Human Genome & Genome Assembly

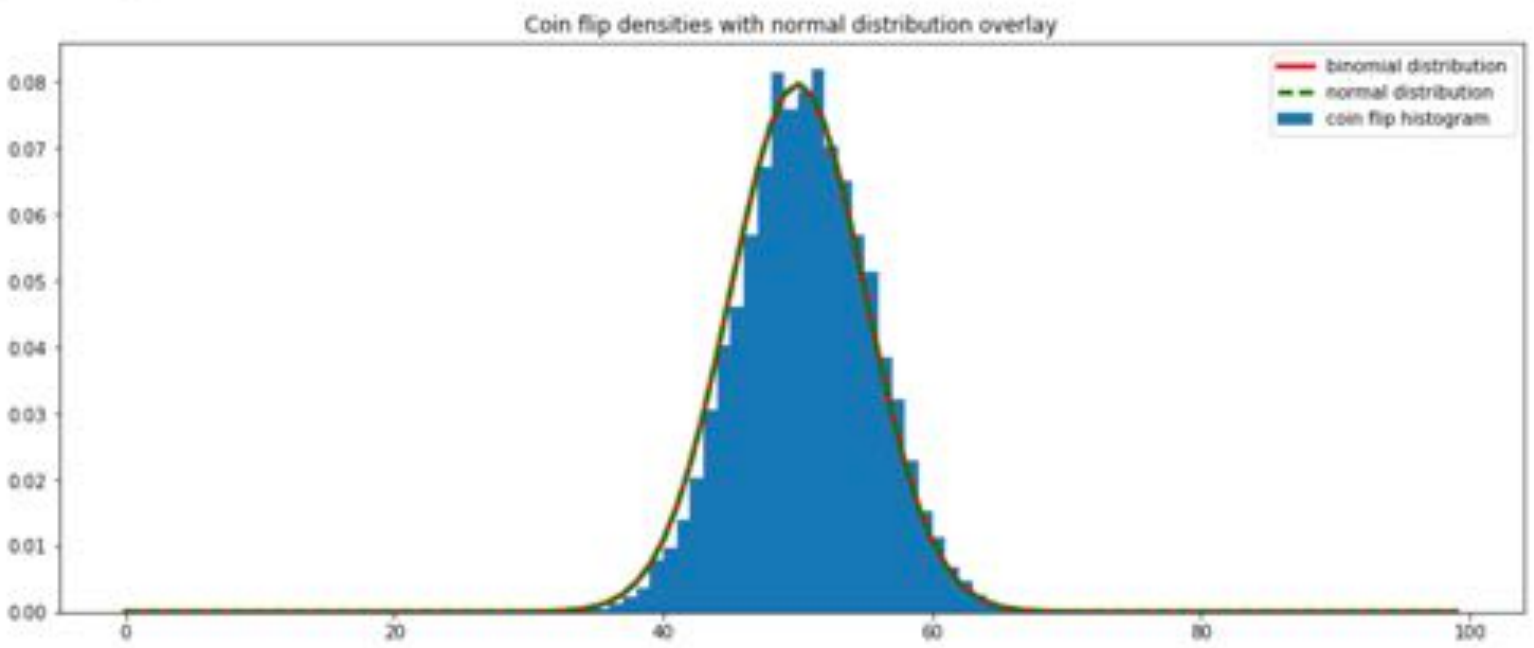
Michael Schatz

Sept 25, 2017 – Lecture 7

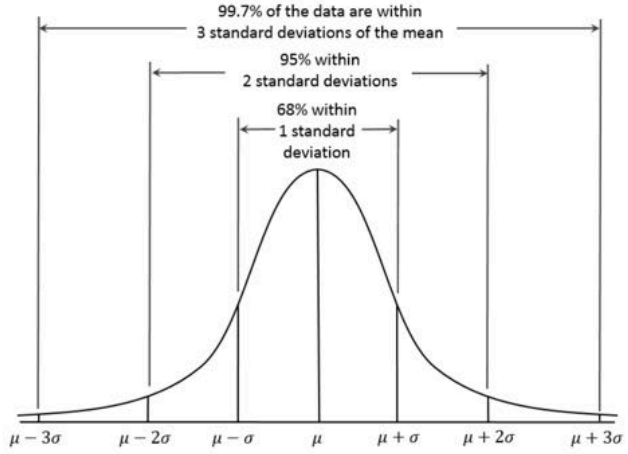
EN.601.452 Computational Biomedical Research

AS.020.415 Advanced Biomedical Research

Exercise 1: Due tonight @ 11:59pm

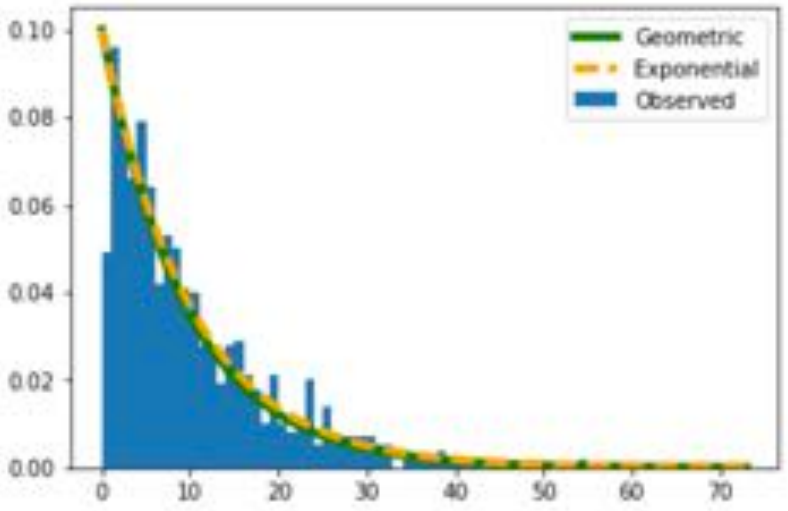


The binomial distribution is closely related to the normal distribution (aka Gaussian distribution)



The probability density for the Gaussian distribution is

$$p(x) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(x-\mu)^2}{2\sigma^2}},$$



Project Proposal: Due in 1 week

Project Proposal

Assignment Date: September 25, 2017
Due Date: Monday, October 2, 2017 @ 11:59pm

Please email a PDF of your project proposal (1/2 to 1 page) to "jhubiomedicalresearch at gmail dot com" by 11:59pm on Monday October 2, 2017.

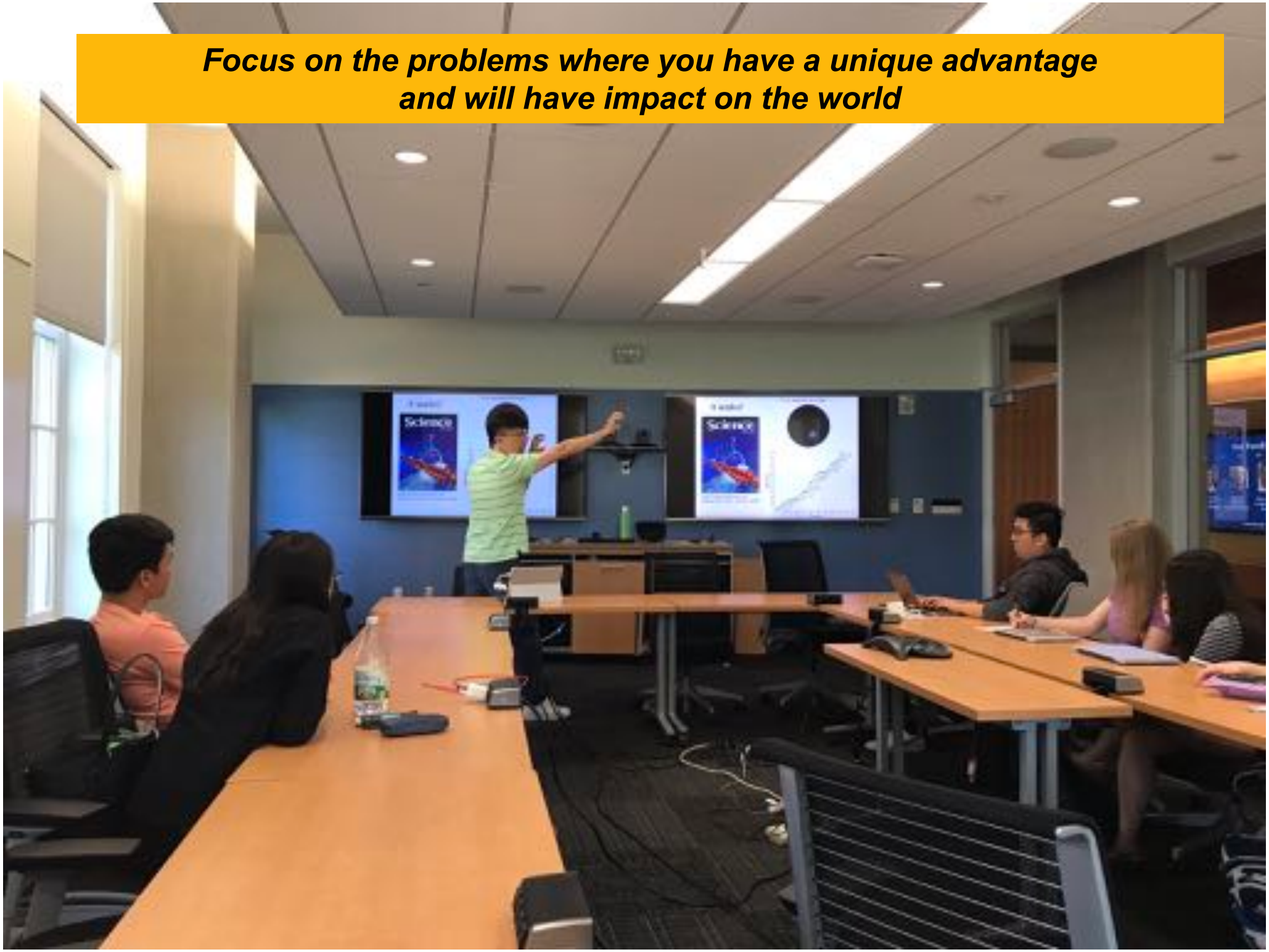
The proposal should have the following components:

- Short title for your proposal
- Your name
- Email addresses
- Description of what you hope to do and how you will do it:
  - What is the key question you hope to address?
  - What data will you use to study it? Are all the data you need available now? When will they be available?
  - What techniques (experimental or computational) will you use to generate and analyze the data?
  - What are the desired results?
- References to relevant papers
- References/URLs to datasets that you will be studying (Note you can also use simulated data)

After submitting your proposal, we will schedule a time to discuss your proposal, especially to ensure you have access to the data that you need and you have a clear path forward for analyzing it. The sooner that you submit your proposal, the sooner we can schedule the meeting. No late days can be used for this project.

Later, you will present your project in class at the the end of the semester. You will also submit a written report (5-7 pages) of your project, formatting as scholarly article with separate sections for the Abstract, Introduction, Methods, Results, Discussion, and References. More details will be provided later in the semester.

Focus on the problems where you have a unique advantage and will have impact on the world



The human genome

The human genome

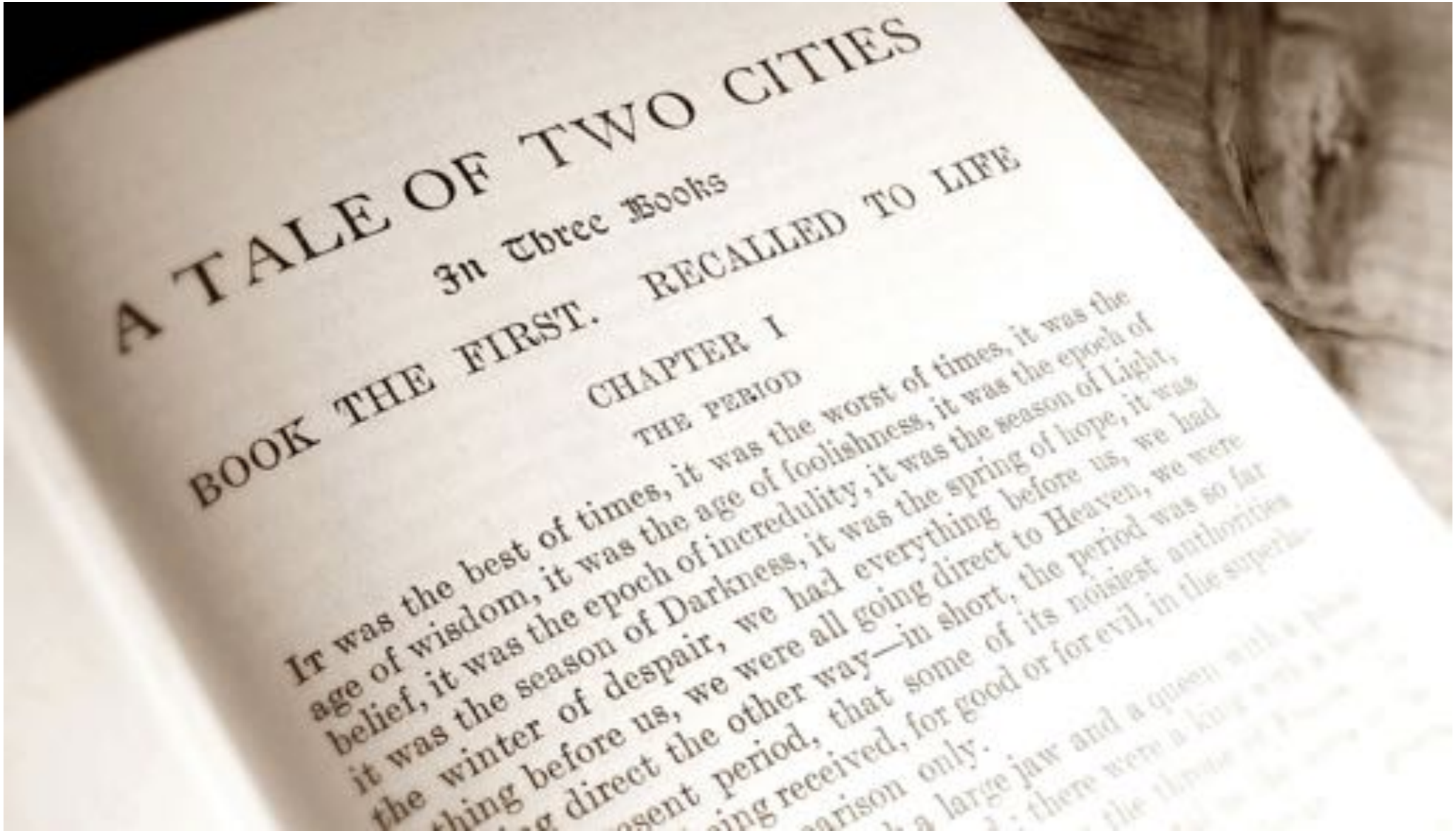
Outline

1. ***Assembly theory***
   - Assembly by analogy

2. ***Practical Issues***
   - Coverage, read length, errors, and repeats

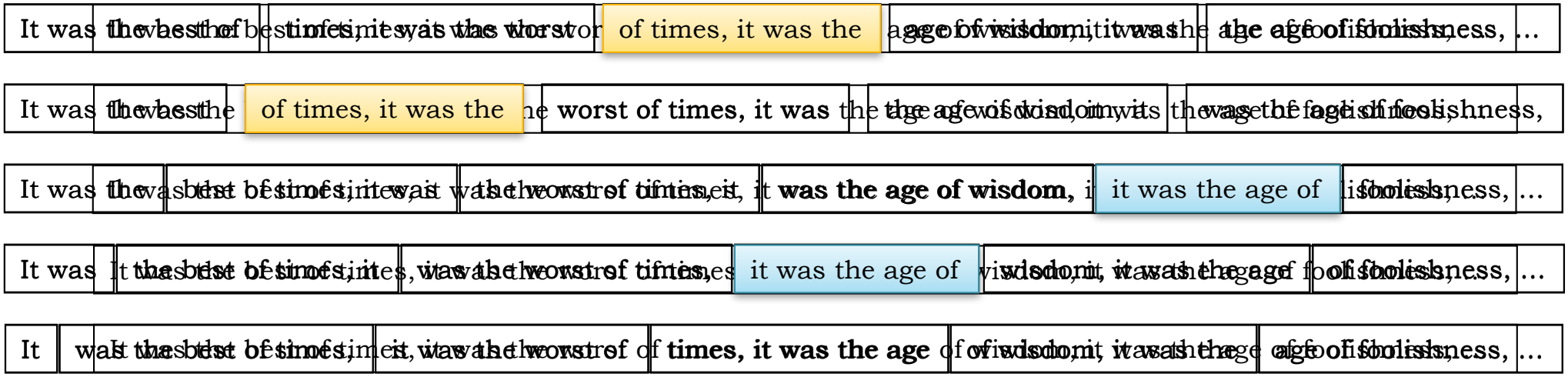
3. ***Next-next-gen Assembly***
   - PacBio/ONT projects

