De novo assembly of complex genomes using 3rd generation sequencing

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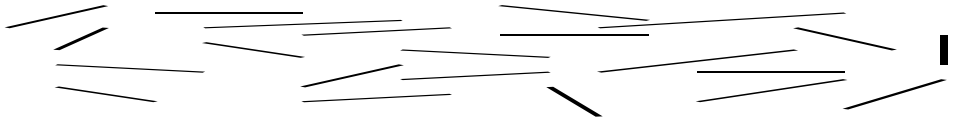
Jan 15, 2012
PAG-XX: Sequencing Complex Genomes

Assembling a Genome

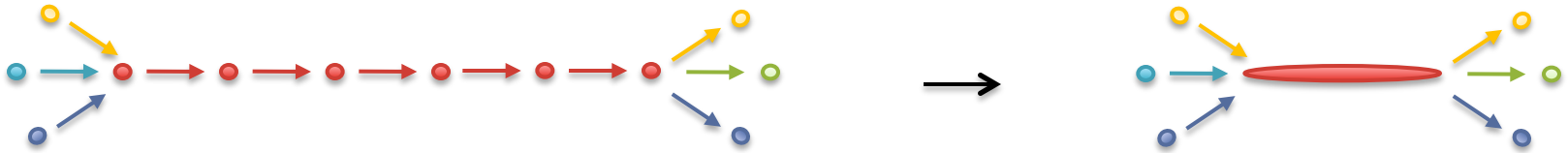
1. Shear & Sequence DNA



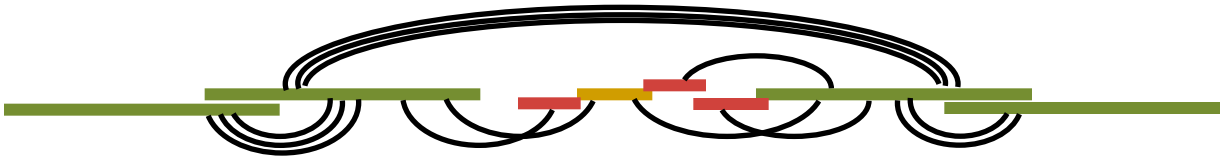
2. Construct assembly graph from overlapping reads

...AGCCTAGACCTACAGGATGCGCGACACGT
GGATGCGCGACACGTCGCATATCCGGT...

3. Simplify assembly graph



4. Detangle graph with long reads, mates, and other links

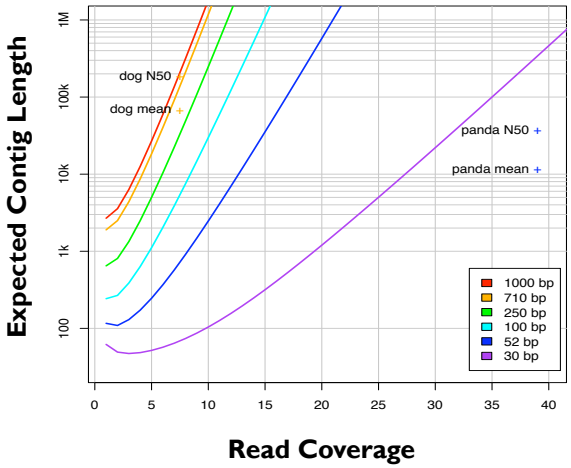


Why are PAG genomes hard to assemble?

1. ***Instrumentation***:
   - (Very) large genomes, imperfect sequencing
2. ***Biological***:
   - (Very) High ploidy, heterozygosity, repeat content
3. ***Computational***:
   - (Very) Large genomes, complex structure
4. ***Accuracy***:
   - (Very) Hard to assess correctness

