Robert Cedergren Bioinformatics Colloquium 2009,
room S1-139, Jean-Coutu Bldg.

On the fifth of November, 2009, 11h00 - 11h30

OpenAssembler: assembly of reads from a mix of high-throughput sequencing technologies

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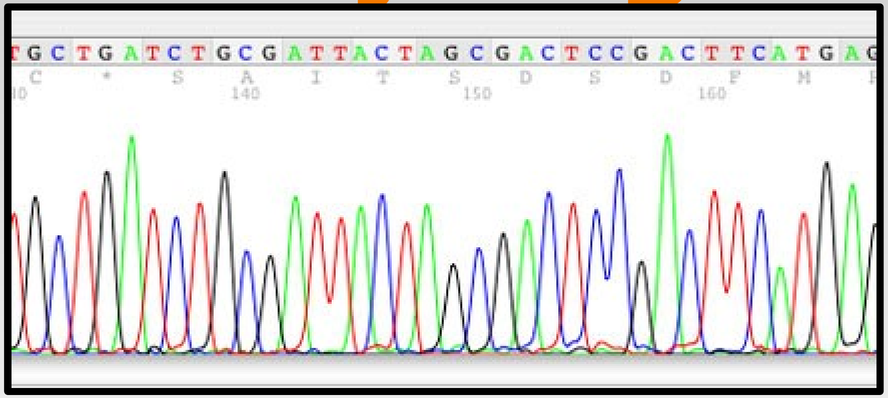
Sequencing and analyzing DNA

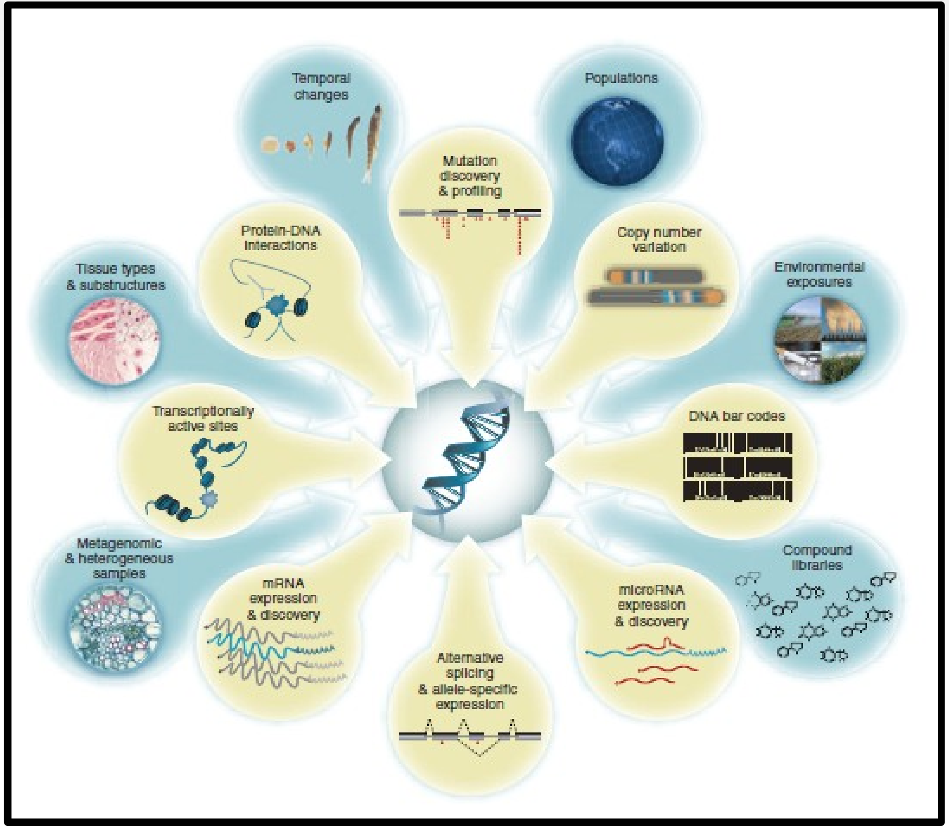


- Sequencing reads DNA
- Determine the primary structure of DNA
- Algorithms can help us!
- Hutchinson (1969) had foreseen the power of graph theory in sequence analysis
- Graph theory is everywhere

Evaluation of polymer sequence fragment data using graph theory.
Hutchinson G.
Bull Math Biophys. 1969 Sep;31(3):541-62.

Why do we decode life?

