De novo assembly of complex genomes

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Oct 3, 2013

Beyond the Genome

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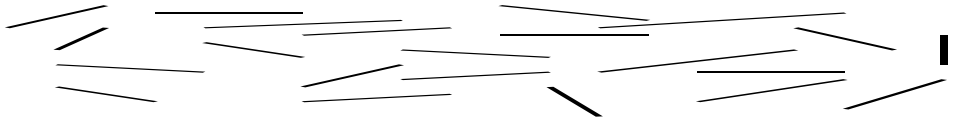
Assembling a Genome

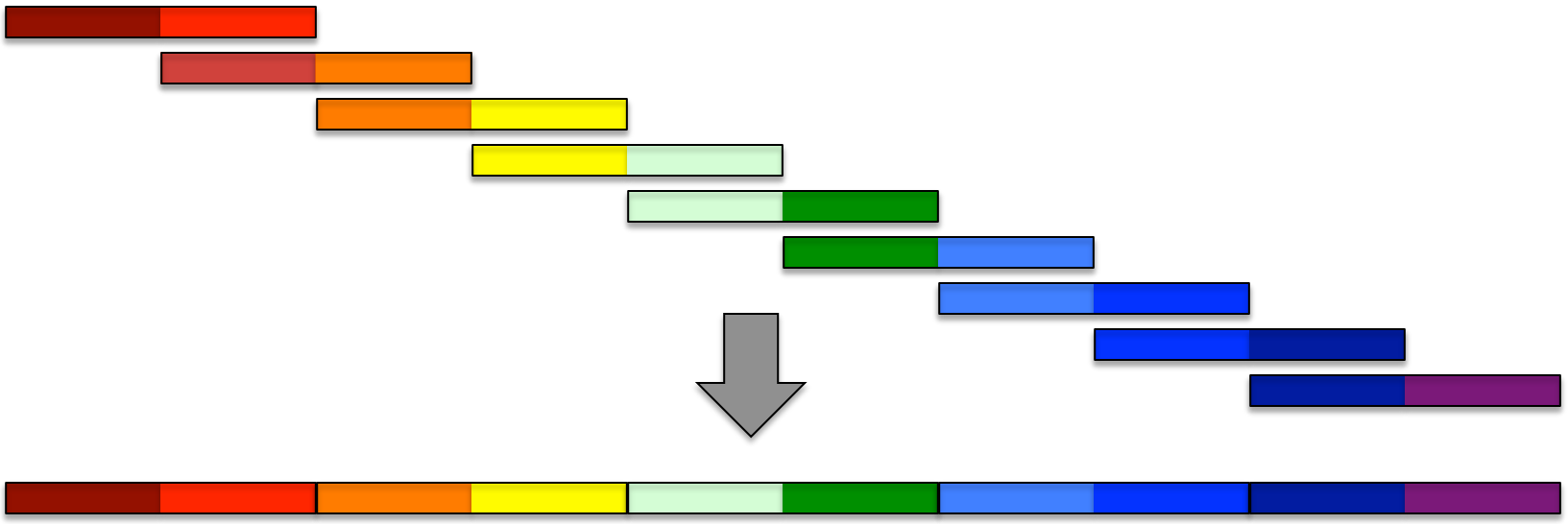
1. Shear & Sequence DNA



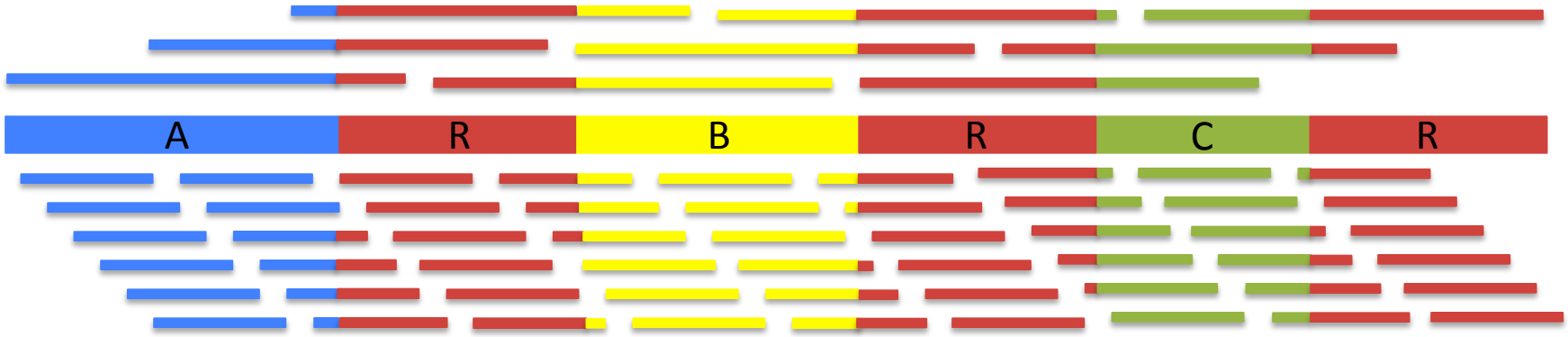
2. Construct assembly graph from overlapping reads

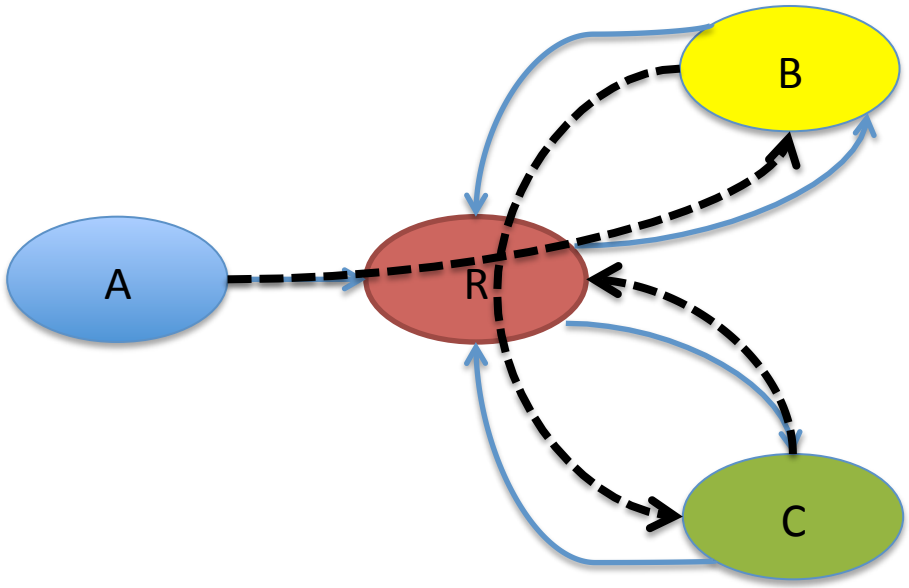
...AGCCTAGGGATGCGCGACACGT
GGATGCGCGACACGTCGCATATCCGGTTTGGTCAACCTCGGACGGAC
CAACCTCGGACGGACCTCAGCGAA...

