A near perfect de novo assembly of a eukaryotic genome using sequence reads of greater than 10 kilobases generated by the Pacific Biosciences RS II

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Disclosures

Orion Genomics – Founder and Shareholder
Cancer epigenetics and plant genomics

Previously Compensated Speaker for Illumina, Inc.

Previously Compensated Speaker for Pacific Biosciences, Inc.

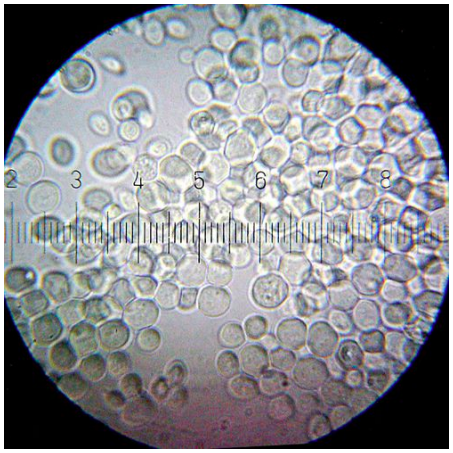
Introduction to the challenge

- Short read NGS has revolutionized resequencing
- *De novo* assembly is possible but not optimal with short reads
- Long reads improve the ability do *de novo* assembly dramatically
- Even in organisms with a good reference, such as humans, resequencing misses some structural differences relative to the reference
- Plant genomes are very large in general
- There are significant structural differences between different strains of the same plant such as rice
- These structural differences contribute to salient biological differences

Potential uses for rice genomes

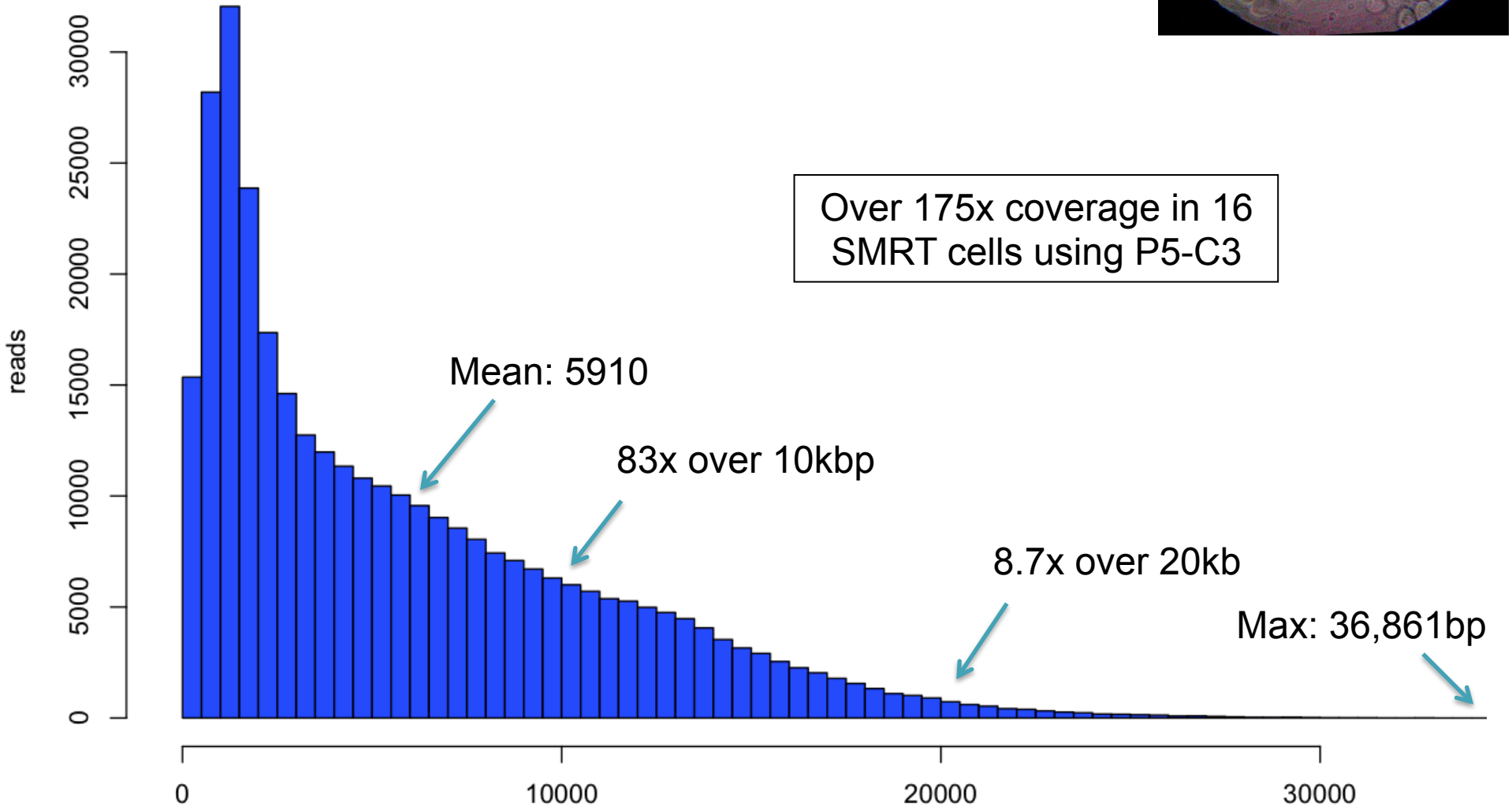
- Understand basis of differences among subpopulations and varieties (duplications, CNVs, etc.)that lead to important phenotypic differences - this requires de novo assemblies - not simple resequencing
- Many of these differences relate to ability to grow in less than optimum conditions
- Low phosphorus
- Submergence
- Drought
- Disease exposure

