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Glomus intraradices

Initial Whole-Genome Shotgun Sequencing and Assembly Results

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DOE Joint Genome Institute

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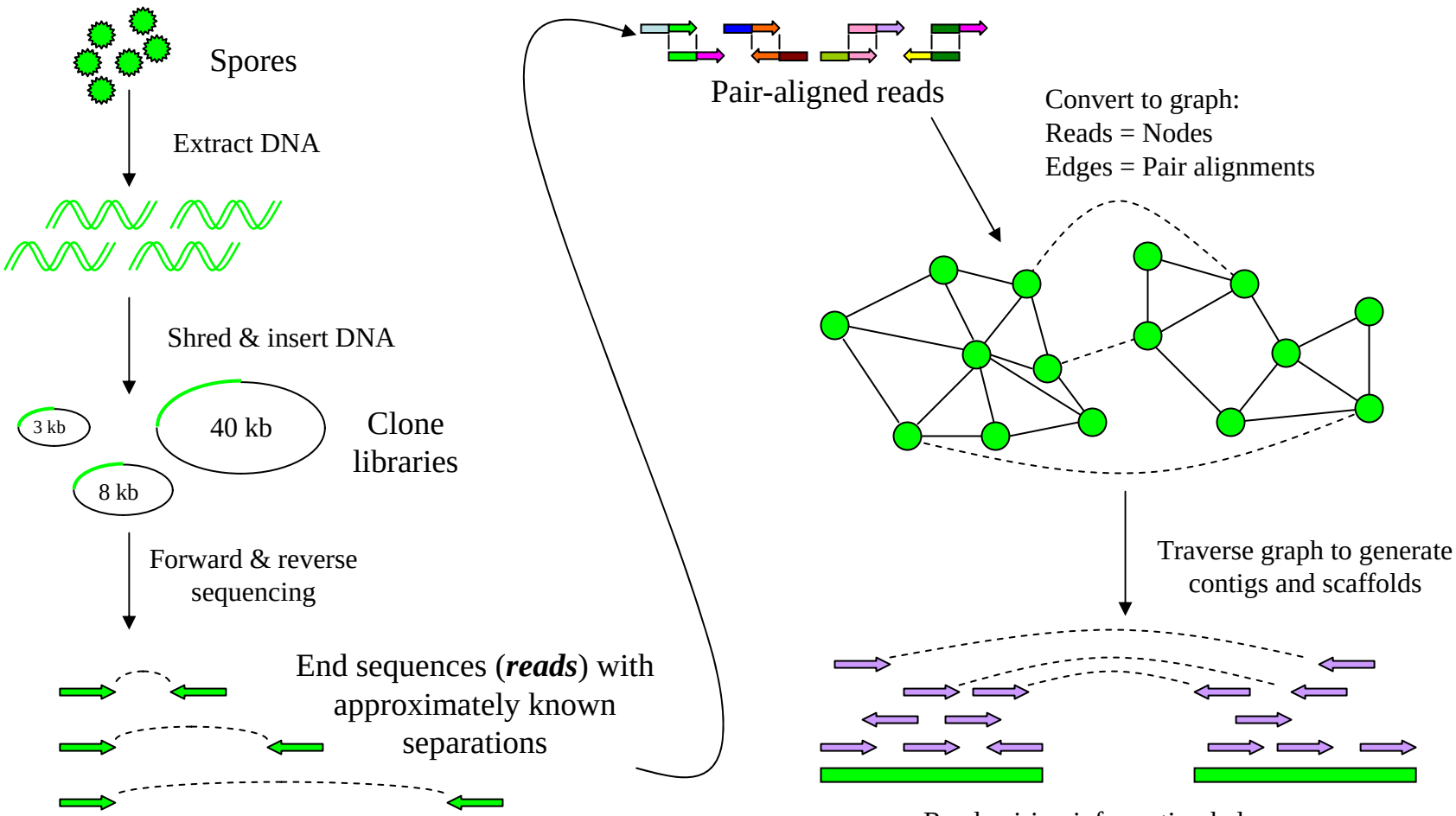
Overview

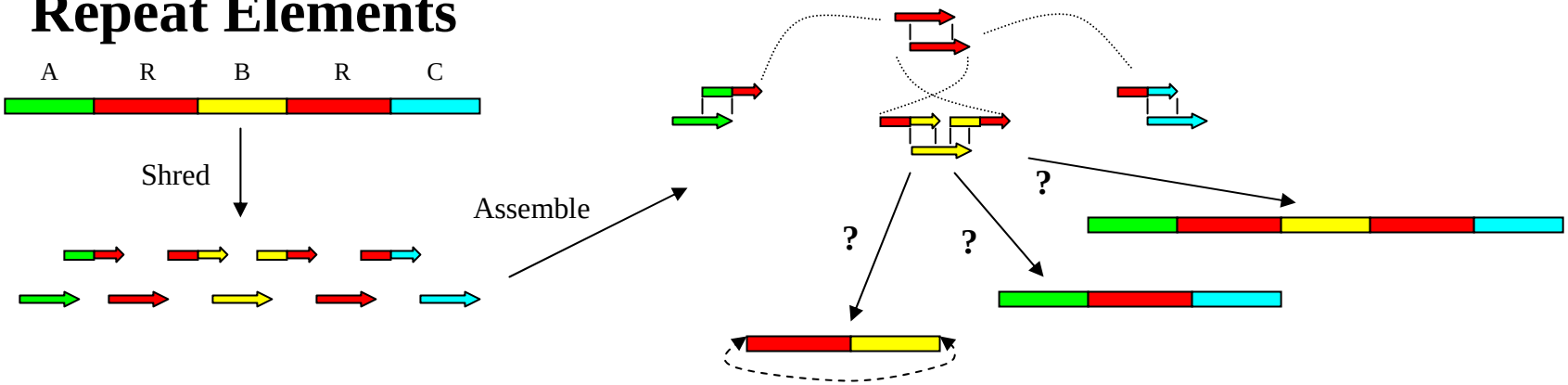
- **Review of the Whole-Genome Shotgun (WGS) assembly procedure**
- **WGS library Quality Control (QC)**
- ***G.intraradices* test assembly**
- **Polymorphism estimates**
- **Data set anomalies**
- **Where to go from here**

Outline of the Assembly Process: JAZZ, the JGI In-House Assembler

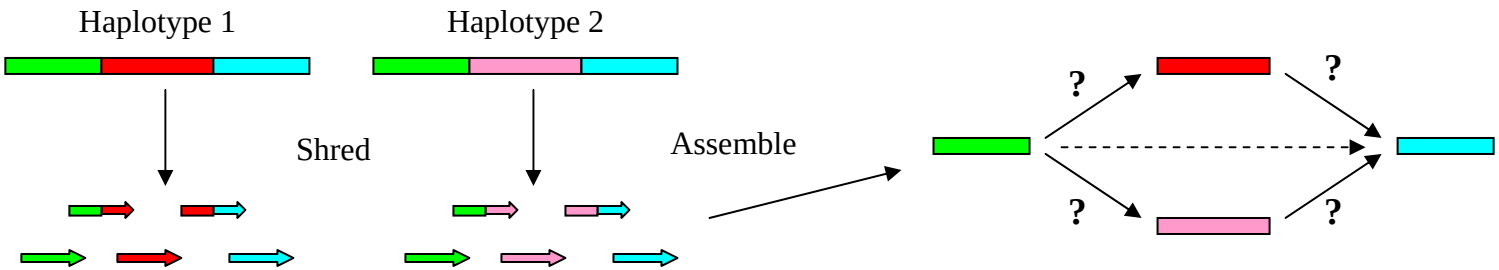


So What Could Go Wrong?

