Algorithms for de novo genome assembly and disease analytics

Michael Schatz

April 7, 2014
Hamilton College

Introductions

Ke Jiang

Transcriptomics and epigenetics

Tomato & Solanaceae

Srividya "Sri" Ramakrishnan

DOE Systems Biology Knowledgebase

Worlds fastest -omics pipelines

Maria Nattestad

Hi-C Chromatin Interactions

Plant Assembly & Analysis

Tyler Garvin

CNV analysis of single cells

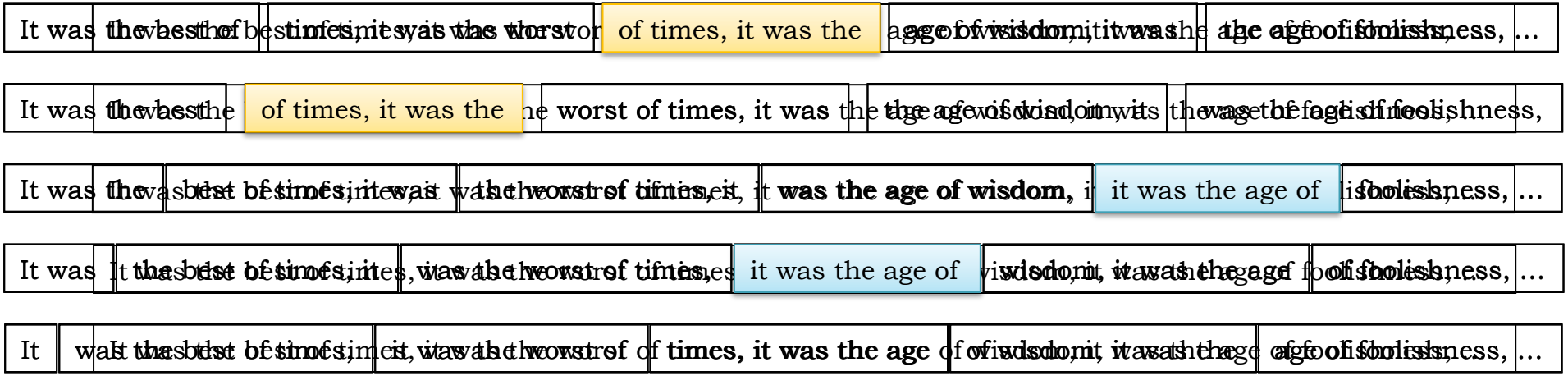
Breast & Prostate Cancer

Outline

1. De novo assembly by analogy
2. Long Read Assembly
3. Disease Analytics

Shredded Book Reconstruction

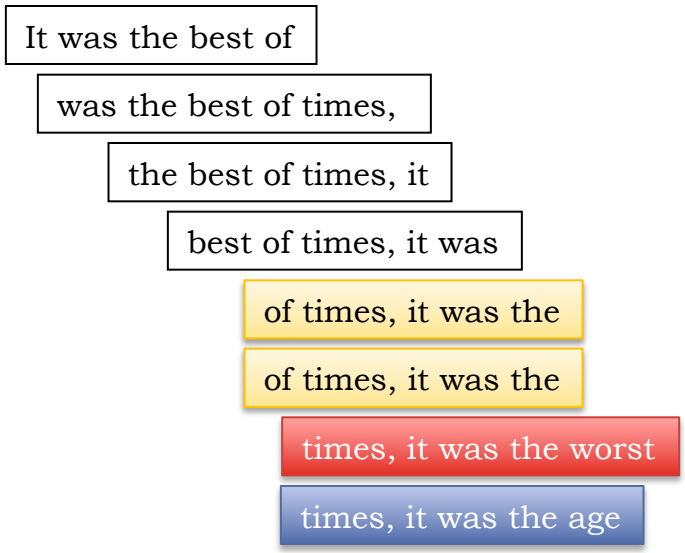
- Dickens accidentally shreds the first printing of A Tale of Two Cities
  - Text printed on 5 long spools



- How can he reconstruct the text?
  - 5 copies x 138, 656 words / 5 words per fragment = 138k fragments
  - The short fragments from every copy are mixed together
  - Some fragments are identical

Greedy Reconstruction

It was the best of
age of wisdom, it was
best of times, it was
it was the age of
it was the age of
it was the worst of
of times, it was the
of times, it was the
of wisdom, it was the
the age of wisdom, it
the best of times, it
the worst of times, it
times, it was the age
times, it was the worst
was the age of wisdom,
was the age of foolishness,
was the best of times,
was the worst of times,
wisdom, it was the age
worst of times, it was



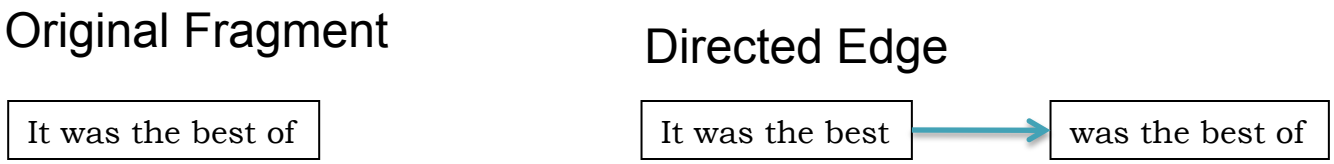
The repeated sequence make the correct reconstruction ambiguous

- It was the best of times, it was the [worst/age]

Model the assembly problem as a graph problem

de Bruijn Graph Construction

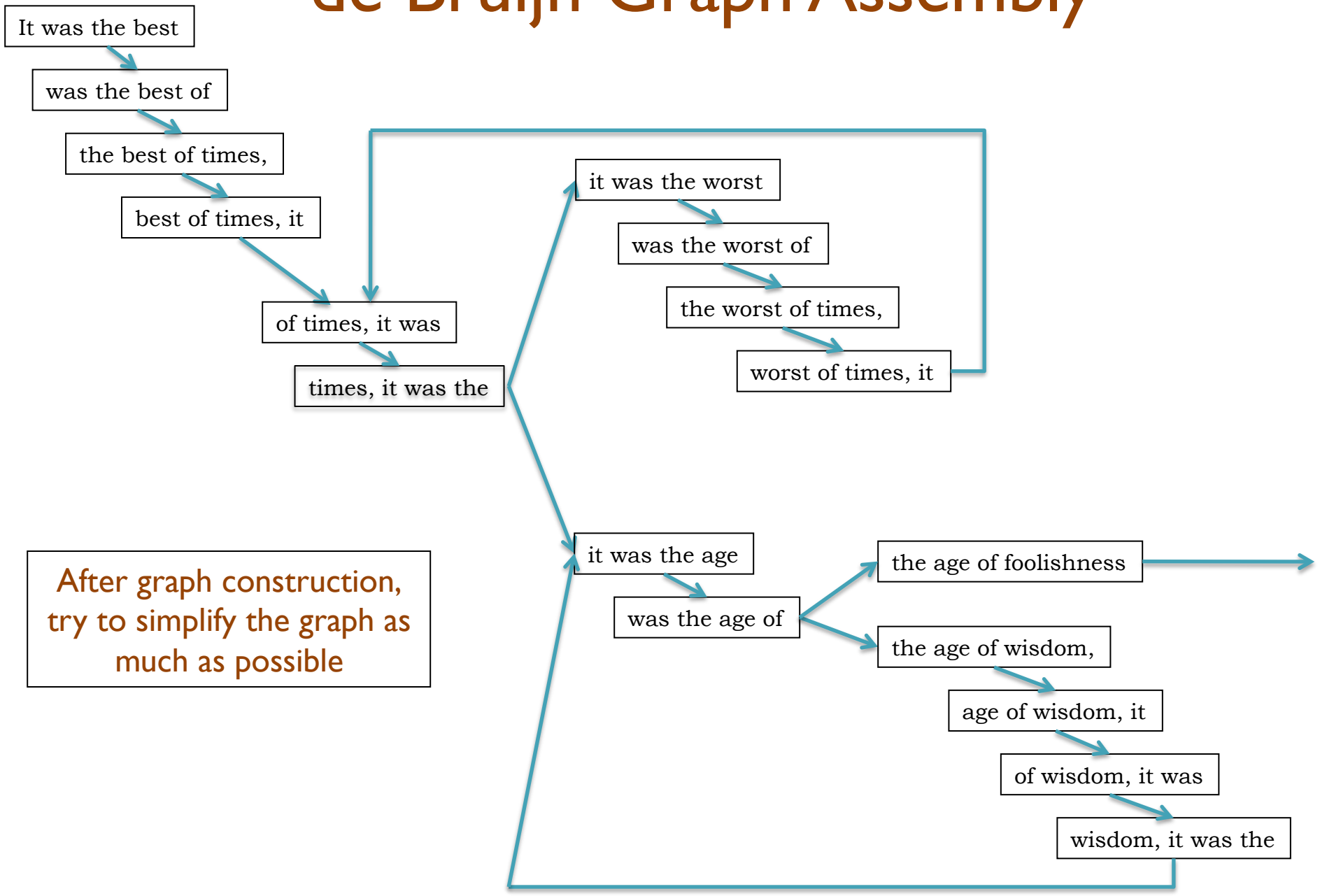
- $D_k = (V,E)$
  - V = All length-k subfragments (k < l)
  - E = Directed edges between consecutive subfragments
    - Nodes overlap by k-1 words



- Locally constructed graph reveals the global sequence structure
  - Overlaps between sequences implicitly computed

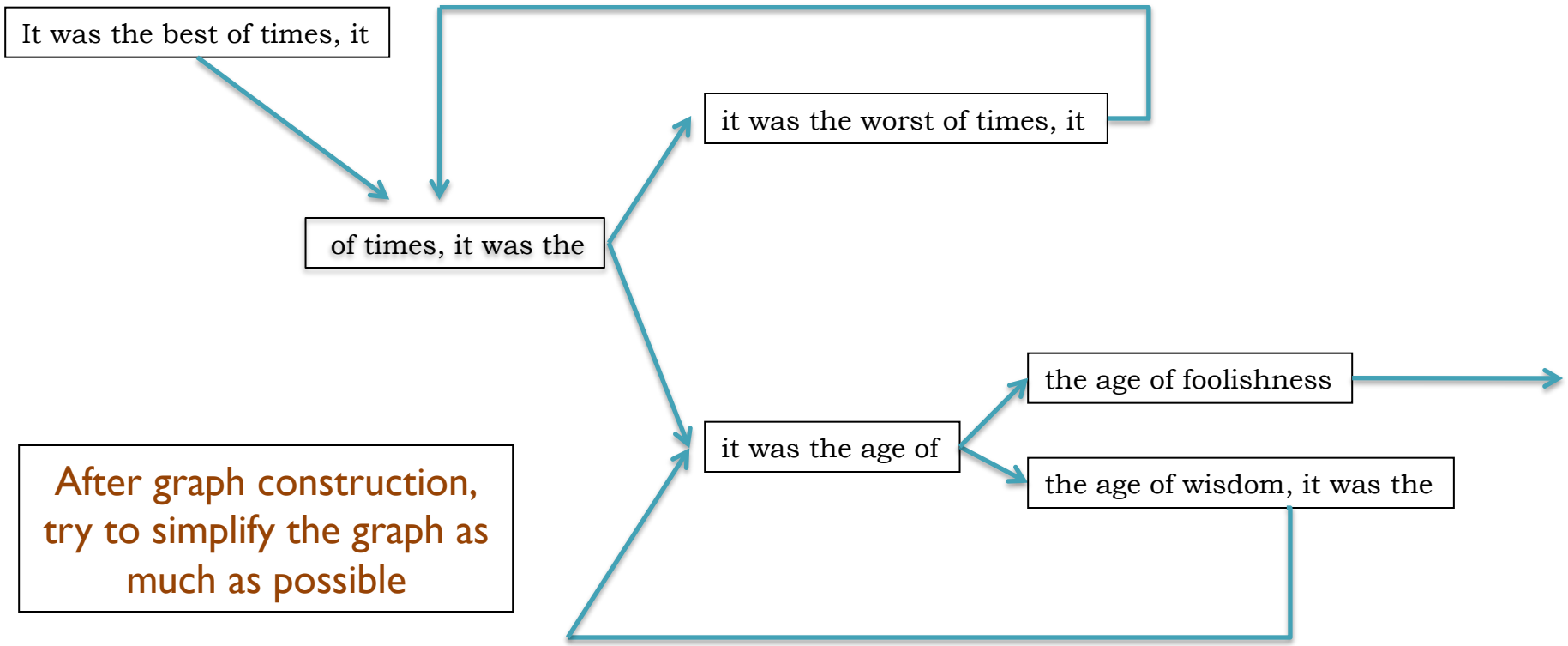
de Bruijn, 1946
Idury and Waterman, 1995
Pevzner, Tang, Waterman, 2001

de Bruijn Graph Assembly



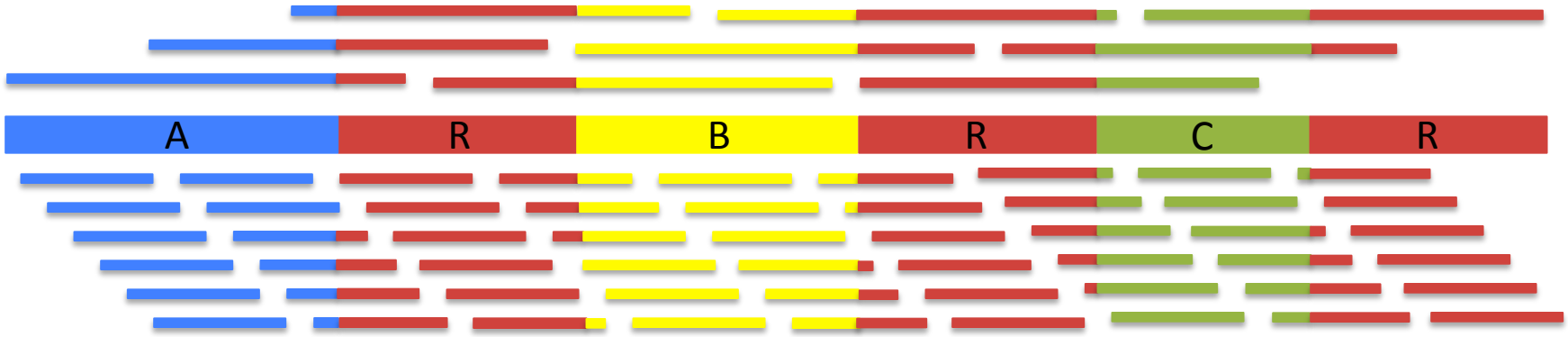
After graph construction, try to simplify the graph as much as possible

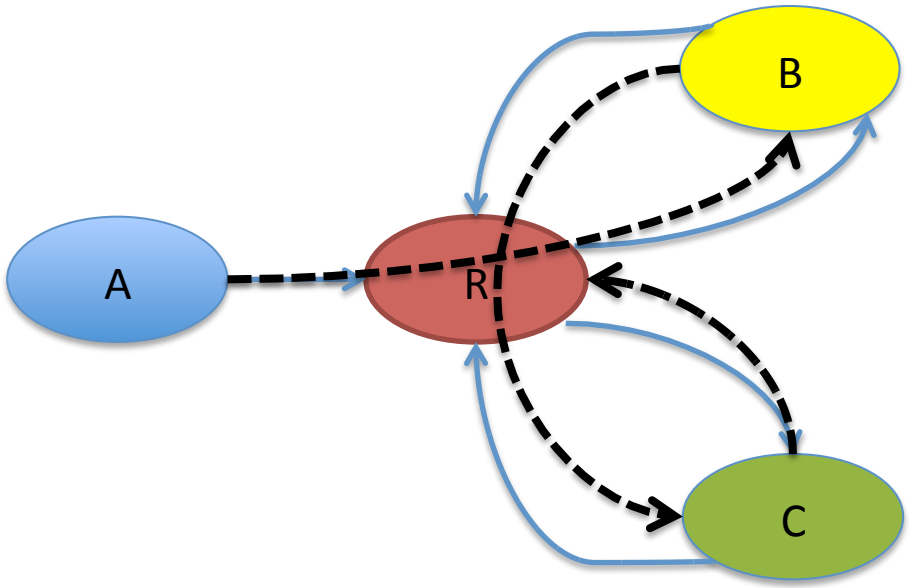
de Bruijn Graph Assembly



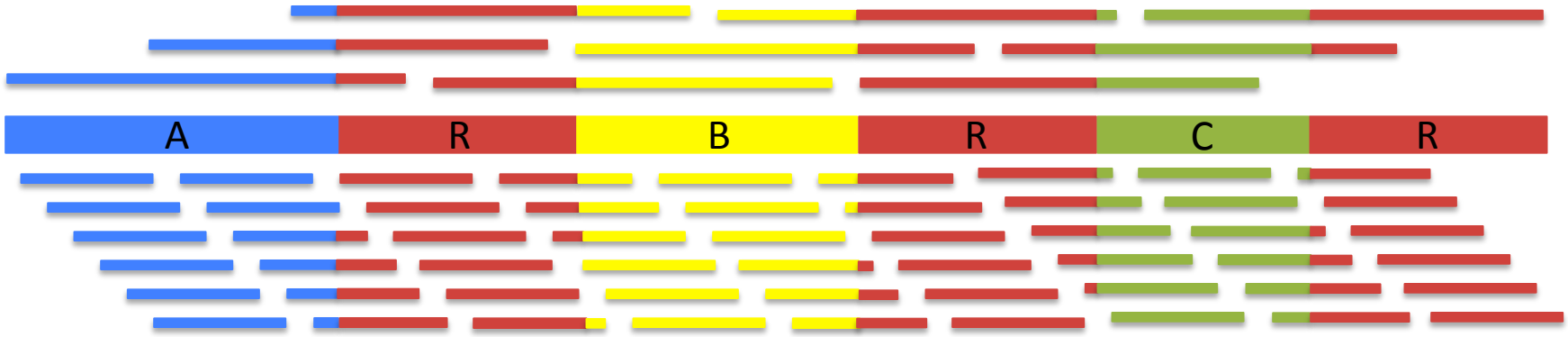
After graph construction, try to simplify the graph as much as possible

