Applications of micro-, mega-, and meta- assembly

Michael Schatz

Nov. 3, 2011

Genome Informatics

micro-

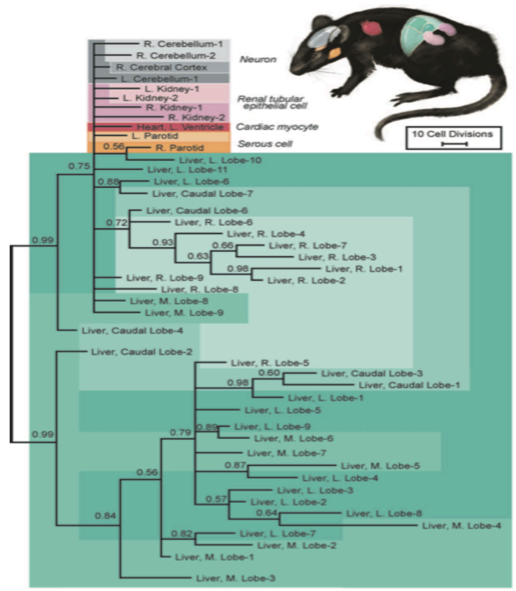
MicroSeq: high-throughput microsatellite genotyping

Mitch Bekritsky, Jennifer Troge, Dan Levy, Michael Wigler, Michael Schatz

- Highly variable simple sequence repeats
  - …GCACACACACAT… = …G(CA)$_5$T…
  - Created and mutate primarily through slippage during replication

- Genotyping with MicroSeq:
  1. Rapidly detect MS sequences
  2. Map reads using a new MS-mapper
  3. Analyze profiles in across cells & populations
     - Loss of heterozygosity, de novo mutations
     - Development of somatic & cancer cells
     - Relations across strains, across species
     - etc…

- Currently being applied to look for de novo mutations associated with autism

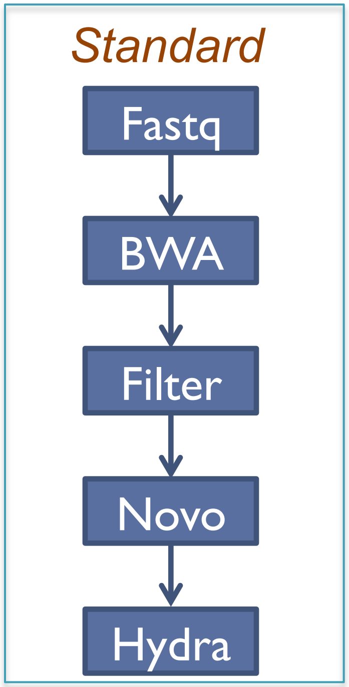


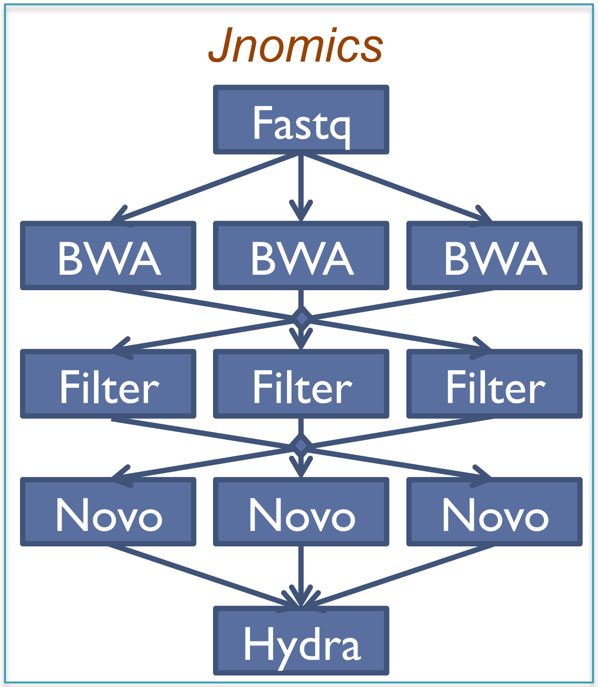
(Salipante *et al.* 2006)

mega- (x2)

Jnomics: Cloud-scale genomics

Matt Titmus, James Gurtowski, Michael Schatz





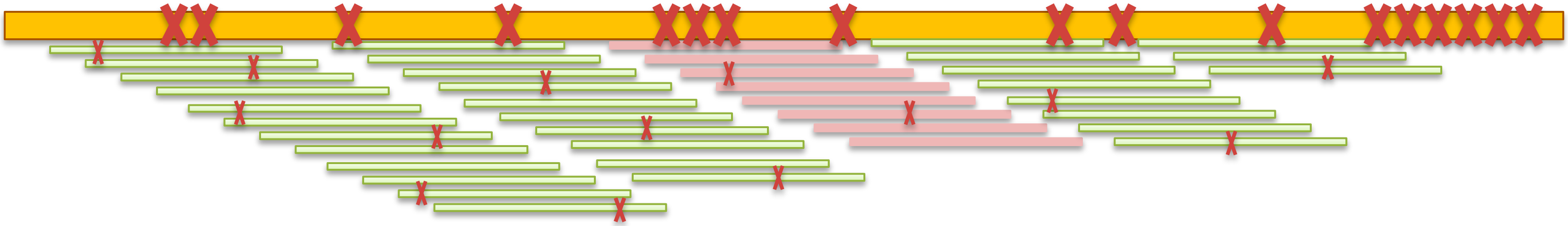
Poster 173

- Rapid parallel execution of NGS analysis pipelines
  - FASTX, BWA, Novoalign, SAMTools, Hydra
- Seamless read/write of common formats
  - BAM, SAM, BED, fastq, fasta
  - Sorting, merging, filtering, selection, etc