- **Repeat Elements**



- **Polymorphism**



Stricter assembly parameters can distinguish (some) repeats, but make it more likely that haplotypes will assembly separately.

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WGS Library Quality Control

- **Identification of insertless clones**
- **Trimming of vector and low quality sequence**
- **Examination of the GC content distribution**
- **BLAST analysis**

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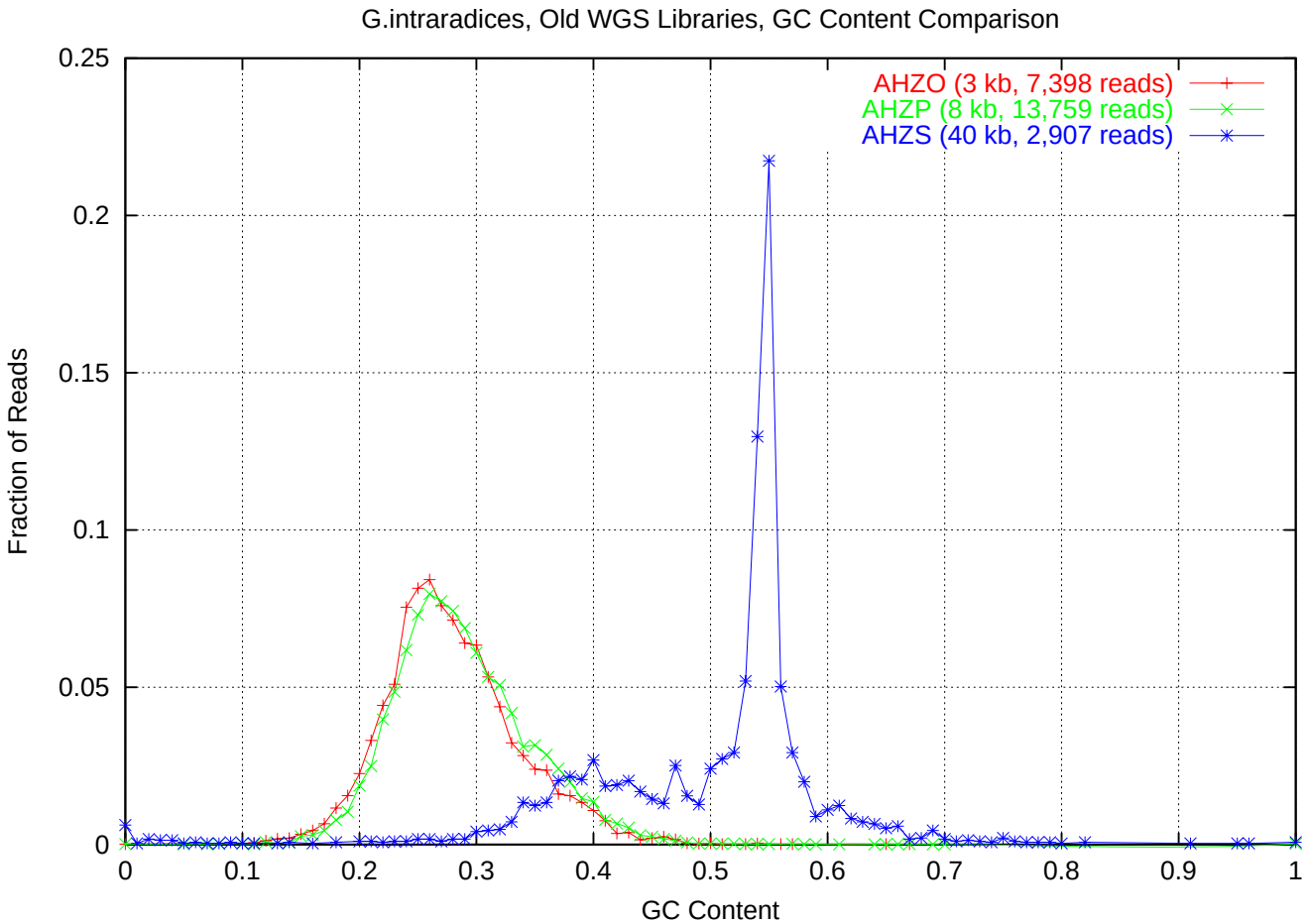
GC Content Comparison: Initial WGS Libraries



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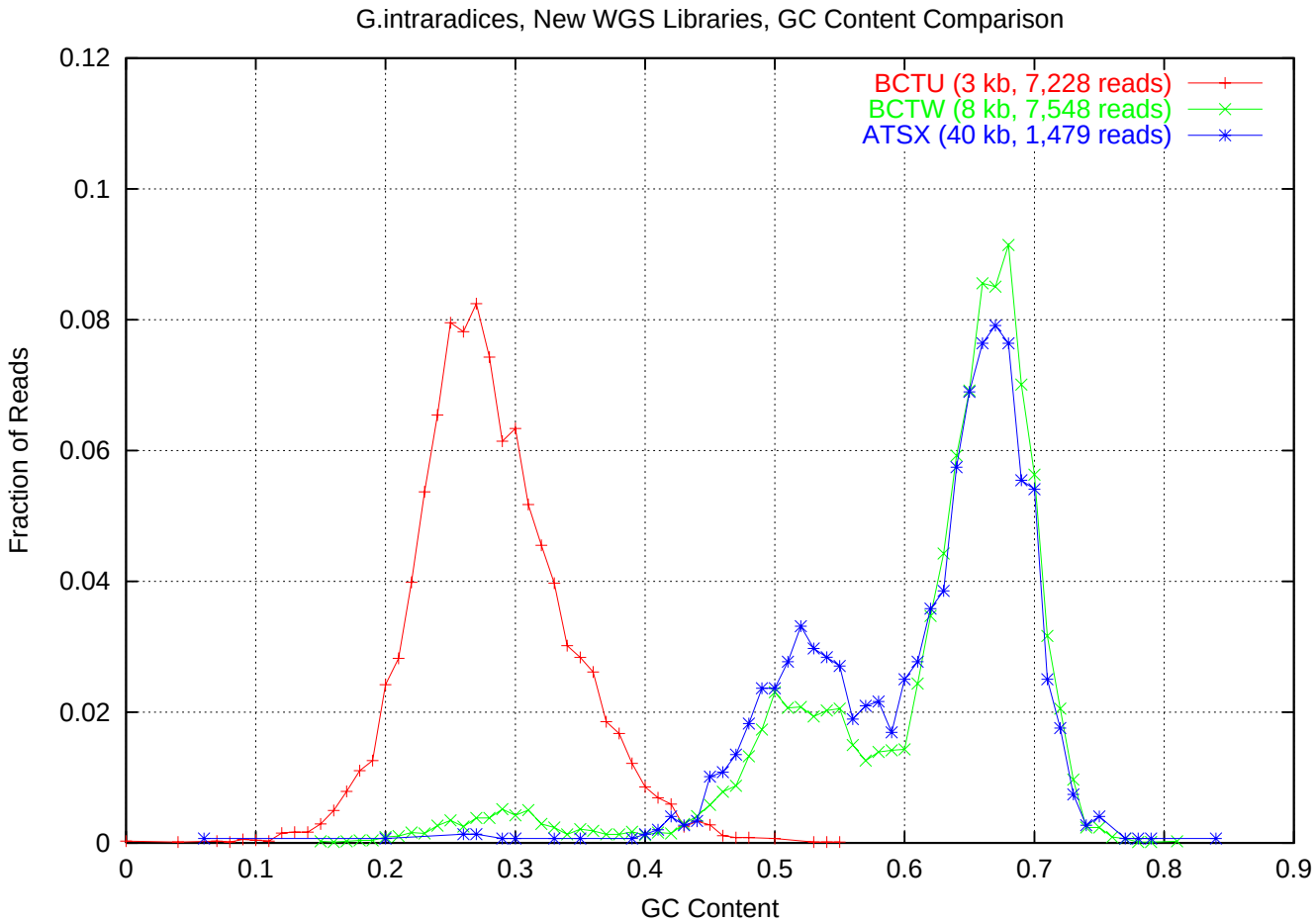
GC Content Comparison: Second WGS Libraries



