Improving Genome Assemblies without Sequencing

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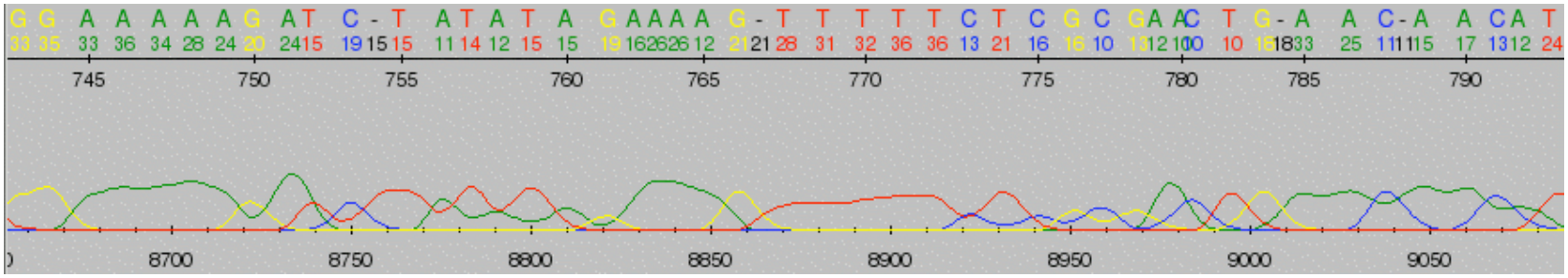
April 25, 2005
TIGR Bioinformatics Seminar

Assembly Pipeline Overview

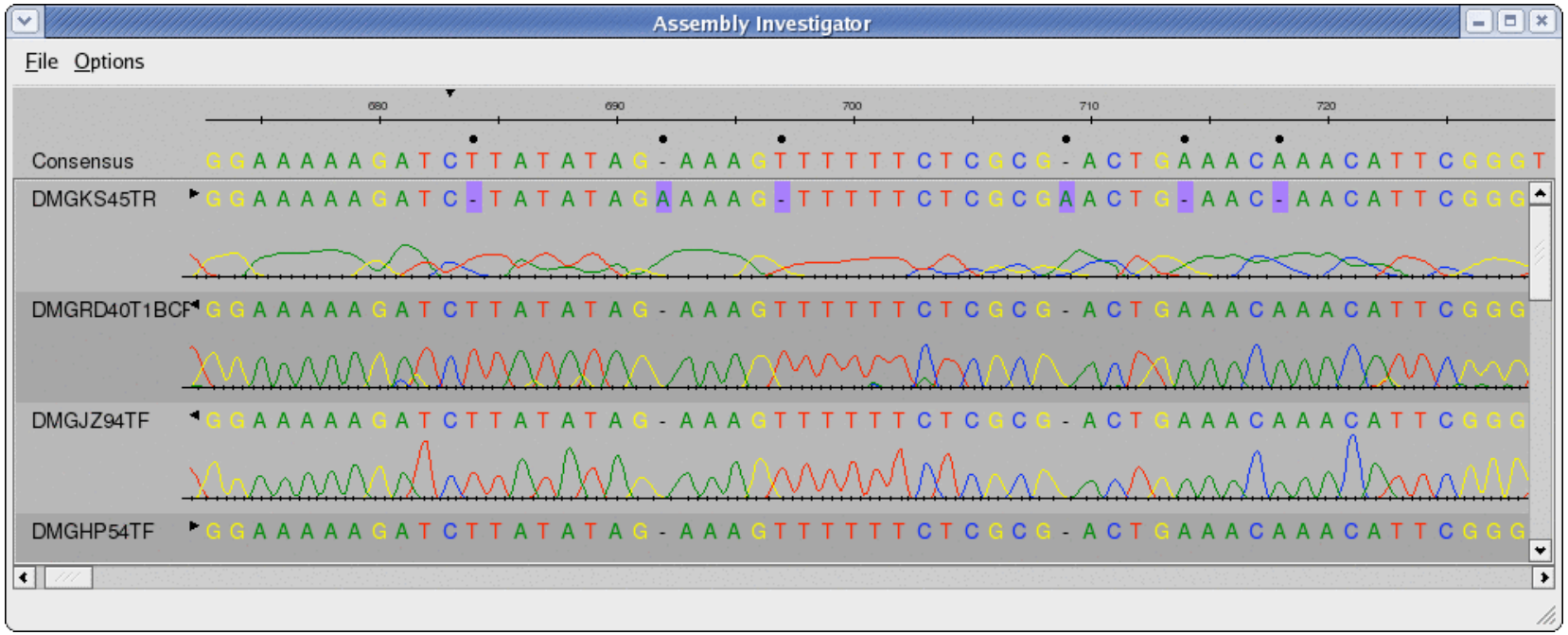
1. Sequence shotgun reads
2. Call Bases *phred/TraceTuner/KB Base Caller*
3. Trim Reads *lucy*
4. Assemble *CA/TA/Arachne*
5. Electronic Finishing
   - Second generation base-caller *AutoEditor*
   - Automatic Gap Closure *AutoJoiner*
   - Research Techniques

AutoEditor

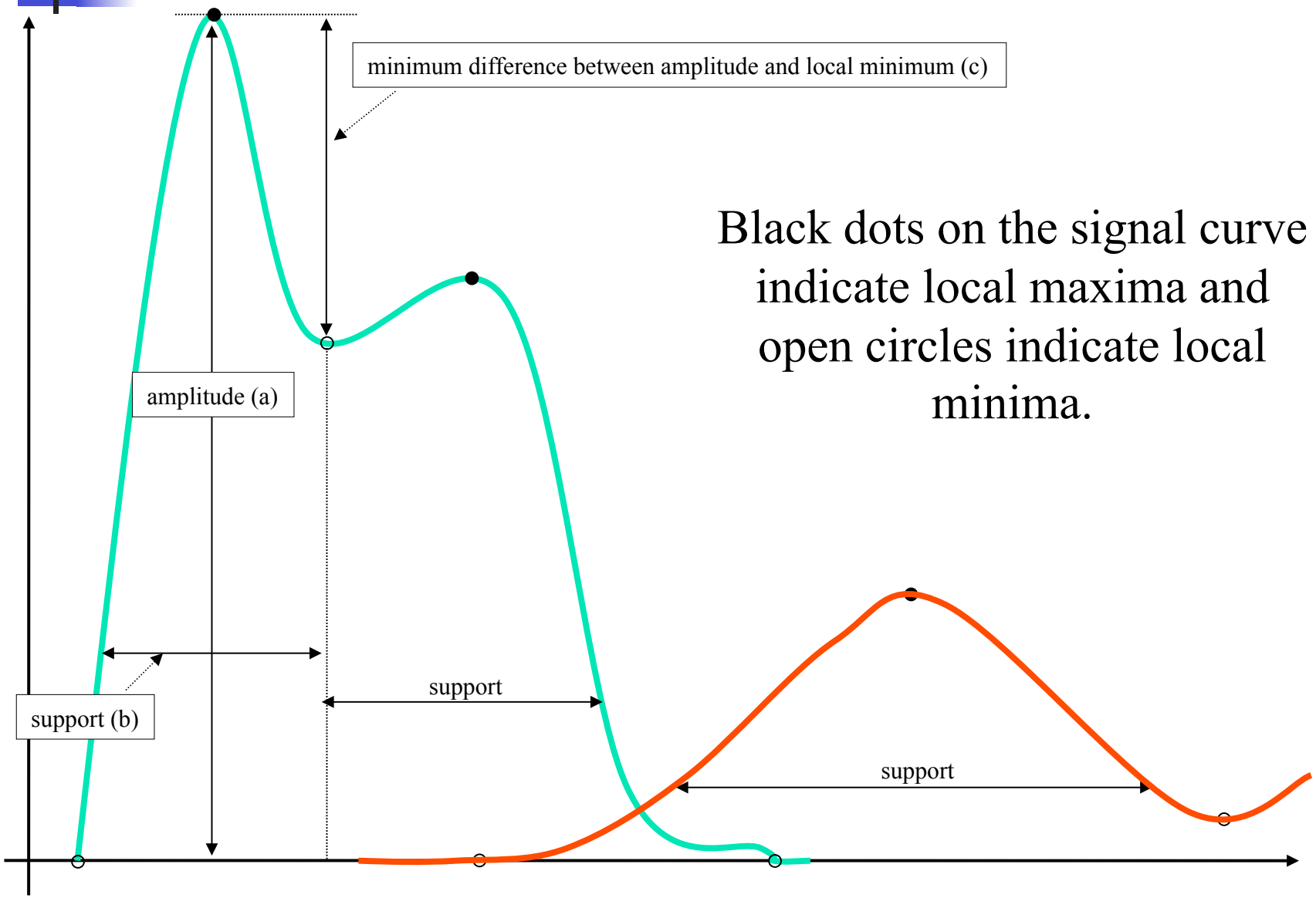
Base-calling in the context of single chromatogram is hard…



but finding base-calling "mistakes" in a multiple alignment is easy.



Signal Parameters



Black dots on the signal curve indicate local maxima and open circles indicate local minima.

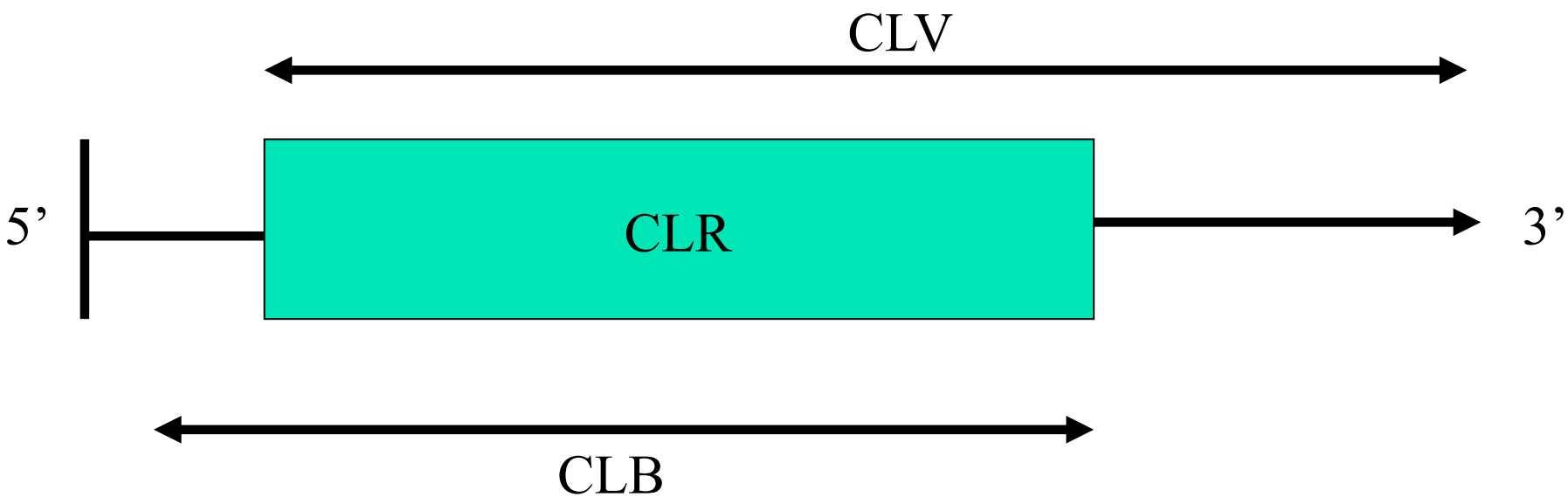
AutoEditor Results

- Corrects 80% of all discrepant base-calls with an error rate better than 1/8800.
- Increase consensus quality, decrease finishing costs
- Remaining discrepancies highlight assembly problem regions or interesting biological events.

