Hybrid Error Correction and De Novo Assembly with Oxford Nanopore

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PAG Bioinformatics

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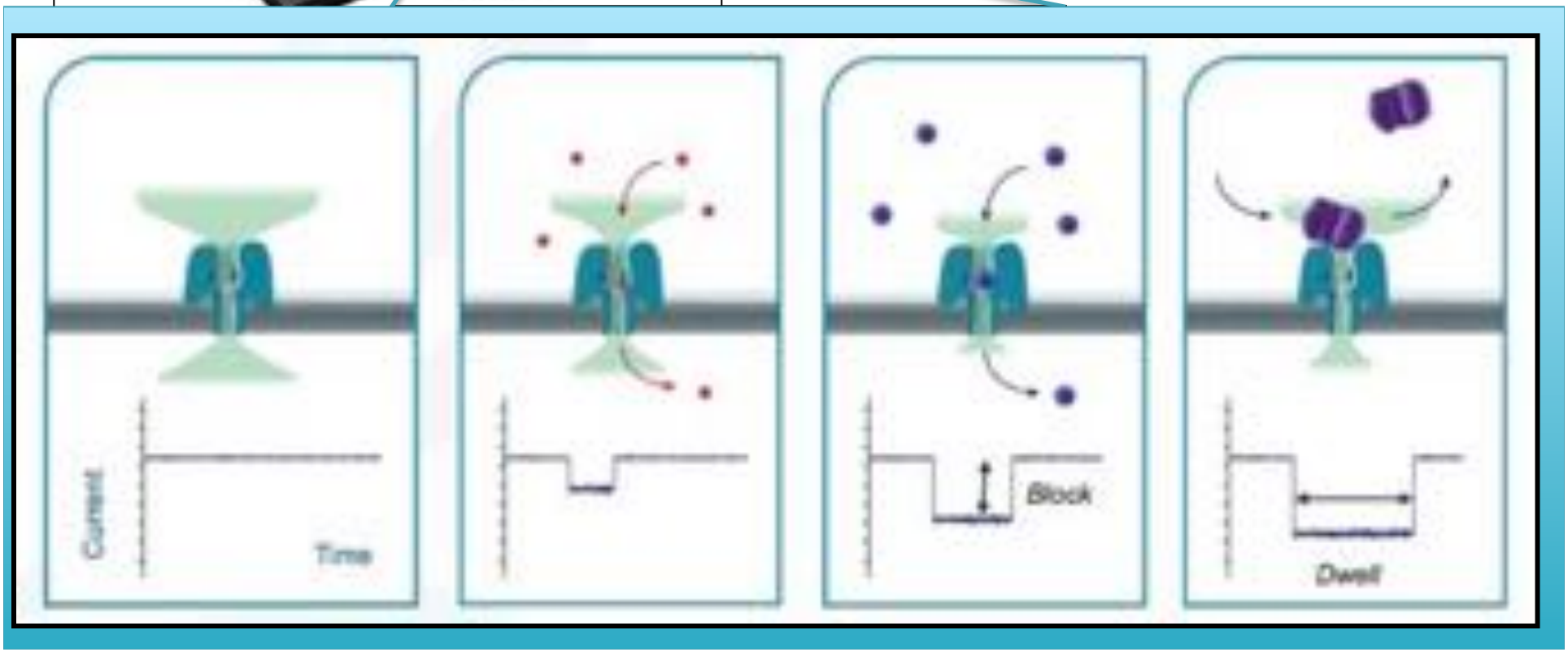
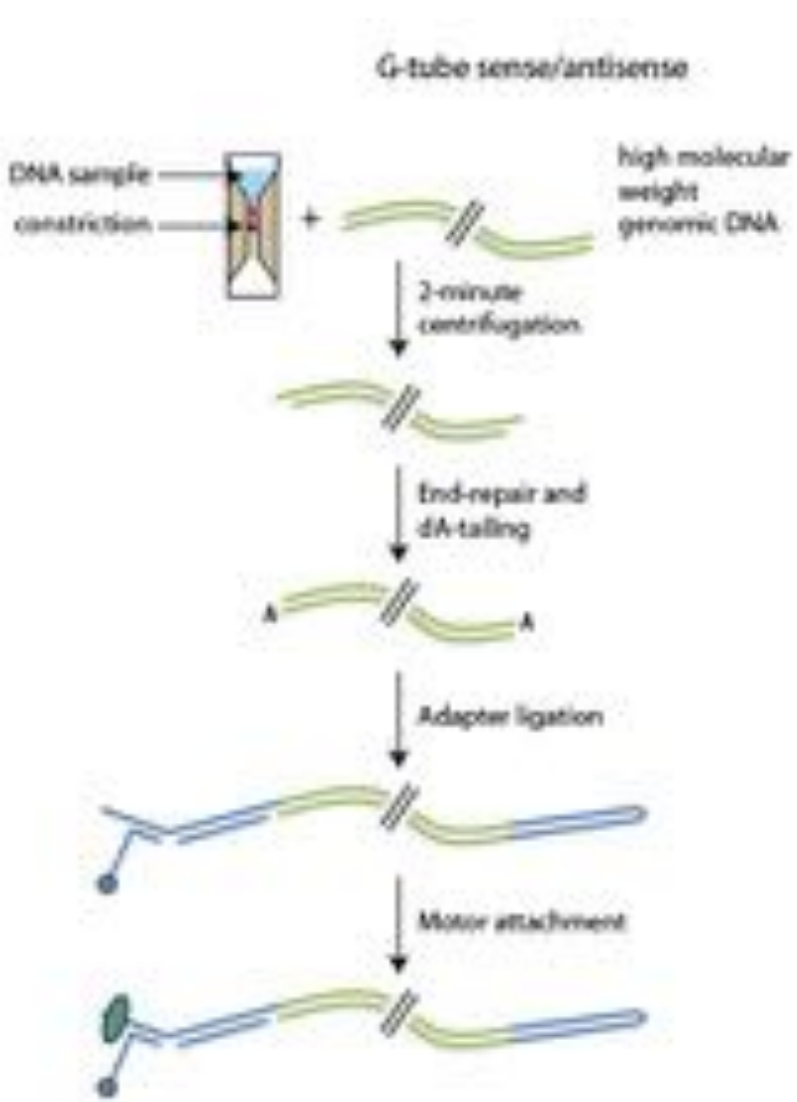
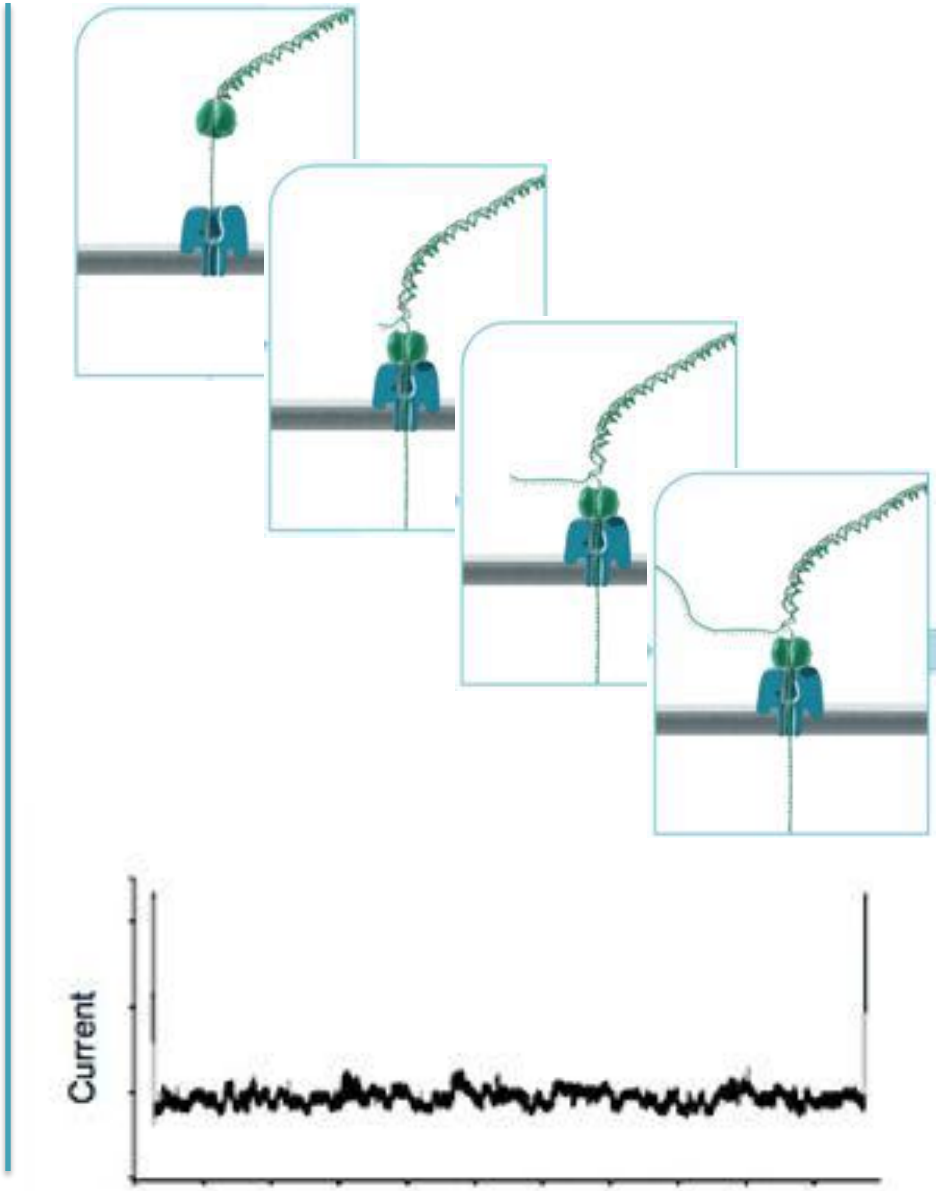
Oxford Nanopore MinION

