Algorithms for genome sequencing and disease analytics

Michael Schatz

Sept 9, 2014
CSHL Special Seminar

Unsolved Questions in Biology

- W
- H
- W
- H
- H
- H
- H
- H
- W
- W
- H
- W
- ***Plus thousands and thousands more***

The instruments provide the data, but none of the answers to any of these questions.

What software and systems will?

And who will create them?

Introductions

Tyler Garvin

CNV and transcriptome analysis of single cells

Tomorrow @ noon!

Srividya "Sri" Ramakrishnan

DOE Systems Biology Knowledgebase

Worlds fastest -omics pipelines

Maria Nattestad

Hi-C Chromatin Interactions

Plant Assembly & Analysis

Aspyn Palatnick

Mobile Genotype Analysis

Flu antiviral analysis

Genome Structure & Function

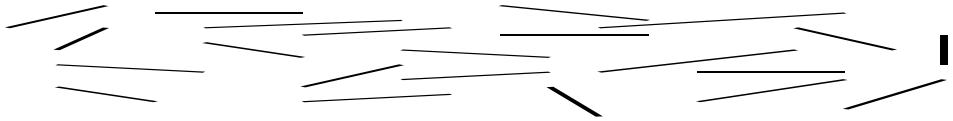
1. **Structure: Sequencing and Assembly**

   "A tale of two sequencers"

2. **Function: Disease Analytics**
   1. Pan-genome analysis
   2. The role of indels in human diseases

Sequencing a Genome

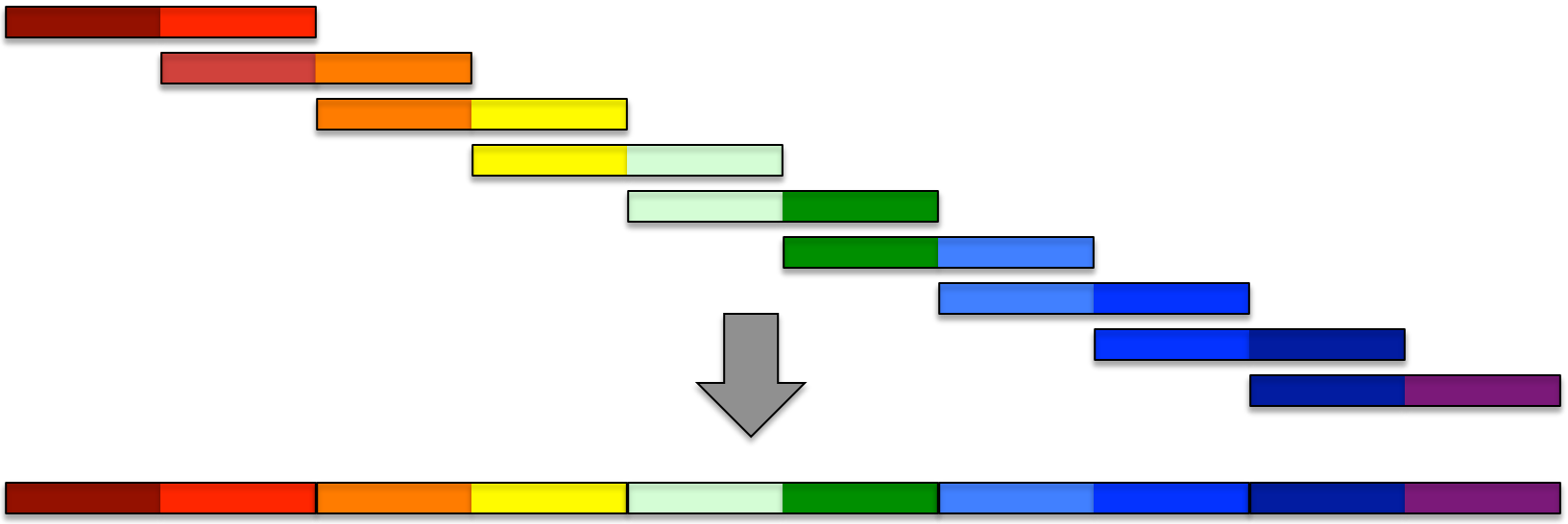
1. Shear & Sequence DNA



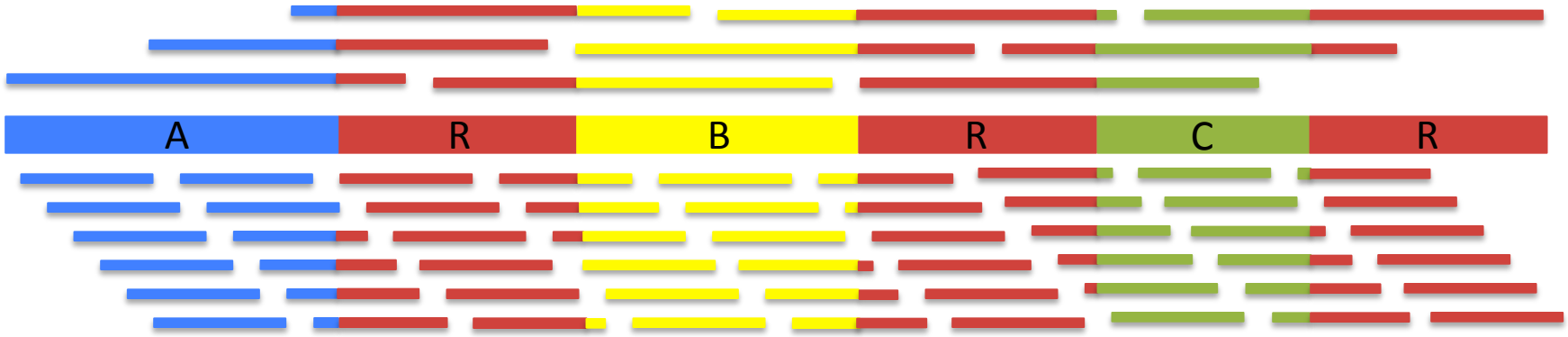
2. Construct assembly graph from overlapping reads

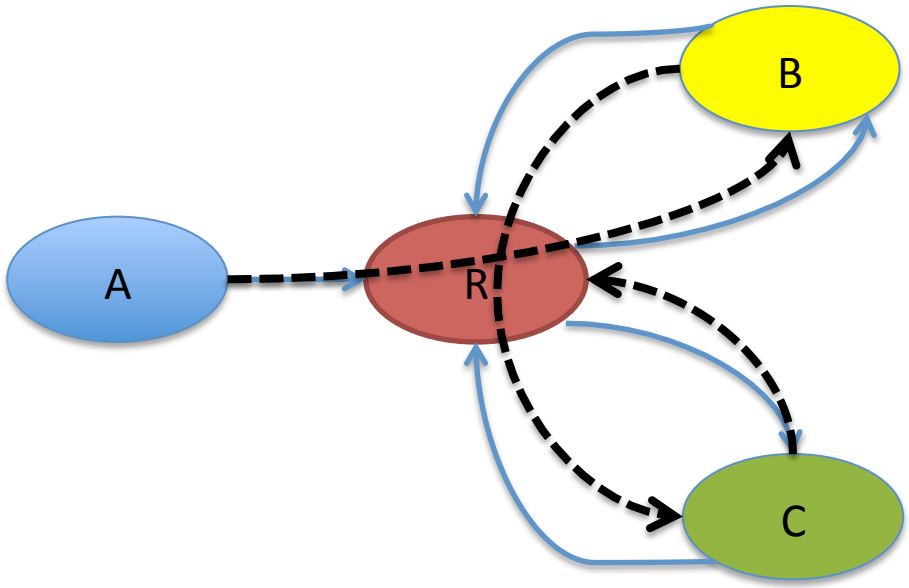
...AGCCTAGGGATGCGCGACACGT
GGATGCGCGACACGTCGCATATCCGGTTTGGTCAACCTCGGACGGAC
CAACCTCGGACGGACCTCAGCGAA...

3. Simplify assembly graph

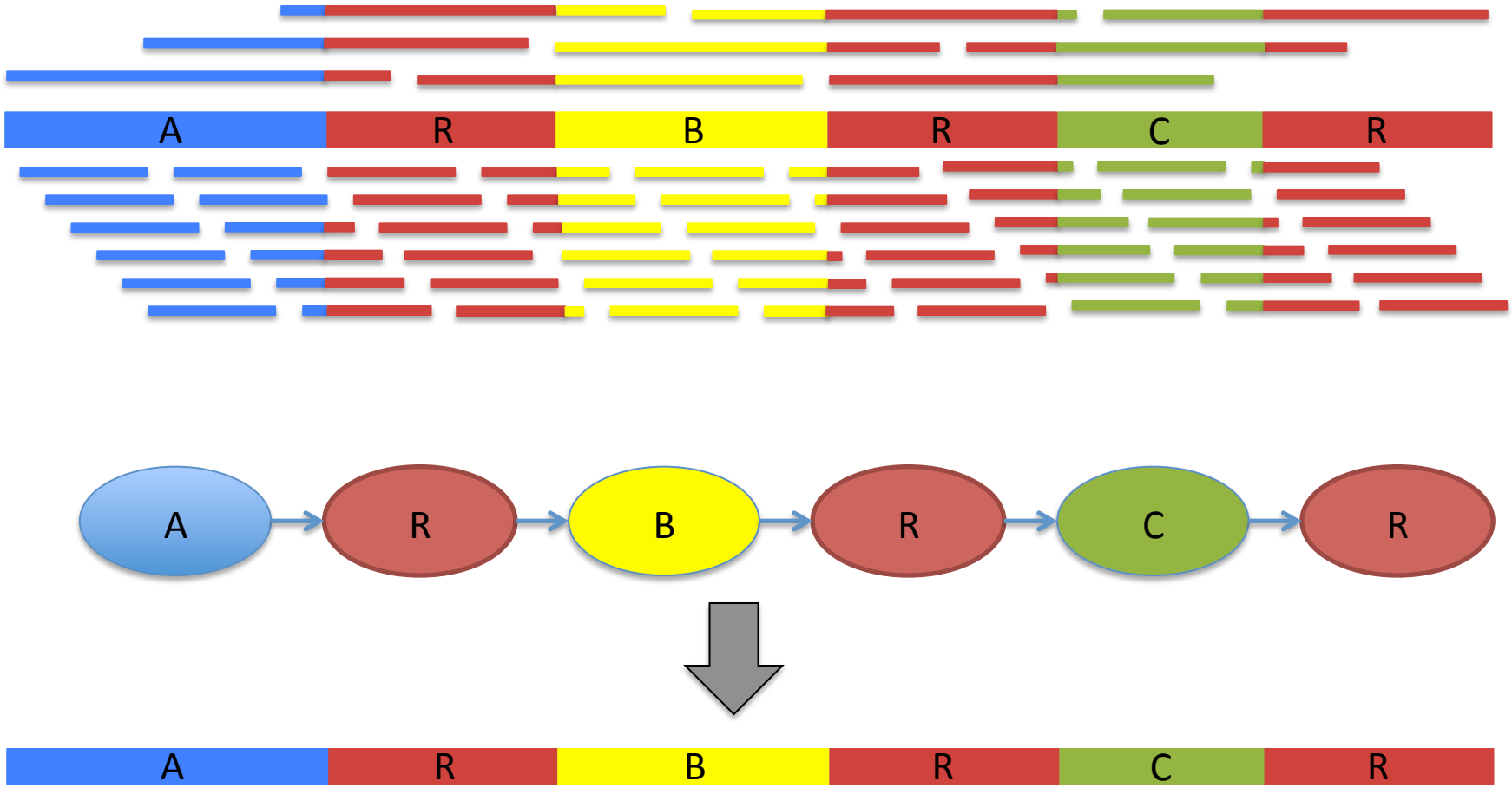


Assembly Complexity





Assembly Complexity

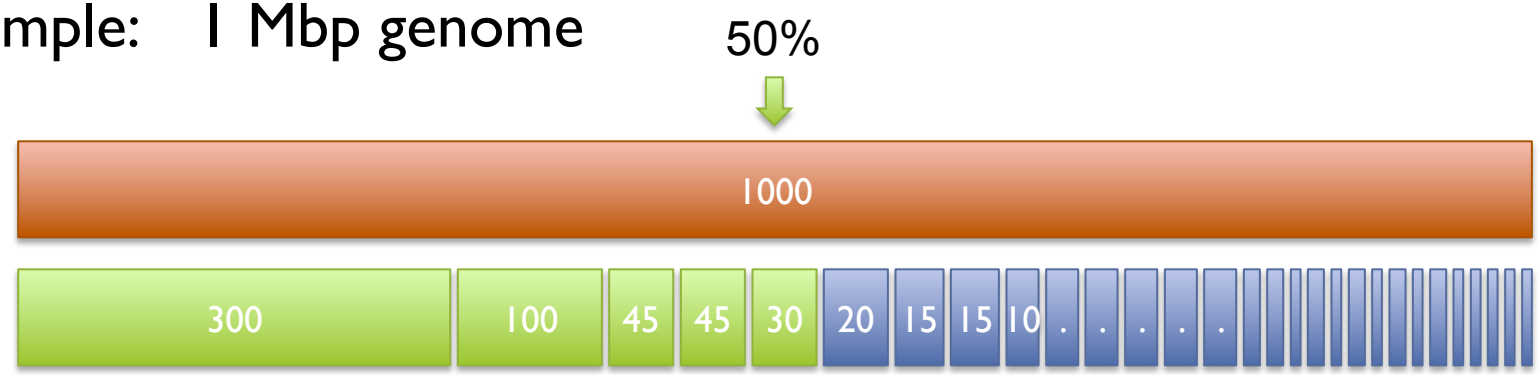


The advantages of SMRT sequencing
Roberts, RJ, Carneiro, MO, Schatz, MC (2013) *Genome Biology*. 14:405

N50 size

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

Example: 1 Mbp genome



N50 size = 30 kbp

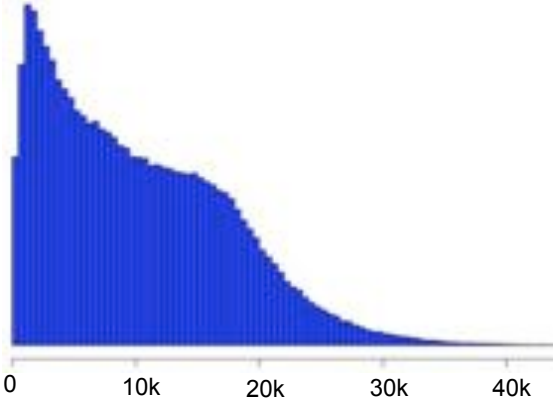
(300k+100k+45k+45k+30k = 520k >= 500kbp)

A greater N50 is indicative of improvement 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

