Whole Genome Alignment

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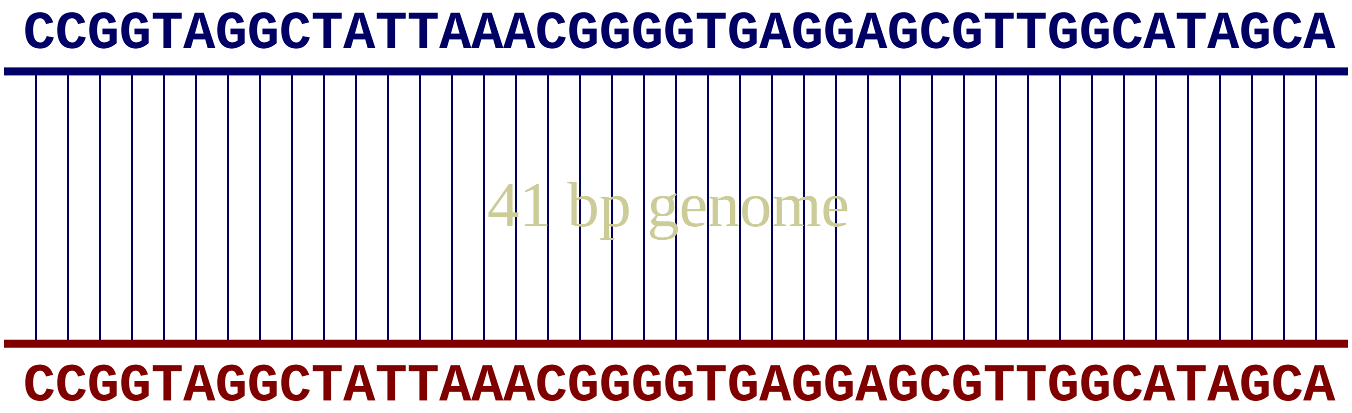
Goal of WGA

- For two genomes, *A* and *B*, find a mapping from each position in *A* to its corresponding position in *B*



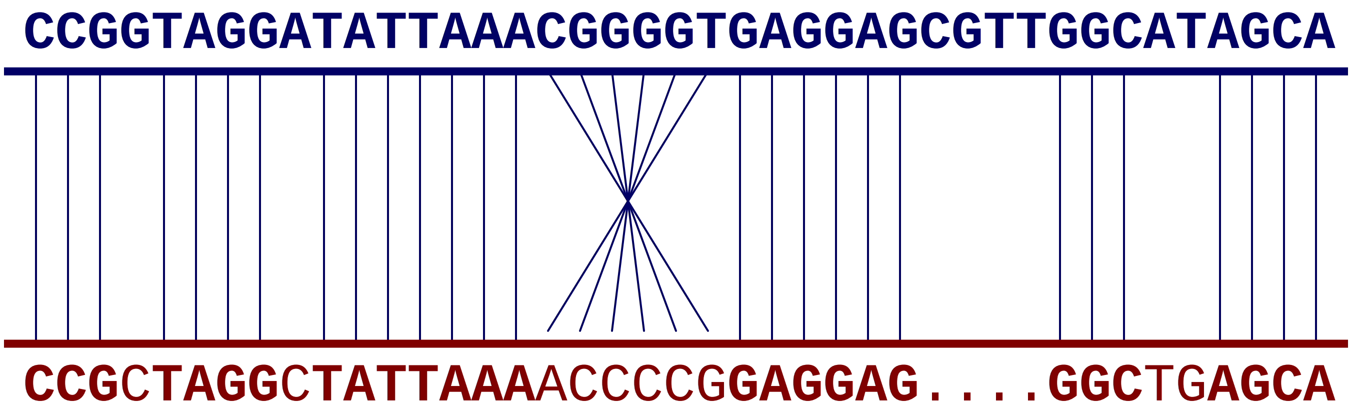
CCGGTAGGCTATTAAACGGGGTGAGGAGCGTTGGCATAGCA

41 bp genome

CCGGTAGGCTATTAAACGGGGTGAGGAGCGTTGGCATAGCA

Not so fast...

- Genome *A* may have insertions, deletions, translocations, inversions, duplications or SNPs with respect to *B* (sometimes all of the above)



CCGGTAGGATATTAAACGGGGTGAGGAGCGTTGGCATAGCA

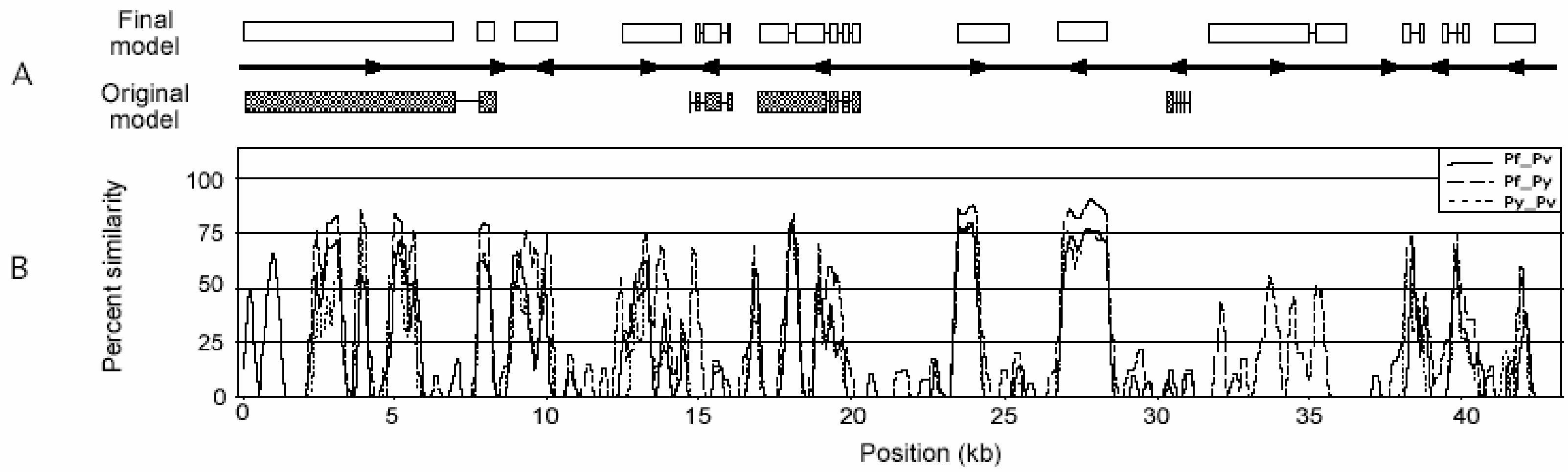
CCGCTAGGCTATTAAAACCCCGGAGGAG....GGCTGAGCA

Sidetrack: Plots

- How can we visualize alignments?

- With an identity plot
  - XY plot
    - Let x = position in genome *A*
    - Let y = %similarity of A_x to corresponding position in *B*
  - Plot the identity function
  - This can reveal islands of conservation, e.g. exons

