Whole Genome Assembly with iPlant

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CSHL Plant Genomes and Biotechnology

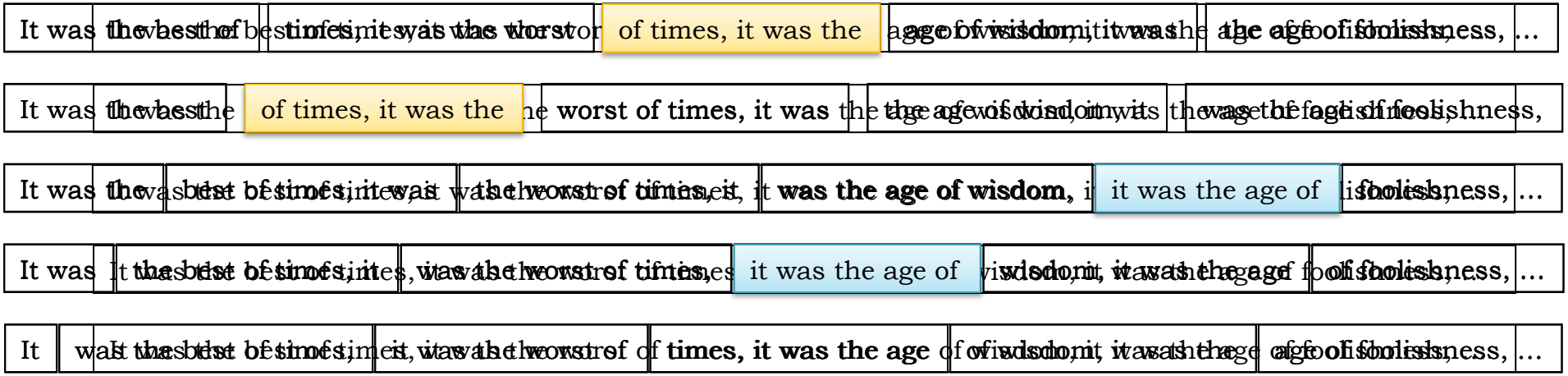
iPlant Collaborative™ *Empowering A New Plant Biology*

Outline

1. Assembly theory
   1. Assembly by analogy
   2. De Bruijn and Overlap graph
   3. Coverage, read length, errors, and repeats
2. Genome assemblers
   1. Assemblathon
   2. ALLPATHS-LG
   3. Celera Assembler
3. Assembly Tutorial with iPlant

Shredded Book Reconstruction

- Dickens accidentally shreds the first printing of <u>A Tale of Two Cities</u>
  - 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

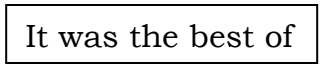
- It was the best of times, it was the [worst/age]

Model the assembly problem as a graph problem

de Bruijn Graph Construction

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

Original Fragment

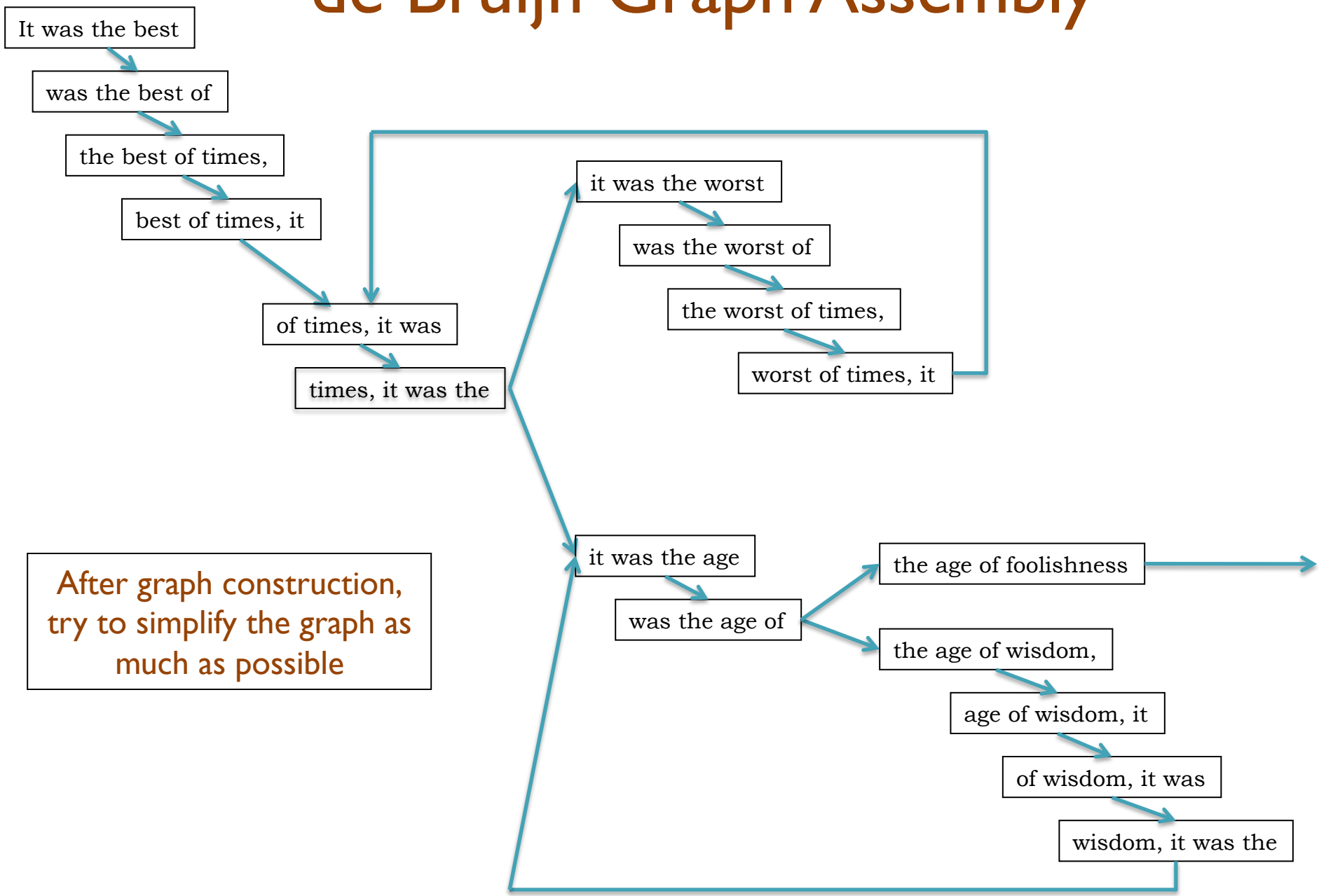


Directed Edge

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

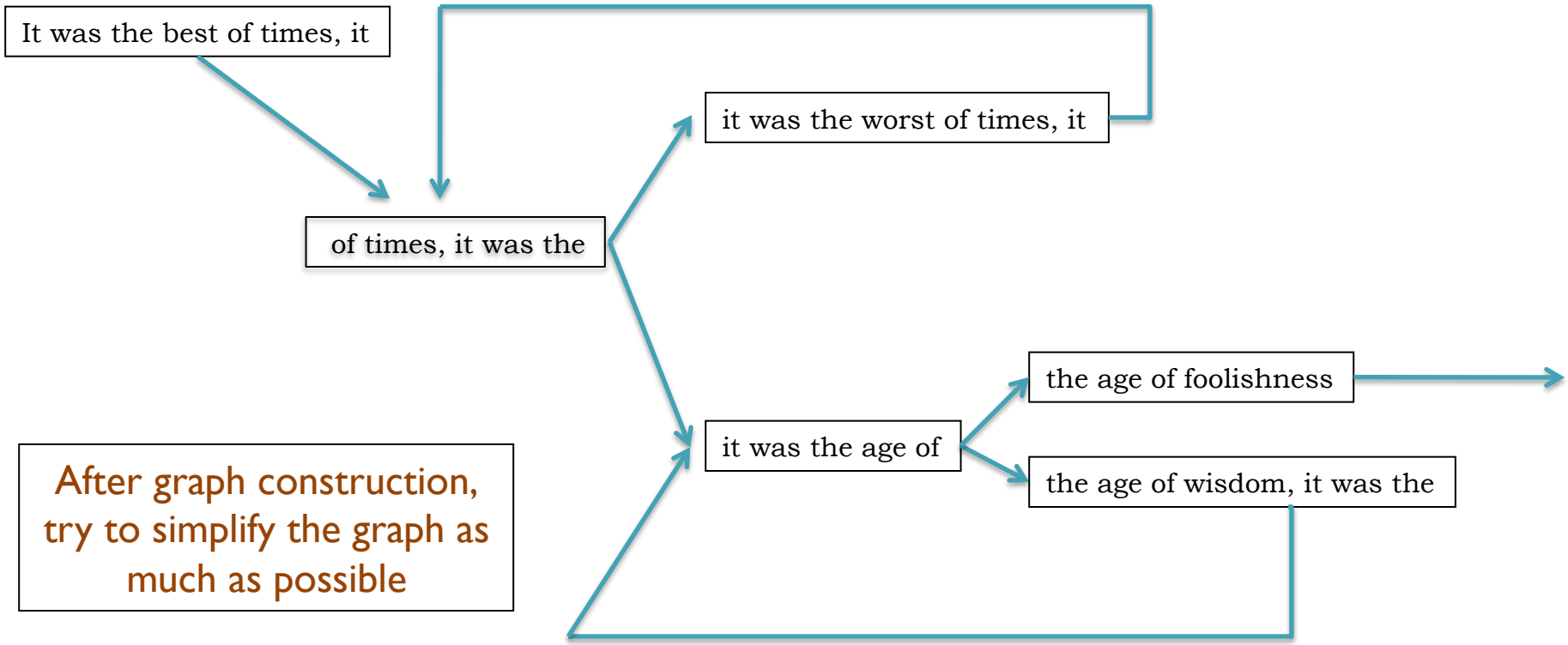
de Bruijn, 1946
Idury and Waterman, 1995
Pevzner, Tang, Waterman, 2001

de Bruijn Graph Assembly



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

de Bruijn Graph Assembly



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

The full tale

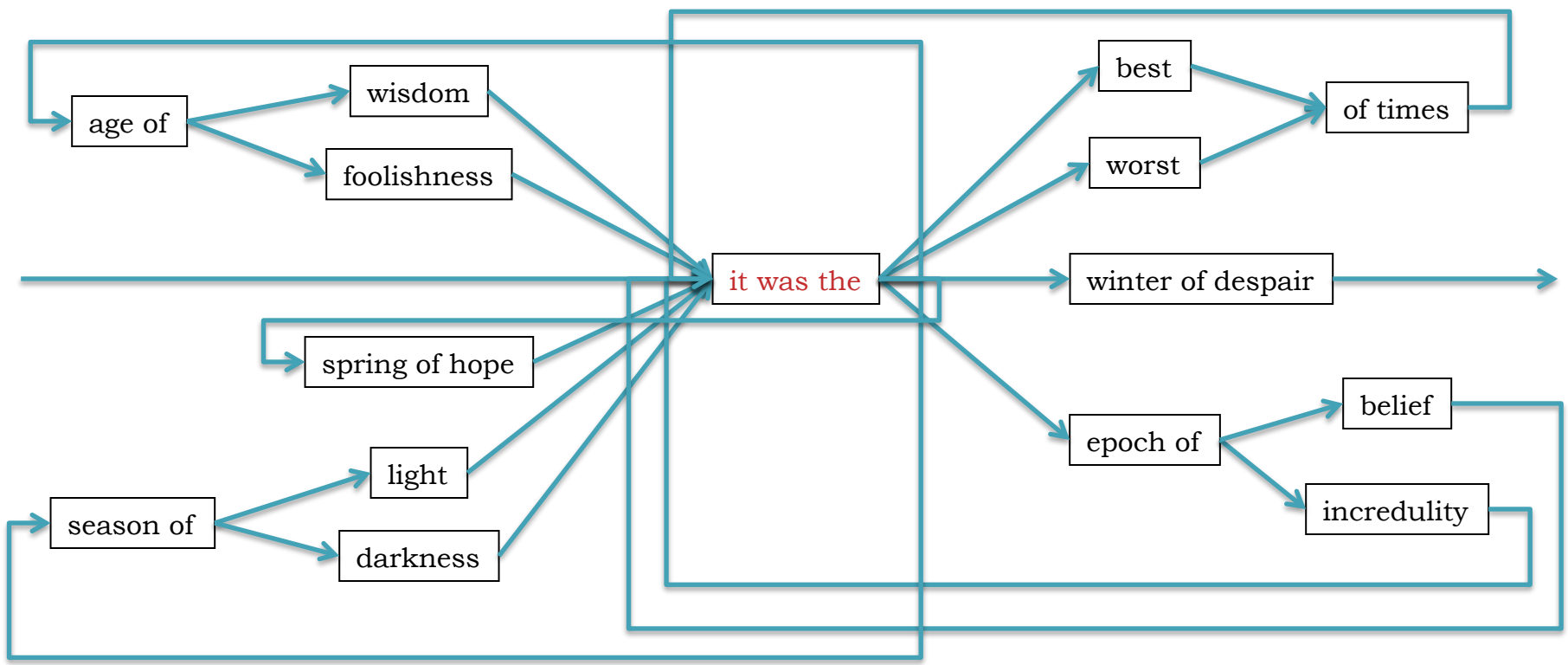
… it was the best of times it was the worst of times …

… 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 …

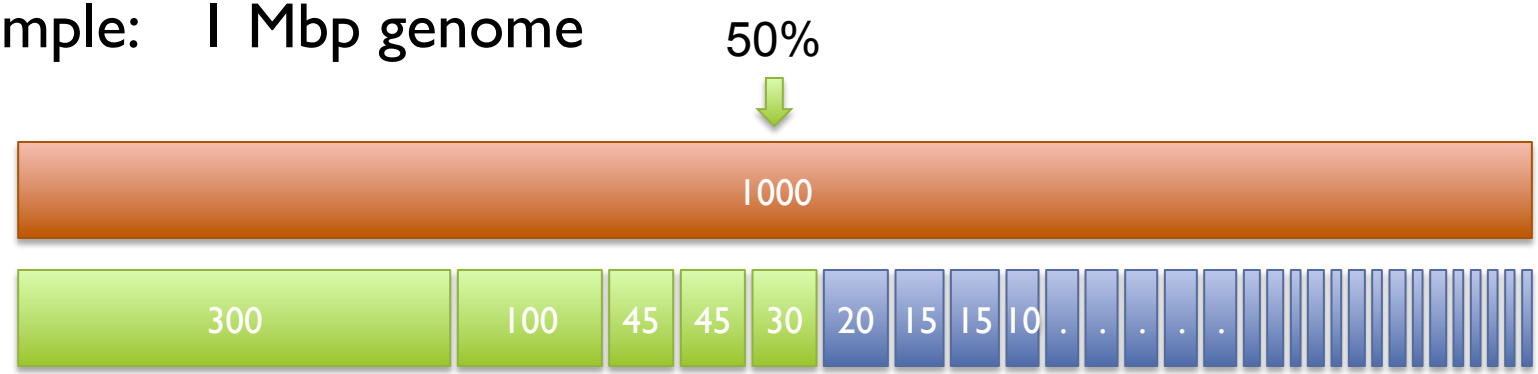
… it was the spring of hope it was the winder of despair …



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:

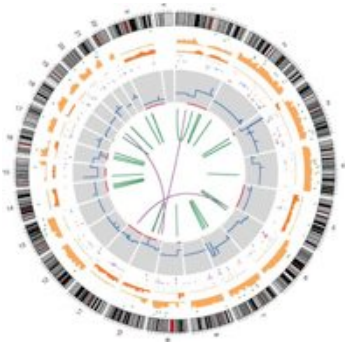
N50 values are only meaningful to compare when base genome size is the same in all cases

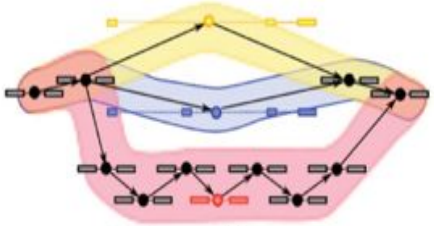
Assembly Applications

- Novel genomes

- Metagenomes

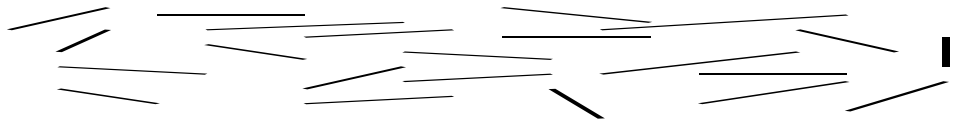
- Sequencing assays
  - Structural variations
  - Transcript assembly
  - …





Assembling a Genome

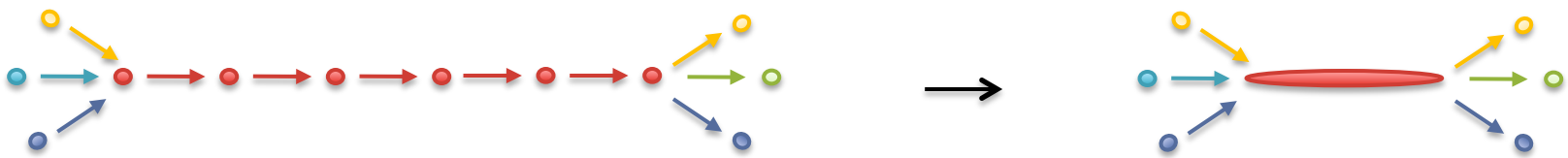
1. Shear & Sequence DNA



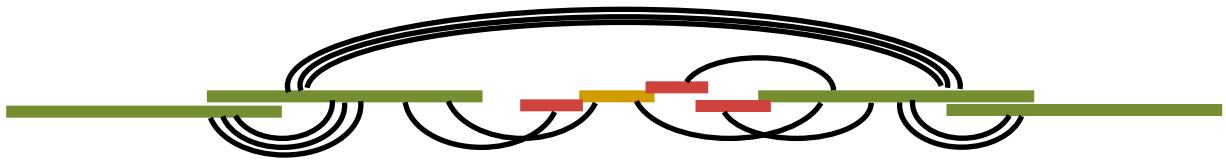
2. Construct assembly graph from overlapping reads

...AGCCTAGGGATGCGCGACACGT
GGATGCGCGACACGTCGCATATCCGGTTTGGTCAACCTCGGACGGAC
CAACCTCGGACGGACCTCAGCGAA...