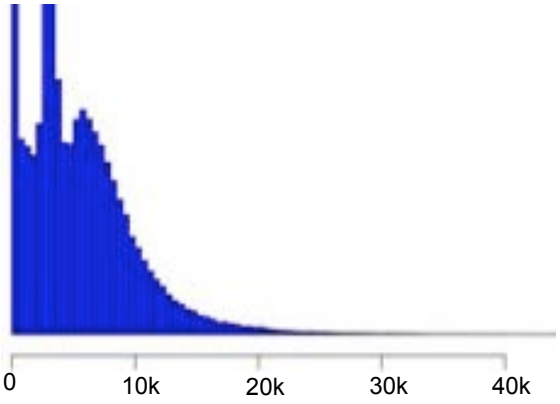
3rd Gen Long Read Sequencing

PacBio RS II



CSHL/PacBio

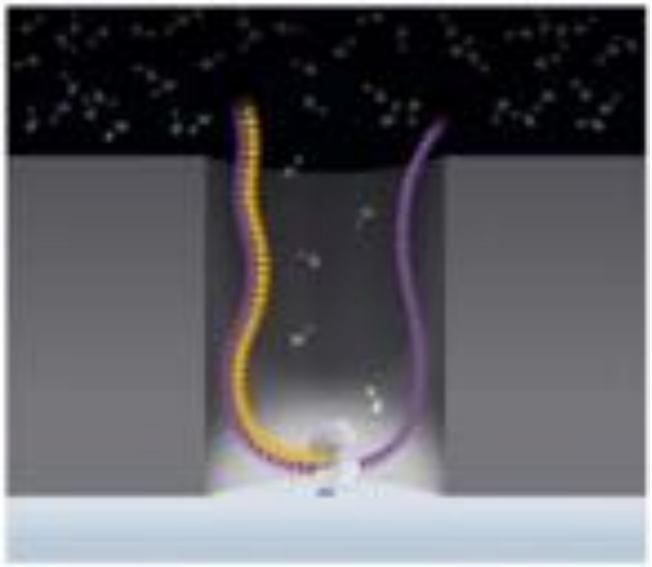
Oxford Nanopore

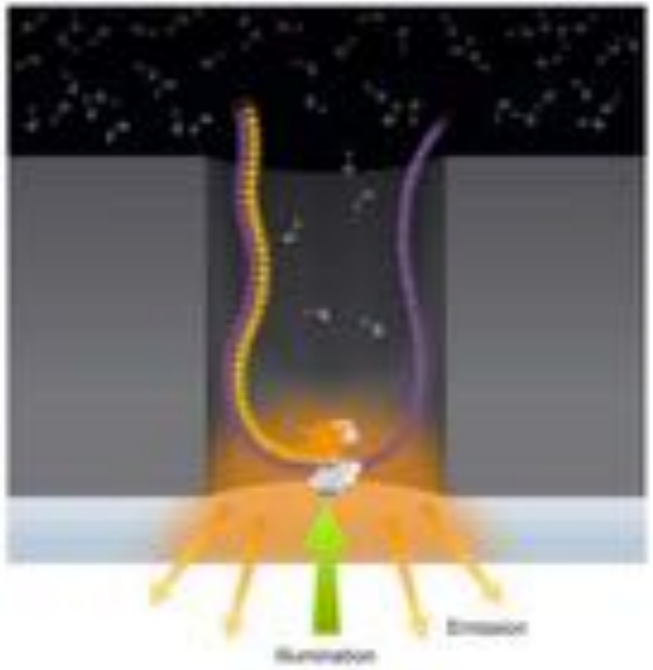


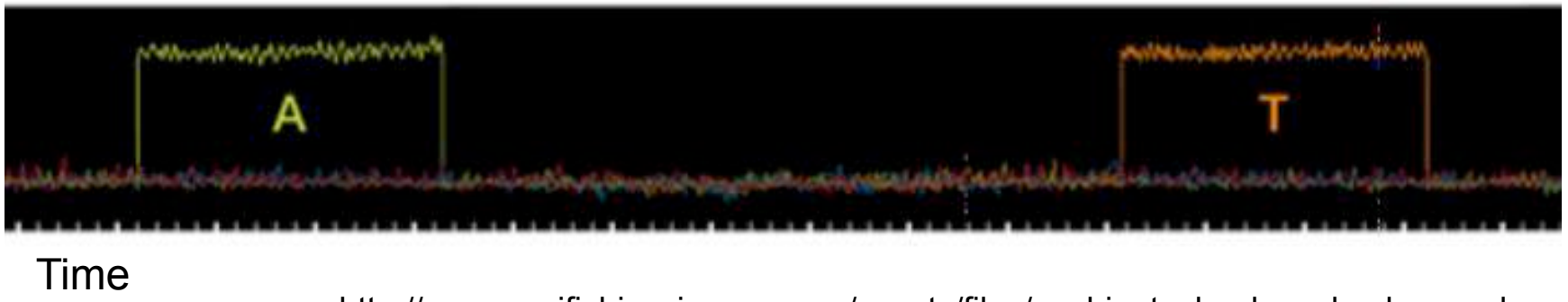
CSHL/ONT

PacBio SMRT Sequencing

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

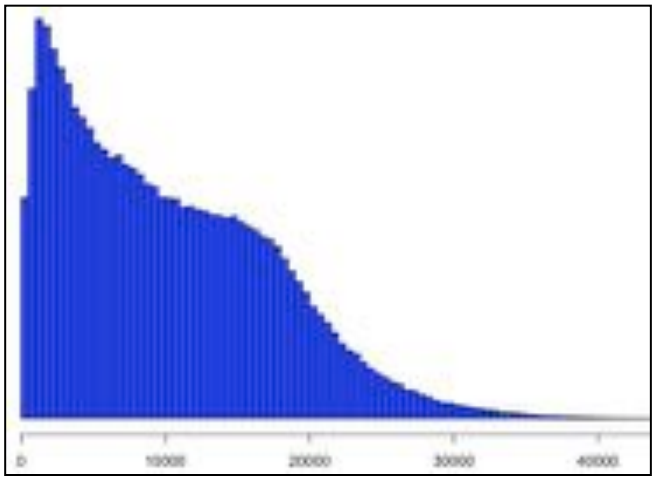






http://www.pacificbiosciences.com/assets/files/pacbio_technology_backgrounder.pdf

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

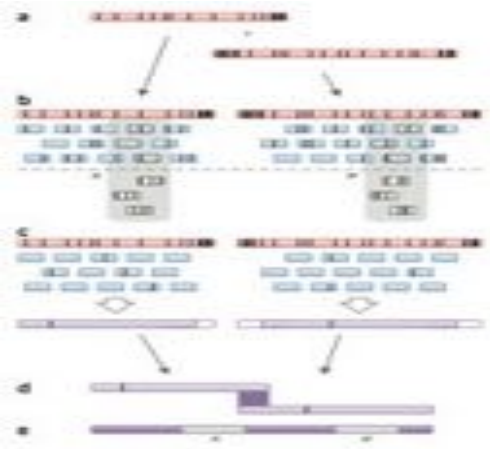
PacBio Assembly Algorithms

PBJelly

Gap Filling and Assembly Upgrade

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

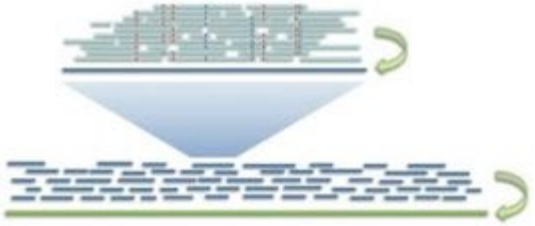
PacBioToCA & ECTools



Hybrid/PB-only Error Correction

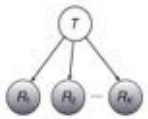
Koren, Schatz, *et al* (2012)
Nature Biotechnology. 30:693–700

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)

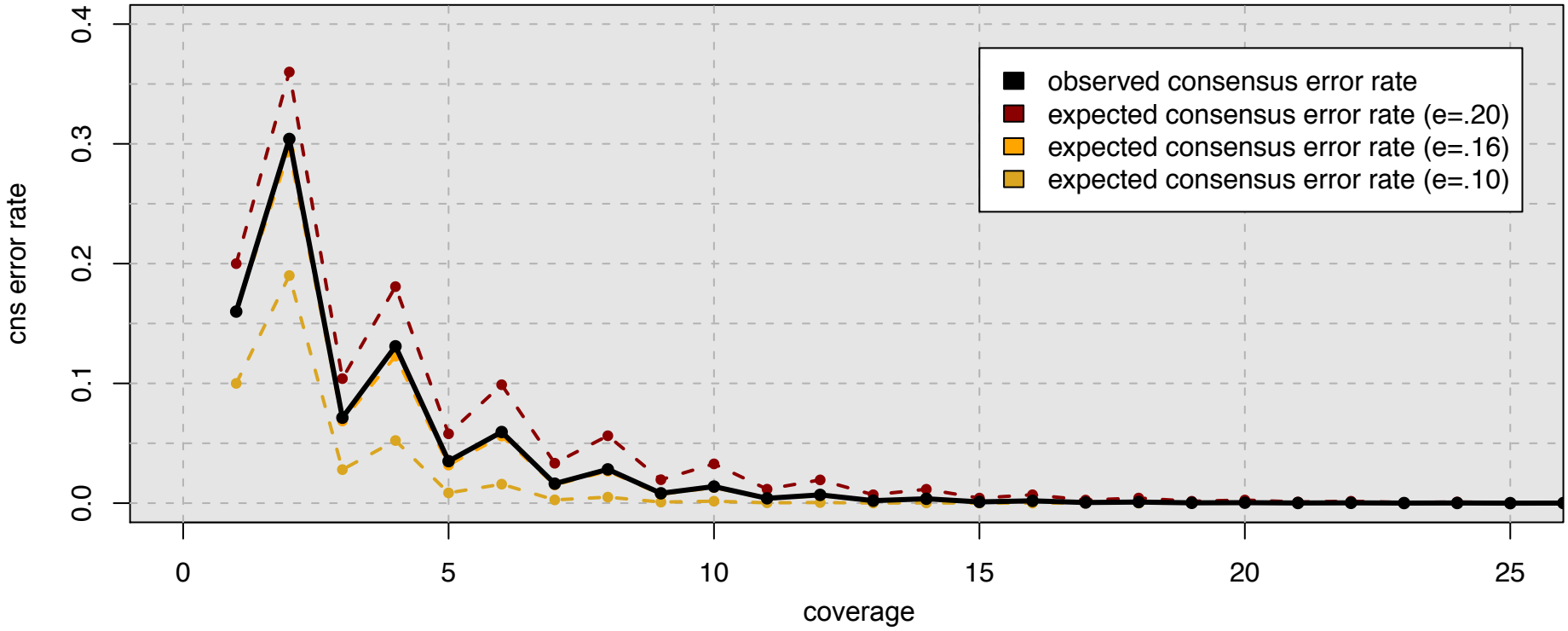
	Initial Assembly	Quiver Consensus
QV	43.4	54.5
Accuracy	99.99540%	99.99964%
Differences	141	11

PB-only Correction & Polishing

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

< 5x PacBio Coverage > 50x

Consensus Accuracy and Coverage



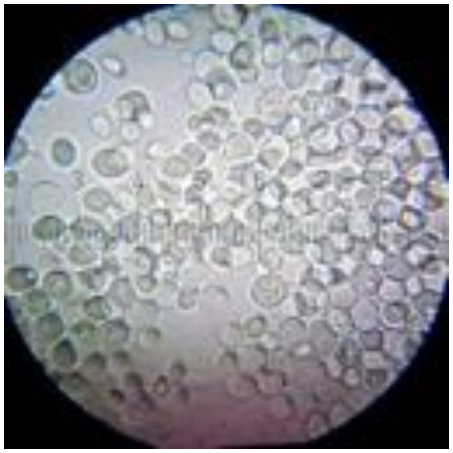
Coverage can overcome random errors

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

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

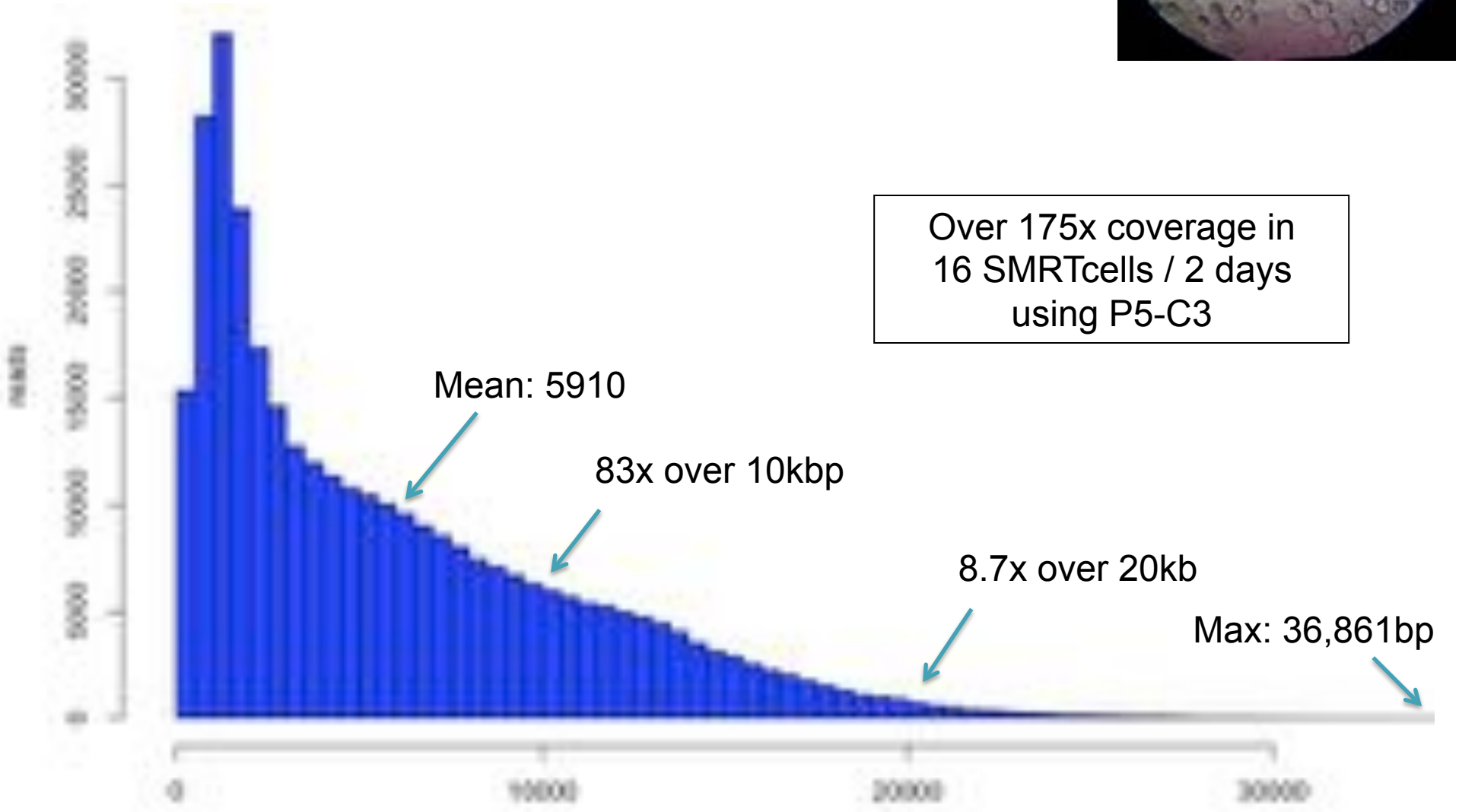
Koren, Schatz, *et al* (2012)
Nature Biotechnology. 30:693–700