Ingredients for a good assembly

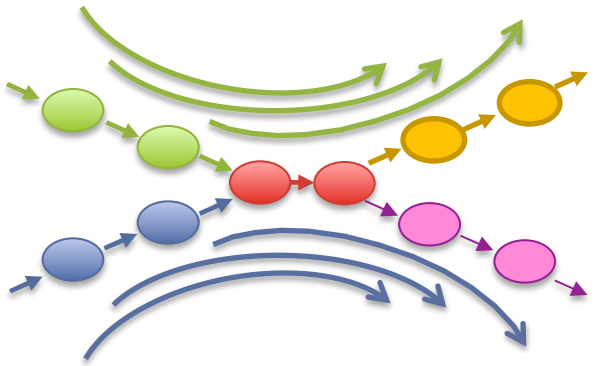
Coverage



High coverage is required

- Oversample the genome to ensure every base is sequenced with long overlaps between reads
- Biased coverage will also fragment assembly

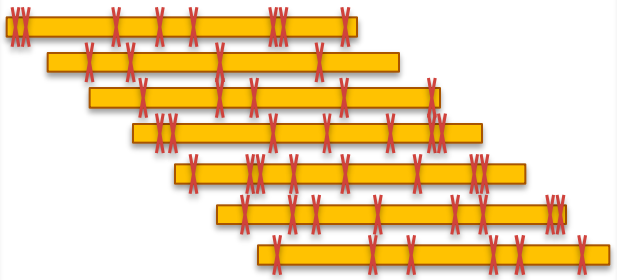
Read Length



Reads & mates must be longer than the repeats

- Short reads will have ***false overlaps*** forming hairball assembly graphs
- With long enough reads, assemble entire chromosomes into contigs

Quality



Errors obscure overlaps

- Reads are assembled by finding kmers shared in pair of reads
- High error rate requires very short seeds, increasing complexity and forming assembly hairballs

Assembly of Large Genomes using Second Generation Sequencing
Schatz MC, Delcher AL, Salzberg SL (2010) *Genome Research.* 20:1165-1173.

Hybrid Sequencing

Illumina

Sequencing by Synthesis

High throughput (60Gbp/day)
High accuracy (~99%)
Short reads (~100bp)