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Test Assembly Specification

- **All 6 WGS libraries were included: ~28.7 MB of trimmed sequence, of which perhaps 4 – 7 MB consisted of various contaminants**
- **Genome size estimate: ~15 MB (Hijri & Sanders, 2004)**
- **Estimated depth: 1 – 1.5x; set to 1.0 for the assembly**
- **Repeats: Maximum copy number of 5 times the estimated depth (5) before they can't be used to seed an alignment**
- **Polymorphism/repeat divergence: used the default value of ~3% different for a "neutral" alignment**

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Summary of Test Assembly Results

- **615 scaffolds, containing 704 KB of scaffold sequence (582 KB contig sequence = 17.3% gap).**
- **After removing short and redundant scaffolds, 165 were left, with 318 KB of scaffold sequence (197 KB contig sequence = 38.1% gap)**
- **Filtered scaffold set was easily partitioned:**
  - **Prokaryotic contaminant: GC content > 0.60**
  - **Cloning artifacts: GC content between 0.50 and 0.60 (with one exception)**
  - **Potential *G.intraradices* scaffolds: GC content < 0.50 (with one exception)**
- **EST alignments supported the identification of the low-GC scaffolds**

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Test Assembly Statistics

Scaffold Set	Number of Scaffolds	Scaffold Sequence Total (KB)	Contig Sequence Total (KB)
Potential *G.intraradices*	126	222.5	147.1
Cloning Artifact	10	14.3	14.3
Prokaryotic Contamination	29	81.6	35.2

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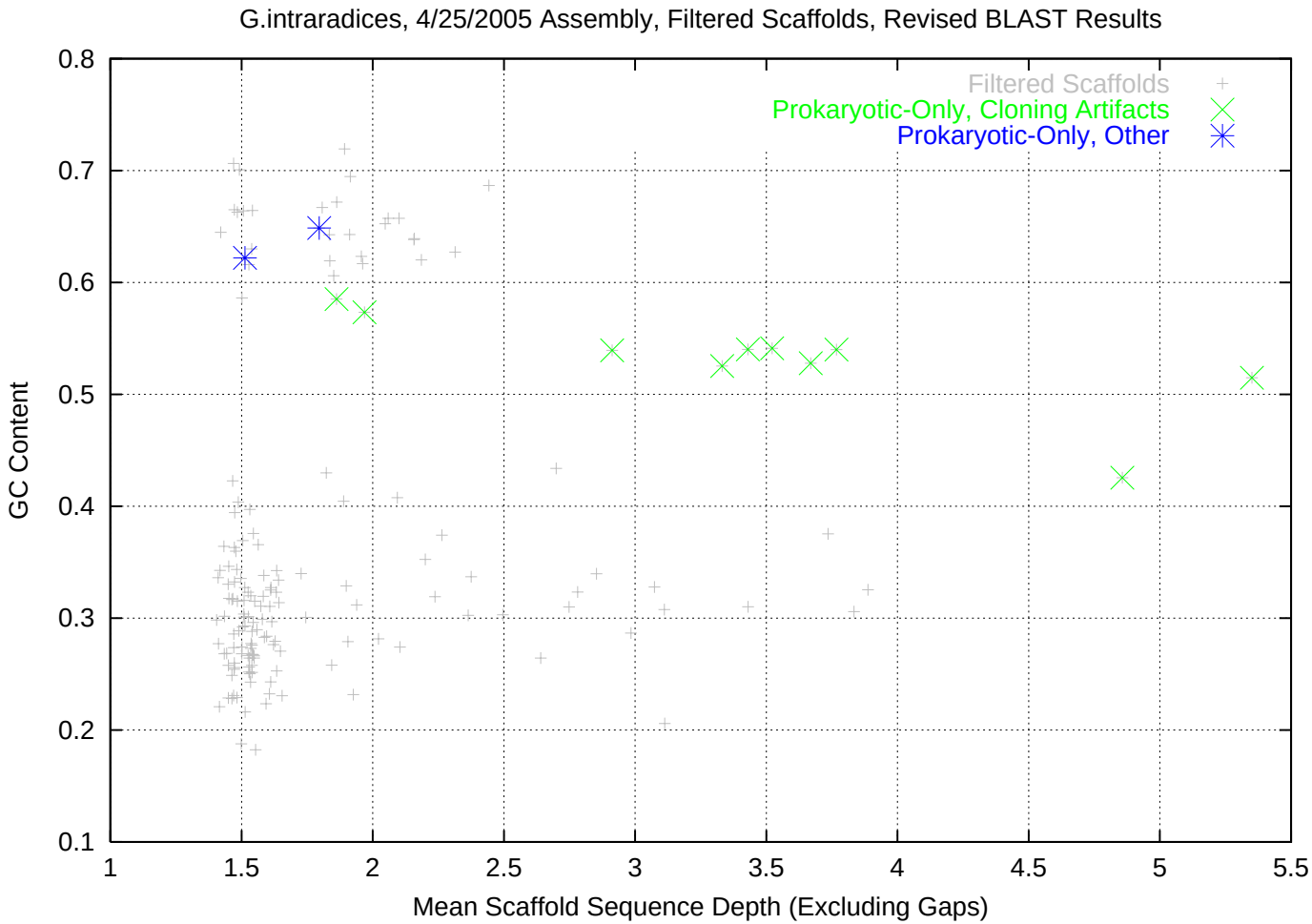
Test Assembly: BLAST Analysis



