Algorithms for de novo genome assembly and disease analytics

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Feb 18, 2014

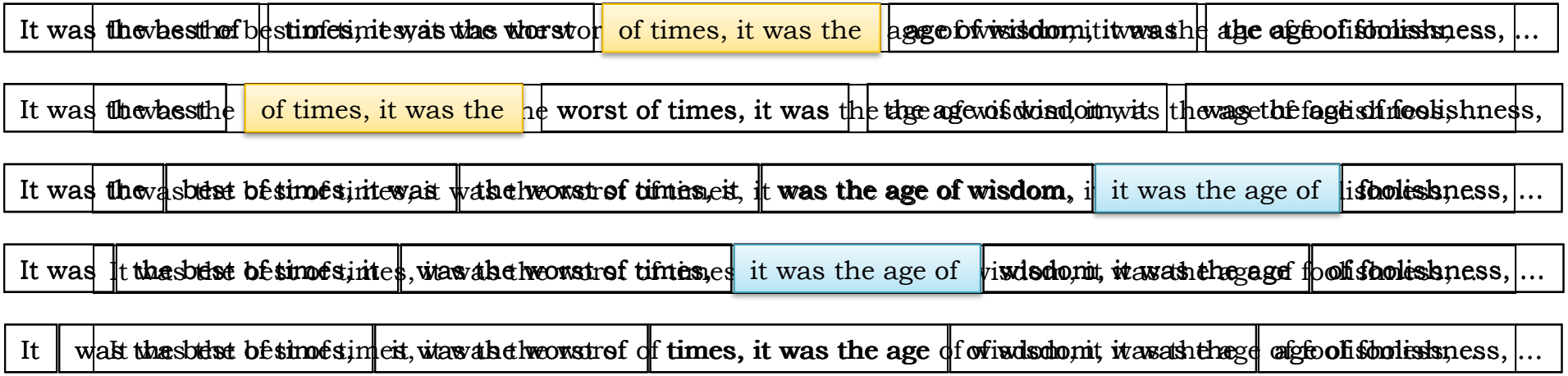
CCMB, Brown University

Outline

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

Shredded Book Reconstruction

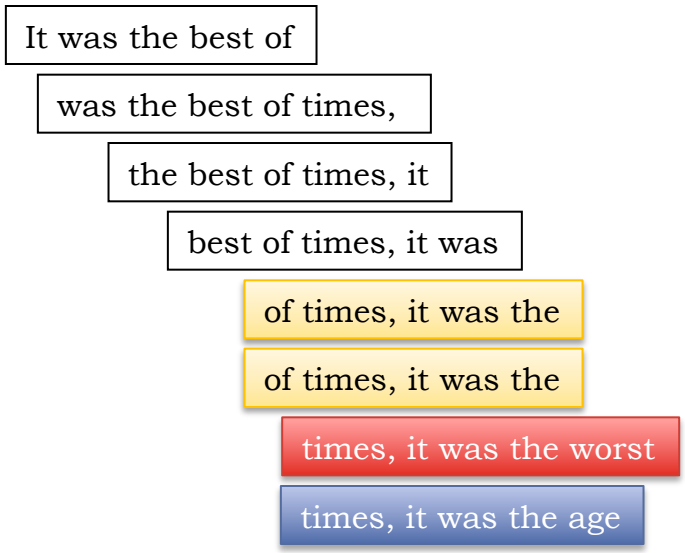
- Dickens accidentally shreds the first printing of A Tale of Two Cities
  - Text printed on 5 long spools



- 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



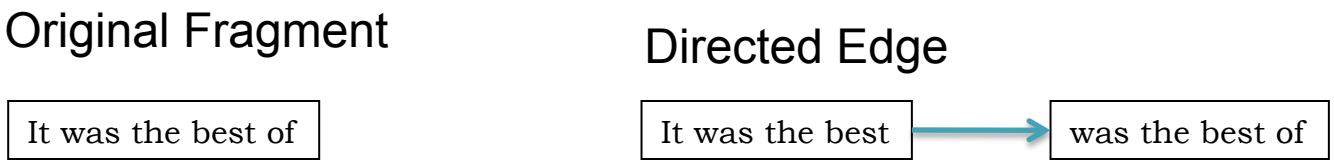
The repeated sequence make the correct reconstruction ambiguous

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

Model the assembly problem as a graph problem

de Bruijn Graph Construction

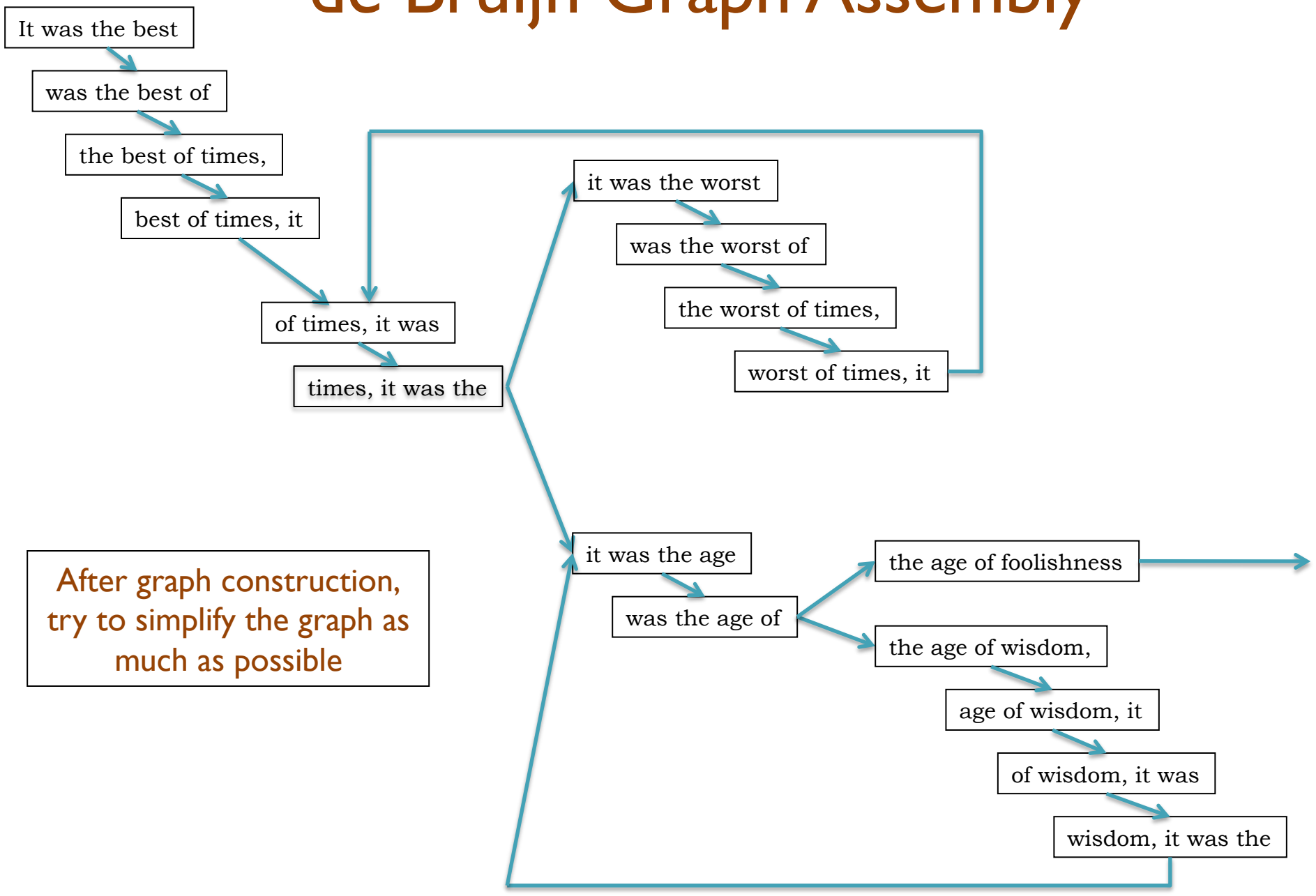
- $D_k = (V,E)$
  - V = All length-k subfragments (k < l)
  - E = Directed edges between consecutive subfragments
    - Nodes overlap by k-1 words



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

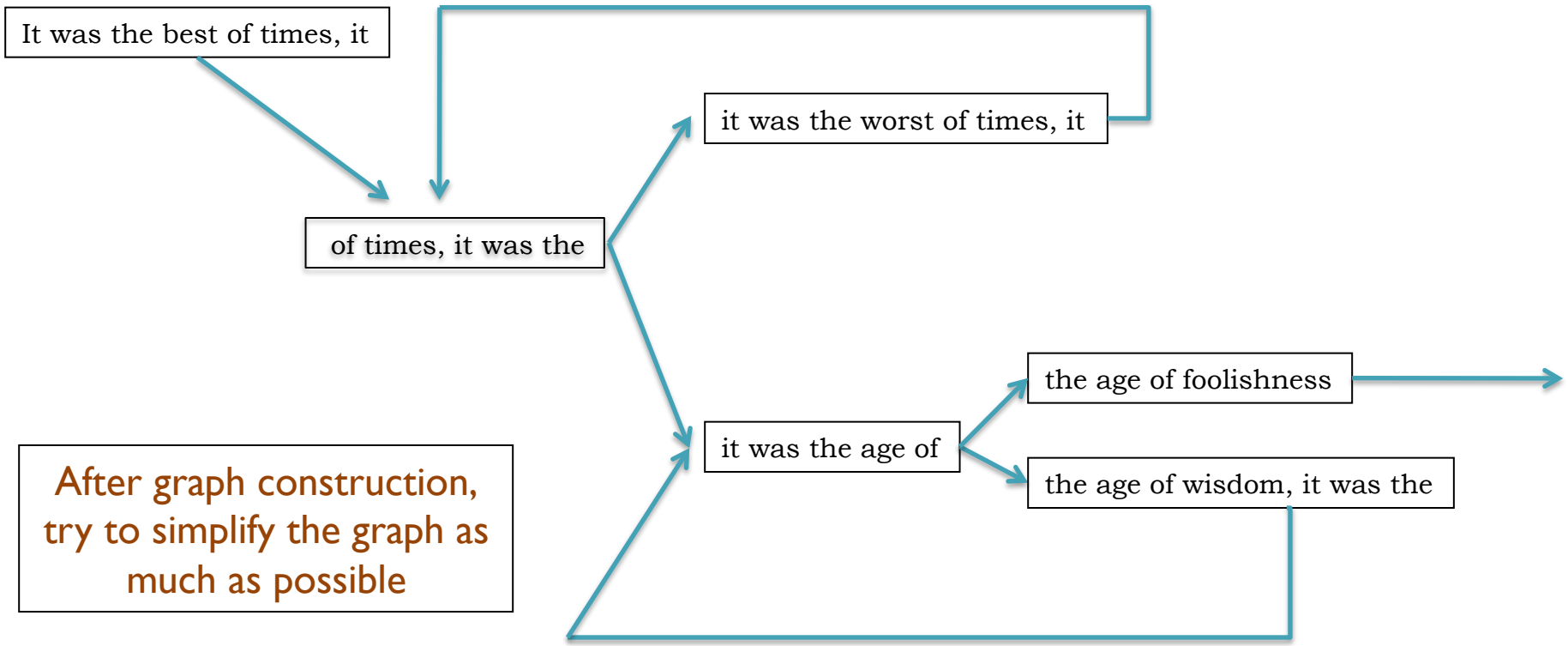
de Bruijn, 1946
Idury and Waterman, 1995
Pevzner, Tang, Waterman, 2001

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

The full tale

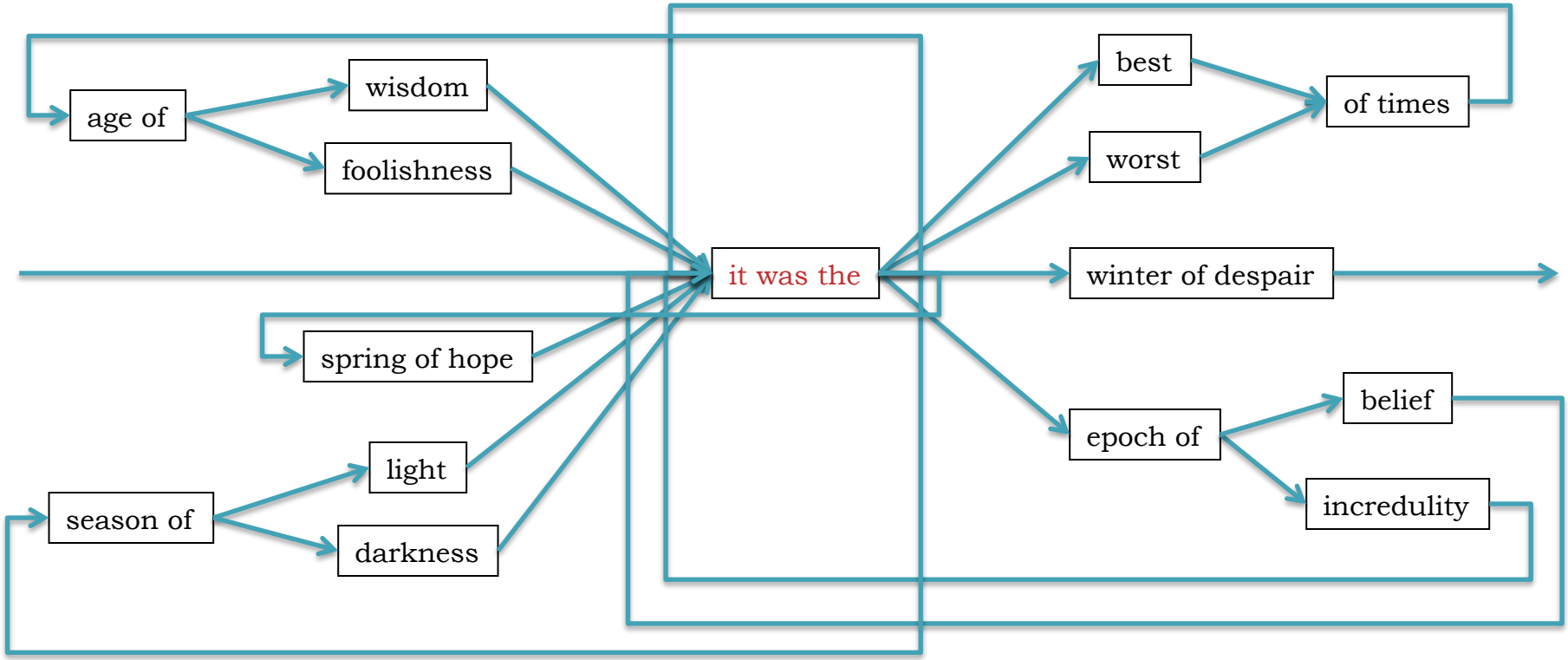
… it was the best of times it was the worst of times …

… it was the age of wisdom it was the age of foolishness …

… it was the epoch of belief it was the epoch of incredulity …

… it was the season of light it was the season of darkness …

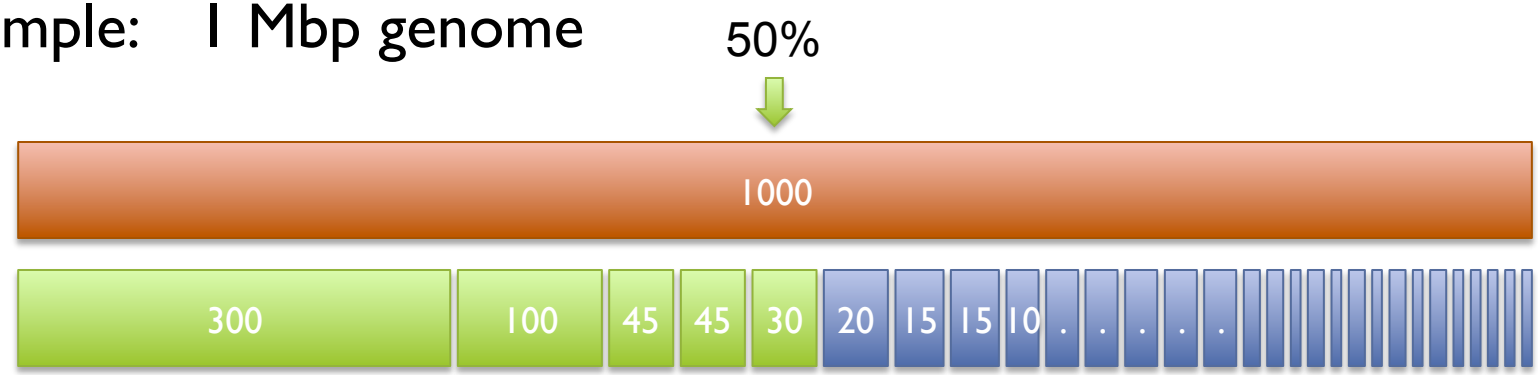
… it was the spring of hope it was the winder of despair …



