Whole Genome Comparison: Project Presentations

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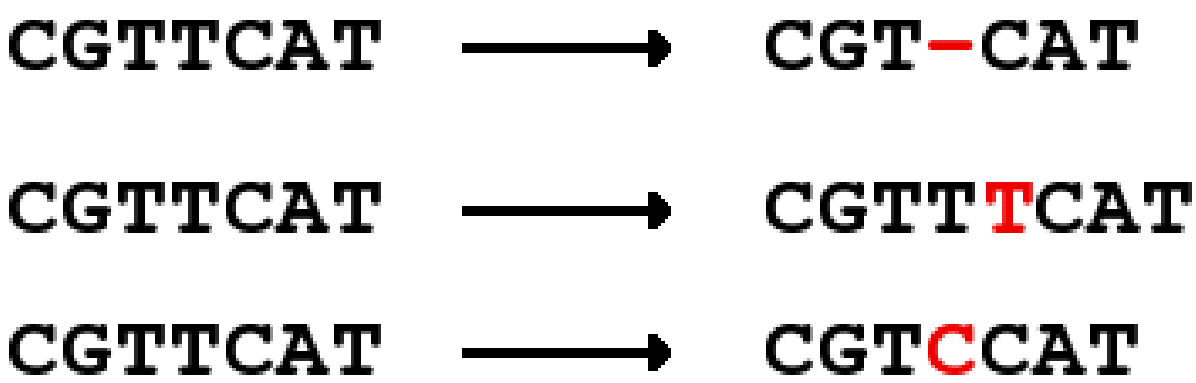
Svenja Specovius, John Wiedenhoeft

July 19, 2010

Outline

Evolutionary events

Nucleotide deletion, insertion and point mutation



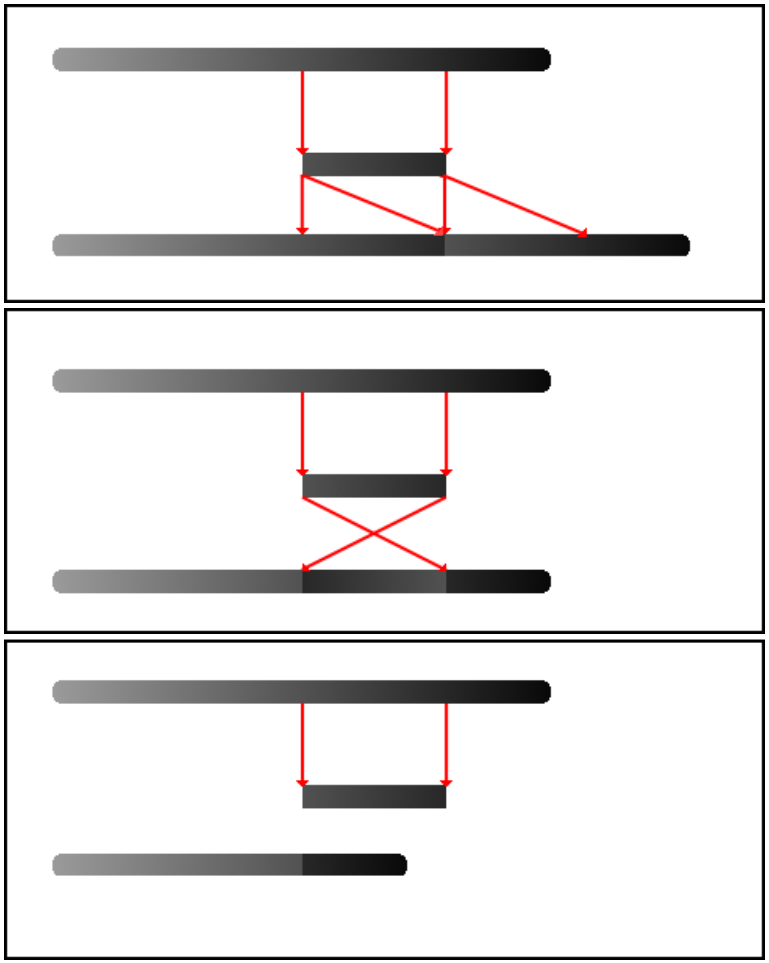
Collinear alignment

Columns of aligned sequences

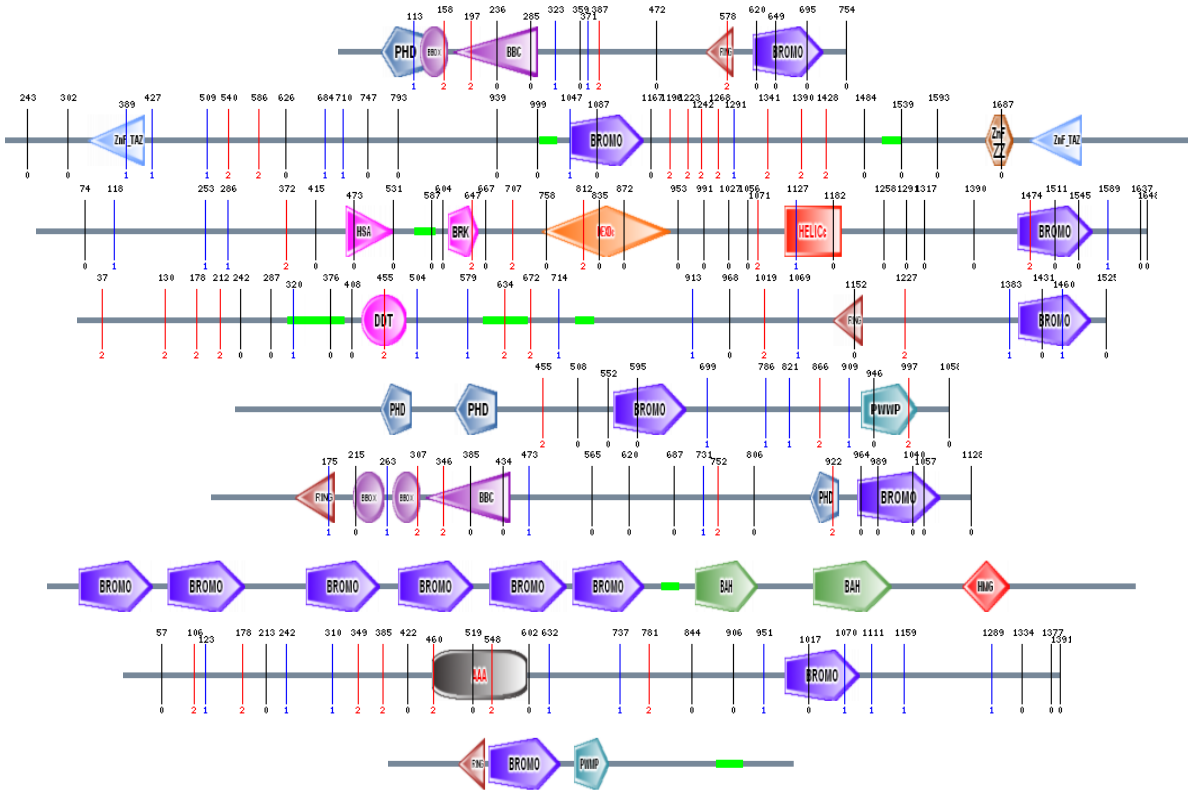
```
CONSENSUS       a.gttcctgc.tgcgtttgctggactgatgtactt.gtttgtgagg.caa
Hs#S663801      a.gttcctgc.tgcgtttgctggacttatgtactt.gtttgtgagg.caa
Hs#S337687      aagttcctgc.tgcgtttgctggactgatgtacttggtttgtgnaggcaa
Hs#S629177      a.gttcctgc.tgcgtttgctggactgatgtactt.gtttgtnagg.caa
Hs#S672957      a.gttcctgc.tgcgtttgct..........................
Hs#S672182      a.gttcctgc.tgcgtttgctggactgatgtactt.gttt.......
Hs#S674099      a.gttcctgc.tgcgtttgctggactgatgtactt.gtttgtgagg.caa
Hs#S196113      a.gttnctgn.tgngtttgctggactgatgtactt.gtttgtgagg.caa
Hs#S994400      ..........................gtacnt.gtttgtgagg.cta
Hs#S80460       a.gttcctgc.tgcgtttgctggactgatgtactt.gtttgtgagg.caa
Hs#S1988018     a.gttcctgc.tgcttttgctggactgatgtactt.gattgtgagg.caa
Hs#S1794113     a.gttcctgc.tgcgcttgctggactgatgtactt.gtttgtgagg.caa
Hs#S4698        a.gttcctgc.tgcgtttgctggactgatgtactt.gtttgtgcgg.caa
Hs#S813765      a.gt.cctgc.g.cgtttgc.ggacggatgtactt.gtt.gtgagg.caa
Hs#S1184845     .........................................g.caa
Hs#S1577463     ..........................................gg.caa
Hs#S914987      ......................ctgatgtactt.gtt.gtgagggcaa
Hs#S1985364     a.gttcctgc.tgcgtttgctggactgatgtactt.gtttgtgagg.caa
Hs#S1465644     ..gttc.tgcctgcgtttgctgaactgatgtactt.gttagt.aag.caa
Hs#S1850471     c.gttactgc.ggggtttgctggactcatg.actttgttngt.agg.caa
```

More evolutionary events

Genome rearrangements: duplication, reversal and deletion of segments

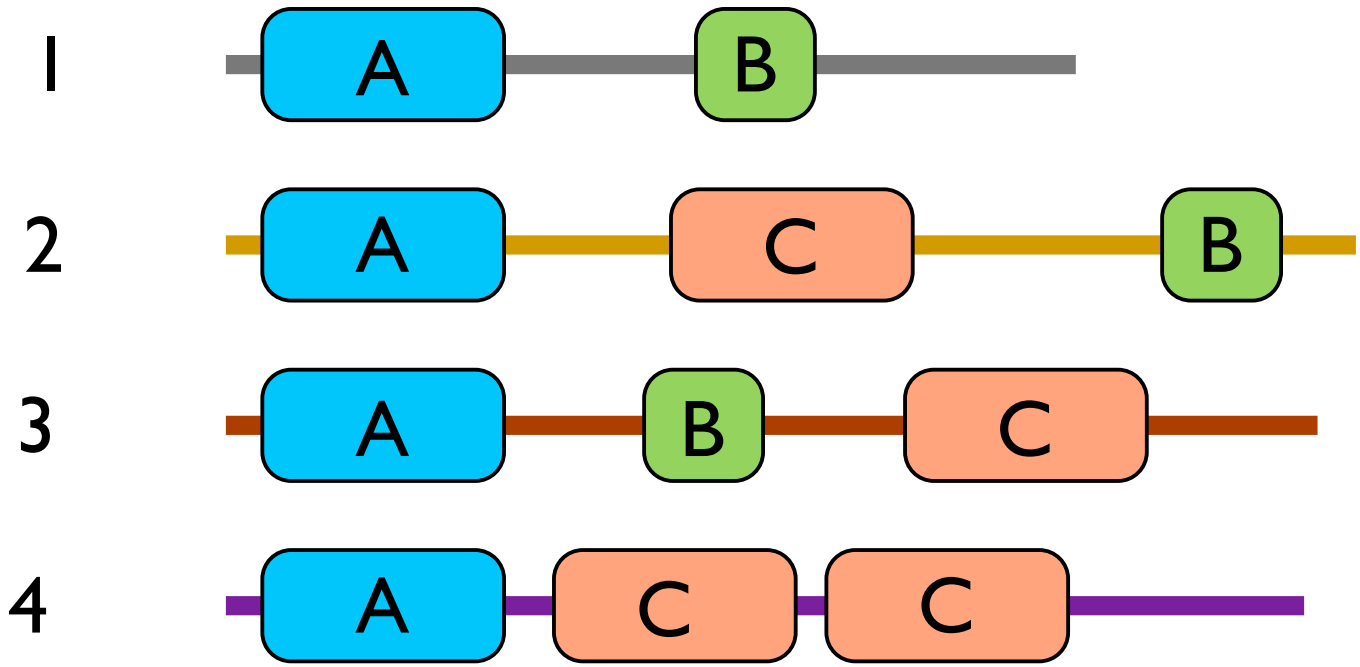


Multidomain proteins

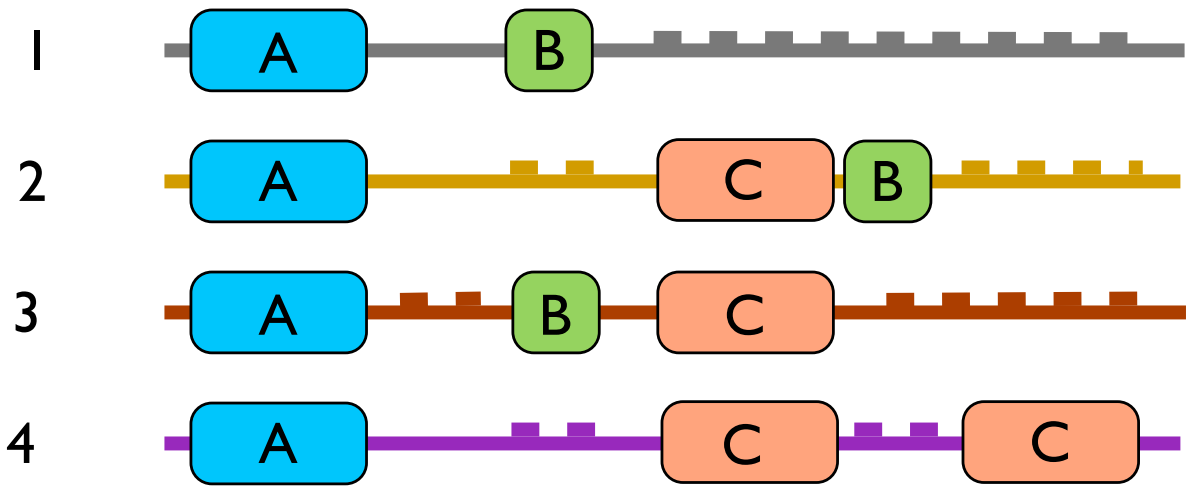


- Diverged by rearrangements of modular units, e.g. domains
- Multidomain proteins (MDPs) difficult to align collinearly

Multidomain protein toy example

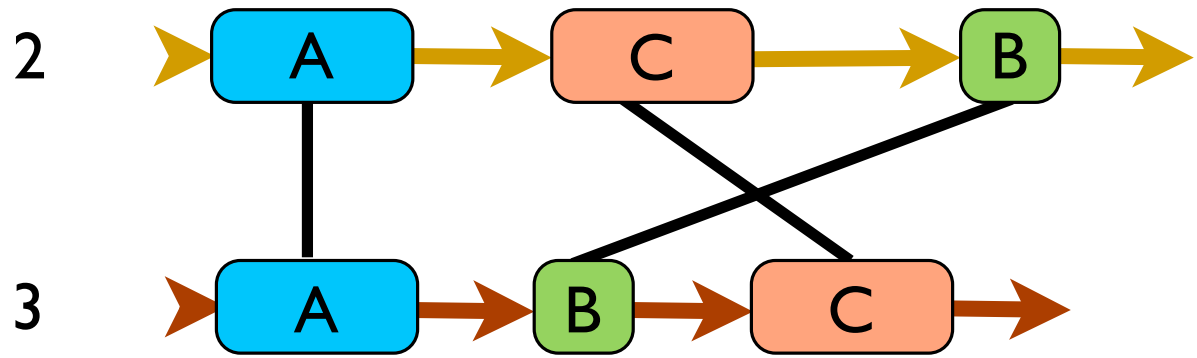


Collinear alignment



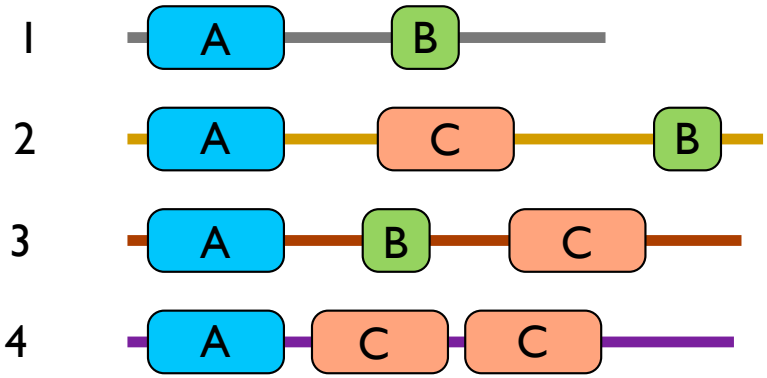
- It's not possible to align all similar domains without reordering

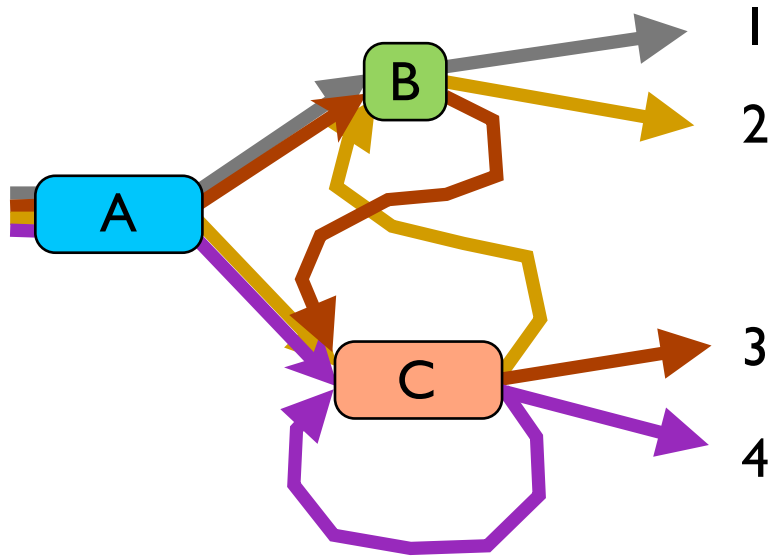
Graph representation of alignments



- Arcs: input sequences
- Edges: matches
- Some edges may be inconsistent: mixed cycles