Genome Assembly by Analogy



Shredded Book Reconstruction

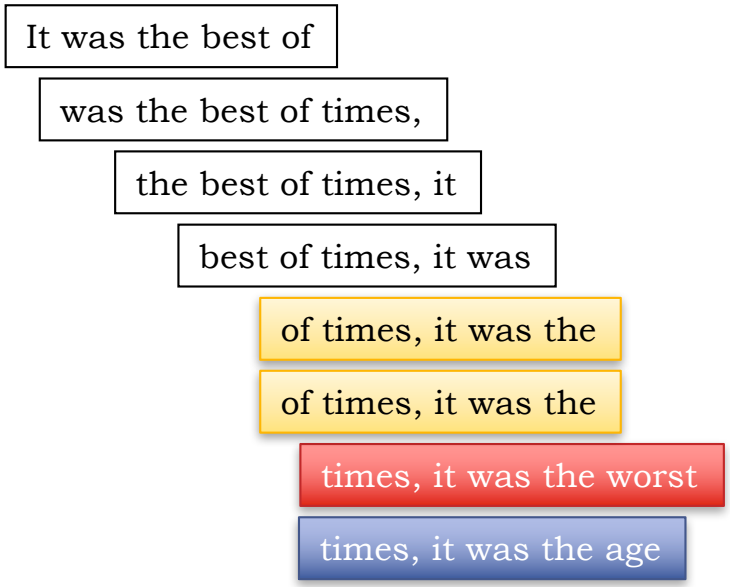
- Dickens accidentally shreds the first printing of A Tale of Two Cities
  - Text printed on 5 long spools



- 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



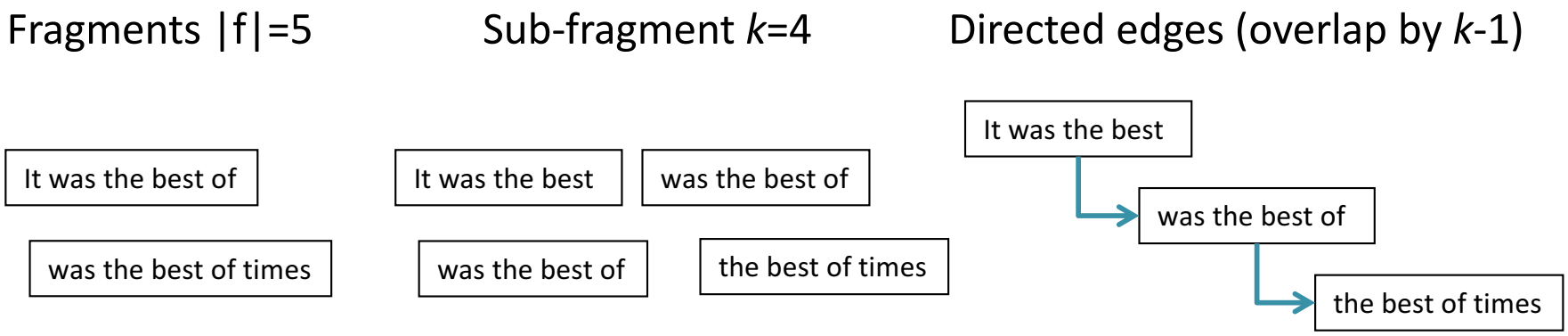
The repeated sequence make the correct reconstruction ambiguous

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

Model the assembly problem as a graph problem

de Bruijn Graph Construction

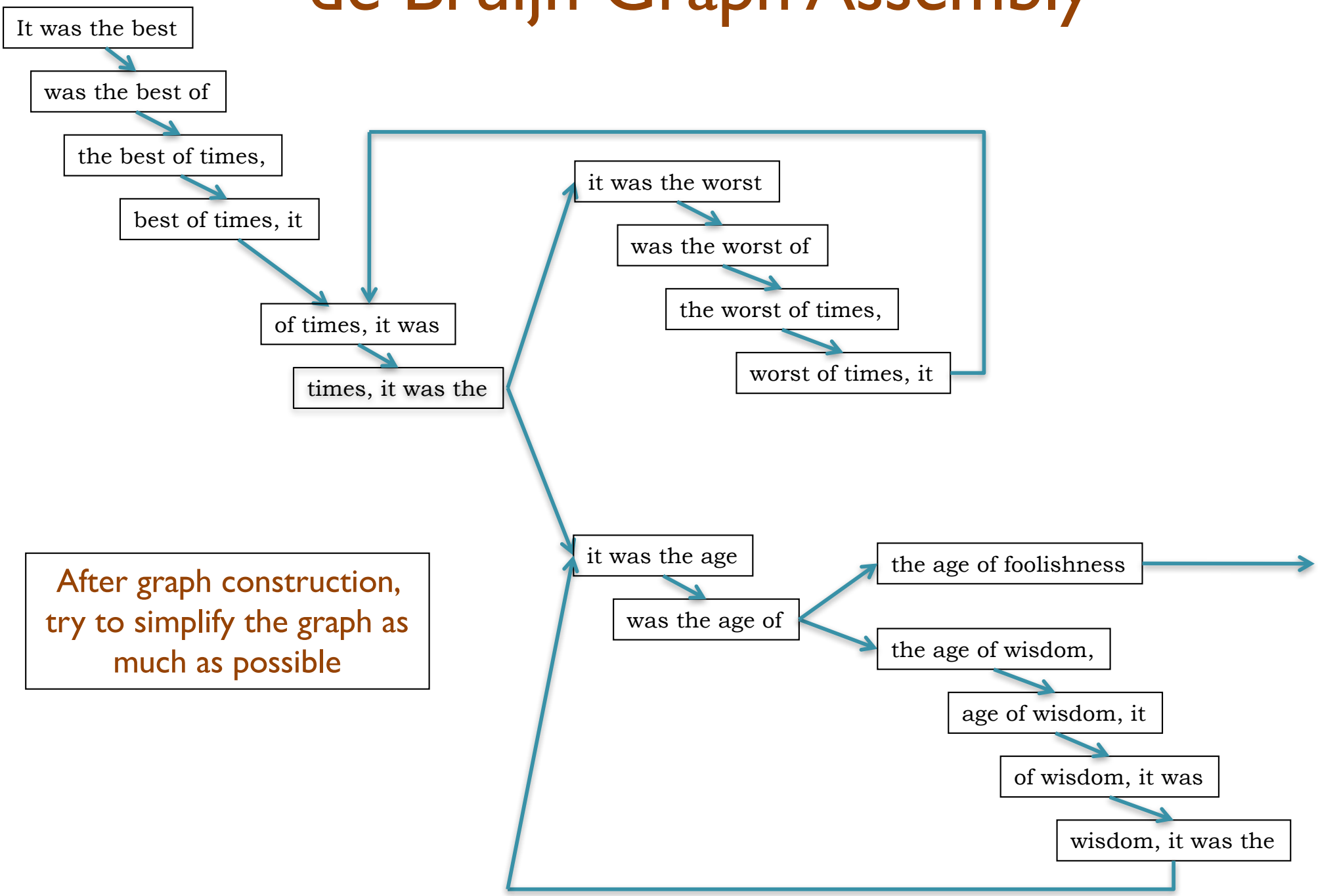
- $G_k = (V,E)$
  - V = Length-*k* sub-fragments
  - E = Directed edges between consecutive sub-fragments
    - Sub-fragments overlap by *k*-1 words



– Overlaps between fragments are implicitly computed

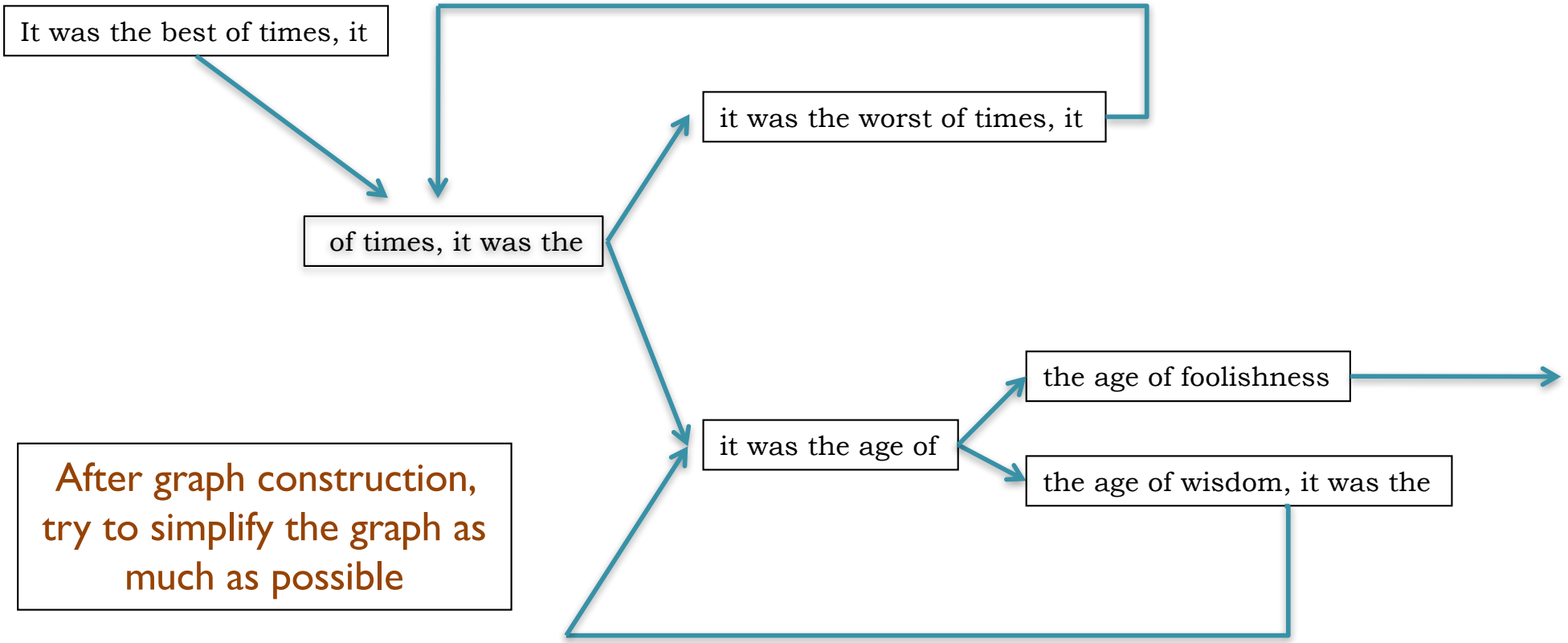
de Bruijn, 1946
Idury et al., 1995
Pevzner et al., 2001

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

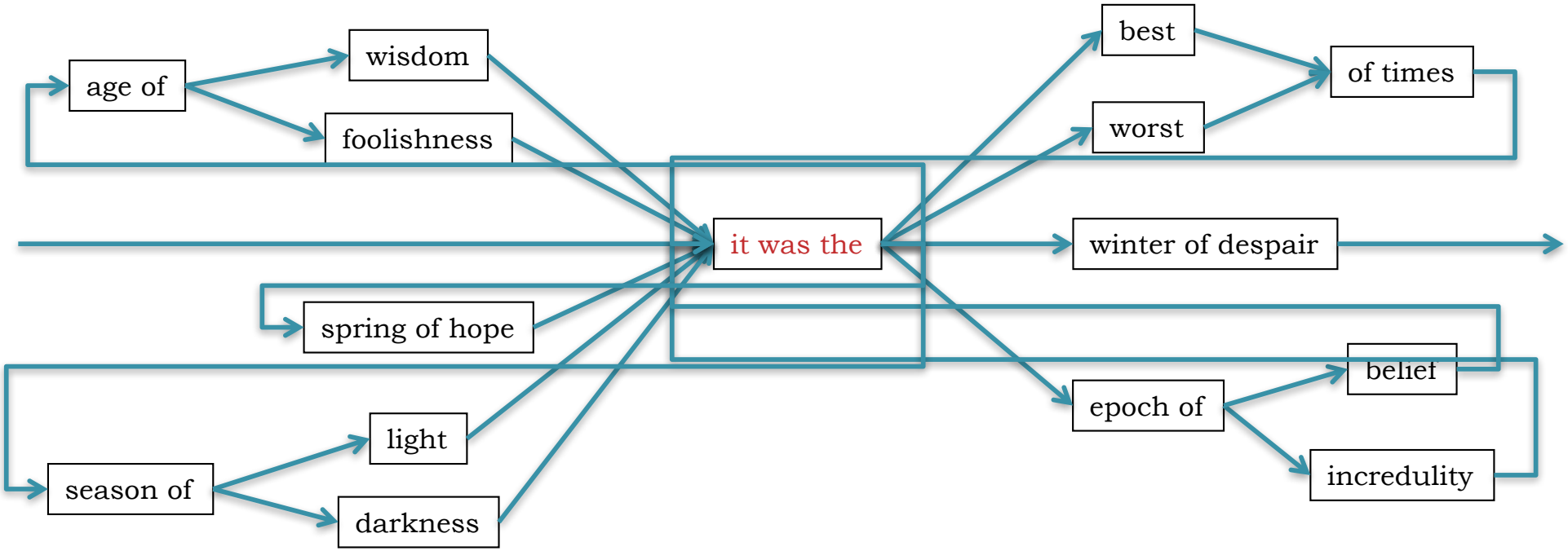
… it was the best of times it was the worst of times …

… 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 …

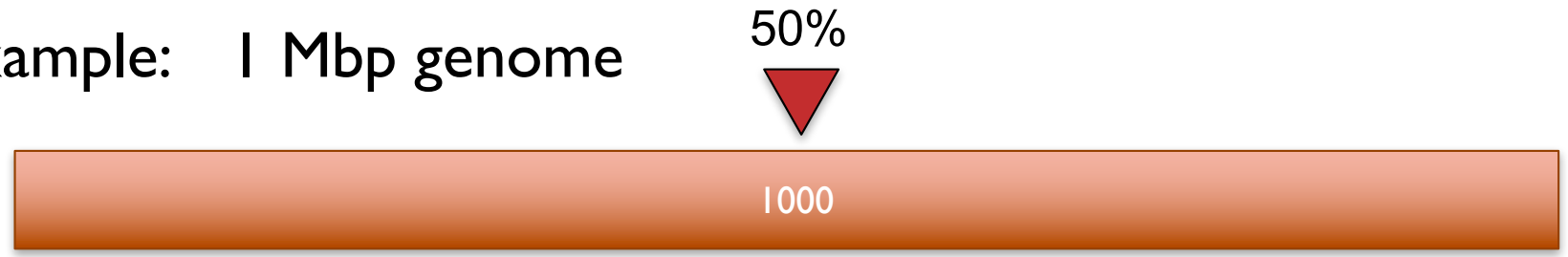
… it was the spring of hope it was the winder of despair …



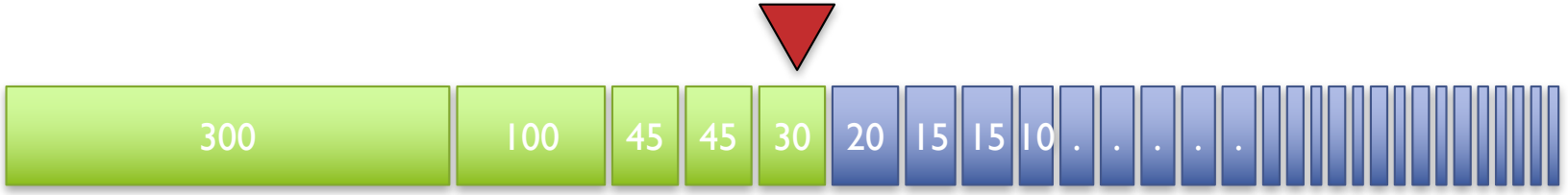
Contig N50

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

Example: 1 Mbp genome

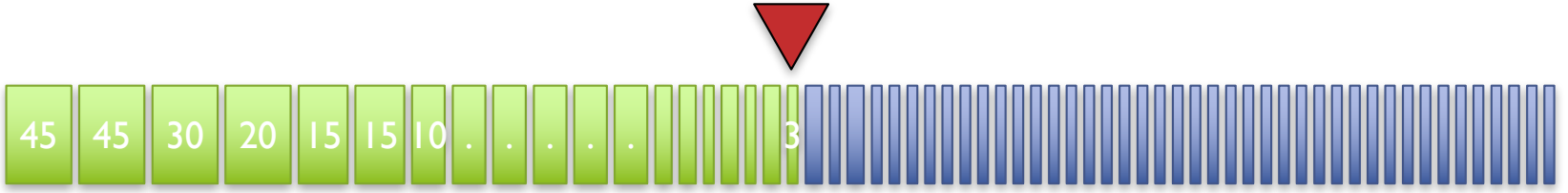


A



N50 size = 30 kbp

B



N50 size = 3 kbp

Contig N50

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

50%

Better N50s improves the analysis 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

Just be careful of N50 inflation!