Organism	Read length	Corrections	AE errors
Listeria monocytogenes	37 420 828	145 274	4
Wolbachia sp.	11 446 011	51 163	0
Burkholderia mallei	47 407 080	99 711	28
Brucella suis	26 629 877	112 359	2
Streptococcus agalactiae	23 485 615	105 878	3
Coxiella burnetii	29 135 115	117 232	30
Campylobacter jejuni	15 013 845	792 37	11
Chlamydophila caviae	10 286 694	36 972	6
Dehalococcoides ethenogenes	10 724 521	46 416	12
Neorickettsia sennetsu Miyayama	8 805 232	37 425	0
Fibrobacter succinogenes	46 463 268	196 150	4
Mycoplasma capricolum	9 353 819	15 444	0
Prevotella intermedia	20 084 365	94 162	3
Pseudomonas syringae	50 369 232	177 897	46
Total	346 625 502	1 315 320	149

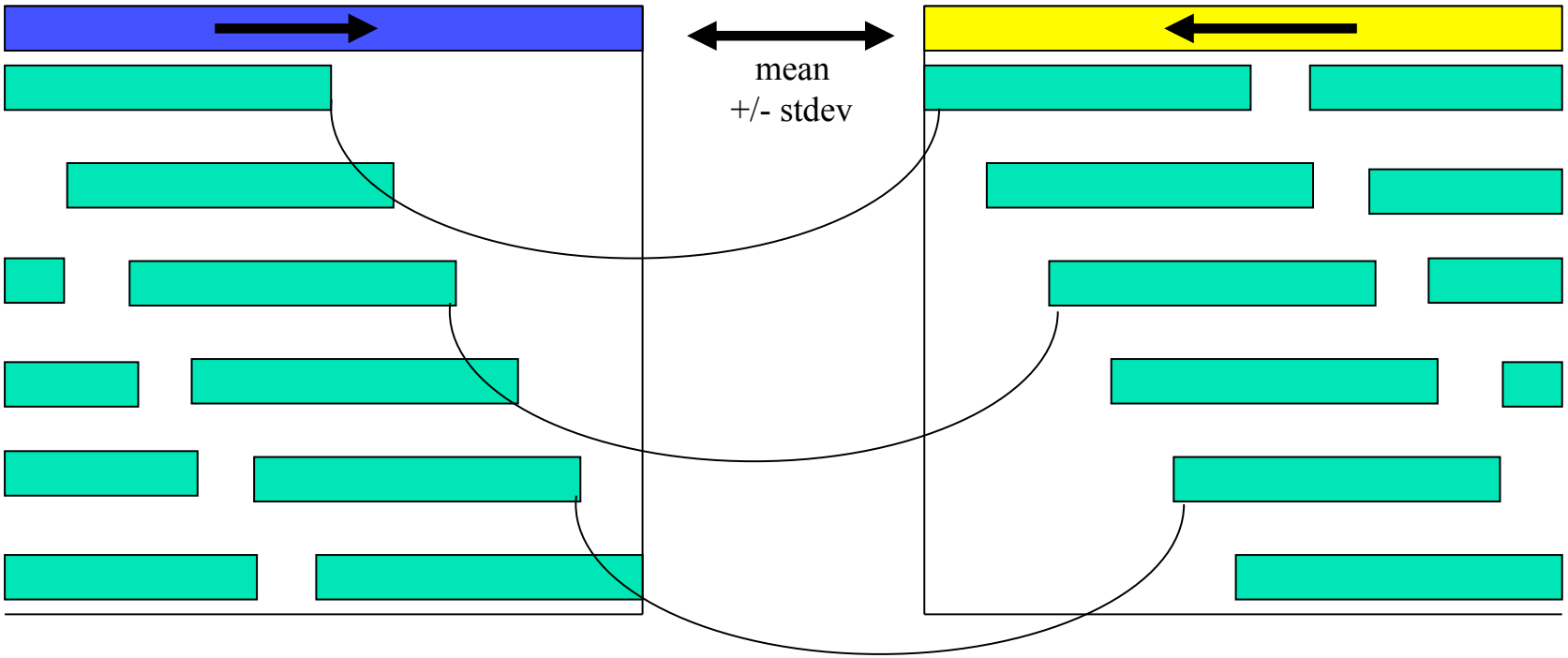
Ask Pawel for more information!

Quick Trimming Review



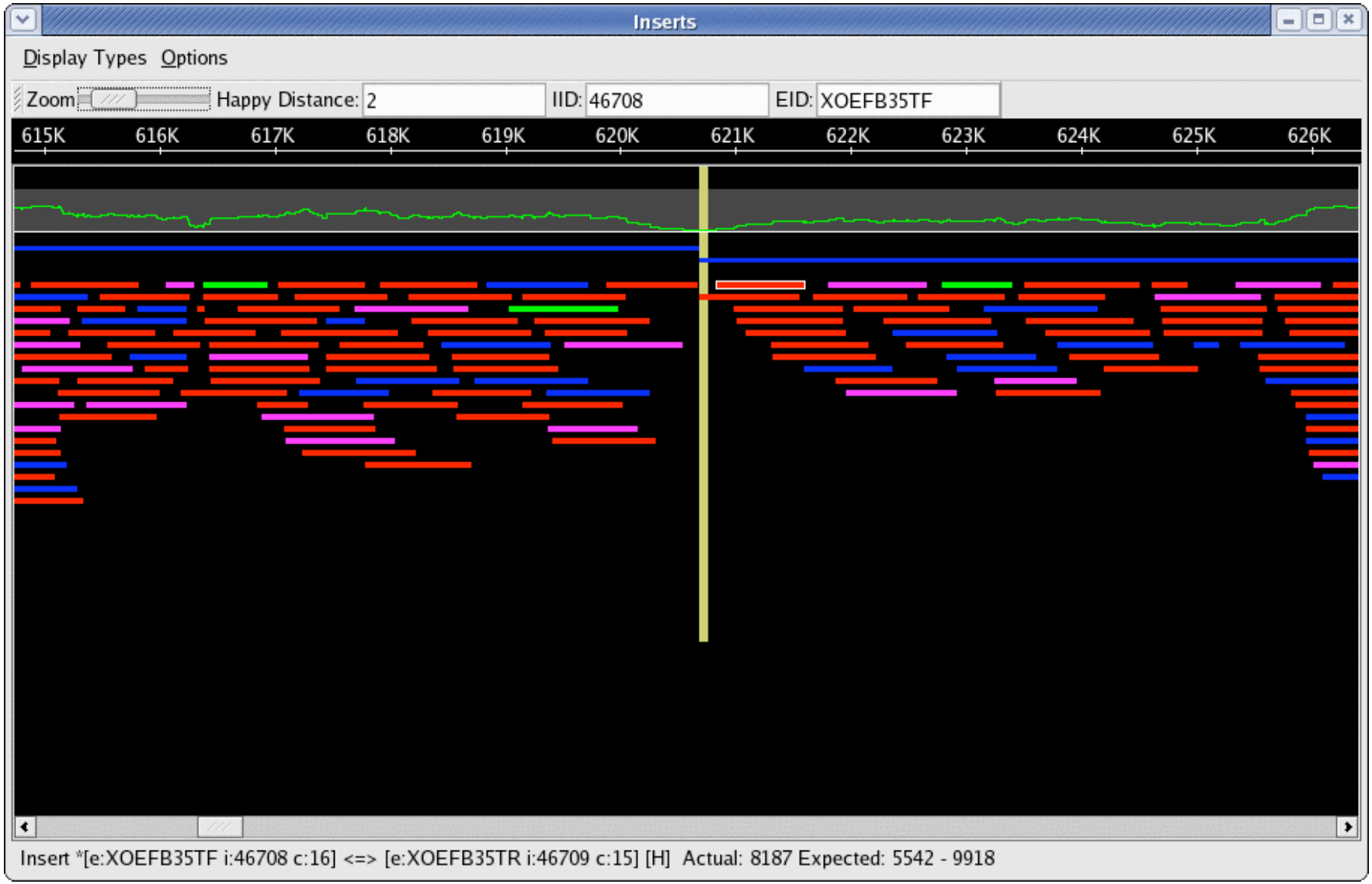
Trimming identifies the regions of good quality for the assembler to use (CLR), as the intersection of the region free of vector (CLV) and the region free of bad quality (CLB).

Quick Assembly Review

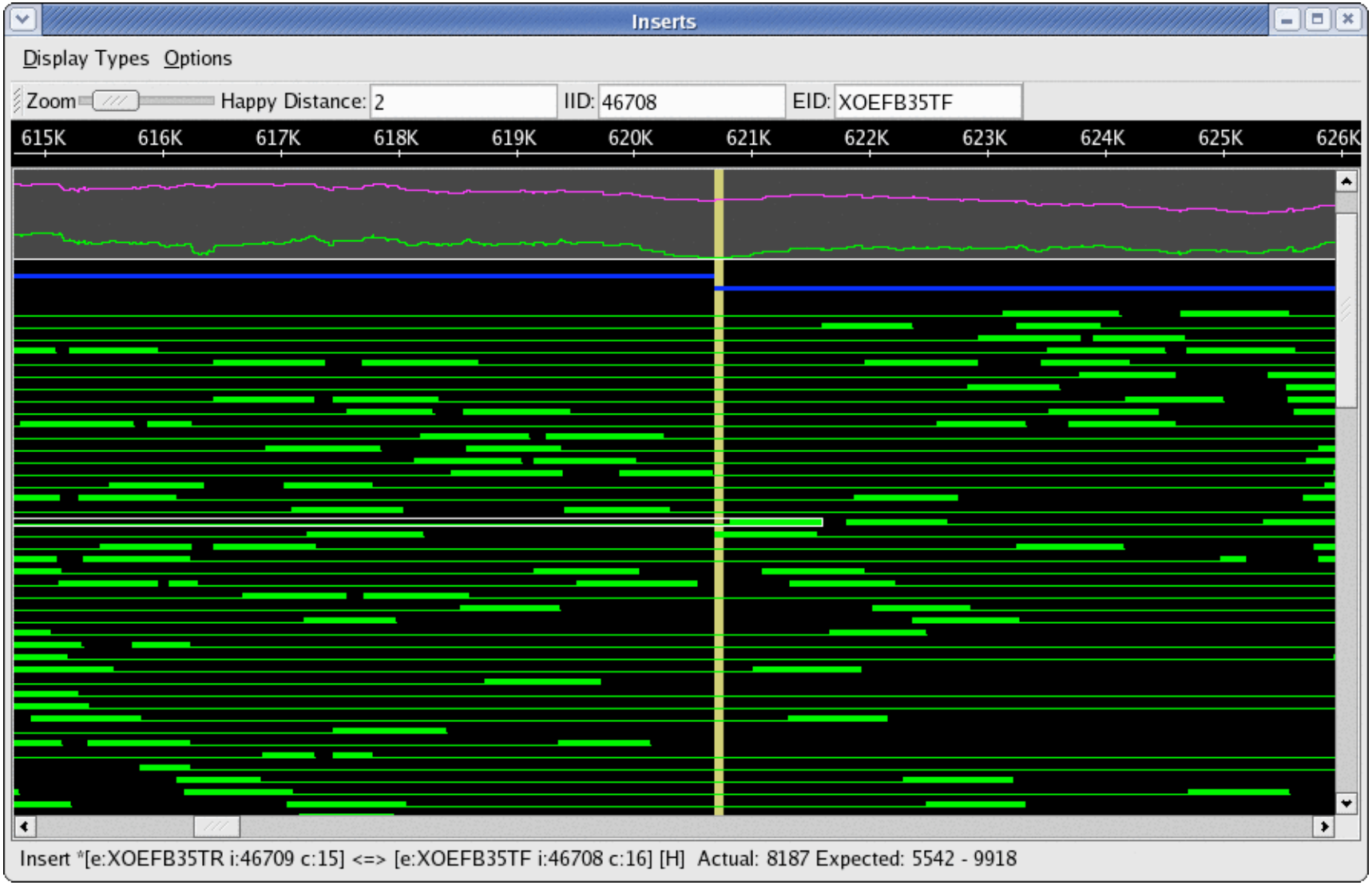


The individual reads (green) have been assembled into 2 contigs (blue & yellow). The mate relationship between the reads allows for the contigs to be oriented and the gap size to be estimated.