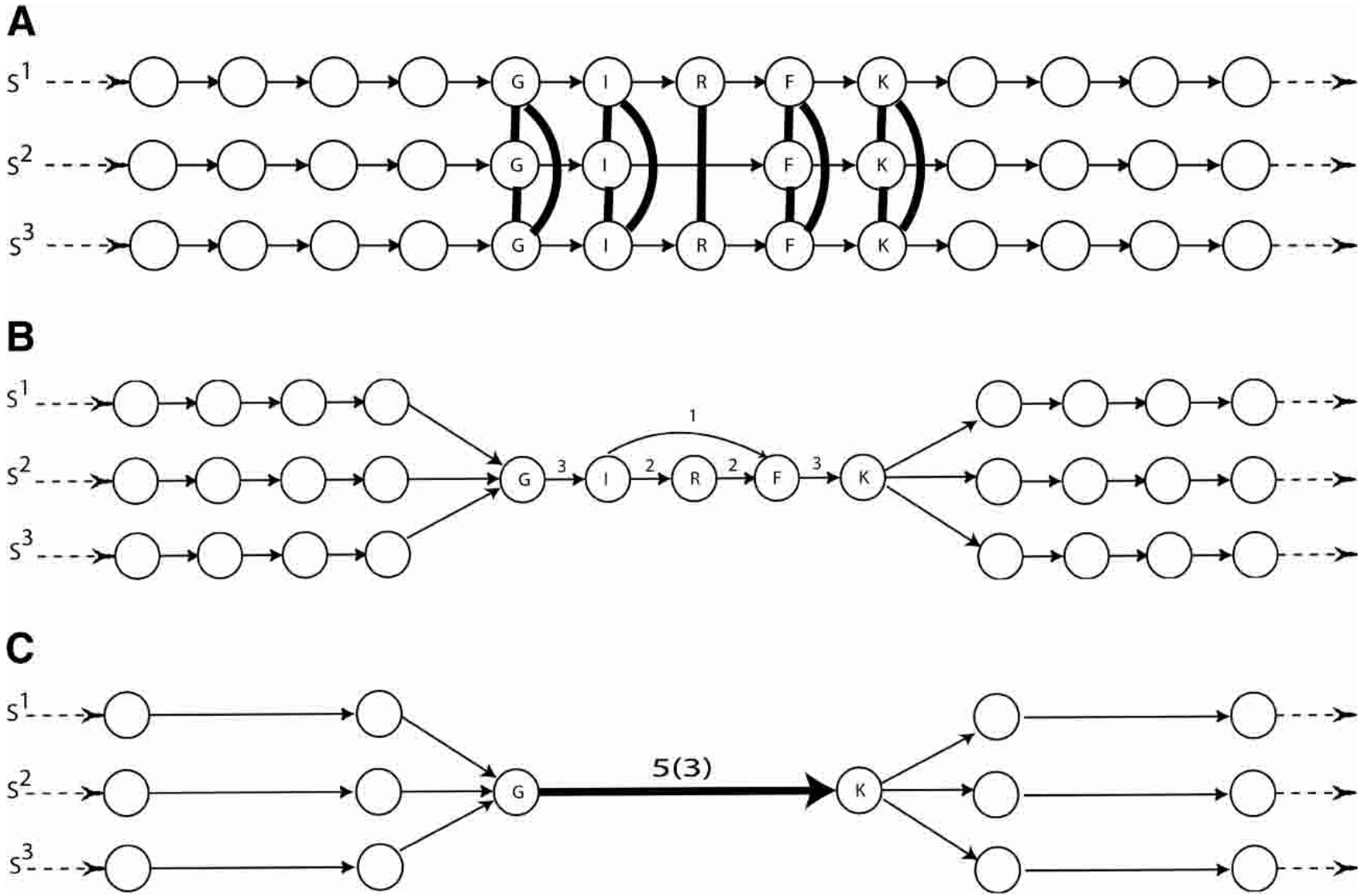
Non-collinear alignment



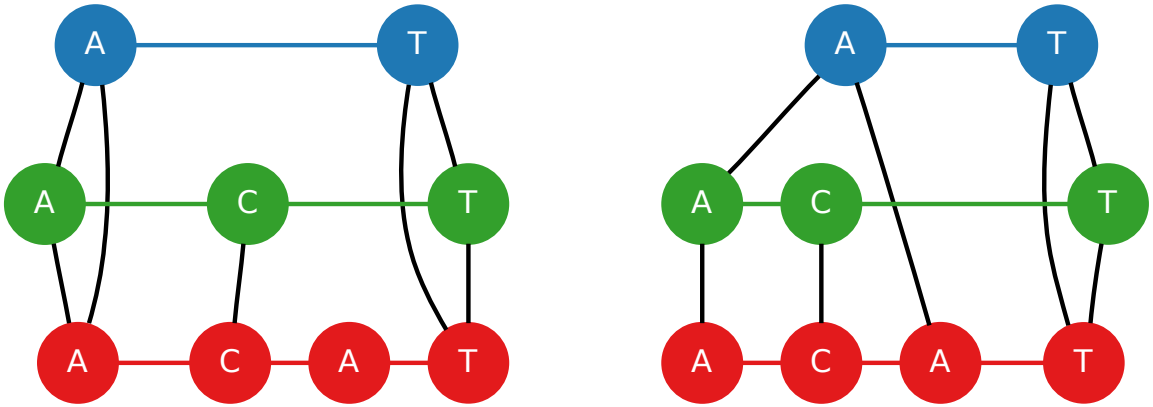


- Allow *large* cycles of *similar* segments

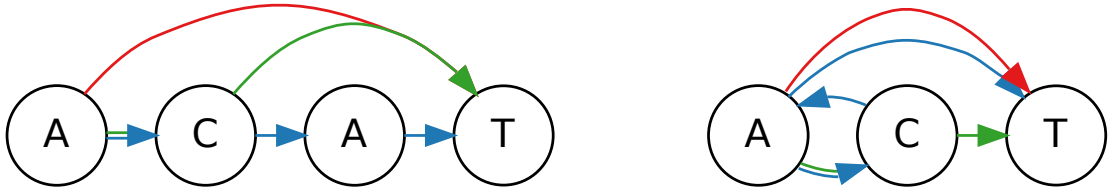
Construction of the A-Bruijn Graph



Whirls and inconsistencies



A-graph



A-Bruijn graph

Evaluating ABA

J. Wiedenhoeft

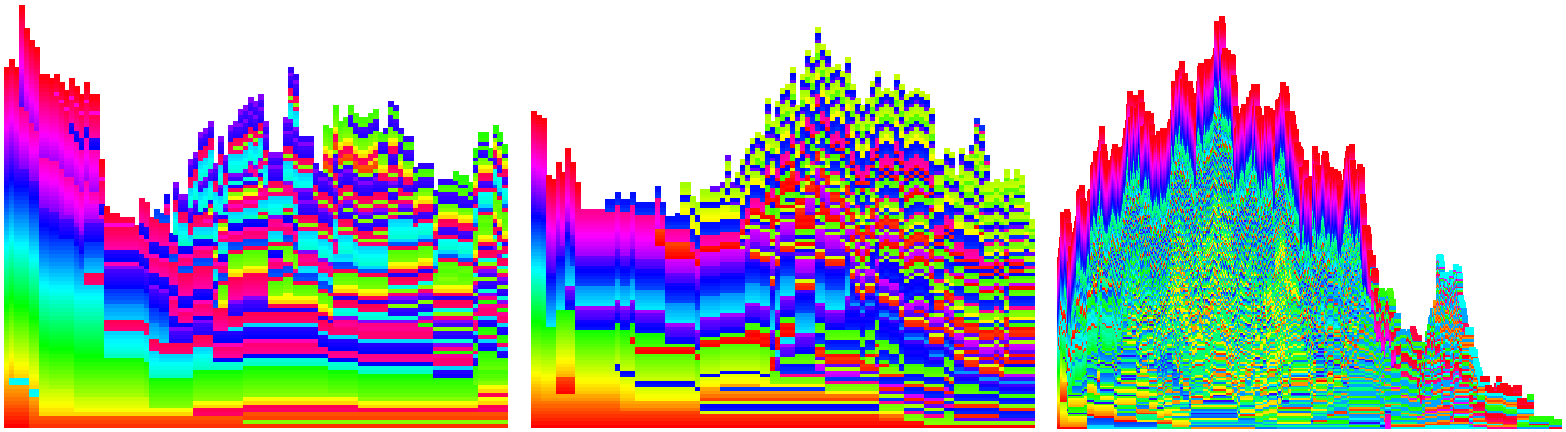
- simulate sequence evolution using PAM (*point accepted mutation*)
- two models of sequence evolution
  - geometric duplication/deletion model
  - rearrangement according to fragility model
- true homology can be tracked to provide a gold standard

PAM sequence evolution

- amino acid substitution modeled as a Markov process
- PAM = transition matrix
- using ABA's BLAST subroutine with PAM30 provides a null model of character homology

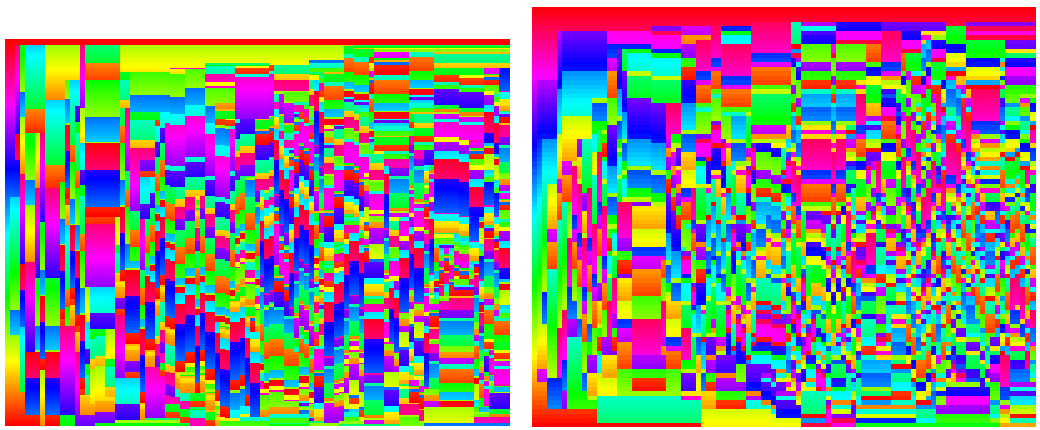
Geometric duplication/deletion model

- pick position by uniform distribution
- determine deletion or duplication by binomial distribution
- determine direction by binomial distribution
- determine length by geometric distribution



Fragility model

- models only translocations
- successful translocation *increases* the chance of a segment being translocated again $\Rightarrow$ models conservation of substructures
- boundaries weighted by length of substructure
- borders of substructures are preferred as insertion spots $\Rightarrow$ prevents disruption of other substructures

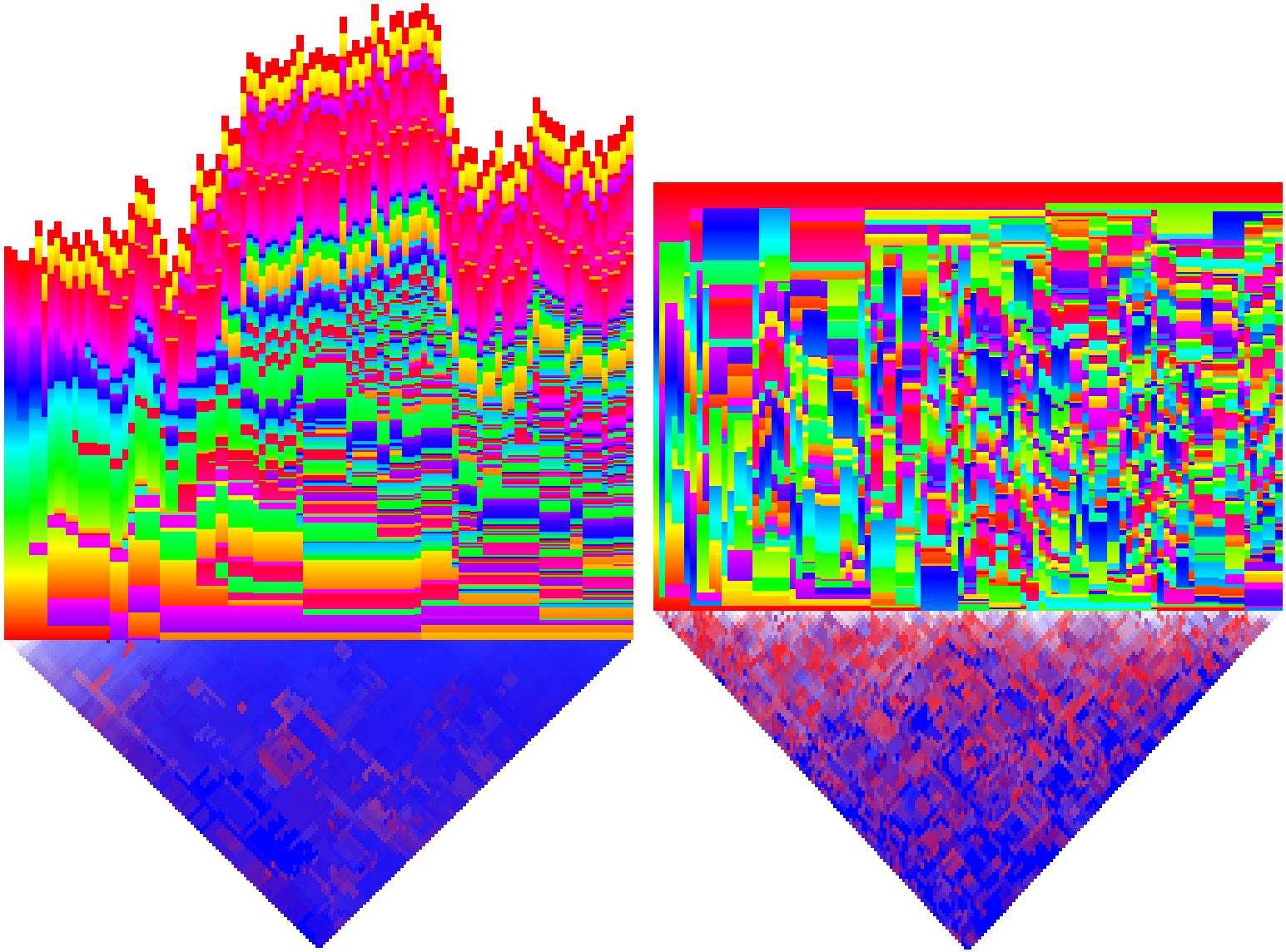


Score

- true negatives are vast due to the low number of paralogs and the alignment bias (BLAST)
- hence precision and accuracy are not suitable measures

$$\frac{\mathrm{FP} + \mathrm{FN}}{\mathrm{FP} + \mathrm{FN} + \mathrm{TP}}$$

Results



Analysing Multidomain Proteins with ABA

M. Homilius

- Noncolinear alignment applied on multidomain proteins (MDPs).
- Histone Deacetylation / Chromatin Remodeling Complexes.

HATs / CRCs

Regulation of gene expression.