3. Simplify assembly graph

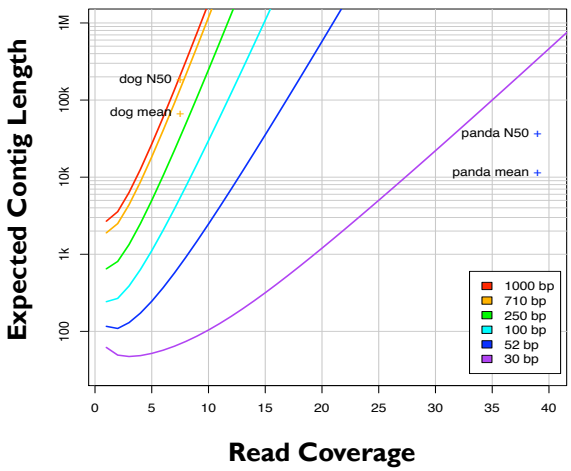


4. Detangle graph with long reads, mates, and other links



Ingredients for a good assembly

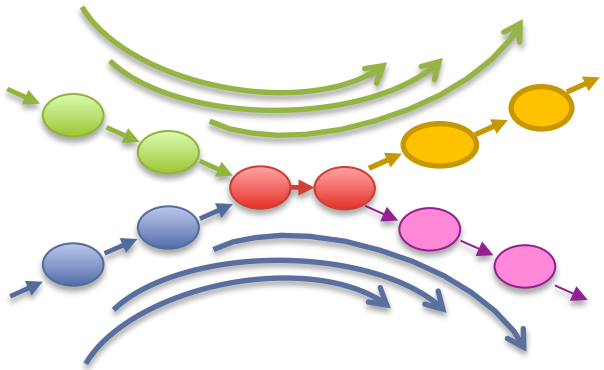
Coverage



High coverage is required

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

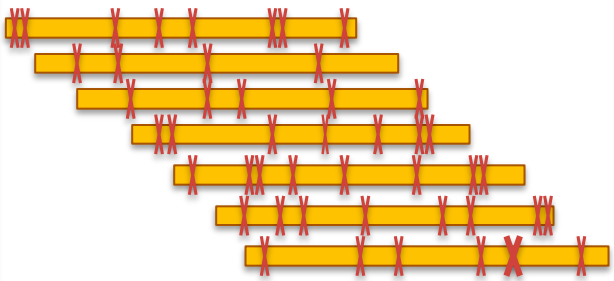
Read Length



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



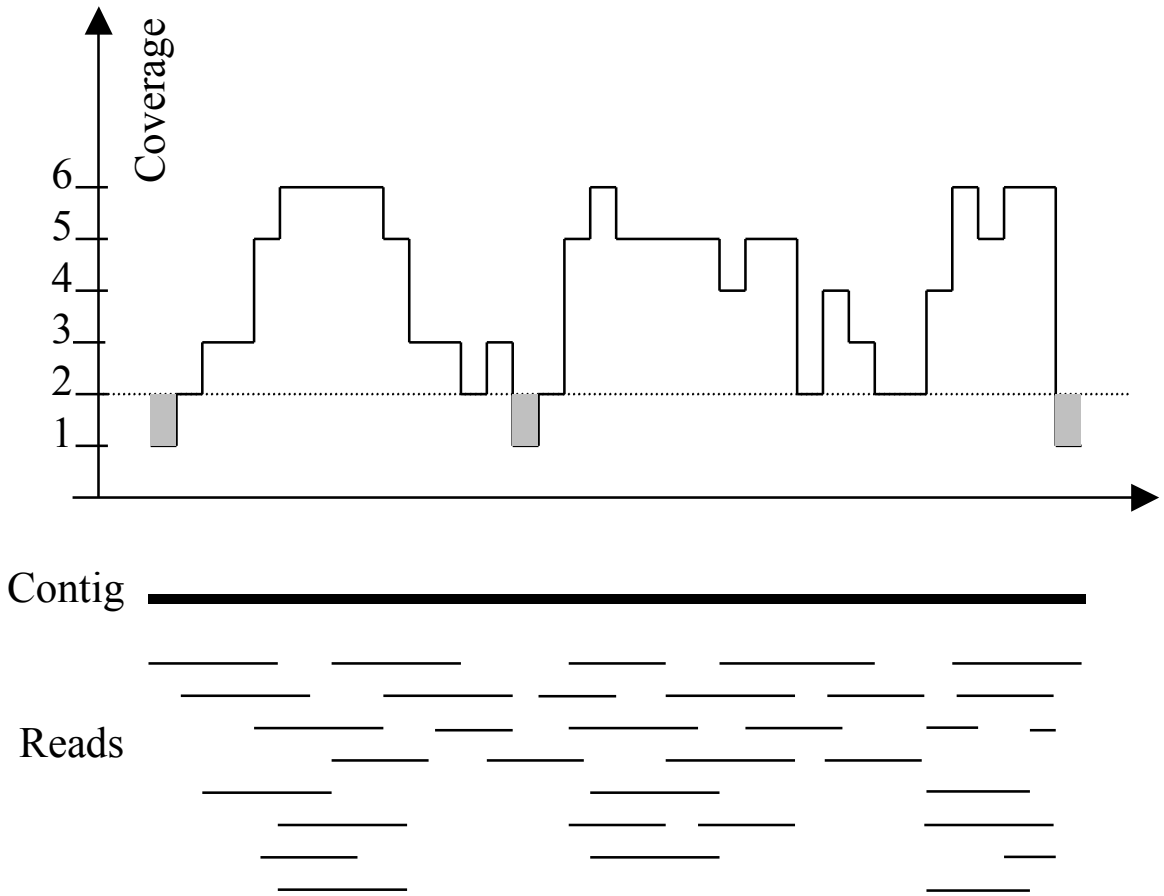
Errors obscure overlaps

- 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

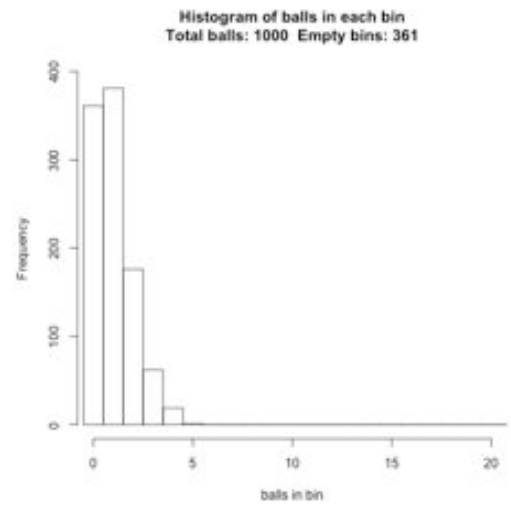
Coverage

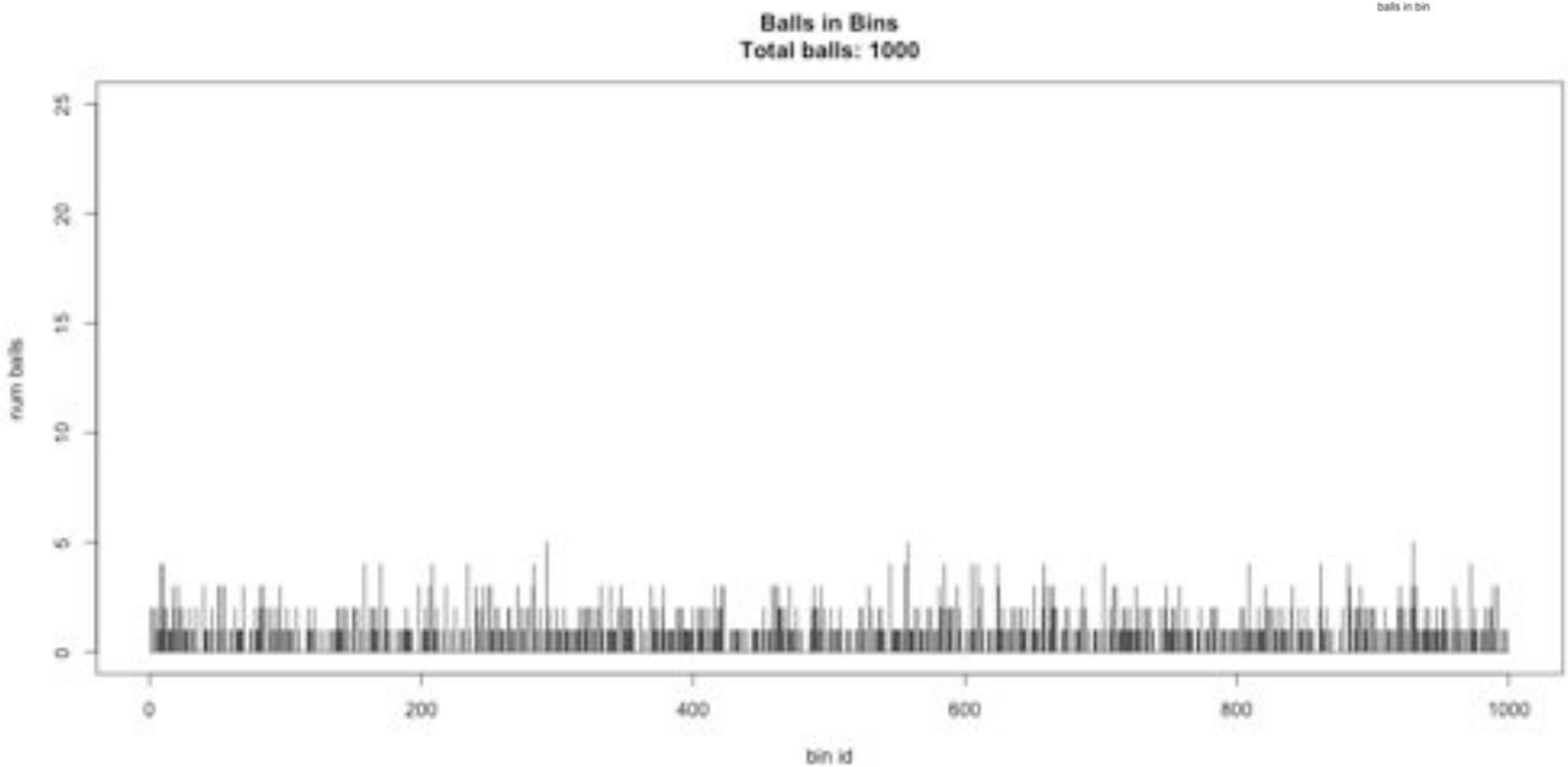
Typical contig coverage



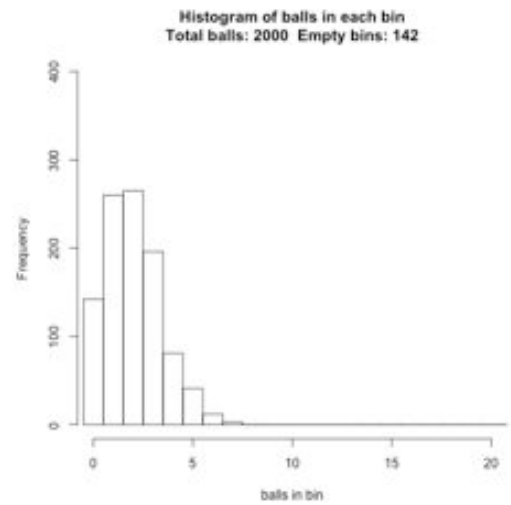
Imagine raindrops on a sidewalk

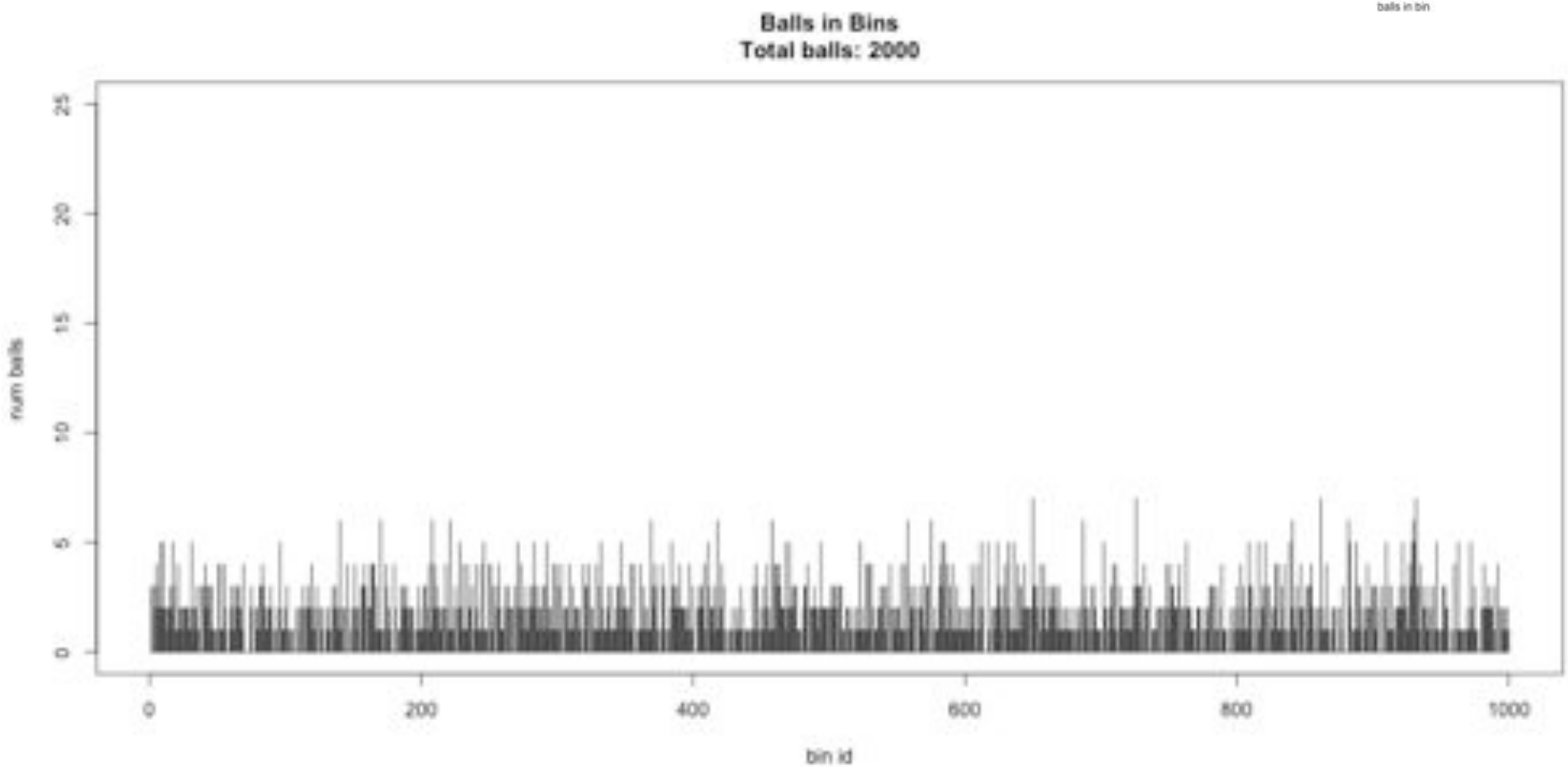
Balls in Bins 1x



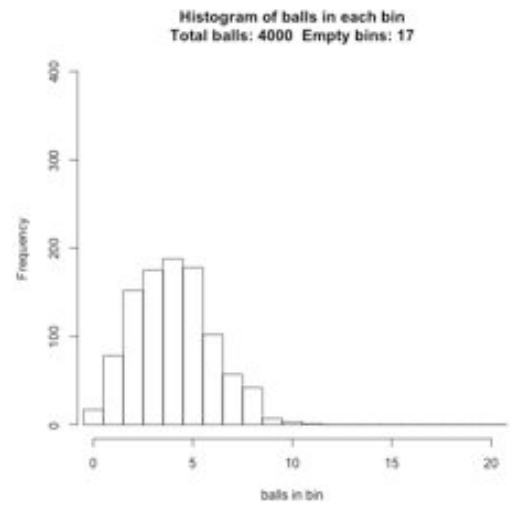


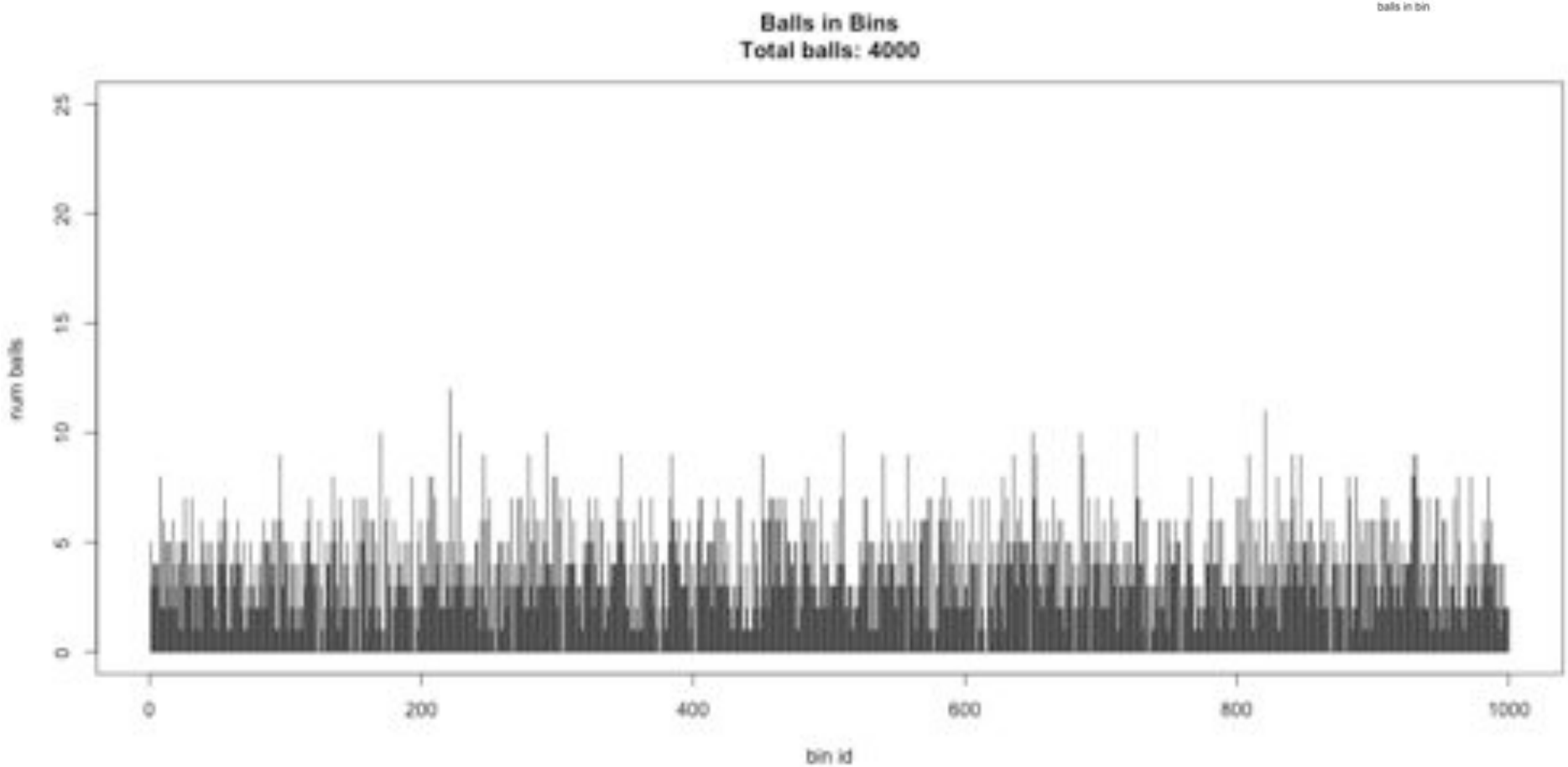
Balls in Bins 2x



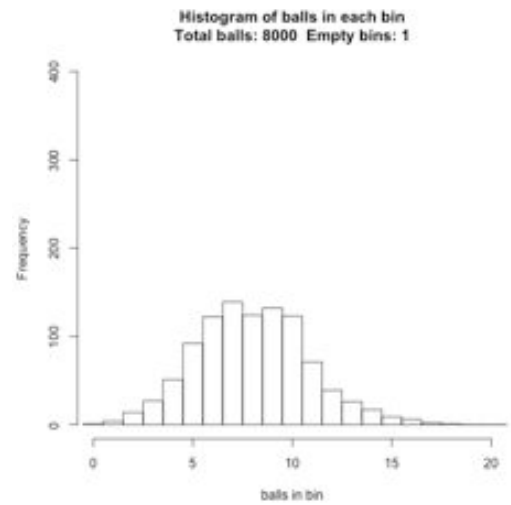


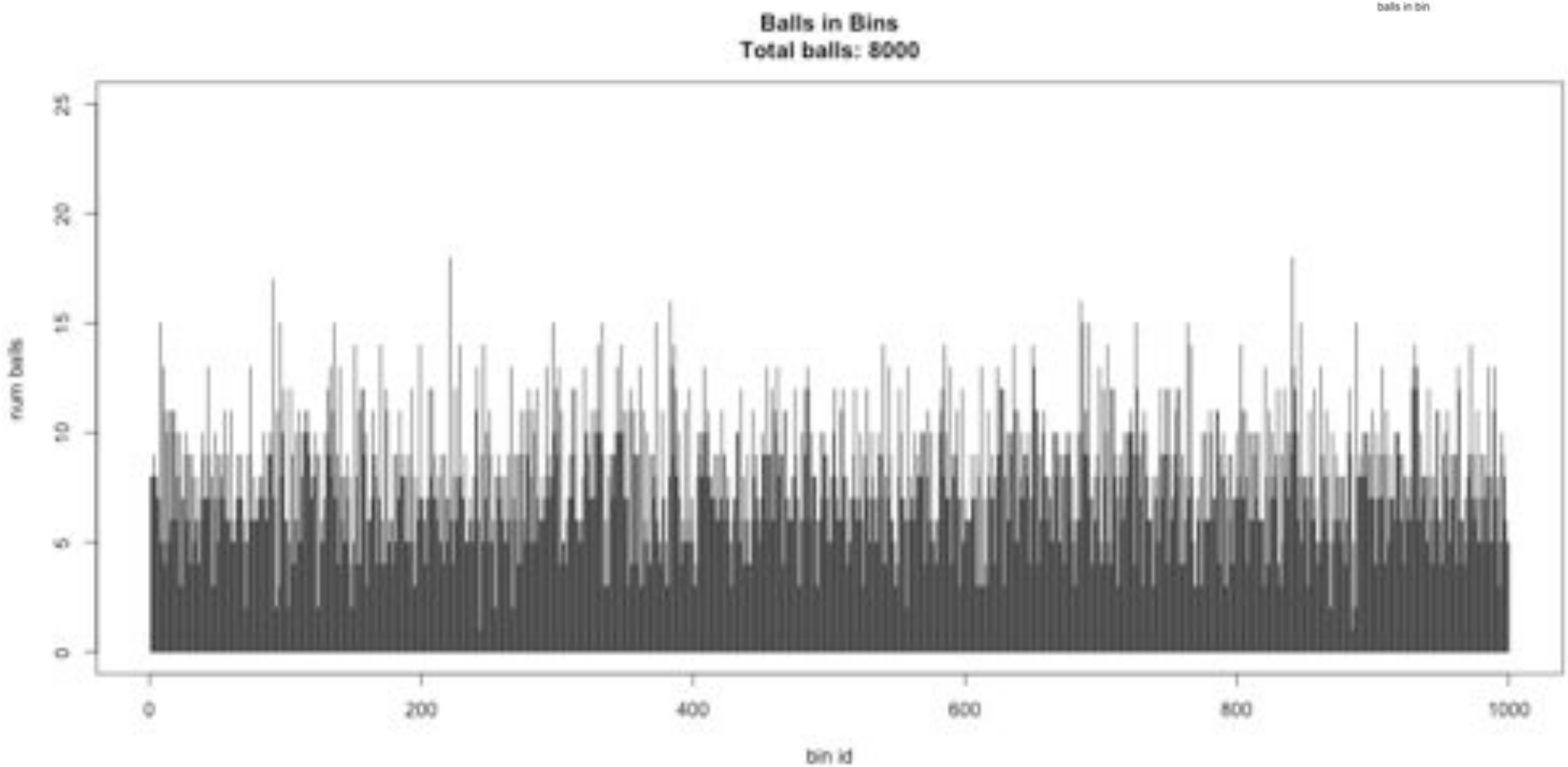
Balls in Bins 4x





Balls in Bins 8x

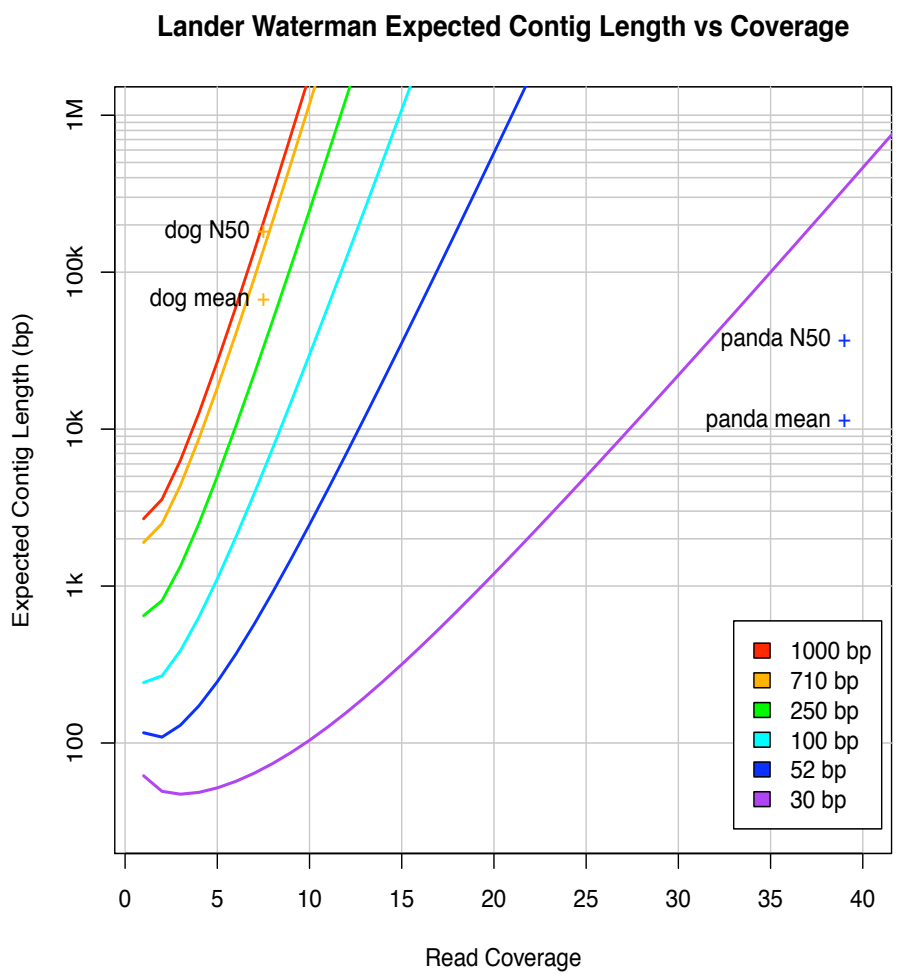




Coverage and Read Length

Idealized Lander-Waterman model

- Reads start at perfectly random positions
- Contig length is a function of coverage and read length
  - Short reads require much higher coverage to reach same expected contig length
- Need even high coverage for higher ploidy, sequencing errors, sequencing biases
  - Recommend 100x coverage