3. Simplify assembly graph

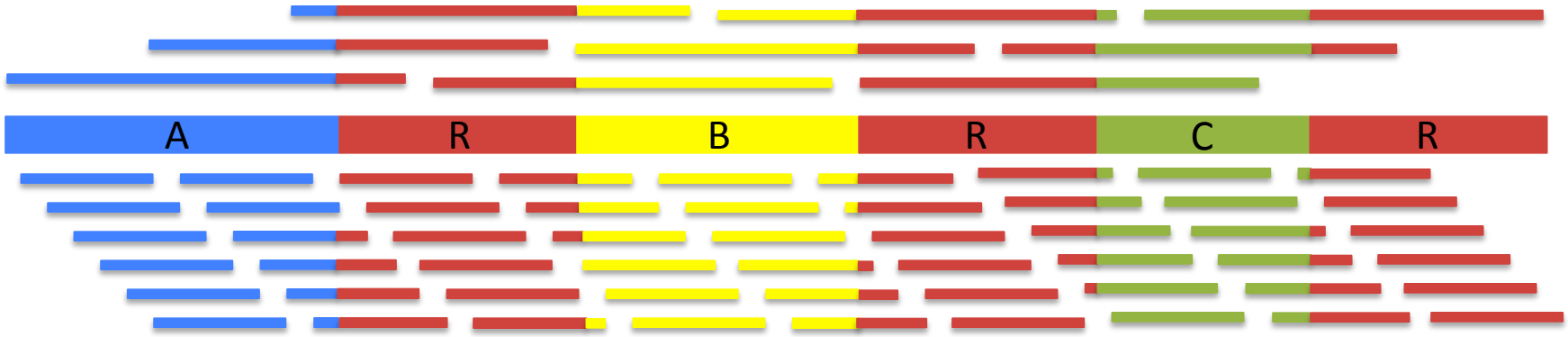


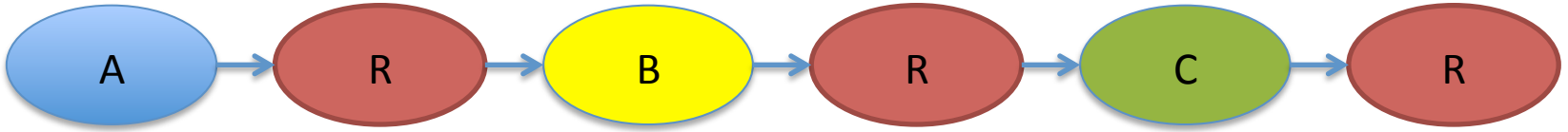
Assembly Complexity





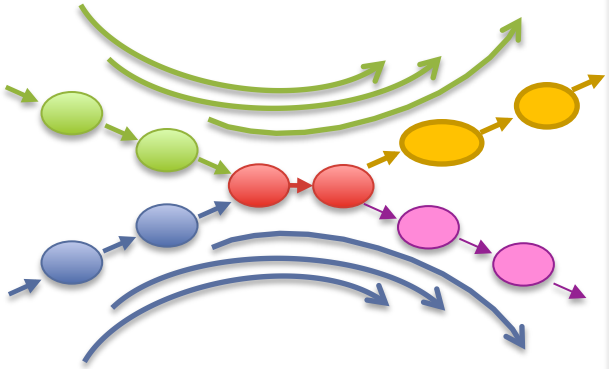
Assembly Complexity





Ingredients for a good assembly

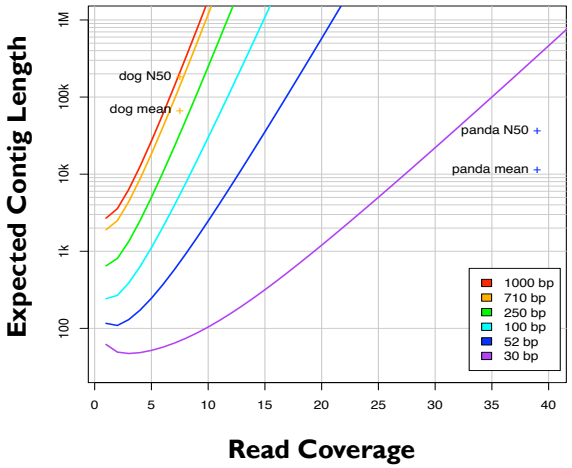
Read Length



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

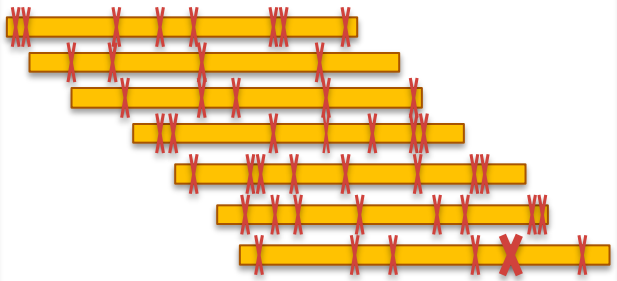
Coverage



High coverage is required

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

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

Current challenges in *de novo* plant genome sequencing and assembly
Schatz MC, Witkowski, McCombie, WR (2012) *Genome Biology*. 12:243