- *A very very very bad assembler in 1 line of bash:*
- *cat *.reads.fa > genome.fa*

N50 size = 3 kbp

Outline

1. ***Assembly theory***
   - Assembly by analogy

2. ***Practical Issues***
   - Coverage, read length, errors, and repeats

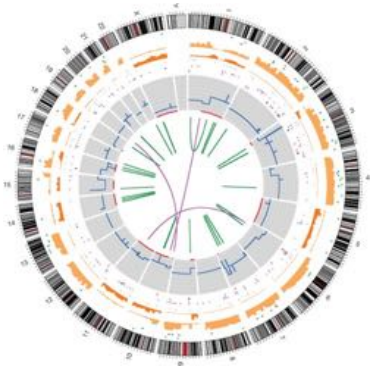
3. ***Next-next-gen Assembly***
   - PacBio/ONT projects

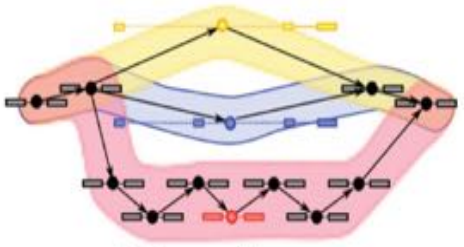
Assembly Applications

- Novel genomes

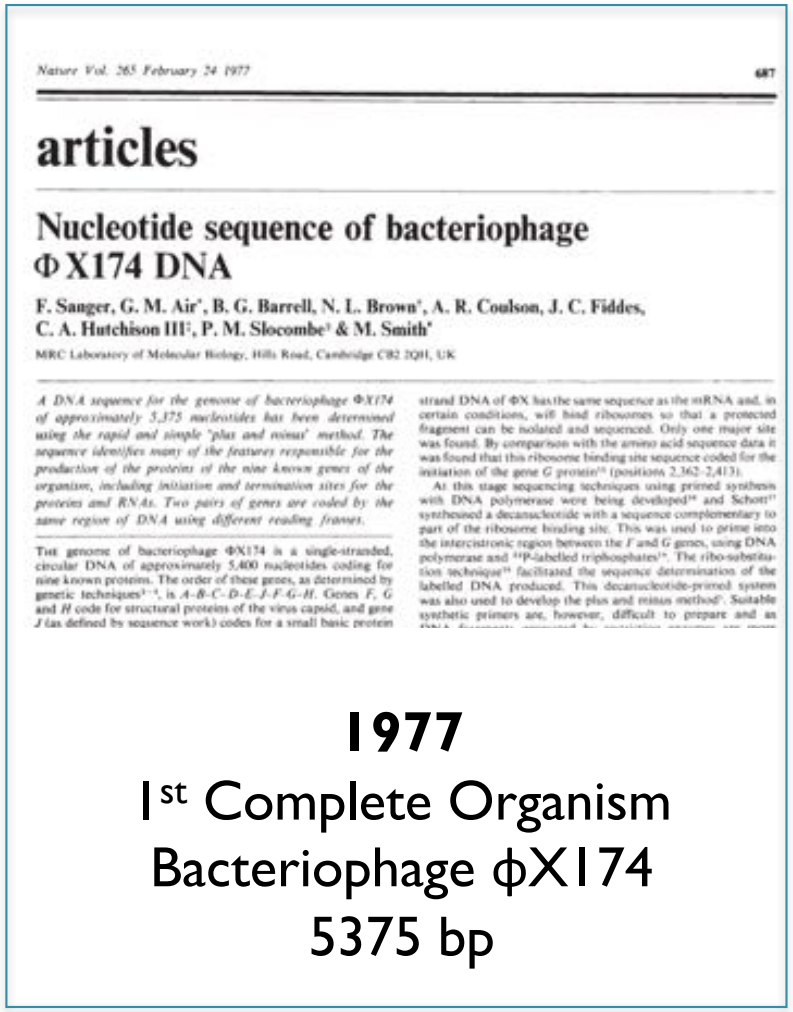
- Metagenomes

- Sequencing assays
  - Structural variations
  - Transcript assembly
  - …





Milestones in Molecular Biology



Nature Vol. 265 February 24 1977 687

articles

Nucleotide sequence of bacteriophage ΦX174 DNA

F. Sanger, G. M. Air*, B. G. Barrell, N. L. Brown*, A. R. Coulson, J. C. Fiddes, C. A. Hutchison III‡, P. M. Slocombe† & M. Smith*

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

A DNA sequence for the genome of bacteriophage ΦX174 of approximately 5,375 nucleotides has been determined using the rapid and simple 'plus and minus' method. The sequence identifies many of the features responsible for the production of the proteins of the nine known genes of the organism, including initiation and termination sites for the proteins and RNAs. Two pairs of genes are coded by the same region of DNA using different reading frames.

THE genome of bacteriophage ΦX174 is a single-stranded, circular DNA of approximately 5,400 nucleotides coding for nine known proteins. The order of these genes, as determined by genetic techniques[1-3], is *A-B-C-D-E-J-F-G-H*. Genes *F*, *G* and *H* code for structural proteins of the virus capsid, and gene *J* (as defined by sequence work) codes for a small basic protein

strand DNA of ΦX has the same sequence as the mRNA and, in certain conditions, will bind ribosomes so that a protected fragment can be isolated and sequenced. Only one major site was found. By comparison with the amino acid sequence data it was found that this ribosome binding site sequence coded for the initiation of the gene *G* protein[15] (positions 2,362–2,413).

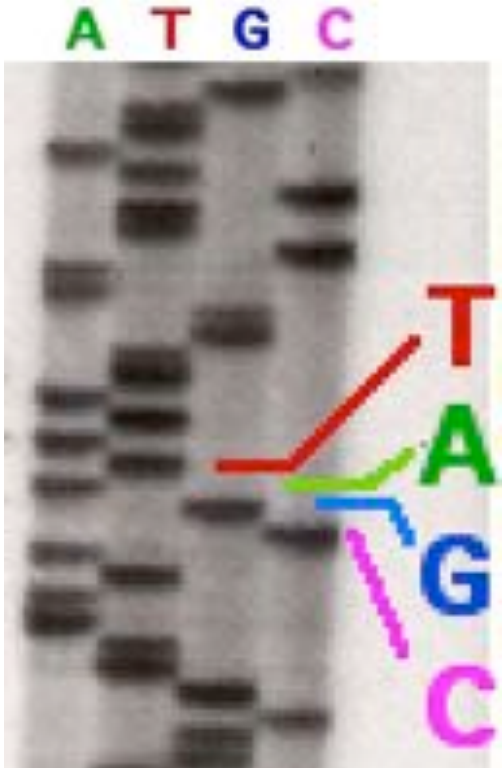
At this stage sequencing techniques using primed synthesis with DNA polymerase were being developed[16] and Schott[17] synthesised a decanucleotide with a sequence complementary to part of the ribosome binding site. This was used to prime into the intercistronic region between the *F* and *G* genes, using DNA polymerase and ^{32}P-labelled triphosphates[18]. The ribo-substitution technique[19] facilitated the sequence determination of the labelled DNA produced. This decanucleotide-primed system was also used to develop the plus and minus method[2]. Suitable synthetic primers are, however, difficult to prepare and as

1977

1st Complete Organism

Bacteriophage ϕX174

5375 bp



Radioactive Chain Termination

5000bp / week / person

http://en.wikipedia.org/wiki/File:Sequencing.jpg
http://www.answers.com/topic/automated-sequencer

Nucleotide sequence of bacteriophage φX174 DNA

Sanger, F. et al. (1977) *Nature. 265: 687 - 695*

Milestones in Molecular Biology

1995
Fleischmann *et al.*
1st Free Living Organism
TIGR Assembler. 1.8Mbp

2000
Myers *et al.*
1st Large WGS Assembly.
Celera Assembler. 116 Mbp

2001
Venter *et al.* / IHGSC
Human Genome
Celera Assembler. 2.9 Gbp

ABI 3700: 500 bp reads x 768 samples / day = 384,000 bp / day.

"The machine was so revolutionary that it could decode in a single day the same amount of genetic material that most DNA labs could produce in a year. " J. Craig Venter

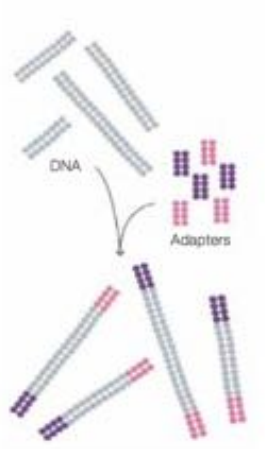
Milestones in Molecular Biology

2004
454/Roche Titanium
Pyrosequencing
1M 400bp reads / run =
1Gbp / day

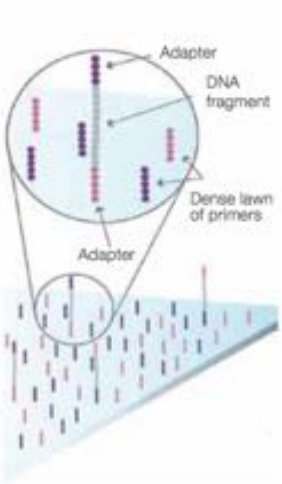
2007
Illumina HiSeq 2000
Sequencing by Synthesis
2.5B 2x100bp reads / run =
60Gbp / day

2017
Illumina NovaSeq
Sequencing by Synthesis
12B 2x150bp reads / run =
>1TB / day

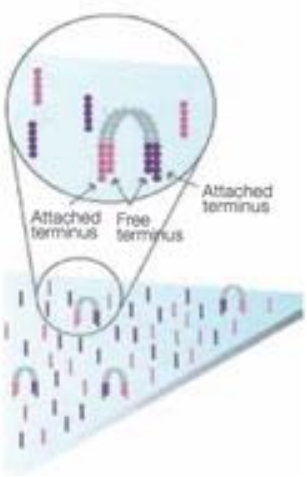
Illumina Sequencing by Synthesis



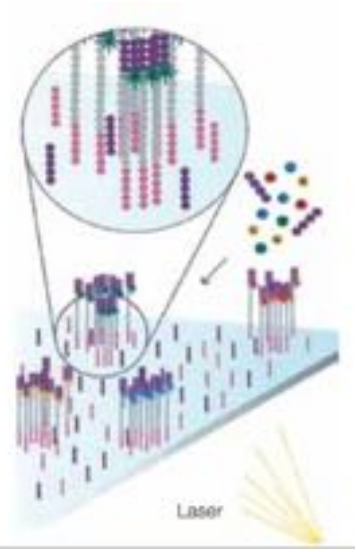
1. Prepare



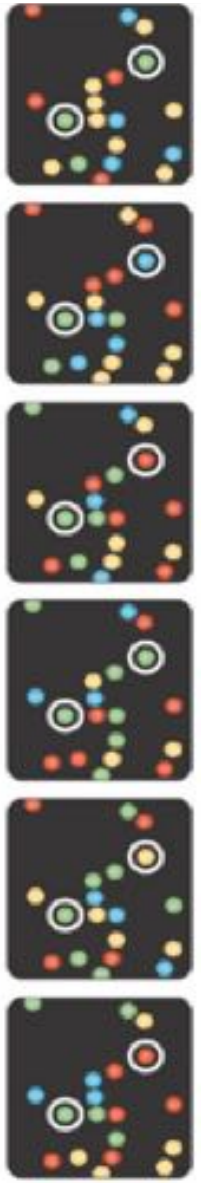
2. Attach



3. Amplify



4. Image

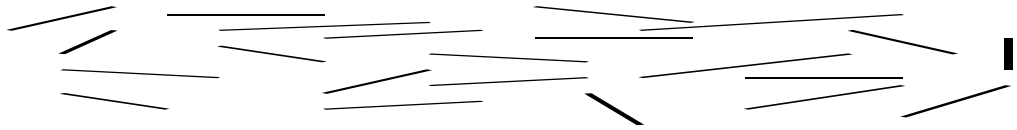


5. Basecall

Metzker (2010) Nature Reviews Genetics 11:31-46
https://www.youtube.com/watch?v=fCd6B5HRaZ8

Assembling a Genome

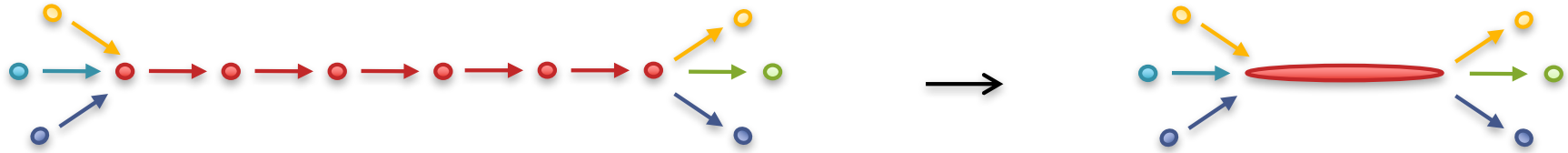
1. Shear & Sequence DNA



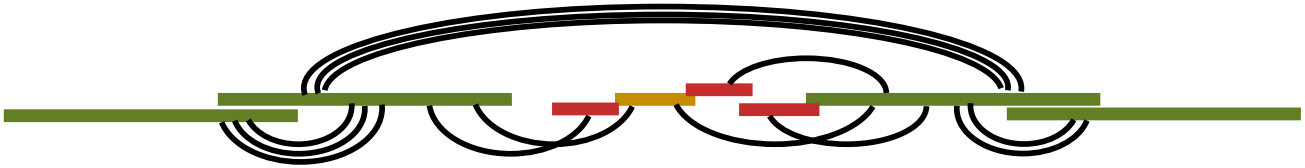
2. Construct assembly graph from reads (de Bruijn / overlap graph)

...AGCCTAGGGATGCGCGACACGT
GGATGCGCGACACGTCGCATATCCGGTTTGGTCAACCTCGGACGGAC
CAACCTCGGACGGACCTCAGCGAA...

3. Simplify assembly graph



4. Detangle graph with long reads, mates, and other links



Why are genomes hard to assemble?

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

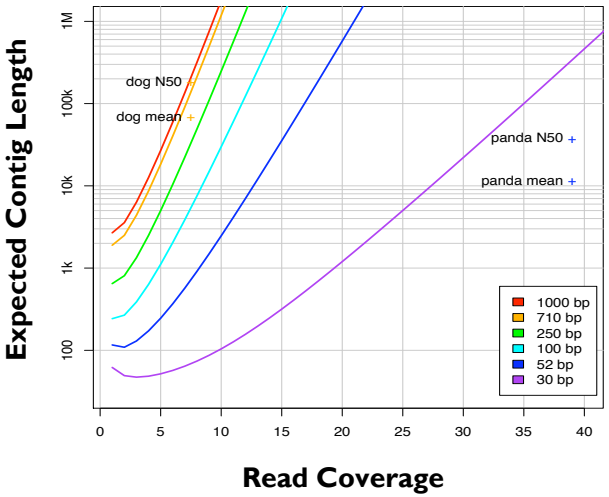
2. ***Sequencing***:
   - (Very) large genomes, imperfect sequencing

