

The Resurgence of Reference Quality Genome Sequence

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ARTICLES

The map-based sequence of the rice genome

International Rice Genome Sequencing Project*

Rice, one of the world's most important food plants, has important genetic relationships with the other cereal species and is a model plant for genome analysis. We report here the map-based sequence of the rice genome, a 389 Mb genome, including 38,500 genes and 100,000 transposable-element insertions. The rice genome is similar in size and structure to that of *Arabidopsis*. In a reciprocal manner, the rice genome is similar in size and structure to that of *Arabidopsis*. Twenty-nine classes of transposable elements are identified in the rice genome. The rice genome is similar in size and structure to that of maize and sorghum genomes. The rice genome is similar in size and structure to that of nuclear chromosomes. The rice genome is similar in size and structure to that of traits. The additional sequences are similar in size and structure to that of accelerate improvements in rice production.

Table 2 | Size of each chromosome based on sequence data and estimated gaps

Chr	Sequenced bases (bp)	Gaps on arm regions No.	Length (Mb)	Telomeric gaps* (Mb)	Centromeric gaps (Mb)	rDNA ₁ (Mb)	Total (Mb)	Coverage (%)
1	43,260,640	5	0.33	0.06	1.40		45.05	99.1
2	35,954,074	3	0.10	0.01	0.72		36.78	99.7
3	36,189,985	4	0.96	0.04	0.18		37.37	97.3
4	35,489,479	3	0.46	0.20			36.15	98.7
5	29,733,216	6	0.22	0.05			30.00	99.3
6	30,731,386	1	0.02	0.03	0.82		31.60	99.8
7	29,643,843	1	0.31	0.01	0.32		30.28	98.9
8	28,434,680	1	0.09	0.05			28.57	99.7
9	22,692,709	4	0.13	0.14	0.62	6.95	30.53	98.8
10	22,683,701	4	0.68	0.13	0.47		23.96	96.6
11	28,357,783	4	0.21	0.04	1.90	0.25	30.76	99.1
12	27,561,960	0	0.00	0.05	0.16		27.77	99.8
All	370,733,456	36	3.51	0.81	6.59	7.20	388.82	98.9

Contig N50: 5.1Mbp
Total projects costs: >\$100M

Initial Assembly Attempts with early Illumina sequencers circa 2007-2008

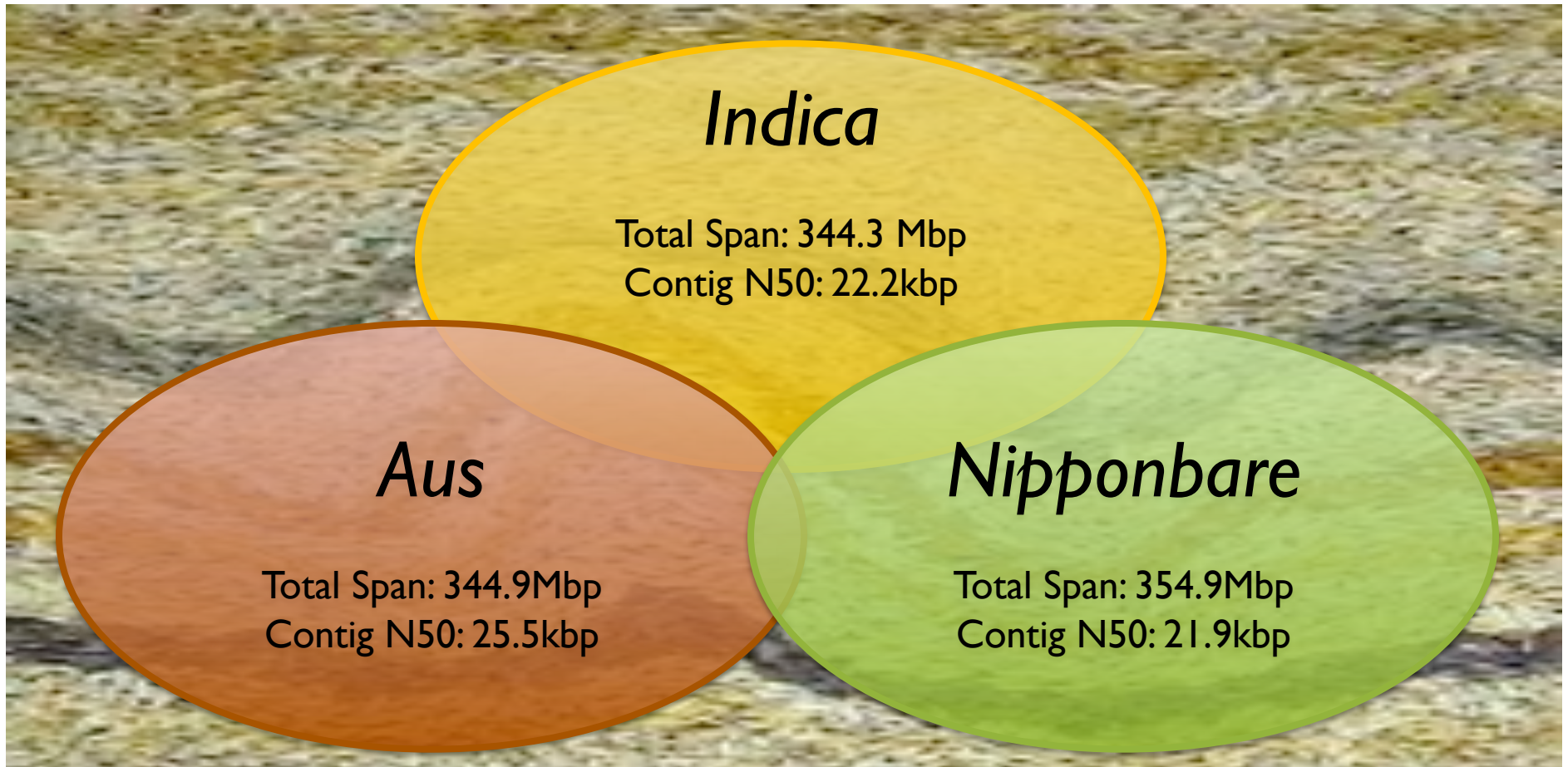
(older Illumina PE76 library with small insert size ~150bp)