A test genome – yeast: S. cerevisiae W303

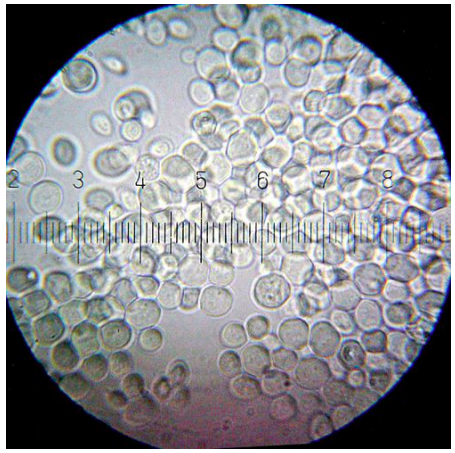


PacBio RS II sequencing at CSHL

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



S. cerevisiae W303

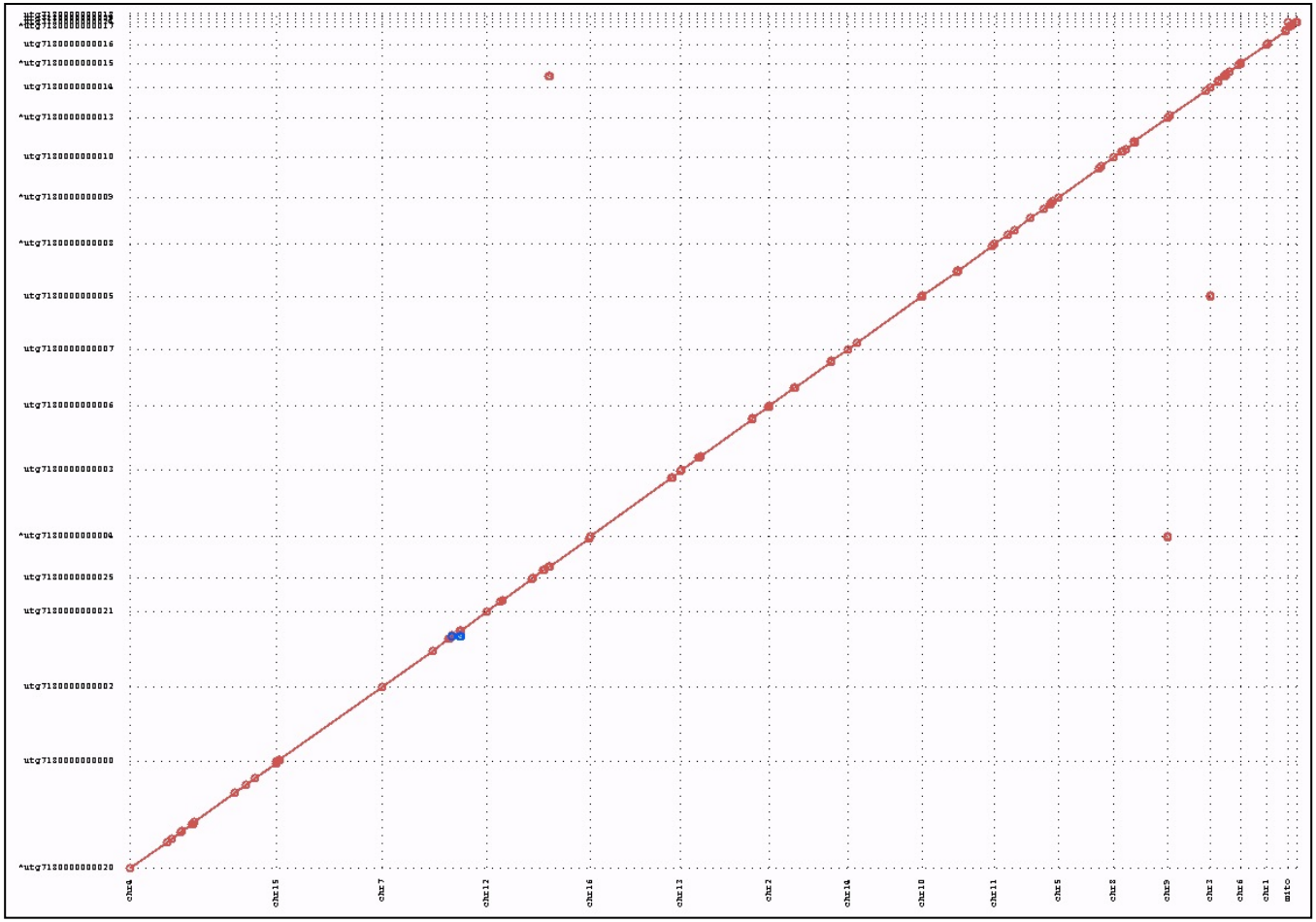


S288C Reference sequence

- 12.1Mbp; 16 chromo + mitochondria; N50: 924kbp

PacBio assembly using HGAP + Celera Assembler