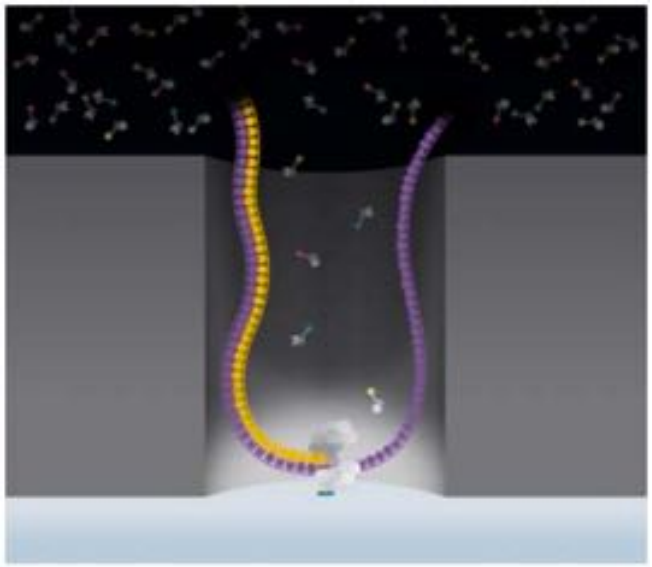
Pacific Biosciences

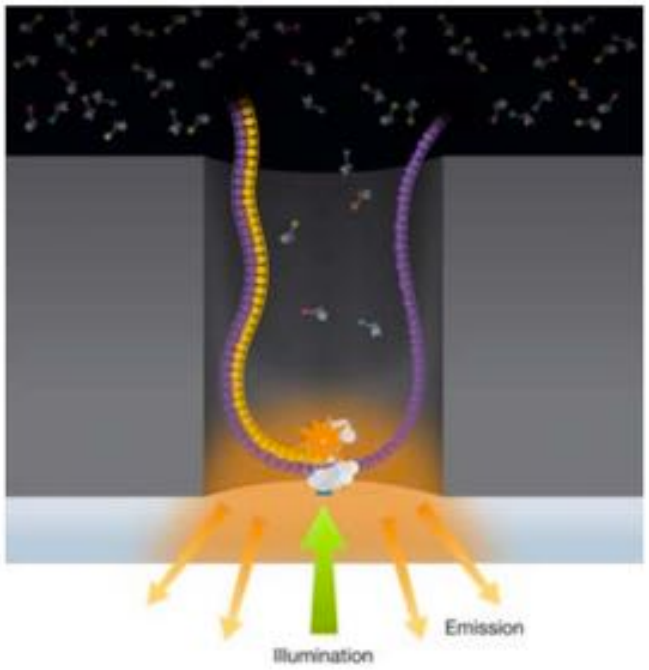
SMRT Sequencing

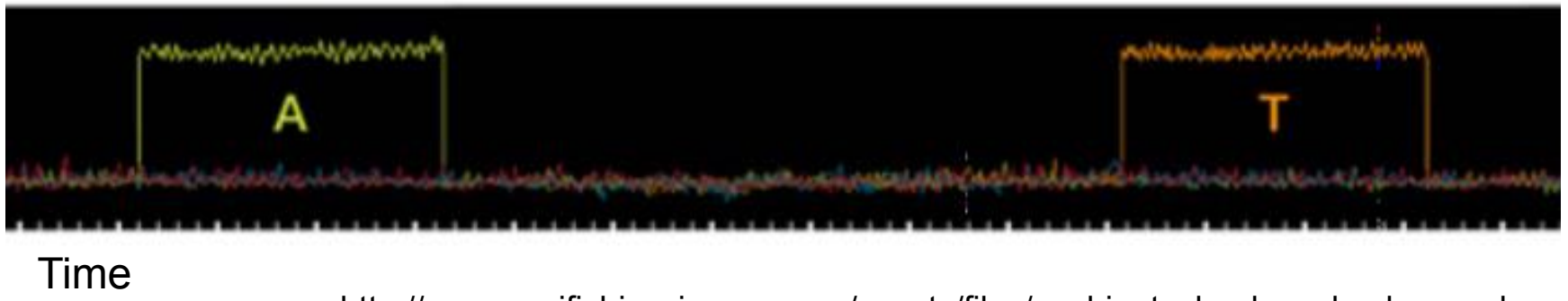
Lower throughput (600Mbp/day)
Lower accuracy (~85%)
Long reads (1-2kbp+)

SMRT Sequencing

Imaging of florescent phospholinked labeled nucleotides as they are incorporated by a polymerase anchored to a Zero-Mode Waveguide (ZMW).