Assembler	Data set	N50 contig size	Max contig size	Total assembly size
Velvet	25X Nipponbare	1049bp	21833bp	325.8 Mbp
Velvet	50X Nipponbare	411bp	23095bp	401.6 Mbp
Abyss	25X Nipponbare	1853bp	12688bp	288.4 Mbp
Abyss	50X Nipponbare	2847bp	34893bp	317.4 Mbp

Total costs: ~\$10k
>1,000x times cheaper, but at what cost scientifically?

W.R. McCombie

Population structure of *Oryza sativa*

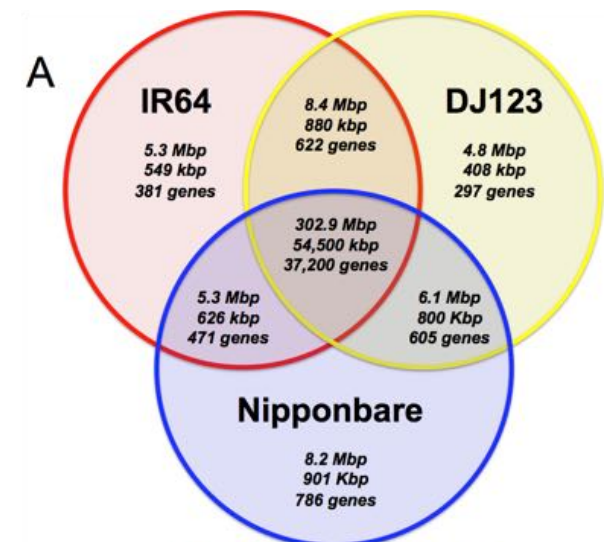


Whole genome de novo assemblies of three divergent strains of rice (*O. sativa*) documents novel gene space of *aus* and *indica*

Schatz, Maron, Stein et al (2014) *Genome Biology*. 15:506 doi:10.1186/s13059-014-0506-z

Oryza sativa Gene Diversity

- Very high quality representation of the “gene-space”
 - Overall identity ~99.9%
 - Less than 1% of exonic bases missing
- Genome-specific genes enriched for disease resistance
 - Reflects their geographic and environmental diversity
- Assemblies fragmented at (high copy) repeats
 - Difficult to identify full length gene models and regulatory features



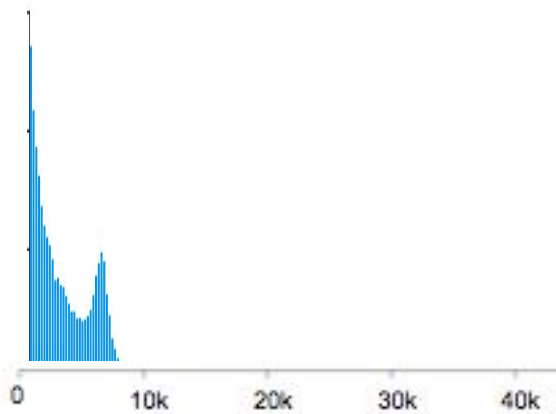
Overall sequence content

In each sector, the top number is the total number of base pairs, the middle number is the number of exonic bases, and the bottom is the gene count. If a gene is partially shared, it is assigned to the sector with the most exonic bases.

Long Read Sequencing Technology

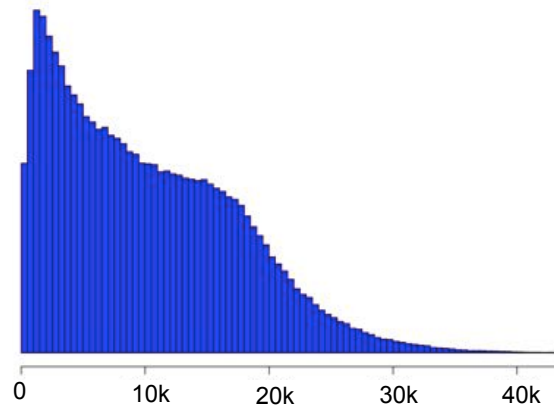
Moleculo

illumina
moleculo



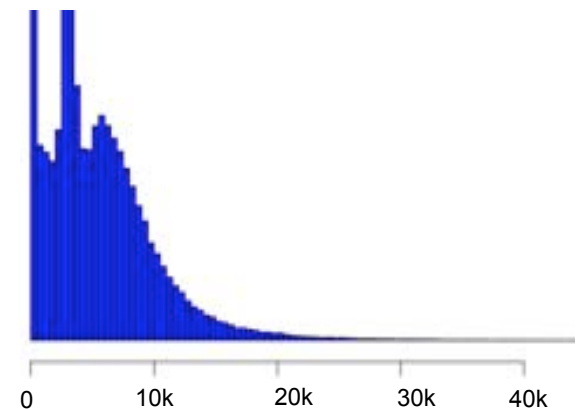
(Voskoboynik et al. 2013)

