



CENTER FOR
BIOINFORMATICS &
COMPUTATIONAL BIOLOGY



AMOS Assembly Validation and Visualization

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Outline

- AMOS Introduction
 - Getting Data into AMOS
- AMOS Validation Pipeline
 - Mate-Based Validation
 - C/E Statistic
 - Read Alignment Validation
 - Read Depth Validation
- AMOS Assembly Investigator
 - Contigs, Inserts, Histograms, SNP Barcode, Features
 - Misassembly Walkthrough
- Demo



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Slides available at:

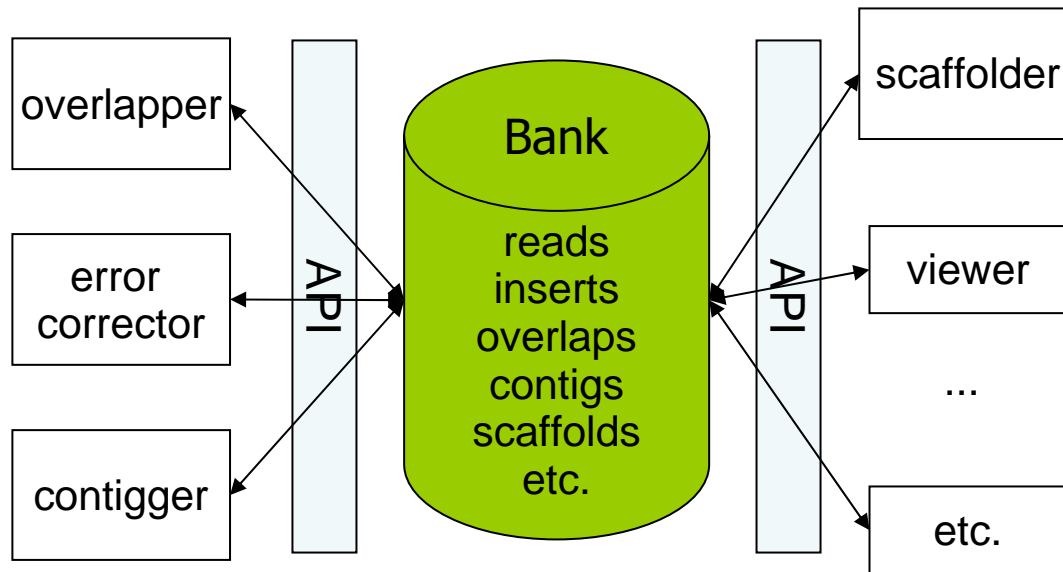
<http://www.cbcb.umd.edu/~mschatz/>



AMOS Goals

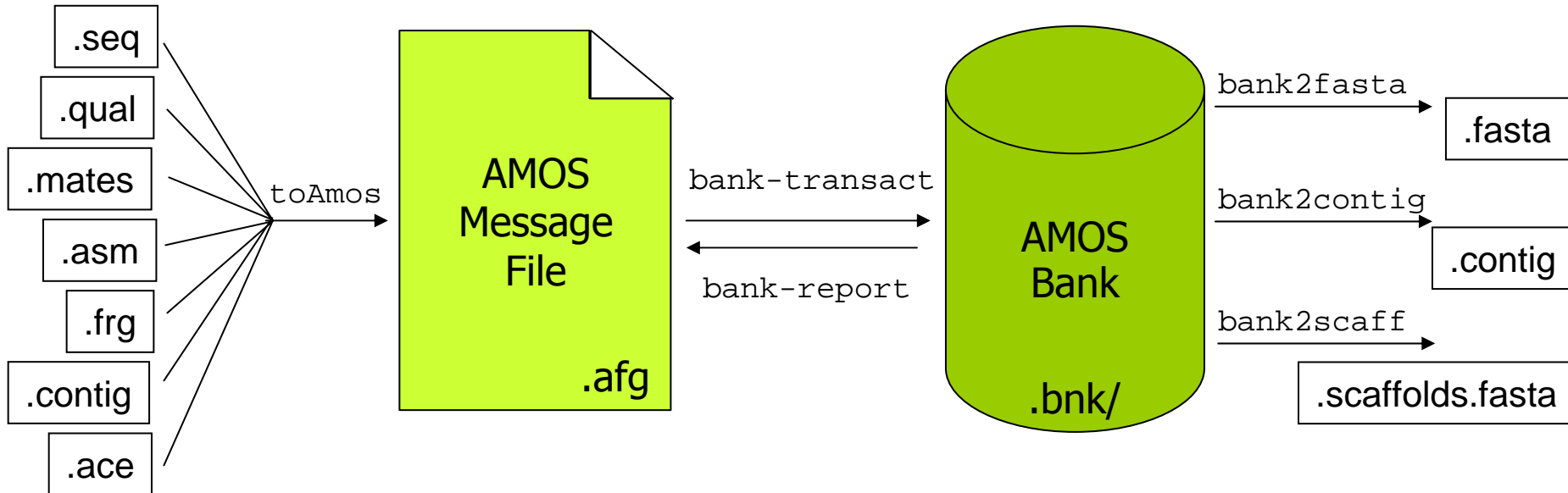
- Open Source Assembly Package
 - <http://amos.sourceforge.net>
- Modular design
- Flexibility in building “pipelines”
- Well defined input/output formats
- General use: does not depend on databases, proprietary data formats, specialized hardware, etc.

Modular Design



- Converters: Celera Assembler, .ACE, TIGR Assembler, Trace Archive
- Overlapper
- Contigger (**Minimus**)
- Consensus caller
- Comparative assembler (**AMOScmp**)
- Mate-pair based QC tool
- Viewer (**Assembly Investigator**)
- Pipeline executor

Assembly Data Conversions



CA Assembly w/ Surrogates to AMOS Message File (.asm, .frg)

```
$ toAmos -a prefix.asm -f prefix.frg -o prefix.afg -S
```

Finished Assembly to AMOS Message File (.contig, .frg)

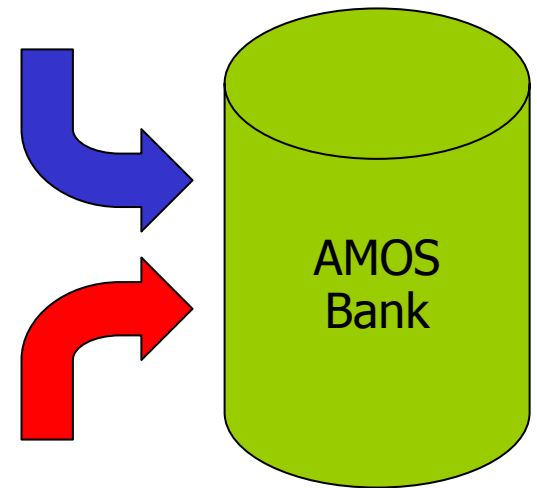
```
$ toAmos -f prefix.frg -c prefix.contig -o prefix.afg
```

AMOS Message File to Bank

```
$ bank-transact -m prefix.afg -b prefix.bnk -c
```

AMOS Validation Pipeline

- Automatically scan an assembly to locate misassembly signatures for further analysis and correction
- cavvalidate prefix (.frg, .asm)
 1. Load CA Assembly Data into Bank
 2. Evaluate Mate Pairs & Libraries
 3. Evaluate Read Alignments
 4. Analyze Depth of Coverage
 5. List Surrogates
 6. Load Misassembly Signatures into Bank
- amosvalidate prefix (.afg)
 - Same as cavvalidate, except skips surrogates





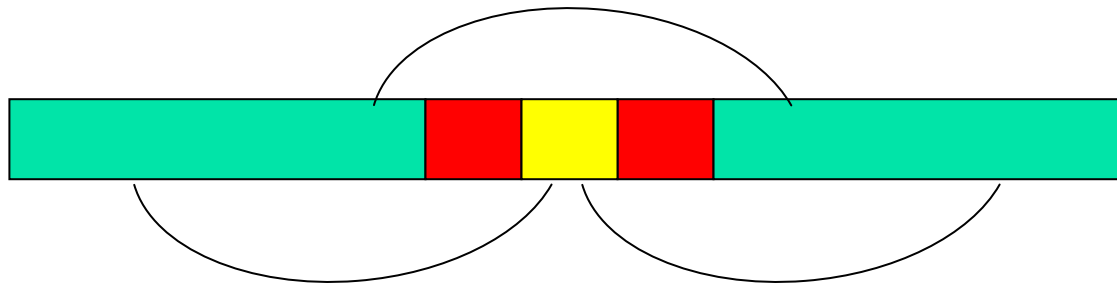
Mate-Happiness: asmQC

- Evaluate mate “happiness” across assembly
 - Happy = Correct orientation and distance
- Finds regions with multiple:
 - Compressed Mates
 - Expanded Mates
 - Invalid same orientation ($\rightarrow \rightarrow$)
 - Invalid outie orientation ($\leftarrow \rightarrow$)
 - Missing Mates
 - Linking mates (mate in a different scaffold)
 - Singleton mates (mate is not in any contig)
- Regions with high C/E statistic

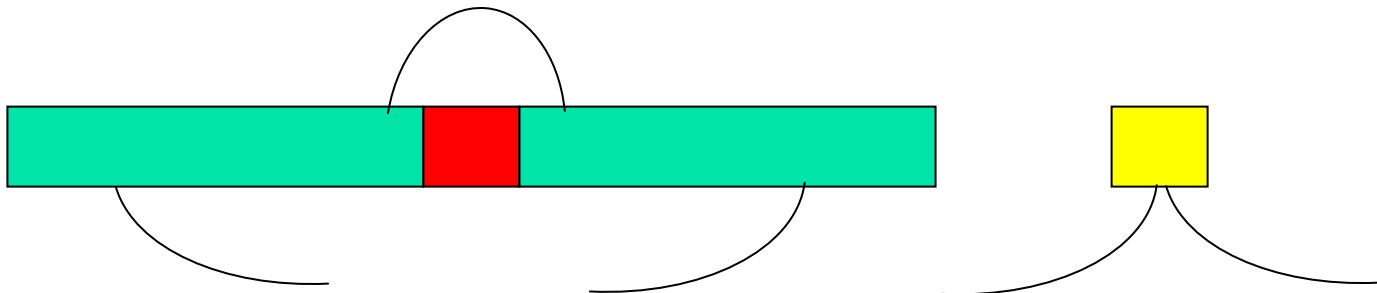
Mate-Happiness: asmQC

- Excision: Skip reads between flanking repeats

- Truth



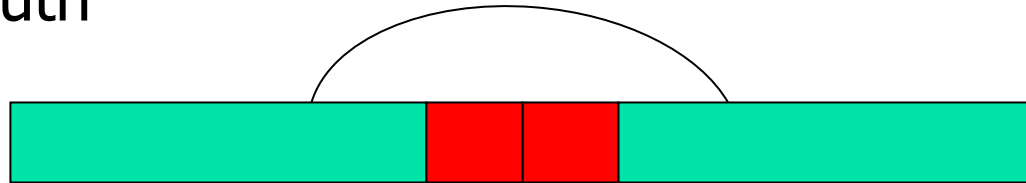
- Misassembly: Compressed Mates, Missing Mates



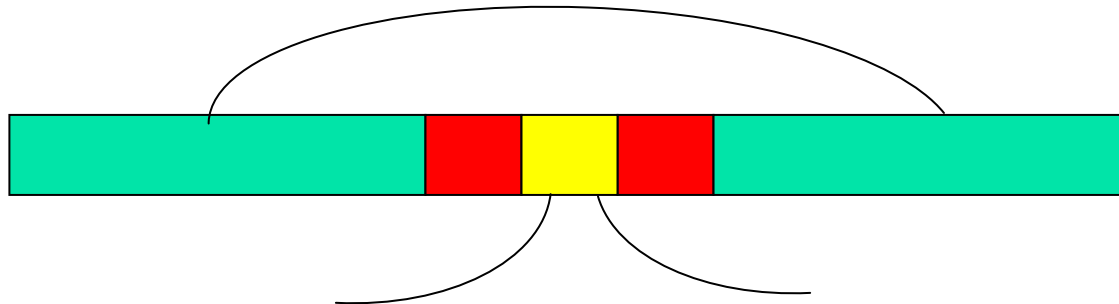
Mate-Happiness: asmQC

- Insertion: Additional reads between flanking repeats

- Truth



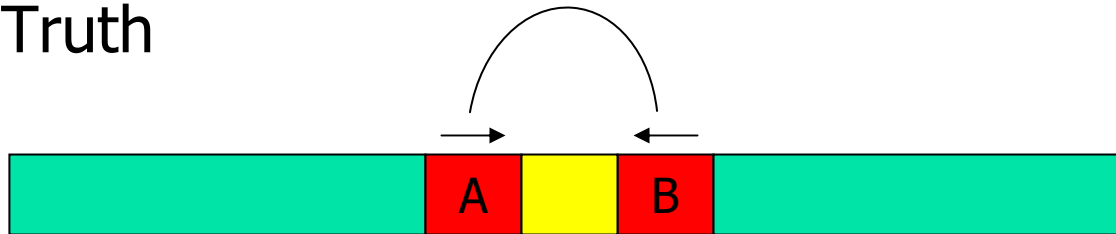
- Misassembly: Expanded Mates, Missing Mates



Mate-Happiness: asmQC

- Rearrangement: Reordering of reads

- Truth



- Misassembly: Misoriented Mates



Note: Unhappy mates may also occur for biological or technical reasons.

