Near perfect de novo assemblies of eukaryotic genomes using PacBio long read sequencing

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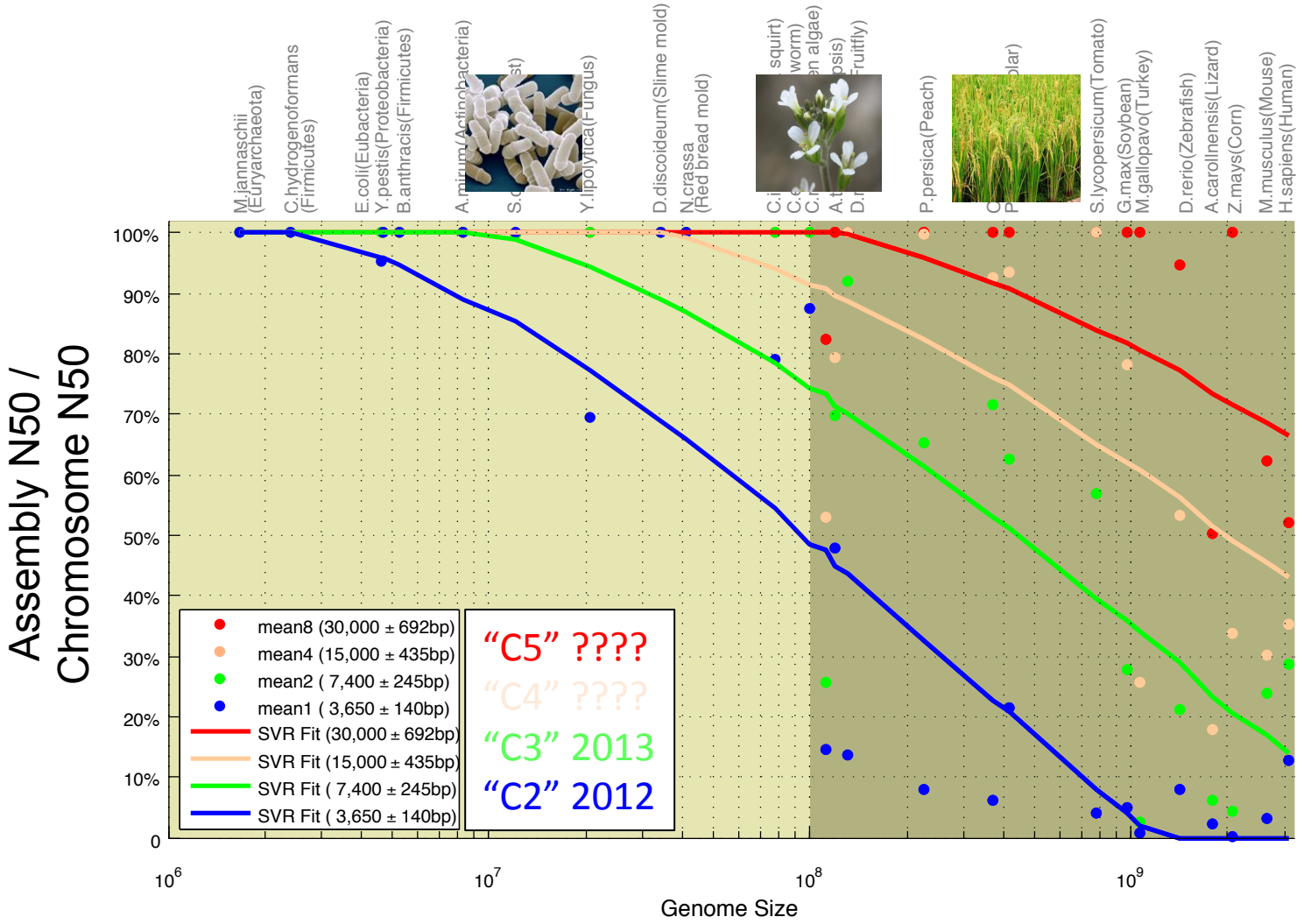
5/29/2014