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

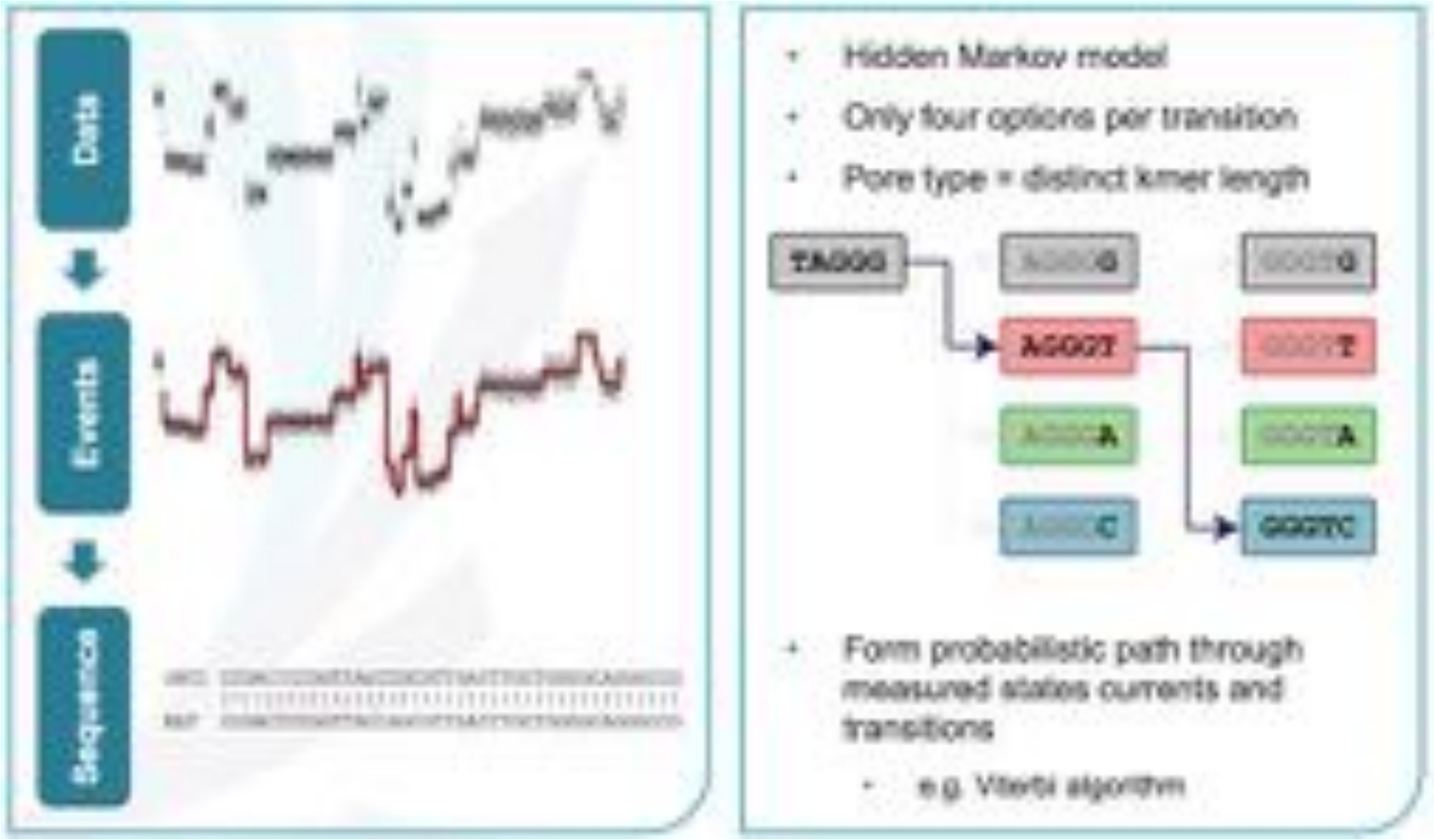


Nanopore Sequencing



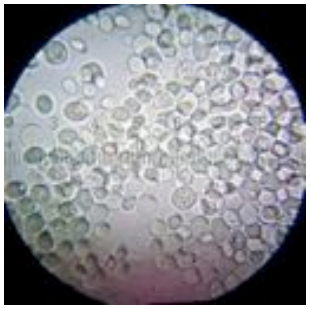


Nanopore Basecalling



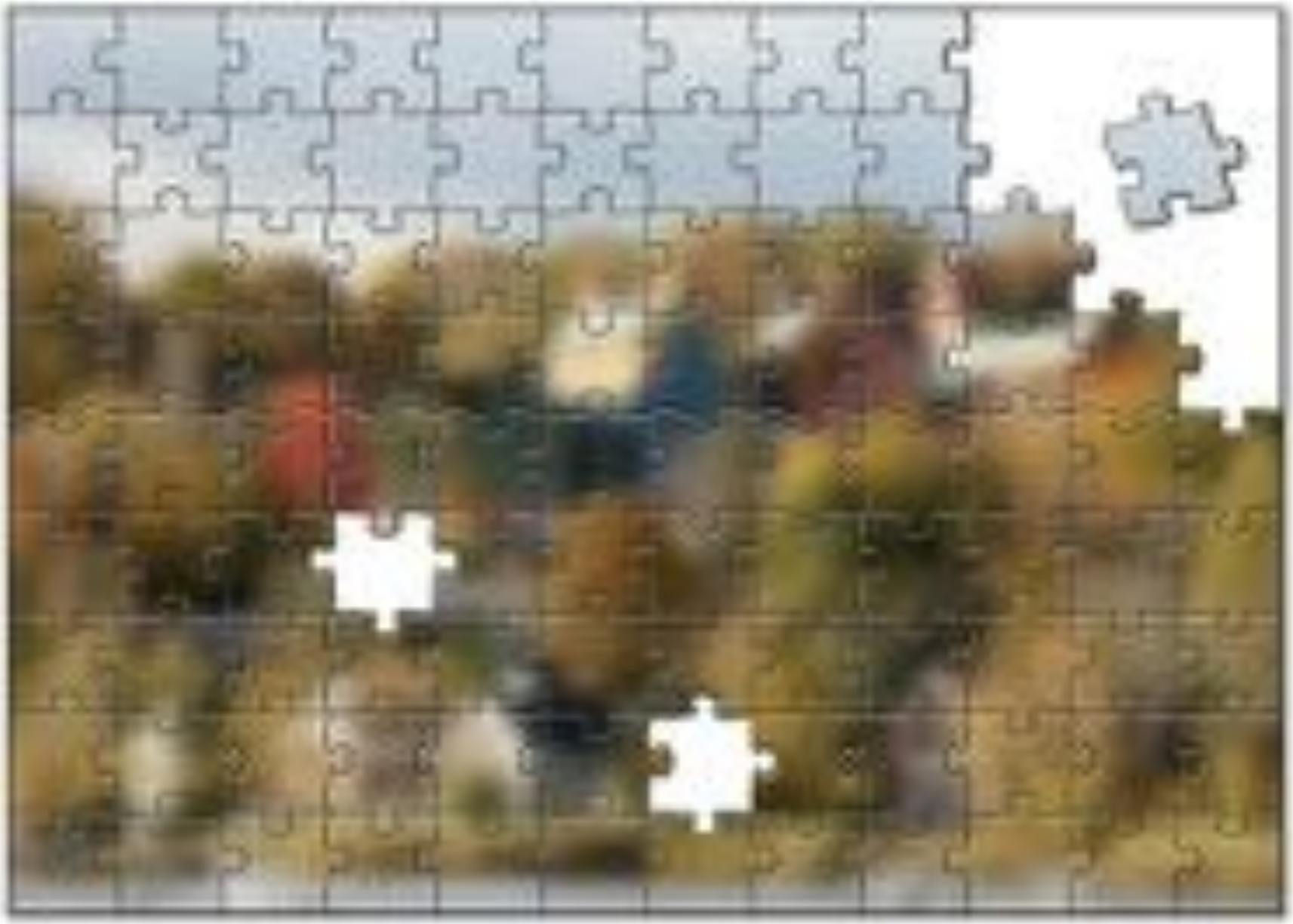
Basecalling currently performed at Amazon with frequent updates to algorithm

