_2 in r.
- Label edge with $(pos(v_2, r) - pos(v_1, r))$.

Observe that $dBG(Reads) = ABruijn(V = \Sigma^k, E = Reads)$

Ex: $ABruijn(V = \{ab, dca, bcd\}, abcdcab)$:

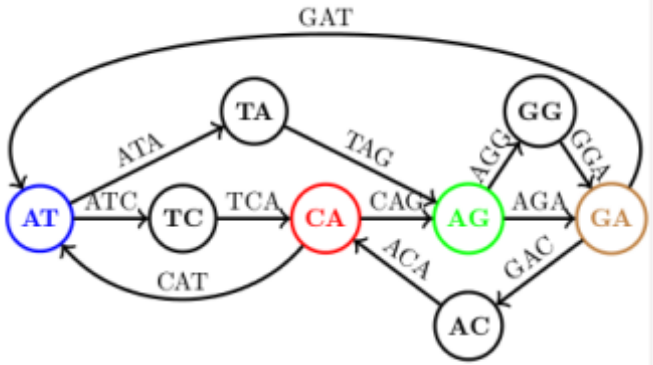


de Bruijn vs A-Bruijn

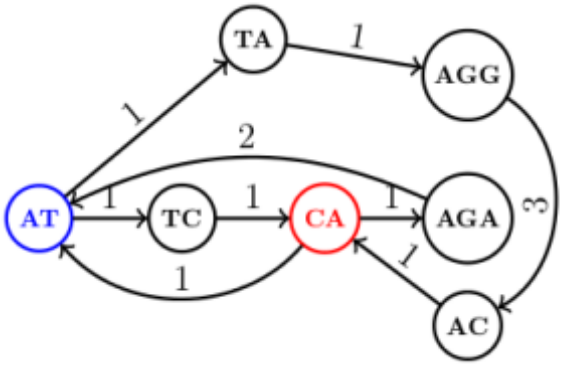
String=CATCAGATAGGGA.
For dBG: $k = 2$,
edge-centric.

For A-Bruijn, V = {CA, AT, TC, AGA, TA, AGG, AC} (V is arbitrary here)

$DB(String, 3)$



$AB(String, V)$



A-Bruijn usages

- Original paper — Sanger, handling repeats in EULER [Pevzner 2004]
- SPAdes (early vers) — Illumina DNA/metaDNA assembler [Bankevich,. . . ,Pevzner 2012]
- ABruijn — Pacbio assembler [Lin,. . . ,Pevzner 2016]

Superstring graph

[Cazaux, Sacomoto, Rivals 2016]

Consider a set of reads **P** (none is contained in another).
Consider all maximal overlaps between these reads, **Ov**(**P**).

Given a set of strings **F** (also none contained), remove all overlaps that are substrings of F. Call this set $\mathbf{Ov}^*(\mathbf{P}, \mathbf{F})$.

E.g.

$P = \{abcc, ccd, cde\}$,

$Ov(P) = \{cc, cd, c\}$.

Now if $F = \{cdef\}$,

$Ov^*(P, F) = \{cc\}$.

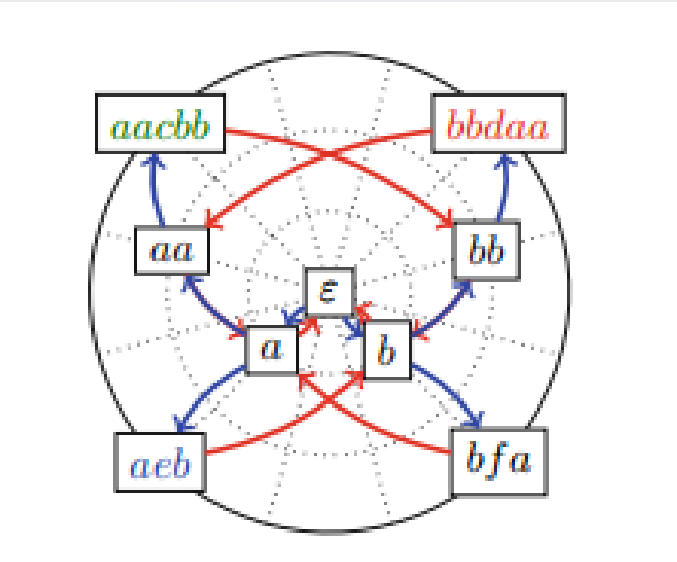
Superstring graph (2)

Recall, $Ov^*(P, F)$ are maximal overlaps between words in P, except substrings in F. Typically $F = \Sigma^{k_{min}-1}$.