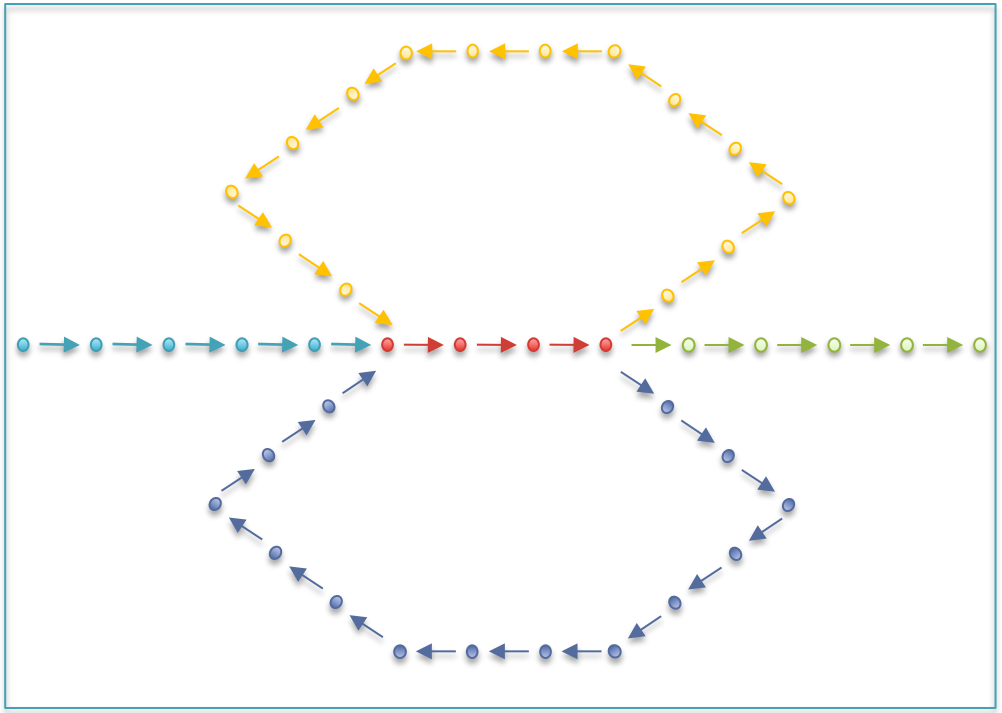


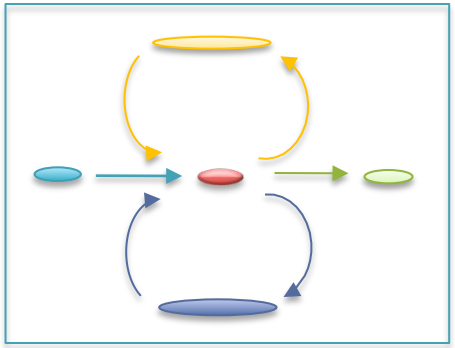
Assembly of Large Genomes using Second Generation Sequencing
Schatz MC, Delcher AL, Salzberg SL (2010) *Genome Research.* 20:1165-1173.

Errors

Unitigging / Unipathing

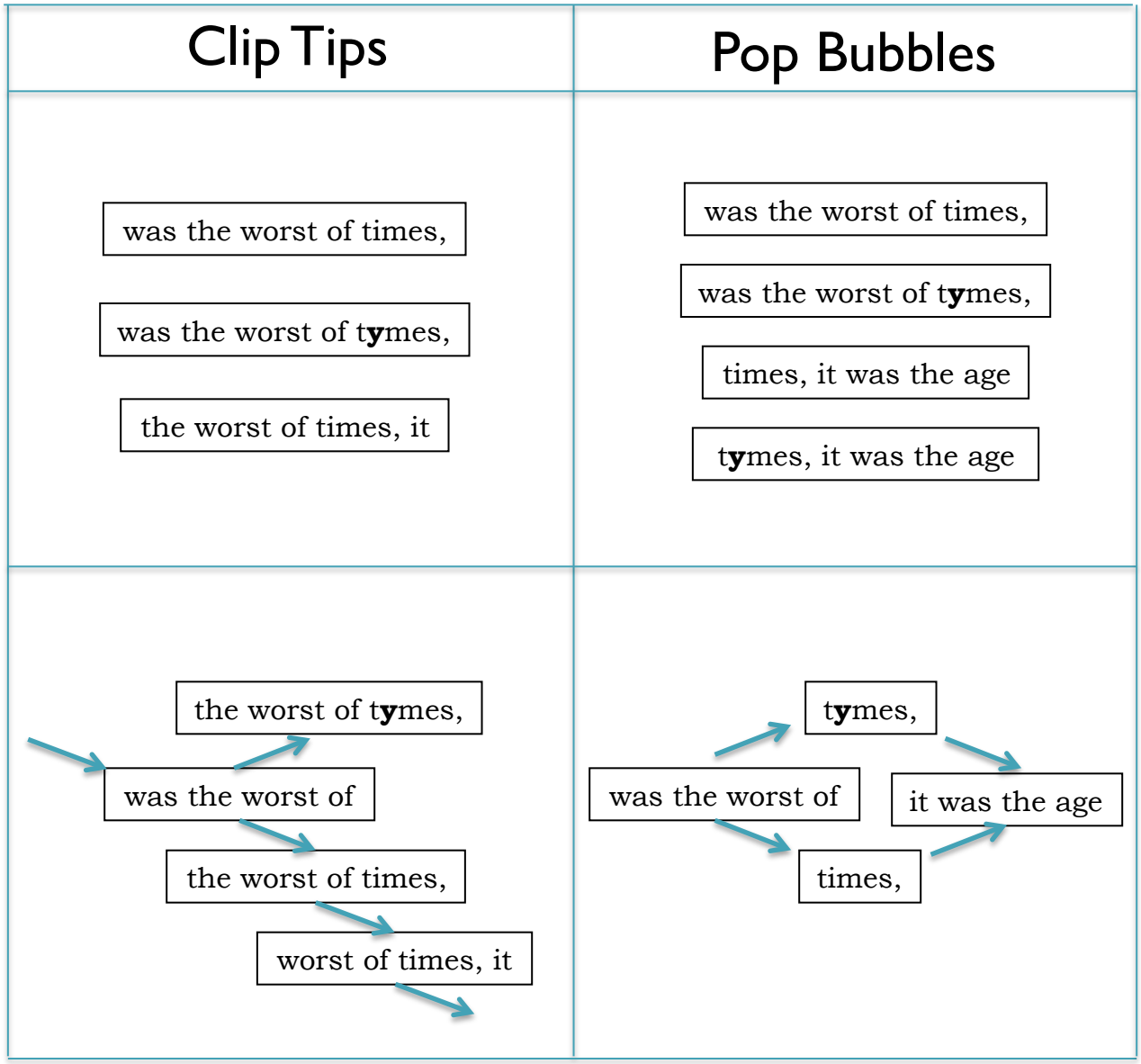
- After simplification and correction, compress graph down to its non-branching initial contigs
  - Aka "unitigs", "unipaths"
  - Unitigs end because of (1) lack of coverage, (2) errors, and (3) repeats





Errors in the graph

(Chaisson, 2009)



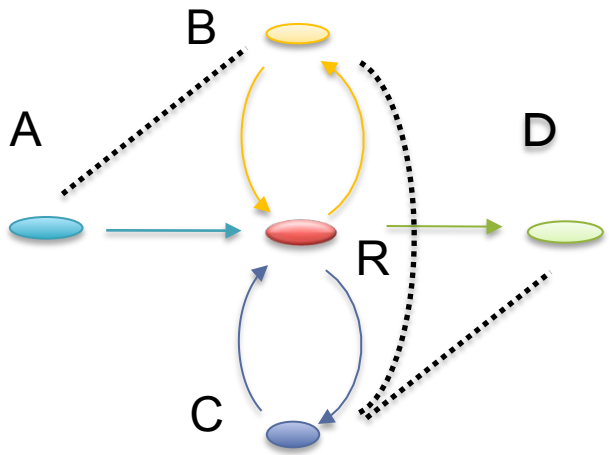
Repetitive regions

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%

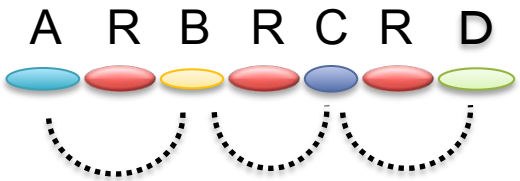
- Over 50% of mammalian genomes are repetitive
  - Large plant genomes tend to be even worse
  - Wheat: 16 Gbp; Pine: 24 Gbp

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

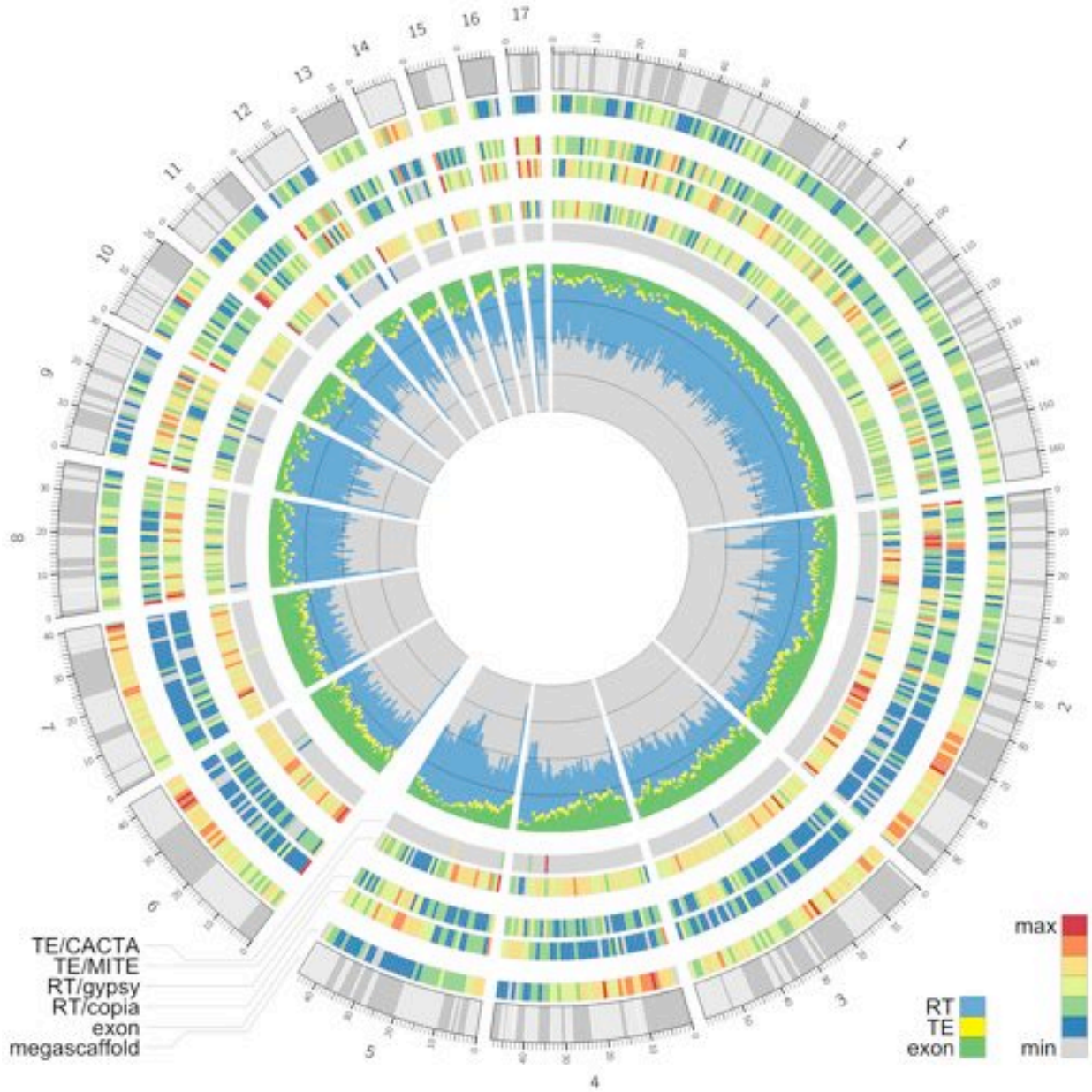


- Final scaffold may have internal gaps called sequencing gaps
  - We know the order, orientation, and spacing, but just not the bases. Fill with Ns instead

Post-assembly Analysis

After assembly:

- Validation
- CEGMA
- BLAST
- Gene Finding
- Repeat mask
- RNA-seq
- *-seq
- …
- Publish! ☺



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   2. ALLPATHS-LG
   3. Celera Assembler
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THE ASSEMBLATHON

- Attempt to answer the question:
  "What makes a good assembly?"