Hybrid Sequencing

Illumina

Sequencing by Synthesis

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

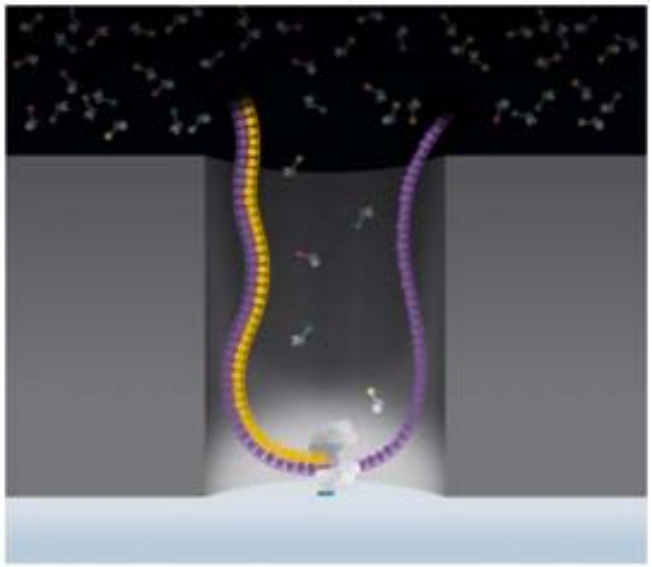
Pacific Biosciences

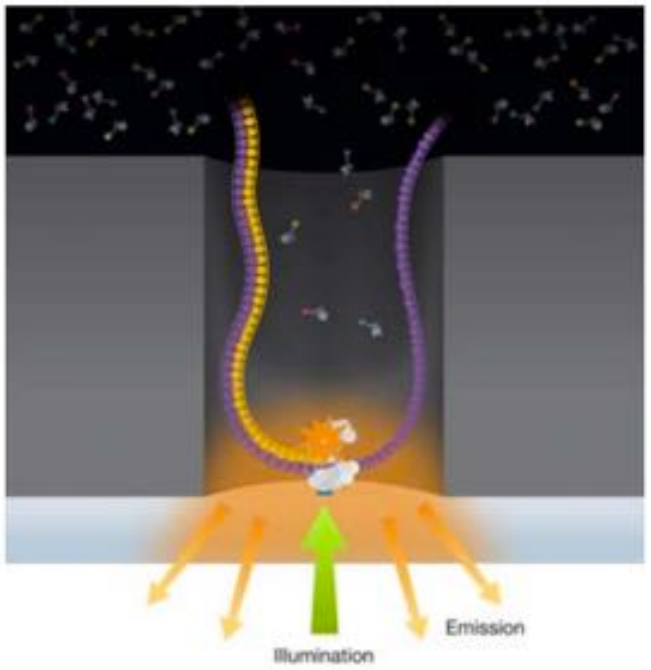
SMRT Sequencing

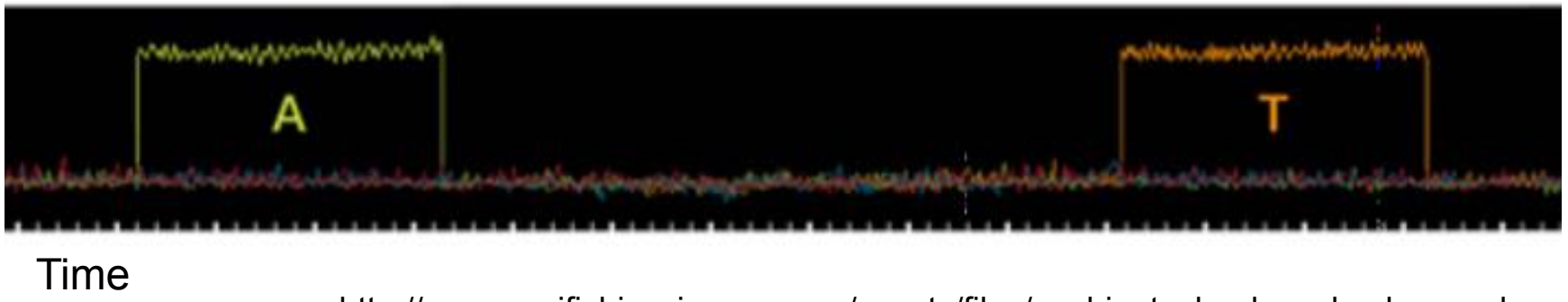
Lower throughput (1Gbp/day)
Lower accuracy (~85%)
Long reads (5kbp+)

SMRT Sequencing

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

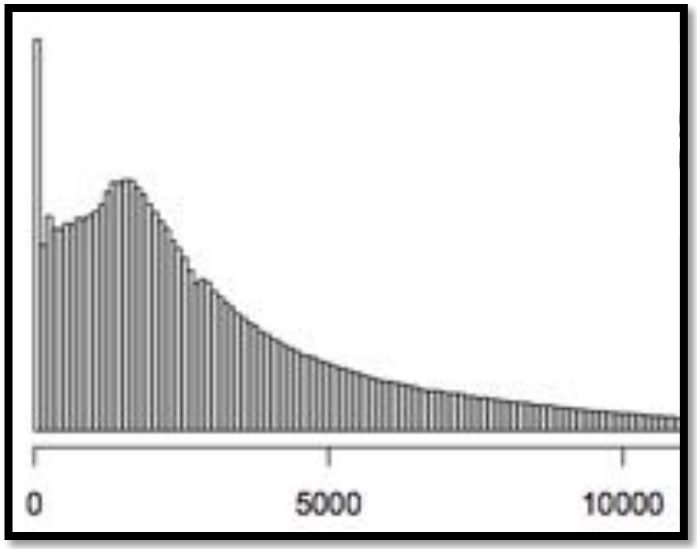






http://www.pacificbiosciences.com/assets/files/pacbio_technology_backgrounder.pdf

