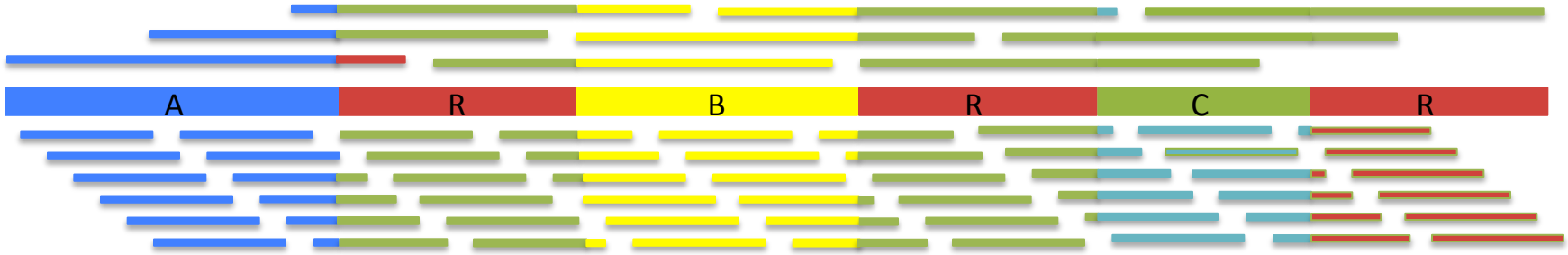
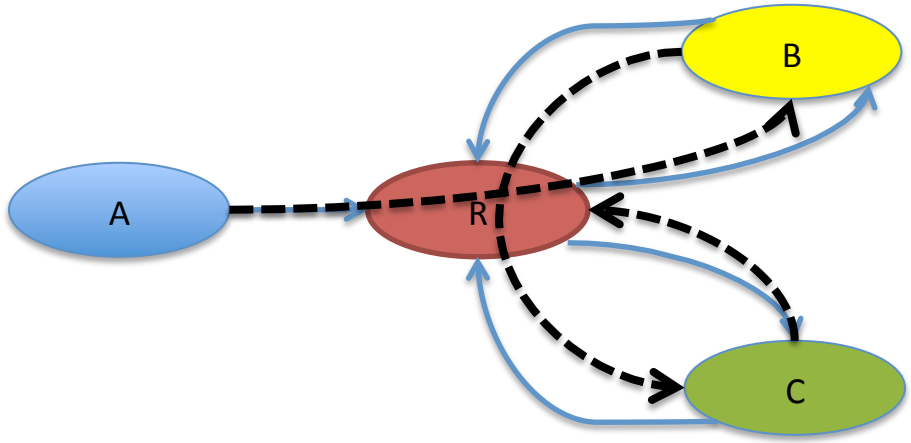
Error Correction and Assembly of Oxford Nanopore Sequencing