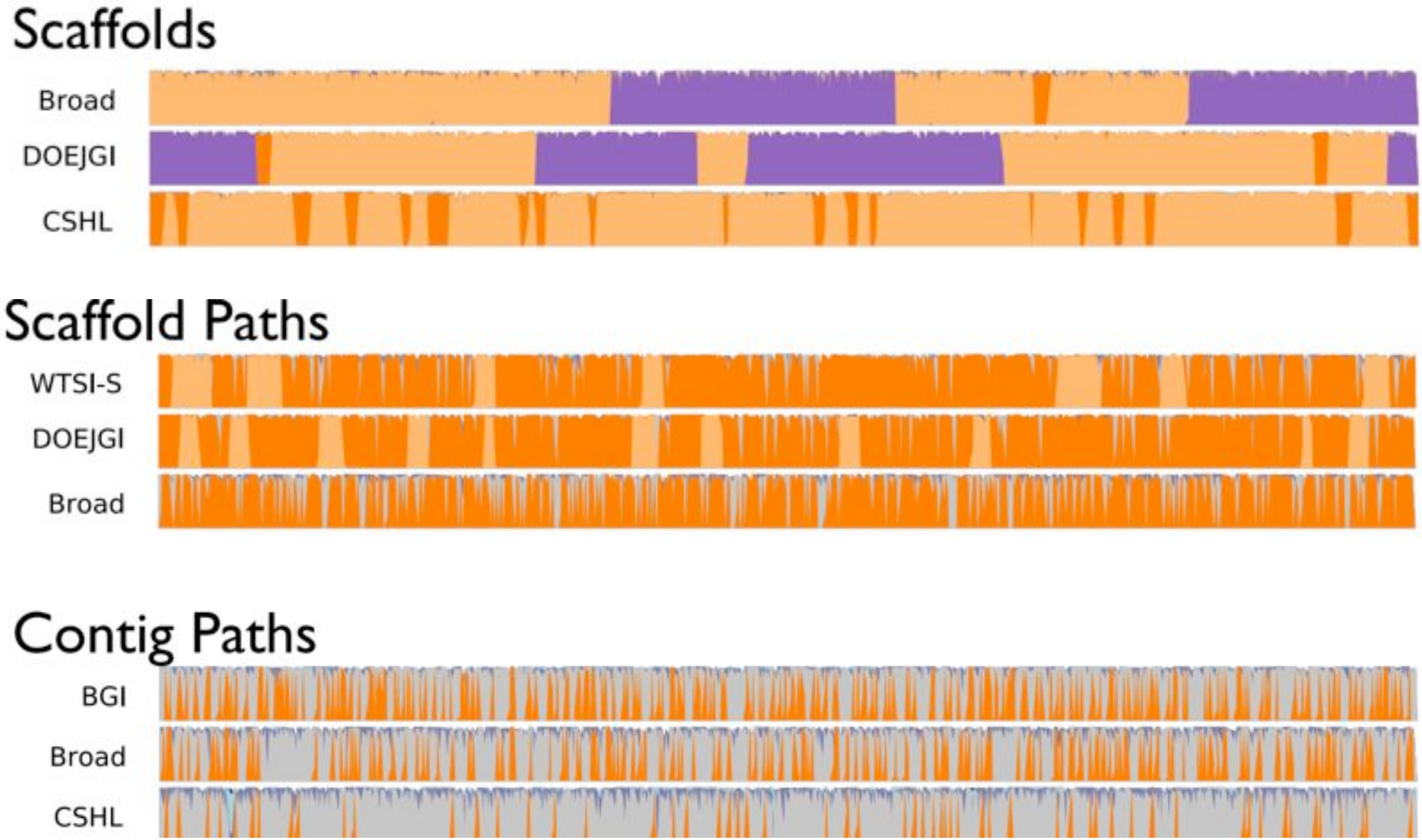
- Organizers provided sequence data to assembly experts around the world
  - Assemblathon 1: ~100Mbp simulated genome
  - Assemblathon 2: 3 vertebrate genomes each ~1GB

- Results demonstrate trade-offs assemblers must make

Assemblathon 1: A competitive assessment of de novo short read assembly methods.
Earl, DA, et al. (2011) Genome Research. doi: 10.1101/gr.126599.111

Assemblathon 2: Evaluating de novo methods of genome assembly in three vertebrate species
Bradnam, KR. et al (2013) GigaScience 2:10 doi:10.1186/2047-217X-2-10

Assembly Results

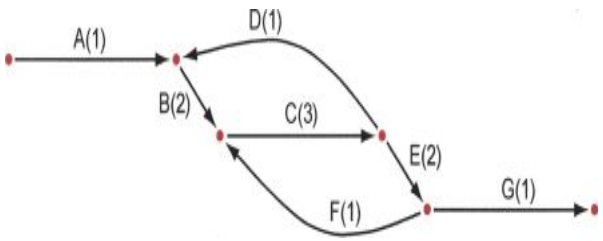


Final Rankings

ID	Overall	CPNG50	SPNG50	Struct.	CC50	Subs.	Copy. Num.	Cov. Tot.	Cov. CDS
BGI	36	★					☆	★	☆
Broad	37	☆	★	★	★				
WTSI-S	46		★	☆	★	★			
CSHL	52	★							☆
BCCGSC	53							☆	★
DOEJGI	56		☆	★	☆	★			
RHUL	58								
WTSI-P	64							☆	
EBI	64						★		
CRACS	64					☆			

- ALLPATHS and SOAPdenovo came out neck-and-neck followed closely behind by Celera Assembler, SGA, and ABySS

- My recommendation for "typical" short read assembly is to use ALLPATHS
- Single molecule sequencing becoming extremely attractive if you have access

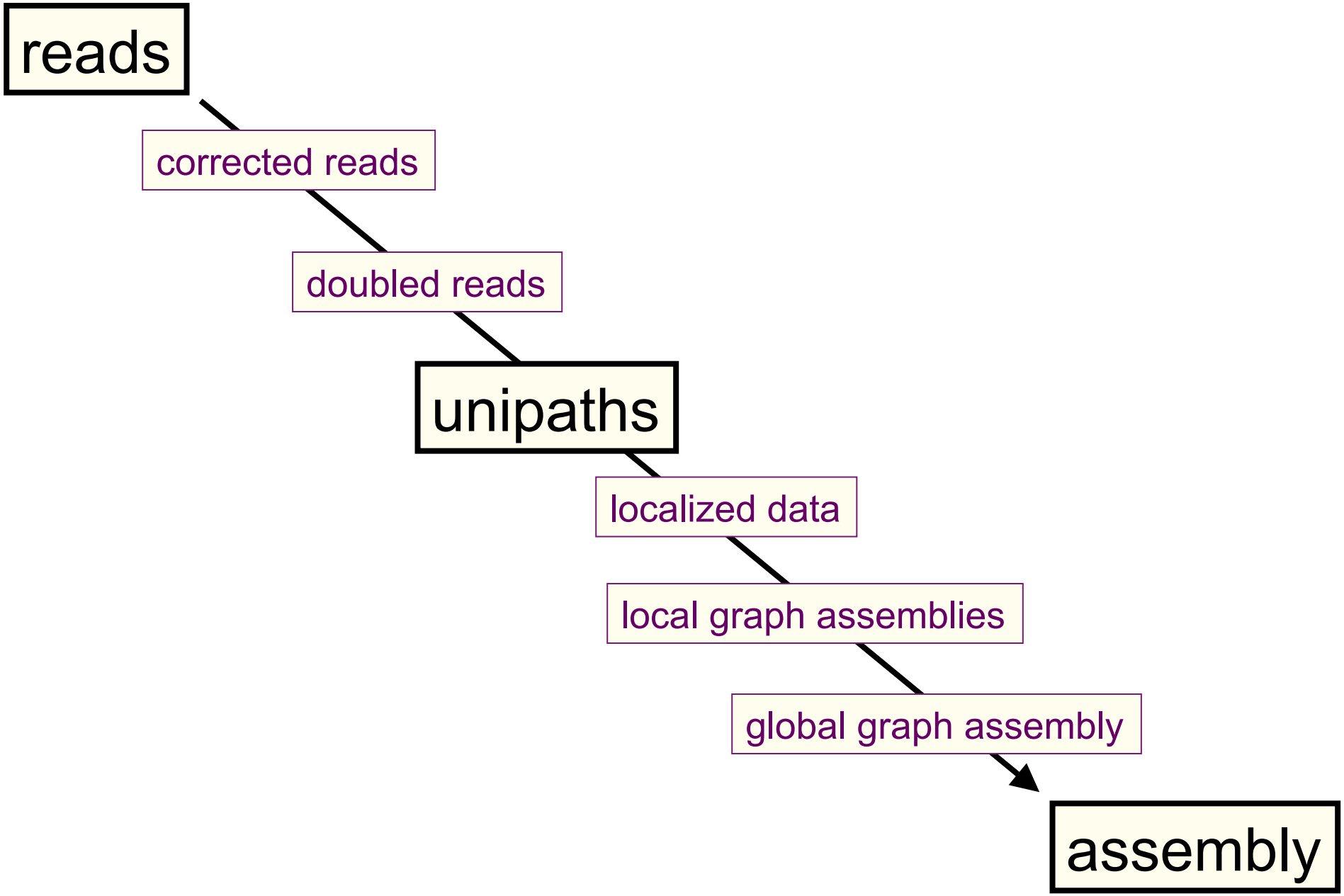


Genome assembly with ALLPATHS-LG

Iain MacCallum

BROAD INSTITUTE

How ALLPATHS-LG works



ALLPATHS-LG sequencing model

Libraries (insert types)	Fragment size (bp)	Read length (bases)	Sequence coverage (x)	Required
Fragment	180*	≥ 100	45	yes
Short jump	3,000	≥ 100 preferable	45	yes
Long jump	6,000	≥ 100 preferable	5	no**
Fosmid jump	40,000	≥ 26	1	no**

*See next slide.

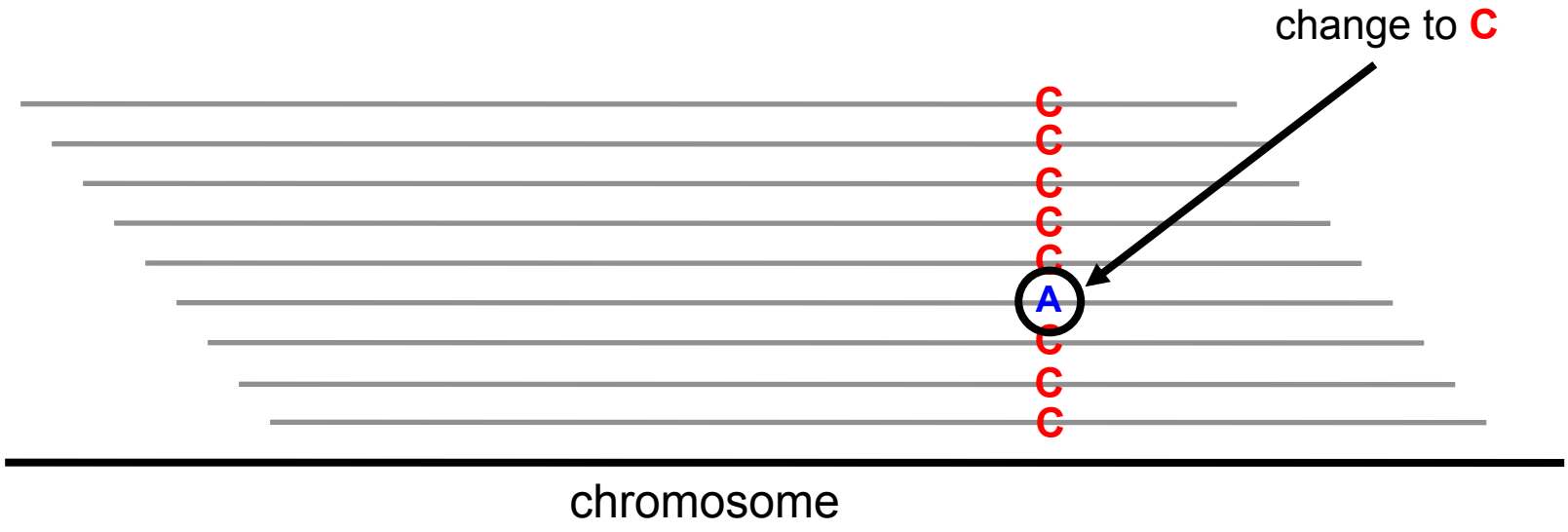
**For best results. Normally not used for small genomes. However essential to assemble long repeats or duplications.

Cutting coverage in half still works, with some reduction in quality of results.

All: protocols are either available, or in progress.

Error correction

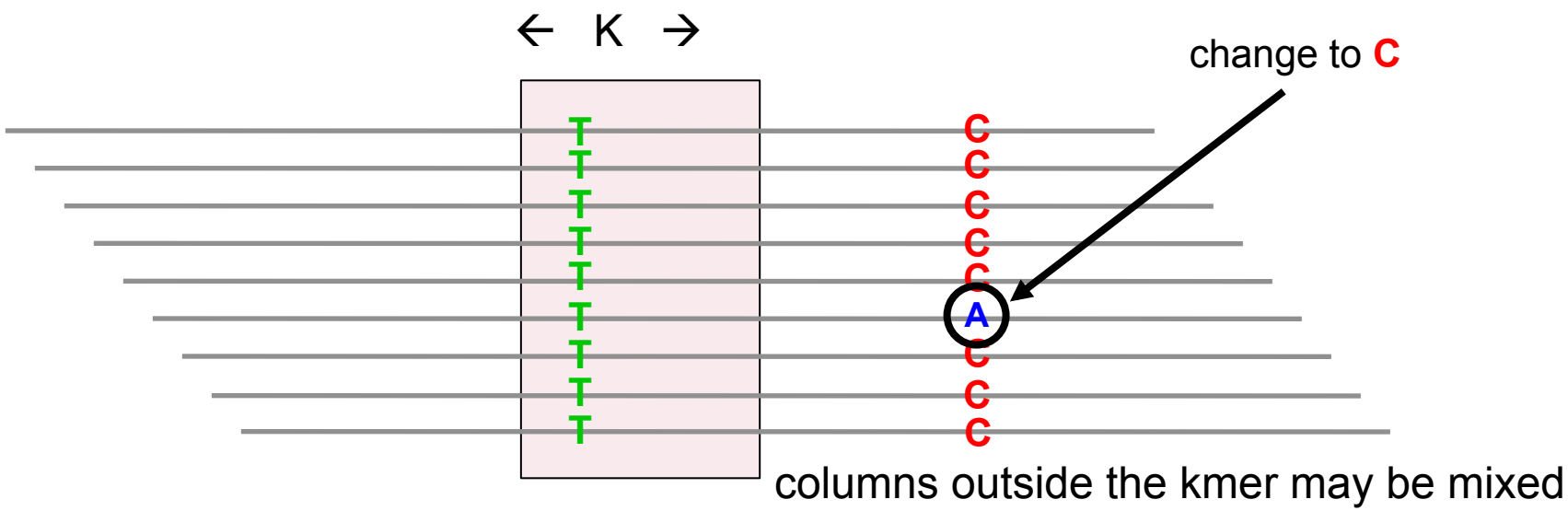
Given a crystal ball, we could stack reads on the chromosomes they came from (with homologous chromosomes separate), then let each column 'vote':



But we don't have a crystal ball....

Error correction

ALLPATHS-LG. For every K-mer, examine the stack of all reads containing the K-mer. Individual reads may be edited if they differ from the overwhelming consensus of the stack. If a given base on a read receives conflicting votes (arising from membership of the read in multiple stacks), it is not changed. (K=24)