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

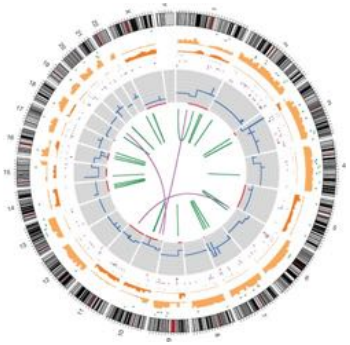
1. De novo assembly by analogy
2. Long read assembly
3. Disease Analytics

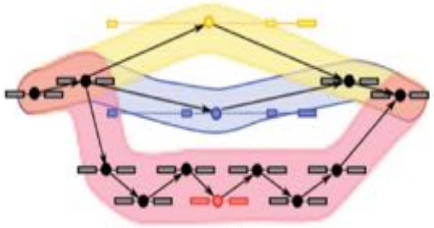
De Novo Genome Assembly

- Novel genomes

- Metagenomes

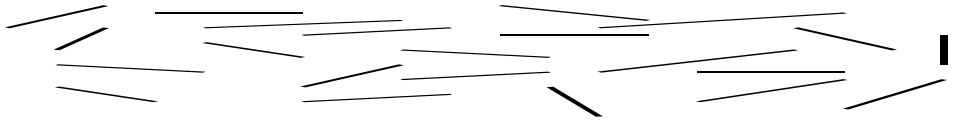
- Sequencing assays
  - Structural variations
  - Transcript assembly
  - …





Assembling a Genome

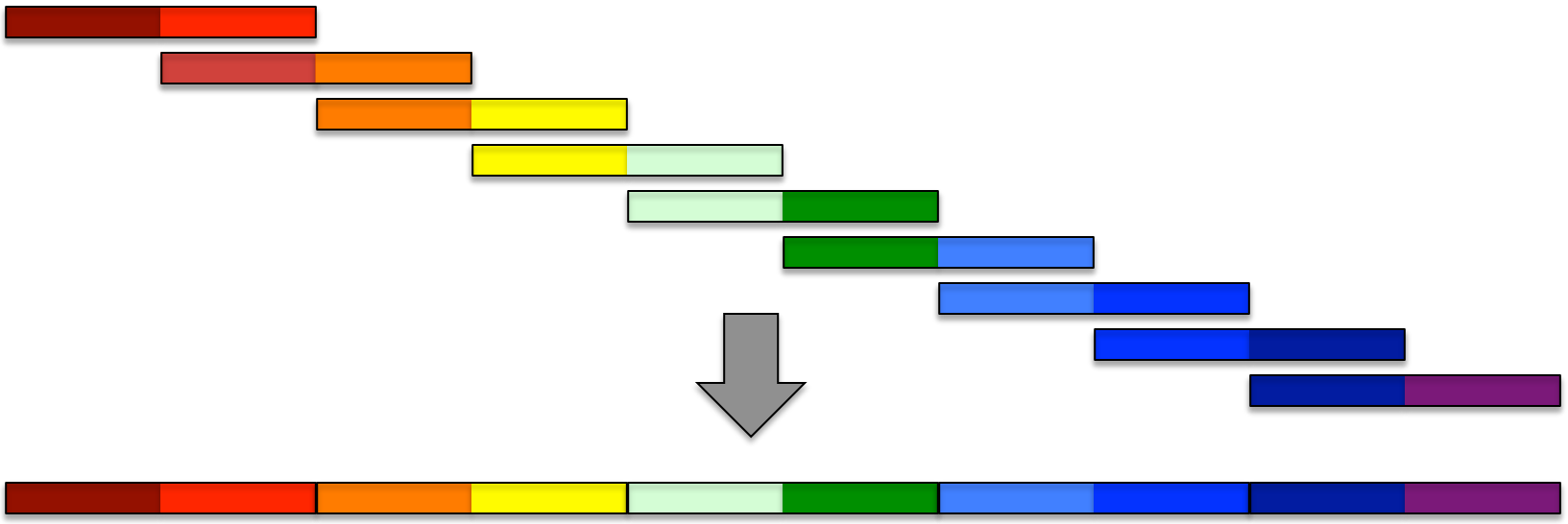
1. Shear & Sequence DNA



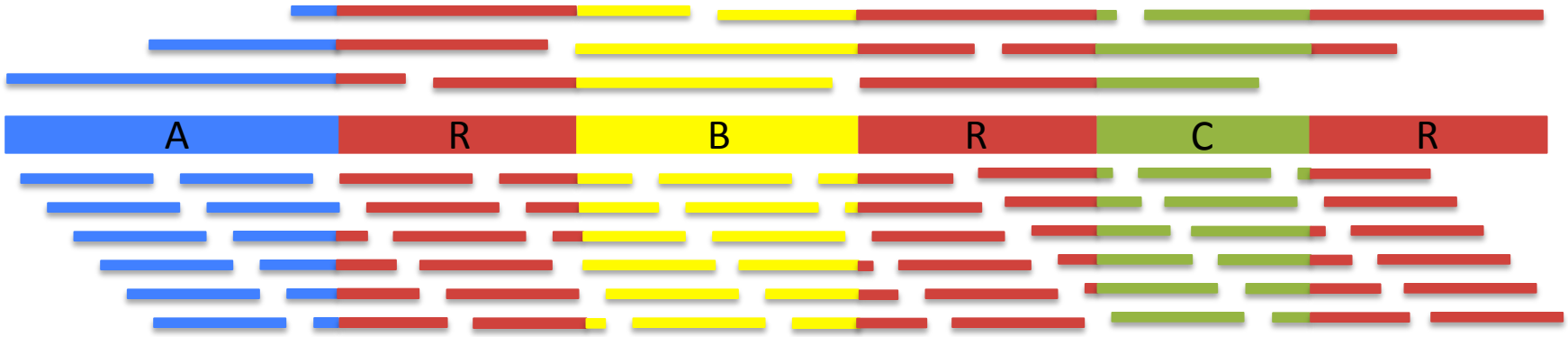
2. Construct assembly graph from overlapping reads

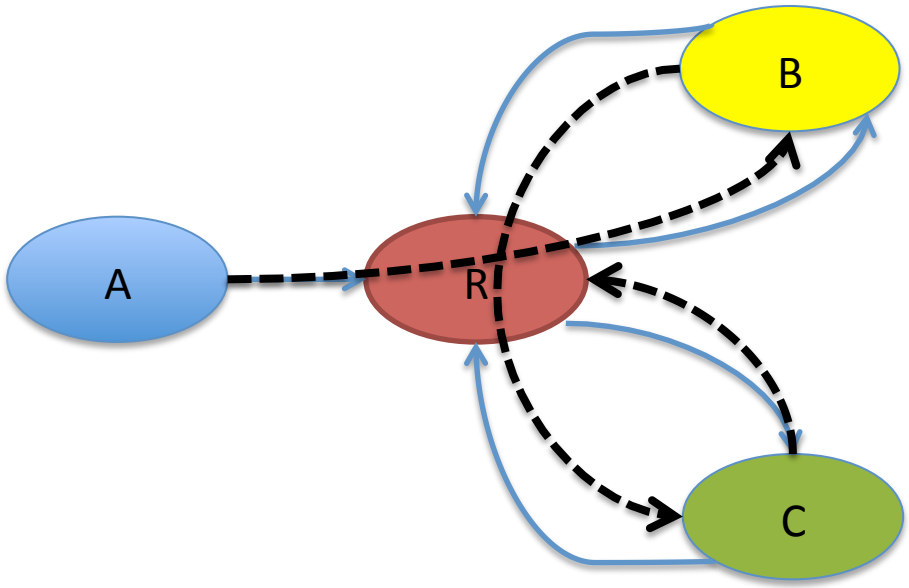
...AGCCTAGGGATGCGCGACACGT
GGATGCGCGACACGTCGCATATCCGGTTTGGTCAACCTCGGACGGAC
CAACCTCGGACGGACCTCAGCGAA...

3. Simplify assembly graph

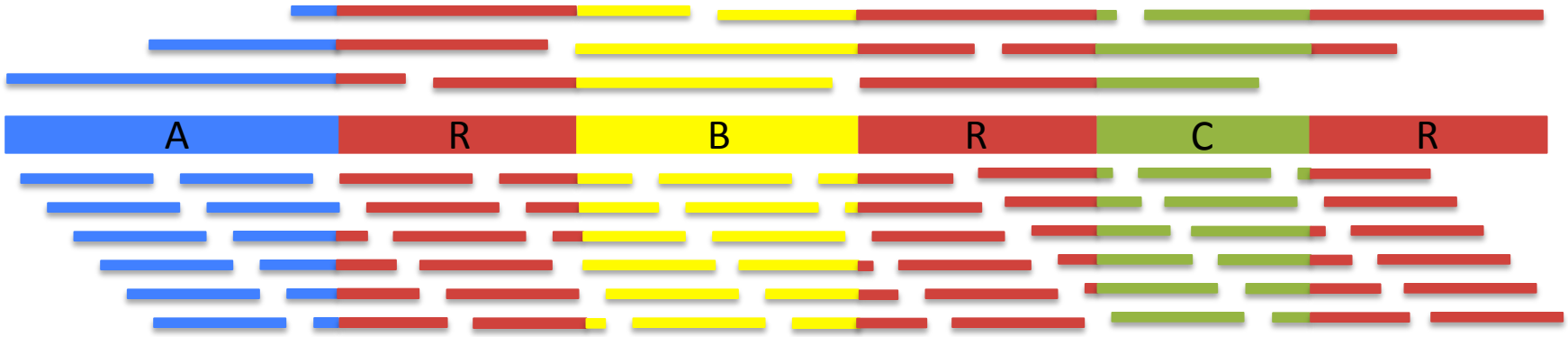


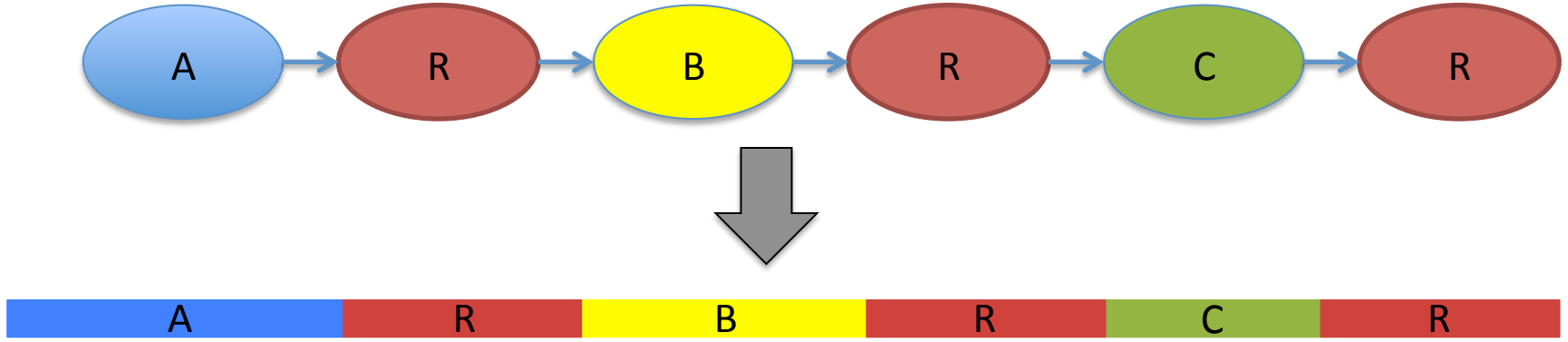
Assembly Complexity





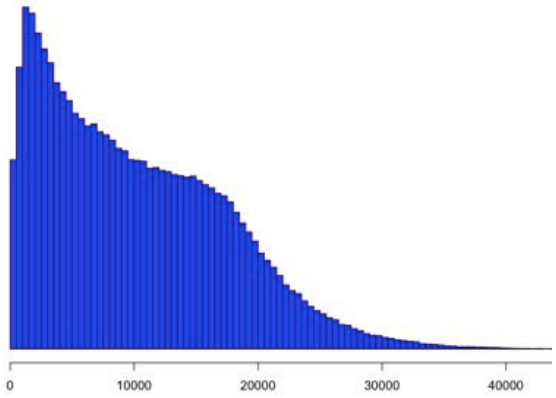
Assembly Complexity





Long Read Sequencing Technology

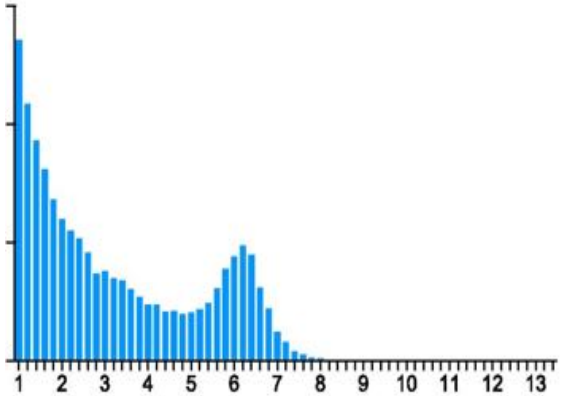
PacBio RS II



CSHL/PacBio

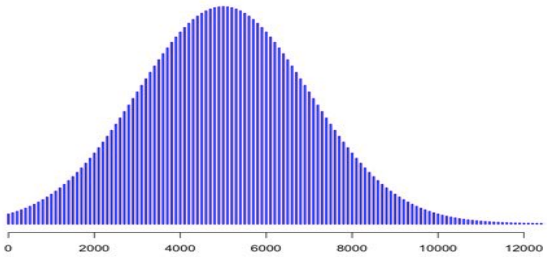
Moleculo

illumina
moleculo



(Voskoboynik et al. 2013)