S. cerevisiae W303

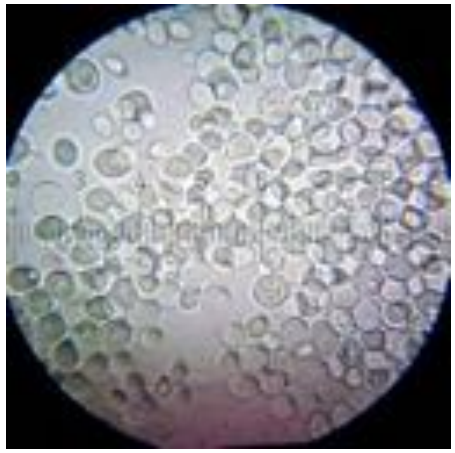


PacBio RS II sequencing at CSHL in the McCombie Lab

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



S. cerevisiae W303

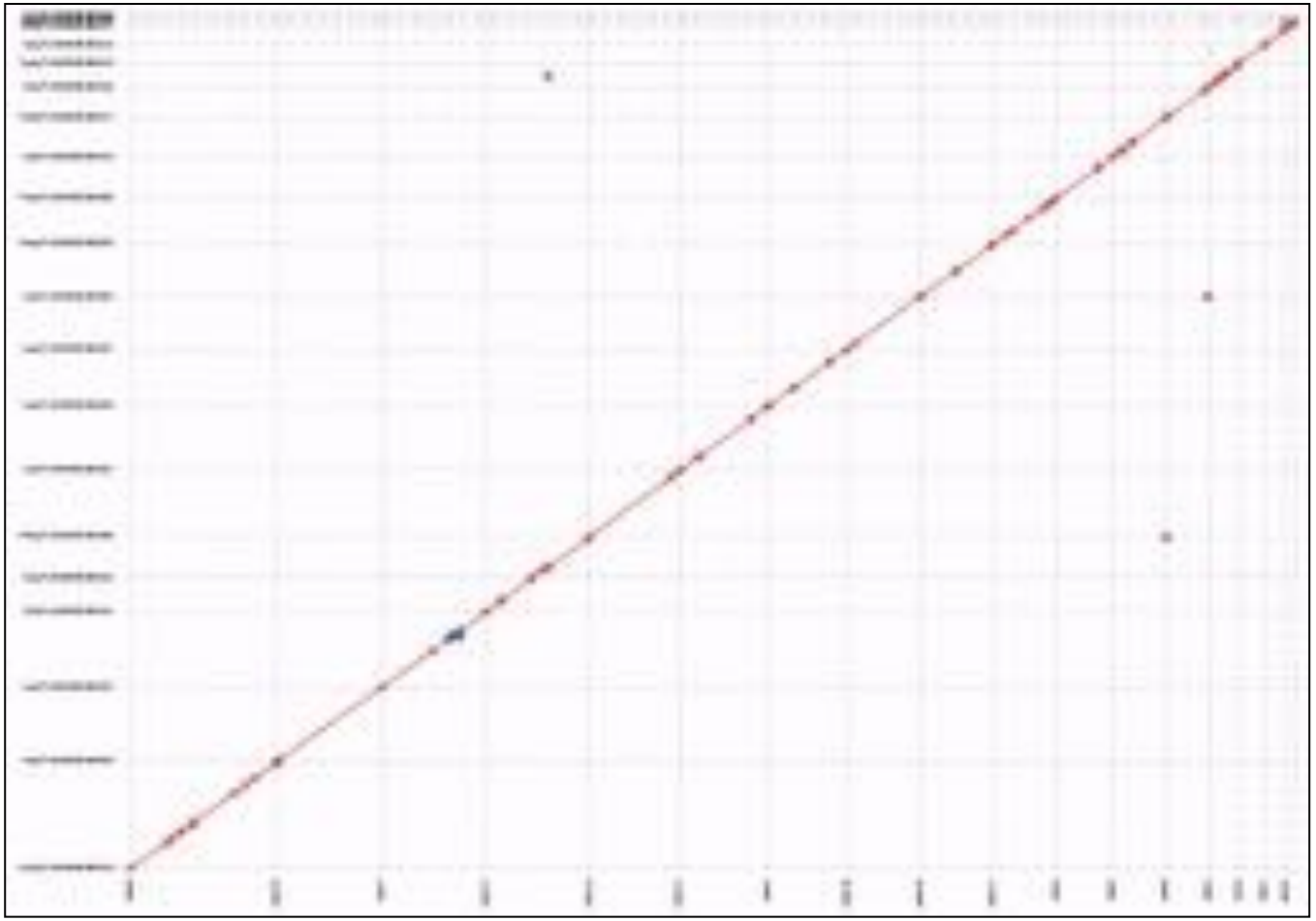


S288C Reference sequence

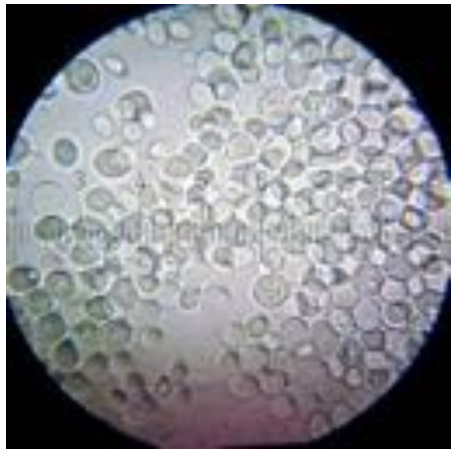
- 12.1Mbp; 16 chromo + mitochondria; N50: 924kbp

PacBio assembly using HGAP + Celera Assembler

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



S. cerevisiae W303

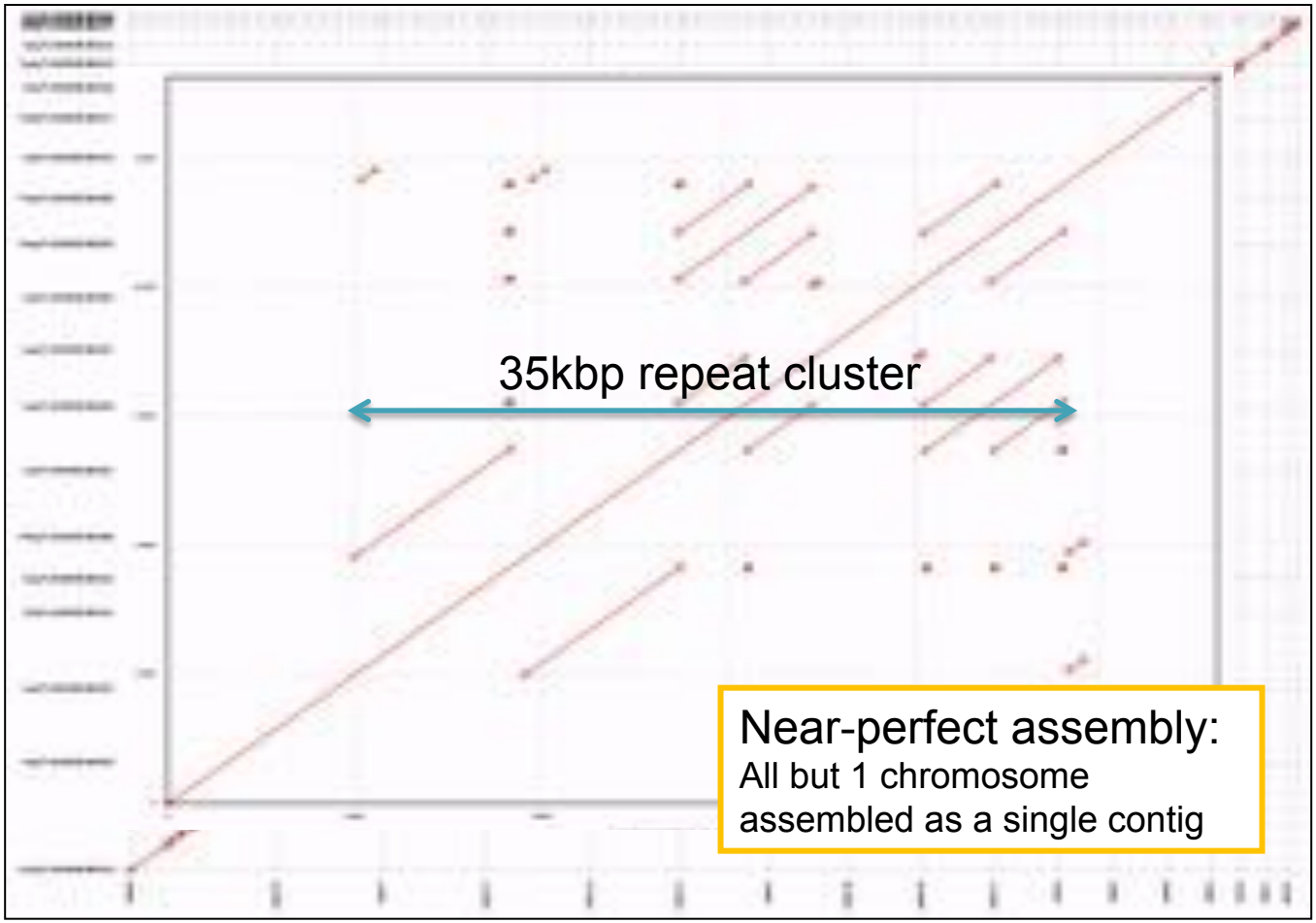


S288C Reference sequence

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

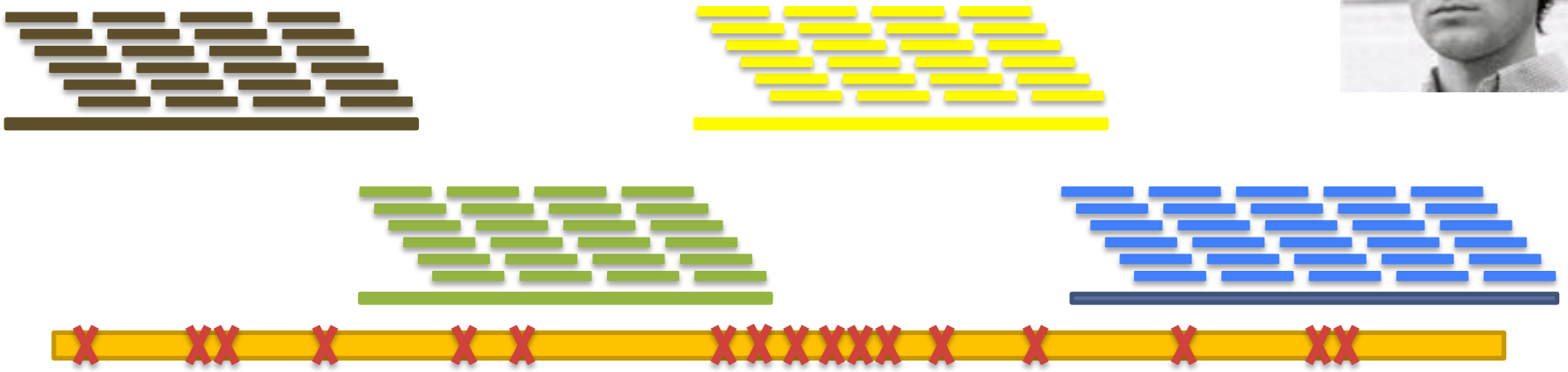
PacBio assembly using HGAP + Celera Assembler

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



ECTools: Hybrid Error Correction for large genomes

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

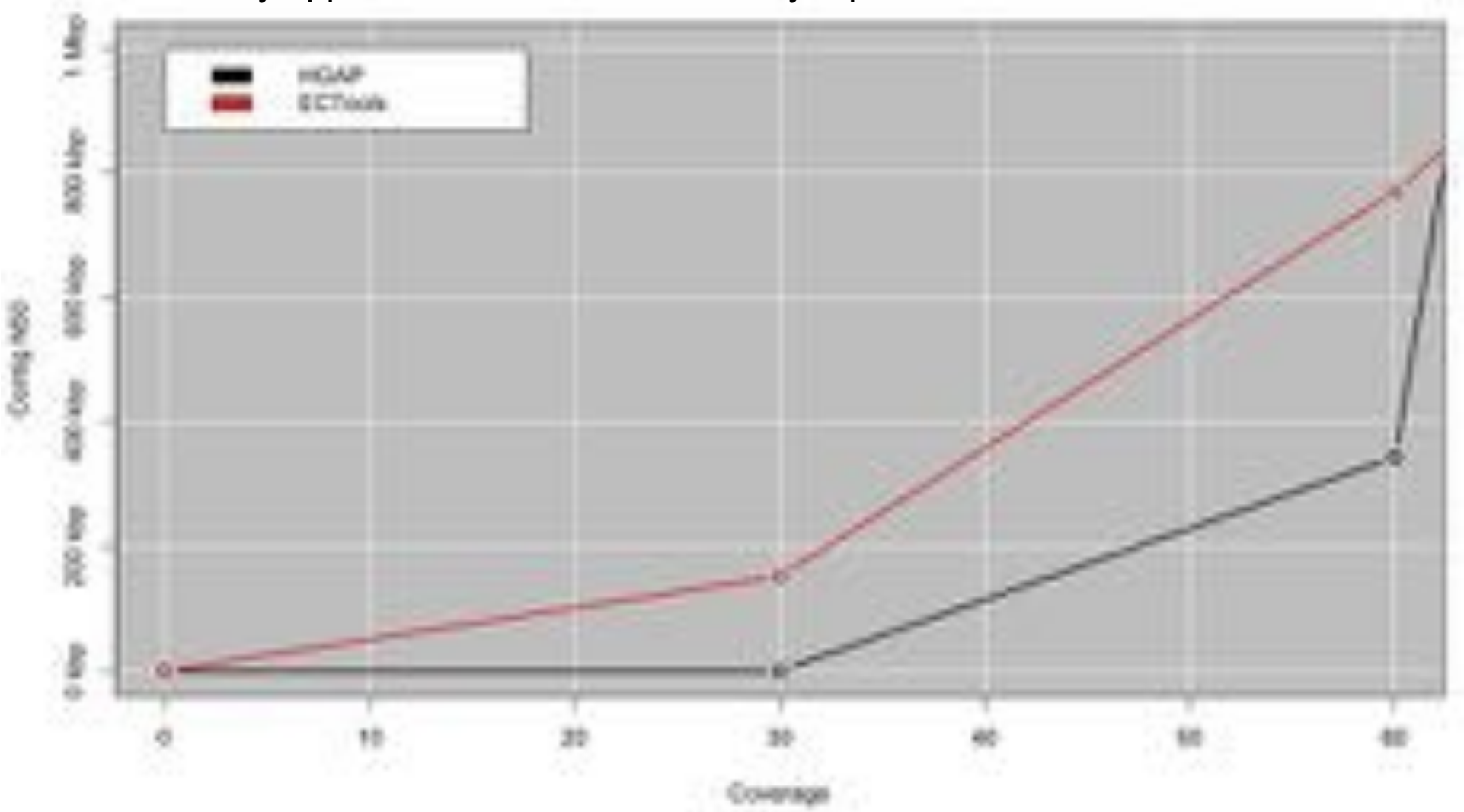
However, cannot overcome Illumina coverage gaps & other biases