Assembly Complexity of Long Reads



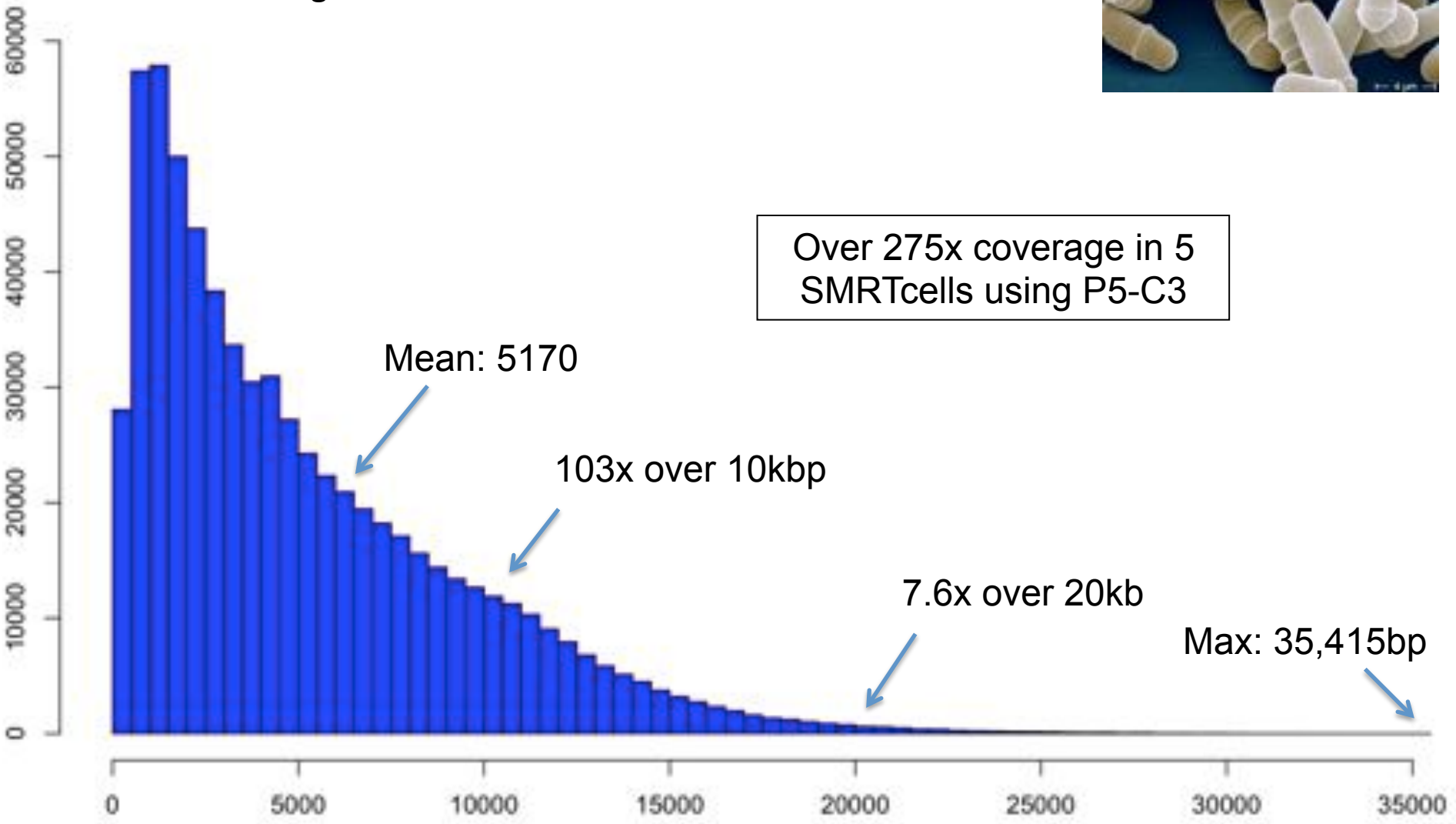
Assembly complexity of long read sequencing

Lee, H*, Gurtowski, J*, Yoo, S, Marcus, S, McCombie, WR, Schatz MC. (2014) *In preparation*

S. pombe dg21

PacBio RS II *sequencing* at CSHL

- Size selection using an 7 Kb elution window on a BluePippin™ device from Sage Science