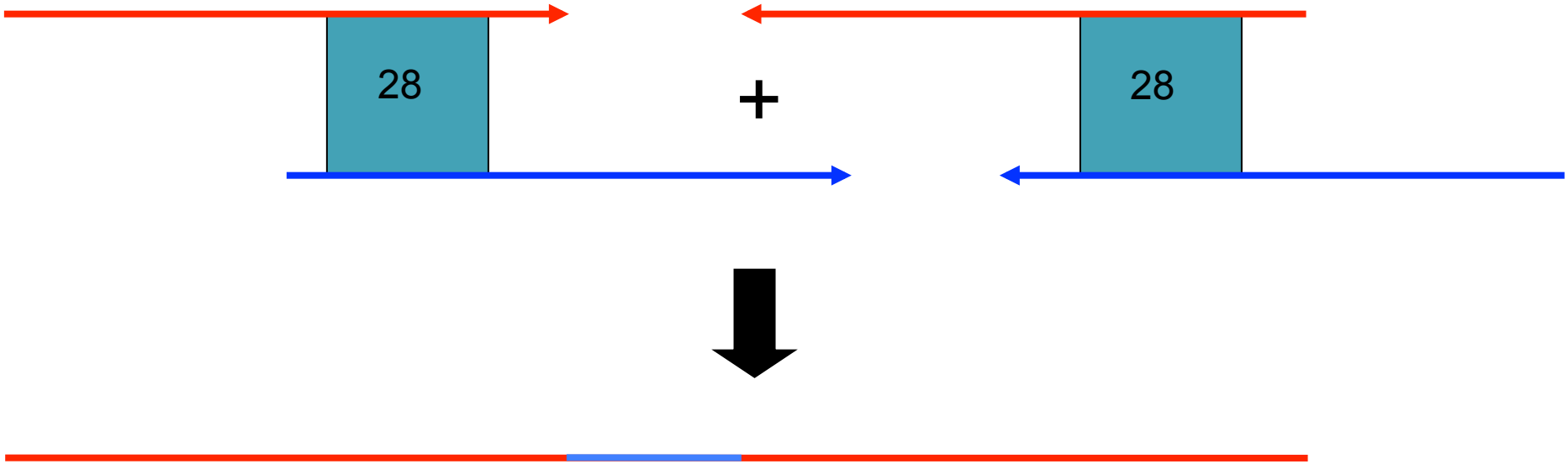
columns outside the kmer may be mixed

columns inside the kmer are homogeneous

Two calls at Q20 or better are enough to protect a base

Read doubling

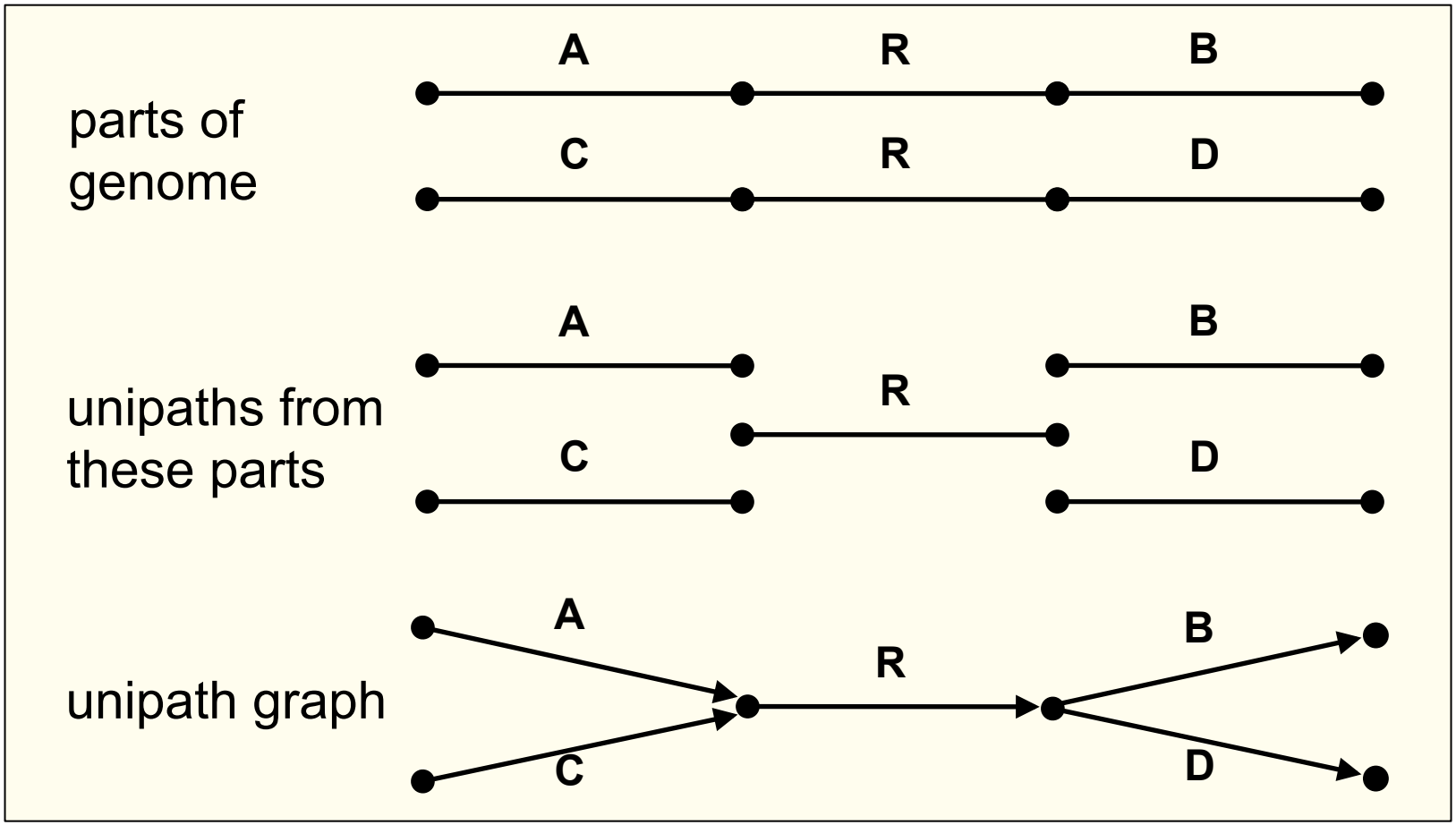
To close a read pair (red), we require the existence of another read pair (blue), overlapping perfectly like this:



More than one closure allowed (but rare).

Unipaths

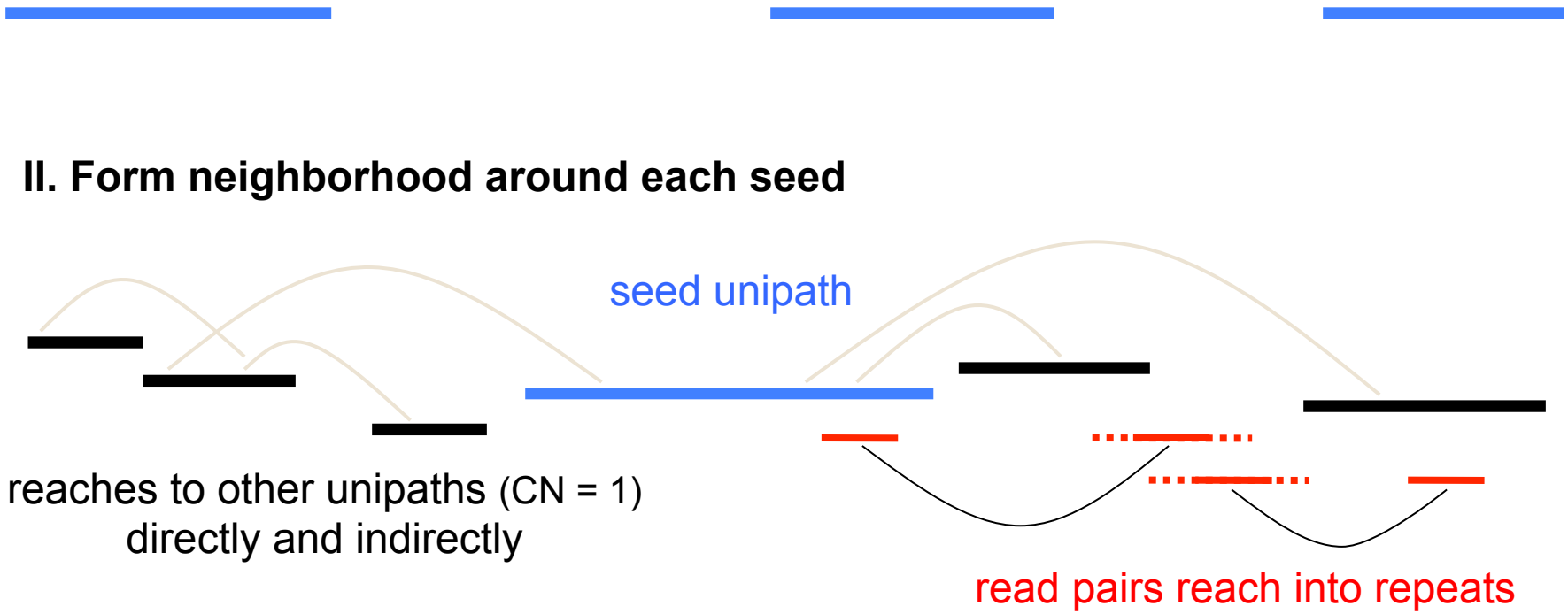
Unipath: unbranched part of genome – squeeze together perfect repeats of size ≥ K



Adjacent unipaths overlap by K-1 bases

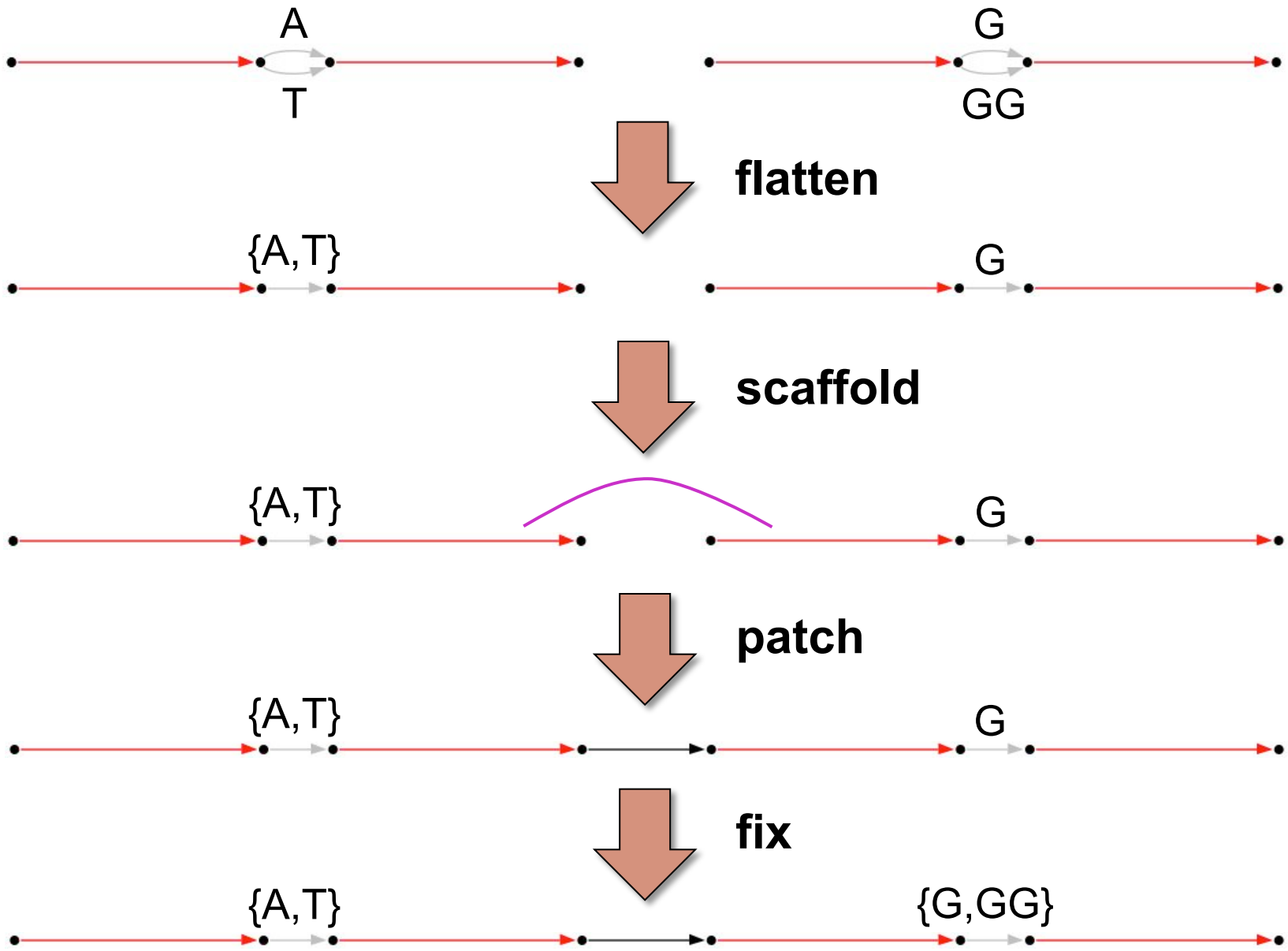
Localization

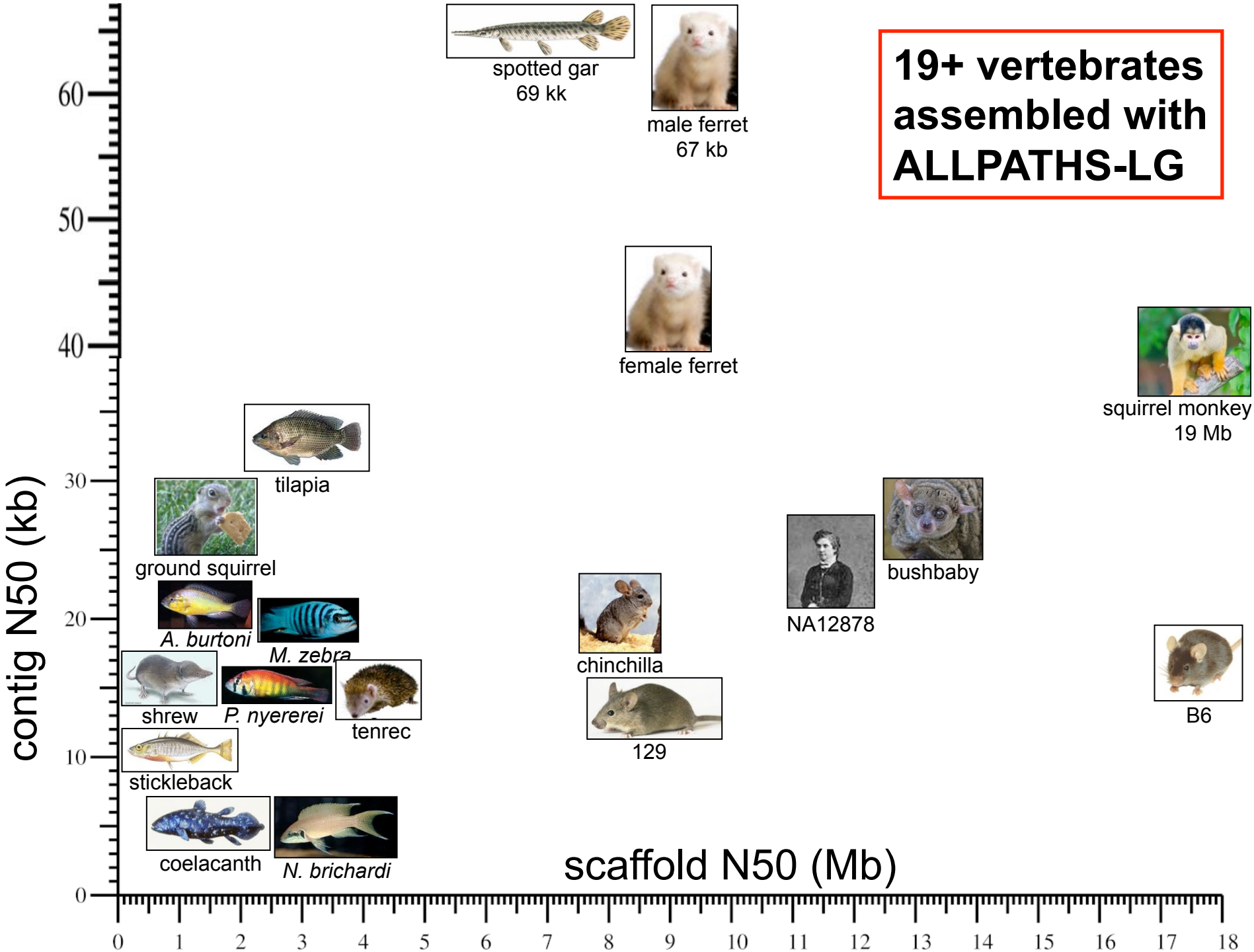
I. Find 'seed' unipaths, evenly spaced across genome
(ideally long, of copy number CN = 1)



II. Form neighborhood around each seed

Create assembly from global assembly graph




19+ vertebrates assembled with ALLPATHS-LG
spotted gar
69 kk
male ferret
67 kb
female ferret
squirrel monkey
19 Mb
tilapia
ground squirrel
bushbaby
NA12878
chinchilla
A. burtoni
M. zebra
shrew
P. nyererei
tenrec
129
B6
stickleback
coelacanth
N. brichardi
contig N50 (kb)
scaffold N50 (Mb)
0
10
20
30
40
50
60
0
1
2
3
4
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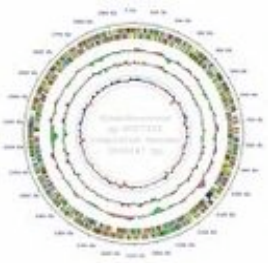
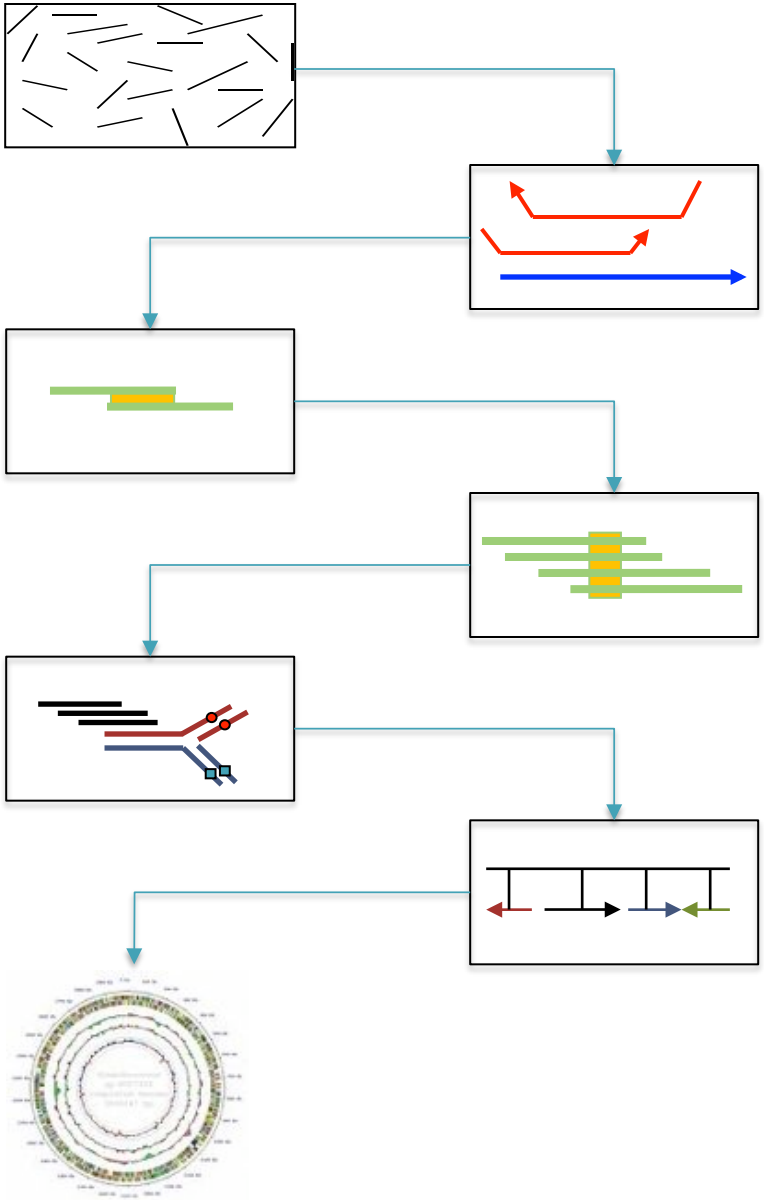


Genome assembly with the Celera Assembler

Celera Assembler

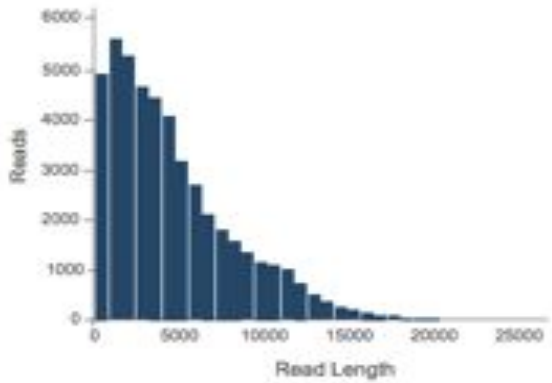
http://wgs-assembler.sf.net

1. Pre-overlap
   - Consistency checks
2. Trimming
   - Quality trimming & partial overlaps
3. Compute Overlaps
   - Find high quality overlaps
4. Error Correction
   - Evaluate difference in context of overlapping reads
5. Unitigging
   - Merge consistent reads
6. Scaffolding
   - Bundle mates, Order & Orient
7. Finalize Data
   - Build final consensus sequences

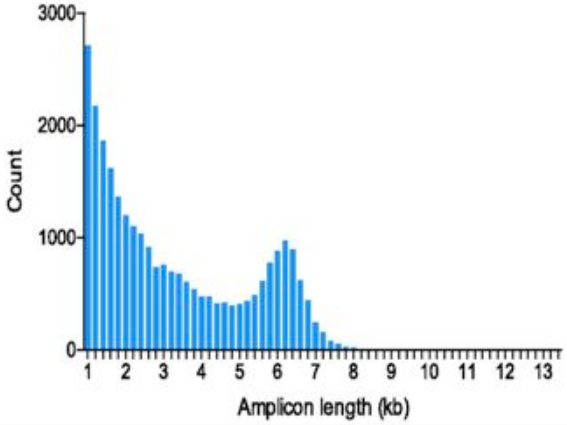


Single Molecule Sequencing Technology

PacBio RS II



Moleculo



Oxford Nanopore

?