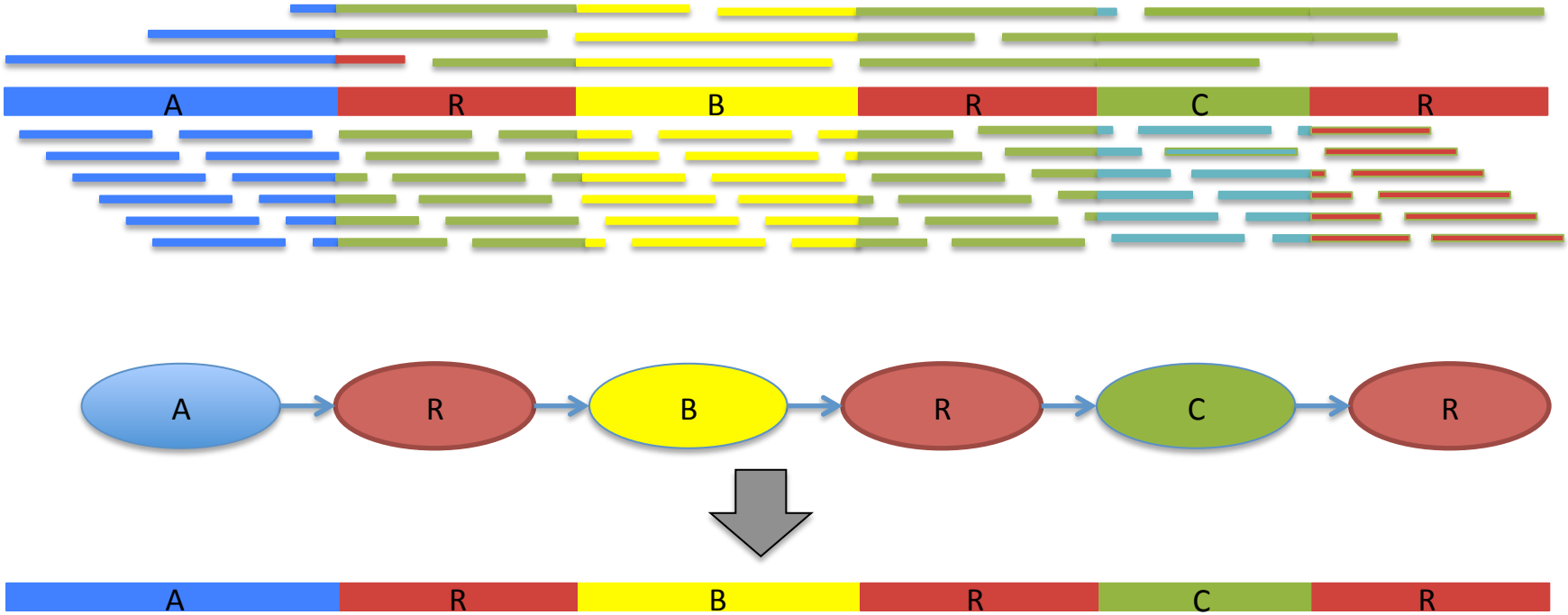
James Gurtowski

Assembly Complexity





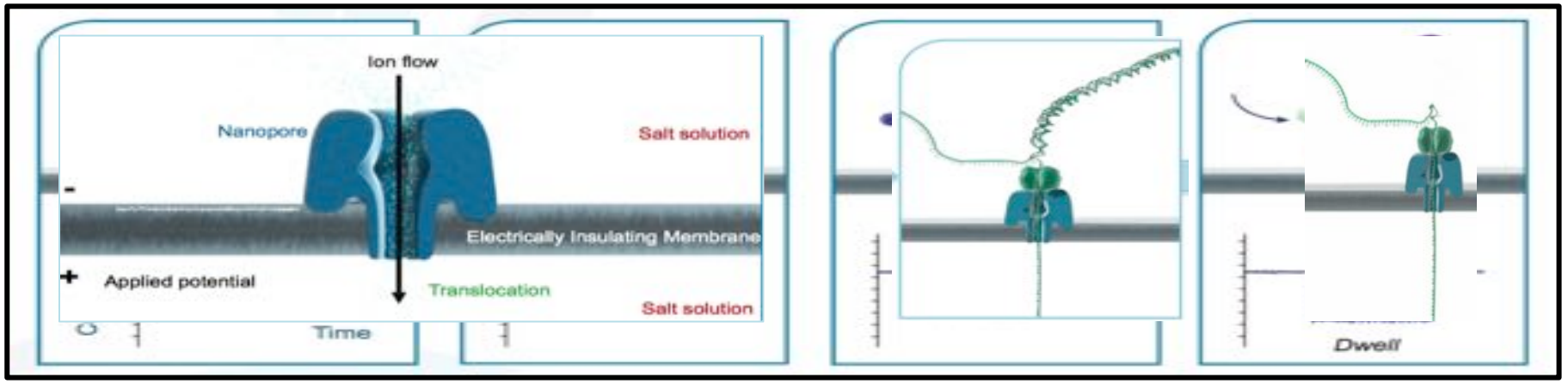
Assembly Complexity



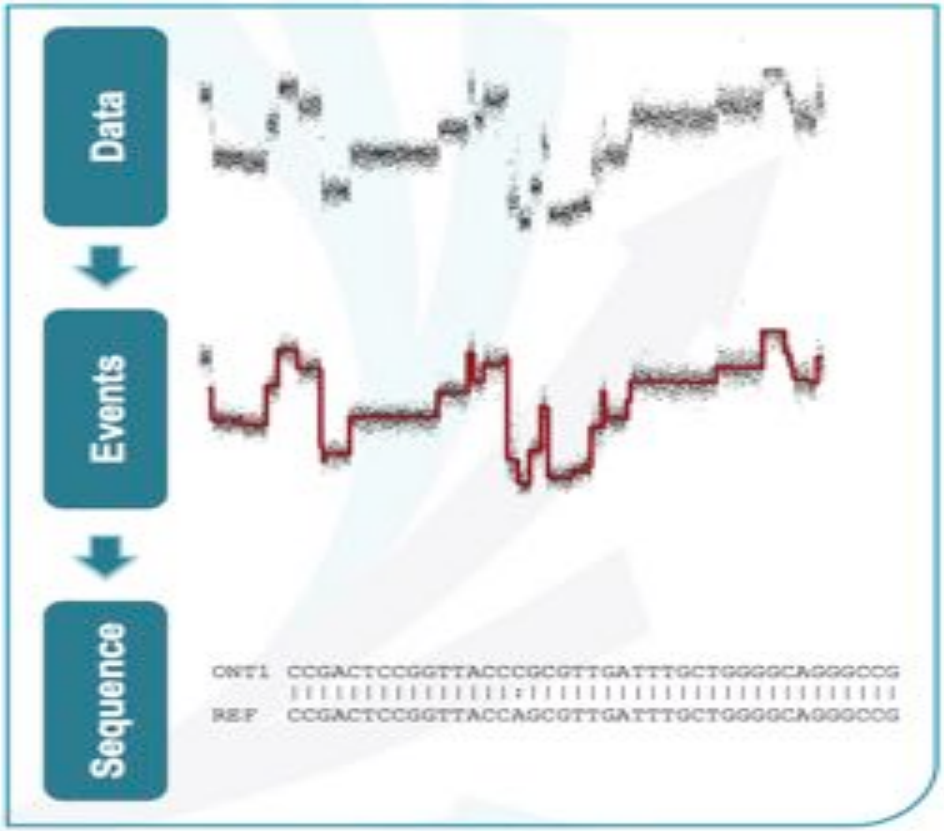
The advantages of SMRT sequencing
Roberts, RJ, Carneiro, MO, Schatz, MC (2013) *Genome Biology.* 14:405

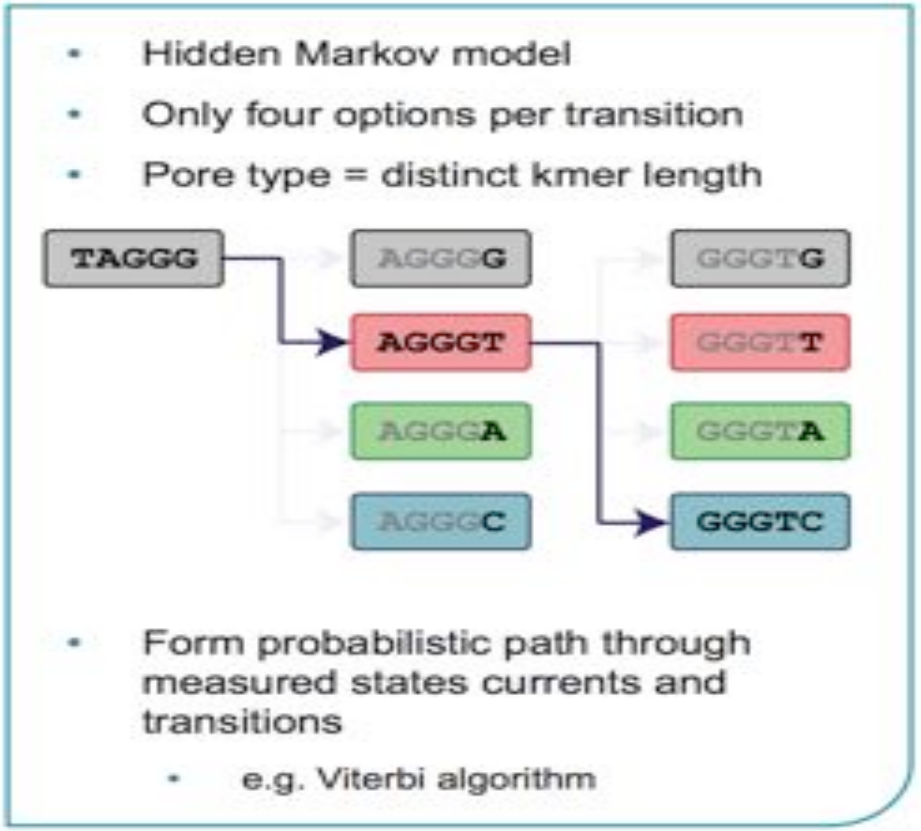
Oxford Nanopore MinION

- Thumb drive sized sequencer powered over USB
- Senses DNA by measuring changes to ion flow
- Reads both DNA Strands (2D)



