De novo assembly of complex genomes

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April 10, 2013
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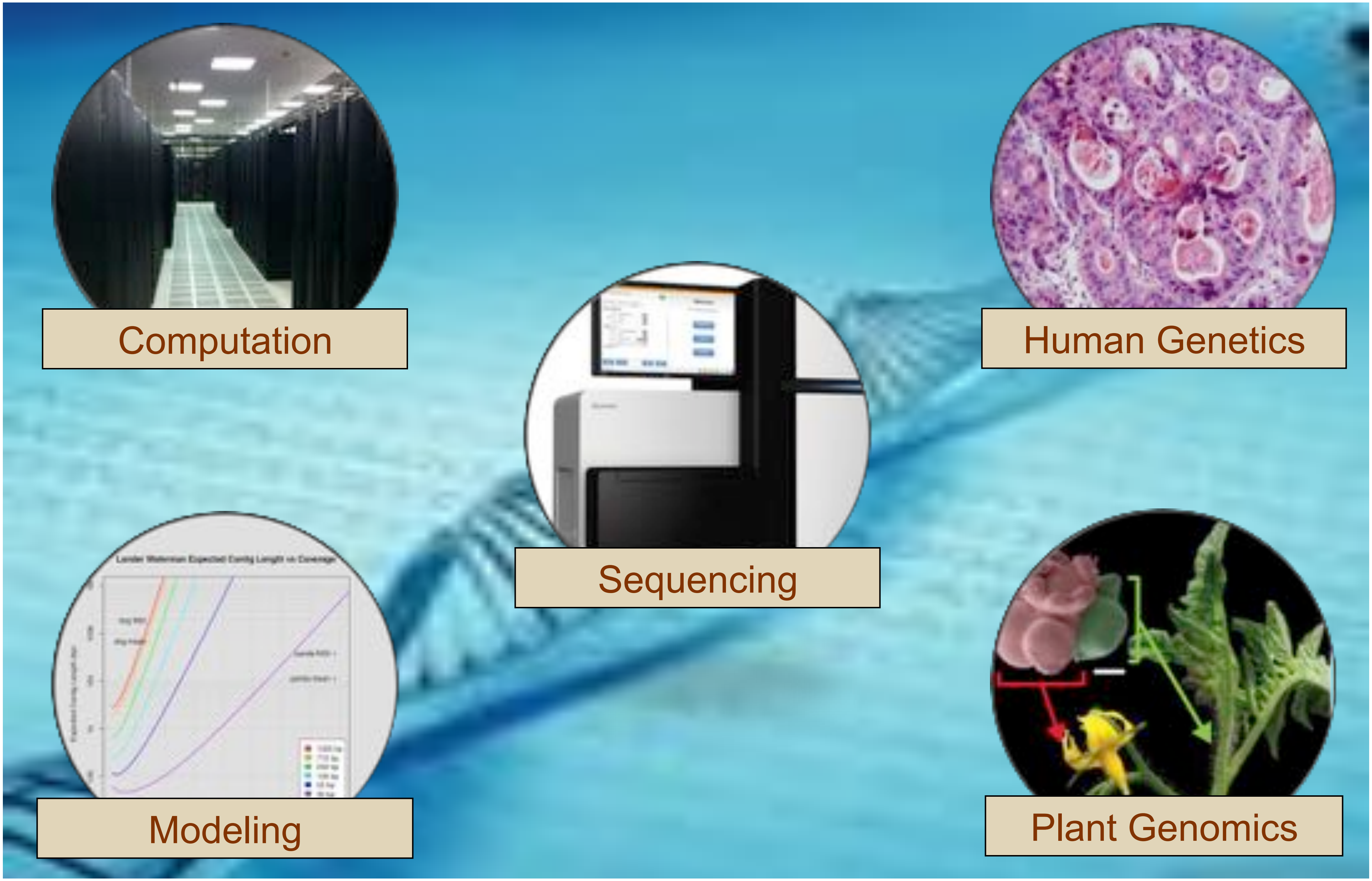
Schatz Lab Overview



Computation

Human Genetics

Sequencing

Modeling

Plant Genomics

Outline

1. Genome assembly by analogy
2. Hybrid error correction and assembly
3. De novo mutations in autism

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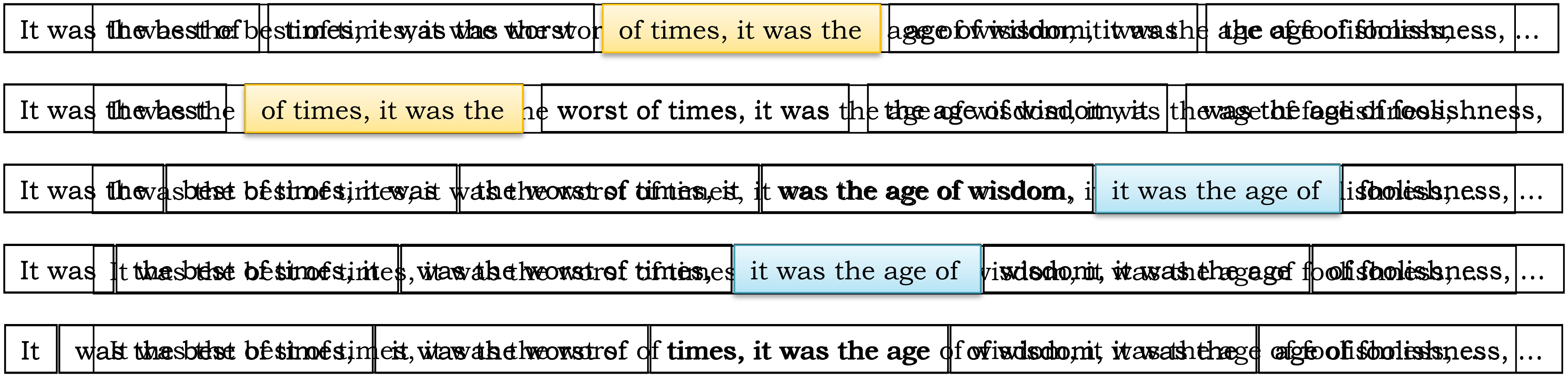
Shredded Book Reconstruction

- 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,

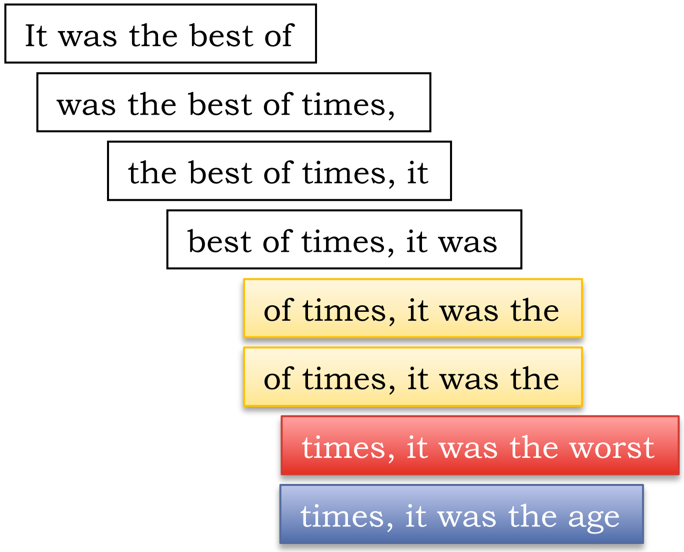
was the age of foolishness,

was the best of times,

was the worst of times,

wisdom, it was the age

worst of times, it was



It was the best of

was the best of times,

the best of times, it

best of times, it was

of times, it was the

of times, it was the

times, it was the worst

times, it was the age

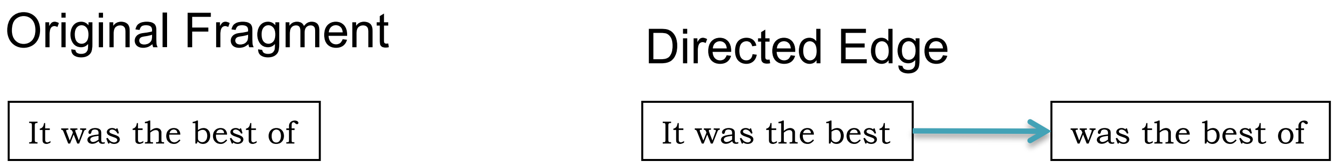
The repeated sequence make the correct reconstruction ambiguous

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

Model sequence reconstruction as a graph problem.

de Bruijn Graph Construction

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



Original Fragment

It was the best of

Directed Edge

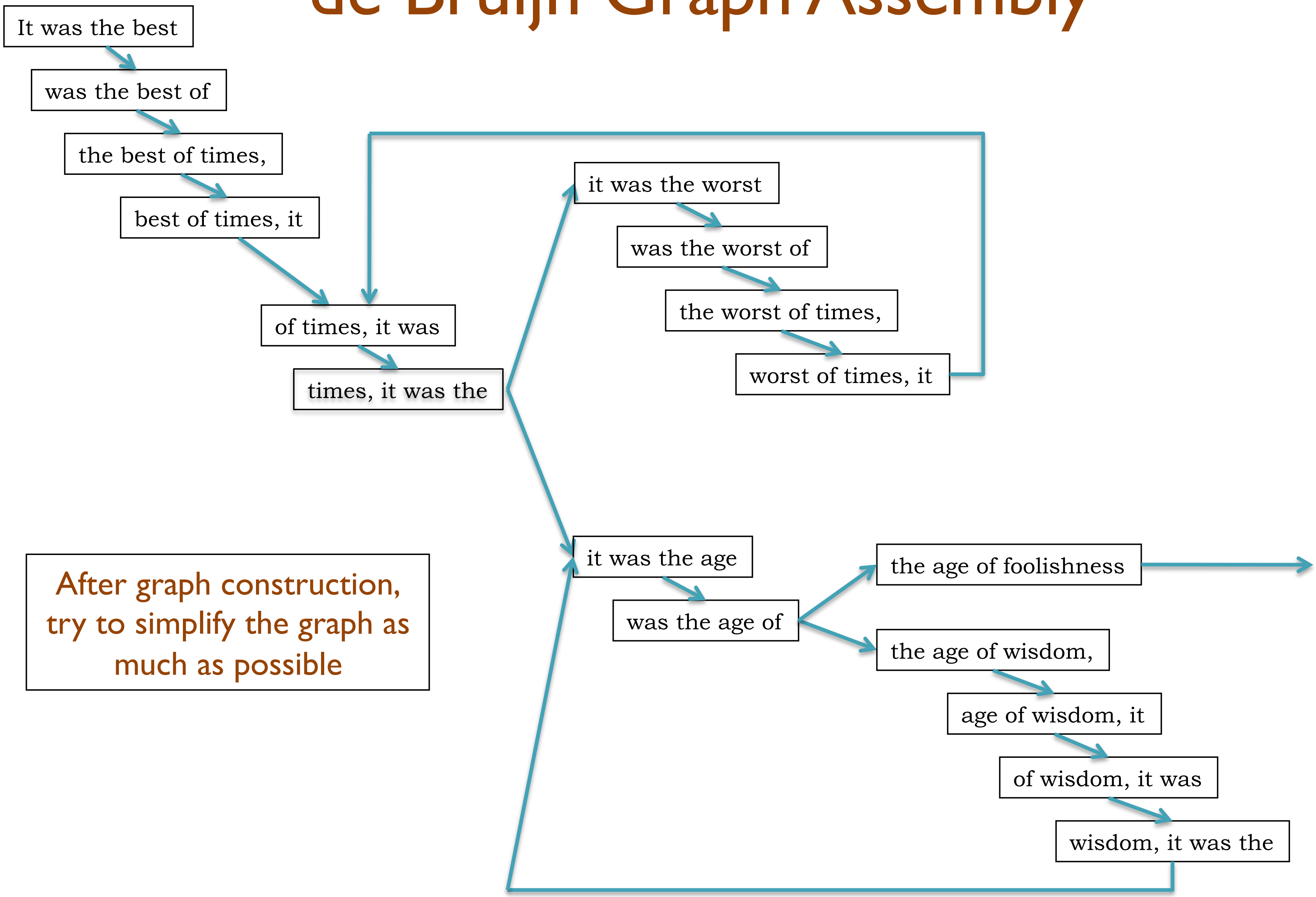
It was the best

was the best of

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

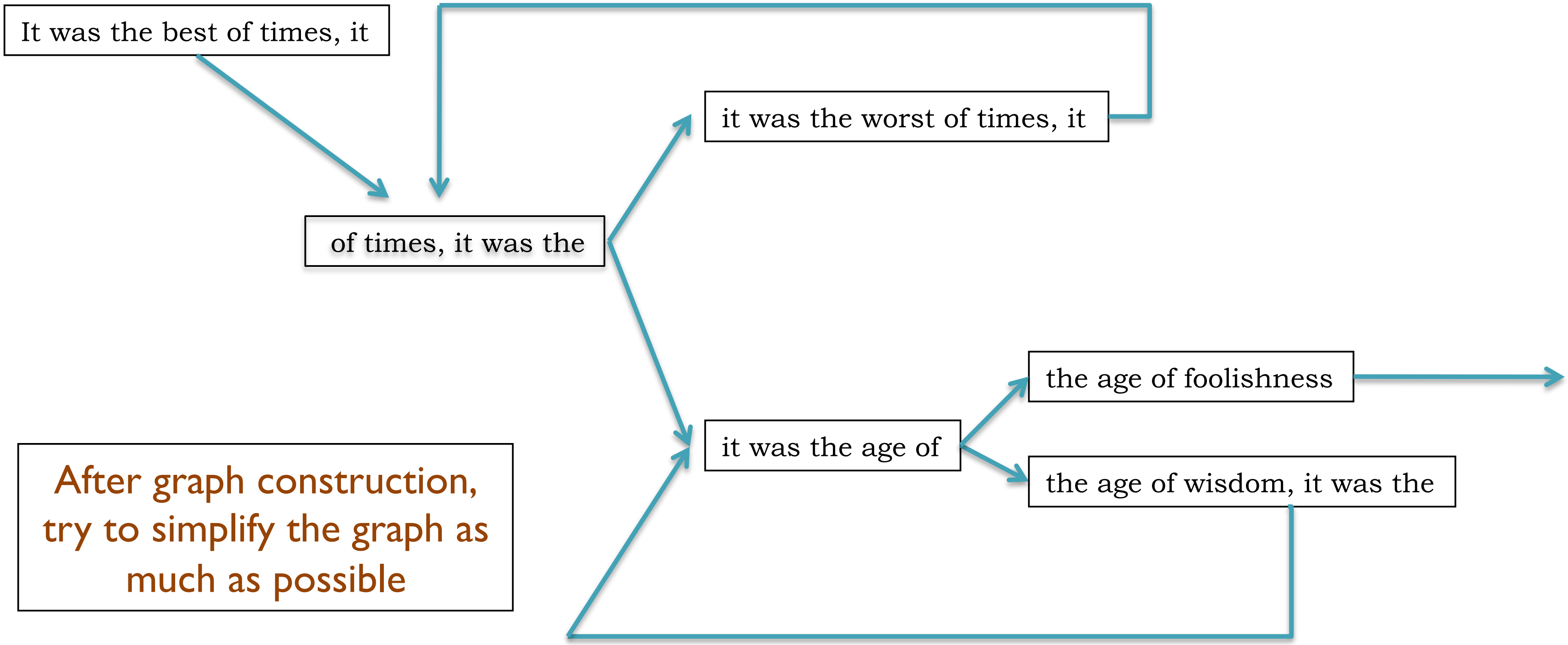
de Bruijn, 1946
Idury and Waterman, 1995
Pevzner, Tang, Waterman, 2001