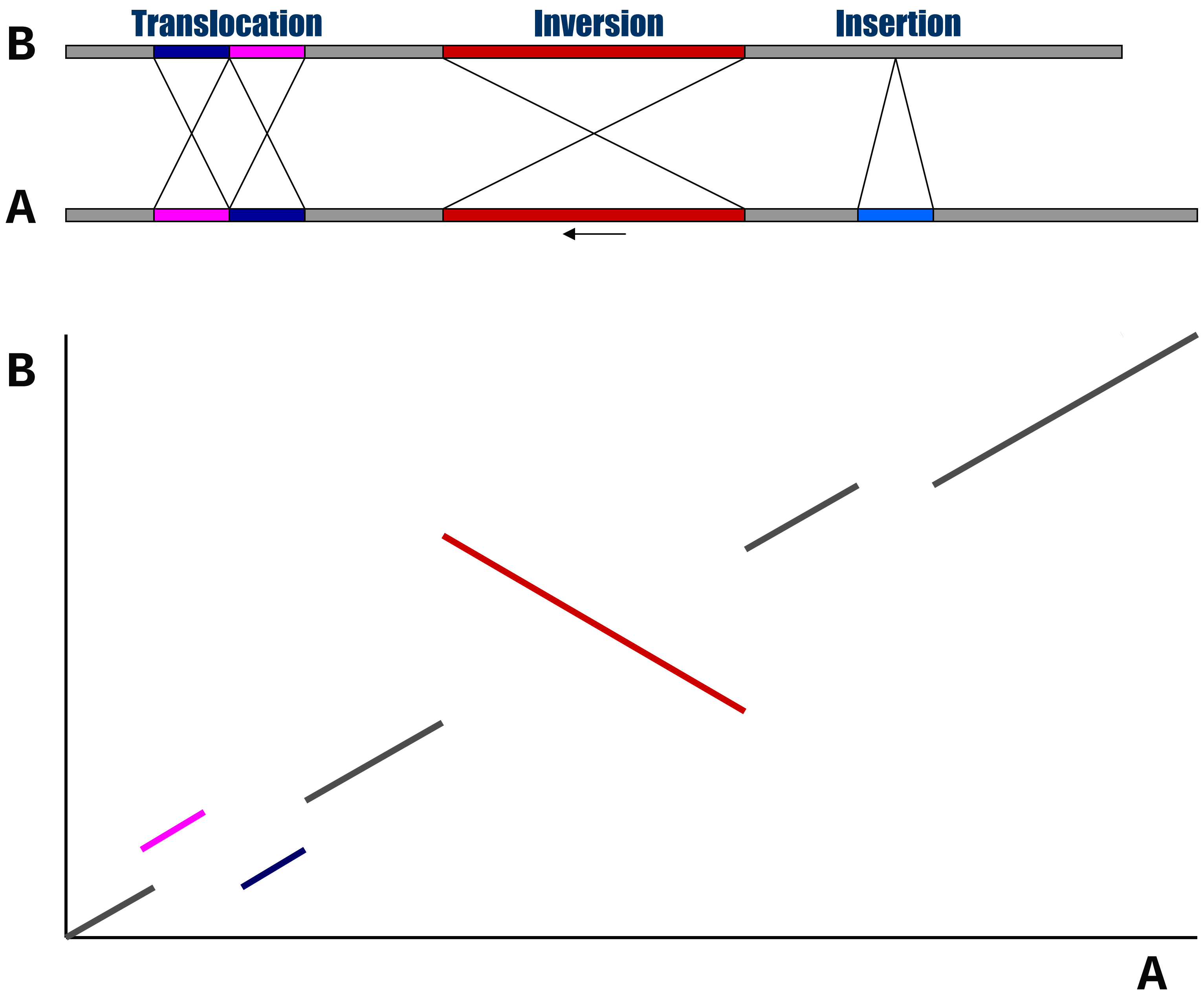
Identity plot example



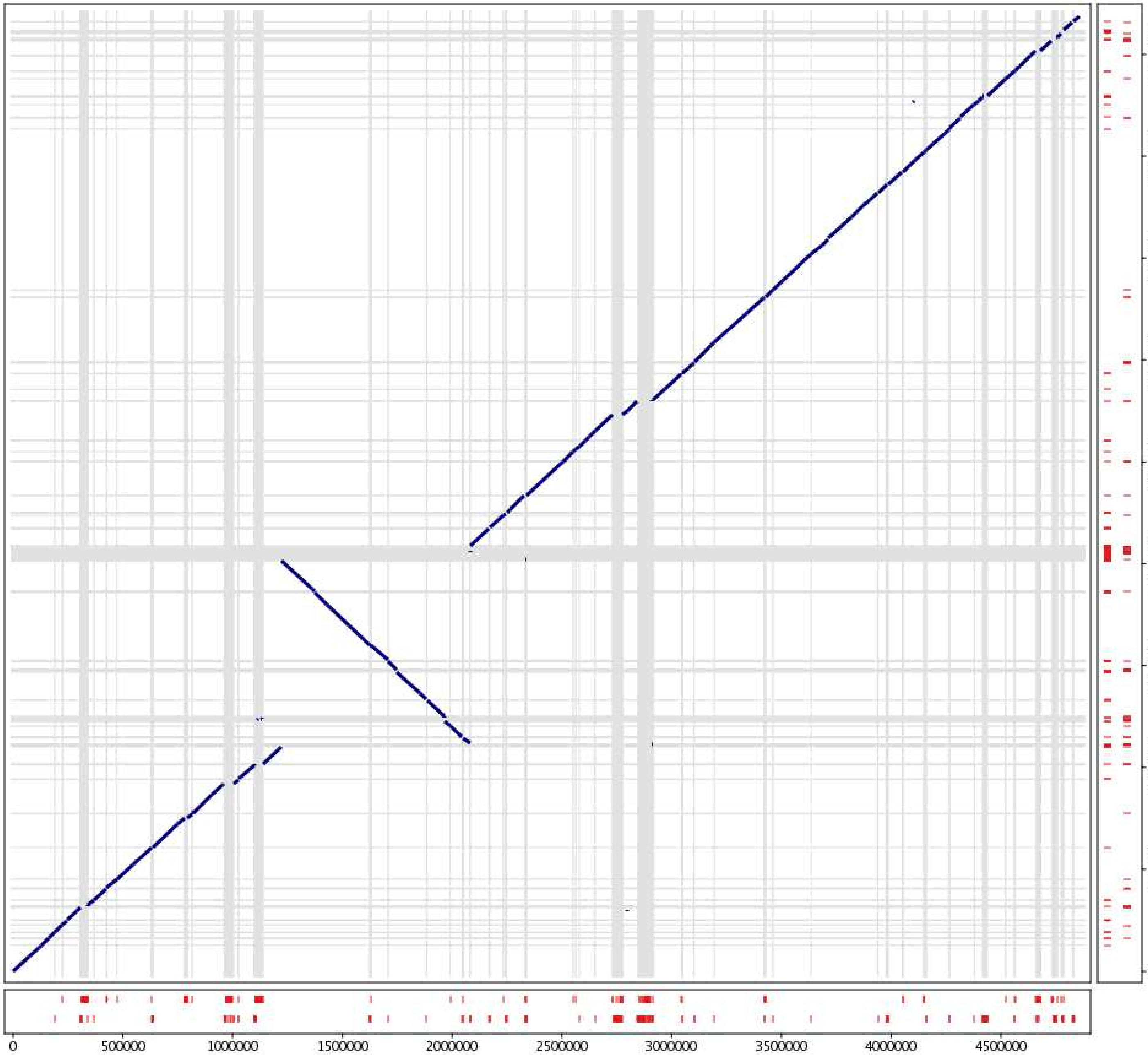
Sidetrack: Plots

- How can we visualize *whole* genome alignments?

- With an alignment dot plot
  - N x M matrix
    - Let i = position in genome A
    - Let j = position in genome B
    - Fill cell (i,j) if A_i shows similarity to B_j
  - A perfect alignment between A and B would completely fill the positive diagonal



http://mummer.sourceforge.net/manual/AlignmentTypes.pdf


0
500000
1000000
1500000
2000000
2500000
3000000
3500000
4000000
4500000
0
500000
1000000
1500000
2000000
2500000
3000000
3500000
4000000
4500000

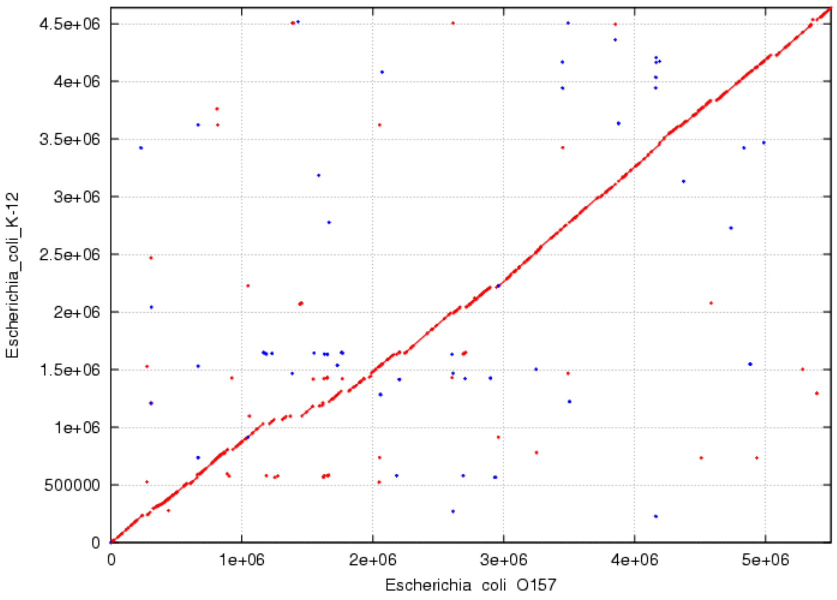
Global vs. Local

- Global pairwise alignment

```
...AAGCTTGGCTTAGCTGCTAGGGTAGGCTTGGG...
...AAGCTGGGCTTAGTTGCTAG..TAGGCTTTGG...
        ^       ^      ^^       ^
```

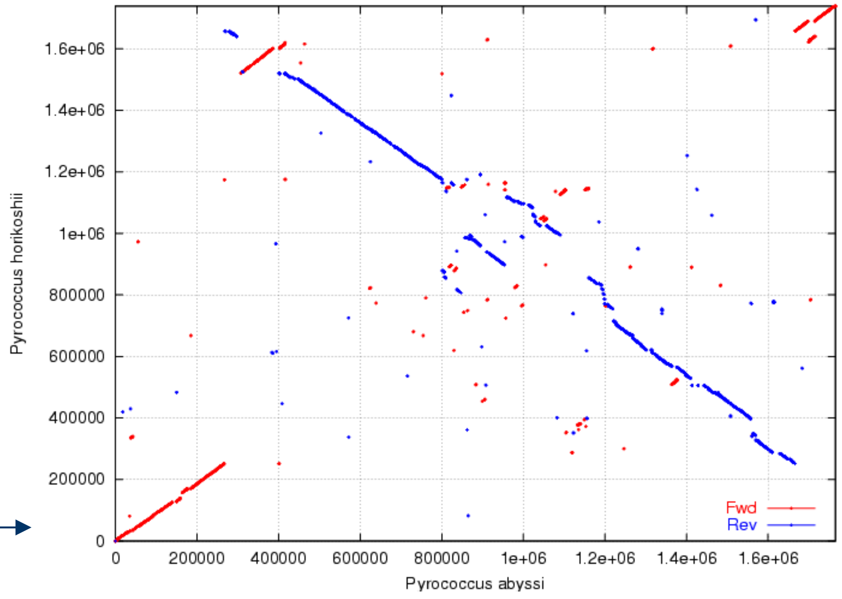
- Whole genome alignment
  - Often impossible to represent as a global alignment
  - We will assume a set of local alignments (g-local)
    - This works great for draft sequence

Global vs. Local



global ok

global no way



Alignment Uses

- **Whole genome alignment**
  - Synteny analysis
  - Polymorphism detection
  - Sequence mapping
- **Multiple genome alignment**
  - Identify conserved sequence, e.g. functional elements (annotation)
  - Polymorphism detection
- **Multiple alignment**
  - Phylogenetics
  - Protein domain/structure analysis
- **Local sequence alignment**
  - Identify a DNA or protein sequence (annotation)
  - Sensitive homology search
  - Anchor a whole genome alignment

Alignment Tools

- **Whole genome alignment**
  - MUMmer*
    - Developed, supported and available at TIGR
  - LAGAN*, AVID
    - VISTA identity plots
- **Multiple genome alignment**
  - MGA, MLAGAN*, DIALIGN, MAVID
- **Multiple alignment**
  - Muscle$^{?}$, ClustalW*
- **Local sequence alignment**
  - BLAST*, FASTA,Vmatch

* open source

MUMmer

- Maximal Unique Matcher (MUM)
    - match
        - exact match of a minimum length
    - maximal
        - cannot be extended in either direction without a mismatch
    - *unique*
        - occurs only once in both sequences (MUM)
        - occurs only once in a single sequence (MAM)
        - occurs one or more times in either sequence (MEM)

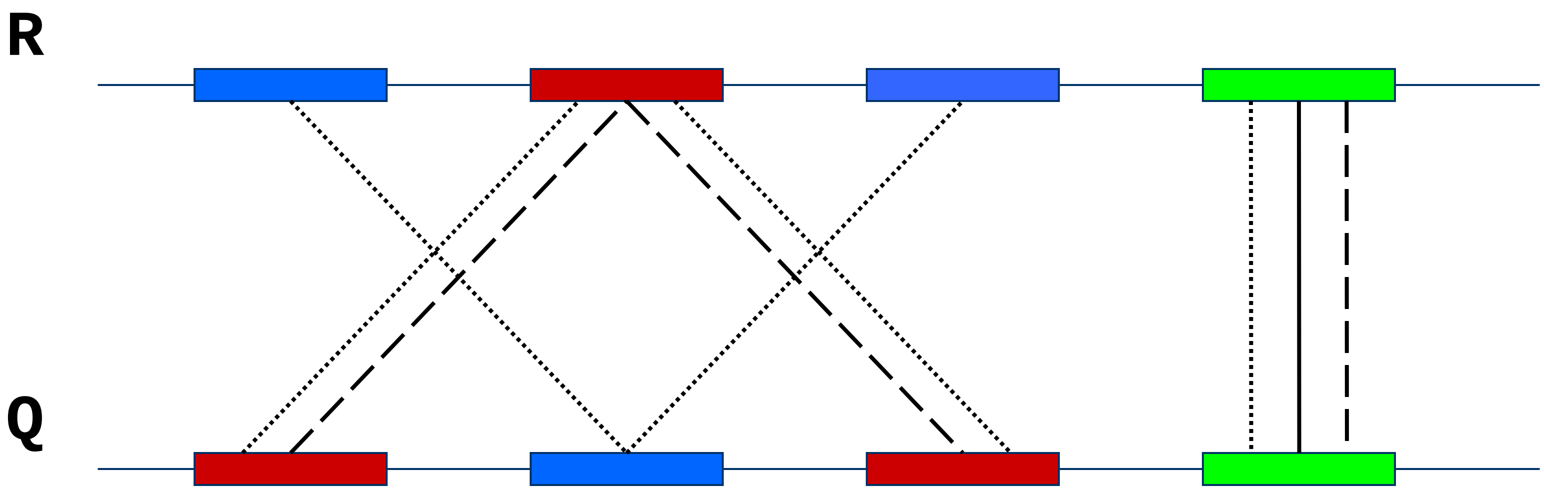
Fee Fi Fo Fum,

is it a MAM, MEM or MUM?