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

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

Ingredients for a good assembly

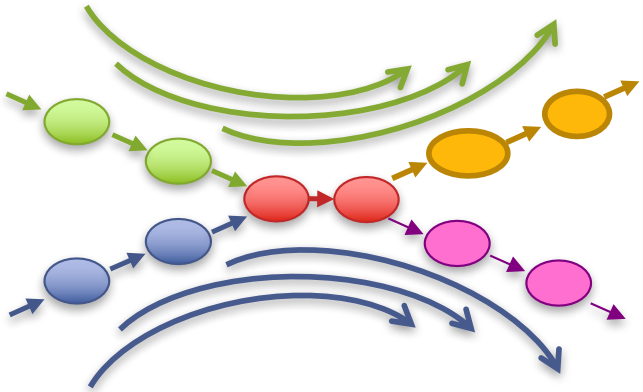
Coverage



High coverage is required

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

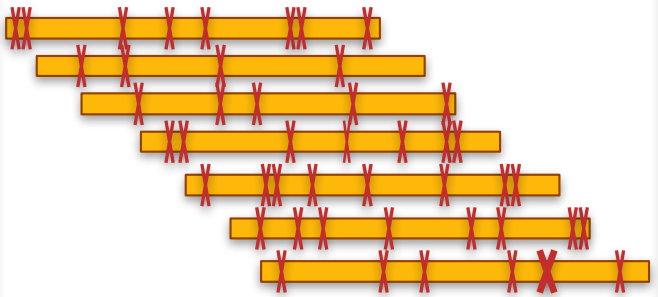
Read Length



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

Quality

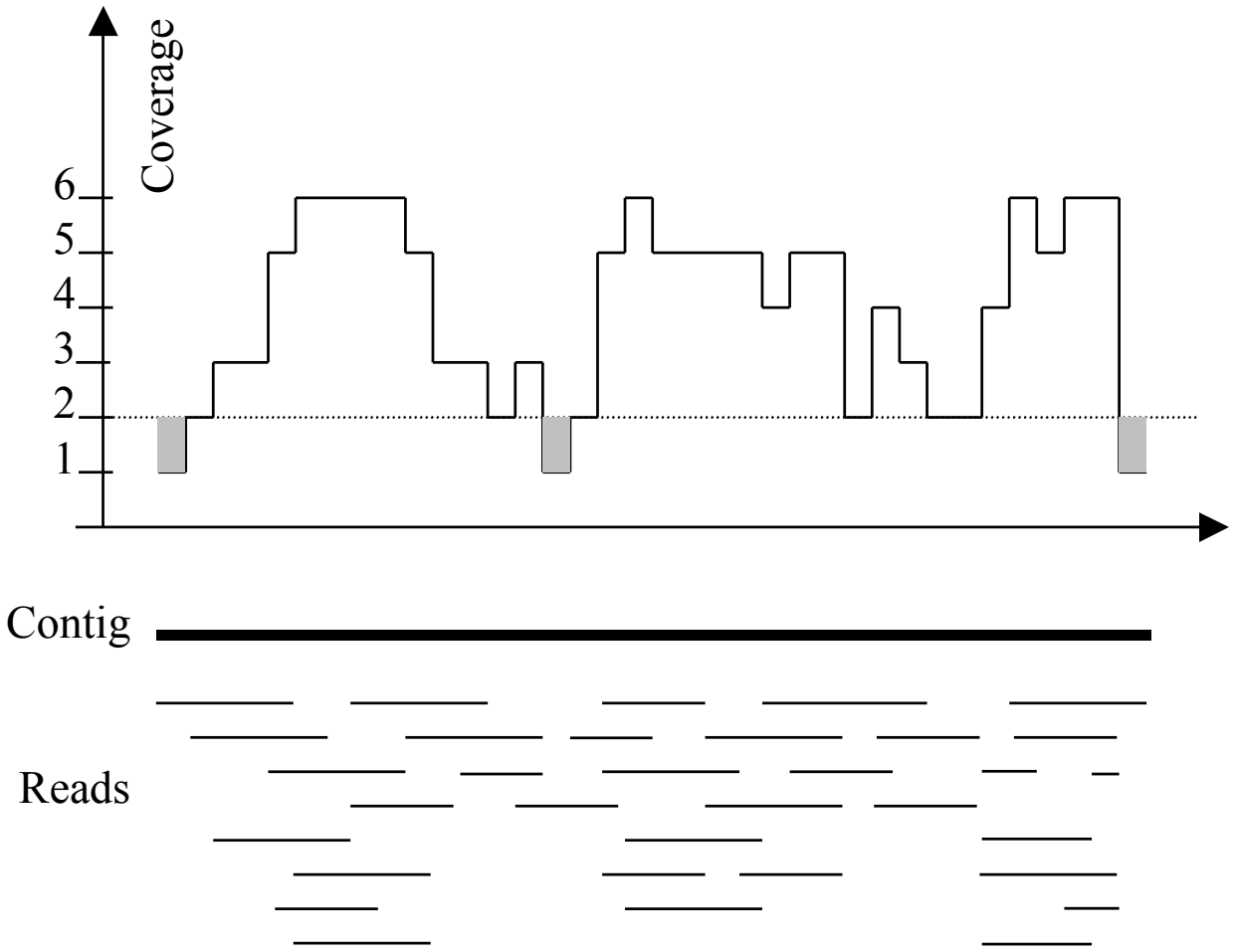


Errors obscure overlaps

- 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

Typical sequencing coverage

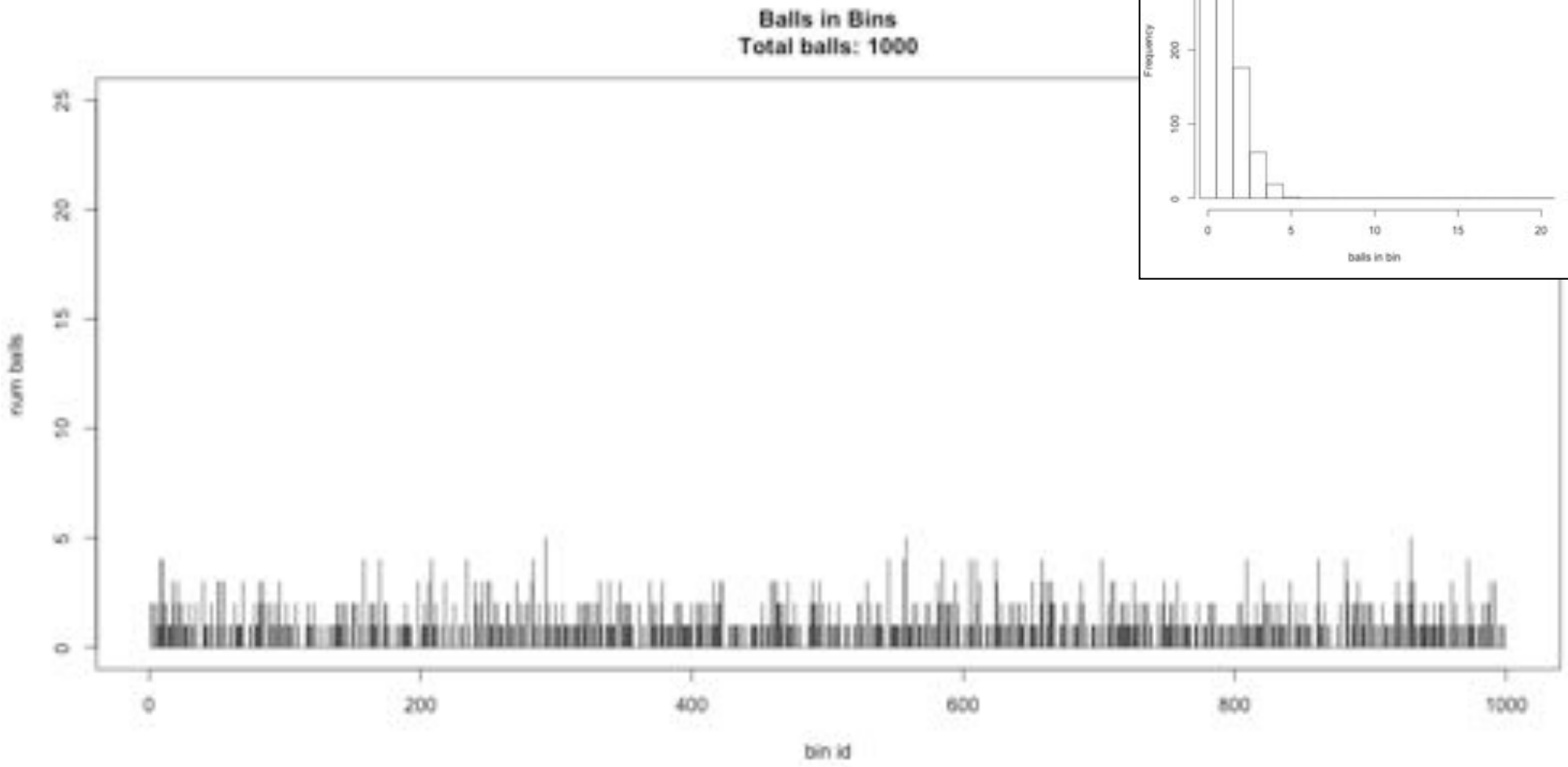


Imagine raindrops on a sidewalk

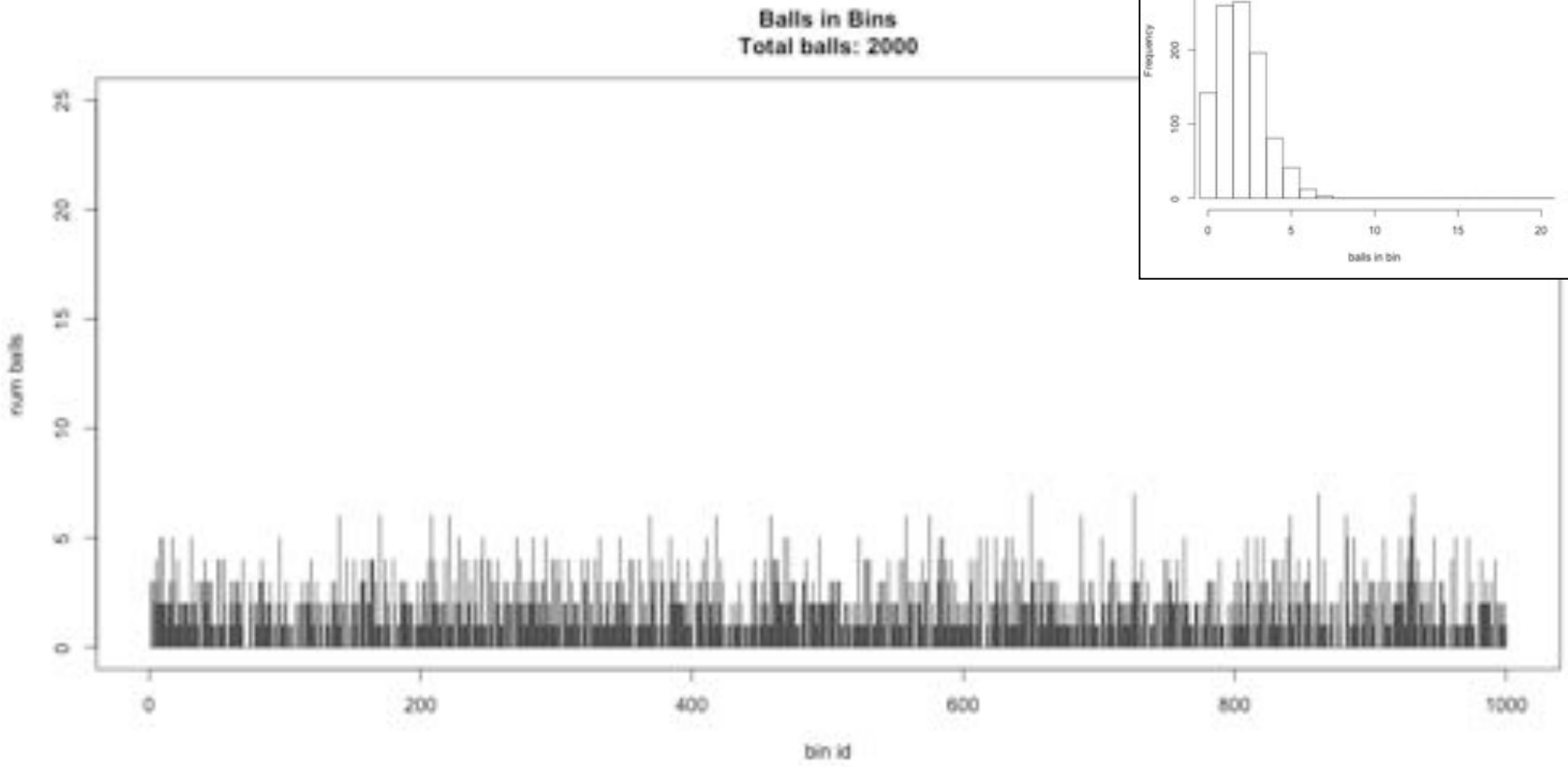
We want to cover the entire sidewalk but each drop costs $1

If the genome is 100 Mbp, should we sequence 1M 100bp reads?

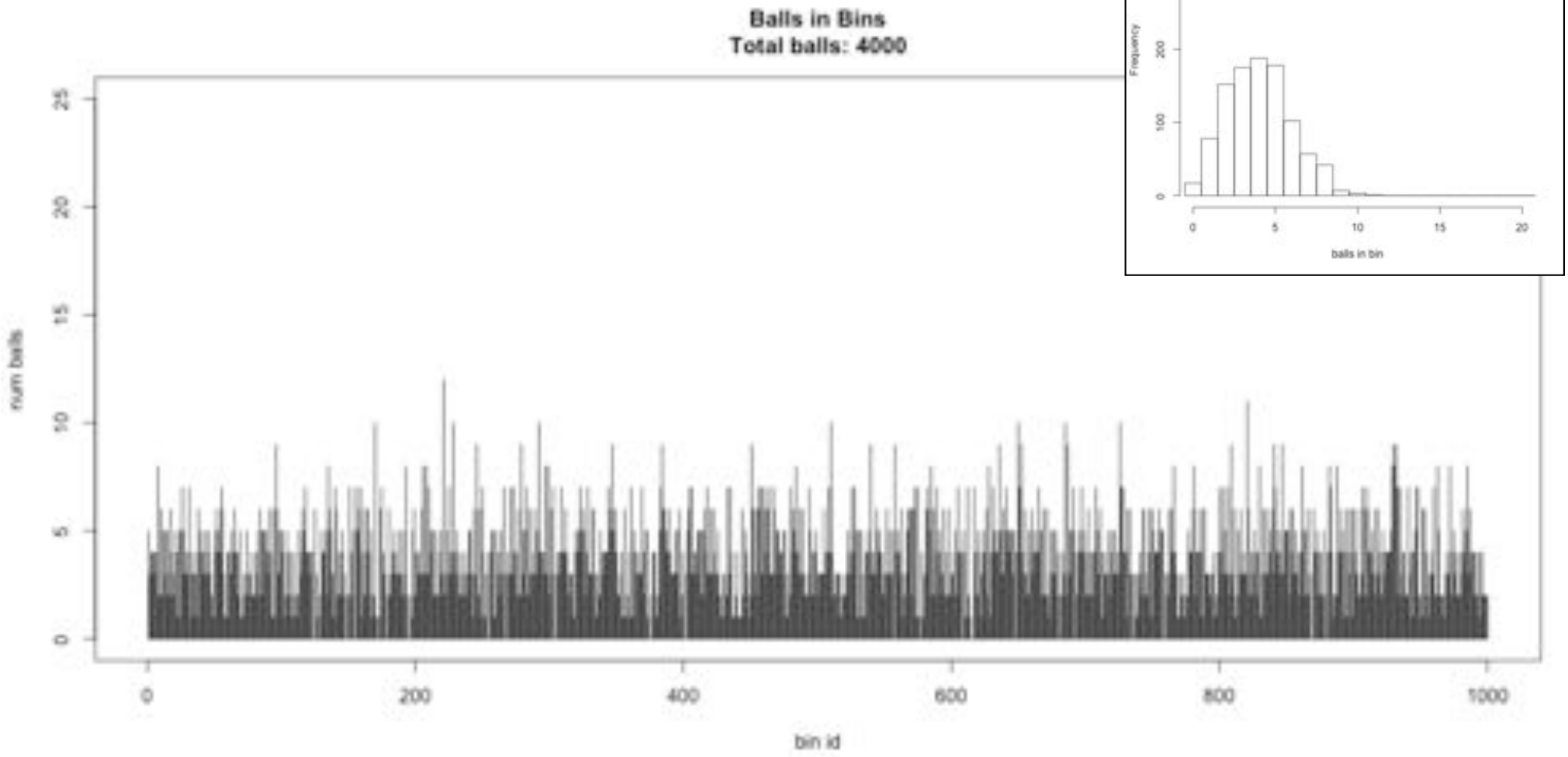
1x sequencing



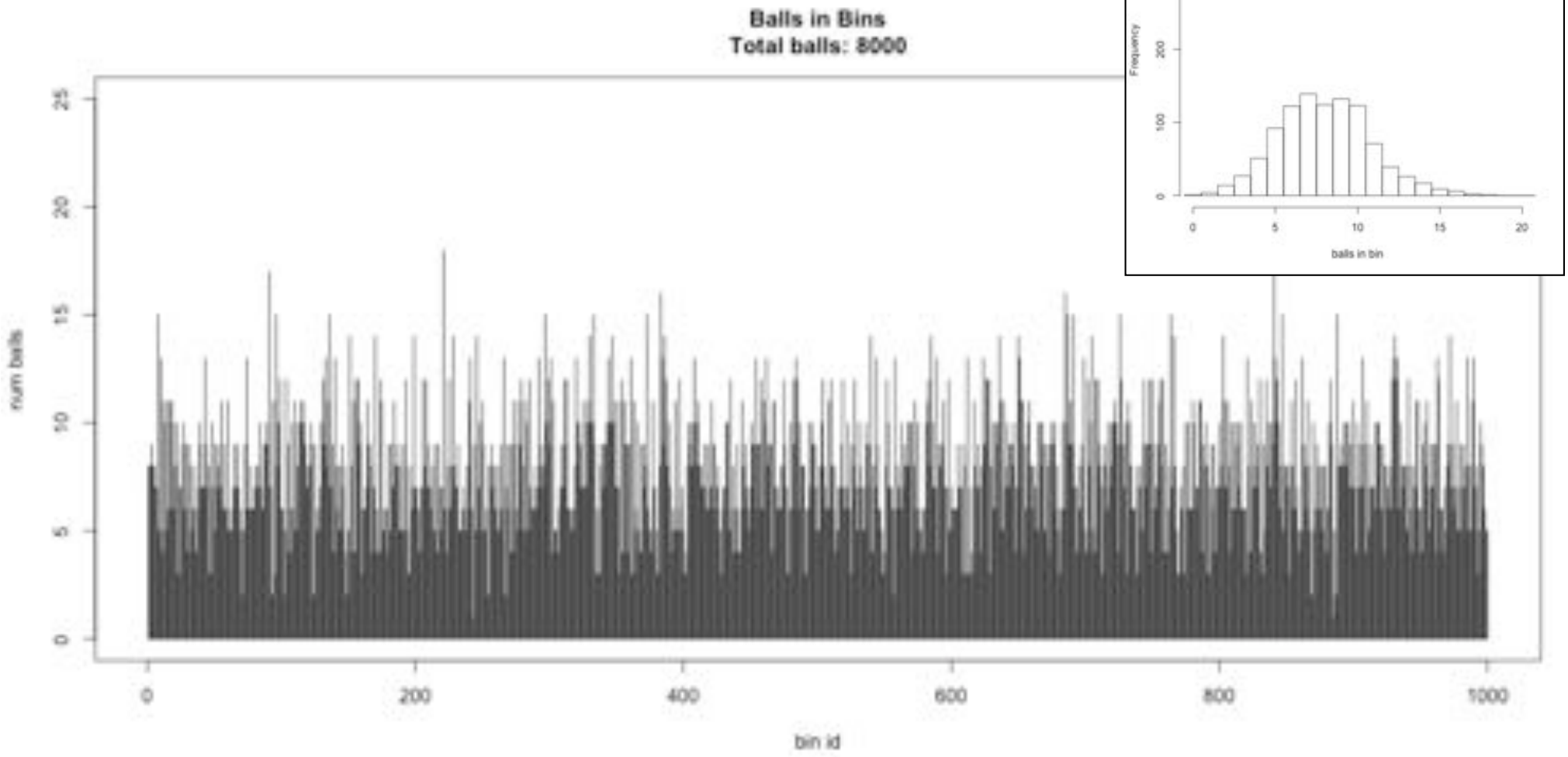
2x sequencing



4x sequencing



8x sequencing



Poisson Distribution

The probability of a given number of events occurring in a fixed interval of time and/or space if these events occur with a known average rate and independently of the time since the last event.