SMRT Sequencing Data



Match	83.7%
Insertions	11.5%
Deletions	3.4%
Mismatch	1.4%

```
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

PacBio Error Correction: HGAP

- With 50-100x of Pacbio coverage, virtually all of the errors can be eliminated
  - Works well for Microbial genomes: single contig per chromosome routinely achieved
  - Difficult to scale up for use with eukaryotic genomes

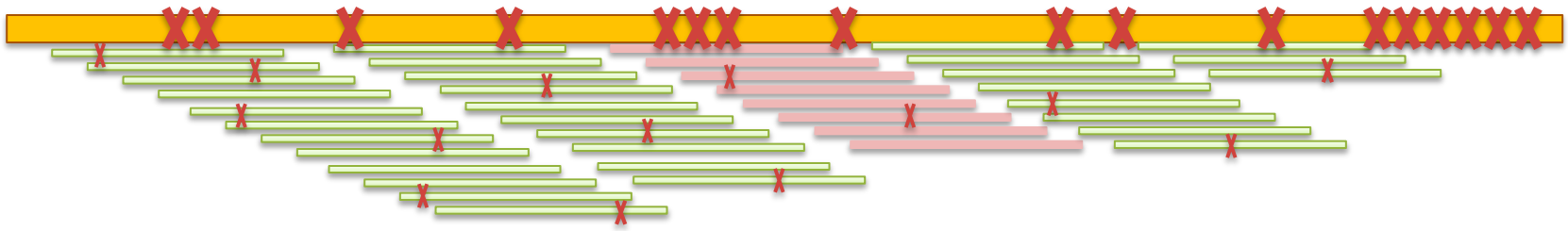
Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data
Chin, CS *et al.* (2013) *Nature Methods*. 10: 563-569

Hybrid Error Correction: PacBioToCA

http://wgs-assembler.sf.net

1. Correction Pipeline
    1. Map short reads to long reads
    2. Trim long reads at coverage gaps
    3. Compute consensus for each long read

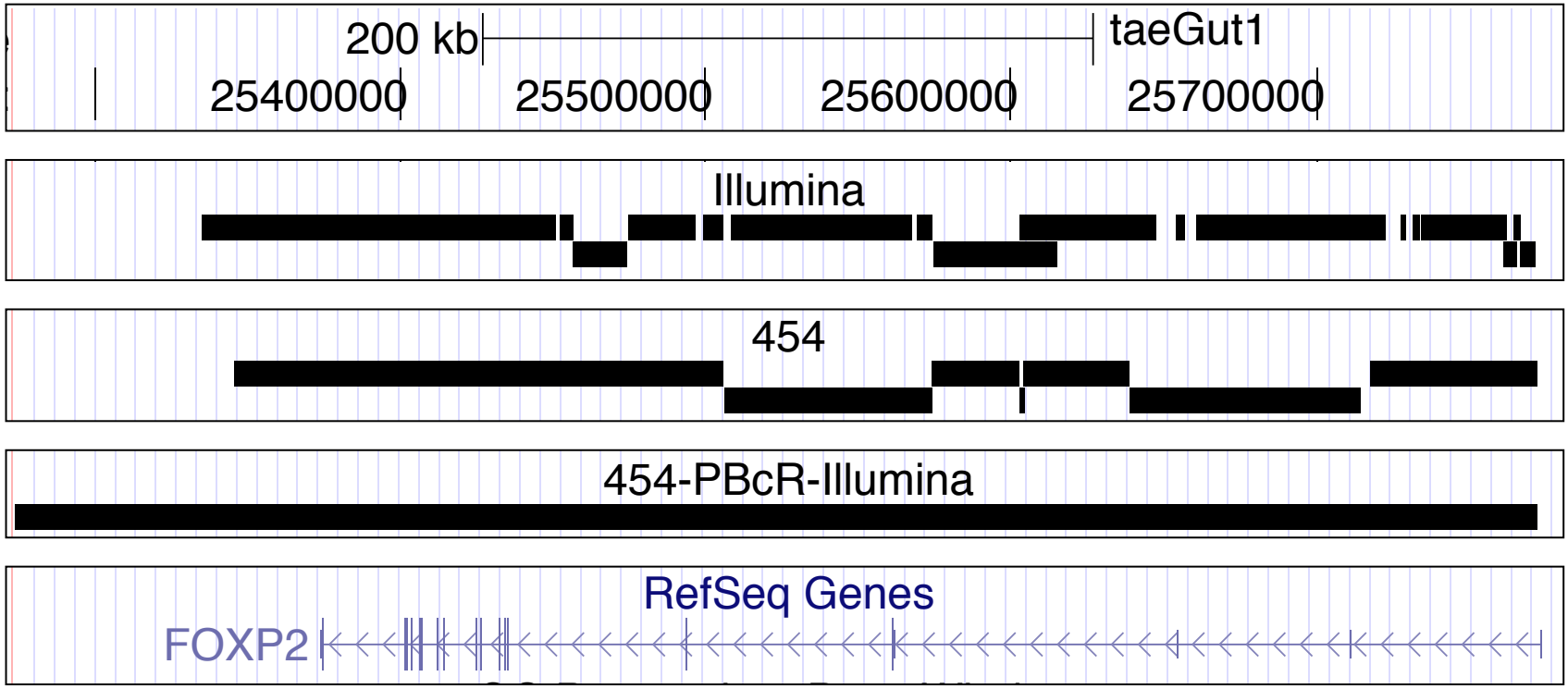
2. Error corrected reads can be easily assembled, aligned



Hybrid error correction and de novo assembly of single-molecule sequencing reads.
Koren, S, Schatz, MC, et al. (2012) *Nature Biotechnology*. doi:10.1038/nbt.2280

Improved Gene Reconstruction