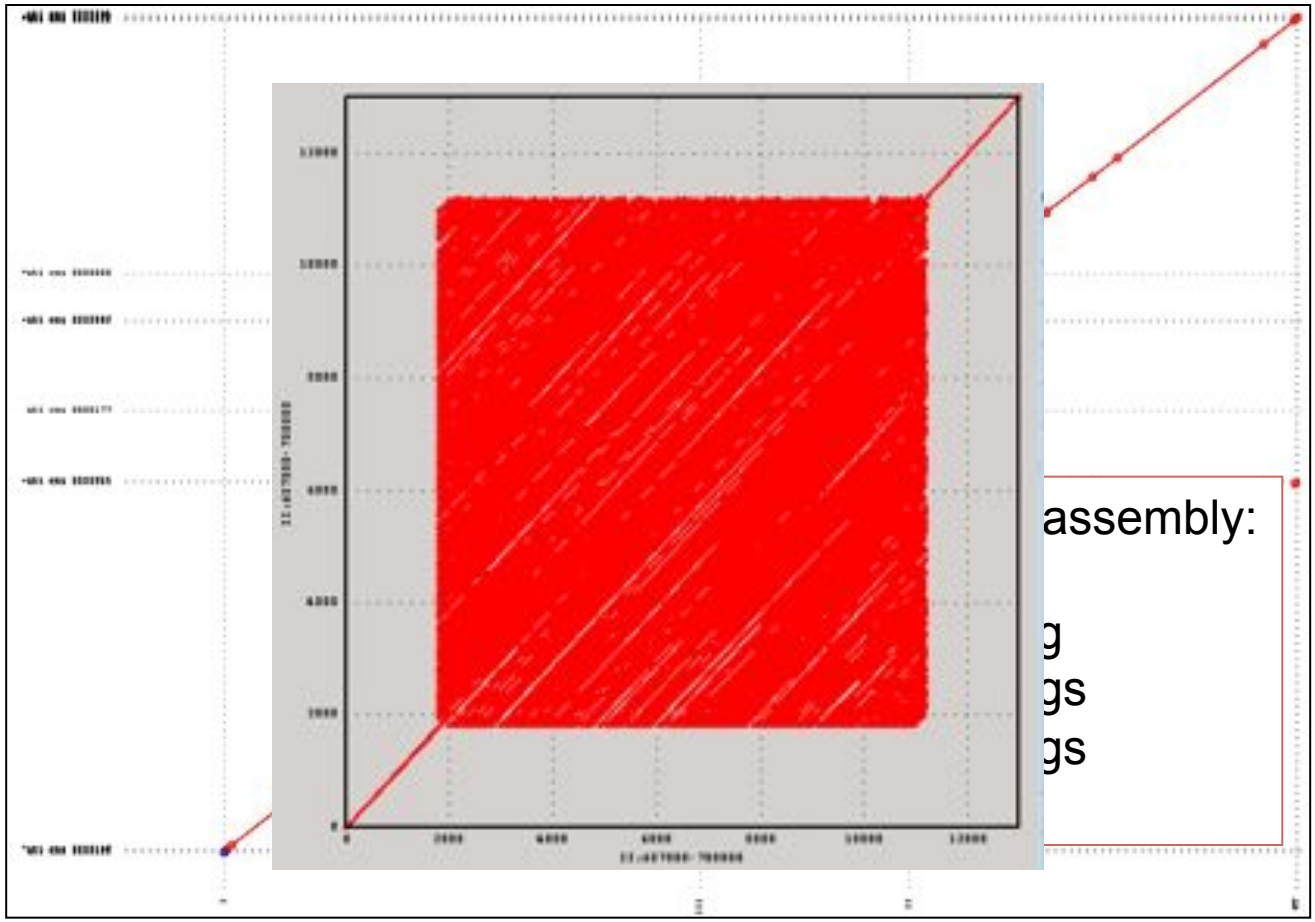
S. pombe dg21

ASM294 Reference sequence

- 12.6Mbp; 3 chromo + mitochondria; N50: 4.53Mbp

PacBio assembly using HGAP + Celera Assembler

- 12.7Mbp; 13 non-redundant contigs; N50: 3.83Mbp; >99.98% id



Spanning vs Standard Coverage

Standard Coverage
(SpanLength = 1bp)

$$\frac{\sum_{reads} Length(read)}{GenomeSize}$$

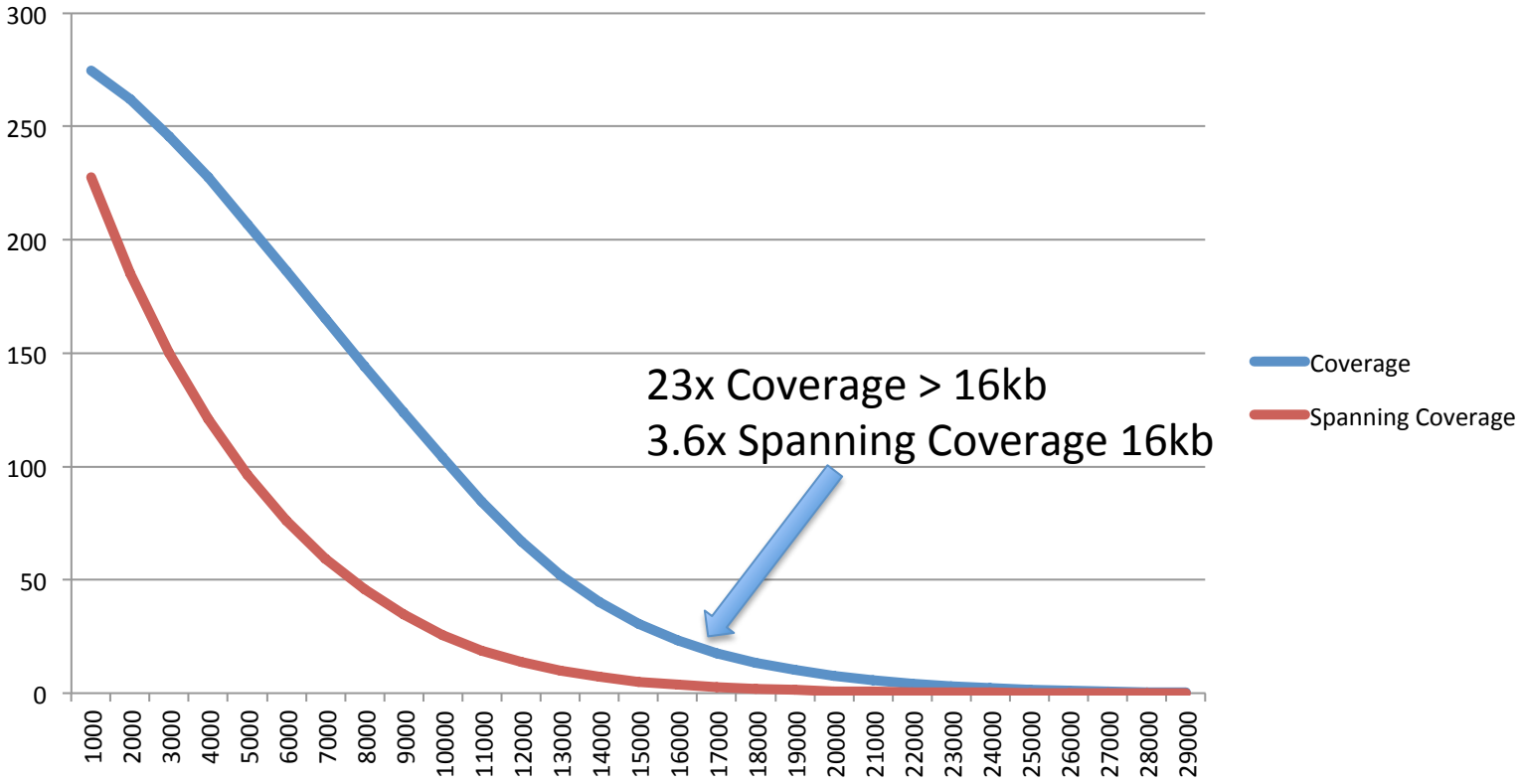
Spanning Coverage
(SpanLength > 1bp)

$$\frac{\sum_{reads} \max(0, Length(read) - SpanLength)}{GenomeSize}$$

Spanning Coverage (S. pombe)