The Truncated Hierarchical Overlap Graph, **THOG**(**P**, **F**) is a graph, where:

- nodes = P and $Ov^*(P, F)$
- edges = whenever a node is the longest suffix or the longest prefix of another node

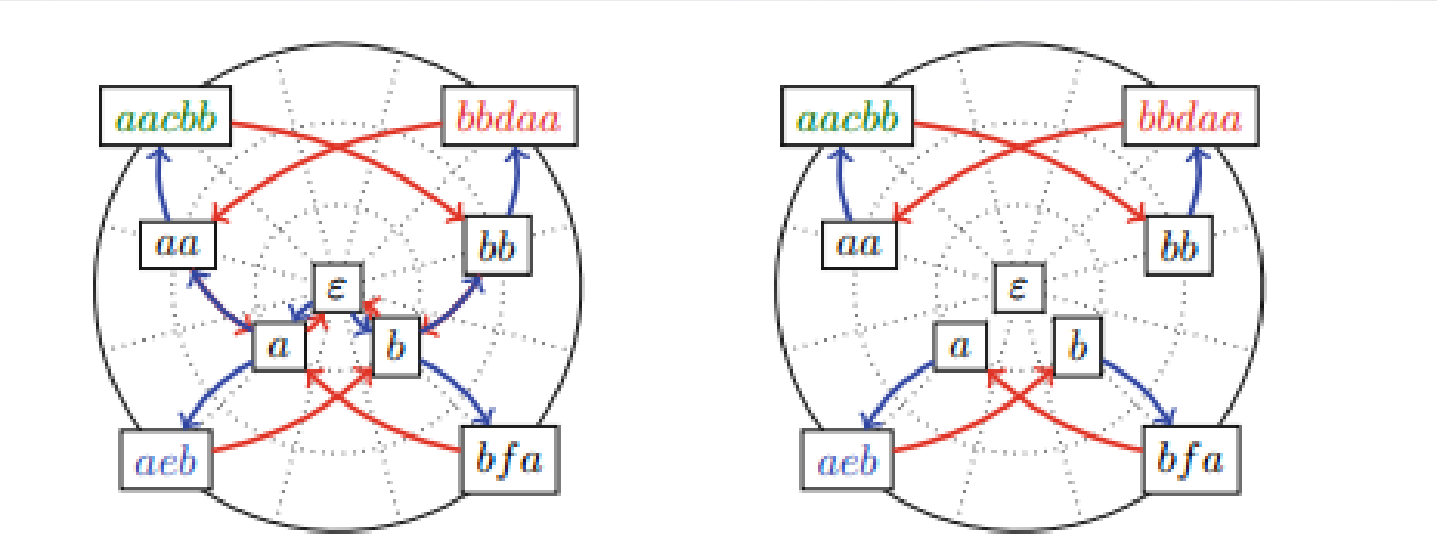
E.g. $THOG(\{aacbb, bbdaa, aeb, bfa\}, \emptyset)$



Superstring graph (3)

The **superstring graph** is defined as a subgraph of *THOG*.

It consists of a set of paths in *THOG* that correspond to a greedy solution of a computational problem (Constrained Shortest Cyclic Cover of Strings).



Applications:

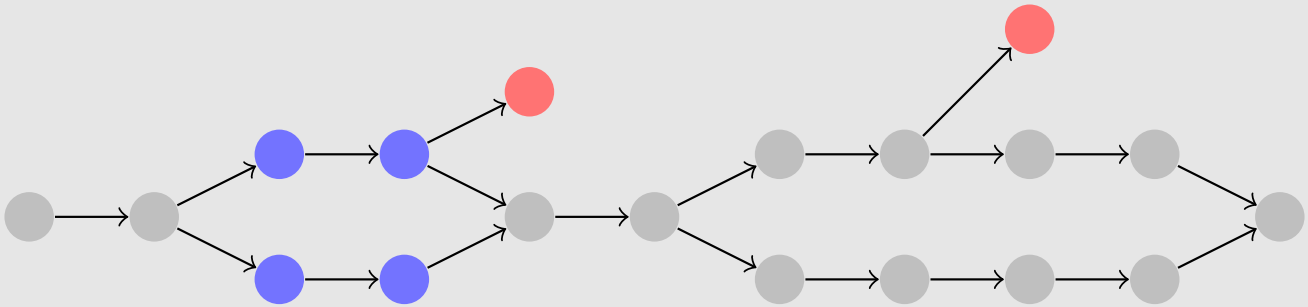
- superstring problems (CompSci)
- multi-k assembly