FOXP2 assembled in a single contig in the PacBio parrot assembly



Hybrid error correction and de novo assembly of single-molecule sequencing reads.
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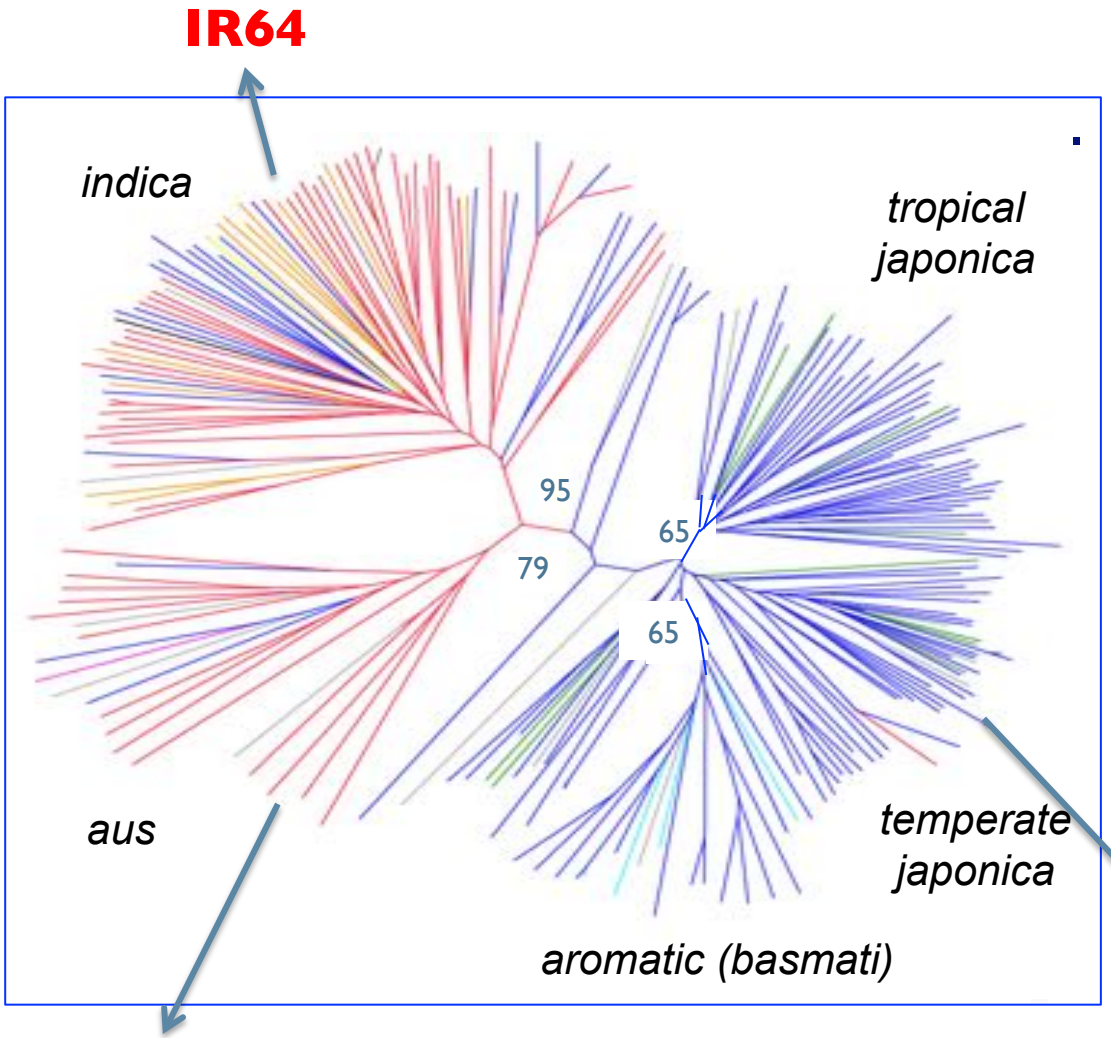
Population structure in *Oryza sativa*

3 varieties selected for *de novo* sequencing

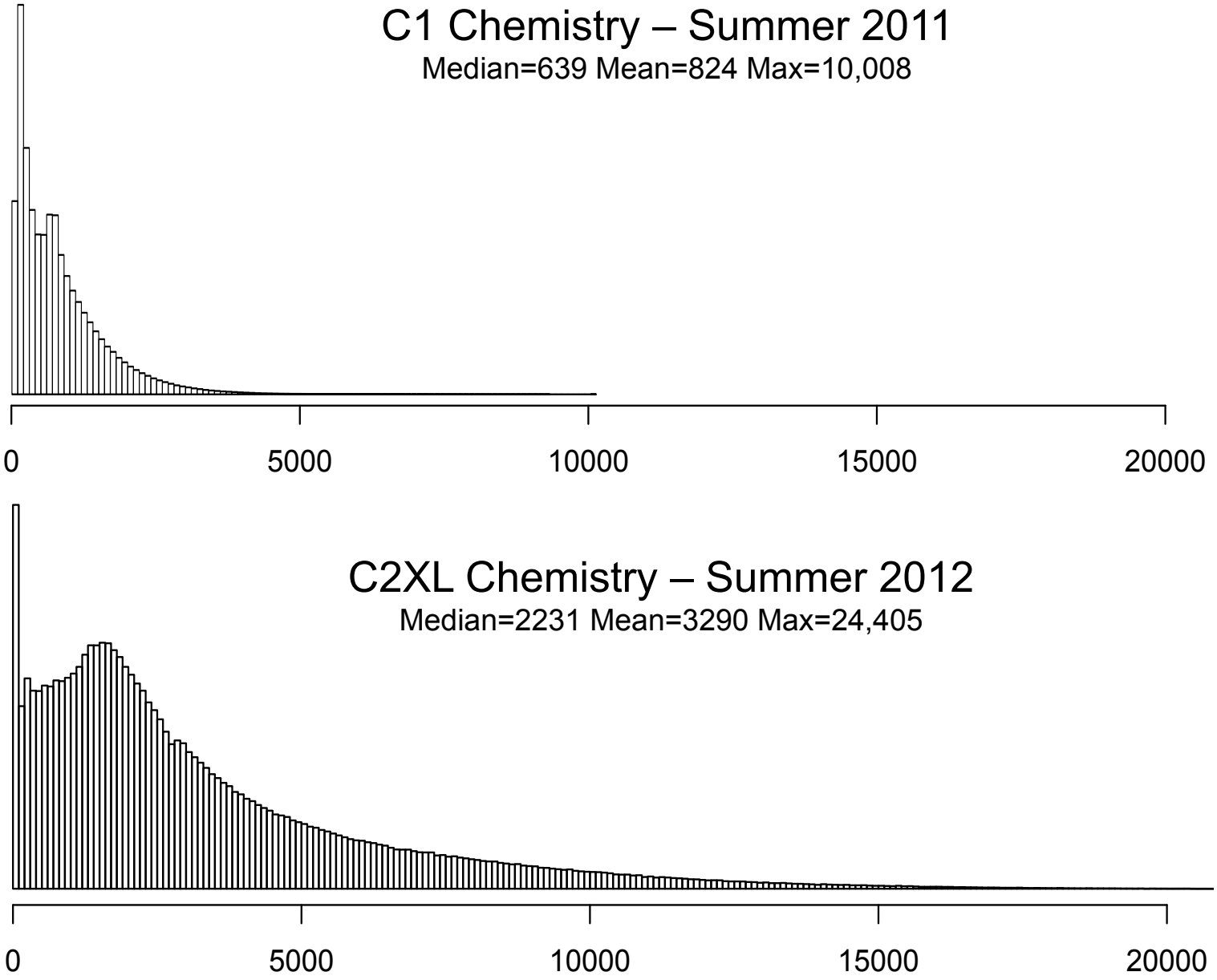


Garris et al. (2005)
Genetics 169: 1631–1638

High quality BAC-by-BAC reference

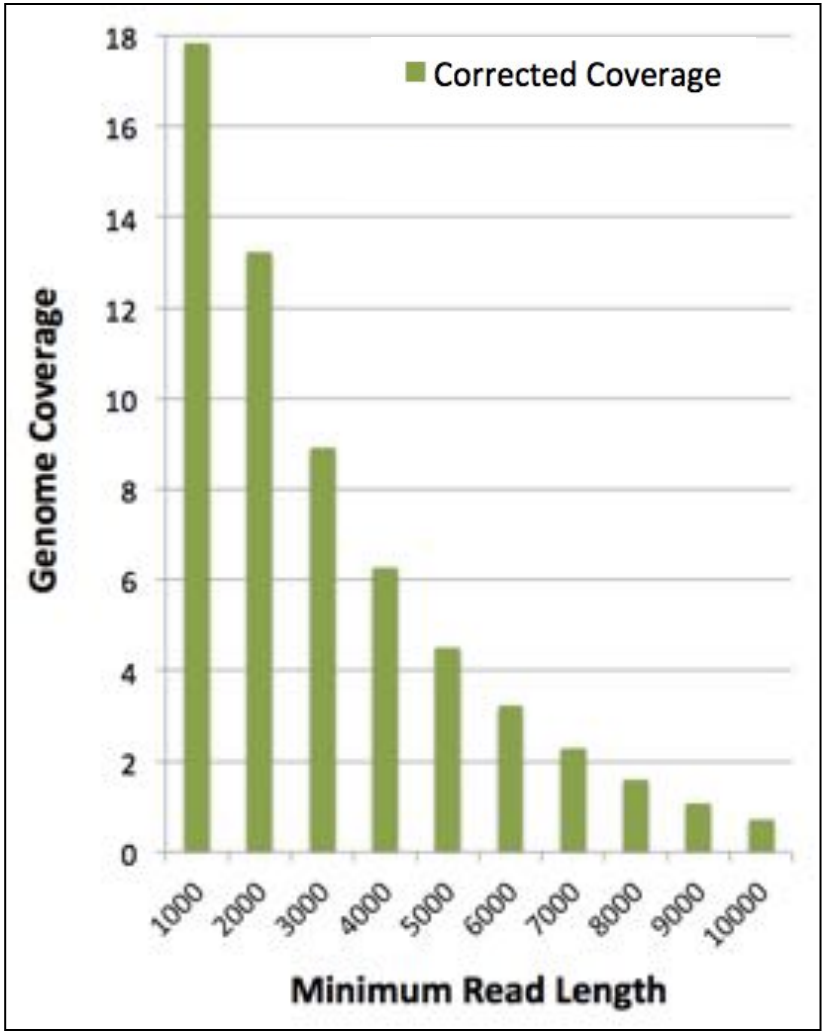
- ~370 Mbp genome in 12 chromosomes
- About 40% repeats:
  - Many 4-8kbp repeats
  - 300kbp max high identity repeat (99.99%)
- Useful model for other cereal genomes

PacBio Long Read Rice Sequencing



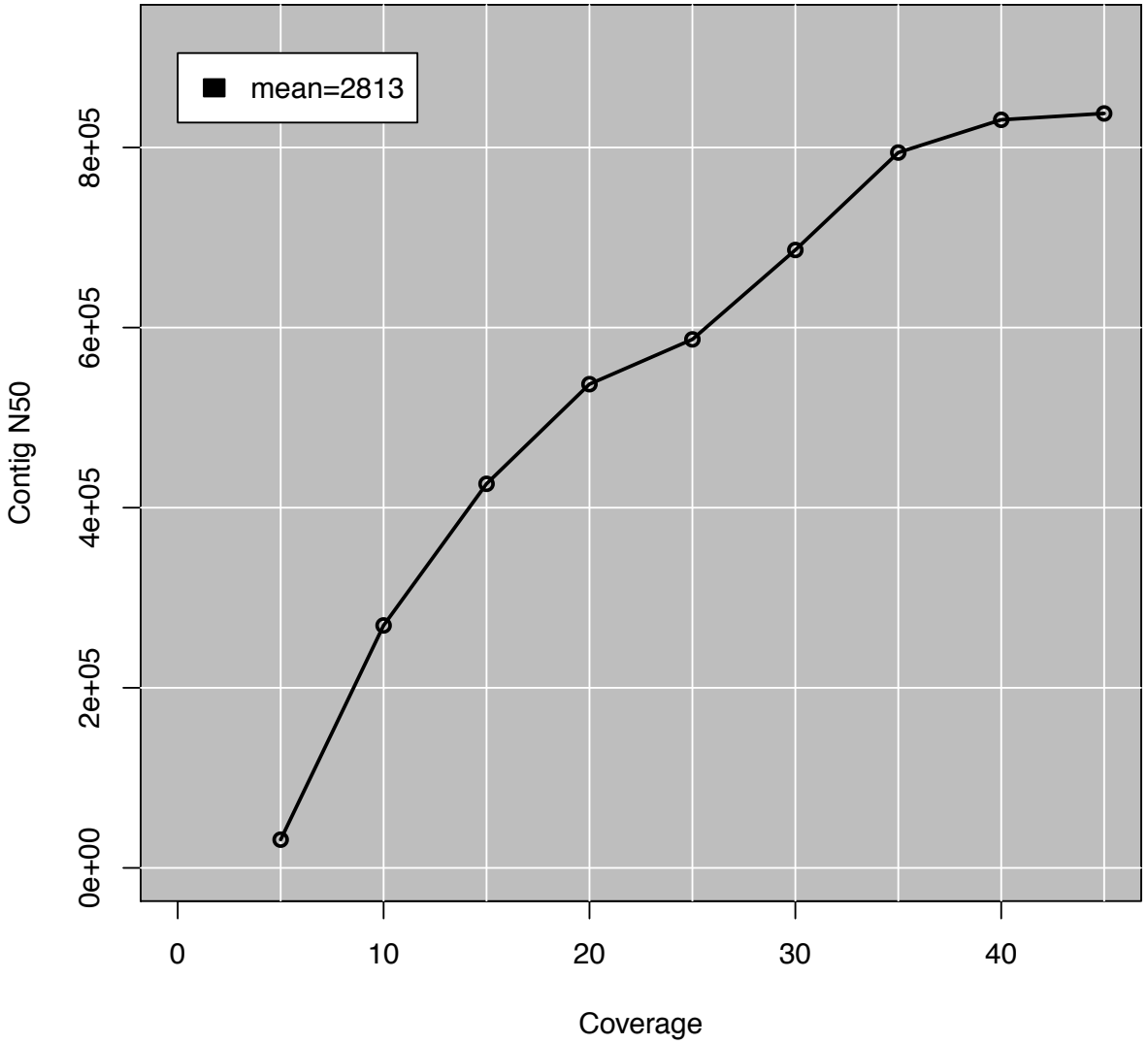
Preliminary Rice Assemblies

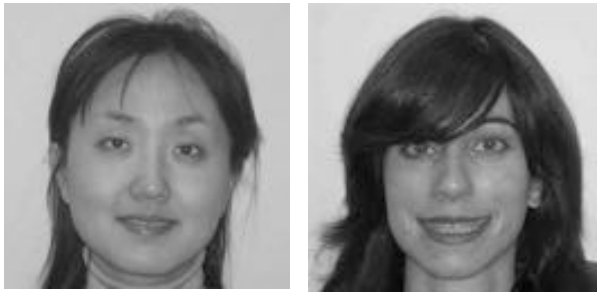
Assembly	Contig NG50
HiSeq Fragments 50x 2x100bp @ 180	3,925
MiSeq Fragments 23x 459bp 8x 2x251bp @ 450	6,332
"ALLPATHS-recipe" 50x 2x100bp @ 180 36x 2x50bp @ 2100 51x 2x50bp @ 4800	18,248
PBeCR Reads 19x @ 3500 ** MiSeq for correction	50,995