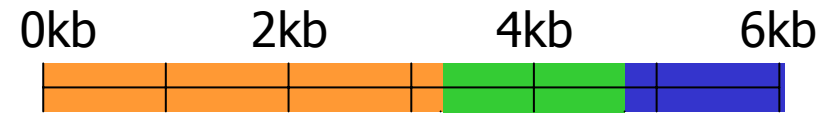
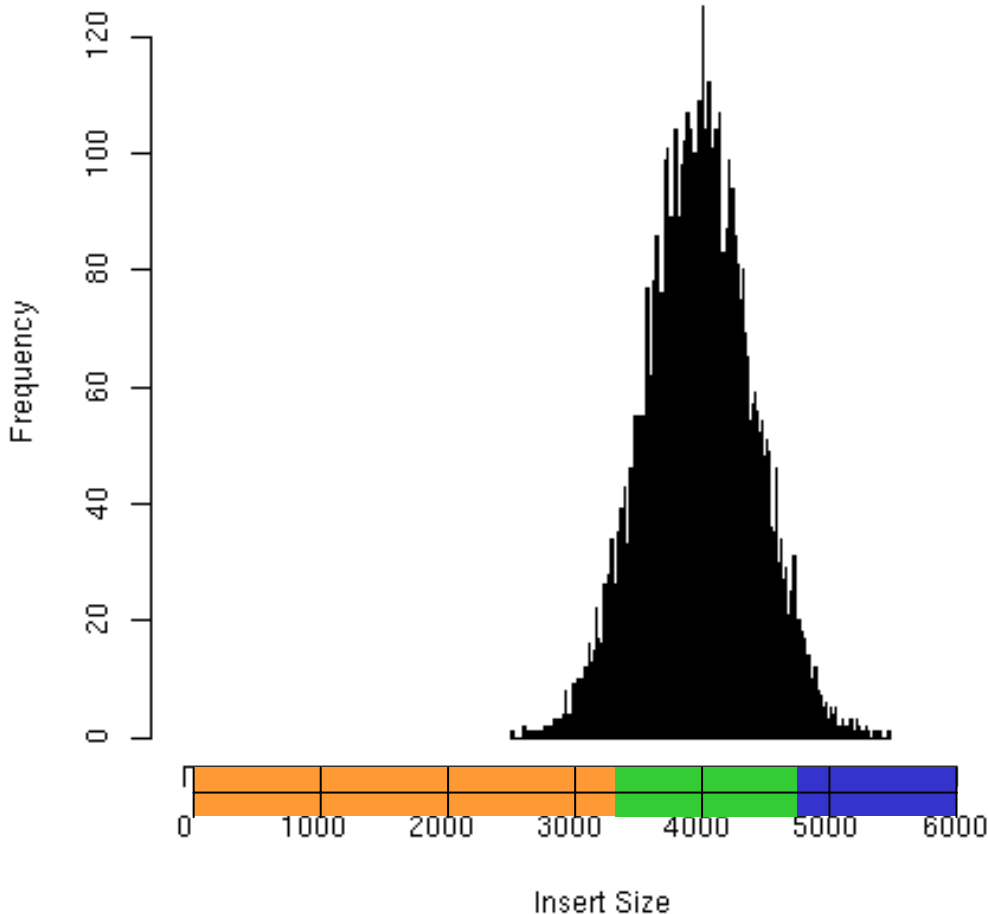


C/E Statistic

- The presence of individual compressed or expanded mates is rare but expected.
- Do the inserts spanning a given position differ from the rest of the library?
 - Flag large differences as potential misassemblies
 - Even if each individual mate is “happy”
- Compute the statistic at all positions
 - $(\text{Local Mean} - \text{Global Mean}) / \text{Scaling Factor}$
- Introduced by Jim Yorke’s group at UMD

Sampling the Genome

Normal Library
Count=10000, Mean=4000, SD=400



8 inserts: 3kb-6kb

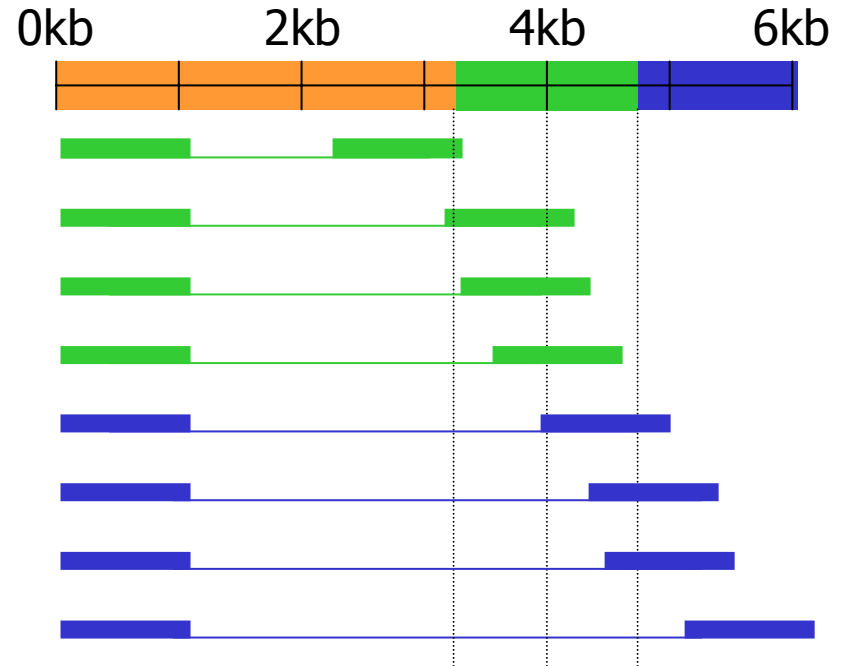
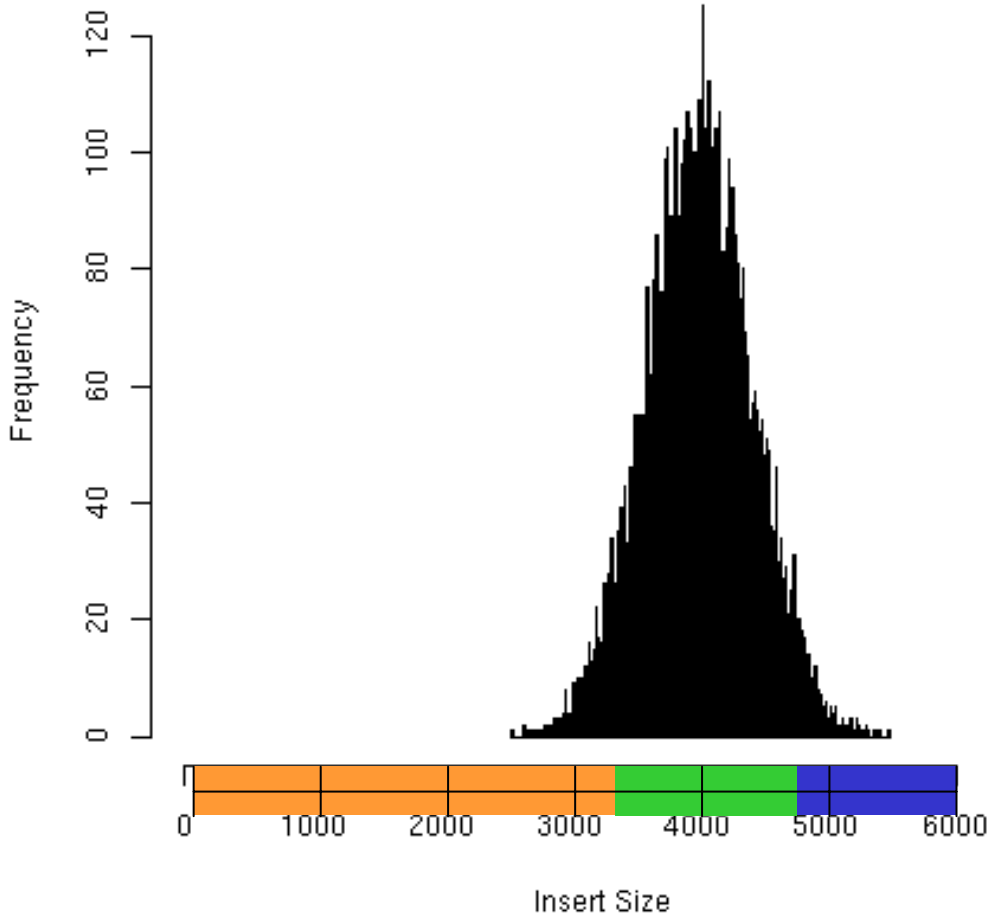
Local Mean: 4048