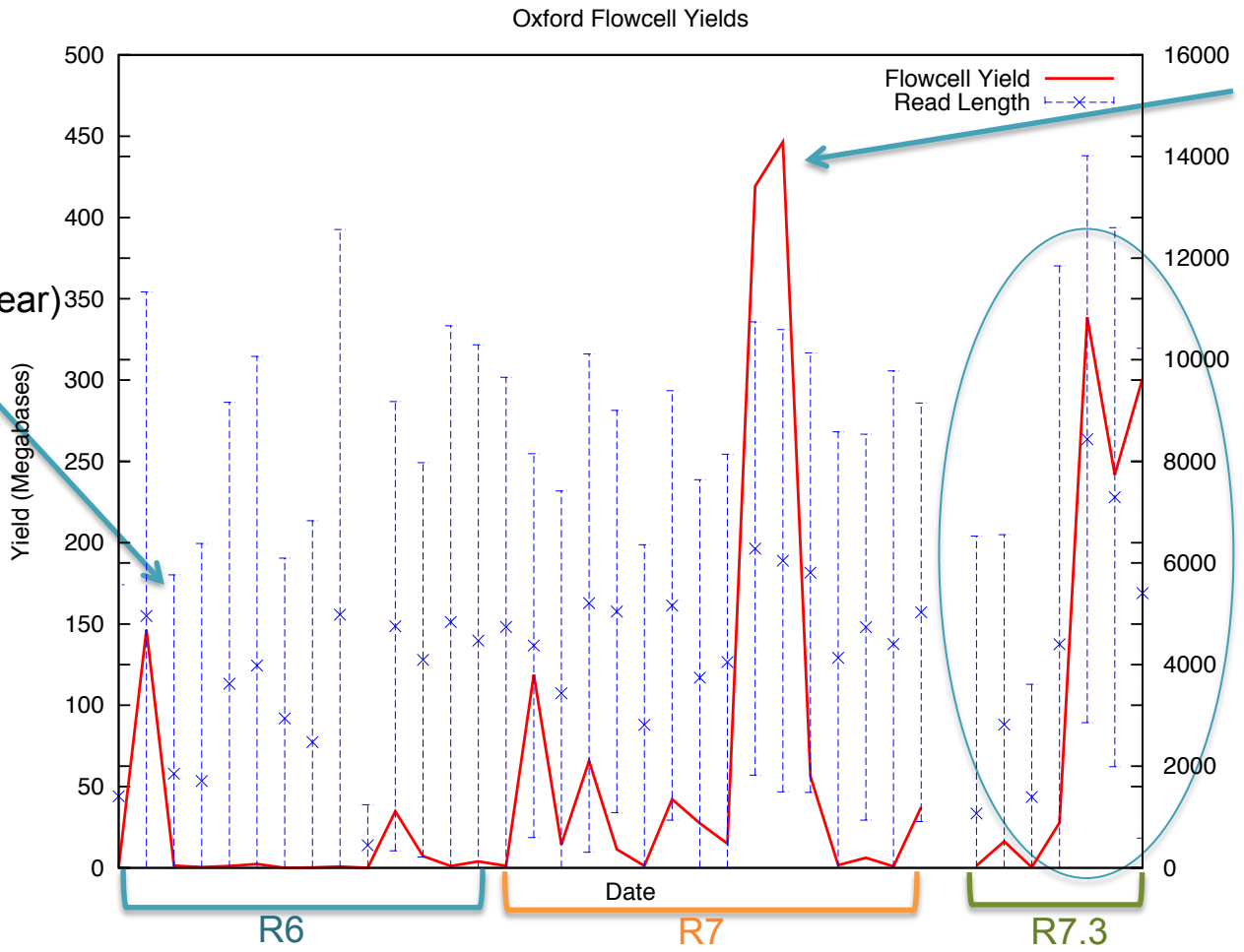
Nanopore Basecalling





Basecalling currently performed at Amazon with frequent updates to algorithm

Our Data - Yeast W303

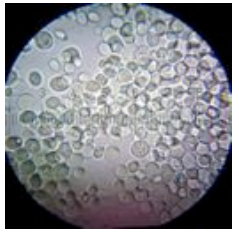


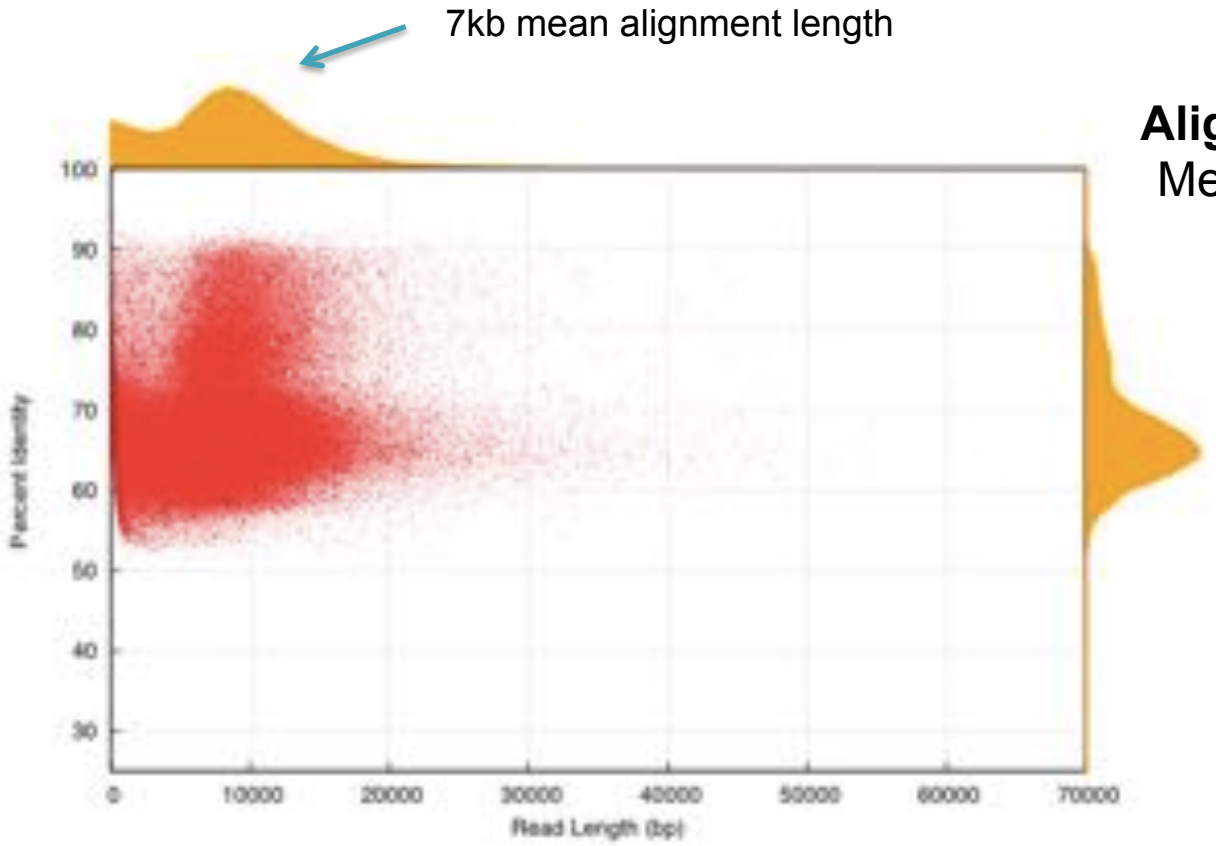
Mean Read Length:
~6kb (10kb shear)