- Explain and treat genetic diseases (dystonia, huntington disease, Alzheimer's disease,...)
- Rapid detection of pathogenic agents (flu, H1N1, *C. difficile*, *S. pneumoniae*,...)
- Study evolution
- Study speciation
- Bridge the proteome and genome
- Study gene splicing
- Study **genome variation**


Temporal changes
Populations
Mutation discovery & profiling
Protein-DNA interactions
Copy number variation
Tissue types & substructures
Environmental exposures
Transcriptionally active sites
DNA bar codes
Metagenomic & heterogeneous samples
mRNA expression & discovery
Alternative splicing & allele-specific expression
microRNA expression & discovery
Compound libraries

What would you do if you could sequence everything?
Kahvejian A, Quackenbush J, Thompson JF.
Nat Biotechnol. 2008 Oct;26(10):1125-33.

Limits of sequencing

- Uneven genome coverage
- Reproducible errors (example: Roche/454's homopolymer-located errors)
- Contaminations
- Read length shorter than genome length

Technology	Read length (in bases)
Sanger	800
Roche/454	400
Illumina	50

The new paradigm of flow cell sequencing.
Holt RA, Jones SJ.
Genome Res. 2008 Jun;18(6):839-46.

Genome assembly

- DNA assemblers piece together reads to build larger contiguous sequences
- NP-Hard (according to Pop 2009)
- Genome finishing is lengthy
- Minimizing assembly errors is relevant (to avoid the laborious finishing step)

Genome assembly reborn: recent computational challenges.
Pop M.
Brief Bioinform. 2009 Jul;10(4):354-66.

Hybrid assemblies

More than one technology...

A Sanger/pyrosequencing hybrid approach for the generation of high-quality draft assemblies of marine microbial genomes.
Goldberg SM et al.
Proc Natl Acad Sci U S A. 2006 Jul 25;103(30):11240-5.

High quality draft sequences for prokaryotic genomes using a mix of new sequencing technologies.
Aury JM et al.
BMC Genomics. 2008 Dec 16;9:603.

De novo genome sequence assembly of a filamentous fungus using Sanger, 454 and Illumina sequence data.
Diguistini S et al.
Genome Biol. 2009 Sep 11;10(9):R94.

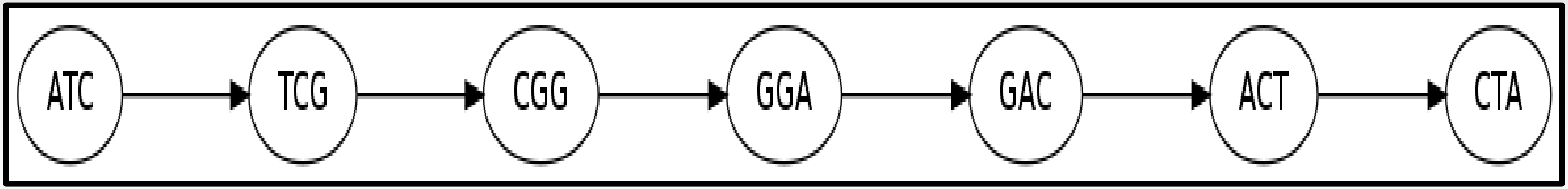
Drawbacks

- These approaches use several tools
- Reads obtained by different technologies are assembled separately
- Each assembler is tailored to a particular technology
- They consider reads from different technologies as being fundamentally different.
- All reads should be born equal!
- Graphs make that possible

de Bruijn and his graphs

Nucleotide space: ATCGGACTA

Graph space (with k=3):



- de Bruijn property: k-1 overlap between adjacent vertices
- Reads naturally induce a de Bruijn graph (with a fixed k)
- An assembly is a set of walks

http://en.wikipedia.org/wiki/De_Bruijn_graph

Assembly with Eulerian paths

- Uses a de Bruijn graph
- Equivalent transformations
- Polynomial
- Very sensitive to errors

An Eulerian path approach to DNA fragment assembly.
Pevzner PA, Tang H, Waterman MS.
Proc Natl Acad Sci U S A. 2001 Aug 14;98(17):9748-53.

De novo fragment assembly with short mate-paired reads: Does the read length matter?
Chaisson MJ, Brinza D, Pevzner PA.
Genome Res. 2009 Feb;19(2):336-46.