PacBio Error Correction & Assembly

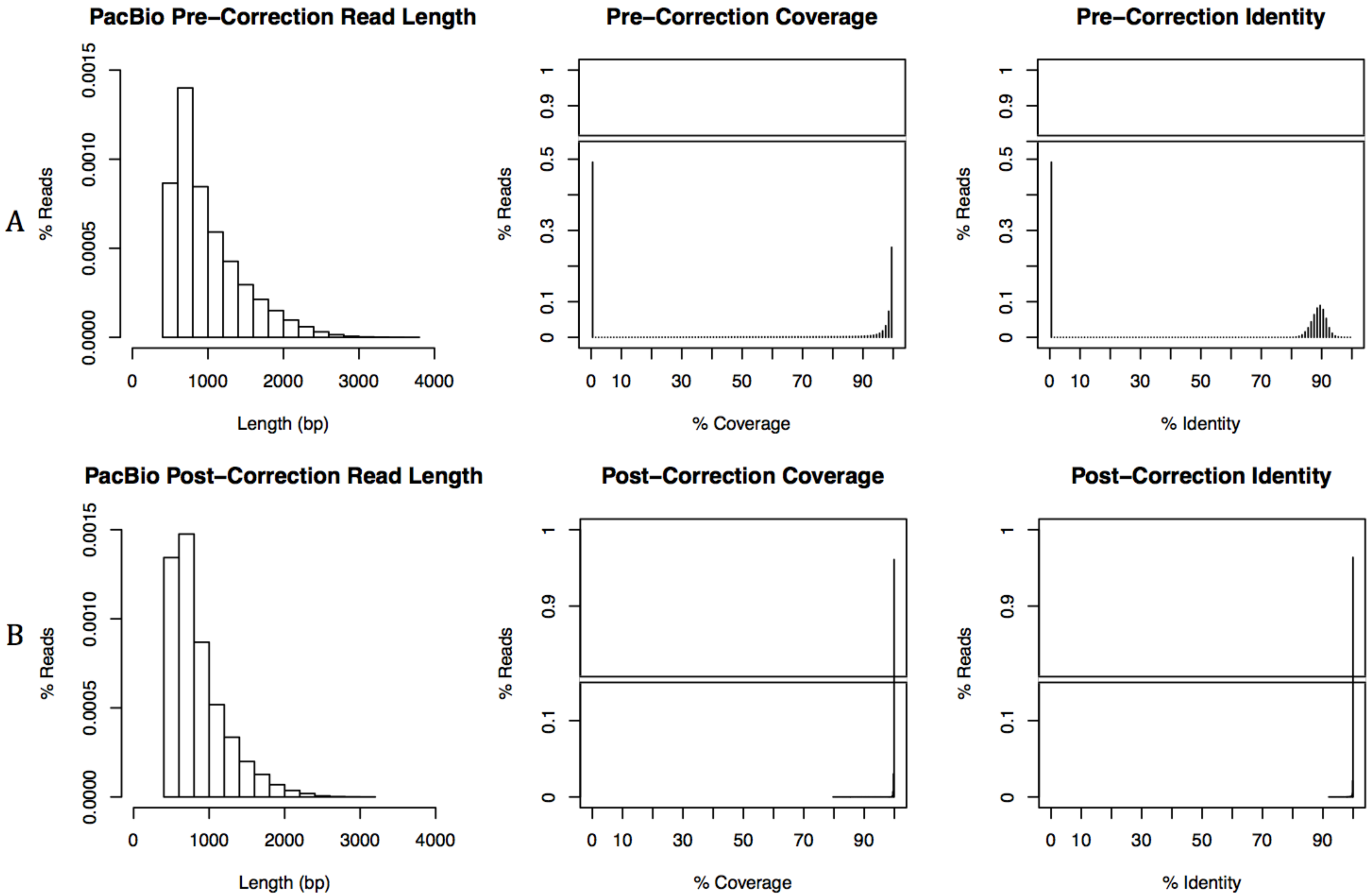
http://wgs-assembler.sf.net

1. Correction Pipeline
   1. Map short reads (SR) to long reads (LR)
   2. Trim LRs at coverage gaps
   3. Compute consensus for each LR
2. Co-assemble corrected LRs and SRs
   – Celera Assembler enhanced to support 32 Kbp reads
3. Assemblies substantially improve with longer reads