de Bruijn Graph Assembly



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

de Bruijn Graph Assembly


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

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

The full tale

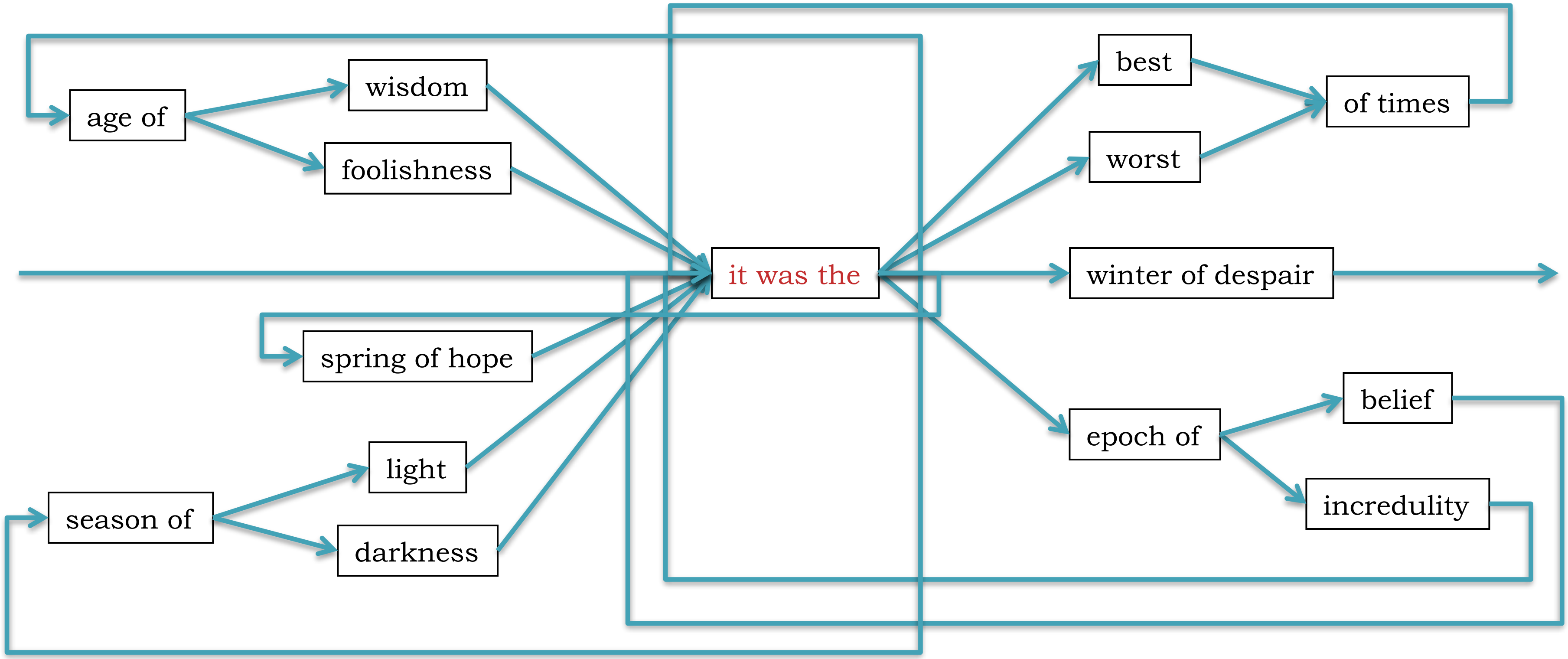
… it was the best of times it was the worst of times …

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

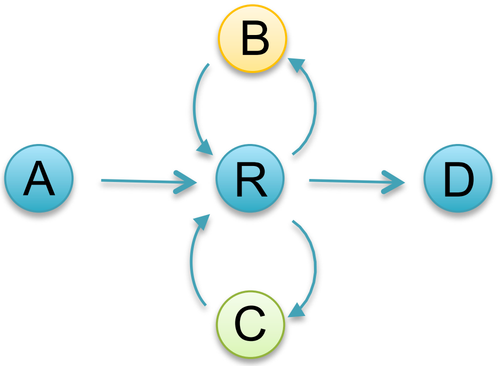
… it was the epoch of belief it was the epoch of incredulity …

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

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



Counting Eulerian Tours



ARBRCRD
or
ARCRBRD

Generally an exponential number of compatible sequences

– Value computed by application of the BEST theorem

$$\mathcal{W}(G,t) = (\det L)\left\{\prod_{u\in V}(r_u - 1)!\right\}\left\{\prod_{(u,v)\in E} a_{uv}!\right\}^{-1}$$

L = n x n matrix with r_u-a_{uu} along the diagonal and -a_{uv} in entry uv

r_u = $d^+(u)+1$ if $u=t$, or $d^+(u)$ otherwise

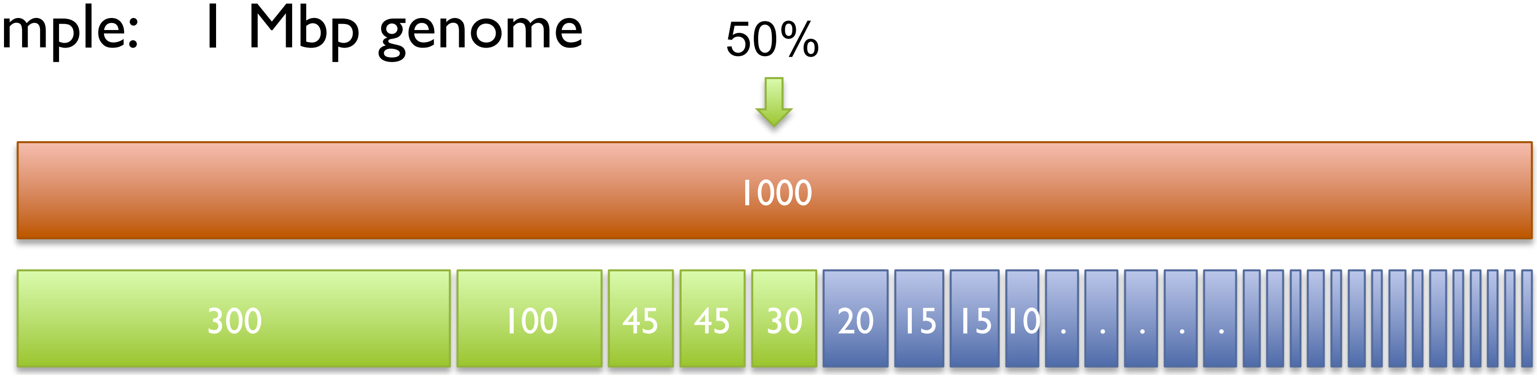
a_{uv} = multiplicity of edge from u to v

Assembly Complexity of Prokaryotic Genomes using Short Reads.
Kingsford C, Schatz MC, Pop M (2010) *BMC Bioinformatics.*

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:

N50 values are only meaningful to compare when base genome size is the same in all cases

Outline

1. Genome assembly by analogy

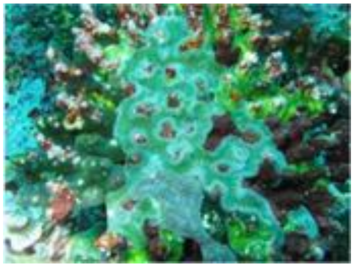
2. Hybrid error correction and assembly

3. De novo mutations in autism

Assembly Applications

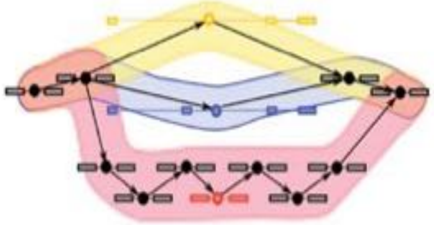
Novel genomes

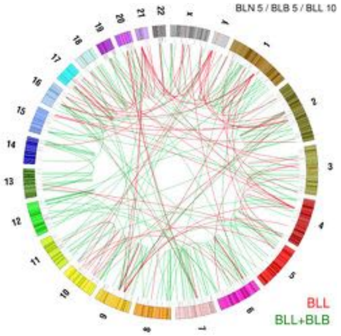
Metagenomes



Sequencing assays

- Transcript assembly
- Structural variations
- Haplotype analysis
- …





Why are genomes hard to assemble?

1. ***Biological***:
   - (Very) High ploidy, heterozygosity, repeat content

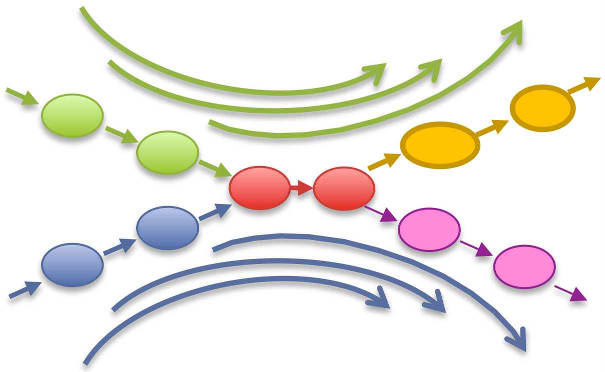
2. ***Sequencing***:
   - (Very) large genomes, imperfect sequencing

3. ***Computational***:
   - (Very) Large genomes, complex structure

4. ***Accuracy***:
   - (Very) Hard to assess correctness

Ingredients for a good assembly

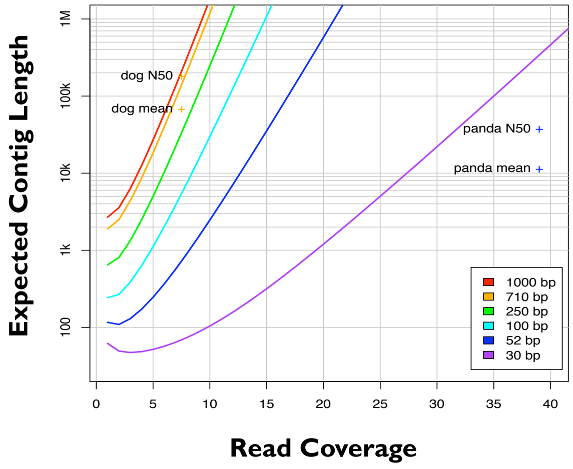
Read Length



Reads & mates must be longer than the repeats

- Short reads will have ***false overlaps*** forming hairball assembly graphs
- With long enough reads, assemble entire chromosomes into contigs

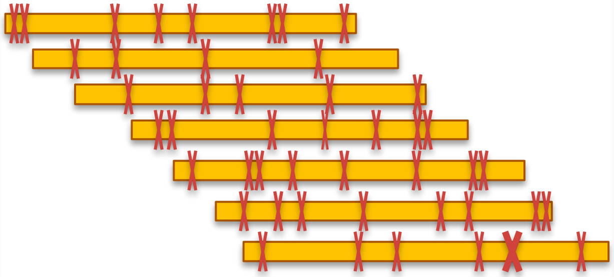
Coverage



High coverage is required

- Oversample the genome to ensure every base is sequenced with long overlaps between reads
- Biased coverage will also fragment assembly

Quality



Errors obscure overlaps

- Reads are assembled by finding kmers shared in pair of reads
- High error rate requires very short seeds, increasing complexity and forming assembly hairballs

Current challenges in *de novo* plant genome sequencing and assembly
Schatz MC, Witkowski, McCombie, WR (2012) *Genome Biology*. 12:243

Hybrid Sequencing

Illumina
Sequencing by Synthesis

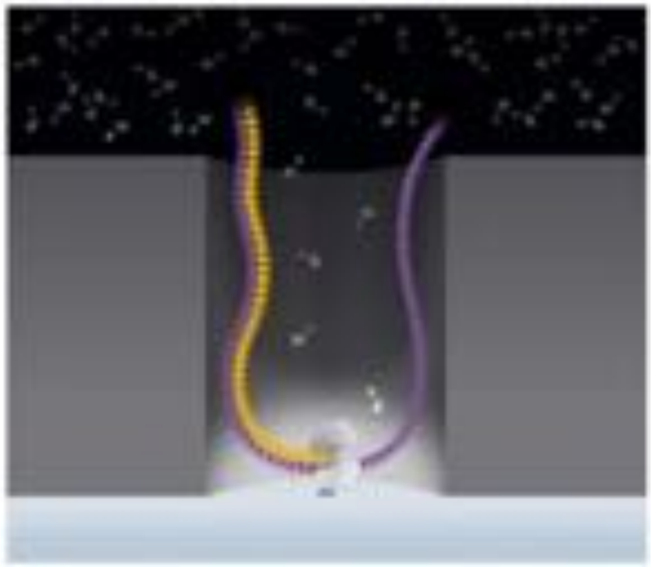
High throughput (60Gbp/day)
High accuracy (~99%)
Short reads (~100bp)

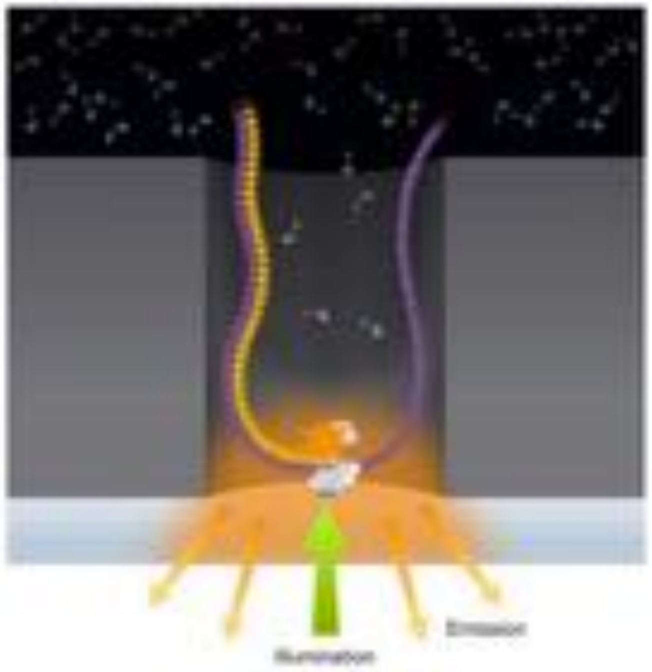
Pacific Biosciences
SMRT Sequencing

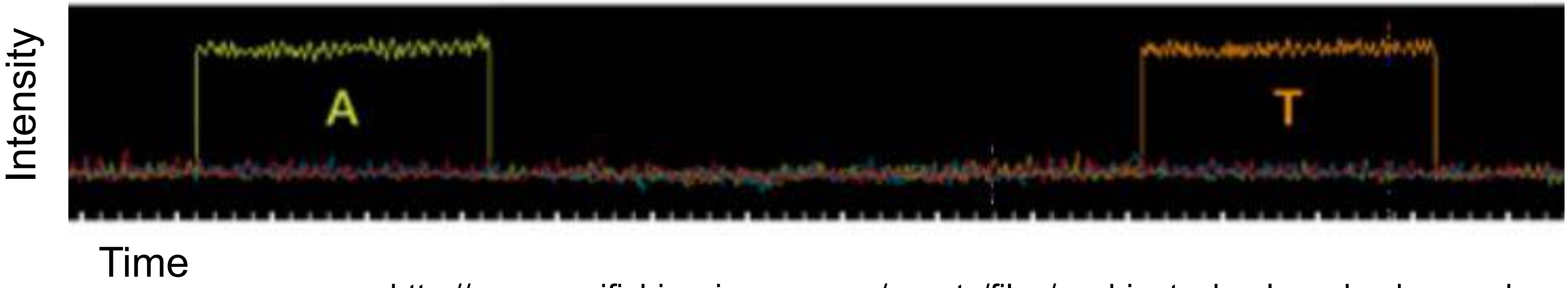
Lower throughput (600Mbp/day)
Lower accuracy (~90%)
Long reads (2-5kbp+)