$$\text{C/E Stat: } \frac{(4048-4000)}{(400 / \sqrt{8})} = +0.33$$

Near 0 indicates overall happiness

C/E-Statistic: Expansion

Normal Library
Count=10000, Mean=4000, SD=400



8 inserts: 3.2kb-6kb

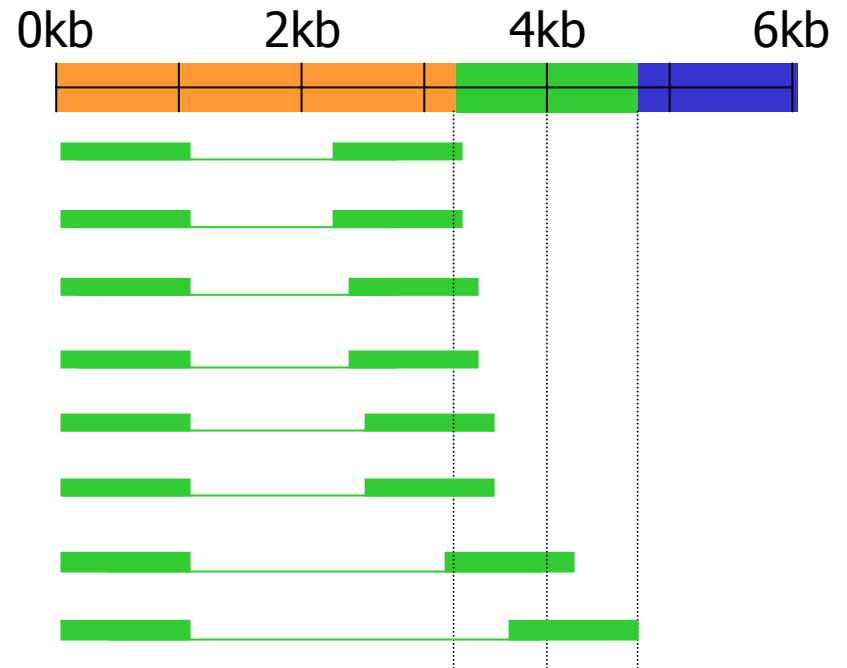
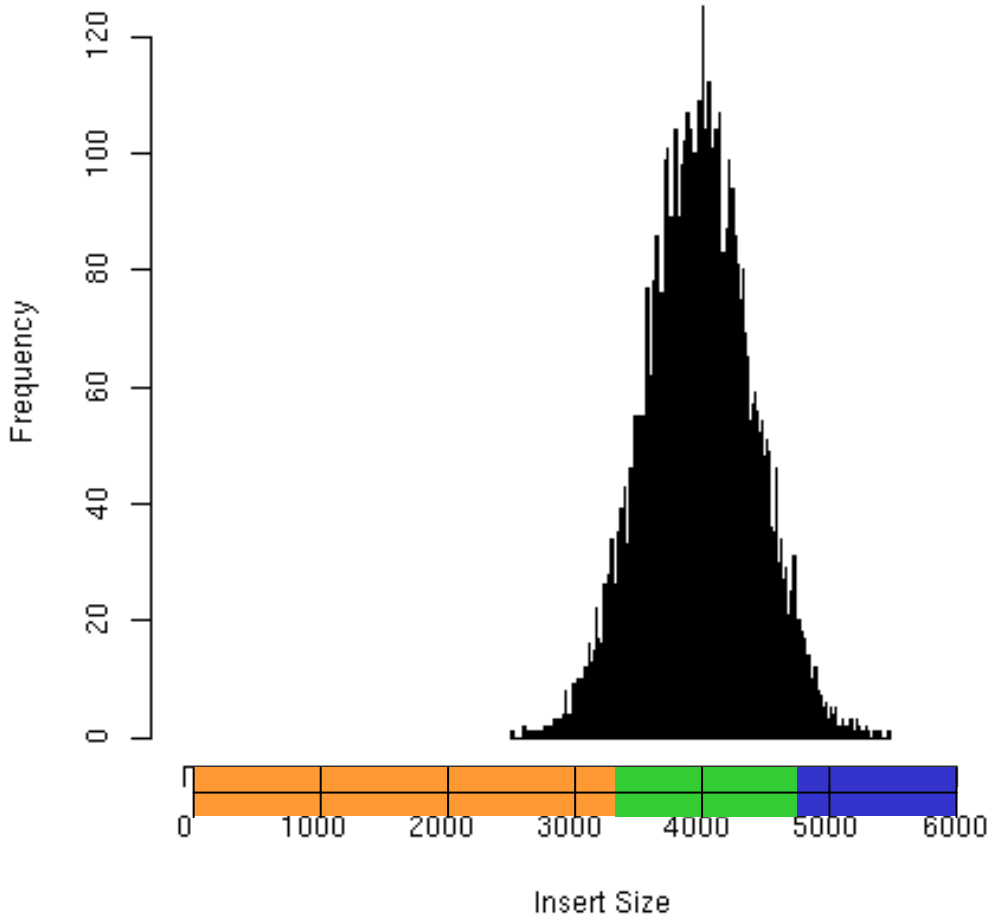
Local Mean: 4461

$$\text{C/E Stat: } \frac{(4461 - 4000)}{(400 / \sqrt{8})} = +3.26$$

C/E Stat ≥ 3.0 indicates Expansion

C/E-Statistic: Compression

Normal Library
Count=10000, Mean=4000, SD=400



8 inserts: 3.2 kb-4.8kb

Local Mean: 3488

$$\text{C/E Stat: } \frac{(3488-4000)}{(400 / \sqrt{8})} = -3.62$$

C/E Stat \leq -3.0 indicates Compression

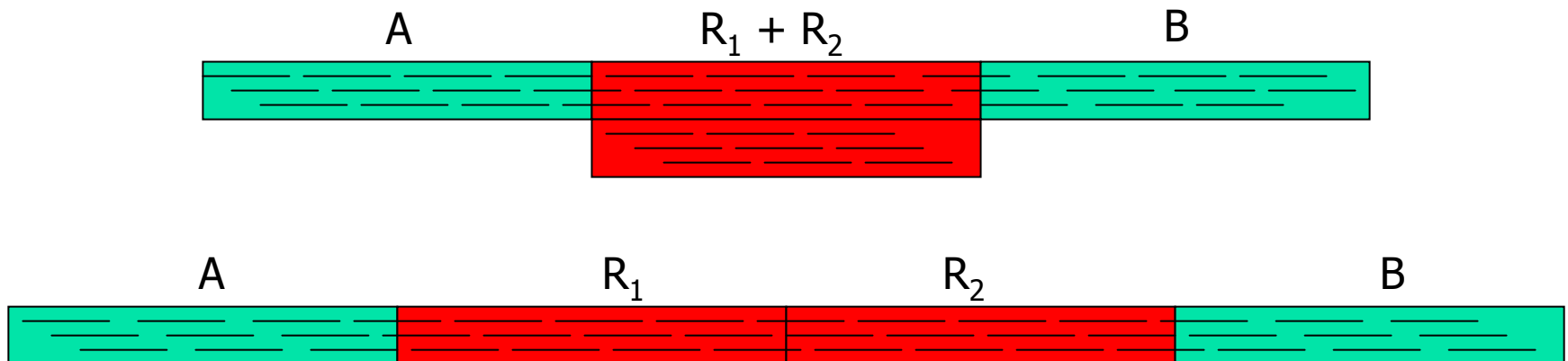
Read Alignment

- Multiple reads with same conflicting base are unlikely
 - 1x QV 30: 1/1000 base calling error
 - 2x QV 30: 1/1,000,000 base calling error
 - 3x QV 30: 1/1,000,000,000 base calling error
- Regions of correlated SNPs are likely to be assembly errors or interesting biological events
 - Highly specific metric
- AMOS Tools: analyzeSNPs & clusterSNPs
 - Locate regions with high rate of correlated SNPs
 - Parameterized thresholds:
 - Multiple positions within 100bp sliding window
 - 2+ conflicting reads
 - Cumulative QV ≥ 40 (1/10000 base calling error)

A	G	C
A	G	C
A	G	C
A	G	C
A	G	C
A	G	C
C	T	A
C	T	A
C	T	A
C	T	A
C	T	A

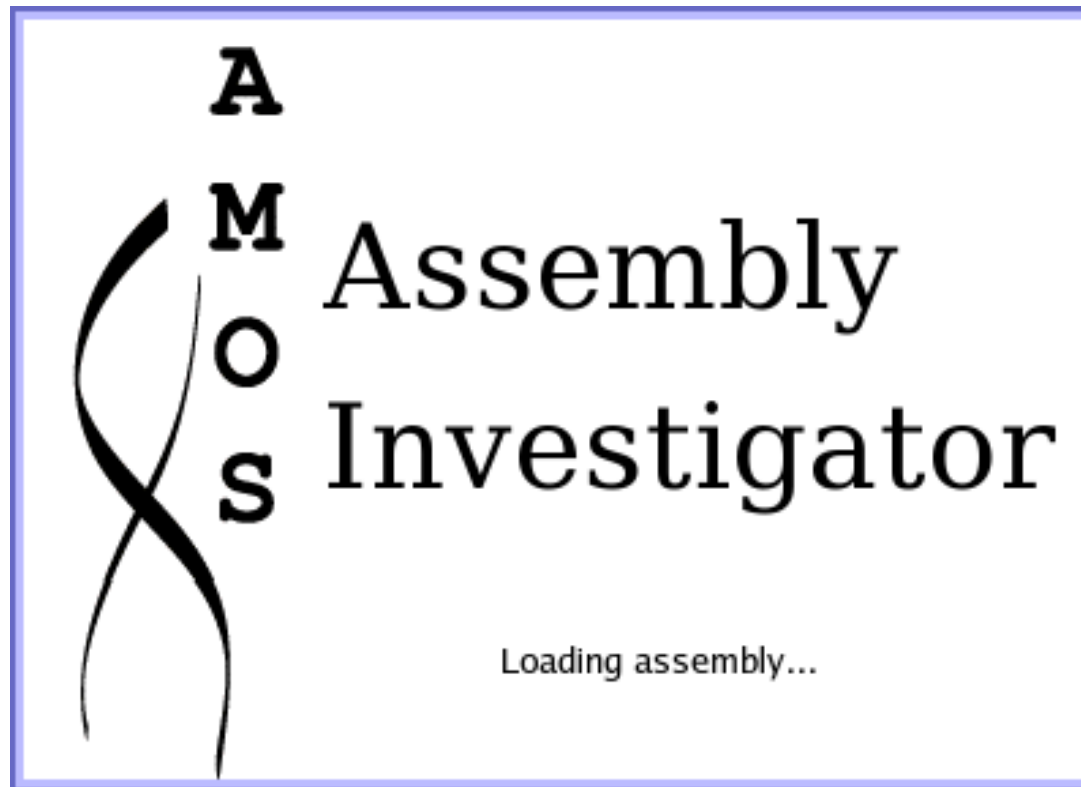
Read Coverage

- Find regions of contigs where the depth of coverage is unusually high
- Collapsed Repeat Signature
 - Can detect collapse of 100% identical repeats
- AMOS Tool: `analyzeReadDepth`
 - 2.5x mean coverage





Assembly Investigator





Assembly Investigator Goals

Interactively explore and analyze

- Libraries
 - Insert Sizes, Read Length, Inserts
- Scaffolds & Contigs
 - Sizes, Composition, Sequence, Multiple Alignment, SNP Barcode
- Inserts
 - Happiness, Coverage, CE Statistic
- Reads
 - Clear Range, Quality Values, Chromatograms
- Features
 - Arbitrary regions of interest
 - Including Misassembly Signatures!!!