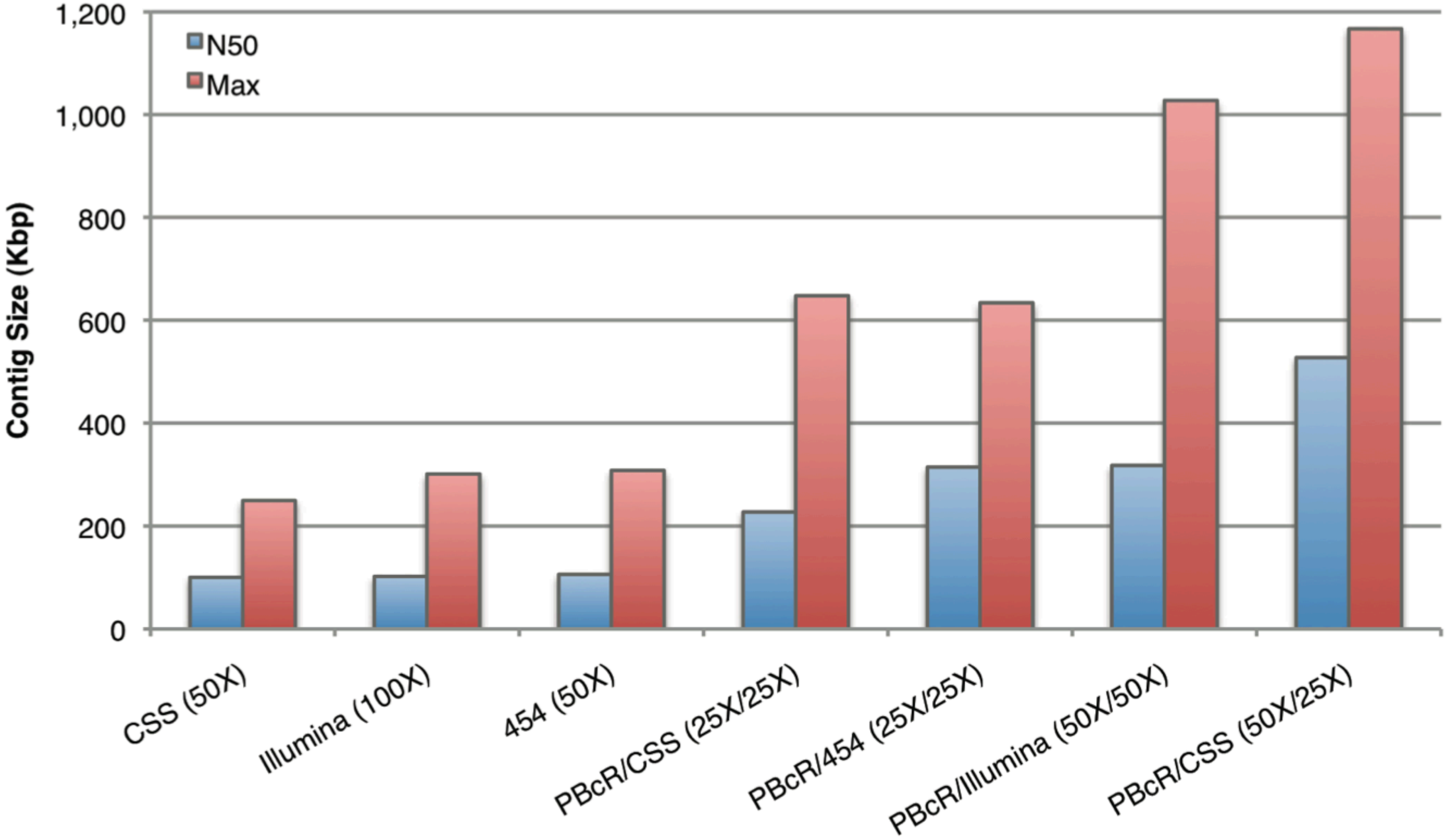
Hybrid error correction and de novo assembly of single-molecule sequencing reads.
Koren, S, Schatz, MC, Walenz, BP, Martin, J, Howard, J, Ganapathy, G, Wang, Z, Rasko, DA, McCombie, WR, Jarvis, ED, Phillippy, AM. (2011) *Under Review*

Error Correction Results



Correction results of 20x PacBio coverage of E. coli K12 corrected using 50x Illumina

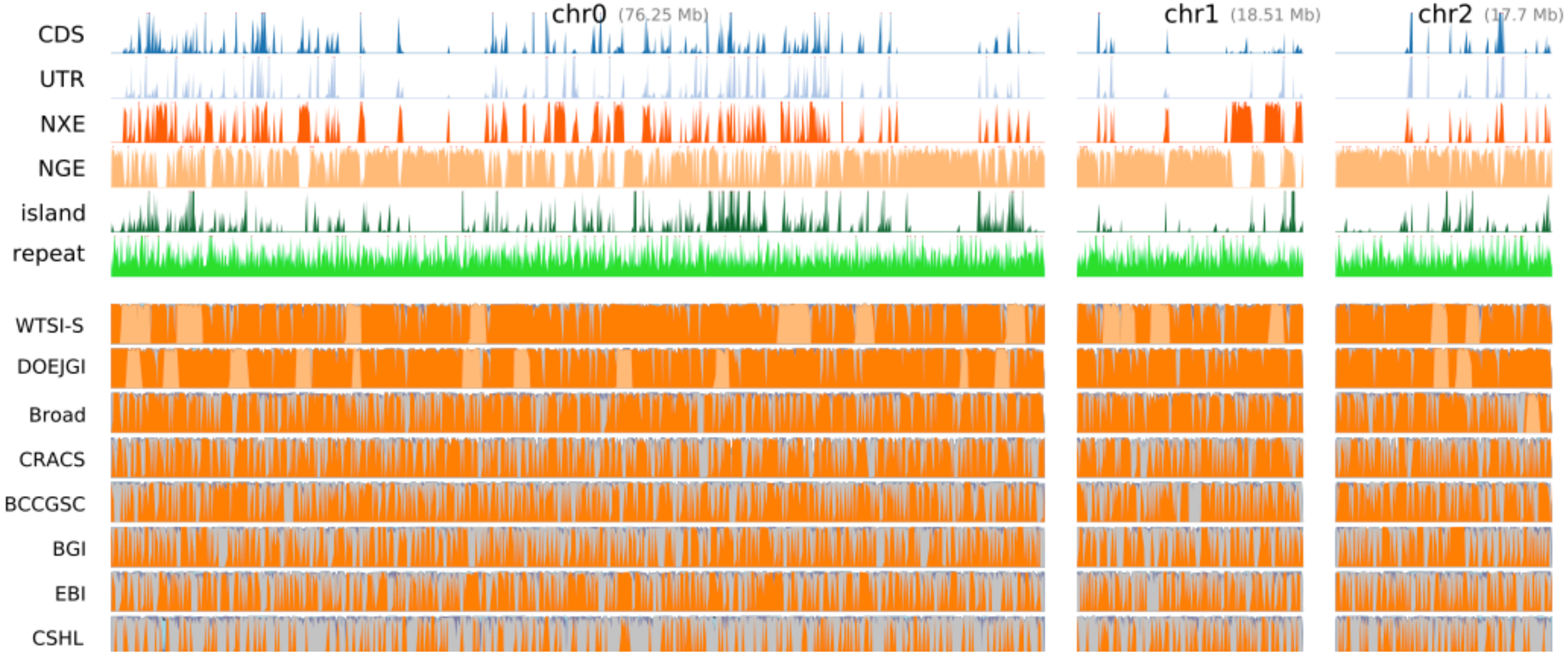
Assembly Results



SMRT-hybrid assembly results of 50x PacBio corrected coverage of E. coli K12
Long reads lead to ***contigs*** over 1Mbp

meta-

Assemblathon 1



- Assembly competition with a known reference genome enables base-by-base comparison to the truth
  - But evaluating an assembly in absence of a reference is difficult
  - Once we identify differences, what can we do about them?