Read Coverage

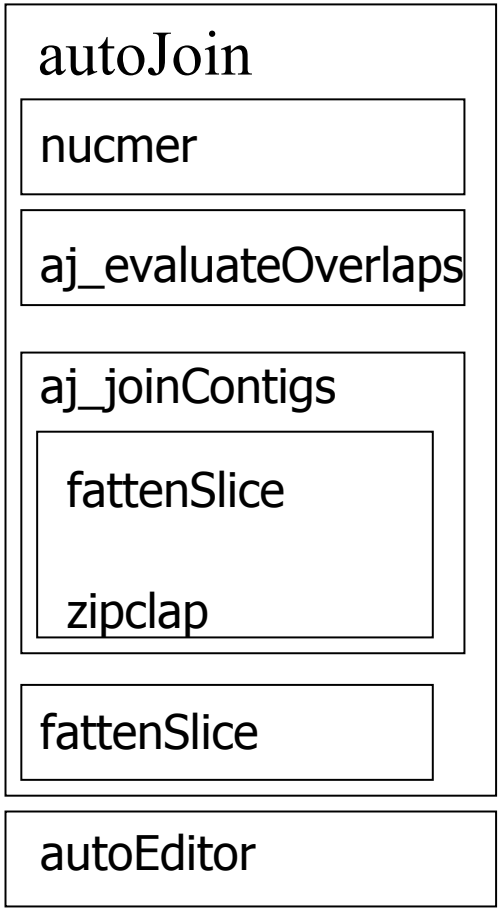


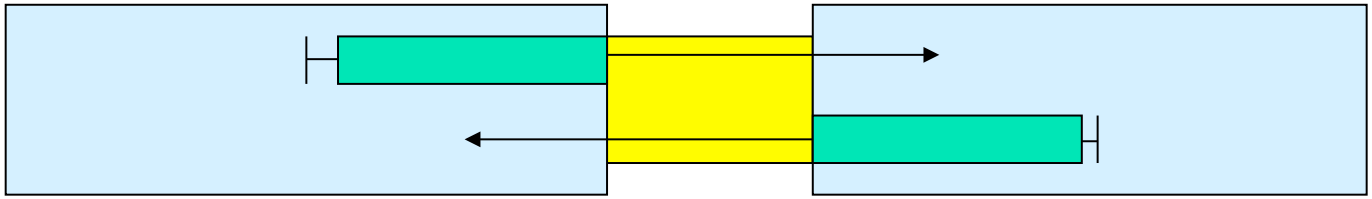
Sequencing Gap



AutoJoiner Architecture

1. All-vs-All Alignment
2. Analyze Alignments
3. Extend
   and
   Join Contigs
4. Contig Fattening
5. AutoEdit Result





All-vs-All Alignment

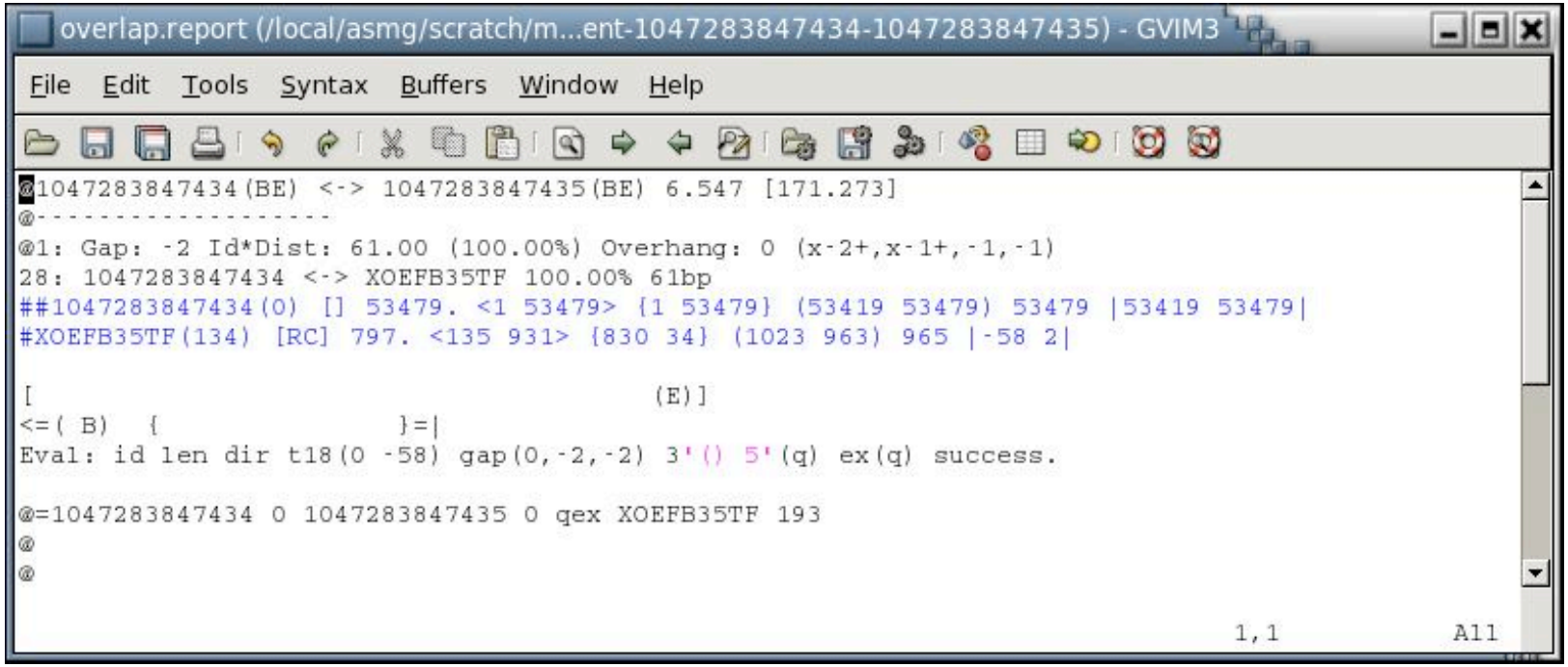
read-alignment.txt + (/local/asmg/scratch/...lignment-1047283847434-1047283847435) - GVIM4

File Edit Tools Syntax Buffers Window Help

```
% show-coords -rcl out.delta | grep XOEFB35TF
   35001    35998  |  1023   14  |  998     1010  |  95.15  | 53479  1023  | 1047283847434  XOEFB35TF
   53419    53479  |  1023  963  |   61       61  | 100.00  | 53479  1023  | 1047283847434  XOEFB35TF
    1012     1133  |   285  405  |  122      121  |  90.24  |  1163  1023  | XODA905TF      XOEFB35TF
     769      980  |  1023  813  |  212      211  |  99.06  |   980  1023  | XOEC861TR      XOEFB35TF

% show-aligns out.delta 1047283847434 XOEFB35TF
============================================================
-- Alignments between 1047283847434 and XOEFB35TF

-- BEGIN alignment [ +1 53419 - 53479 | -1 1023 - 963 ]


53419      tcgttcgcgtctggagaaccagatcgacttgcgccatccgctggtccag
1023       tcgttcgcgtctggagaaccagatcgacttgcgccatccgctggtccag


53468      ctgagccatcgg
974        ctgagccatcgg



--   END alignment [ +1 53419 - 53479 | -1 1023 - 963 ]

============================================================
                                                      25,60          Top
```

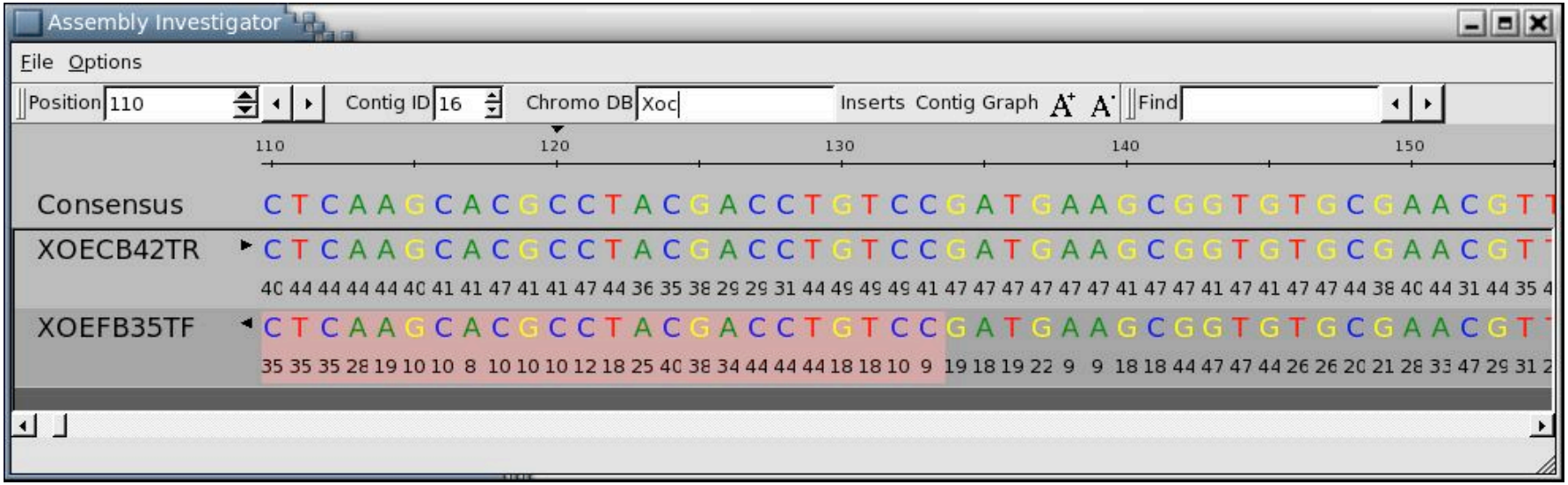
The first AutoJoiner!

Alignment Reports



Why did AutoJoiner make this join?