MUM : maximal unique match ────────

MAM : maximal almost-unique match – – – – – – – –

MEM : maximal exact match ················



Seed and Extend

- How can we make MUMs **BIGGER?**

1. Find MUMs
   - using a suffix tree
2. Cluster MUMs
   - using size, gap and distance parameters
3. Extend clusters
   - using modified Smith-Waterman algorithm

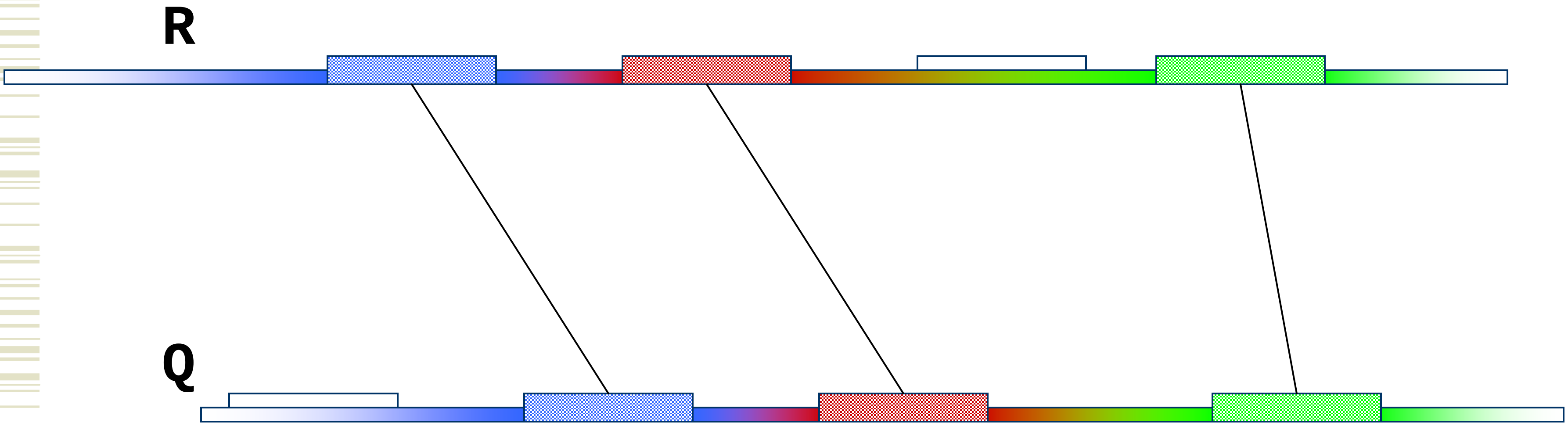
Seed and Extend

visualization

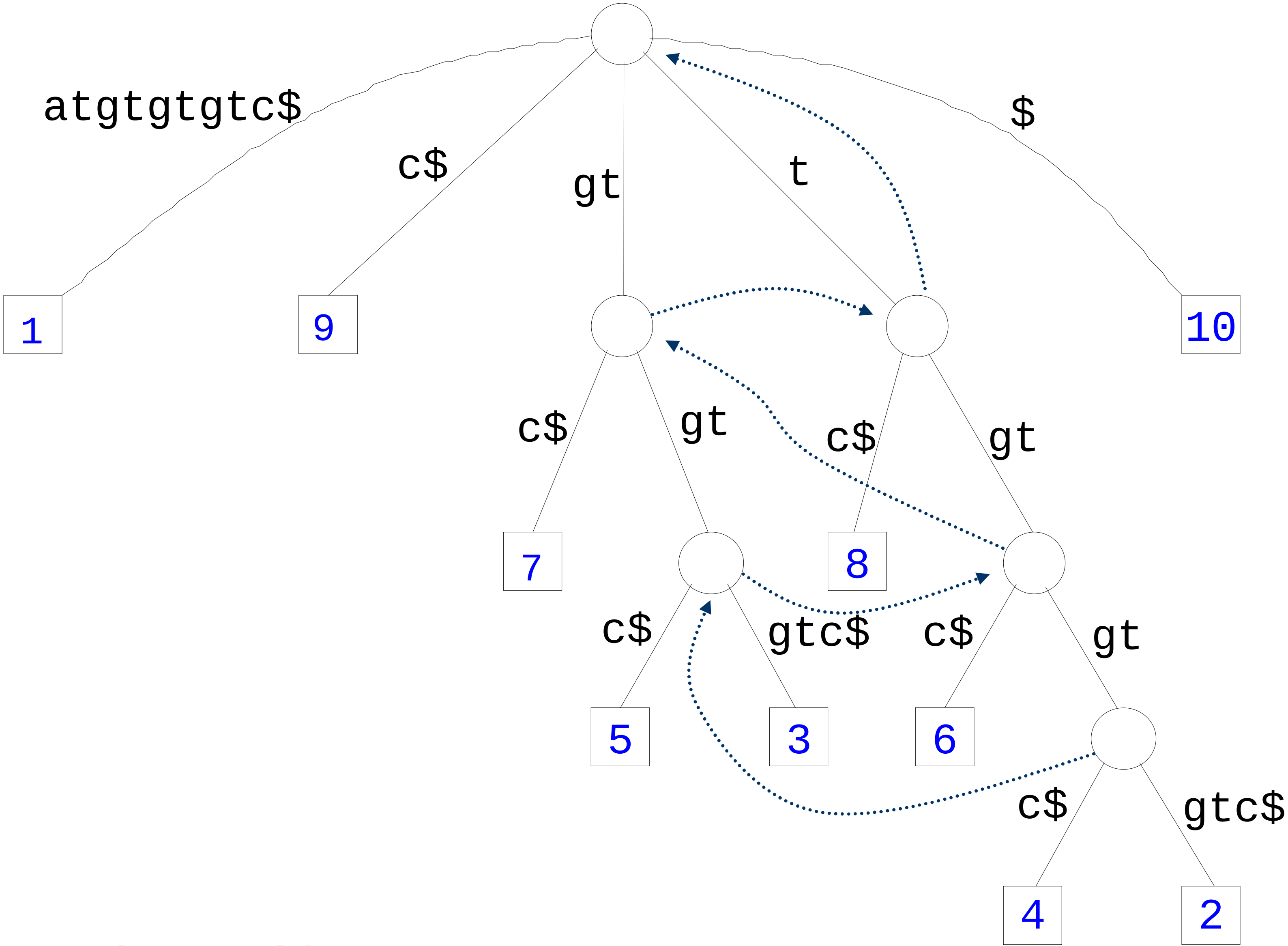
FIND all MUMs

CLUSTER consistent MUMs

EXTEND alignments



Suffix Tree for atgtgtgtc$



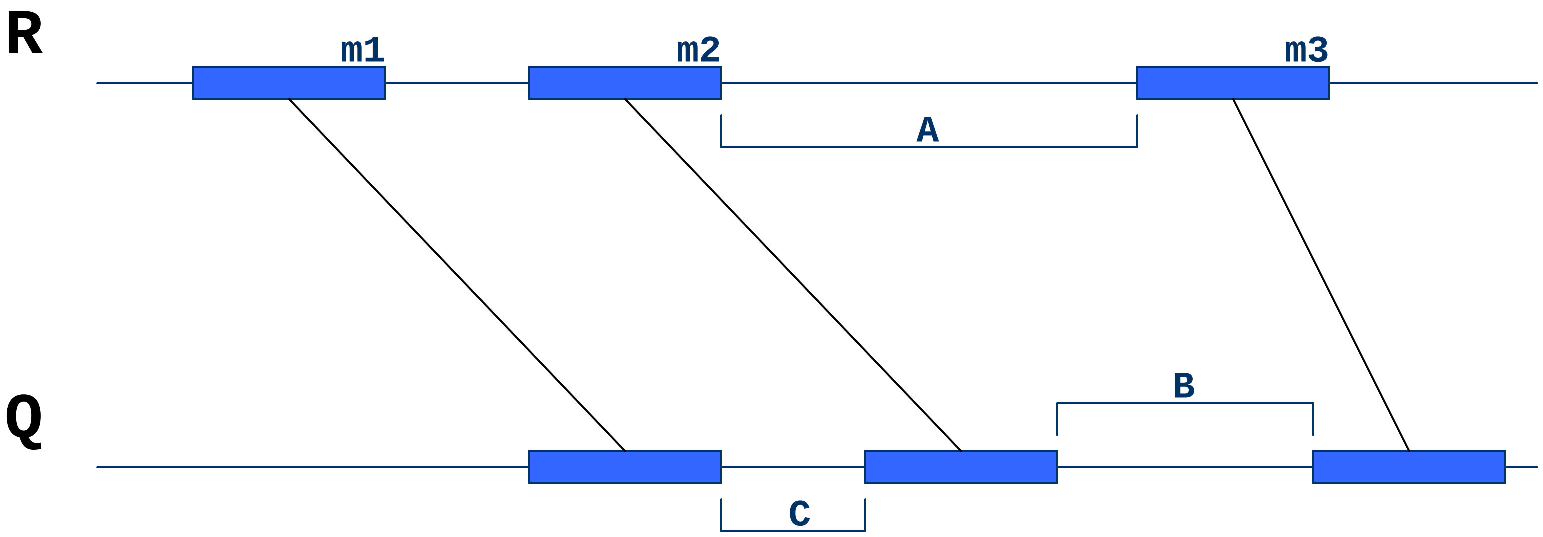
Drawing credit: Art Delcher

Clustering

cluster length = Σm_i

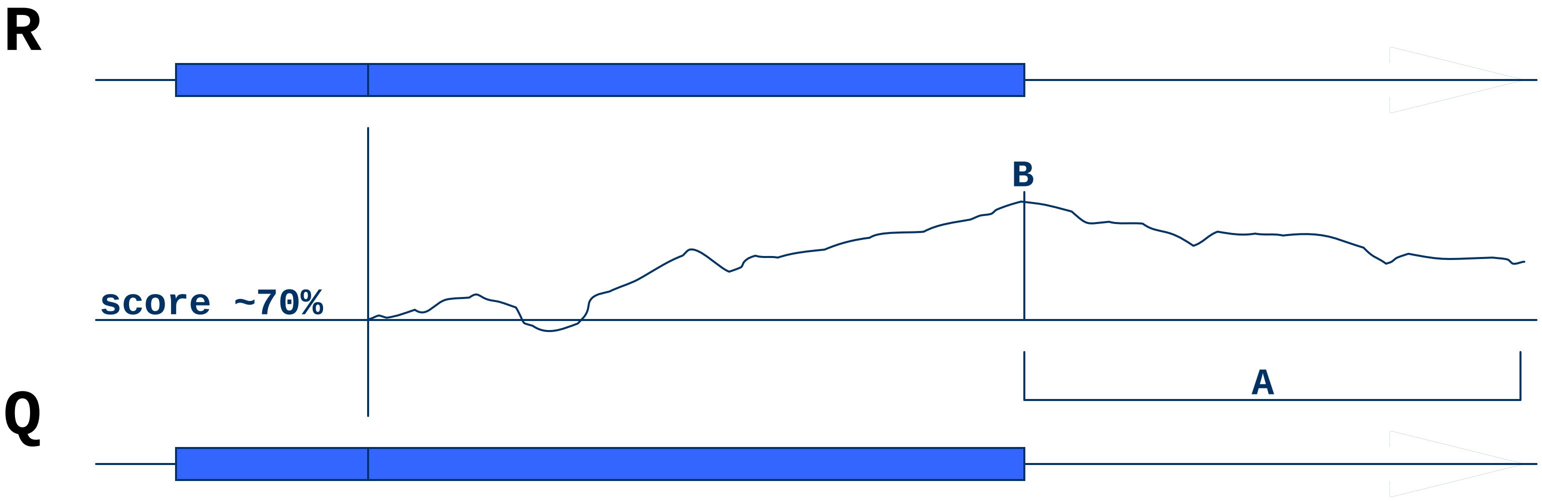
gap distance = C

indel factor = |B – A| / B *or* |B – A|



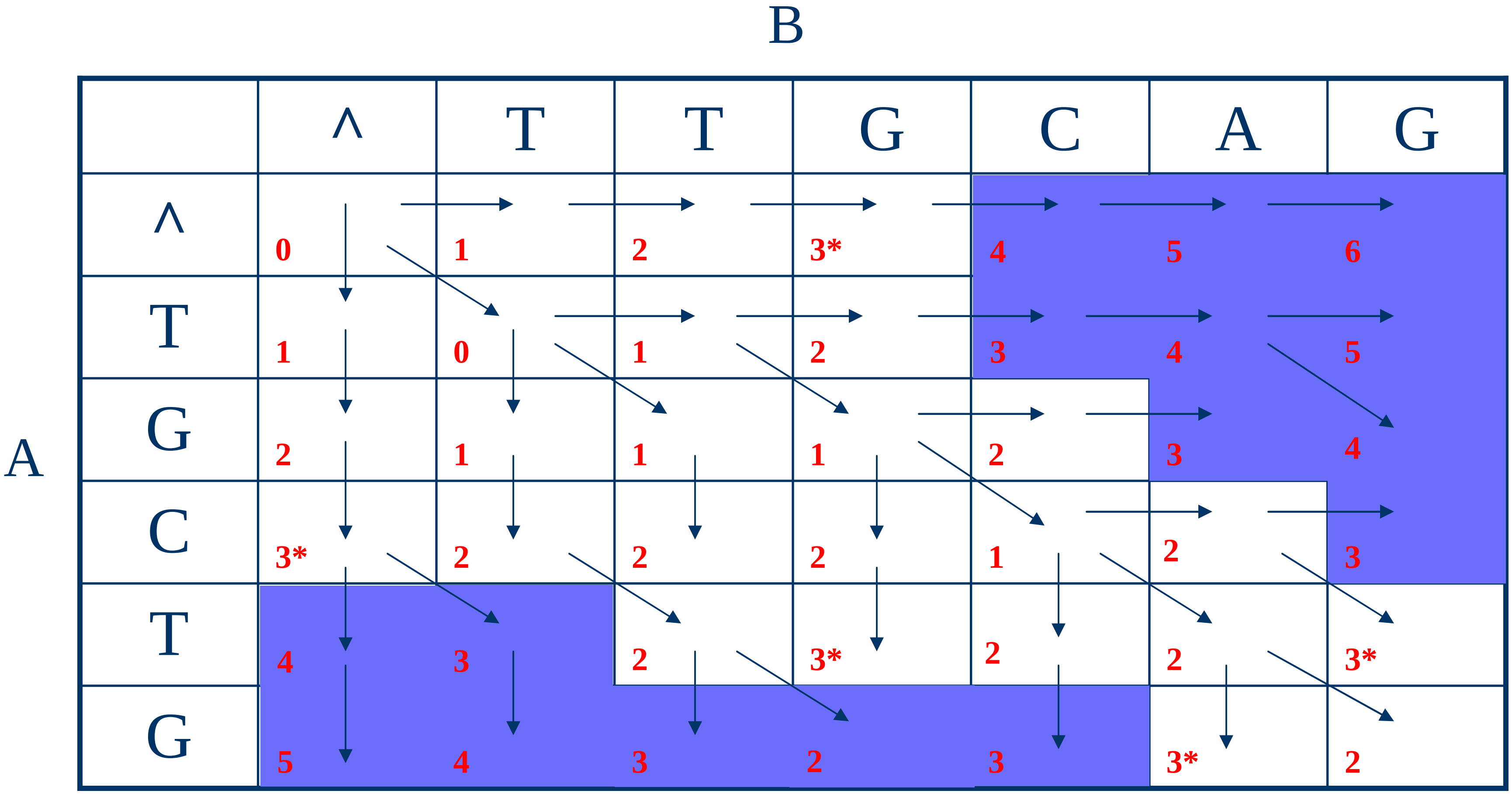
Extending

break point = B



break length = A

Banded Alignment



B

A

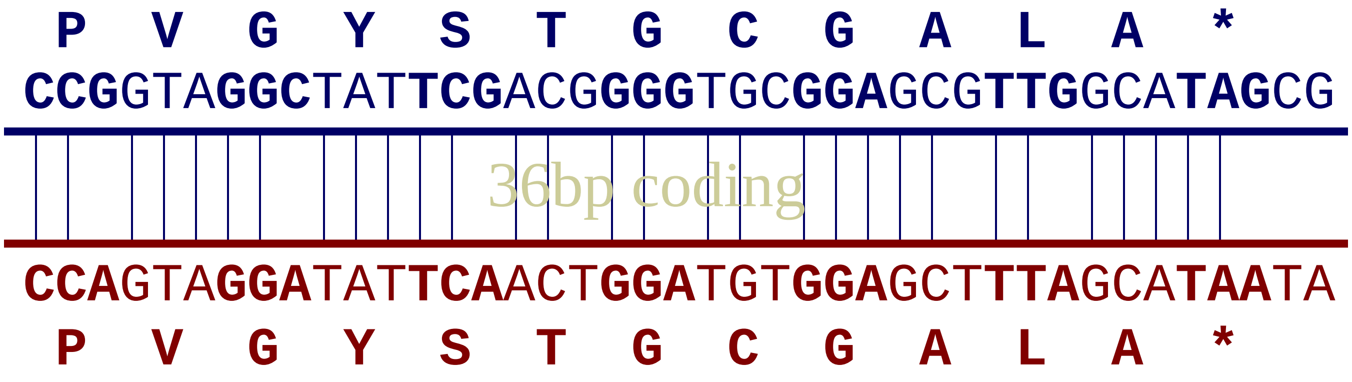
	^	T	T	G	C	A	G
^	0	1	2	3*	4	5	6
T	1	0	1	2	3	4	5
G	2	1	1	1	2	3	4
C	3*	2	2	2	1	2	3
T	4	3	2	3*	2	2	3*
G	5	4	3	2	3	3*	2

Adjustables

Matching	**nuc/promer options**
match length	-l
mum, mam, mem	-mum, -mumreference, -maxmatch
Clustering	
cluster length	-c
gap distance	-g
indel factor	-d
Extending	
search length	-b
scoring matrix	-x

Seedless Genes

- Single base pair substitution
  - non-synonymous mutation
  - synonymous mutation
    - 80% AT *Plasmodium falciparum*
    - 55% AT *Plasmodium vivax*



P V G Y S T G C G A L A *

CCGGTA**GGC**TAT**TCG**ACG**GGG**TGC**GGA**GCG**TTG**GCA**TAG**CG