Main Window: Contig View

Assembly Investigator

File Options

Position 116659 Contig ID 738 Chromo DB GB6 Inserts Contig Graph A+ A-

Find

110000 110070 110080 110090

Consensus C A T G G C C T G A C C C C G G A C C A G G T G A T G A C C A T C G

XO1HX22TF	C A T G G C C T G A C C C C G G A C C A G G T G A T G A C C A T C
XO1GL75TR	C A T G G C C T G A C C C C G G A C C A G G T G G T G G C C A T C
XO1G181TR	C A T G G C C T G A C C C C G G A C C A G G T G A T G A C C A T C
XO1D260TF	C A T G G C C T G A C C C C G G A C C A G G T G A T G A C C A T C
XO1EE84TR	C A T G G C C T G A C C C C G G A C C A G G T G A T G A C C A T C
XO1GA32TF	C A T G G C C T G A C C C C G G A C C A G G T G A T G A C C A T C
XO1BR60TR	C A T G G C C T G A C C C C G G A C C A G G T G G T G G C C A T C
XO1I240TR	C A T G G C C T G A C C C C G G A C C A G G T G G T G G C C A T C
XO1DK48TR	C A T G G C C T G A C C C C G G A C C A G G T G A T G A C C A T C
XOOA531TF	C A T G G C C T G A C C C C G G A C C A G G T G G T G G C C A T C
XOOAF19TR	C A T G G C C T G A C C C C G G A C C A G G T G A T G A C C A T C
XO1G100TF	C A T G G C C T G A C C C C G G A C C A G G T G A T G A C C A T C

Viewing Xoo.bnk/ with 776 contigs Contig Id:738 Size: 119783 Reads: 1114

Main Window: Contig View

Discrepancy Navigation Contig Quick Select Discrepancy

Regular Expression Consensus Search

Consensus & Position

Scrollable Read Tiling

Summary

Read Orientation Discrepancy Highlight

Assembly Investigator

File Options

Position 116659 Contig ID 738 Chromo DB GB6 Inserts Contig Graph A+ A-

Find

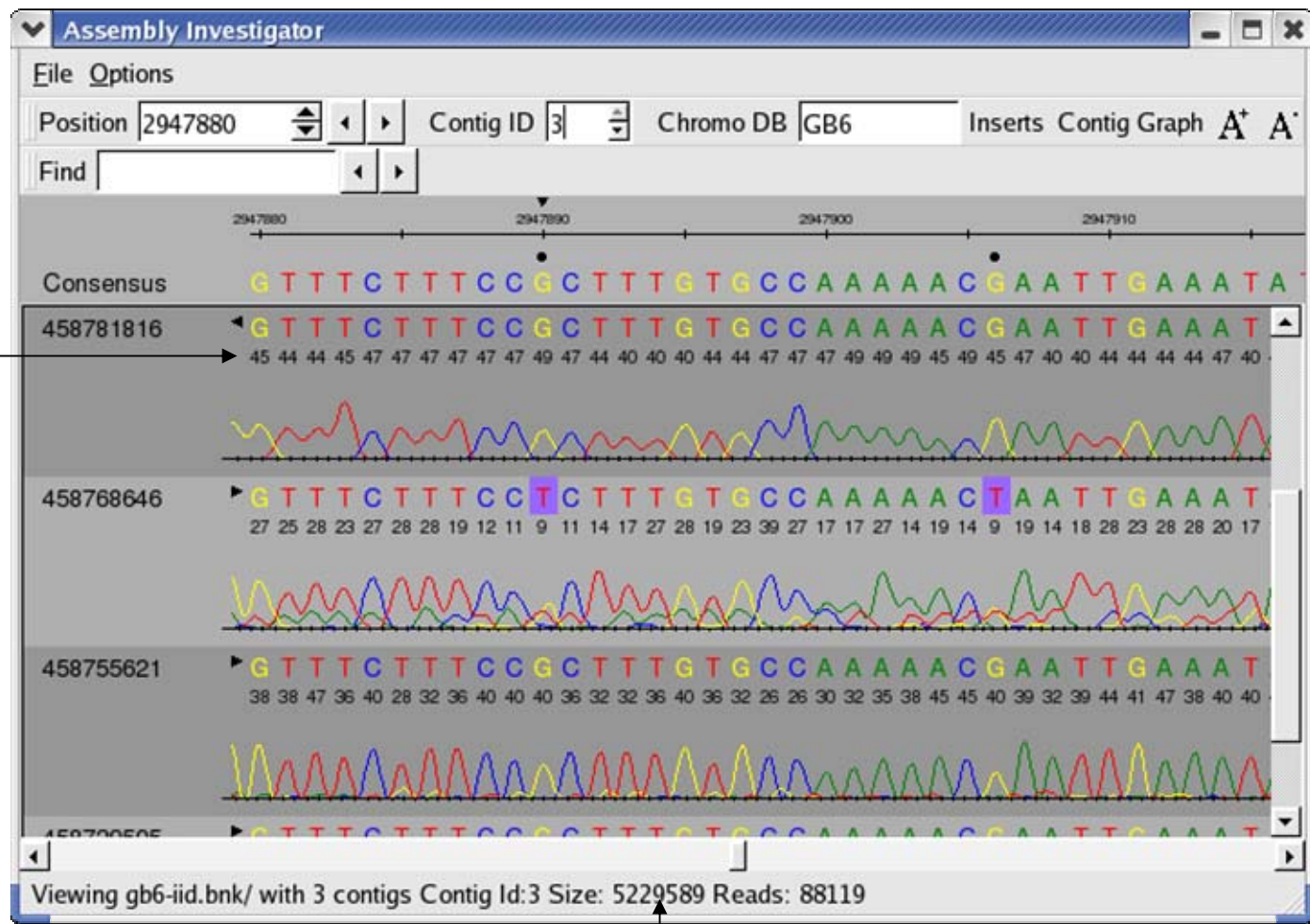
110000 110070 110080 110090

Consensus C A T G G C C T G A C C C C G G A C C A G G T G A T G A C C A T C G

XO1HX22TF	◀ C A T G G C C T G A C C C C G G A C C A G G T G A T G A C C A T C
XO1GL75TR	◀ C A T G G C C T G A C C C C G G A C C A G G T G G T G G C C A T C
XO1G181TR	▶ C A T G G C C T G A C C C C G G A C C A G G T G A T G A C C A T C
XO1D260TF	◀ C A T G G C C T G A C C C C G G A C C A G G T G A T G A C C A T C
XO1EE84TR	▶ C A T G G C C T G A C C C C G G A C C A G G T G A T G A C C A T C
XO1GA32TF	◀ C A T G G C C T G A C C C C G G A C C A G G T G A T G A C C A T C
XO1BR60TR	◀ C A T G G C C T G A C C C C G G A C C A G G T G G T G G C C A T C
XO1I240TR	◀ C A T G G C C T G A C C C C G G A C C A G G T G G T G G C C A T C
XO1DK48TR	▶ C A T G G C C T G A C C C C G G A C C A G G T G A T G A C C A T C
XOOA531TF	▶ C A T G G C C T G A C C C C G G A C C A G G T G G T G G C C A T C
XOOAF19TR	▶ C A T G G C C T G A C C C C G G A C C A G G T G T G A C C A T C
XO1G100TF	◀ C A T G G C C T G A C C C C G G A C C A G G T G T G A C C A T C

Viewing Xoo.bnk/ with 776 contigs Contig Id:738 Size: 119783 Reads: 1114

Contig View Expanded



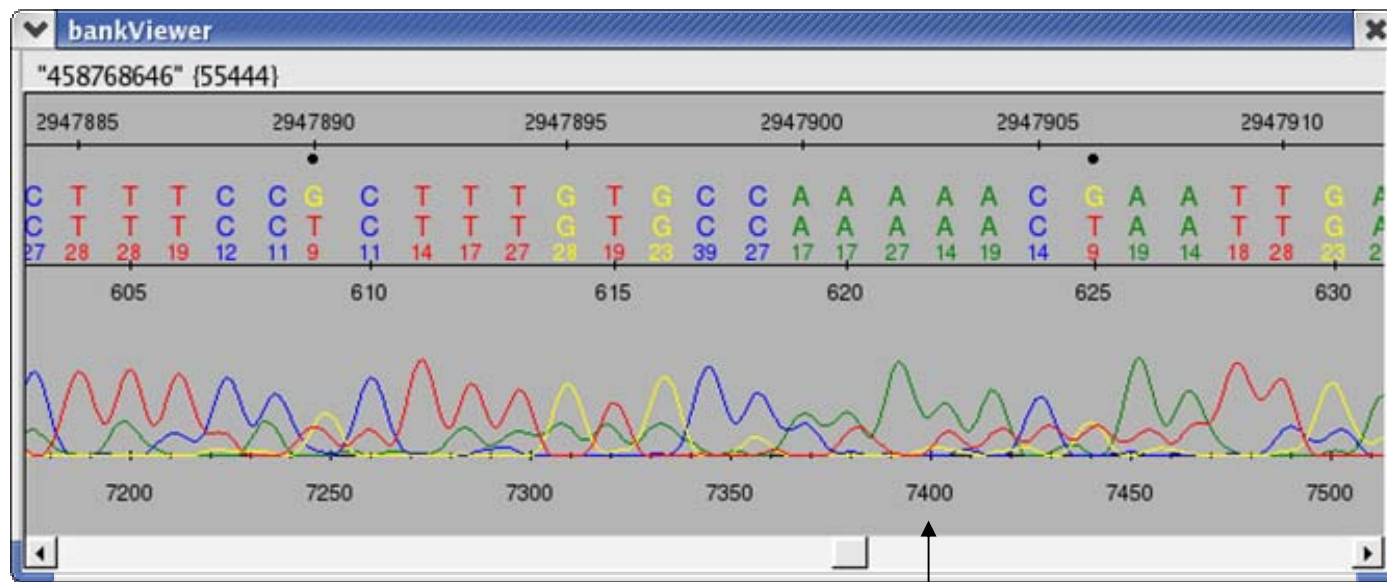
Quality Values

Normalized Chromatogram

No size restrictions

Chromatogram View

Read EID, IID
Consensus
Read
Raw
Chromatogram



Chromatogram Position

Chromatograms are loaded from specified directories,
or on demand from Trace Archive.

Main Window: Contig View

Display Inserts

The screenshot shows the 'Assembly Investigator' window. At the top, there is a menu bar with 'File' and 'Options'. Below the menu bar, there are several input fields: 'Position' (116659), 'Contig ID' (738), and 'Chromo DB' (GB6). To the right of these fields is a 'Display Inserts' button, which is highlighted by an arrow from the text 'Display Inserts' above it. Below the input fields is a 'Find' field. The main area of the window displays a sequence alignment. At the top of this area is a scale bar with markers at 110000, 110070, 110080, and 110090. Below the scale bar is a 'Consensus' sequence: C A T G G C C T G A C C C C G G A C C A G G T G A T G A C C A T C G. Below the consensus sequence are several rows of sequence alignments, each starting with a read ID (e.g., XO1HX22TF, XO1GL75TR, etc.) and followed by a sequence of colored letters (C, A, T, G, G, C, C, T, G, A, C, C, C, C, G, G, A, C, C, A, G, G, T, G, A, T, G, A, C, C, A, T, C). The alignment for XO1GL75TR shows several 'G' characters highlighted in purple, indicating insertions. At the bottom of the window, there is a status bar that reads: 'Viewing Xoo.bnk/ with 776 contigs Contig Id:738 Size: 119783 Reads: 1114'.