Best: 446Mb

New Flowcells
7k Avg Read Length

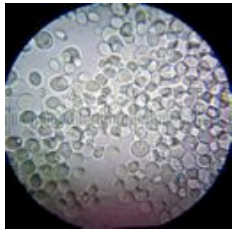
Nanopore Alignments





Alignment Statistics (BLASTN)
Mean alignment length at ~7kbp
Shearing targeted 10kbp
255k reads align (64%)
174x coverage

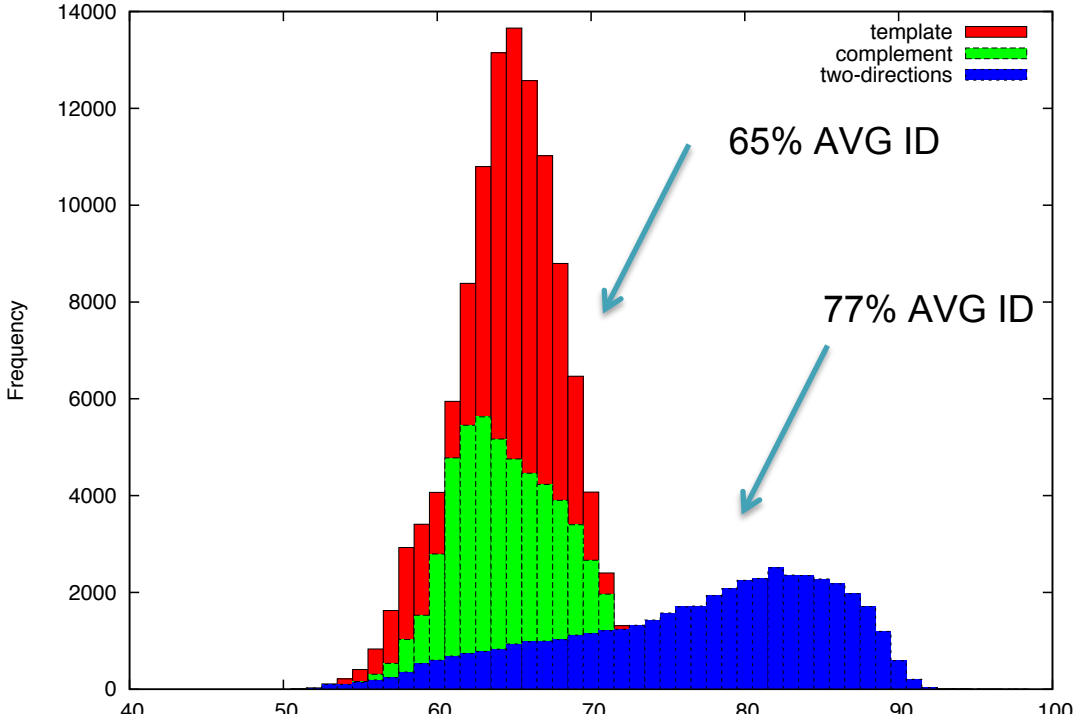
Nanopore Accuracy



Alignment Quality (BLASTN)

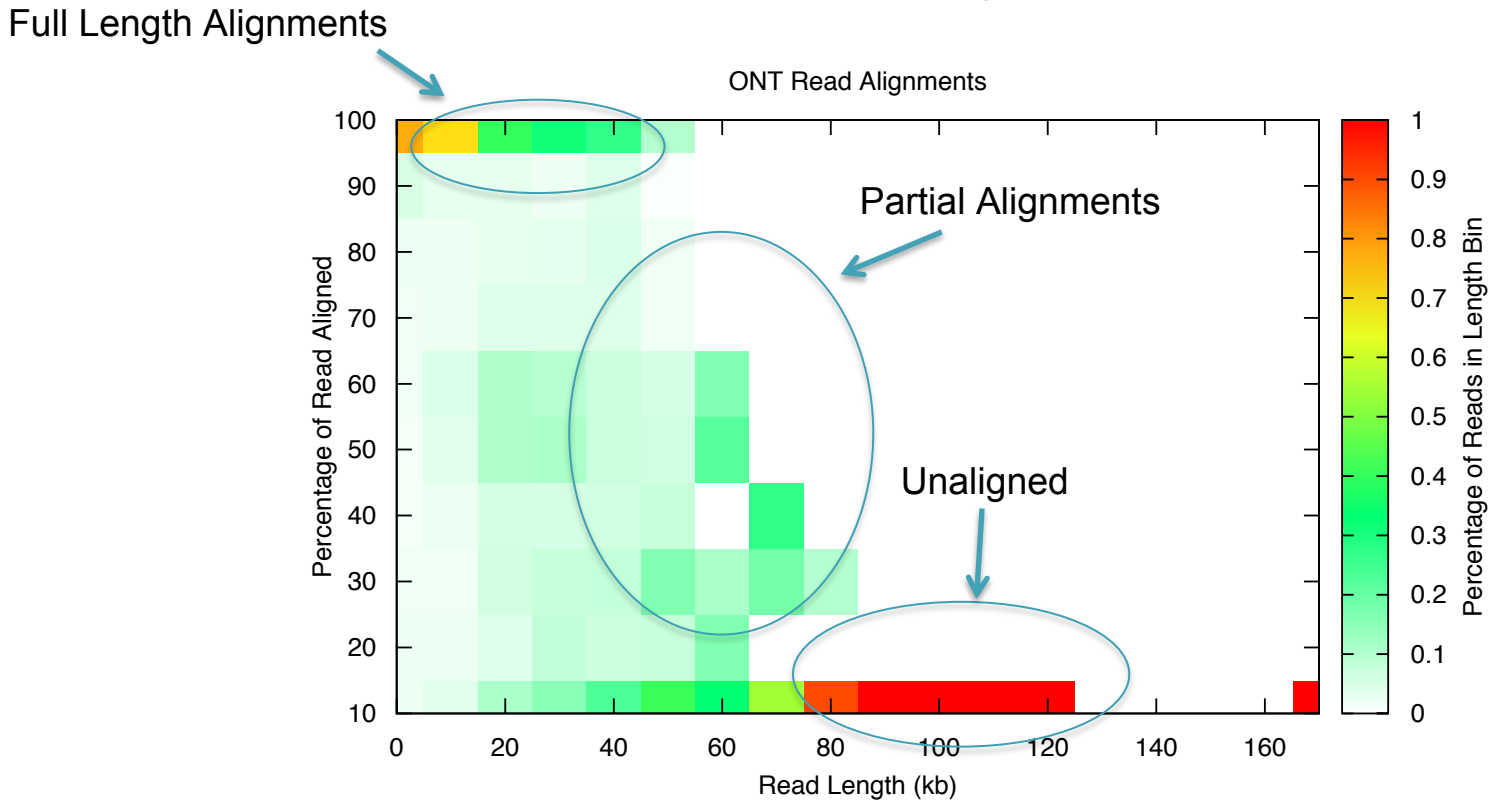
Of reads that align, average ~65% identity