- 12.4Mbp; 21 non-redundant contigs; N50: 811kbp; >99.8% id



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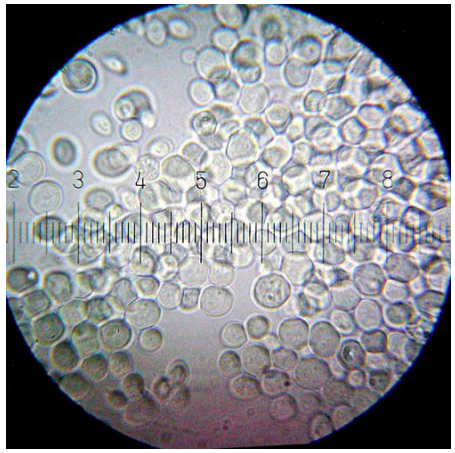

S288C Reference sequence

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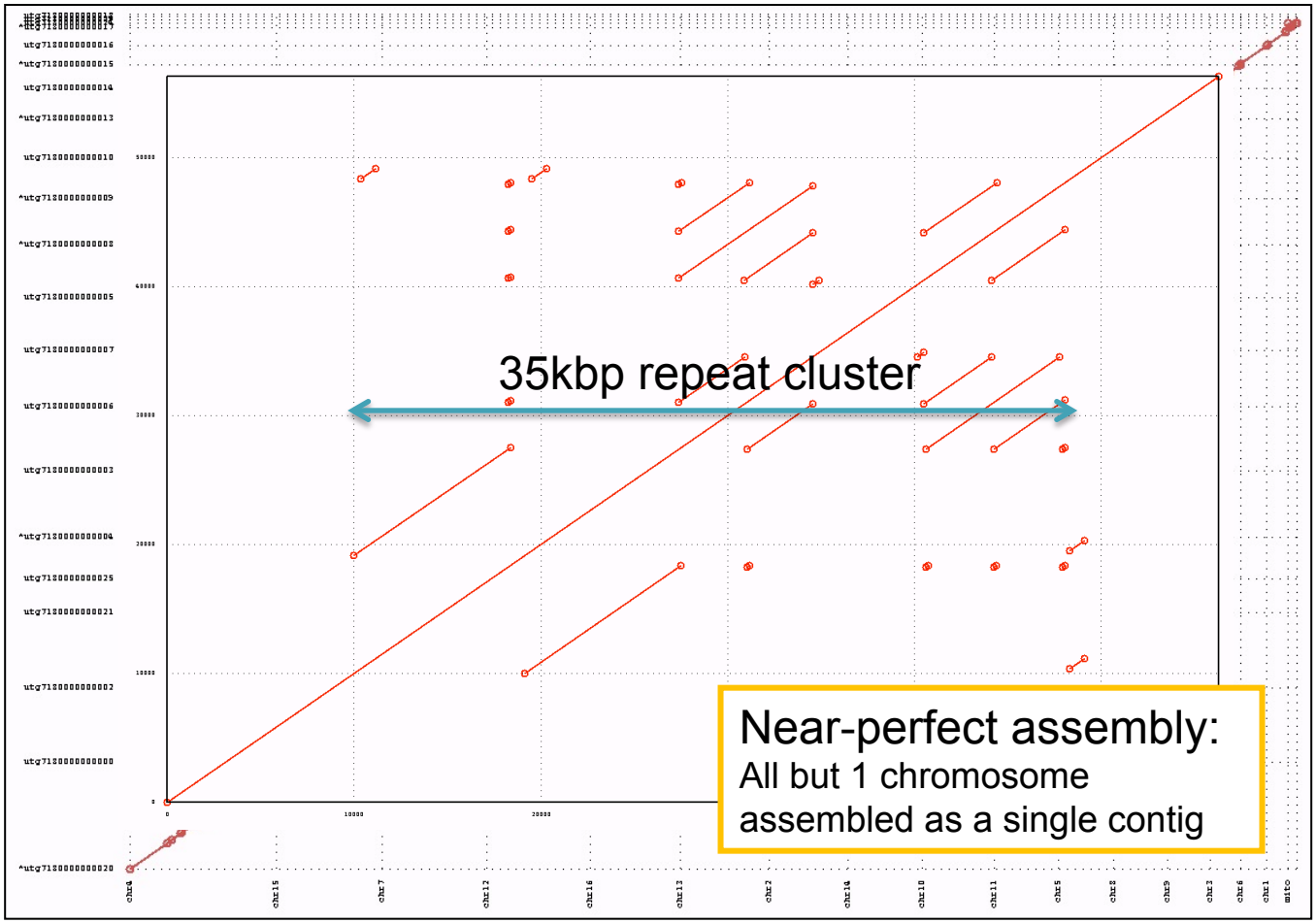
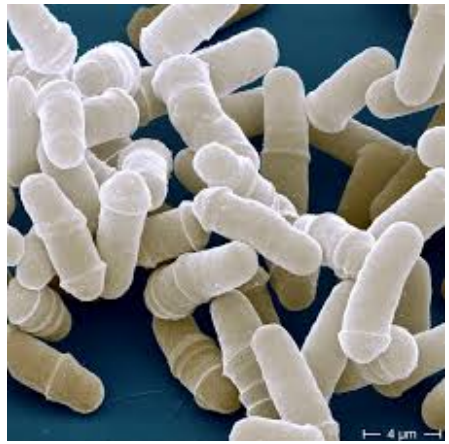
PacBio assembly using HGAP + Celera Assembler