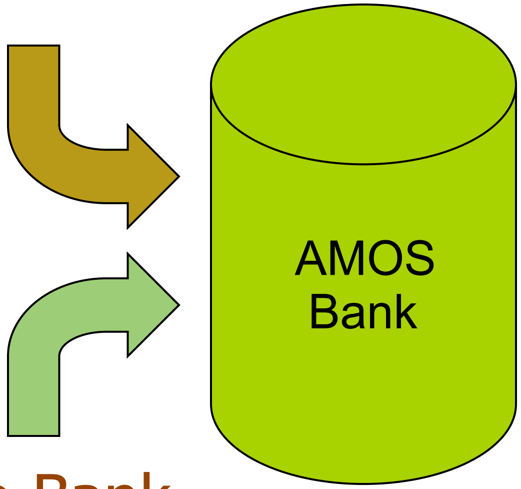
Forensics Pipeline

Computationally scan an assembly for mis-assemblies.

- Data inconsistencies are indicators for mis-assembly
- Some inconsistencies are merely statistical variations

AMOSvalidate

1. Load Assembly Data into Bank
2. Analyze Mate Pairs & Libraries
3. Analyze Depth of Coverage
4. Analyze Read Alignments
5. Analyze Read Breakpoints
6. Load Mis-assembly Signatures into Bank

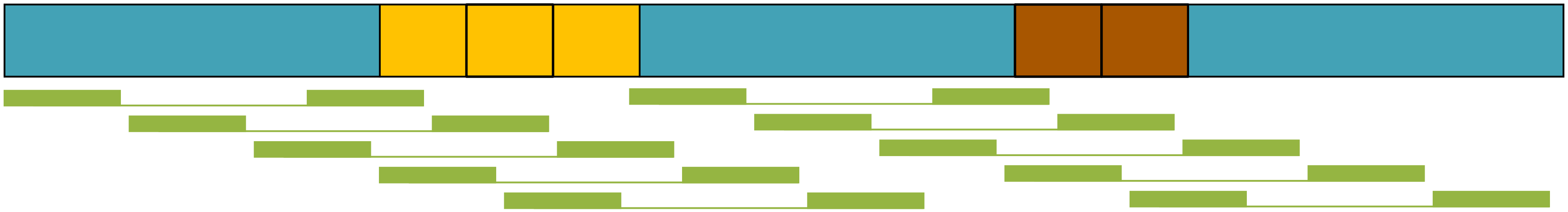


Genome Assembly forensics: finding the elusive mis-assembly.
Phillippy, AM, Schatz, MC, Pop, M. (2008) Genome Biology 9:R55.