PacBio RS II



CSHL/PacBio

Oxford Nanopore



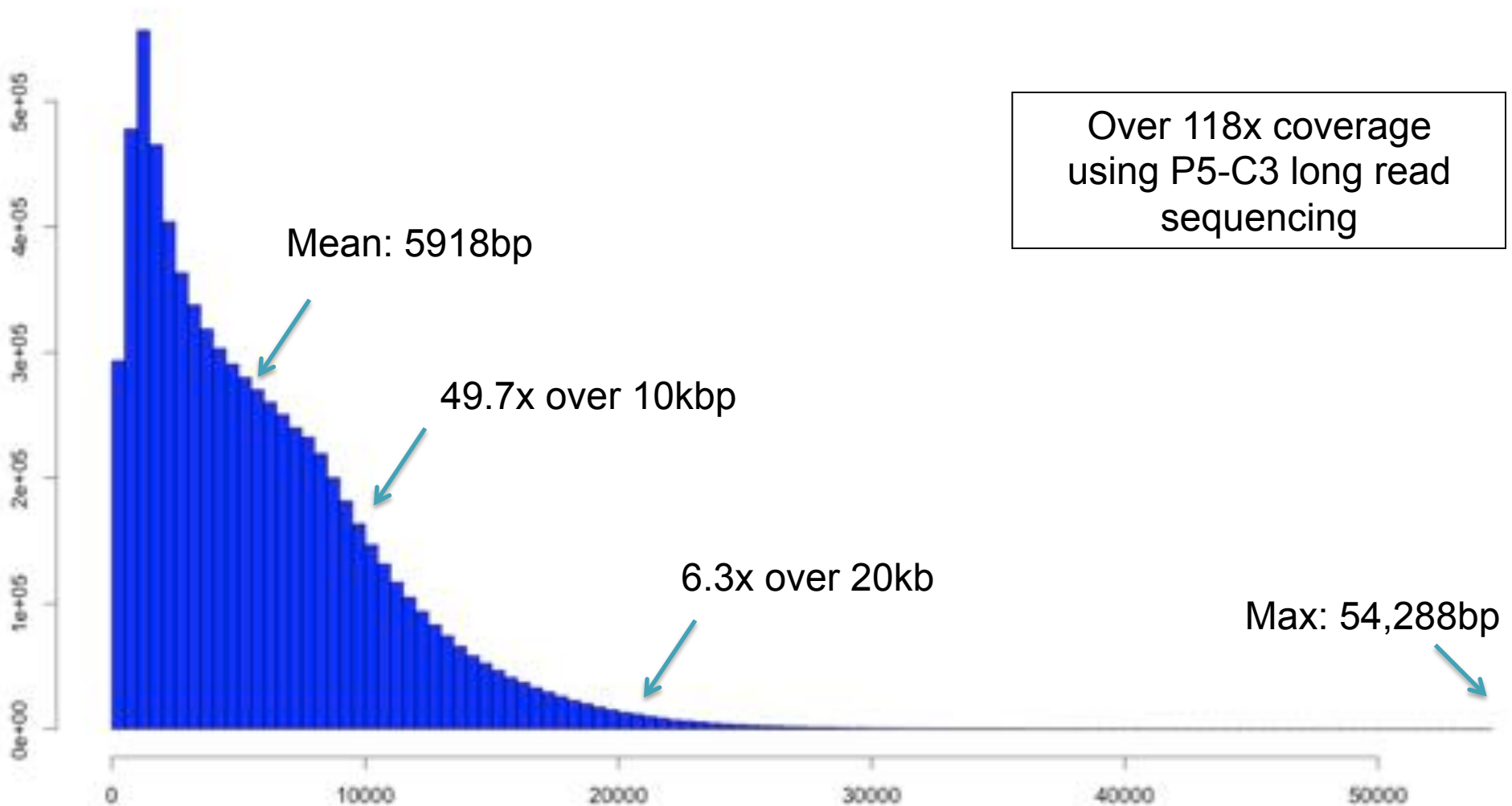
CSHL/ONT

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

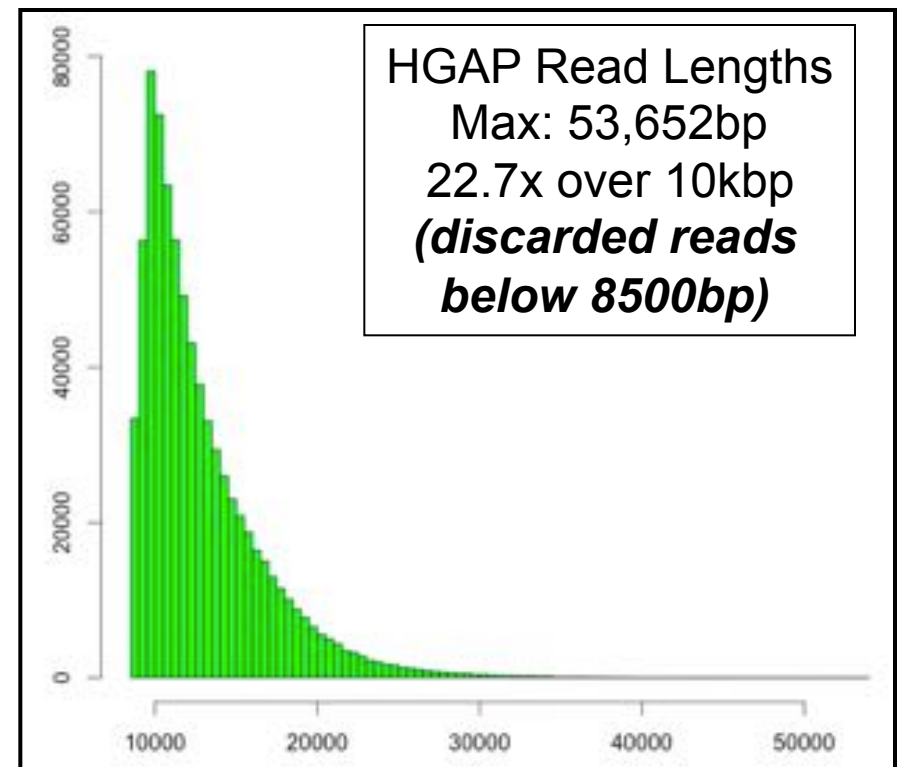


O. sativa pv Indica (IR64)

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



Assembly	Contig NG50
MiSeq Fragments 25x 456bp (3 runs 2x300 @ 450 FLASH)	19 kbp
“ALLPATHS-recipe” 50x 2x100bp @ 180 36x 2x50bp @ 2100 51x 2x50bp @ 4800	18 kbp
HGAP + CA 22.7x @ 10kbp	4.0 Mbp
Nipponbare BAC-by-BAC Assembly	5.1 Mbp