“2D base-calling” improves to ~77% identity



Nanopore Alignment Summary

64% of the data map using BLASTN



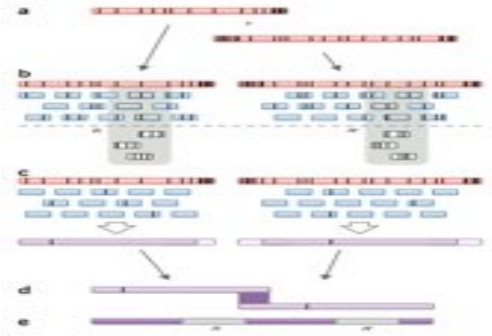
Long Read Correction Algorithms

PBJelly

Gap Filling and Assembly Upgrade

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

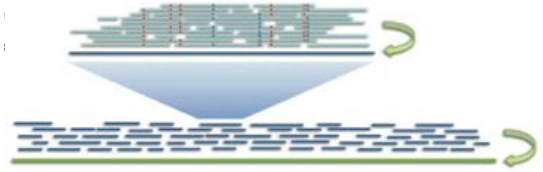
PacBioToCA & ECTools



Hybrid Error Correction

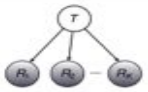
Koren, Schatz, *et al* (2012)
Nature Biotechnology. 30:693–700

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

LR-only Correction & Polishing

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

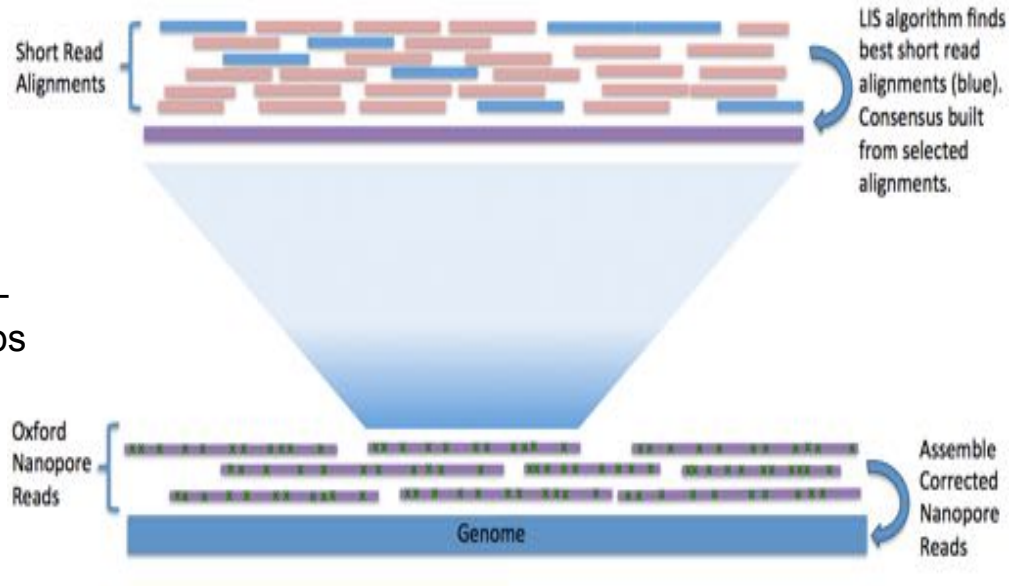
< 5x Long Read Coverage > 50x

NanoCorr: Nanopore-Illumina Hybrid Error Correction

1. BLAST Miseq reads to all raw Oxford Nanopore reads

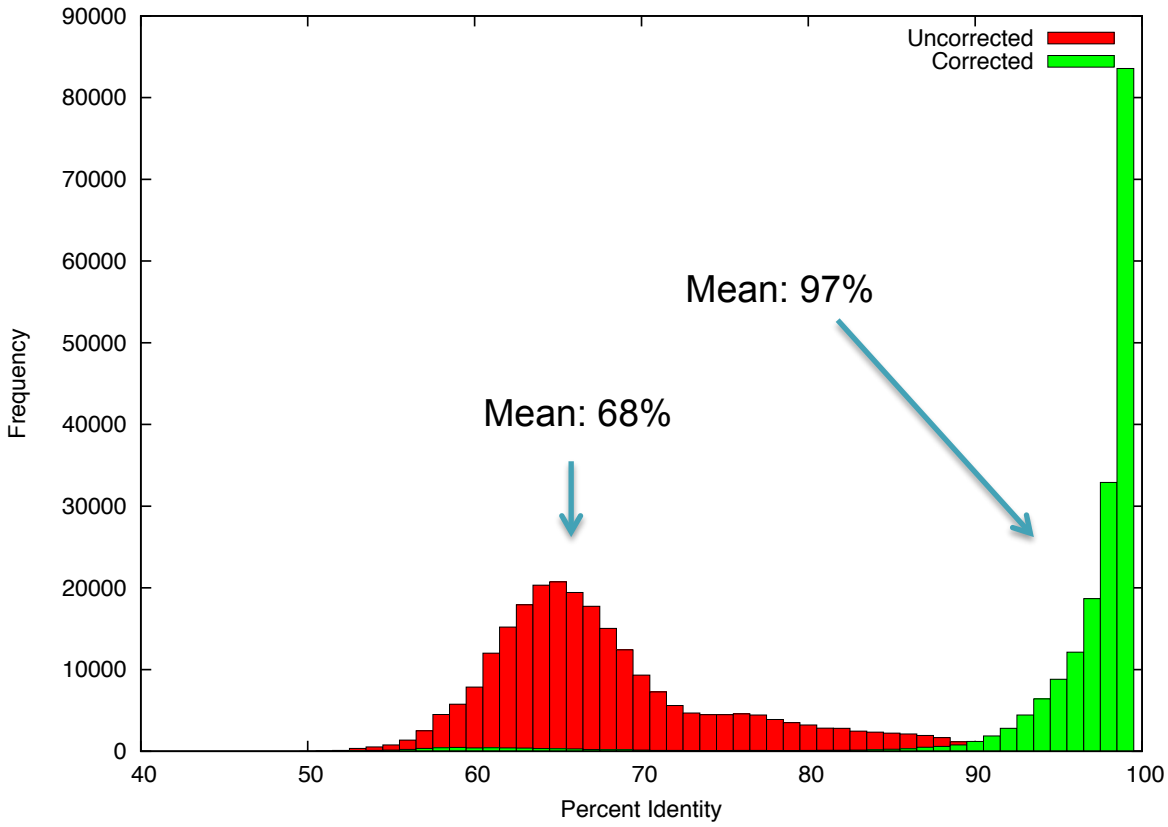
2. Select non-repetitive alignments
   - First pass scans to remove “contained” alignments
   - Second pass uses Dynamic Programming (LIS) to select set of high-identity alignments with minimal overlaps