SMRT Sequencing

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

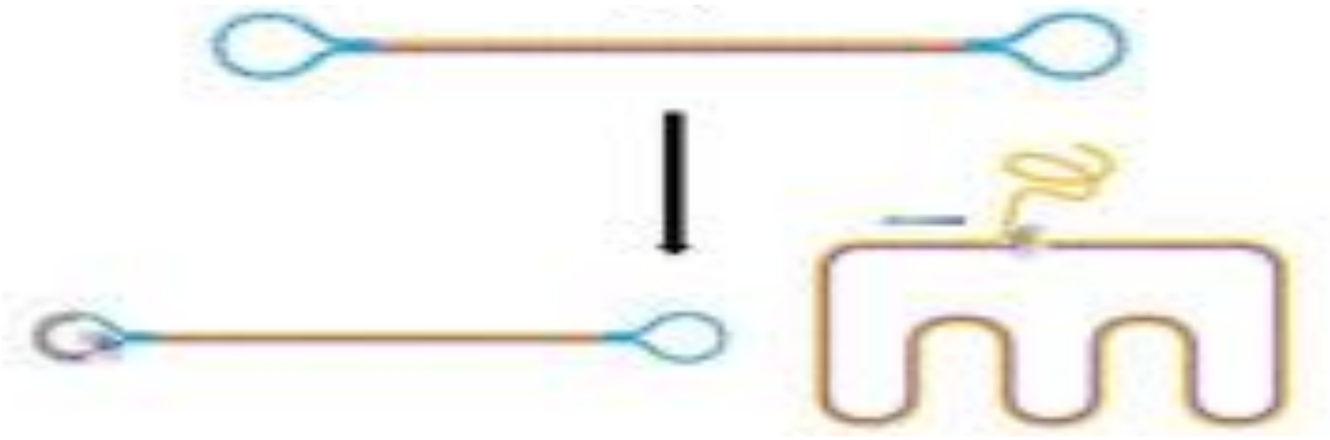






http://www.pacificbiosciences.com/assets/files/pacbio_technology_backgrounder.pdf

SMRT Read Types

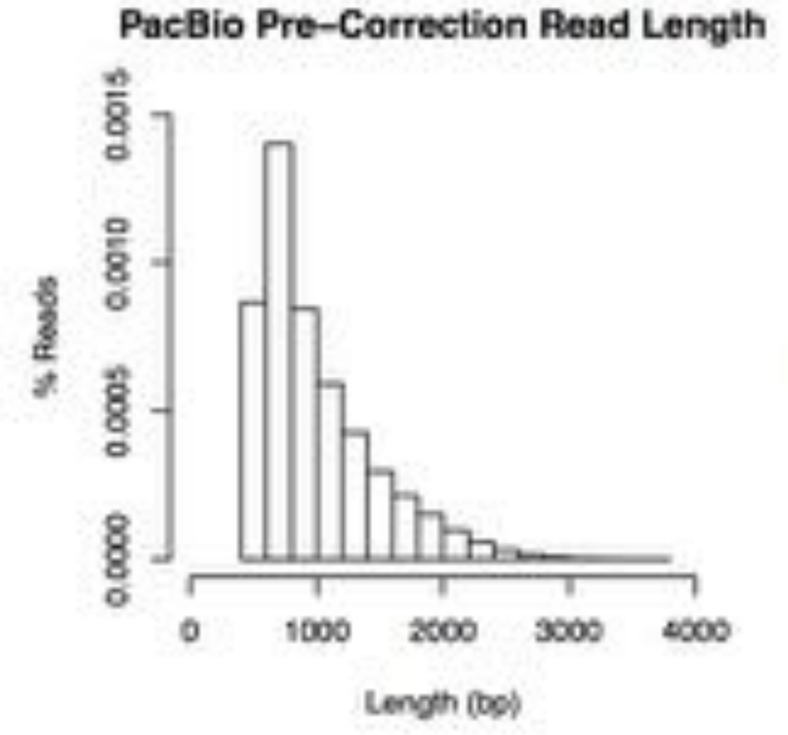


- ***Standard sequencing***
  - Long inserts so that the polymerase can synthesize along a single strand

- ***Circular consensus sequencing***
  - Short inserts, so polymerase can continue around the entire SMRTbell multiple times and generate multiple sub-reads from the same single molecule.
  - Barbell sequence: ATCTCTCTCttttcctcctcctccgttgttgttgttGAGAGAGAT

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

SMRT Sequencing Data



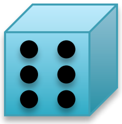
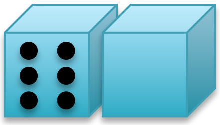
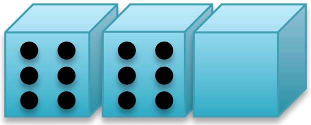
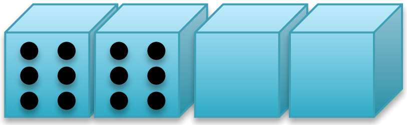
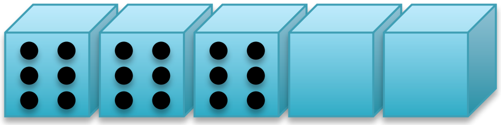
Match	83.7%
Insertions	11.5%
Deletions	3.4%
Mismatch	1.4%

```
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
```

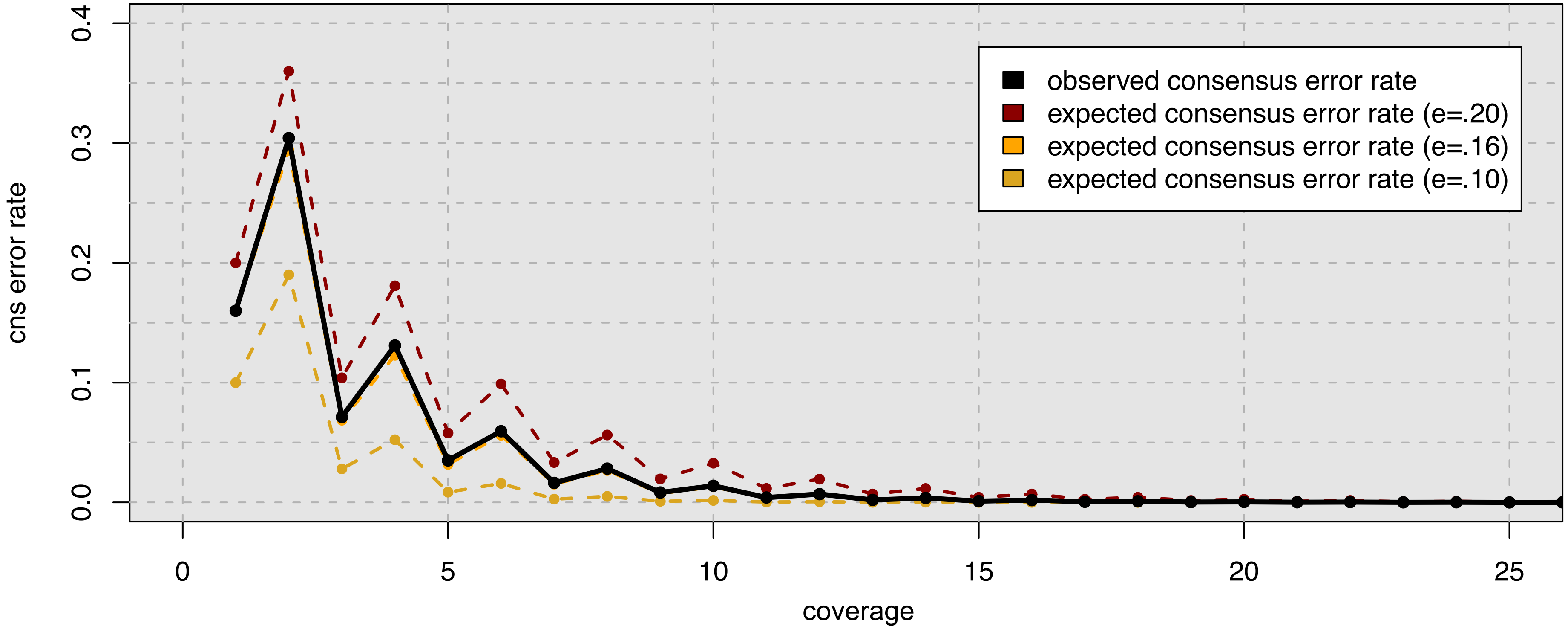
Sample of 100k reads aligned with BLASR requiring >100bp alignment

Consensus Quality: Probability Review

Roll *n* dice => What is the probability that at least half are 6's

n	*Min to Lose*	*Losing Events*	*P(Lose)*
1		*1/6*	*16.7%*
2		*P(1of 2) + P(2 of 2)*	*30.5%*
3		*P(2 of 3) + P(3 of 3)*	*7.4%*
4		*P(2 of 4) + P(3 of 4) + P(4 of 4)*	*13.2%*
5		*P(3 of 5) + P(4 of 5) + P(5 of 5)*	*3.5%*
n	*ceil(n/2)*	$\sum_{i=\lceil n/2\rceil}^{n} P(i\ \ of\ \ n) = \sum_{i=\lceil n/2\rceil}^{n}\binom{n}{i}(p)^{i}(1-p)^{n-i}$	

Consensus Accuracy and Coverage



Coverage can overcome random errors

- Dashed: error model from binomial sampling; solid: observed accuracy
- For same reason, CCS is extremely accurate when using 5+ subreads

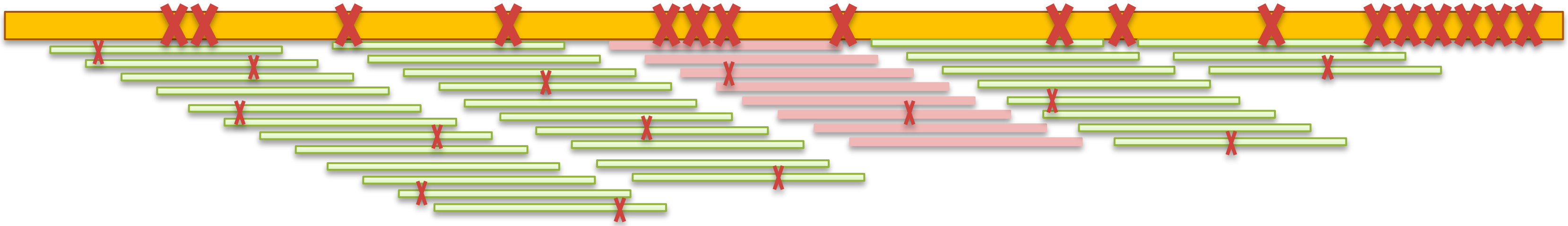
$$CNS\ Error = \sum_{i=\lceil c/2 \rceil}^{c} \binom{c}{i} (e)^{i} (1-e)^{n-i}$$

PacBio Error Correction

http://wgs-assembler.sf.net

1. Correction Pipeline
   1. Map short reads to long reads
   2. Trim long reads at coverage gaps
   3. Compute consensus for each long read

2. Error corrected reads can be easily assembled, aligned



Hybrid error correction and de novo assembly of single-molecule sequencing reads.
Koren, S, Schatz, MC, et al. (2012) *Nature Biotechnology*. doi:10.1038/nbt.2280

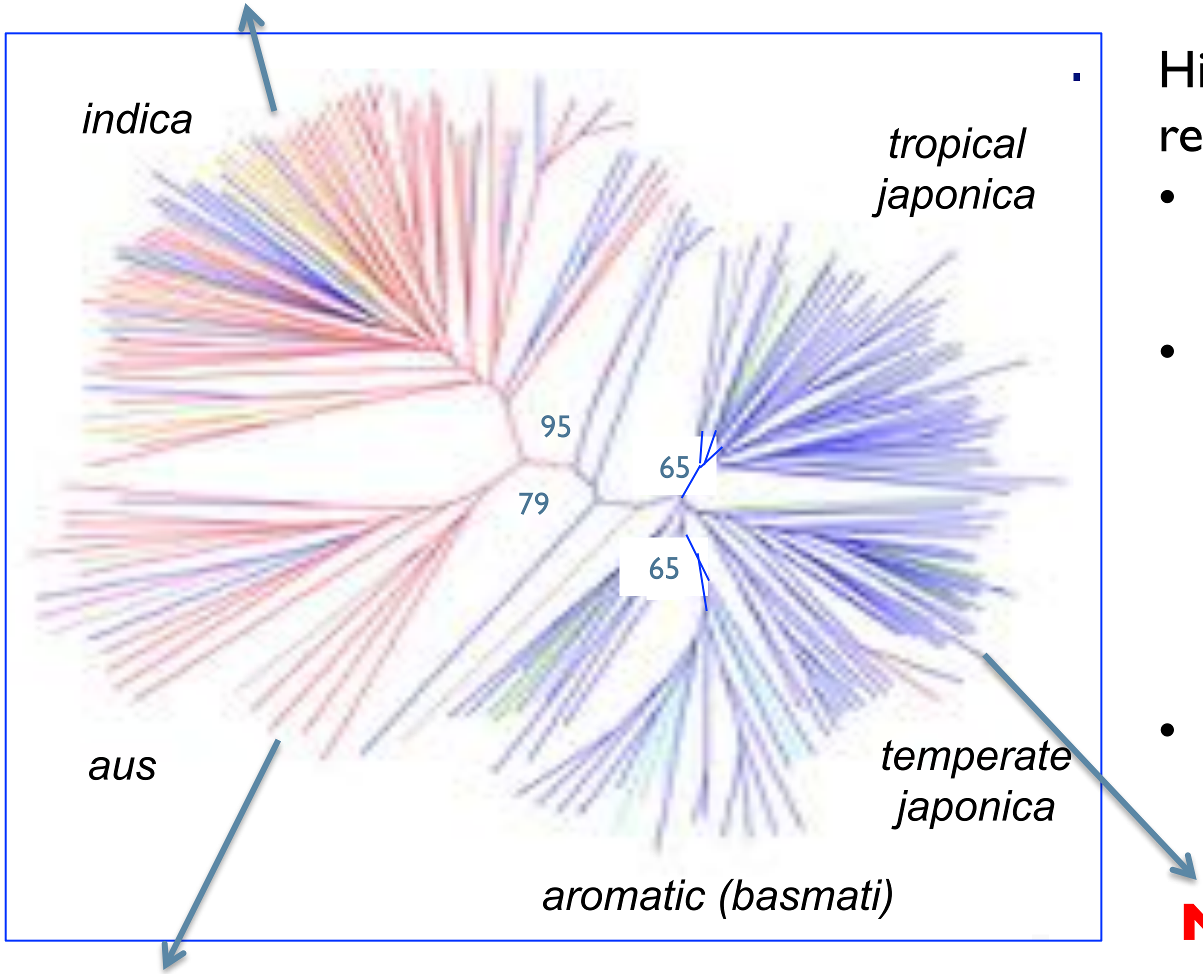
Plant Genomics

- Motivations
  - 15 crops provide 90% of the world's food
  - Responsible for maintaining the balance of the carbon cycles, soil from erosion
  - Promising sources of renewable energy
  - Plant byproducts used in many medicines
  - Model organisms for studying biological systems