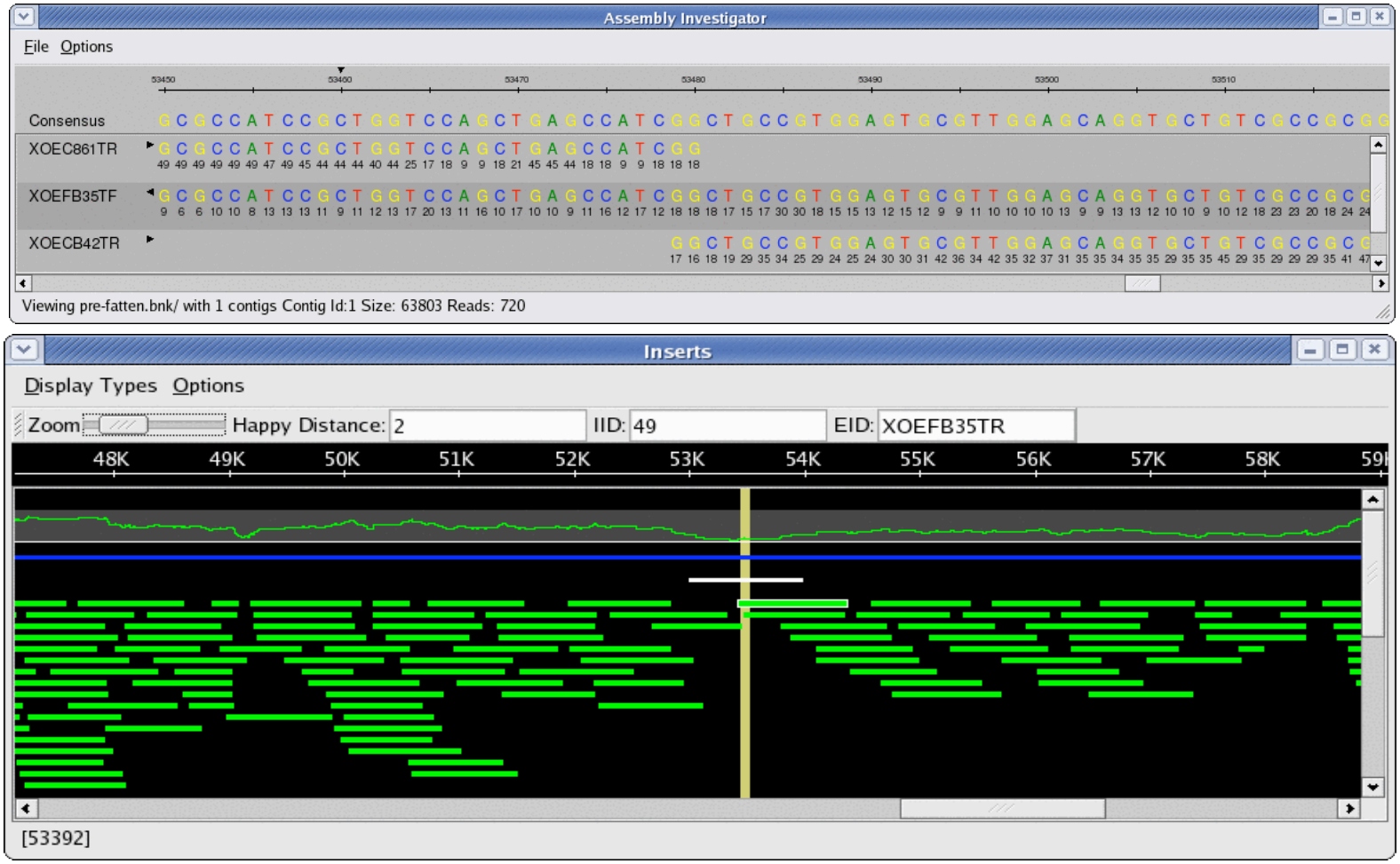
Contig Extension



Extension Procedure:

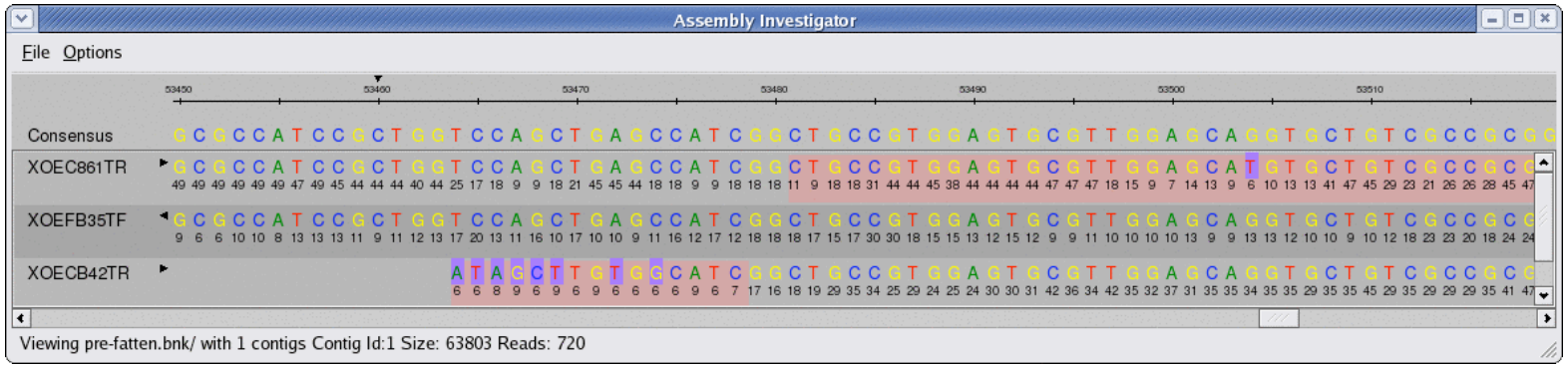
- If necessary, extended selected read by aligning trimmed bases to existing consensus.
- Untrim to desired base, promote untrimmed bases to consenus, shift offsets.

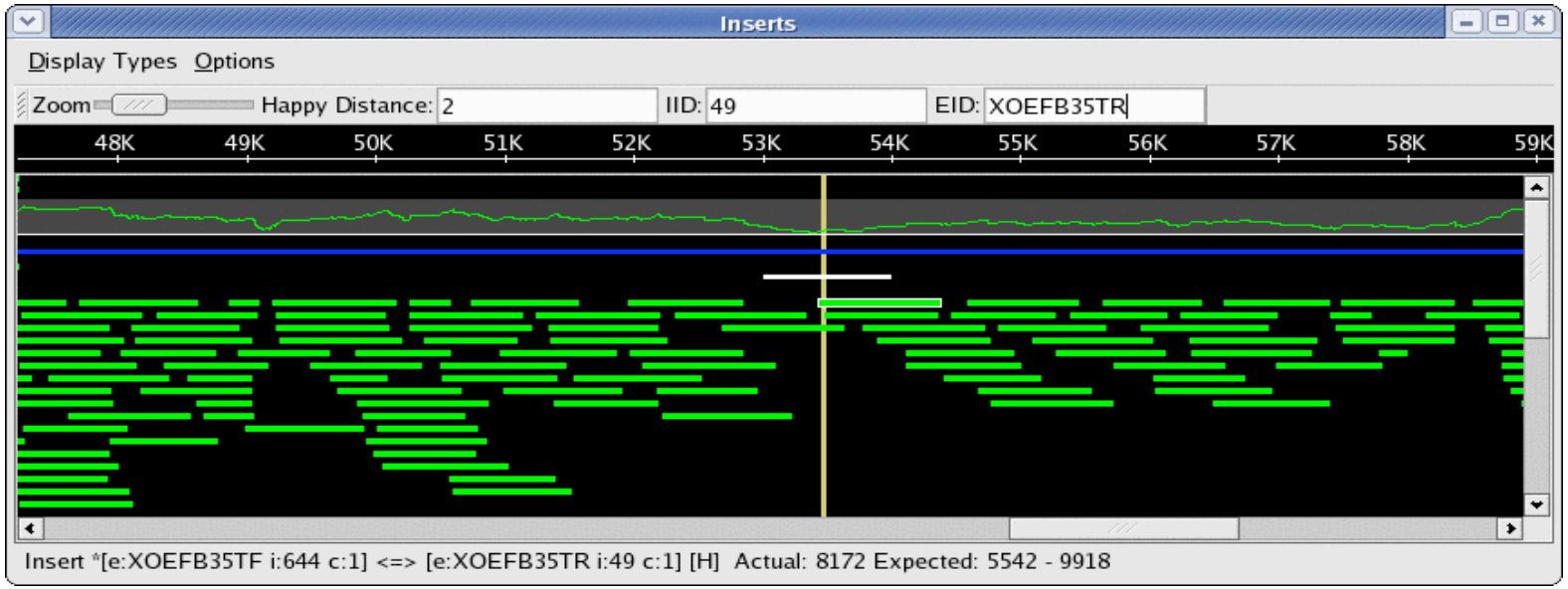
Contig Joining



"Zip" together contigs by pairwise alignment between consensi.

Contig Fattening





“Fatten” addition reads in the join region to increase coverage.

Join Report

blasted.joinreport (/local/asmg...c/Blasted/autojoin/results) - GVIM

File Edit Tools Syntax Buffers Window Help

```
[autoJoin]
NumSequenceGaps=39
NumJoined=6
NumDoubleExtend=0
NumSingleExtend=5
NumNoExtend=1
PercJoin=0.154
ExpectedGap.Range=-20.000,15.034
ExpectedGap.Mean=-4.39
ExpectedGap.SD=14.25
ExpectedGap.Median=-3.95
ExpectedGap.Mad=19.67
ActualGap.Range=-82,67
ActualGap.Mean=19.83
ActualGap.SD=54.92
ActualGap.Median=42.75
ActualGap.Mad=23.72

~
~
~
~
"blasted.joinreport" 18L, 338C
                                                    1,1           All
```

What did AutoJoiner do?