- 12.4Mbp; 21 non-redundant contigs; N50: 811kbp; >99.9% id



S. pombe dg21



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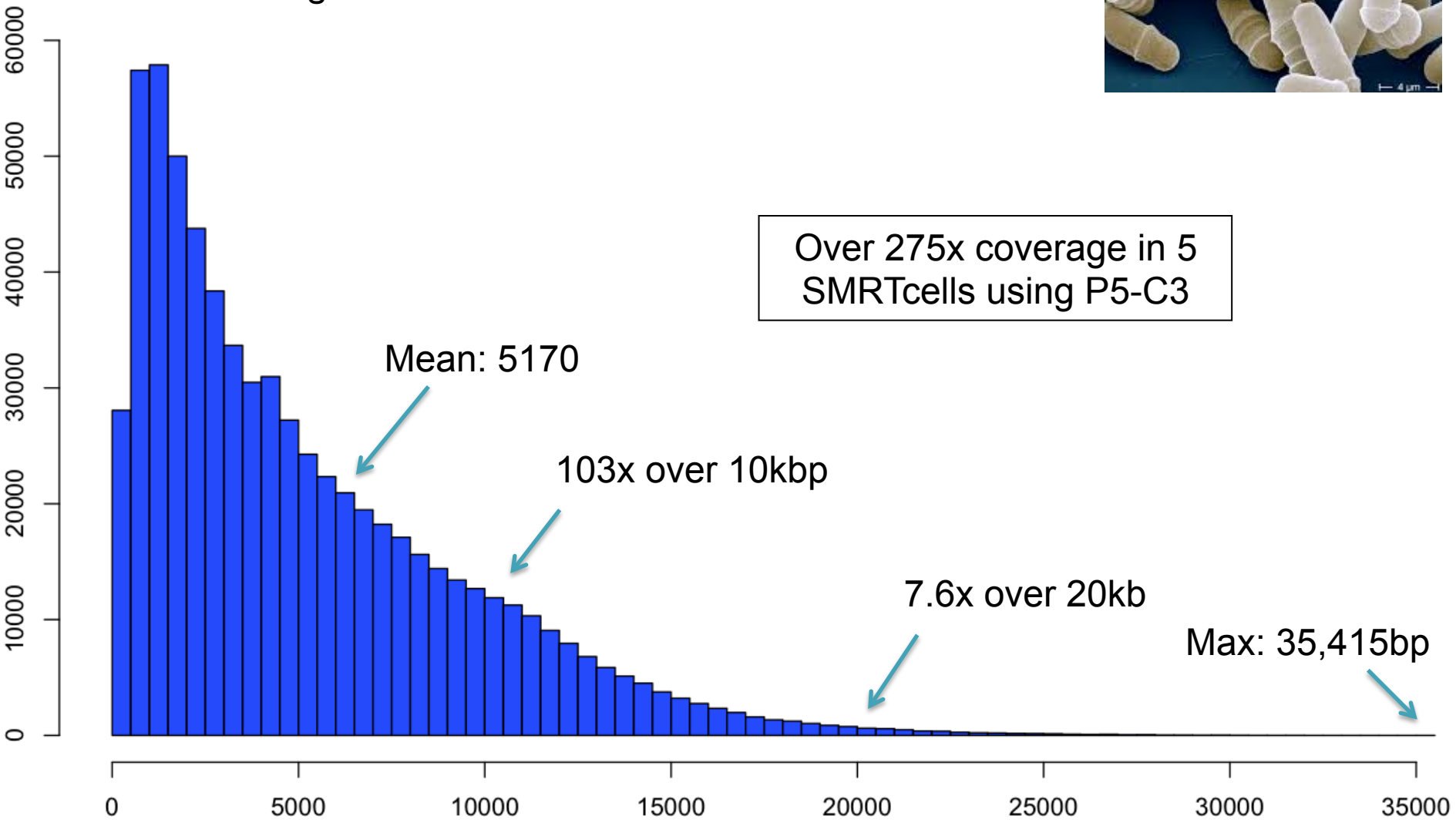
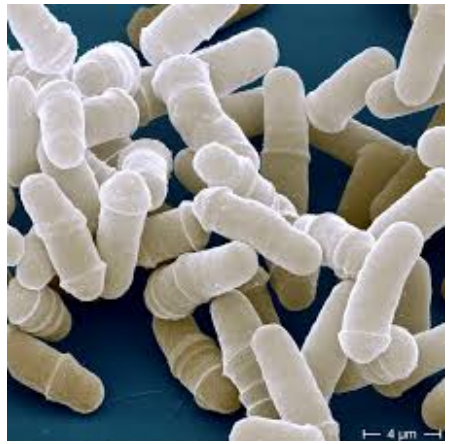