CCAGTA**GGA**TAT**TCA**ACT**GGA**TGT**GGA**GCT**TTA**GCA**TAA**TA

P V G Y S T G C G A L A *

Sidetrack: MUMmer suite

- `mummer`
  - exact matching
- `nucmer`
  - DNA multi-FastA input
  - whole genome alignment
- `promer`
  - DNA multi-FastA input
  - whole genome alignment
- `run-mummer1*`
  - FastA input
  - global alignment
- `run-mummer3*`
  - FastA input w/ draft
  - whole genome alignment
- `exact-tandems`
  - FastA input
  - exact tandem repeats

- NUCmer / PROmer utilities
  - `mapview*`
    - alignment plotter
    - draft sequence mapping
  - `delta-filter`
    - alignment filter
  - `mummerplot`
    - dot plotter
  - `show-aligns`
    - pairwise alignments
  - `show-coords`
    - alignment summary
  - `show-snps`
    - snp reporting
  - `show-tiling*`
    - draft sequence tiling
- System utilities
  - `gnuplot`
  - `xfig`

* outdated

mummer

- Primary uses
  - exact matching (seeding)
  - dot plotting
- Pros
  - very efficient *O*(n) time and space
    - ~17 bytes per bp of reference sequence
    - *E. coli K12* vs. *E. coli O157:H7* (~5Mbp each)
      - 17 seconds using 77 MB RAM
  - multi-FastA input
- Cons
  - exact matches only

nucmer & promer

- Primary uses
  - whole genome alignment and analysis
  - draft sequence alignment
- Pros
  - multi-FastA inputs
  - well suited for genome and contig mapping
  - convenient helper utilities
    - `show-coords, show-snps, show-aligns`
    - `mummerplot`
- Cons
  - low sensitivity (w\ default parameters) with respect to BLAST