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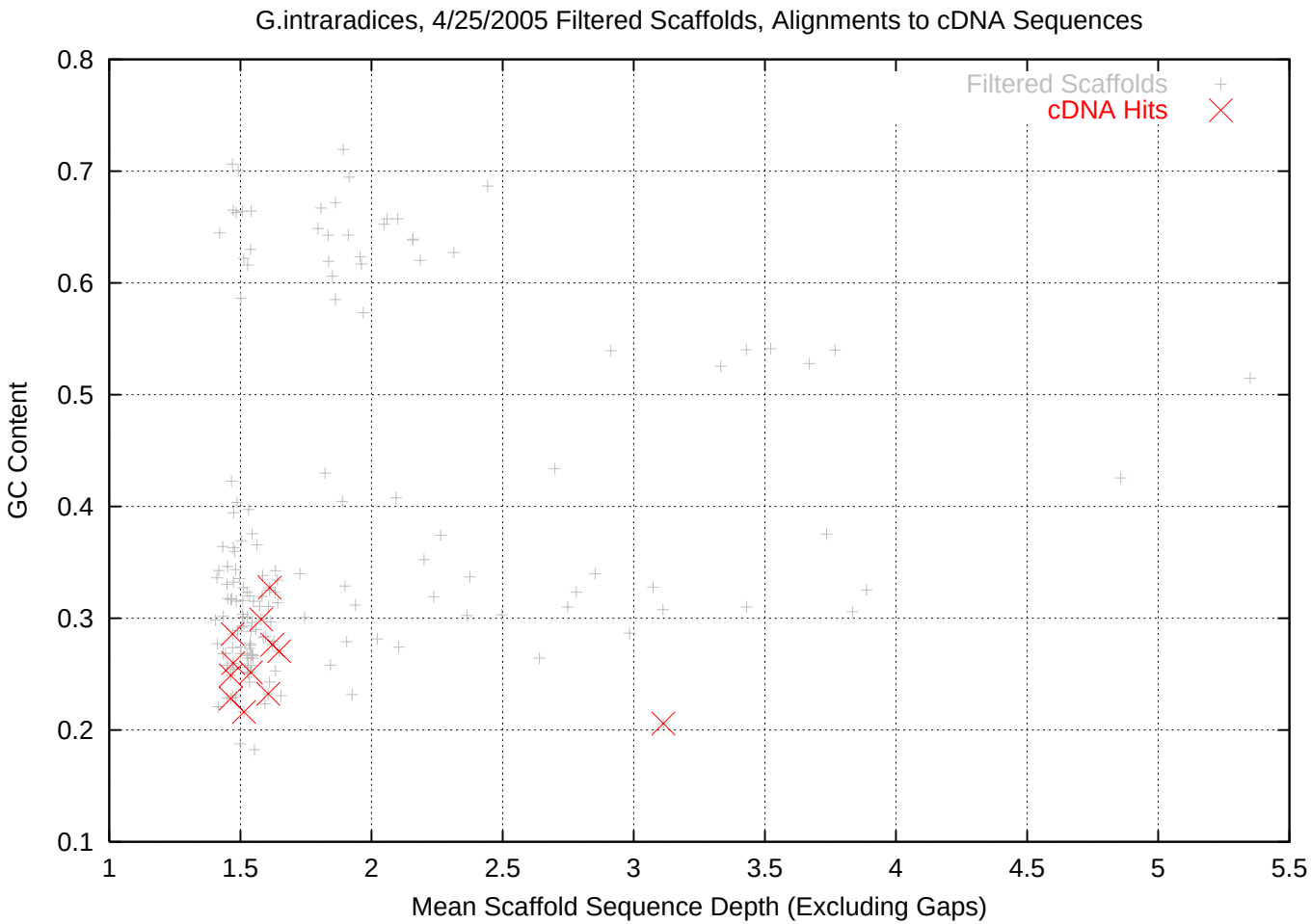
Test Assembly: EST Analysis



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Polymorphism Estimation Procedure

- **Generation of reference sequence: draft & finished subcloned fosmids**
- **Alignment of WGS & EST reads to the reference sequence**
- **Identification of "good" alignments**
  - **Reads with unique alignments to the reference fosmids**
  - **Greater than 97% of the read covered by the alignment**
- **Polymorphism calculations**
  - **% ID of the good alignments (Number of matches/alignment footprint)**
  - **Mismatch % of the good alignments (Number of mismatches/read length)**

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Subcloned Fosmid Creation

- **First batch: 15 randomly selected fosmids from the AHZS library. Consisted almost entirely of cloning artifacts.**
- **Second batch: 10 targeted fosmids from the AHZS library. Due to cloning artifacts and sequencing problems, this yielded 5 finished fosmids, containing about 181 KB of reference sequence.**
- **Third batch: 10 targeted fosmids from the ATSX library. All 10 yielded draft sequences.**

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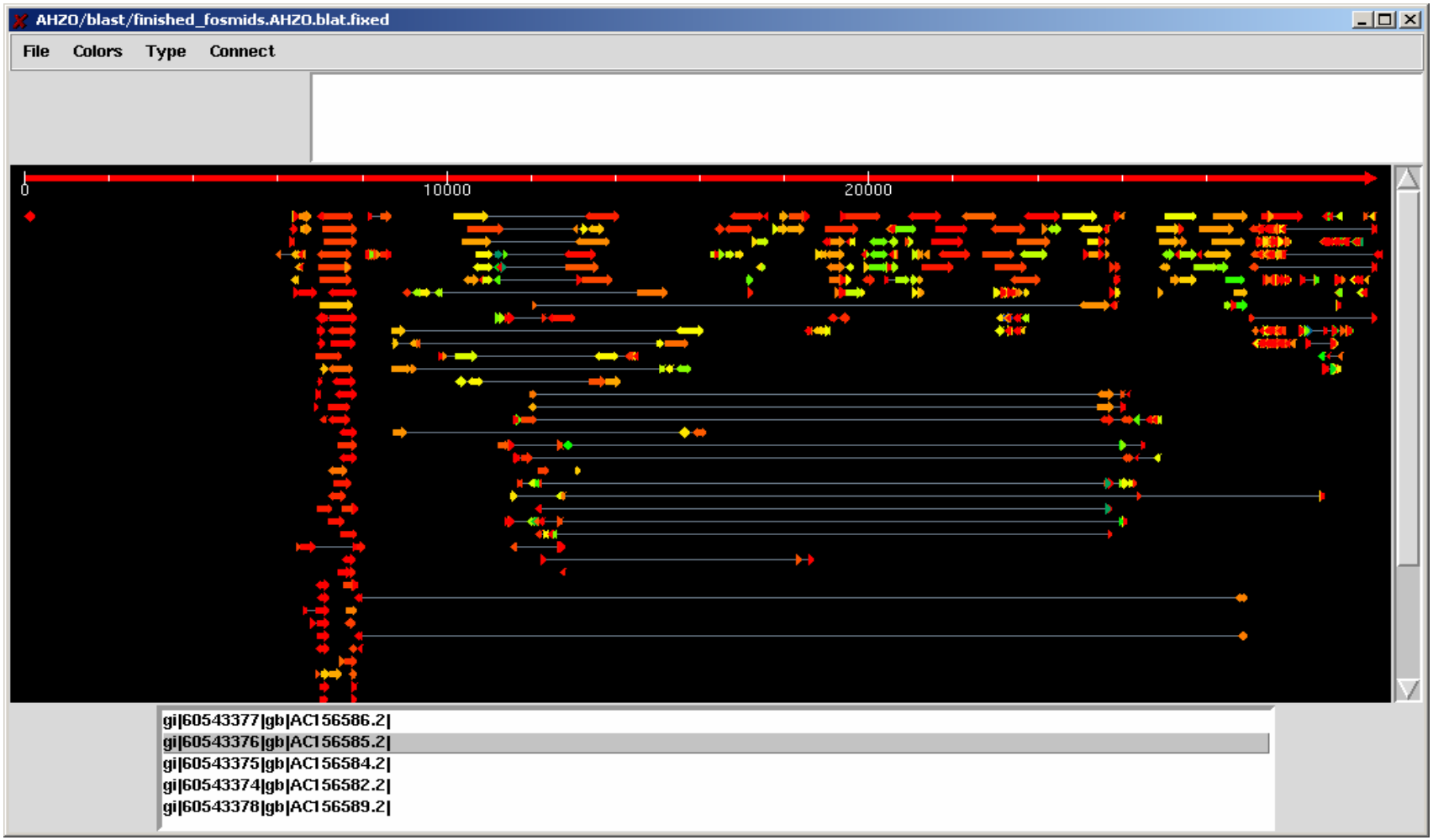
Sample Finished Fosmid WGS Alignments