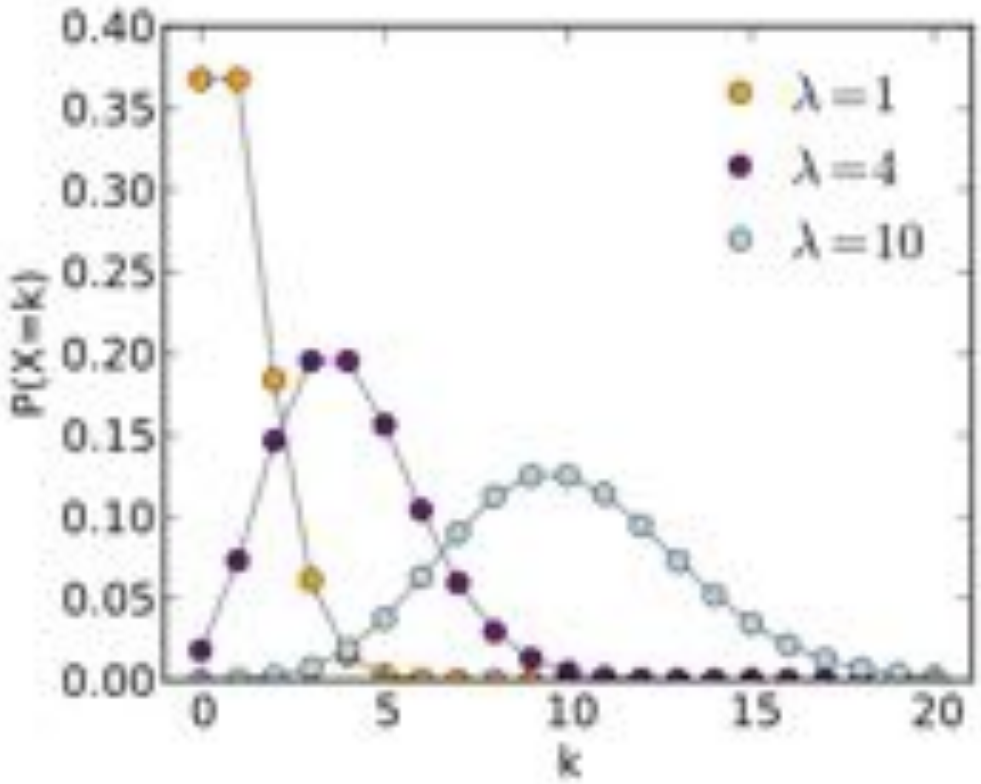
$$P(k) = \frac{\lambda^k}{k!} e^{-\lambda}$$

Formulation comes from the limit of the binomial equation

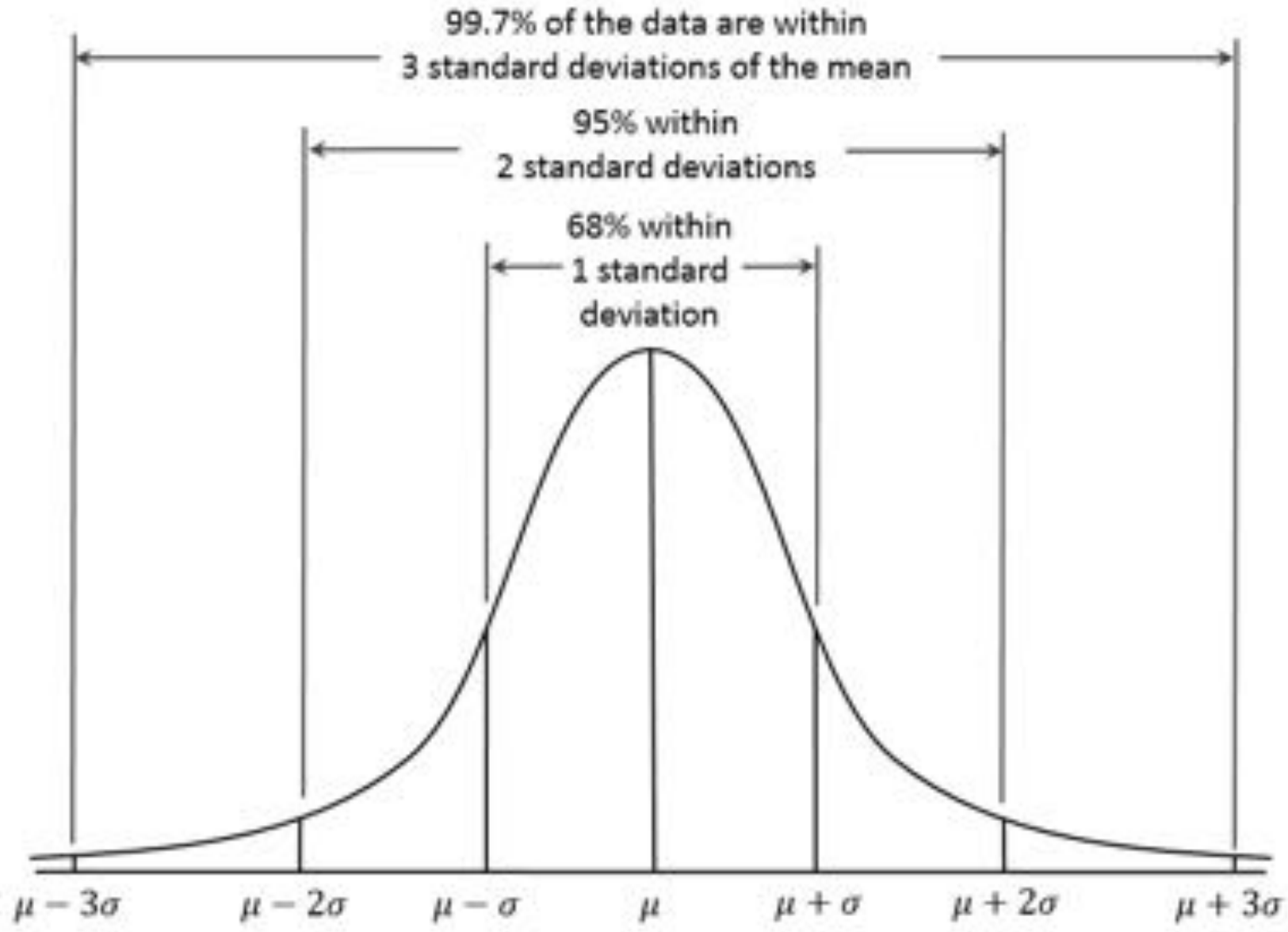
Resembles a normal distribution, but over the positive values, and with only a single parameter.

Key properties:

- ***The standard deviation is the square root of the mean.***
- ***For mean > 5, well approximated by a normal distribution***



Normal Approximation



Can estimate Poisson distribution as a normal distribution when $\lambda > 10$

Pop Quiz!

I want to sequence a 10Mbp genome to 24x coverage.
How many 150bp reads do I need?

I need 10Mbp x 24x = 240Mbp of data
240Mbp / 150bp / read = 1.6M reads

I want to sequence a 10Mbp genome so that
>97.5% of the genome has at least 24x coverage.
How many 150bp reads do I need?

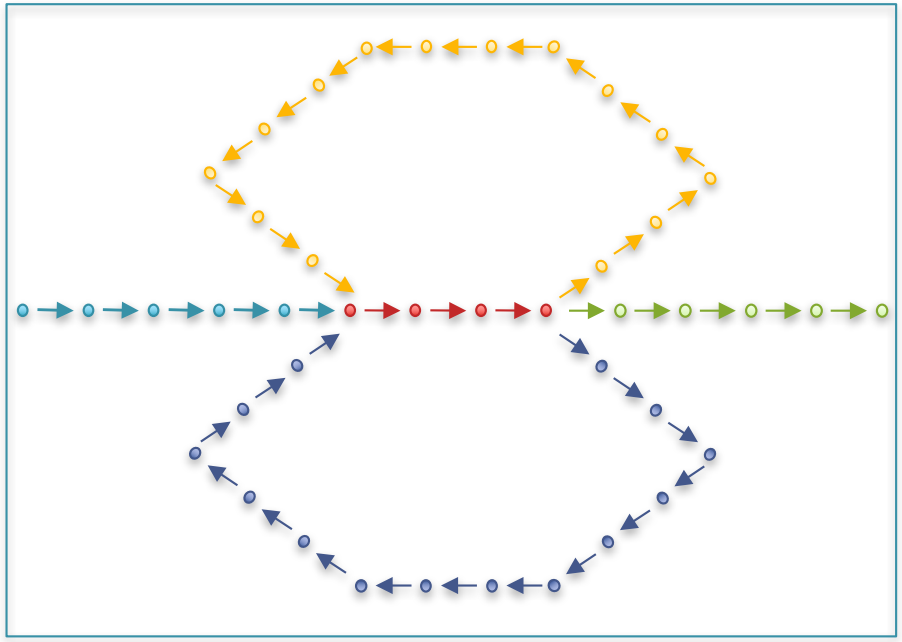
Find X such that X-2*sqrt(X) = 24

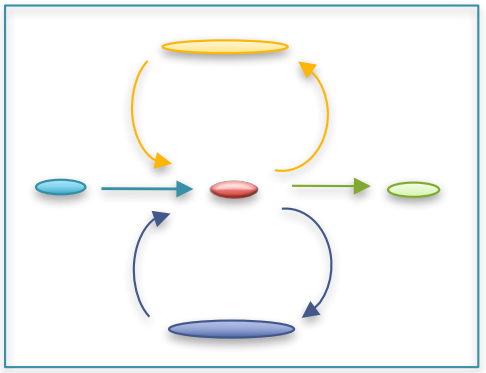
36-2*sqrt(36) = 24

I need 10Mbp x 36x = 360Mbp of data
360Mbp / 150bp / read = 2.4M reads

Unitigging / Unipathing

- After simplification and correction, compress graph down to its non-branching initial contigs
  - Aka "unitigs", "unipaths"
  - Unitigs end because of (1) lack of coverage, (2) errors, (3) heterozygosity and (4) repeats



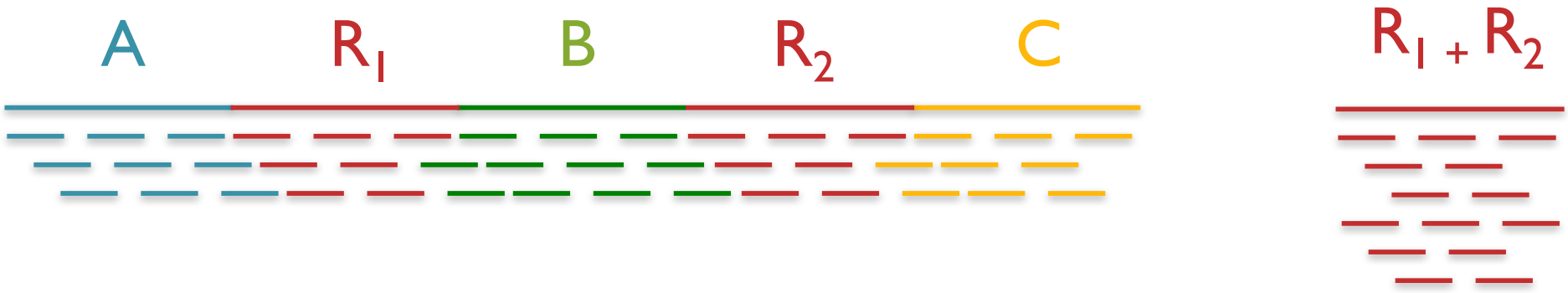


Repetitive regions

Repeat Type	Definition / Example	Prevalence
Low-complexity DNA / Microsatellites	$(b_1 b_2 \ldots b_k)^N$ where $1 \leq k \leq 6$ CACACACACACACACACACA	2%
SINEs (Short Interspersed Nuclear Elements)	*Alu* sequence (~280 bp) Mariner elements (~80 bp)	13%
LINEs (Long Interspersed Nuclear Elements)	~500 – 5,000 bp	21%
LTR (long terminal repeat) retrotransposons	Ty1-copia, Ty3-gypsy, Pao-BEL (~100 – 5,000 bp)	8%
Other DNA transposons		3%
Gene families & segmental duplications		4%

- Over 50% of mammalian genomes are repetitive
  - Large plant genomes tend to be even worse
  - Wheat: 16 Gbp; Pine: 24 Gbp

Repeats and Coverage Statistics



- If *n* reads are a uniform random sample of the genome of length *G*, we expect $k = n\Delta/G$ reads to start in a region of length Δ.
  - If we see many more reads than k (if the arrival rate is > A) , it is likely to be a collapsed repeat

$$\Pr(X - copy) = \binom{n}{k}\left(\frac{X\Delta}{G}\right)^{k}\left(\frac{G - X\Delta}{G}\right)^{n-k}$$

$$A(\Delta,k) = \ln\left(\frac{\Pr(1-copy)}{\Pr(2-copy)}\right) = \ln\left(\frac{\frac{(\Delta n/G)^{k}}{k!}e^{\frac{-\Delta n}{G}}}{\frac{(2\Delta n/G)^{k}}{k!}e^{\frac{-2\Delta n}{G}}}\right) = \frac{n\Delta}{G} - k\ln 2$$

The fragment assembly string graph
Myers, EW (2005) Bioinformatics. 21(suppl 2): ii79-85.

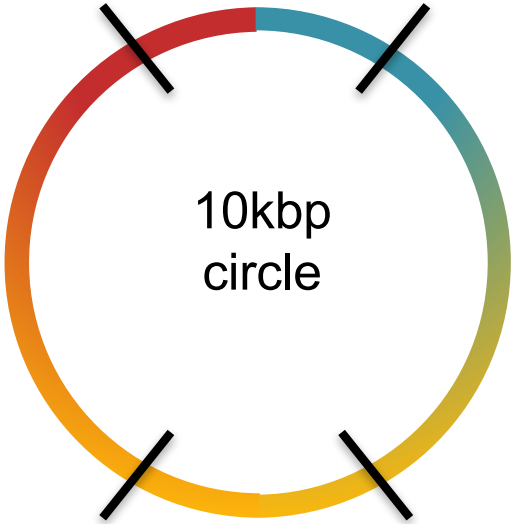
Paired-end and Mate-pairs

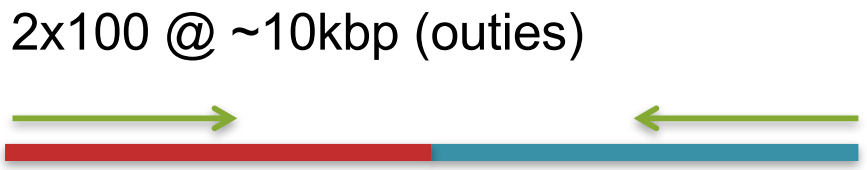
Paired-end sequencing

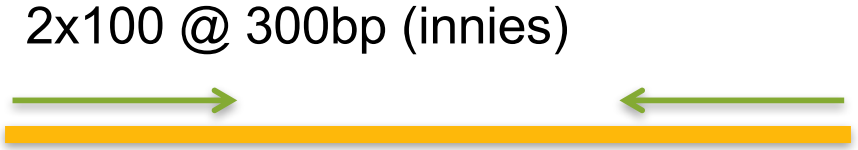
- Read one end of the molecule, flip, and read the other end
- Generate pair of reads separated by up to 500bp with inward orientation

Mate-pair sequencing

- Circularize long molecules (1-10kbp), shear into fragments, & sequence
- Mate failures create short paired-end reads

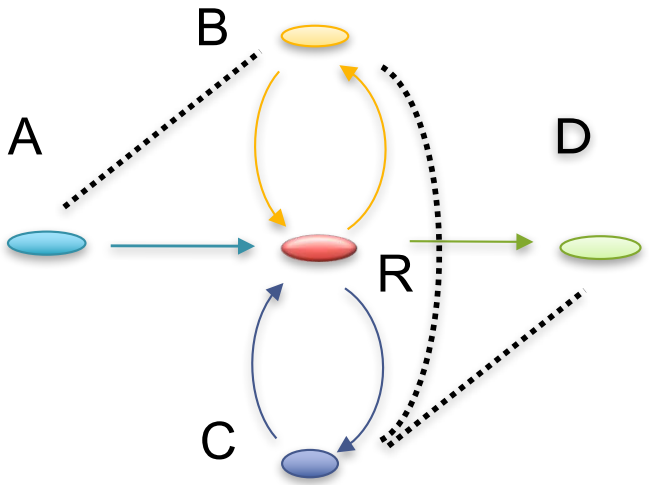




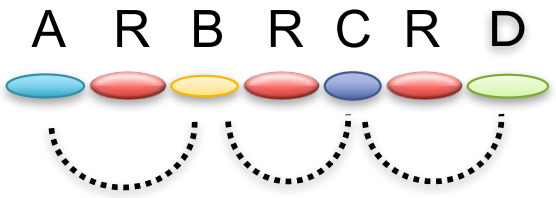


Scaffolding

- Initial contigs (*aka* unipaths, unitigs) terminate at
  - *Coverage gaps*: especially extreme GC
  - *Conflicts*: errors, repeat boundaries