How an assembler works

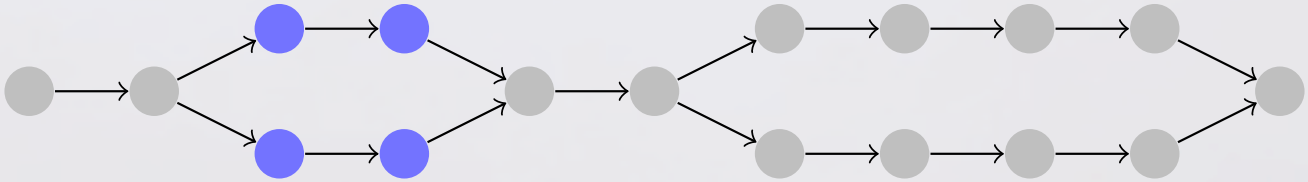
[SPAdes, Velvet, ABySS, SOAPdenovo, SGA, Megahit, Minia, .., HGAP, FALCON]

1) Maybe correct the reads. (SPAdes, HGAP, SGA, FALCON)
2) Construct a graph from the reads.

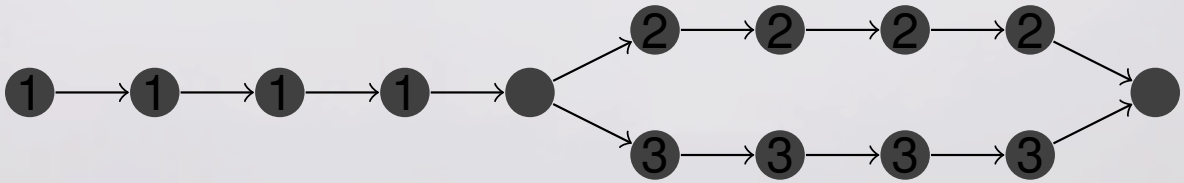
Assembly graph with variants & errors



3) Likely sequencing errors are removed. (not in FALCON)



3) Known biological events are removed. (not in FALCON)
4) Finally, **simple paths** (i.e. contigs) are returned.



The choice of k

Choice of k is critical in dBG applications:

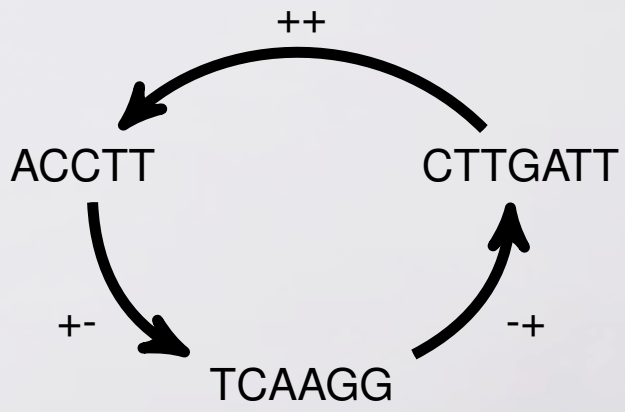
- k-mers with sequencing errors are noise
- only *non-erroneous* k-mers matter
- $k < \log_4(|\text{genome}|)$: nearly complete graph, uninformative
- small k: collapses repeats, more non-erroneous k-mers
- large k: less repeat collapsing, less non-erroneous k-mers (due to error and shortness of reads)

Generally, $k \geq 20$.
(Compare 4^k to the genome size.)
Higher sequencing coverage means larger k values can be used.

Graph formats

- FASTG
- **GFA**
- GFA2

```
H    VN:Z:1.0
S    11   ACCTT
S    12   TCAAGG
S    13   CTTGATT
L    11   +    12   -    4M
L    12   -    13   +    5M
L    11   +    13   +    3M
P    14   11+,12-,13+ 4M,5M
```



Conclusion

We saw many graphs

- Overlap graph
- String graph
- De Bruijn graph
- A-Bruijn graph
- Superstring graph

Those slides will be available at `http://rayan.chikhi.name`

Enjoy the workshop!