S5 Hybrid Sterility Locus



Sanger	...ACCCTGATATTCTGAGTTACAAGGCATT C AGCTACTGCTTGCCCCACTGACGAGACC...
Illumina	...ACCCTGATATTCTGAGTTACAAGGCATT C AGCTACTGCTTGCCCCACTGACGAGACC...
PacBio	...ACCCTGATATTCTGAGTTACAAGGCATT C AGCTACTGCTTGCCCCACTGACGAGACC...

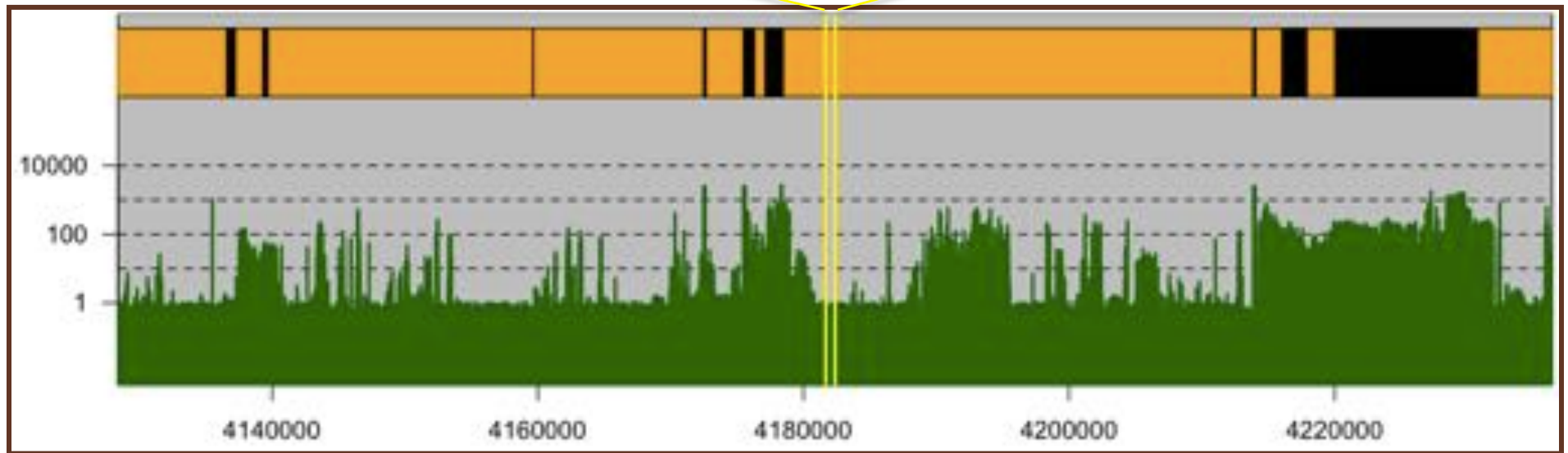
S5 is a major locus for hybrid sterility in rice that affects embryo sac fertility.

- Genetic analysis of the S5 locus documented three alleles: an indica (S5-i), a japonica (S5-j), and a neutral allele (S5-n)
- Hybrids of genotype S5-i/S5-j are mostly sterile, whereas hybrids of genotypes consisting of S5-n with either S5-i or S5-j are mostly fertile.
- Contains three tightly linked genes that work together in a 'killer-protector'-type system: ORF3, ORF4, ORF5
- The ORF5 indica (ORF5+) and japonica (ORF5-) alleles differ by only **two nucleotides**

S5 Hybrid Sterility Locus



Sanger	...ACCCTGATATTCTGAGTTACAAGGCATT C AGCTACTGCTTGCCCACTGACGAGACC...
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PacBio	...ACCCTGATATTCTGAGTTACAAGGCATT C AGCTACTGCTTGCCCACTGACGAGACC...