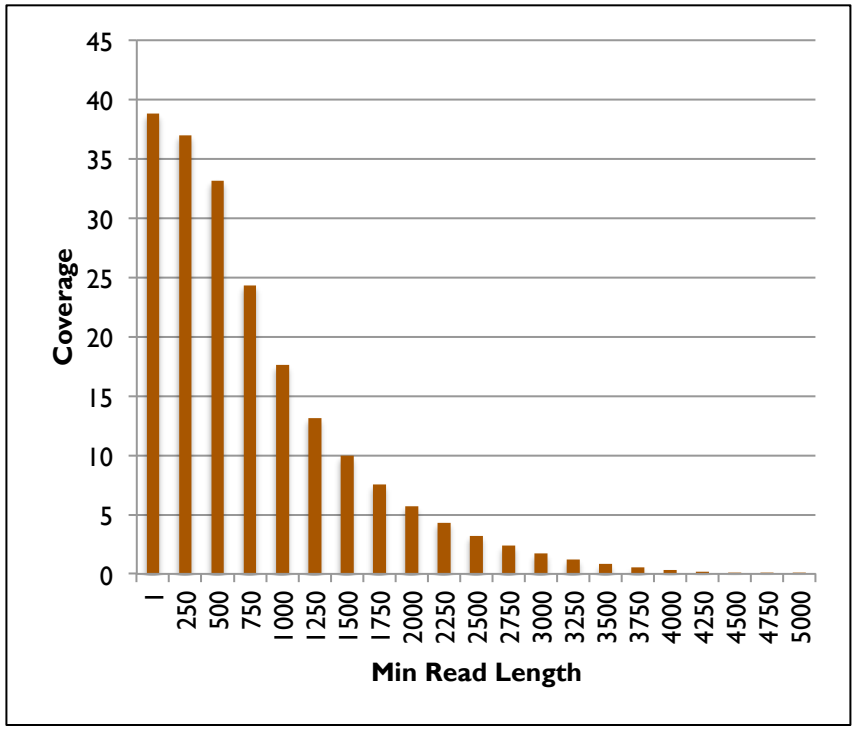


http://www.pacificbiosciences.com/assets/files/pacbio_technology_backgrounder.pdf

SMRT Sequencing Data

Yeast
(12 Mbp genome)

65 SMRT cells
734,151 reads after filtering
Mean: 642.3 +/- 587.3
Median: 553 Max: 8,495




```
TTGTAAGCAGTTGAAAACTATGTGTGGATTTAGAATAAAGAACATGAAAG
|||||||||||||||||||||||||| ||||||| |||||||||||| |||
TTGTAAGCAGTTGAAAACTATGTGT-GATTTAG-ATAAAGAACATGGAAG

ATTATAAA-CAGTTGATCCATT-AGAAGA-AAACGCAAAAGGCGGCTAGG
| |||||| |||||||||||||| |||| | |||||| |||||| |||||||
A-TATAAATCAGTTGATCCATTAAGAA-AGAAACGC-AAAGGC-GCTAGG

CAACCTTGAATGTAATCGCACTTGAAGAACAAGATTTTATTCCGCGCCCG
| |||||| |||| ||  ||||||||||||||||||||||||||||||||
C-ACCTTG-ATGT-AT--CACTTGAAGAACAAGATTTTATTCCGCGCCCG

TAACGAATCAAGATTCTGAAAACACAT-ATAACAACCTCCAAAA-CACAA
| ||||||| |||||||||||||| || ||    |||||||||| |||||
T-ACGAATC-AGATTCTGAAAACA-ATGAT----ACCTCCAAAAGCACAA

-AGGAGGGGAAAGGGGGGAATATCT-ATAAAAGATTACAAATTAGA-TGA
 ||||||   ||     |||||||| || |||||||||||||| || |||
GAGGAGG---AA-----GAATATCTGAT-AAAGATTACAAATT-GAGTGA

ACT-AATTCACAATA-AATAACACTTTTA-ACAGAATTGAT-GGAA-GTT
||| ||||||||| | |||||||||||| ||| ||||||| |||| |||
ACTAAATTCACAA-ATAATAACACTTTTAGACAAAATTGATGGGAAGGTT

TCGGAGAGATCCAAAACAATGGGC-ATCGCCTTTGA-GTTAC-AATCAAA
|| ||||||||| ||||||| ||| |||| |||||| ||||| |||||||
TC-GAGAGATCC-AAACAAT-GGCGATCG-CTTTGACGTTACAAATCAAA

ATCCAGTGGAAAATATAATTTATGCAATCCAGGAACTTATTCACAATTAG
||||||| ||||||||  |||||| ||||| ||||||||||||||||||
ATCCAGT-GAAAATATA--TTATGC-ATCCA-GAACTTATTCACAATTAG
```