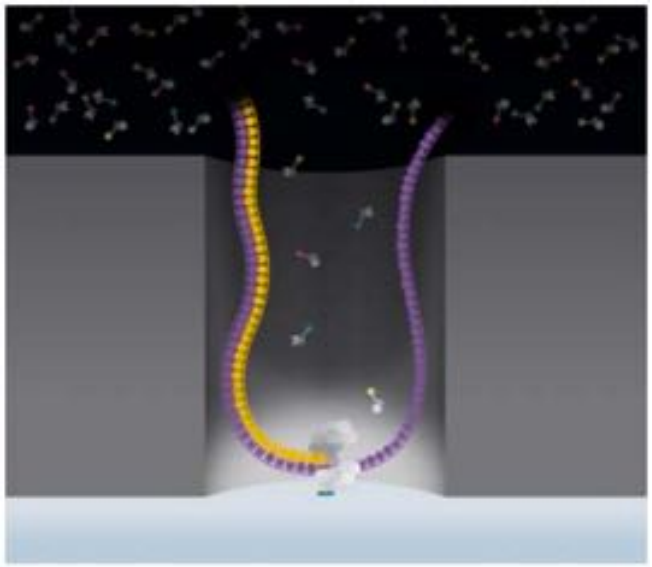
Oxford Nanopore

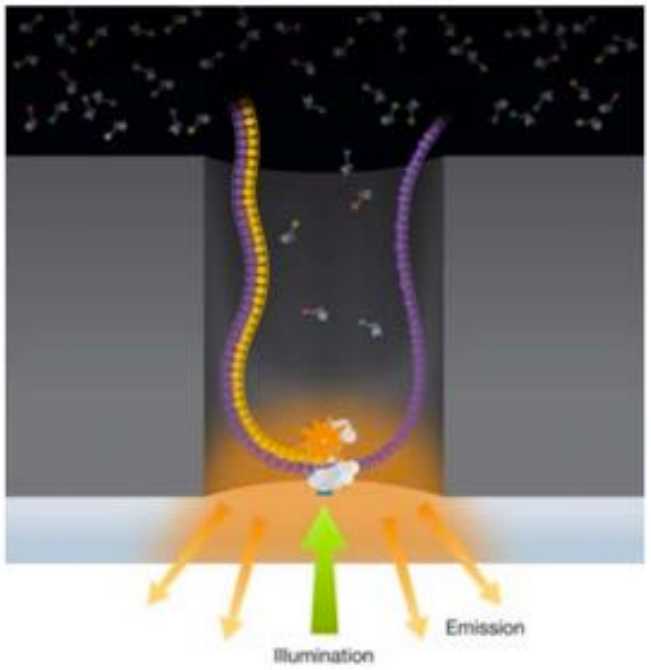


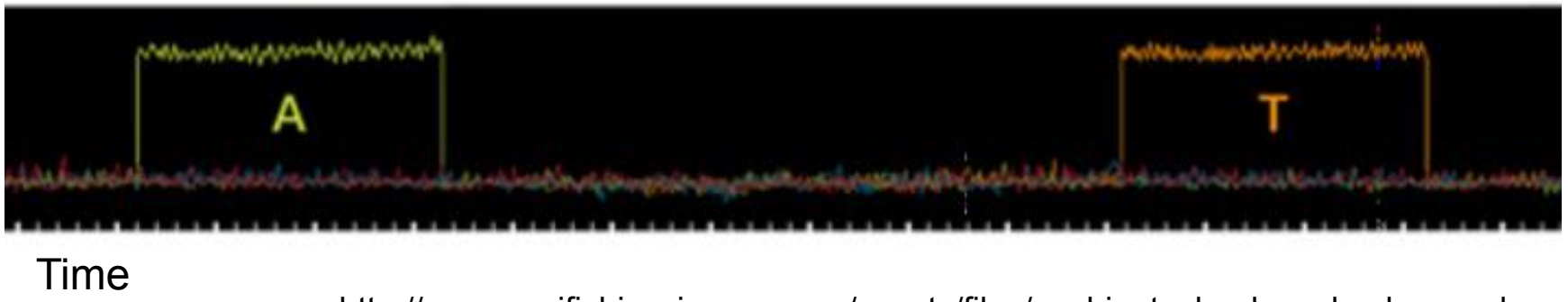
Broad/OxNano @ AGBT

SMRT Sequencing

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

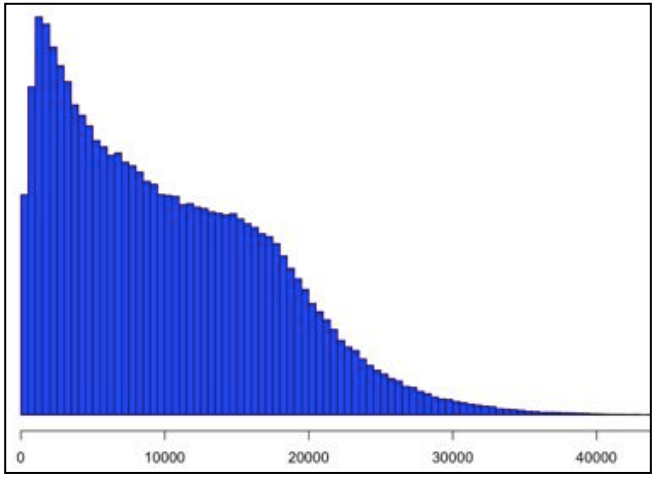






http://www.pacificbiosciences.com/assets/files/pacbio_technology_backgrounder.pdf