Velvet

- Tailored for Illumina
- Similar to EULER-SR
- Error correction
- Very fast

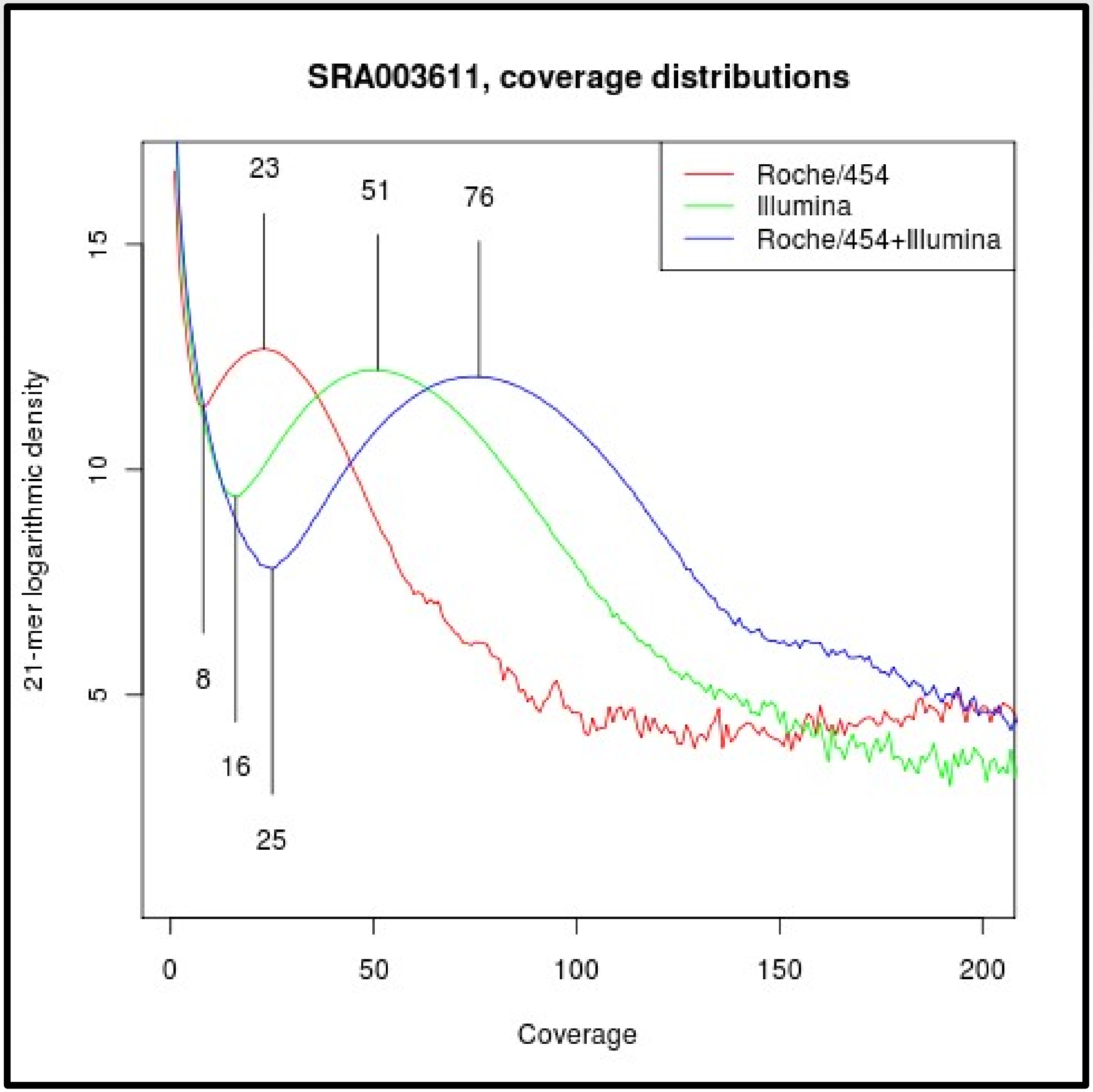
Velvet: algorithms for de novo short read assembly using de Bruijn graphs.
Zerbino DR, Birney E.
Genome Res. 2008 May;18(5):821-9.

OpenAssembler

- No eulerian paths
- No equivalent transformations
- Greedy (owing to the NP-hard nature of the problem)
- All reads have the same rights.