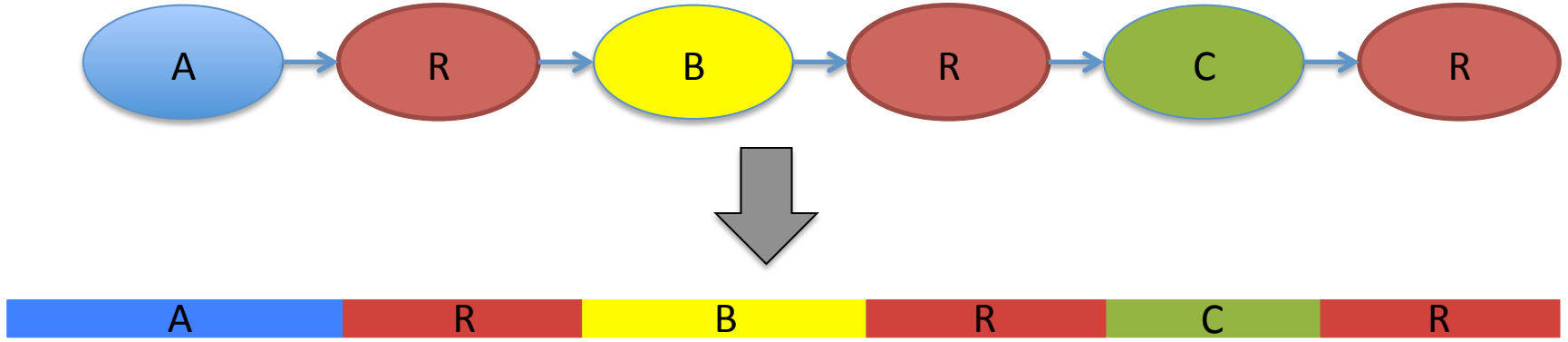
Assembly Complexity





Assembly Complexity

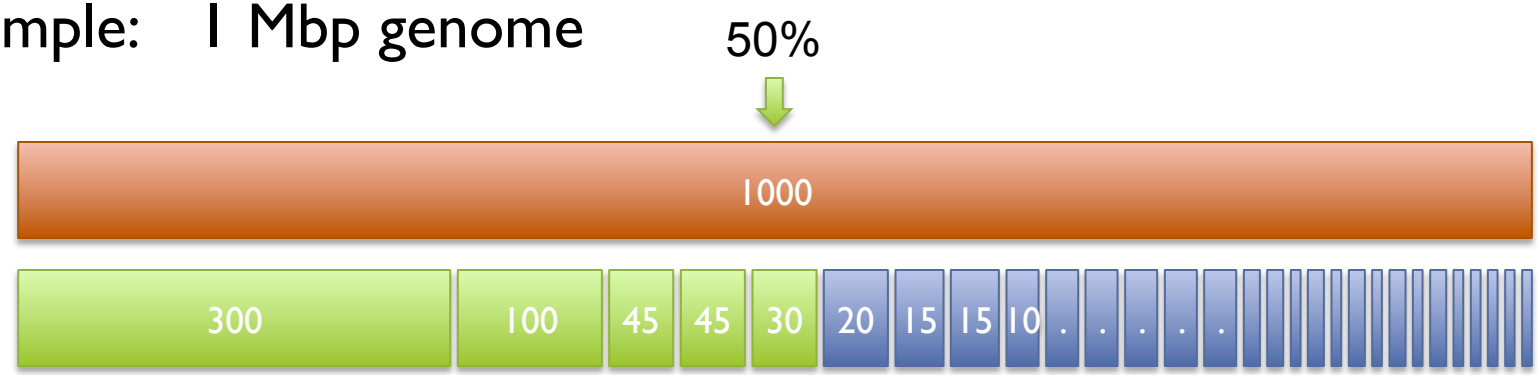




N50 size

Def: 50% of the genome is in contigs as large as the N50 value

Example: 1 Mbp genome



N50 size = 30 kbp

(300k+100k+45k+45k+30k = 520k >= 500kbp)

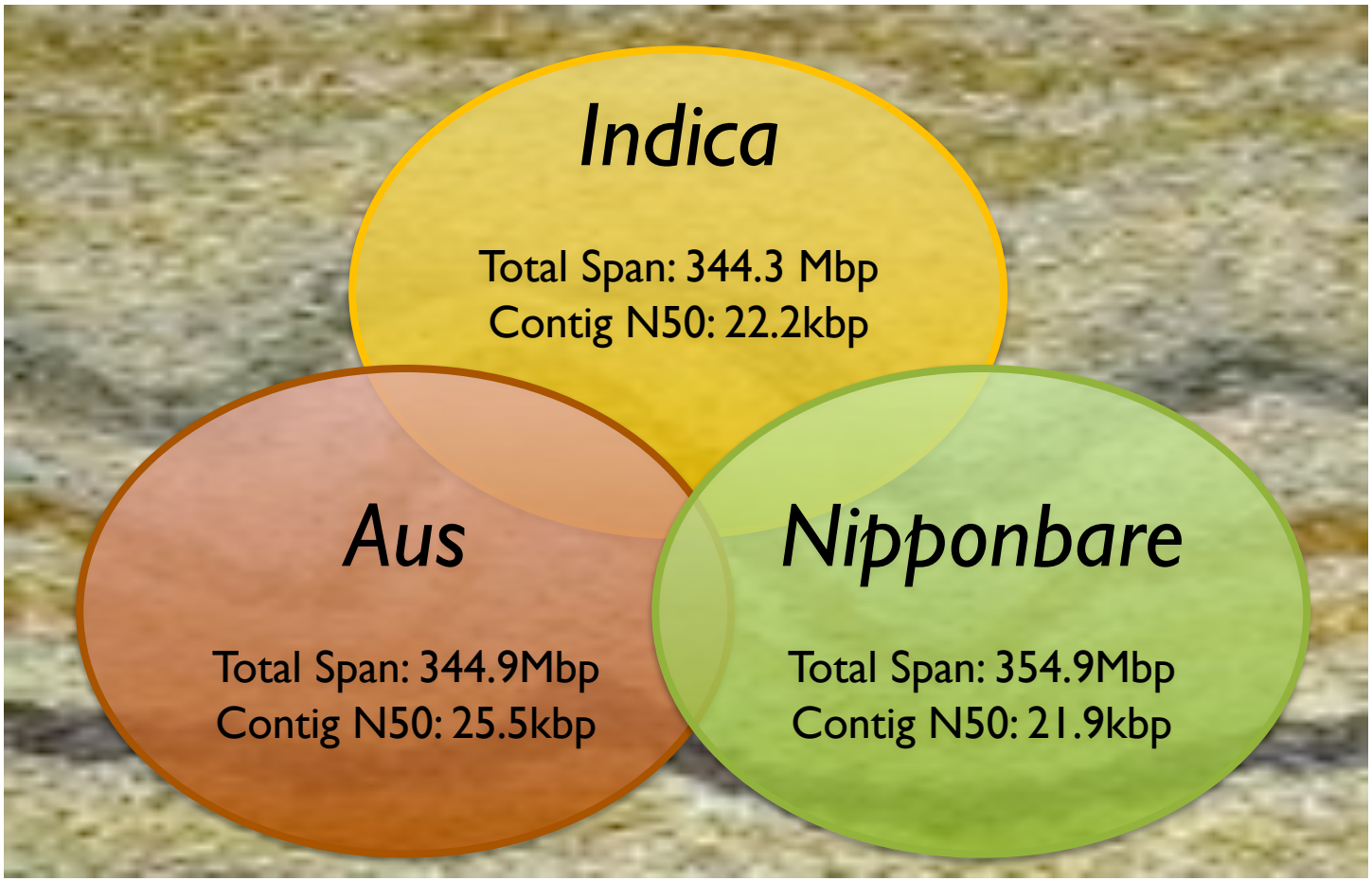
Note:

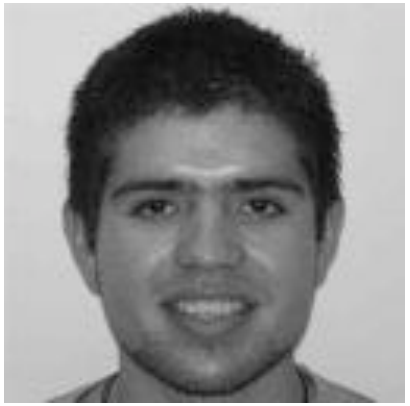
A "good" N50 size is a moving target relative to other recent publications. 10-20kbp contig N50 is currently a typical value for most "simple" genomes.

Outline

1. De novo assembly by analogy
2. Long read assembly
3. Disease Analytics

Population structure of *Oryza sativa*

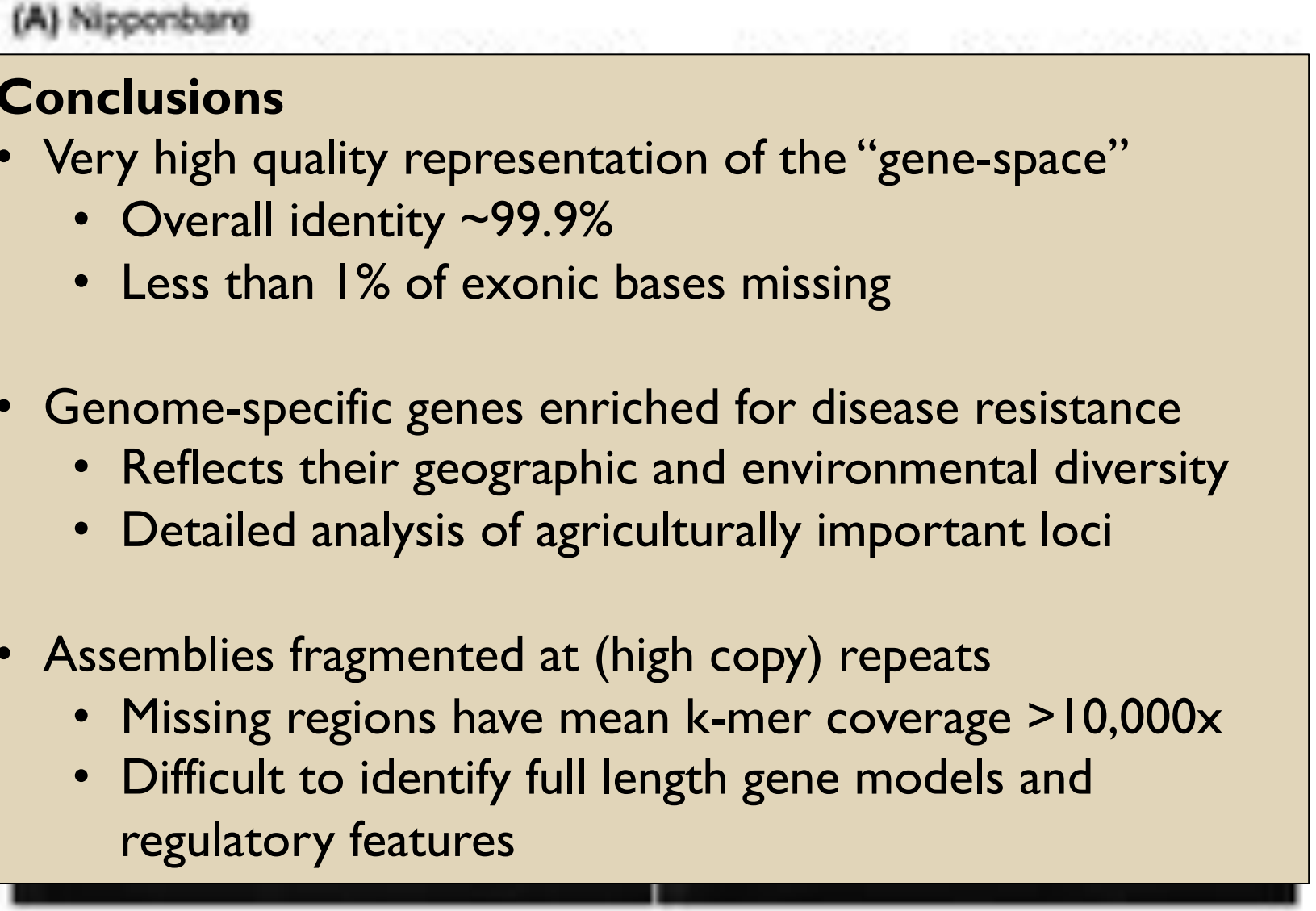




New whole genome de novo assemblies of three divergent strains of rice (*O. sativa*) documents novel gene space of *aus* and *indica*

Schatz, MC, Maron, L, Stein, *et al* (2014) *Under Review.*

Strain specific regions

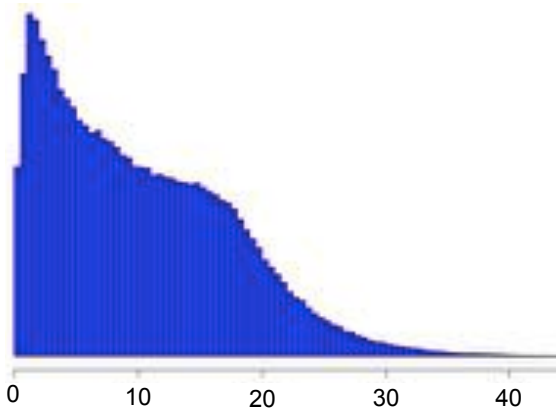


Conclusions

- 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
  - Detailed analysis of agriculturally important loci
- Assemblies fragmented at (high copy) repeats
  - Missing regions have mean k-mer coverage >10,000x
  - Difficult to identify full length gene models and regulatory features

Long Read Sequencing Technology

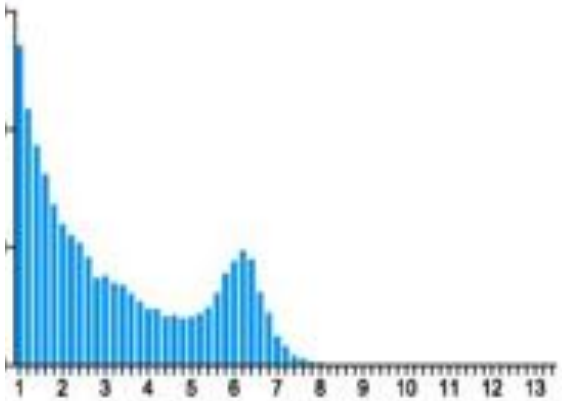
PacBio RS II



CSHL/PacBio

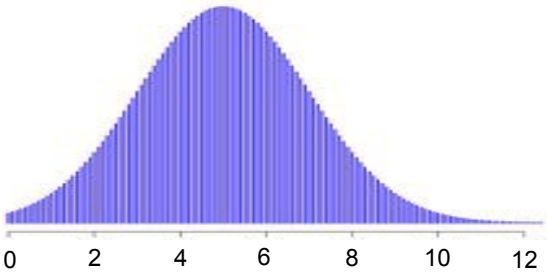
Moleculo

illumina
moleculo



(Voskoboynik et al. 2013)