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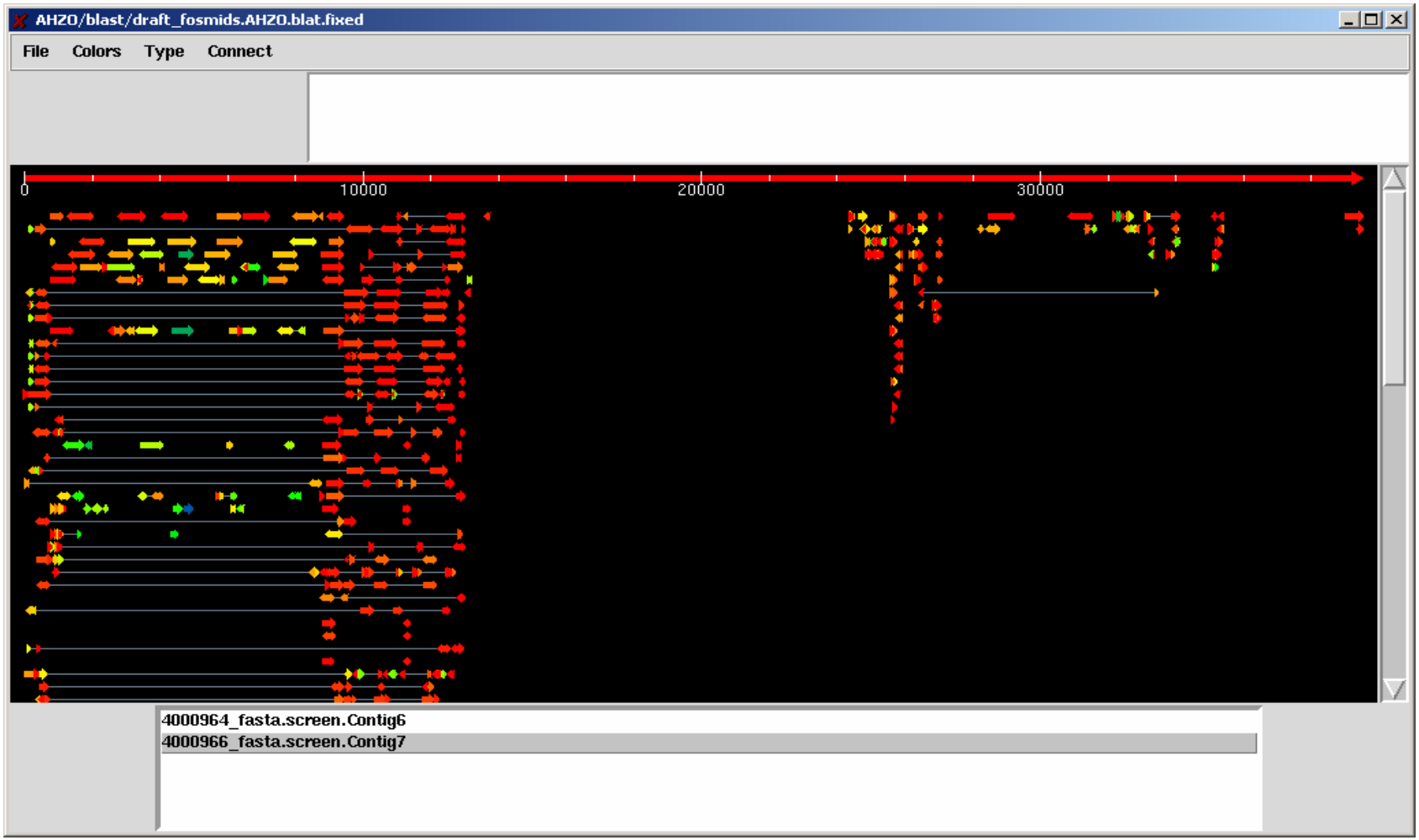
Sample Draft Fosmid WGS Alignments



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Summary of the WGS Polymorphism Results

		Polymorphism Estimate	
Data Set	**Number of Reads**	**% ID Method**	**Mismatch Method**
AHZO	52	5.1% +/- 4.9%	3.5% +/- 2.9%
AHZP	91	3.5% +/- 4.2%	2.6% +/- 2.7%
BCTU	68	3.7% +/- 6.1%	2.3% +/- 2.5%
Overall	**211**	**4.0% +/- 5.1%**	**N/A**

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Details of the WGS Polymorphism Results

- **With the % ID Method:**
  - **All three WGS libraries had their main polymorphism peak at 0%-1%.**
  - **All three WGS libraries had a secondary peak at 7%-8%.**
  - **AHZO may have had an additional secondary peak at 5%.**
- **With the Mismatch Method:**
  - **All three WGS libraries had their main polymorphism peak at 0%-1%.**
  - **AHZO and BCTU still had secondary peaks at 7% - 8%.**
  - **AHZO still had a secondary peak at 5%.**
  - **AHZP no longer had any secondary peaks, instead having its mismatch rate decline smoothly to a background value.**