A. thaliana Ler-0

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

Downsampling experiment

Accuracy approaches 100% but limited by repeats



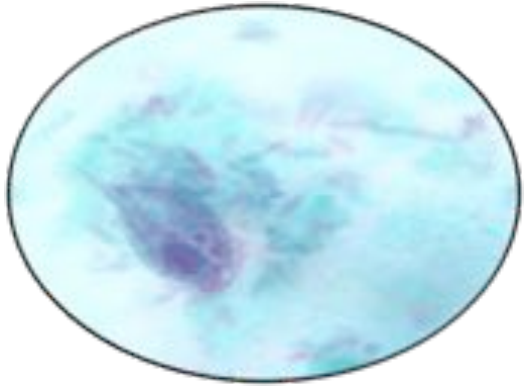
Current Collaborations

Indica & Aus Rice
McCombie/Ware/McCouch

Pinapple
UIUC

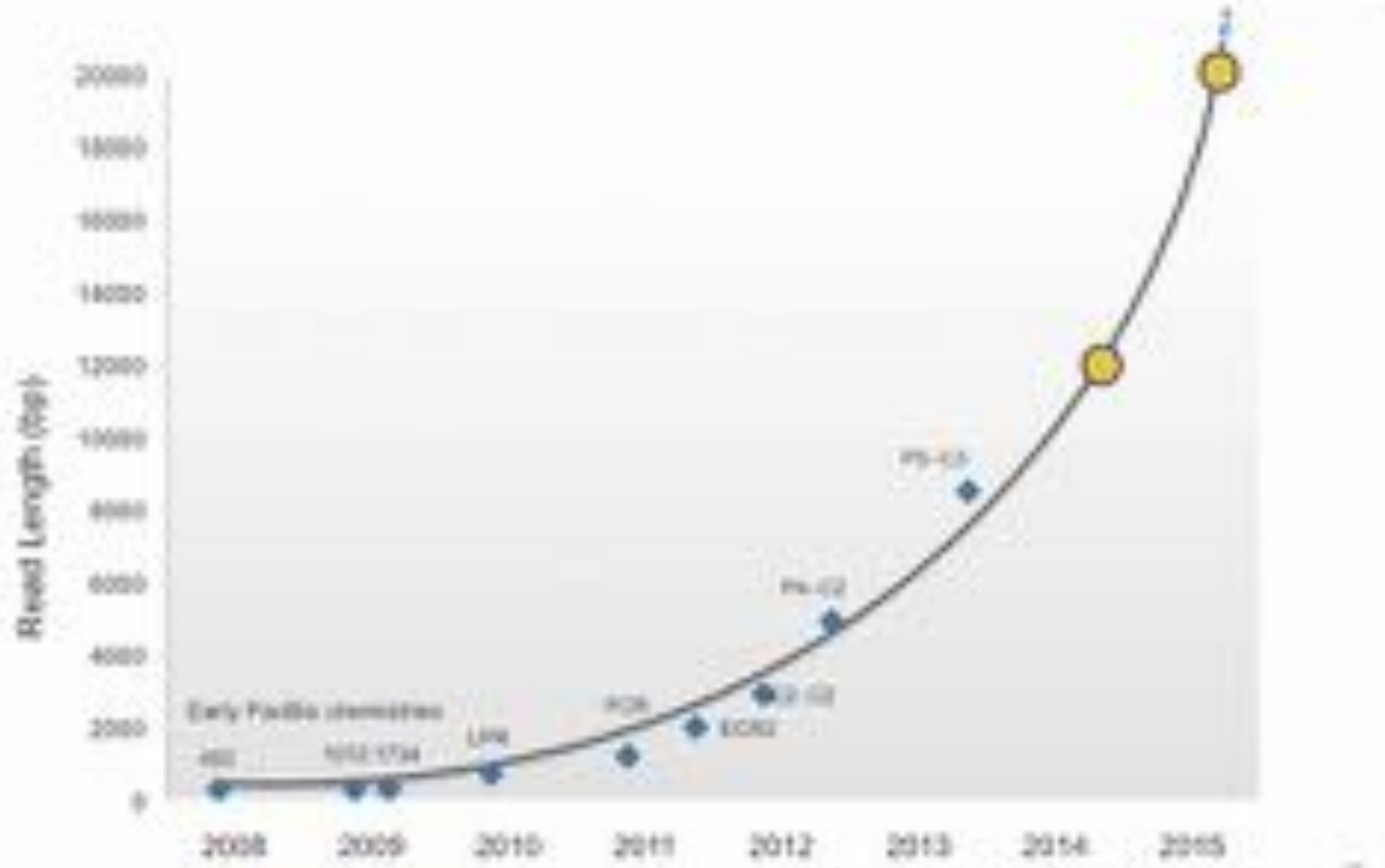
M. ligano
Hannon

Asian Sea Bass
Temasek Life Sciences Laboratory



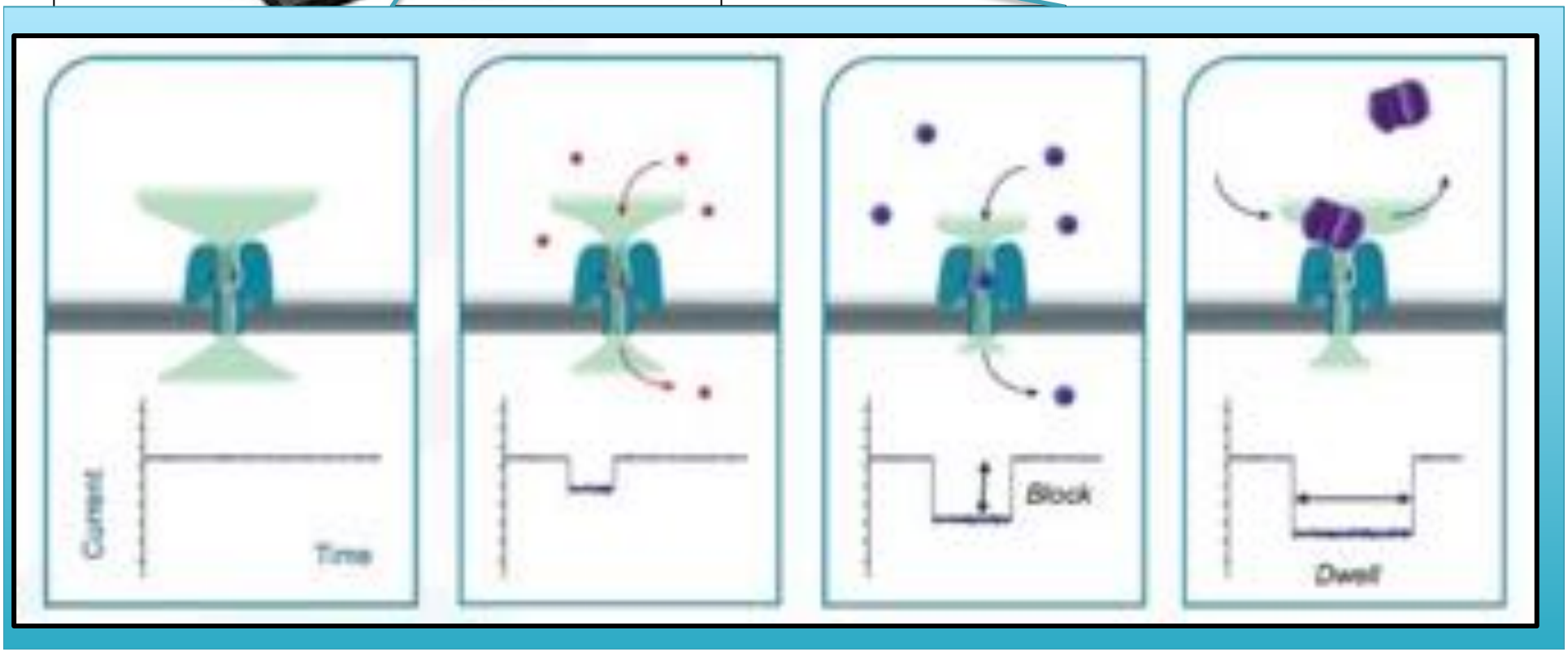
P. hominis
NYU

PacBio® Advances in Read Length

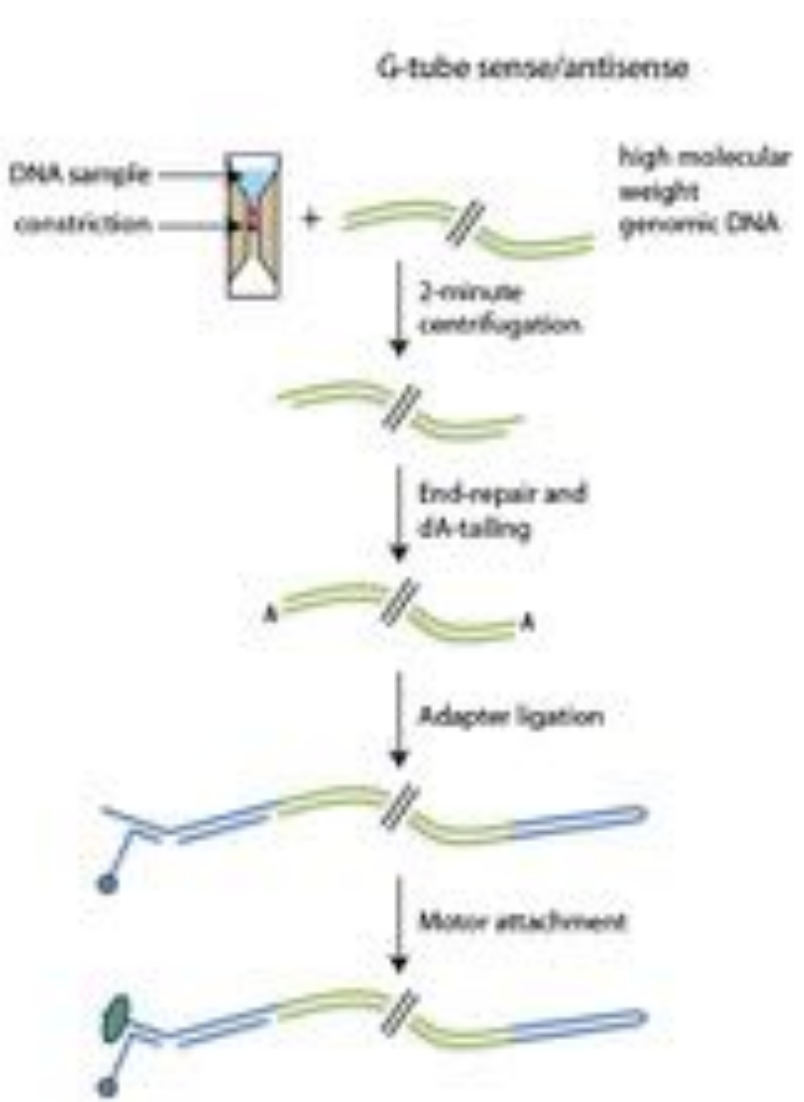


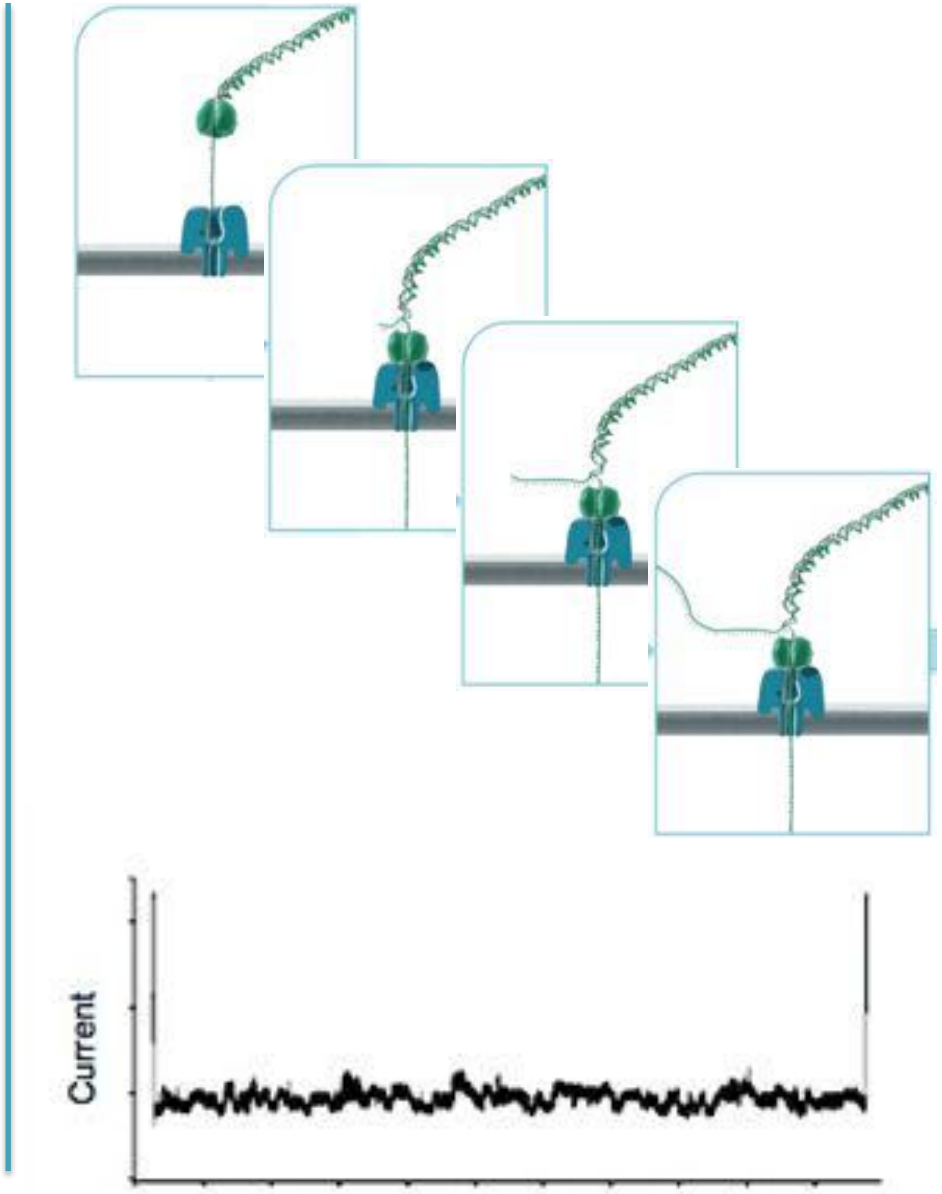
Oxford Nanopore MinION

- Thumb drive sized sequencer powered over USB
- Capacity for 512 reads at once
- Senses DNA by measuring changes to ion flow

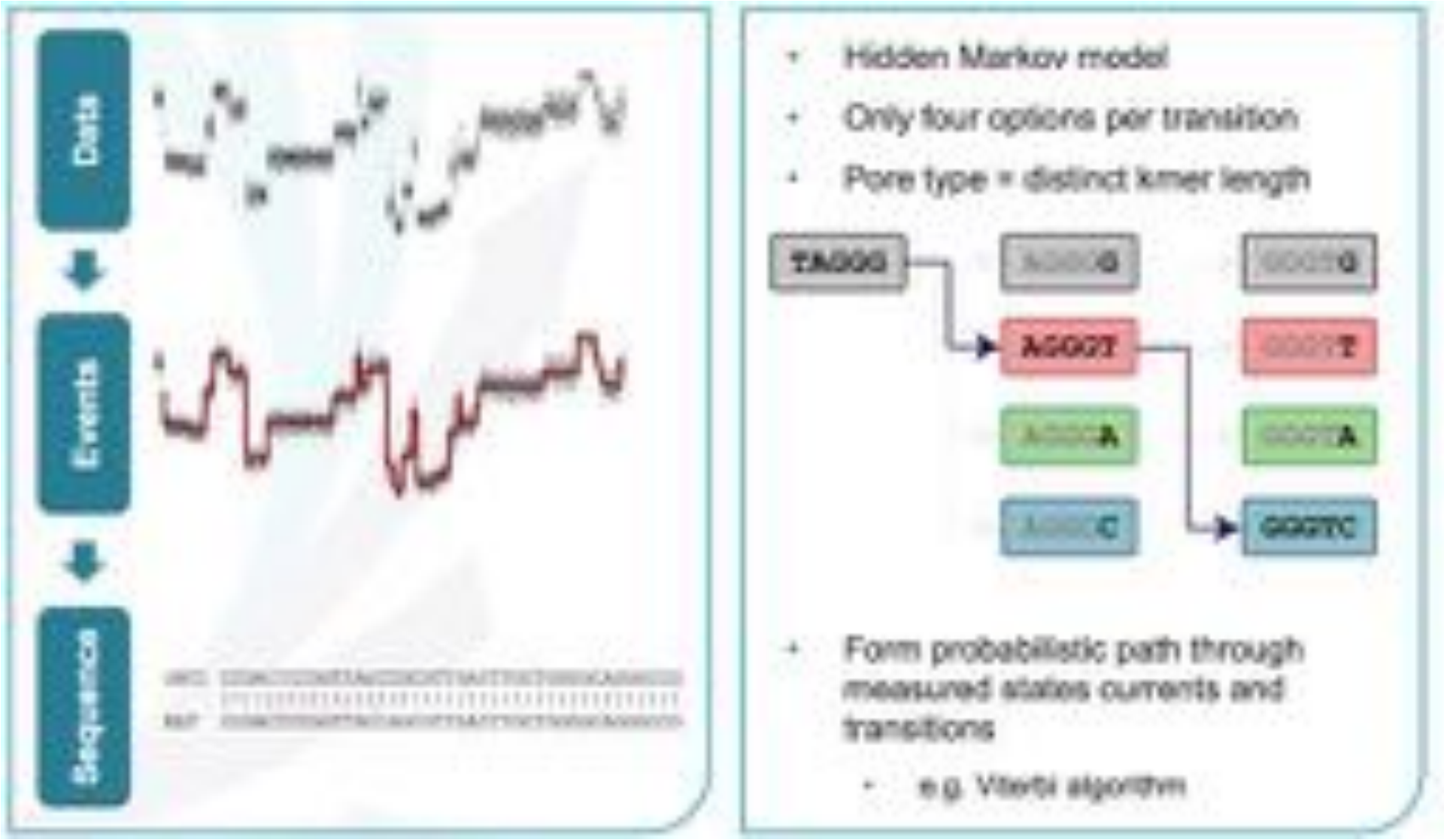


Nanopore Sequencing



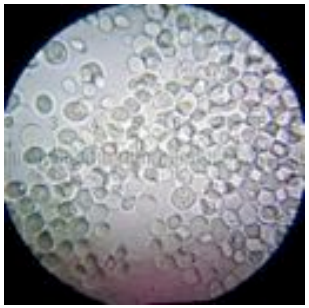


Nanopore Basecalling



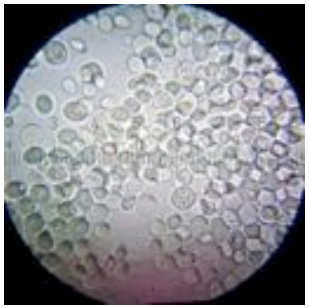
Basecalling currently performed at Amazon with frequent updates to algorithm