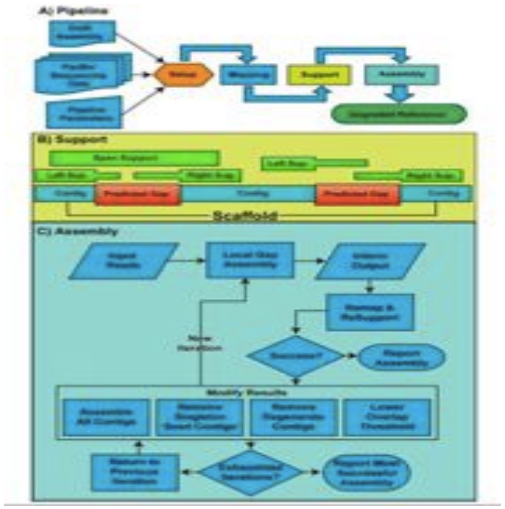
- **How many reads span a particular 16kb region?**

  23x Coverage of reads > 16kb, but only expect **3.6** reads to span a particular 16kb region



PacBio Correction/Assembly Algorithms

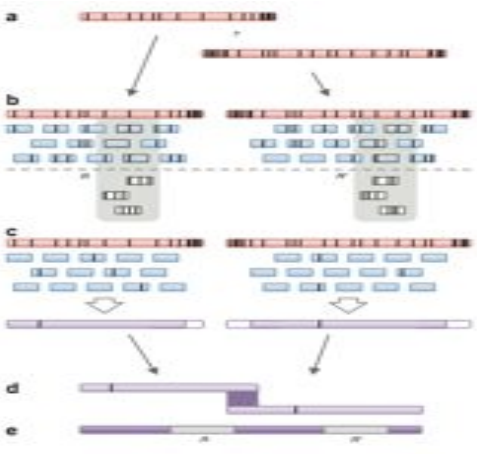
PBJelly



Gap Filling and Assembly Upgrade

English *et al* (2012)
PLOS One. 7(11): e47768

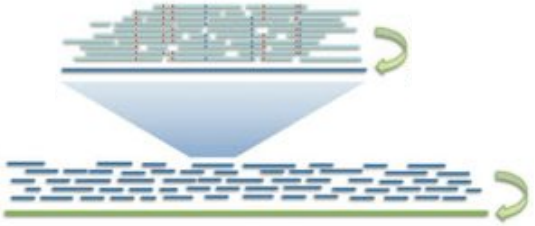
PacBioToCA & ECTools



Hybrid/PB-only Error Correction

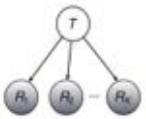
Koren, Schatz, *et al* (2012)
Nature Biotechnology. 30:693–700

HGAP & Quiver



$$\Pr(\mathbf{R} \mid T)$$

$$\Pr(\mathbf{R} \mid T) = \prod_k \Pr(R_k \mid T)$$



Quiver Performance Results
Comparison to Reference Genome
(M. ruber ; 3.1 MB ; SMRT® Cells)

	Initial Assembly	Quiver Consensus
QV	43.4	54.5
Accuracy	99.99540%	99.99964%
Differences	141	11

PB-only Correction & Polishing

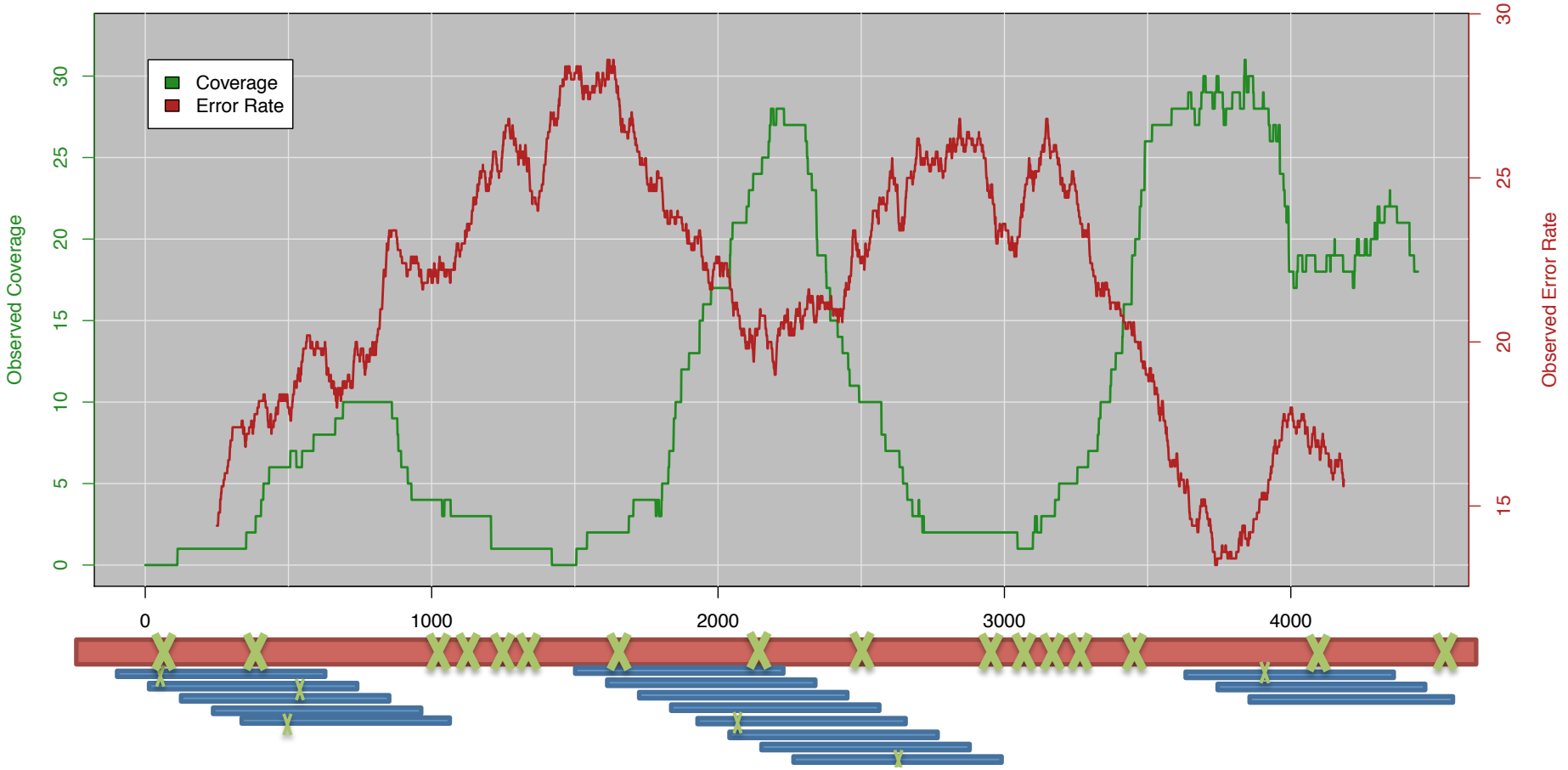
Chin *et al* (2013)
Nature Methods. 10:563–569