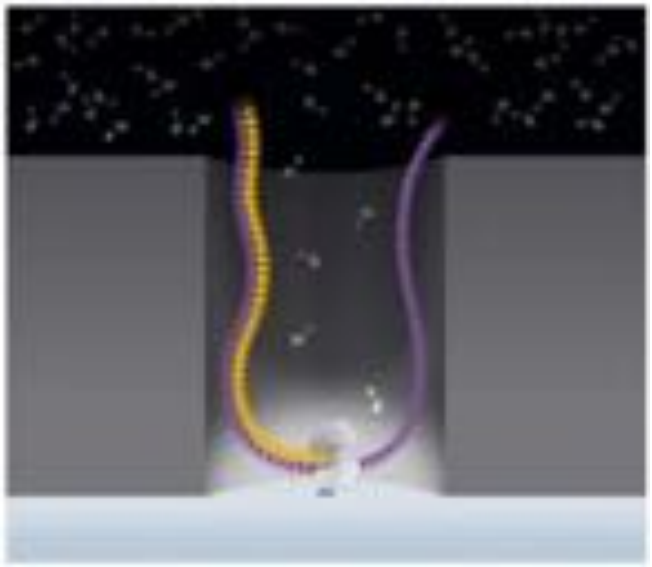
Oxford Nanopore

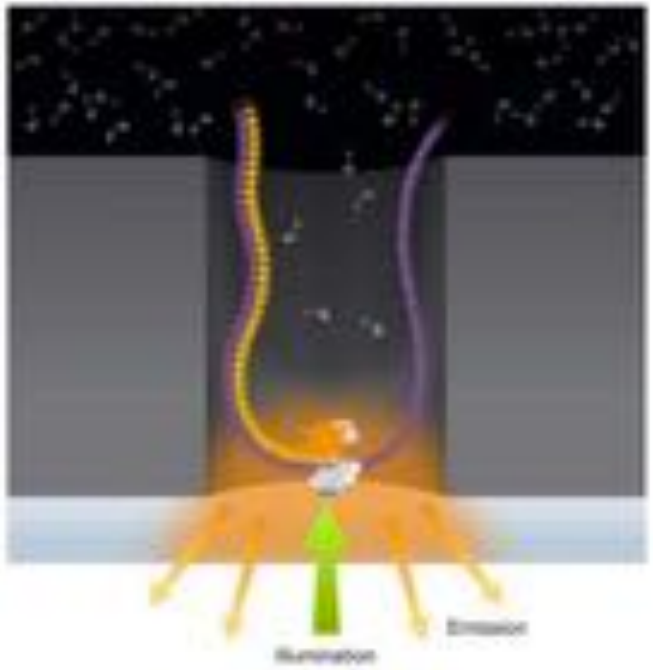


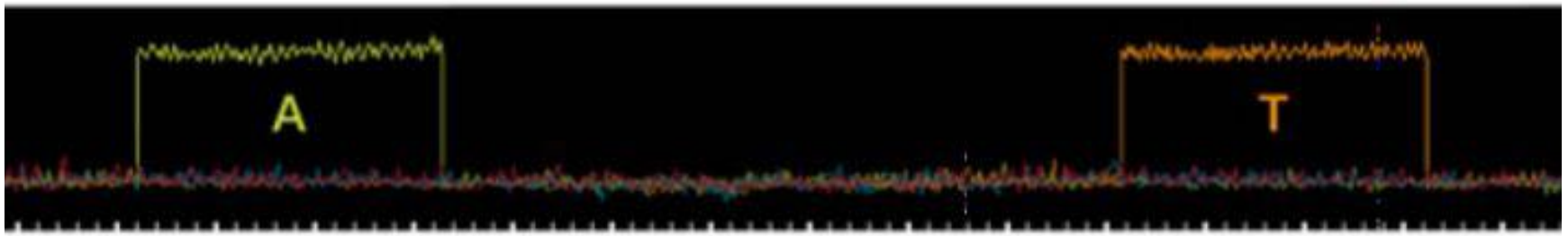
Broad/OxNano @ AGBT ***

SMRT Sequencing

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





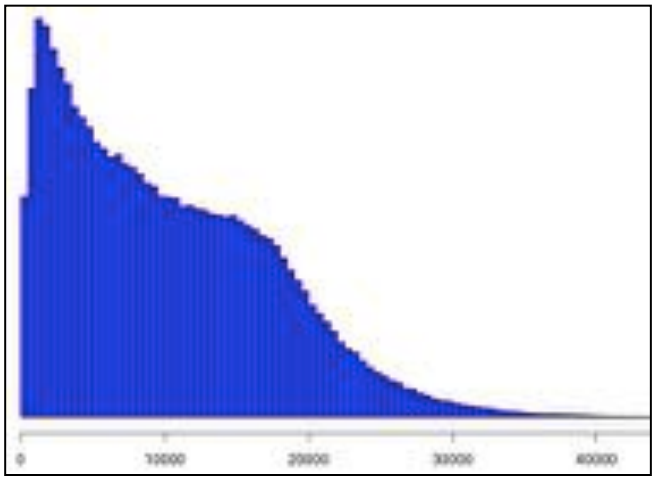


Intensity

Time

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

SMRT Sequencing Data

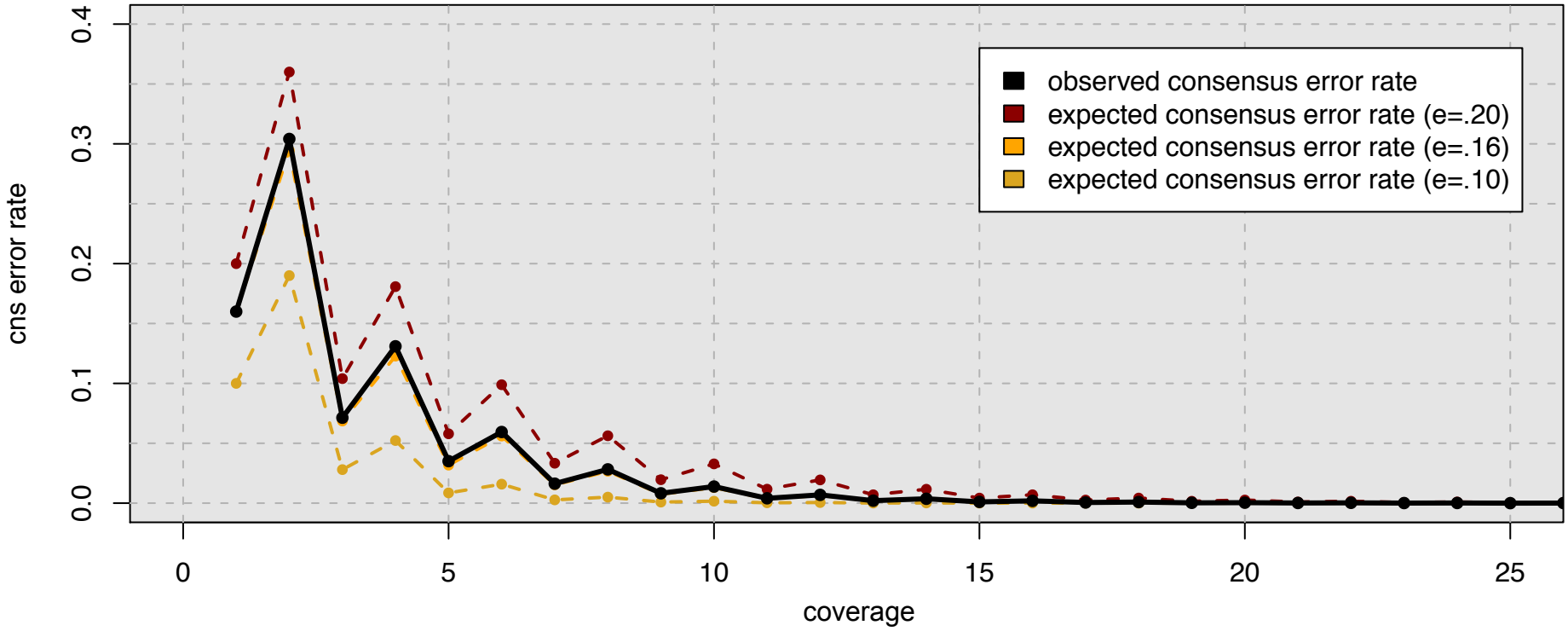


Match	83.7%
Insertions	11.5%
Deletions	3.4%
Mismatch	1.4%

```
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

Consensus Accuracy and Coverage



Coverage can overcome random errors

- Dashed: error model from binomial sampling
- Solid: observed accuracy

$$CNS\ Error = \sum_{i=\lceil c/2 \rceil}^{c} \binom{c}{i} (e)^{i} (1-e)^{n-i}$$

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

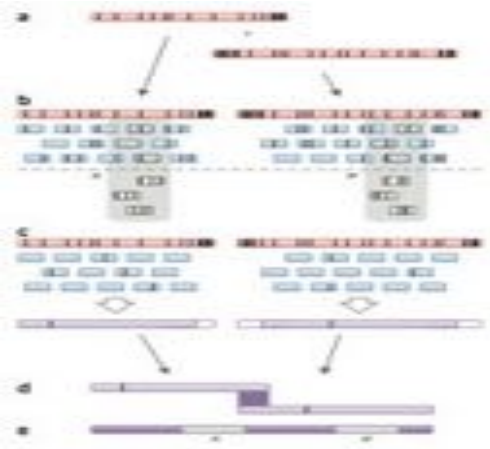
PacBio Assembly Algorithms

PBJelly

Gap Filling and Assembly Upgrade

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

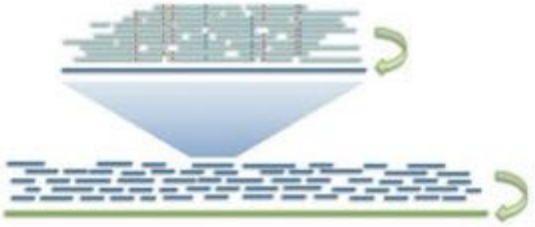
PacBioToCA & ECTools



Hybrid/PB-only Error Correction

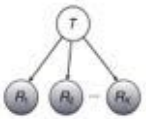
Koren, Schatz, *et al* (2012)
Nature Biotechnology. 30:693–700

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)

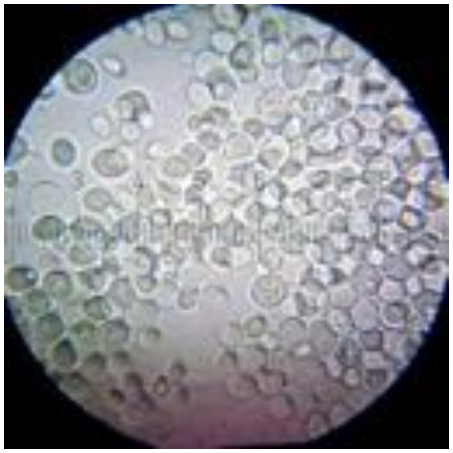
	Initial Assembly	Quiver Consensus
QV	43.4	54.5
Accuracy	99.99540%	99.99964%
Differences	141	11

PB-only Correction & Polishing

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

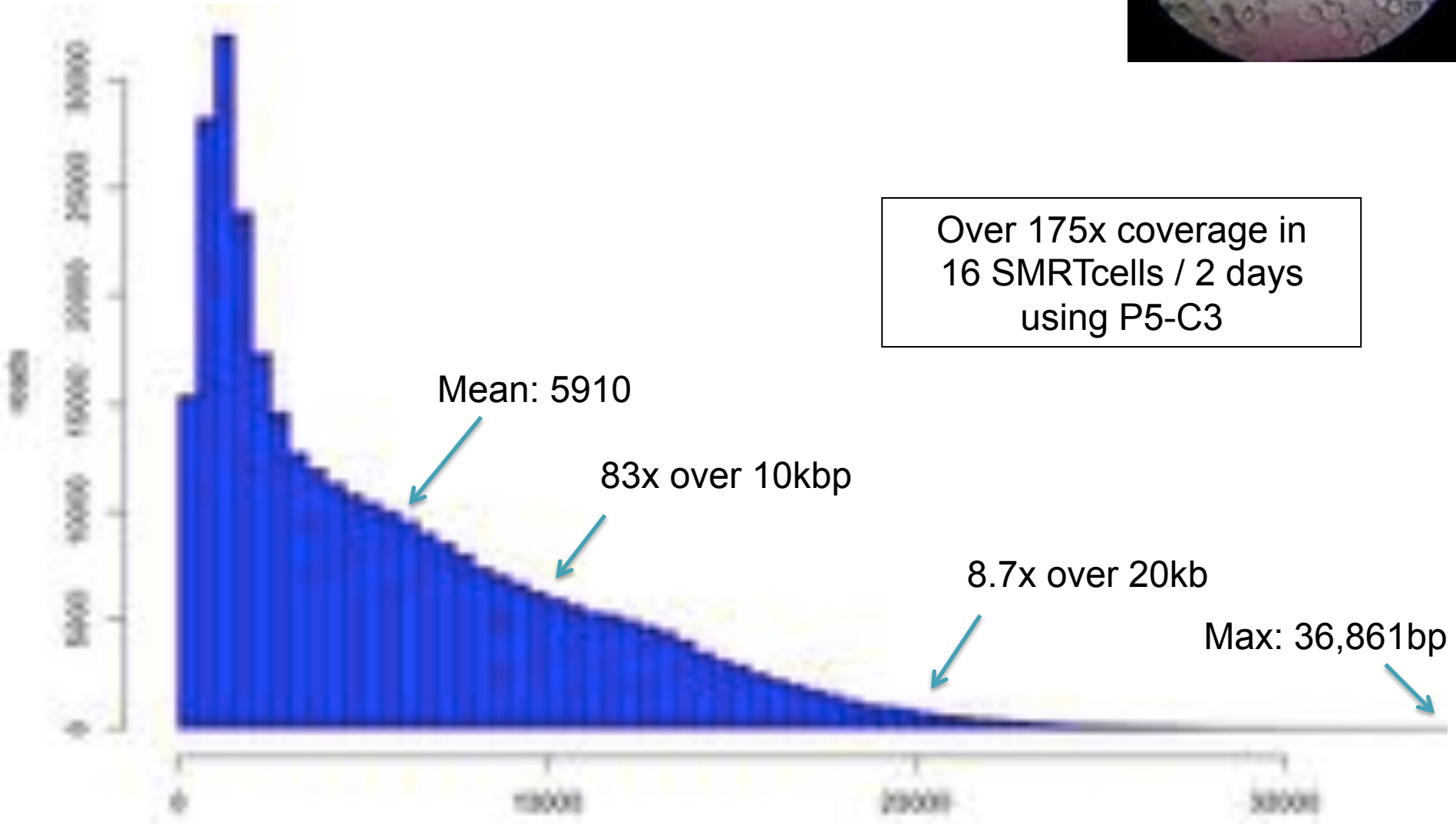
< 5x PacBio Coverage > 50x

S. cerevisiae W303

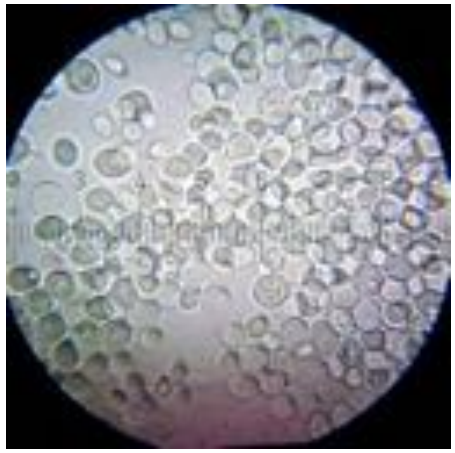


PacBio RS II sequencing at CSHL by Dick McCombie

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



S. cerevisiae W303

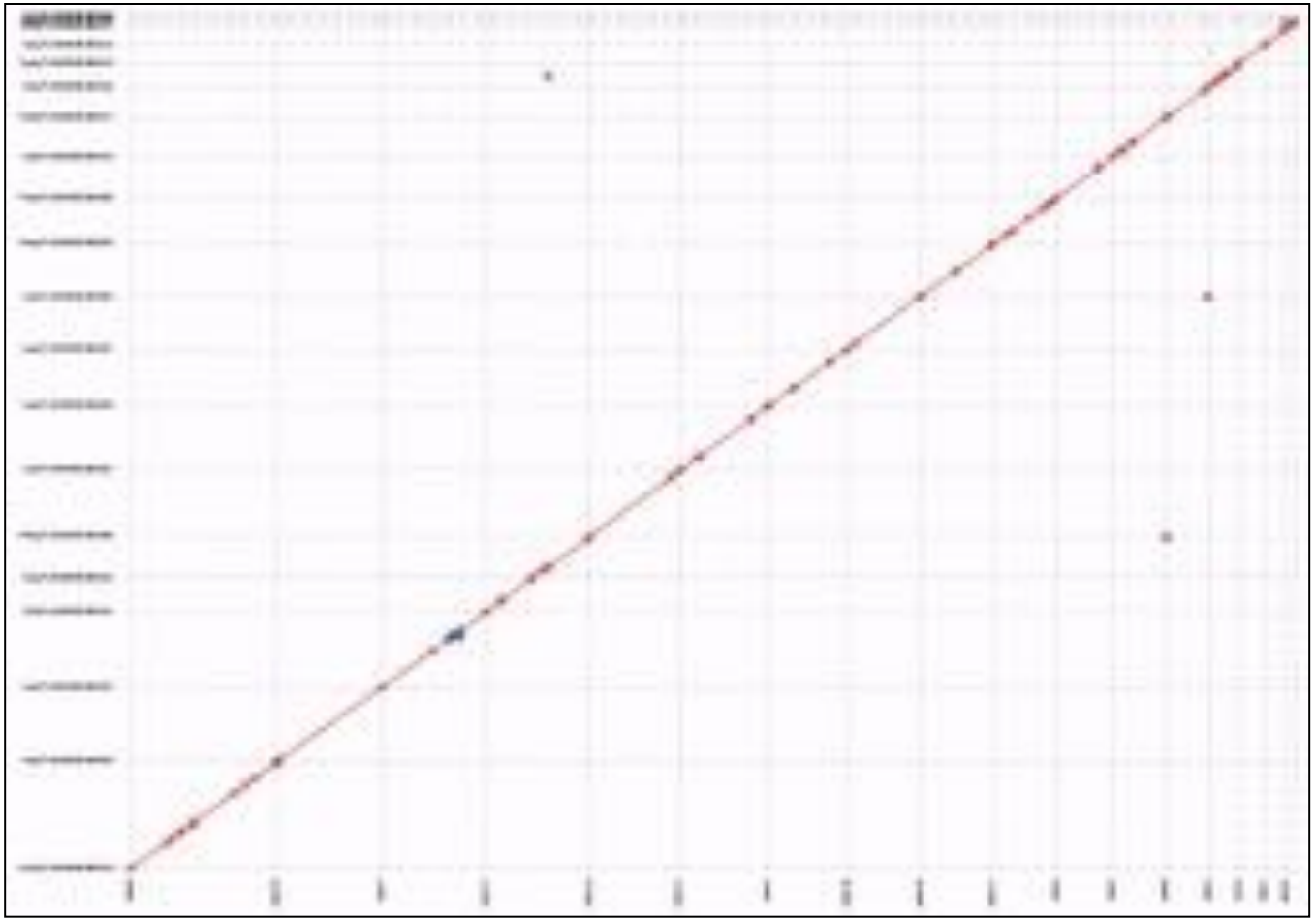


S288C Reference sequence

- 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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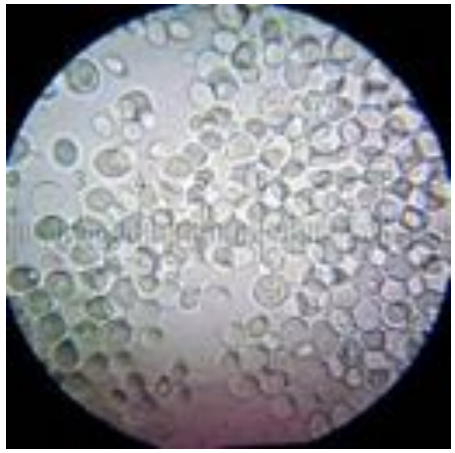