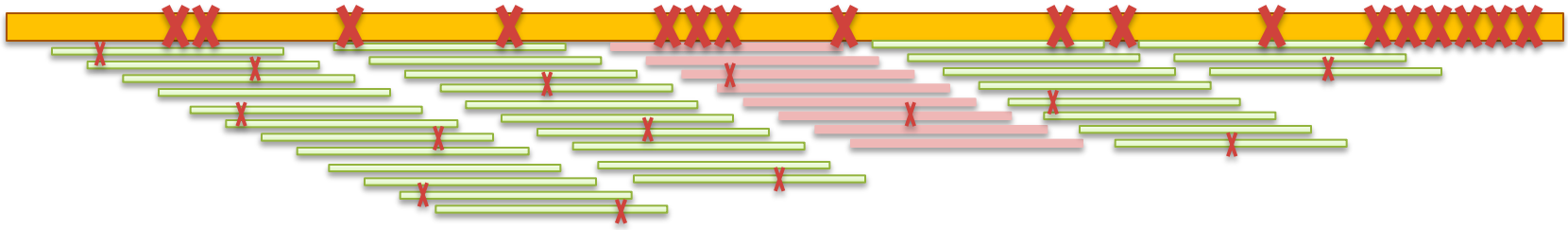
Sample of 100k reads aligned with BLASR requiring >100bp alignment
Average overall accuracy: 83.7%, 11.5% insertions, 3.4% deletions, 1.4% mismatch

PacBio Error Correction

http://wgs-assembler.sf.net

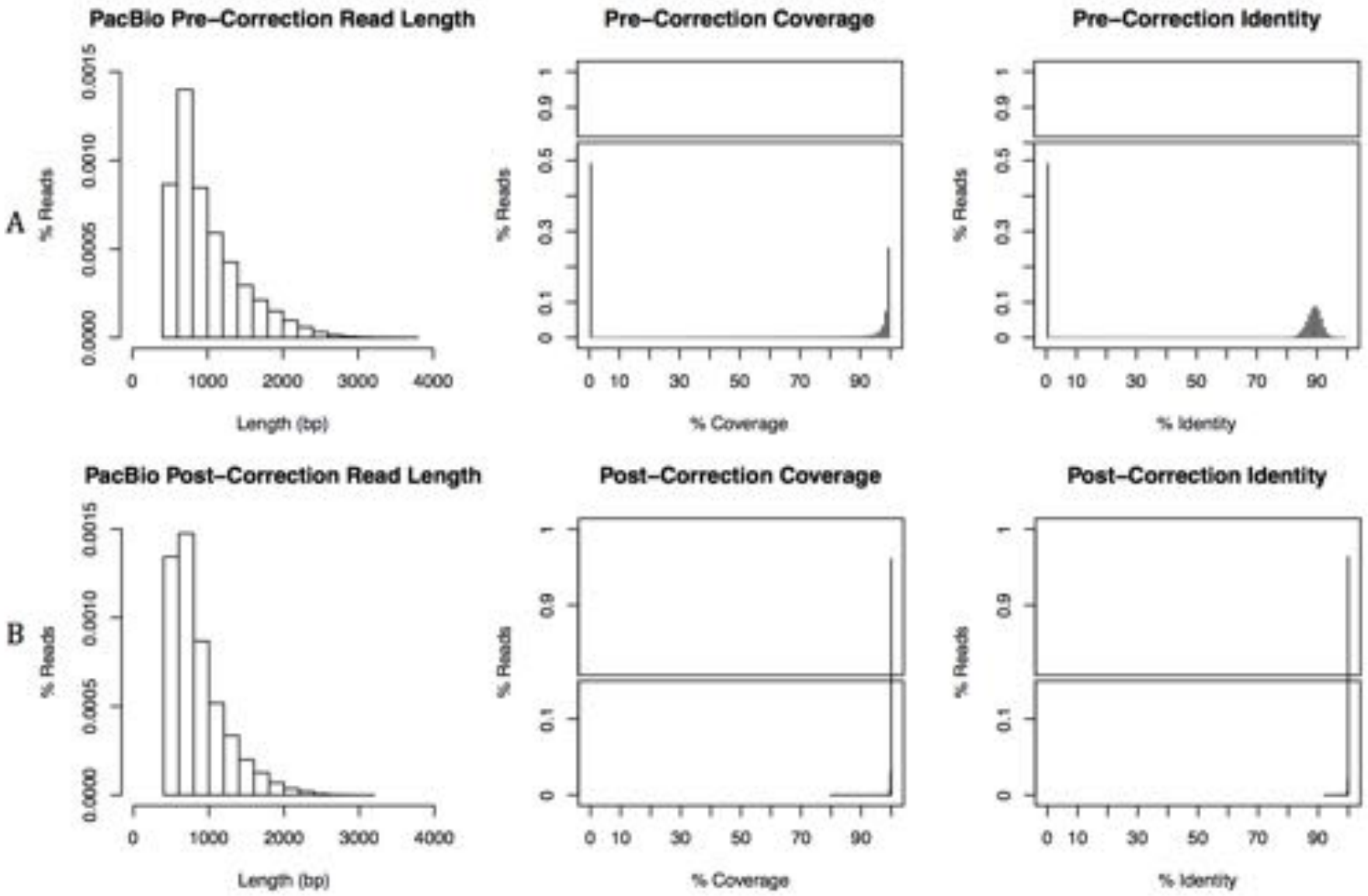
1. Correction Pipeline
   1. Map short reads (SR) to long reads (LR)
   2. Trim LRs at coverage gaps
   3. Compute consensus for each LR