- Challenges
  - Very large genomes, some many times larger than human
  - High repeat content, especially high copy retrotransposons
  - High ploidy, high heterozygosity

Population structure in *Oryza sativa*

3 varieties selected for *de novo* sequencing

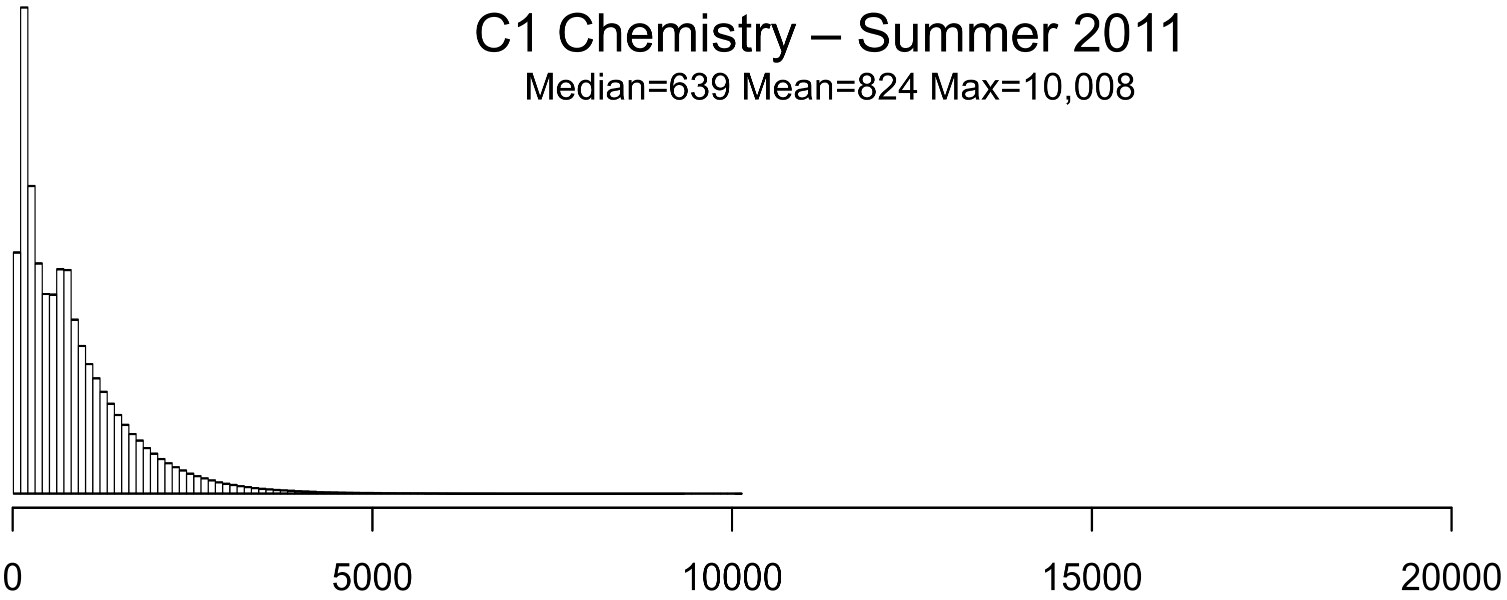


Garris et al. (2005)
Genetics 169: 1631–1638

High quality BAC-by-BAC reference

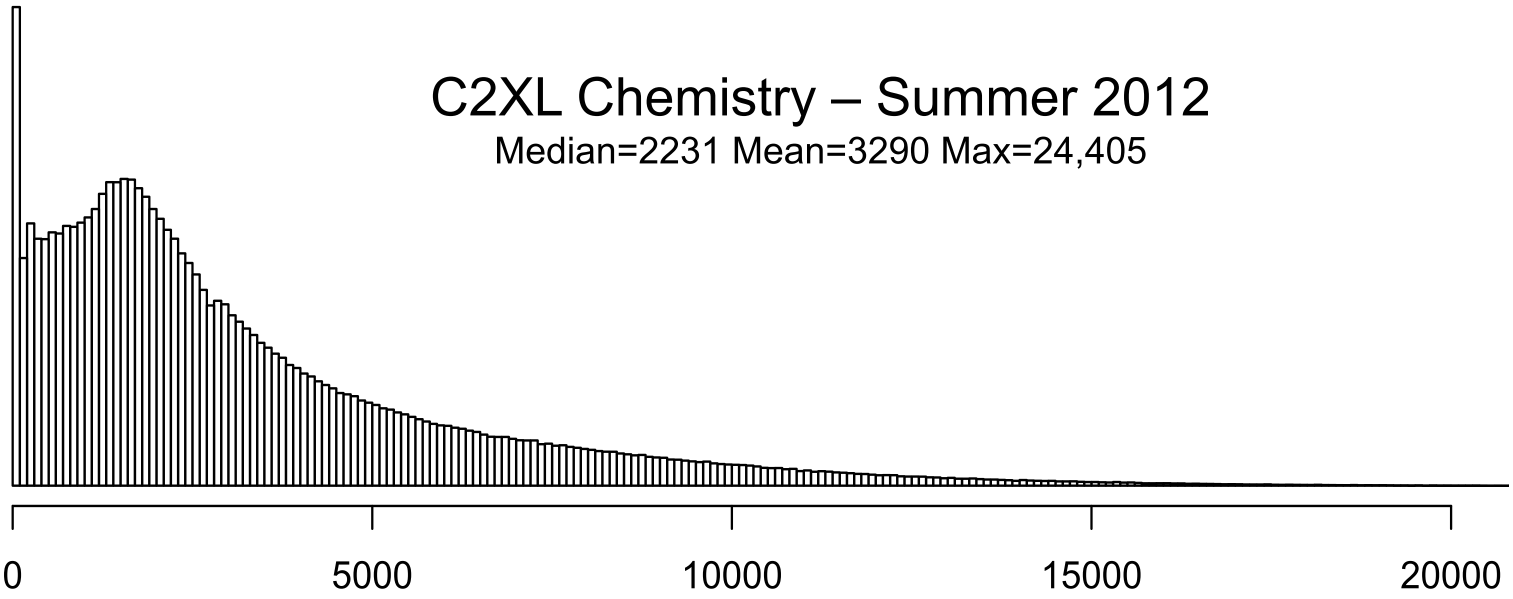
- ~370 Mbp genome in 12 chromosomes
- About 40% repeats:
  - Many 4-8kbp repeats
  - 300kbp max high identity repeat (99.99%)
- Useful model for other cereal genomes

PacBio Long Read Rice Sequencing



C1 Chemistry – Summer 2011

Median=639 Mean=824 Max=10,008

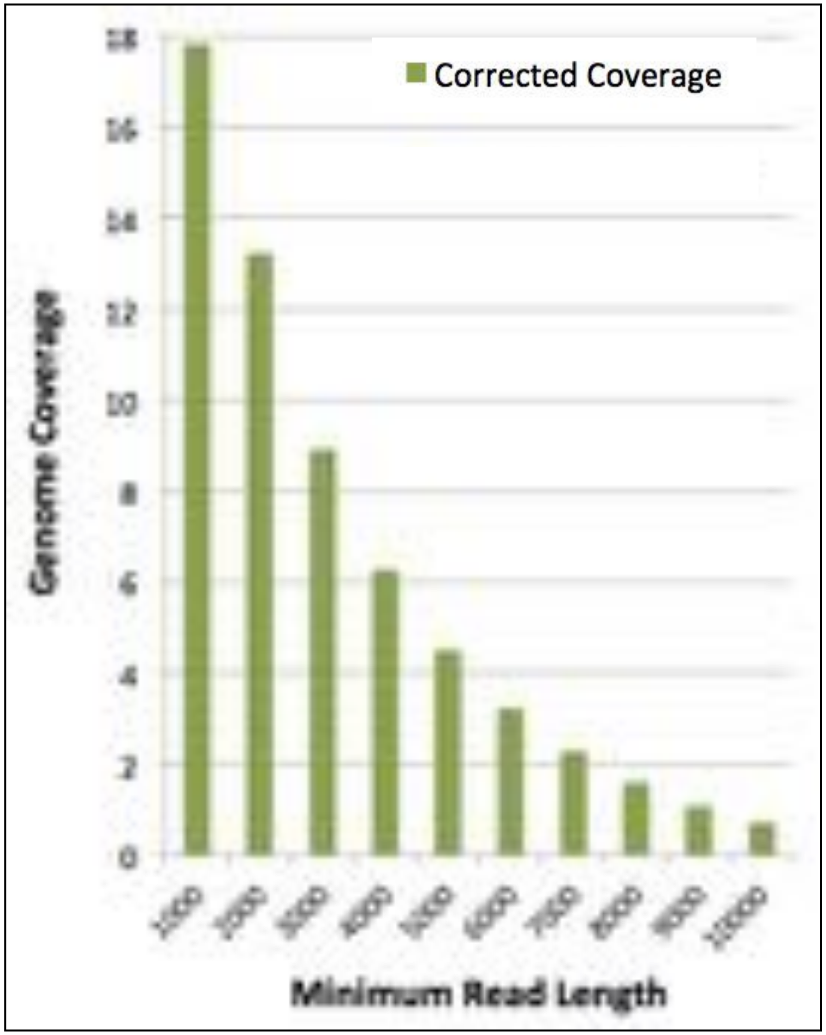


C2XL Chemistry – Summer 2012

Median=2231 Mean=3290 Max=24,405

Preliminary Rice Assemblies

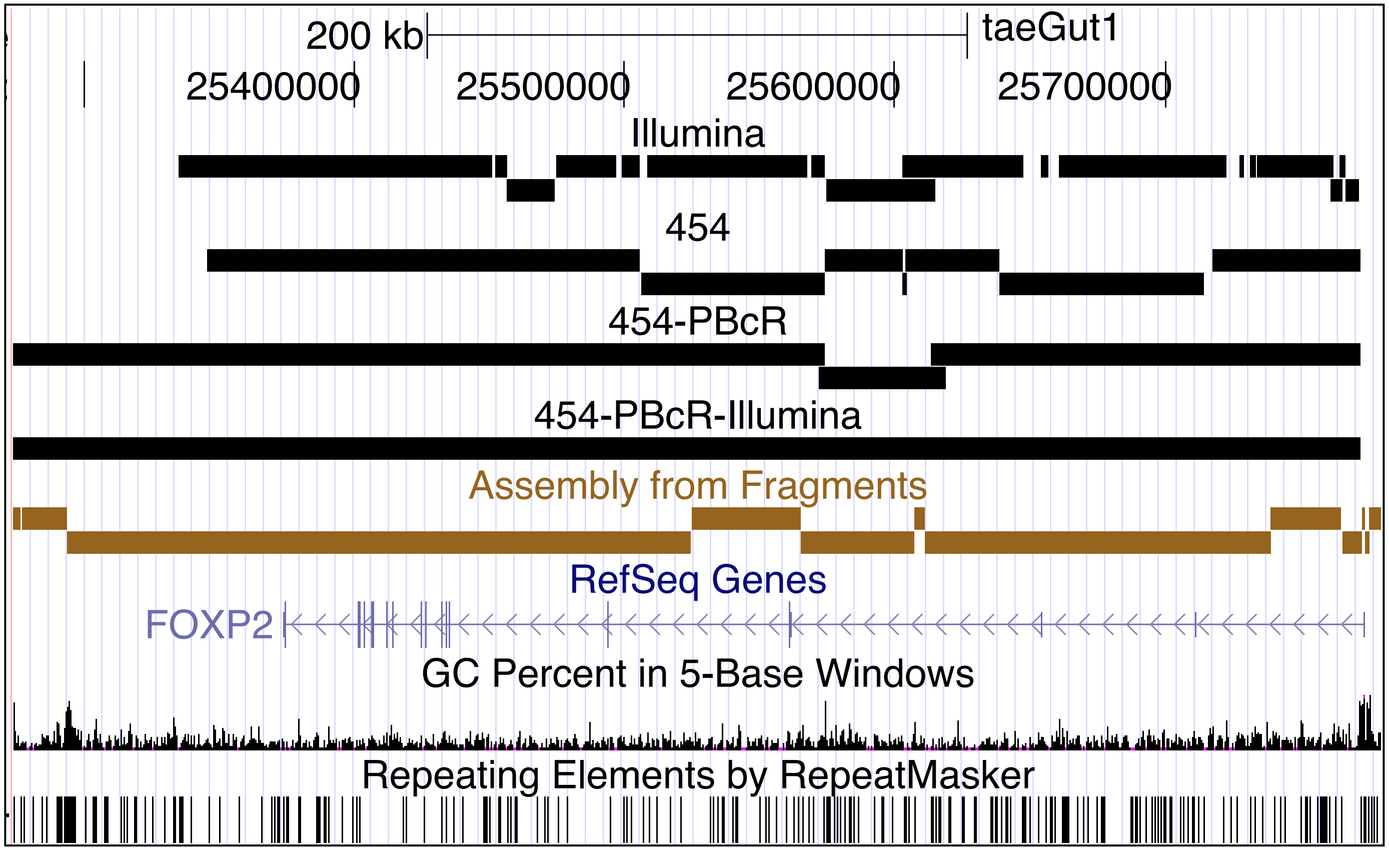
Assembly	Contig NG50
HiSeq Fragments 50x 2x100bp @ 180	3,925
MiSeq Fragments 23x 459bp 8x 2x251bp @ 450	6,332
"ALLPATHS-recipe" 50x 2x100bp @ 180 36x 2x50bp @ 2100 51x 2x50bp @ 4800	18,248
PBeCR Reads 7x @ 3500 ** MiSeq for correction	50,995
PBeCR + Illumina Shred 7x @ 3500 ** MiSeq for correction 5x @ 3000bp shred	59,695