Hybrid Sequencing

Illumina
Sequencing by Synthesis

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

Pacific Biosciences
SMRT Sequencing

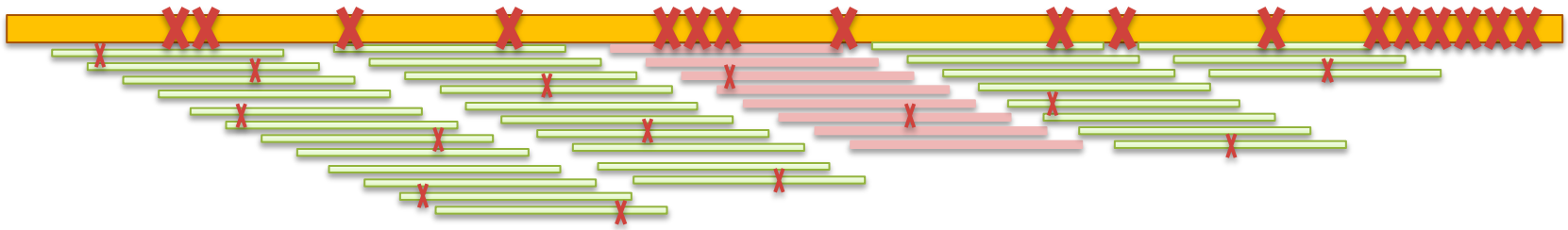
Lower throughput (1Gbp/day)
Lower accuracy (~85%)
Long reads (5kbp+)

Hybrid Error Correction: PacBioToCA

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

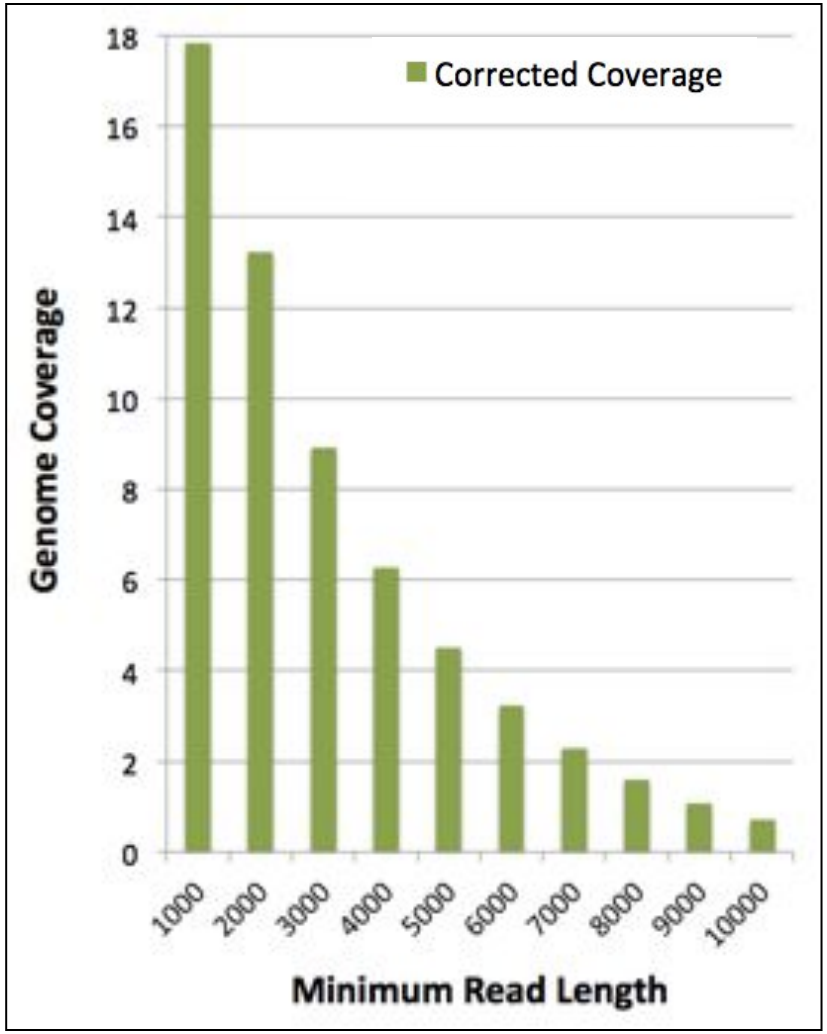
2. Error corrected reads can be easily assembled, aligned



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

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



In collaboration with McCombie & Ware labs @ CSHL

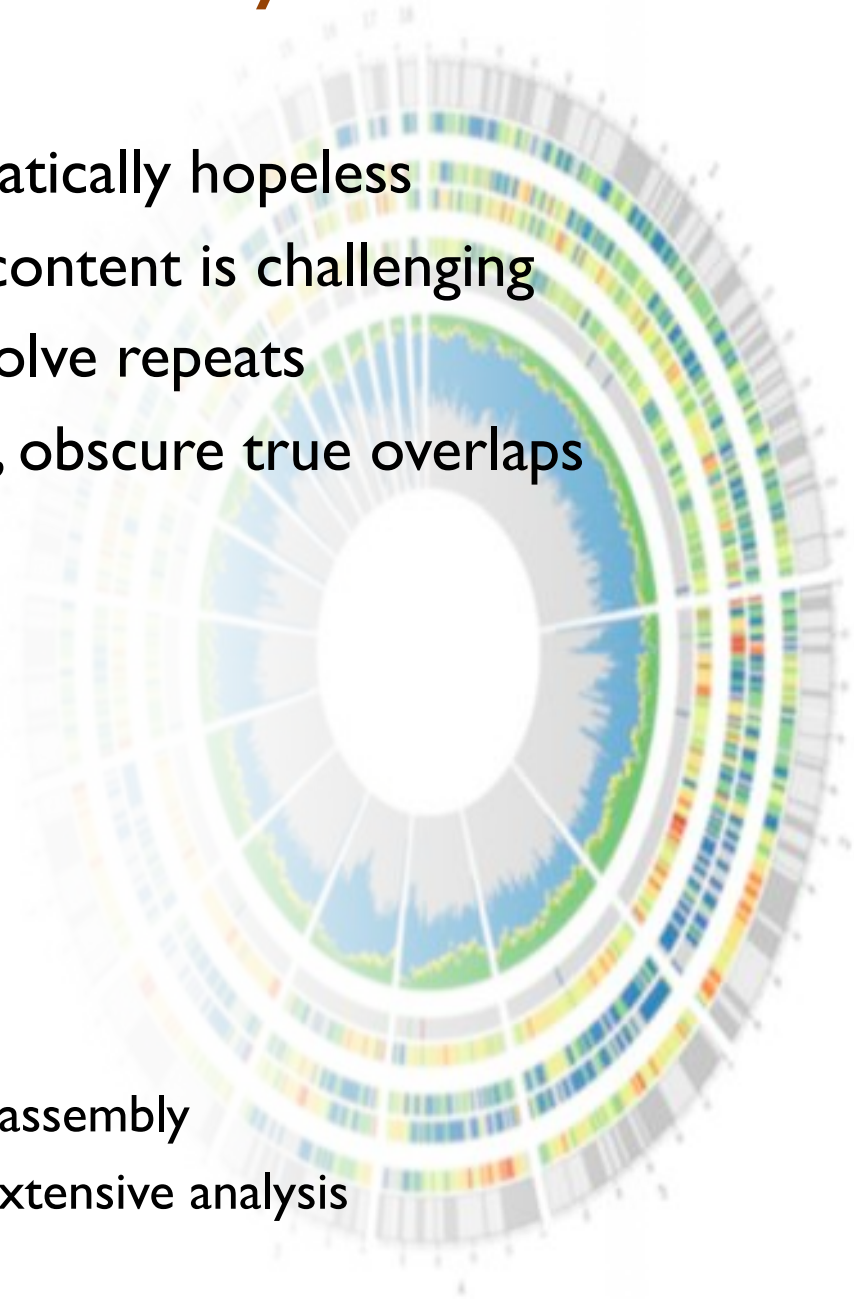
Assembly Summary

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
  - Reads -> unitigs -> mates -> scaffolds
    -> optical / physical / genetic maps
    -> chromosomes

- Recommendations:
  - ALLPATH-LG for Illumina-only
  - HGAP for PacBio-only, CA for Hybrid assembly
  - See Assemblathon papers for a more extensive analysis



Outline

1. Assembly theory
   1. Assembly by analogy
   2. De Bruijn and Overlap graph
   3. Coverage, read length, errors, and repeats
2. Genome assemblers
   1. Assemblathon
   2. ALLPATHS-LG
   3. Celera Assembler
3. Assembly Tutorial with iPlant

Assembly with ALLPATHS-LG

0. Download and install ALLPATHS-LG source code

```
% wget ftp://ftp.br adinstitute.org/pub/crd/ LPATHS/Release-LG/
% configure && ma  make install
```

1. Collect the BAM that you w Create a

```
in_libs.c  ribe  roups.csv
```

metadata file t

2. Prepare input files

```
% cd /tmp/cshl/asm
% PrepareAllPathsInpu
    DATA_DIR=`pwd`
```

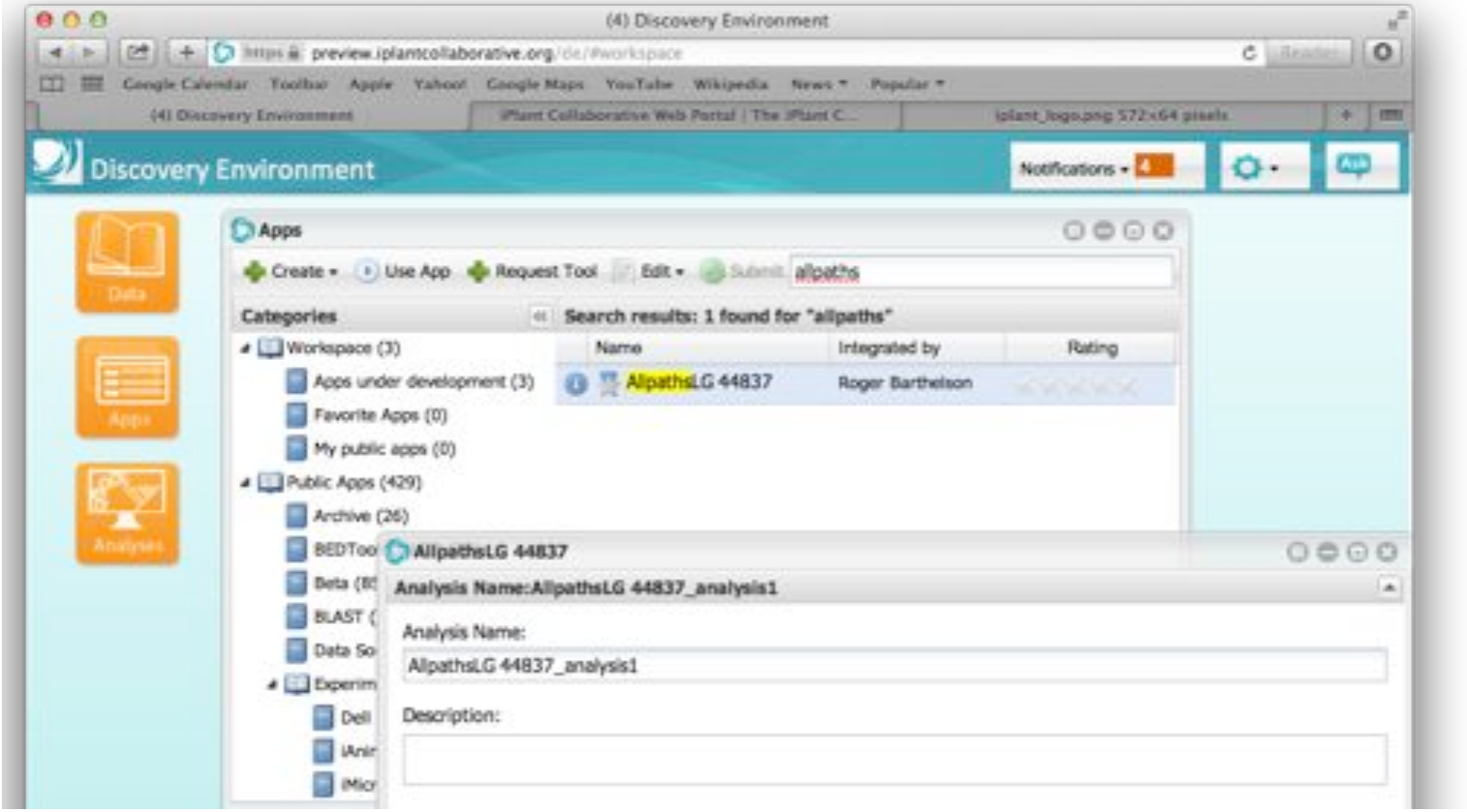
3. Assemble.

```
% RunAllPa
    PRE=/t  -cshl
    DATA_SUB  efault >&
```

4. Get the results (fou

```
% cd /tmp/cshl/as /default/ASSEMBLIES/te t/
% less final.{assembly,contigs}.{fasta,efasta}
```

Assembly with iPlant



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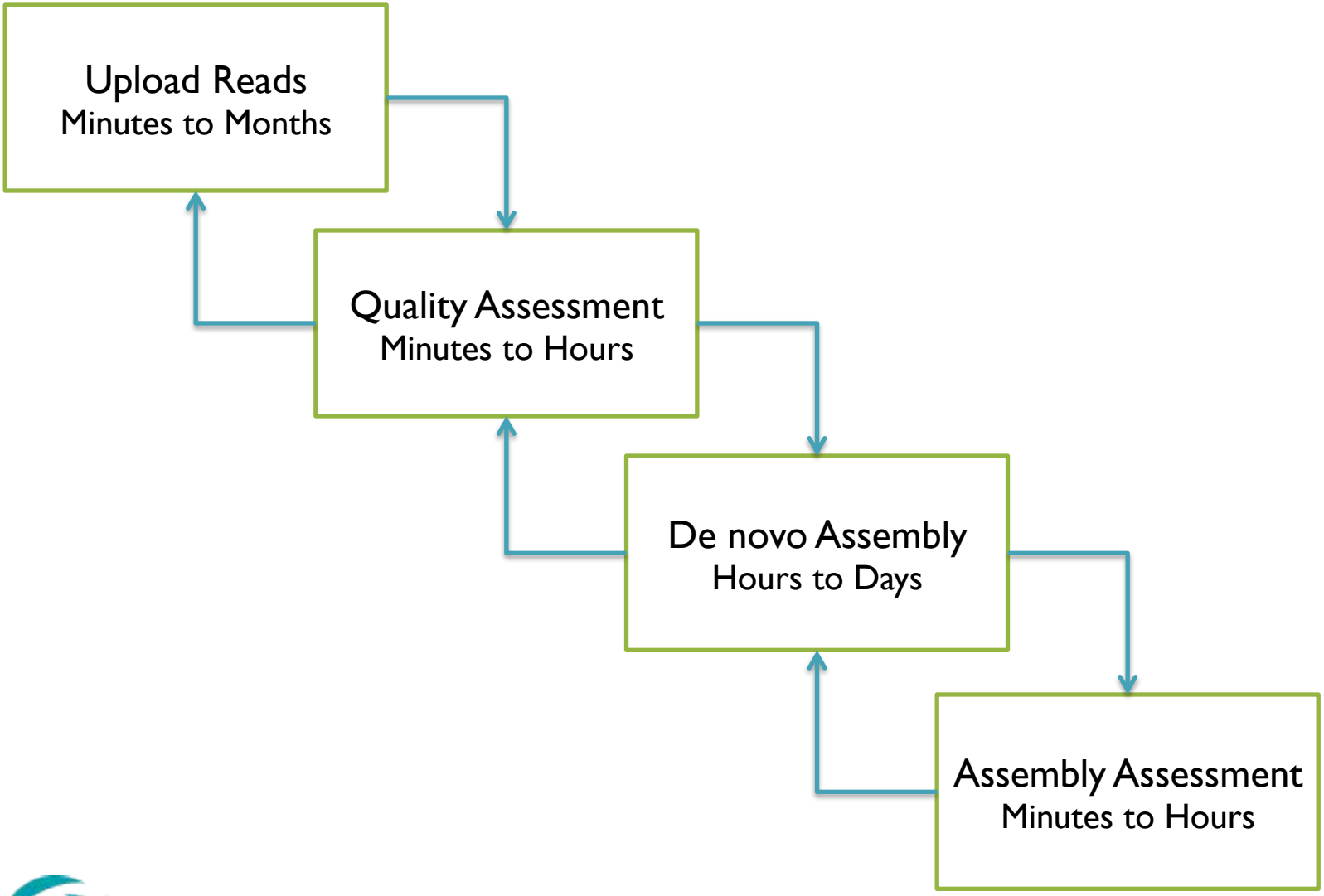
Assembly Workflow