Coverage

- Each vertex of the graph has its depth of coverage – its number of occurences in reads



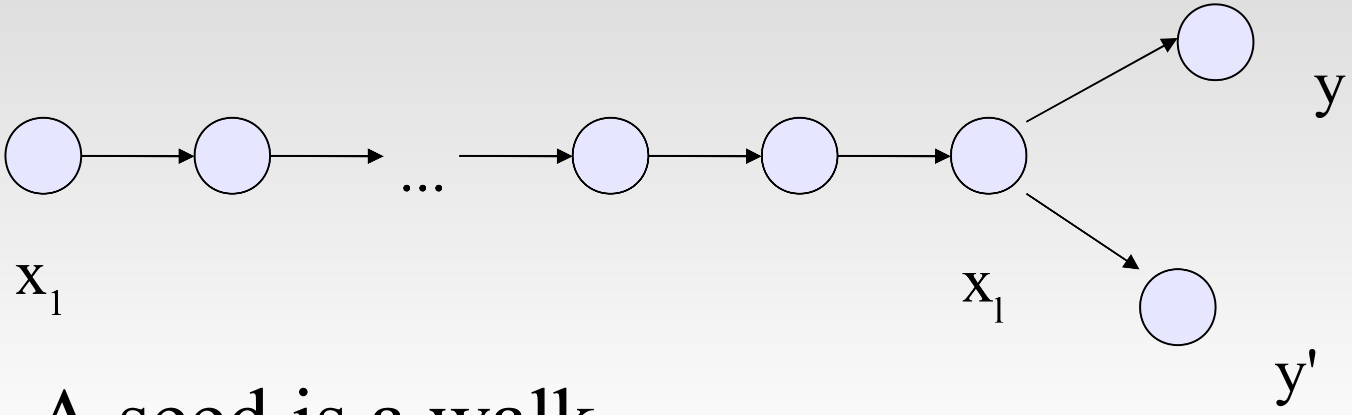
Mixing 454 and Illumina Improves the distribution.

Minimum and peak coverages are important.

Priming the assembly

- Seed coverage: average between minimum and peak coverages
- Seeds: maximal walks with only vertices of in-degree 1 and out-degree 1, and with a depth of coverage a least ”seed coverage”