In collaboration with McCombie & Ware labs @ CSHL

Improved Gene Reconstruction

FOXP2 assembled in a single contig in the PacBio parrot assembly

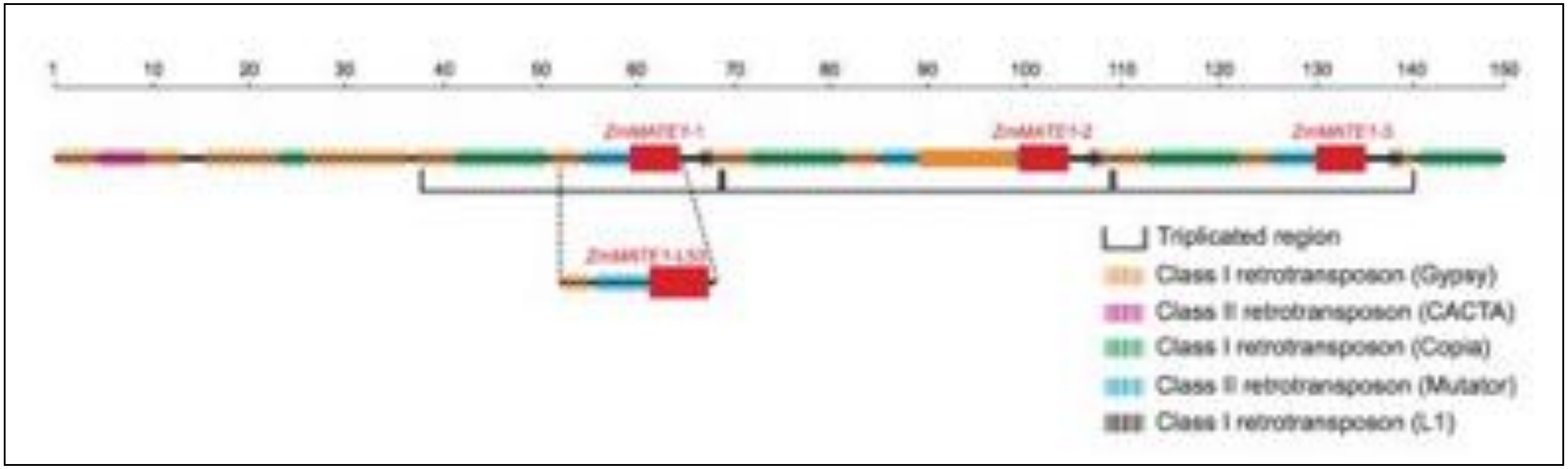


Hybrid error correction and de novo assembly of single-molecule sequencing reads.
Koren, S, Schatz, MC, et al. (2012) *Nature Biotechnology*. doi:10.1038/nbt.2280

Long Read CNV Analysis

Aluminum tolerance in maize is important for drought resistance and protecting against nutrient deficiencies

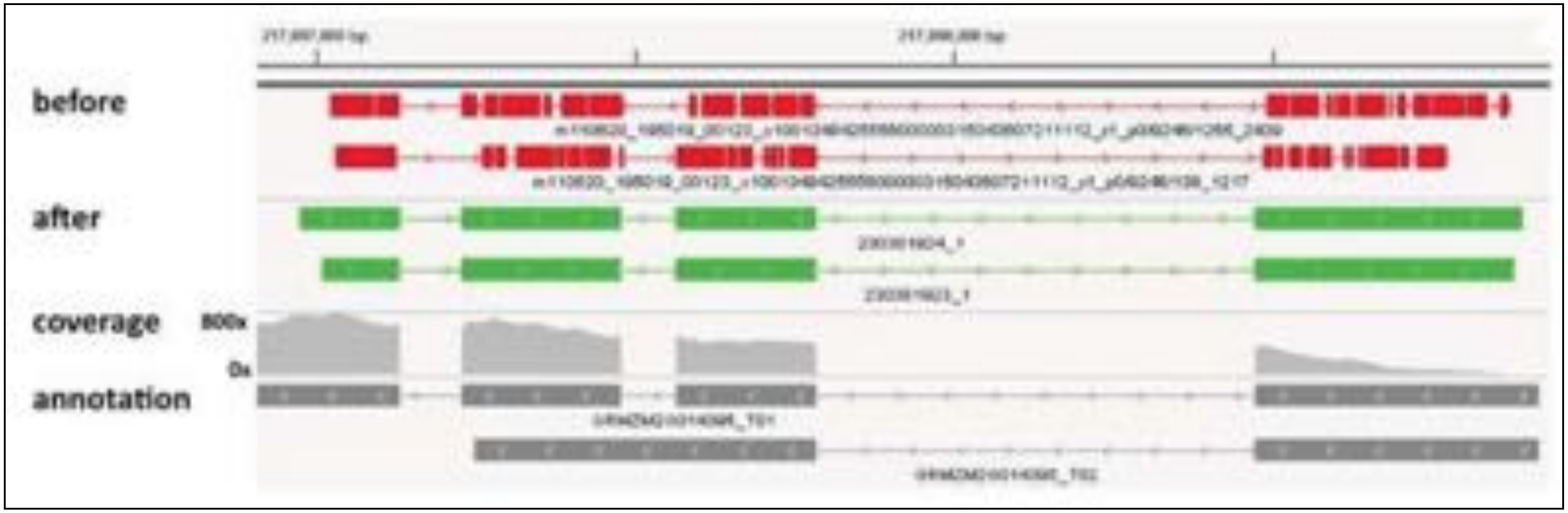
- Segregating population localized a QTL on a BAC, but unable to genotype with Illumina sequencing because of high repeat content and GC skew
- Long read PacBio sequencing corrected by CCS reads revealed a triplication of the ZnMATE1 membrane transporter


1
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
ZmMATE1-1
ZmMATE1-2
ZmMATE1-3
ZmMATE1-L537
Triplicated region
Class I retrotransposon (Gypsy)
Class II retrotransposon (CACTA)
Class I retrotransposon (Copia)
Class II retrotransposon (Mutator)
Class I retrotransposon (L1)

A rare gene copy-number variant that contributes to maize aluminum tolerance and adaptation to acid soils

Maron, LG *et al.* (2012) *PNAS.* doi: 10.1073/pnas.1220766110

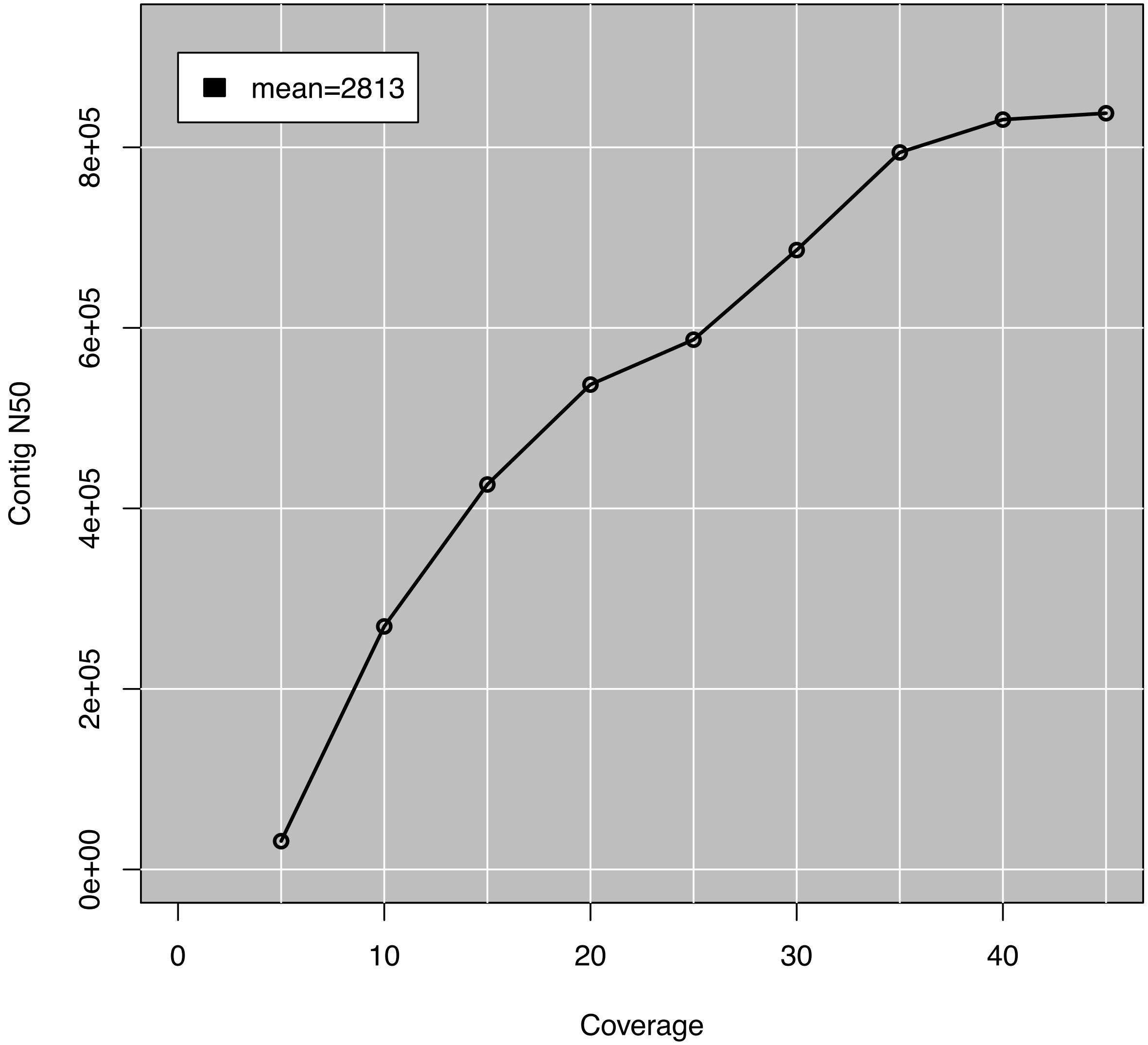
Transcript Alignment



- Long-read single-molecule sequencing has potential to directly sequence full length transcripts
  - Raw reads and raw alignments (red) have many spurious indels inducing false frameshifts and other artifacts
  - Error corrected reads almost perfectly match the genome, pinpointing splice sites, identifying alternative splicing
- New collaboration with Gingeras Lab looking at splicing in human

Hybrid error correction and de novo assembly of single-molecule sequencing reads.
Koren, S, Schatz, MC, et al. (2012) *Nature Biotechnology*. doi:10.1038/nbt.2280

Assembly Coverage Model

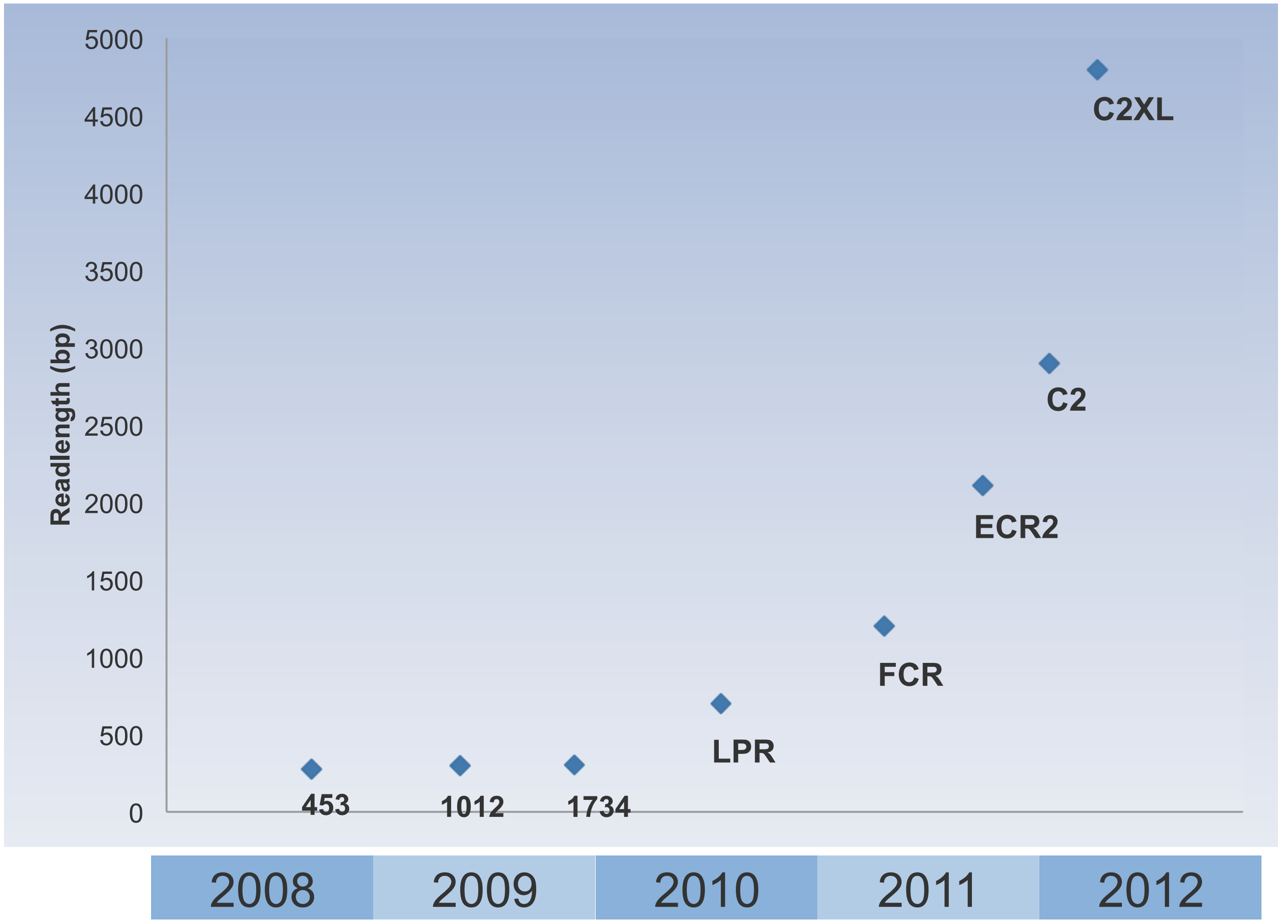


Simulate PacBio-like reads to predict how the assembly will improve as we add additional coverage

Only 8x coverage is needed to sequence every base in the genome, but 40x improves the chances repeats will be spanned by the longest reads

Assembly complexity of long read sequencing
Marcus, S, Lee, H, Gurtowski, J, Schatz MC et al. (2013) *In preparation*

PacBio Technology Roadmap



Internal Roadmap has made steady progress towards improving read length and throughput

Very recent improvements:

1. Improved enzyme:

   Maintains reactions longer

2. "Hot Start" technology:

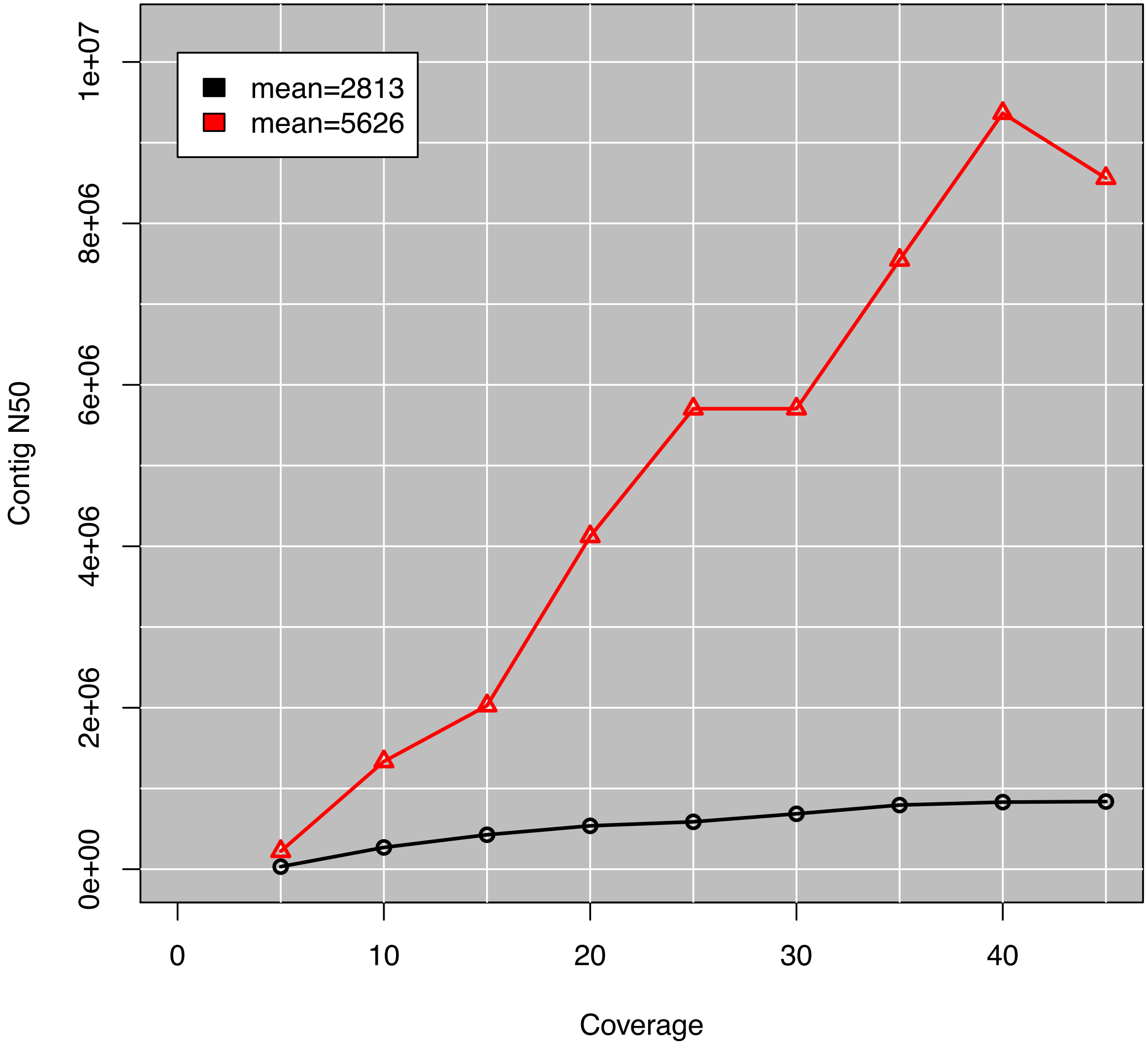
   Maximize subreads

3. MagBead loading:

   Load longest fragments

PacBio Users Meeting, June 18, Frederick MD

Speculation for 2014



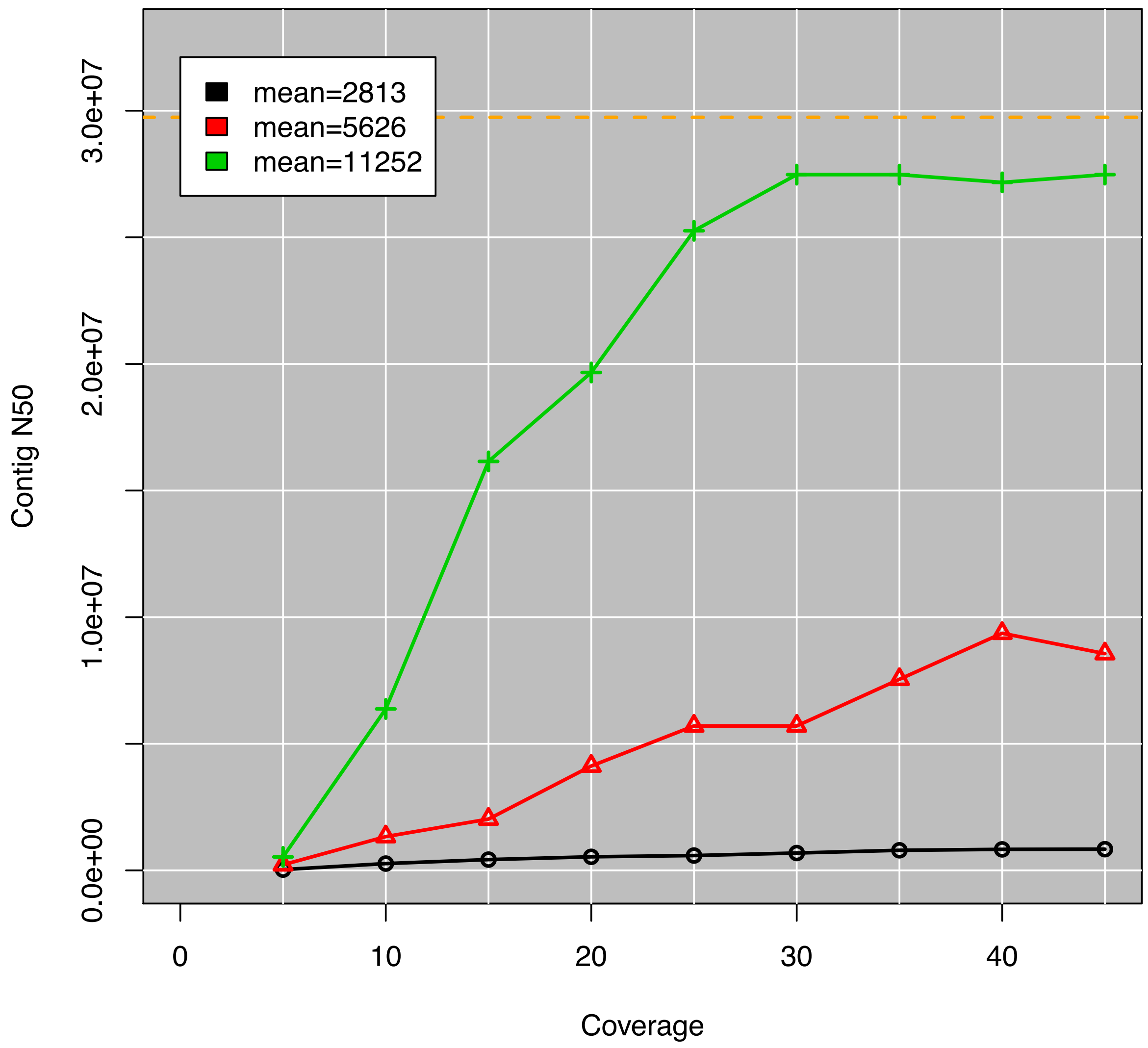
Doubling the average read length dramatically improves the assembly quality

- Able to span a larger repeats and lock contigs together

Expect to see contig N50 values over 1Mbp very soon, even in very complicated plant and animal species

- Megabase contig N50 already routine in microbial assembly with PacBio sequencing

Speculation for 2014

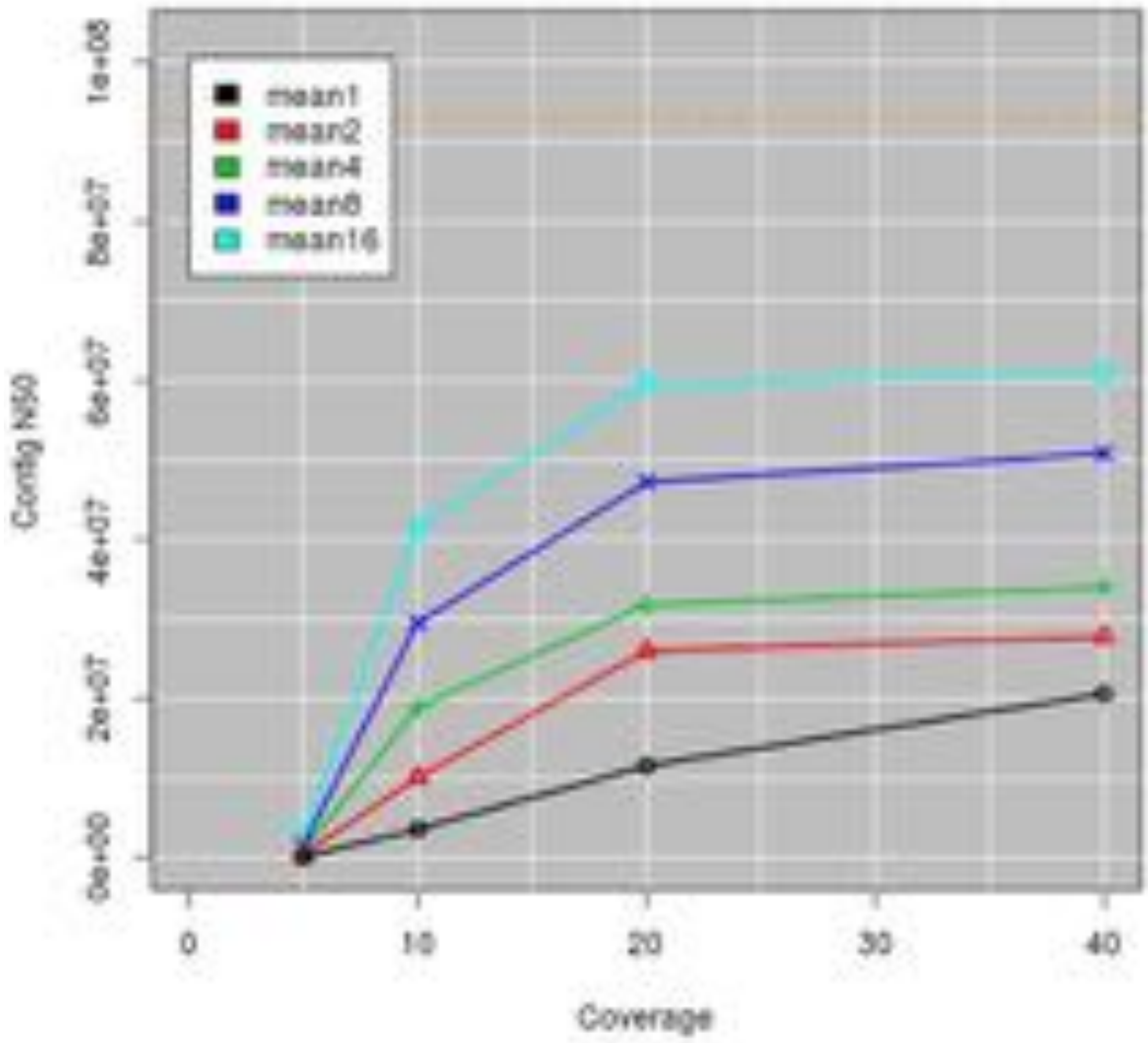


With PacBio-like reads averaging 11.2kbp (4x current), we should be able to assemble almost every chromosome arm of rice into single contigs

- The 300kbp near perfect repeat is the only exception

Even with the current assembly, we are seeing new genes and other sequences missing in the "high quality" BAC-by-BAC reference genome

Speculation for 2015



For human, it will still take a few more rounds of read length doubling before we should expect to see single contig chromosome arms

However, we can still learn a lot of interesting biology about the ~13% of the human genome that is currently inaccessible

Genomic Dark Matter: The reliability of short read mapping illustrated by the GMS.
Lee, H., Schatz, M.C. (2012) *Bioinformatics*. 10.1093/bioinformatics/bts330

Outline

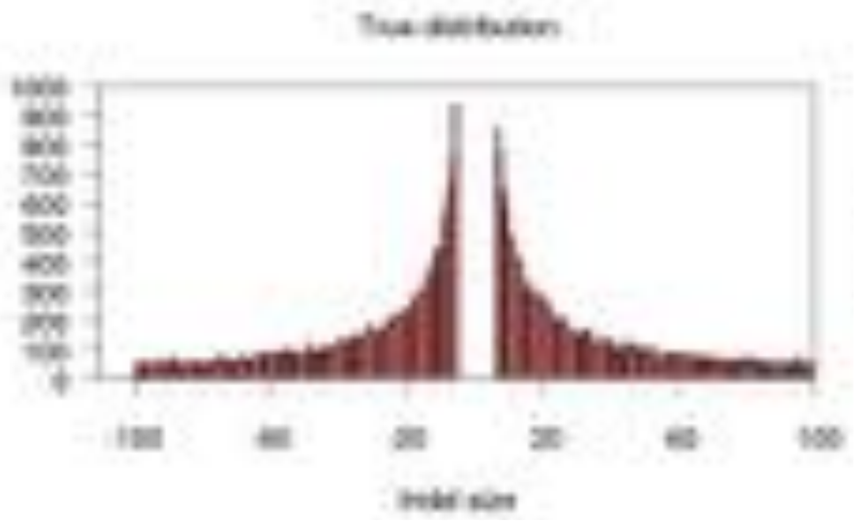
1. Genome assembly by analogy
2. Hybrid error correction and assembly
3. De novo mutations in autism

Variation Detection Complexity

SNPs + Short Indels

High precision and sensitivity

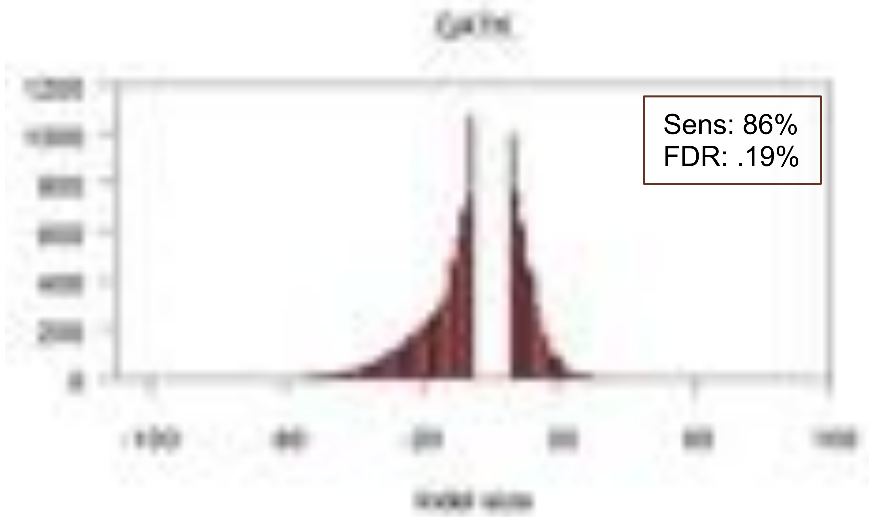
```
..TTTAGAATAG-CGAGTGC...
     ||||||| ||||
     AGAATAGGCGAG
```



"Long" Indels (>5bp)