Insert View



Insert View

Toolbar

Position

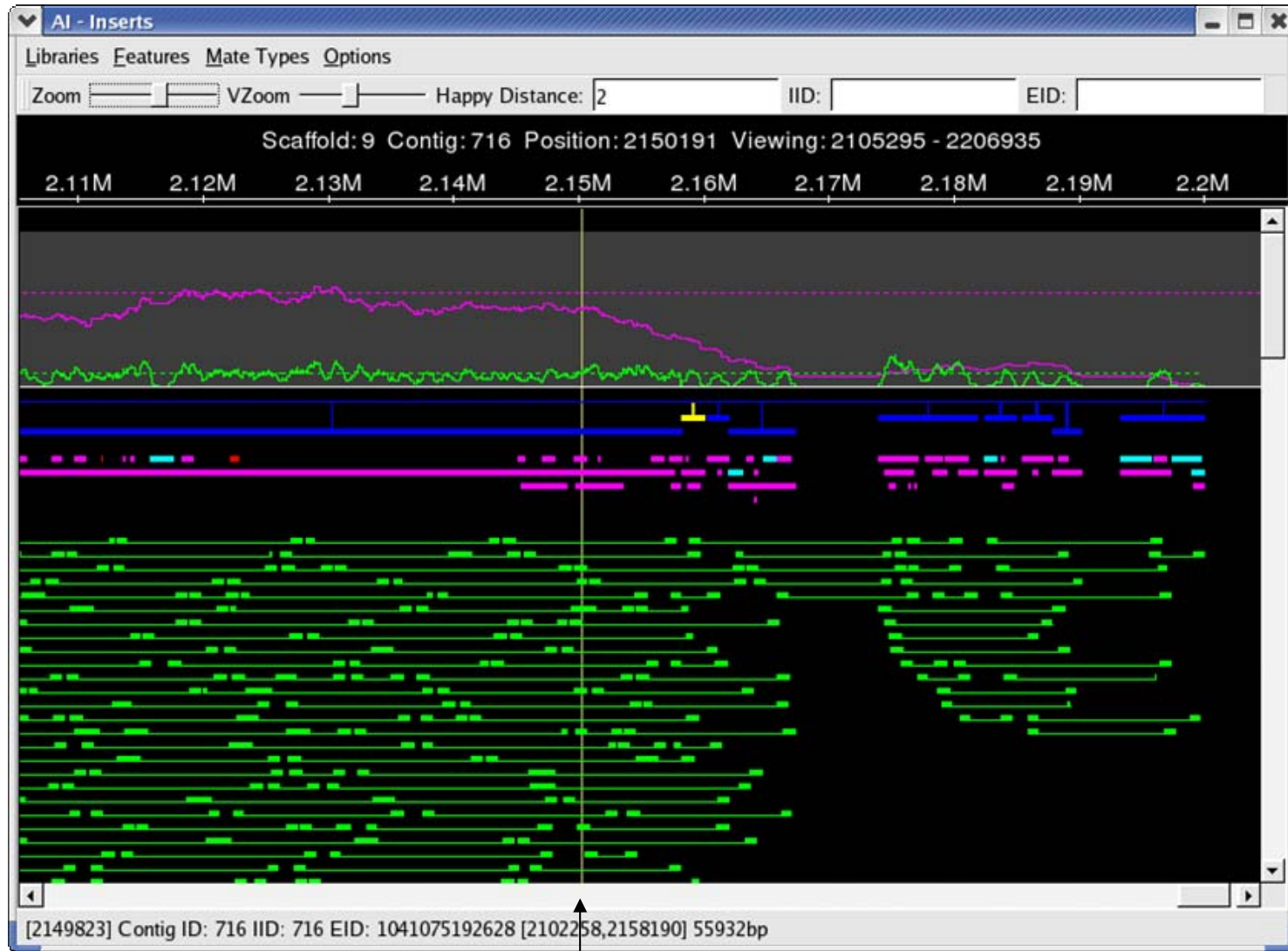
Insert and
Read Coverage

Scaffold

Features

Inserts

Details



Current Contig Position



Standard Feature Types

 **[B] Breakpoint**

Alignment ends at this position

 **[C] Coverage**

Location of unusual mate coverage (asmQC)

 **[S] SNPs**

Location of Correlated SNPs

 **[U] Unitig**

Used to report location of surrogate unitigs in CA assemblies

 **[X] Other**

All other Features

Loading Features:

```
$ loadFeatures bankname featfile
```

Featfile format:

```
Contigid type end5 end3 comment
```

Insert Happiness

Both mates present



Happy

- Oriented Correctly &&
- $|\text{Insert Size} - \text{Library.mean}| \leq \text{Happy-Distance} * \text{Library.sd}$



Stretched

- Oriented Correctly &&
- $\text{Insert Size} > \text{Library.mean} + \text{Happy-Distance} * \text{Library.sd}$



Compressed

- Oriented Correctly &&
- $\text{Insert Size} < \text{Library.mean} - \text{Happy-Distance} * \text{Library.sd}$



Misoriented

- Same or Outies

Only 1 read present



Linking

- Read's mate is in some other scaffold



Singleton

- Read's mate is a singleton

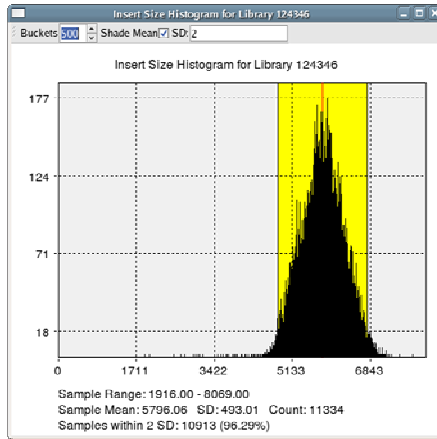


Unmated

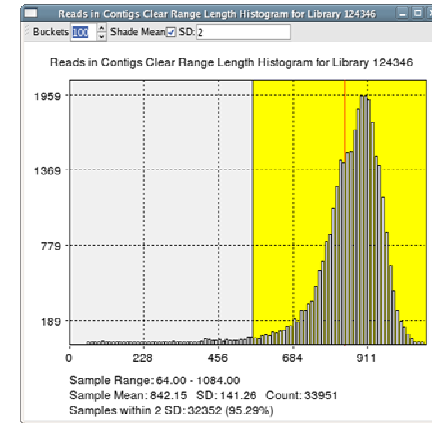
- No mate was provided for read

Histograms & Statistics

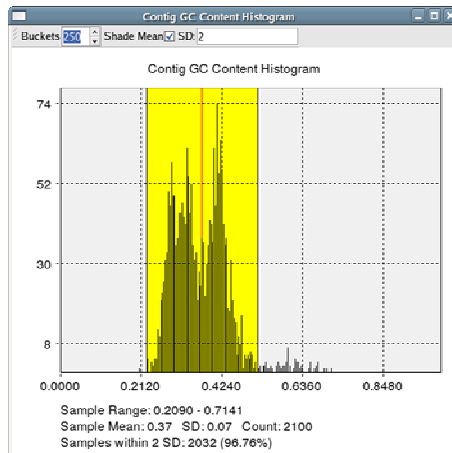
Insert Size



Read Length



GC Content



Overall Statistics

Field	Value
[Scaffolds]	
TotalScaffolds	1076
TotalContigsInScaffolds	1396
MeanContigsPerScaffold	1.30
MinContigsPerScaffold	1
MaxContigsPerScaffold	15
[Bases]	
TotalBasesInScaffolds	7511900
MeanBasesInScaffolds	6981.32
MaxBasesInScaffolds	279040
N50ScaffoldBases	75935
[Spans]	
TotalSpanOfScaffolds	7804540
MeanSpanOfScaffolds	7253.29
MinScaffoldSpan	1007
MaxScaffoldSpan	285205
IntraScaffoldCaps	320
2KbScaffolds	200
2KbScaffoldSpan	6464092
2KbScaffoldPercent	82.82
MeanSequenceGapSize	-866.37
[Contigs]	
TotalContigs	2100

This table displays various assembly statistics for library 124346. It is organized into sections for Scaffolds, Bases, Spans, and Contigs. The total number of scaffolds is 1076, and the total number of contigs is 2100. The total number of bases is 7,511,900, with a mean of 6,981.32 bases per scaffold. The N50 scaffold bases is 75,935. The total span of scaffolds is 7,804,540, with a mean span of 7,253.29. The table also shows the distribution of contigs per scaffold, with a mean of 1.30 and a maximum of 15. The mean sequence gap size is -866.37.

- Bird's eye view of data and assembly quality

Assembly Reports

Contigs

Id	IID	EID	Status	Length	Reads	GC Content
144	144	1047283847442	P	519090	6280	0.6399
141	141	1047283847439	P	326218	3784	0.6391
160	160	1047283847458	P	315606	3611	0.6372
152	152	1047283847450	P	259589	3402	0.6422
171	171	1047283847469	P	254579	2555	0.6459
148	148	1047283847446	P	253482	3415	0.6423
147	147	1047283847445	P	228649	2914	0.6475
140	140	1047283847438	P	220970	2386	0.6435
156	156	1047283847454	P	200997	2630	0.6445
151	151	1047283847440	P	106066	2660	0.6272

Select from 172 contigs in xoc4.bnk

Features

EID	Type	Source Type	Source IID	Dir	Start	End	Length	Comment
B	C		164	F	3259	3260	1	END_BREAK: 175763
B	C		145	F	1563	1564	1	END_BREAK: 22996
B	C		156	F	197501	197502	1	END_BREAK: 3244
B	C		130	F	5853	5854	1	END_BREAK: 60701
B	C		144	F	512056	512057	1	END_BREAK: 6420
B	C		159	F	87187	87188	1	END_BREAK: 690
D	C		23	F	2055	3454	1399	HIGH_READ_COVERAGE 32
D	C		84	F	899	2463	1564	HIGH_READ_COVERAGE 32
D	C		41	F	634	1675	1041	HIGH_READ_COVERAGE 35
D	C		28	F	4463	5735	1272	HIGH_READ_COVERAGE 36
P	C		2	F	299	1393	1094	HIGH_SNP 10.121.67
P	C		23	F	1561	3317	1756	HIGH_SNP 10.195.22
P	C		164	F	29745	30597	852	HIGH_SNP 10.94.78
P	C		153	F	21586	22457	871	HIGH_SNP 10.96.89
P	C		37	F	772	2506	1734	HIGH_SNP 12.157.73
P	C		124	F	268	1196	928	HIGH_SNP 12.84.45

Select from 171 features

Reads

IID	EID	MateType	Offset	End Offset	Length	Dir	CLR Begin	CLR End	Lib ID	GC Content
38852	XOEDL61TF	71	342	1308	967	F	28	994	86919	0.5890
8396	XODA243TF	71	720	1686	967	F	985	20	86918	0.5896
40100	XOEA209TR	71	795	1711	917	R	933	16	86919	0.5911
8007	XODAG50TF	71	748	1710	963	F	20	982	86918	0.5946
121	XOCA015TFB	71	344	1198	855	F	23	877	86920	0.6090
36894	XOEDC38TR	71	291	1206	916	F	19	934	86919	0.6055
42027	XOEDT12TF	71	284	1056	773	F	74	947	86919	0.6080
17934	XOEA62TR	71	135	1140	1006	R	1035	40	86919	0.6151
52159	XOEF111TF	71	169	1106	938	R	963	27	86919	0.6154
43894	XOEF980TR	71	199	1140	942	R	976	36	86919	0.6170
24879	XOECN79TR	71	232	1040	809	R	830	22	86919	0.6225
18209	XOEA132TR	71	86	1082	997	R	1015	22	86919	0.6234
28687	XOEBN27TF	71	163	1050	888	F	21	907	86919	0.6253
4238	XOCAN73TF	71	92	970	879	F	29	906	86920	0.6271

Select from 23 reads

Scaffolds

Id	IID	EID	Offset	Span	Contigs
1	173	1047283847471		2559	1
2	174	1047283847472		2725904	25
3	175	1047283847473		2111083	24
152	152	1047283847450	0	259589	BE
153	153	1047283847451	259820	61666	BE
154	154	1047283847452	321466	24156	BE
155	155	1047283847453	345602	73623	BE
156	156	1047283847454	419250	200997	BE
75	75	1047283847329	620227	8956	BE
157	157	1047283847455	629163	14699	BE
158	158	1047283847456	643842	15947	BE
159	159	1047283847457	659769	88018	BE
160	160	1047283847458	747786	315606	BE
161	161	1047283847459	1063385	86827	BE

Select from 10 scaffolds in xoc4.bnk

- Full Integration: "Double click takes you there"

Assembly Reports

Misassembly Walkthrough: Correlated SNPs

Contigs

Id	IID	EID	Status	Length	Reads	GC Content
144	144	1047283847442	P	519090	6280	0.6399
141	141	1047283847439	P	326218	3784	0.6391
160	160	1047283847458	P	315606	3611	0.6372
152	152	1047283847450	P	259589	3402	0.6422
171	171	1047283847469	P	254579	2555	0.6459
148	148	1047283847446	P	253482	3415	0.6423
147	147	1047283847445	P	228649	2914	0.6475
140	140	1047283847438	P	220970	2386	0.6435
156	156	1047283847454	P	200997	2630	0.6445
151	151	1047283847440	P	106066	2660	0.6272