Function of chromatin-remodeling complexes

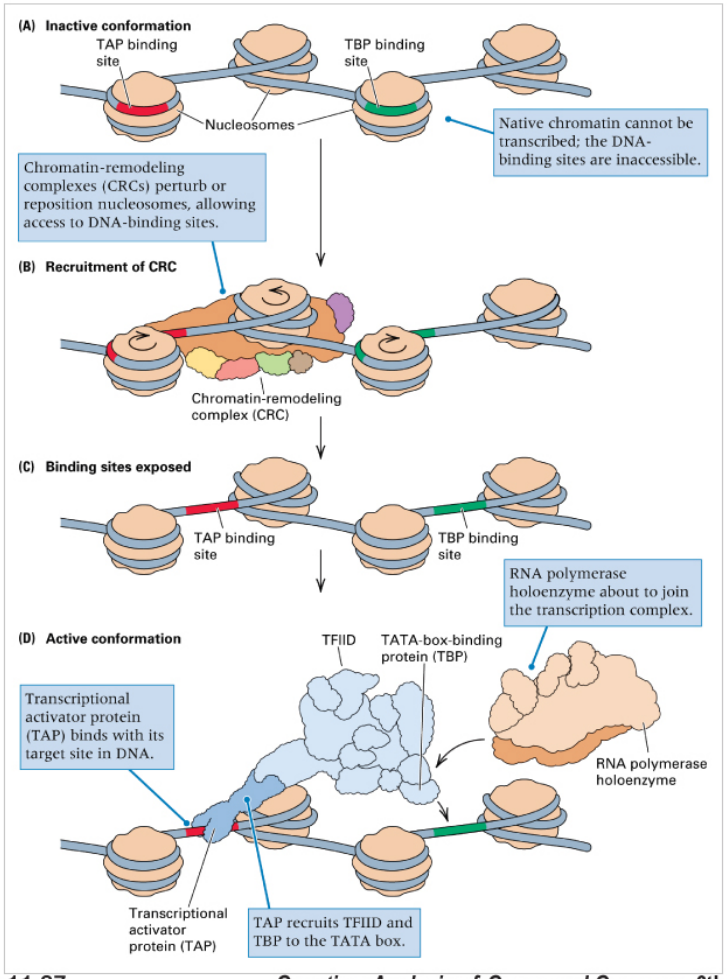


Figure 11.27 *Genetics: Analysis of Genes and Genomes*, 6th Edition
Hartl, Jones

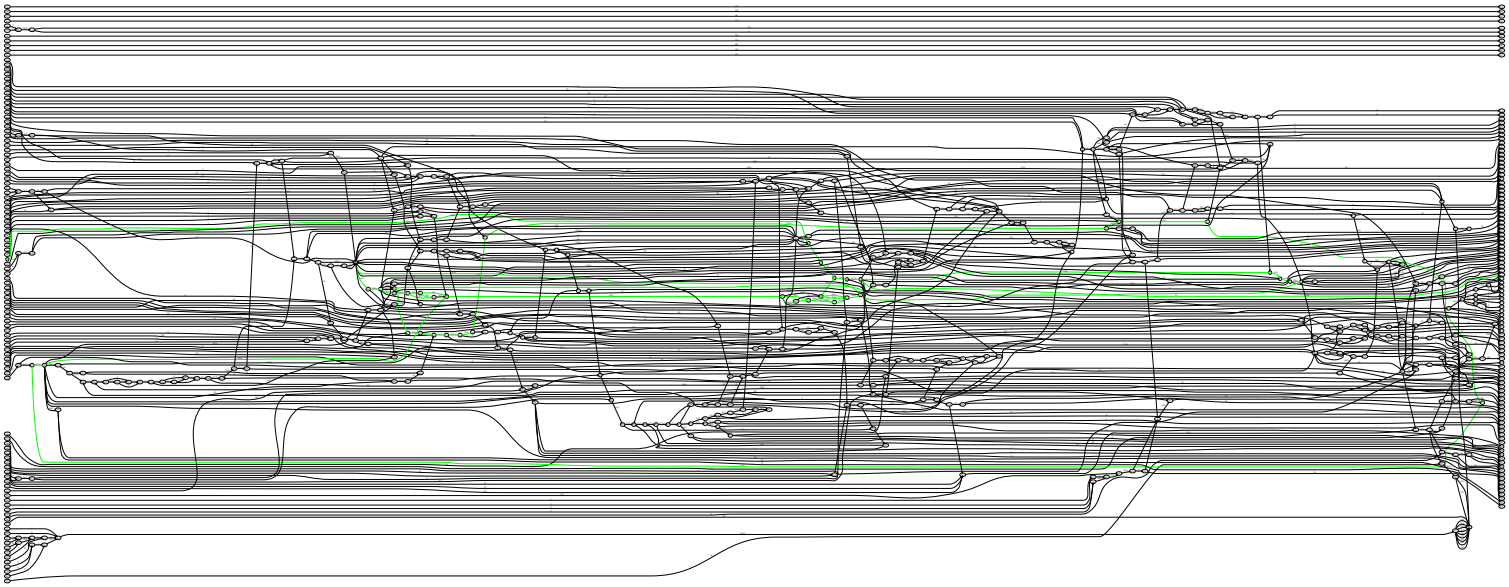
Dataset

- 262 proteins found in literature and manually annotated.
- Thanks to Sebastian, Ivan and Christoph!
- From *S. cervisiae*, *S. pombe*, *D. melanogaster* and *H. Sapiens*

Questions

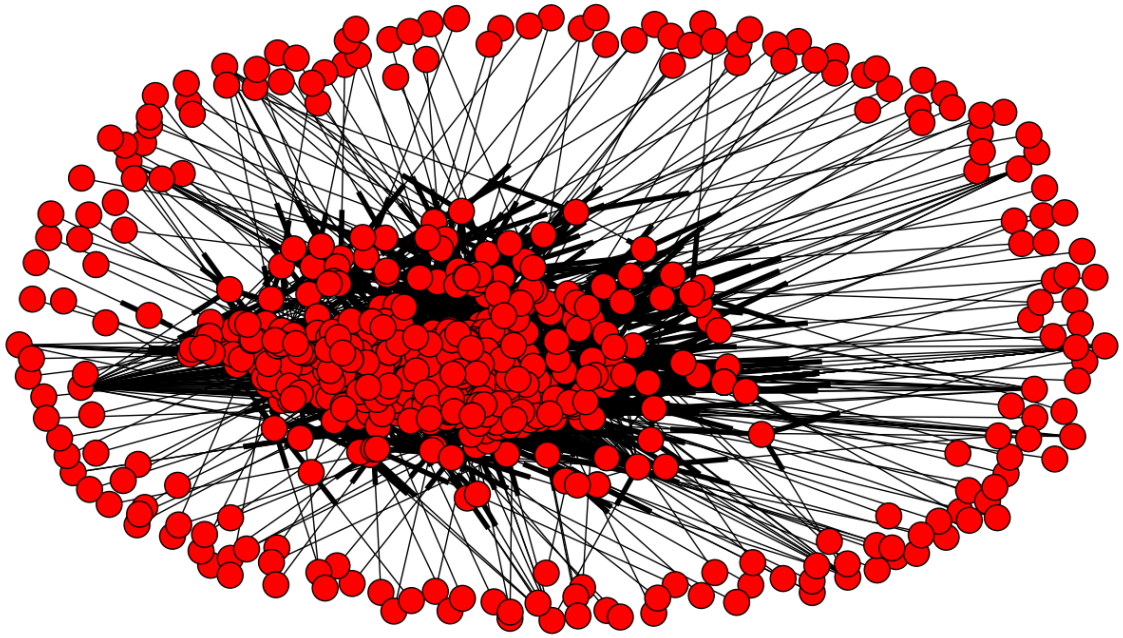
- Can ABA recognize domain-like structures?
- Do domains move around in the complexes?
- What structures occur often?

Output of ABA



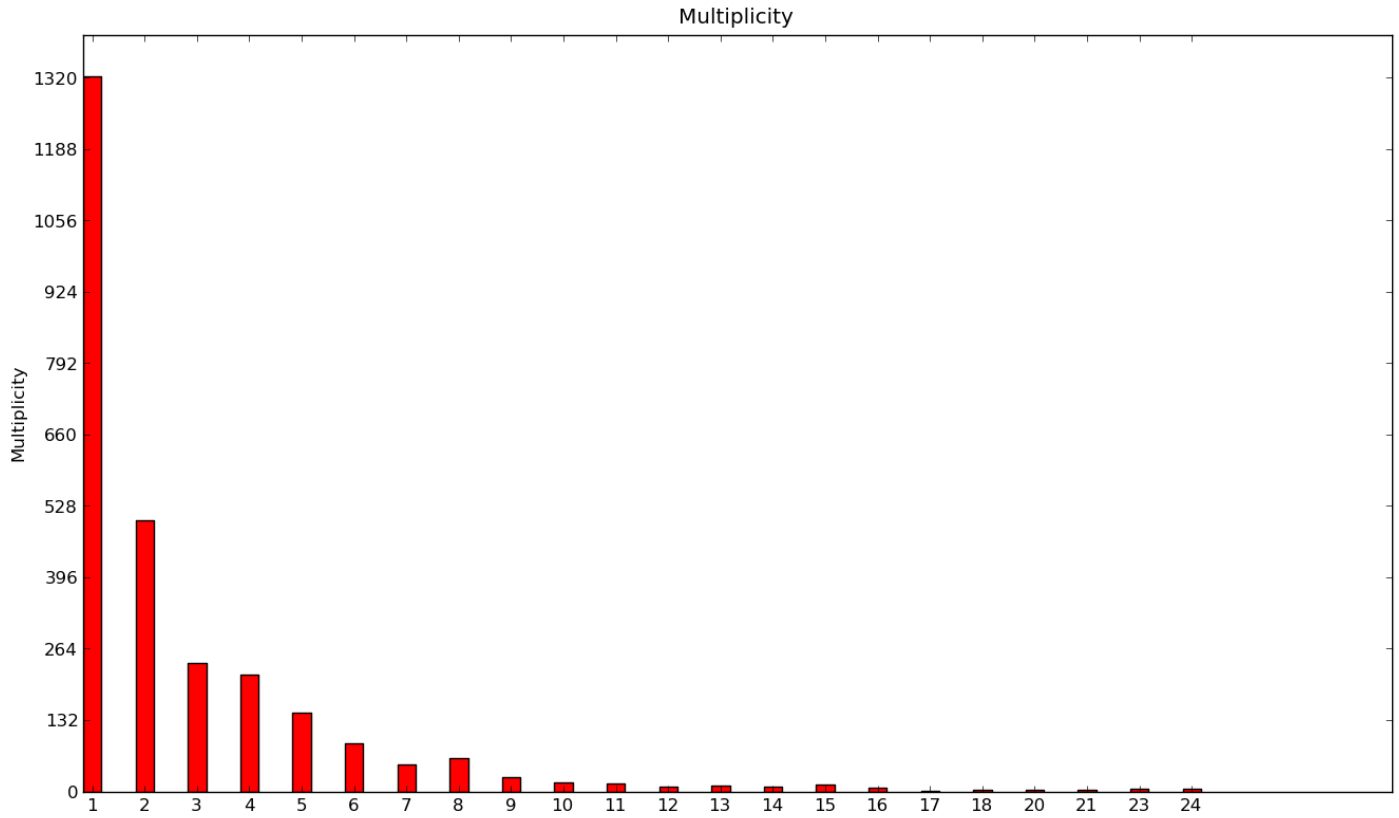
- Applied to only 2 species.
- Rendering takes a long time.
- Hard to interprete (manually).

Parsing output of ABA



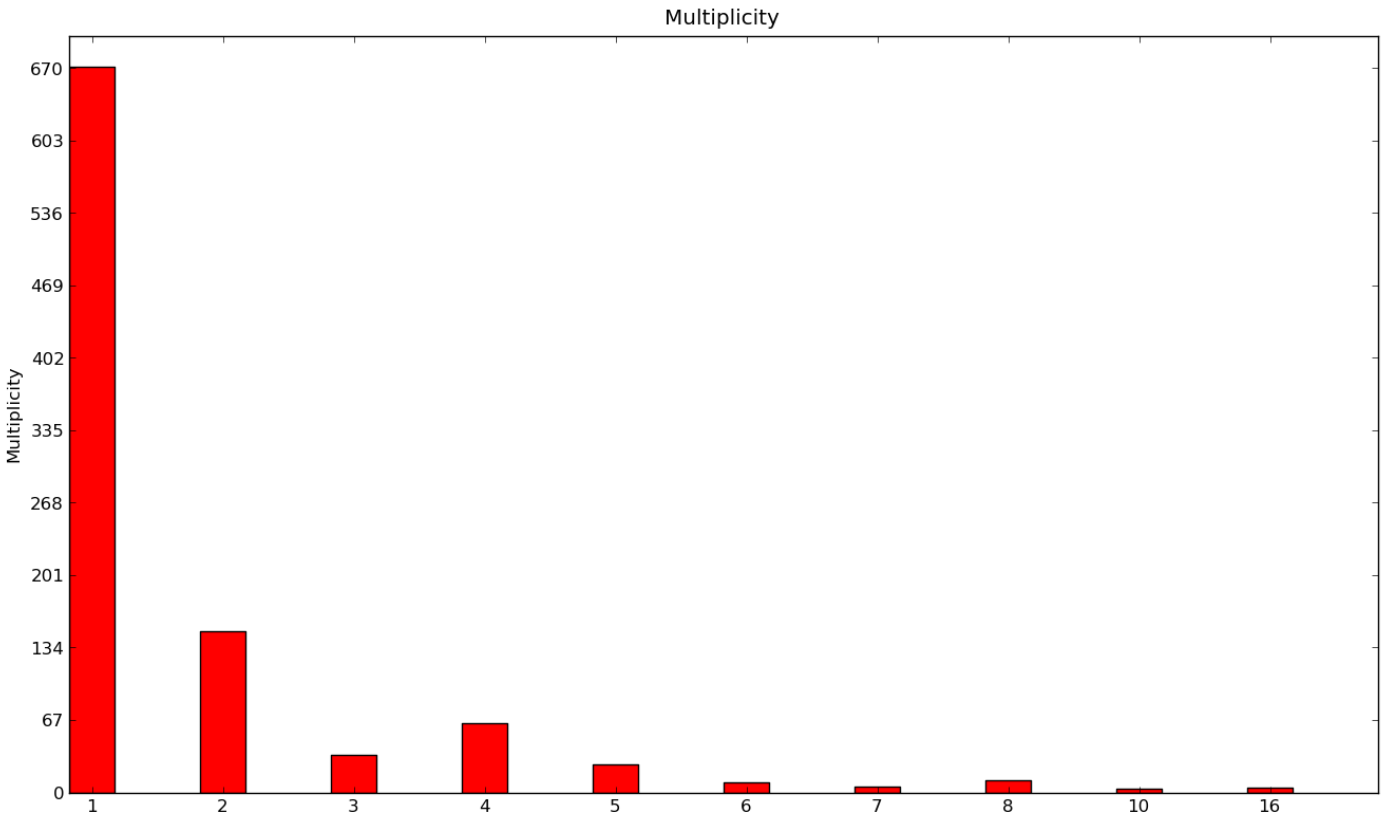
- Applied to 4 species.
- Reconstructed A-Bruijn Graph from ABA-Output.

Distribution of edge multiplicity



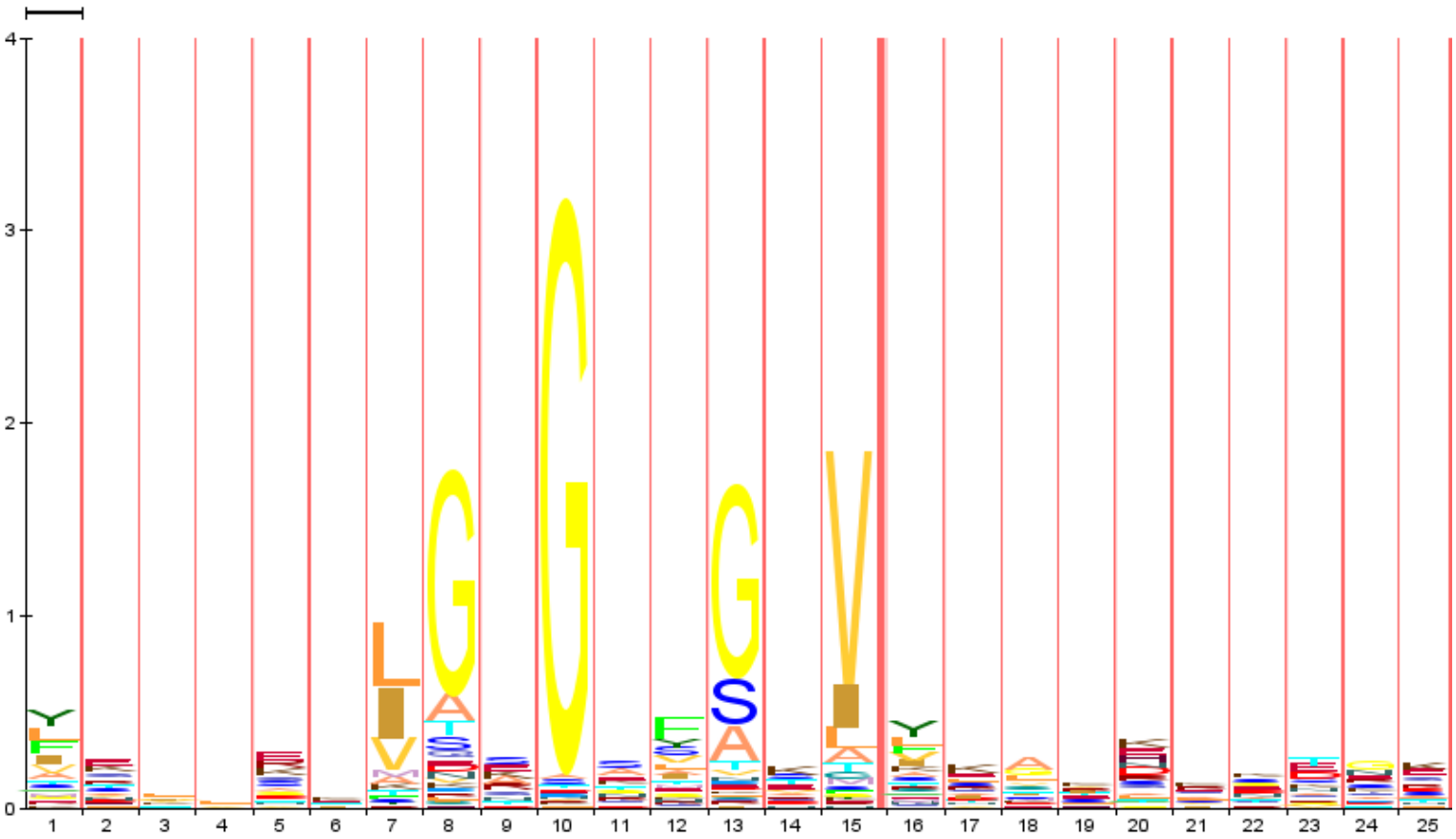
- High-weight edges point out to conserved and repeated elements.
- Within and across proteins.
- (Girth parameter did not seem to work.)

Distribution of edge multiplicity (filtered)



- Filtered distribution of the multiplicity of edges (length > 40).