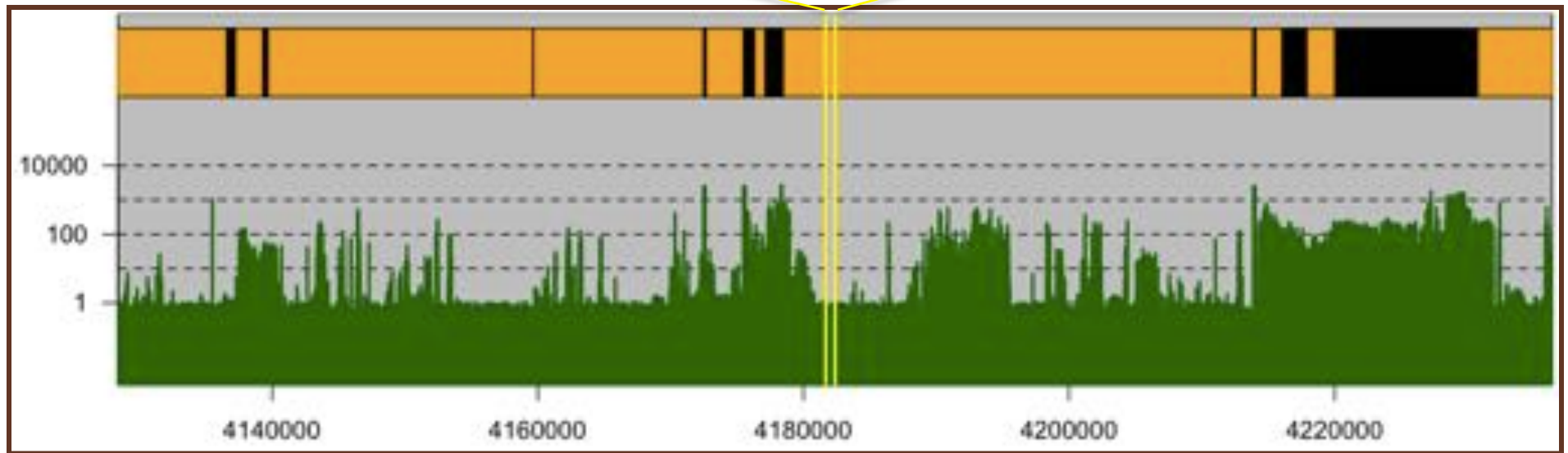


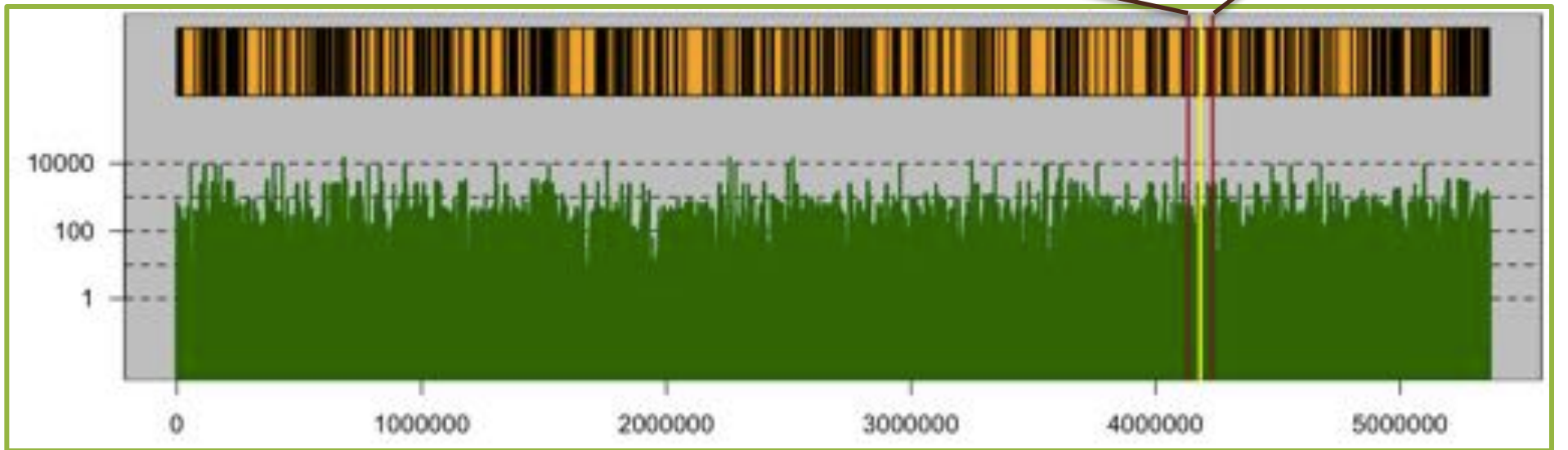
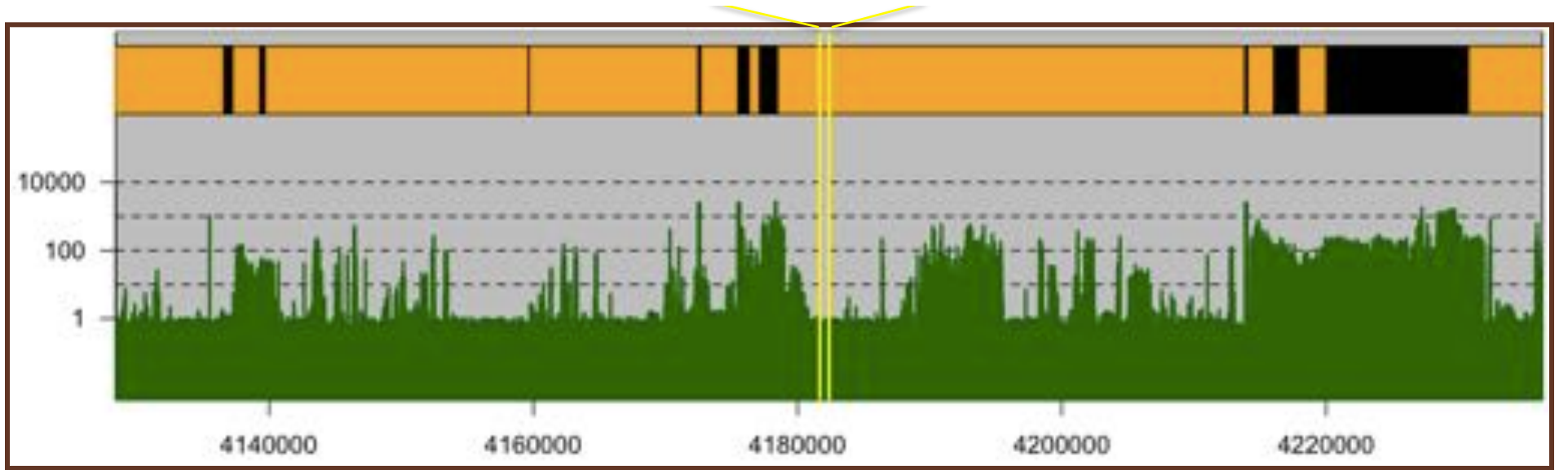
100kbp