S. pombe dg21

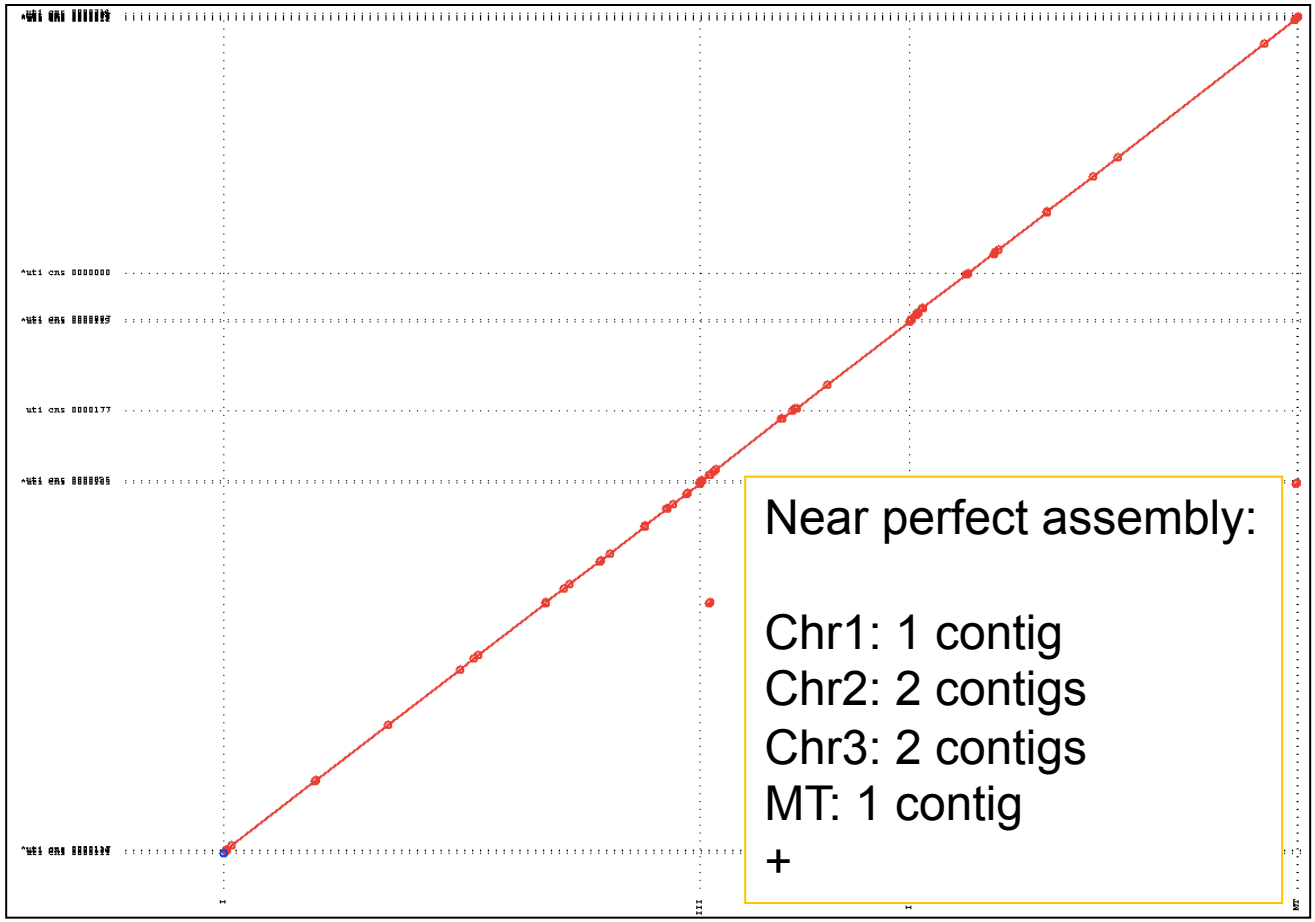


ASM294 Reference sequence

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

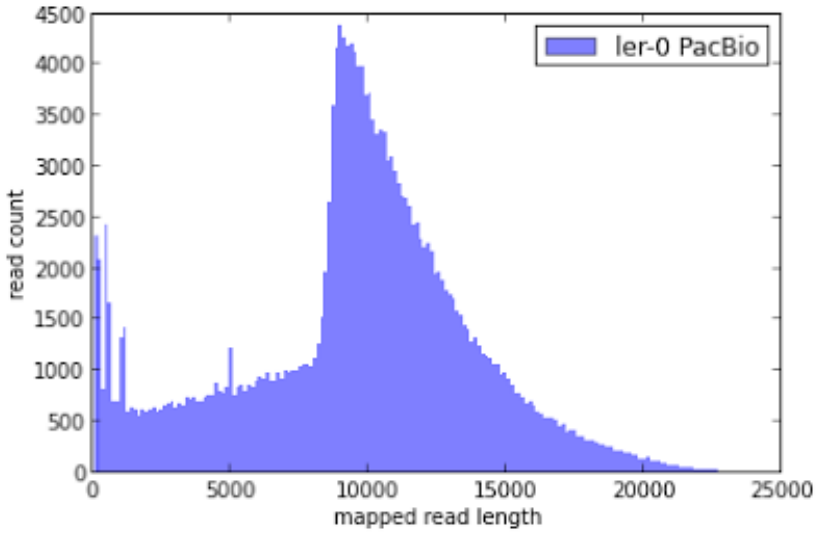
PacBio assembly using HGAP + Celera Assembler