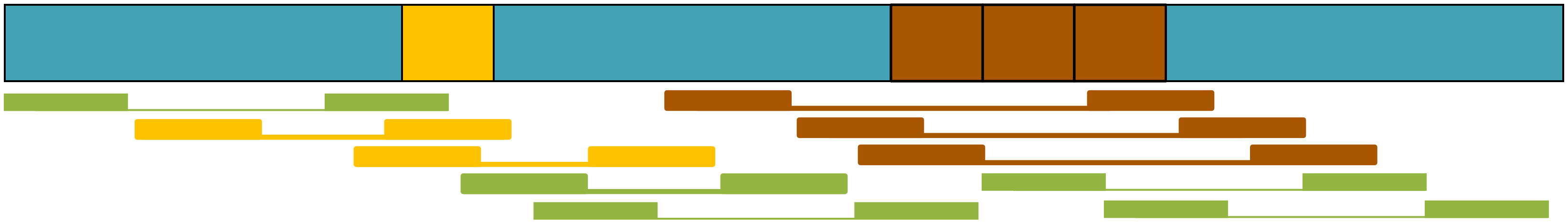
Forensics

Mate Evaluation

- Correct: mates have expected orientation and separation



- Mis-assembled: mates have incorrect orientation and separation



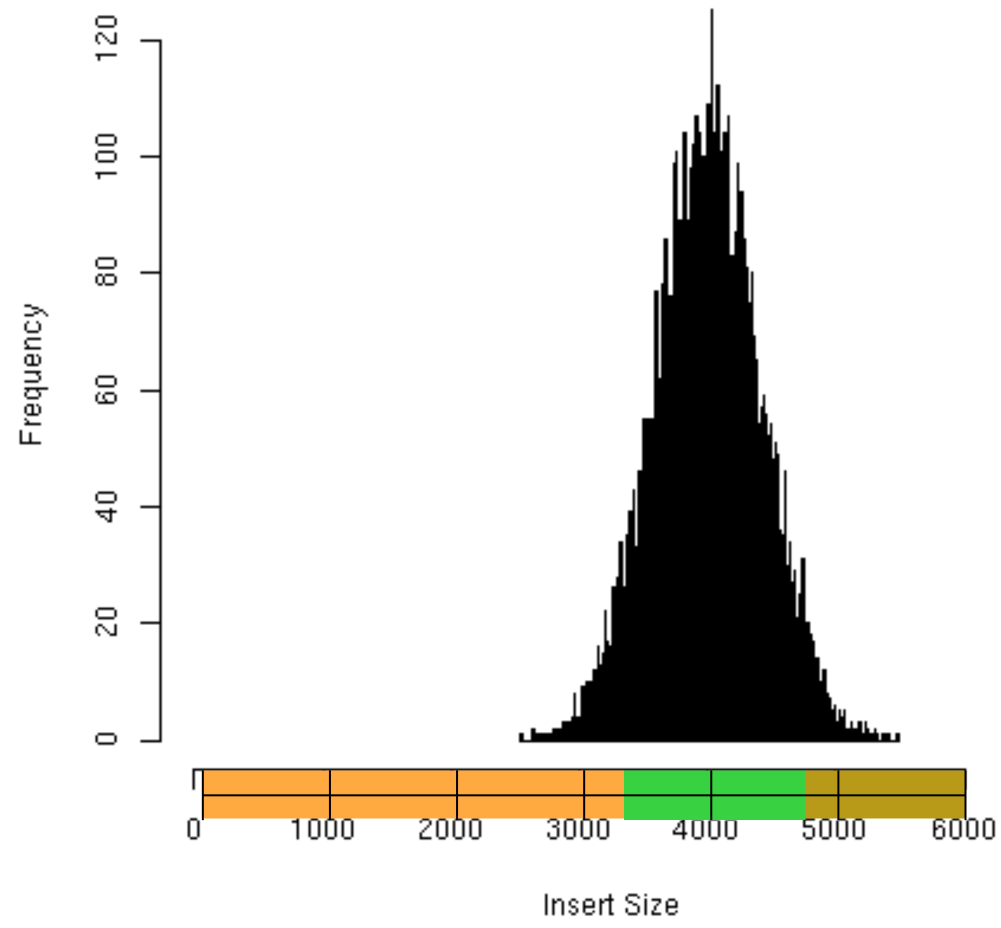
- Slightly compressed/expanded mates are expected because mates are sampled from a distribution of fragments

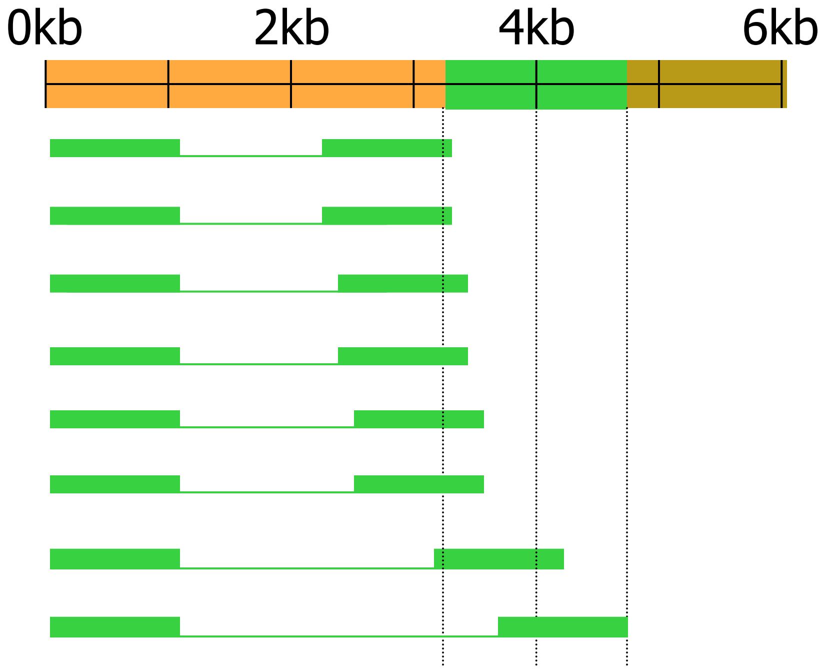
Forensics

Hidden Compression

Library size distribution

Mean: 4000, SD: 400





8 inserts: 3.2 kb-4.8kb

Local Mean: 3488

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

C/E Stat ≤ -3.0 indicates Compression

Assemblathon 2: Metassembly

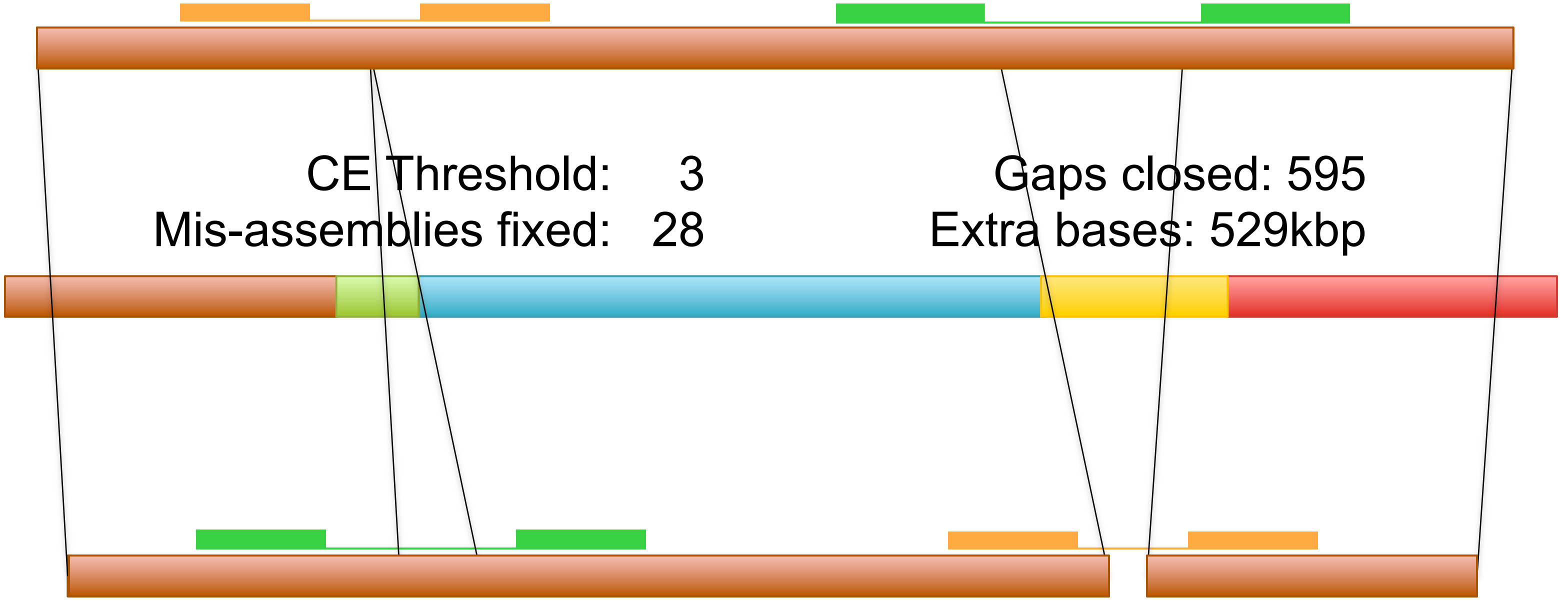
Paul Baranay, Scott Emrich, Michael Schatz

