De Novo Repeat Classification and Fragment Assembly (Pevzner, et. al. 2004)

Presenter: Taher Mun

Introduction

Tool presented: RepeatGluer

Concepts introduced: A-bruijn graph

Motivation: Need a good way to represent repeats in genomes while **preserving their mosaic structure** and **repeat-boundaries**

Why is repeat representation important?

- Many problems require boundaries of sub-repeats to be defined:
  - Finding mobile elements
  - Determining nature of segmental duplication - evolution
  - Assembly from short reads
  - (in my case) - alignment to repeat regions

Previous work

- RepeatMasker, MaskerAid
  - Use repeat libraries defined by RepBase
- RepeatMatch, REPuter
  - Simply lists repeats
  - Doesn’t show any underlying structures

(A) Genomic dot-plot of an imaginary sequence with repeats containing sub-repeats.

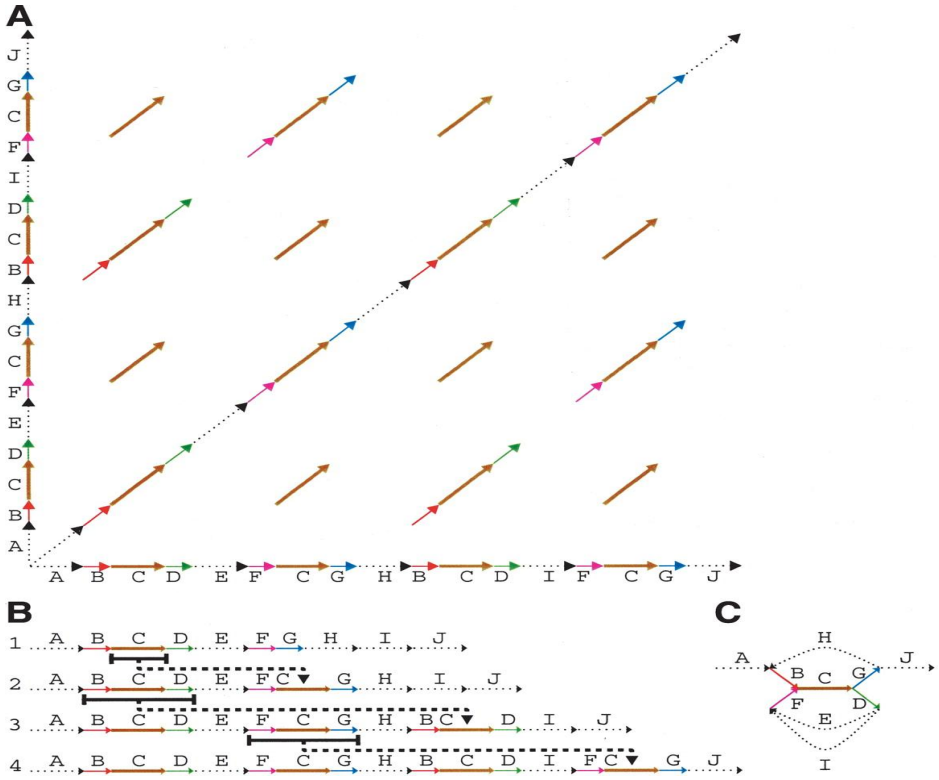

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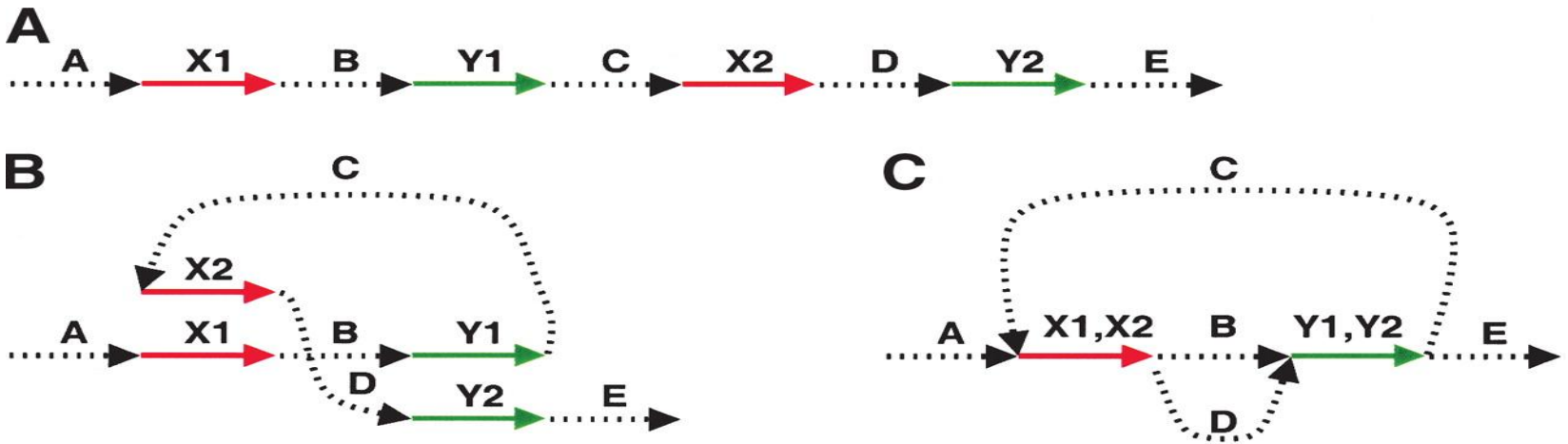
Figure 1

- Sub-repeats within repeats
  - Determining repeat boundaries?
  - Determining repeat structure?
- De-bruijn graph good way to represent (1C)
  - sub-repeats only occur once per edge
  - Though, only good for “perfect” repeats
  - Real genomic repeats are not perfect - indels, mismatches
- How to allow for imperfect pairwise alignments?
  - A-bruijn graph

(A) A hypothetical DNA sequence with unique regions A, B, C, D, E and repeats X (appearing twice as X1 and X2) and Y (appearing twice as Y1 and Y2).



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Mosaic repeat organization of BAC from human Chromosome Y. For purposes of illustration, only sufficiently long and very conservative repeats are shown.