Comparison with PFAM-Annotation

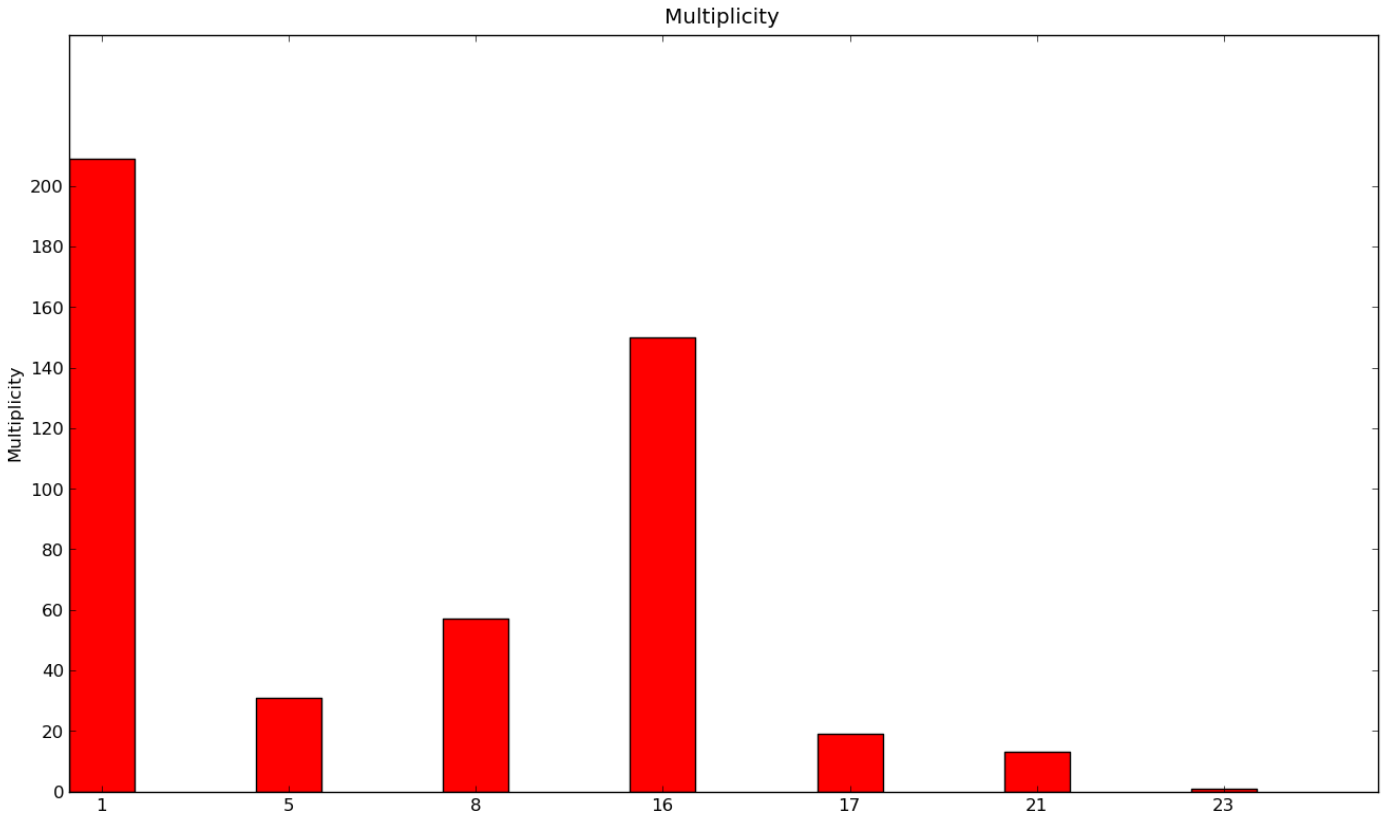


- Hidden markov models learned from multiple sequence alignments.

Comparison with PFAM-Annotation

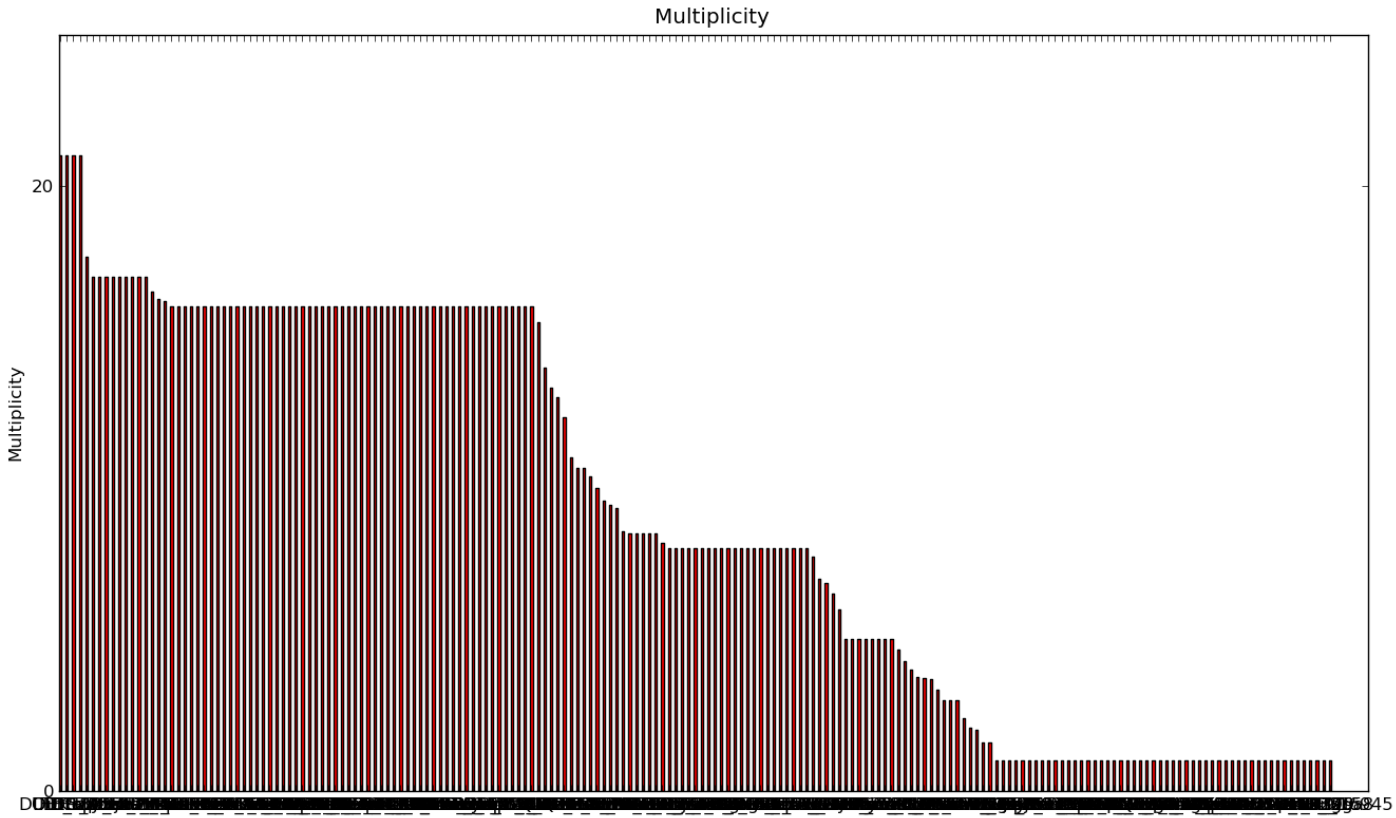
- Annotated all proteins with PFAM/HMMER.
- Detected 561 domains (not unique).

Distribution of edges with domains



- $\approx$ 210 edges of multiplicity 1.
- $\approx$ 150 edges of multiplicity 16.

Repeated domains



- Domains seem to share edges in ABA-graph.

Repeated domains

Domain	Average Multiplicity
DUF1679	21.0
Elf1	21.0
DUF1825	21.0
Fib_alpha	21.0
ZZ	17.7
Otopetrin	17.0
CDK5_activator	17.0
...	...
RFX_DNA_binding	1.0
zfC5HC2	1.0
DUF1542	1.0
Rep_N	1.0
DUF3619	1.0
TIP49	1.0
HTH_Mga	1.0

Whats next?

- Do ABA-edges correlate with found domains?
- Apply real null model. Significance tests.
- Can ABA be used to complement the domains found with HMMER?

Non-Collinear Alignment: Reannotation of genomes.

Carosonella ruddii: an interesting thing

- unclassified γ-proteobacteria. (Like e.g. *E.Coli*)
- Sequenced 2006.

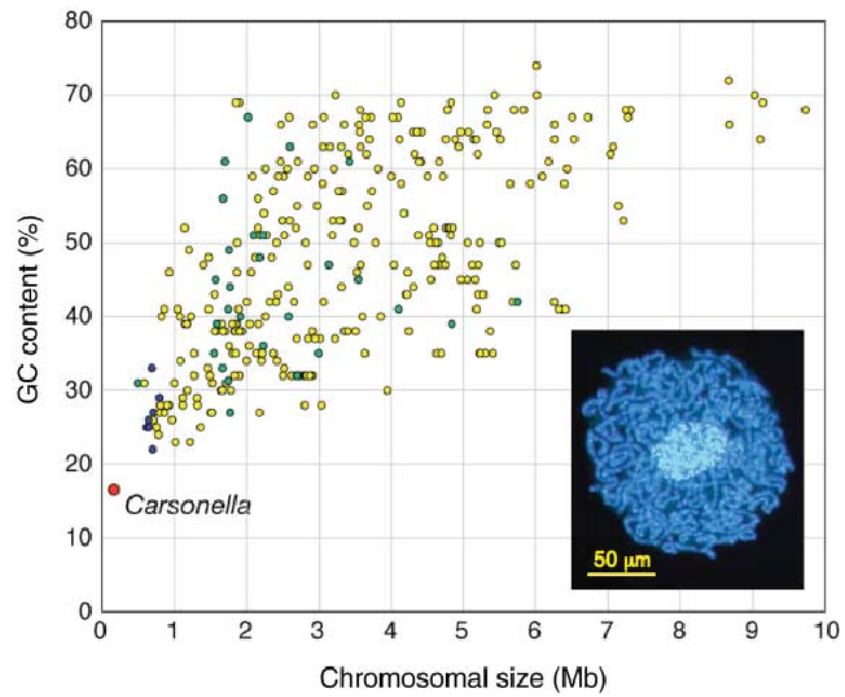
Carosonella ruddii

what is it?

- Smallest bacterial genome known.→ 160 Mb (!). *E.Coli* has 4,5 Gb

Smallest genome before Carsonella

- 362 protein-coding genes in Buchnera aphidicola BCc



Carosonella ruddii

what is it?

- CG-Content: Very low (16%). *E.coli*: (50%)

GC-Content

GC Content is defined as: GC-content (or guanine-cytosine content), in molecular biology, is the percentage of specific bases on a DNA molecule which are either guanine or cytosine.

Carosonella ruddii

what is it?

- CG-Content: Very low (16%). *E.coli*: (50%)
- First annotation: 213 genes. *E.coli*: 4400 genes

Minimal set of genes for life

- : Moya A. et al. proposed 2003 that the minimal gene set for a endosymbiotic life is close to 313.

Interesting question

- DNA replication and repair system is strongly degraded.

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- DNA replication and repair system is strongly degraded.
- Transcriptioin machinery is reduced to core subunits of RNA Polymerase (no promotor-recognition)
- Translation machinery is highly reduced. (three essential rRNAs are present)
- No Shine-Dalgarno sequence present (the way it is defiend)

16S rRNA and Shine-Dalgarno Sequence

- Shine-Dalgarno (SD) is a regulatory sequence strongly involved in translation of bacterial poly-cystronic mRNAs.

Interesting question

Is Carsonella ruddii a living cell?

- 9 aminoa-cyl-tRNA synthetases and 15 out of 50 essential ribosomal protein are **missing** or degraded.

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Two different theories

- C.ruddii is a bacteria which undergoes the change to endosymbiont.
- C.ruddii is an former primary endosymbiont, is being driven towards its extinction and replacement by a new symbiont.

Current Annotation

What has been done until now

- 2006: First annotaion (213 genes)
- 2007: Second annotation
- Both teams used well known Gene-prediction algorithms + **collinear** alignment

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Current Annotation

What has been done until now

- 2006: First annotaion (213 genes)
- 2007: Second annotation
- Both teams used well known Gene-prediction algorithms + **collinear** alignment
- Problem: Over-annotation of function of genes. Many genes that are believed to be orthologous are much shorter and therefore deffer in their function.

My goal

use an non-collinear alignment algorithm to reannotate the whole genome of C.ruddii

Reannotation

Algorithms

- SuperMap + S-LAGAN
- A-Bruijn Alignment (ABA)

S-LAGAN

Species used

- Carsonella Ruddii PV (160 kb genome, 213 genes)
- Buchnera aphidicola BCc (Cc) (+ a plasmid) : 450 kb. (397 genes)
- Candidatus Blochmannia floridanus: 705 kb. (631 genes).
- Wigglesworthia glossinidia (+ a plasmid): 698 kb. (651 genes)
- Baumannia cicadellinicola str. Hc: 686 kb (651 genes)

S-LAGAN

plus Supermap

- A guiding tree (evolutionary tree) was build out of 16S-rRNAs of the species.

S-LAGAN

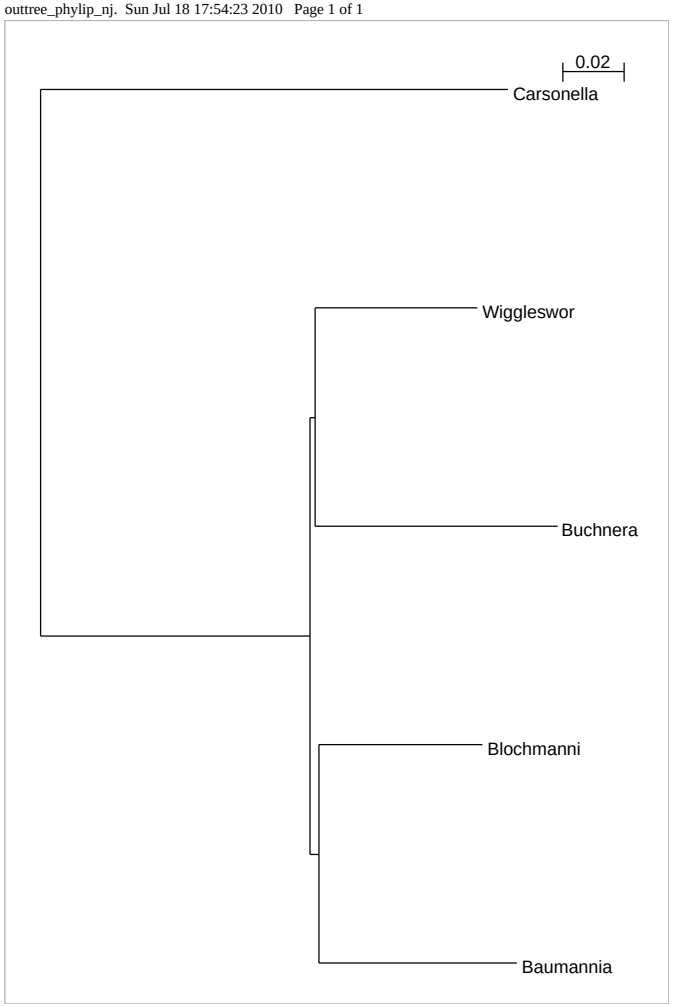
plus Supermap

- A guiding tree (evolutionary tree) was build out of 16S-rRNAs of the species.
- Neighbor joining tree
- Maximum likelyhood tree

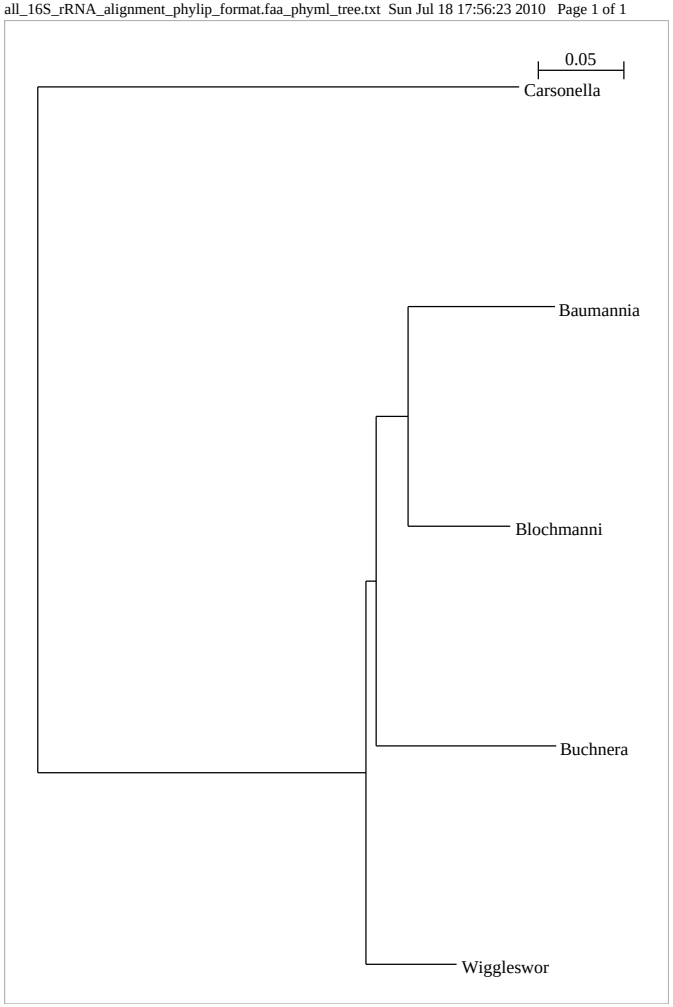
Trees

of 16S-rRNA sequence

outtree_phylip_nj. Sun Jul 18 17:54:23 2010 Page 1 of 1

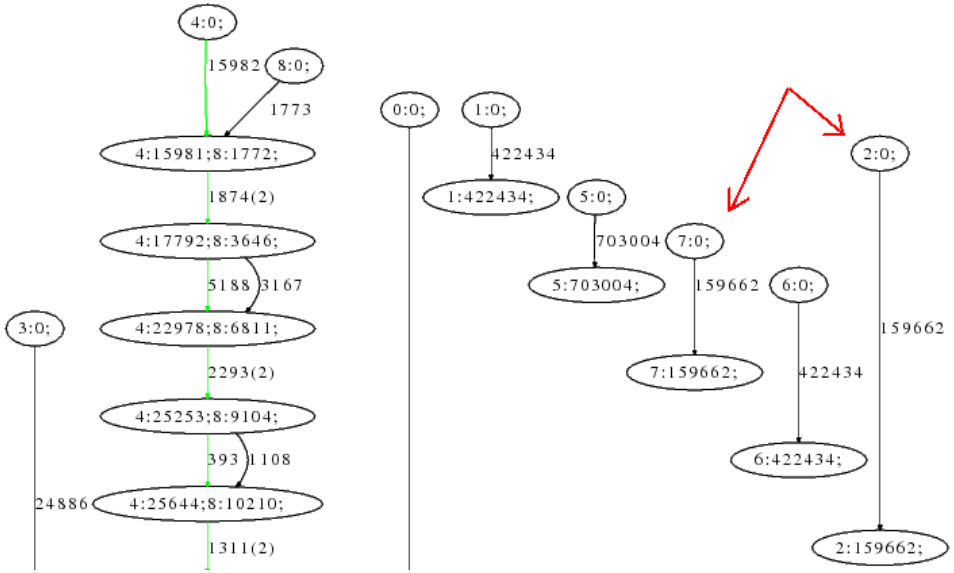


all_16S_rRNA_alignment_phylip_format.faa_phyml_tree.txt Sun Jul 18 17:56:23 2010 Page 1 of 1