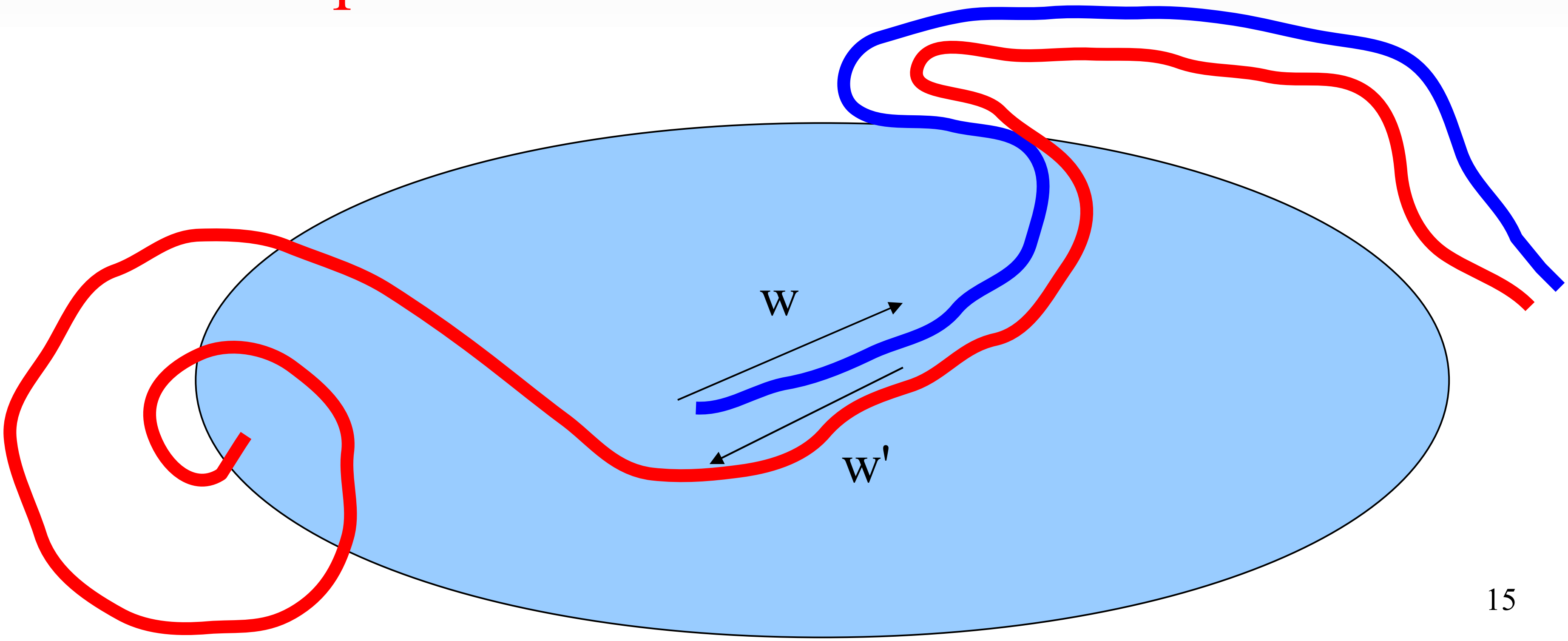
When a seed becomes a grown-up contig



- A seed is a walk.
- Given a walk $<x_1,x_2,\ldots,x_l>$, and two arcs $<x_l,y>$ and $<x_l,y'>$, our algorithm decides which vertex (y or y') is the next to visit
- If the choice is deemed as 'too risky', the extension is stopped.

Bilateral growth

- Each walk w is associated to its reverse-complement walk w'
- Extend w (call the result **w***), and then extend the reverse-complement of w*



OpenAssembler at a glance

- Load reads
- Build the de Bruijn graph (k=21)
- Compute the seeds
- Extend each seed in both directions
- Skip any previously encountered seed
- Write the assembly

- Implemented in c++

The assembler championship

- Two sets of competitions: simulated and real
- Five contenders
- Stringent metrics

Metrics

- Number of contiguous sequences
- Number of bases
- Mean contig length
- Largest contig length
- Genome coverage
- Number of incorrect (chimeric) contigs
- Number of mismatches
- Number of insertions and deletions

Contenders

- The “parallel” AbySS
- The “Eulerian” EULER-SR
- The “commercial” 454 Newbler
- The “greedy” OpenAssembler
- The “fast” Velvet

Living in a virtual world – simulated datasets

- Simulation offers great control – we know the reference sequence.
- SpSim: S. pneumoniae, 50-nt reads, 50 X
- SpErSim: S. pneumoniae, 50-nt reads, 50 X, 1% random mismatch
- SpPairedSim: S. pneumoniae, 50-nt reads, 50 X, paired (fragment length=200)
- EcoliSim: E. coli, 400-nt reads, 50 X