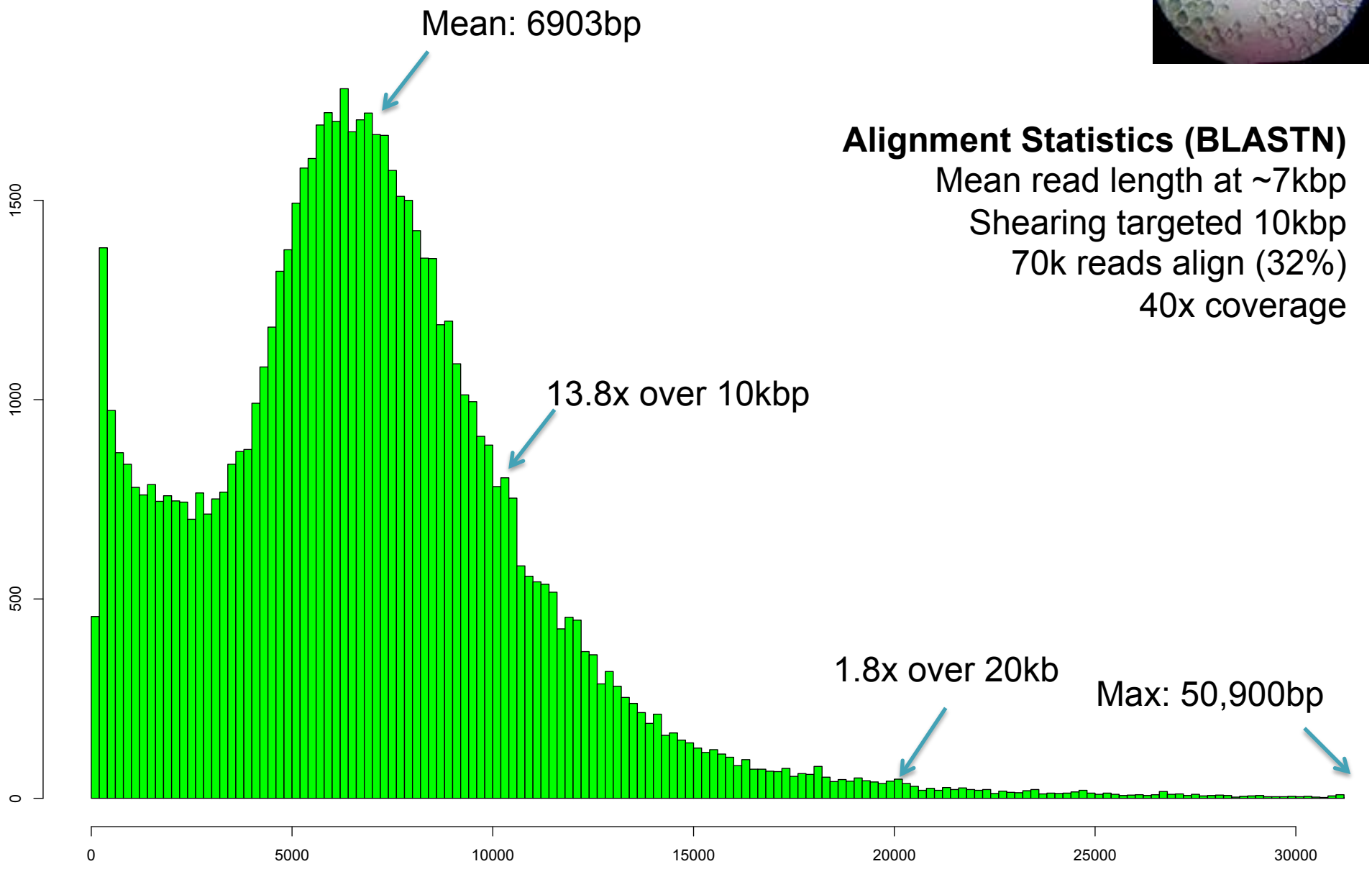
Nanopore Readlengths



Oxford Nanopore Sequencing at CSHL
30 runs, 267k reads, 122x total coverage
Between 11 and 73k reads per run!
Mean flow cell: 50 Mbp in 2 days
Max flow cell: 446Mbp in 2 days

Nanopore Alignments





Alignment Statistics (BLASTN)

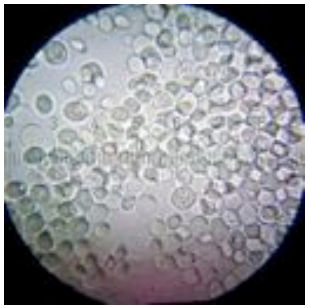
Mean read length at ~7kbp

Shearing targeted 10kbp

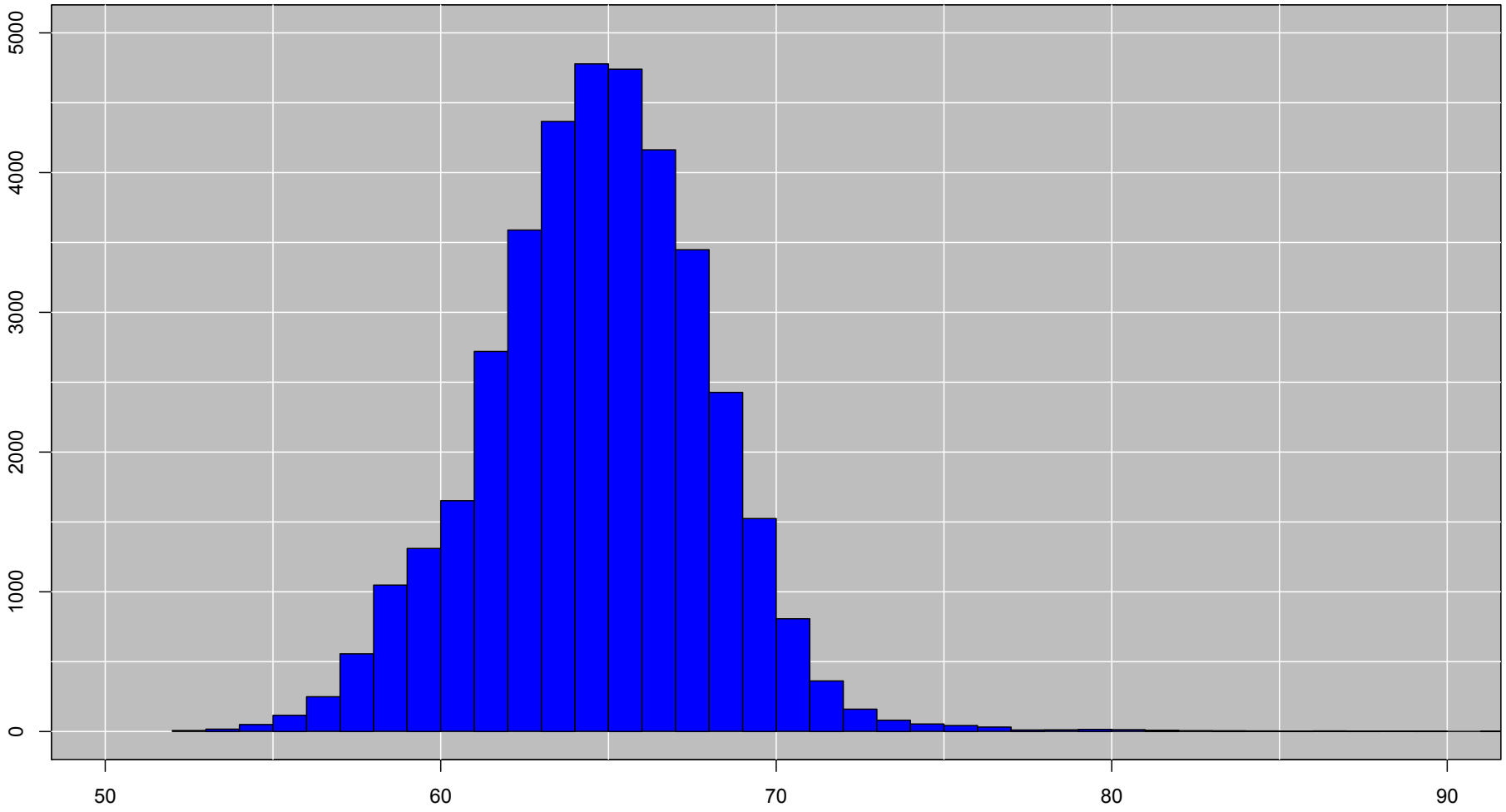
70k reads align (32%)

40x coverage

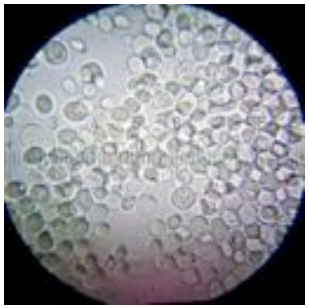
Nanopore Accuracy



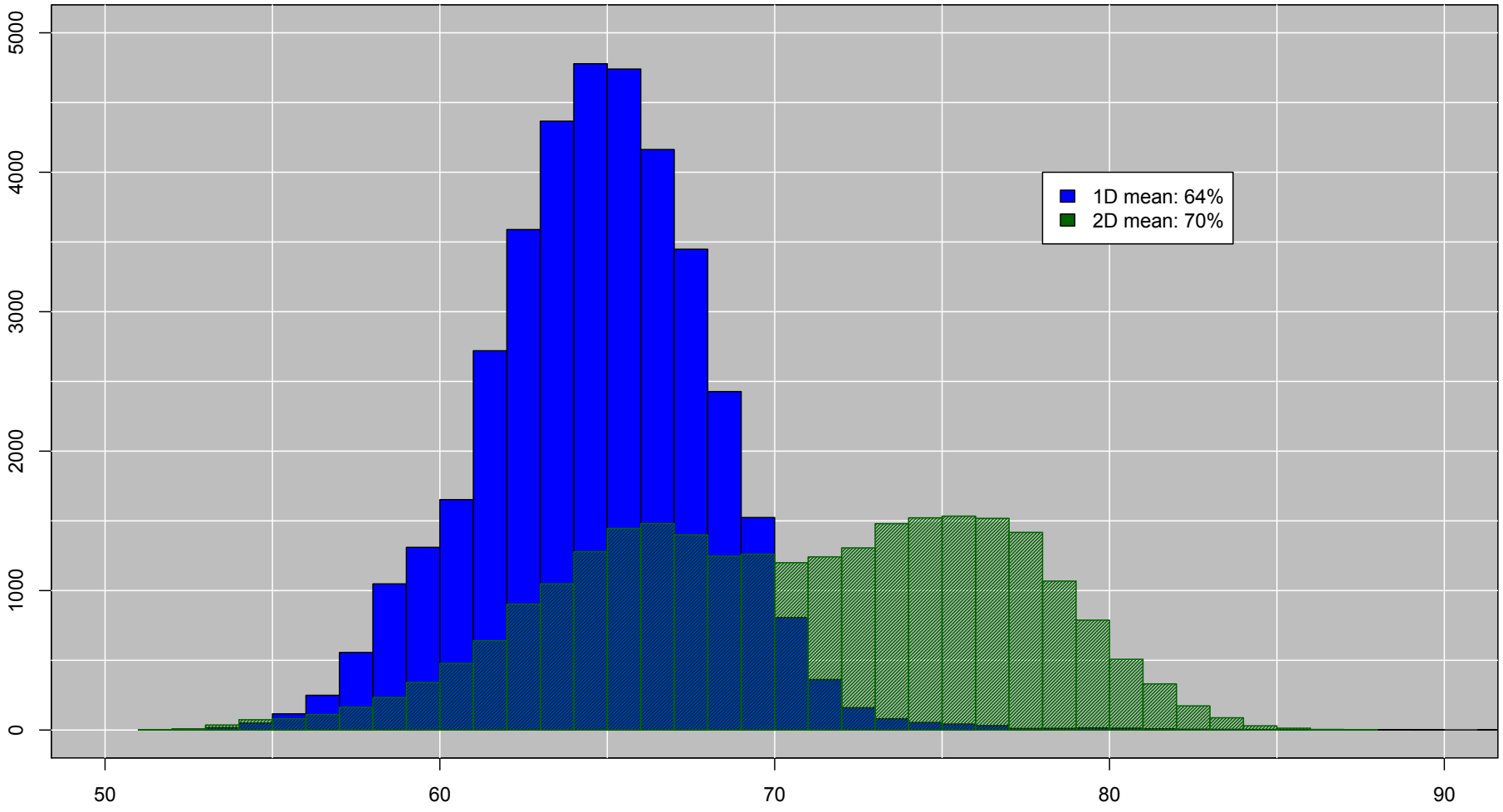
Alignment Quality (BLASTN)
Of reads that align, average ~64% identity



Nanopore Accuracy



Alignment Quality (BLASTN)
Of reads that align, average ~64% identity
“2D base-calling” improves to ~70% identity



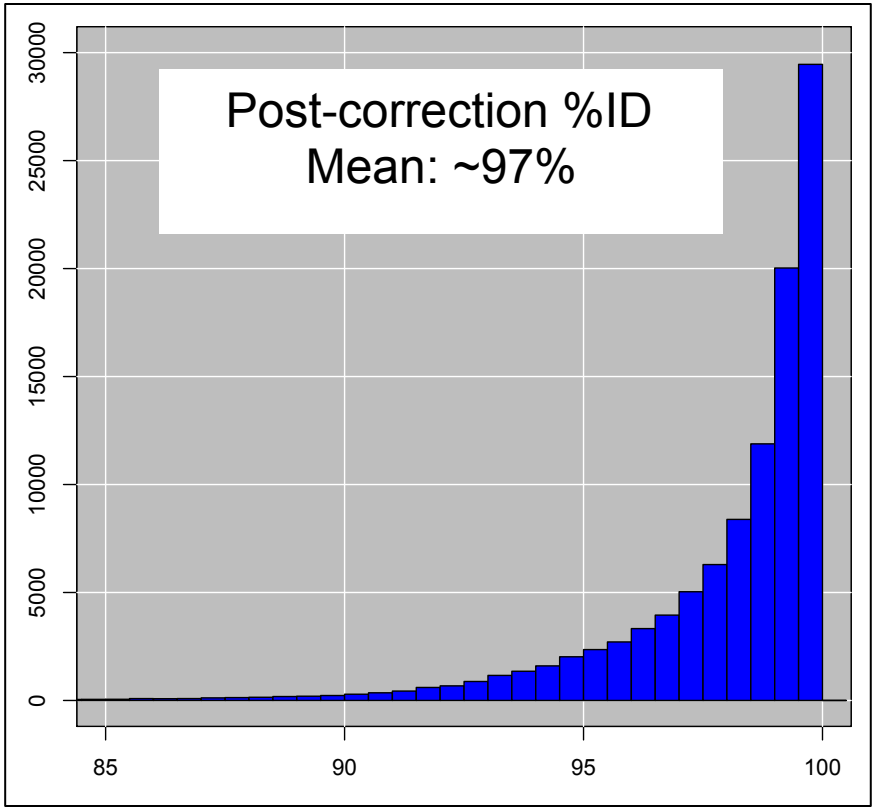
NanoCorr: Nanopore-Illumina Hybrid Error Correction

https://github.com/jgurtowski/nanocorr

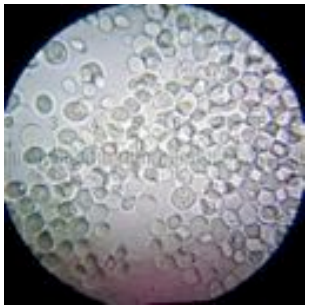
1. BLAST Miseq reads to all raw Oxford Nanopore reads

2. Select non-repetitive alignments
   - First pass scans to remove "contained" alignments
   - Second pass uses Dynamic Programming (LIS) to select set of high-identity alignments with minimal overlaps