SMRT Sequencing Data

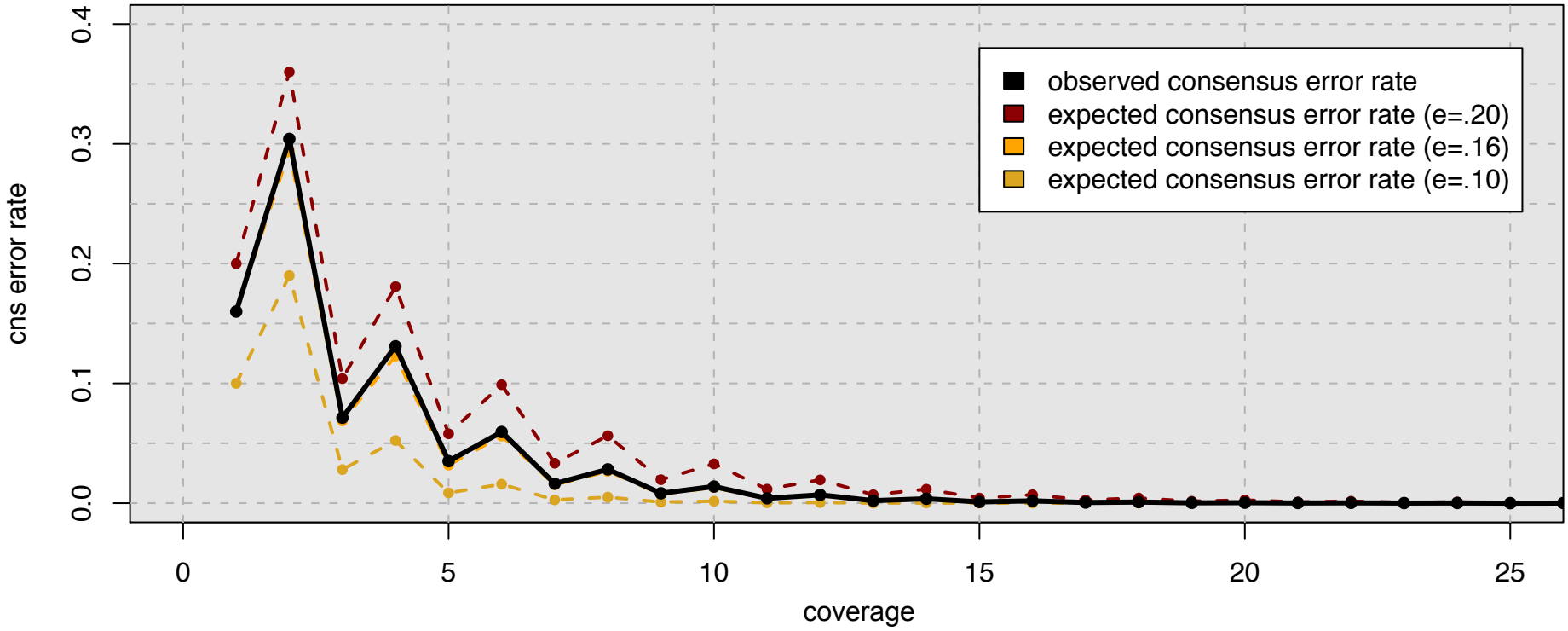


Match	83.7%
Insertions	11.5%
Deletions	3.4%
Mismatch	1.4%

```
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

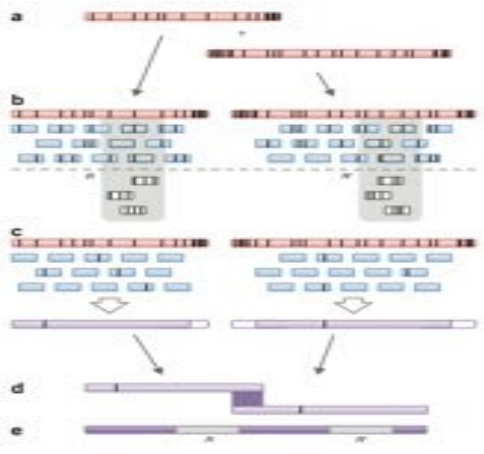
PacBio Assembly Algorithms

PBJelly

Gap Filling and Assembly Upgrade

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

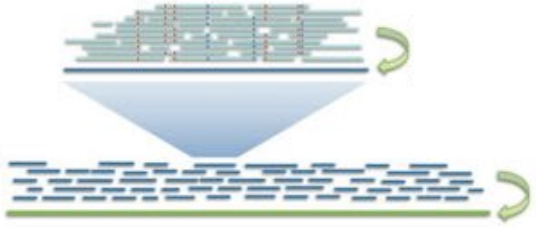
PacBioToCA & ECTools



Hybrid/PB-only Error Correction

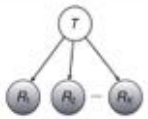
Koren, Schatz, *et al* (2012)
Nature Biotechnology. 30:693–700

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)

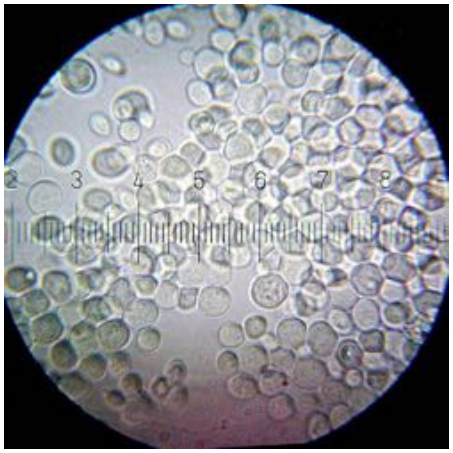
	Initial Assembly	Quiver Consensus
QV	43.4	54.5
Accuracy	99.99540%	99.99964%
Differences	141	11

PB-only Correction & Polishing

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

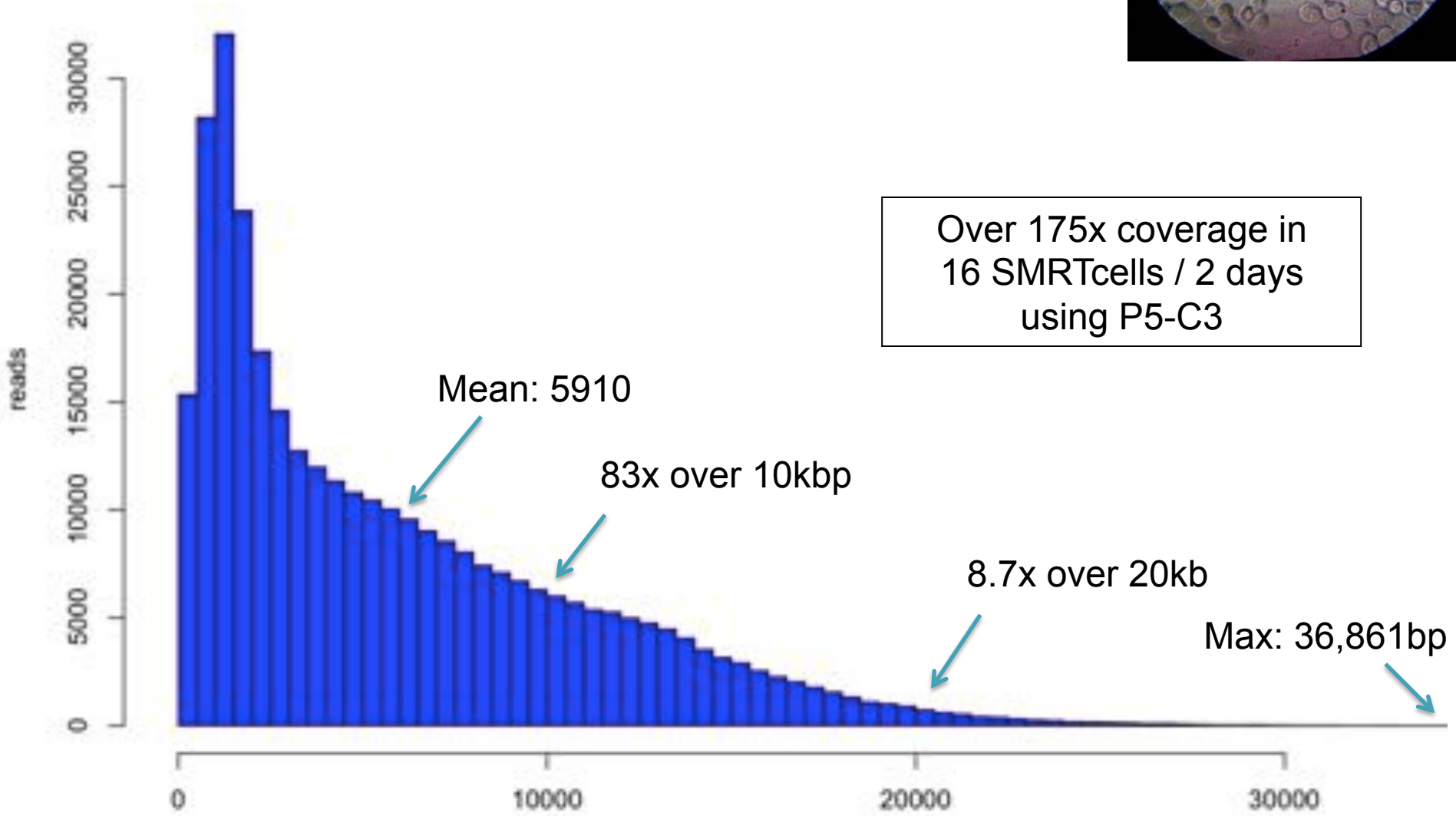
< 5x PacBio Coverage > 50x

S. cerevisiae W303



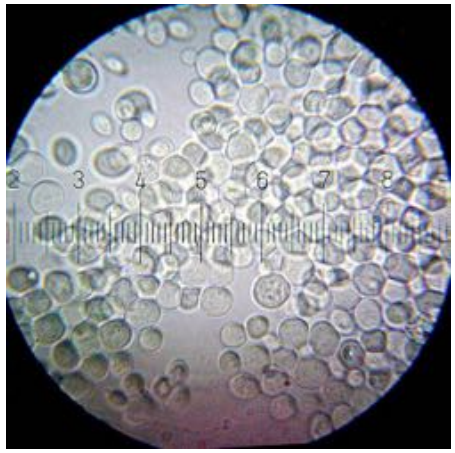
PacBio RS II sequencing at CSHL by Dick McCombie

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



Over 175x coverage in
16 SMRTcells / 2 days
using P5-C3

S. cerevisiae W303

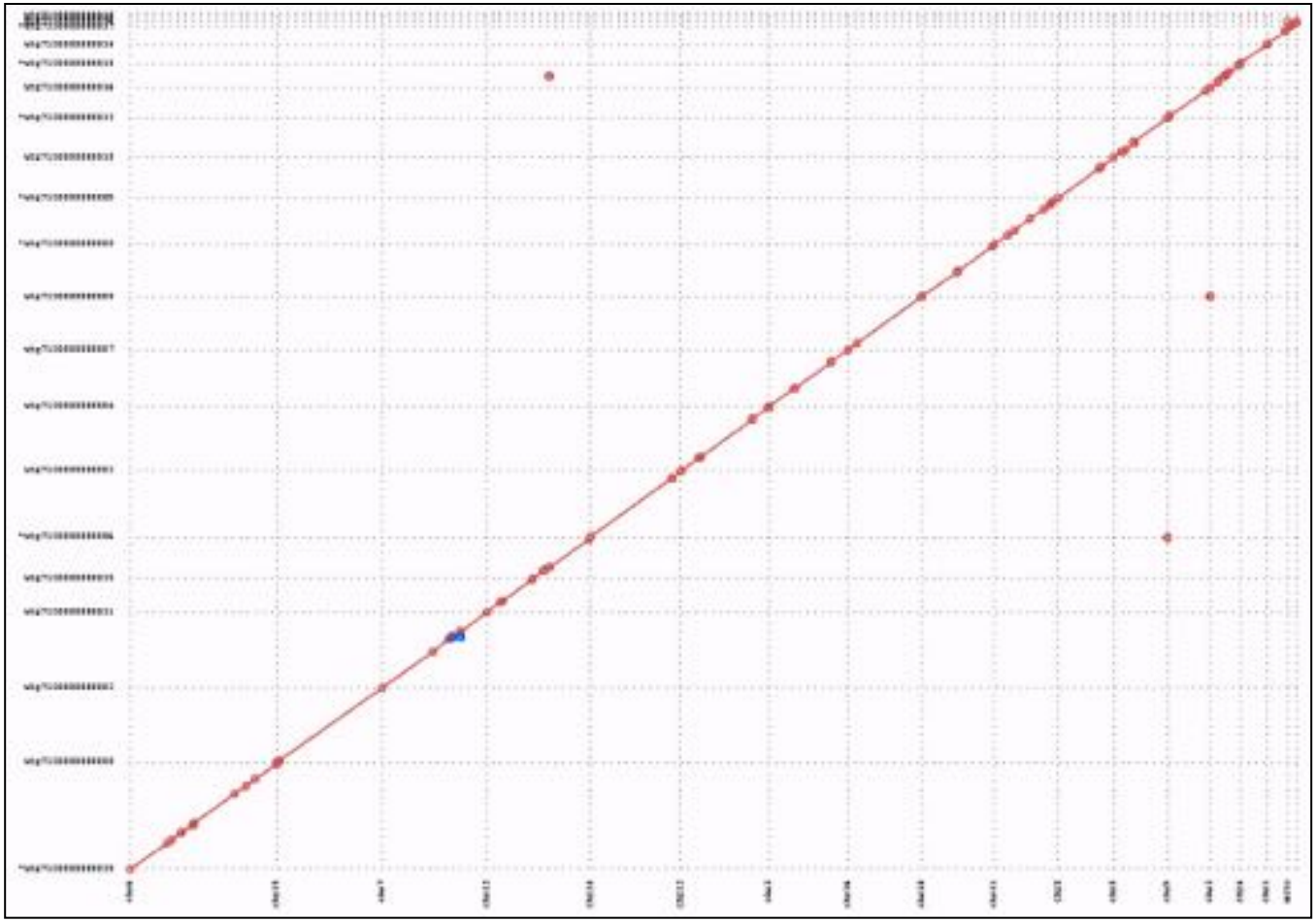


S288C Reference sequence

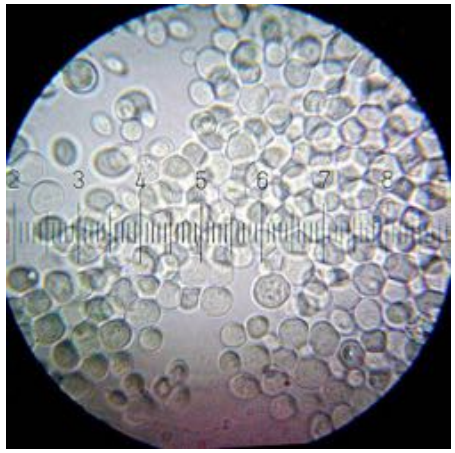
- 12.1Mbp; 16 chromo + mitochondria; N50: 924kbp

PacBio assembly using HGAP + Celera Assembler

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



S. cerevisiae W303



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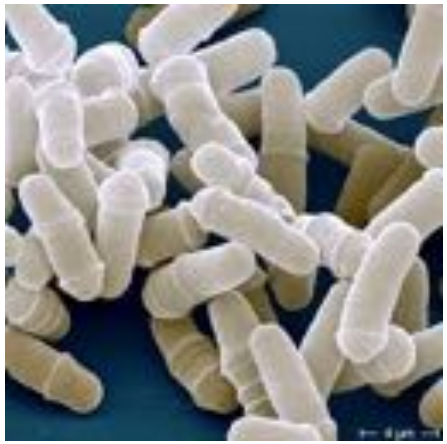