ABA

Using “my” 5 Species



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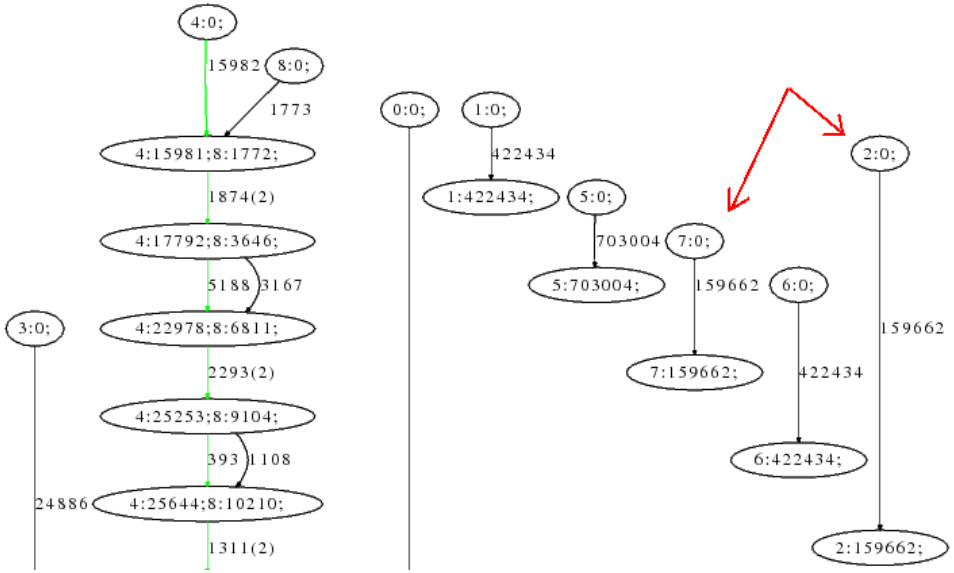
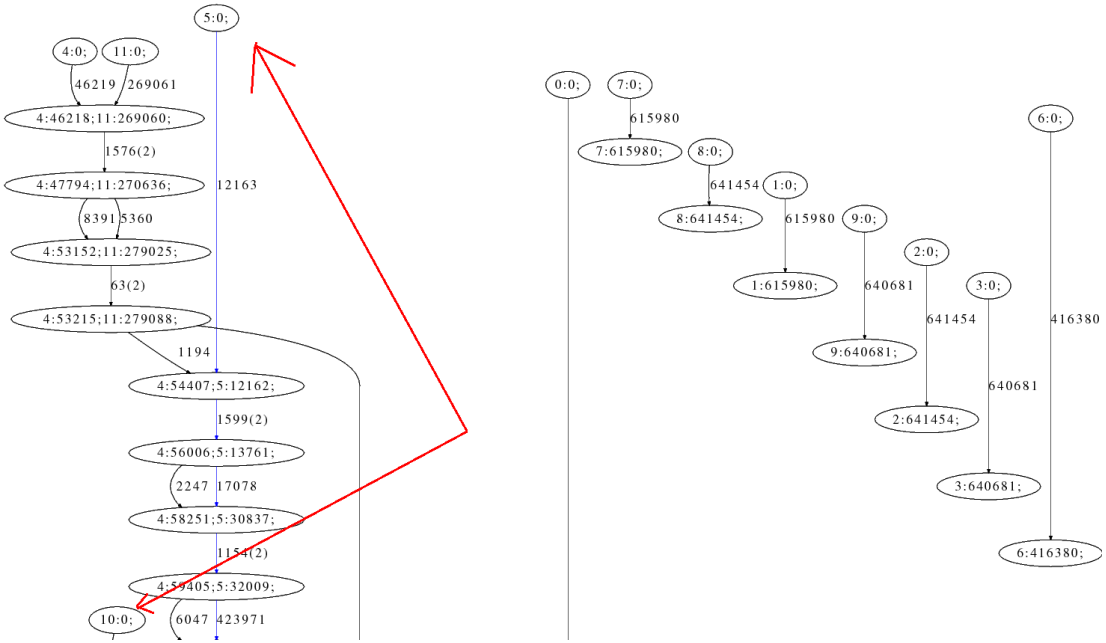


- Species
  - 0 and 5: Wigglesworthia
  - 1 and 6: Buchnera aphidicola
  - 2 and 7: Carsonella Ruddii
  - 3 and 8: Blochmannia
  - 4 and 9: Baumannia

ABA

Using “Moya’s” Species



ABA

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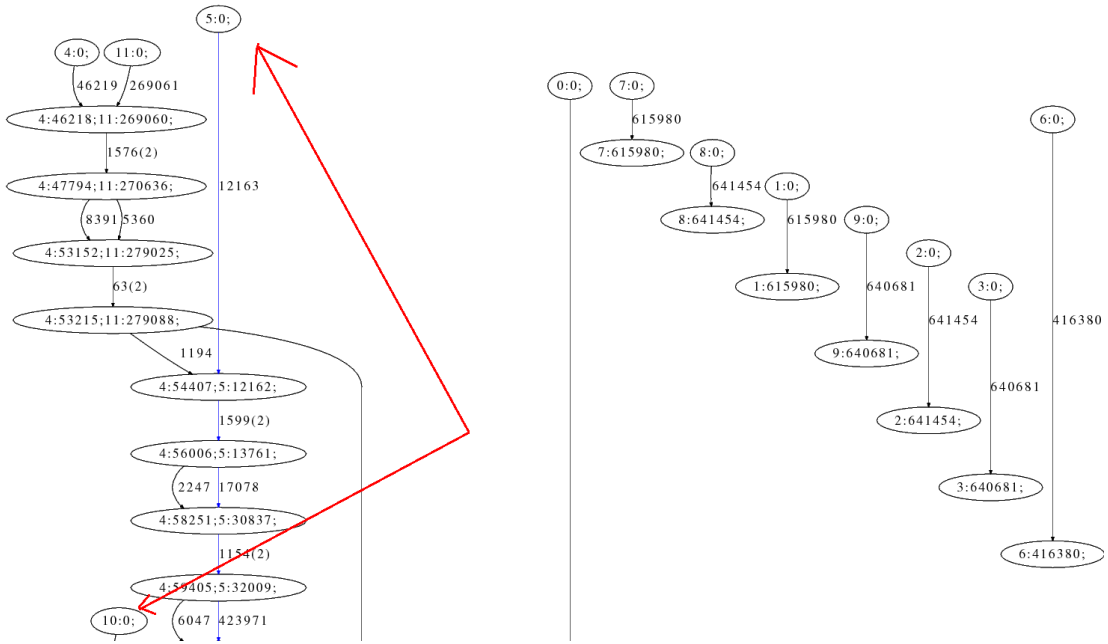

- Species
  - 0 and 6: Buchnera aphidicola str. Cc
  - 1 and 7: Buchnera aphidicola str. Bp
  - 2 and 8: Buchnera aphidicola str. Sg
  - 3 and 9: Buchnera aphidicola str. APS
  - 4 and 10: Carsonella ruddii
  - 5 and 11: E.Coli

ABA

Only using Carsonella and E.Coli

2 Species (Carsonella and E.Coli) produce the same alignment as 6 Species from Moya paper

ABA

Gene prediction

7 genes of 213 were cut by the prediction in **C.ruddii**. 22 genes of 4494 were cut by the prediction in **E.Coli**.

ABA

Gene prediction

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Example

region 0 - 46219 : 56 genes
region 46219 - 47795 : 0 genes
region 47795 - 53155 : 10 genes
region 53155 - 53218 : 0 genes
region 53218 - 54412 : 4 genes
region 54412 - 56011 : 0 genes
region 56011 - 58258 : 4 genes
region 58258 - 59412 : 0 genes
region 59412 - 65459 : 8 genes
region 65459 - 67041 : 1 genes
region 67041 - 70177 : 4 genes

ABA

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A possible future

- There are still at least 29 genes with no assigned function.
- Insightes into the possibility to create symbiotic life.

Project: Reimplementation of S-LAGAN Using SeqAn

F. Heeger, S. Specovius

1. Introduction to S-LAGAN
2. Implementation and Problems
3. Results

S-LAGAN

Shuffle-Limited Area Global Alignment of Nucleotides

- S-LAGAN computes **glocal** alignments of 2 sequences

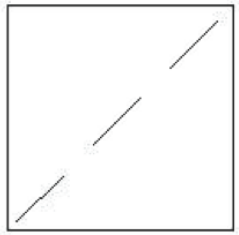
  $\rightarrow$ Set of local alignments which cover the whole sequence

- S-LAGAN is able to handle rearrangements

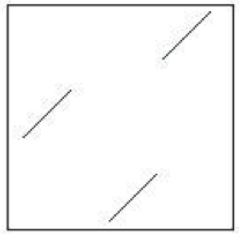
S-LAGAN

Rearrangements

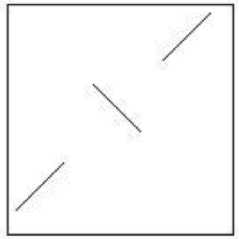
- No rearrangements



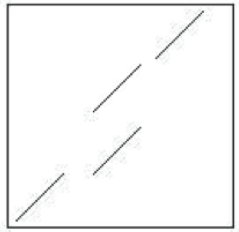
- Translocation



- Inversion



- Duplication



S-LAGAN

Overview

1. Computation of local alignments
2. Chaining
3. Realignment of consistent subchains

S-LAGAN

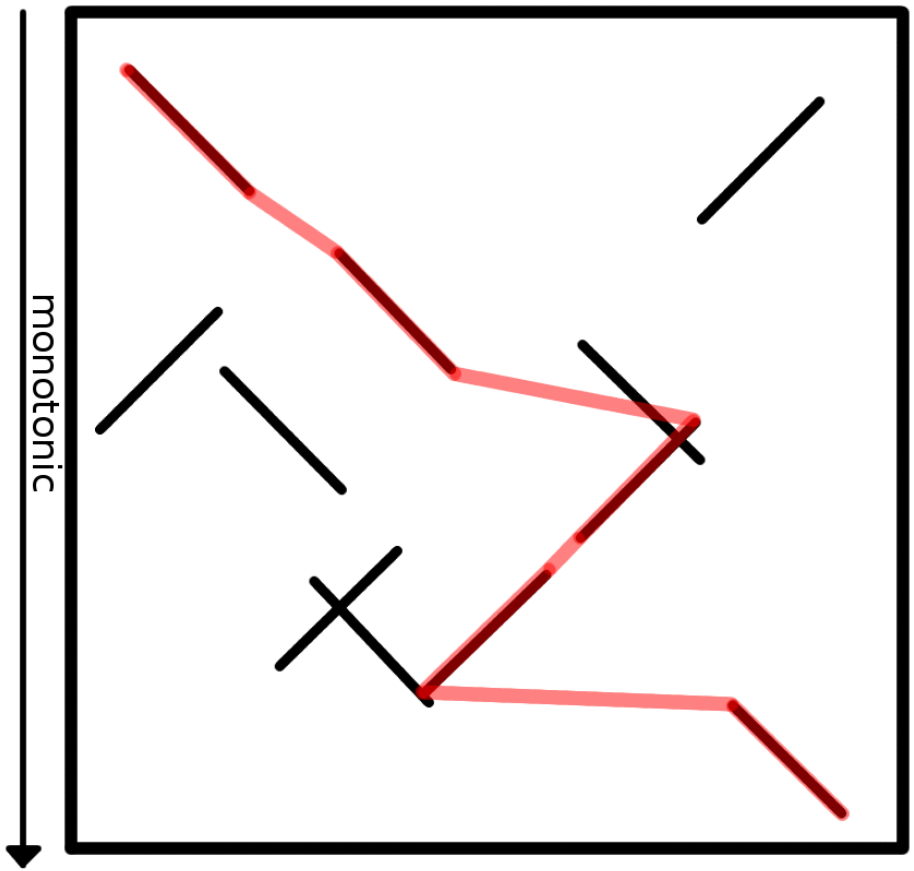
1. Computation of local alignments

- S-LAGAN uses CHAOS for this step
- Applies CHAOS twice
  - $\rightarrow$ Sequence 1 with sequence 2
  - $\rightarrow$ Sequence 1 with reverse complement of sequence 2

S-LAGAN

2. Chaining

1-monotonic



S-LAGAN

3. Realignment of consistent subchains

- Consistent (co-linear) subchains are globally aligned
- S-LAGAN uses LAGAN for this step

Implementation and Problems

Goal

- Implementation in SeqAn
- Extract Chaos from SeqAn implementation of LAGAN
- Implement 1-monotonic chaining
- Use existing SeqAn implementation of LAGAN

Implementation and Problems

Local Alignments

- Find seeds with q-gram index
- Merge overlapping seeds
- Chain seeds with Chaos algorithm

  $\rightarrow$ Segmentation Fault on certain data

  $\rightarrow$ Only gap-free local matches

Implementation and Problems

Chaining

- Graph with nodes representing local matches
- Edges to all matches, which can be chained 1-monotonic
  $\rightarrow$ Heaviest path (Bellman-Ford Algorithm)
- $\mathcal{O}(n^3)$

Implementation and Problems

Realign Consistent Subchains

- Find consistent subchains
- Align them with global alignment algorithm
- LAGAN runs into an endless loop on certain data

  $\rightarrow$ Use Needleman-Wunsch Algorithm

Results

Our implementation...

- is very slow
- can be used on small data, like virus genomes ($\sim$ 5000 bp)
- finds manually inserted rearrangements

Introduction OSL

Motivation

Assume there are two assemblies obtained from different assemblers:

Introduction OSL

Whole **G**enome **S**hotgun Approach (WGS)

Aim: Assemble a genome sequence from given reads.

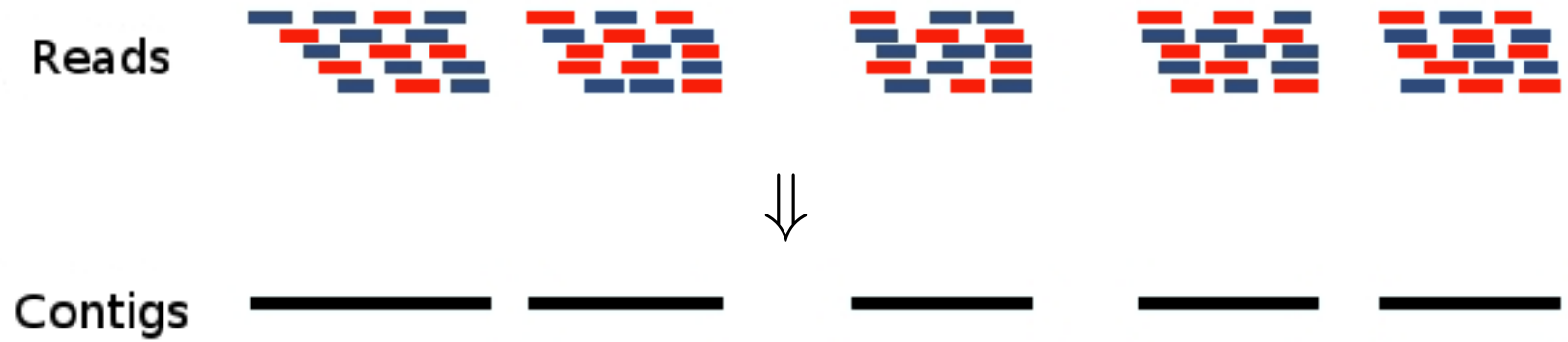
- **Reads**
  - → Collection of short sequences
  - → Obtained from an automated sequencer
  - → Orientation is not known

Introduction OSL

Whole **G**enome **S**hotgun Approach (WGS)

Assemble overlapping reads together to obtain contigs.