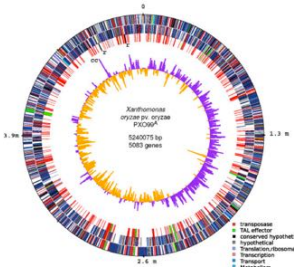


- Use mate-pairs to resolve correct order through assembly graph
  - Place sequence to satisfy the mate constraints
  - Mates through repeat nodes are tangled



- Final scaffold may have internal gaps called sequencing gaps
  - We know the order, orientation, and spacing, but just not the bases. Fill with Ns instead

Assembly Summary



Assembly quality depends on

1. ***Coverage***: low coverage is mathematically hopeless
2. ***Repeat composition***: high repeat content is challenging
3. ***Read length***: longer reads help resolve repeats
4. ***Error rate***: errors reduce coverage, obscure true overlaps

- Assembly is a hierarchical, starting from individual reads, build high confidence contigs/unitigs, incorporate the mates to build scaffolds
  - Extensive error correction is the key to getting the best assembly possible from a given data set

- Watch out for collapsed repeats & other misassemblies
  - Globally/Locally reassemble data from scratch with better parameters & stitch the 2 assemblies together

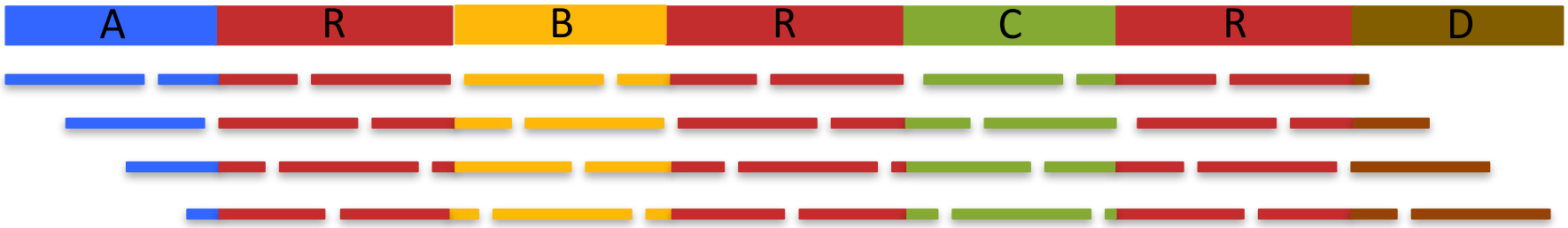
Outline

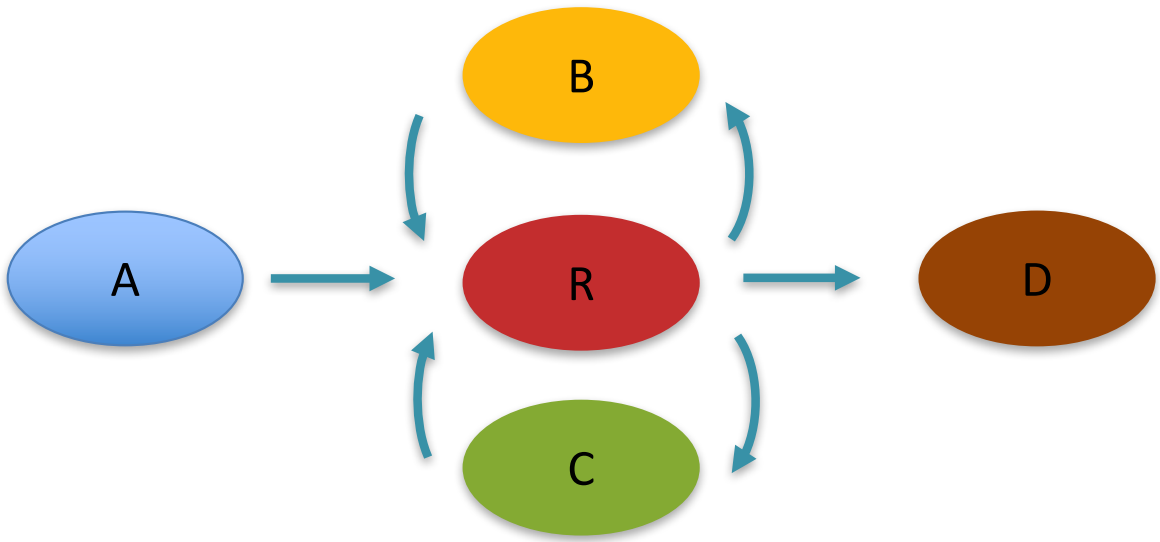
1. ***Assembly theory***
   - Assembly by analogy

2. ***Practical Issues***
   - Coverage, read length, errors, and repeats

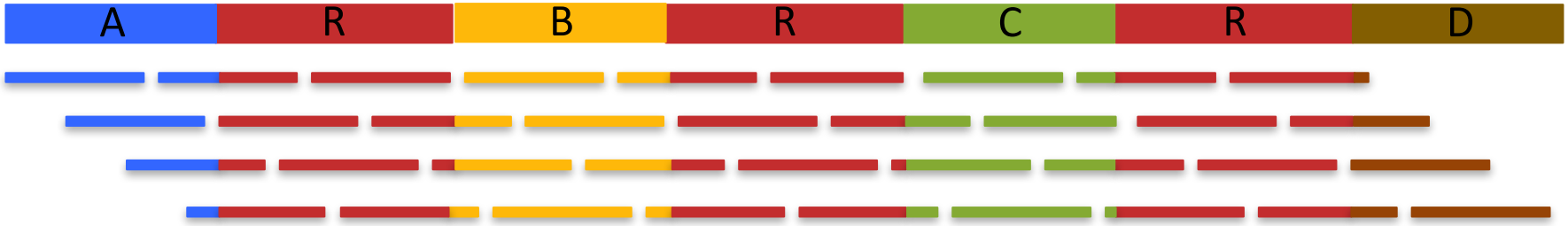
3. ***Next-next-gen Assembly***
   - PacBio/ONT projects

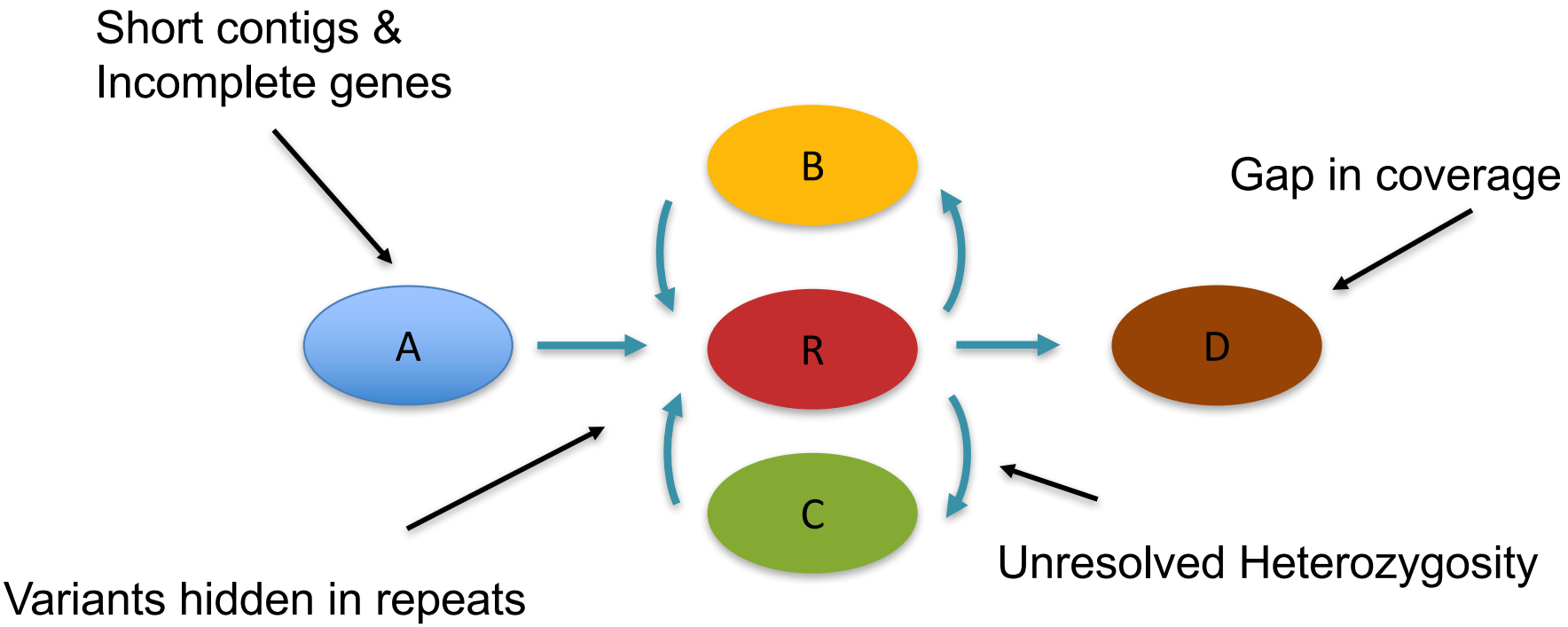
Assembly Complexity



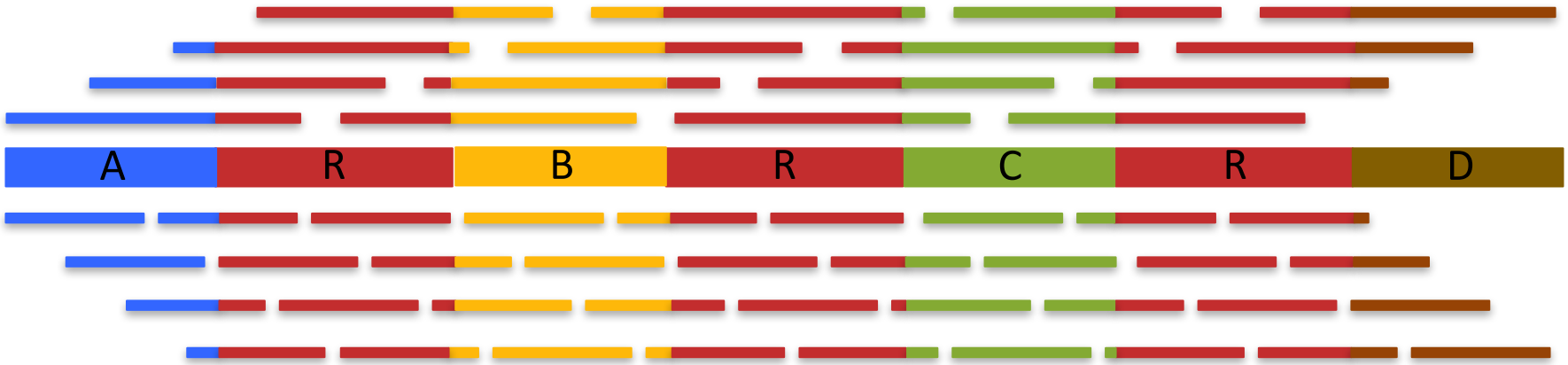


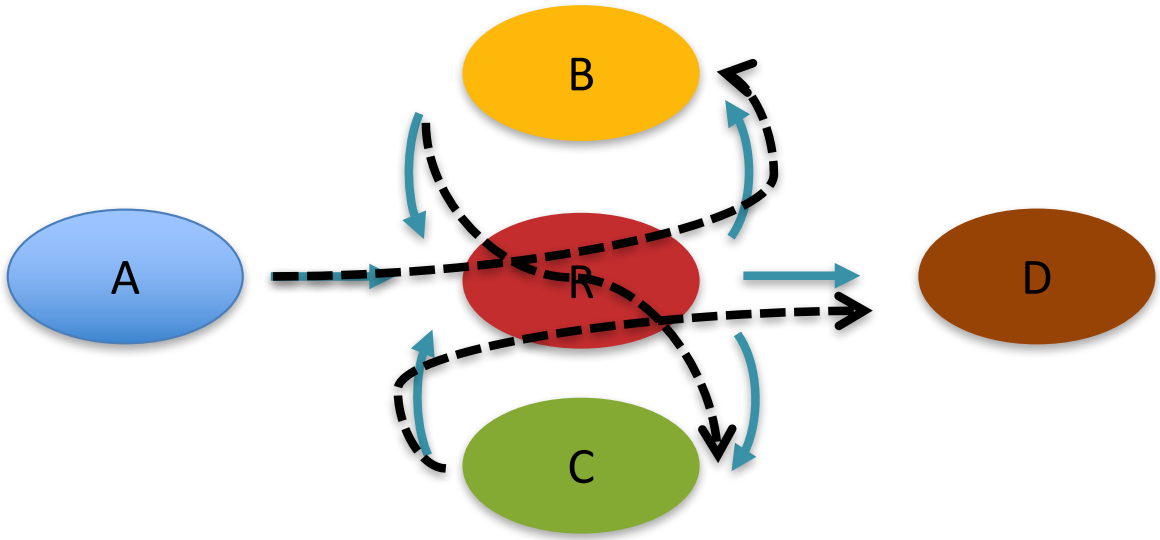
Assembly Complexity



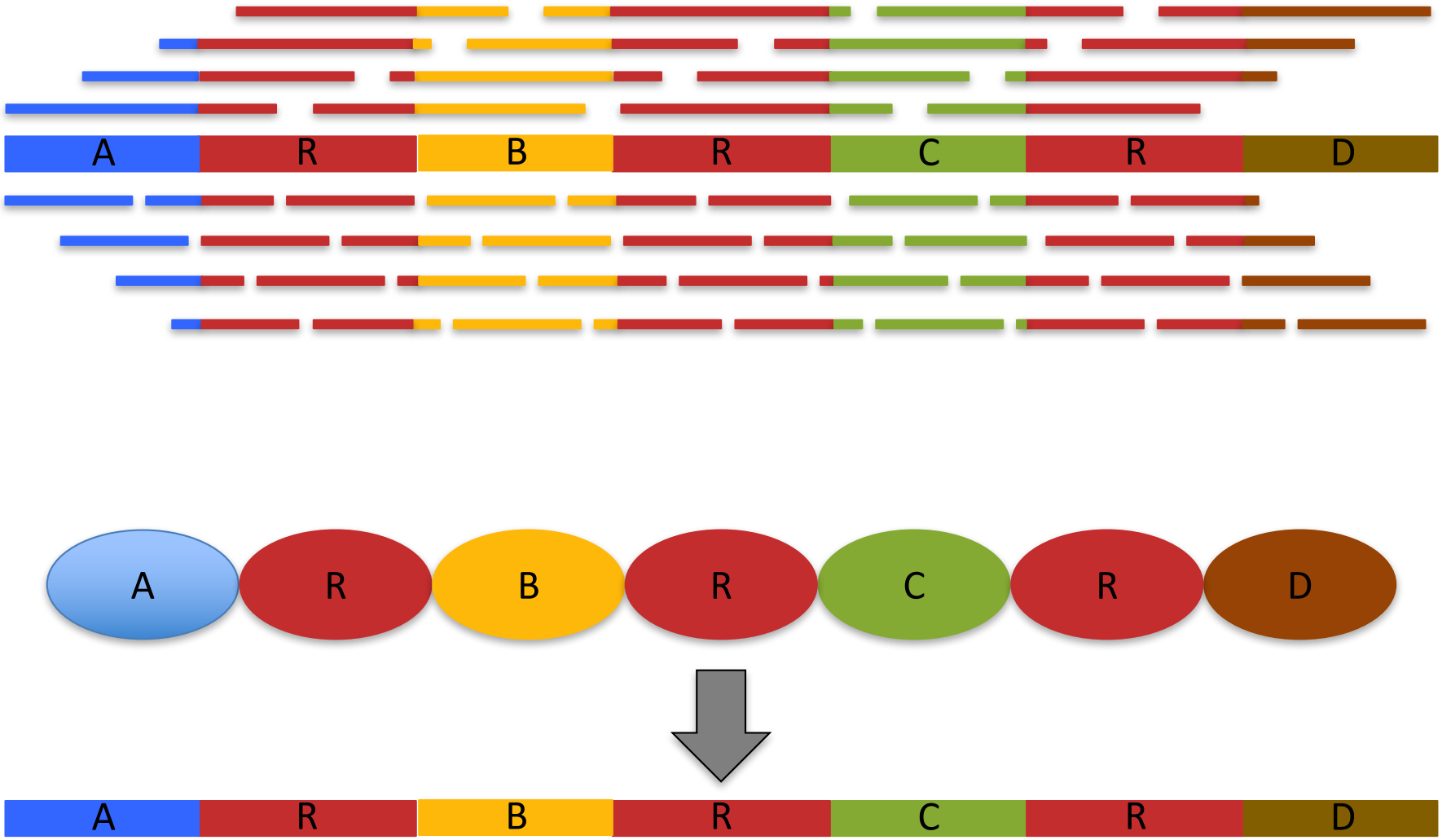


Assembly Complexity





Assembly Complexity



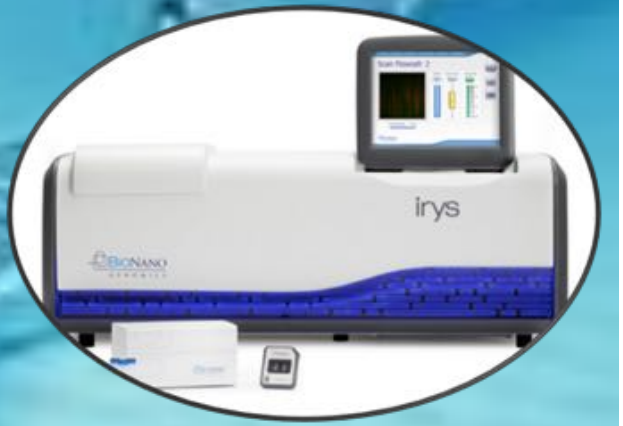
The advantages of SMRT sequencing
Roberts, RJ, Carneiro, MO, Schatz, MC (2013) *Genome Biology*. 14:405