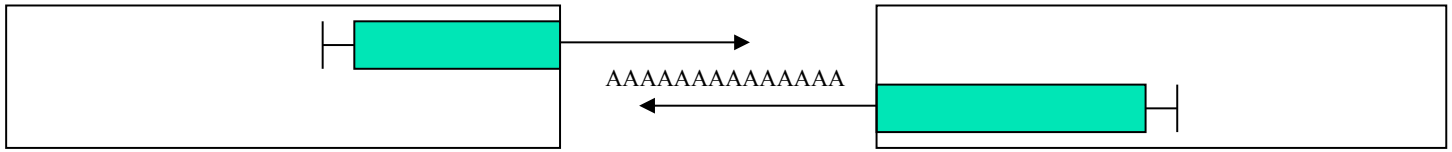
AutoJoiner Validation

Project	Gaps	Joined	%	Invalid	Degenerate	Gap Size	Mean	Join ID	Mean
blm	106	14	13.20%	0	0	-200.5:156	21.18	98.39:100.00	99.26
dmg	52	13	25.00%	0	3	-666.5:36.5	-140.73	98.99:100.00	99.64
gb6	32	10	31.20%	0	0	-13:146	42.3	98.50:99.87	99.31
gba	110	38	34.50%	0	0	-452:229	29.18	97.18:100.00	99.36
gbm	43	6	14.00%	0	0	-17:22	-4.67	99.24:100.00	99.78
gbr	32	11	34.40%	0	0	-62.5:103.5	15.36	98.89:99.92	99.44
gbs	31	5	16.10%	0	0	-5:32.5	10.9	98.99:99.65	99.33
gcb	10	2	20.00%	0	0	-37.5:-4.5	-21	99.38:99.81	99.59
gcj	22	11	50.00%	0	0	-53:139	27.45	98.71:99.81	99.45
gcp	25	8	32.00%	0	4	-555:184.5	-75.31	99.03:99.85	99.42
gde	82	17	20.70%	0	1	-113:203.5	22.29	97.04:99.93	99.14
ges	27	17	63.00%	0	0	-779:-302	-586.71	100.00:100.00	100
gfs	131	33	25.20%	0	6	-182.5:212	21.79	98.81:100.00	99.51
gmcap	10	2	20.00%	0	0	-11.5:171	79.75	98.67:99.84	99.25
gpi	150	52	34.70%	0	0	-231.5:181	20.3	97.96:99.93	99.4
gps	162	43	26.50%	0	0	-1069.5:213.5	-36.13	98.76:100.00	99.51
gsa	262	32	12.20%	0	0	-618:136	-43.44	94.90:100.00	99.25
crypt_1	20	8	40.00%	0	0	-36:186.5	63.62	98.74:99.88	99.43
crypt_2	7	5	71.40%	1	0	-39:148	27.8	98.63:99.56	99.18
crypt_3	21	8	38.10%	0	0	-93:67.5	-3.06	97.83:100.00	99.31
crypt_4	25	7	28.00%	0	0	-90:159	45.21	98.94:100.00	99.52
crypt_5	23	8	34.80%	0	0	-111.5:249	35.12	98.98:99.92	99.43
crypt_6	14	7	50.00%	0	0	-14:192	37.21	98.41:99.93	99.58
crypt_7	17	6	35.30%	0	0	-3.5:230.5	66.67	99.09:100.00	99.62
crypt_8	15	6	40.00%	0	0	-19:57.5	15	99.20:100.00	99.74
crypt_9	12	6	50.00%	0	0	-423:34	-120.5	99.16:100.00	99.82
crypt_10	14	7	50.00%	1	0	-777:124	-91.21	95.23:100.00	99.04
crypt_11	12	2	16.70%	0	0	-6:69.5	31.75	99.63:99.69	99.66
crypt_12	10	4	40.00%	0	0	-340:77.5	-69.88	99.77:100.00	99.86
crypt_13	13	7	53.80%	1	0	-213.5:144	19.07	99.38:100.00	99.7
Composite	**1490**	**395**	**26.51%**	**3**	**14**	**-1069.5:249**	**-25.89**	**94.90:100.00**	**99.45**

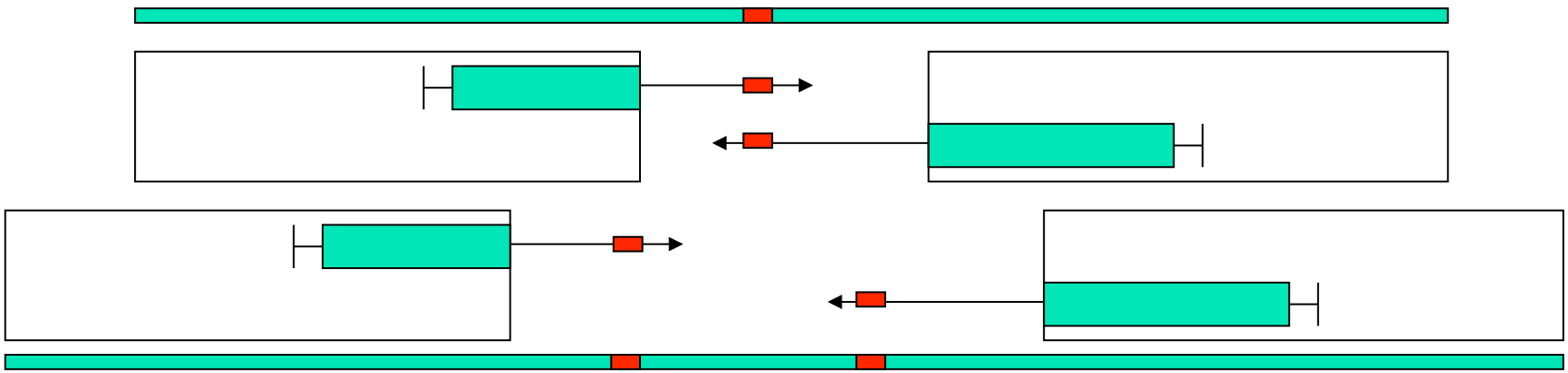
25%+ of all sequencing gaps closed with 3 mistakes.

Complicating Issues

- Poly-monomer tails
  - Use dust to filter low complexity sequence



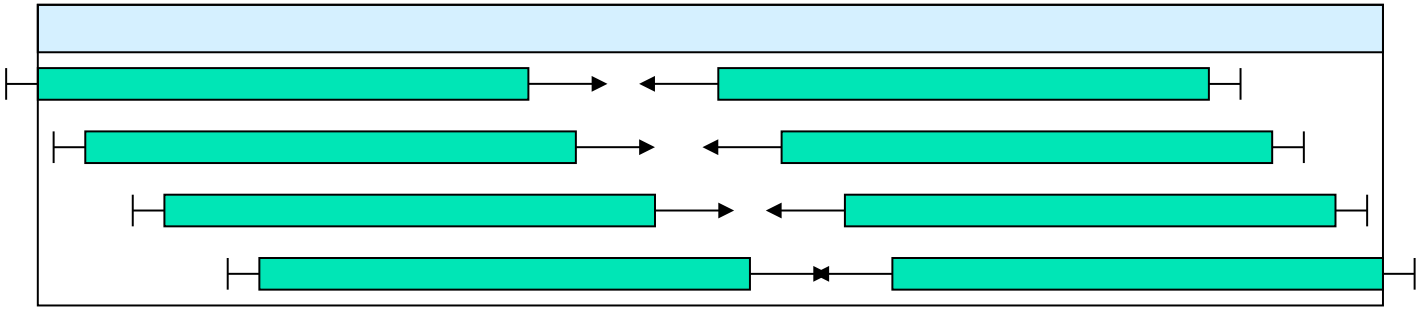
- Undetected repeats
  - Require strict agreement with scaffold



- Chimeric reads / Hard Stops
  - Good: Require high alignment similarity.
  - Better: Recognize hard stops by coverage gradients, other clues.
  - Best: Recognize unreliable sequence at chromatogram level.

Pre-Production Techniques

- Contig Fattening
  - TVG coverage increased from 5.83X to 6.10X (mean extension: 80.5bp)



- Contig Growing
  - Extended 6144 edges in TVG (mean extension: 59.0bp)

Measuring Assembly Quality

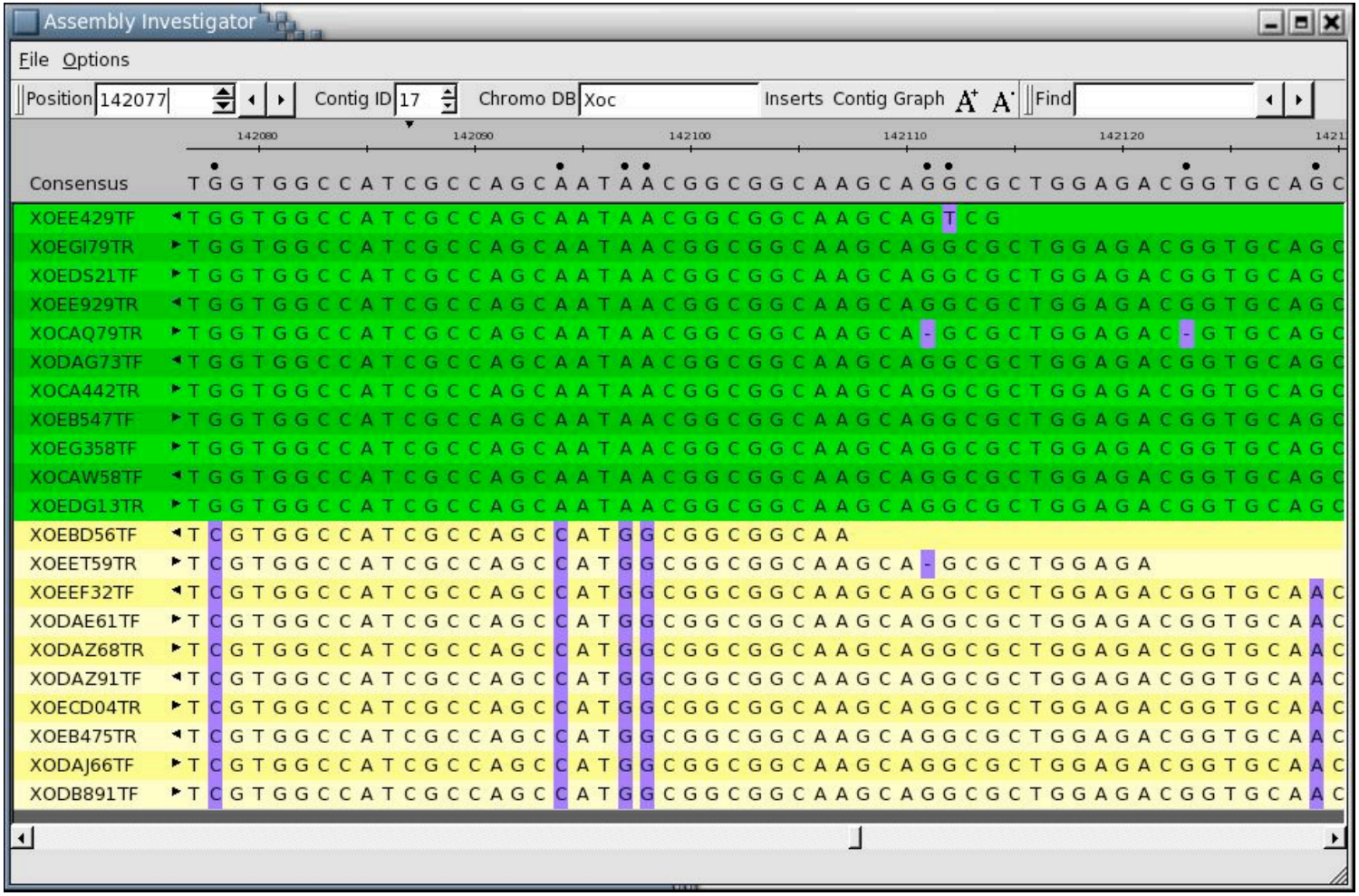
- Gross Status — QC file
  - scaffold & contig sizes
- Connectedness Status — Cloe, AssemblyViewer, getCoverage, cvgChop, asmQC
  - read & clone coverage
- Insert Status — QC file, Assembly Viewer, asmQC
  - mate happiness,
  - library randomness
- Consensus Status — Cloe, getCoverage, getqc
  - Quality Class
  - Consensus Quality Value
- Read Status — findTcovSnps
  - Correlated SNPs