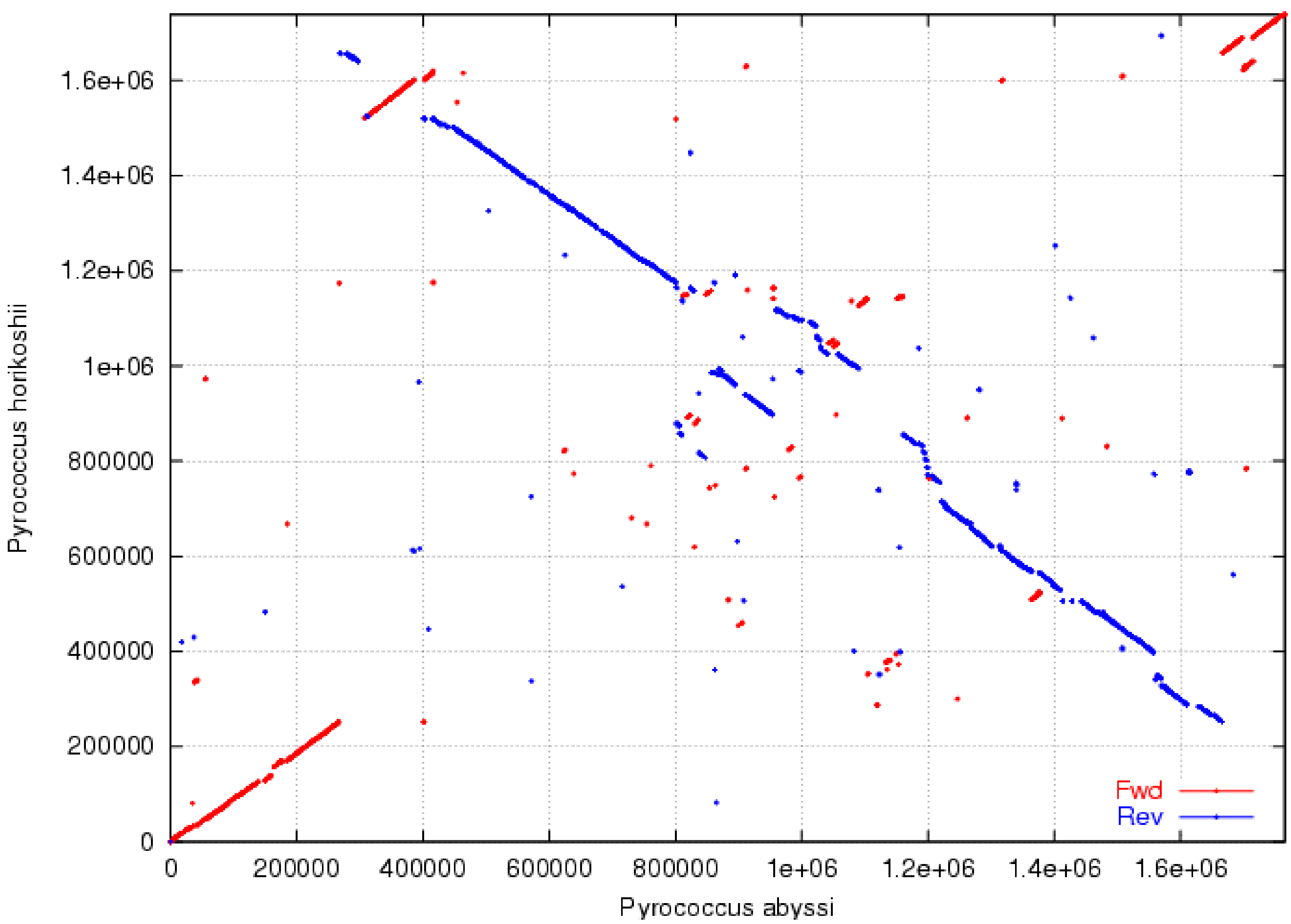
Applied MUMing

- Comparative genomics
  - dot plotting
  - synteny analysis
  - SNP detection
- Genome sequencing
  - draft sequence comparision
  - comparative scaffolding
  - contig and BAC overlaps
- Repeat detection
  - genomic repeats

WGA Example

- *Pyrococcus abyssi* vs. *horikoshii*
  - Hyperthermophilic Archaea
    - 100 °C / 200 bar
  - ~1.7 Mbp circular chromosome
  - ~58% unique genes at time of publication (1998)
  - Chromosome shuffling
    - "Pyrococcus genome comparison evidences chromosome shuffling-driven evolution." Zivanovic Y, Lopez Philippe, Philippe H, Forterre P, *Nucleic Acids Res.* 2002 May 1;30(9):1902-10.
  - See DAGchainer (B. Hass, *et al.*)
    - *Arabidopsis thaliana* segmental duplications

dotplot from promer-based mummerplot



COMMAND

dotplot

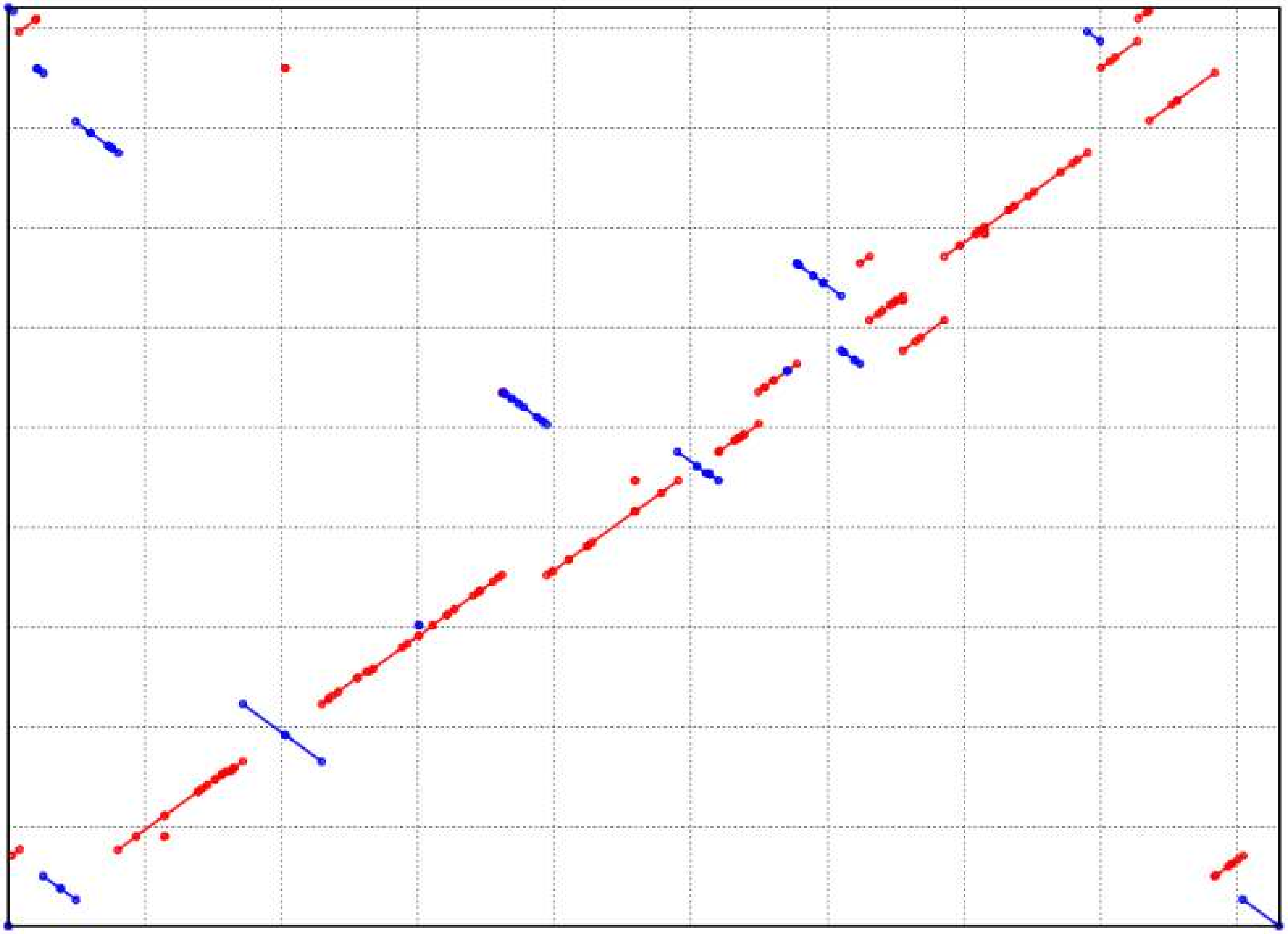
```
promer –mum –l 5 PABY.fasta PHOR.fasta
-mum                 Find maximal unique matches (MUMs)
-l                   Minimum match length (amino acids)

mummerplot –postscript out.delta
-postscript          Generate a postscript format plot
```

OR

```
mummer –mum –l 20 –b –c PABY.fasta PHOR.fasta > out.mums
mummerplot out.mums
```

SNP Example

- *Yersina pestis CO92* vs. *Yersina pestis KIM*
  - High nucleotide similarity, 99.86%
  - Extensive genome shuffling
    - Global alignment will not work
  - Highly repetitive
    - Will confuse local alignment (e.g. BLAST)



COMMAND

SNP detection

```
nucmer –maxmatch CO92.fasta KIM.fasta
-maxmatch      Find maximal exact matches (MEMs)
```

```
delta-filter –r -q out.delta > out.filter
-r             Filter out repetitive reference alignments
-q             Filter out repetitive query alignment
```

```
show-snps –r –I –T –x 10 out.filter > out.snps
-r             Sort SNPs by reference position
-I             Do not output indels
-T             Tab delimited output
-x 10          Output 10bp context for each SNP
```

show-snps output

- **[P1]** position of the SNP in the reference
- **[SUB]** reference base
- **[SUB]** query base
- **[P2]** position of the SNP in the query
- **[BUFF]** distance to the nearest polymorphism
- **[DIST]** distance to the nearest end of sequence
- **[R]** number of overlapping reference alignments (repeats)
- **[Q]** number of overlapping query alignments (repeats)
- **[LEN R]** length of the reference sequence
- **[LEN Q]** length of the query sequence
- **[CTX R]** context surrounding the reference base
- **[CTX Q]** context surrounding the query base
- **[FRM]** alignment orientation, 1 or -1 for forward or reverse
- **[TAGS]** the reference and query FastA IDs respectively

- All output coordinates and lengths are relative to the forward strand

COMMAND

BAC overlapping

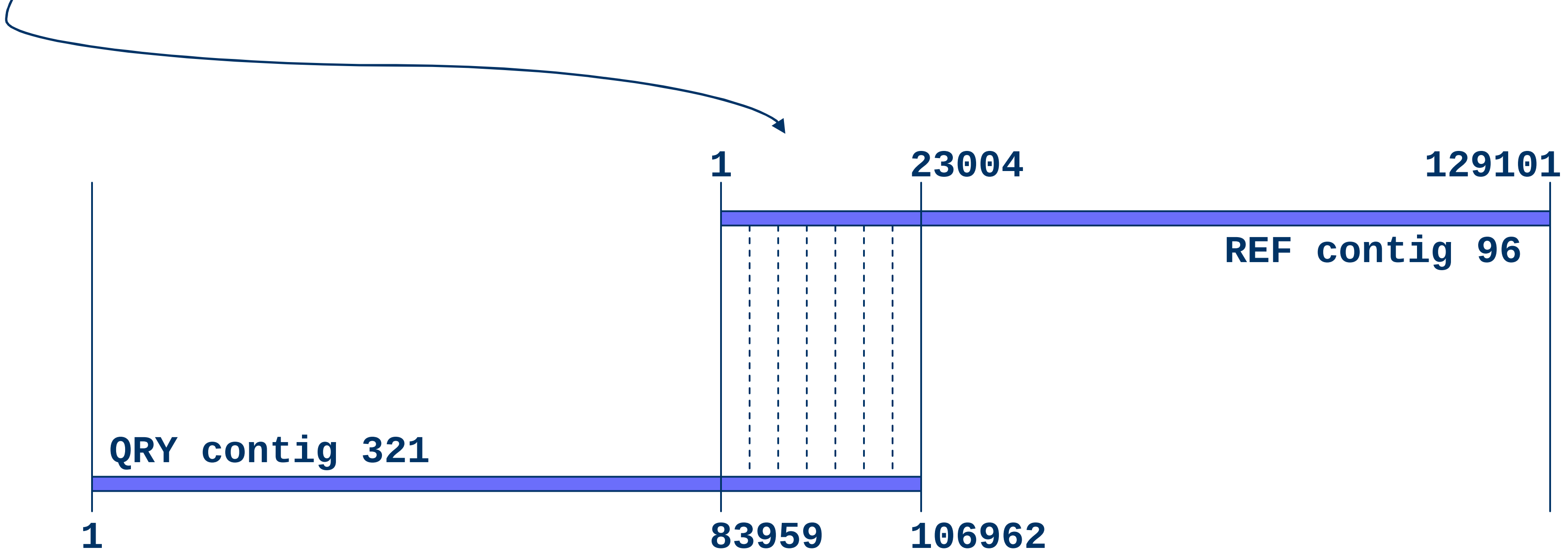
```
nucmer –maxmatch BACS.fasta BACS.fasta
-maxmatch        Find maximal exact matches (MEMs)
```

```
show-coords –rcloT out.delta > out.coords
-r               Sort alignments by reference
-c               Display alignment coverage percentage
-l               Display sequence length
-o               Annotate overlaps between contigs
-T               Tabular output
```

```
show-aligns –r out.delta REF_ID QRY_ID
-r               Sort alignments by reference
```