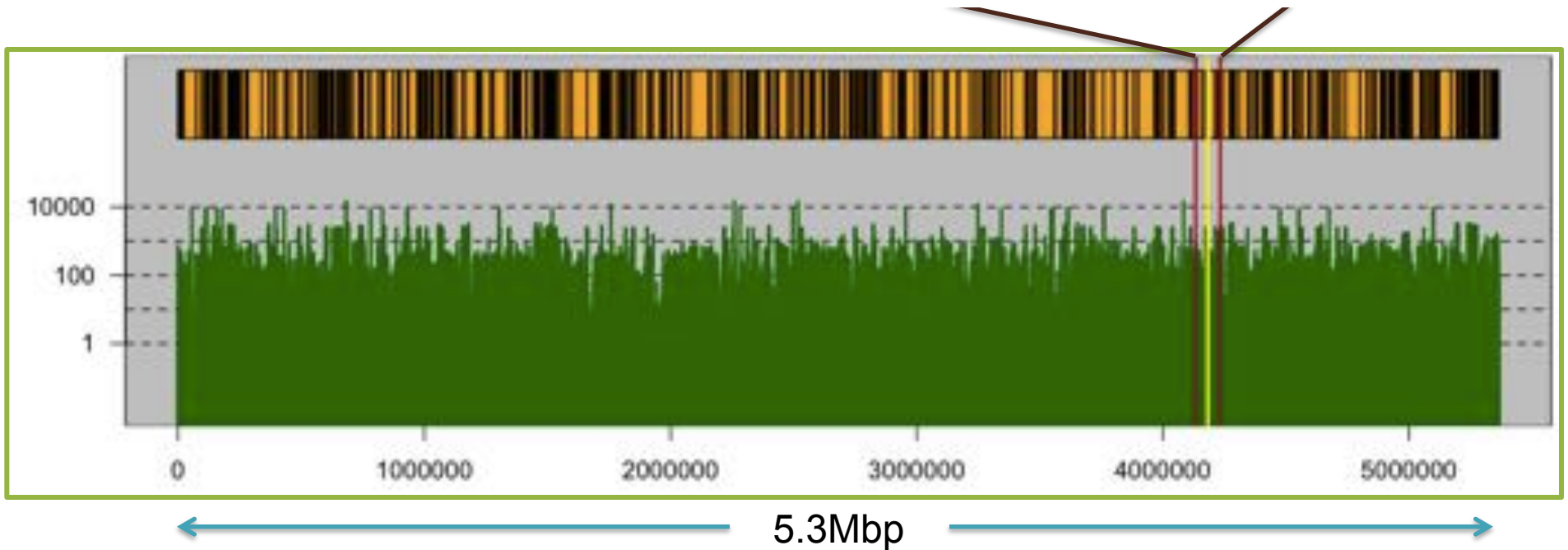
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Improvements from 20kbp to 4Mbp contig N50:

- Over 20 Megabases of additional sequence
 - Extremely high sequence identity (>99.9%)
 - Thousands of gaps filled, hundreds of mis-assemblies corrected
- Complete gene models, promoter regions for nearly every gene
 - True representation of transposons and other complex features
- Opportunities for studying large scale chromosome evolution
 - Largest contigs approach complete chromosome arms