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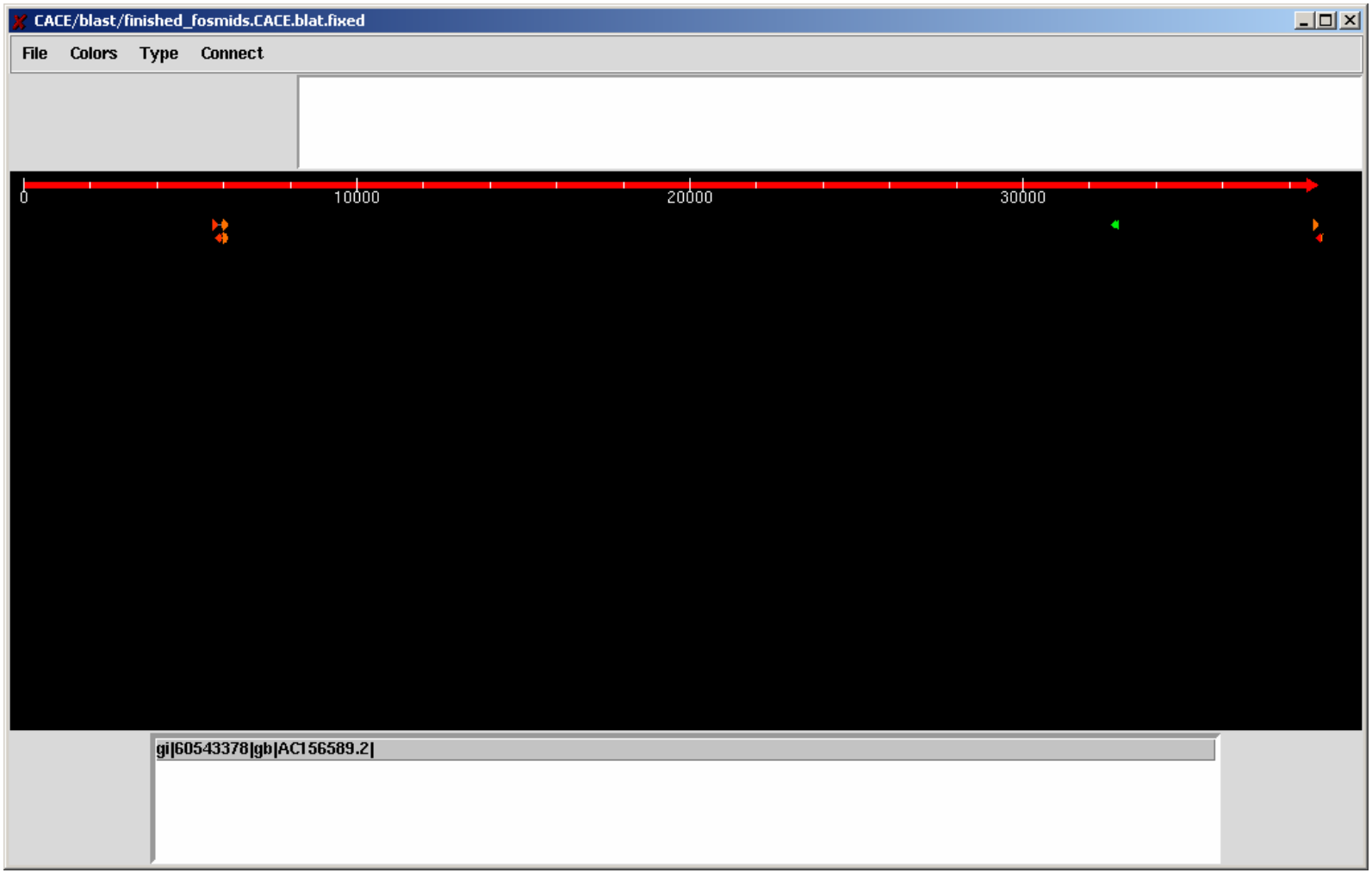
WGS Data Set Issues

- **Only produced plausible alignments to 2 of the 10 subcloned fosmids in the latest batch**
- **Very non-uniform coverage of some of the draft and finished fosmids**
- **Potential causes:**
  - **Mis-assembled or –finished fosmids**
  - **Chimeric fosmid clones**
  - **Undetected non-Glomus contamination**
  - **Library bias**
  - **Statistical artifact**

Sample Finished Fosmid EST Alignments



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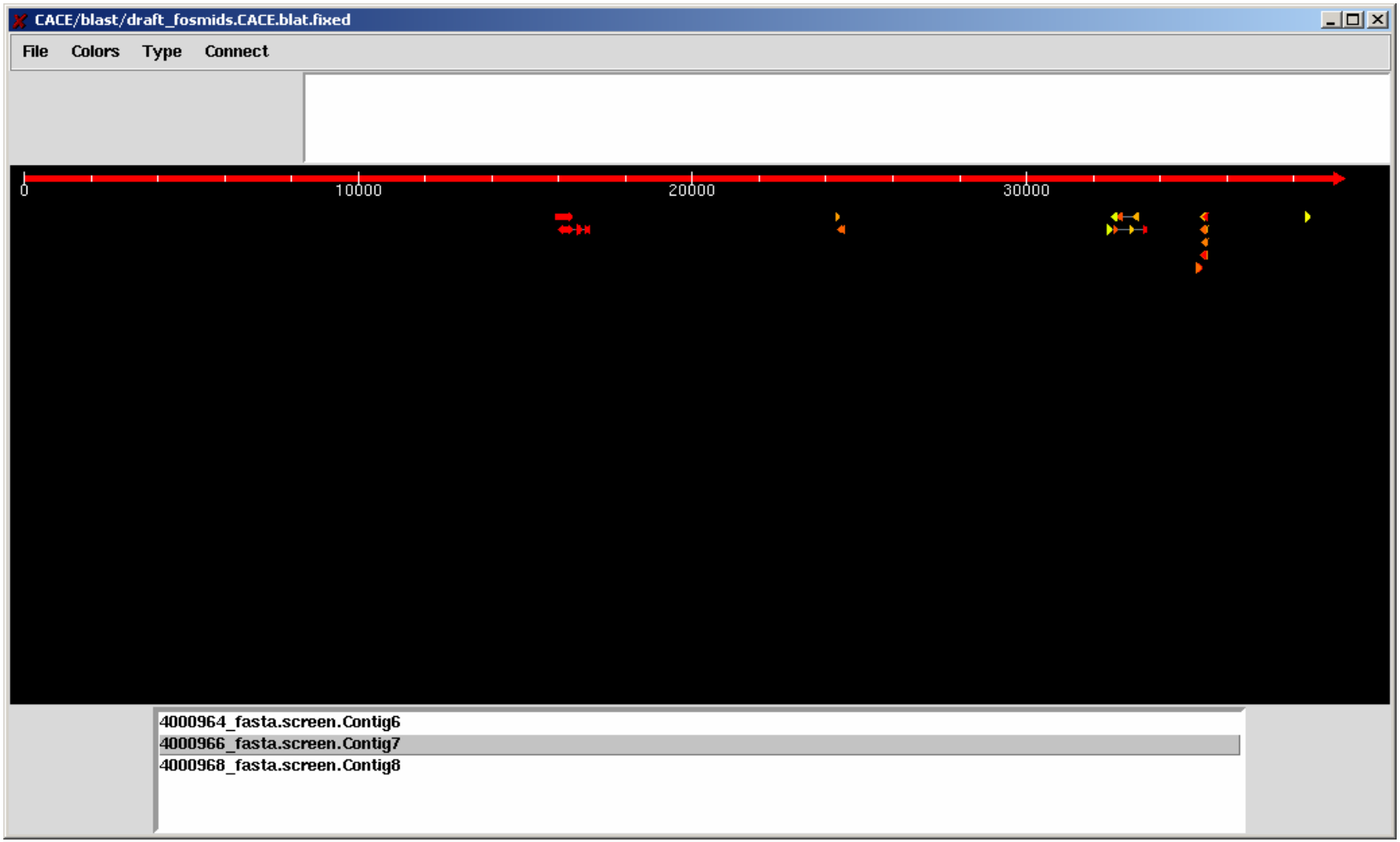
Sample Draft Fosmid EST Alignments