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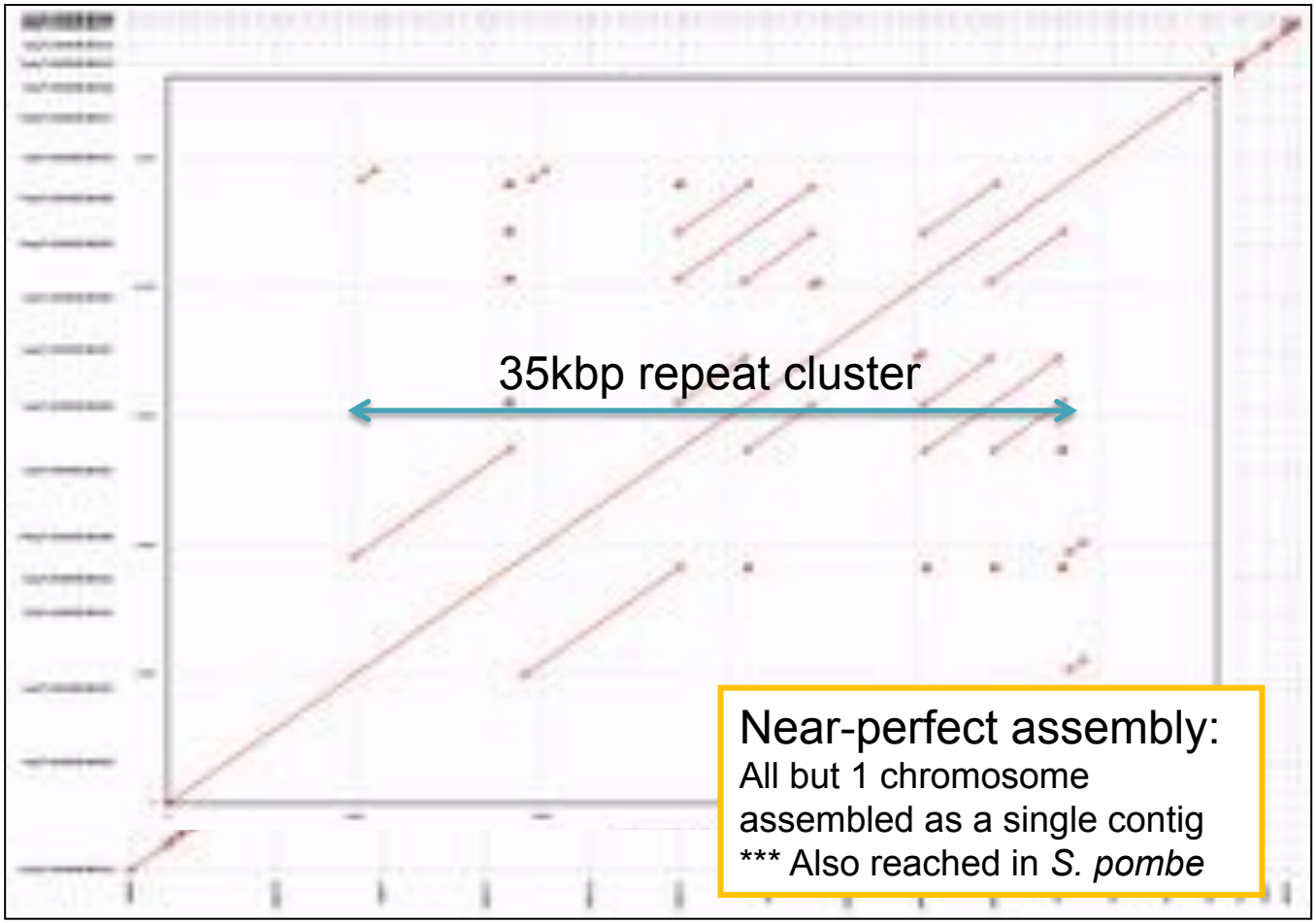
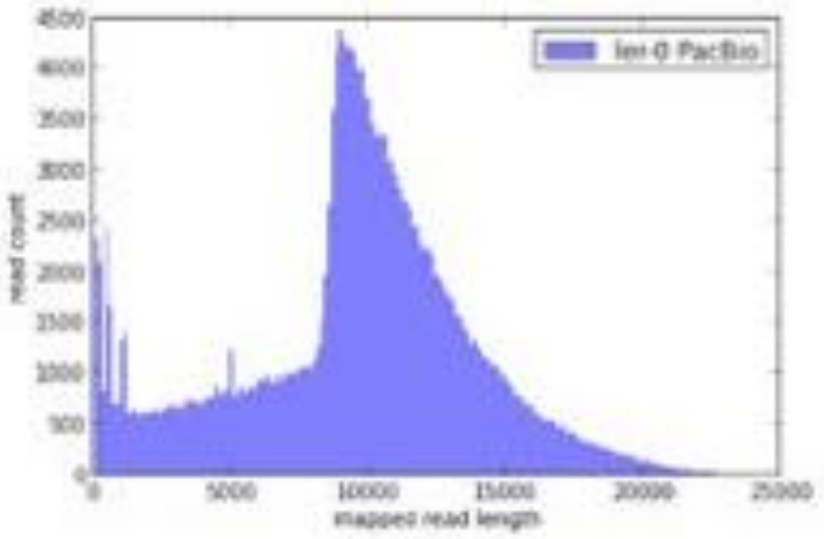


A. thaliana Ler-0

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



A. thaliana Ler-0 sequenced at PacBio

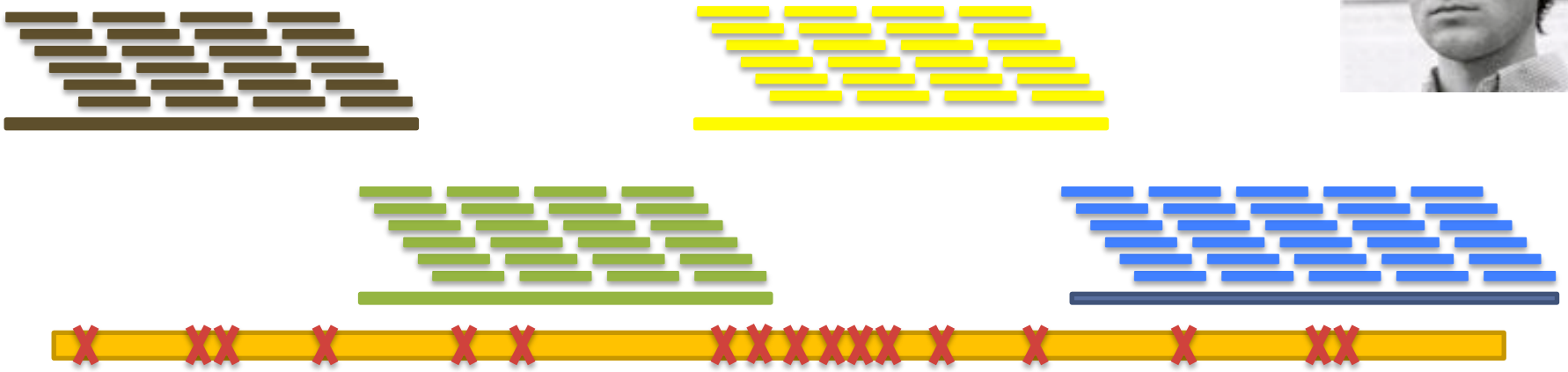
- Sequenced using the previous P4 enzyme and C2 chemistry
- Size selection using an 8 Kb to 50 Kb elution window on a BluePippin™ device from Sage Science
- Total coverage >119x

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

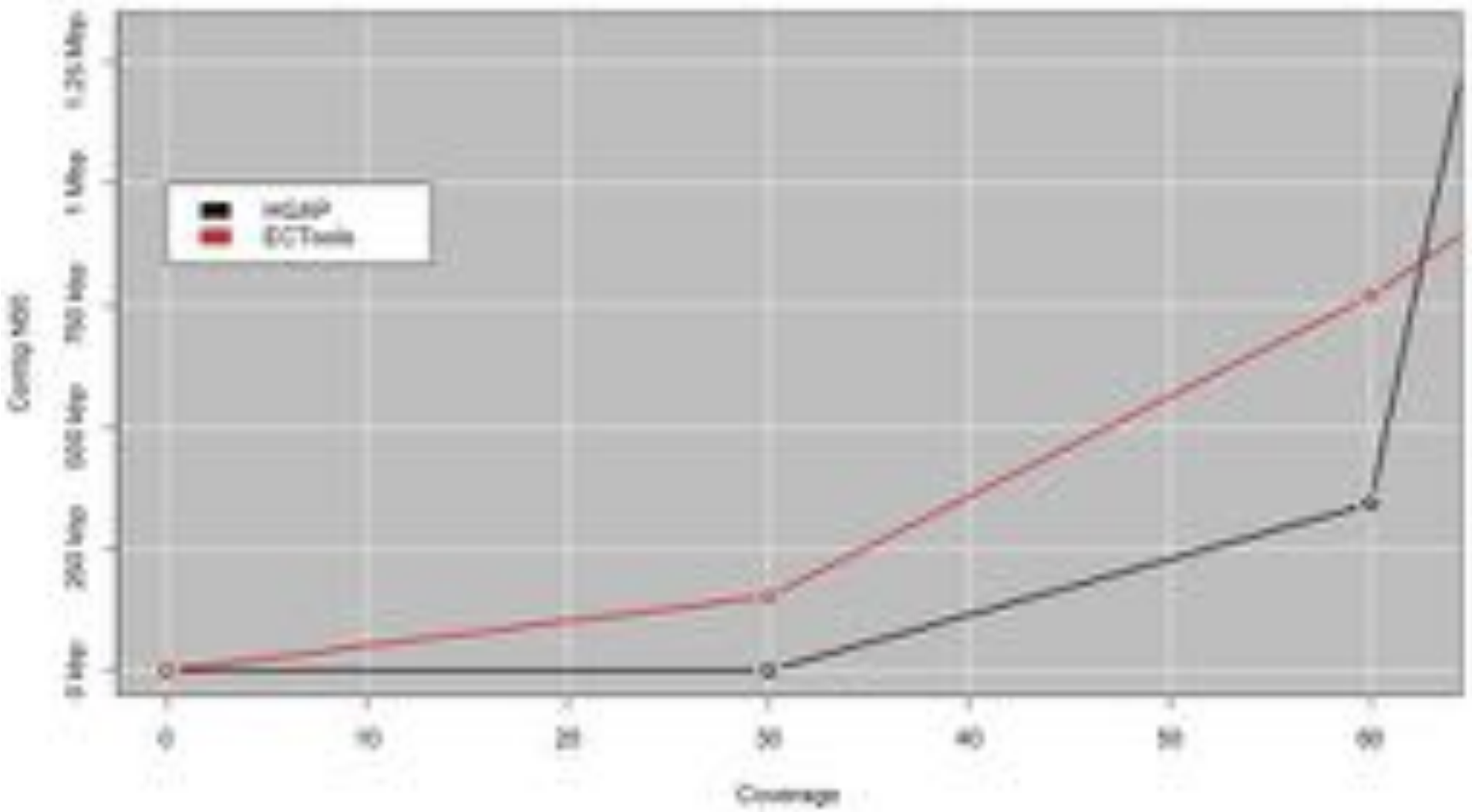
Can Help us overcome:

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

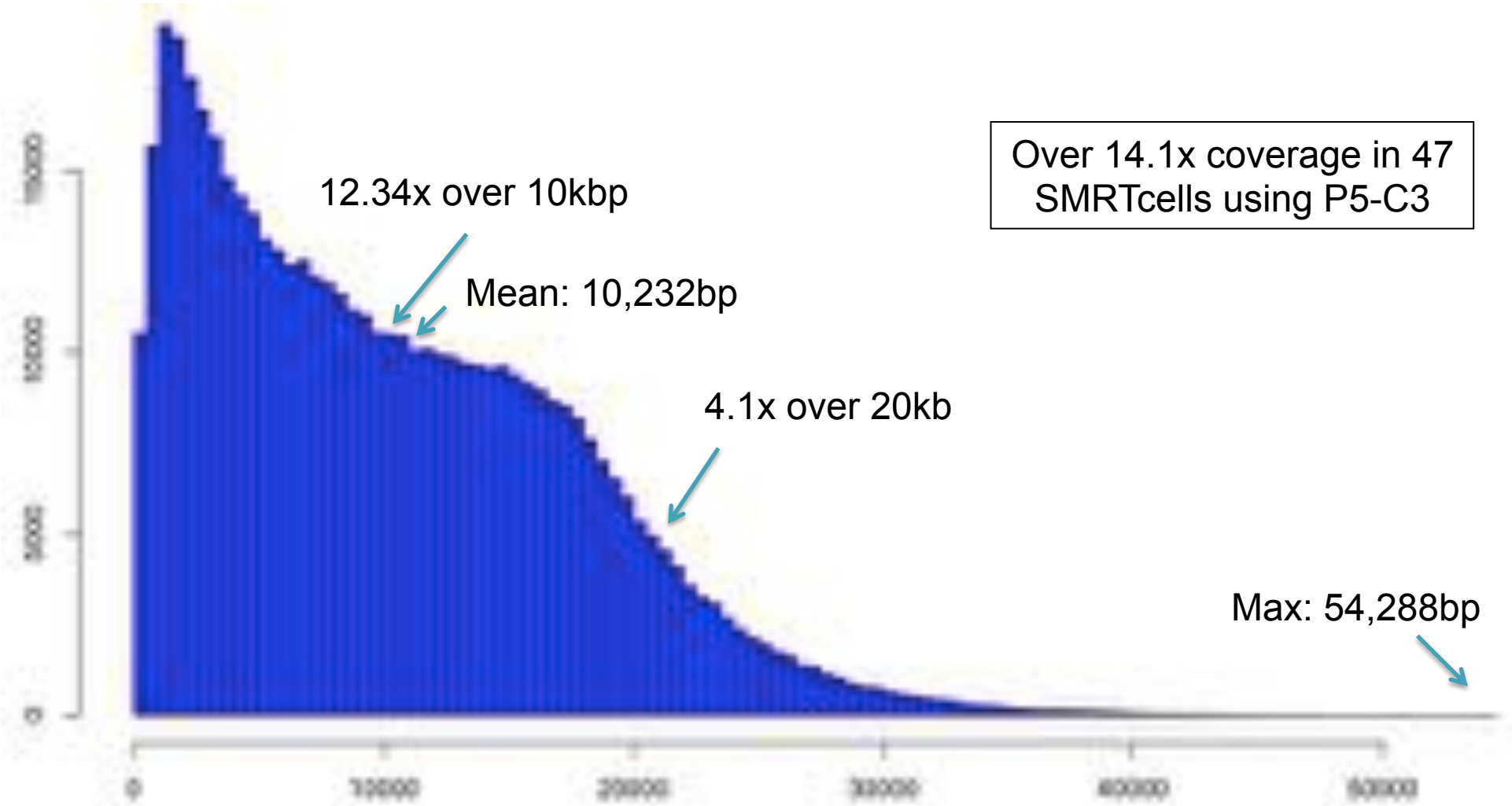
http://blog.pacificbiosciences.com/2013/08/new-data-release-arabidopsis-assembly.html



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

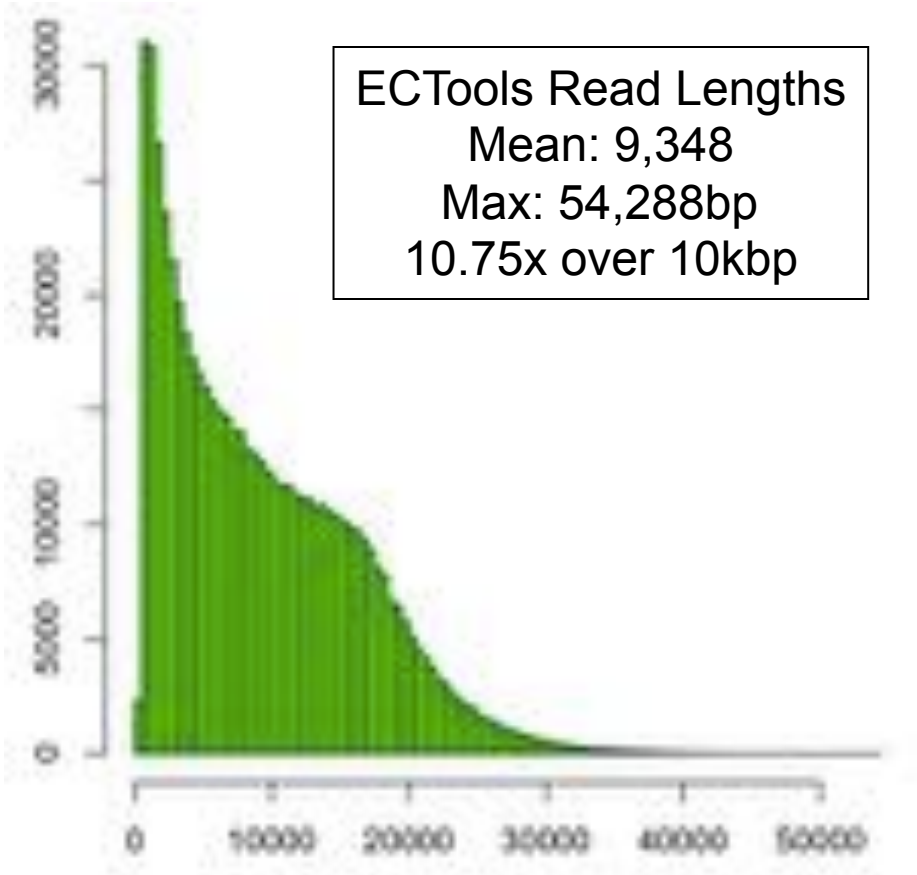


Over 14.1x coverage in 47 SMRTcells using P5-C3

O. sativa pv Indica (IR64)

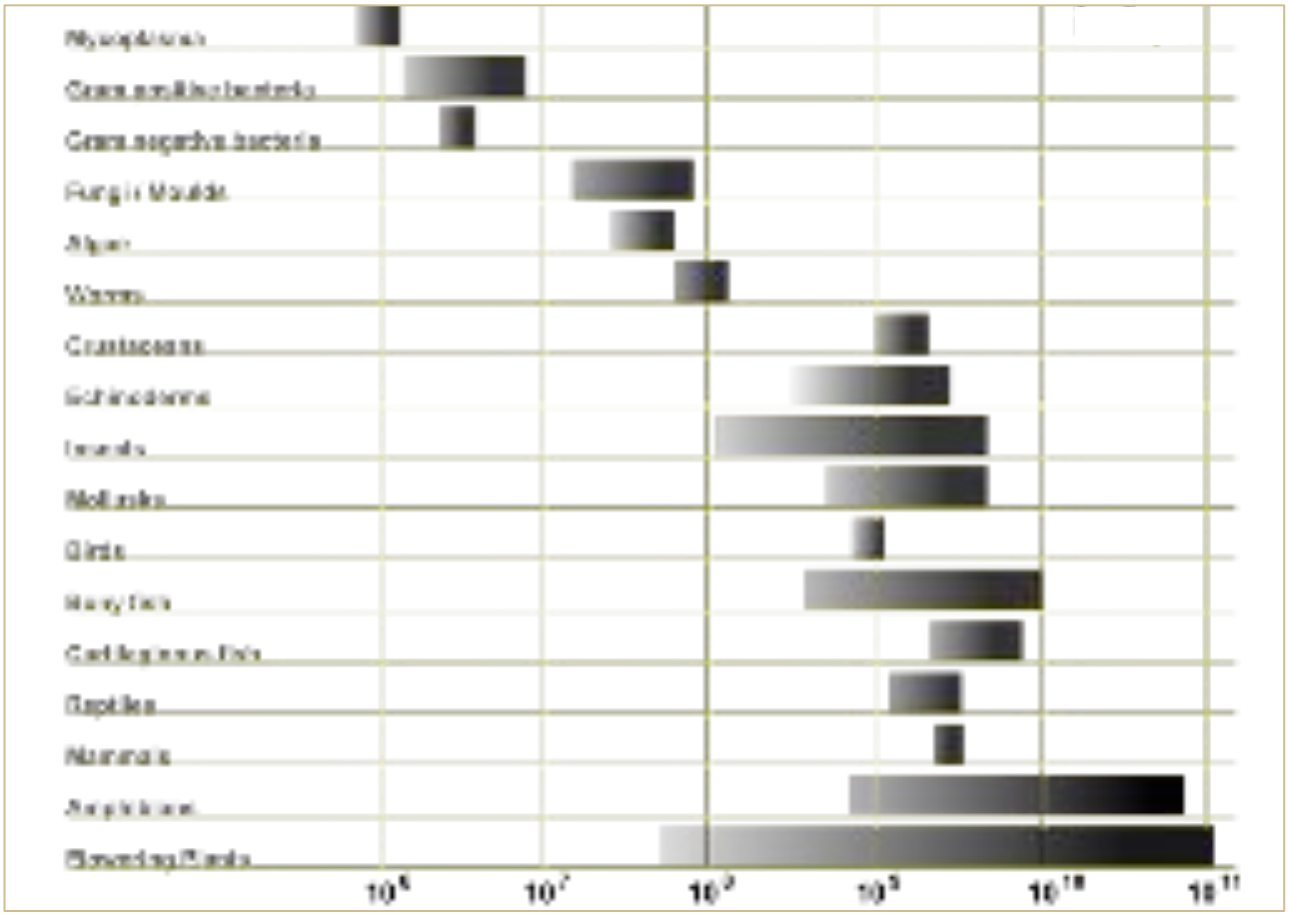
Genome size: ~370 Mb
Chromosome N50: ~29.7 Mbp

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



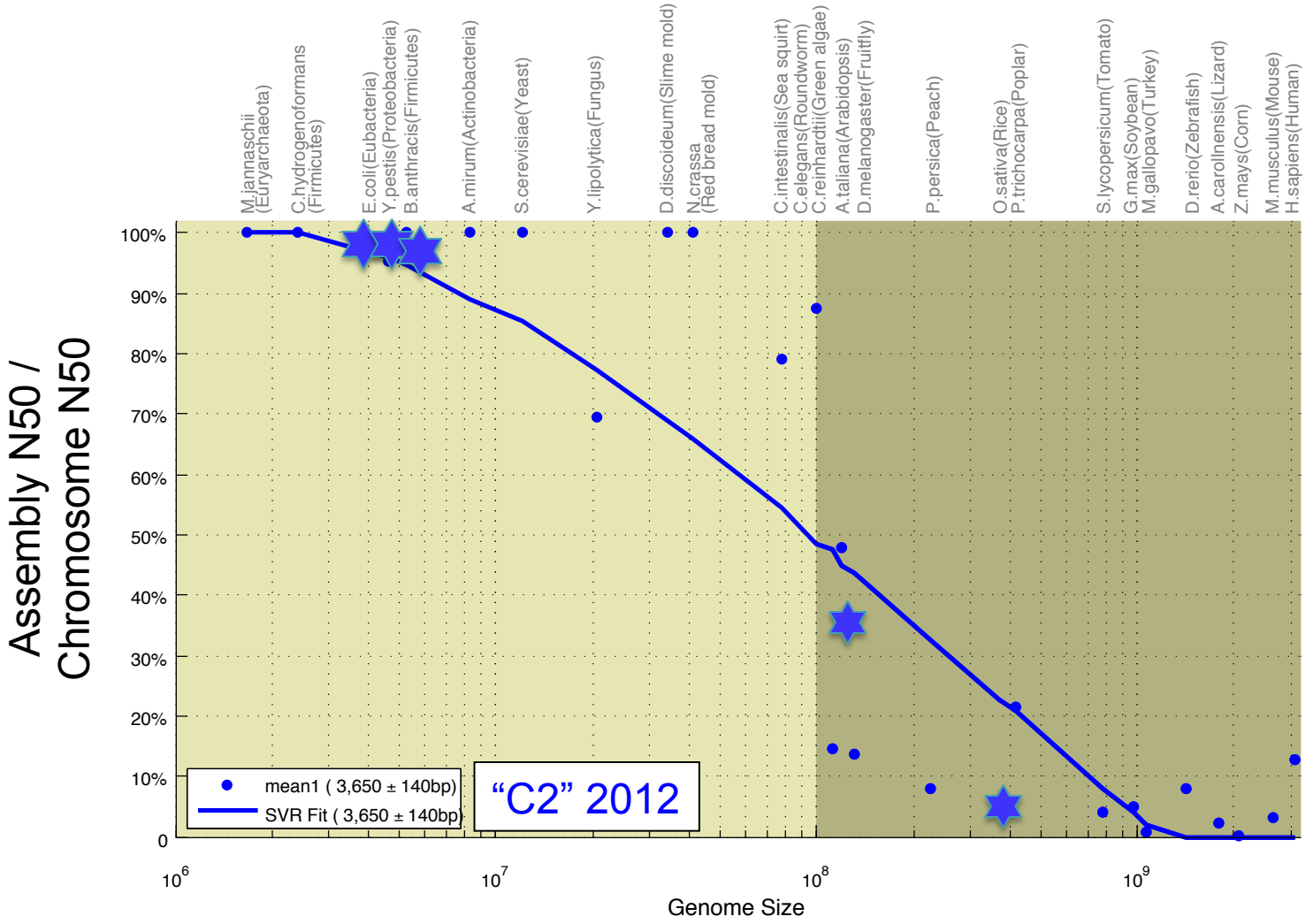
ECTools Read Lengths
Mean: 9,348
Max: 54,288bp
10.75x over 10kbp

What should we expect from an assembly?



https://en.wikipedia.org/wiki/Genome_size

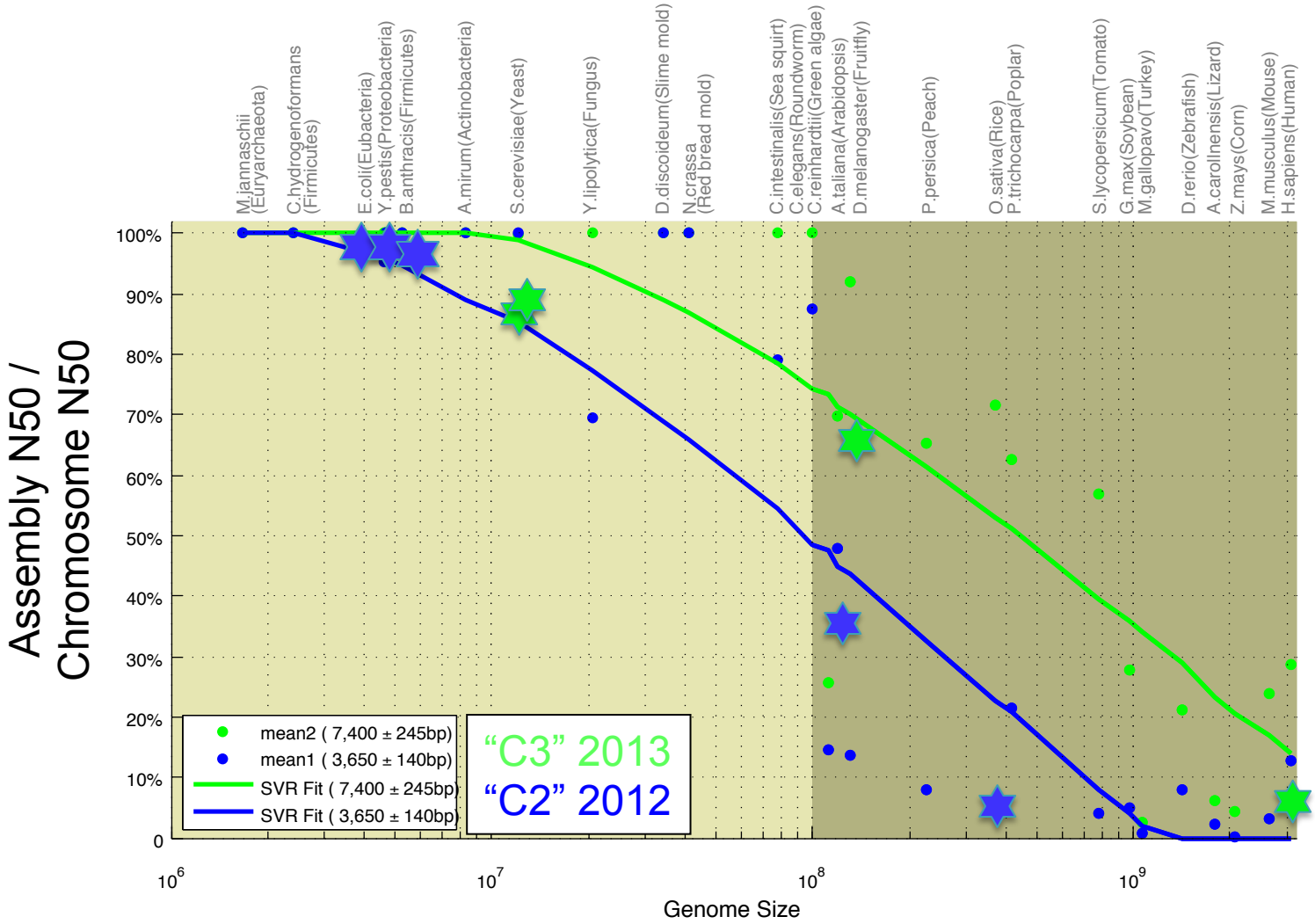
Assembly Complexity of Long Reads



Assembly complexity of long read sequencing

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

Assembly Complexity of Long Reads



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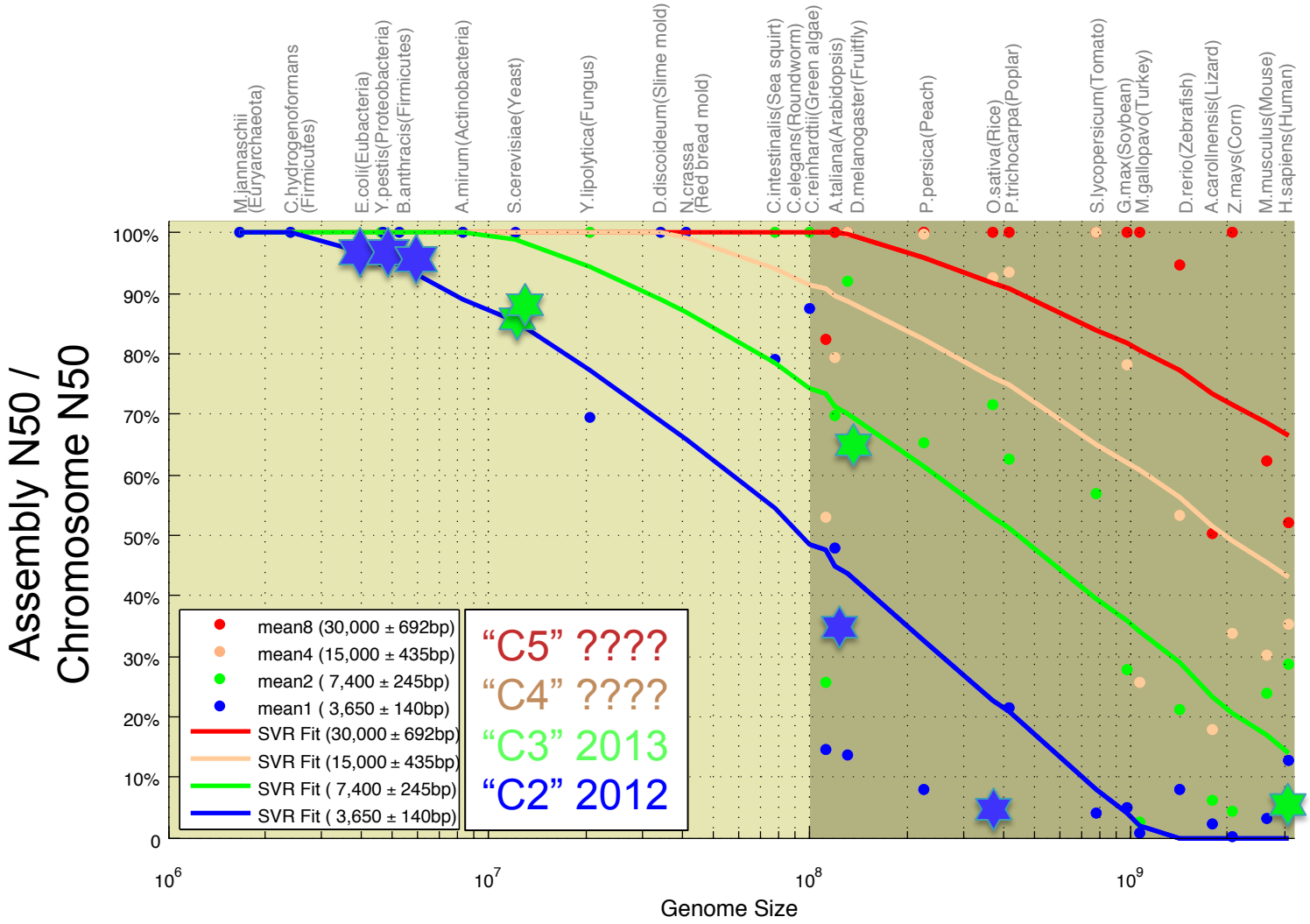
Lee, H*, Gurtowski, J*, Yoo, S, Marcus, S, McCombie, WR, Schatz MC (2014) *In preparation*

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*

Assembly Recommendations

- **Long read sequencing of eukaryotic genomes is here**