Nanopore Readlengths

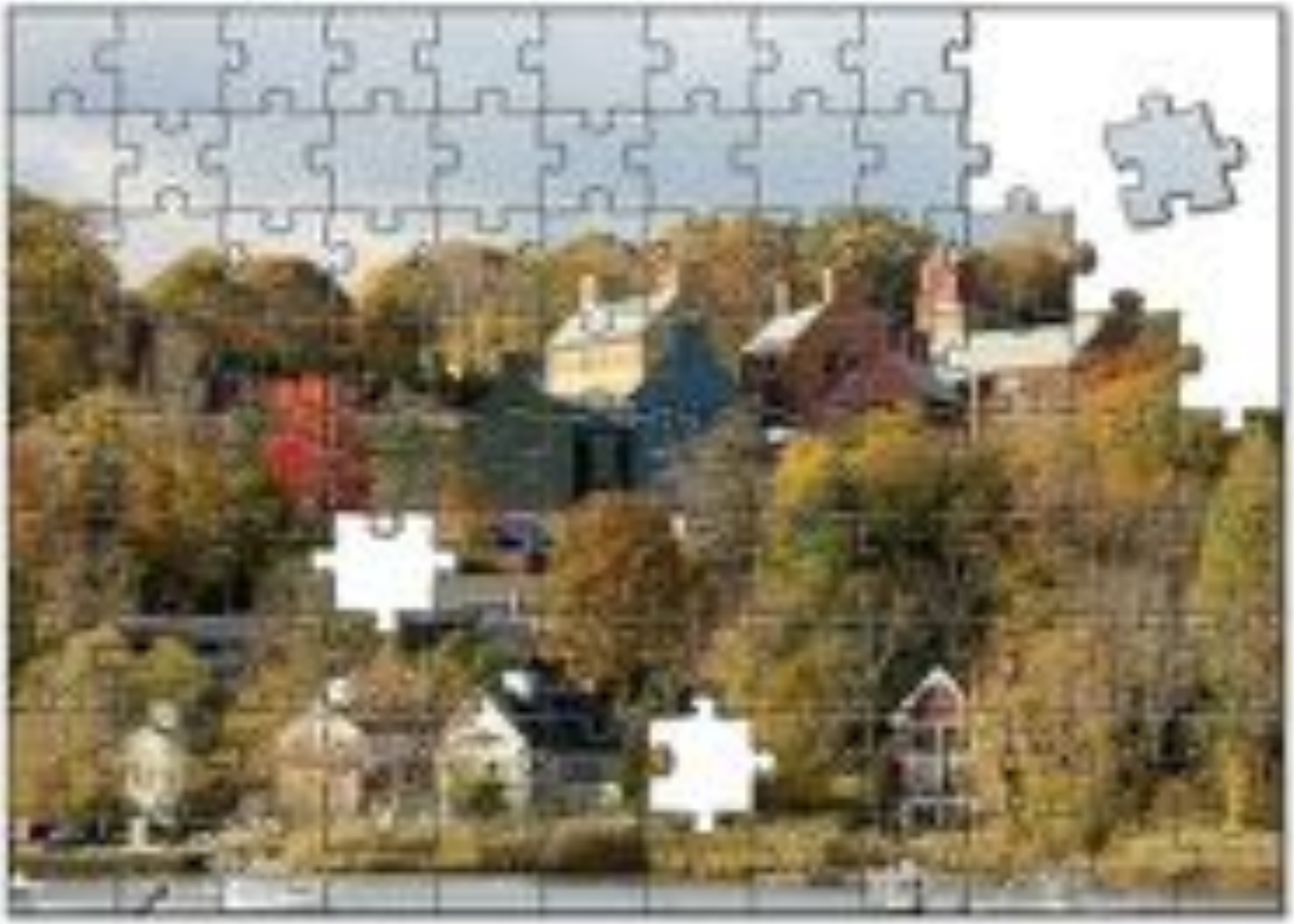


Oxford Nanopore Sequencing at CSHL
30 runs, 267k reads, 122x total coverage
Between 11 and 73k reads per run!
Mean flow cell: 50 Mbp in 2 days
Max flow cell: 446Mbp in 2 days

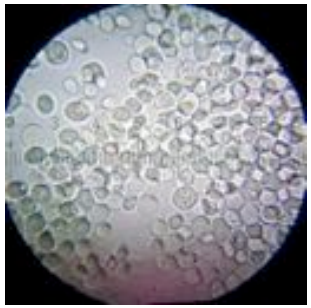
Nanopore Sequences



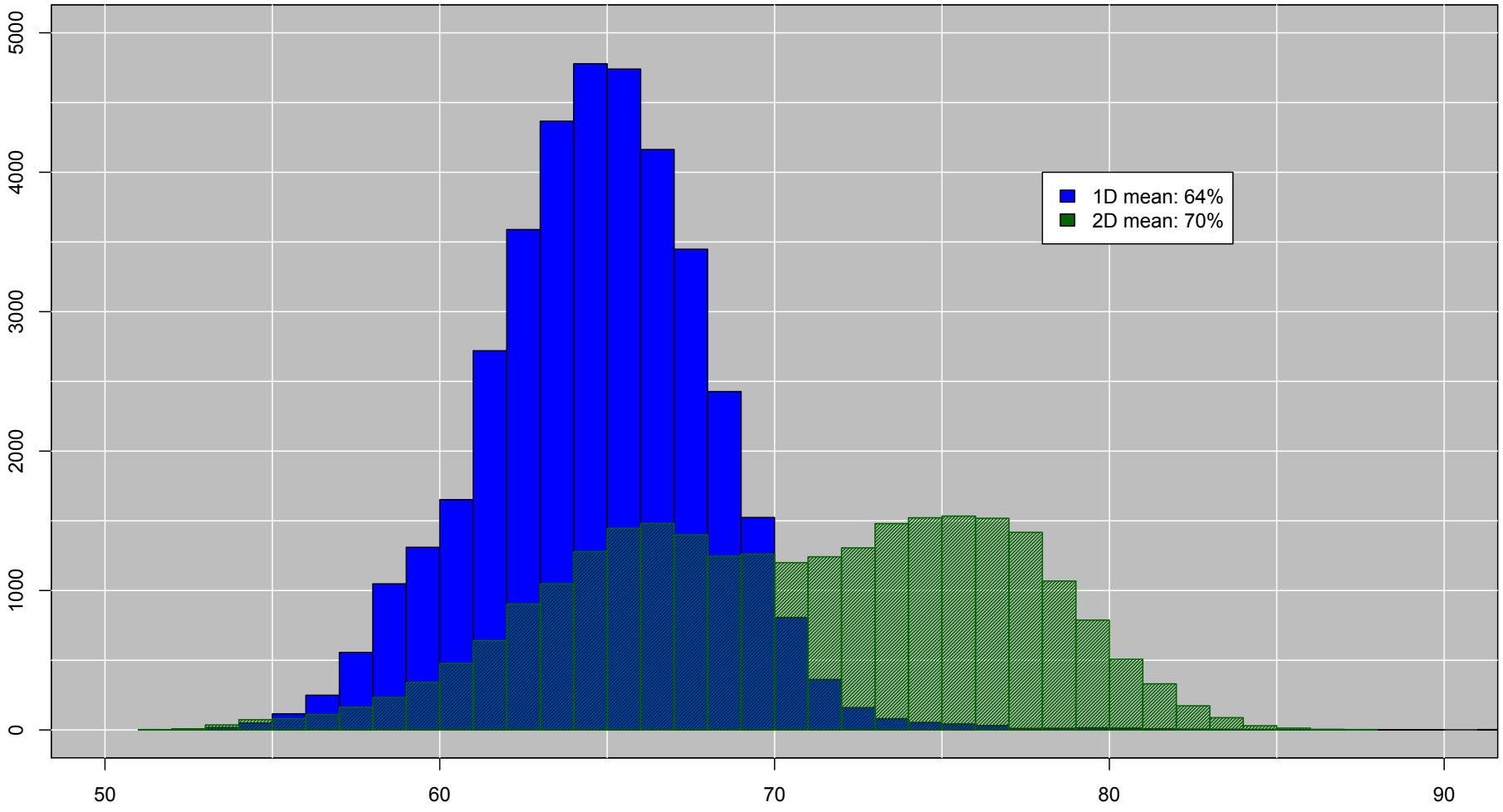
“Corrective Lens” for Sequencing



Nanopore Accuracy



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



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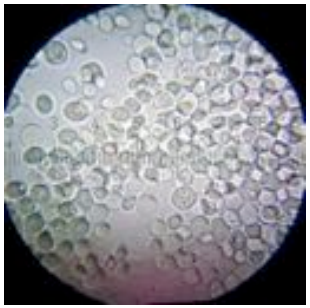