BAC overlaps found by nucmer

```
[S1]    [E1]    [S2]    [E2]    [LEN 1] [LEN 2] [% IDY] [LEN R] [LEN Q] [COV R] [COV Q] [TAGS]
===================================================================================================
77793   127472  121884  72202   49680   49683   99.95   127472  121884  38.97   40.76   61      45      [END]
1       67053   56621   123672  67053   67052   99.91   127375  123672  52.64   54.22   72      18      [BEGIN]
1       111255  1       111255  111255  111255  99.99   111255  111255  100.00  100.00  74      75      [IDENTITY]
1       111255  1       111255  111255  111255  99.99   111255  111255  100.00  100.00  75      74      [IDENTITY]
107096  114214  116998  109898  7119    7101    98.08   114214  116998  6.23    6.07    76      332     [END]
55298   112695  1       57399   57398   57399   100.00  112695  130043  50.93   44.14   8       90      [END]
42551   116775  139969  65746   74225   74224   99.99   116775  139969  63.56   53.03   87      126     [END]
100319  101839  1       1521    1521    1521    99.41   125220  1521    1.21    100.00  89      561     [CONTAINS]
1       57399   55298   112695  57399   57398   100.00  130043  112695  44.14   50.93   90      8       [BEGIN]
1       23004   83959   106962  23004   23004   100.00  129101  106962  17.82   21.51   96      321     [BEGIN]
```



show-coords output

- **[S1]** start of the alignment region in the reference sequence
- **[E1]** end of the alignment region in the reference sequence
- **[S2]** start of the alignment region in the query sequence
- **[E2]** end of the alignment region in the query sequence
- **[LEN 1]** length of the alignment region in the reference sequence
- **[LEN 2]** length of the alignment region in the query sequence
- **[% IDY]** percent identity of the alignment
- **[% SIM]** percent similarity of the alignment
- **[% STP]** percent of stop codons in the alignment
- **[LEN R]** length of the reference sequence
- **[LEN Q]** length of the query sequence
- **[COV R]** percent alignment coverage in the reference sequence
- **[COV Q]** percent alignment coverage in the query sequence
- **[FRM]** reading frame for the reference and query sequence alignments respectively
- **[TAGS]** the reference and query FastA IDs respectively.

- All output coordinates and lengths are relative to the forward strand

show-aligns output

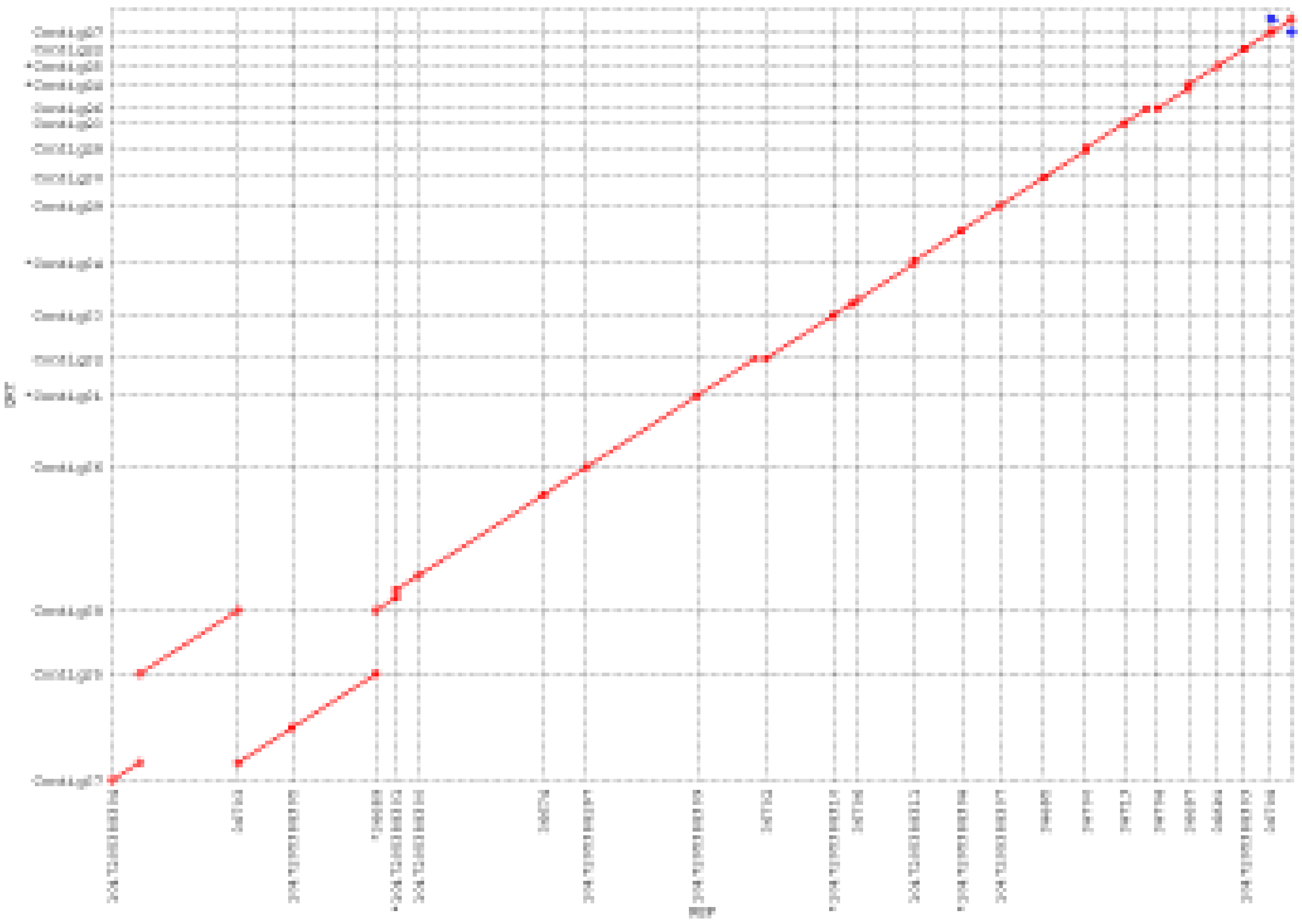
```
-- BEGIN alignment [ +1 1 - 15407 | +1 1 - 15390 ]

1          agcttttcattctgactgcaacgggcaatatgtctctgtgtggattaaaaaaagagtctctgacagcagcttctgaactggttacctgc
1          agcttttcattctgactgcaacgggcaatatgtctctgtgtggattaaaaaaagagtgtctgatagcagcttctgaactggttacctgc
                                                                    ^     ^

90         cgtgagtaaattaaaattttattgacttaggtcactaaatactttaaccaatataggcatagcgcacagacagataaaaattacagagt
90         cgtgagtaaattaaaattttattgacttaggtcactaaatactttaaccaatataggcatagcgcacagacagataaaaattacagagt


179        acacaacatccatgaaacgcattagcaccaccattaccaccaccatcaccaccaccatcaccattaccattaccacaggtaacggtgcg
179        acacaacatccatgaaacgcattagcaccaccattaccaccaccatcacc..................attaccacaggtaacggtgcg
                                                             ^^^^^^^^^^^^^^^^^^

268        ggctgacgcgtacaggaaacacagaaaaaagcccgcacctgacagtgcgggcttttttt.tcgaccaaaggtaacgaggtaacaaccat
250        ggctgacgcgtacaggaaacacagaaaaaagcccgcacctgacagtgcgggctttttttttcgaccaaaggtaacgaggtaacaaccat
                                                                      ^
```

COMMAND

draft sequence comparison

```
nucmer –maxmatch ASM1.fasta ASM2.fasta
-maxmatch        Use maximal exact matches (MEMs)

mummerplot –layout –large -filter out.delta
-layout          Permute alignment matrix for better viewing
-large           Big X11 (or postscript) plot
-filter          Auto-run ‘delta-filter –r –q’

   X11 Navigation:
   left-mouse: position
   middle-mouse: ruler
   right-mouse-drag: zoom-box
   N,P,U keys: next, previous, and un-zoom
```