Reduced precision and sensitivity

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



Sens: 86%
FDR: .19%

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

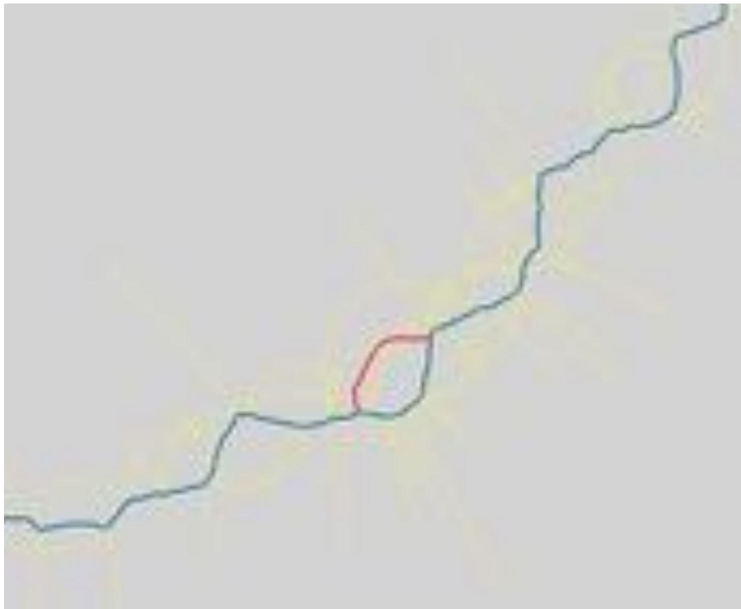
Scalpel: Haplotype Microassembly

G. Narzisi, D. Levy, I. Iossifov, J. Kendall, M. Wigler, M. Schatz

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

Features

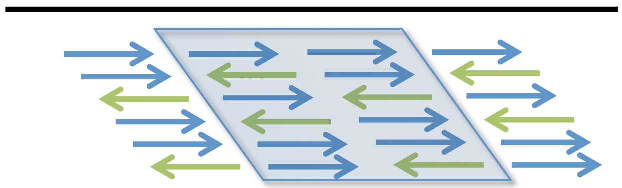
1. Combine mapping and assembly
2. Exhaustive search of haplotypes
3. De novo mutations



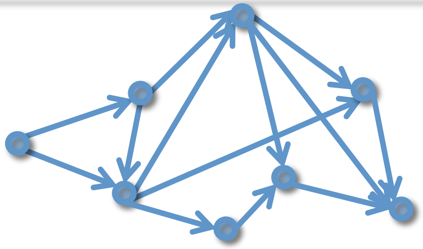
NRXN1 *de novo* SNP
(auSSC12501 chr2:50724605)

Scalpel Pipeline

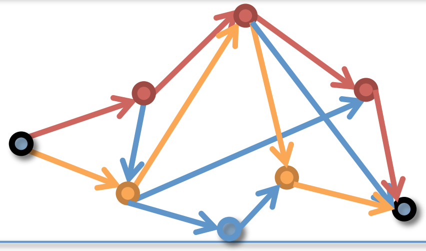
Extract reads mapping within the exon including (1) well-mapped reads, (2) soft-clipped reads, and (3) anchored pairs



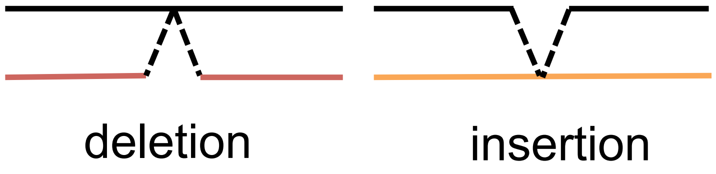
Decompose reads into overlapping *k*-mers and construct de Bruijn graph from the reads



Find end-to-end haplotype paths spanning the region

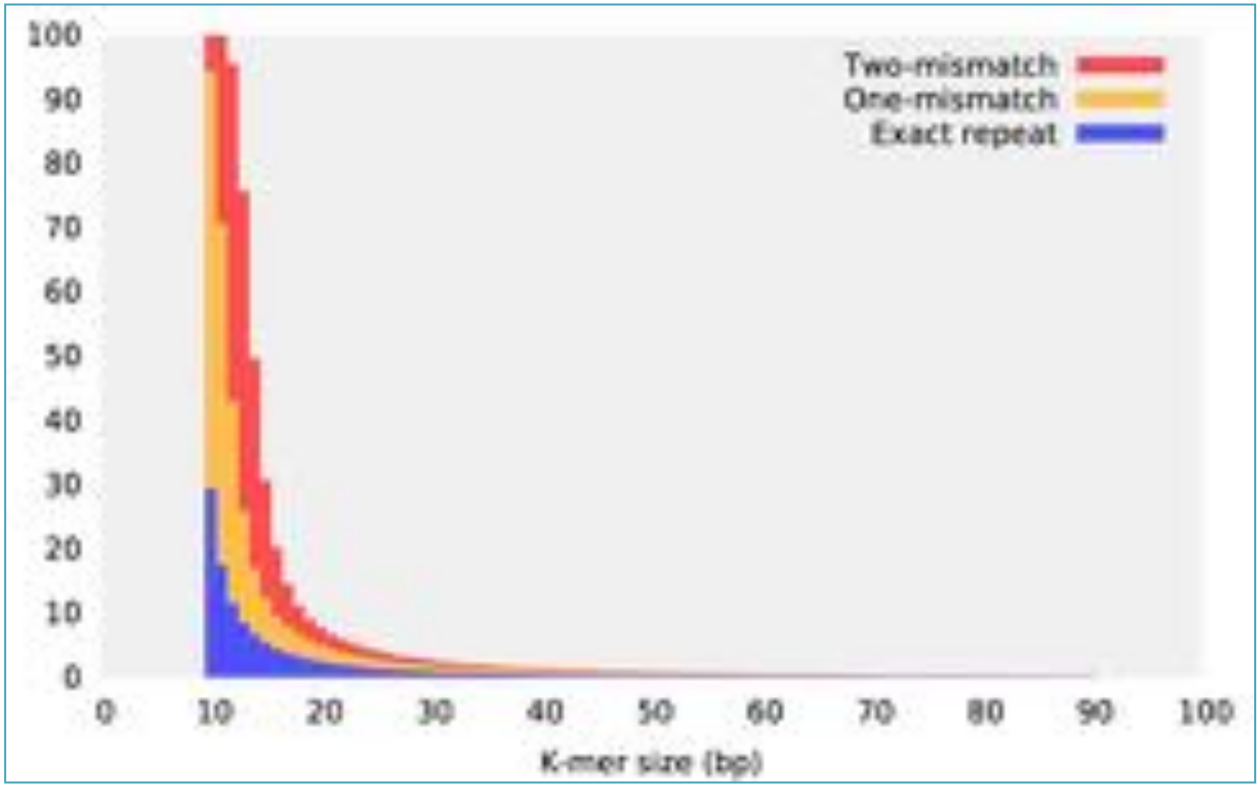


Align assembled sequences to reference to detect mutations



Repeats in the Genome

Specificity Challenge: 30% of exons have a perfect 10bp or larger repeat



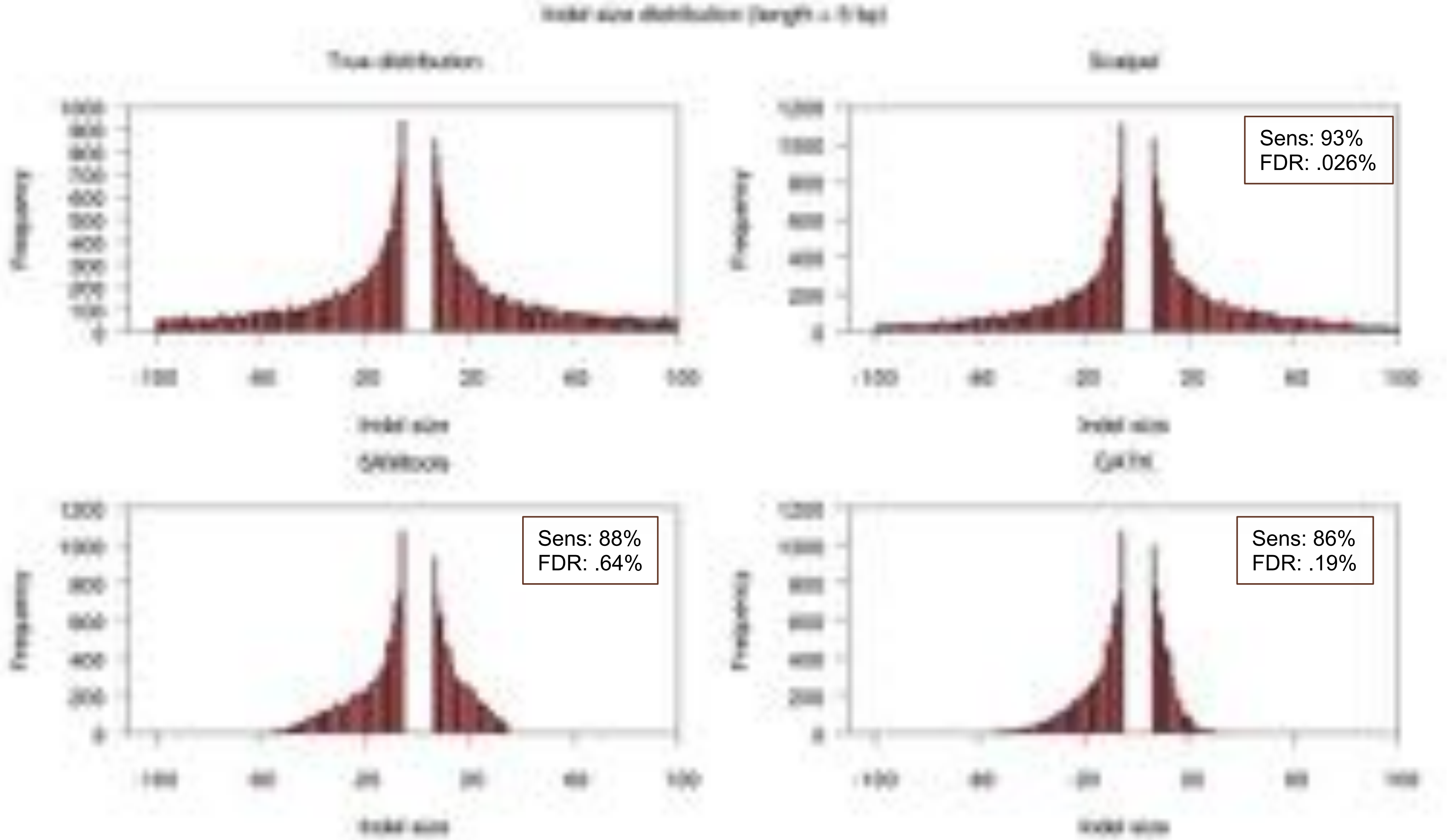
Reference Exon: Localized repeat sequence

TTTAGCAAGGGTTGAAGAA C GGCTAAAAGCTTTGCCACATTTTTGTAGGGTTTCTCTCCAGT A TGAATTCTCTTATG TTTAGCAAGGGTTGAAGAA T GGCTAAAAGCTTTGCCACATTTTTGTAGGGTTTCTCTCCAGT G TGTATCCTCTT

AGGGTTGAAGAA C GGCTAAAAGCTTTGCCACATTTTTGTAGGGTTTCTCTCCAGT G TGTATCCTCTT

Variant Read: Large deletion or critical snp?