3. Compute consensus of each Oxford Nanopore read
   - Currently using Pacbio's pbdagcon



Long Read Assembly



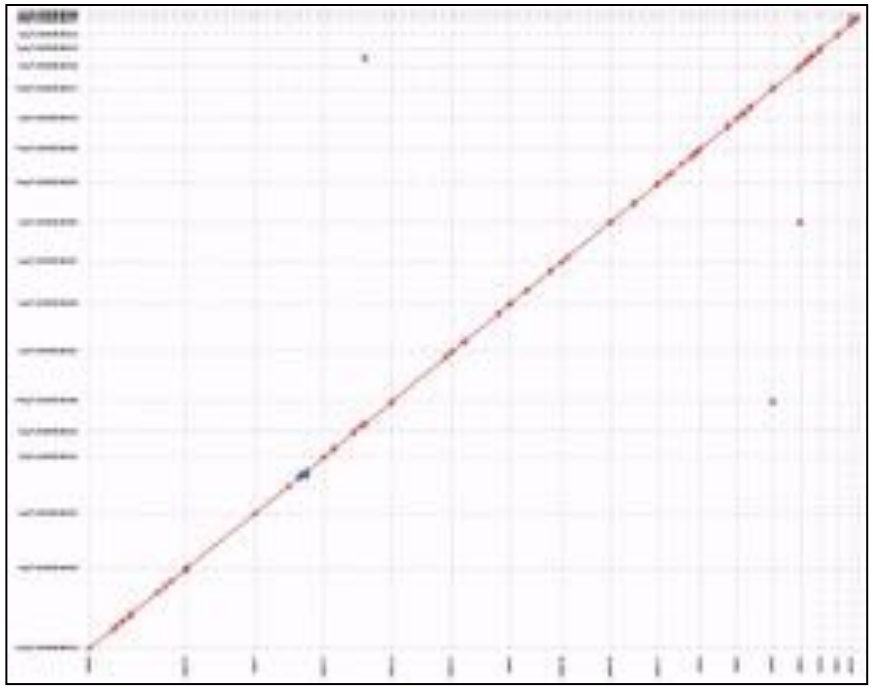
S288C Reference sequence

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

Pacific Biosciences

HGAP + Celera Assembler

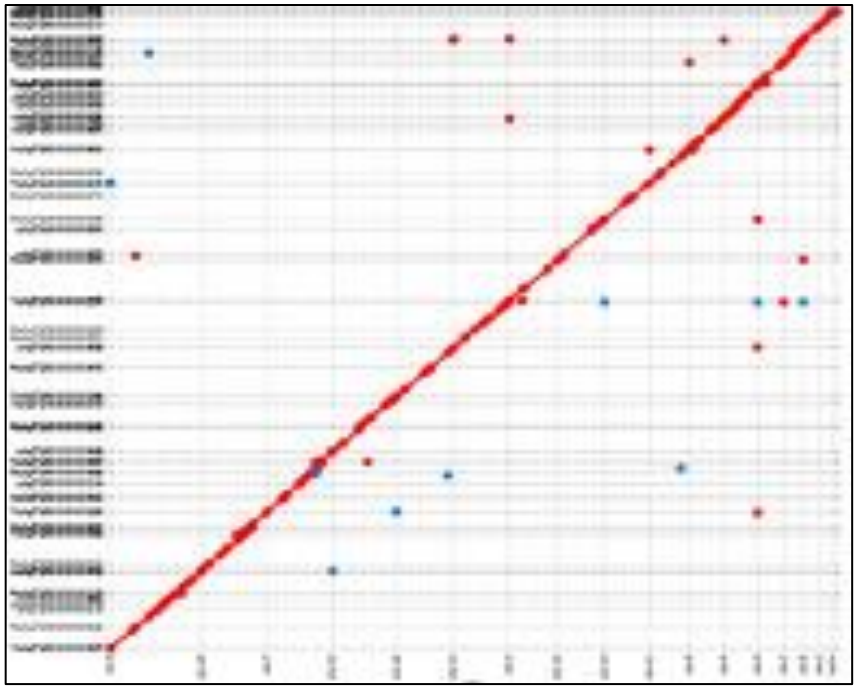
- 21 non-redundant contigs
- N50: 811kbp >99.8% id



Oxford Nanopore

NanoCorr + Celera Assembler

- 234 non-redundant contigs
- N50: 362kbp >99.78% id




Platform Technology:
Instruments for scaled nanopore analysis
MinION
GridION
Miniaturised USB device
One instrument:
Scalable, groupable, networked, real-time
Entry / Field / Small runs
Desktop
Mid range installation
Supercenter
NANOPORE

What should we expect from an assembly?

Analysis of dozens of genomes from across the tree of life with real and simulated data

Summary & Recommendations

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

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

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

> 5GB: Email mschatz@cshl.edu

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

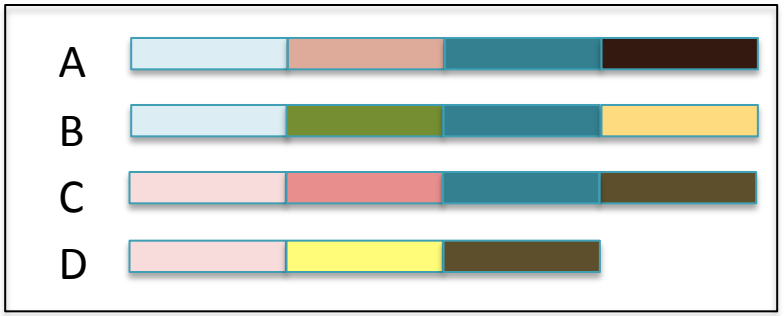
Genome Structure & Function

1. **Structure: Sequencing and Assembly**

   "A tale of two sequencers"

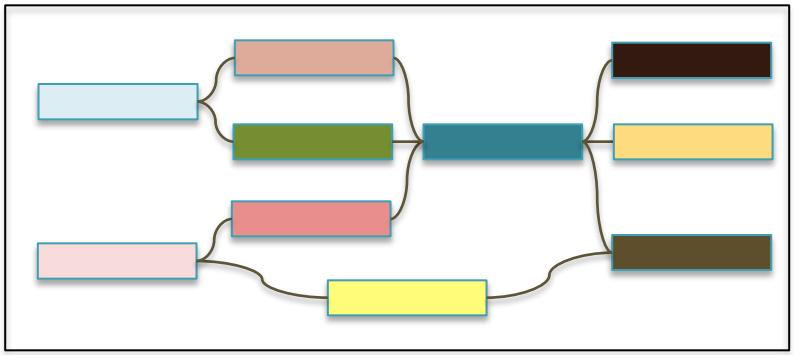
2. **Function: Disease Analytics**
   1. Pan-genome analysis
   2. The role of indels in human diseases

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

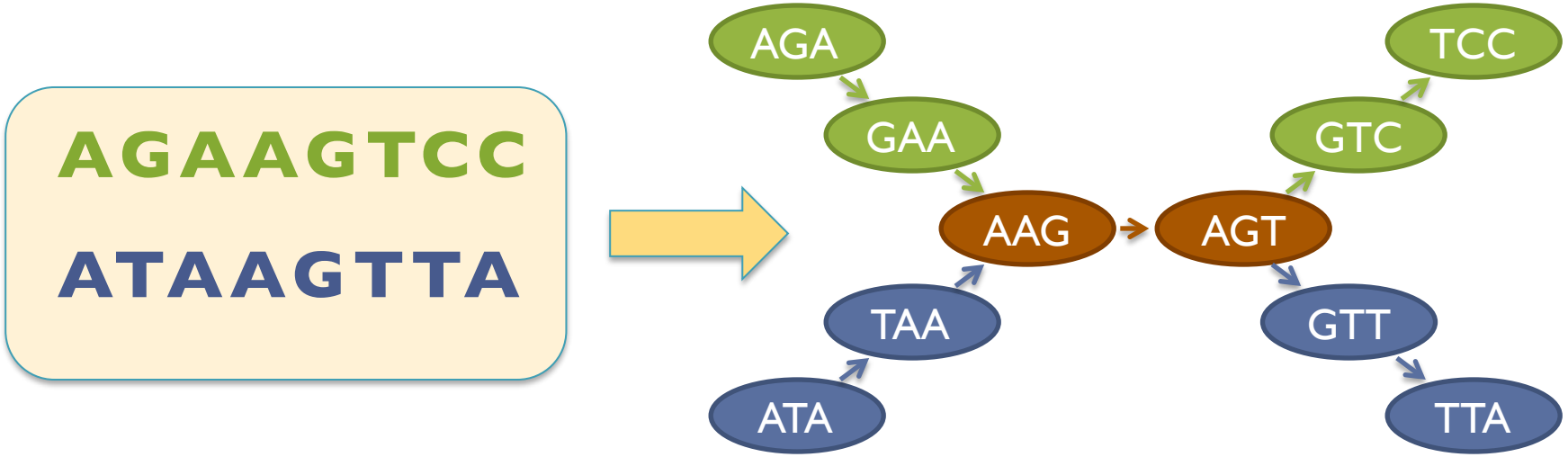
- Encodes all the sequence relationships between the genomes
- How well conserved is a given sequence?
- What are the pan-genome network properties?

SplitMEM: Graphical pan-genome analysis with suffix skips
Marcus, S, Lee, H, Schatz, MC
http://biorxiv.org/content/early/2014/04/06/003954

Graphical pan-genome analysis

Colored de Bruijn graph

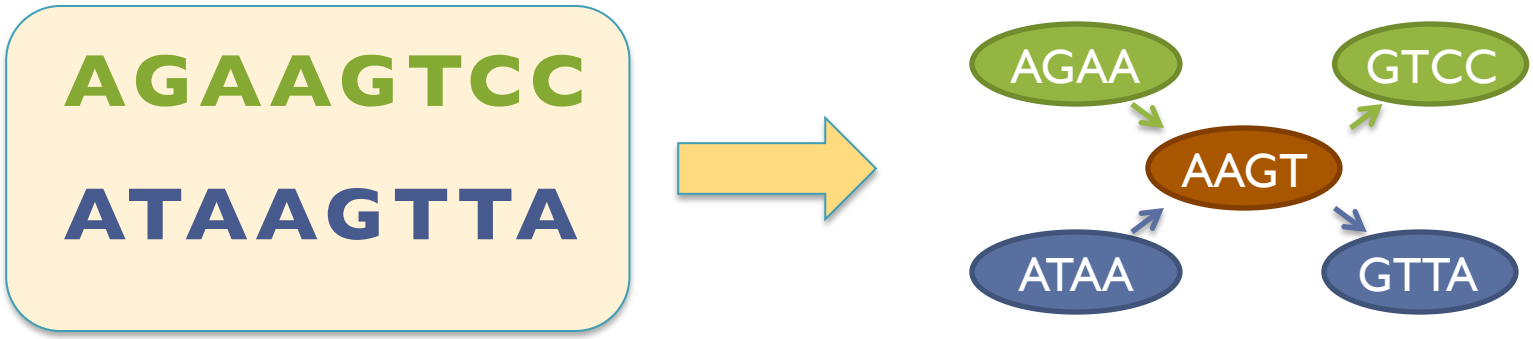
- Node for each distinct kmer
- Directed edge connects consecutive kmers
- Nodes overlap by k-1 bp



Graphical pan-genome analysis

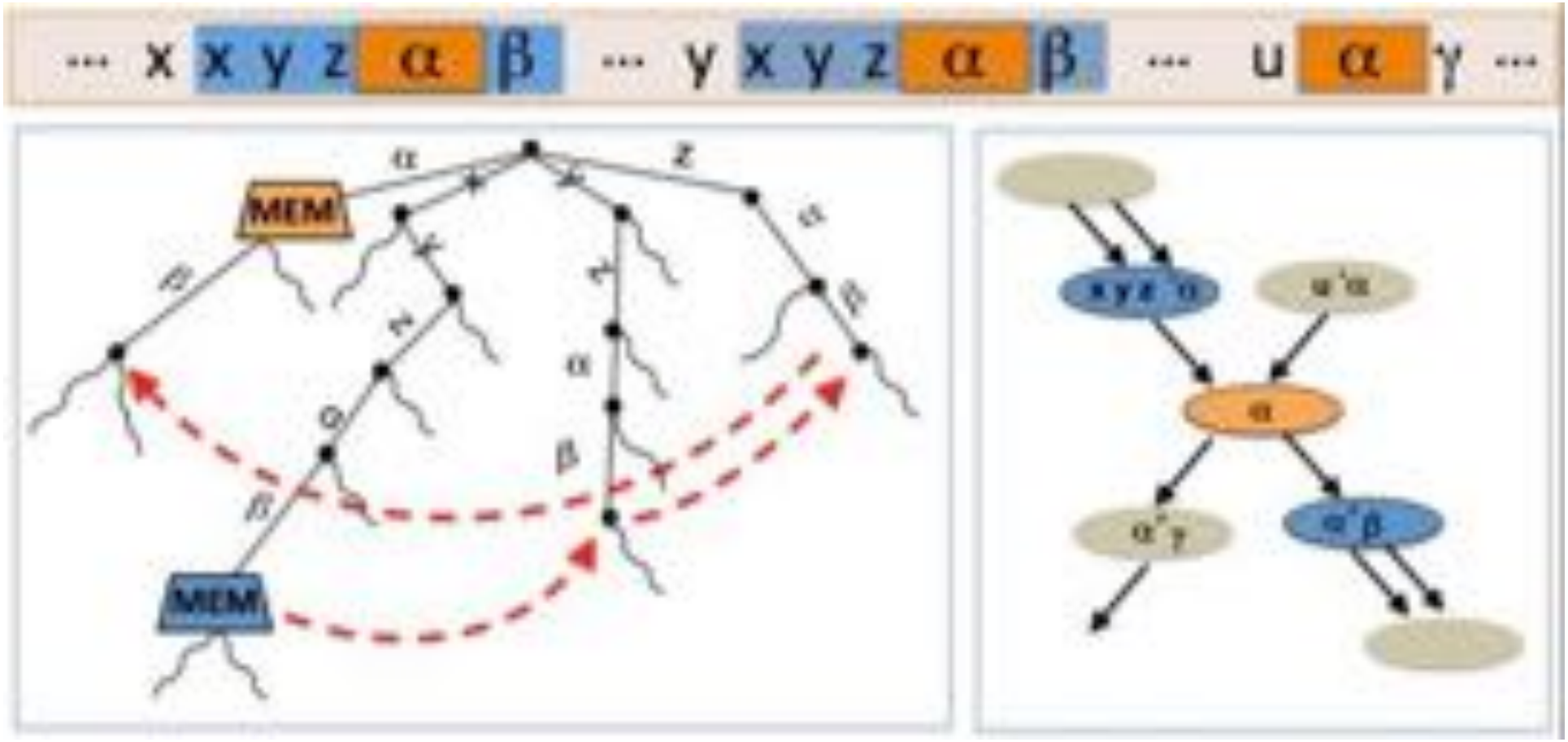
Colored de Bruijn graph

- Node for each distinct kmer
- Directed edge connects consecutive kmers
- Nodes overlap by k-1 bp



Other approaches all start from the raw de Bruijn graph, we aim to directly build the compressed graph as quickly as possible

Suffix Trees & de Bruijn Graphs



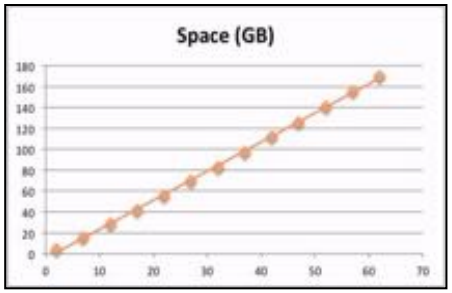
Key concepts:

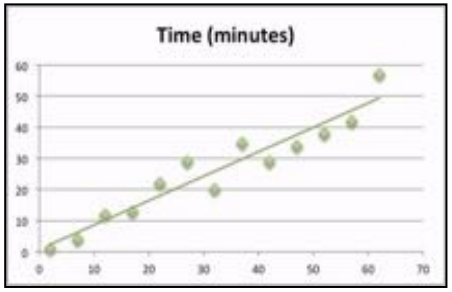
- Shared sequences form repeats called "maximal exact matches" (MEM)
- Easy to identify MEMs in a suffix tree, but may be nested within other MEMs
- Use "suffix skips" to quickly decompose MEMs, add in the missing nodes and edges