Simulated reads

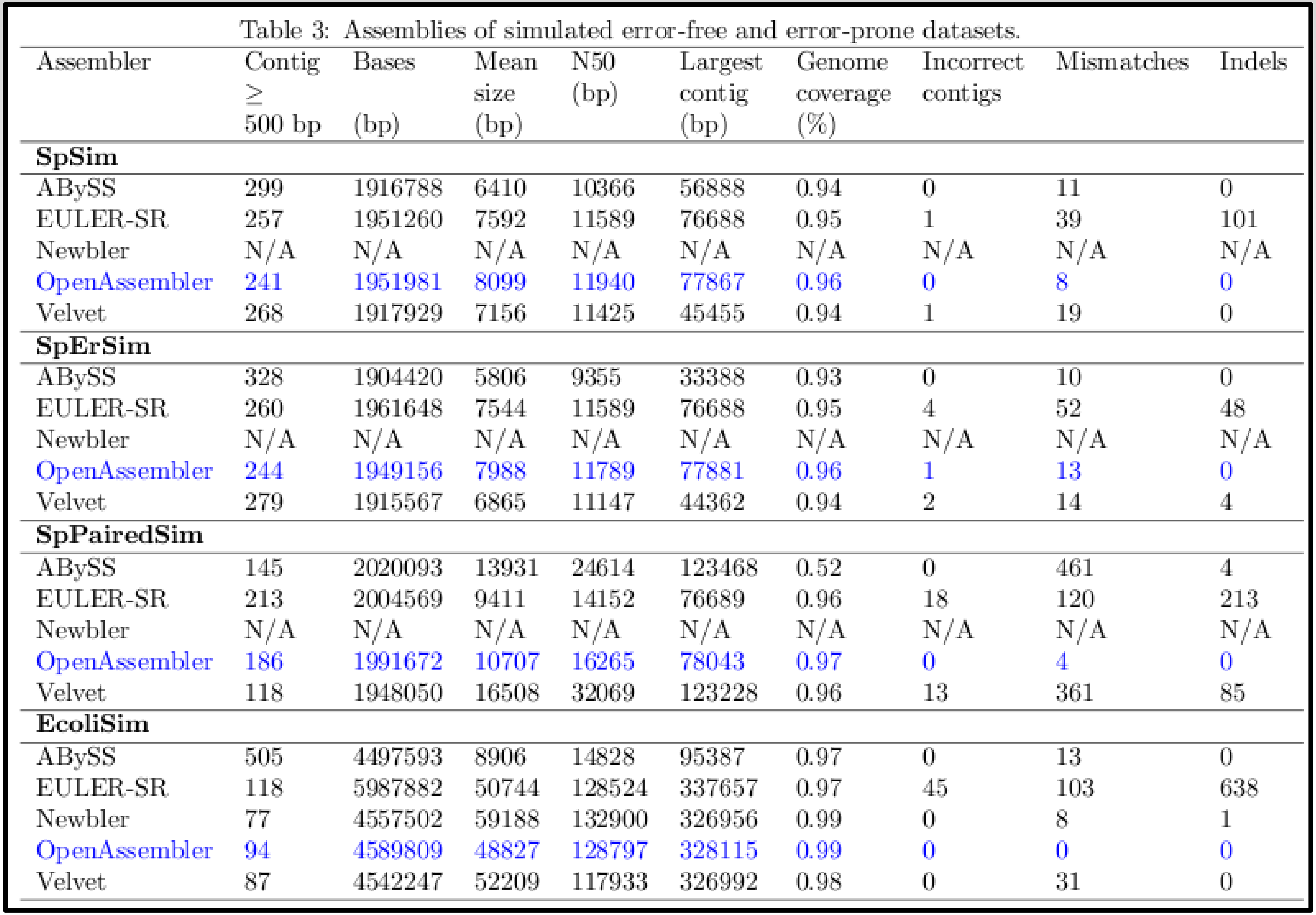
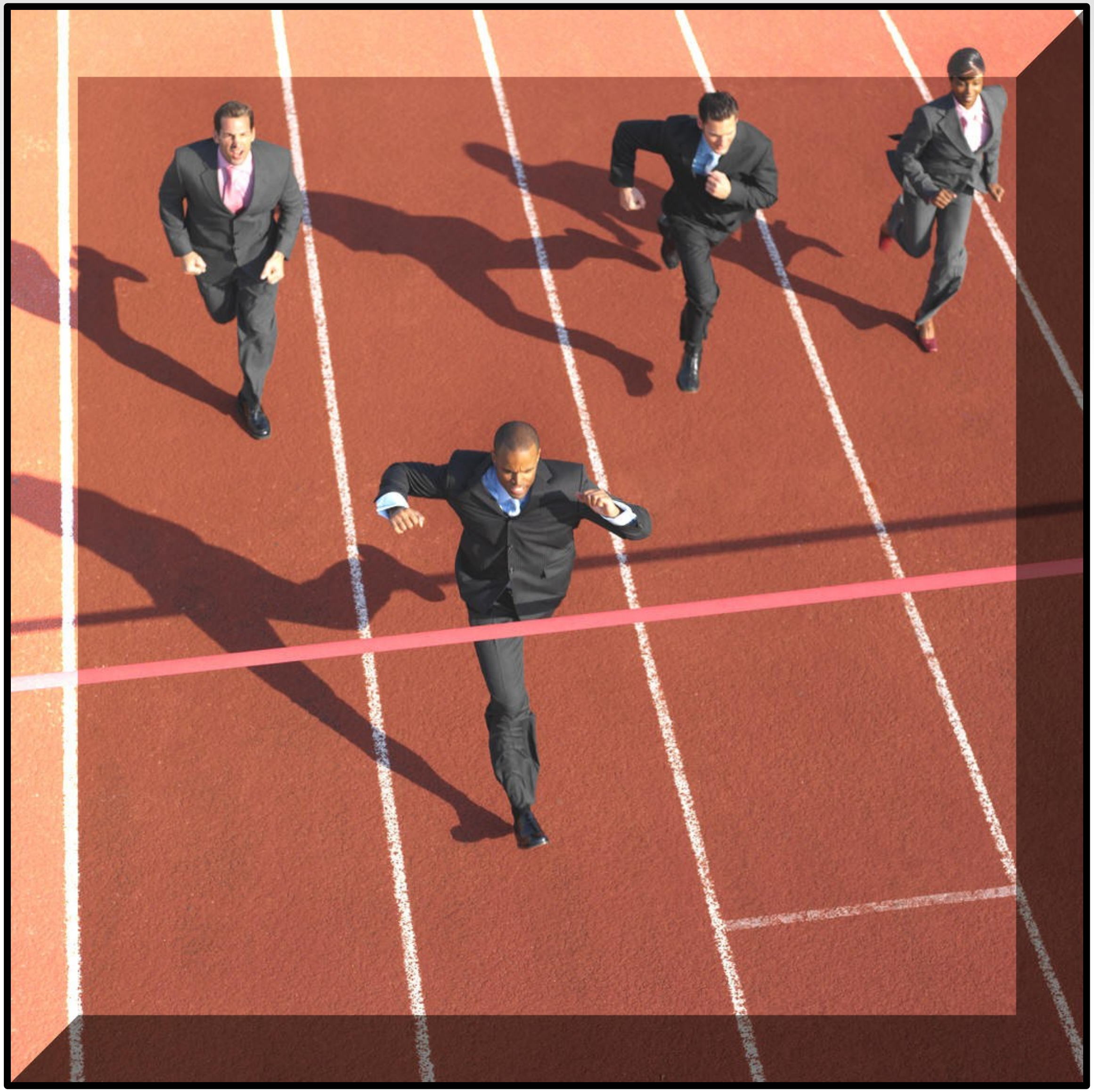