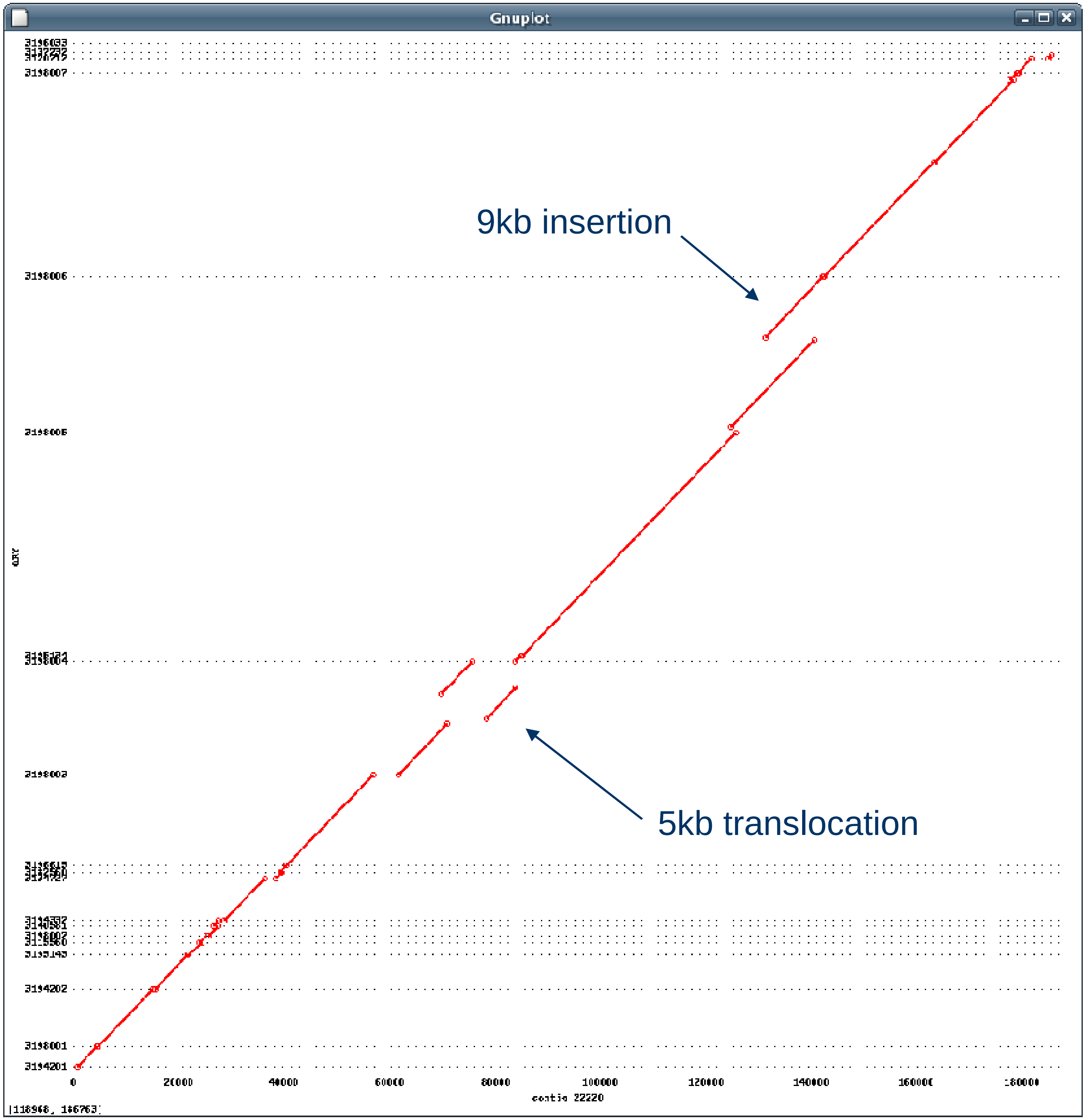
Multiple contig alignment by nucmer



Arachne vs. CA

D. virilis assemblies

Arachne contig (X) mapping to multiple CA contigs (Y). Two macroscopic differences are highlighted, hundreds were found.



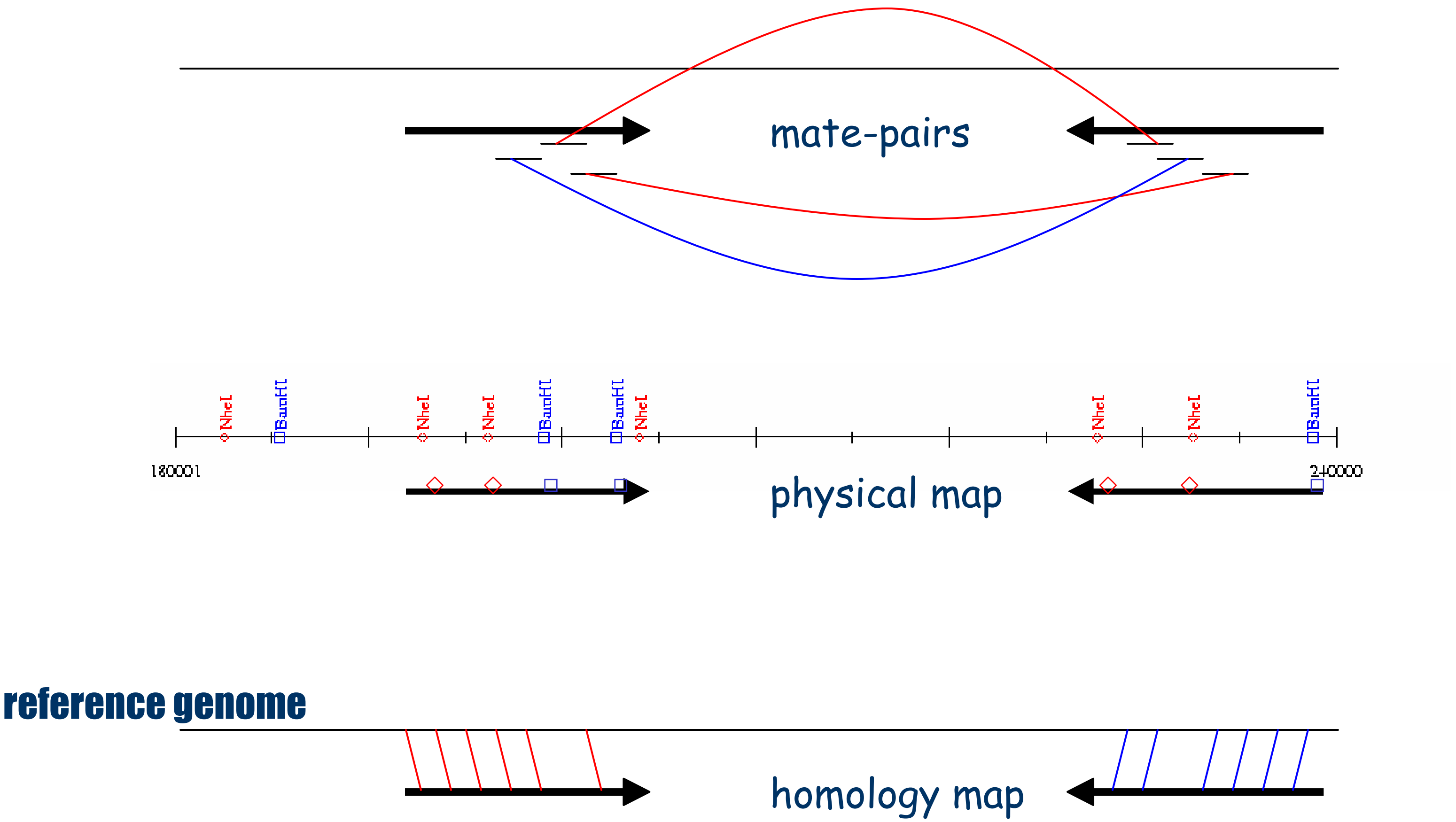
9kb insertion

5kb translocation

Comparative Scaffolding

- Scaffolding
  - order and orient draft contigs
    - using WGS mate-pair information
    - using physical map information
- Comparative Scaffolding
  - order and orient draft contigs
    - using a reference genome and alignment mapping
      - nucmer
  - very useful for physical gaps
  - can instantly close some sequencing gaps (overlapping contigs)

Comparative Scaffolding



mate-pairs

physical map

reference genome

homology map

COMMAND

contig mapping

```
nucmer –maxmatch REF.fasta CTGS.fasta
```

```
-maxmatch    Find maximal exact matches (MEMs)
```

```
delta-filter –q out.delta > out.delta.filter
```

```
-q           Filter out repetitive query alignments
```

```
show-coords –rcl out.delta > out.coords
```

```
-r           Sort alignments by reference
-c           Display alignment coverage percentage
-l           Display sequence length
```

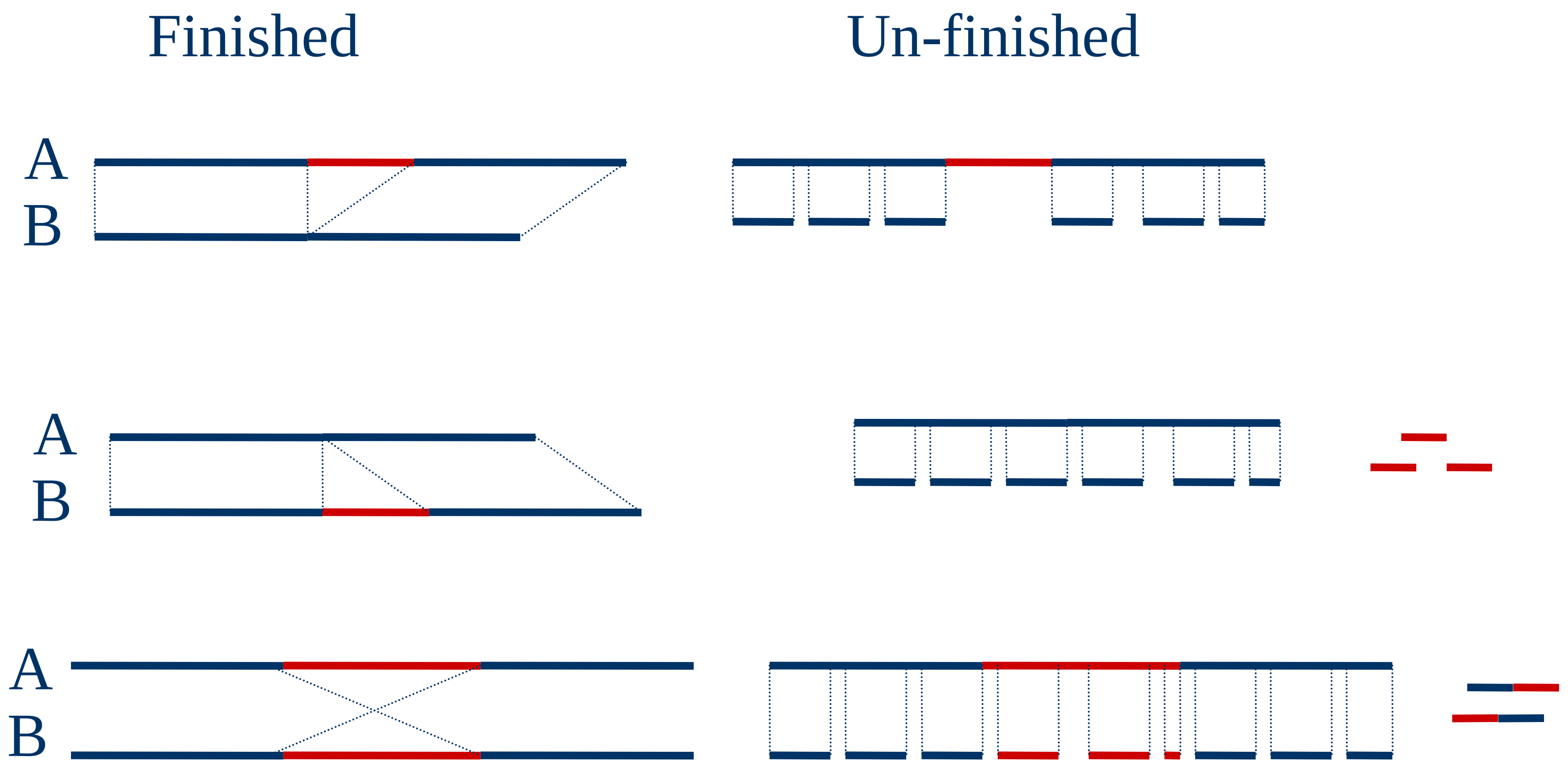
Read Mapping

- Comparative assembly
  - Neanderthal genome, NY Times
    - 454 pyrosequencing
      - 100bp reads
      - no mate-pairs

```
nucmer –maxmatch –l 15 –c 40
delta-filter –q
show-coords -q
```