2. Error corrected reads can be easily assembled, aligned



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. (2012) *Under Review*

Error Correction Results



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

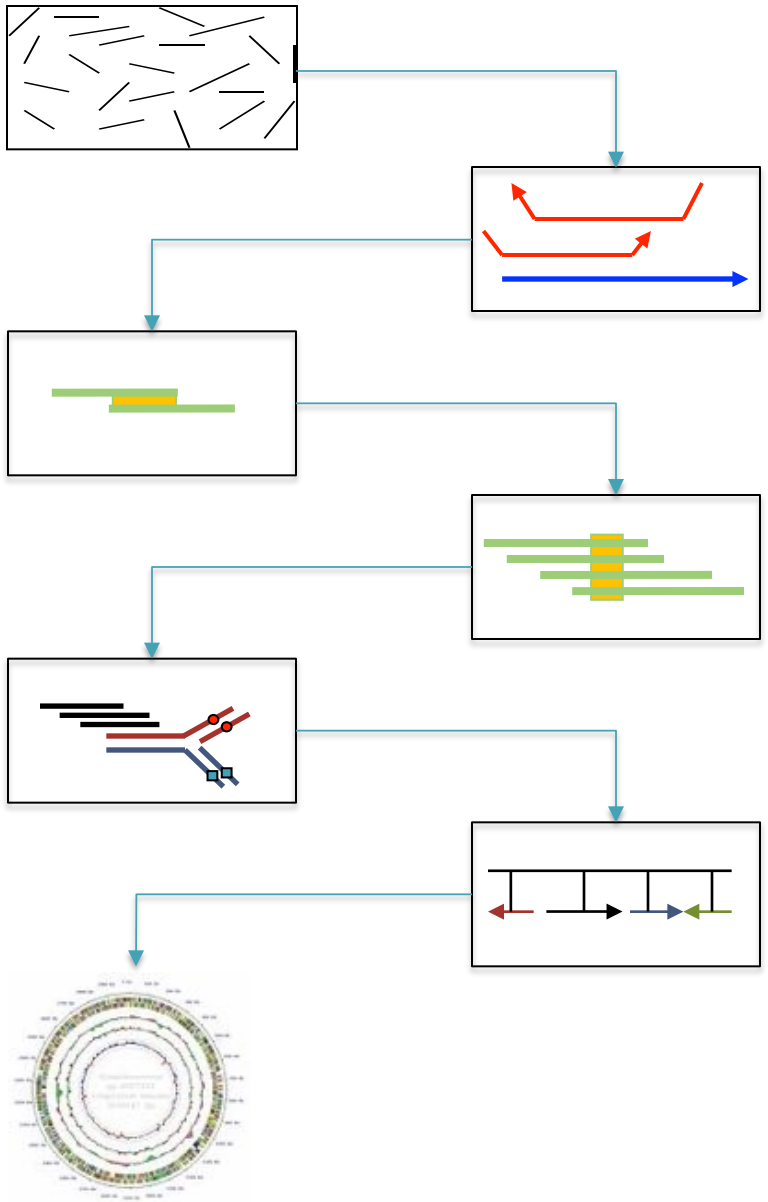
SMRT-assembly

- Co-assemble error corrected long reads with short reads
  - Long reads natively span repeats (red)
  - Guards against mis-assemblies in draft assembly
  - Use all available data at once
- Challenges
  - Assembler must supports a wide mix of read lengths