Table 3: Assemblies of simulated error-free and error-prone datasets.

Assembler	Contig ≥ 500 bp	Bases (bp)	Mean size (bp)	N50 (bp)	Largest contig (bp)	Genome coverage (%)	Incorrect contigs	Mismatches	Indels
SpSim									
ABySS	299	1916788	6410	10366	56888	0.94	0	11	0
EULER-SR	257	1951260	7592	11589	76688	0.95	1	39	101
Newbler	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
OpenAssembler	241	1951981	8099	11940	77867	0.96	0	8	0
Velvet	268	1917929	7156	11425	45455	0.94	1	19	0
SpErSim									
ABySS	328	1904420	5806	9355	33388	0.93	0	10	0
EULER-SR	260	1961648	7544	11589	76688	0.95	4	52	48
Newbler	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
OpenAssembler	244	1949156	7988	11789	77881	0.96	1	13	0
Velvet	279	1915567	6865	11147	44362	0.94	2	14	4
SpPairedSim									
ABySS	145	2020093	13931	24614	123468	0.52	0	461	4
EULER-SR	213	2004569	9411	14152	76689	0.96	18	120	213
Newbler	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
OpenAssembler	186	1991672	10707	16265	78043	0.97	0	4	0
Velvet	118	1948050	16508	32069	123228	0.96	13	361	85
EcoliSim									
ABySS	505	4497593	8906	14828	95387	0.97	0	13	0
EULER-SR	118	5987882	50744	128524	337657	0.97	45	103	638
Newbler	77	4557502	59188	132900	326956	0.99	0	8	1
OpenAssembler	94	4589809	48827	128797	328115	0.99	0	0	0
Velvet	87	4542247	52209	117933	326992	0.98	0	31	0

Competition results

- OpenAssembler wins



Facing reality – real datasets

- Simulated reads are useless for real-life applications
- EcoliIllumina: Illumina paired reads, lots of coverage
- A. baylyi ADP1 data: Ab454, AbIllumina, and AbMix
- **Is the mix worth it?**