AMOS Assembly Investigator

Finding Suspicious Regions

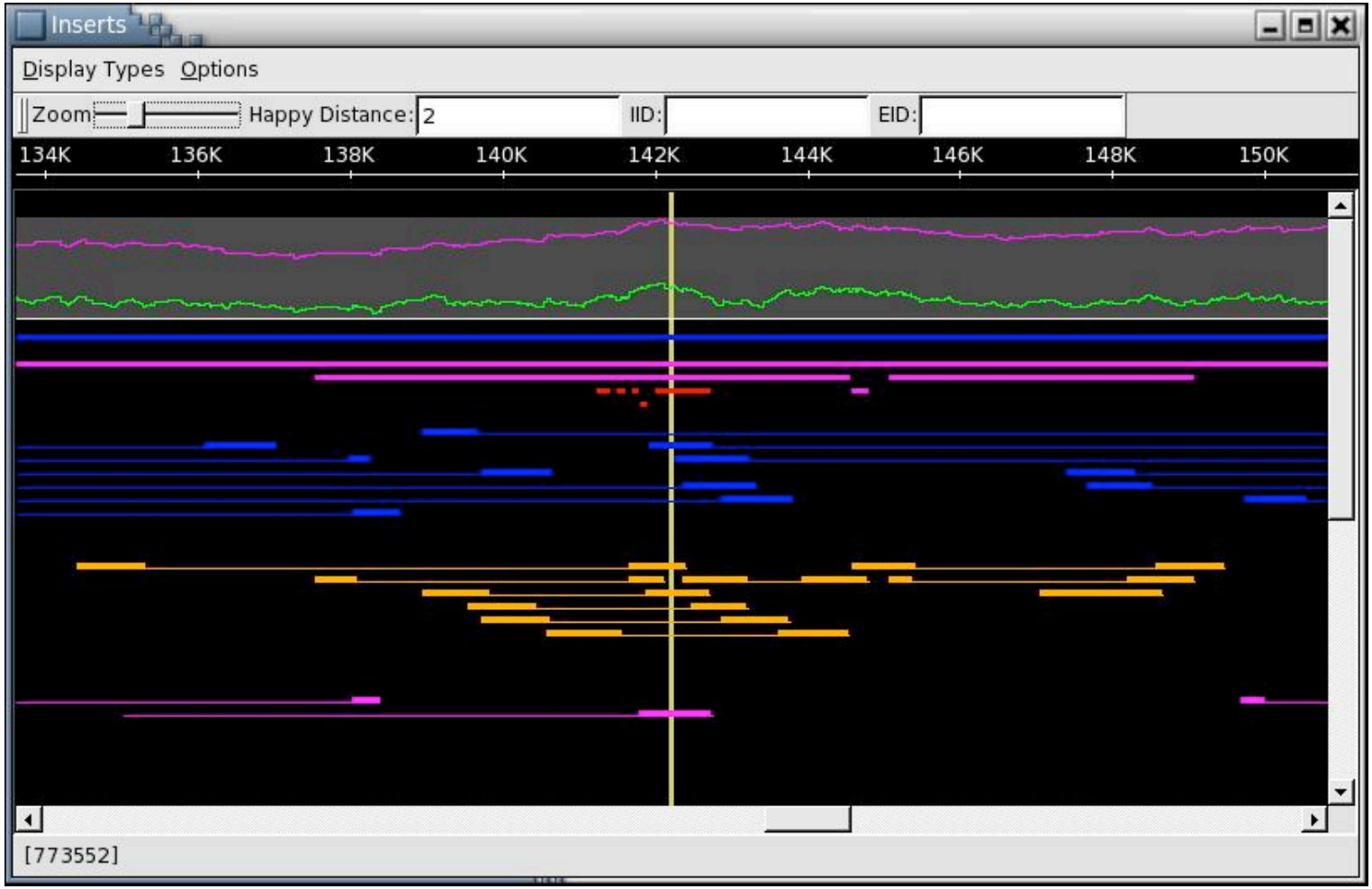
```
% ls
blasted.frg blasted.asm
% /local/asmg/Linux/bin/cavalidate blasted
Doing step 10:  toAmos
Doing step 20:  bank-transact
Doing step 30:  asmQC
Doing step 40:  bank2contig
Doing step 50:  getCoverage
Doing step 60:  findTcovSnps
Doing step 70:  ClusterSnps
Doing step 80:  Load SNP Features
Doing step 90:  Find Surrogates
Doing step 100:  Load Surrogates
Doing step 1000:  Dump Features
Doing step 1010:  Get Suspicious Features
Doing step 1020:  Create Suspicious Regions
% sort -nrk 6 blasted.snp.feat | head
1047283847436 P HIGH_SNP 141991 142712 46 15.67
```

Overcollapsed Repeat?



For a bacterial sample, correlated discrepancies strongly suggest a repeat has been collapsed.

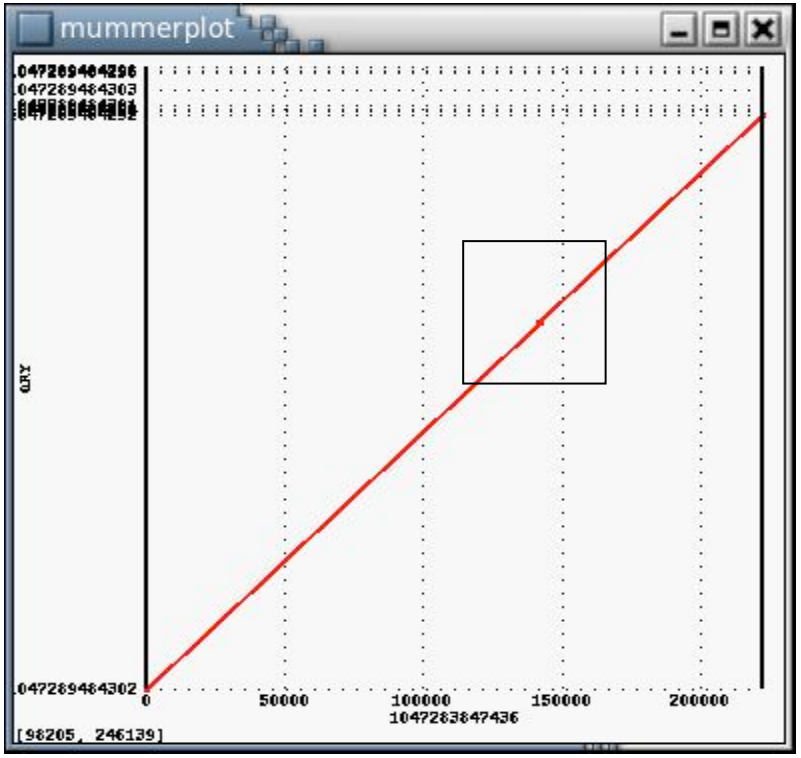
Mate View

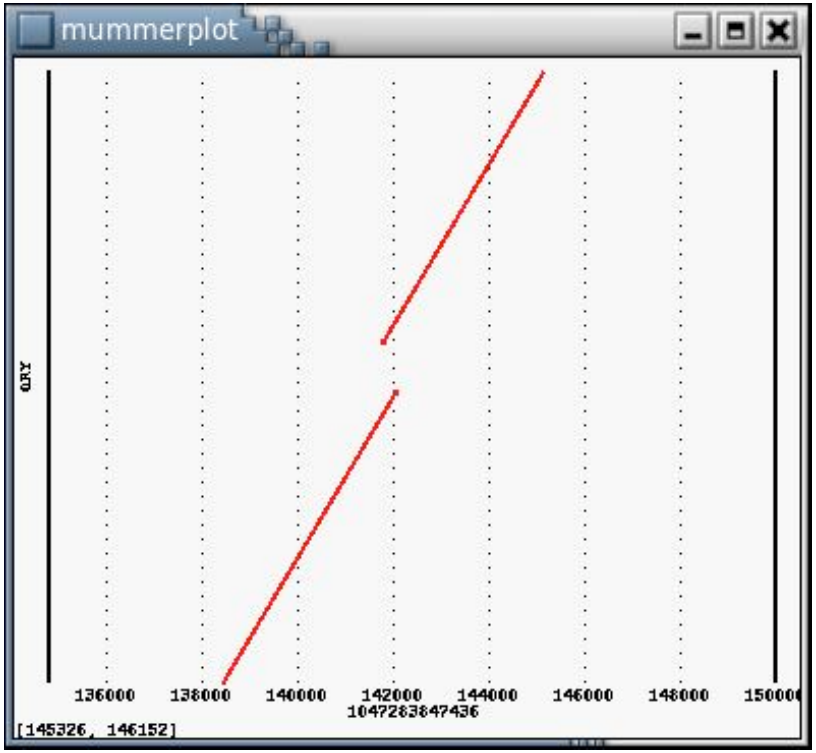


The shrunken mates (orange) suggest the assembly has a deletion from the true sequence.

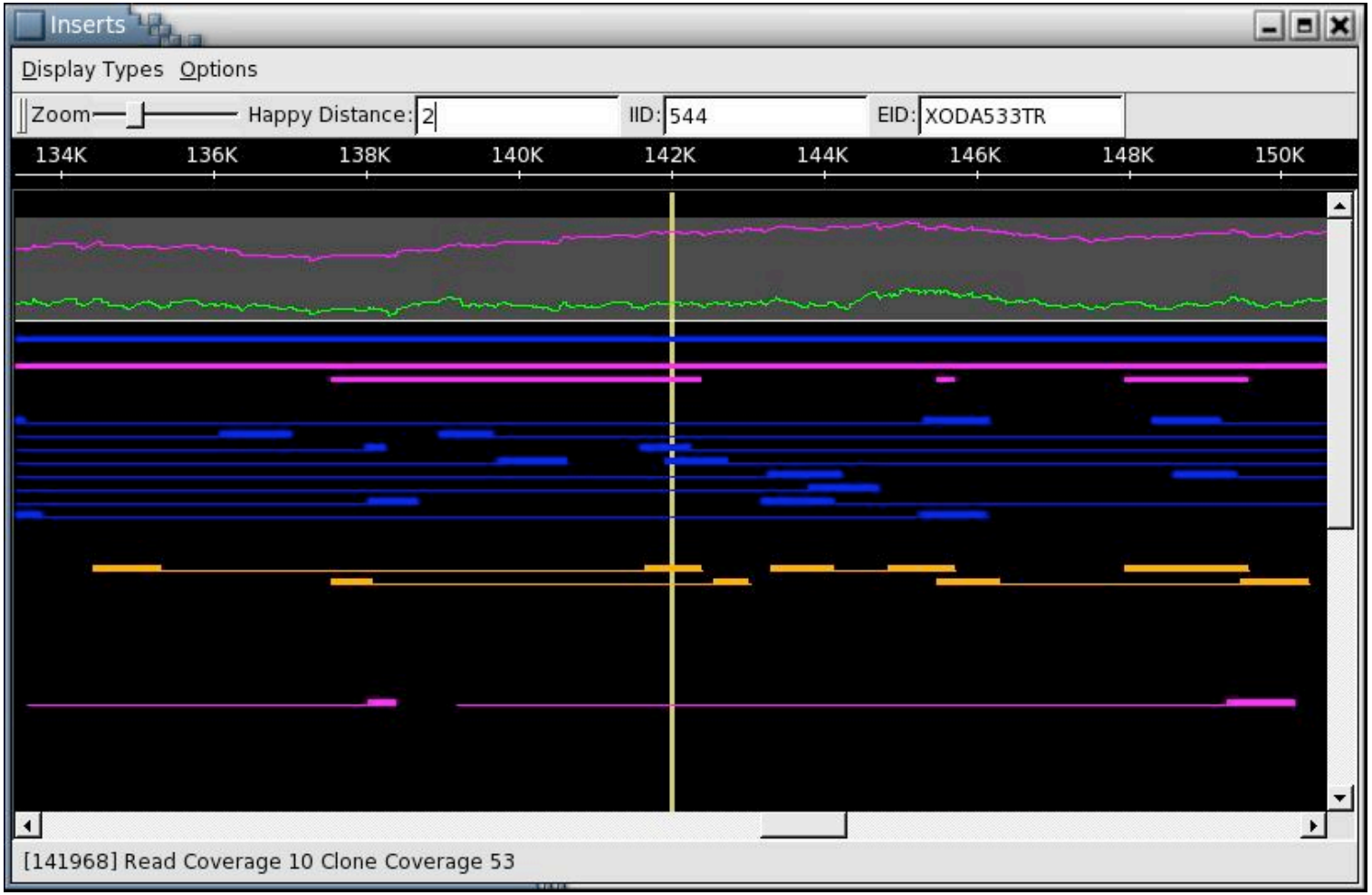
Local Assembly

```
% run_CA -local -noedit -noupload local.frg -dir ca-0.003 -e 0.003
% nucmer 1047283847436.fasta ca-0.003/local.fasta
% /local/asmg/Linux/bin/mummerplot out.delta -R 1047283847436.fasta -Q ca-0.003/
    local.fasta -layout -filter
```





Resolved Repeat



Unfortunately, size violated mates are only a clue.
Ask Mihai for current research techniques.