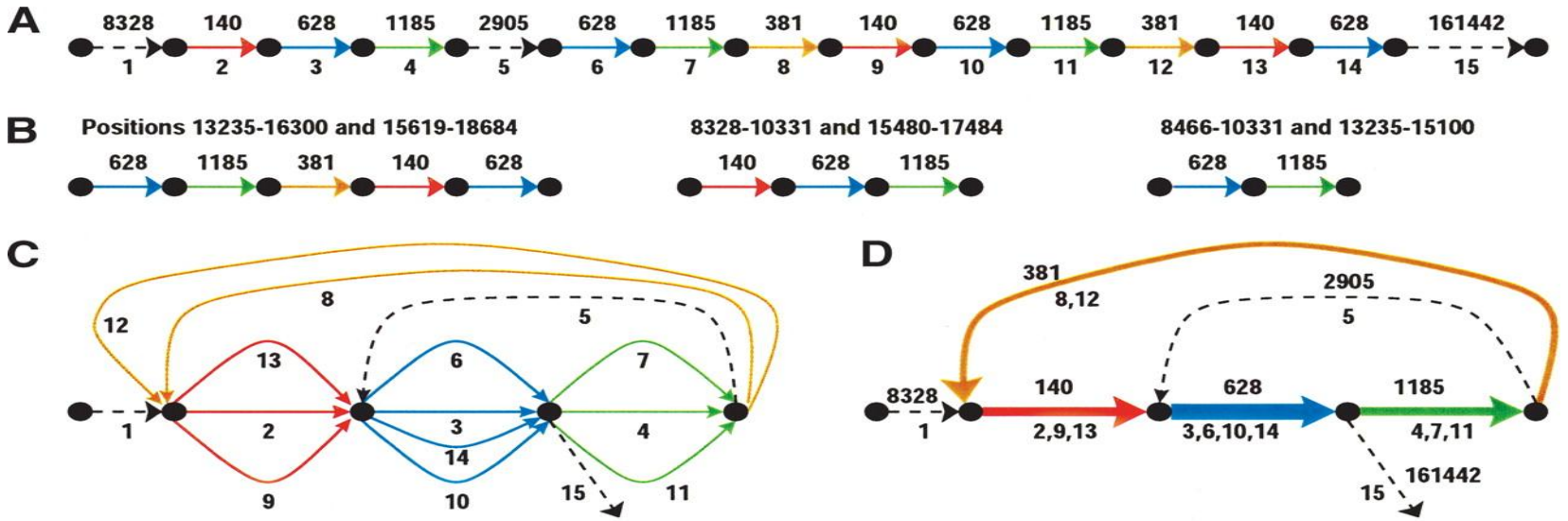

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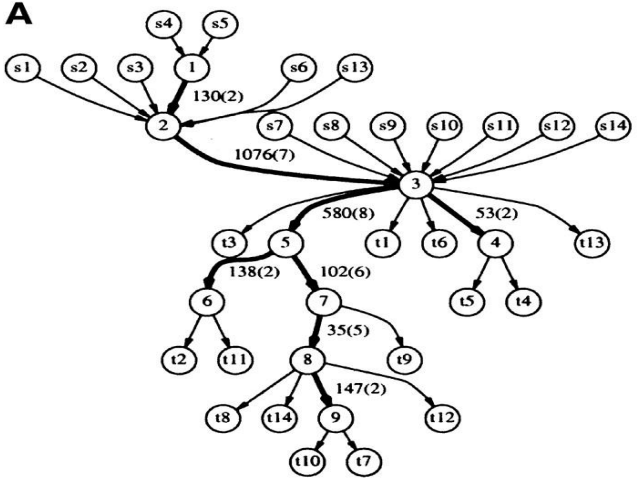
Figure 3

- Real world example - BAC from Chromosome Y
- Repeats + subrepeats present (3A)
- REPuter doesn’t capture sub-repeats (3B)
- A-bruijn graph approach does (3CD)

RepeatGluer representation of a 14-copy transposase IS30 repeat family in the N. meningitidis genome as a mosaic of eight sub-repeats >30 bp (shown by bold edges).

A



B

	Repeats		Sub-repeats w/in a repeat	
	# of repeat copies	Maximum length of repeat copy	# sub-repeats longer than 30bp	Maximum length of a sub-repeat
1	20	4371	37	1695
2	14	1794	8	1076
3	4	6317	3	6091
4	4	740	1	740
5	3	1475	3	641
6	3	620	4	180
7	3	838	4	387
8	2	2775	1	2775
9	2	2700	1	2700
10	2	523	1	523
11	2	494	1	494
12	2	474	1	474
13	2	456	1	456
14	2	446	1	446
15	2	420	1	420
16	2	415	1	415
17	2	410	1	410
18	2	406	1	406