S. pombe dg21



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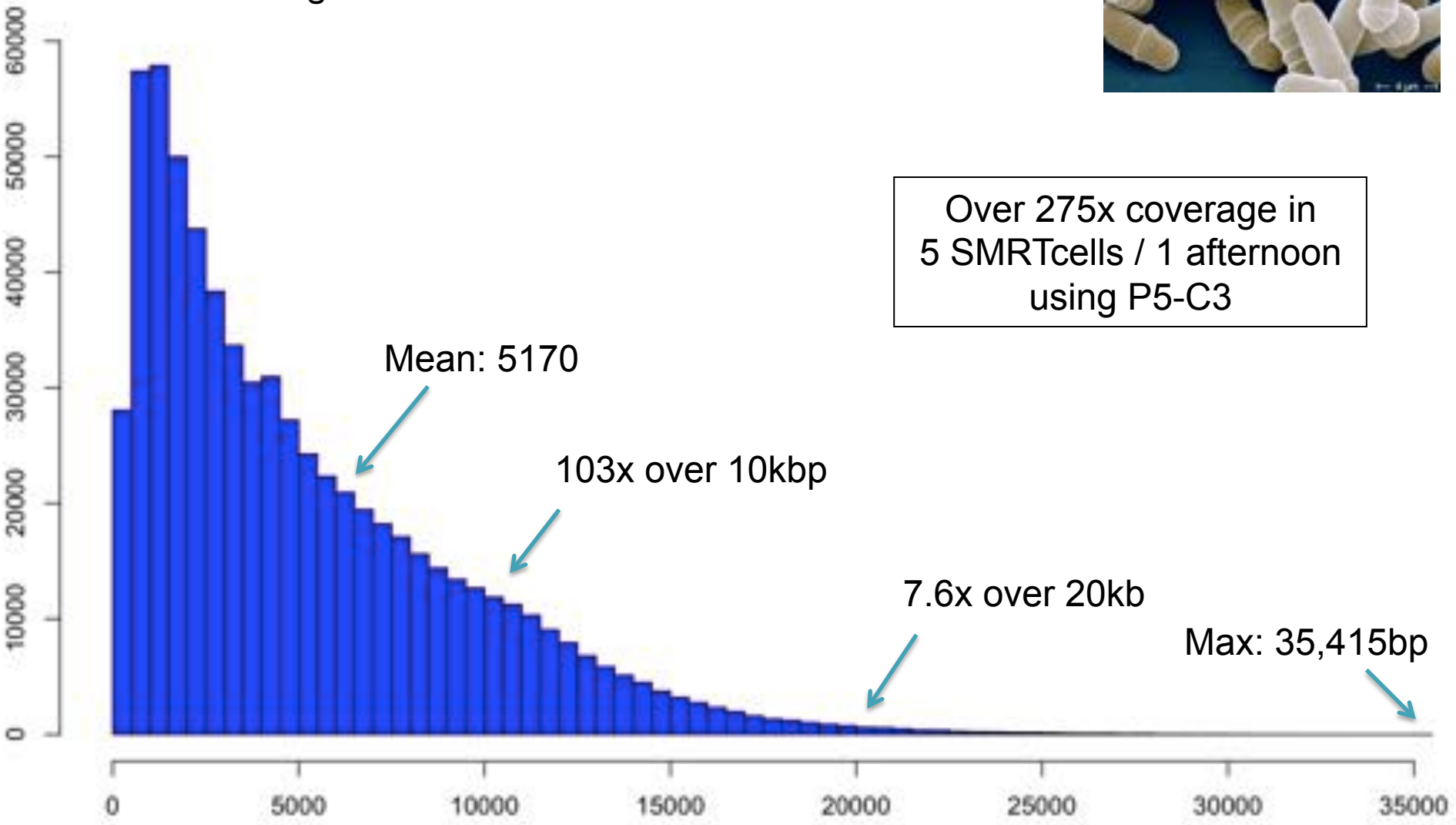
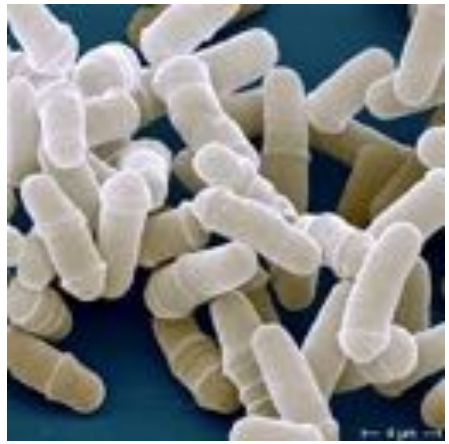


Over 275x coverage in
5 SMRTcells / 1 afternoon
using P5-C3

S. pombe dg21

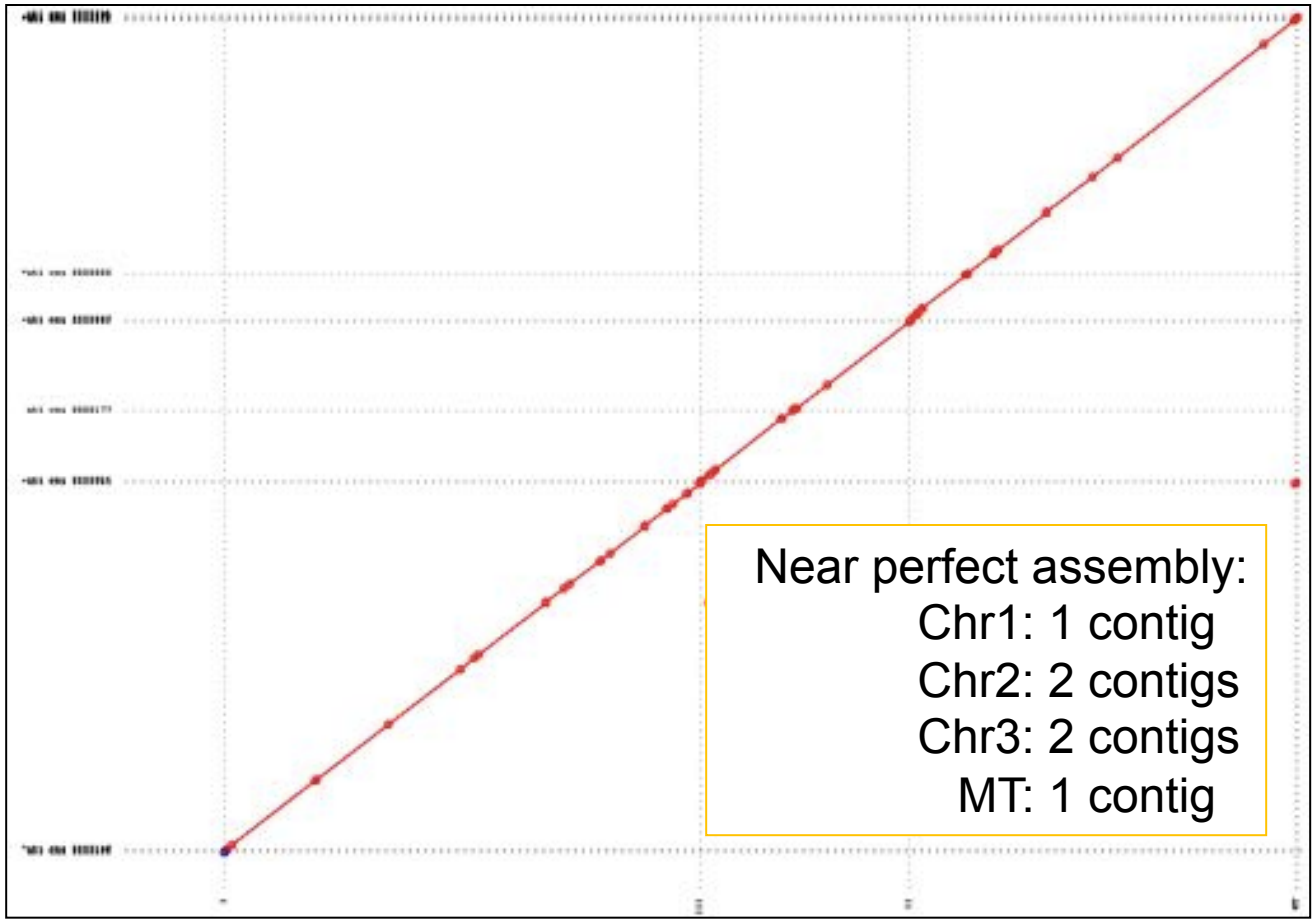


ASM294 Reference sequence

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

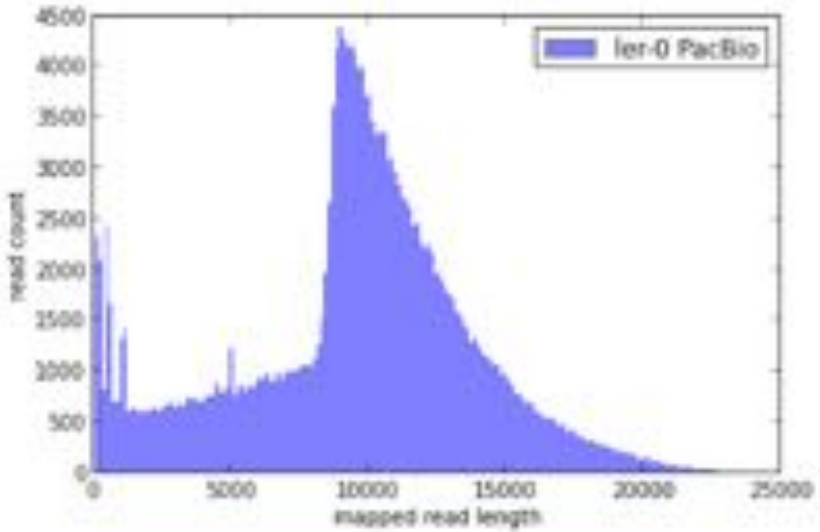
PacBio assembly using HGAP + Celera Assembler

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



A. thaliana Ler-0

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



A. thaliana Ler-0 sequenced at PacBio

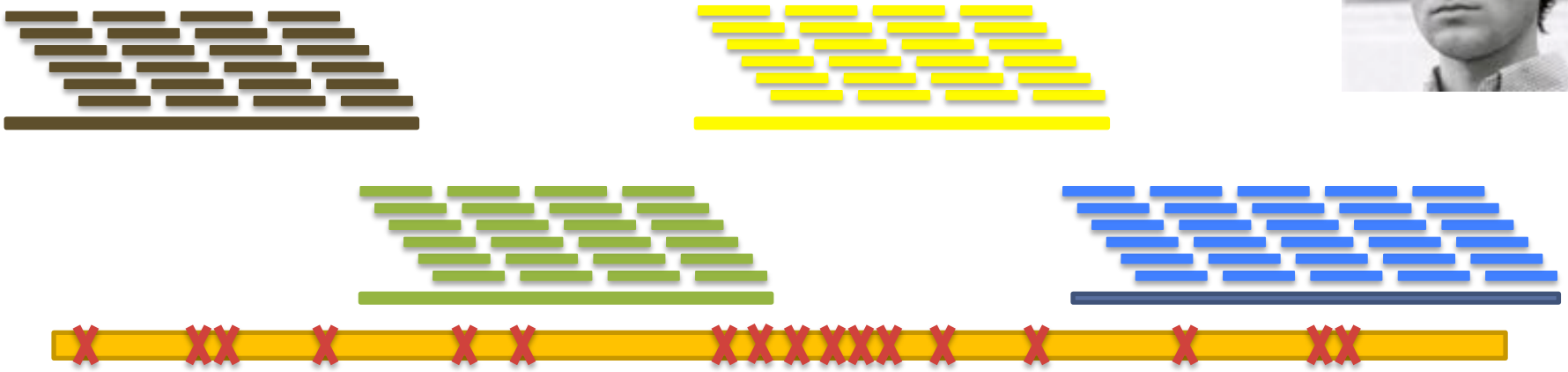
- Sequenced using the previous P4 enzyme and C2 chemistry
- Size selection using an 8 Kb to 50 Kb elution window on a BluePippin™ device from Sage Science
- Total coverage >119x

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

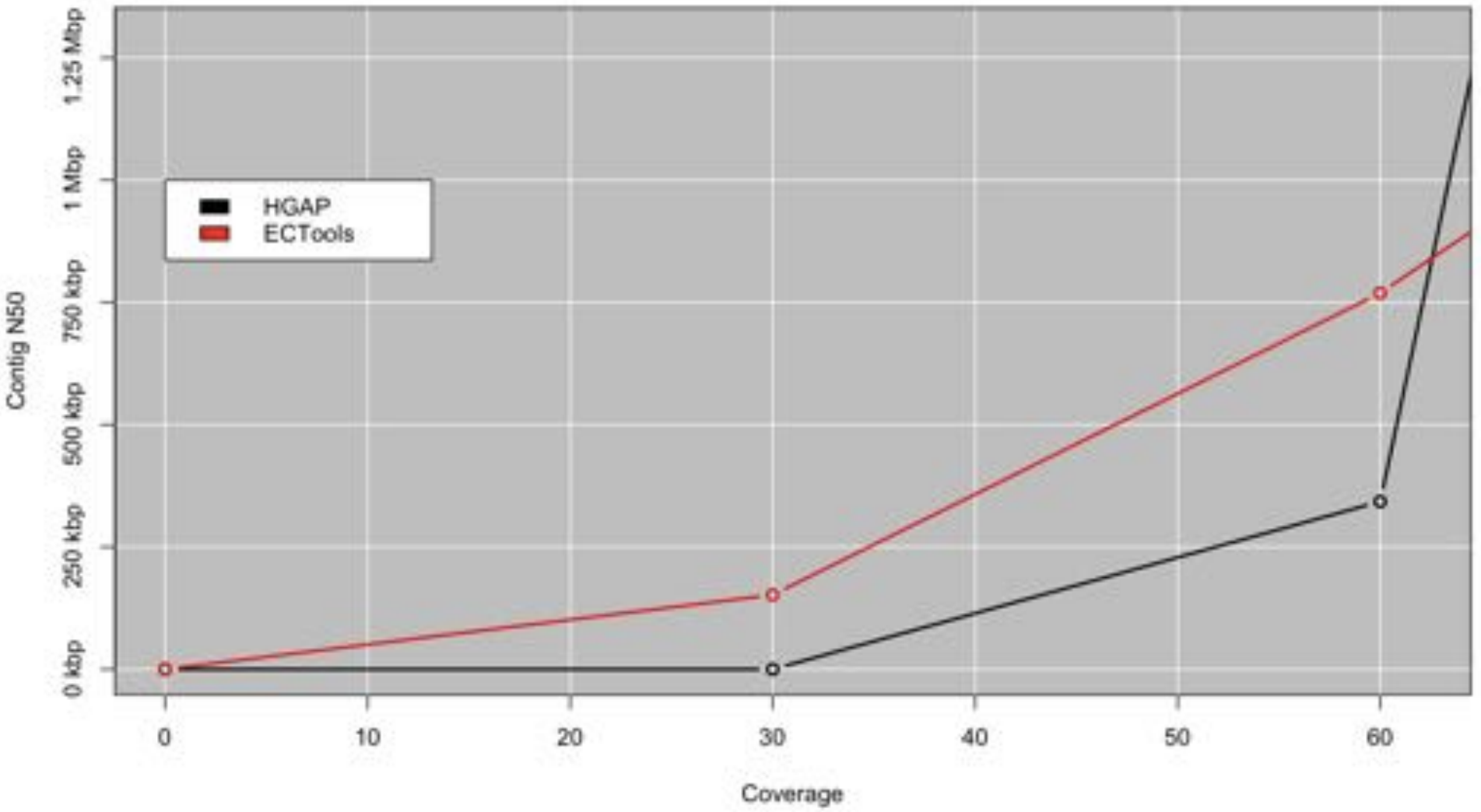
Can Help us overcome:

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

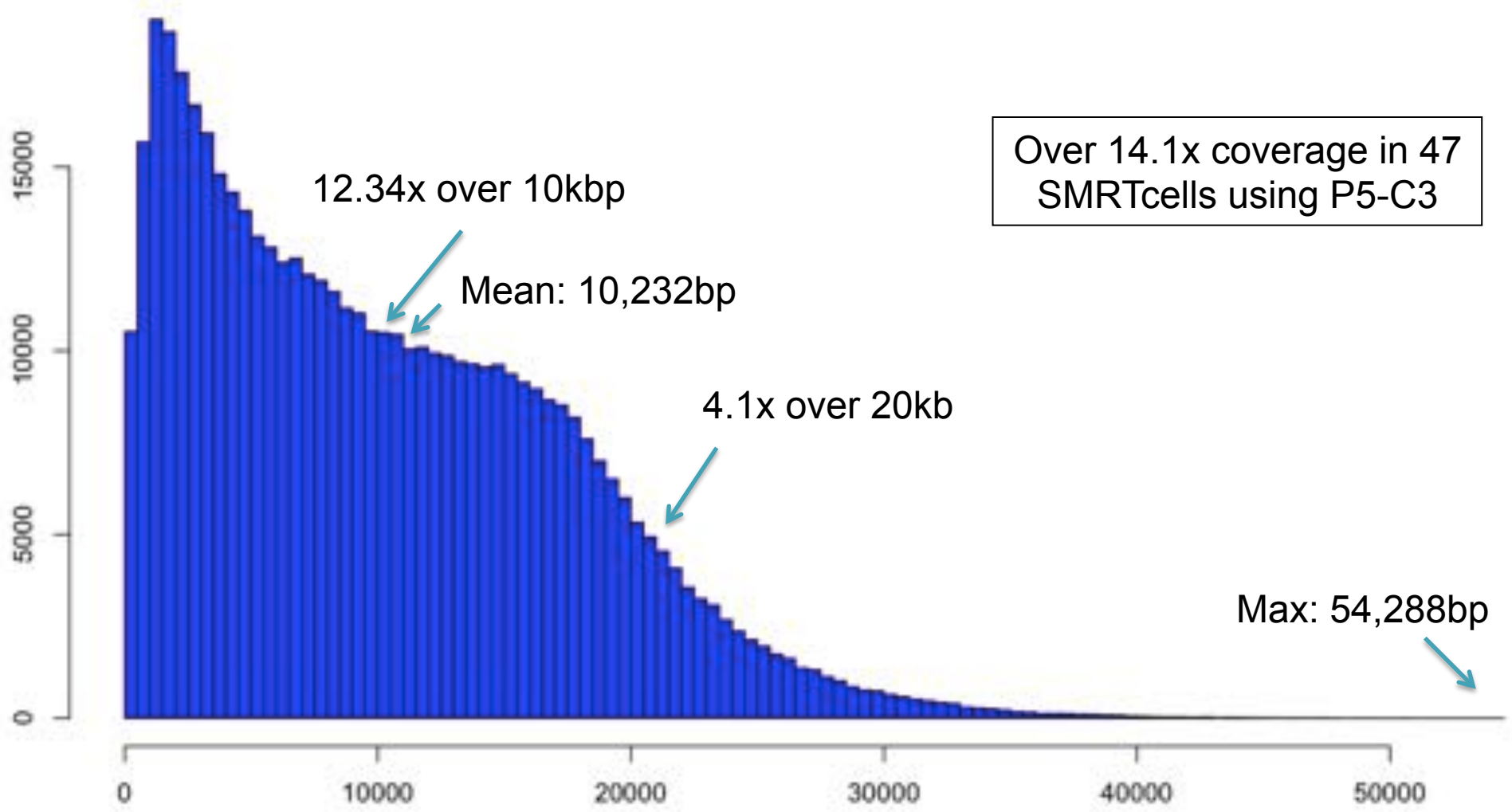
http://blog.pacificbiosciences.com/2013/08/new-data-release-arabidopsis-assembly.html



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

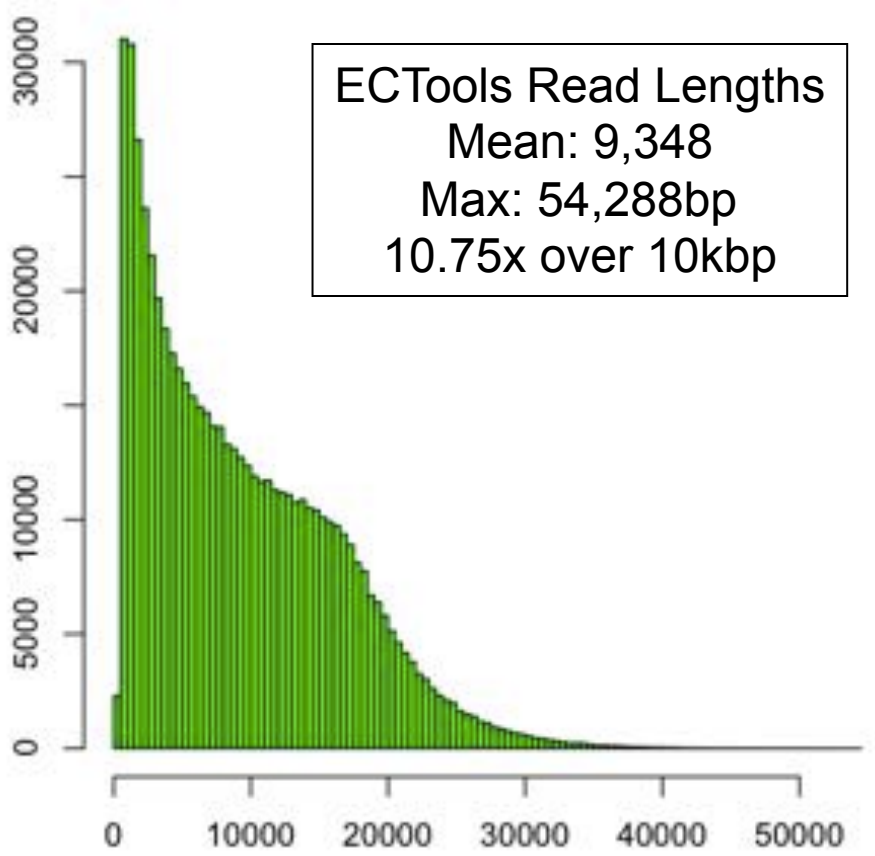


Over 14.1x coverage in 47 SMRTcells using P5-C3

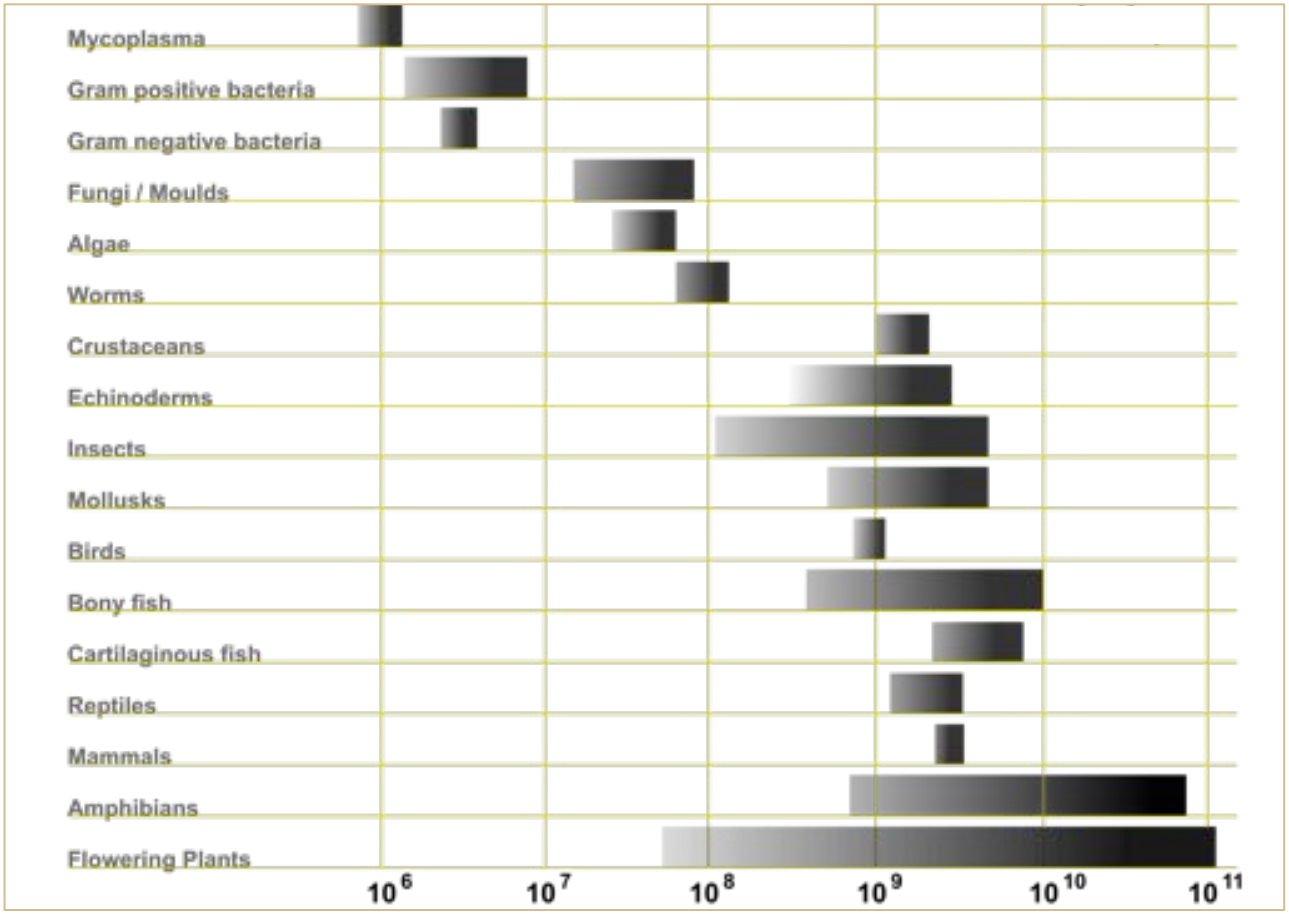
O. sativa pv Indica (IR64)

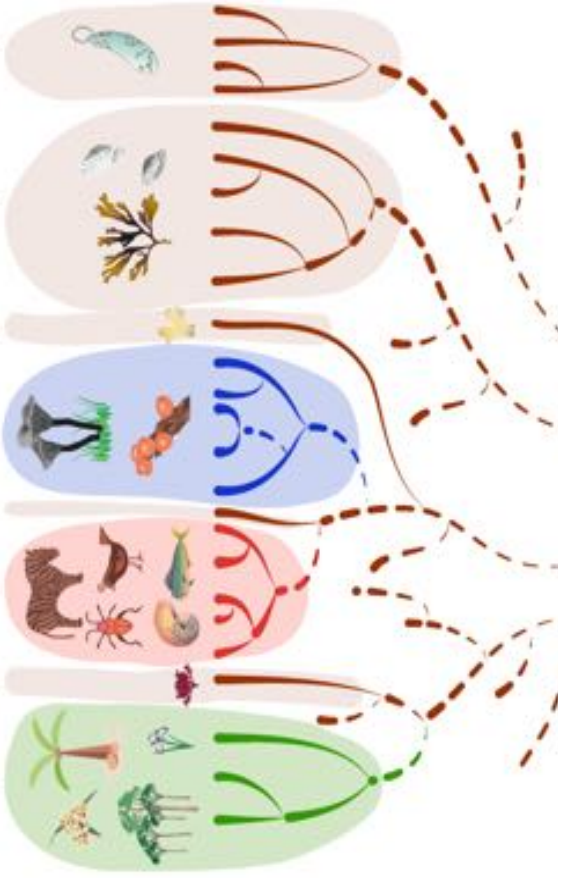
Genome size: ~370 Mb
Chromosome N50: ~29.7 Mbp

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



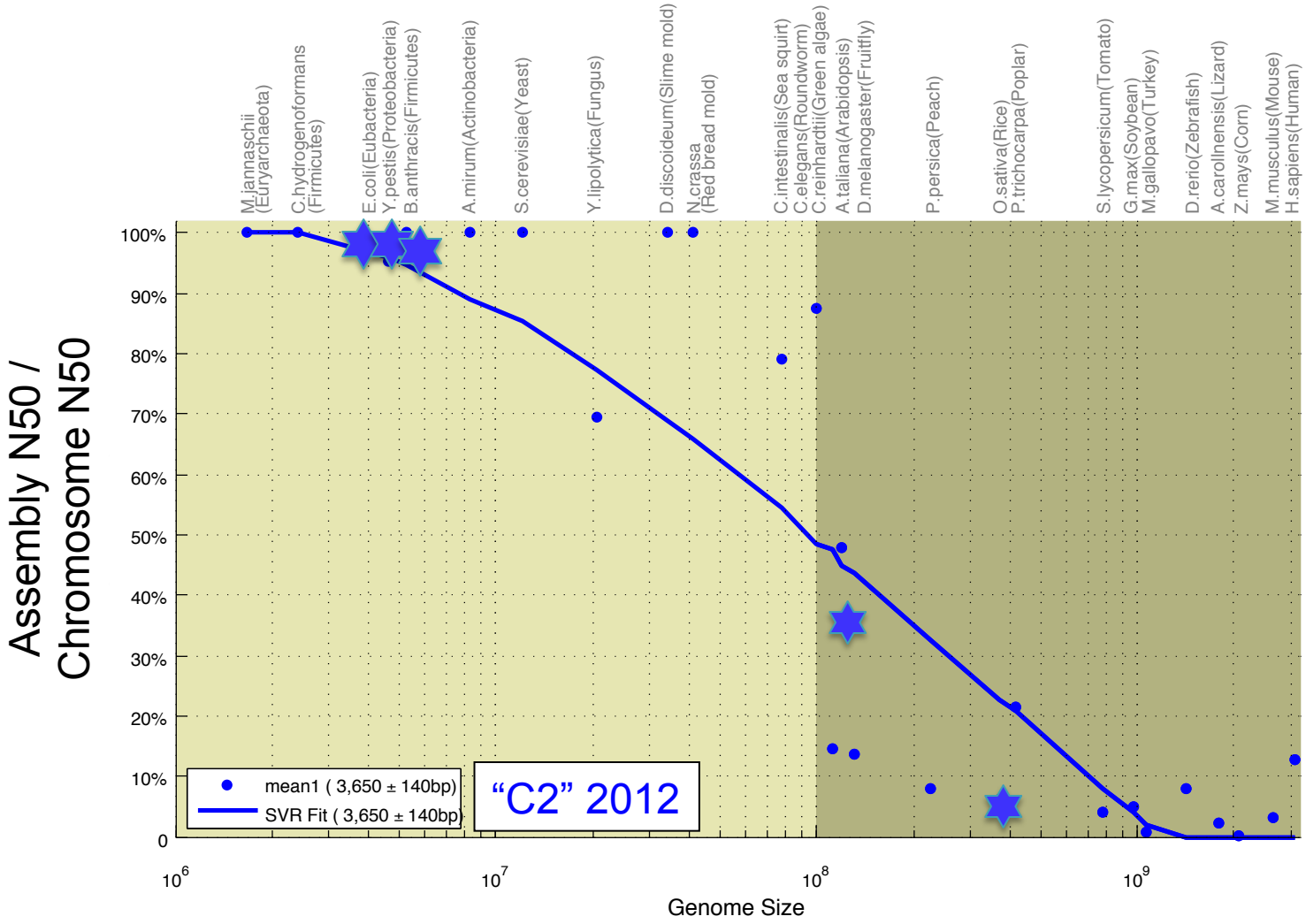