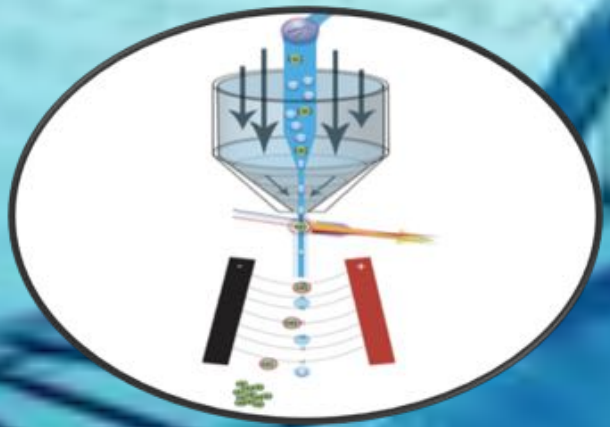
Genomics Arsenal in the year 2017

Sample Preparation

Sequencing

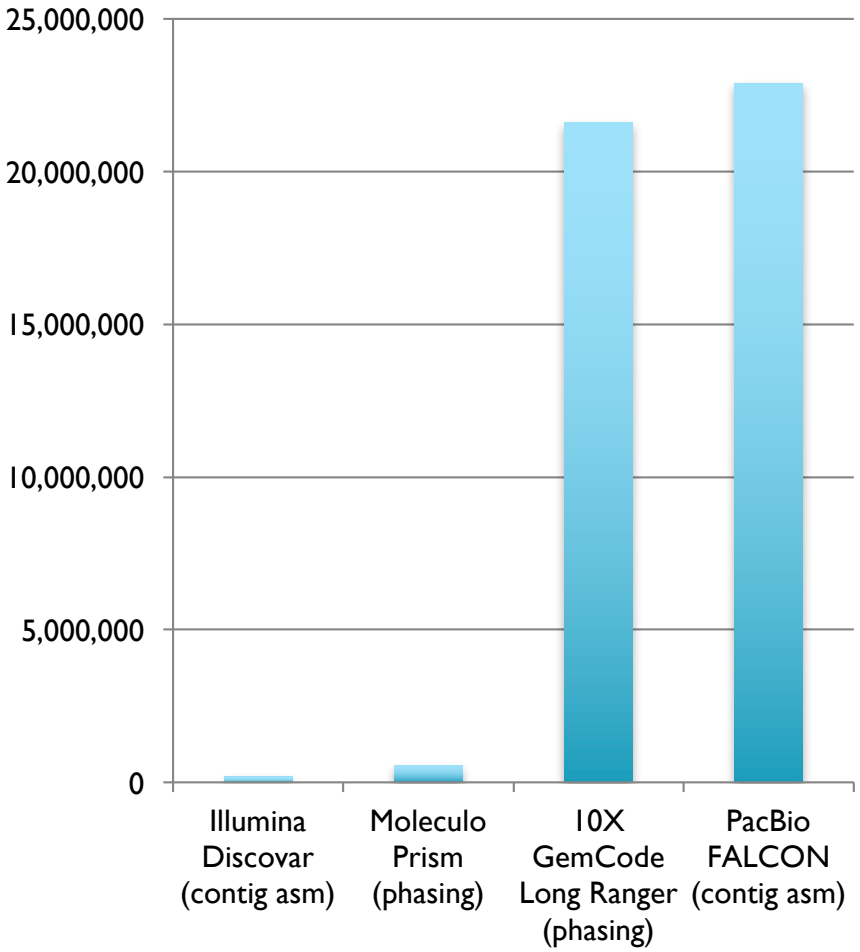
Chromosome Mapping





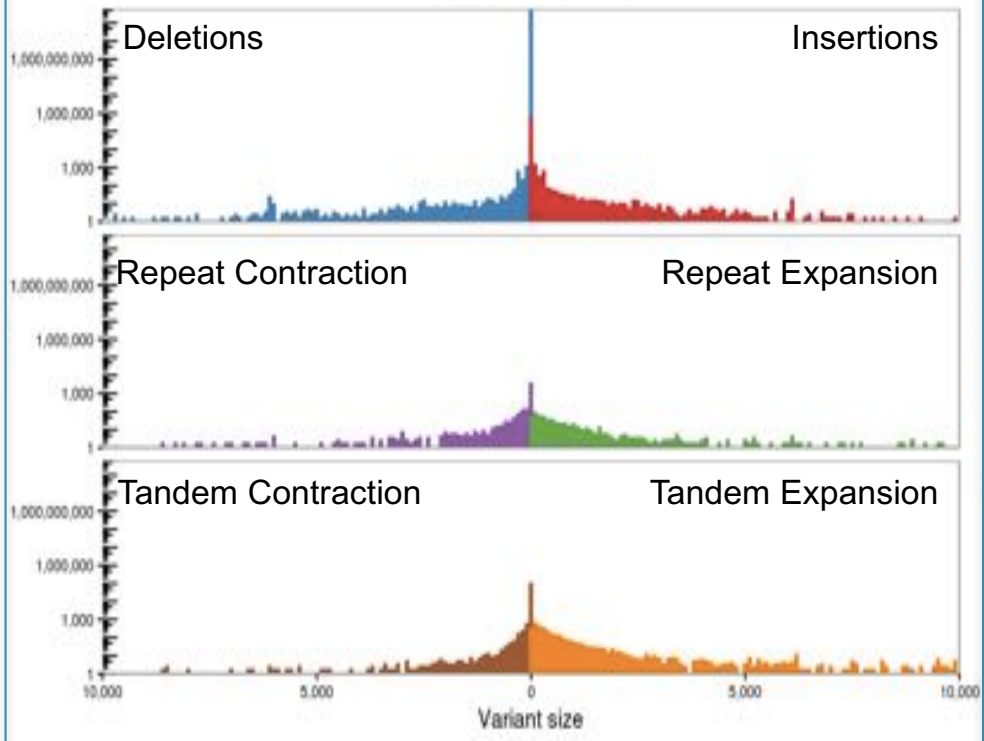
Recent Long Read Assemblies

Human Analysis N50 Sizes



Third-generation sequencing and the future of genomics
Lee et al (2016) *bioRxiv*
doi: http://dx.doi.org/10.1101/048603

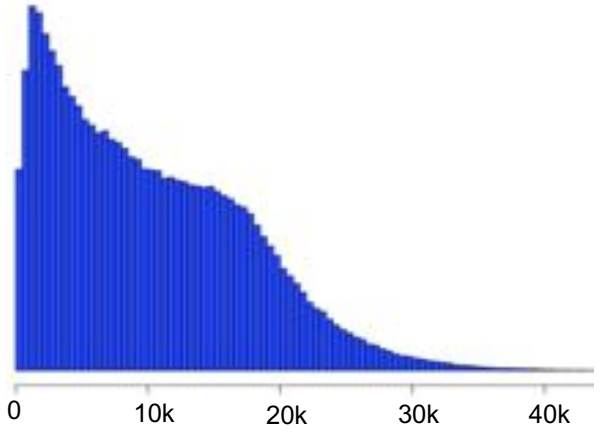
Structural Variants in CHM1



Assemblytics: a web analytics tool for the detection of variants from an assembly
Nattestad & Schatz (2016) *Bioinformatics.*
doi: 10.1093/bioinformatics/btw369

Single Molecule Sequencing

PacBio RS II

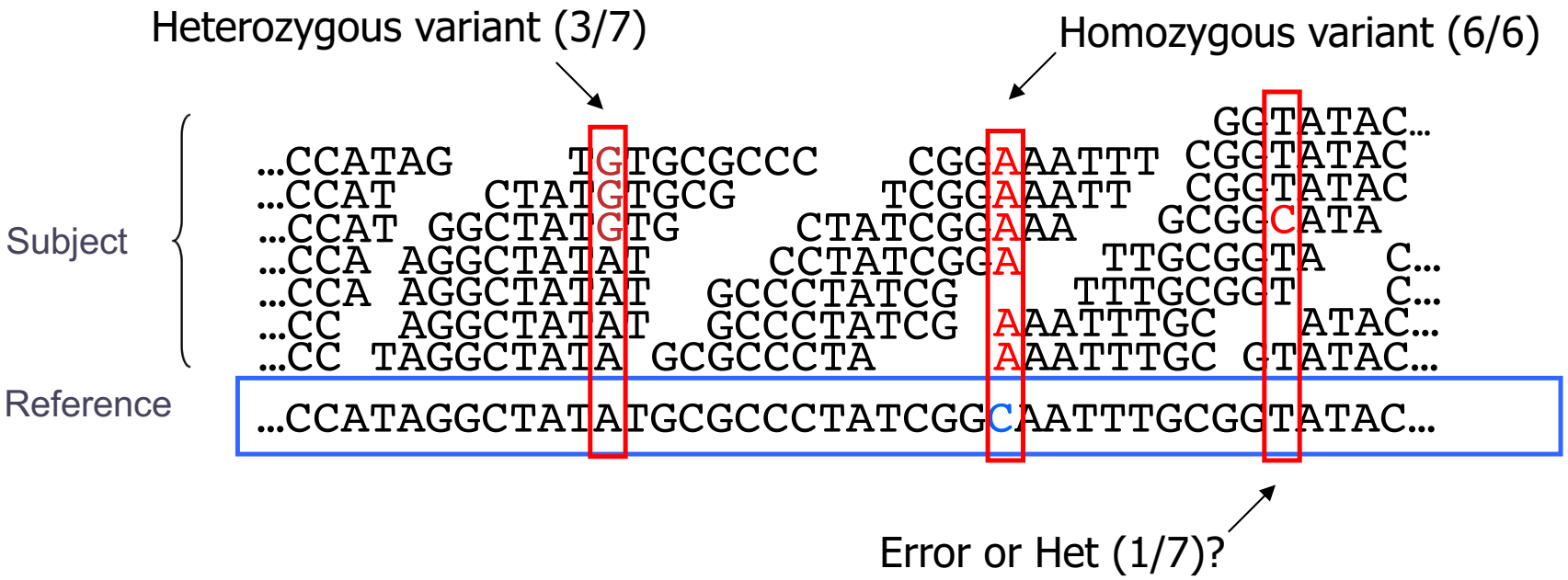


CSHL/PacBio

```
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
```

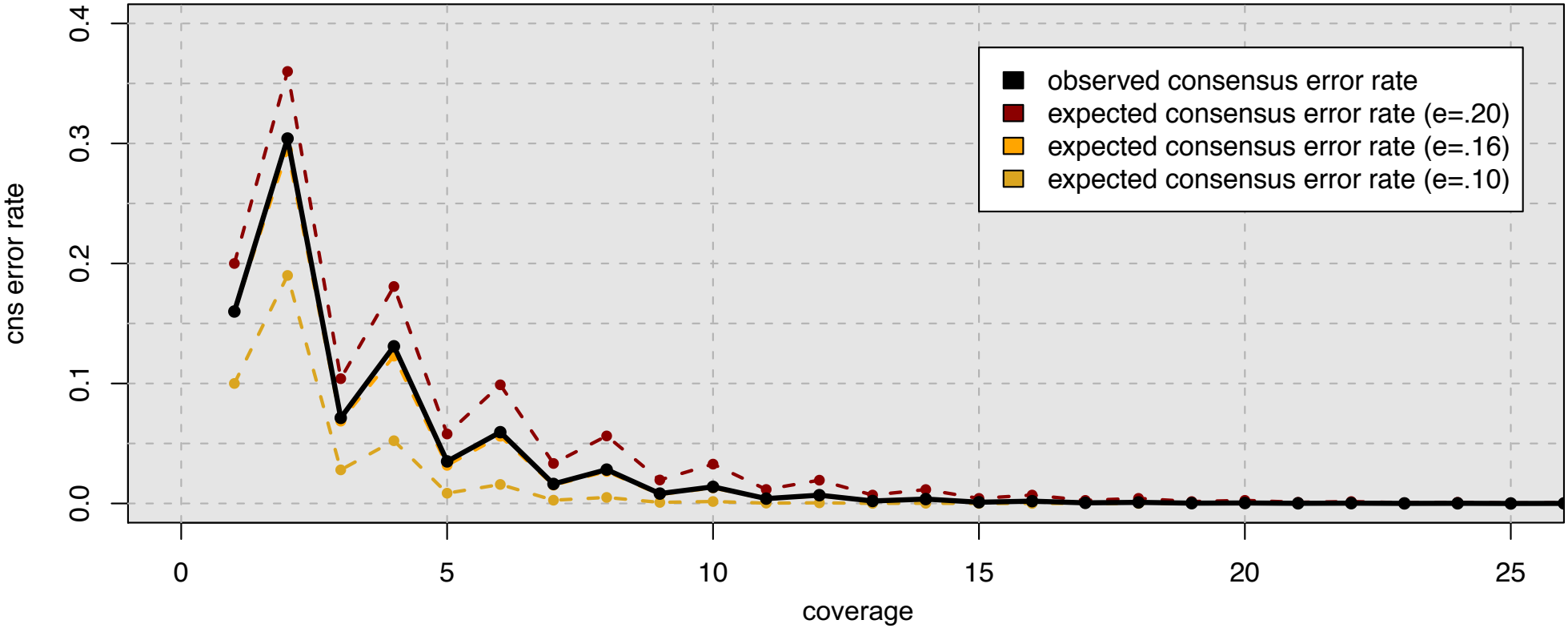
Sample of 100k reads aligned with BLASR requiring >100bp alignment
Average overall accuracy 83.7%: 11.5% insertions, 3.4% deletions, 1.4% mismatch

Genotyping Theory



- If there were no sequencing errors, identifying SNPs would be trivial:
  - Any time a read disagrees with the reference, it must be a variant!
- A single read of many differing from the reference is probably just an error, but it becomes more likely to be real as we see it multiple times
  - Use binomial test to evaluate prob. of heterozygosity vs. prob of error
  - Coverage (oversampling) is our main tool to improve accuracy

Consensus Accuracy and Coverage



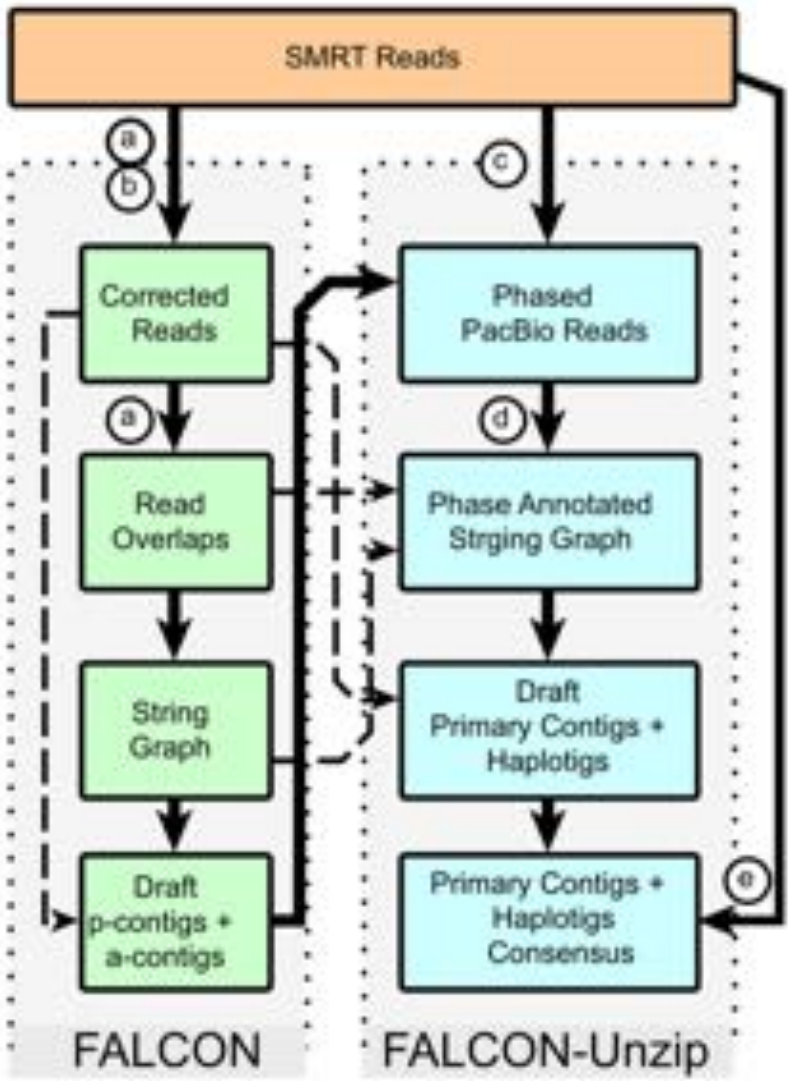
Coverage can overcome random errors

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

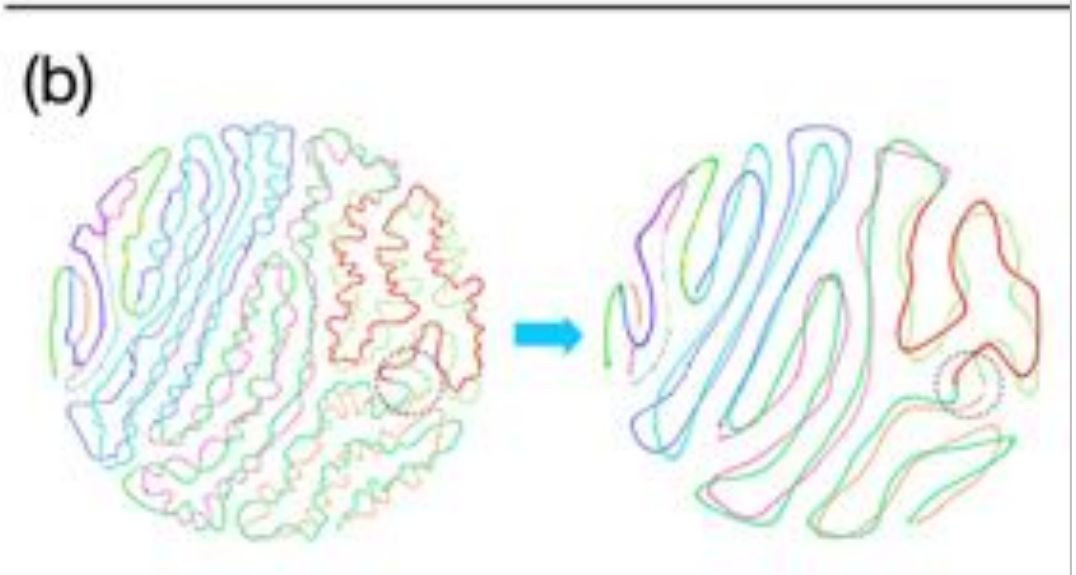
$$CNS\ Error = \sum_{i=\lceil c/2 \rceil}^{c} \binom{c}{i} (e)^{i} (1-e)^{n-i}$$