- **Recommendations**

  < 100 Mbp: HGAP/PacBio2CA @ 100x PB C3-P5
  expect near perfect chromosome arms

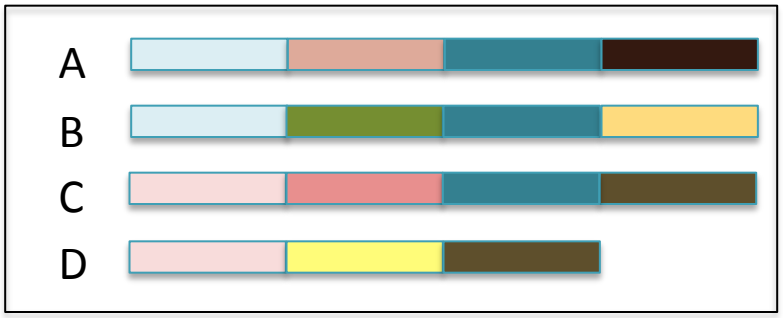
  < 1GB: HGAP/PacBio2CA @ 100x PB C3-P5
  expect high quality assembly: contig N50 over 1Mbp

  > 1GB: hybrid/gap filling
  expect contig N50 to be 100kbp – 1Mbp

  > 5GB: Email mschatz@cshl.edu

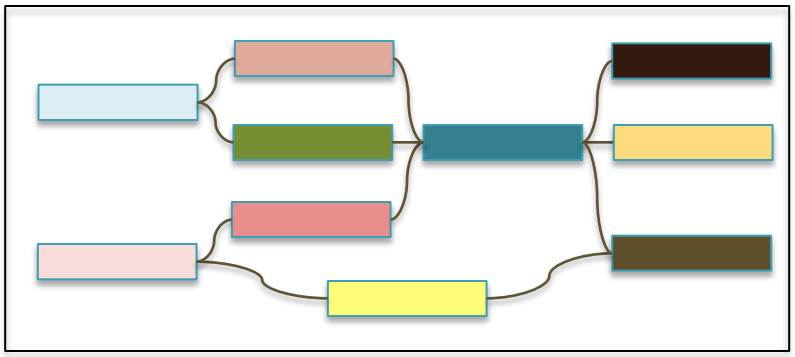
- **Caveats**
  - Model only as good as the available references (esp. haploid sequences)
  - Technologies are quickly improving, exciting new scaffolding technologies

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

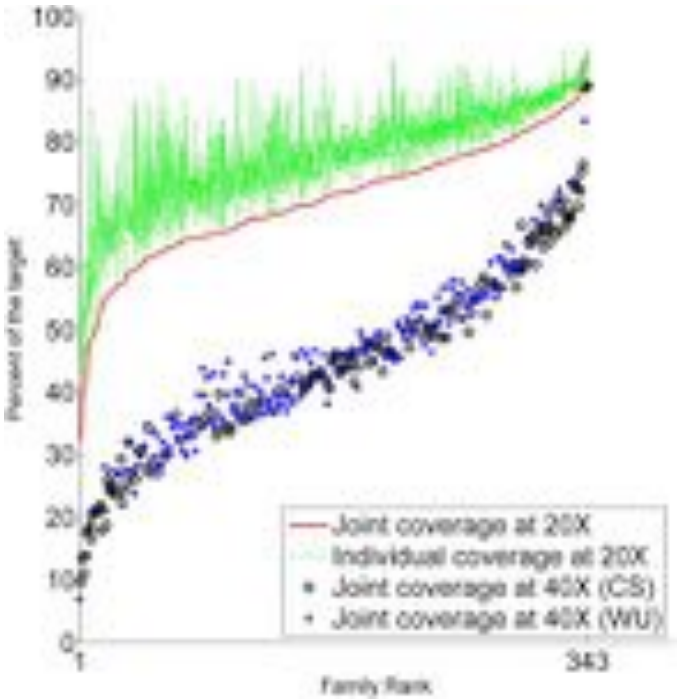
- Encodes all the sequence relationships between the genomes
- How well conserved is a given sequence?
- What are the pan-genome network properties?

SplitMEM: Graphical pan-genome analysis with suffix skips
Marcus, S, Lee, H, Schatz, MC (2014) *Under Review*

Outline

1. De novo assembly by analogy
2. Long Read Assembly
3. Disease Analytics

Exome sequencing of the SSC



Last year saw 3 reports of >593 families from the Simons Simplex Collection

- Parents plus one child with autism and one non-autistic sibling
- All attempted to find mutations enriched in the autistic children
- Iossifov (343) and O'Roak (50) used GATK, Sanders (200) didn't attempt to identify indels

De novo gene disruptions in children on the autism spectrum
Iossifov *et al.* (2012) *Neuron*. 74:2 285-299

De novo mutations revealed by whole-exome sequencing are strongly associated with autism
Sanders *et al.* (2012) *Nature*. 485, 237–241.

Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations
O'Roak *et al.* (2012) *Nature*. 485, 246–250.

Scalpel: Haplotype Microassembly

DNA sequence **micro-assembly** pipeline for accurate detection and validation of *de novo* mutations (SNPs, indels) within exome-capture data.

Features

1. Combine mapping and assembly