Final Results

	Original Assembly	Reassembled
TotalScaffolds	21	5
TotalSpanOfScaffolds	4770228	4819528
IntraScaffoldGaps	51	33
MeanSequenceGapSize	410.18	96.94
[Top5Scaffolds]		
1	25:2156009:2164583:357.25	34:4810208:4813407:96.94
2	13:826284:830667:365.25	1:2558:2558:0.00
3	3:437076:439209:1066.50	1:1473:1473:0.00
4	3:333768:334012:122.00	1:1056:1056:0.00
5	5:310971:311756:196.25	1:1034:1034:0.00
TotalContigsInScaffolds	72	38
N50ContigBases	151430	253084
TotalDegenContigs	125	1
DegenContigLength	129182	959
MeanDegenContigSize	1033.46	959
[Top5Contigs]		
1	5939:536280	6291:516881
2	2751:259499	6203:436501
3	3005:238048	3805:385244
4	2199:220720	4292:364829
5	2509:196450	4025:355502

Expected Genome Size: 4.85Mb - 5.04Mb

Research Directions

- AutoEditor 2.0: Better results, better engineering
- Context Based trimming
  - Partial Overlaps
  - Reference sequence
- Advanced CA Techniques
  - Contained Stones bug fixes
  - Blasting Degenerate and Surrogate Unitigs
  - Assembling in the gap
- Arachne & Other Assemblers
- Assembler Reconciliation
- AMOS Framework
- Assembly Forensics
- Assembly Visualization / Navigation

Conclusions

- Overriding strategy: Start conservatively, and iteratively build as more information becomes available.
- 95.5% - 99.2% of genome in a single scaffold not typical yet, but it could be.
  - Be aware of potential size/quality tradeoffs, though.
- Assembly is complicated by genome structure, repeat characteristics, data quality, data management- one size does not fit all, ask for help.
  - Use Data Support!

Acknowledgements

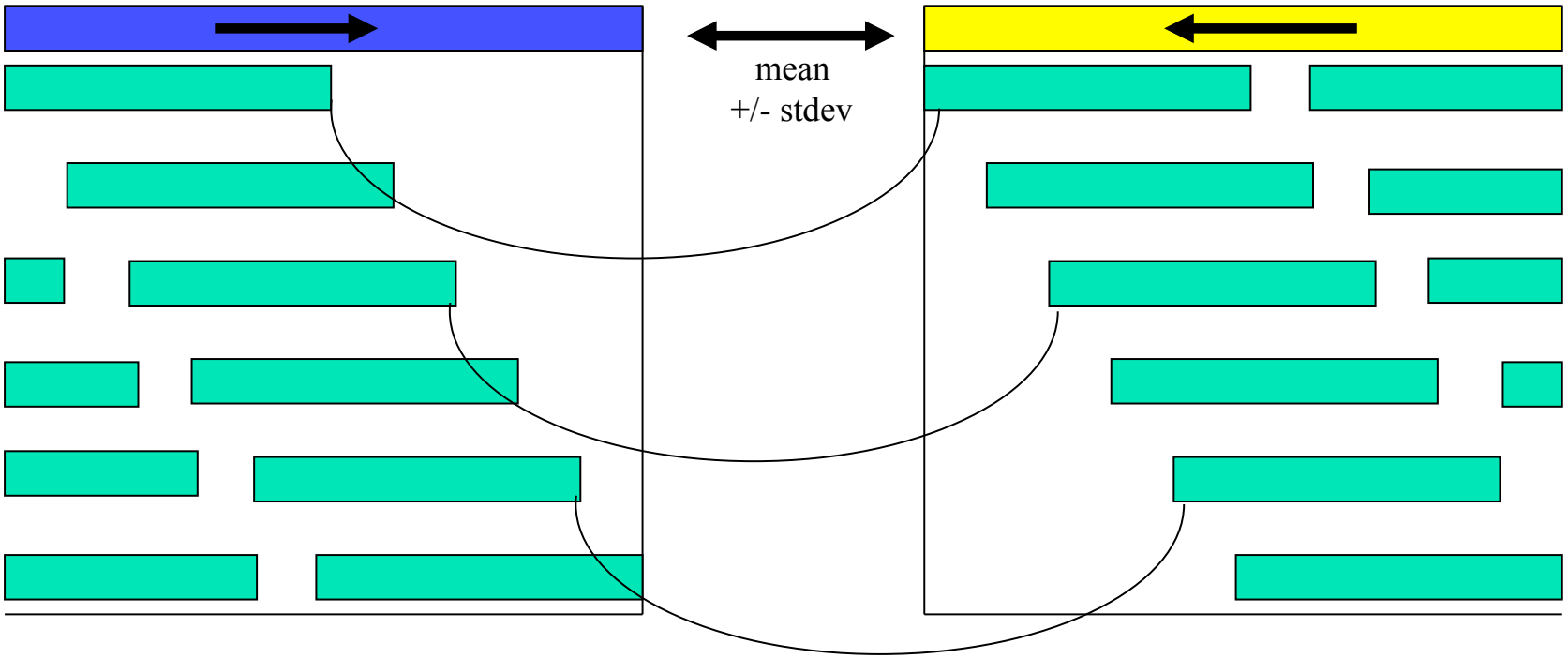
- Steven Salzberg
- Martin Shumway
- Jason Miller

- WGA
- SE
- Data Support

- Pawel Gajer
- Art Delcher
- Mihai Pop
- Adam Phillippy

- Jane Carlton
- Vish Nene

Sequencing Gap



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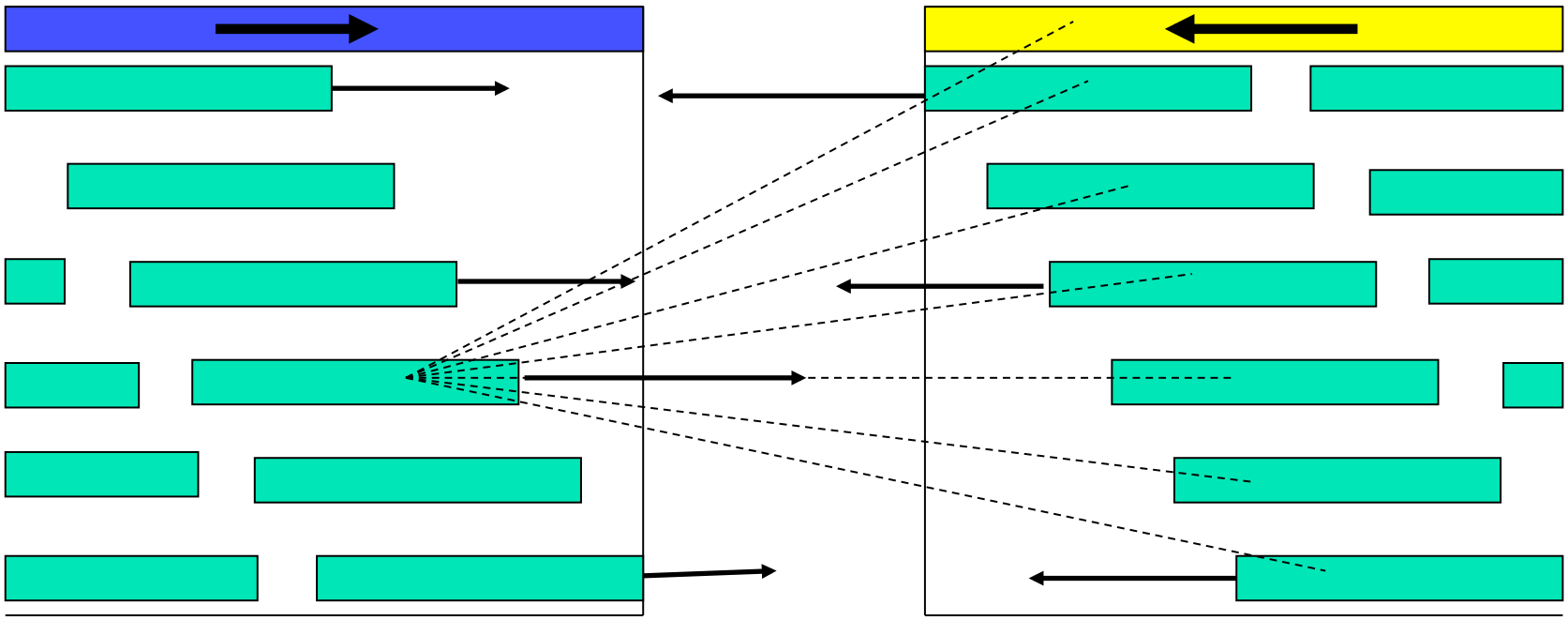
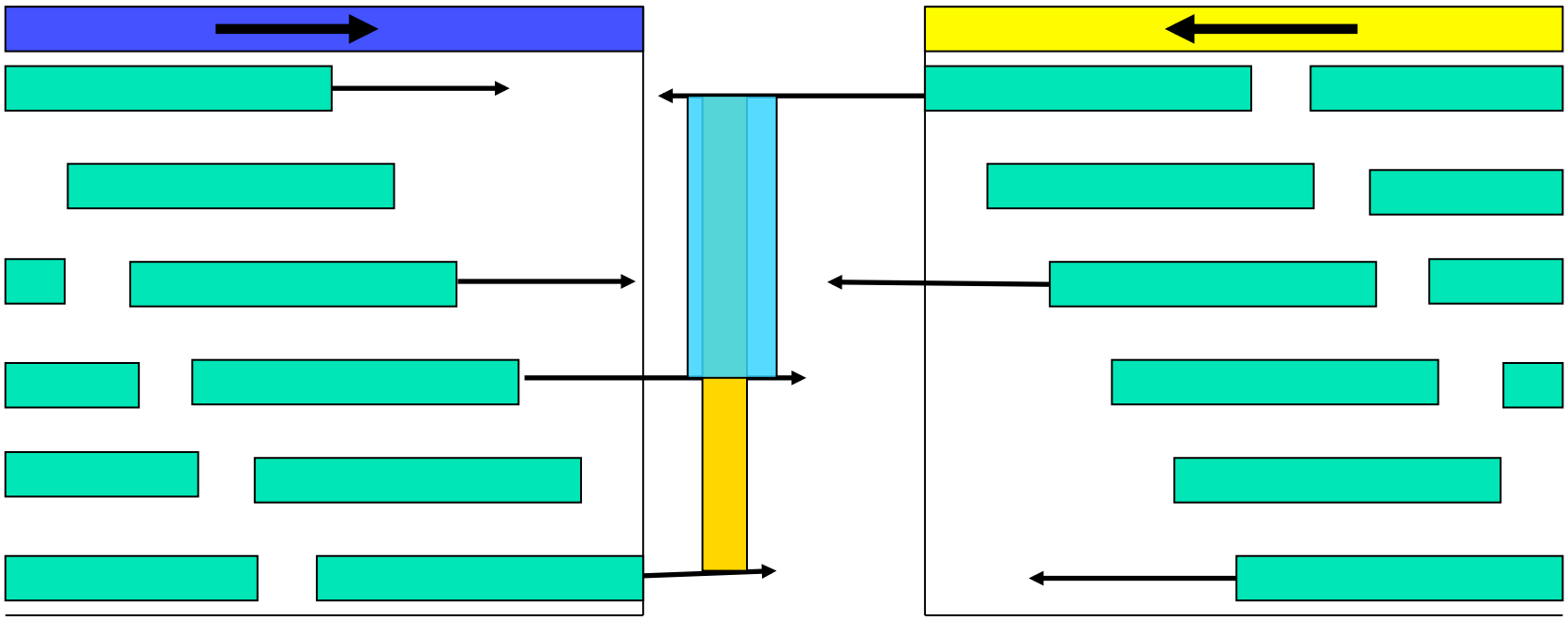
All-vs-all Alignment