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EST Polymorphism Results

- **Very few EST sequences aligned to the subcloned fosmid sequences.**
  - **Only 1 of the 5 finished fosmids had any alignments**
  - **Only 2 of the 10 draft fosmids had plausible alignments**
- **The longest alignments all seemed to be missing sections of the ESTs, on the order of hundreds of bases.**
- **Due to the small number of alignments and the anomalies associated with them, it was not possible to estimate the polymorphism rate using this data set.**

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EST Data Set Issues

- **Only produced plausible alignments to 2 of the 10 subcloned fosmids in the latest batch**
- **Very small number of alignments overall**
- **Alignments that were present did not cover large portions of the ESTs**
- **Potential causes:**
  - **Mis-assembled or –finished fosmids**
  - **Chimeric fosmid clones**
  - **Undetected non-Glomus contamination**
  - **Library bias**
  - **Spurious alignments**
  - **The haplotypes actually differ greatly from each other**
  - **Statistical artifact**

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Where Do We Go From Here?

- **Investigation of the WGS and EST subcloned fosmid anomalies**
- **Creation of a third round of WGS libraries**
- **Further attempts at WGS assembly**
  - **Requires the production of a good set of WGS libraries**
  - **Analysis of the possible repeats, which is complicated by high polymorphism**
  - **Initially strict parameters to force the haplotypes to assemble separately**
  - **Distribution of polymorphic elements might require a relaxed-parameter assembly, to try to force the haplotypes to assemble together**
  - **Pre-screening of repeat elements may allow some sidestepping of the repeat/polymorphism trade-off**

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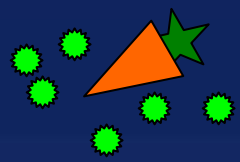
Acknowledgements



- **Joint Genome Institute**
  - **Library Creation: Eileen Dalin, Chris Detter, Paul Richardson**
  - **Production Sequencing: Tijana Glavina, Miranda Harmon-Smith, Susan Lucas**
  - **Genome Assembly: Nik Putnam, Jarrod Chapman, Isaac Ho, Dan Rokhsar**
  - **ESTs: Erika Lindquist, Peter Brokstein**
  - **Subcloned Fosmids: Jane Grimwood**
- **Peter Lammers & his group**
- **Ian Sanders & his group**

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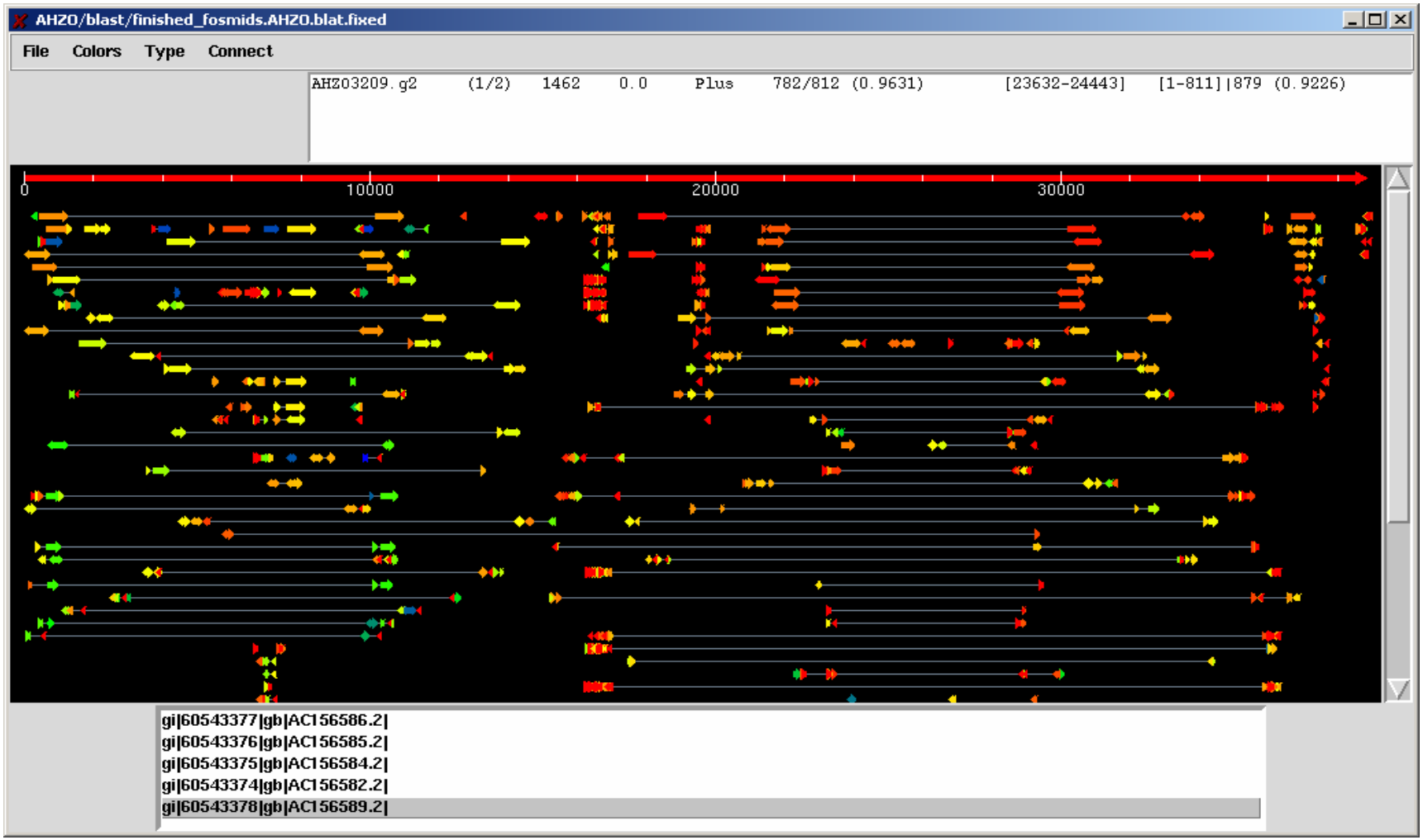
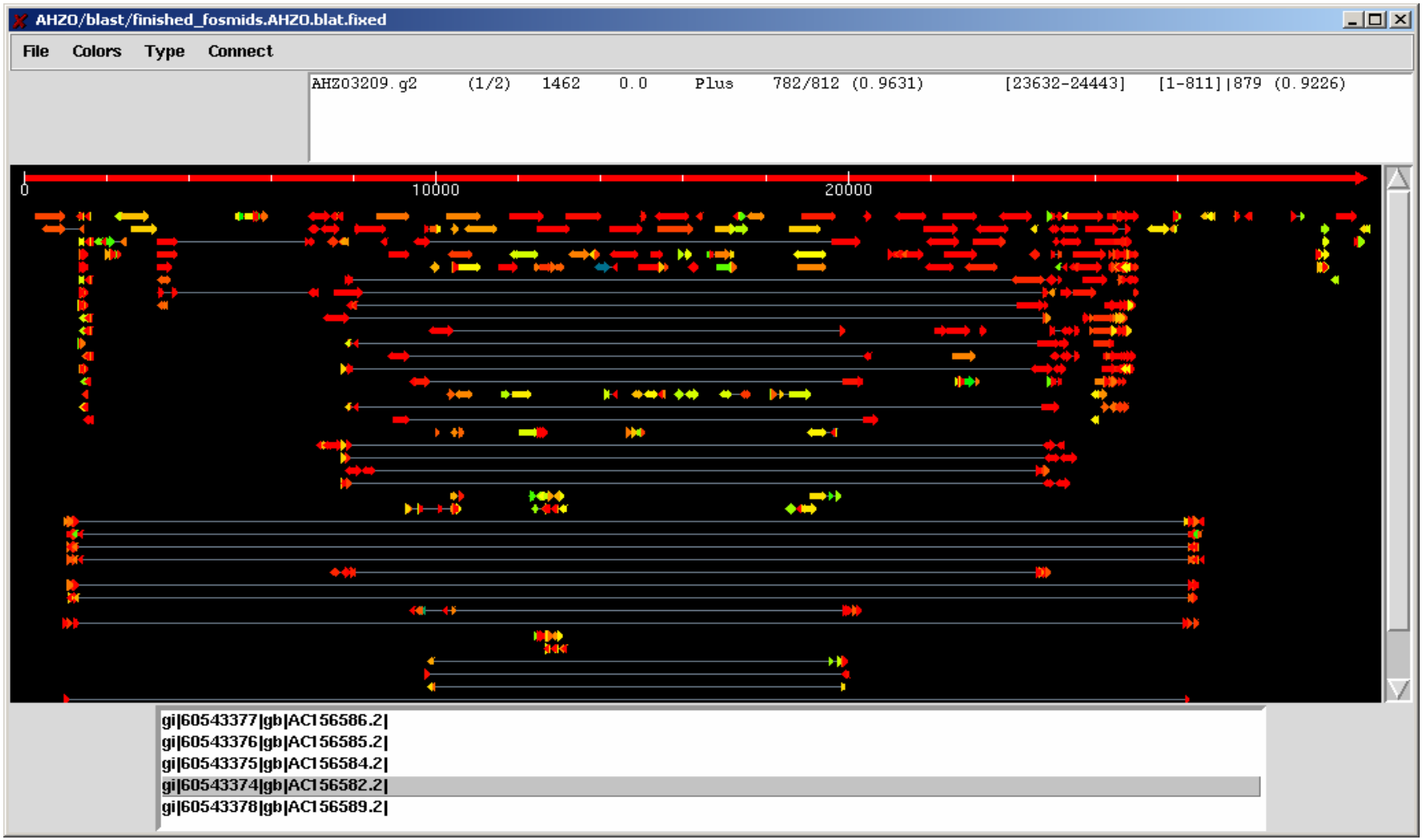
AHZO: ATYW (Finished Fosmid) Alignments