Select from 172 contigs in xoc4.bnk

Features

EID	Type	Source Type	Source IID	Dir	Start	Length	Comment
B	C		164	F	3259	1	END_BREAK: 175763
B	C		145	F	1563	1	END_BREAK: 22996
B	C		156	F	197501	1	END_BREAK: 3244
B	C		130	F	5853	5854	END_BREAK: 60701
B	C		144	F	512056	512057	END_BREAK: 6420
B	C		159	F	87187	87188	END_BREAK: 690
D	C		23	F	2055	3454	HIGH_READ_COVERAGE 32
D	C		84	F	899	2463	HIGH_READ_COVERAGE 32
D	C		41	F	634	1675	HIGH_READ_COVERAGE 35
D	C		28	F	4463	5735	HIGH_READ_COVERAGE 36
P	C		2	F	299	3317	HIGH_SNP 10 121.67
P	C		2	F	1561	3317	HIGH_SNP 10 195.22
P	C		164	F	29745	30597	HIGH_SNP 10 94.78
P	C		153	F	21586	22457	HIGH_SNP 10 96.89
P	C		37	F	772	2506	HIGH_SNP 12 157.73
P	C		124	F	268	1196	HIGH_SNP 12 84.45

Select from 171 features

Reads

IID	EID	MateType	Offset	End Offset	Length	Dir	CLR Begin	CLR End	Lib ID	GC Content
38852	XOEDL61TF	71	342	1308	967	F	28	994	86919	0.5890
8396	XODA243TF	71	720	1686	967	F	985	20	86918	0.5896
40100	XOEA209TR	71	795	1711	917	R	933	16	86919	0.5911
8007	XODAG50TF	71	748	1710	963	F	20	982	86918	0.5946
121	XOCA015TFB	71	344	1198	855	F	23	877	86920	0.6090
36894	XOEDC38TR	71	291	1206	916	F	19	934	86919	0.6055
42027	XOEDT12TF	71	284	1056	773	F	74	947	86919	0.6080
17934	XOEA62TR	71	135	1140	1006	R	1035	40	86919	0.6151
52159	XOEF111TF	71	169	1106	938	R	963	27	86919	0.6154
43894	XOEF980TR	71	199	1140	942	R	976	36	86919	0.6170
24879	XOECN79TR	71	232	1040	809	R	830	22	86919	0.6225
18209	XOEA132TR	71	86	1082	997	R	1015	22	86919	0.6234
28687	XOEBN27TF	71	163	1050	888	F	21	907	86919	0.6253
4238	XOCAN73TF	71	92	970	879	F	29	906	86920	0.6271

Select from 23 reads

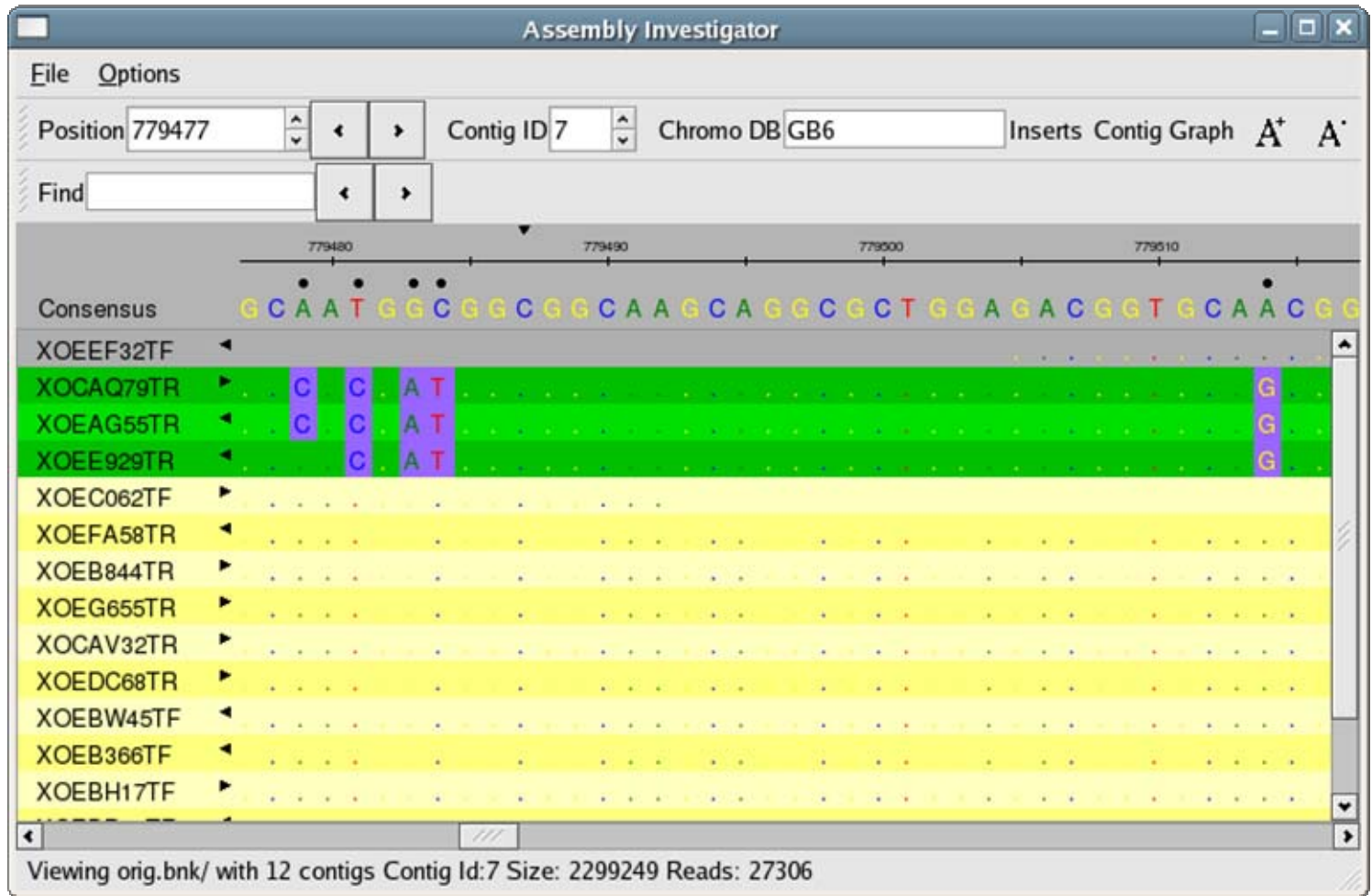
Scaffolds

Id	IID	EID	Offset	Span	Contigs
1	173	1047283847471		2559	1
2	174	1047283847472		2725904	25
3	175	1047283847473		2111083	24
152	152	1047283847450	0	259589	BE
153	153	1047283847451	259820	61666	BE
154	154	1047283847452	321466	24156	BE
155	155	1047283847453	345602	73623	BE
156	156	1047283847454	419250	200997	BE
75	75	1047283847329	620227	8956	BE
157	157	1047283847455	629163	14699	BE
158	158	1047283847456	643842	15947	BE
159	159	1047283847457	659769	88018	BE
160	160	1047283847458	747786	315606	BE
161	161	1047283847459	1063385	86827	BE

Select from 10 scaffolds in xoc4.bnk

- Full Integration: "Double click takes you there"