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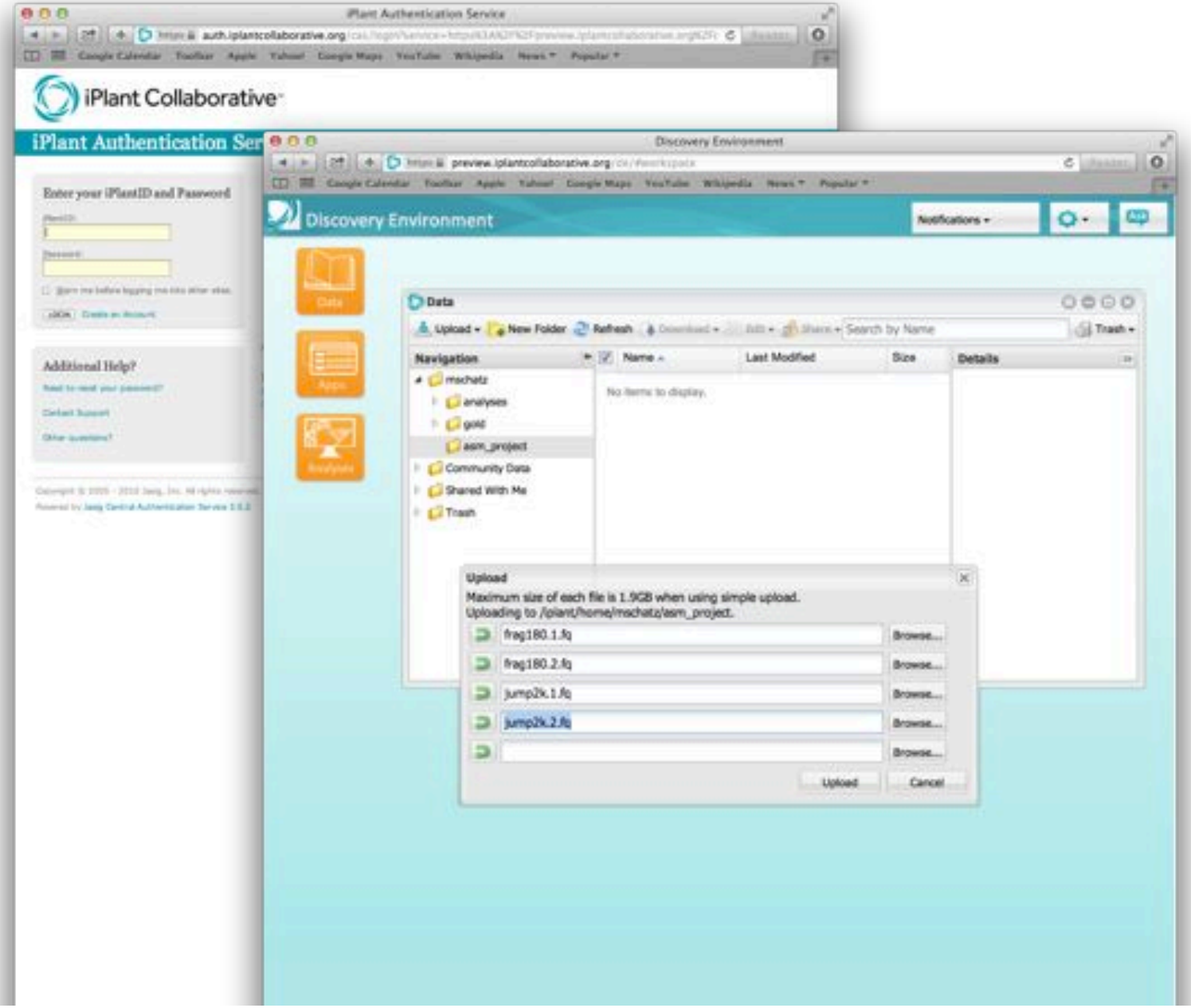
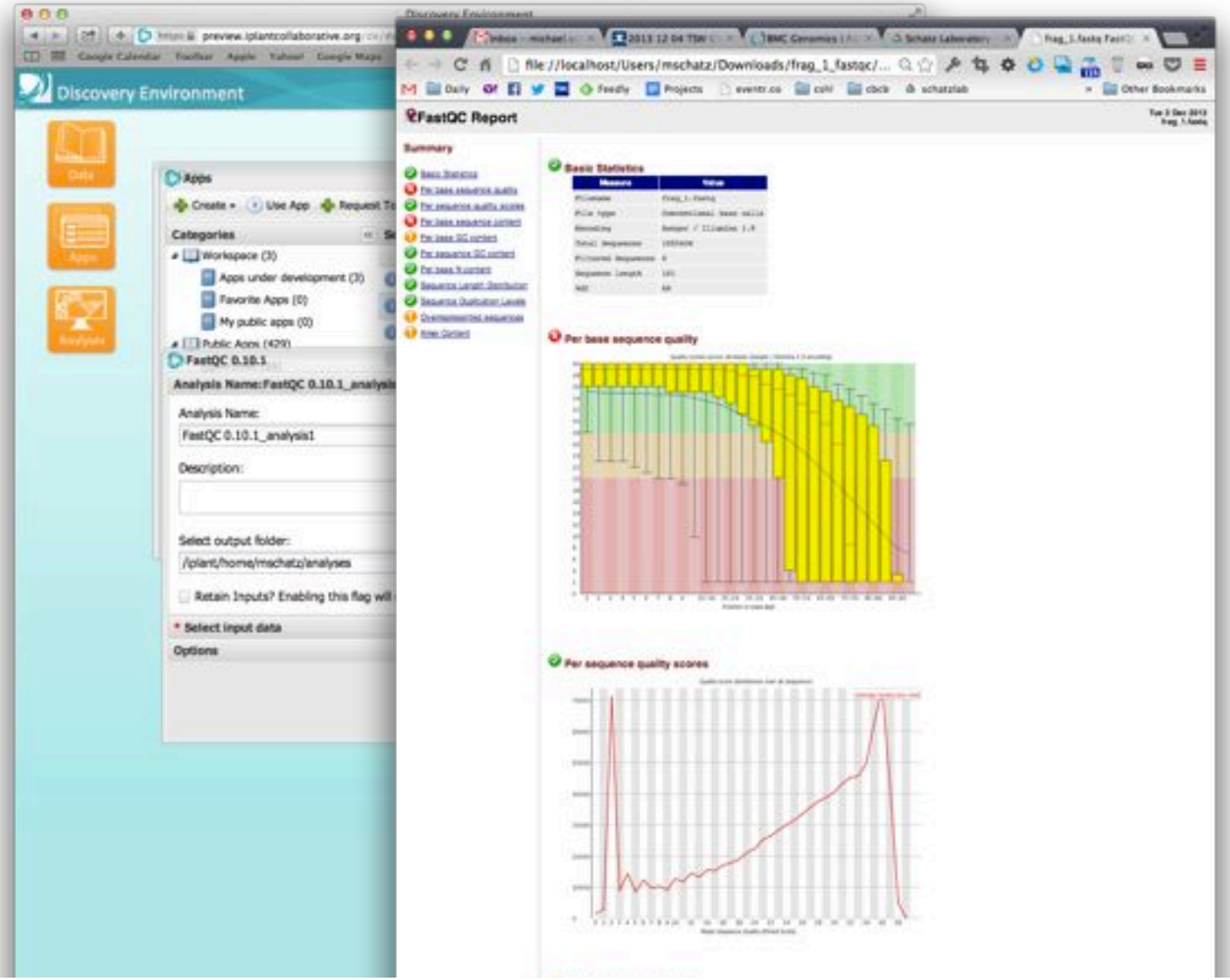
Upload Reads



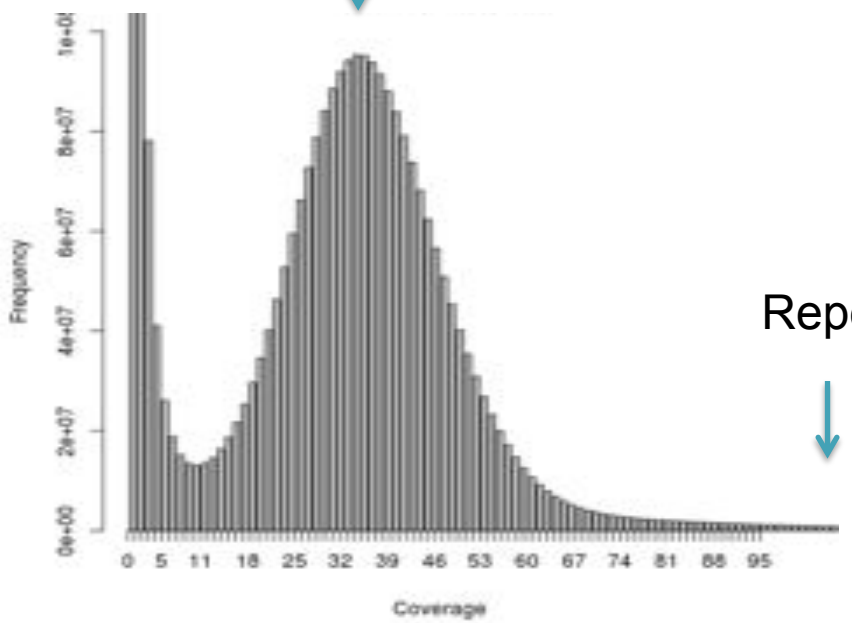
QC: FastQC


Discovery Environment
Apps
Categories
Workspace (3)
Apps under development (3)
Favorite Apps (0)
My public apps (0)
Public Apps (429)
FastQC 0.10.1
Analysis Name:
FastQC 0.10.1_analysis1
Description:
Select output folder:
/plant/home/mschatz/analyses
Select input data
Options
FastQC Report
Summary
Basic Statistics
Per base sequence quality
Per sequence quality scores