What should we expect from an assembly?





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

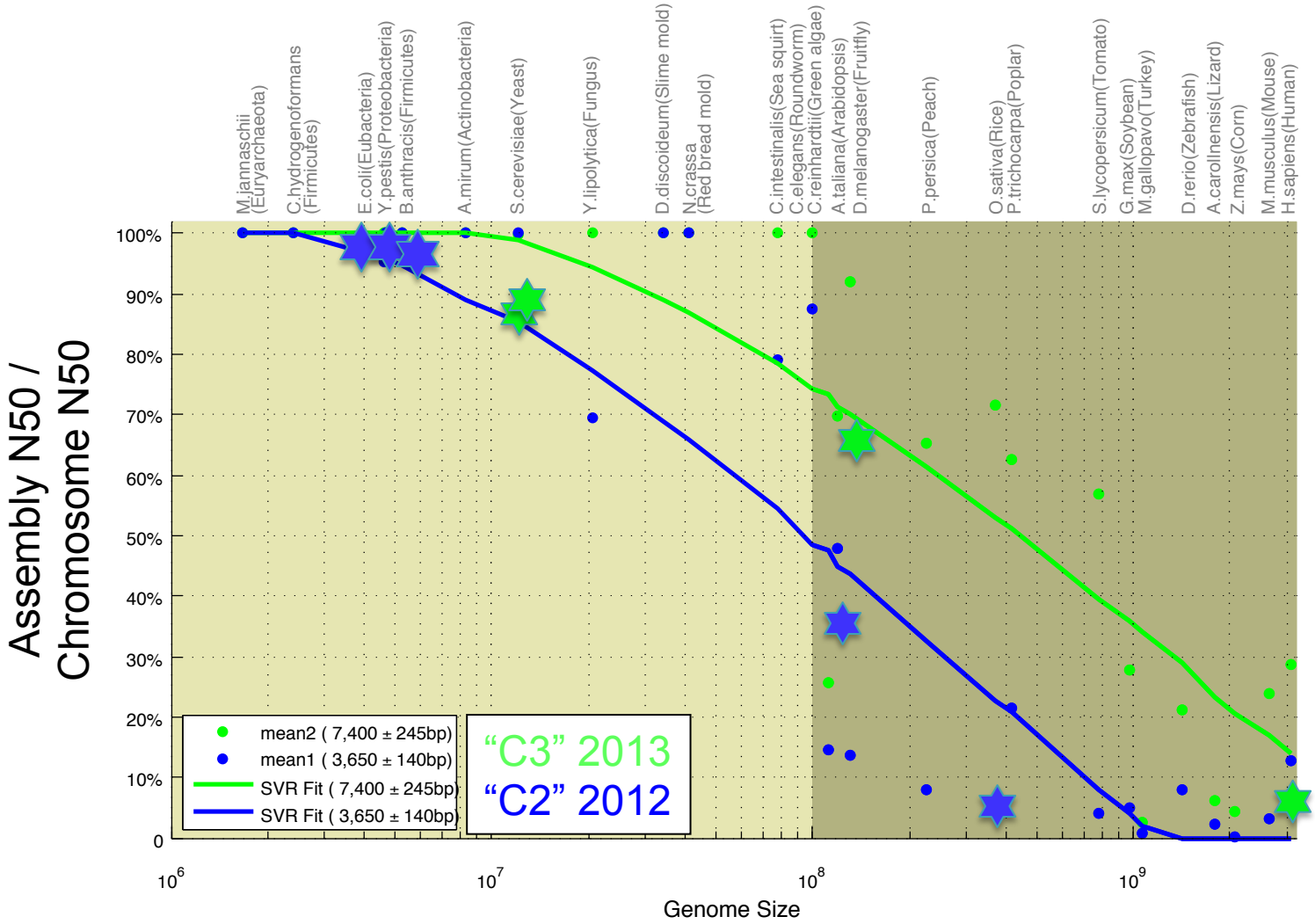
Assembly Complexity of Long Reads



Assembly complexity of long read sequencing

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

Assembly Complexity of Long Reads



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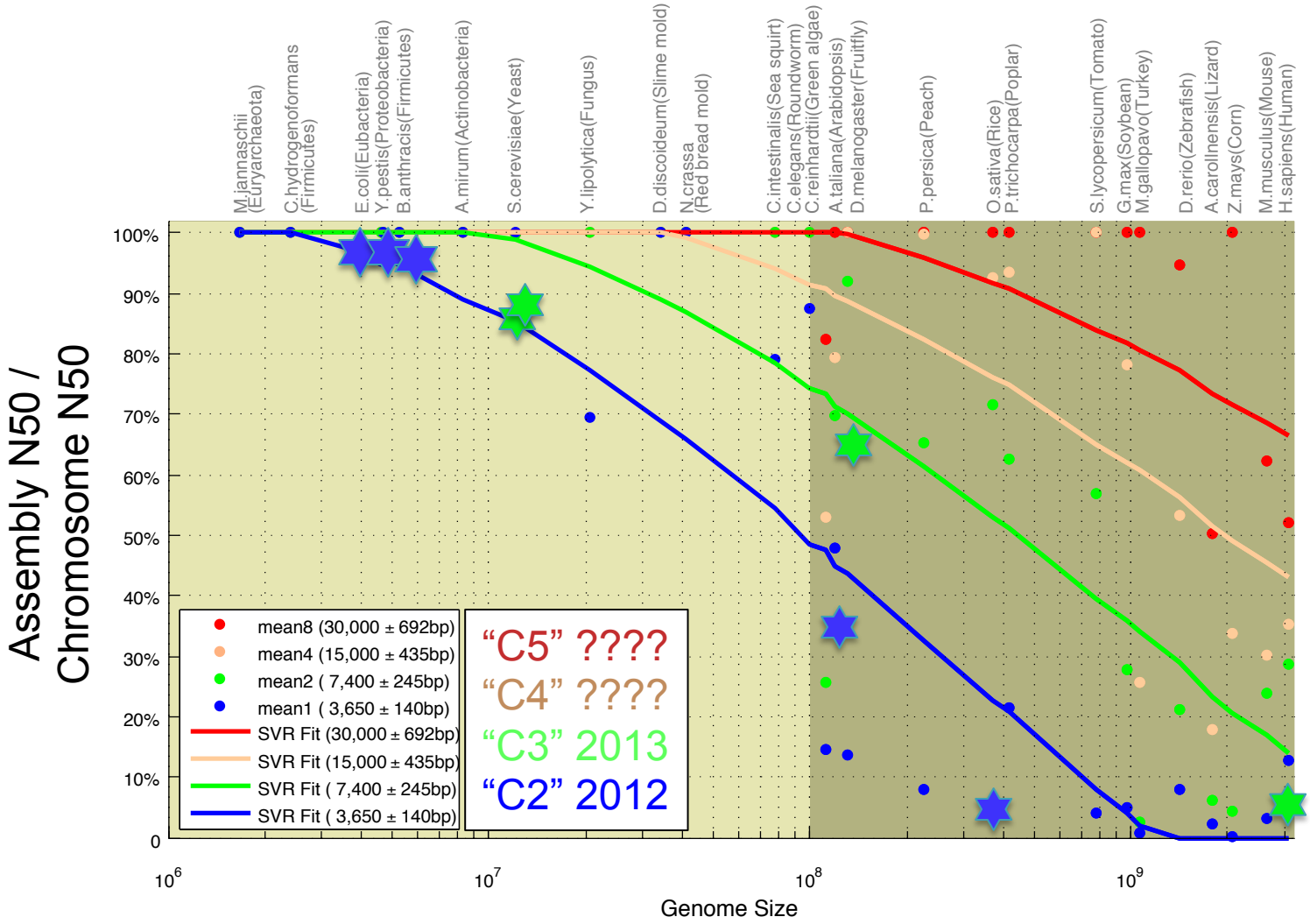
Assembly Complexity of Long Reads



Assembly complexity of long read sequencing

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

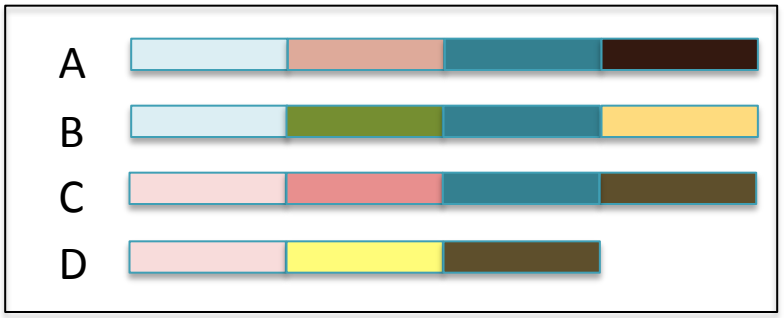
  < 1GB: HGAP/PacBio2CA @ 100x PB C3-P5
  expect high quality assembly: contig N50 over 1Mbp

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

  > 5GB: Email mschatz@cshl.edu

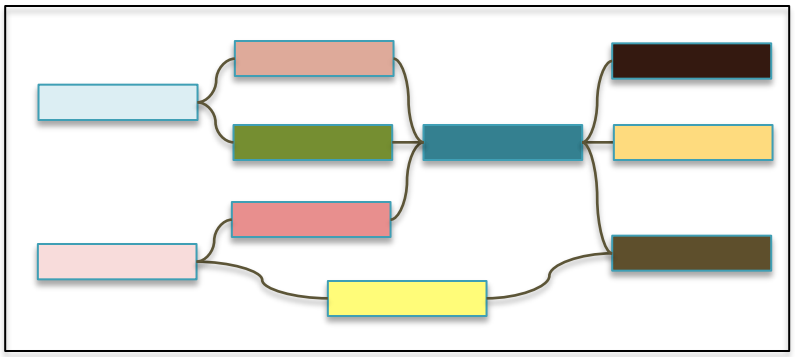
- **Caveats**
  - Model only as good as the available references (esp. haploid sequences)
  - Technologies are quickly improving, exciting new scaffolding technologies

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?

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

Outline

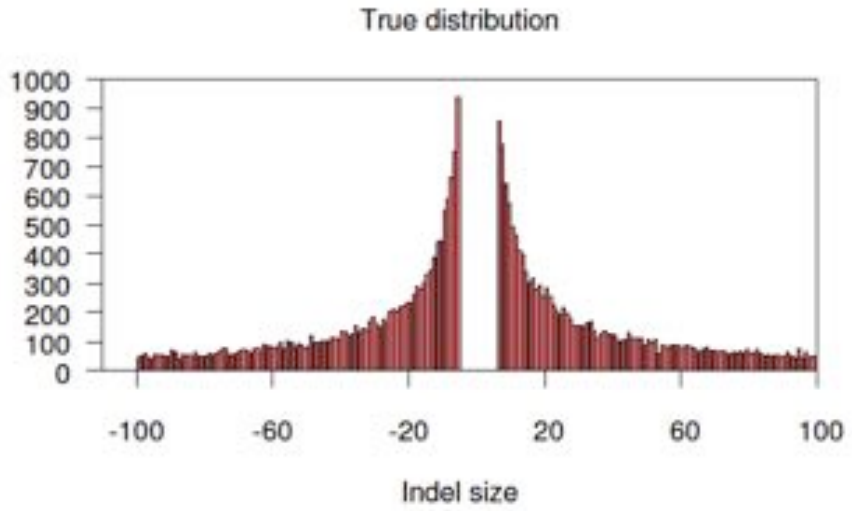
1. De novo assembly by analogy
2. Long Read Assembly
3. Disease Analytics

Variation Detection Complexity

SNPs + Short Indels

High precision and sensitivity

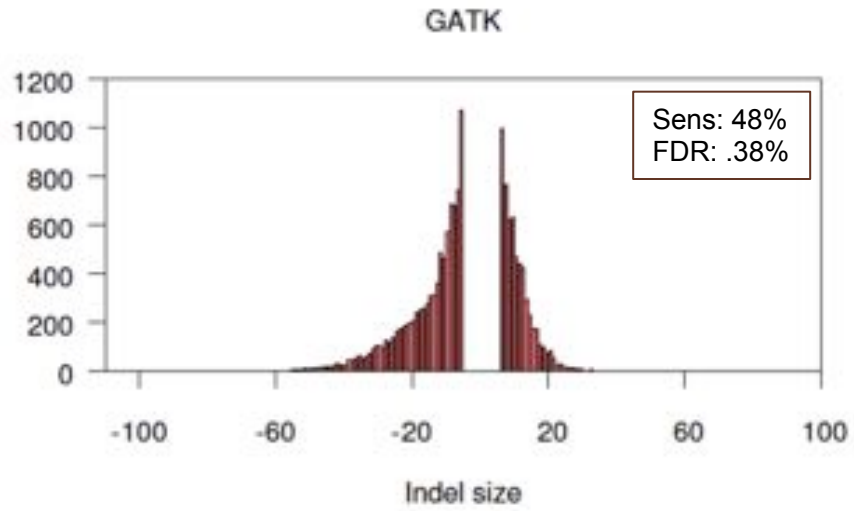
```
..TTTAGAATAG-CGAGTGC...
      ||||||| ||||
      AGAATAGGCGAG
```



"Long" Indels (>5bp)

Reduced precision and sensitivity

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