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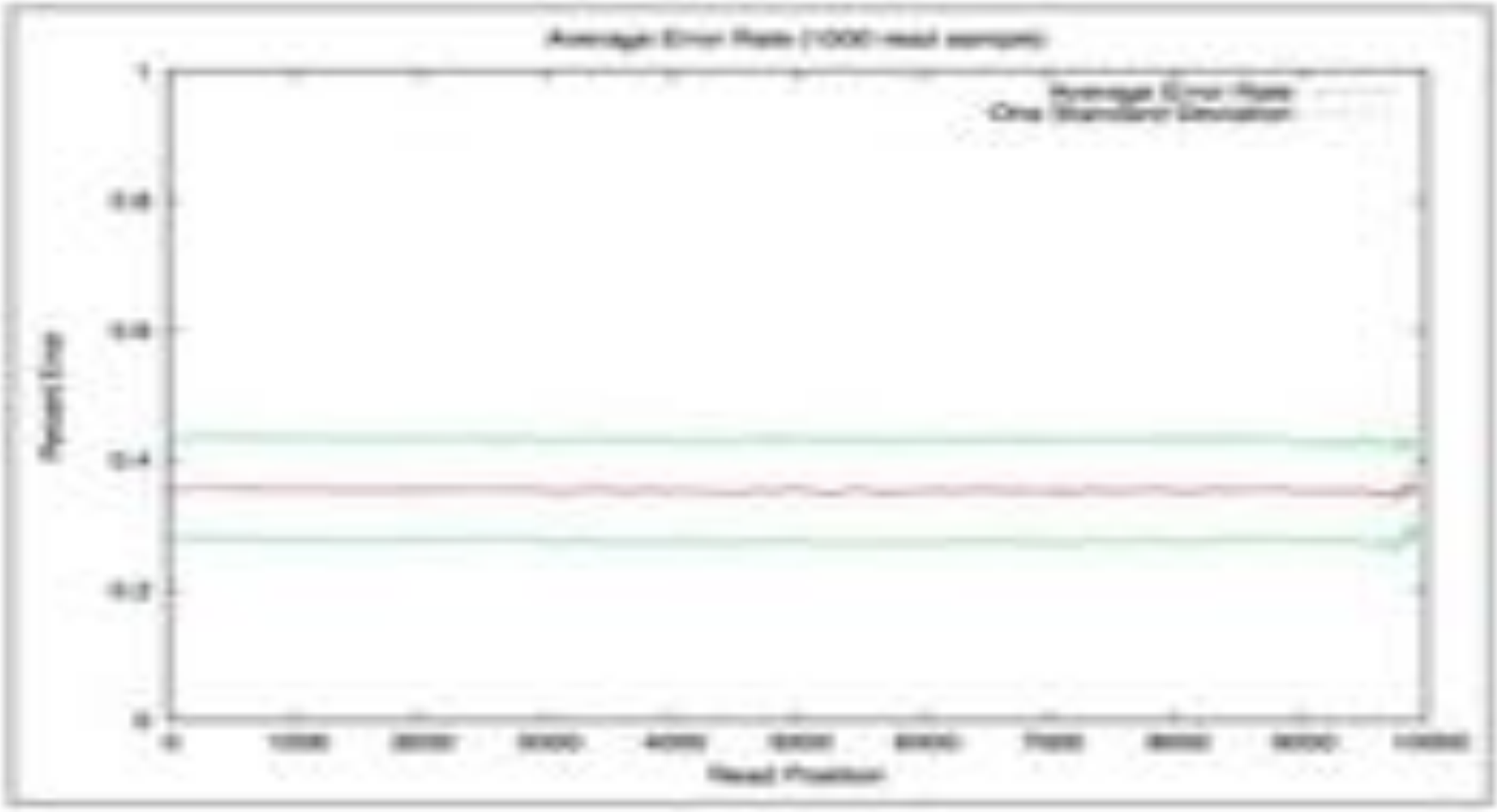

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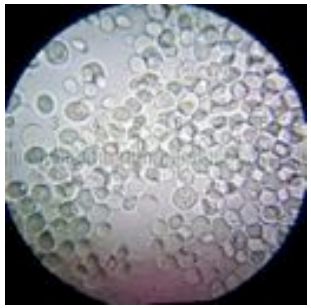