2. Exhaustive search of haplotypes
3. De novo mutations

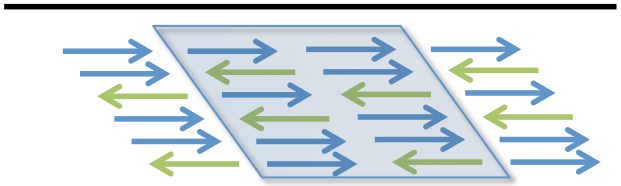
NRXN1 *de novo* SNP
(auSSC12501 chr2:50724605)

Accurate detection of de novo and transmitted INDELs within exome-capture data using micro-assembly

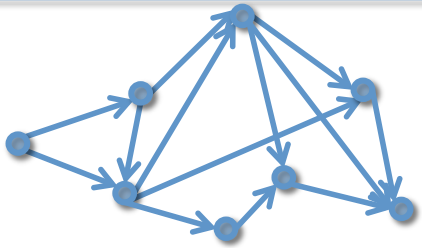
Narzisi, G, O'Rawe, J, Iossifov, I, Lee, Y, Wang, Z, Wu, Y, Lyon, G, Wigler, M, Schatz, MC (2014) *Under review.*

Scalpel Pipeline

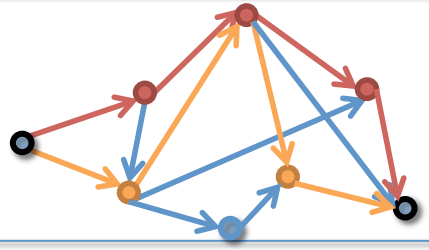
Extract reads mapping within the exon including (1) well-mapped reads, (2) soft-clipped reads, and (3) anchored pairs



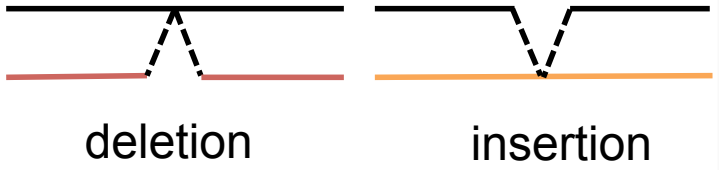
Decompose reads into overlapping *k*-mers and construct de Bruijn graph from the reads



Find end-to-end haplotype paths spanning the region



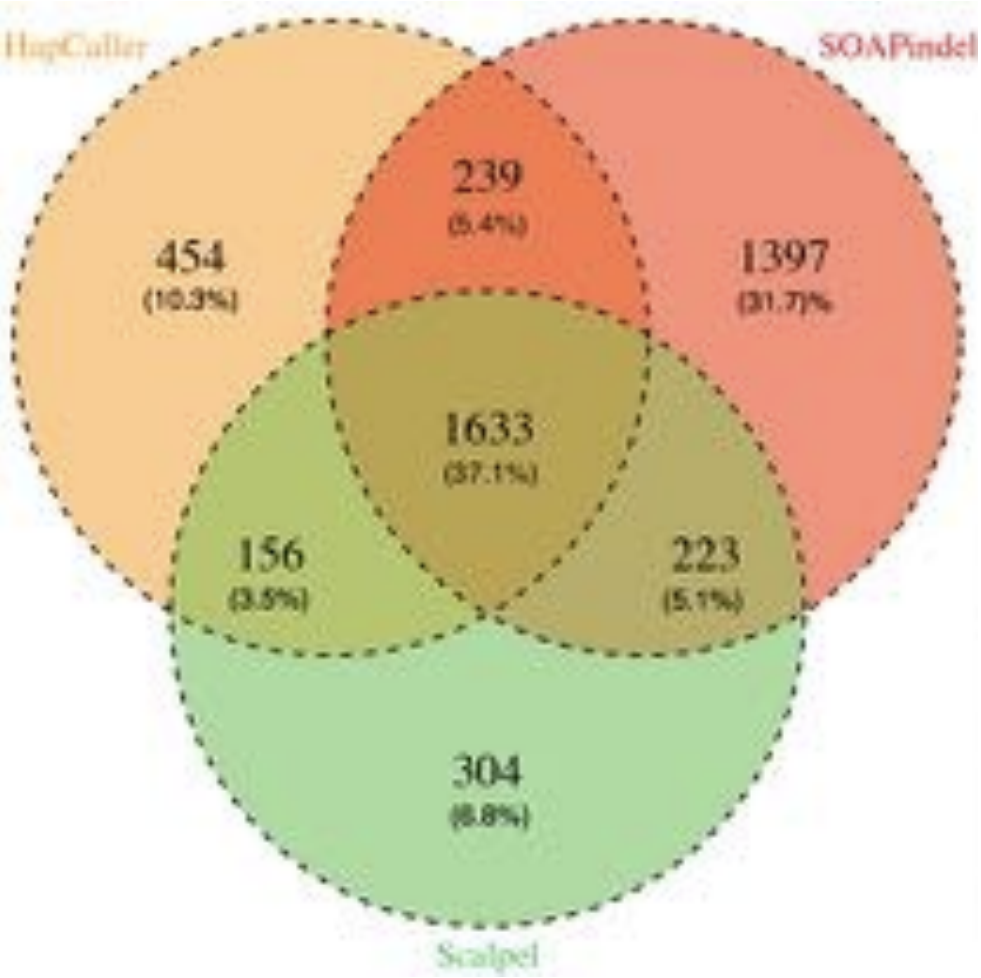
Align assembled sequences to reference to detect mutations



Experimental Analysis & Validation

Selected one deep coverage exome for deep analysis

- Individual was diagnosed with ADHD and turrets syndrome
- 80% of the target at >20x coverage
- Evaluated with Scalpel, SOAPindel, and GATK Haplotype Caller



1000 indels selected for validation

- 200 Scalpel
- 200 GATK Haplotype Caller
- 200 SOAPindel
- 200 within the intersection
- 200 long indels (>30bp)

Experimental Analysis & Validation

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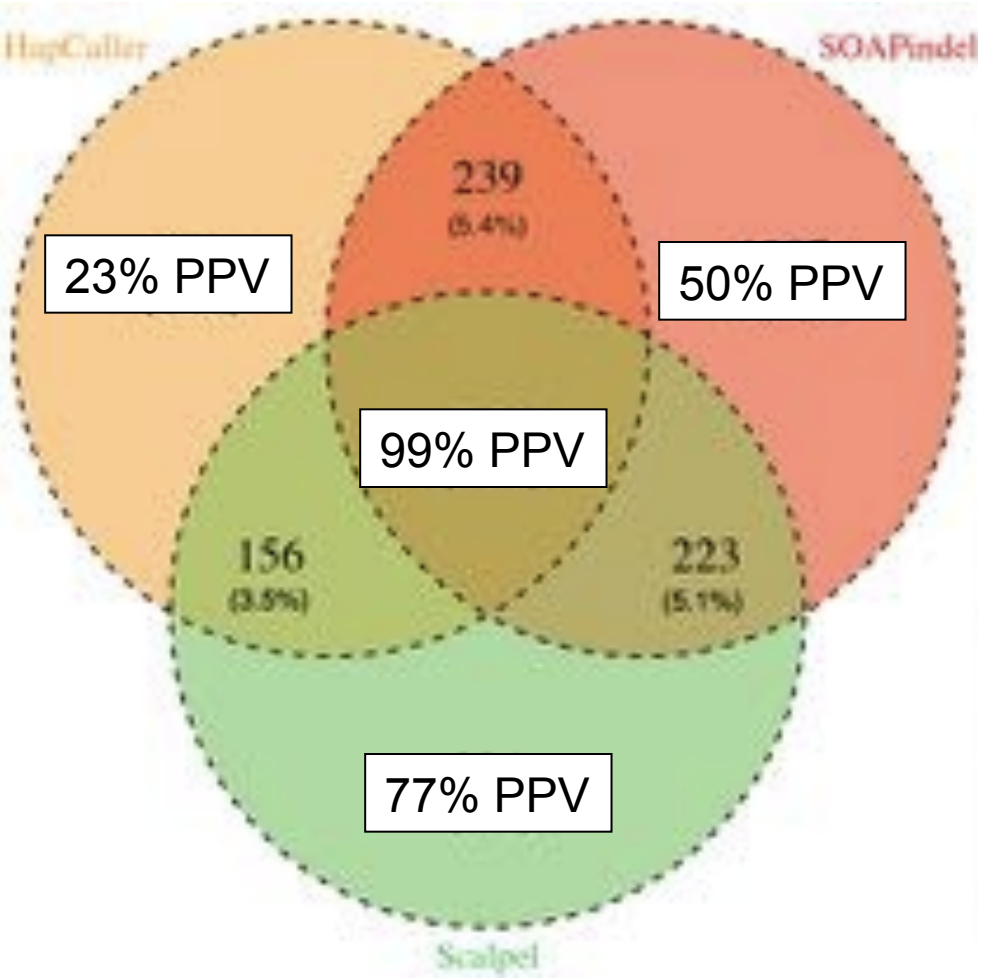

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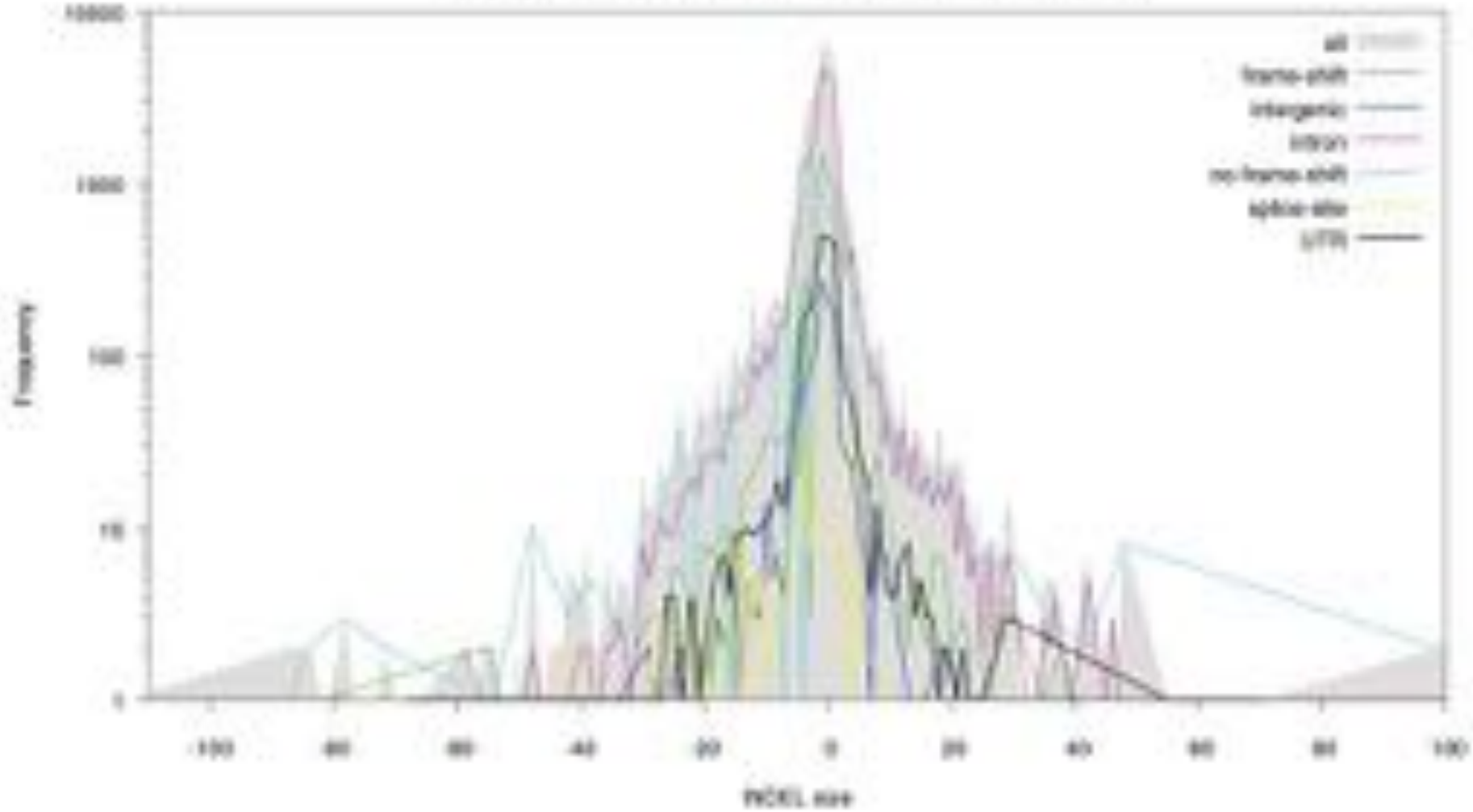
Revised Analysis of the SSC

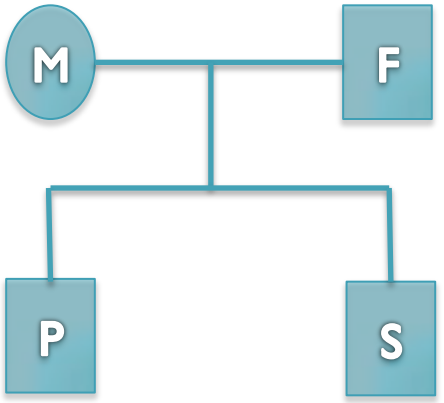


Constructed database of >1M transmitted and de novo indels
Many new gene candidates identified, population analysis underway

De novo mutation discovery and validation

Concept: Identify mutations not present in parents.

Challenge: Sequencing errors in the child or low coverage in parents lead to false positive de novos