In collaboration with McCombie & Ware labs @ CSHL

Assembly Coverage Model





Simulate PacBio-like reads to predict how the assembly will improve as we add additional coverage

Only 8x coverage is needed to sequence every base in the genome, but 40x improves the chances repeats will be spanned by the longest reads

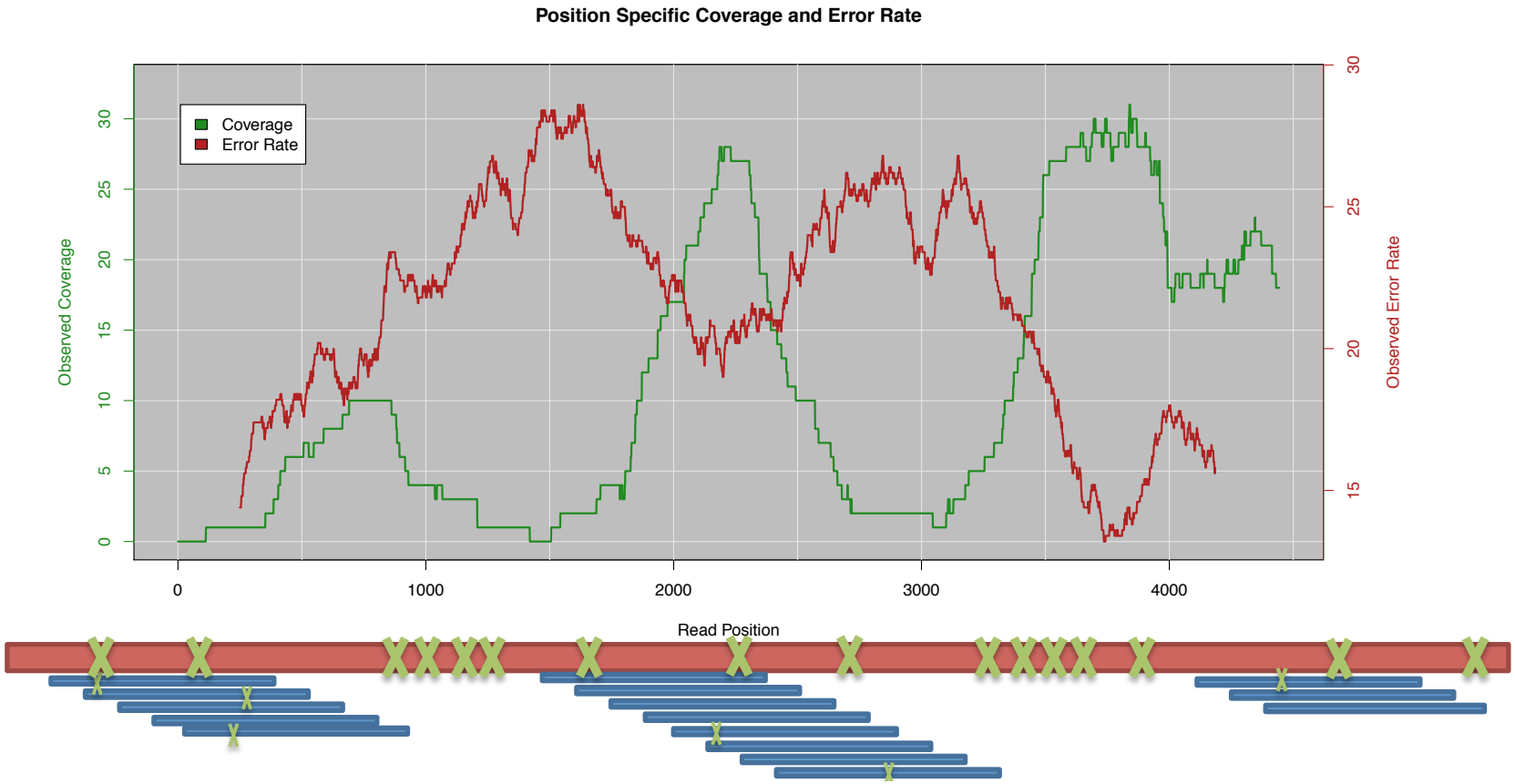
Assembly complexity of long read sequencing

Lee, H*, Gurtowski, J*, Yoo, S, Marcus, S, McCombie, WR, Schatz MC et al. (2013) *In preparation*

Enhanced PacBio Error Correction

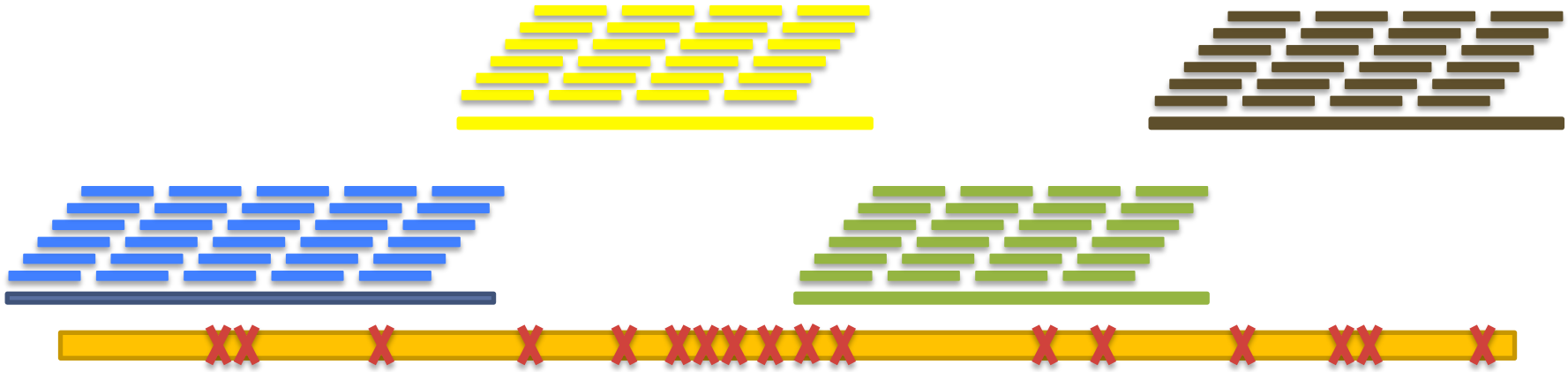
PacBioToCA fails in complex regions

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



Error Correction with pre-assembled Illumina reads

https://github.com/jgurtowski/pbtools



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

Unitigs:

High quality contigs formed from unambiguous, unique overlaps of reads
Each read is placed into a single unitig

Can Help us overcome:

1. **Simple Repeats – Kmer Frequency Too High to Seed Overlaps**