- **Contigs**
  $\rightarrow$ Large, contiguous fragments of assembled reads



Problem

- Order and orientation of contigs is unknown

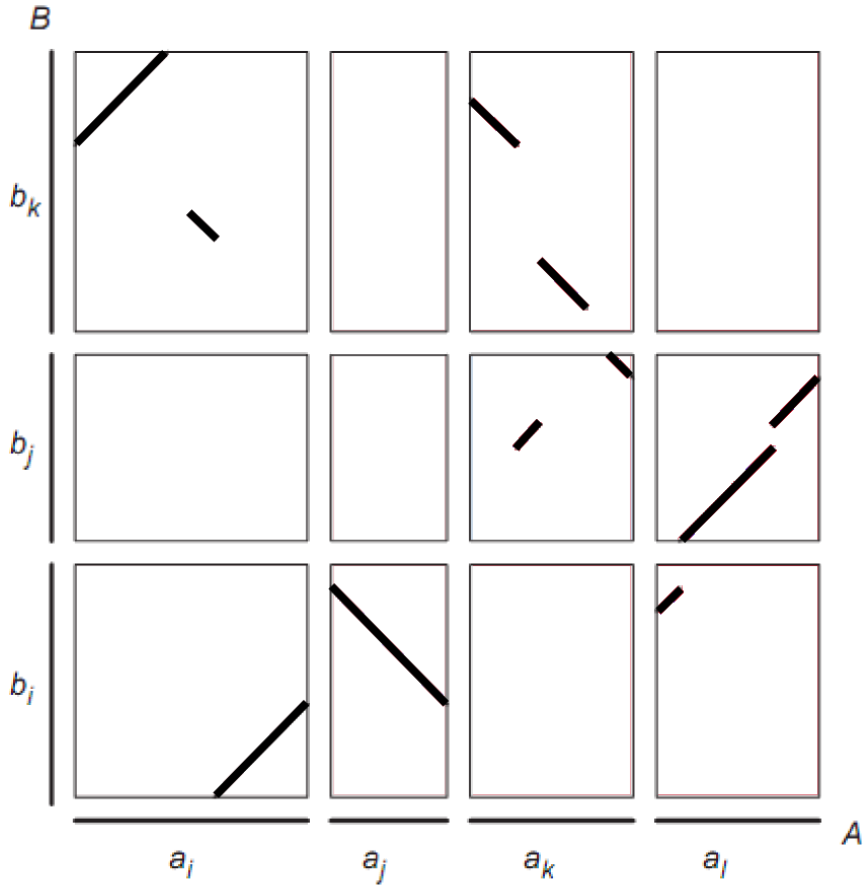
↓

Search for a good assembly layout !

Optimal **S**yntenic **L**ayout of unfinished assemblies

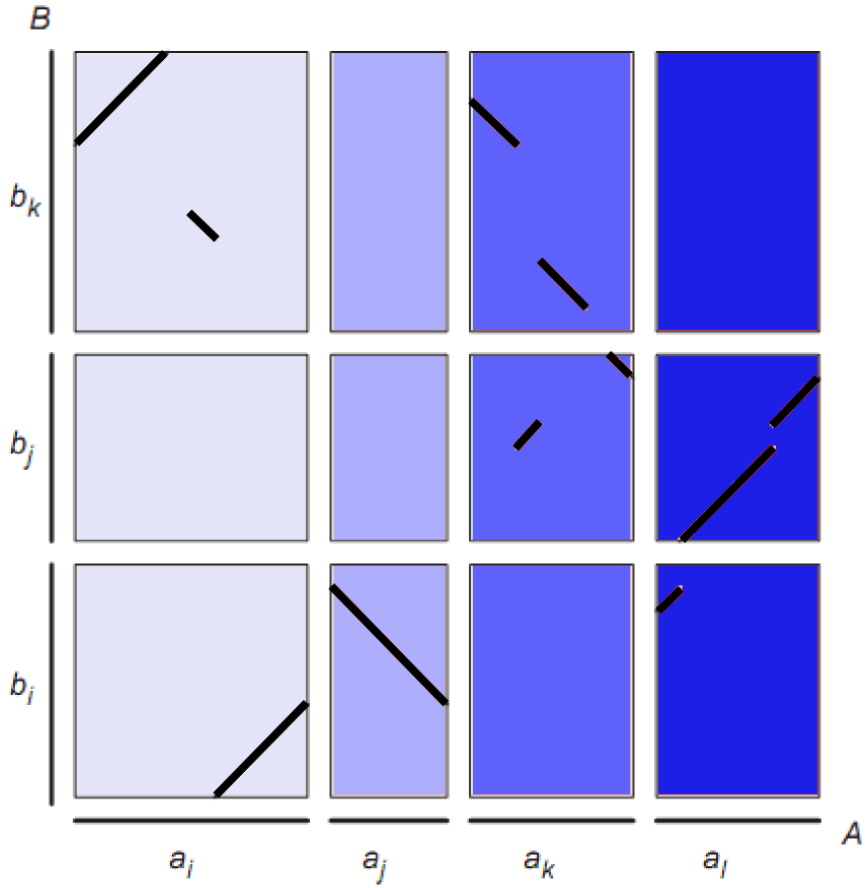
OSL Idea

- Maximize no. of extended local diagonals



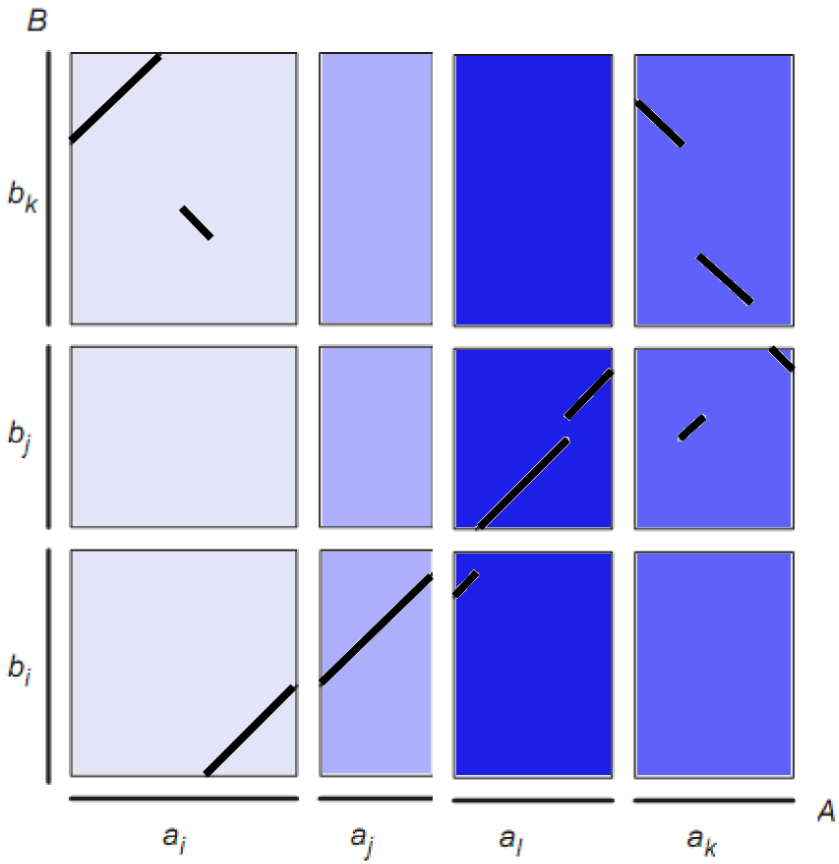
OSL Idea

- Maximize no. of extended local diagonals
- permute and flip contigs of assembly A



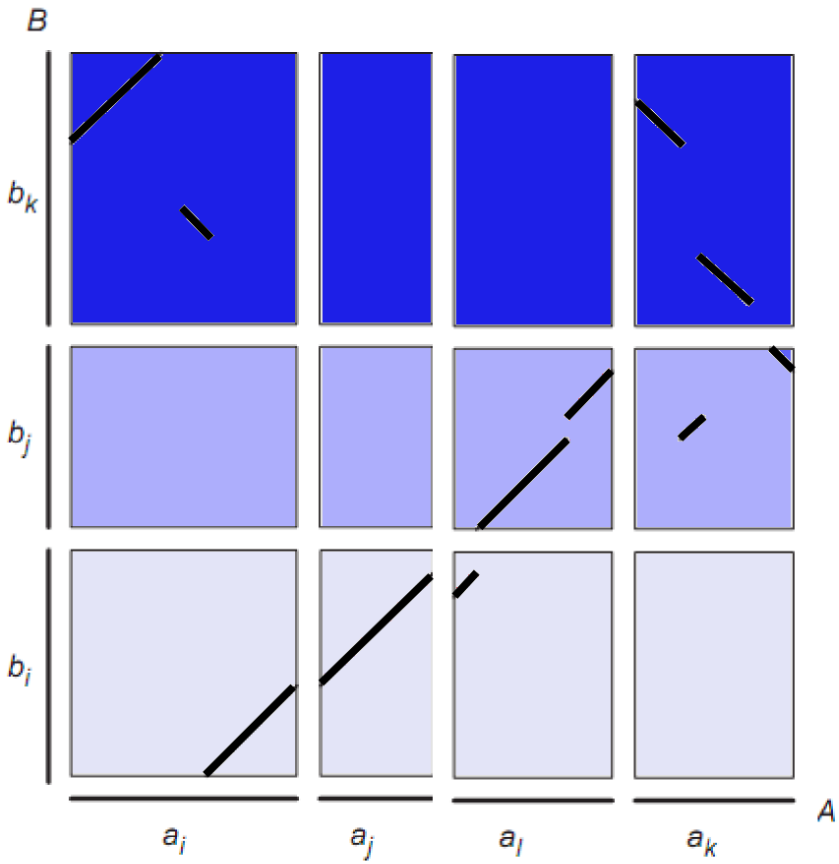
OSL Idea

- Maximize no. of extended local diagonals
- permute and flip contigs of assembly A



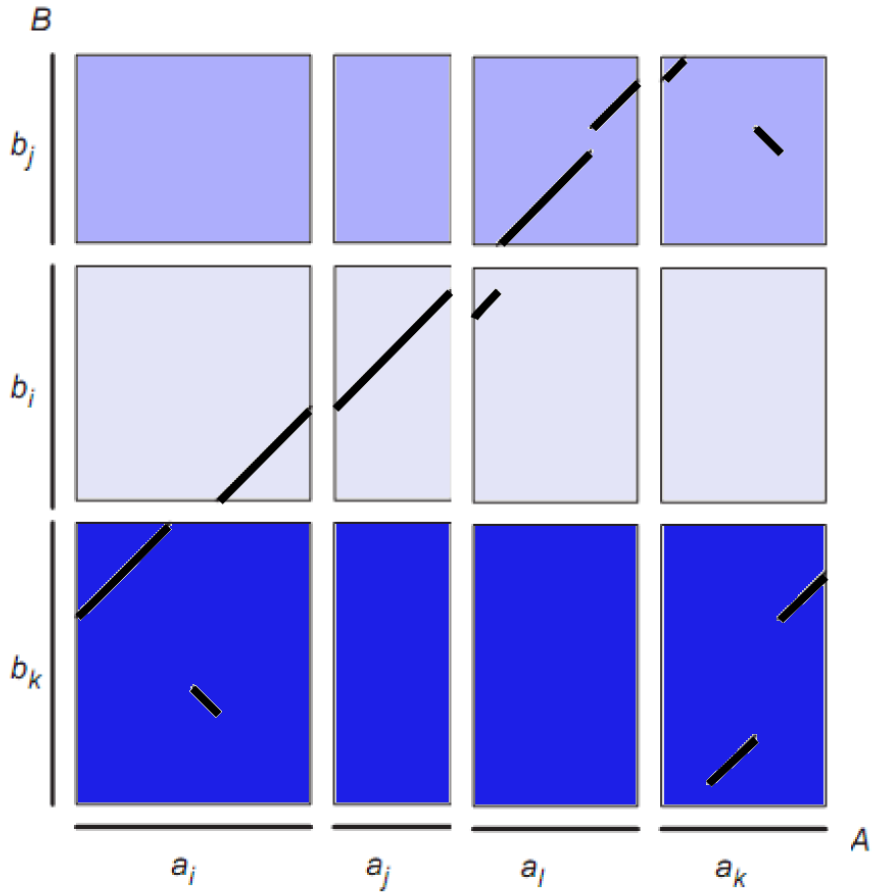
OSL Idea

- Maximize no. of extended local diagonals
- permute and flip contigs of assembly A
- switch roles of A and B



OSL Idea

- Maximize no. of extended local diagonals
- permute and flip contigs of assembly A
- switch roles of A and B



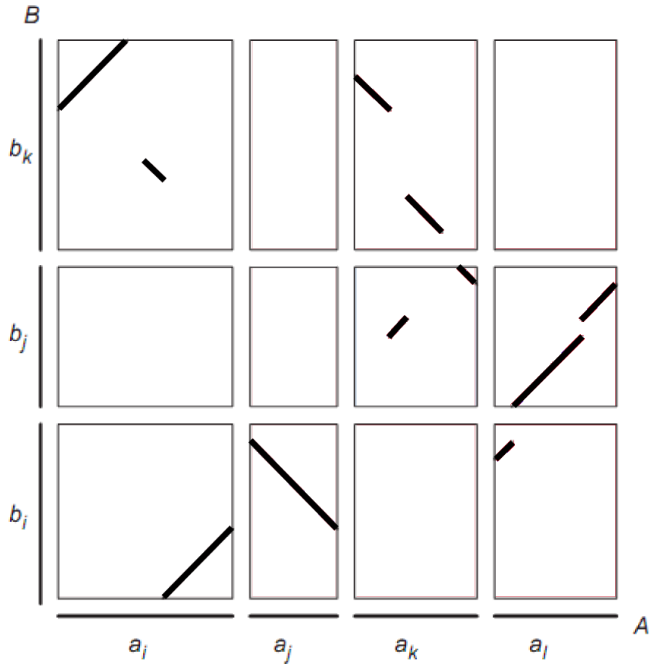
Independency in constructing the layouts of A and B !

The OSL Problem

Basics

Assemblies

$A = (a_1, \ldots, a_p)$
$B = (b_1, \ldots, b_q)$



The OSL Problem

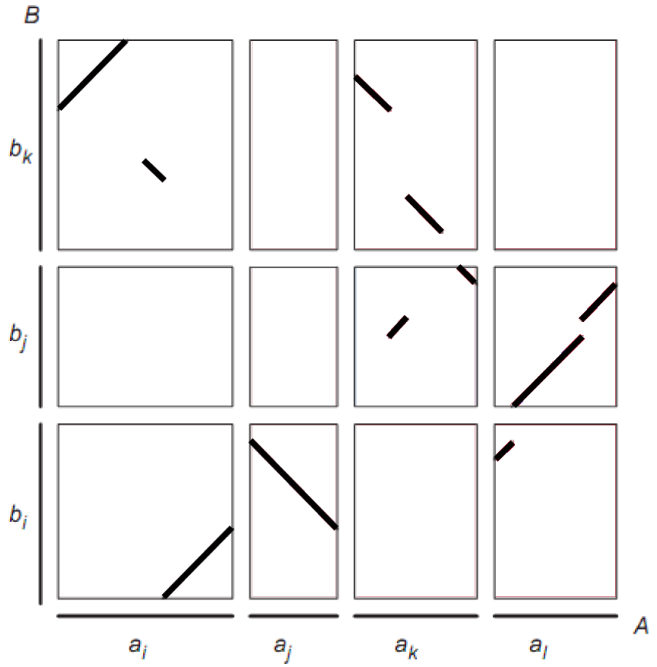
Basics

Assemblies

$A = (a_1, \ldots, a_p)$
$B = (b_1, \ldots, b_q)$

Set of Matches

$M = (m_1, \ldots, m_r)$

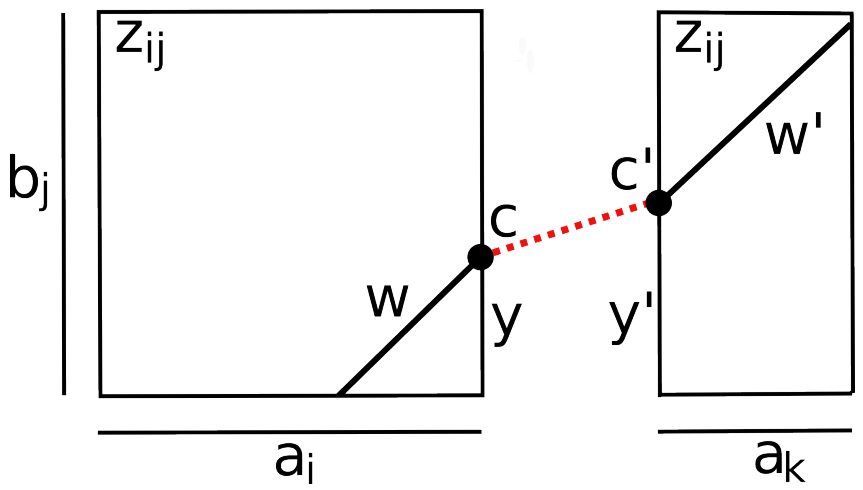


The OSL Problem

Layout

Local diagonal extension

c and c' form a *local diagonal extension* iff $y \sim y'$ and $o = o'$



The OSL Problem

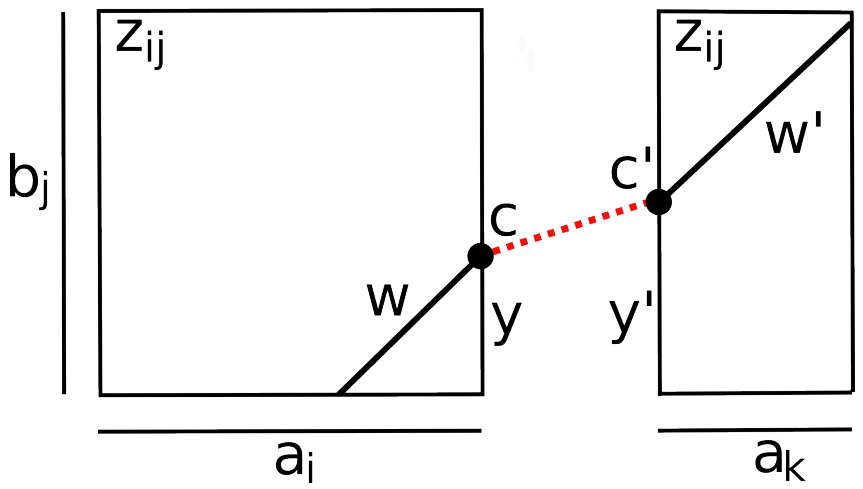
Layout

Local diagonal extension

c and c' form a *local diagonal extension* iff $y \sim y'$ and $o = o'$

Weight of extension

$w + w' - \mid y - y' \mid$



Project: Assembly Comparison

Goal

1. Assemble a set of reads with two different Assemblers
2. Compare the results using Layout Software

   $\rightarrow$ OSLay

Project: Assembly Comparison

1. Assemble a set of reads with two different Assemblers

   - Reads of Chromosom 21
   - Assembler: Mira and Celera (WGS)