19	2	402	1	402

C

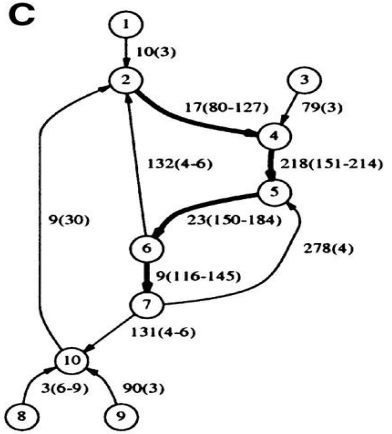


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Figure 4

- *N. meningitis* genome
- Evolutionary history of duplication captured in A-bruijn graph (4A)
  - One repeat family shown - each member contains 1-4 subrepeats
- No. of repeat families and their subrepeats (4B)
- Alu repeats in ChrX (4C)

A-bruijn graph

- Generalization of DBG for imperfect repeats
- Terms:

  Let S = genome of length n

  Let A = (a_ij) = n x n similarity matrix

  Let α = all pairwise local alignments from S to S

  a_ij = 1 iff pos. i and j are aligned somewhere in α, 0 otherwise.

A-bruijn graph, cont’d

A-graph is graph based on adjacency matrix formed by A

V = set of all connected components in A-graph

A-bruijn graph is G(V, E) -

- V - all the connected components collapsed into single vertices
- E - Eulerian path from 1->n in A-graph.