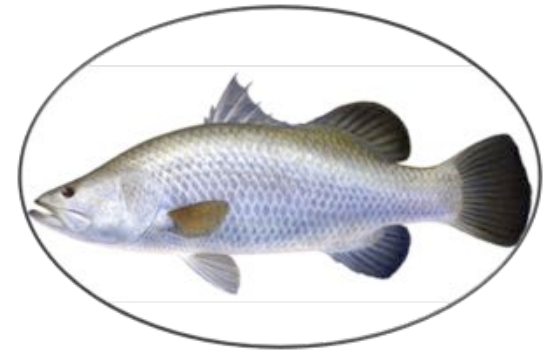
Current Collaborations



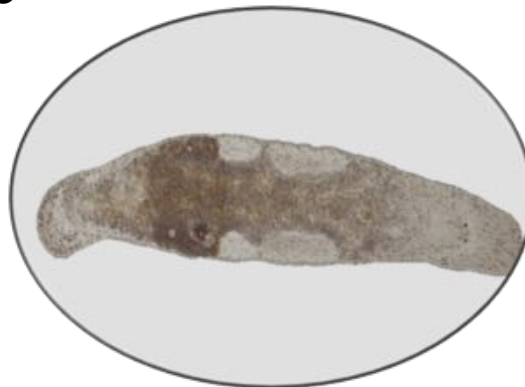
Pineapple
UIUC



Human
CSHL/OICR



Asian Sea Bass
Temasek Life Sciences

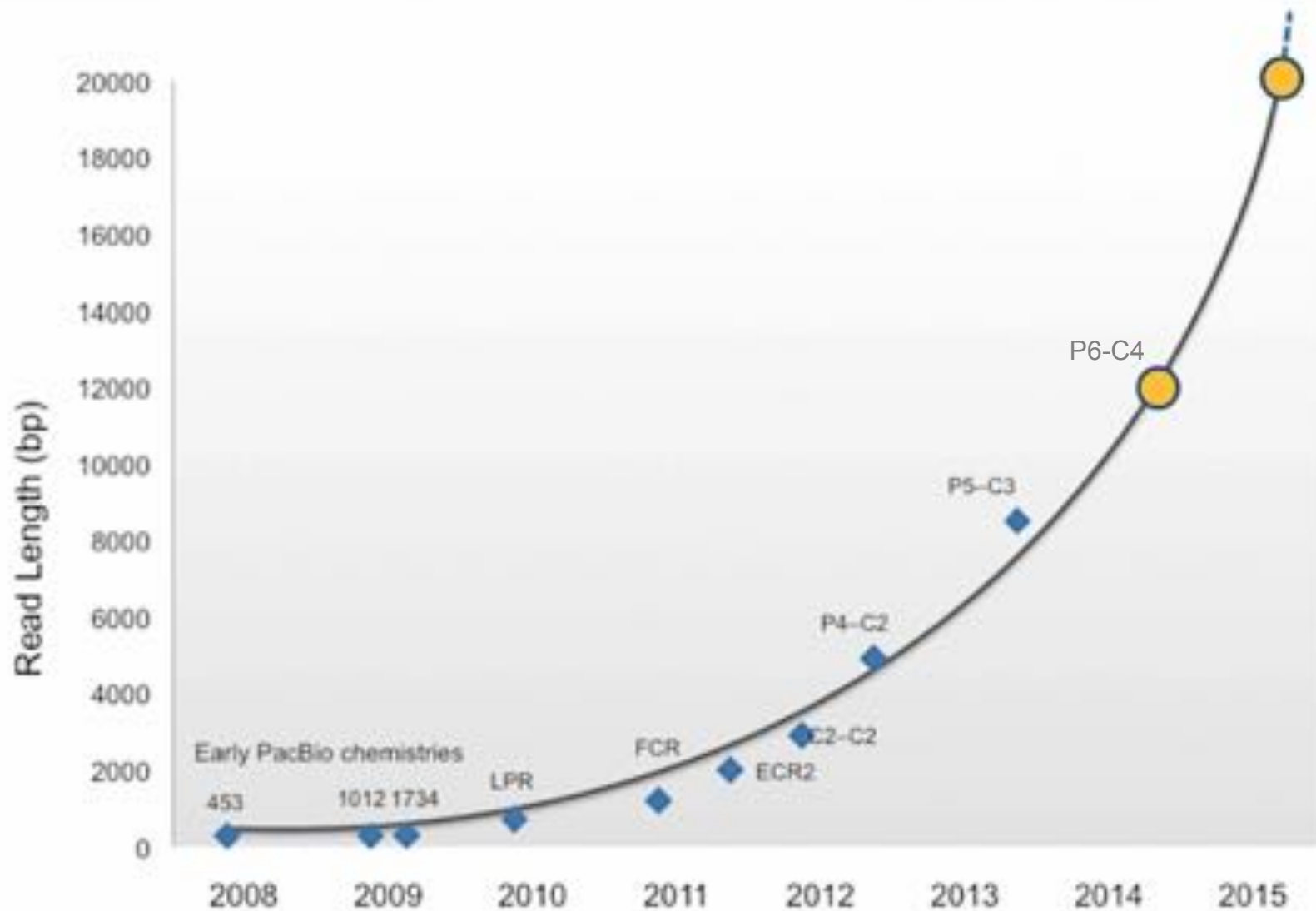


M. ligano
Hannon

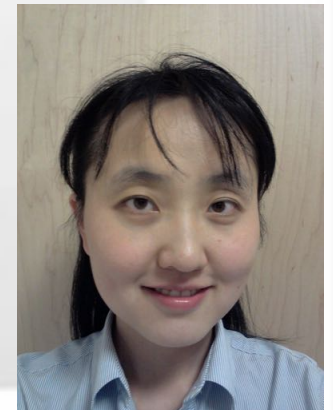
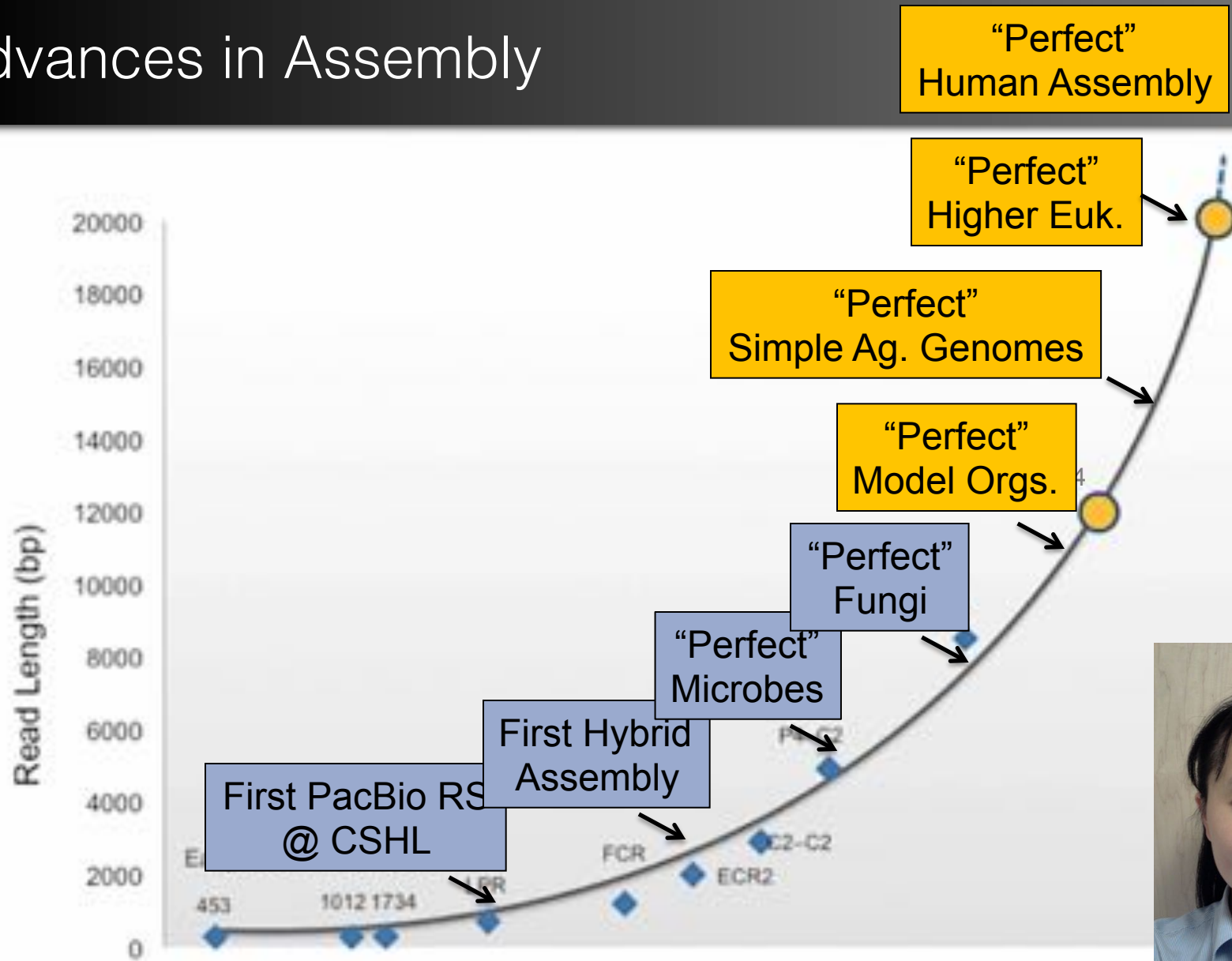