```
Reference:   ...TCAAATCCTTTTAATAAAGAAGAGCTGACA...

Father:      ...TCAAATCCTTTTAATAAAGAAGAGCTGACA...
Mother:      ...TCAAATCCTTTTAATAAAGAAGAGCTGACA...
Sibling:     ...TCAAATCCTTTTAATAAAGAAGAGCTGACA...
Proband(1):  ...TCAAATCCTTTTAATAAAGAAGAGCTGACA...
Proband(2):  ...TCAAATCCTTTTAAT****AAGAGCTGACA...
```

4bp heterozygous deletion at chr15:93524061 CHD2

De novo Genetics of Autism

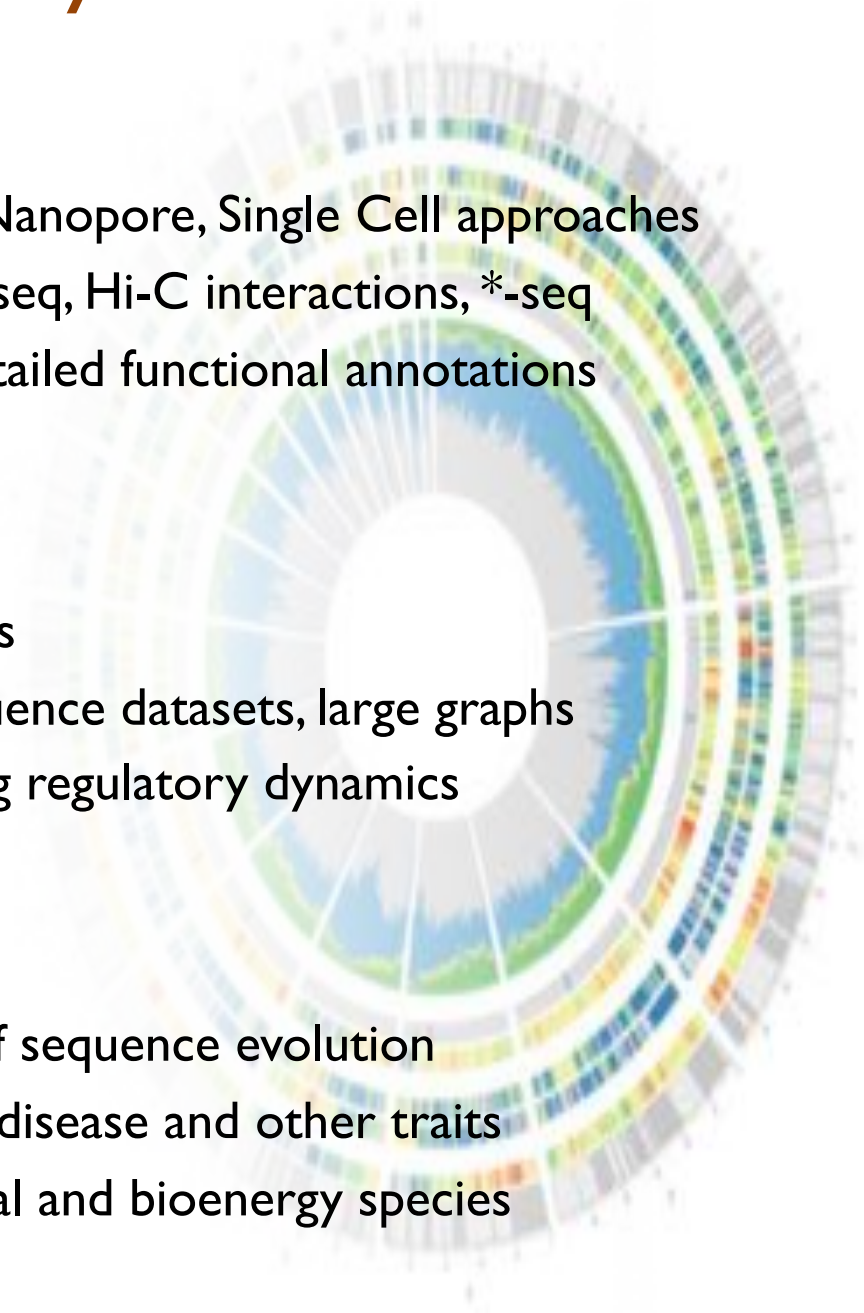
- In 593 family quads so far, we see significant enrichment in de novo ***likely gene killers*** in the autistic kids
  - Overall rate basically 1:1
  - 2:1 enrichment in nonsense mutations
  - 2:1 enrichment in frameshift indels
  - 4:1 enrichment in splice-site mutations
  - Most de novo originate in the paternal line in an age-dependent manner (56:18 of the mutations that we could determine)

- Observe strong overlap with fragile X protein (FMPR) network
  - Related to neuron development and synaptic plasticity
  - Also strong overlap with chromatin remodelers

Accurate detection of de novo and transmitted INDELs within exome-capture data using micro-assembly

Narzisi, G, O'Rawe, J, Iossifov, I, Lee, Y, Wang, Z, Wu, Y, Lyon, G, Wigler, M, Schatz, MC (2014) *Under review.*

Summary



Biotechnology

- Sequencing: Illumina, PacBio, Oxford Nanopore, Single Cell approaches
- Biochemical assays: RNA-seq, Methyl-seq, Hi-C interactions, *-seq
- More accurate assemblies & more detailed functional annotations

Algorithmics

- Highly scalable algorithms and systems
- Indexing and analyzing very large sequence datasets, large graphs
- Constructing Pan-genomes & inferring regulatory dynamics

Comparative Genomics

- Cross species comparisons, models of sequence evolution
- Identifying mutations associated with disease and other traits
- Genotype-to-phenotype of agricultural and bioenergy species

Acknowledgements

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Alejandro Wences
Greg Vurture
Eric Biggers
Aspyn Palatnick

CSHL
McCombie Lab
Wigler Lab
Lyon Lab

Hannon Lab
Gingeras Lab
Jackson Lab
Hicks Lab
Iossifov Lab
Levy Lab
Lippman Lab
Martienssen Lab
Tuveson Lab
Ware Lab

Pacific Biosciences

SFARI
SIMONS FOUNDATION AUTISM RESEARCH INITIATIVE

National Human Genome Research Institute

U.S. DEPARTMENT OF ENERGY

NSF

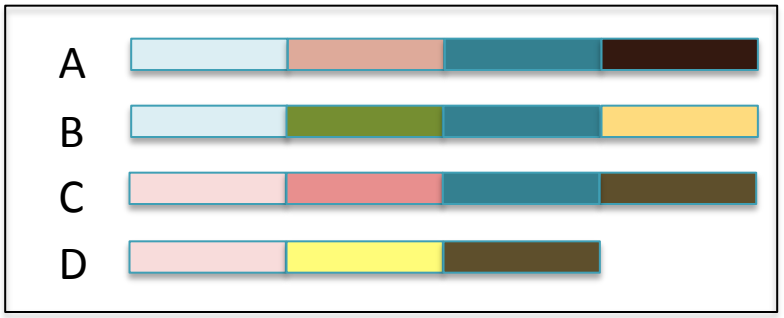
Biological Data Sciences
Cold Spring Harbor Laboratory, Nov 5 - 8, 2014

Thank you

http://schatzlab.cshl.edu

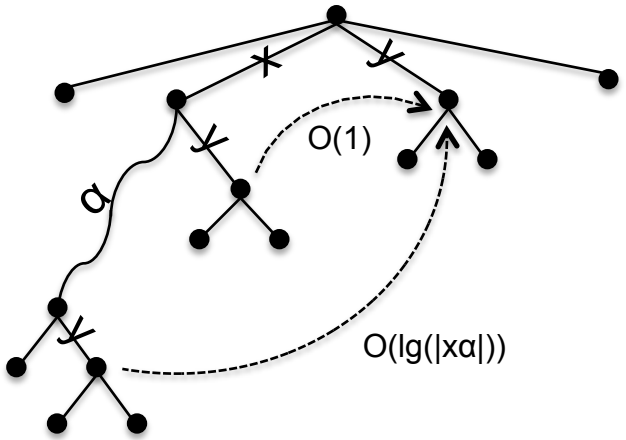
@mike_schatz

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



Align the genomes using a suffix tree augmented with "suffix skips"

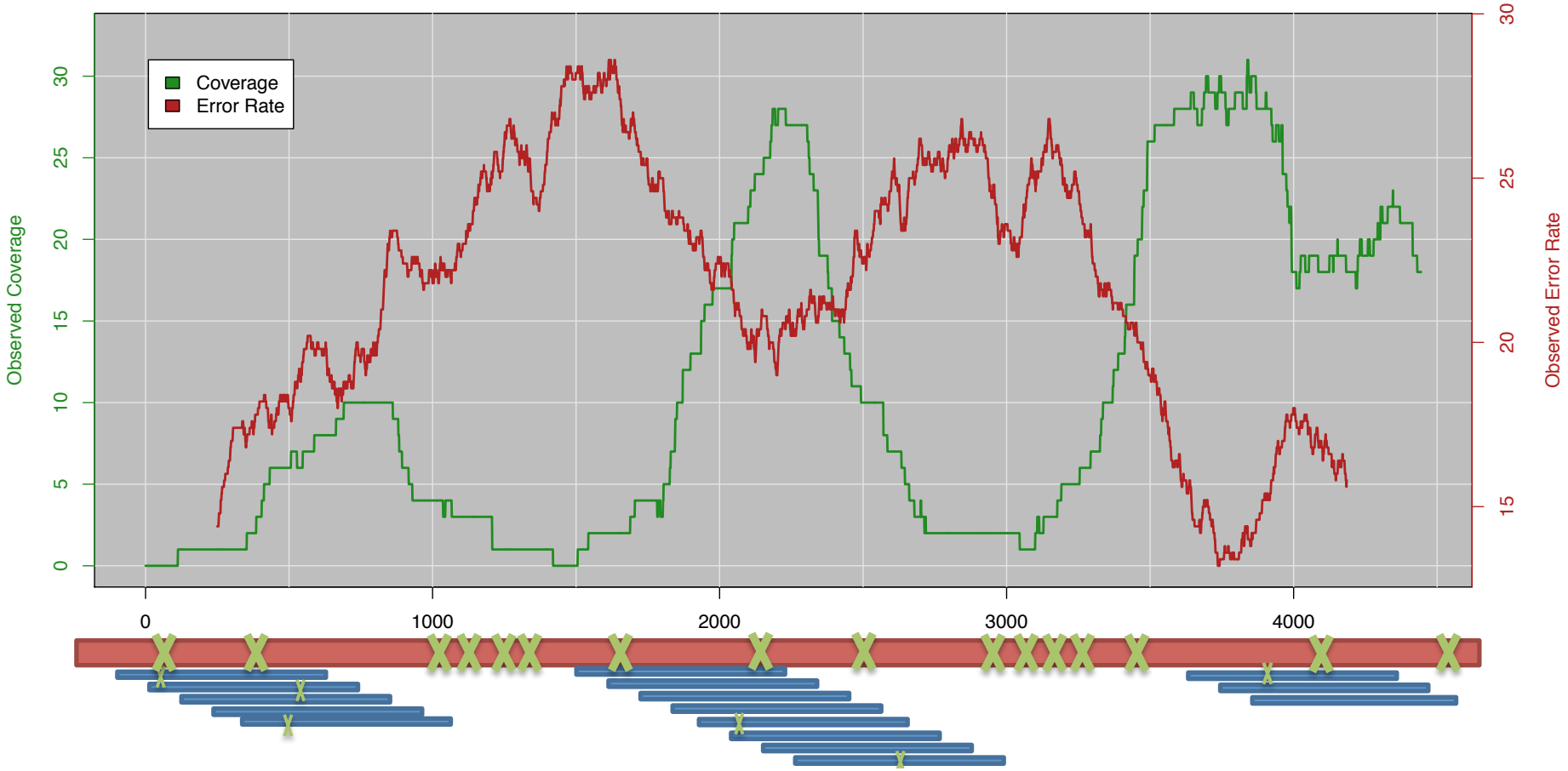
- Similar to suffix links, but navigate between distant suffixes in O(lg |p|)
- Uses pointer doubling techniques to rapidly add additional links

Rapid pan genome analysis with suffix skips
Marcus, S, Schatz, MC (2014) *In preparation*

Hybrid Approaches for Larger Genomes

PacBioToCA fails in complex regions

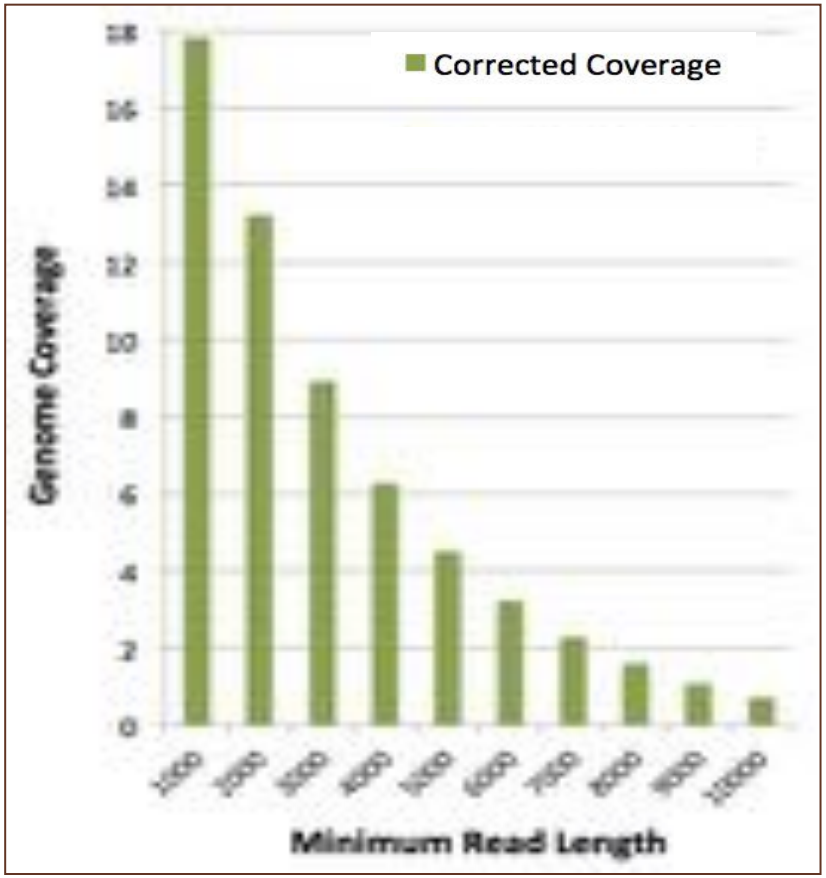
1. Error Dense Regions – Difficult to compute overlaps with many errors
2. Simple Repeats – Kmer Frequency Too High to Seed Overlaps
3. Extreme GC – Lacks Illumina Coverage



O. sativa pv Nipponbare

Genome size: 370 Mb
Chromosome N50: 29.7 Mbp
19x PacBio C2XL sequencing at CSHL from Summer 2012

Assembly	Contig NG50
MiSeq Fragments 23x 459bp 8x 2x251bp @ 450	6,332
"ALLPATHS-recipe" 50x 2x100bp @ 180 36x 2x50bp @ 2100 51x 2x50bp @ 4800	18,248
PacBioToCA 19x @ 3500 ** MiSeq for correction	50,995
ECTools 19x @ 3500 ** MiSeq for correction	155,695

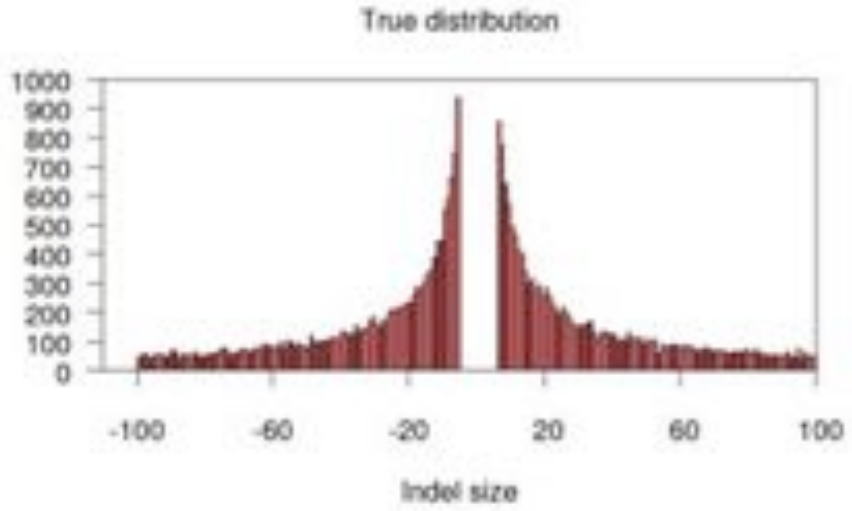


Variation Detection Complexity

SNPs + Short Indels

High precision and sensitivity

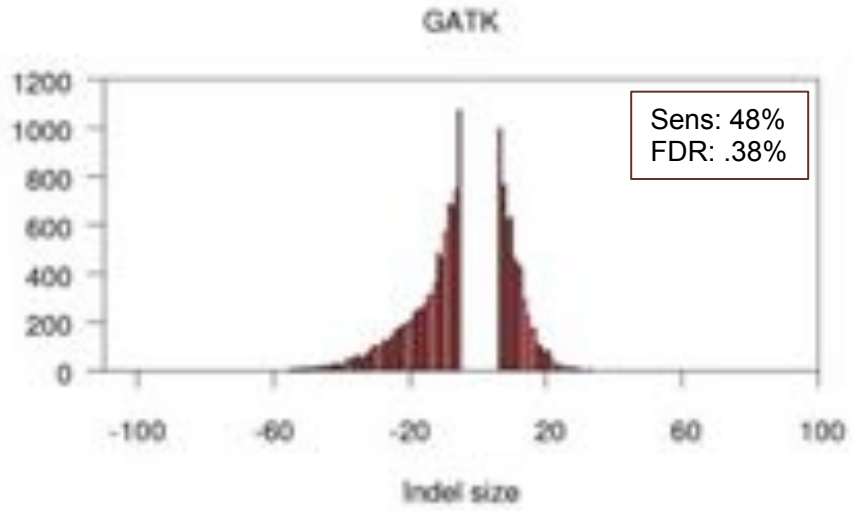
```
..TTTAGAATAG-CGAGTGC...
      ||||||| ||||
      AGAATAGGCGAG
```



"Long" Indels (>5bp)

Reduced precision and sensitivity