A-bruijn graph, cont’d

- α can be any arbitrary set of pw alignments
    - Ex. a DBG is an ABG where α is all k-mers in S (!)
    - Allows ABGs to be extended in cools ways (explored later)
- Here, edges represent multiple sequence alignments.
- Analysis + simplification complicated by Whirls and Bulges

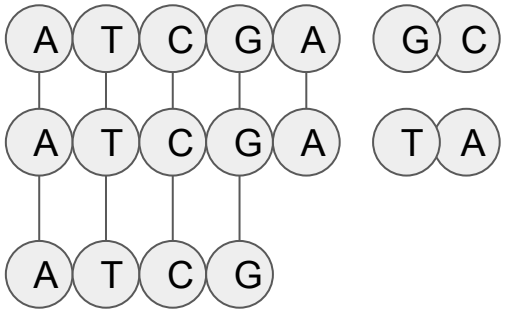
A-bruijn graph example

ATCGAGC**ATCGA**TA**ATCG**

Alignments:

ATCGA	ATCGA	ATCGA
ATCGA	ATCG	ATCG

Create A: (edges only between 1s in A matrix)



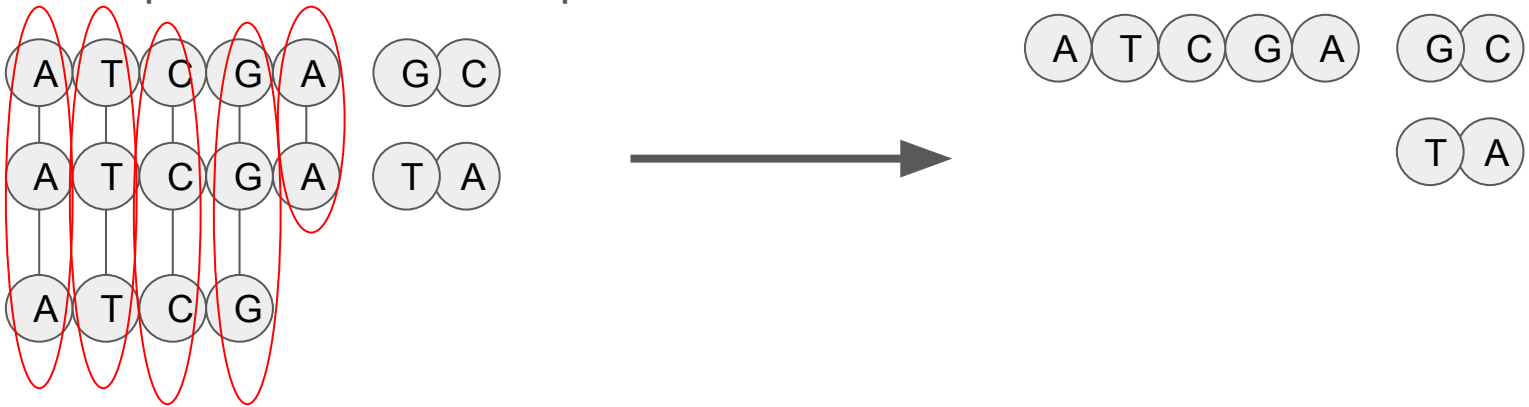
A-bruijn graph example

ATCGAGC**ATCGA**TA**ATCG**

Alignments:

ATCGA	ATCGA	ATCGA
ATCGA	ATCG	ATCG

Collapse connected components



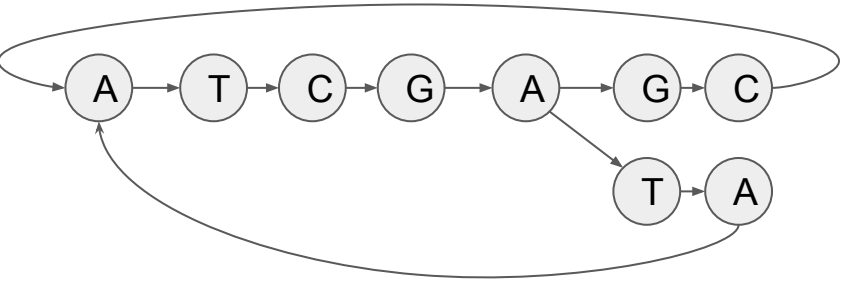
A-bruijn graph example

ATCGAGC**ATCGA**TA**ATCG**

Alignments:

ATCGA	ATCGA	ATCGA
ATCGA	ATCG	ATCG

Create edges



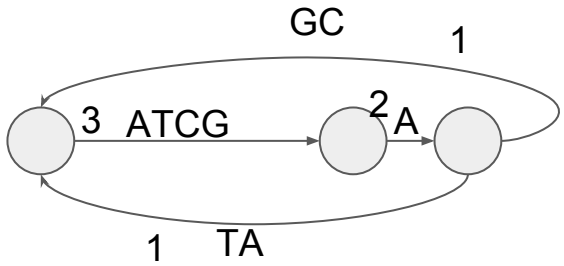
A-bruijn graph example

ATCGAGC**ATCGA**TA**ATCG**

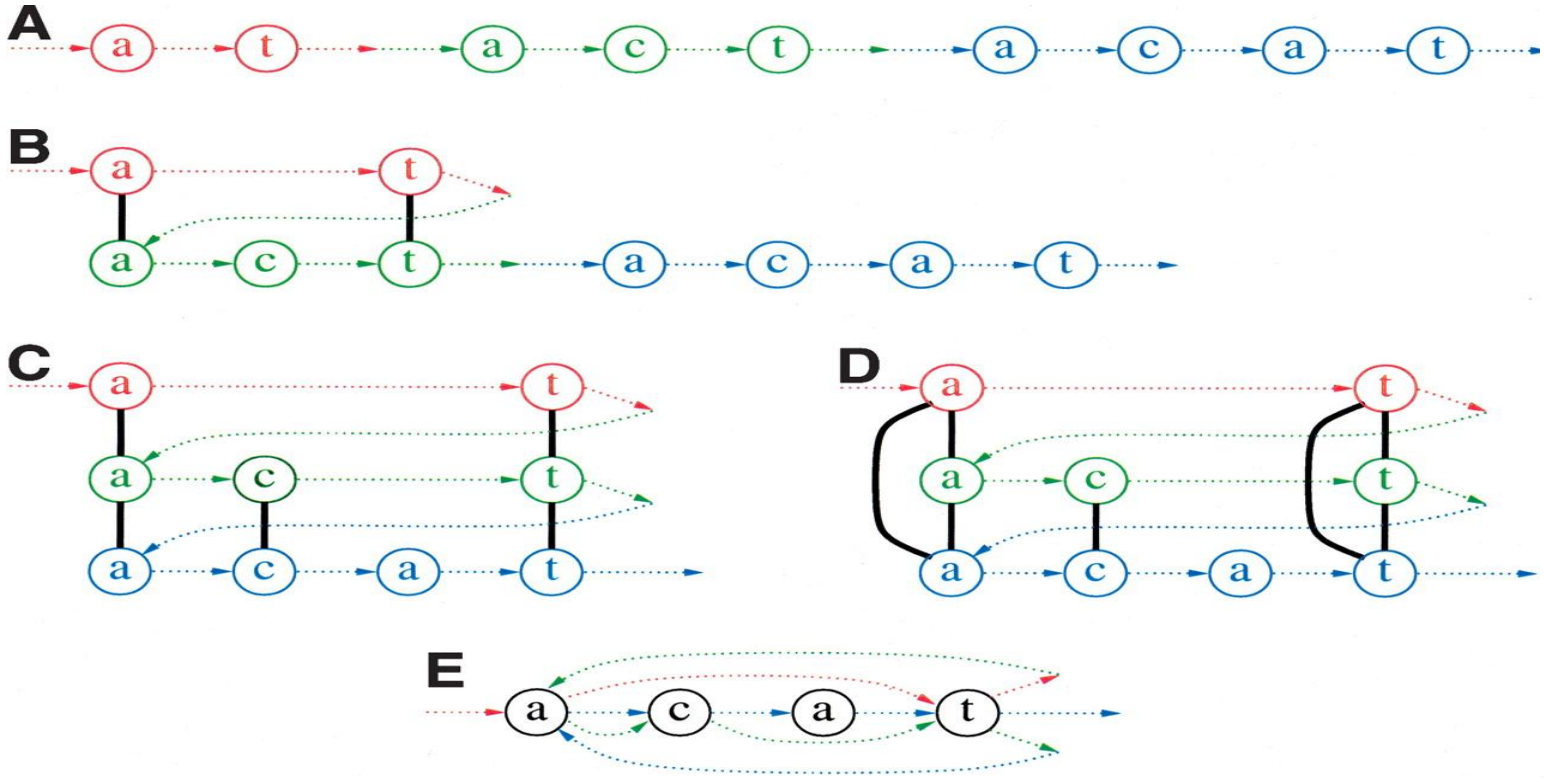
Alignments:

ATCGA	ATCGA	ATCGA
ATCGA	ATCG	ATCG

simplify



(A) Construction of the A-graph from the sequence...at...act...acat by applying three pairwise alignments (B) a-t versus act, (C) act versus acat, and (D) a-t versus acat.