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

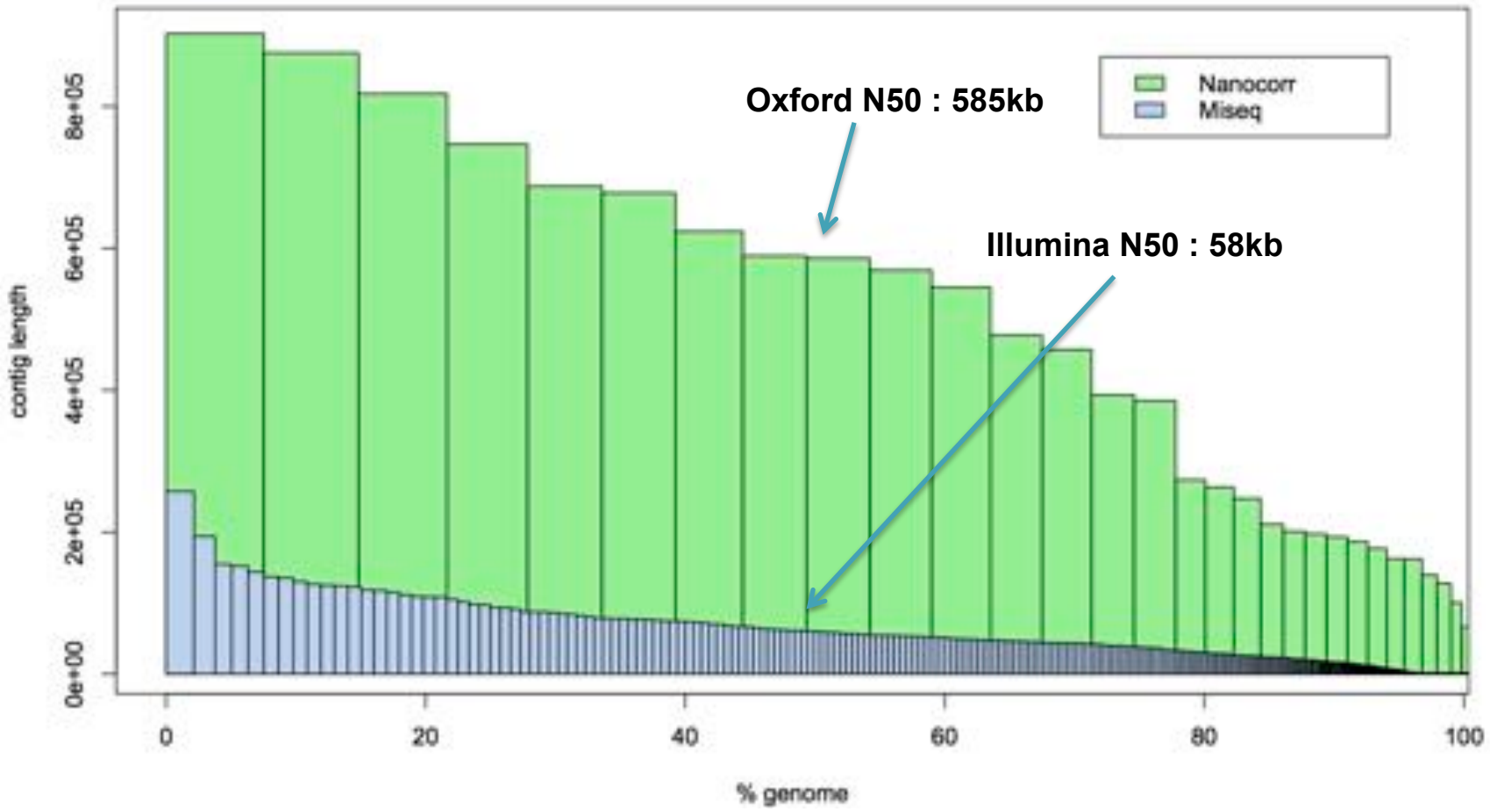


https://github.com/jgurtowski/nanocorr

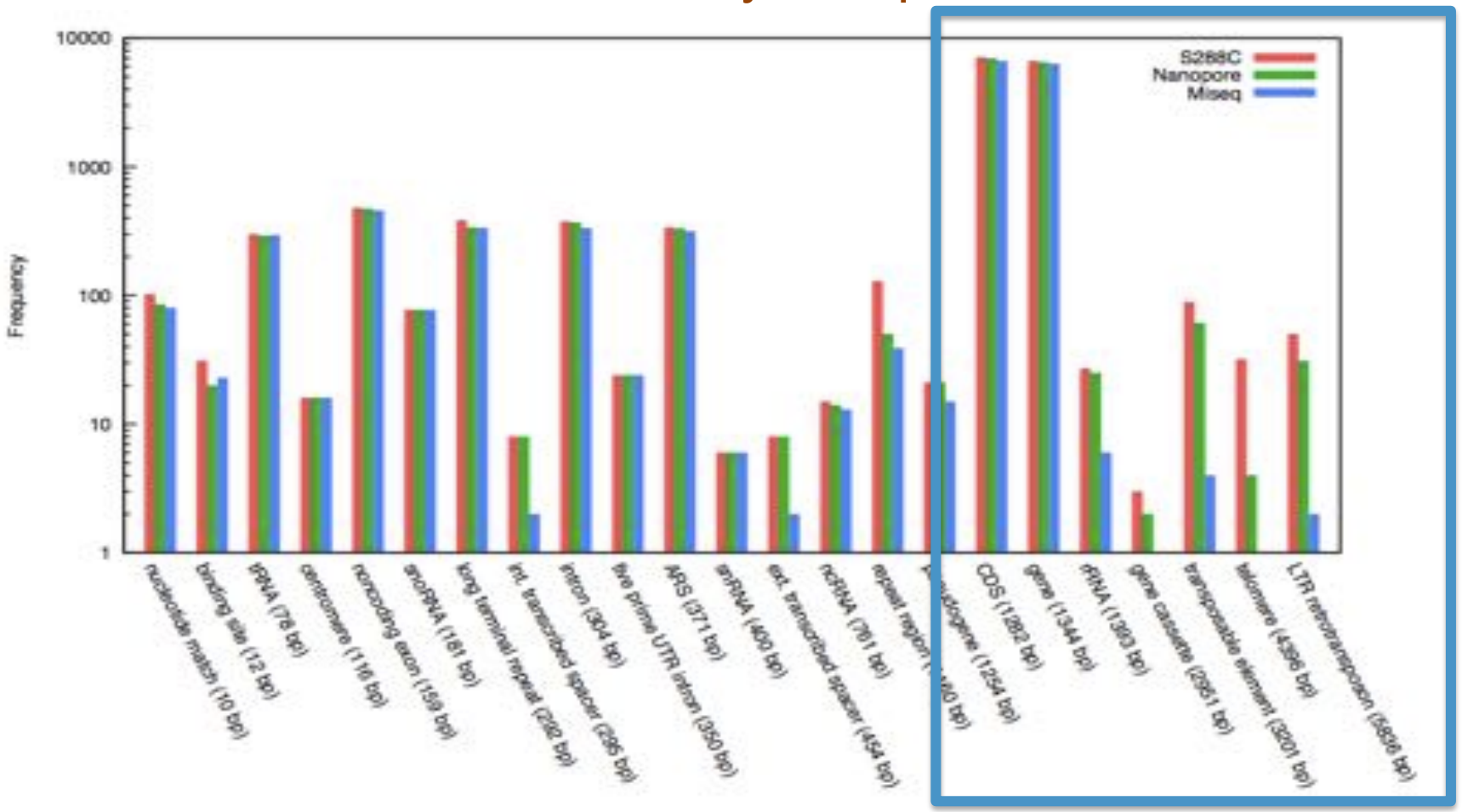
Post Correction Identity



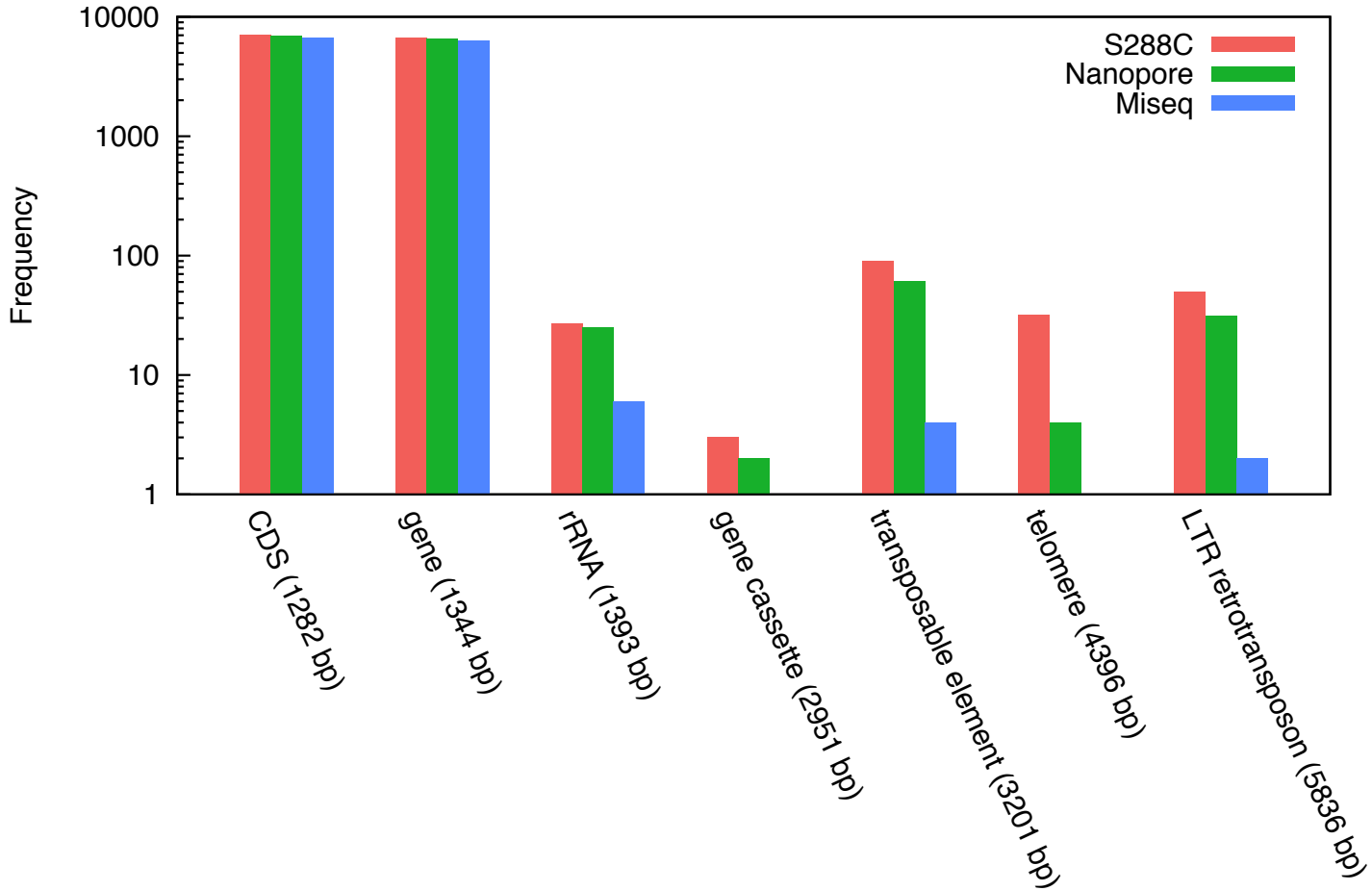
ONT vs Illumina Assembly



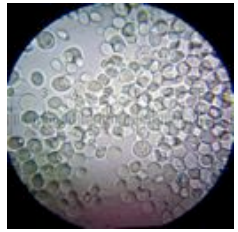
ONT Assembly Completeness



ONT Assembly Completeness



Long Read Assembly



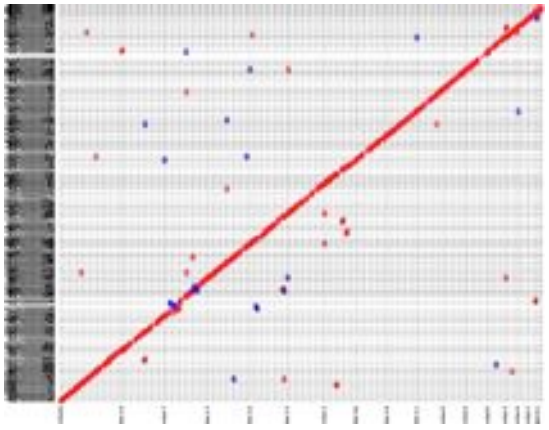
S288C Reference sequence

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

Illumina MiSeq

30x, 300bp PE (Flashed)
Celera Assembler

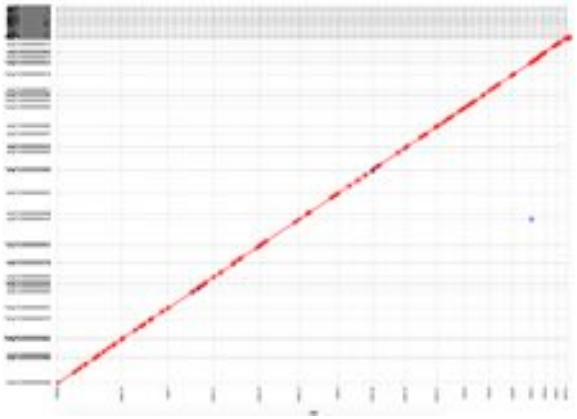