```
..TTTAG--------AGTGC...
  |||||         |||||
  TTTAGAATAGGC  |||||
        ATAGGCGAGTGC
```

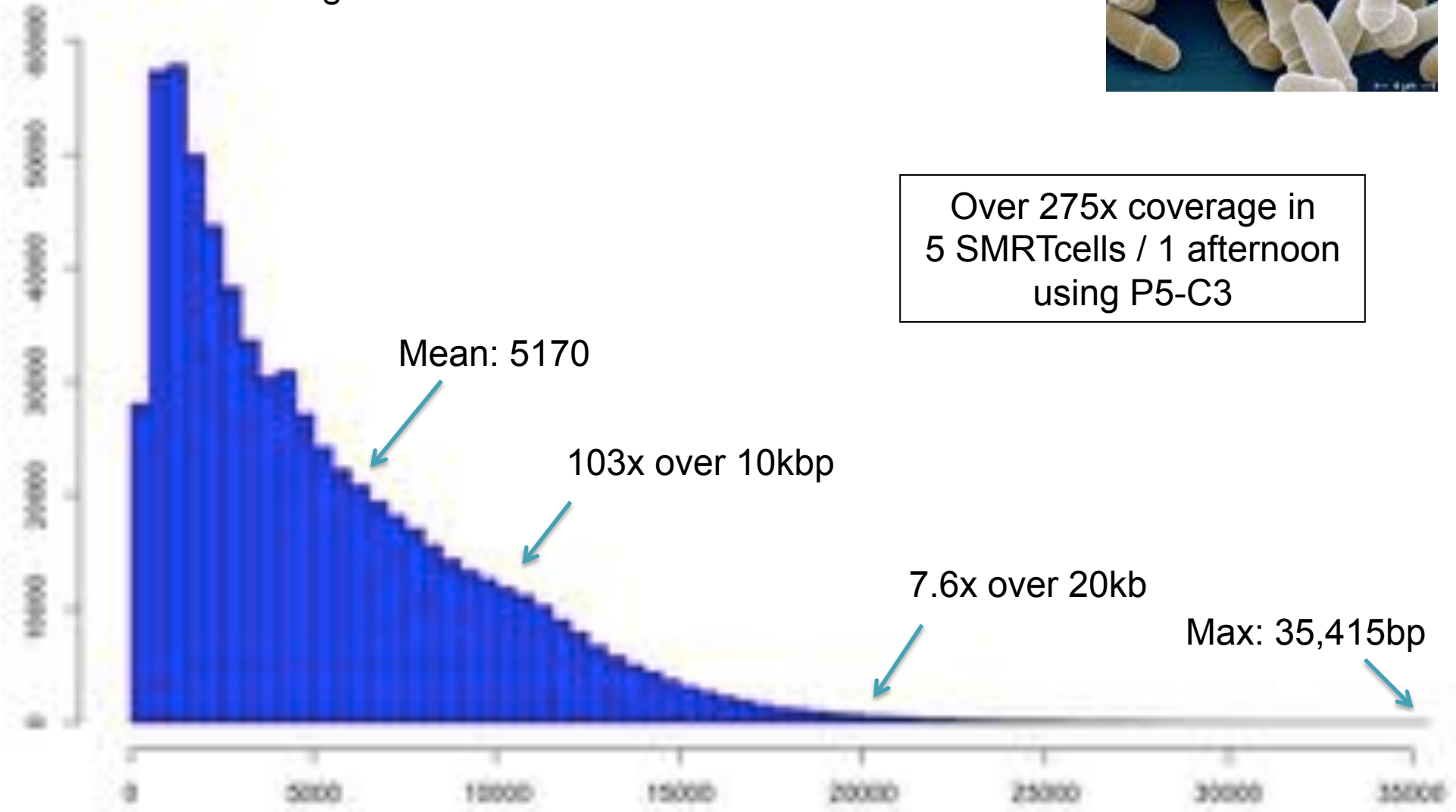


Analysis confounded by sequencing errors, localized repeats, allele biases, and mismapped reads

S. pombe dg21

PacBio RS II sequencing at CSHL

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



Over 275x coverage in
5 SMRTcells / 1 afternoon
using P5-C3

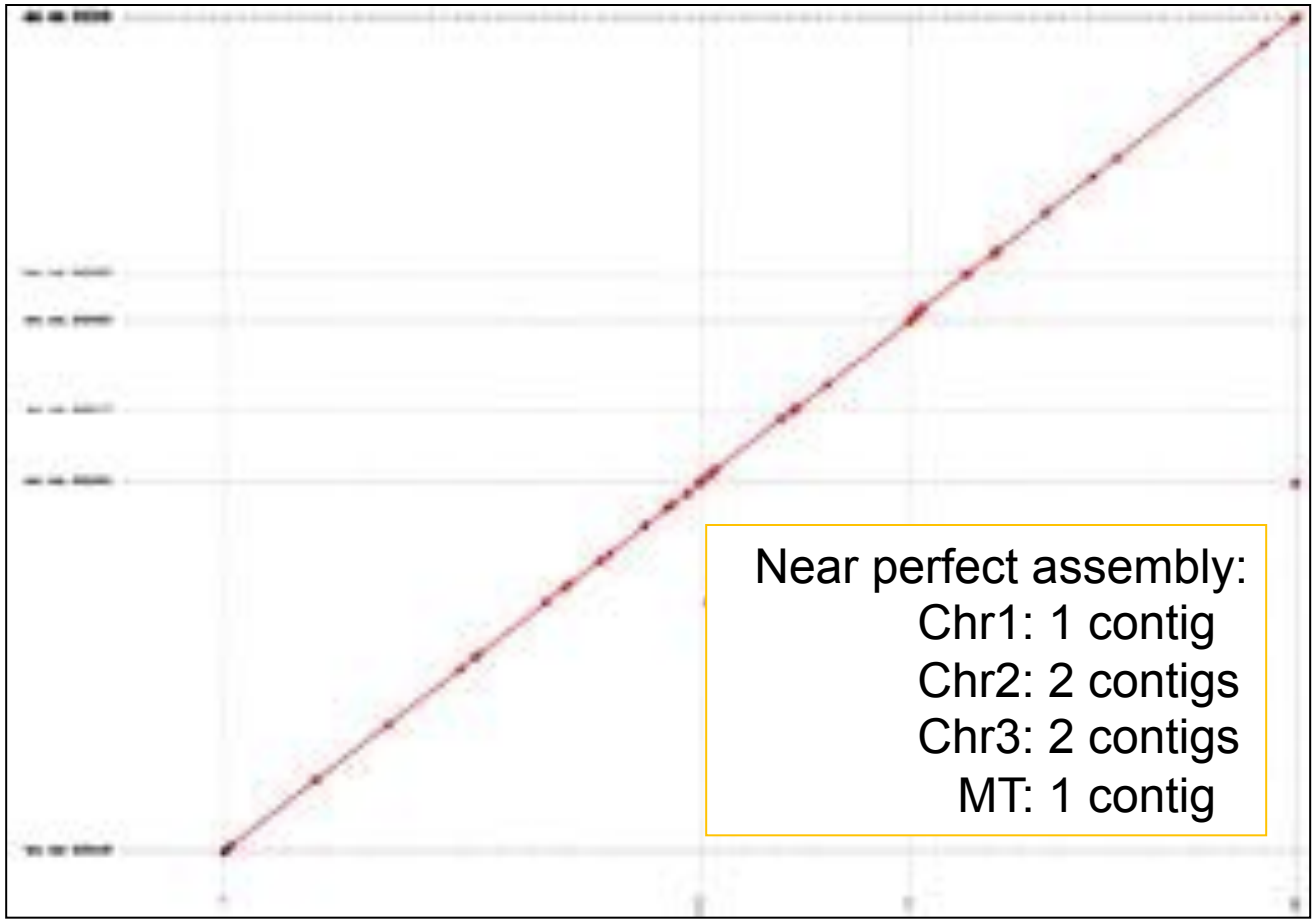
S. pombe dg21

ASM294 Reference sequence

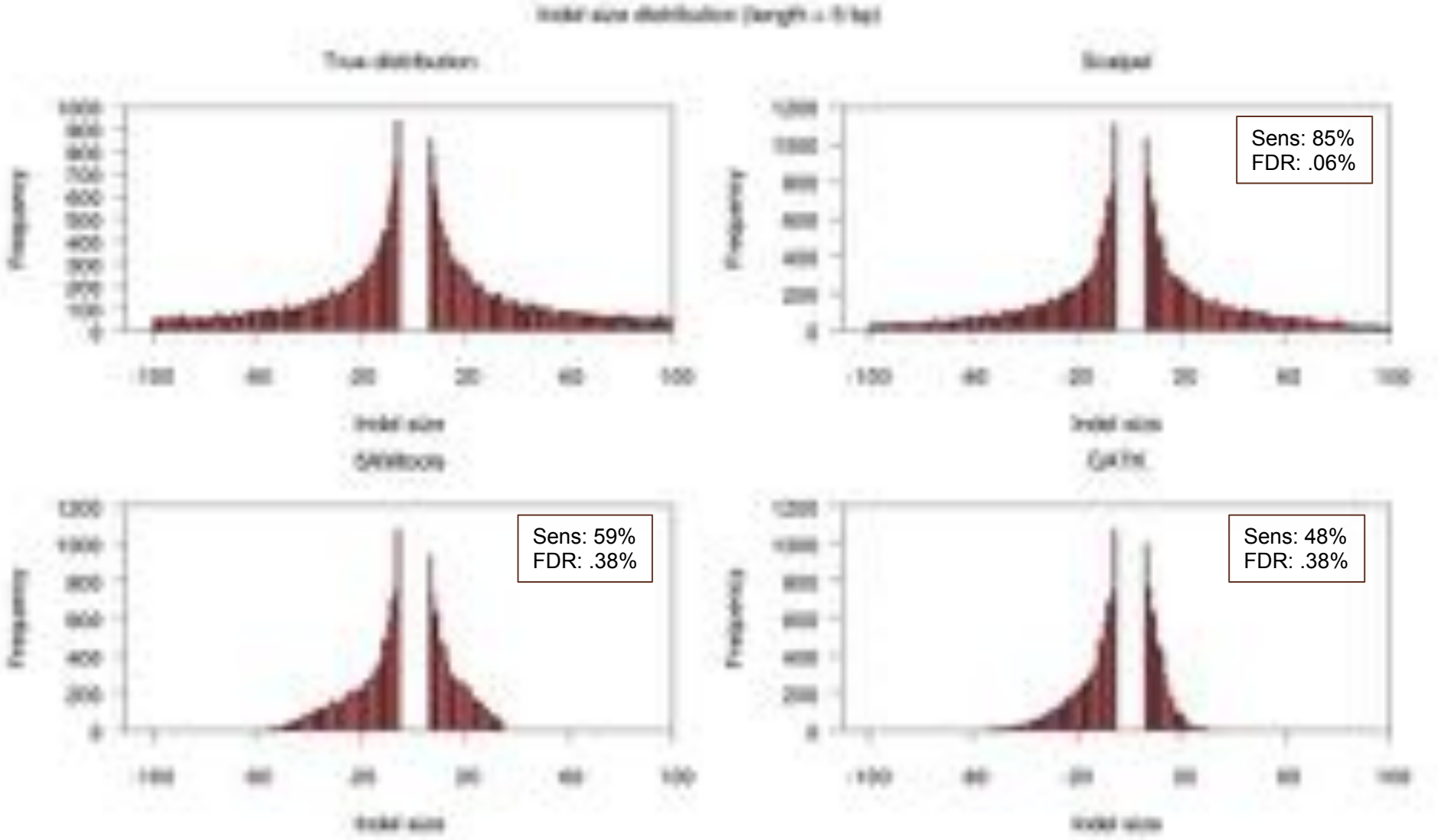
- 12.6Mbp; 3 chromo + mitochondria; N50: 4.53Mbp

PacBio assembly using HGAP + Celera Assembler

- 12.7Mbp; 13 non-redundant contigs; N50: 3.83Mbp; >99.9% id

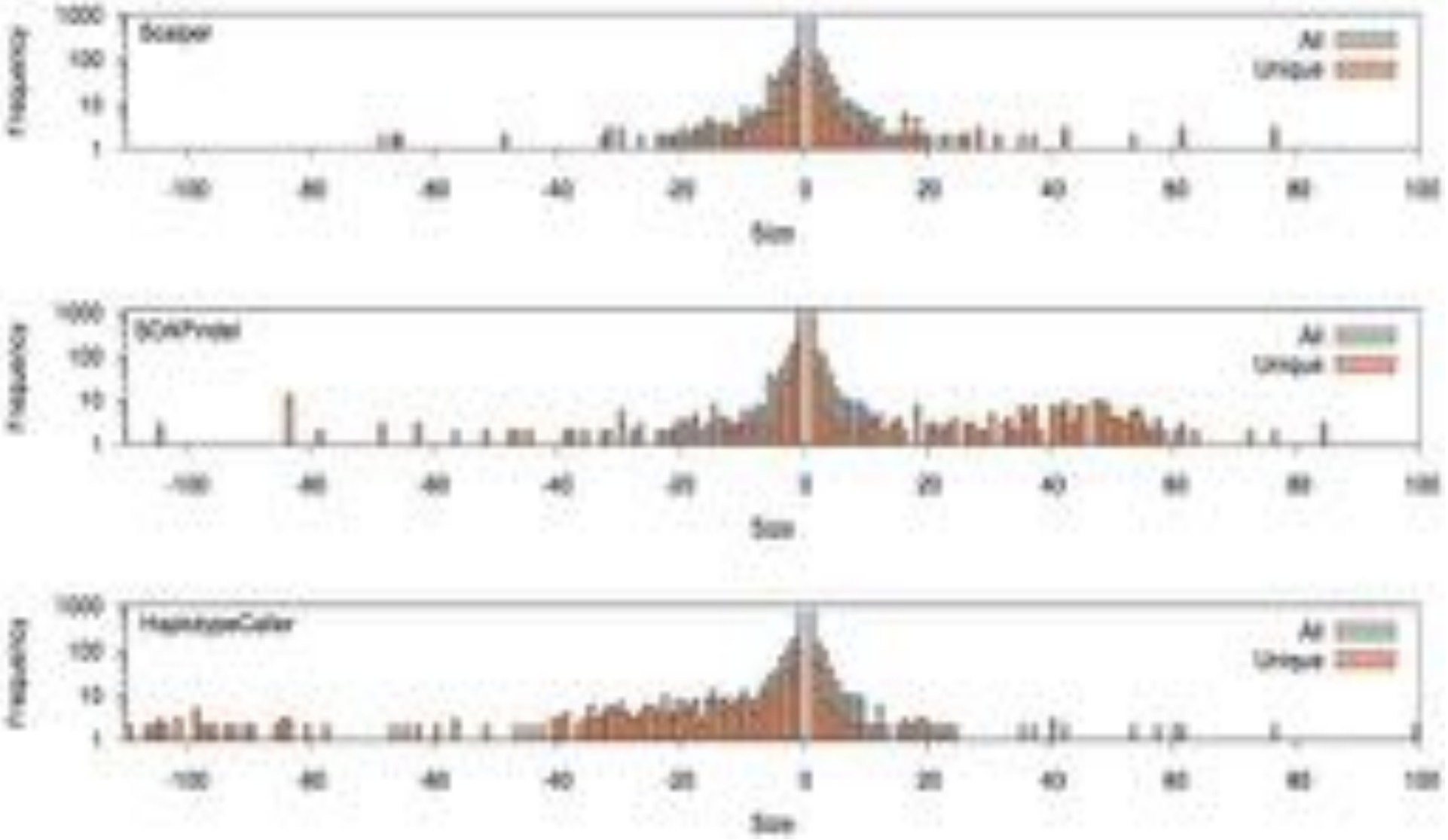


Simulation Analysis

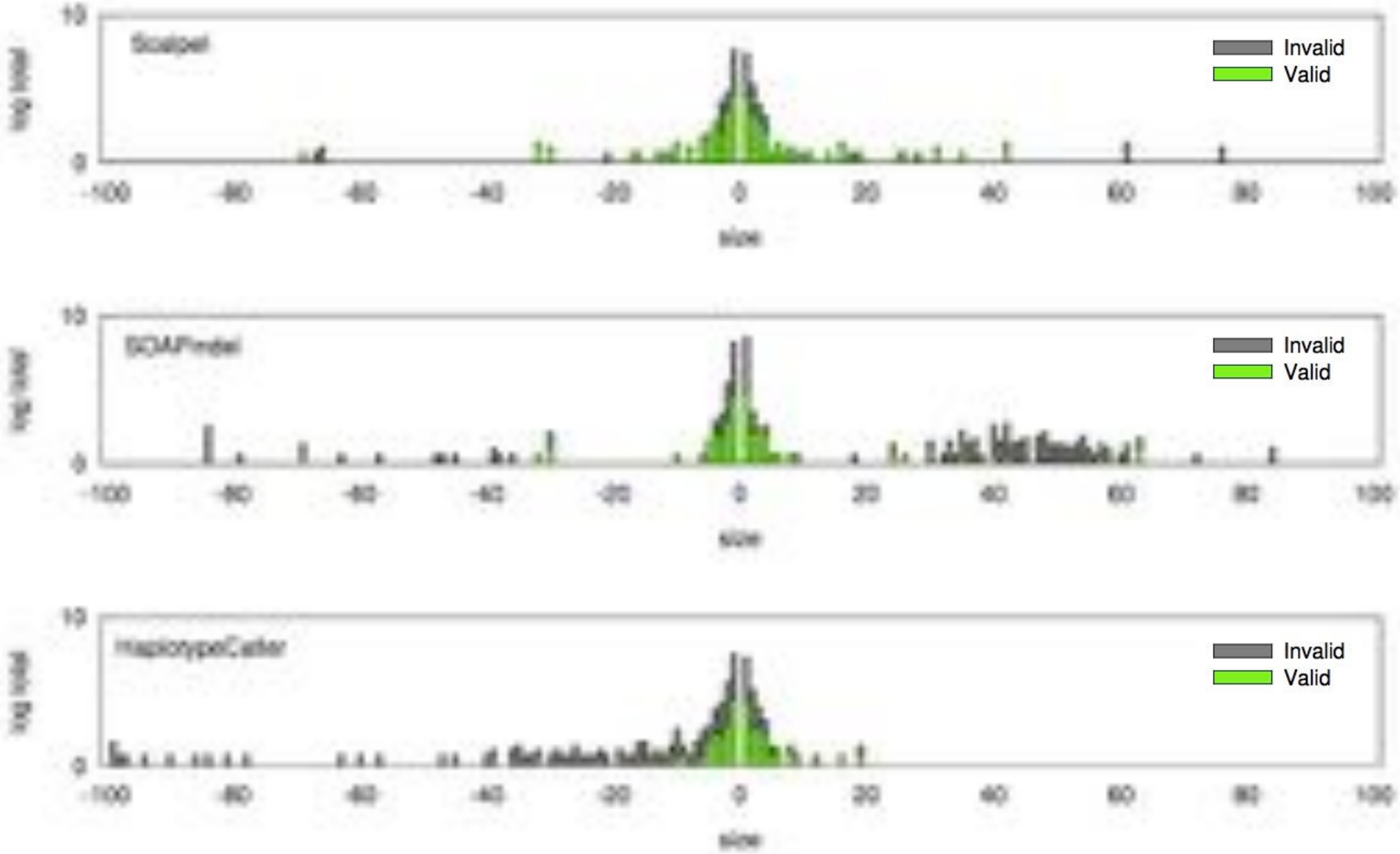


Simulated 10,000 indels in a exome from a known log-normal distribution

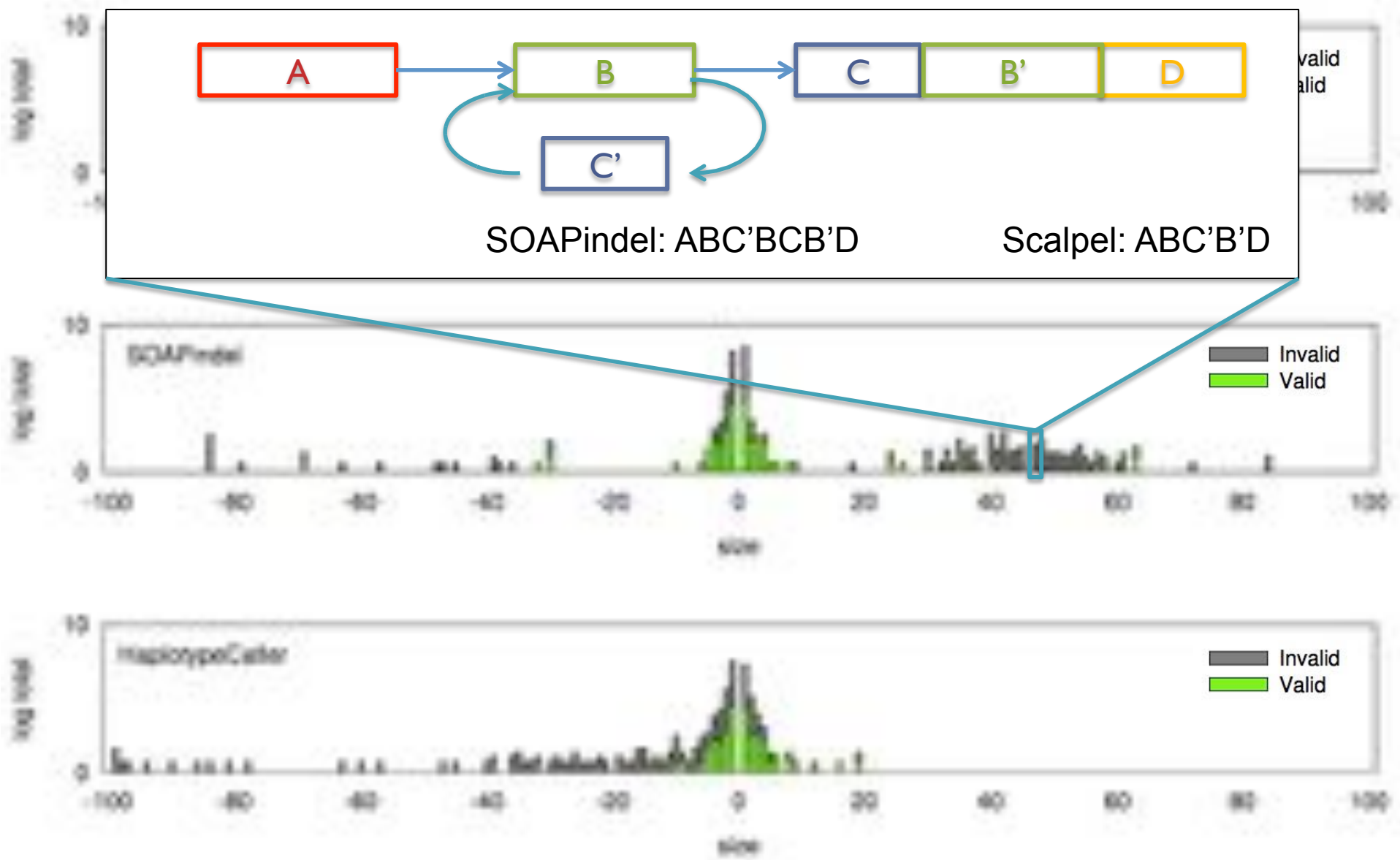
Scalpel Indel Discovery



Scalpel Indel Discovery



Scalpel Indel Discovery



Scalpel Indel Discovery