2. **Error Dense Regions – Difficult to compute overlaps with many errors**

Preliminary Rice Assemblies

Assembly	Contig NG50
HiSeq Fragments 50x 2x100bp @ 180	3,925
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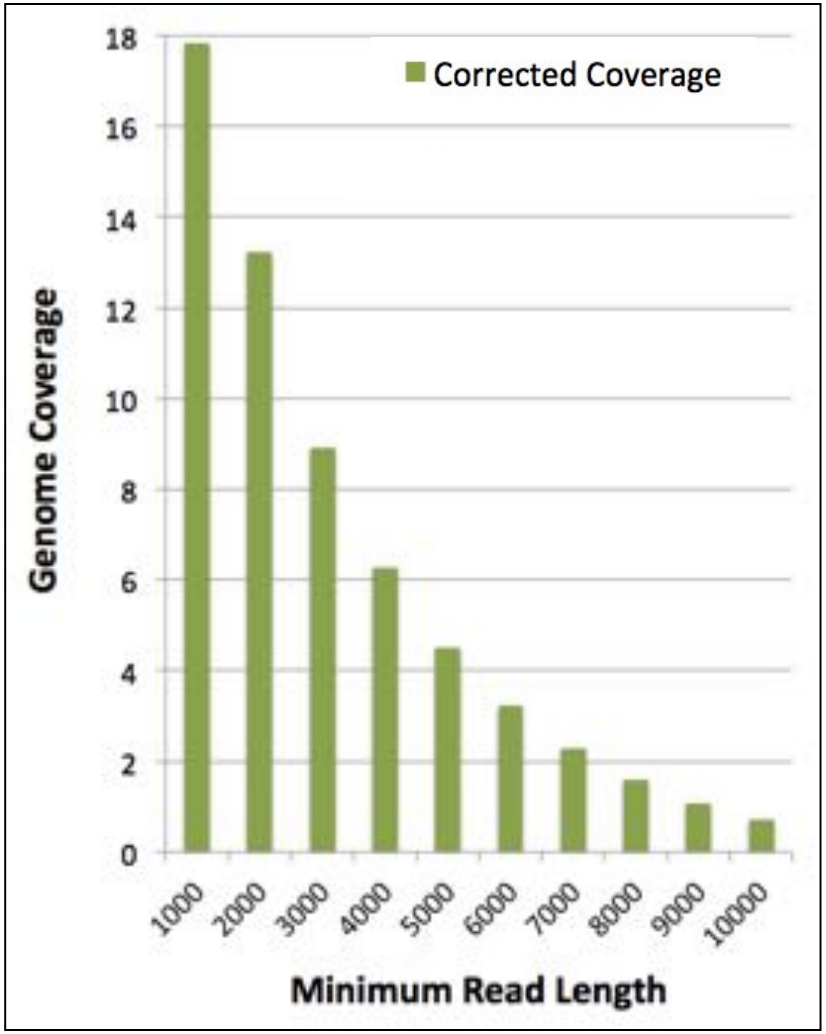
"ALLPATHS-recipe" 50x 2x100bp @ 180 36x 2x50bp @ 2100 51x 2x50bp @ 4800	18,248
PBeCR Reads 19x @ 3500 ** MiSeq for correction	50,995
Enchanced PBeCR 19x @ 3500 ** MiSeq for correction	155,695



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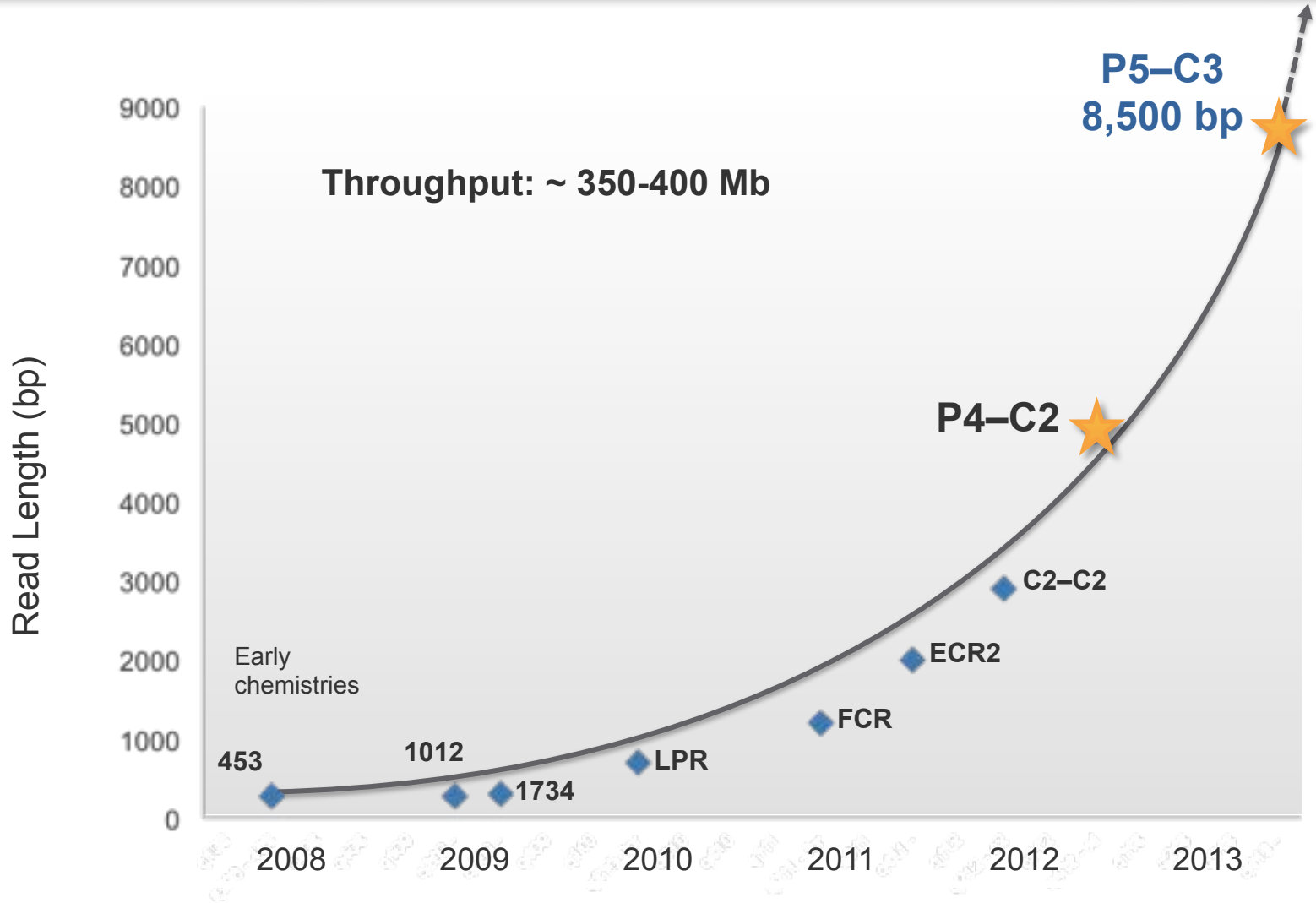
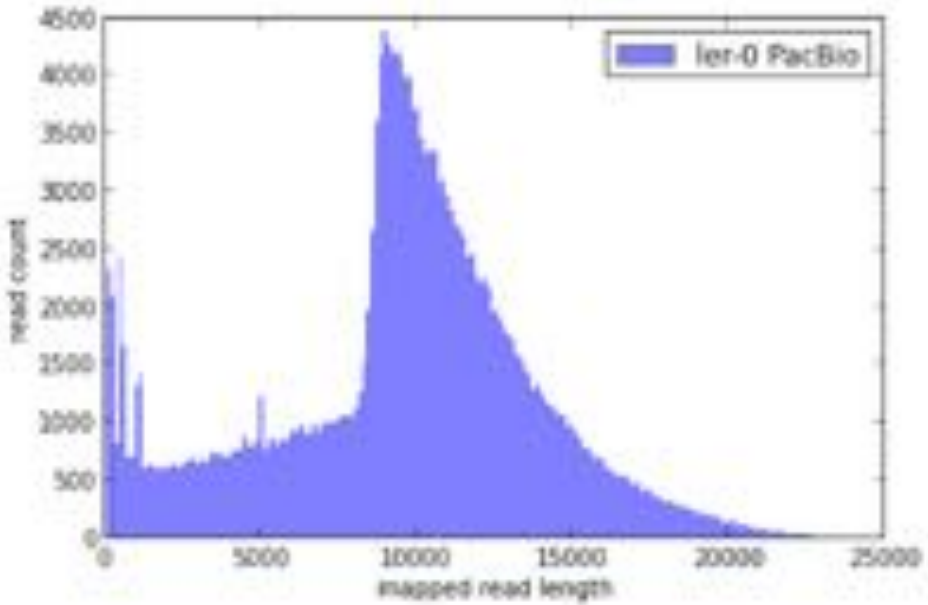
P5-C3 Chemistry Read Lengths



De novo assembly of Arabidopsis

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

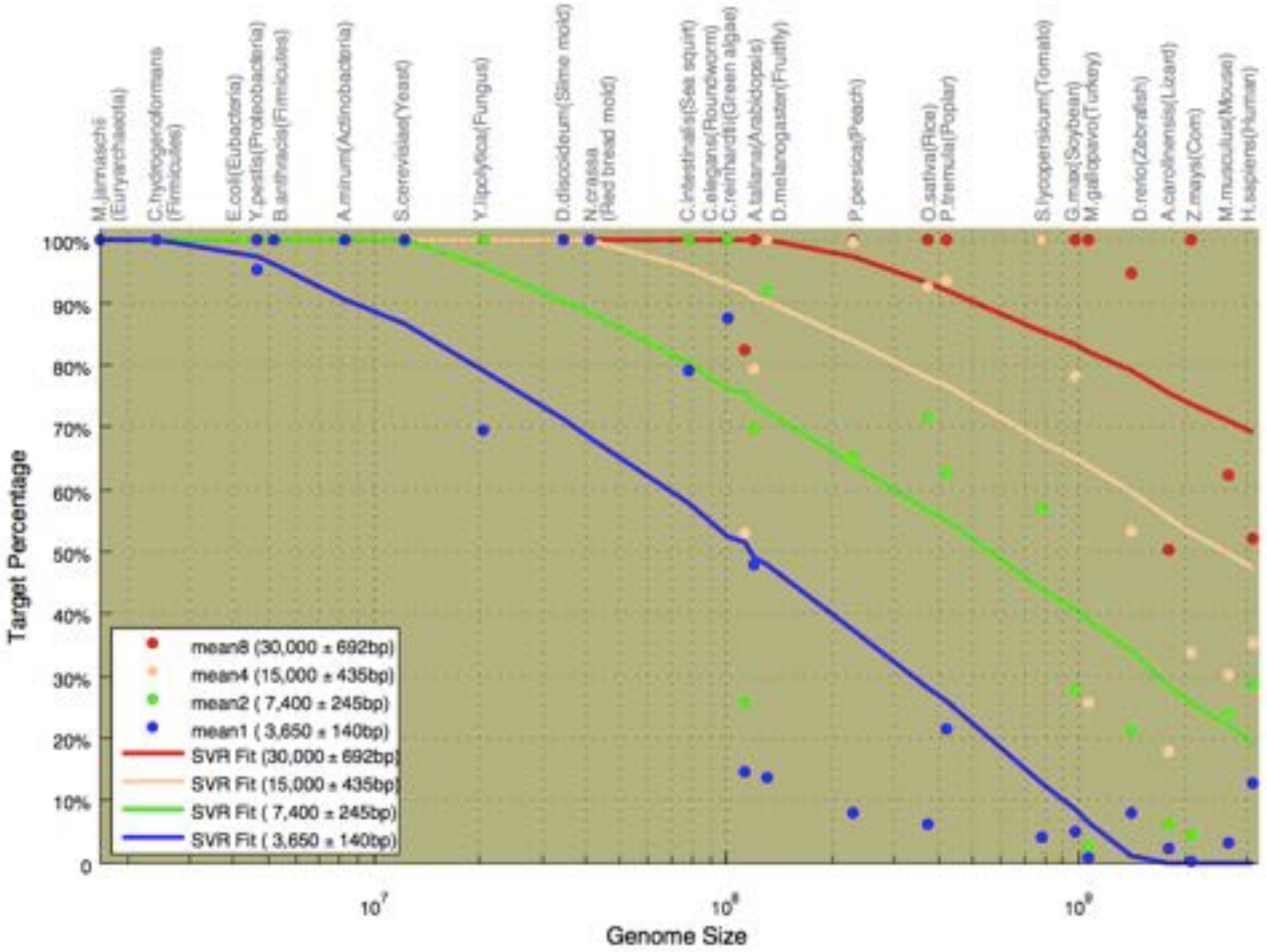


A. thaliana Ler-0 sequenced at PacBio

- Sequenced using the latest 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 >100x

Genome size:	124.6 Mb	Sum of Contig Lengths:	149.5Mb
GC content:	33.92%	Number of Contigs:	1788
Raw data:	11 Gb	Max Contig Length:	12.4 Mb
Assembly coverage:	15x over 9kbp	N50 Contig Length:	8.4 Mb

Assembly Complexity of Long Reads



Summary

- Hybrid assembly let us combine the best characteristics of 2nd and 3rd gen sequencing
  - Better repeat resolution and error correction by pre-assembling Illumina reads into unitigs
- Long reads and good coverage are the keys to a high quality de novo assembly
  - Single contig de novo assemblies of entire microbial chromosomes are now routine
  - Single contig de novo assemblies of entire plant and animal chromosomes on the horizon
- We are starting to apply these technologies to discover significant biology that is otherwise impossible to measure
  - Expect to see results in smaller genomes scale up over the next few years

Acknowledgements

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Levy Lab
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Lyon Lab
Martienssen Lab
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Wigler Lab

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NSF

Thank You!

http://schatzlab.cshl.edu

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