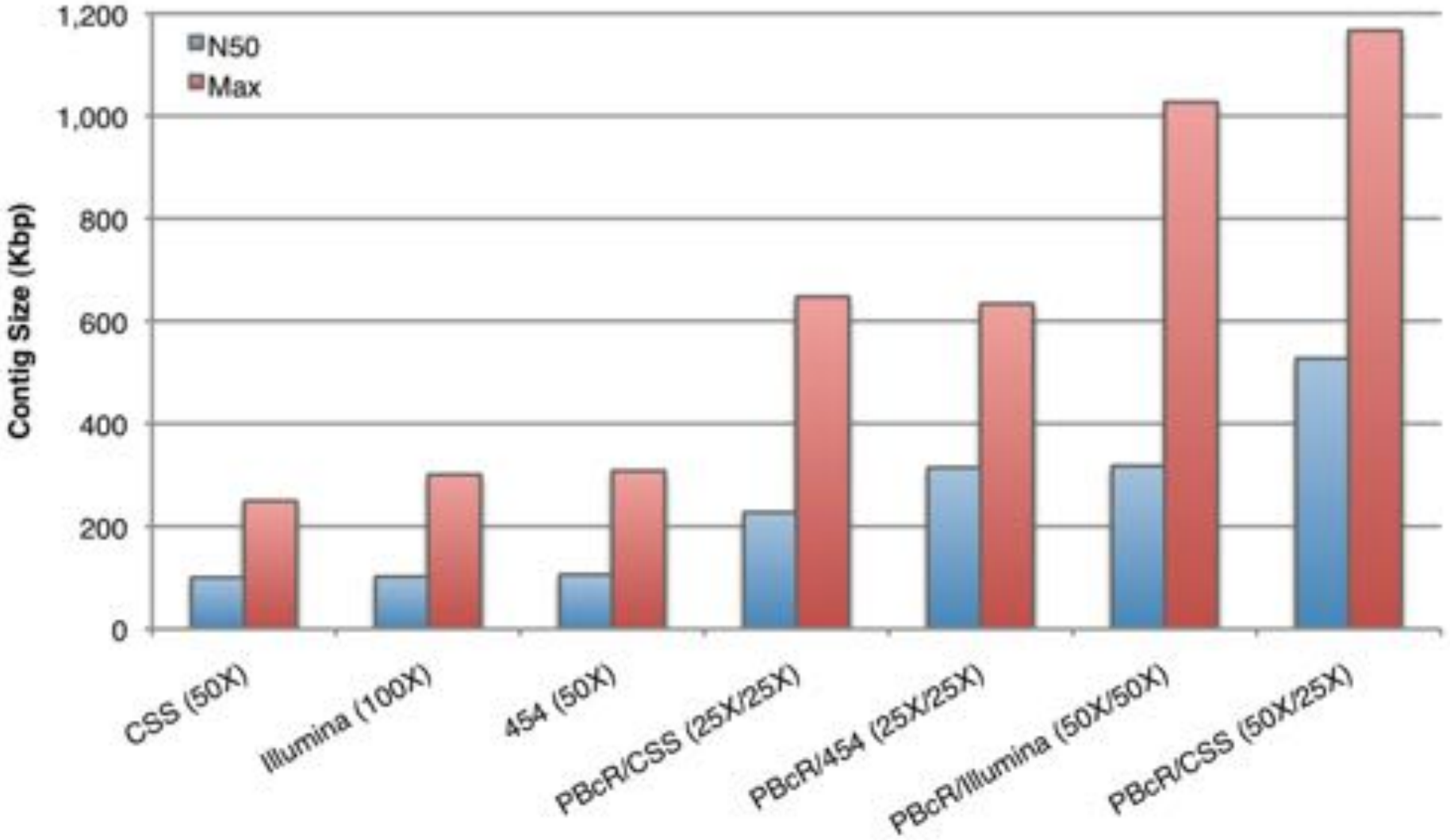
Celera Assembler

http://wgs-assembler.sf.net

1. Pre-overlap
   - Consistency checks
2. Trimming
   - Quality trimming & partial overlaps
3. Compute Overlaps
   - Find high quality overlaps
4. Error Correction
   - Evaluate difference in context of overlapping reads
5. Unitigging
   - Merge consistent reads
6. Scaffolding
   - Bundle mates, Order & Orient
7. Finalize Data
   - Build final consensus sequences



Assembly Results



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

SMRT-Assembly Results

Organism	Technology	Reference bp	Assembly bp	# Contigs	Max Contig Length	N50
Lambda NEB3011	Illumina 100X 200bp	48 502	48 492	1	48 492 / 48 492	48 492 / 48 492 (100%) *
(median: 727 max: 3 280)	PacBio PBcR 25X		48 440	1	48 444 / 48 444	48 444 / 48 440 (100%) *
E. coli K12	Illumina 100X 500bp	4 639 675	4 462 836	61	221 615 / 221 553	100 338 / 83 037 (82.76%) *
(median: 747 max: 3 068)	PacBio PBcR 18X		4 465 533	77	239 058 / 238 224	71 479 / 68 309 (95.57%) *
	Both 18X PacBio PBcR + Illumina 50X 500bp		4 576 046	65	238 272 / 238 224	93 048 / 89 431 (96.11%) *
E. coli C227-11	PacBio CCS 50X	5 504 407	4 917 717	76	249 515	100 322
(median: 1 217 max: 14 901)	PacBio 25X PBcR (corrected by 25X CCS)		5 207 946	80	357 234	98 774
	Both PacBio PBcR 25X + CCS 25X		5 269 158	39	647 362	227 302
	PacBio 50X PBcR (corrected by 50X CCS)		5 445 466	35	1 076 027	376 443
	Both PacBio PBcR 50X + CCS 25X		5 453 458	33	1 167 060	527 198
	Manually Corrected ALLORA Assembly[a]		5 452 251	23	653 382	402 041