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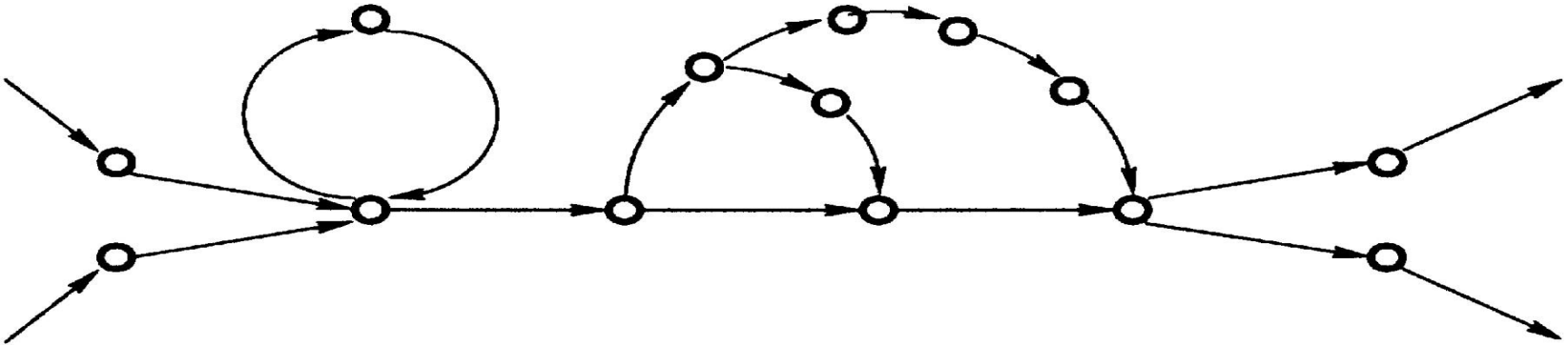
Whirls and Bulges

- Types of short cycles
- Whirl - edges all oriented the same dir.
  - Caused by inconsistent MSAs, tandem repeats
- Buldge - edges oriented in both dirs
  - Caused by gaps - gap of length g -> bulge of length g + 2

A repeat region in an A-Bruijn graph in which alignment inconsistencies have caused a whirl and a network of bulges.



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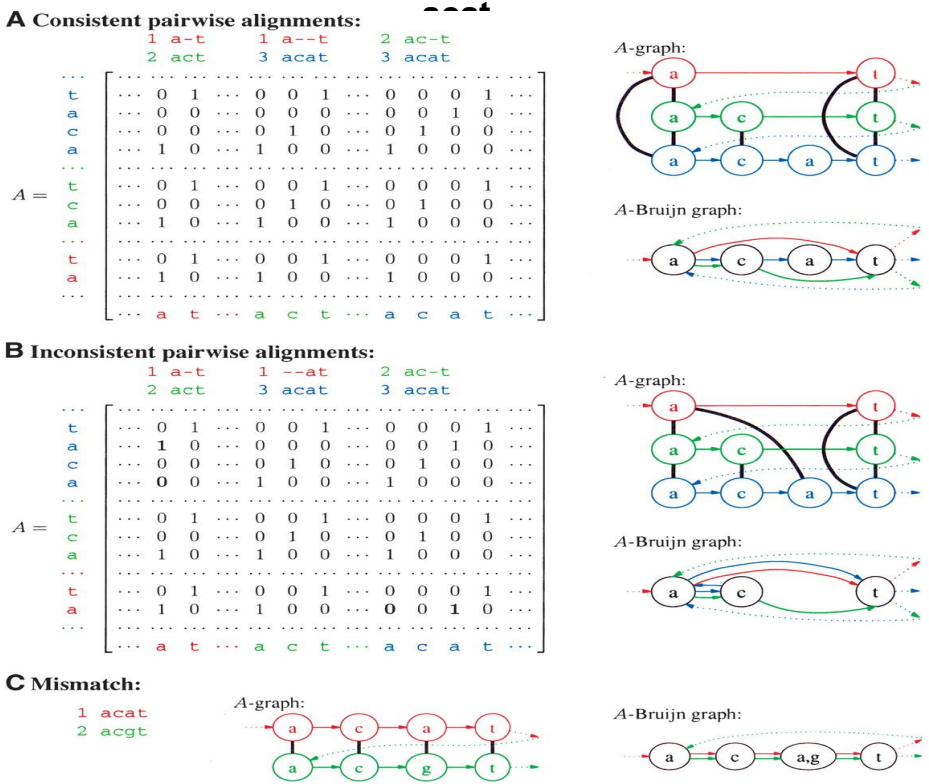
Construction of A-Bruijn graphs from (A) consistent pairwise alignments and (B) inconsistent pairwise alignments, for the genomic sequence...at...act...acat..with a repeat represented by three copies: at, act, acat