< 5x PacBio Coverage > 50x

Hybrid Approaches for Larger Genomes

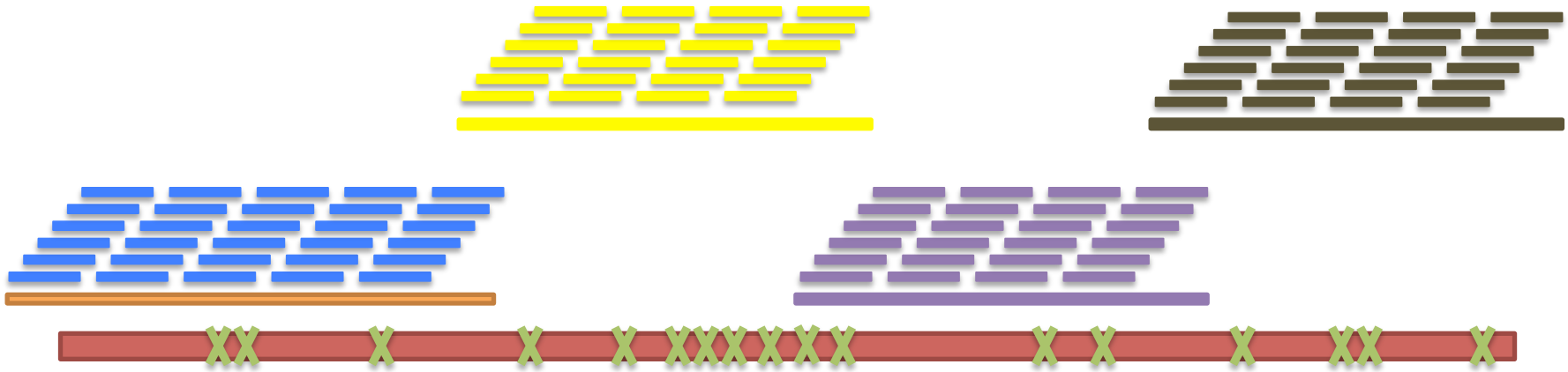
PacBioToCA fails in complex regions

1. Error Dense Regions – Difficult to compute overlaps with many errors
2. Simple Repeats – Kmer Frequency Too High to Seed Overlaps
3. Extreme GC – Lacks Illumina Coverage



ECTools: Error Correction with pre-assembled reads

https://github.com/jgurtowski/ectools



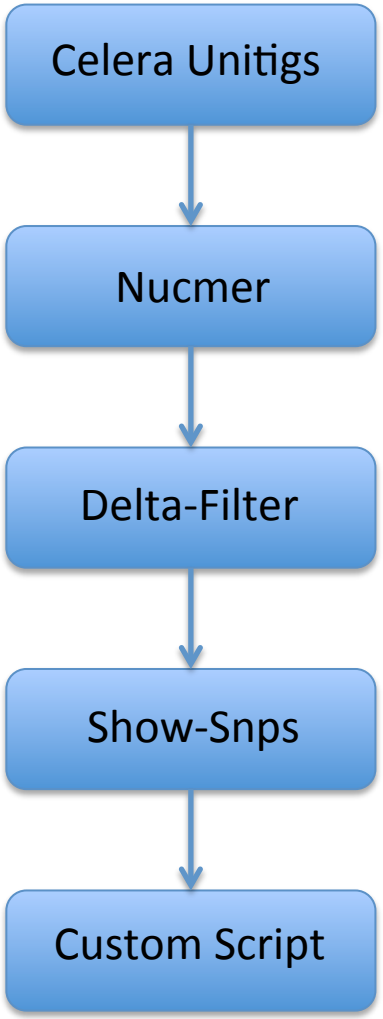
Short Reads -> Assemble Unitigs -> Align & Select - > Error Correct

Can Help us overcome:

1. Error Dense Regions – Longer sequences have more seeds to match
2. Simple Repeats – Longer sequences easier to resolve