Scalpel Indel Discovery



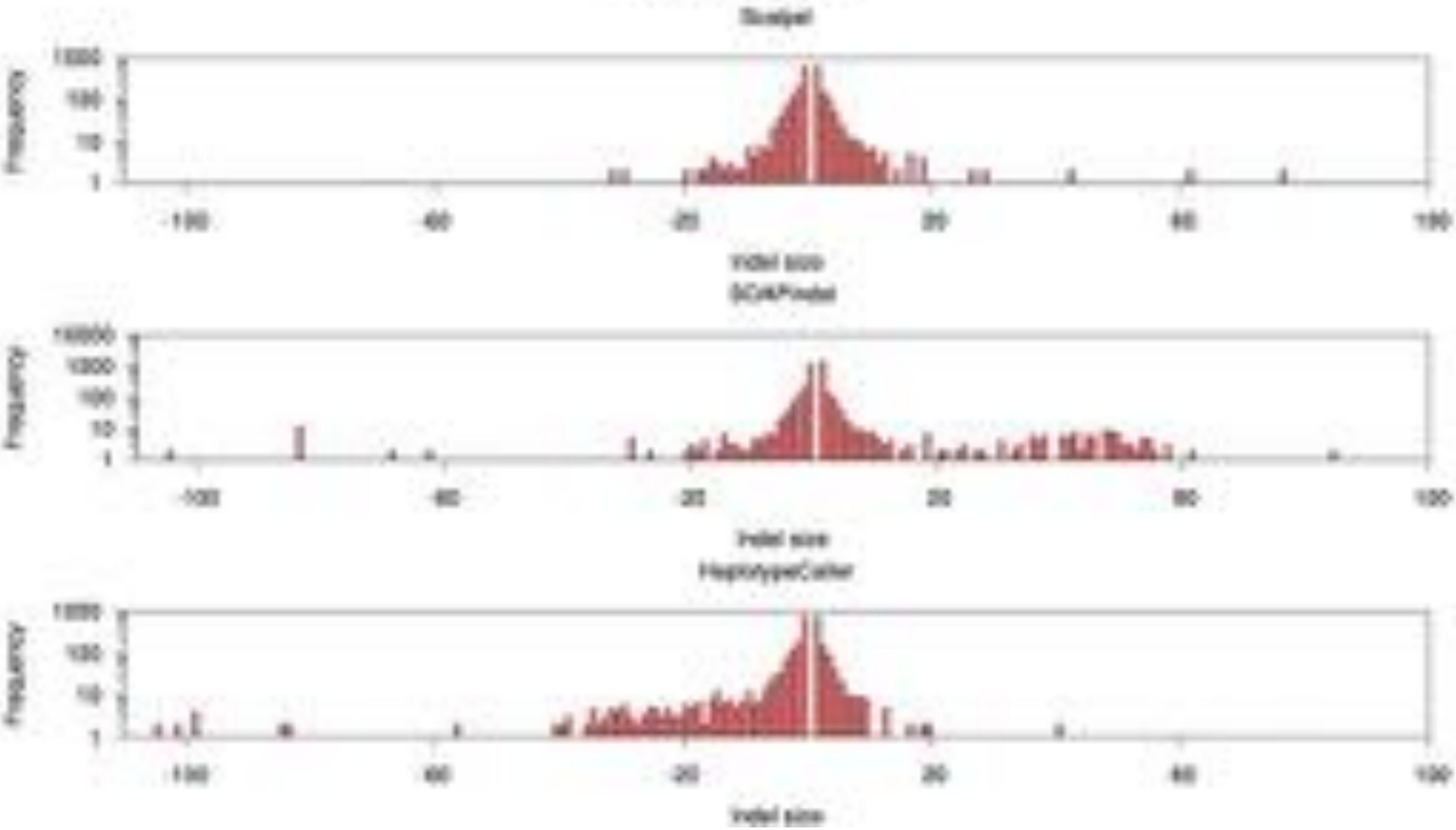
Sens: 93%
FDR: .026%

Sens: 88%
FDR: .64%

Sens: 86%
FDR: .19%

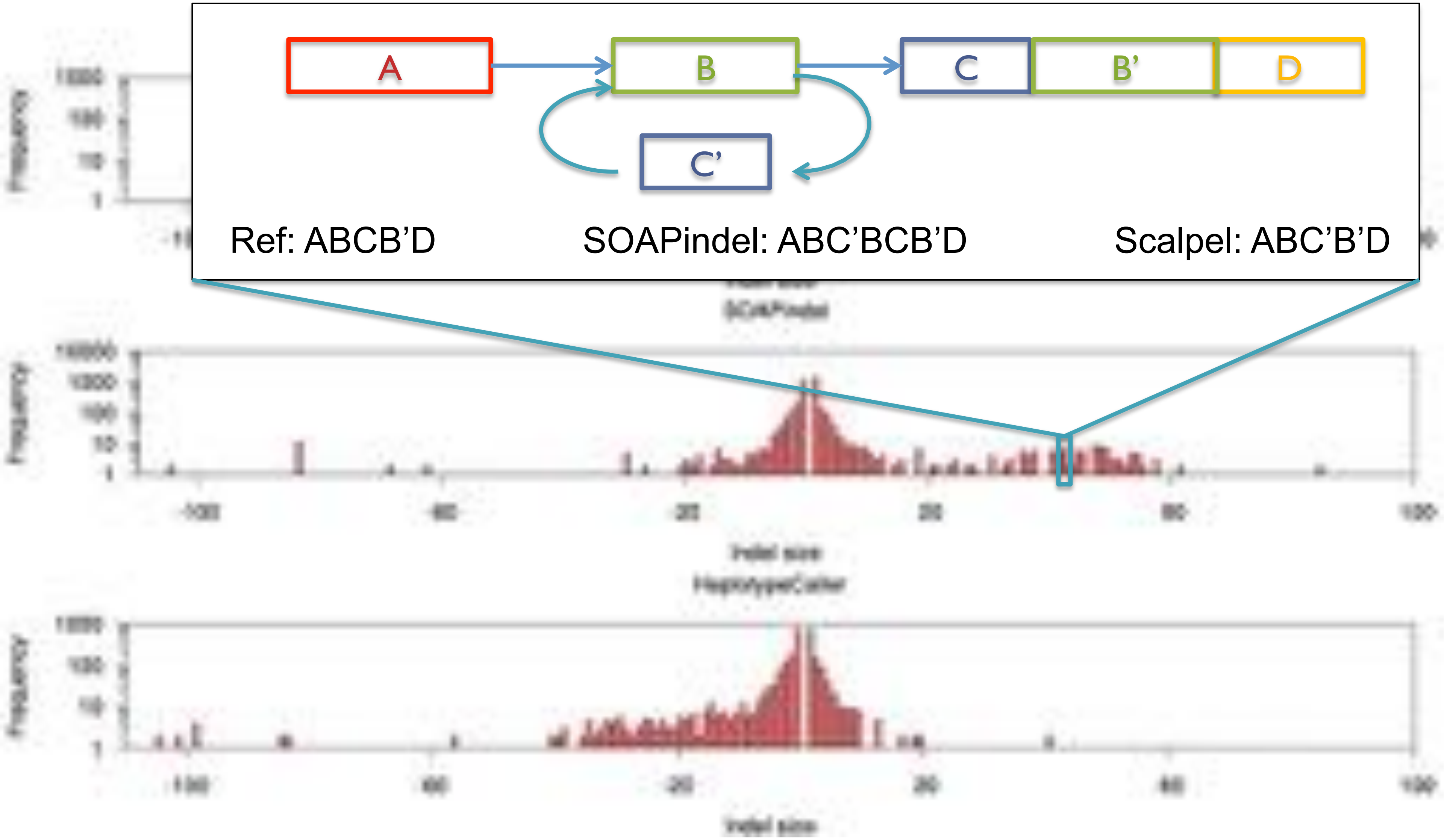
Detection of de novo mutations in exome-capture data using micro-assembly
Narzisi *et al.* (2013) *In preparation*

Scalpel Indel Discovery



Detection of de novo mutations in exome-capture data using micro-assembly
Narzisi *et al.* (2013) *In preparation*

Scalpel Indel Discovery



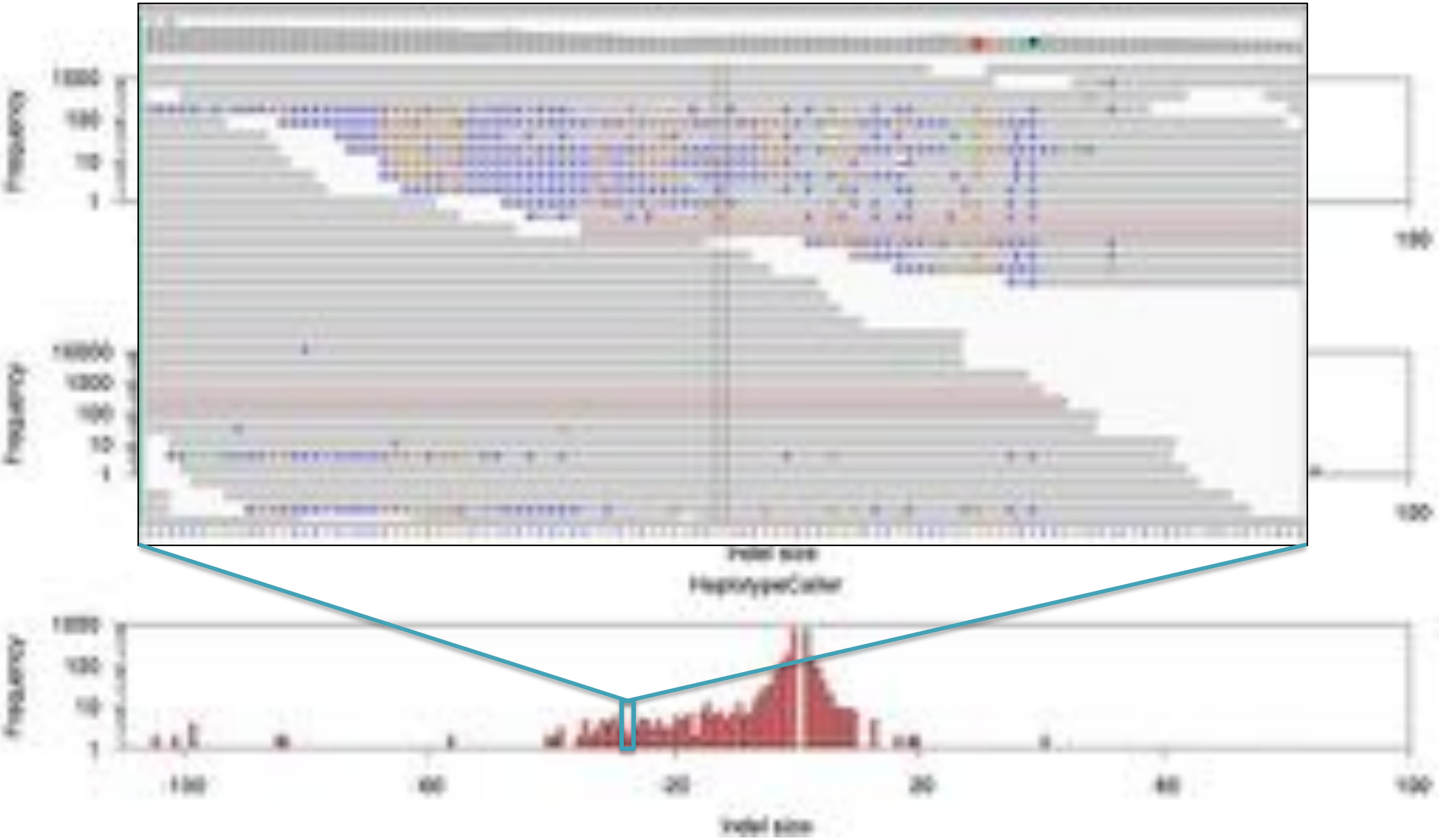
Ref: ABCB’D

SOAPindel: ABC’BCB’D

Scalpel: ABC’B’D

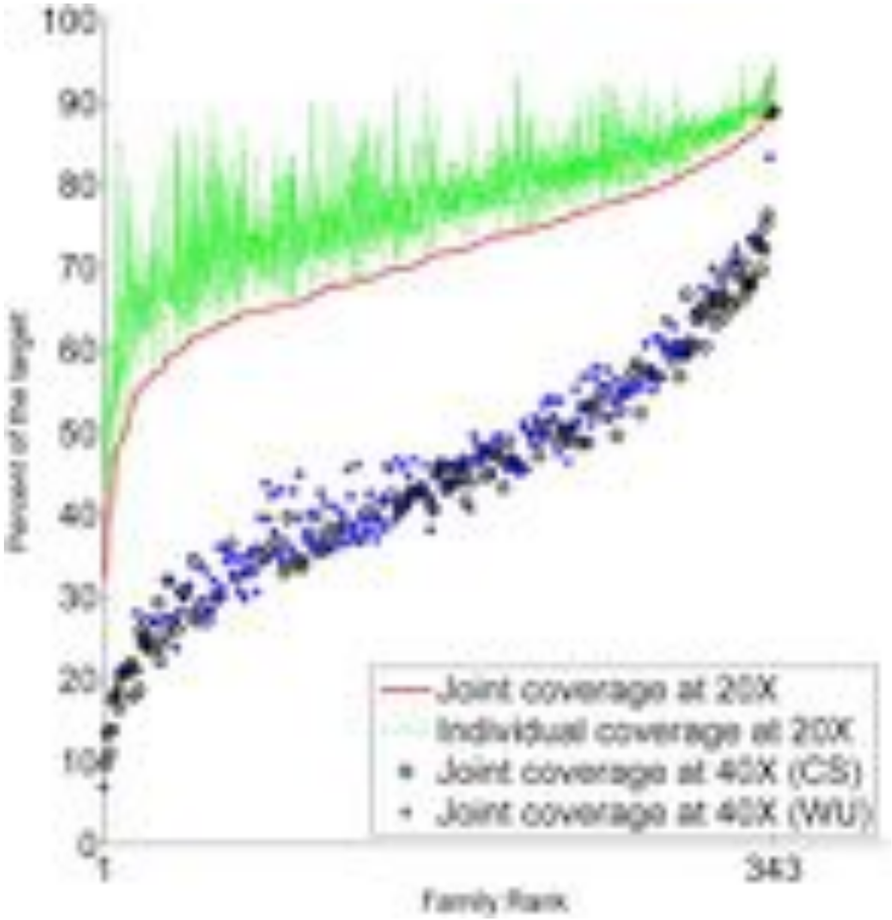
Detection of de novo mutations in exome-capture data using micro-assembly
Narzisi *et al.* (2013) *In preparation*

Scalpel Indel Discovery



Detection of de novo mutations in exome-capture data using micro-assembly
Narzisi *et al.* (2013) *In preparation*

Exome sequencing of the SSC



Sequencing of 343 families from the Simons Simplex Collection

- Parents plus one child with autism and one non-autistic sibling
- Enriched for higher-functioning individuals

Families prepared and captured together to minimize batch effects

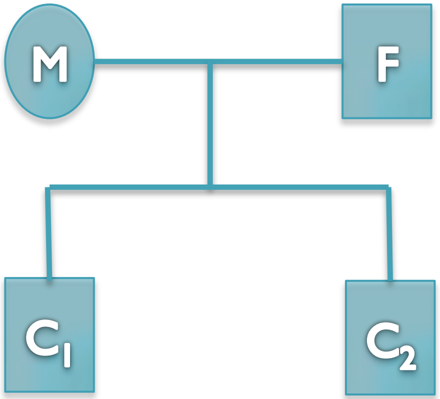
- Exome-capture performed with NimbleGen SeqCap EZ Exome v2.0 targeting 36 Mb of the genome.
- ~80% of the target at >20x coverage with ~93bp reads

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

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



```
Ref:     ...TCAGAACAGCTGGATGAGATCTTAGCCAACTACCAGGAGATTGTCTTTGCCCGGA...

Father:  ...TCAGAACAGCTGGATGAGATCTTAGCCAACTACCAGGAGATTGTCTTTGCCCGGA...
Mother:  ...TCAGAACAGCTGGATGAGATCTTAGCCAACTACCAGGAGATTGTCTTTGCCCGGA...
Sib:     ...TCAGAACAGCTGGATGAGATCTTAGCCAACTACCAGGAGATTGTCTTTGCCCGGA...
Aut(1):  ...TCAGAACAGCTGGATGAGATCTTAGCCAACTACCAGGAGATTGTCTTTGCCCGGA...
Aut(2):  ...TCAGAACAGCTGGATGAGATCTTACC------CCGGGAGATTGTCTTTGCCCGGA...
```

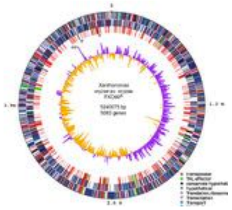
6bp heterozygous deletion at chr13:25280526 ATP12A

De novo Genetics of Autism

- In 343 family quads so far, we see significant enrichment in de novo ***likely gene killers*** in the autistic kids
  - Overall rate basically 1:1 (432:396)
  - 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 the 842 genes known to be associated with fragile X protein FMPR
  - Related to neuron development and synaptic plasticity
  - Also strong overlap with chromatin remodelers

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



Summary

- Hybrid assembly let us combine the best characteristics of 2nd and 3rd gen sequencing
  - Long reads and good coverage are the keys to a good de novo assembly
  - Single contig de novo assemblies of entire microbial chromosomes are now routine; Single contig de novo assemblies of entire plant and animal chromosomes on the horizon

- Assembly is the missing link towards high accuracy indel mutation discovery
  - Allows the algorithm to break free from the expectations of the reference
  - Pinpointing de novo mutations require both high sensitivity and specificity

- We are starting to apply these technologies to discover significant biology that is otherwise impossible to measure

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Varrun Ramani
Piyush Kansal
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CSHL

Hannon Lab
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Iossifov Lab
Levy Lab
Lippman Lab
Lyon Lab
Martienssen Lab
McCombie Lab
Ware Lab
Wigler Lab

IT Department

NBACC

Adam Phillippy
Sergey Koren

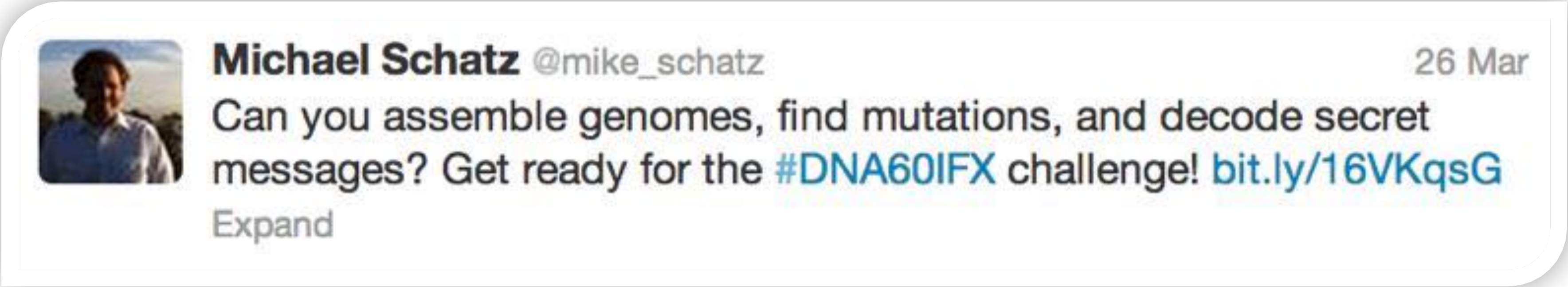
SFARI
SIMONS FOUNDATION
AUTISM RESEARCH INITIATIVE

National Human
Genome Research
Institute

U.S. DEPARTMENT OF
ENERGY

NSF

Thank You!



Michael Schatz @mike_schatz 26 Mar

Can you assemble genomes, find mutations, and decode secret messages? Get ready for the #DNA60IFX challenge! bit.ly/16VKqsG

Expand