Poster 30

ALLPATHS-LG

Scaffold N50: 3,710,017
#>1000: 2,791

Contig N50: 20,183
#>1000: 68,591



CE Threshold: 3
Mis-assemblies fixed: 28

Gaps closed: 595
Extra bases: 529kbp

SOAPdenovo
+ FLASH
+ Quake
+ AMOS

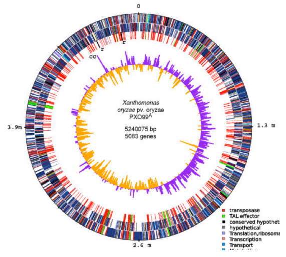
Scaffold N50: 285,413
#>1000: 29,119

Contig N50: 1,607
#>1000: 218,643

Inspired by Zimin *et al*. (2007) *Assembly Reconciliation*. Bioinformatics. 42(1) 42-45

Summary

- Assembly is moving to increasingly more complex and more diverse data types and organisms
  - PacBio error correction is my 3rd iteration of this problem
  - Assembly is useful in many different contexts, requires specialization and tuning
- There is a fundamental tension between connectivity and correctness
  - N50 is useful for evaluating connectivity but says nothing about correctness
  - CE can measure correctness at "gene-length" scale
- Metassembly is very promising for advancing assembly
  - Allows one to construct a consensus superior to the individual submissions
  - Enables one to select a locally optimal threshold



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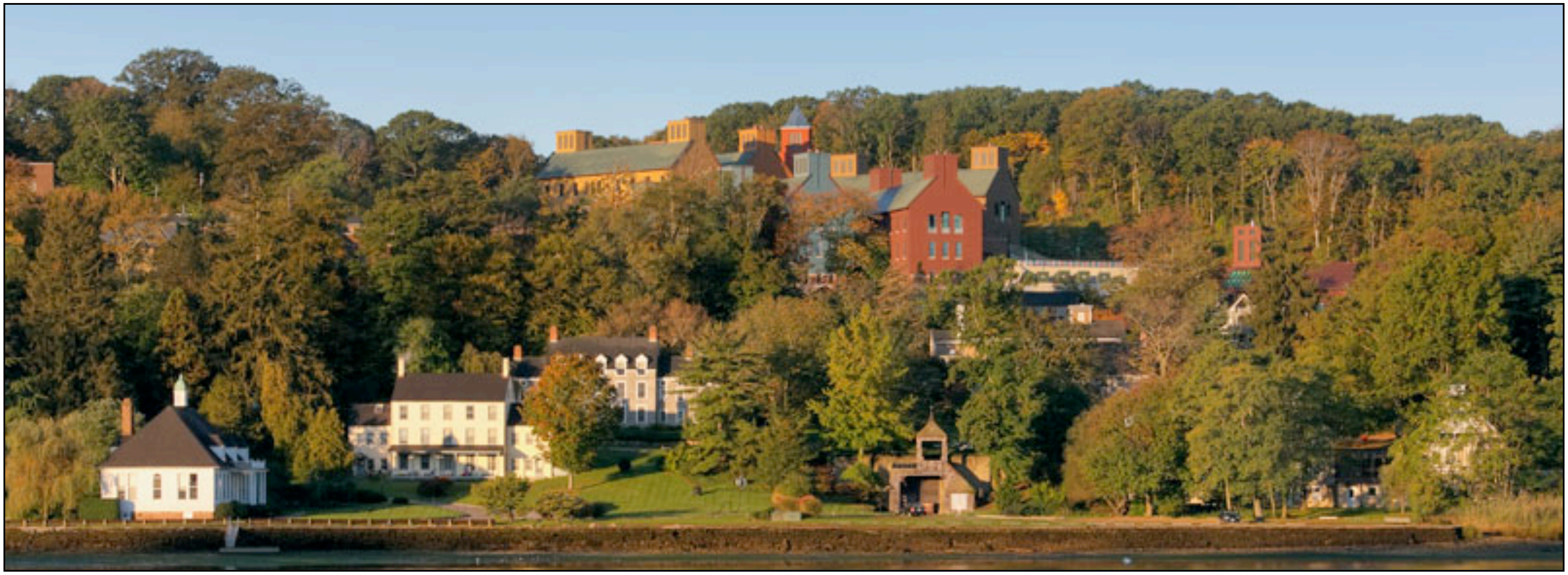
ALLPATHS team
SOAPdenovo team