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



A. thaliana Ler-0

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



A. thaliana Ler-0 sequenced at PacBio

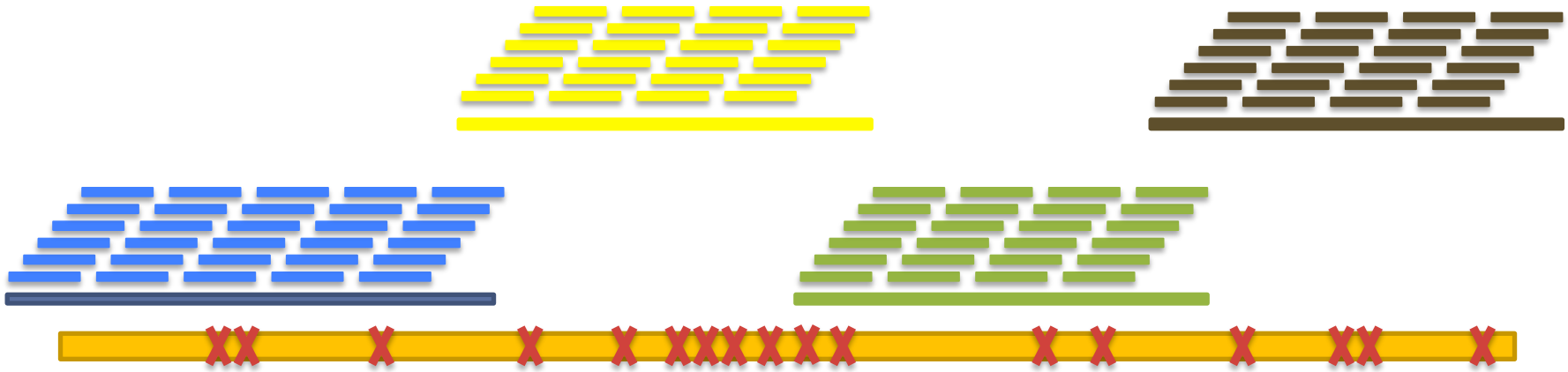
- Sequenced using the previous P4 enzyme and C2 chemistry
- Size selection using an 8 Kb to 50 Kb elution window on a BluePippin™ device from Sage Science
- Total coverage >119x

Genome size:	124.6 Mbp	Sum of Contig Lengths:	149.5Mb
Chromosome N50:	23.0 Mbp	N50 Contig Length:	8.4 Mb
Corrected coverage:	20x over 10kb	Number of Contigs:	1788