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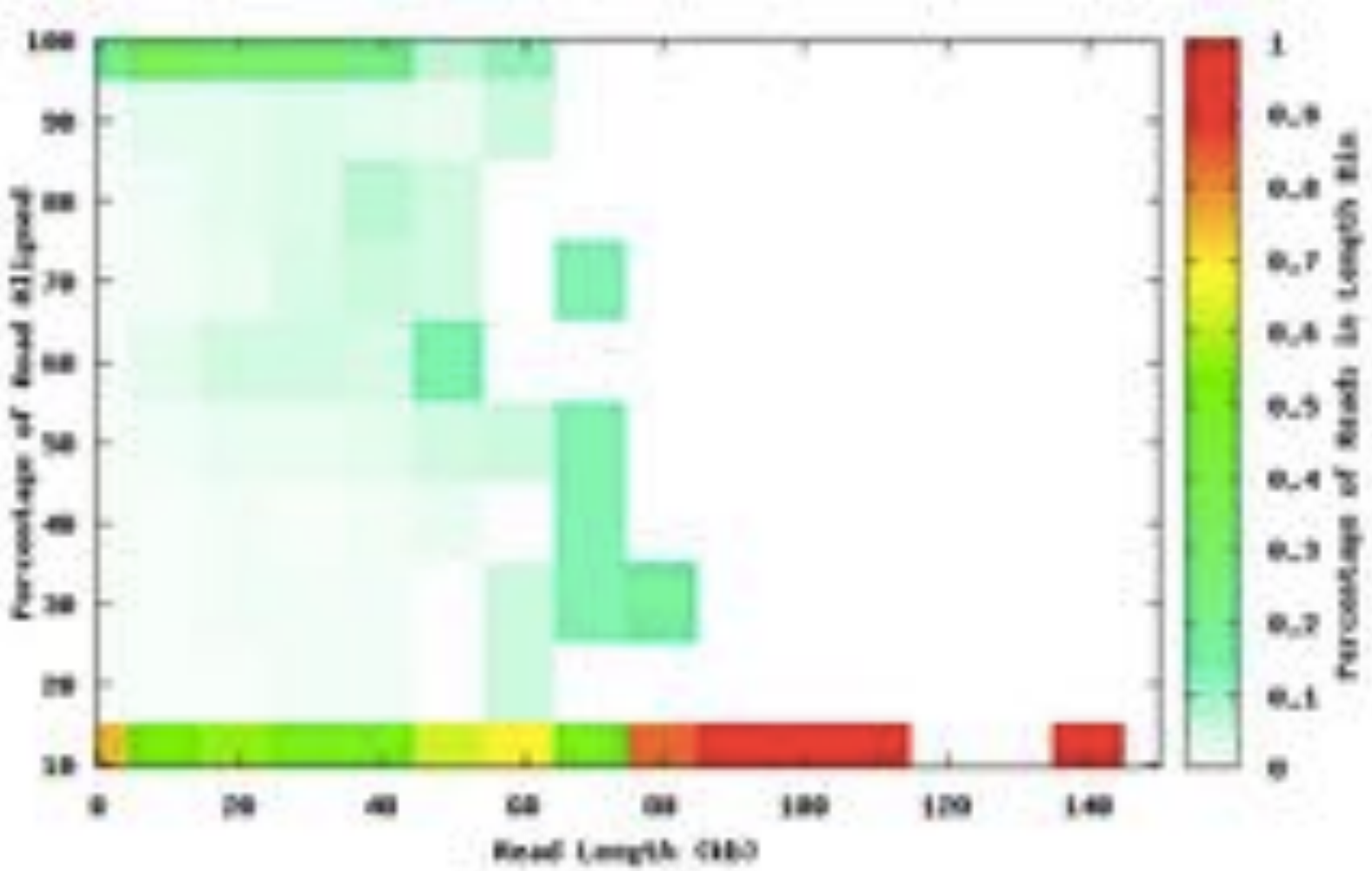


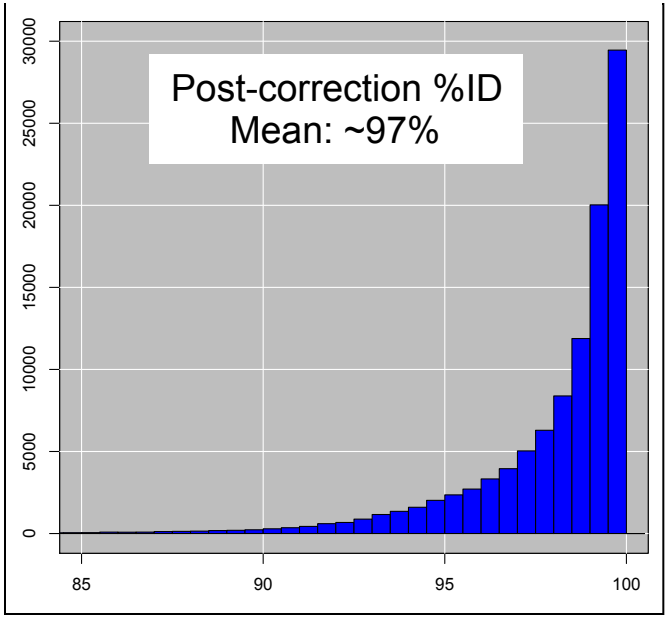
NanoCorr: Nanopore-Illumina Hybrid Error Correction

https://github.com/jgurtowski/nanocorr

1. BLAST Miseq reads to all raw Oxford Nanopore reads

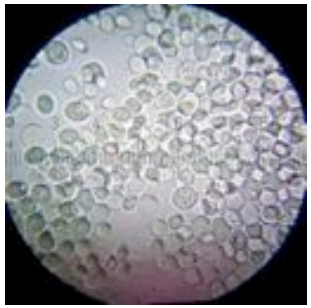
2. Select non-repetitive alignments
   - First pass scans to remove "contained" alignments
   - Second pass uses Dynamic Programming (LIS) to select set of high-identity alignments with minimal overlaps

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



Oxford Nanopore Sequencing and de novo Assembly of a Eukaryotic Genome
Goodwin, S, Gurtowski, J *et al.* (2015) bioRxiv doi: http://dx.doi.org/10.1101/013490

Long Read Assembly



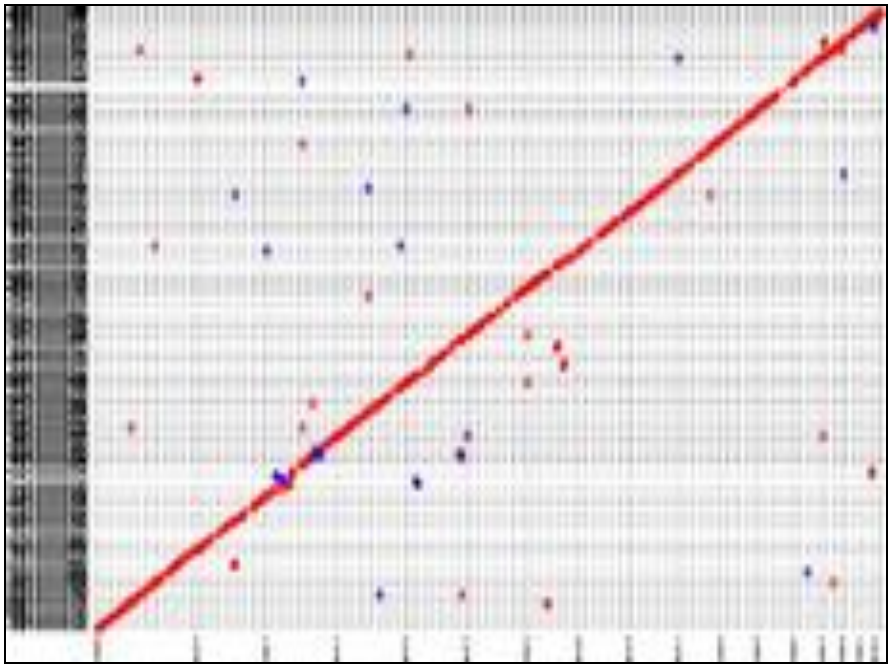
S288C Reference sequence

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

Illumina MiSeq

30x, 300bp PE (Flashed)