- 6953 non-redundant contigs
- N50: 59kb >99.9% id



Oxford Nanopore

28x corrected reads > 7kb
NanoCorr + Celera Assembler

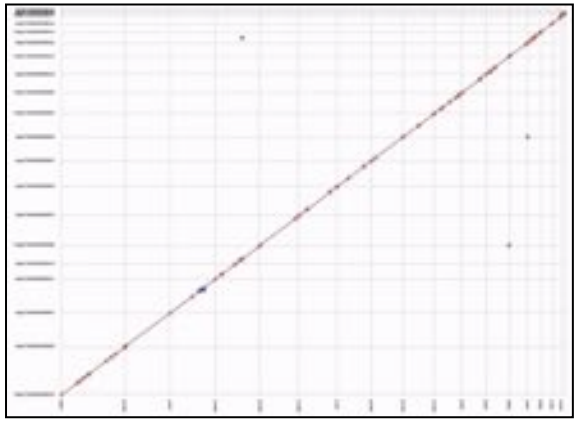
- 95 non-redundant contigs
- N50: 585kbp >99.78% id



Pacific Biosciences

25x corrected reads > 10kb
HGAP + Celera Assembler

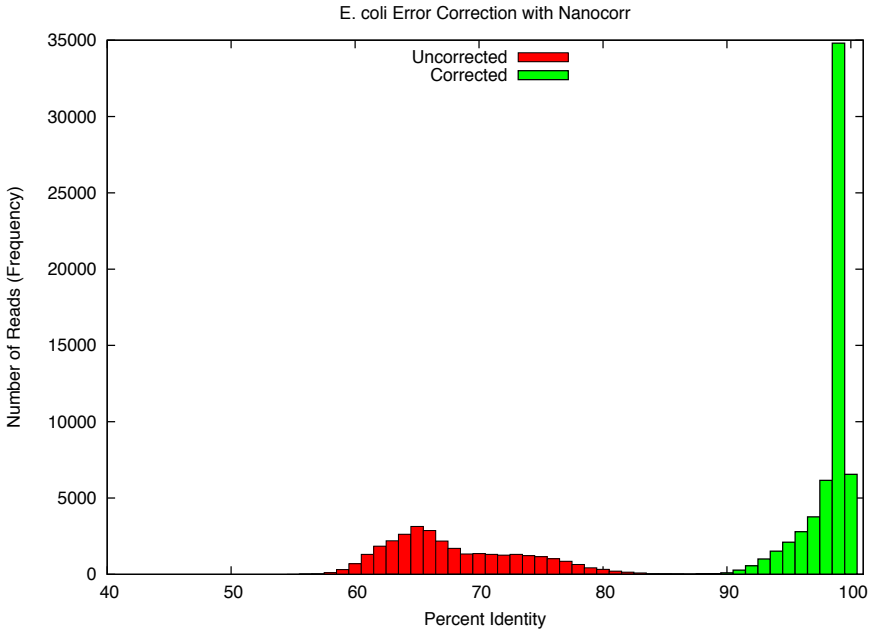
- 21 non-redundant contigs
- N50: 811kb >99.8% id



E. Coli K12 Single Contig Assembly with MinION

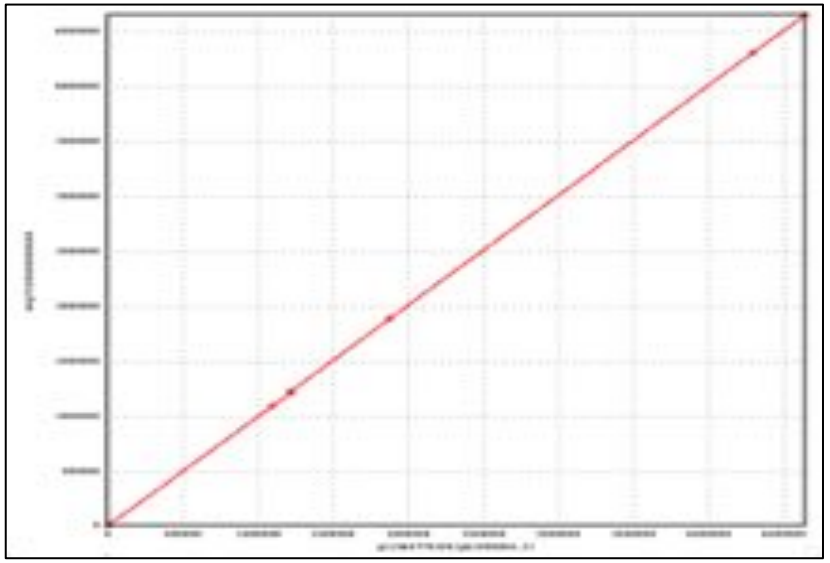
Nanocor Correction Results

145x Oxford Nanopore X 35x MiSeq



Single Contig Assembly

99.99% Identity (Pilon polishing)



Sequencing Data From:

A reference bacterial genome dataset generated on the MinION™ portable single-molecule nanopore sequencer

Joshua Quick, Aaron R Quinlan and Nicholas J Loman

Future of Oxford Nanopore

Zamin Iqbal and 5 others retweeted

GenomeWeb InSequence @InSequence · Oct 20

Oxford Nanopore shows off **PromethION** at **ASHG**. #ASHG14 #nanopore

Acknowledgements

Michael Schatz

Dick McCombie

Sara Goodwin

Schatz Lab

Oxford Nanopore Sequencing and de novo Assembly of a Eukaryotic Genome
Sara Goodwin , James Gurtowski , Scott Ethe-Sayers , Panchajanya Deshpande , Michael Schatz , W Richard McCombie
doi: http://dx.doi.org/10.1101/013490