However, cannot overcome Illumina coverage gaps & other biases

ECTools Pipeline



Generate Unitigs with Celera Using Illumina or another high identity sequencing technology

Align Unitigs to Pacbio Reads With Nucmer

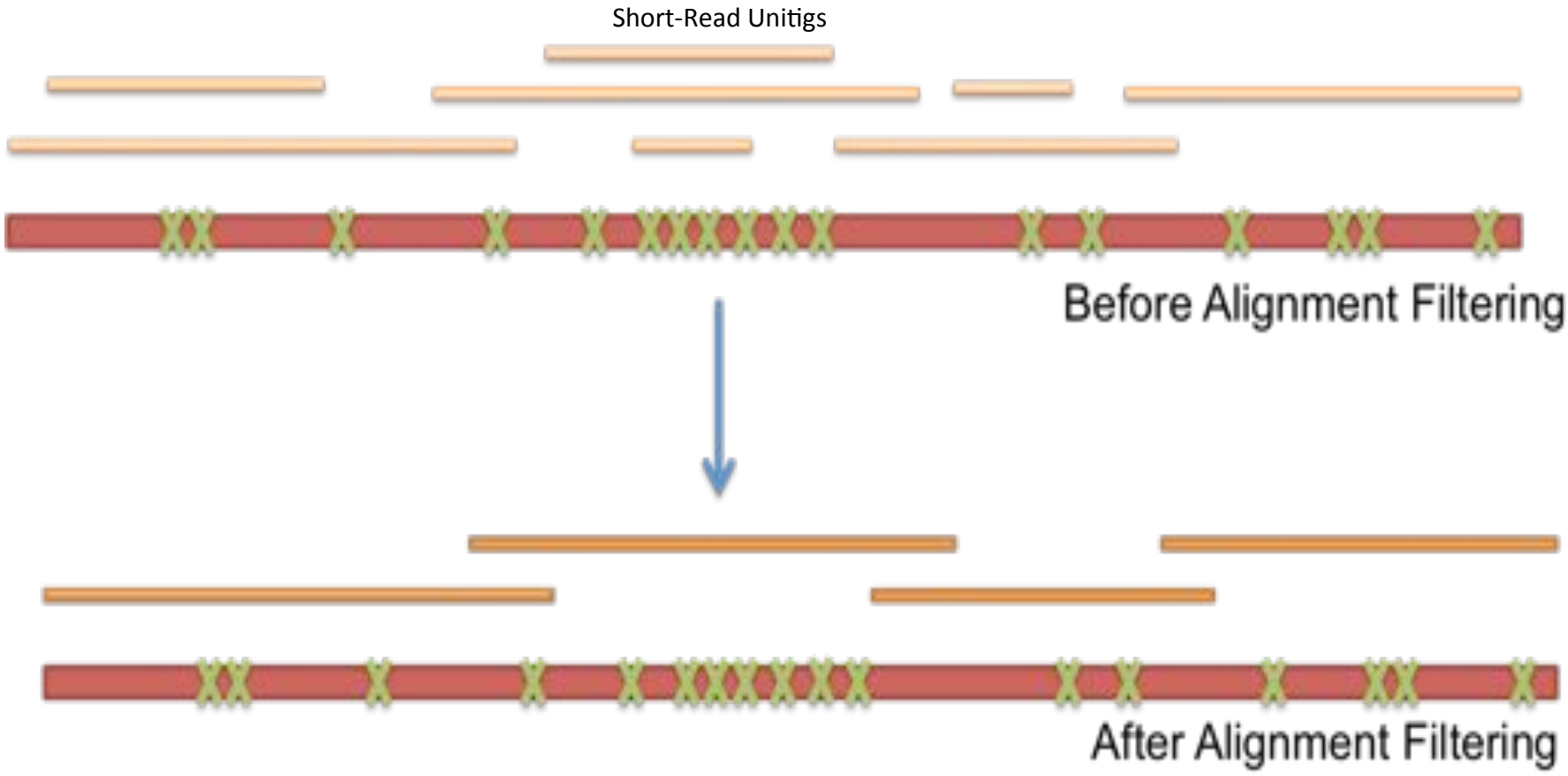
Use Delta-Filter to Generate Unitig Layout

Show-Snps shows differences between trusted Illumina Unitig Sequence and Pacbio Read