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

ECTools: Error Correction with pre-assembled reads

https://github.com/jgurtowski/ectools



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

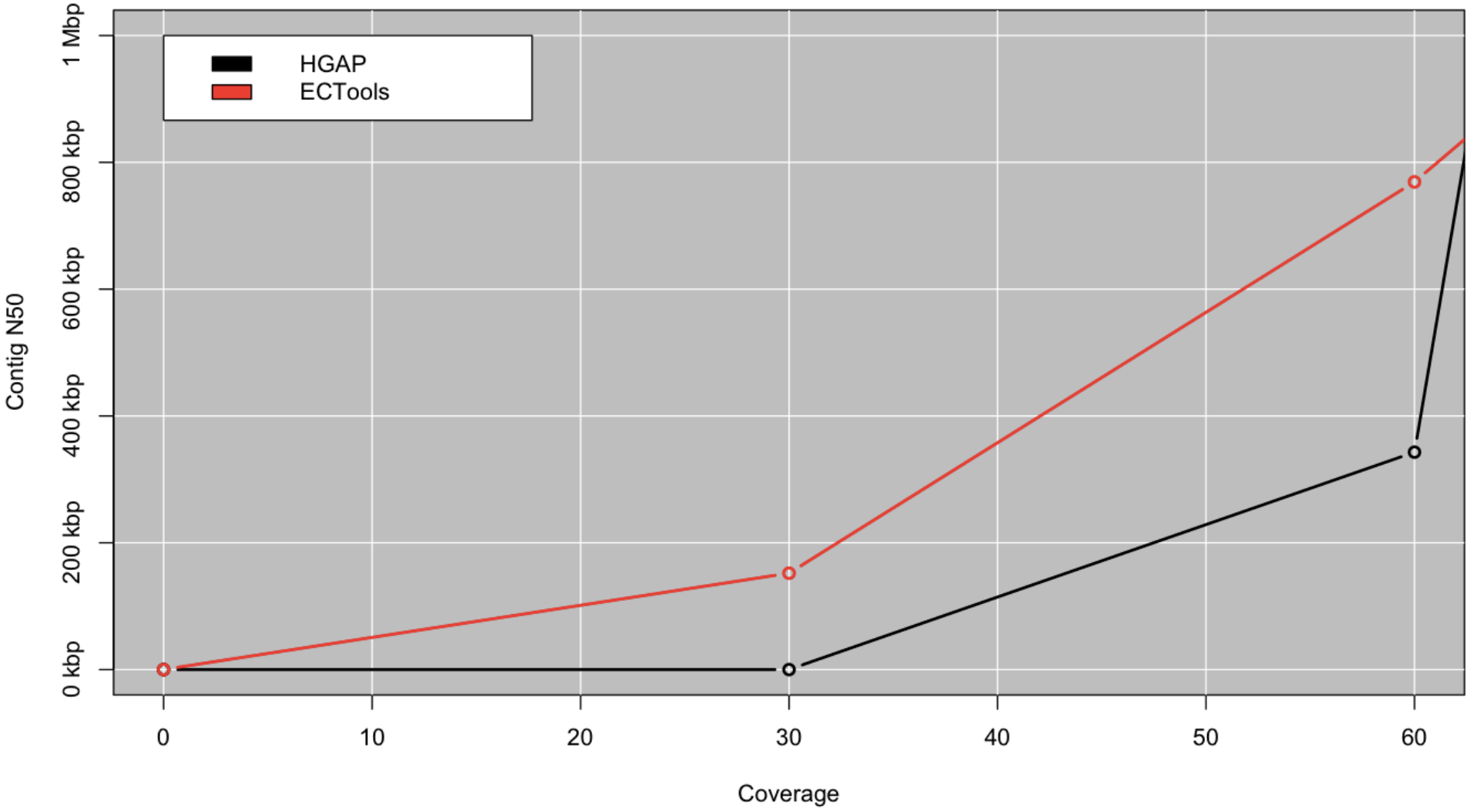
Can Help us overcome:

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

A. thaliana Ler-0

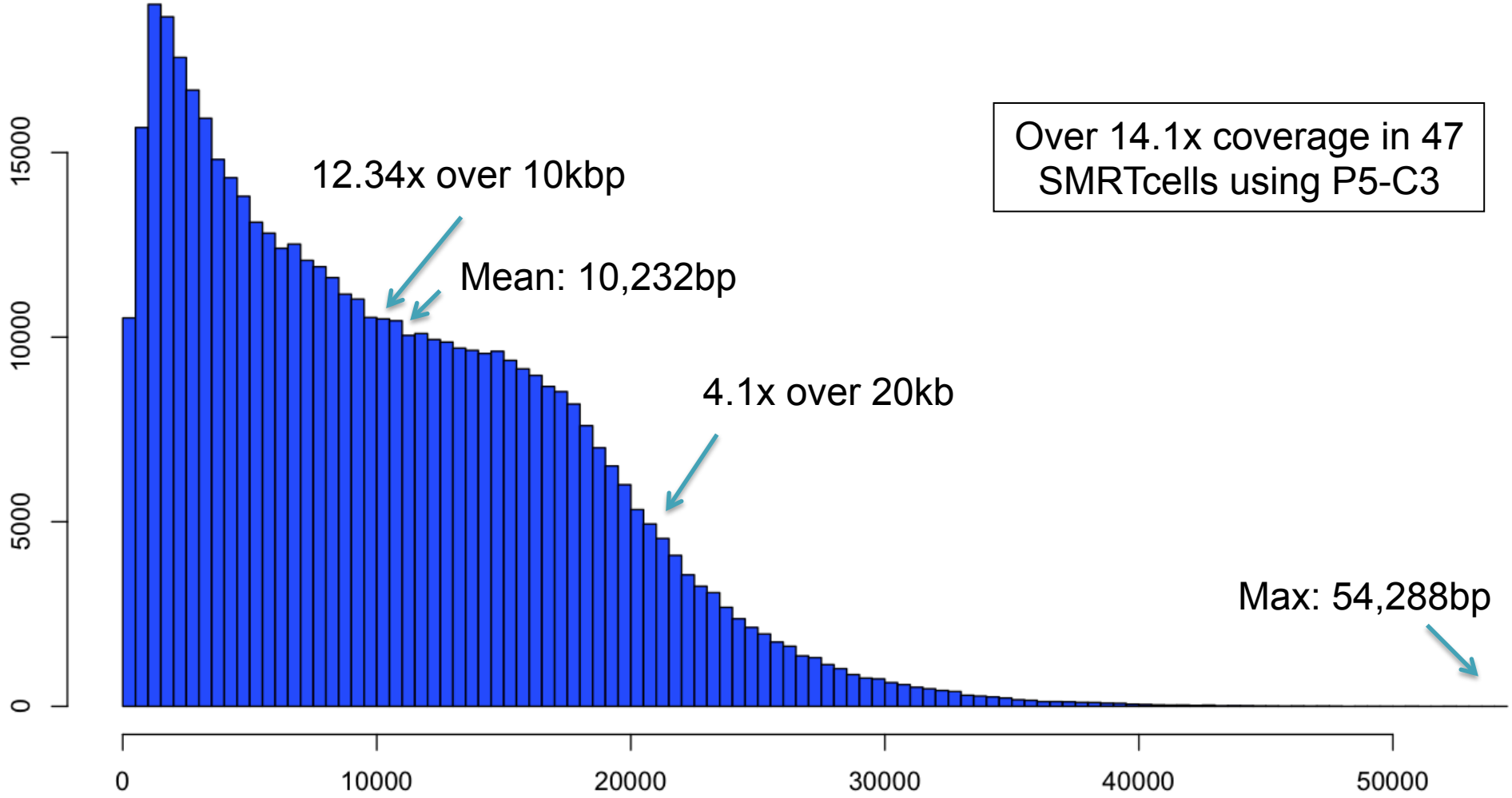
http://blog.pacificbiosciences.com/2013/08/new-data-release-arabidopsis-assembly.html