AHZO: ATYY (Finished Fosmid) Alignments



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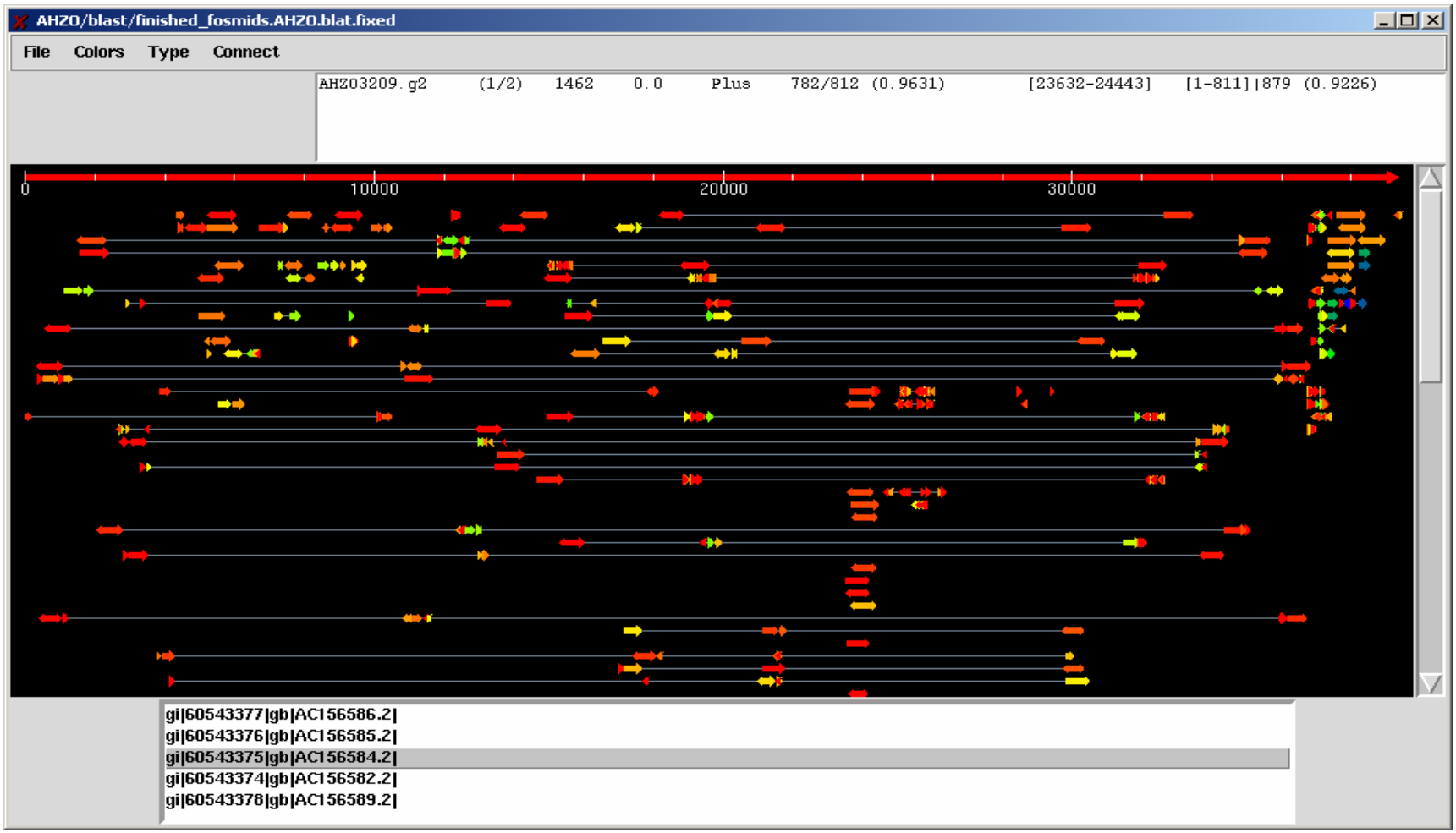
AHZO: ATYZ (Finished Fosmid) Alignments



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AHZO: ATZC (Finished Fosmid) Alignments