Script to “Correct” Pacbio Read

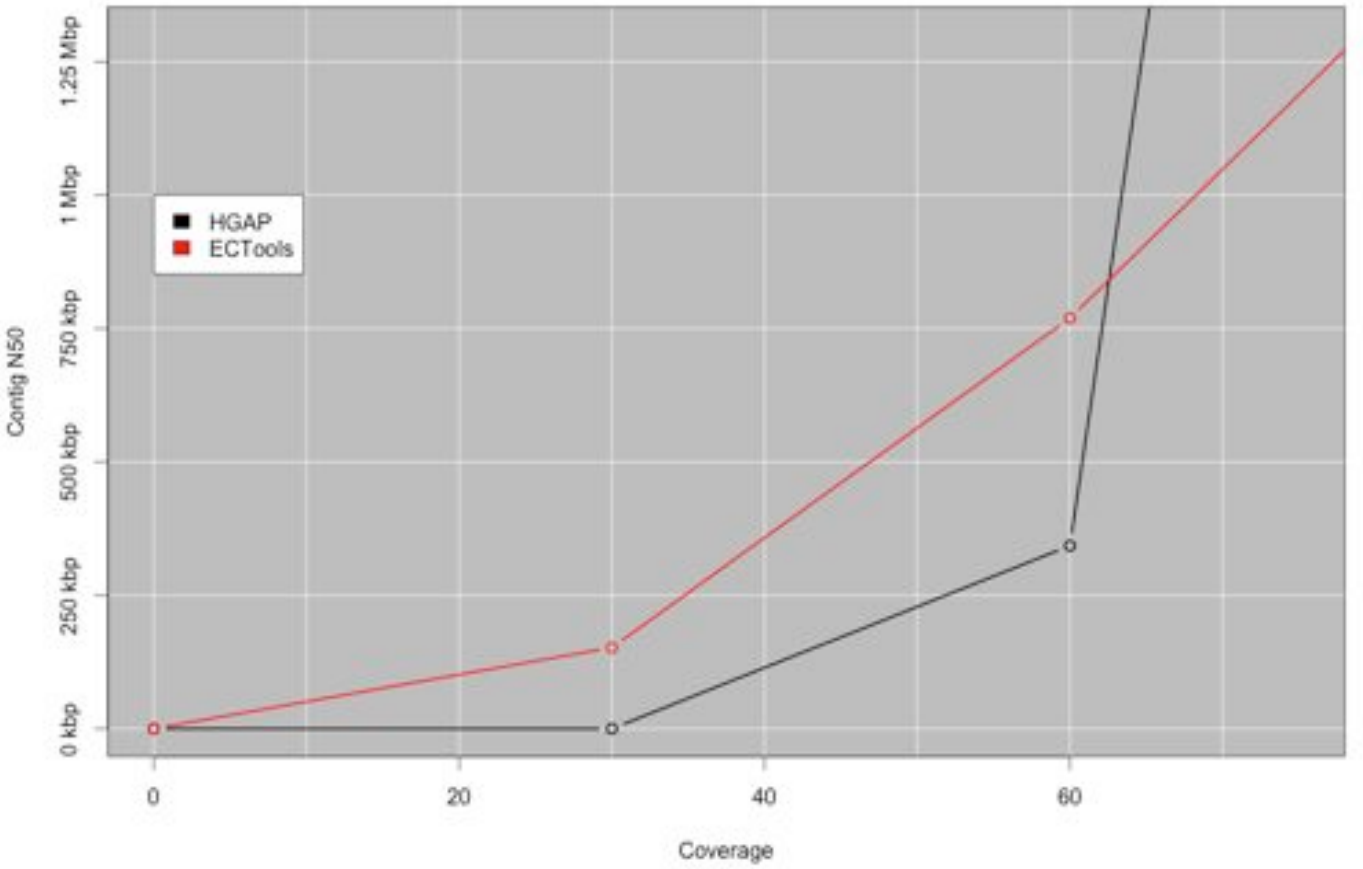
Note: Reads are never split or trimmed

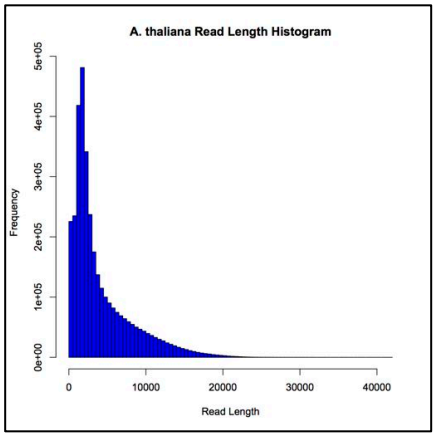
Delta-Filter Alignment filtering



A. thaliana Ler-0

http://blog.pacificbiosciences.com/2013/08/new-data-release-arabidopsis-assembly.html





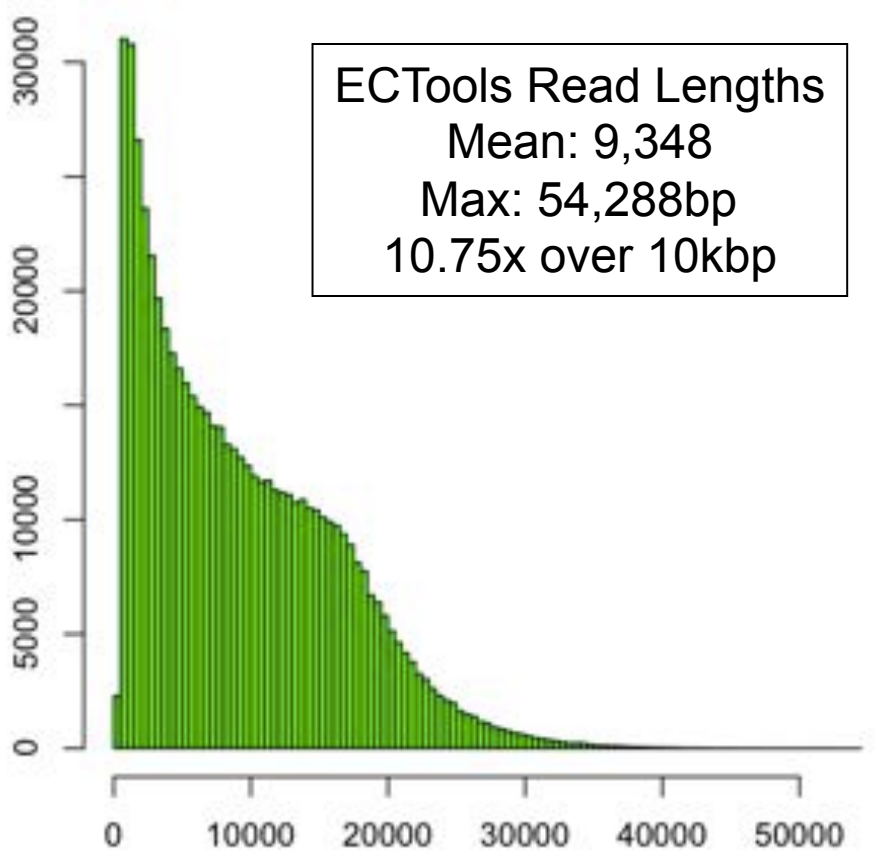
Mean: 4,137bp
Max: 41,753bp
Cov: 118x

High quality assembly of chromosome arms
Assembly Performance: 8.4Mbp/23Mbp = 36%
MiSeq assembly: 63kbp/23Mbp = .2%