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

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

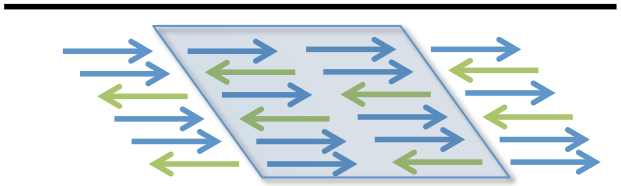
NRXN1 *de novo* SNP
(auSSC12501 chr2:50724605)

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

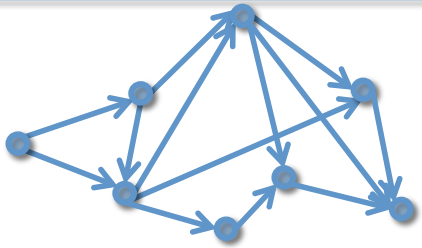
Narzisi, G, O'Rawe, J, Iossifov, I, Lee, Y, Wang, Z, Wu, Y, Lyon, G, Wigler, M, Schatz, MC (2014) *Under review.*

Scalpel Pipeline

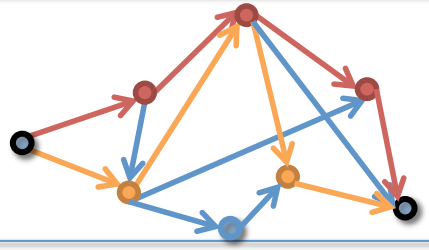
Extract reads mapping within the exon including (1) well-mapped reads, (2) soft-clipped reads, and (3) anchored pairs



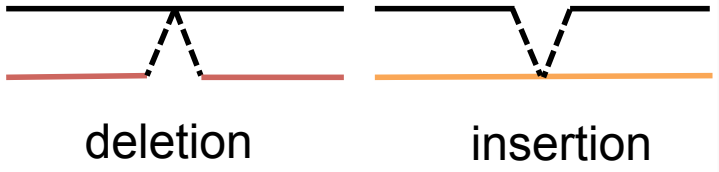
Decompose reads into overlapping *k*-mers and construct de Bruijn graph from the reads



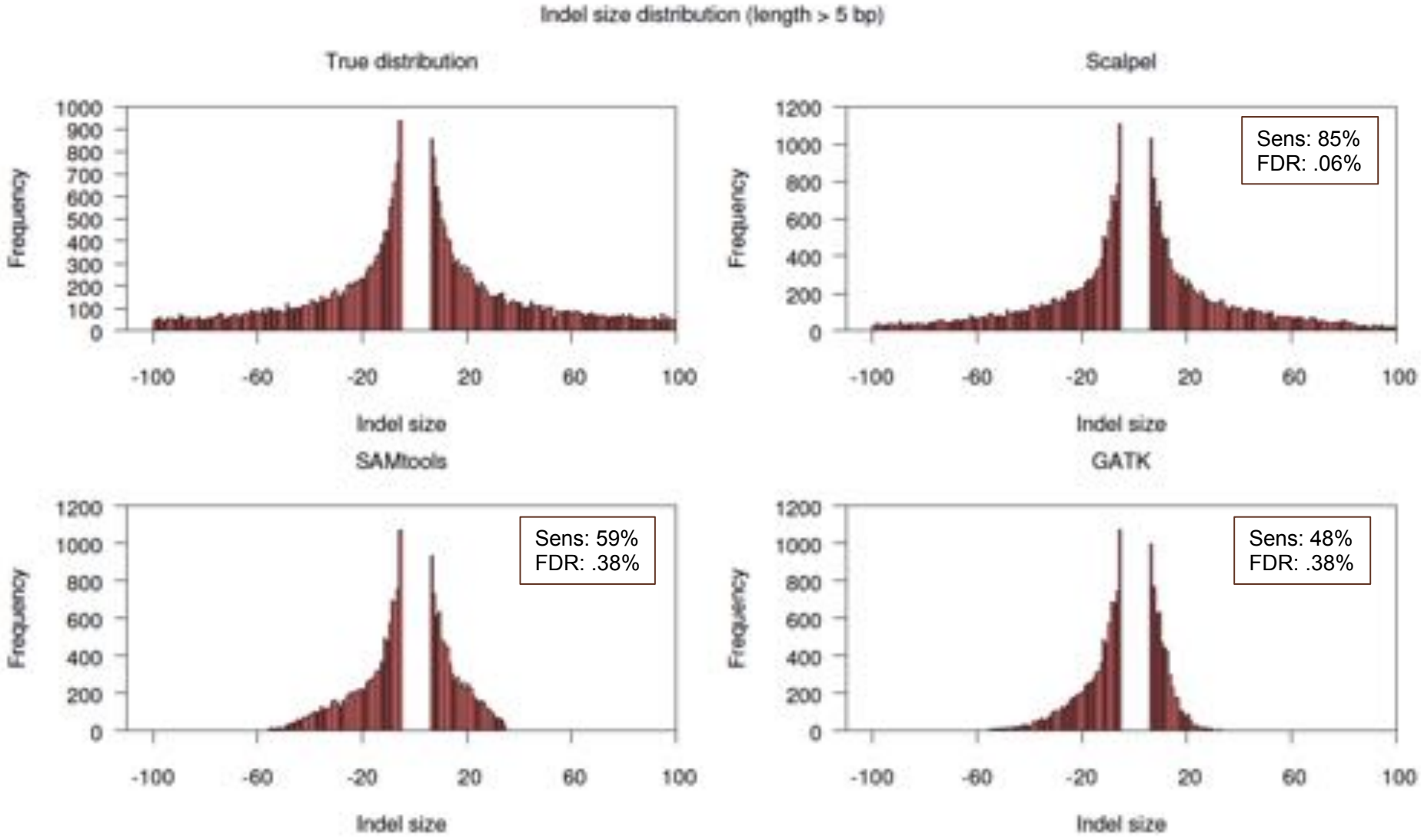
Find end-to-end haplotype paths spanning the region



Align assembled sequences to reference to detect mutations



Simulation Analysis



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

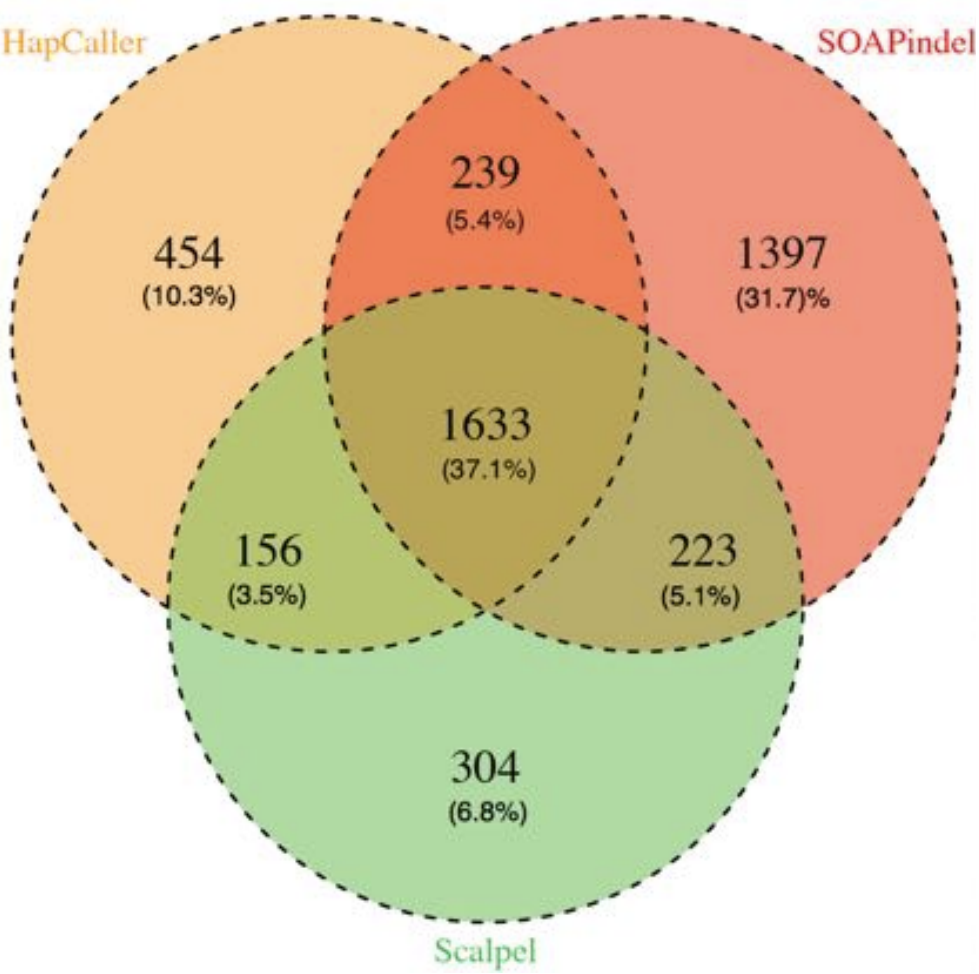
Experimental Analysis & Validation

Selected one deep coverage exome for deep analysis

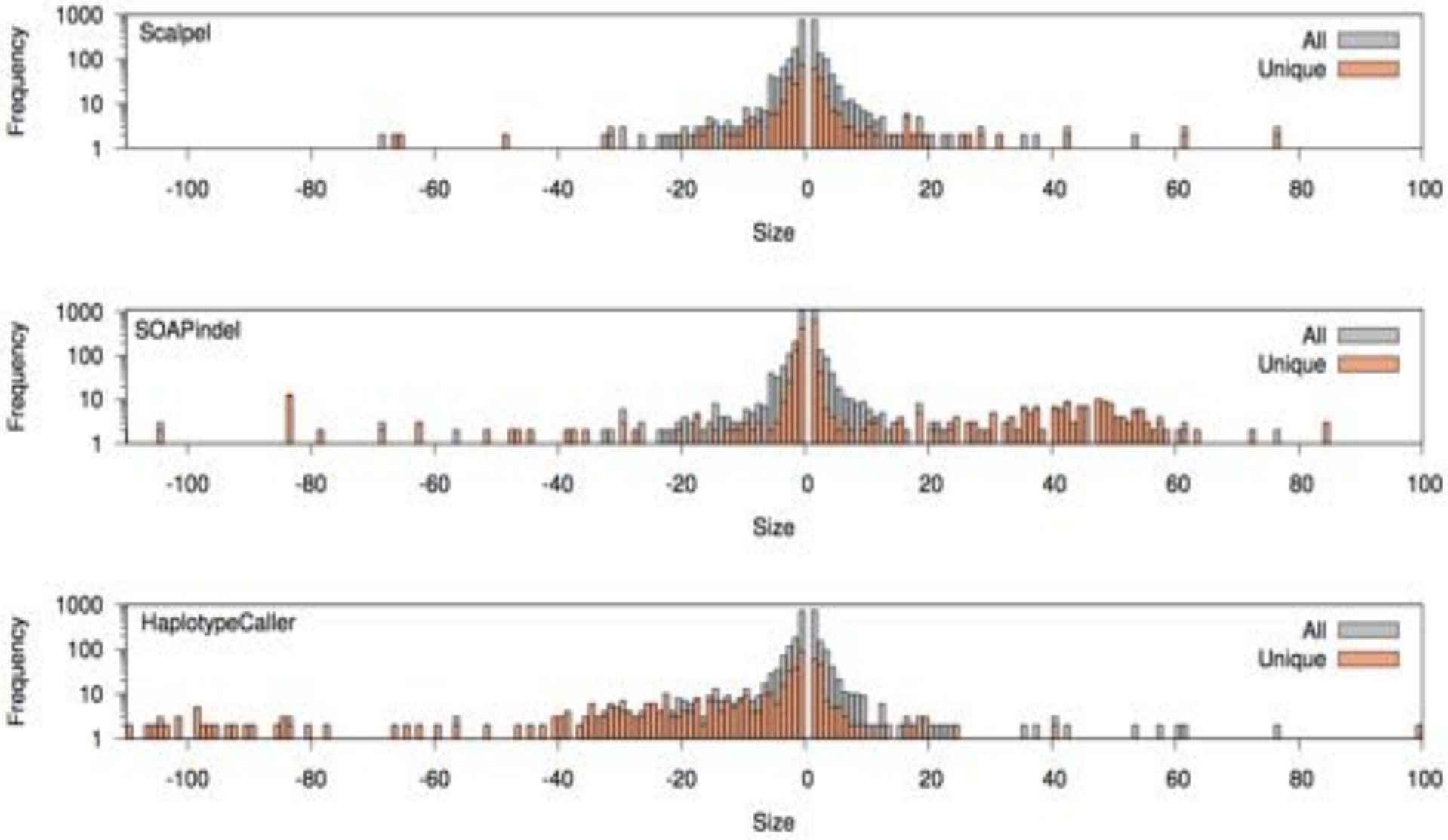
- Individual was diagnosed with ADHD and turrets syndrome
- 80% of the target at >20x coverage
- Evaluated with Scalpel, SOAPindel, and GATK Haplotype Caller

1000 indels selected for validation

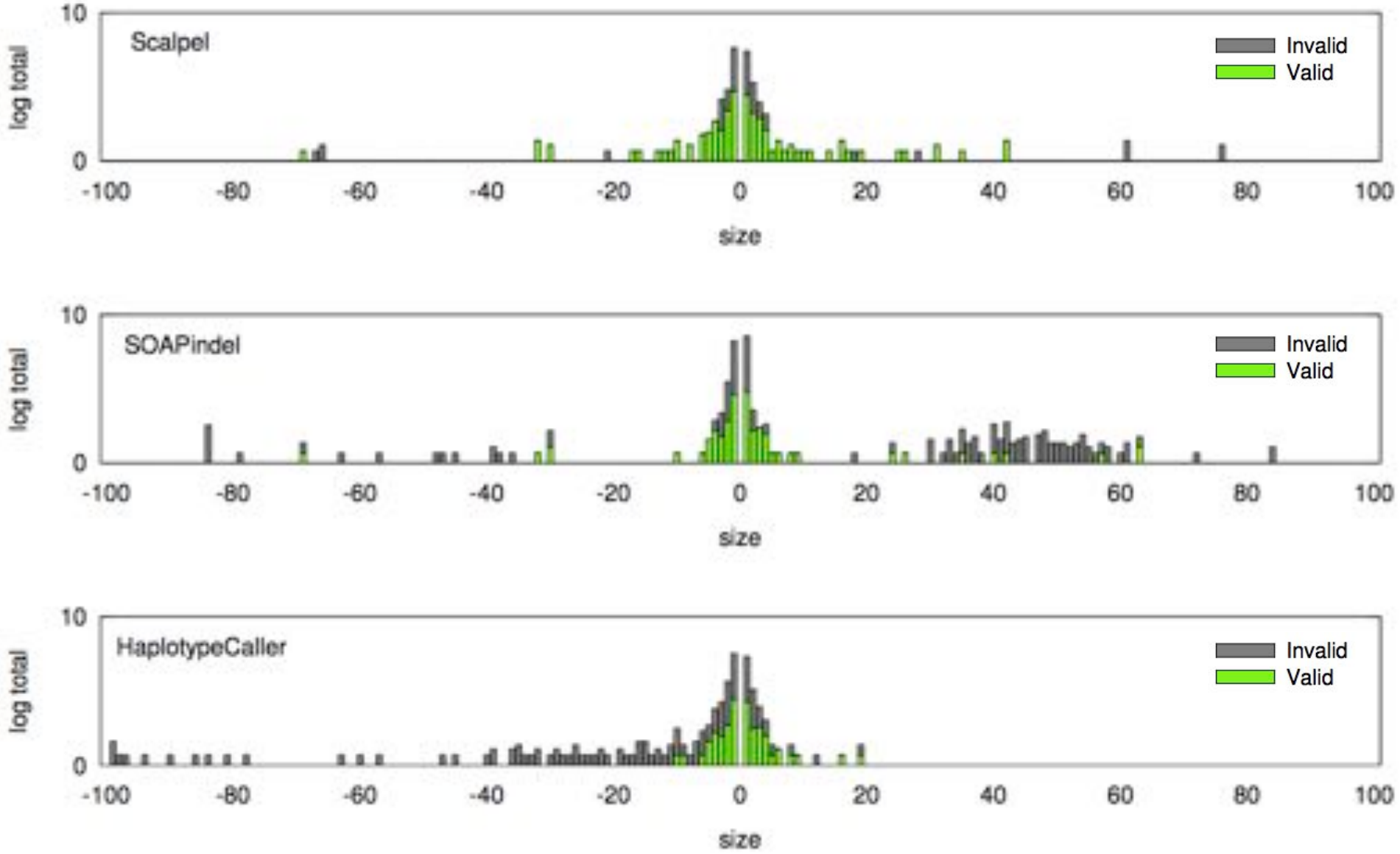
- 200 Scalpel
- 200 GATK Haplotype Caller
- 200 SOAPindel
- 200 within the intersection
- 200 long indels (>30bp)



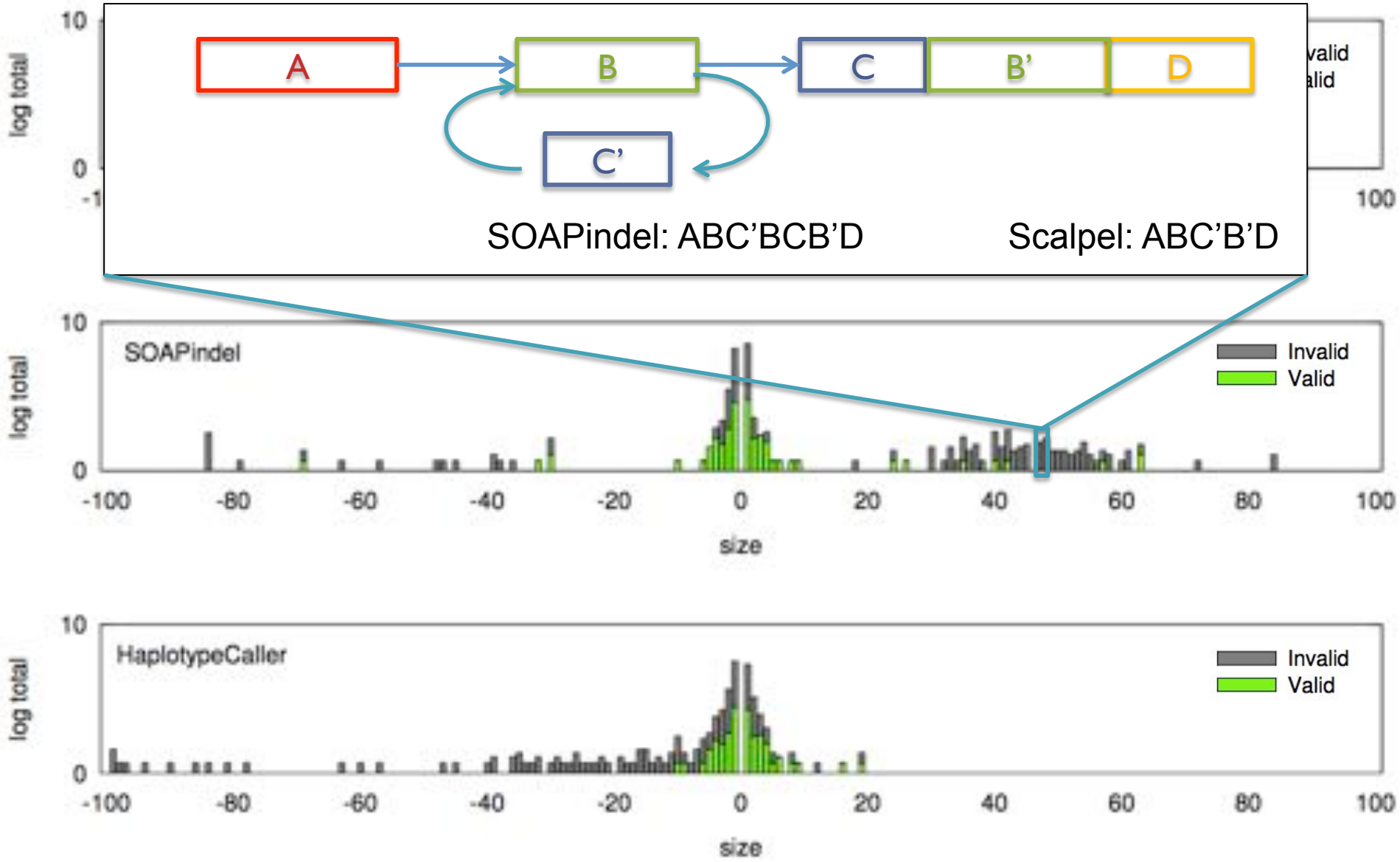