SNP View

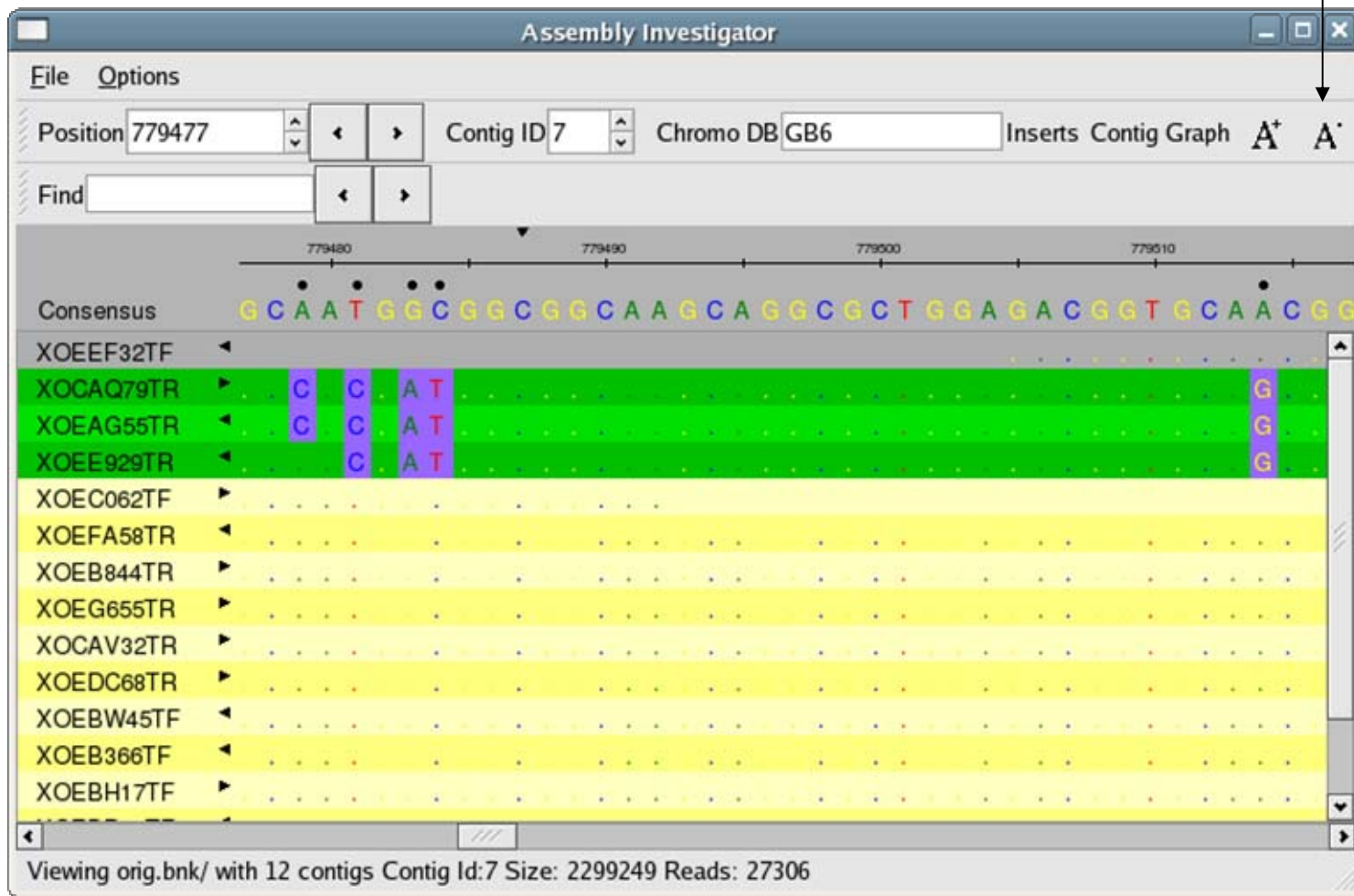


SNP Sorted Reads

Polymorphism View

SNP View

Zoom Out

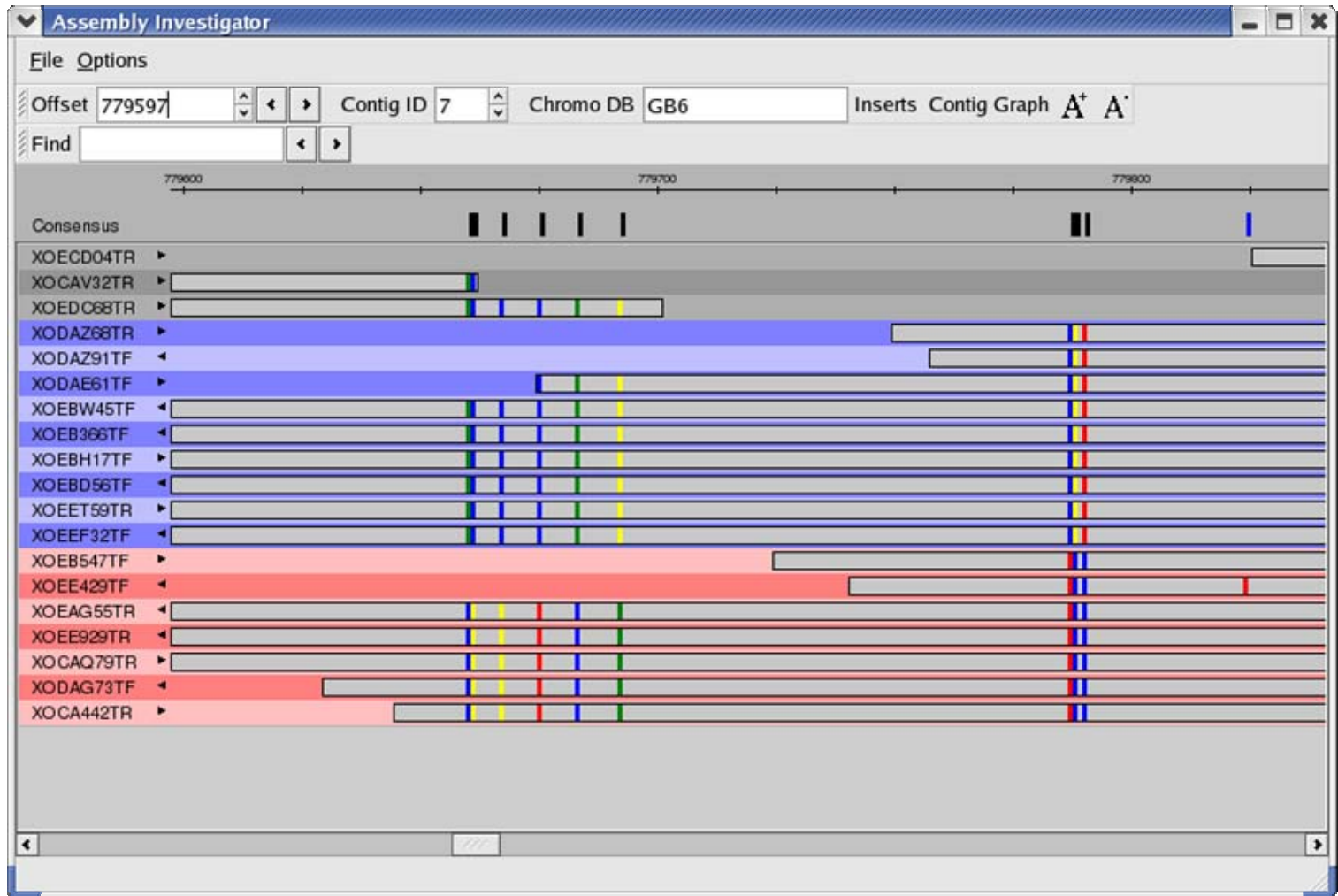


SNP Sorted
Reads

Polymorphism
View

SNP Barcode

SNP Sorted Reads

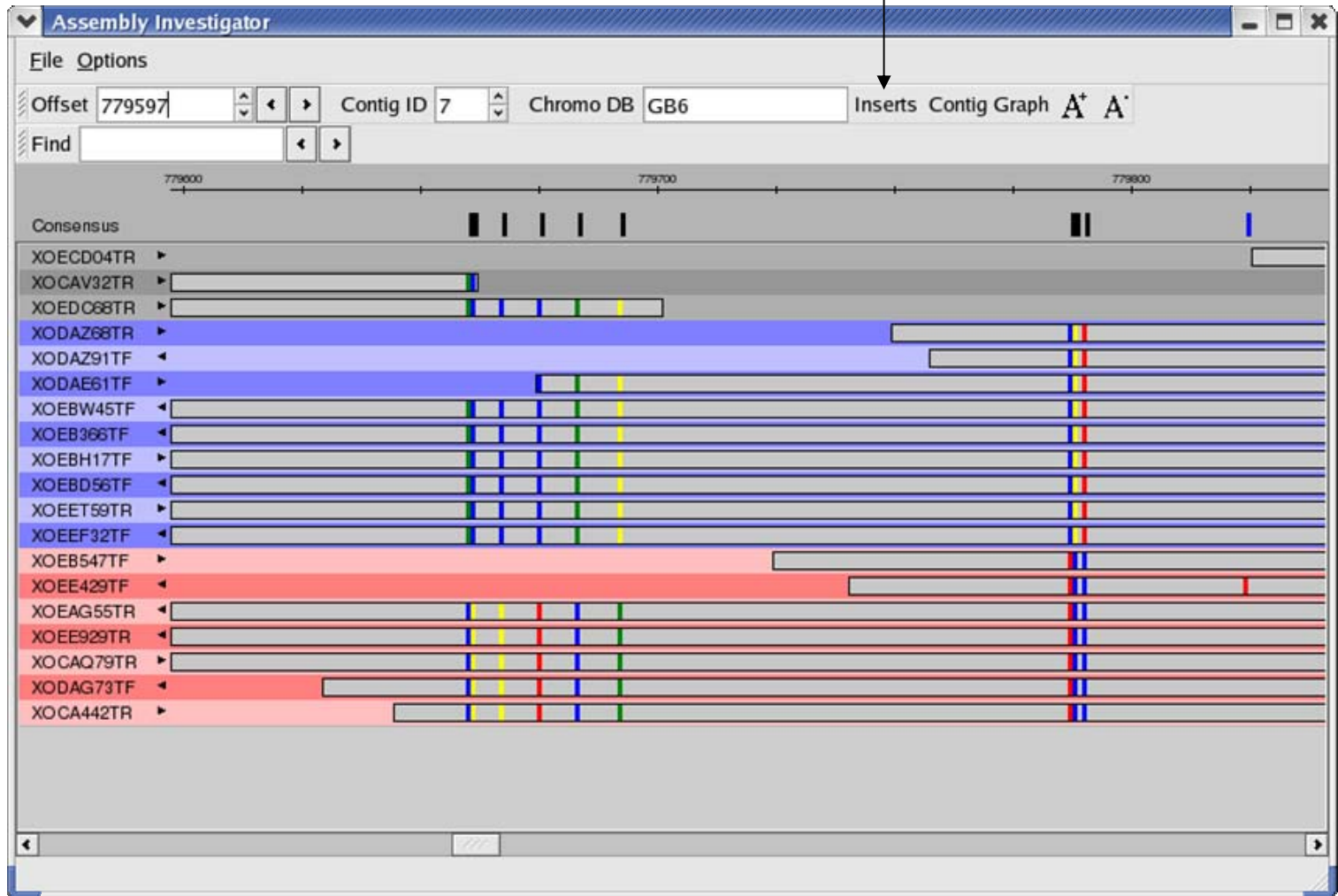


Colored Rectangle indicate the positions and composition of the SNPs

SNP Barcode

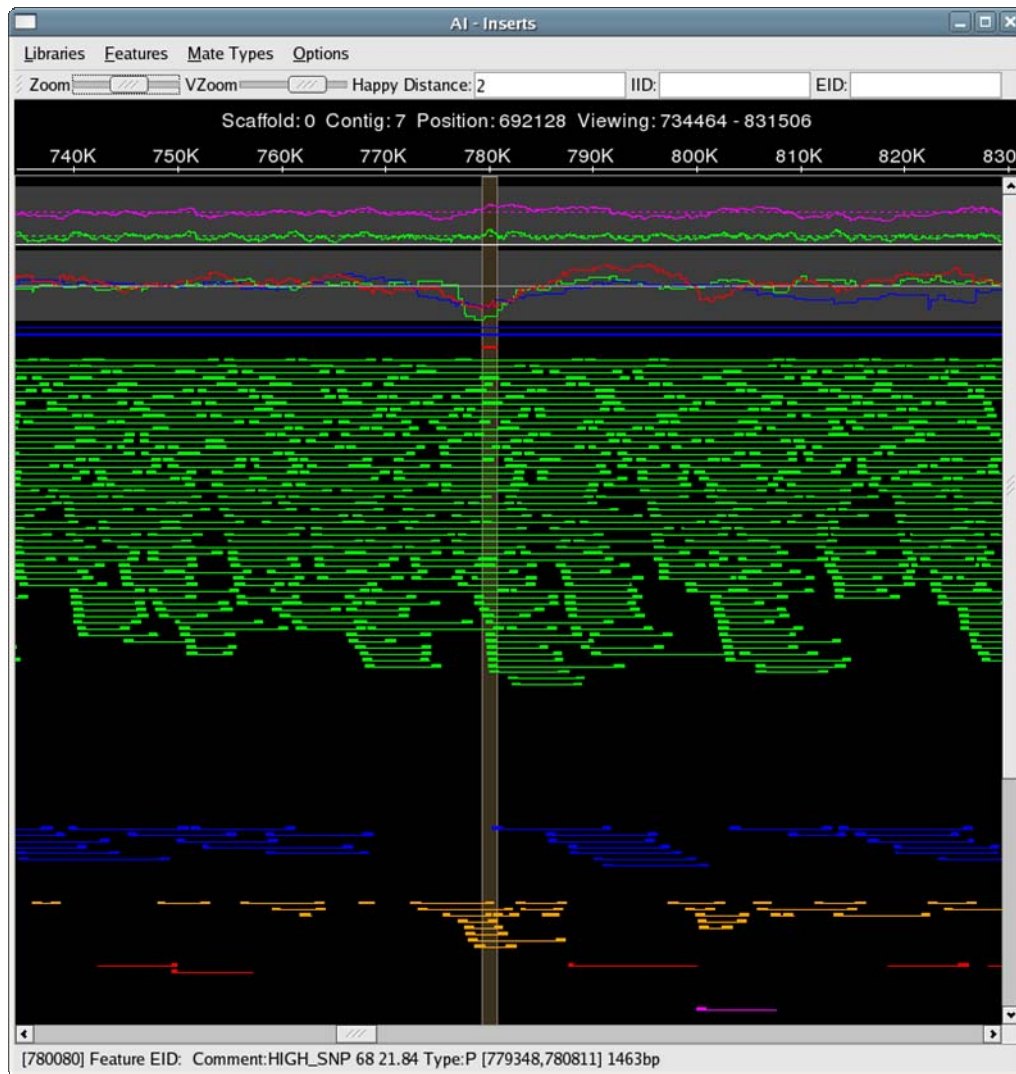
Mate Happiness

SNP Sorted Reads



Colored Rectangle indicate the positions and composition of the SNPs

Insert View



Coverage
CE Statistic

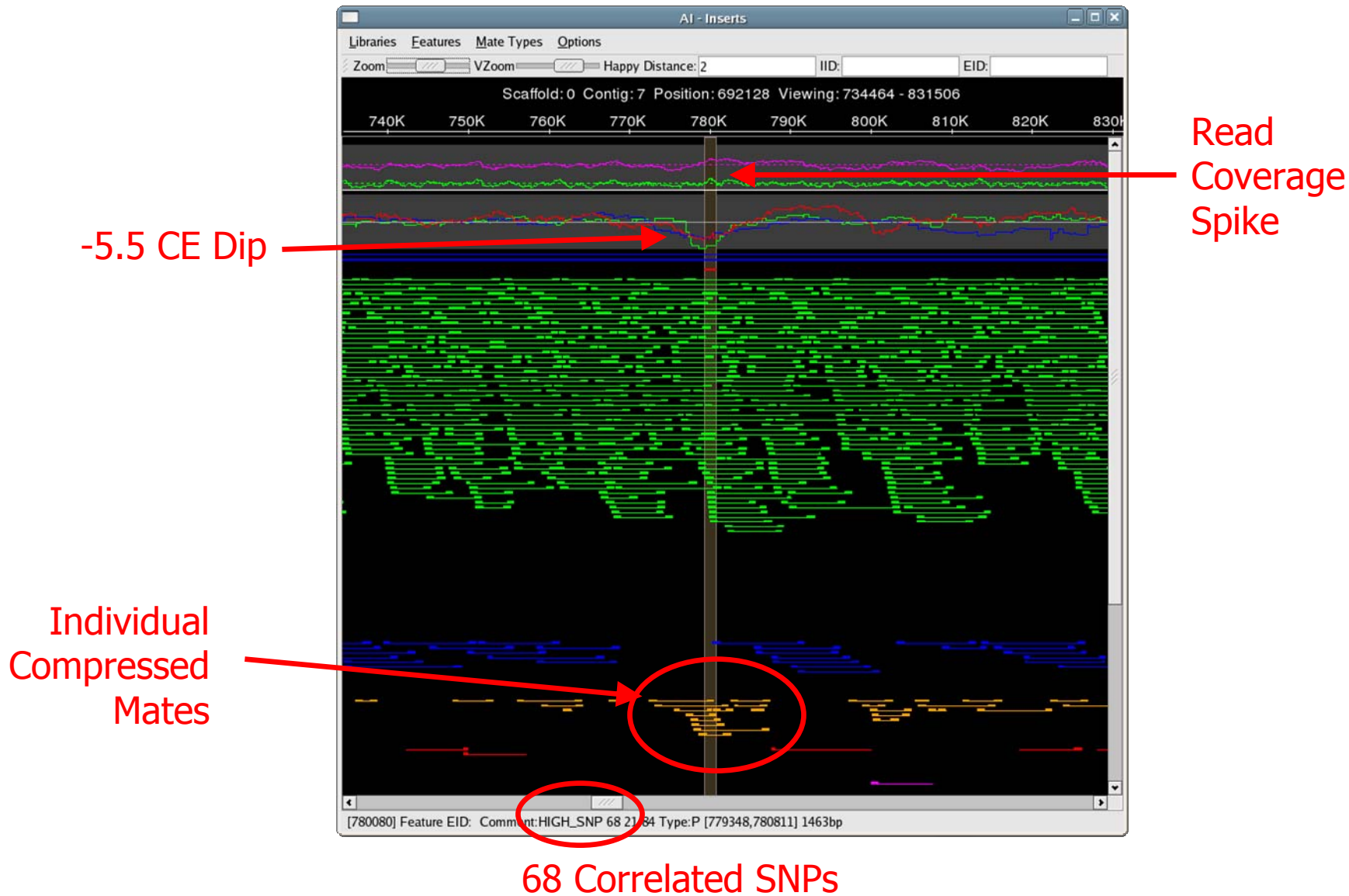
Happy

Stretched
Compressed
Misoriented

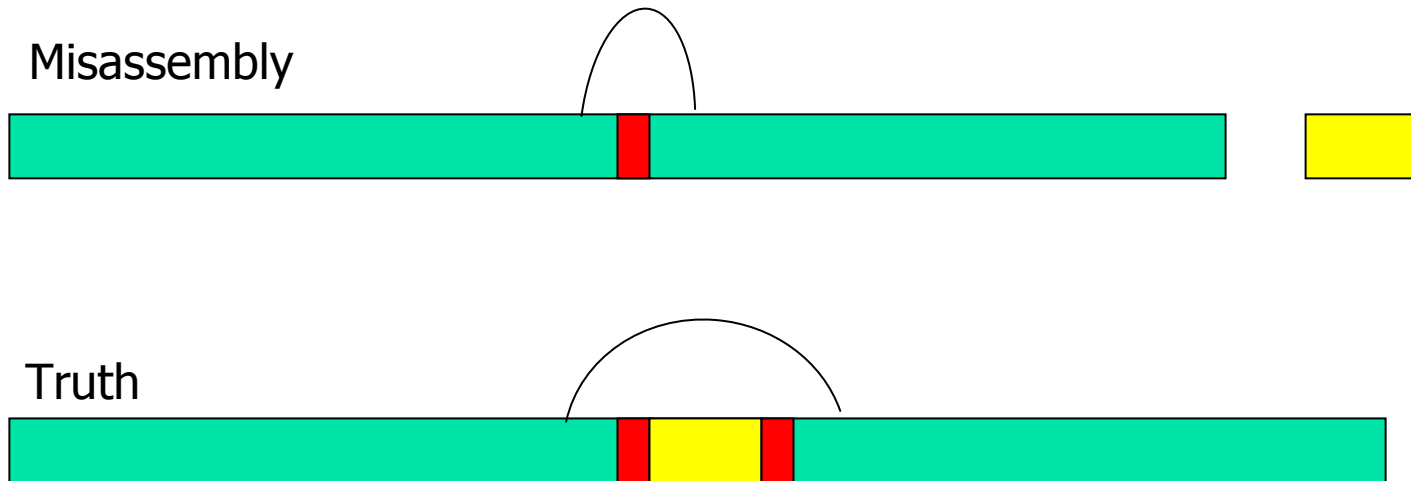
SNP Feature

Linking

Collapsed Repeat



Confirmed Misassembly



Collapsed repeat

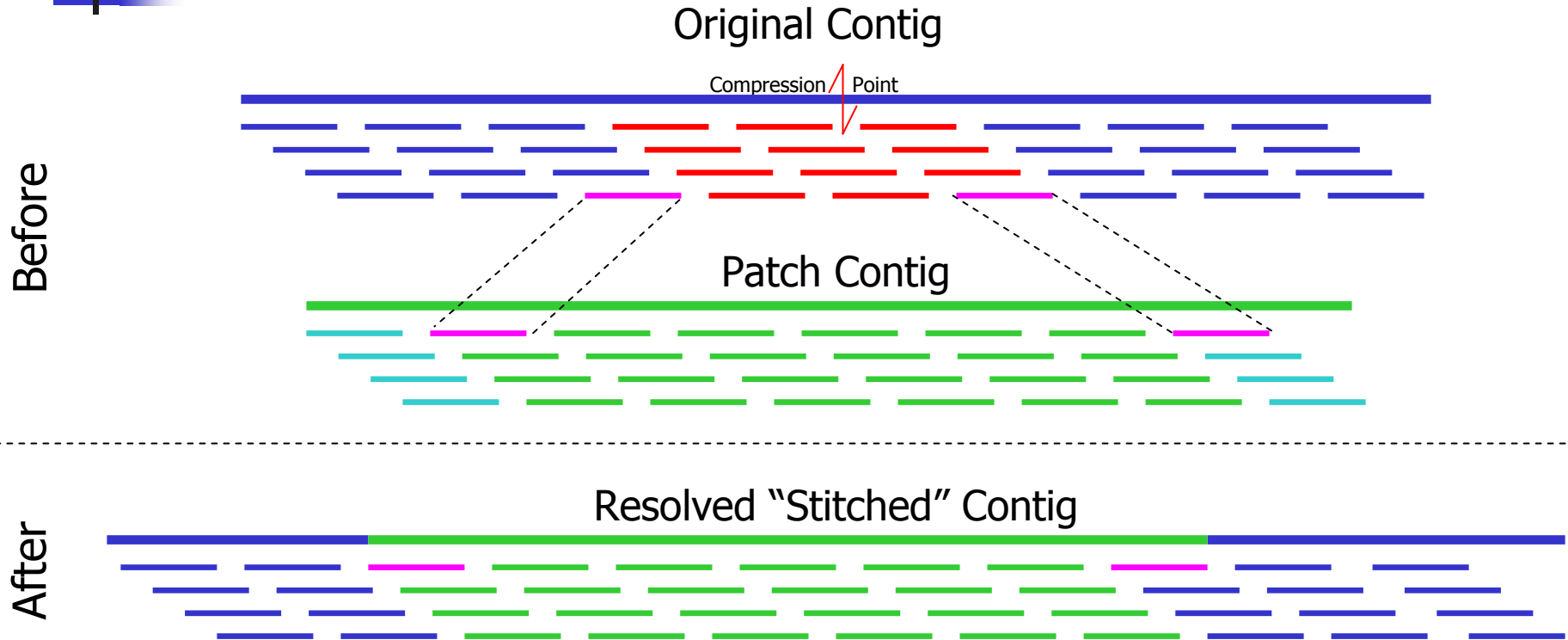
- Compressed mates (-5.5 CE Stat)
- Correlated SNPs (68 Positions within 1400bp)
- Spike in Read Coverage



Fixing collapsed repeats with AMOS

1. Select reads and mates in region of collapse.
 - AMOS: findMissingMates, select-reads
2. Reassemble those reads with stricter parameters.