Comparative Mapping

caveats



Finished

Un-finished

A

B

A

B

A

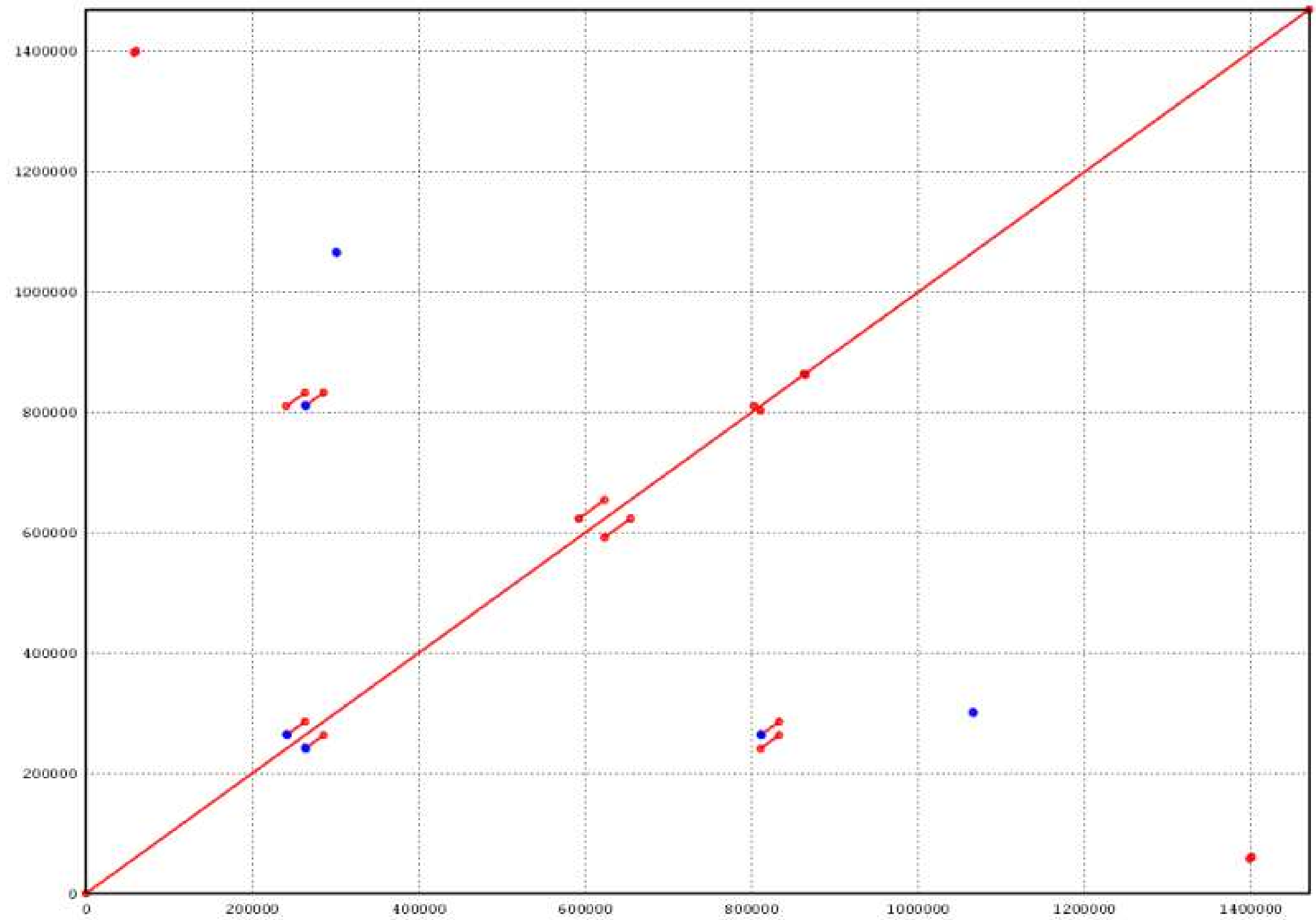
B

…RepeatsRepeatsRepeats…

- Exact repeats, palandromes, tandems, etc.
  - Use Vmatch
    - http://www.vmatch.de
- Long, inexact repeats
  - Use nucmer
    - genomic repeats `-maxmatch` **`-nosimplify`**
    - contig / BAC overlaps `-maxmatch`

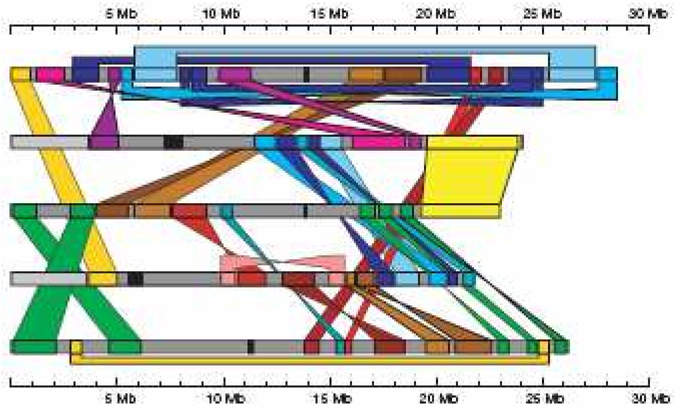
genomic repeats found by ‘nucmer --maxmatch --nosimplify’

```
    [S1]     [E1]  |     [S2]     [E2]  |  [LEN 1]  [LEN 2]  |  [% IDY]  | [TAGS]
=====================================================================================

   57832    60483  |  1398170  1400821  |     2652     2652  |    99.89  | gde:6876     gde:6876
  240759   242028  |   264386   263117  |     1270     1270  |   100.00  | gde:6876     gde:6876
  240759   263123  |   810529   832893  |    22365    22365  |    99.99  | gde:6876     gde:6876
  242022   263123  |   264380   285481  |    21102    21102  |    99.99  | gde:6876     gde:6876

  263117   264386  |   811798   810529  |     1270     1270  |   100.00  | gde:6876     gde:6876

  264380   285490  |   811792   832902  |    21111    21111  |    99.99  | gde:6876     gde:6876
  300630   301615  |  1066580  1065595  |      986      986  |    98.88  | gde:6876     gde:6876
  592225   623250  |   623236   654262  |    31026    31027  |    99.99  | gde:6876     gde:6876

  803061   803126  |   810475   810540  |       66       66  |   100.00  | gde:6876     gde:6876

  862678   863090  |   864053   864465  |      413      413  |    78.74  | gde:6876     gde:6876
```


1400000
1200000
1000000
800000
600000
400000
200000
0
0
200000
400000
600000
800000
1000000
1200000
1400000

References

- Documentation
  - http://mummer.sourceforge.net
    - publication listing
  - http://mummer.sourceforge.net/manual
    - thorough documentation
  - http://mummer.sourceforge.net/examples
    - Walkthroughs
- Email
  - mummer-help (at) lists.sourceforge.net
  - mummer-users (at) lists.sourceforge.net


5 Mb
10 Mb
15 Mb
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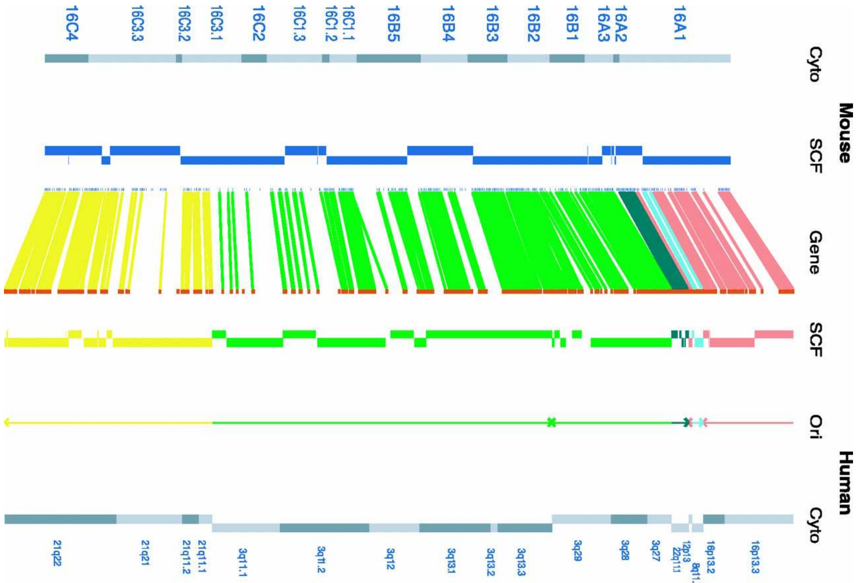
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16B3
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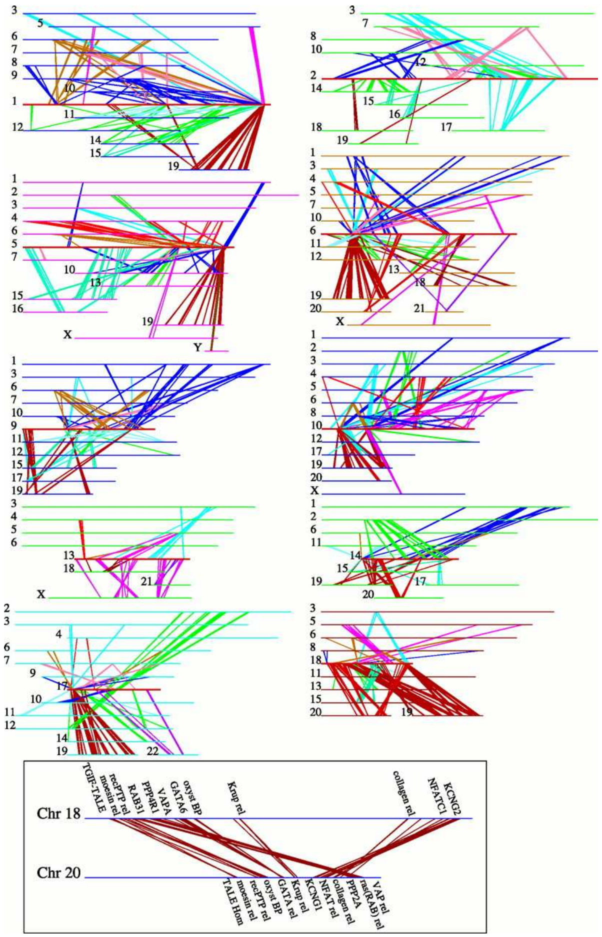
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Chr 18
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TGIF-TALE
moesin rel
recPTP rel
RAB31
PPP4R1
VAPA
GATA6
oxyst BP
Krup rel
collagen rel
NFATC1
KCNG2
TALE Hom
moesin rel
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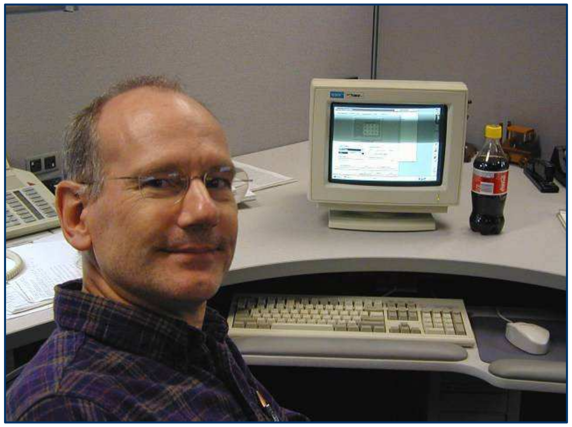
VAP rel

Acknowledgements

Art Delcher



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