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

FALCON Accuracy

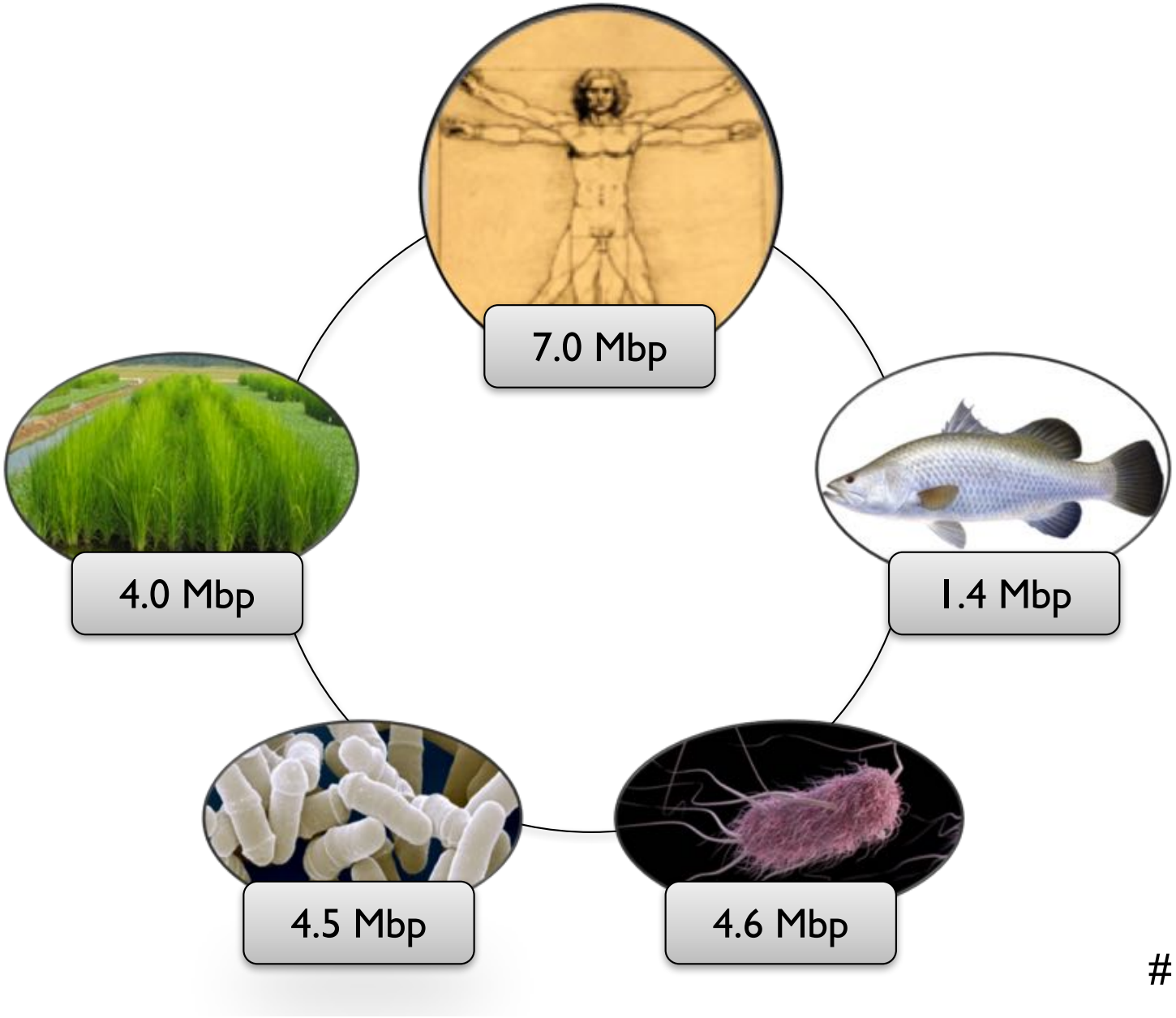


"The overall base-to-base concordance rate is about 99.99% (QV40 in Phred scale) in the F1 FALCON-Unzip assembly. The insertion and deletion (indel) concordances to the parental lines were lower (about QV40) than the SNP concordance rate (about QV50), with most residual errors concentrated in long homopolymer sequences"



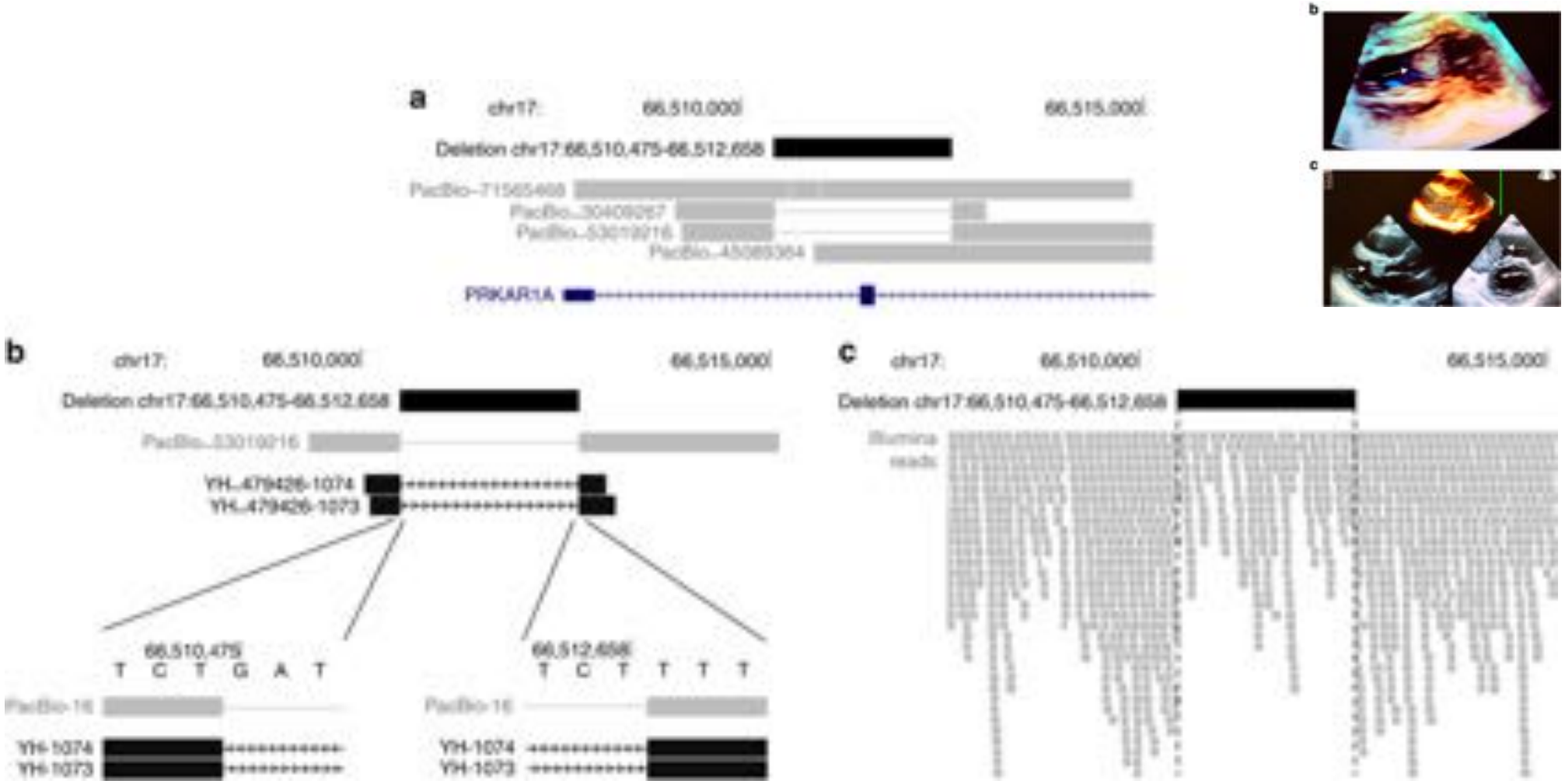
Phased Diploid Genome Assembly with Single Molecule Real-Time Sequencing
Chin et al (2016) *Nature Methods. doi:10.1038/nmeth.4035.*

(A few) Recent PacBio Assemblies



#1mbctgclub

Structural Variations in Human Disease

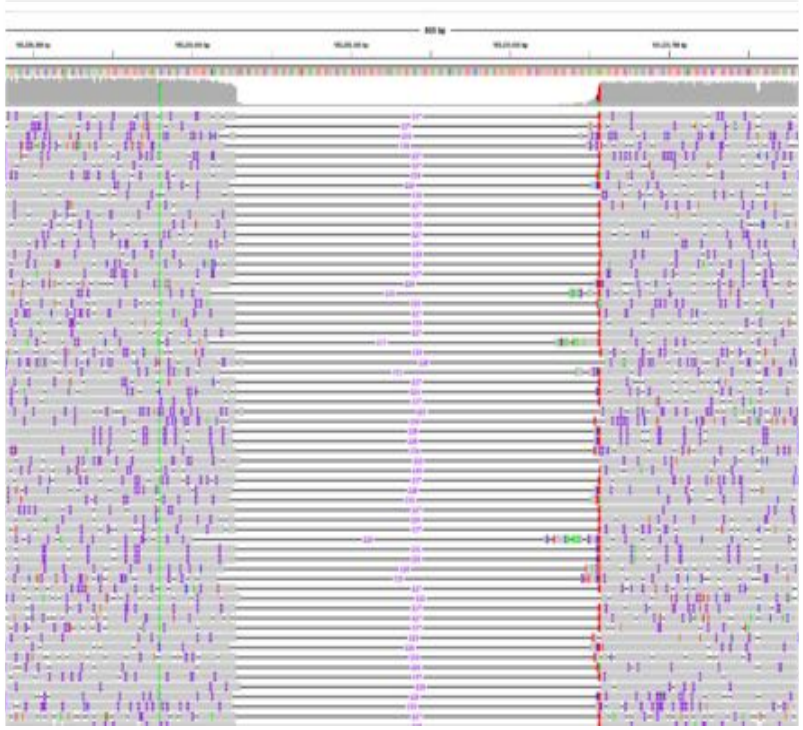


Long-read genome sequencing identifies causal structural variation in a Mendelian disease
Merker et al (2017) *Genetics in Medicine. doi:10.1038/gim.2017.86*

NGMLR + Sniffles

BWA-MEM:

NGMLR:



Accurate detection of complex structural variations using single molecule sequencing
Sedlazeck, Rescheneder et al (2017) *In preparation*

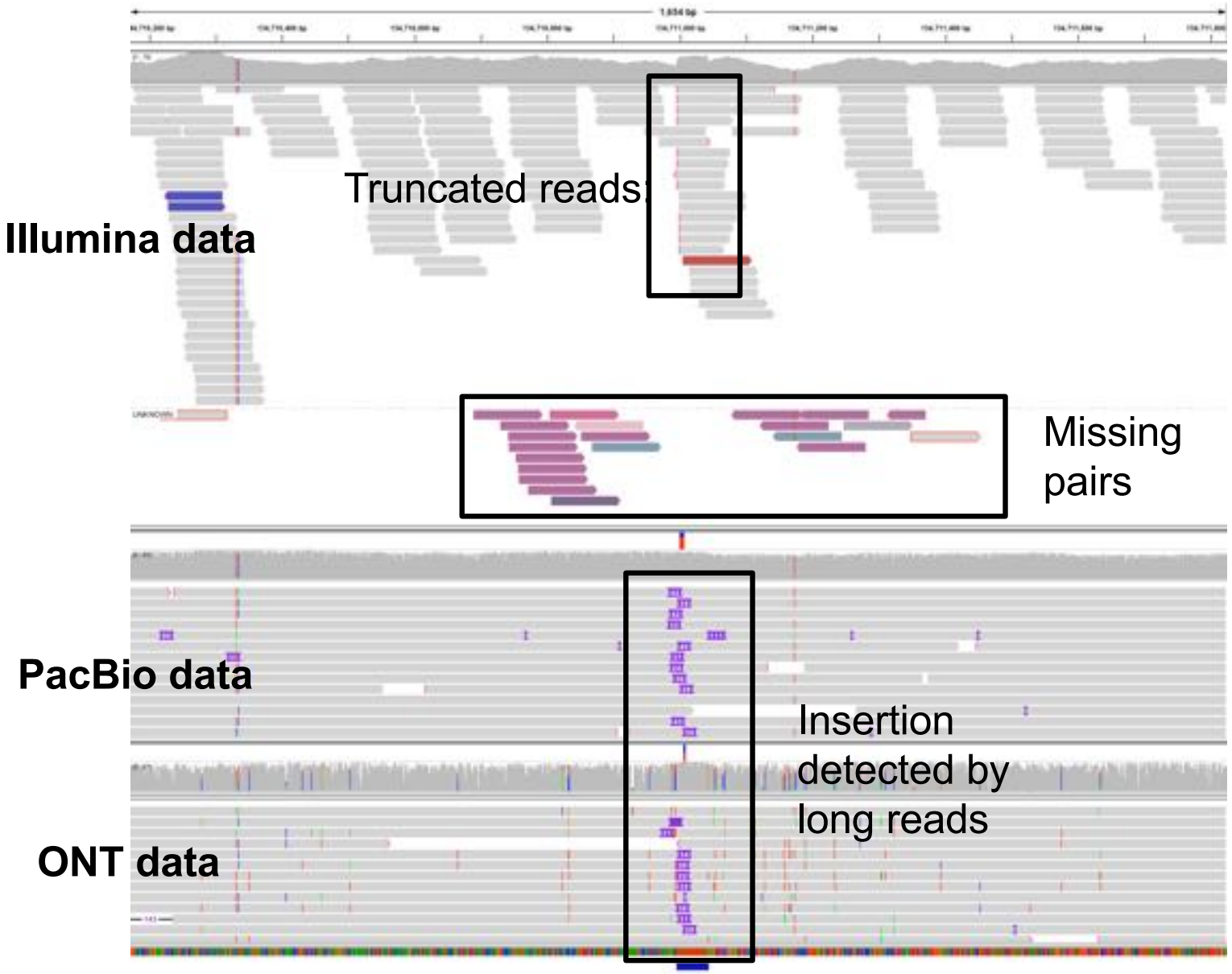
NGMLR + Sniffles

BWA-MEM:

NGMLR:

Accurate detection of complex structural variations using single molecule sequencing
Sedlazeck, Rescheneder et al (2017) *In preparation*

No more false positives!



Accurate detection of complex structural variations using single molecule sequencing
Sedlazeck, Rescheneder et al (2017) *In preparation*

Illumina Roadmap

Illumina Novaseq

$850k instrument cost
~$1k / human @ 50x
Short reads, high throughput

10X Chromium

$125k instrument costs
~$2k / human
Linked reads, medium throughput

PacBio Roadmap

PacBio Sequel

$350k instrument cost
~$30k / human @ 50x
Long reads, Medium throughput

SMRTcell v2

1M Zero Mode Waveguides
~15kb average read length
~$1000 / SMRTcell

Oxford Nanopore

MinION

$1k / instrument
~$30k / human @ 50x
Long reads, Low throughput

PromethION

$75k / instrument
>>100GB / day
??? / human @ 50x

Oxford Nanopore sequencing, hybrid error correction, and de novo assembly of a eukaryotic genome
Goodwin, S, Gurtowski, J, Ethe-Sayers, S, Deshpande, P, Schatz MC* McCombie, WR* (2015) Genome Research doi: 10.1101/gr.191395.115