Real data

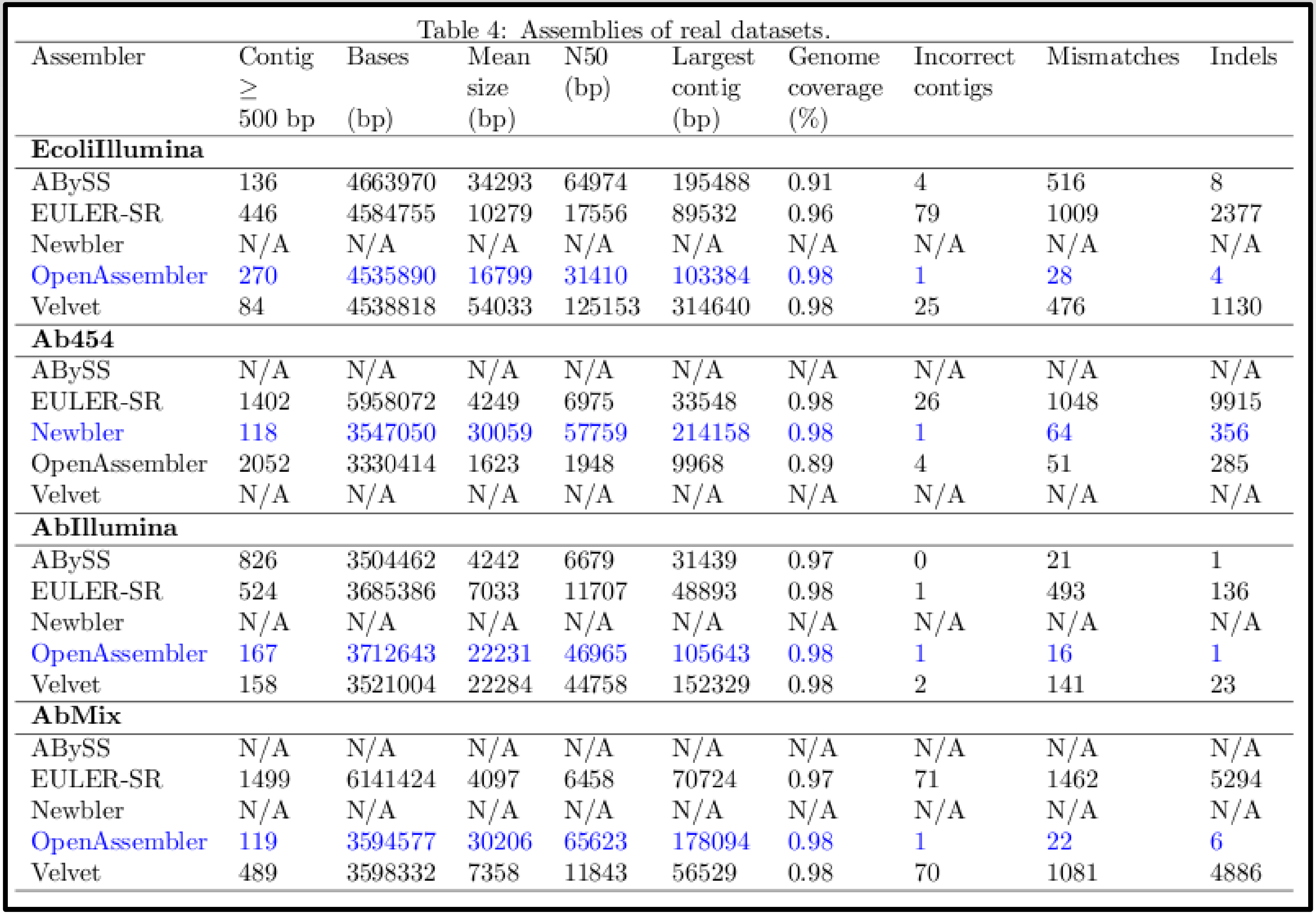


Table 4: Assemblies of real datasets.

Assembler	Contig	Bases	Mean	N50	Largest	Genome	Incorrect	Mismatches	Indels
	≥		size	(bp)	contig	coverage	contigs		
	500 bp	(bp)	(bp)		(bp)	(%)			
EcoliIllumina									
ABySS	136	4663970	34293	64974	195488	0.91	4	516	8
EULER-SR	446	4584755	10279	17556	89532	0.96	79	1009	2377
Newbler	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
OpenAssembler	270	4535890	16799	31410	103384	0.98	1	28	4
Velvet	84	4538818	54033	125153	314640	0.98	25	476	1130
Ab454									
ABySS	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
EULER-SR	1402	5958072	4249	6975	33548	0.98	26	1048	9915
Newbler	118	3547050	30059	57759	214158	0.98	1	64	356
OpenAssembler	2052	3330414	1623	1948	9968	0.89	4	51	285
Velvet	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
AbIllumina									
ABySS	826	3504462	4242	6679	31439	0.97	0	21	1
EULER-SR	524	3685386	7033	11707	48893	0.98	1	493	136
Newbler	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
OpenAssembler	167	3712643	22231	46965	105643	0.98	1	16	1
Velvet	158	3521004	22284	44758	152329	0.98	2	141	23
AbMix									
ABySS	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
EULER-SR	1499	6141424	4097	6458	70724	0.97	71	1462	5294
Newbler	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
OpenAssembler	119	3594577	30206	65623	178094	0.98	1	22	6
Velvet	489	3598332	7358	11843	56529	0.98	70	1081	4886

Who survived?

- 454 is Newbler's ecological niche.
- OpenAssembler is not the winner on 454
- OpenAssembler's excels with Illumina data.
- Mixing is OpenAssembler's specialty.

A. baylyi					
	Genome coverage	**Reads**	**Contigs**	**Mismatches**	**Indels**
Newbler	98%	454	118	64	356
OpenAssembler	98%	Mixed	119	22	6

Closing remarks

- OpenAssembler runs on mixes -- not the others
- OpenAssembler improves the quality of genome drafts
- Quality is important
- One (easy-to-use) tool to rule them all
- Paper submitted

Genome project standards in a new era of sequencing.
Chain PS et al.
Science. 2009 Oct 9;326(5950):236-7.

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