T. vaginalis
NYU

PacBio® Advances in Read Length



Advances in Assembly

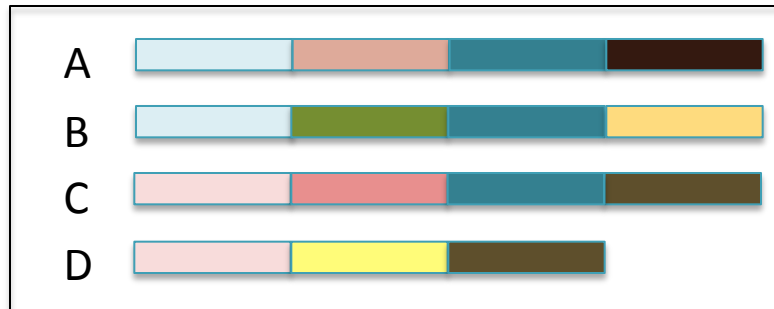


Error correction and assembly complexity of single molecule sequencing reads.

Lee, H*, Gurtowski, J*, Yoo, S, Marcus, S, McCombie, WR, Schatz, MC

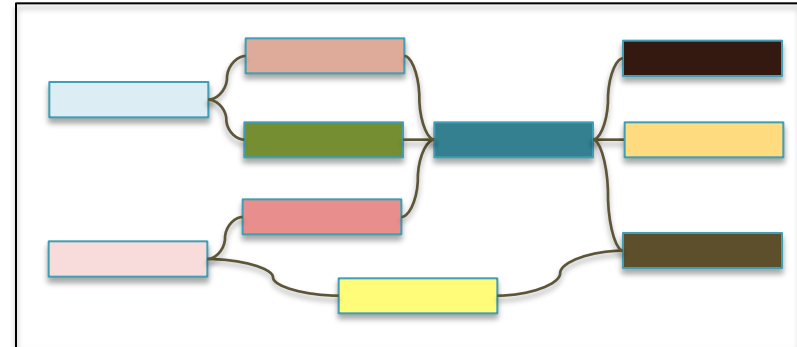
<http://www.biorxiv.org/content/early/2014/06/18/006395>

Pan-Genome Alignment & Assembly



Time to start considering problems for which N complete genomes is the input to study the “pan-genome”

- Available today for many microbial species, near future for higher eukaryotes



Pan-genome colored de Bruijn graph

- Encodes all the sequence relationships between the genomes
- How well conserved is a given sequence?
- What are the pan-genome network properties?

SplitMEM: A graphical algorithm for pan-genome analysis with suffix skips

Marcus, S, Lee, H, Schatz MC (2014) *Bioinformatics*. doi: 10.1093/bioinformatics/btu756

Extending reference assembly models

Church, D. et al. (2015) *Genome Biology*. In Press.

Summary & Recommendations

Reference quality genome assembly is here

- Use the longest possible reads for the analysis
- Don't fear the error rate, coverage and algorithmics conquer most problems

Megabase N50 improves the analysis in every dimension

- Better resolution of genes and flanking regulatory regions
- Better resolution of transposons and other complex sequences
- Better resolution of chromosome organization
- Better sequence for all downstream analysis

The year 2015 will mark the return to reference quality genome sequence

Acknowledgements

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Greg Vulture

Alejandro Wences

CSHL

Hannon Lab

Gingeras Lab

Jackson Lab

Hicks Lab

Iossifov Lab

Levy Lab

Lippman Lab

Lyon Lab

Martienssen Lab

McCombie Lab

Tuveson Lab

Ware Lab

Wigler Lab

IT & Meetings Depts.

Pacific Biosciences

Oxford Nanopore



National Human
Genome Research
Institute



U.S. DEPARTMENT OF
ENERGY

SFARI

SIMONS FOUNDATION
AUTISM RESEARCH INITIATIVE



Thank you

<http://schatzlab.cshl.edu>
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