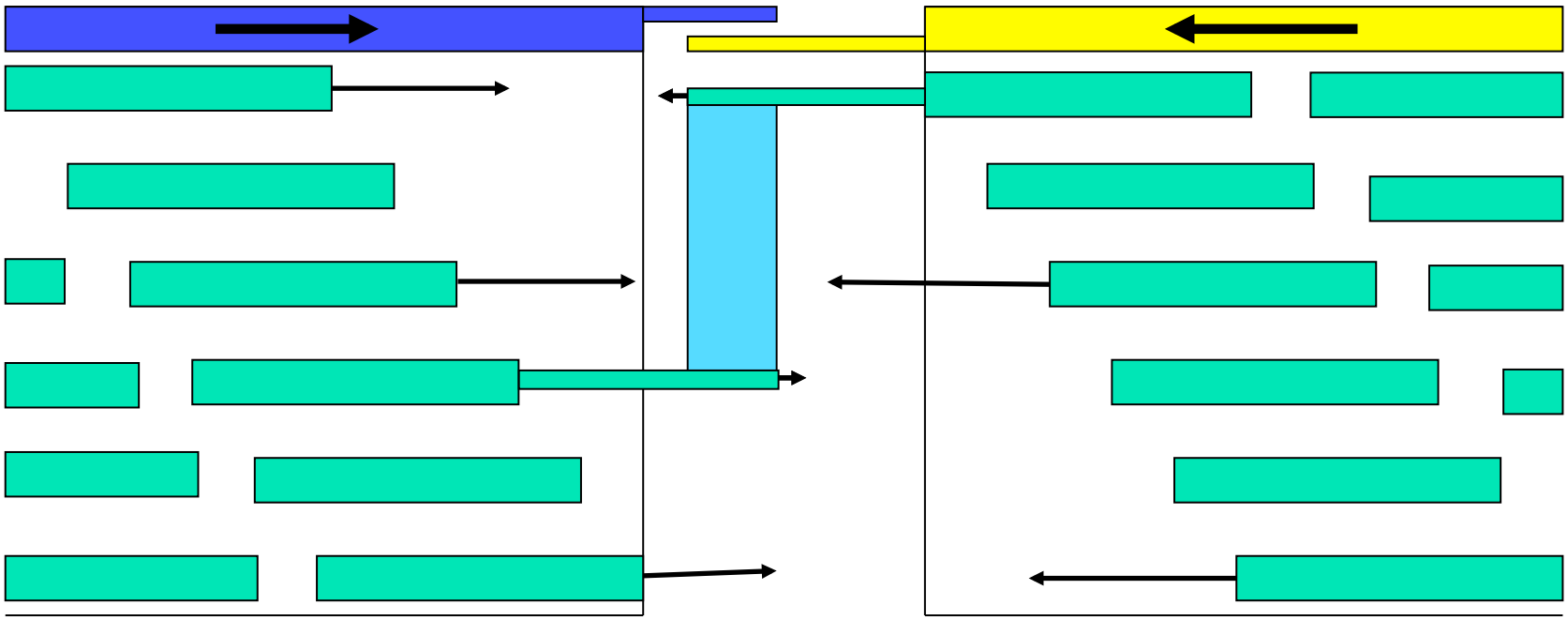
1. An all-vs-all pairwise alignment between the full range sequences from the flanking contigs is computed.

Alignment Analysis



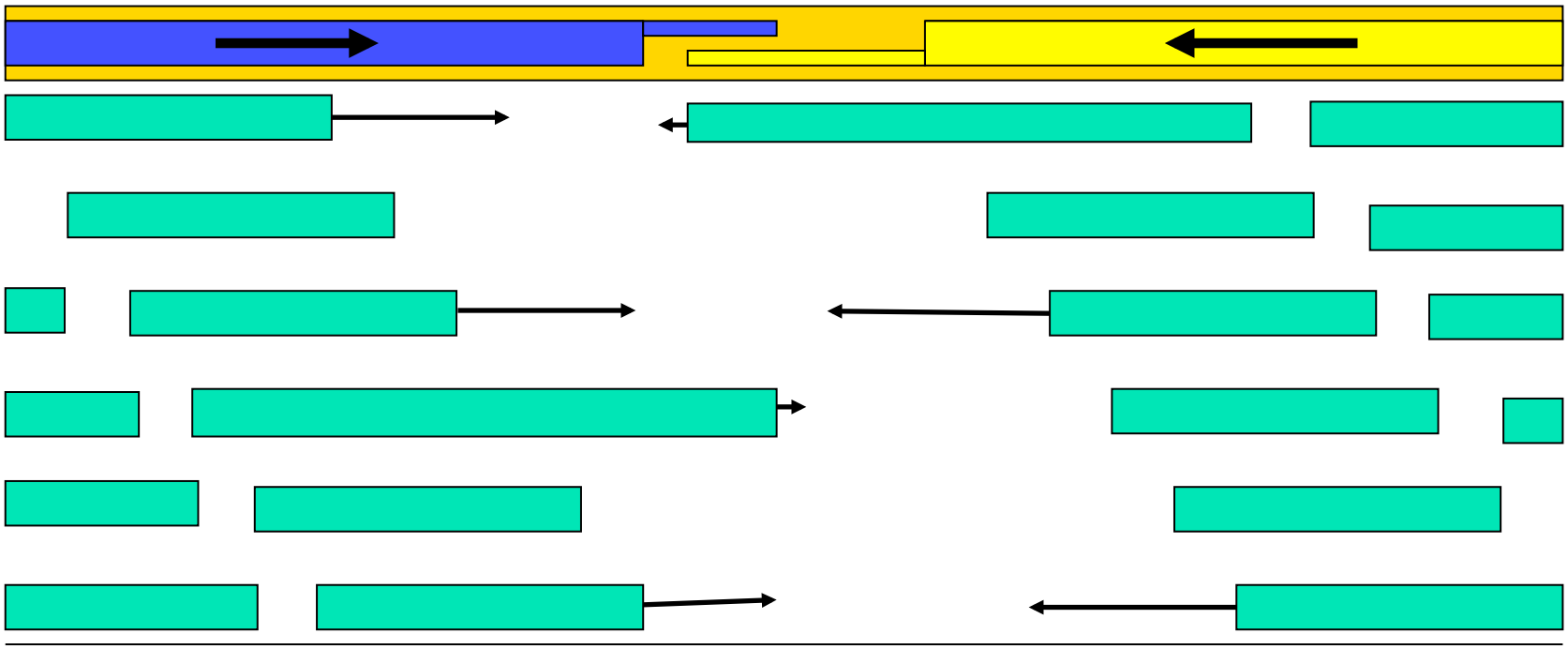
2. The alignments are tested for consistency with the scaffold and for being of sufficient quality. If any alignments satisfy the requirements, the best alignment (blue) is selected for joining the contigs.

Contig Extension



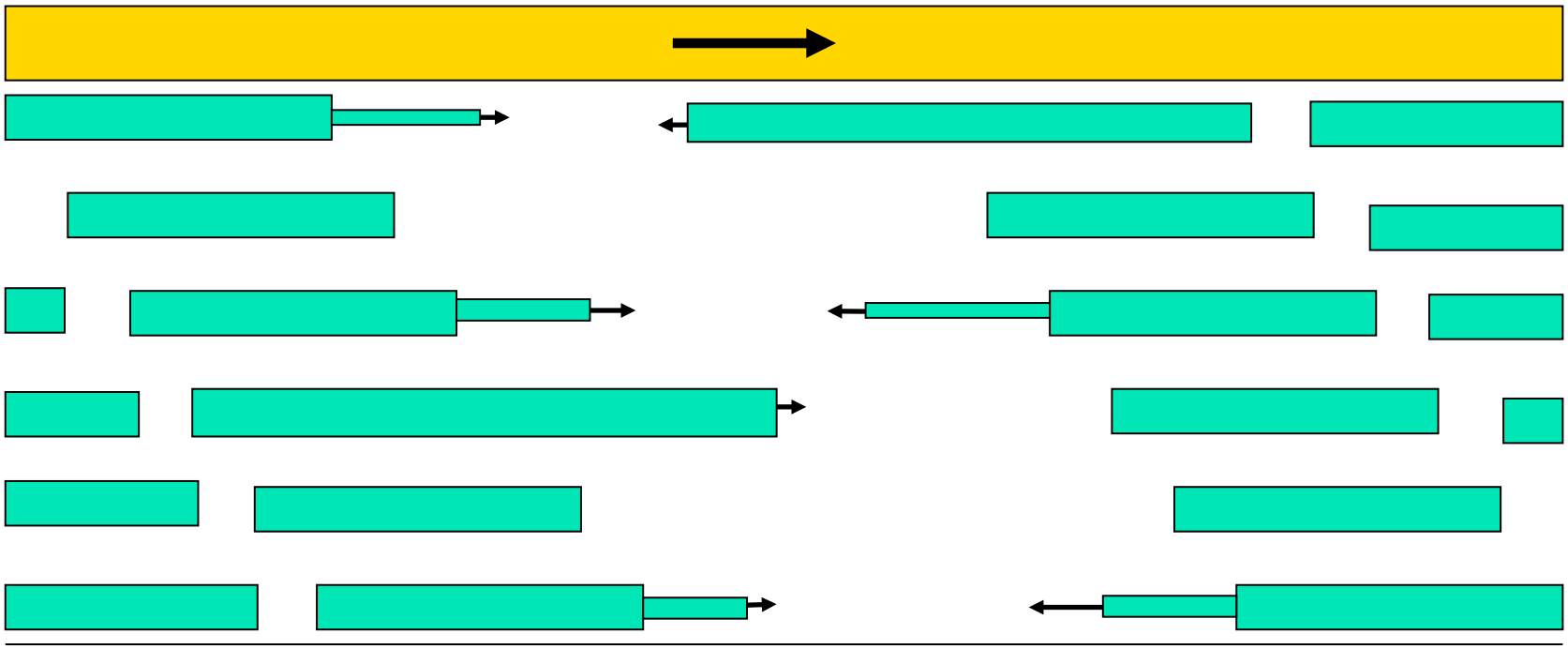
3. The contigs are extended by extending the selected reads beyond their original clear range to the desired position. If necessary, the reads are first aligned to the existing consensus.

Contig Joining



4. The contigs are joined by aligning the newly extended consensi. Alignment gaps inserted into the conseni are promoted into the appropriate positions in the underlying multiple alignment. The joined contig (orange) replaces the original two in the scaffold.

Contig Fattening



5. The join region is fattened to increase the depth of coverage and enhance the consensus quality.