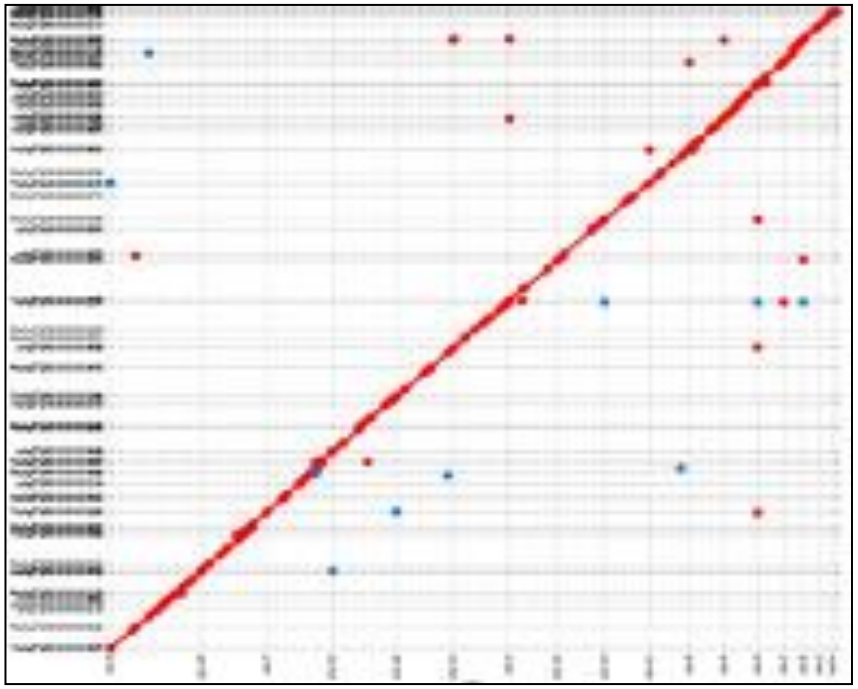
- 6953 non-redundant contigs
- N50:59kbp >99.9% id



Oxford Nanopore

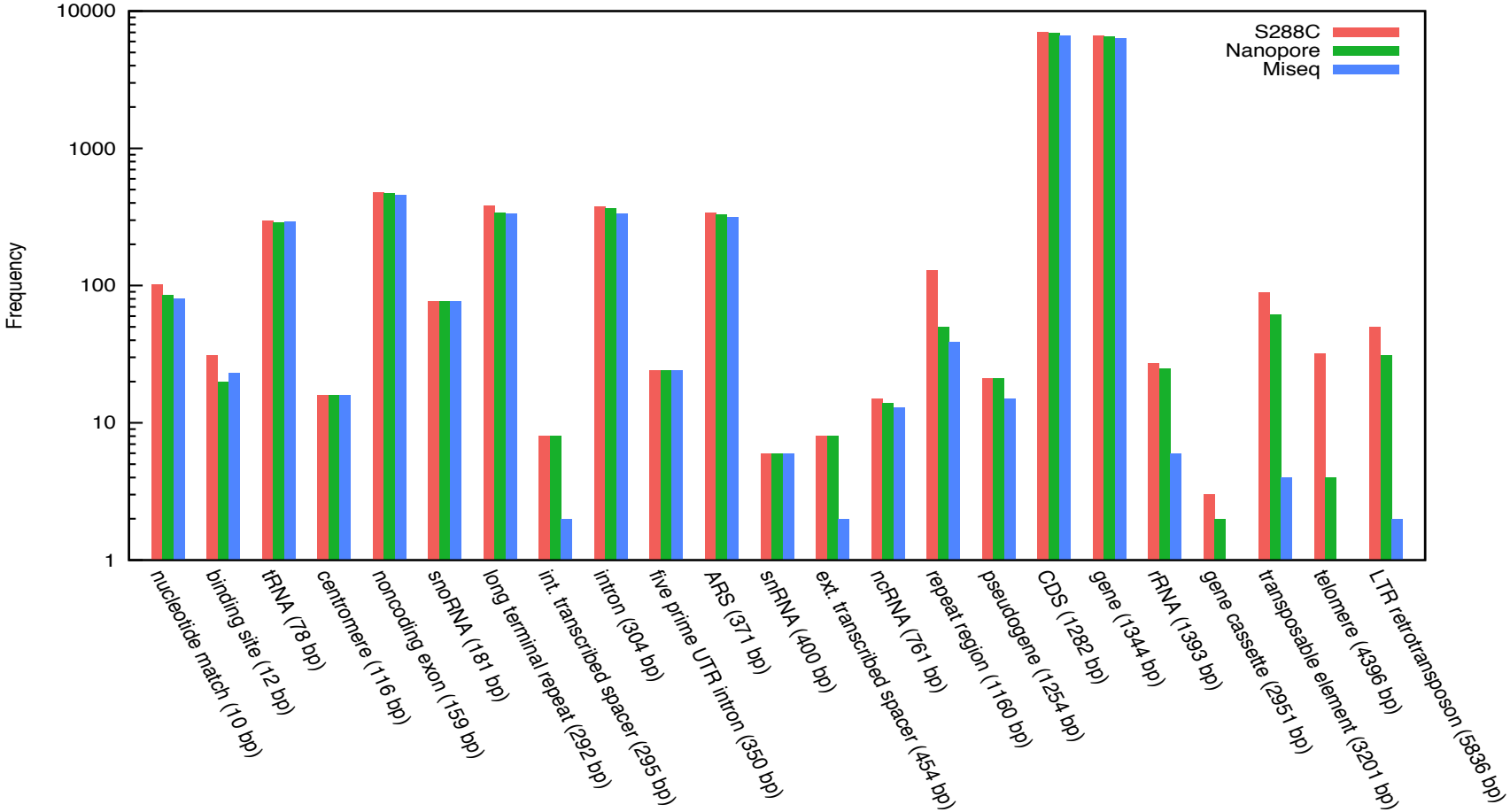
NanoCorr + Celera Assembler

- 214 non-redundant contigs
- N50: 472kbp >99.78% id



Advantages of Long Reads

In yeast, Nanopore-based assembly is ~10x more contiguous
In E. coli, Nanopore-based assembly is basically perfect