O. sativa pv Indica (IR64)

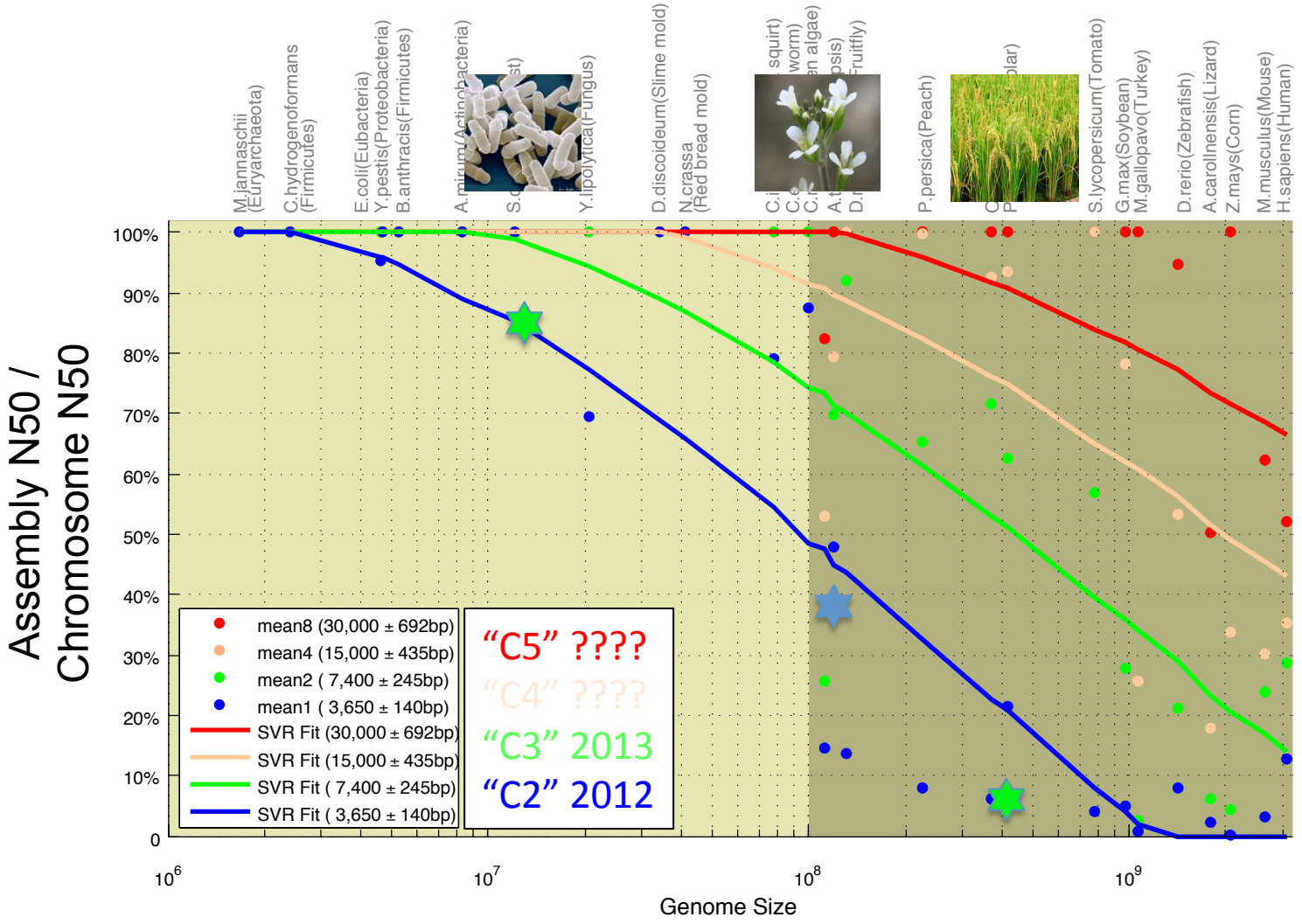
Genome size: ~370 Mb
Chromosome N50: ~29.7 Mbp

Assembly	Contig NG50
"ALLPATHS-recipe" 50x 2x100bp @ 180 36x 2x50bp @ 2100 51x 2x50bp @ 4800	18,450
MiSeq Fragments 25x 456bp (3 runs 2x300 @ 450 FLASH)	19,078
PacbioToCA – 47 SMRTCells 10.7x @ 10kbp	144,042
ECTools - 47 SMRTCells 10.7x @ 10kbp	272,137
NEW HGAP – 114 SMRTCells 29.2x @ 10kbp	600,021



ECTools Read Lengths
Mean: 9,348
Max: 54,288bp
10.75x over 10kbp

Real Data Results



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Acknowledgements

Cold Spring Harbor Laboratory

Schatz Lab
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ECTools:

https://github.com/jgurtowski/ectools