Scalpel Indel Discovery



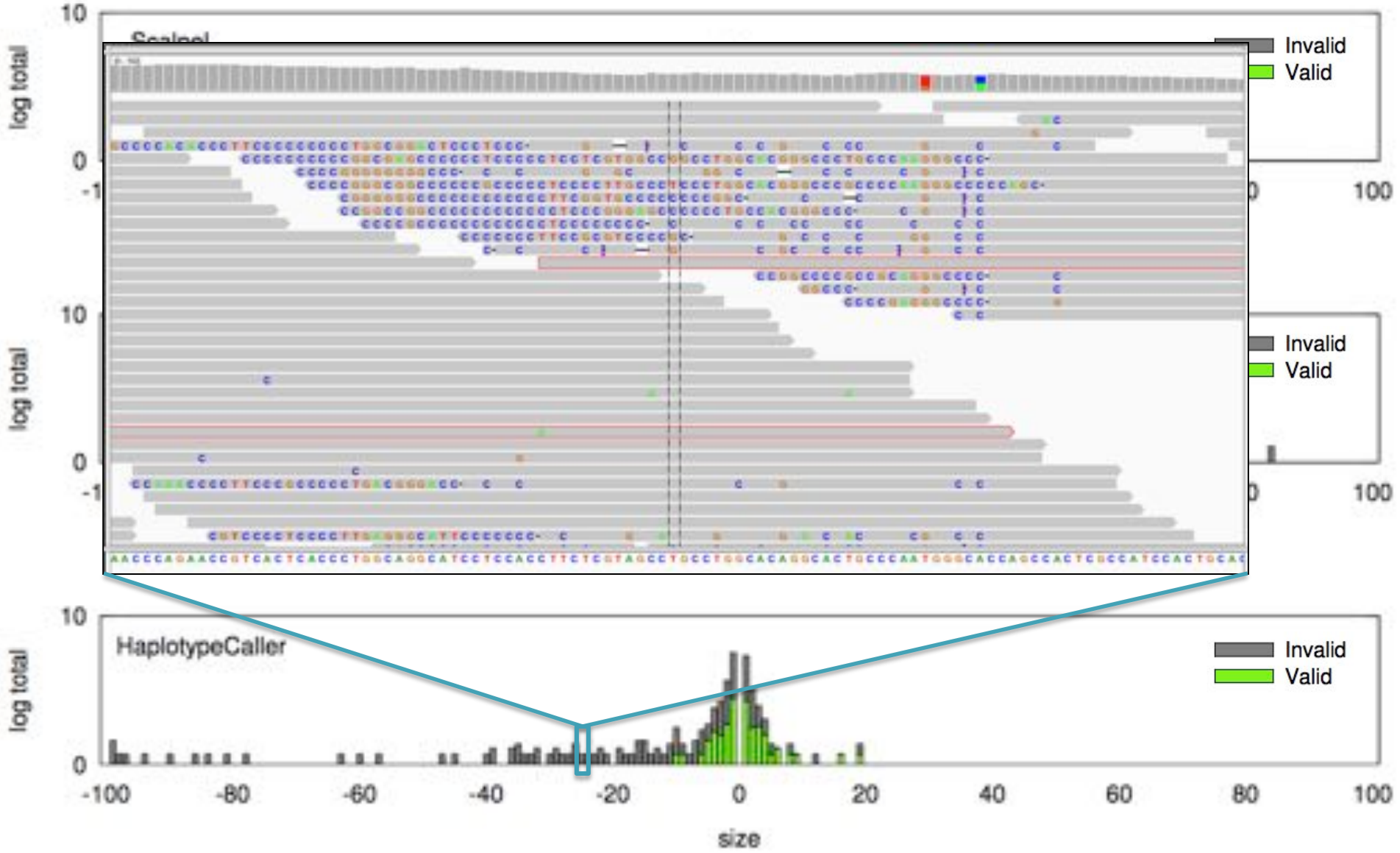
Scalpel Indel Discovery



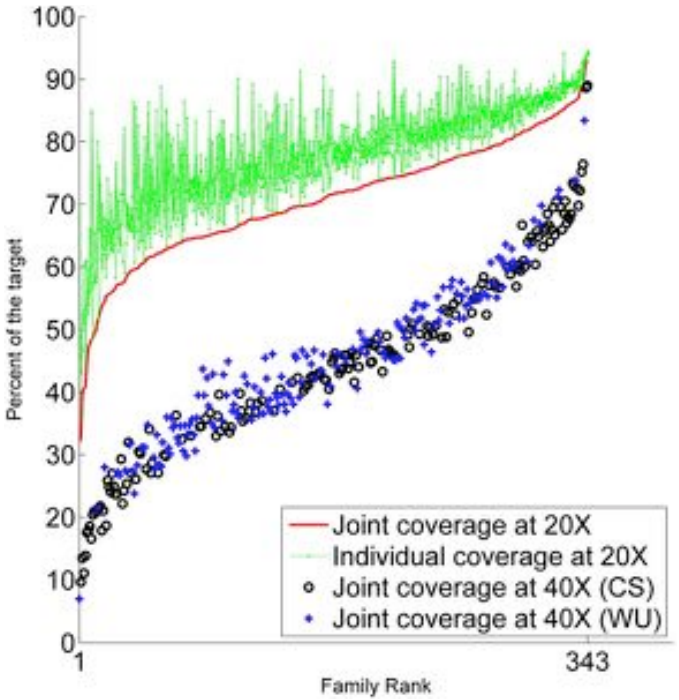
Scalpel Indel Discovery



Scalpel Indel Discovery



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

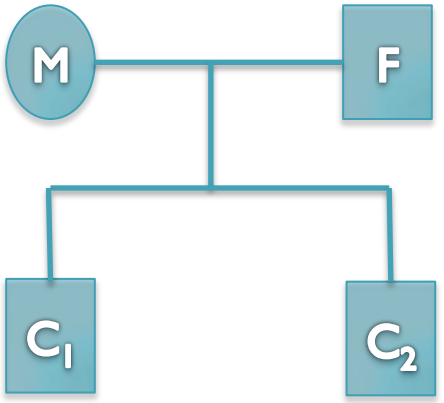
De novo mutations revealed by whole-exome sequencing are strongly associated with autism
Sanders *et al.* (2012) *Nature*. 485, 237–241.

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

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

New Biotechnology

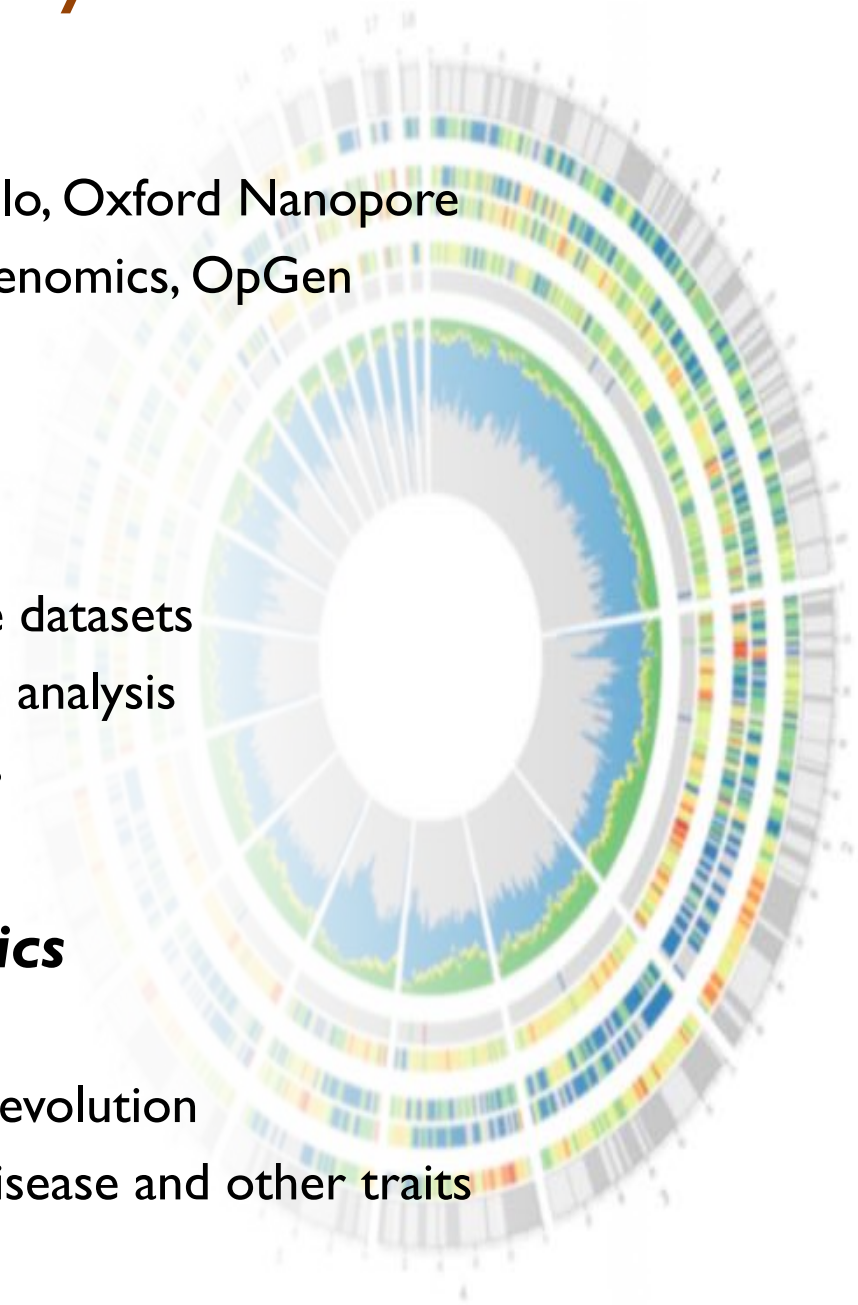
- Sequencing: Pacific Biosciences, Moleculo, Oxford Nanopore
- Mapping: Hi-C interactions, BioNanoGenomics, OpGen
- Faster/Cheaper/Better assemblies

Algorithmics

- Indexing and compressing of very large datasets
- Improved error correction, large graph analysis
- Networks and populations of genomes

Annotation & Comparative Genomics

- Identifying functional elements
- Cross species comparisons, models of evolution
- Identifying mutations responsible for disease and other traits



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Aspyn Palatnick

CSHL

McCombie Lab

Wigler Lab

Hannon Lab

Gingeras Lab

Jackson Lab

Hicks Lab

Iossifov Lab

Levy Lab

Lippman Lab

Lyon Lab

Martienssen Lab

Tuveson Lab

Ware Lab

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NSF

Biological Data Sciences
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http://schatzlab.cshl.edu

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