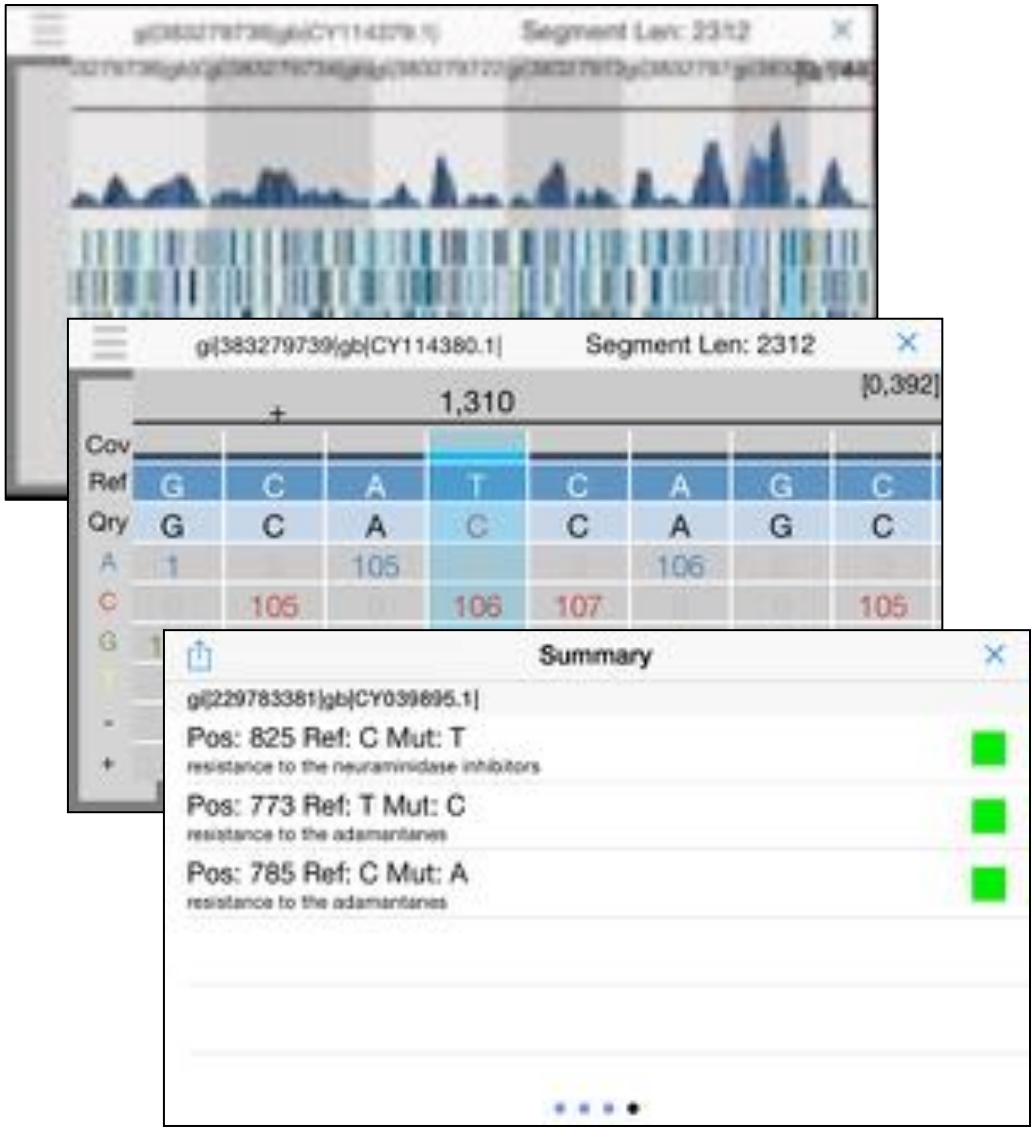
Oxford Nanopore Sequencing and de novo Assembly of a Eukaryotic Genome.
Goodwin, S*, Gurtowski, J*, Ethe-Sayers, S, Deshpande, P, Schatz, MC†, McCombie WR† (2014) *Under review.*

Genomic Futures?

Genomic Futures?

iGenomics: Mobile Sequence Analysis

Aspyn Palatnick, Elodie Ghedin, Michael Schatz



The worlds first genomics analysis app for iOS devices

First application:

- Handheld diagnostics and therapeutic recommendations for influenza infections
- In a few seconds, iGenomics tells you which antivirals to take or avoid
- Coming soon to the App Store

Future applications

- Pathogen detection
- Food safety
- Biomarkers
- etc..

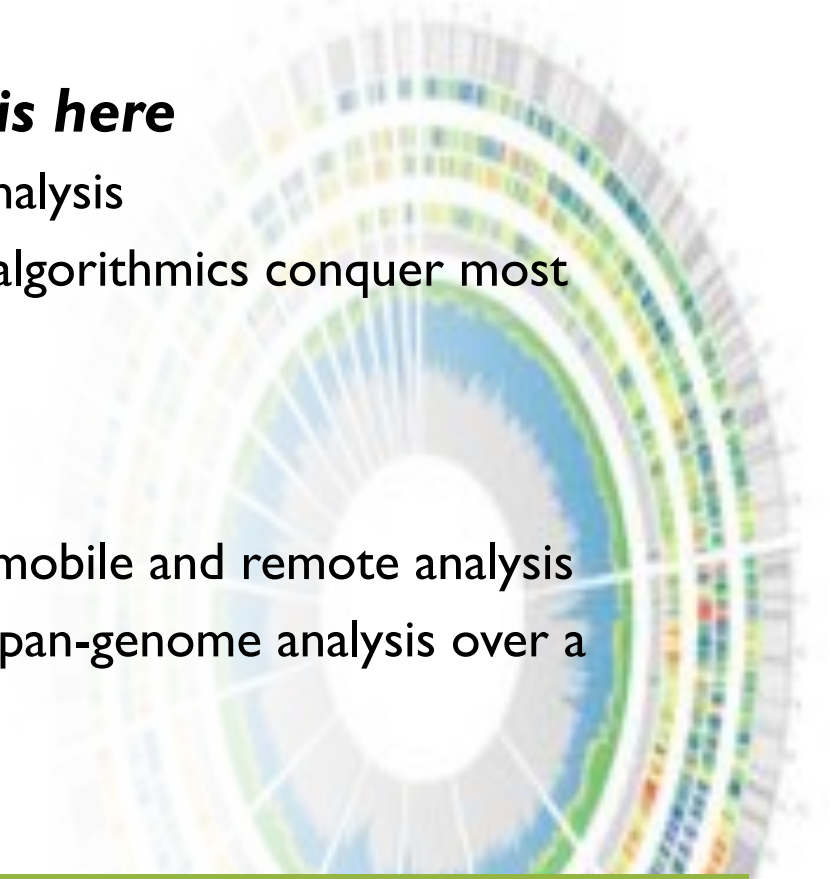
Summary & Recommendations

Reference quality genome assembly is here

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

Trends in Algorithmics

- Exciting developments in the future for mobile and remote analysis
- Now is the time to start thinking about pan-genome analysis over a large number of genomes



The resurgence of reference quality genome sequence

Michael Schatz, Ian Korf, Dan Rokhsar

Tuesday @ 4pm, Pacific Salon 1

Acknowledgements

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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

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SFARI
SIMONS FOUNDATION AUTISM RESEARCH INITIATIVE

Thank you

http://schatzlab.cshl.edu

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