O. sativa pv Indica (IR64)

PacBio RS II sequencing at PacBio

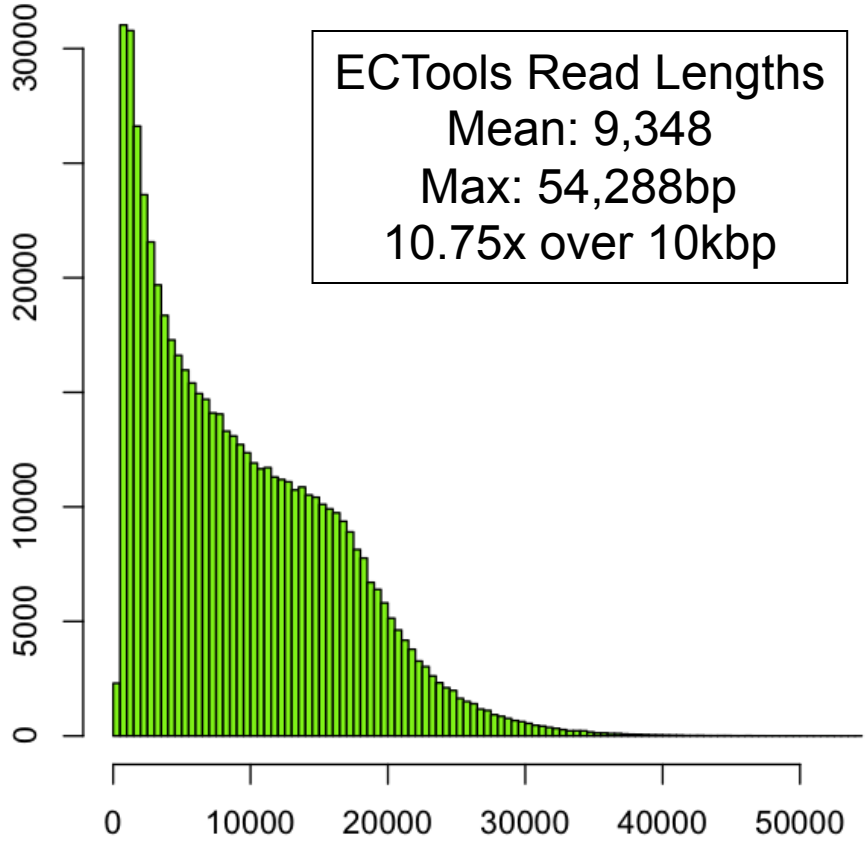
- Size selection using an 10 Kb elution window on a BluePippin™ device from Sage Science



O. sativa pv Indica (IR64)

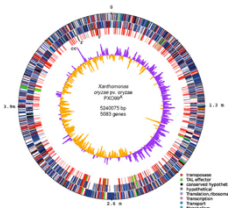
Genome size: ~370 Mb
Chromosome N50: ~29.7 Mbp

Assembly	Contig NG50
MiSeq Fragments 25x 456bp (3 runs 2x300 @ 450 FLASH)	19,078
"ALLPATHS-recipe" 50x 2x100bp @ 180 36x 2x50bp @ 2100 51x 2x50bp @ 4800	18,450
ECTools 10.7x @ 10kbp	271,885



Next steps

- Optimization of large fragment isolation and purification
- Optimization of loading SMRT cells – efficiency and consistency
- Target other yeast genomes – fermentation strains and various mutation containing strains
- Complete coverage of rice IR64
- Complete coverage of rice DJ123 (aus group)



Summary

- **Long read sequencing of eukaryotic genomes is here**
- **Technologies are quickly improving, exciting new scaffolding technologies**

- **Recommendations**
  - < 100 Mbp: HGAP/PacBio2CA @ 100x PB C3-P5
    expect near perfect chromosome arms
  - < 1GB: HGAP/PacBio2CA @ 100x PB C3-P5
    expect high quality assembly: contig N50 over 1Mbp
  - > 1GB: hybrid/gap filling
    expect contig N50 to be 100kbp – 1Mbp
  - ➢ 5GB: Email mschatz@cshl.edu
  - ➢ Poster Schatz Poster #221 @ 5:00pm

Acknowledgements

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