S. cerevisiae S228c	Illumina 100X 300bp	12 157 105	11 034 156	192	266 528 / 227 714	73 871 / 49 254 (66.68%) *
(median: 674 max: 5 994)	PacBio PBcR 13X		11 110 420	224	224 478 / 217 704	62 898 / 54 633 (86.86%) *
	Both PacBio PBcR 13X + Illumina 50X 300bp		11 286 932	177	262 846 / 260 794	82 543 / 59 792 (72.44%) *
Melopsittacus undulatus	Illumina 194X (220/500/800 paired-end 2/5/10Kb mate-pairs)	1.23 Gbp	1 023 532 850	24 181	1 050 202	47 383
	454 15.4X (FLX + FLX Plus + 3/8/20Kbp paired-ends)		999 168 029	16 574	751 729	75 178
(median 997, max 13 079)	454 15.4X + PacBio PBcR 3.75X		1 071 356 415	15 081	1 238 843	99 573

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

Transcript Alignment

- Long-read single-molecule sequencing has potential to directly sequence full length transcripts
  - Raw reads and raw alignments (red) have many spurious indels inducing false frameshifts and other artifacts
  - Error corrected reads almost perfectly match the genome, pinpointing splice sites, identifying alternative splicing
- New collaboration with Gingeras Lab looking at splicing in human

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3. ***Computational***:
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4. ***Accuracy***:
   - (Very) Hard to assess correctness

With new sequencing technologies and improved algorithms we can address these challenges

=> Cautiously optimistic

Acknowledgements

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Mihai Pop
Art Delcher
Jimmy Lin
David Kelley
Dan Sommer
Cole Trapnell

Thank You

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

More Discussion @ PacBio
Workshop 1:30 Tuesday