 - AMOS: minimus
3. Inspect new assembly to ensure misassembly was corrected.
 - AMOS: amosvalidate, Assembly Investigator
4. Patch the collapsed region of the original assembly with corrected version.
 - AMOS: stitchContigs

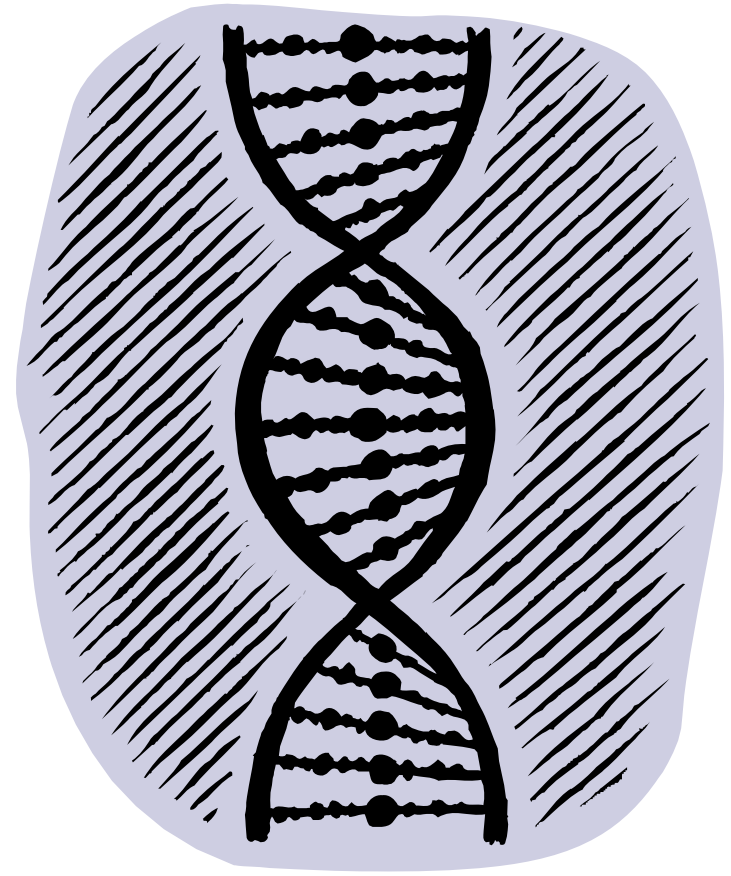
stitchContigs



- Replace the reads between the stitch reads in the original contig with corresponding region in the patch contig.
- Can also close gaps or fix contig ends

Current Research

- Misassembly signature detection
 - Read alignment breaks
 - Singleton / Missing mate analysis
 - Integrated & Dynamic Thresholds of detection
- Automated assembly improvement
 - Automatic contig patching
 - Automatic repeat separation
 - Automatic parameter tuning
- Exotic Assembly
 - Multiple haplotypes
 - Metagenomic assembly
 - 454 & Sanger Sequencing Hybrids



More Information

- Contact AMOS

- <http://amos.sourceforge.net>
- [amos-help \[at \] lists.sourceforge.net](mailto:amos-help@lists.sourceforge.net)

A

M

O

S

- AMOS Team

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