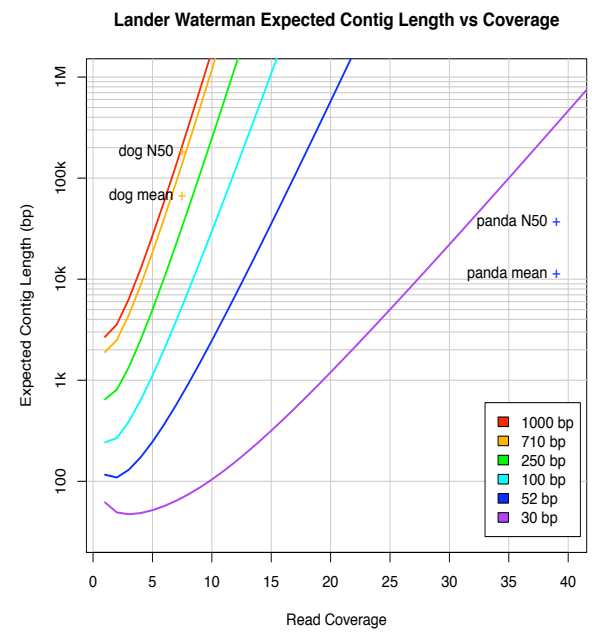
QC: Read Coverage

Errors

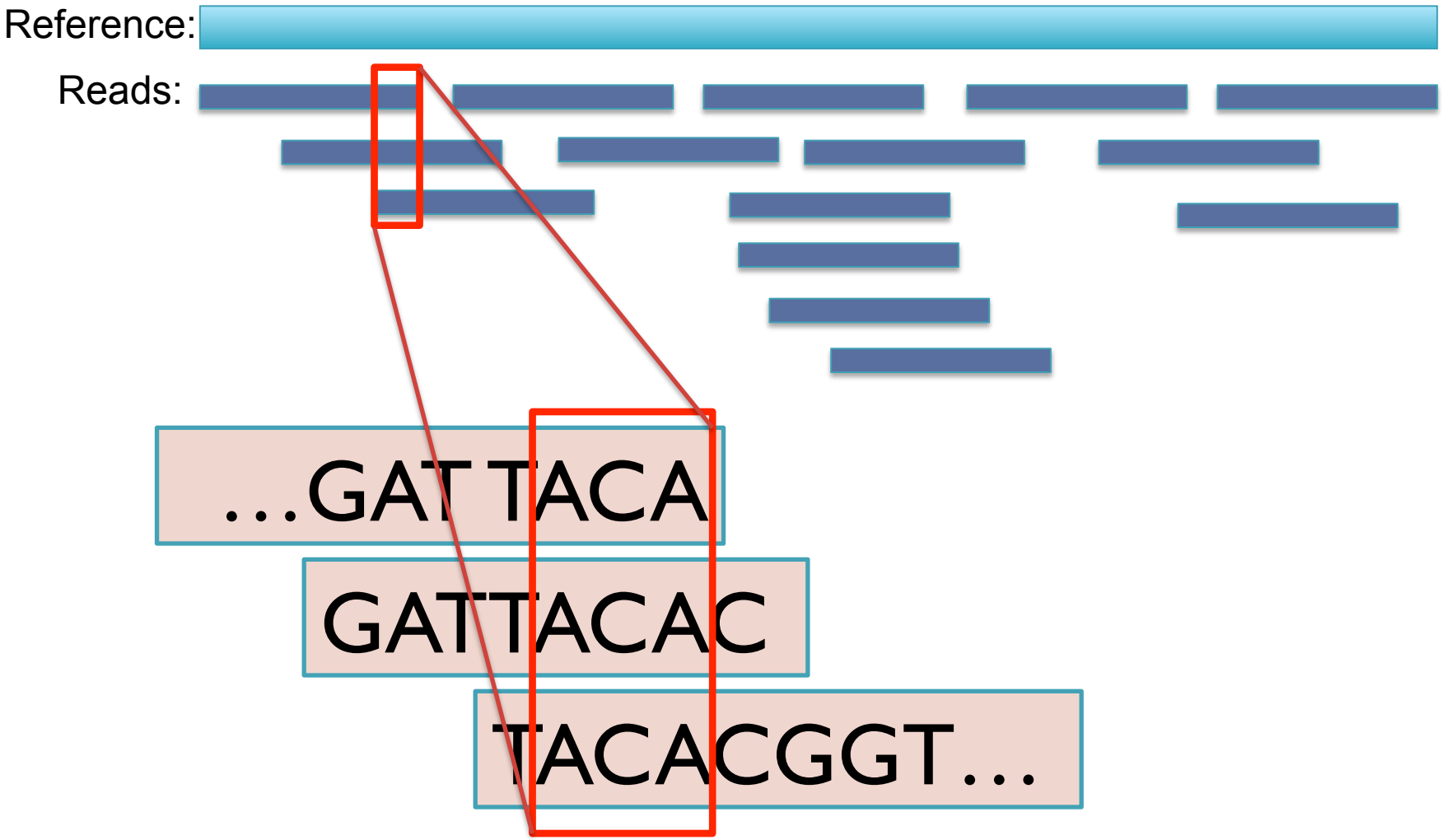
Coverage



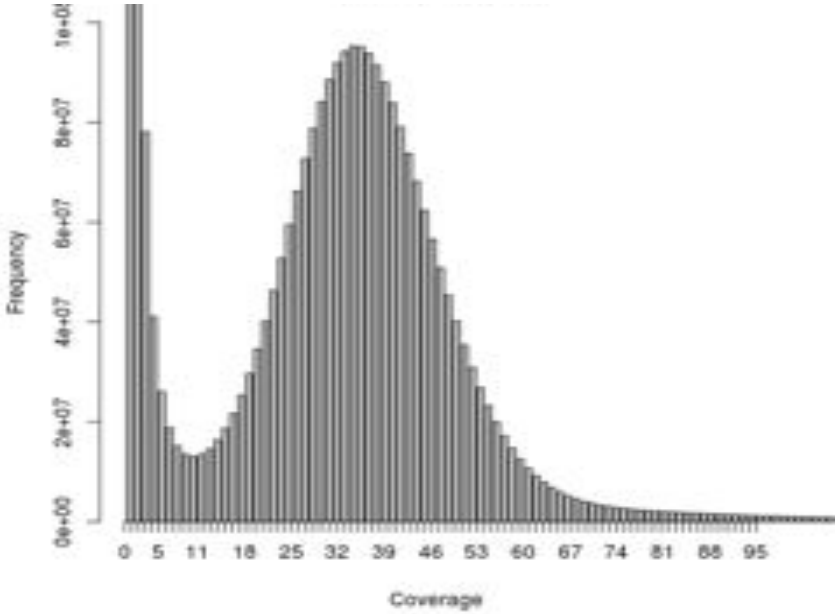
Repeats

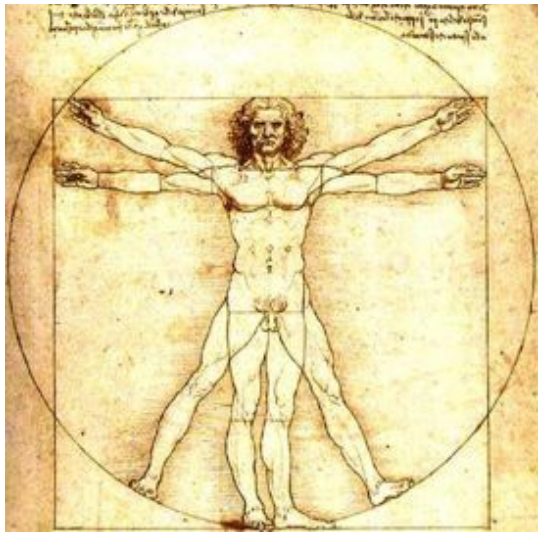


Estimating coverage with Kmers



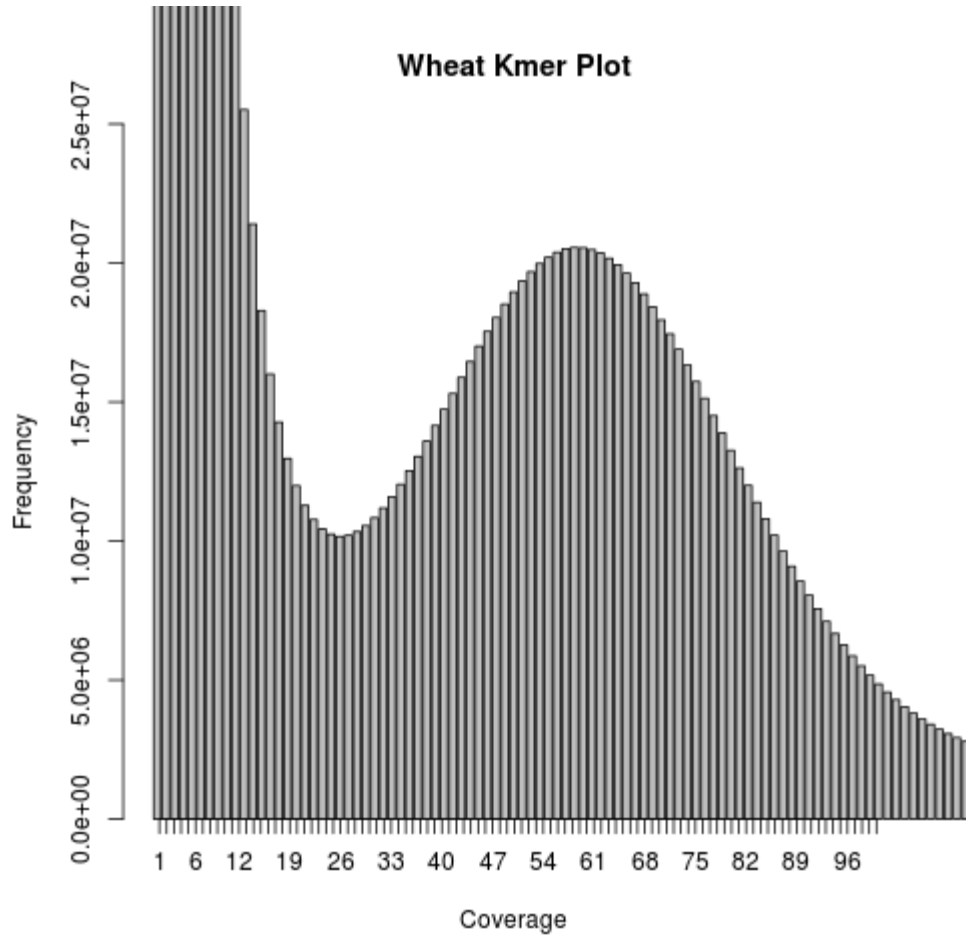
Estimating coverage with Kmers



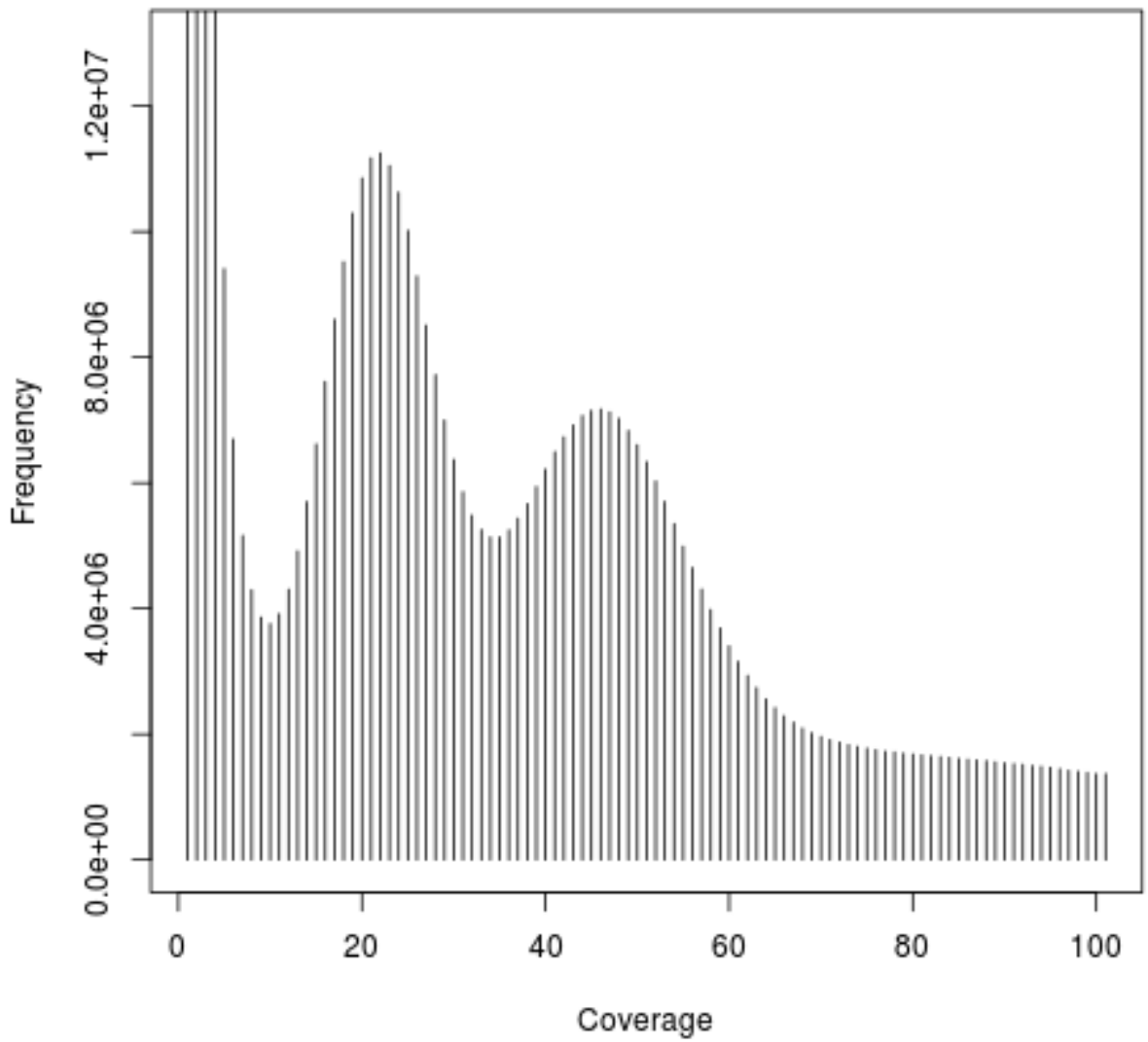


NA12878

Wheat Genome
(A. tauschi / CSHL)

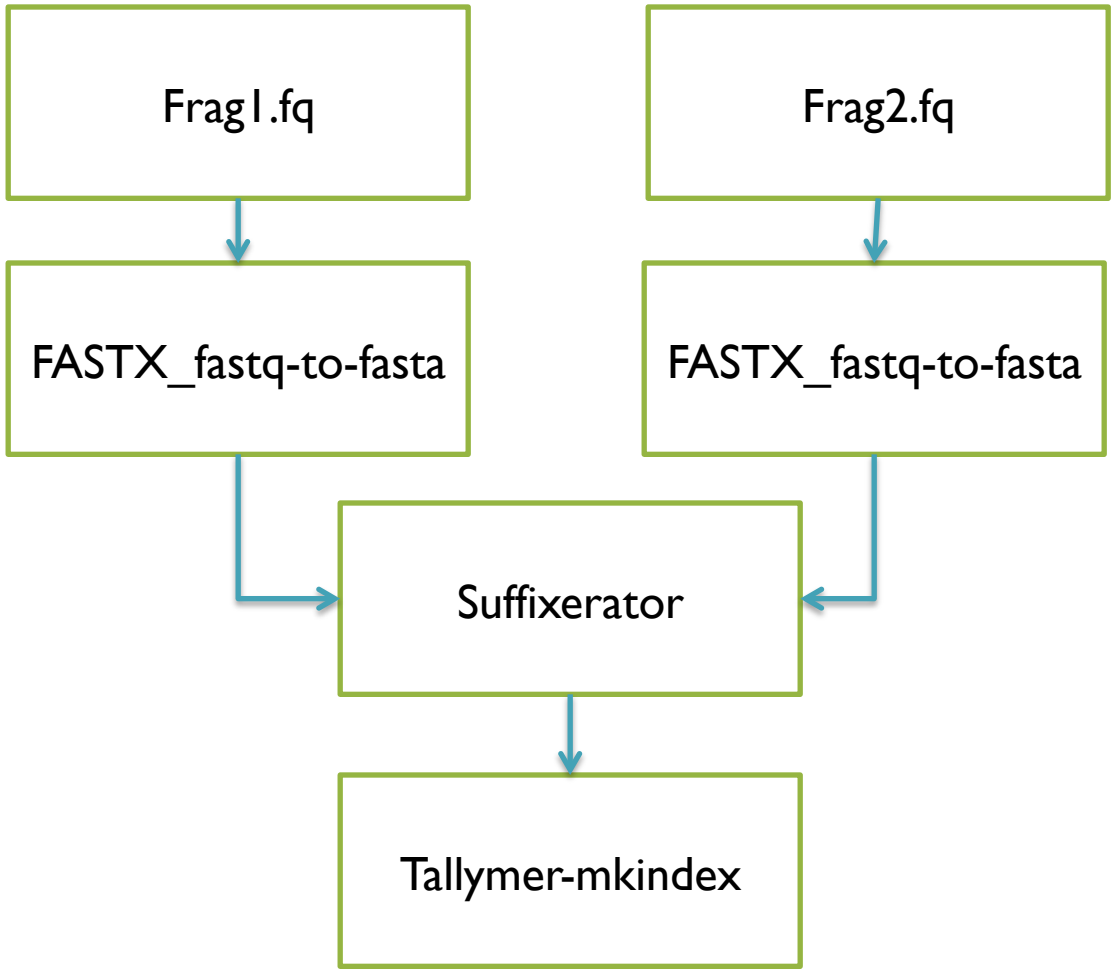


Heterozygous Genome



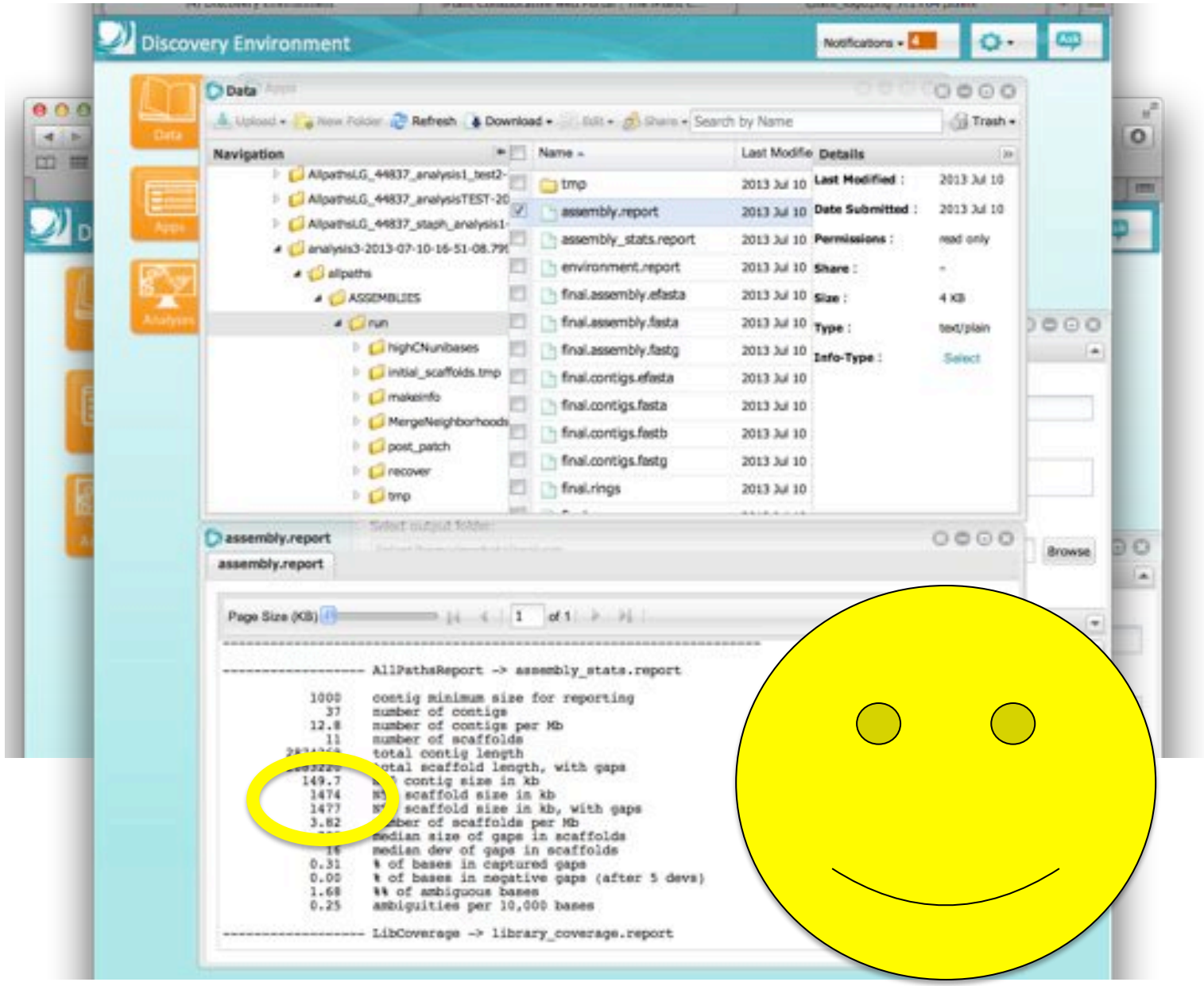
Contact: @mike_schatz

QC: Mer counts

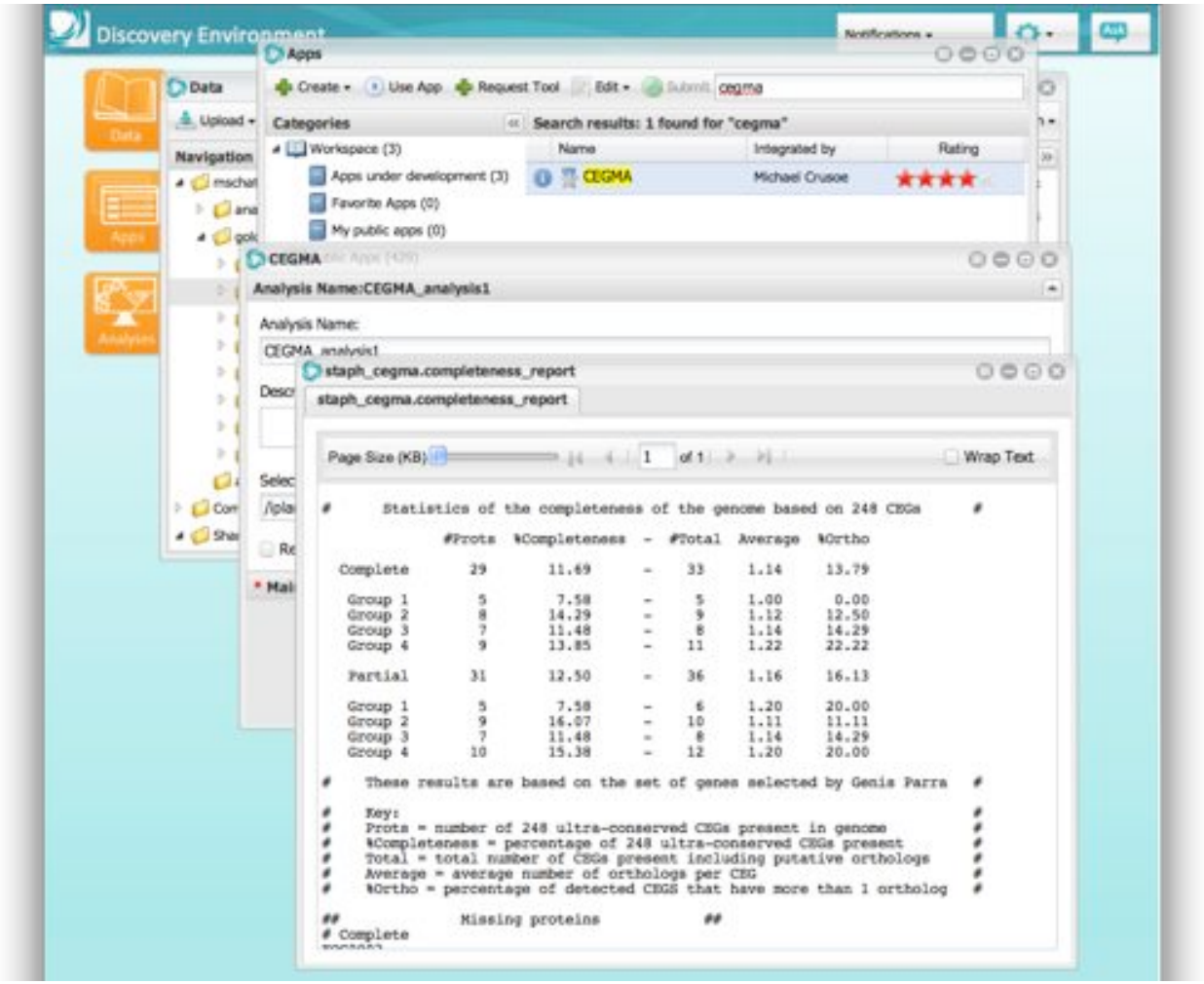


A new method to compute K-mer frequencies and its application to annotate large repetitive plant genomes
Kurtz S. Narechania A, Stein JC, Ware D. (2008) BMC Genomics. 9:517

Running ALLPATHS-LG

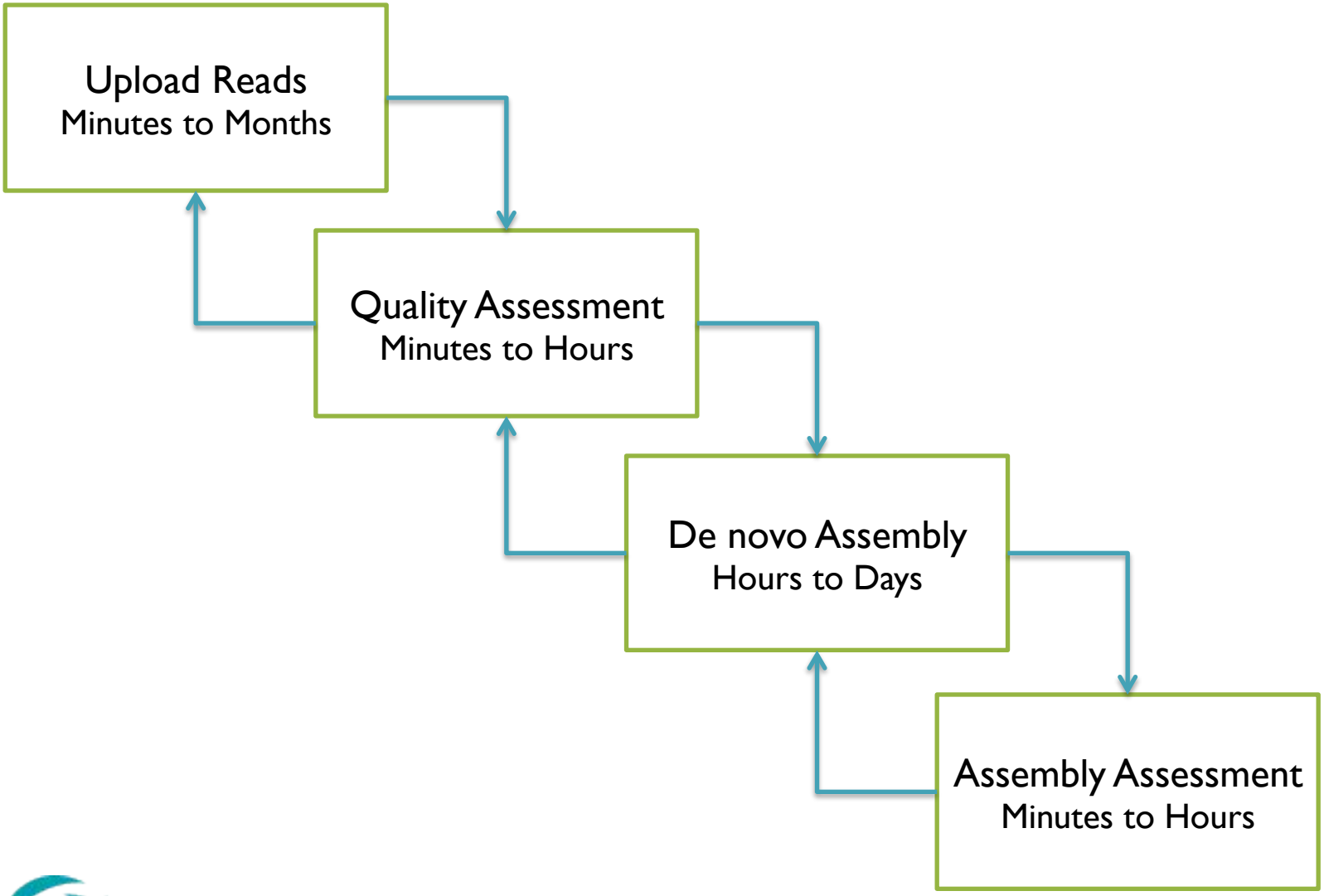


Post-QC: CEGMA



CEGMA: a pipeline to accurately annotate core genes in eukaryotic genomes
Parra G, Bradnam K, Korf I. (2007) Bioinformatics. 23 (9): 1061-1067.

Assembly Workflow



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Resources

- iPlant
  - http://www.iplantcollaborative.org/
- Assembly Competitions
  - Assemblathon: http://assemblathon.org/
  - GAGE: http://gage.cbcb.umd.edu/
- Assembler Websites:
  - ALLPATHS-LG: http://www.broadinstitute.org/software/allpaths-lg/blog/
  - SOAPdenovo: http://soap.genomics.org.cn/soapdenovo.html
  - Celera Assembler: http://wgs-assembler.sf.net
- Tools:
  - FastQC: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/
  - Tallymer: http://www.zbh.uni-hamburg.de/?id=211
  - CEGMA: http://korflab.ucdavis.edu/datasets/cegma/

Acknowledgements

<u>Special Thanks</u>
Shoshana Marcus
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Stephen Goff
Nicole Hopkins
Dan Stanzione
Joshua Stein
Matthew Vaughn
Doreen Ware
Jason Williams

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[illegible]	Research Associate	University of Texas, Austin
[illegible]	Bioinformatics Analyst	University of Arizona
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Questions?

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