Microbial Pan-Genomes

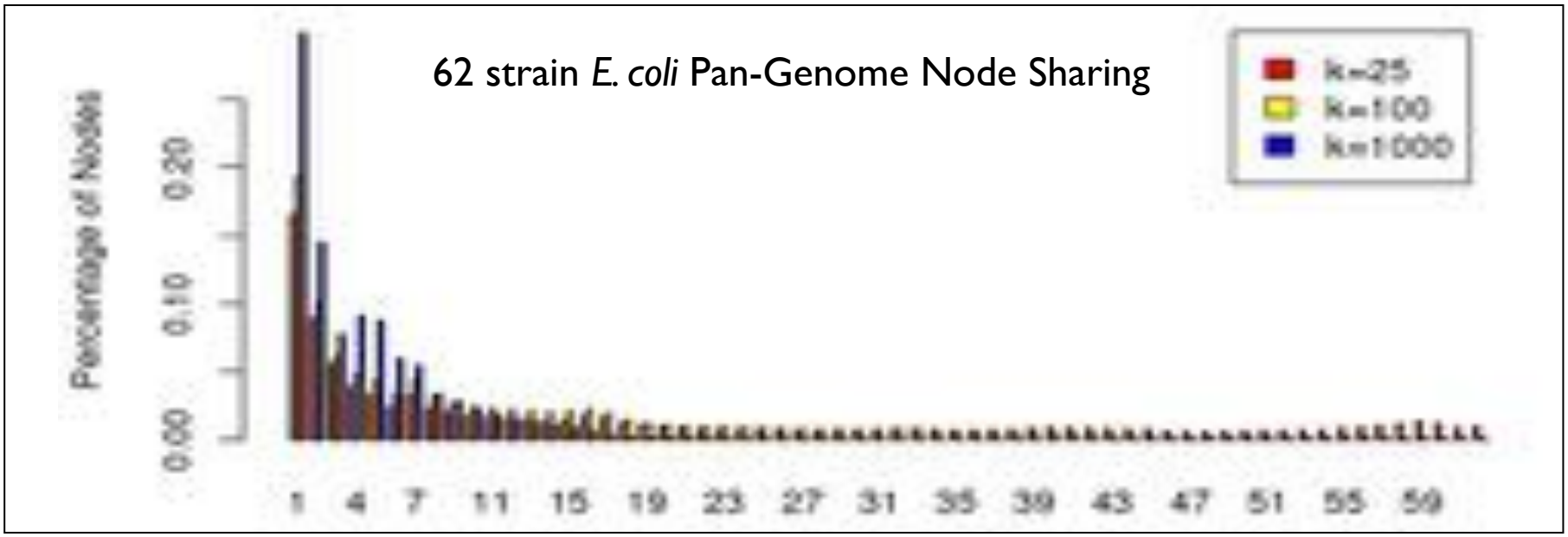
E. coli (62) and B. anthracis (9) pan-genome analysis

- Analyzed all available strains in Genbank
- Space and time are linear in the number of genomes
  - O(n log g) where g is the length of the longest genome





- Many possible applications:
  - Identifying "core" genes present in all strains
  - Characterizing highly variable regions
  - Cataloging sequences shared by pathogenic varieties



Searching for the genetics behind human disorders and plant phenotypes

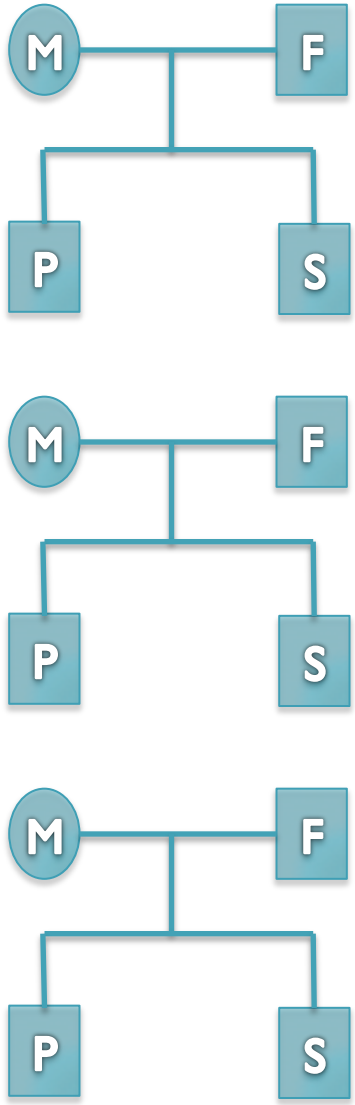
Search Strategy

- Currently uses WGS or WES short read resequencing for economic reasons

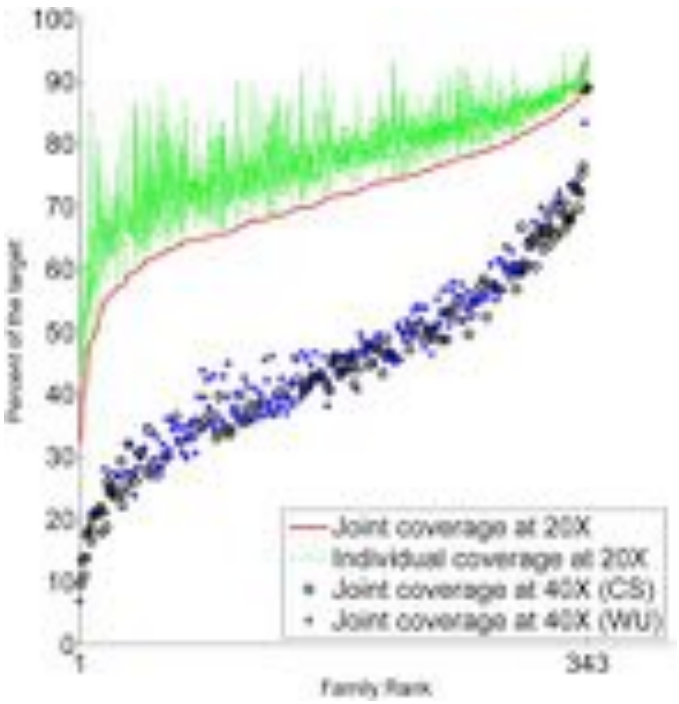
- Collaborate with Lyon, McCombie, Tuveson, and Wigler labs to examine the genetic basis of cancer, ASD, and other psychiatric disorders

- Also collaborating with the Lippman, Ware, and Gingeras labs to study high value crops

Are there any genetic variants present in affected individuals, that are not present or are present at a substantially reduced rate in their relatives?



Exome sequencing of the SSC



The year 2012 was an exciting year for autism genetics

- 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
- ***All used poor or no tools for indels:***
  - Iossifov (343 families) and O'Roak (50 families) used GATK UnifiedGenotype
  - Sanders (200 families) didn't attempt

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

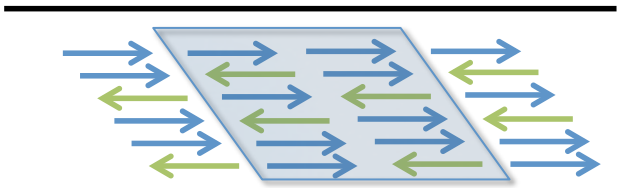
1. Combine mapping and assembly
2. Exhaustive search of haplotypes
3. De novo mutations

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

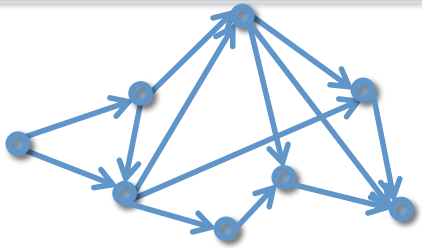
Accurate de novo and transmitted indel detection in exome-capture data using microassembly.
Narzisi, G, O'Rawe, JA, Iossifov, I, Fang, H, Lee, YH, Wang, Z, Wu, Y, Lyon, G, Wigler, M, Schatz MC (2014) *Nature Methods*. doi:10.1038/nmeth.3069

Scalpel Algorithm

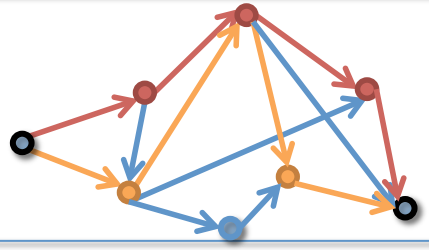
Extract reads mapping within the exon including (1) well-mapped reads, (2) soft-clipped reads, and (3) anchored pairs