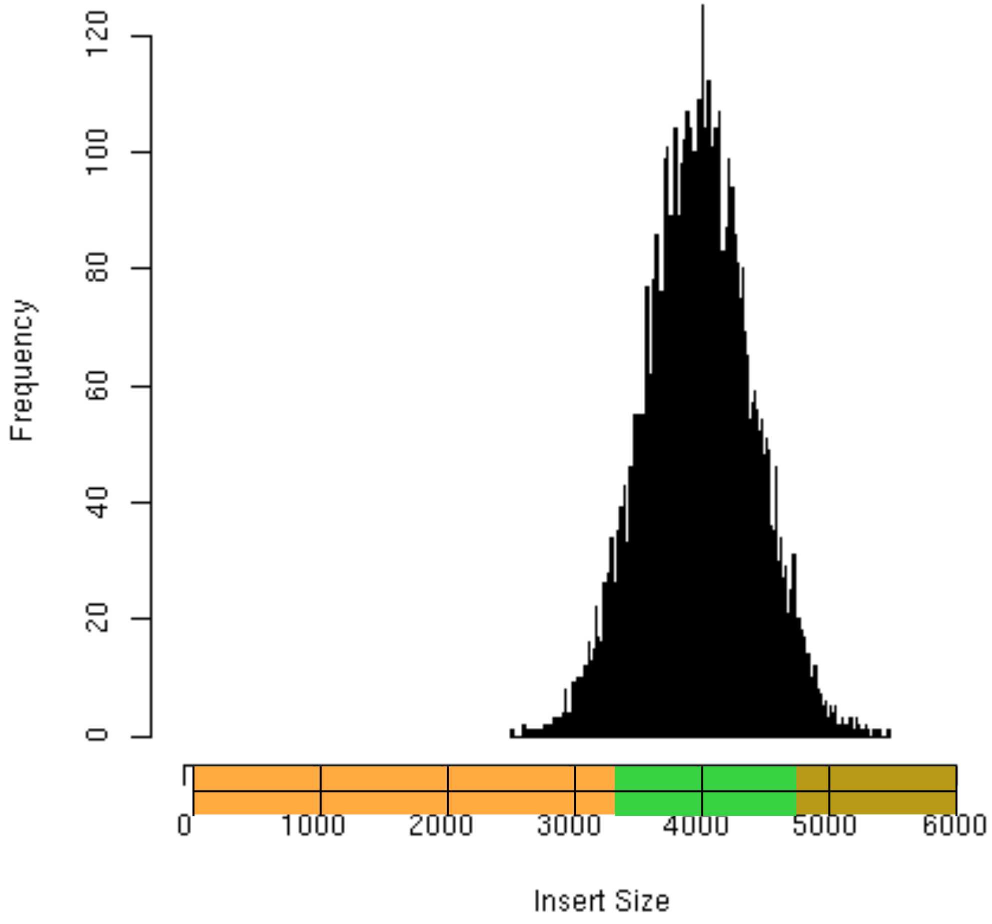


Thank You!

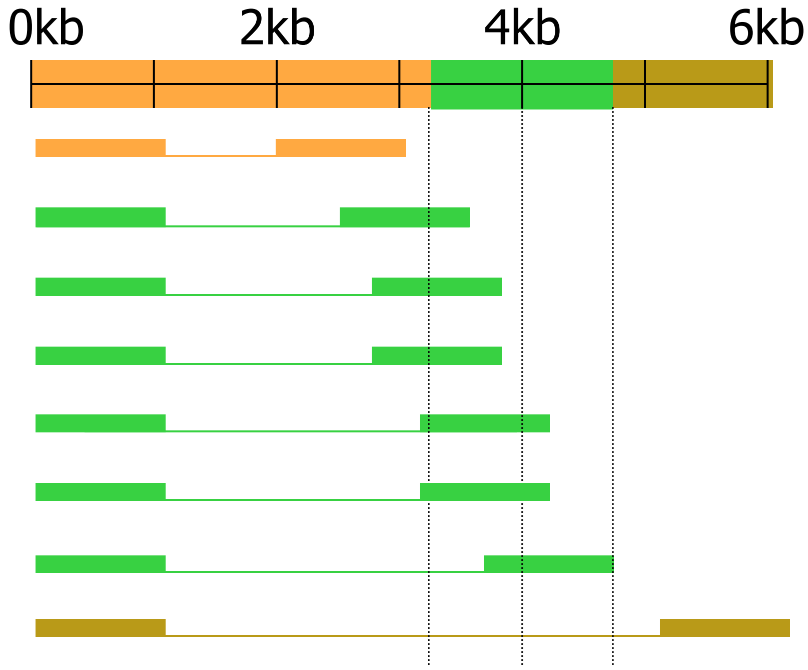
http://schatzlab.cshl.edu
@mike_schatz / #GI2011