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Removing whirls

- 1 vertex represents a set of positions (P(v))
- A composite vertex contains positions that are “near” in genome
- Get max-multiplicity edge M connecting composite to non-composite vs
- Split composite v according to positions defined by M, delete edges from A accordingly.
- Repeat until no composite vertices exist
- This process separates “close” repeats and removes whirls

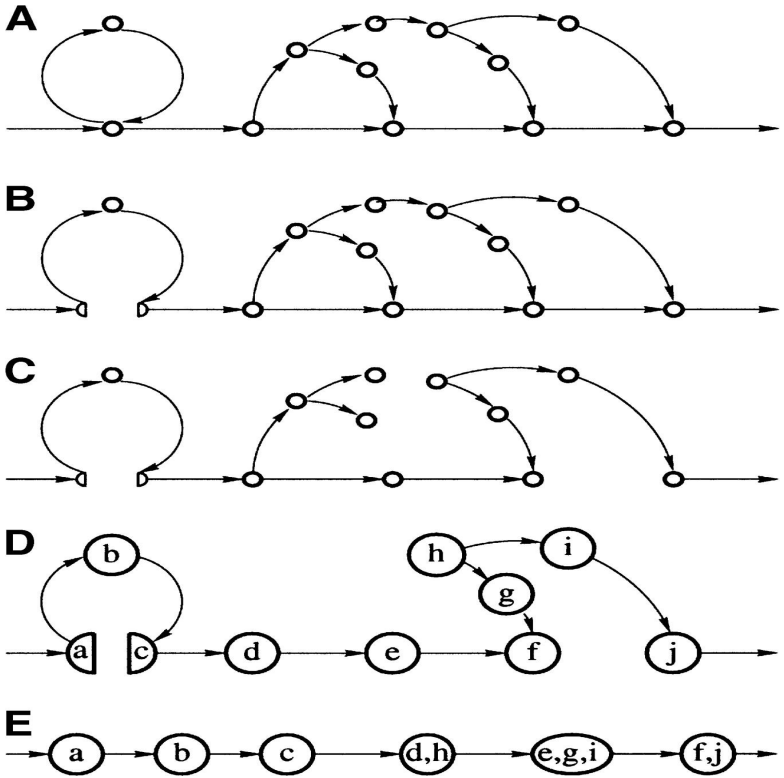
Removing bulges

- Fixed by removing a single edge
  - Which edge do we remove?
- Solve the Maximum Subgraph with Large Girth problem
  - (MST problem with arbitrary minumum cycle length)
  - This is hard, so they use an approximation
- Find a MST T, and add largest multiplicity edges possible, such that no short cycles are formed

Other clean-up procedures

- Erosion - remove leaves left by bulge removal
- Straighten zigzags
  - Erosion -> long simple paths.
  - These paths might not be unidirectional (zigzag)
  - Fix this by uniting vertices w/ same consensus nucleotide
- Threading
  - Graph now disjoint
  - “Thread” graph together using positions in S represented in each vertex v
  - This creates a “consensus” sequence for each edge

(A) Initial A-Bruijn graph (weighted graph representation instead of multigraph).



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Applications:

- Multiple alignments - concatenate all sequences, create de-bruijn graph
  - Partial order alignment
  - Order-independent
  - Raphael, B., Zhi, D., Tang, H., and Pevzner, P. (2004). A novel method for multiple alignment of sequences with repeated and shuffled elements. Genome Res. 14, 2336–2346.
- Fragment assembly - constructing ABG without similarity matrix
  - Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V.M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., et al. (2012). SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. Journal of Computational Biology 19, 455–477.
- Structural Variation Discovery - by alignment to A-bruijn graph
  - Lee, H., Popodi, E., Foster, P.L., and Tang, H. (2014). Detection of Structural Variants Involving Repetitive Regions in the Reference Genome. Journal of Computational Biology 21, 219–233.

Code location

https://github.com/COL-IU/RepGraph