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

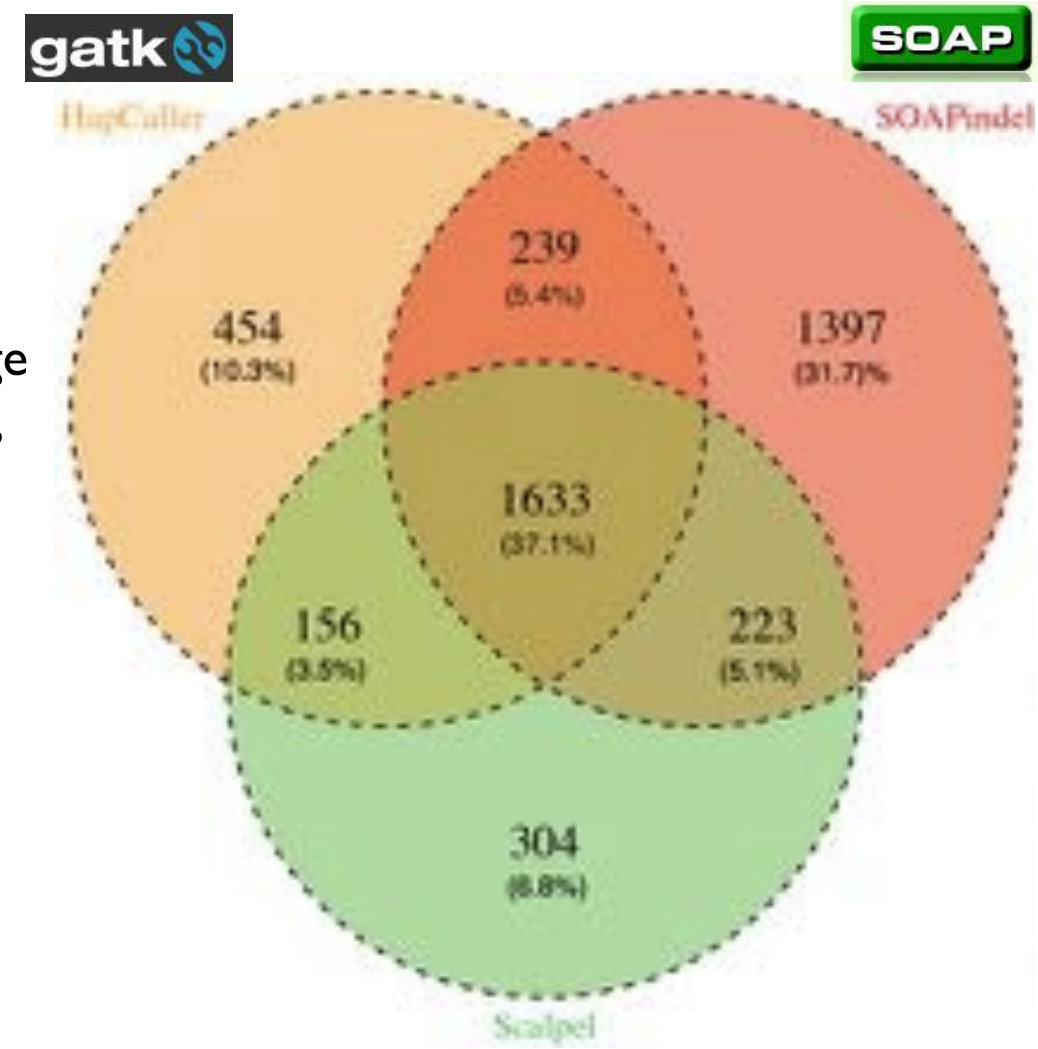
Experimental Analysis & Validation

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

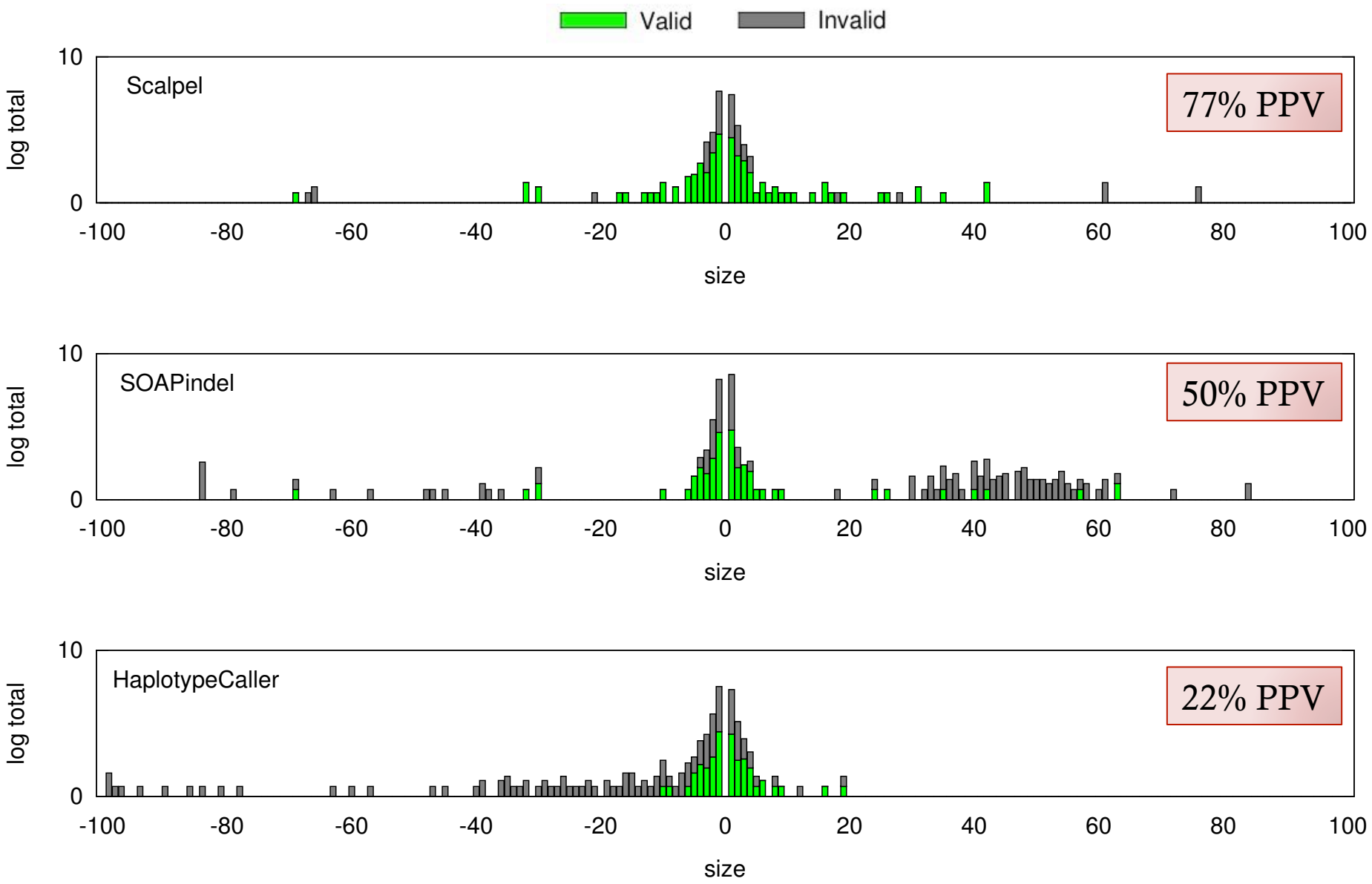
1000 indels selected for validation

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

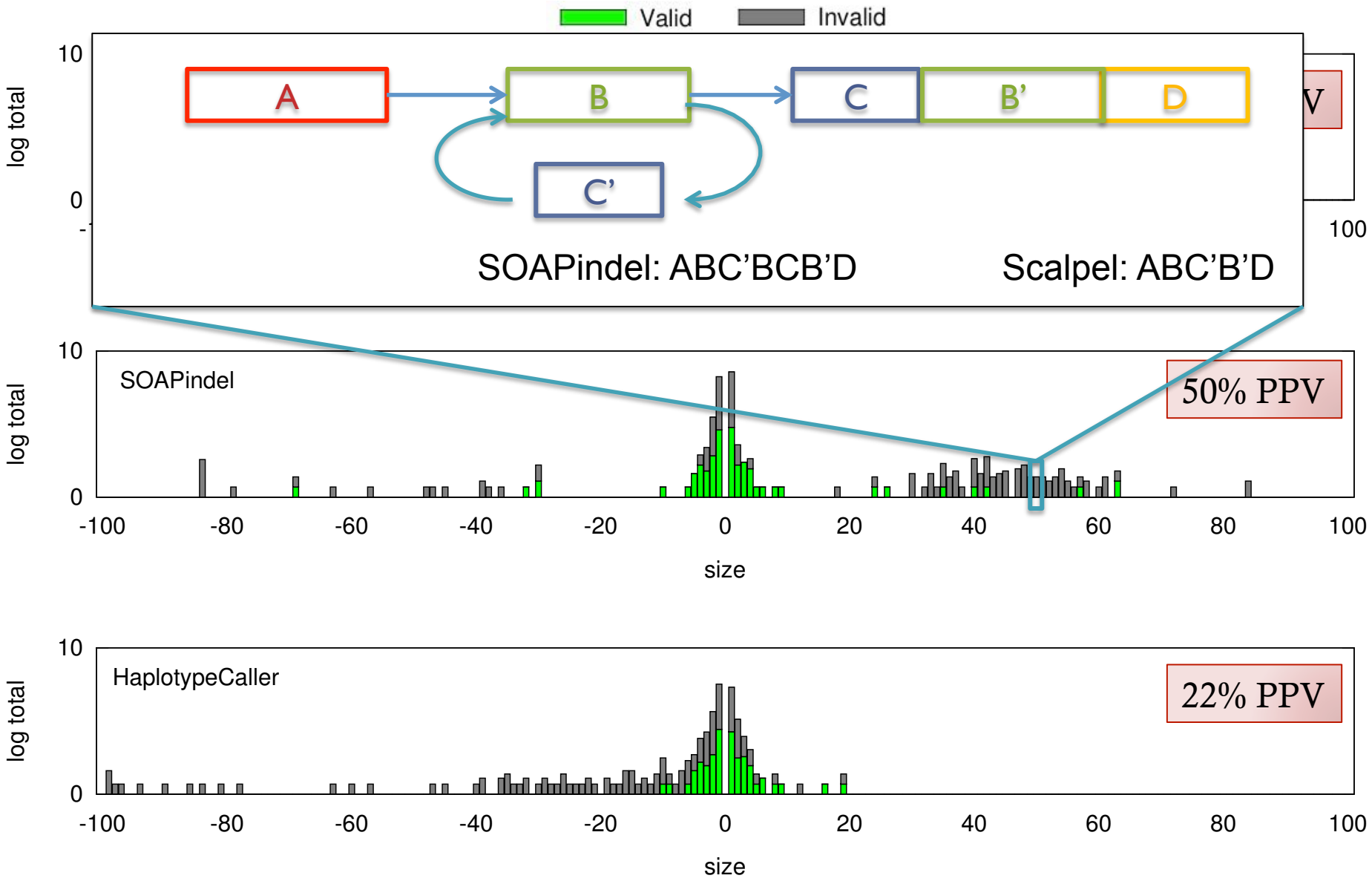


CSH

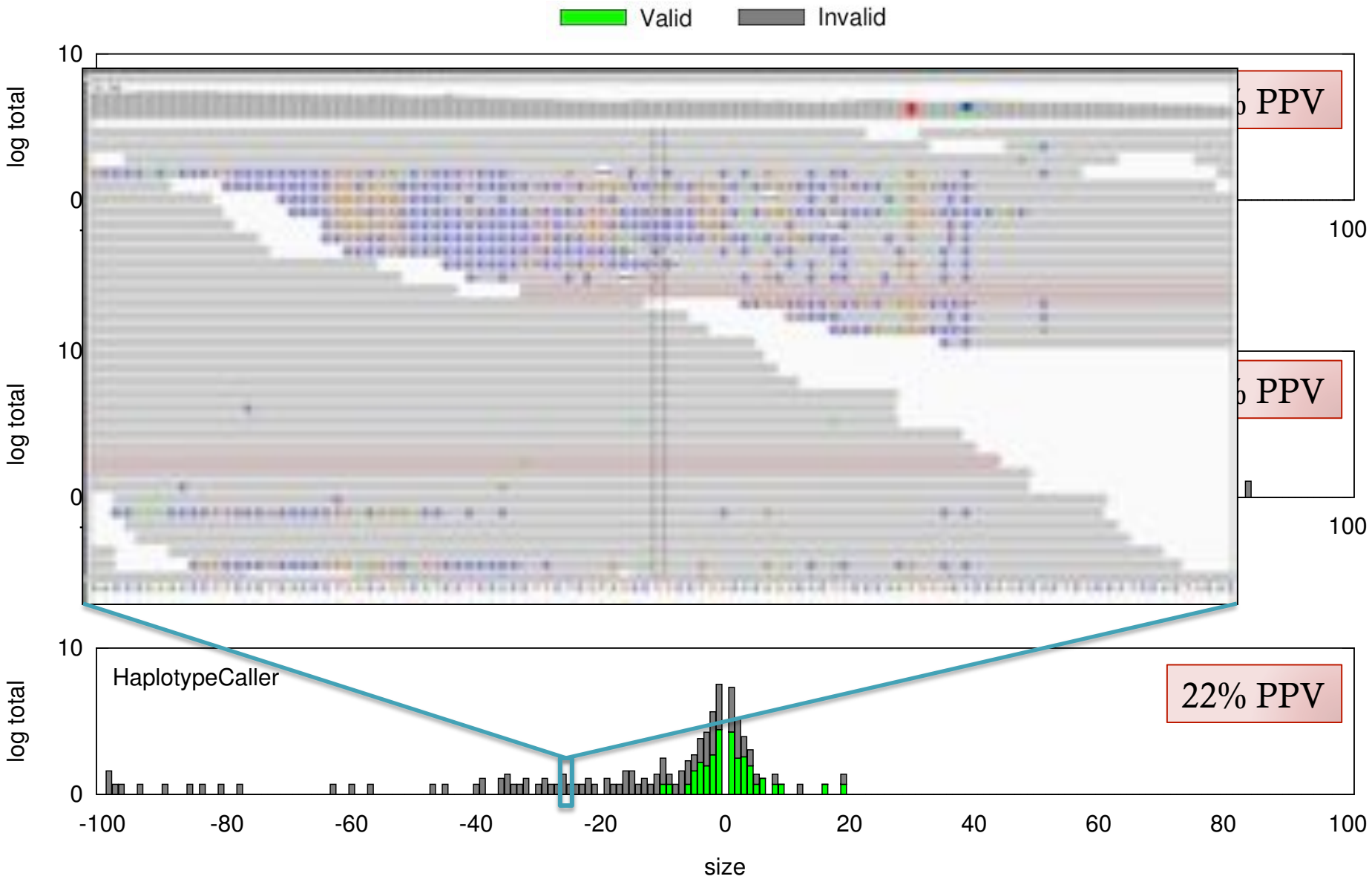
Scalpel Indel Validation



Scalpel Indel Validation



Scalpel Indel Validation



Refined indel analysis

Examine sources of indel errors

- Experimental validation of indels called from 30x whole genome vs. 110x whole exome of the same sample
- Most of the errors due to short microsatellite errors introduced during exome capture, also misses most long indels
- Recommend WGS for indel analysis instead

	All INDELs	Valid	PPV	INDELs >5bp	Valid (>5bp)	PPV (>5bp)
Intersection	160	152	95.0%	18	18	100%
WGS	145	122	84.1%	33	25	75.8%
WES	161	91	56.5%	1	1	100%

Reducing INDEL calling errors in whole-genome and exome sequencing data
Fang, H, Wu, Y, Narzisi, G, O'Rawe, JA, Jimenez Barrón LT, Rosenbaum, J, Ronemus, M, Iossifov I, Schatz, MC§, Lyon, GL§
http://www.biorxiv.org/content/early/2014/06/10/006148

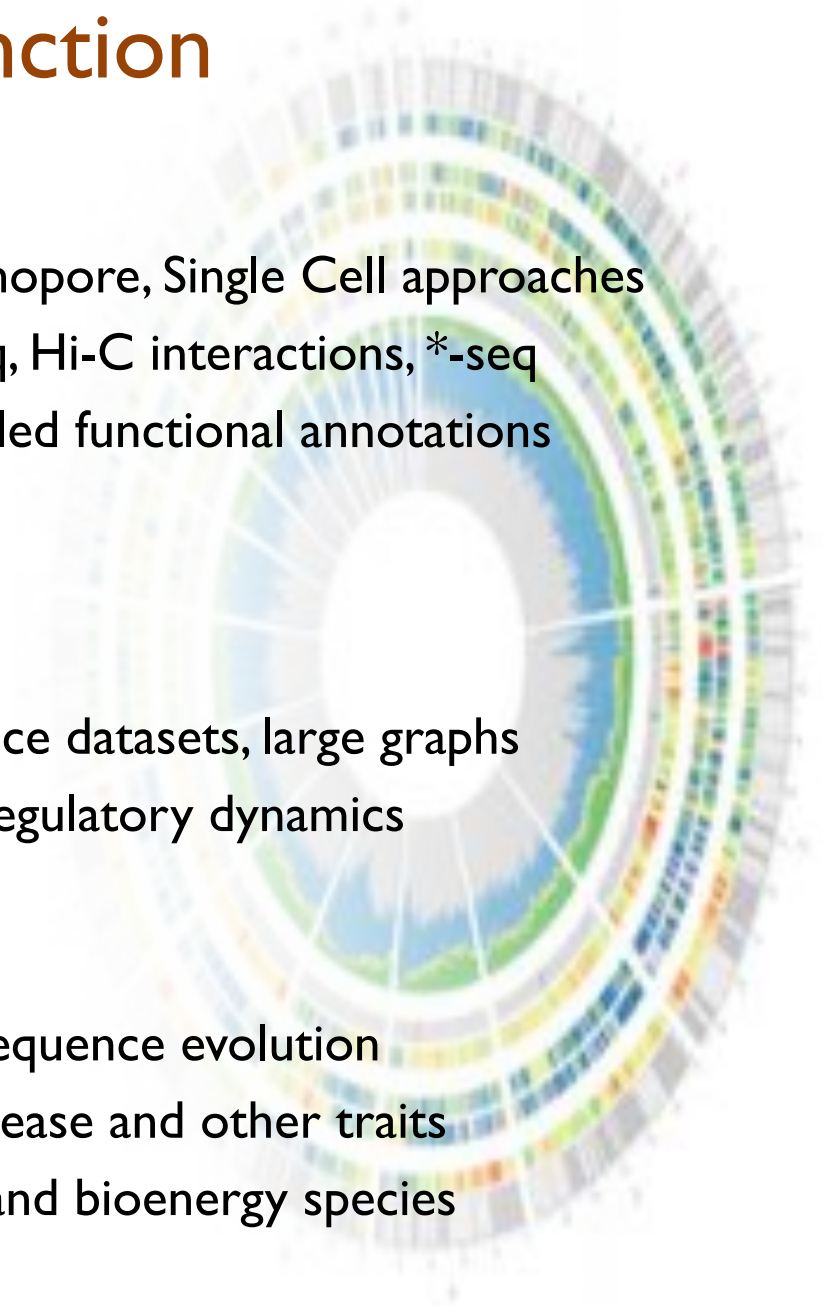
De novo Genetics of Autism

- In 593 family quads so far, we see significant enrichment in de novo ***likely gene disruptions (LGDs)*** in the autistic kids
  - Overall rate basically 1:1
  - 2:1 enrichment in frameshift indels (35:16)
- Confirmed trends observed in previous studies, contributed dozens of new autism candidate genes.
  - 8 out of 35 indel LGDs in autistic children overlapped with the 842 FMRP-associated genes
  - Trends further confirmed in larger study over the entire collection that is currently under review

Accurate de novo and transmitted indel detection in exome-capture data using microassembly.
Narzisi et al. (2014) *Nature Methods* doi:10.1038/nmeth.3069

The burden of de novo coding mutations in autism spectrum disorders.
Iossifov *et al* (2014) *Under review.*

Understanding Genome Structure & Function



Biotechnology

- Sequencing: Illumina, PacBio, Oxford Nanopore, Single Cell approaches
- Biochemical assays: RNA-seq, Methyl-seq, Hi-C interactions, *-seq
- More accurate sequencing & 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

Schatz Lab

Rahul Amin
Tyler Gavin
James Gurtowski
Han Fang
Hayan Lee
Maria Nattestad
Aspyn Palatnick
Srividya Ramakrishnan

Eric Biggers
Ke Jiang
Shoshana Marcus
Giuseppe Narzisi
Rachel Sherman
Greg Vurture
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

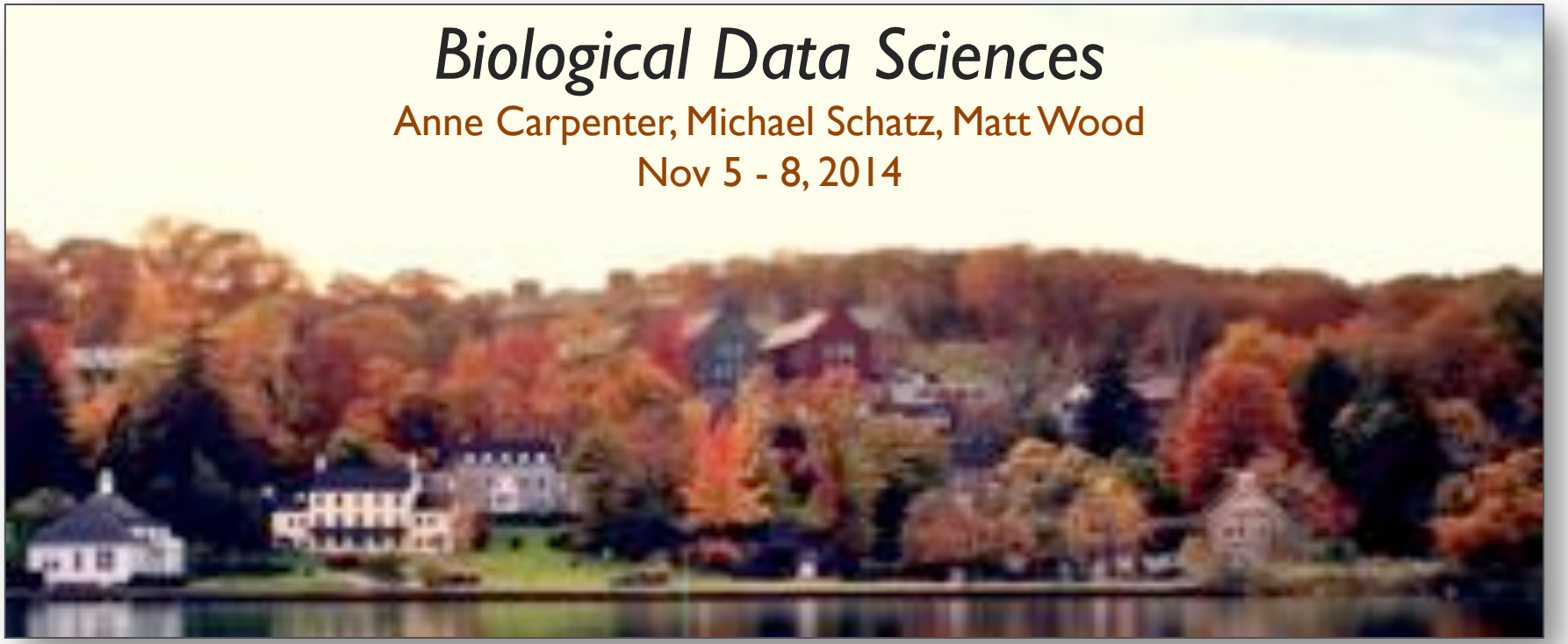
Pacific Biosciences
Oxford Nanopore

NSF

National Human Genome Research Institute

U.S. DEPARTMENT OF ENERGY

SFARI
SIMONS FOUNDATION AUTISM RESEARCH INITIATIVE



Thank you

http://schatzlab.cshl.edu

@mike_schatz