Forensics

Compression/Expansion Statistic


Library size distribution

Mean: 4000, SD: 400



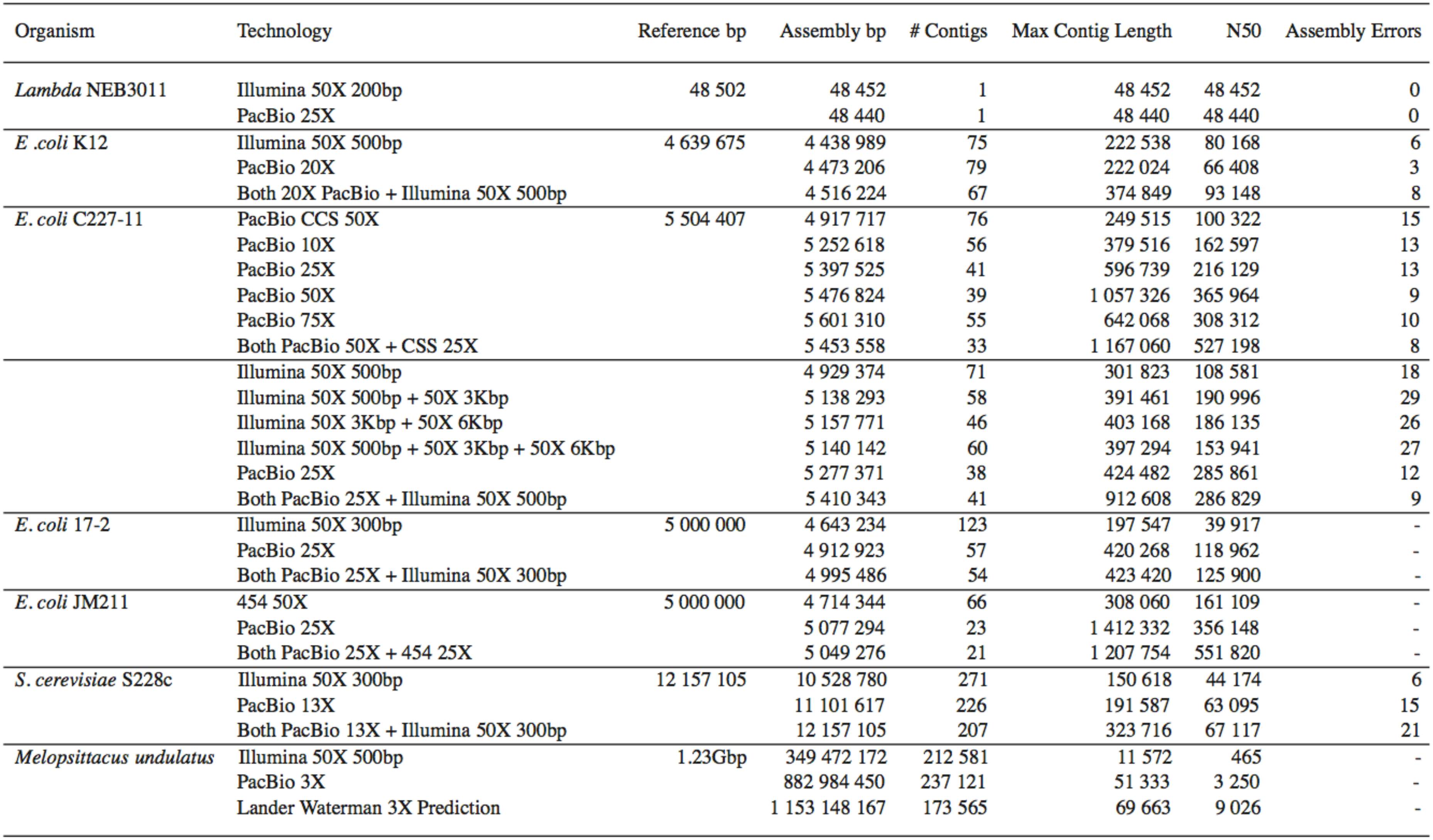
8 inserts: 3kb-6kb

Local Mean: 4048

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

Near 0 indicates overall happiness

Hybrid Assembly Results



Organism	Technology	Reference bp	Assembly bp	# Contigs	Max Contig Length	N50	Assembly Errors
Lambda NEB3011	Illumina 50X 200bp	48 502	48 452	1	48 452	48 452	0
	PacBio 25X		48 440	1	48 440	48 440	0
E .coli K12	Illumina 50X 500bp	4 639 675	4 438 989	75	222 538	80 168	6
	PacBio 20X		4 473 206	79	222 024	66 408	3
	Both 20X PacBio + Illumina 50X 500bp		4 516 224	67	374 849	93 148	8
E. coli C227-11	PacBio CCS 50X	5 504 407	4 917 717	76	249 515	100 322	15
	PacBio 10X		5 252 618	56	379 516	162 597	13
	PacBio 25X		5 397 525	41	596 739	216 129	13
	PacBio 50X		5 476 824	39	1 057 326	365 964	9
	PacBio 75X		5 601 310	55	642 068	308 312	10
	Both PacBio 50X + CSS 25X		5 453 558	33	1 167 060	527 198	8
	Illumina 50X 500bp		4 929 374	71	301 823	108 581	18
	Illumina 50X 500bp + 50X 3Kbp		5 138 293	58	391 461	190 996	29
	Illumina 50X 3Kbp + 50X 6Kbp		5 157 771	46	403 168	186 135	26
	Illumina 50X 500bp + 50X 3Kbp + 50X 6Kbp		5 140 142	60	397 294	153 941	27
	PacBio 25X		5 277 371	38	424 482	285 861	12
	Both PacBio 25X + Illumina 50X 500bp		5 410 343	41	912 608	286 829	9
E. coli 17-2	Illumina 50X 300bp	5 000 000	4 643 234	123	197 547	39 917	-
	PacBio 25X		4 912 923	57	420 268	118 962	-
	Both PacBio 25X + Illumina 50X 300bp		4 995 486	54	423 420	125 900	-
E. coli JM211	454 50X	5 000 000	4 714 344	66	308 060	161 109	-
	PacBio 25X		5 077 294	23	1 412 332	356 148	-
	Both PacBio 25X + 454 25X		5 049 276	21	1 207 754	551 820	-
S. cerevisiae S228c	Illumina 50X 300bp	12 157 105	10 528 780	271	150 618	44 174	6
	PacBio 13X		11 101 617	226	191 587	63 095	15
	Both PacBio 13X + Illumina 50X 300bp		12 157 105	207	323 716	67 117	21
Melopsittacus undulatus	Illumina 50X 500bp	1.23Gbp	349 472 172	212 581	11 572	465	-
	PacBio 3X		882 984 450	237 121	51 333	3 250	-
	Lander Waterman 3X Prediction		1 153 148 167	173 565	69 663	9 026	-

Hybrid assembly results using error corrected PacBio reads
Meets or beats Illumina-only or 454-only assembly in every case