Project: Assembly Comparison

1. Assemble a set of reads with two different Assemblers

   - Reads of Chromosom 21
   - Assembler: Mira and Celera (WGS)

Project: Assembly Comparison

Problems:

- WGS Assembler doesn't work with given reads

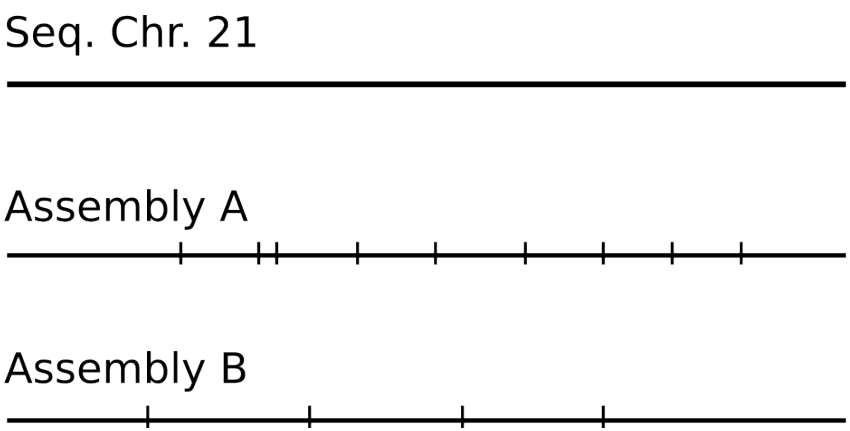
↓

Plan B:

- Take given sequence of chr. 21
- Create artificial contigs

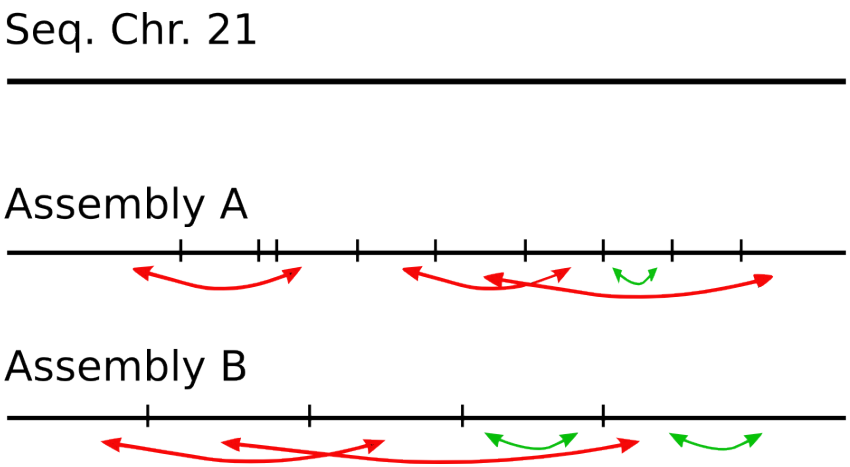
Project: Assembly Comparison

Create artificial contigs:



Project: Assembly Comparison

Create artificial contigs:



Project: Assembly Comparison

BLAST

Assemblies are from the same sequence

↓

Megablast

Project: Assembly Comparison

OSLay

OSLay is the implementation of the OSL algorithm.

Input:

- target assembly
- reference assembly
- matches (e.g. BLAST)

Output:

- original layout
- new layout

Project: Assembly Comparison

OSLay

Problem:

- Input too large for OSLay
- Chr. 21 $\sim$ 34 MB

$\downarrow$

Plan B:

- segment of 210 KB

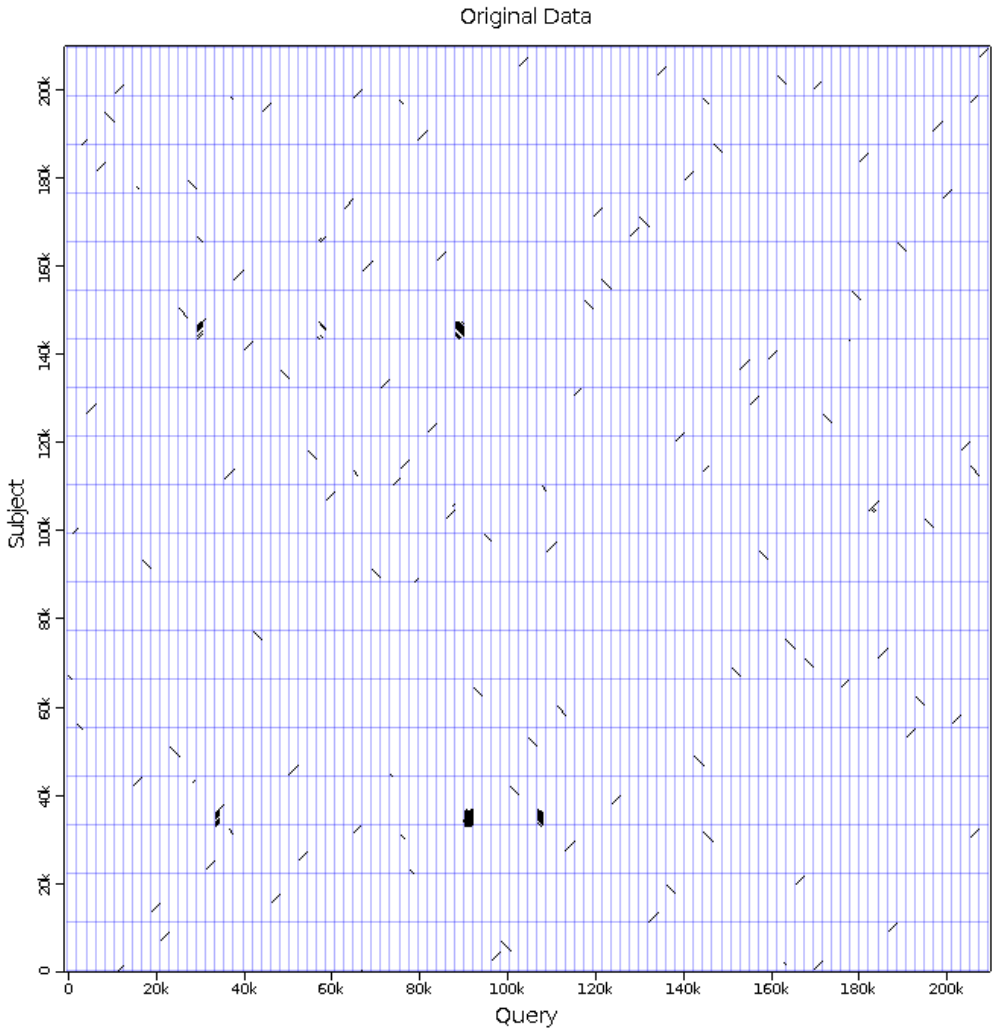
Project: Assembly Comparison

OSLay

- Assembly A: sequence divided by 100
- Assembly B: sequence divided by 19

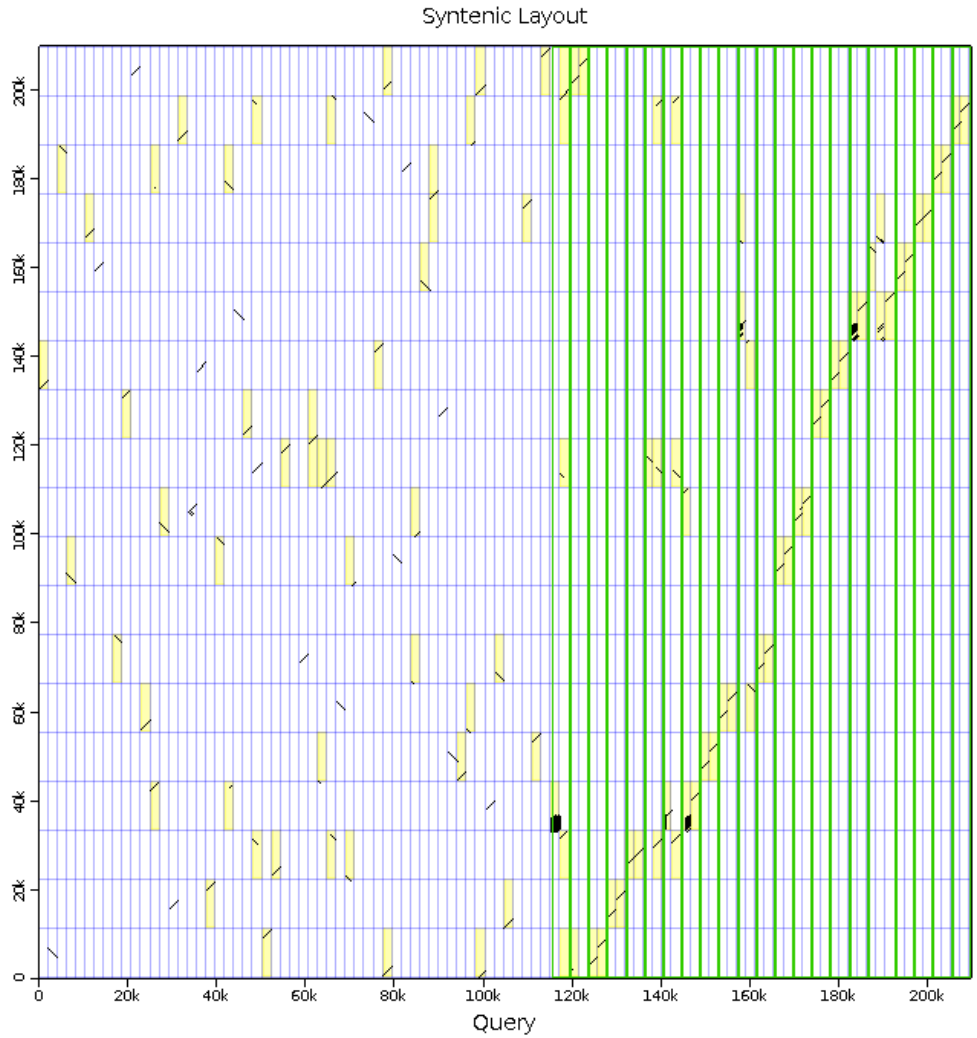
Project: Assembly Comparison

OSLay



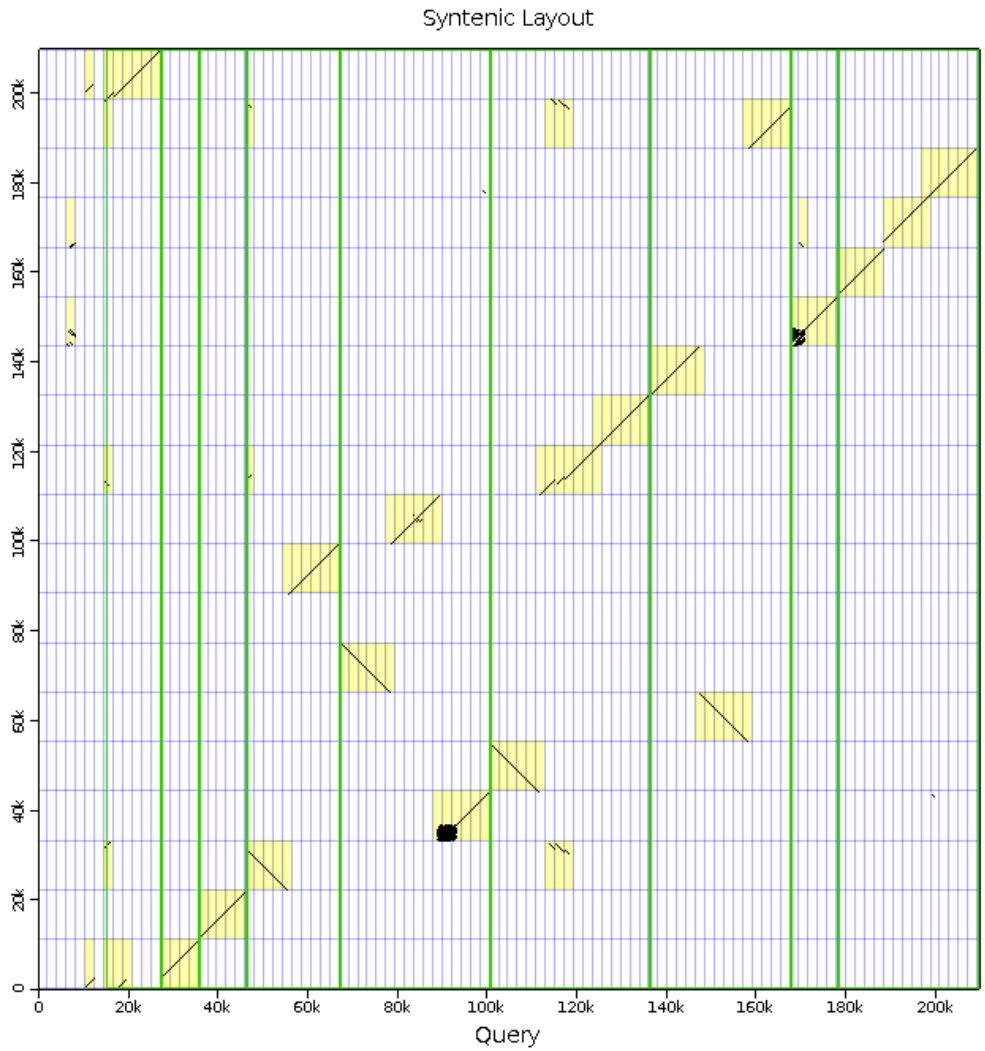
Project: Assembly Comparison

OSLay



Project: Assembly Comparison

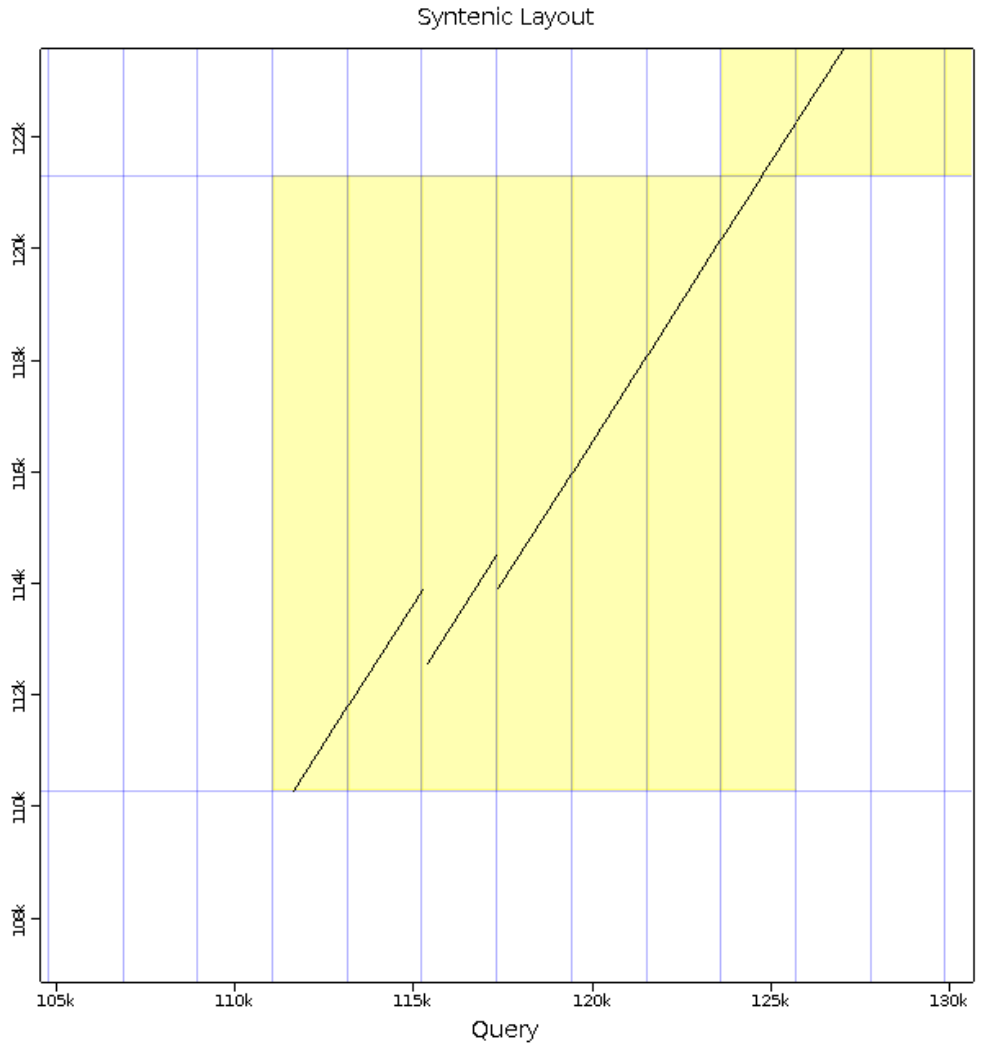
OSLay



Project: Assembly Comparison

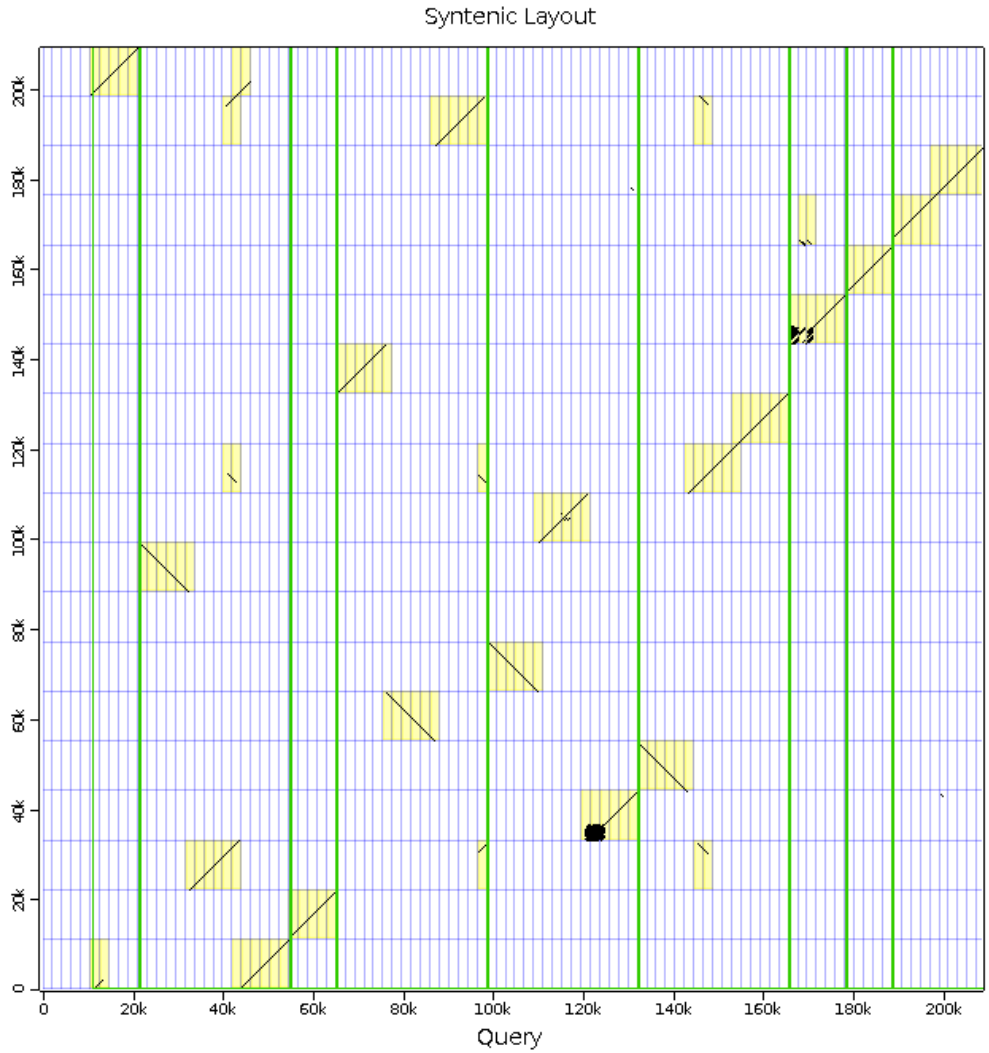
OSLay

False connections:



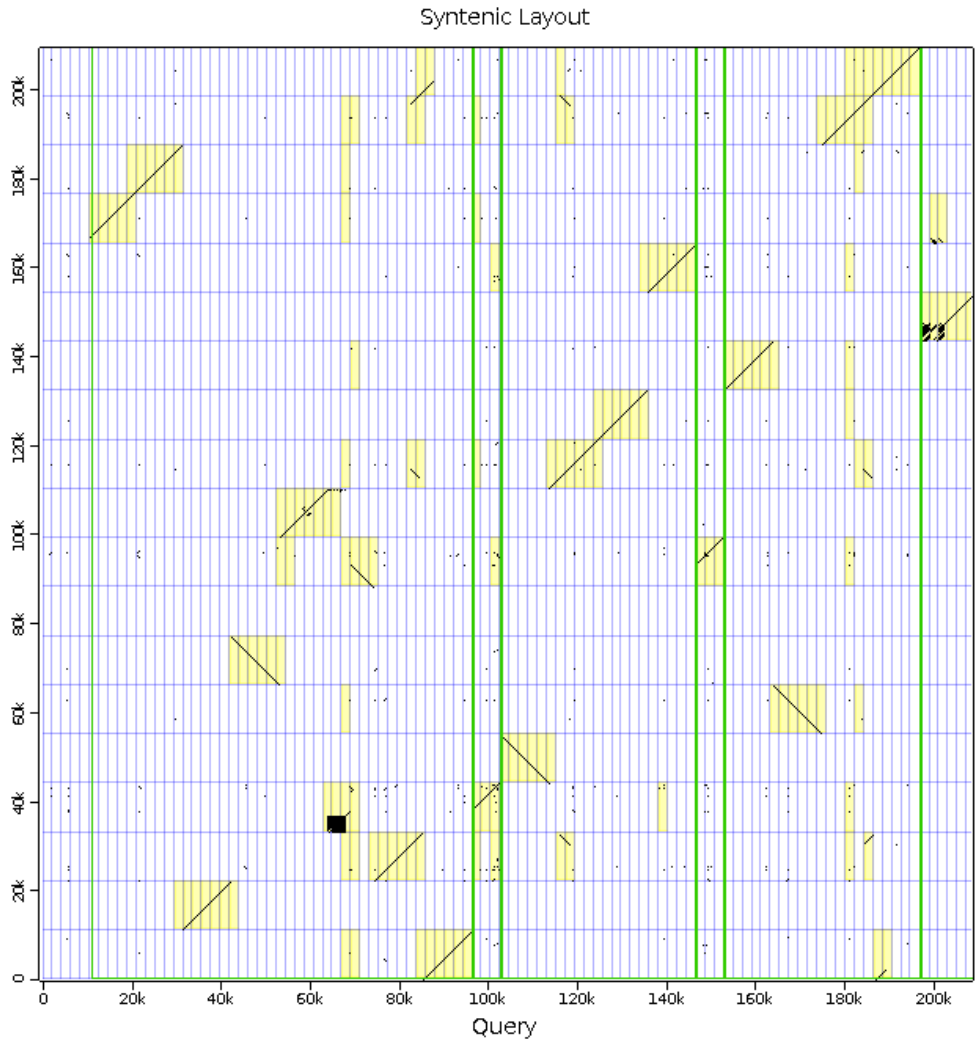
Project: Assembly Comparison

OSLay



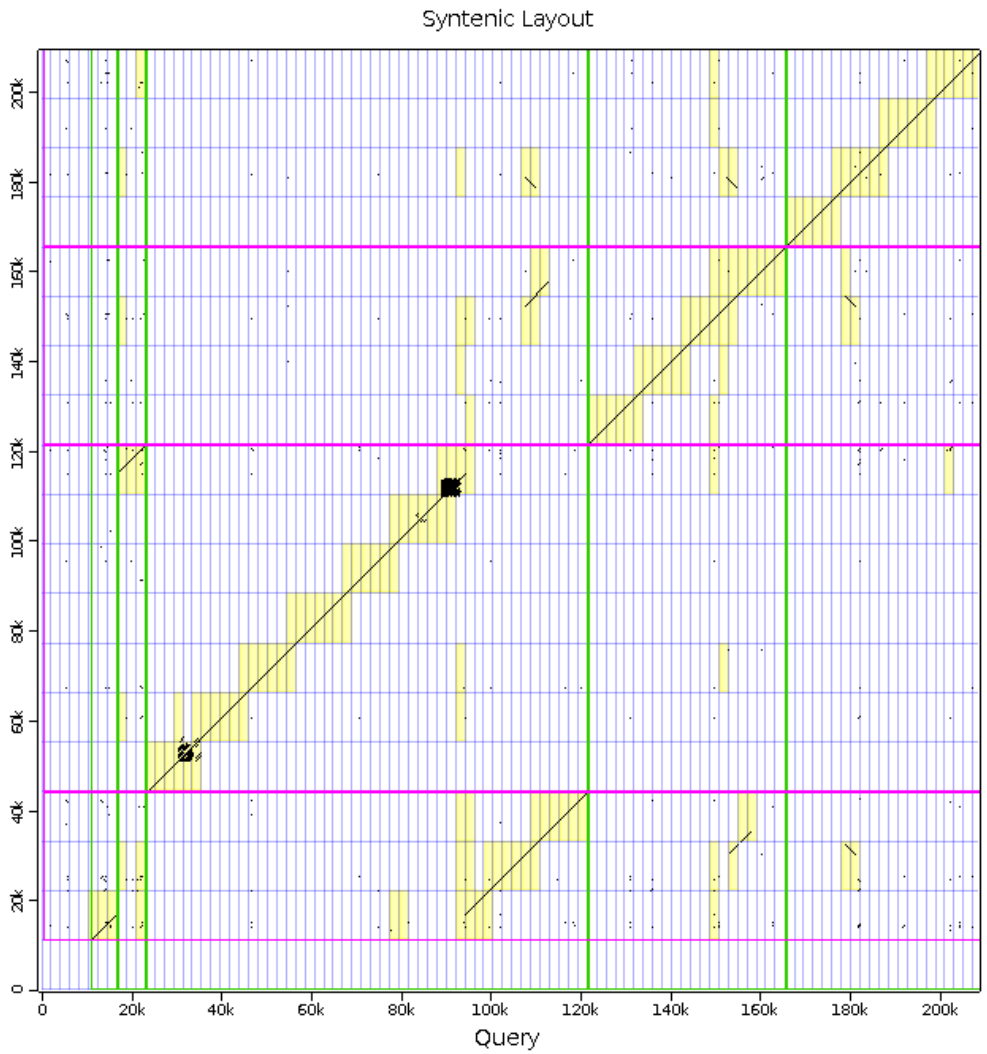
Project: Assembly Comparison

OSLay



Project: Assembly Comparison

OSLay



Project: Assembly Comparison

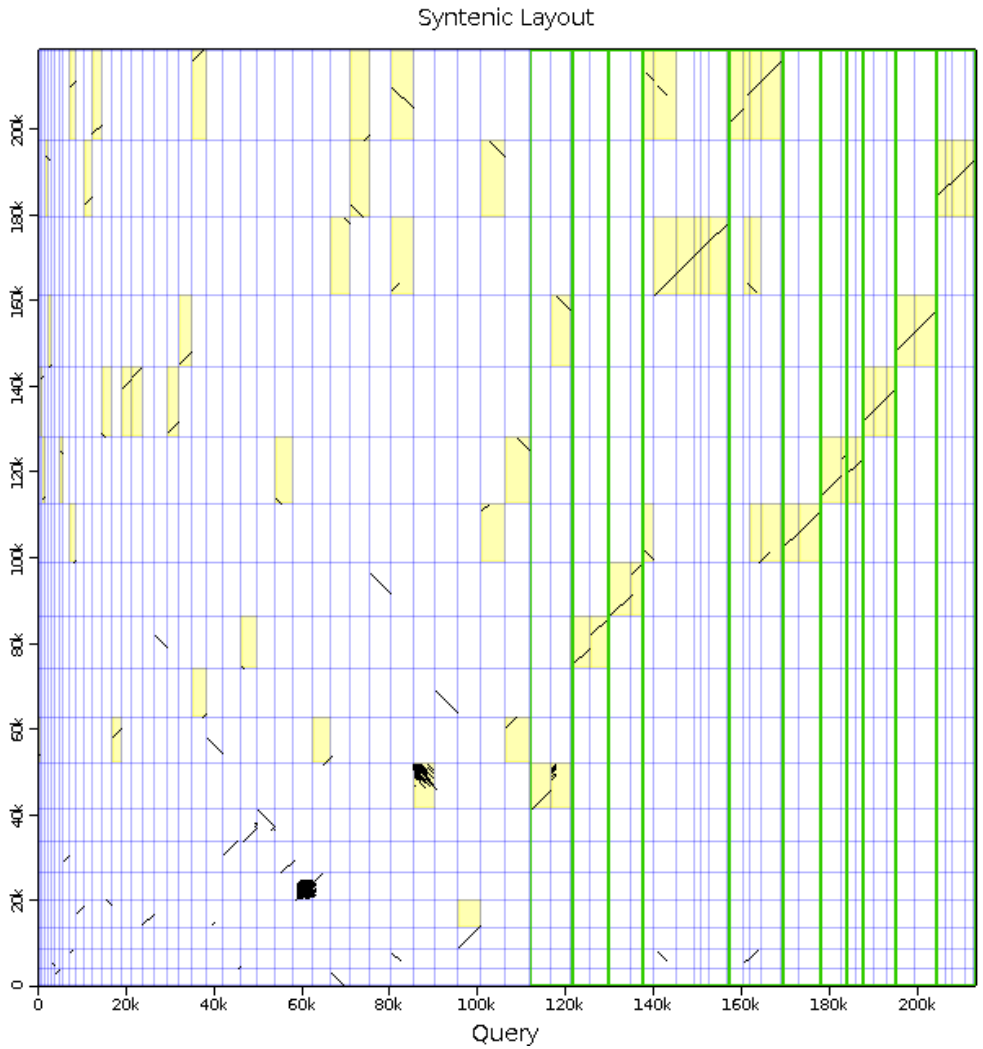
OSLay

Create contigs with random length:

- Assembly A: lengths between 500 and 5000 bp ($\sim$ 100 contigs)
- Assembly B: lengths between 1000 and 200000 bp ($\sim$ 20 contigs)

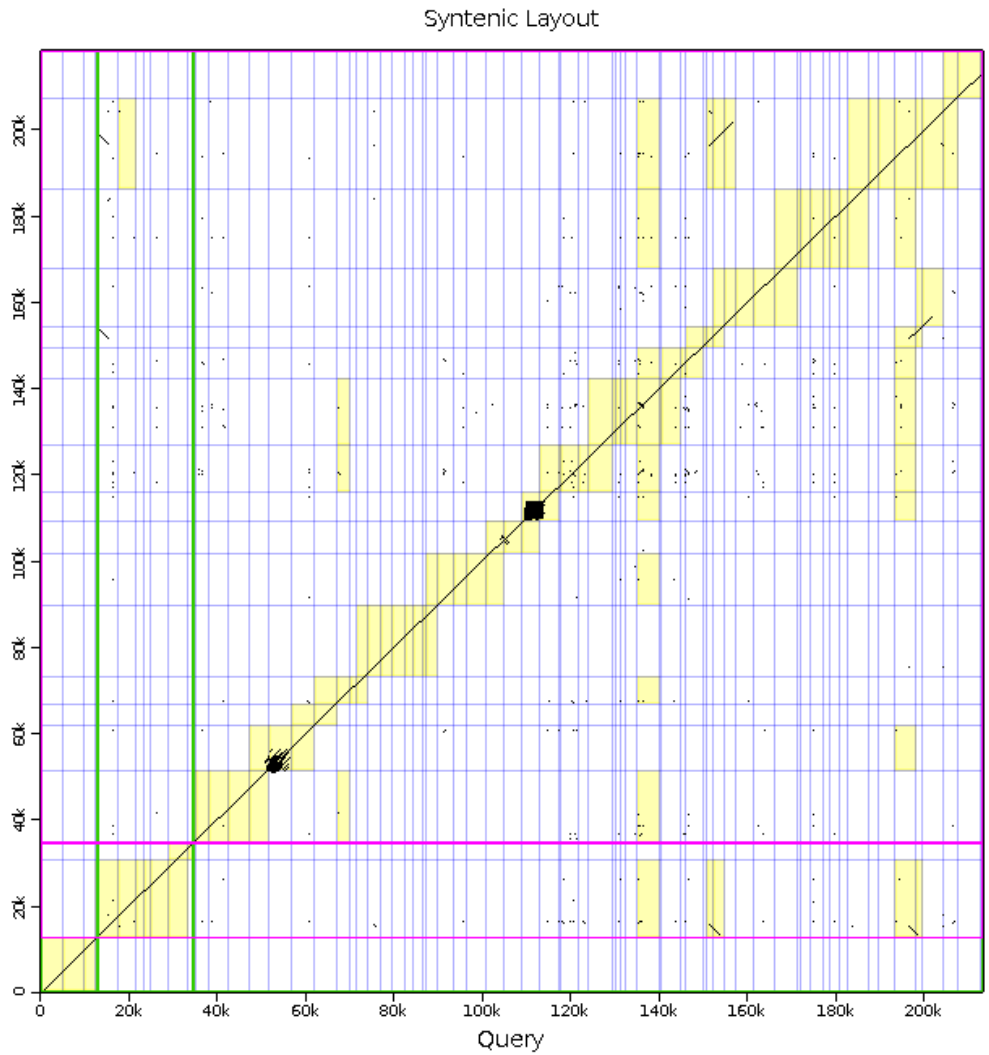
Project: Assembly Comparison

OSLay



Project: Assembly Comparison

OSLay



Project: Assembly Comparison

OSLay

Discussion

- Works only with similar sequences
- But: Contig borders of Assemblies should be different
- Just for small genomes

References

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