Genome assembly

Bioinformatics Applications (PLPTH813)

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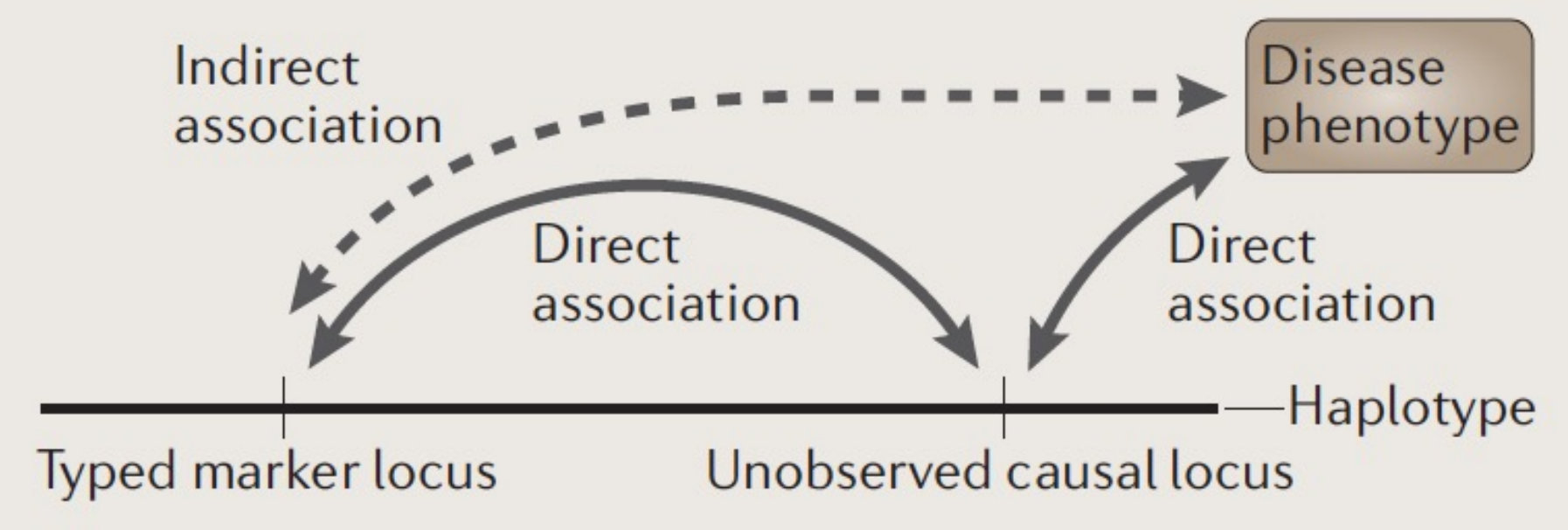
3/25/2021

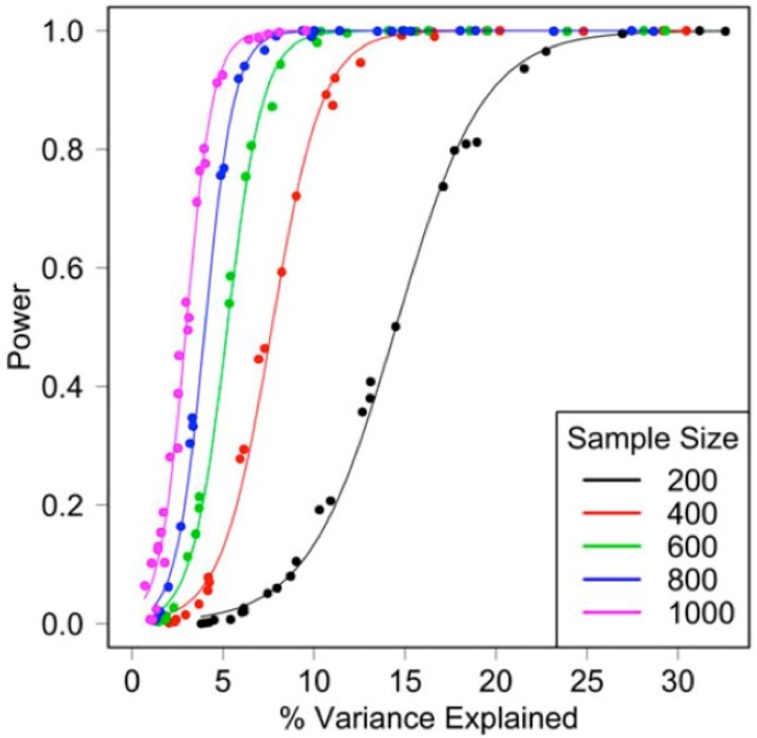
Linkage disequilibrium (LD)

Linkage disequilibrium (LD): a non-random association of alleles at different loci; genotyping data at two loci have some level of correlations



Association is NOT equal to causality

The lower the effect of causal sites, the larger the sample size is needed



Species	year	Journal	Population size	Trait
Cotton	2017	NG	318	Fiber and yield
Cotton	2017	NG	352	Fiber quality
Rice	2016	NG	176	Agronomic traits
Rice	2016	NG	342	Grain shape
Rice	2016	Nature	10,074	Heterotic effects

from previous students (2019)
Ruolin, Upasana, Diriba

Outline

- Genome assembly: concept
- Assembly algorithms: OLC/De Bruijn graph
- Error correction (k-mer counts)
- Assembly strategy to cope with the repeat problem
- Assembly evaluation (N50, comparison to a reference genome)
- Long-read genome assembly
- Genome annotation
- Case study

Whole genome shotgun (WGS) sequencing

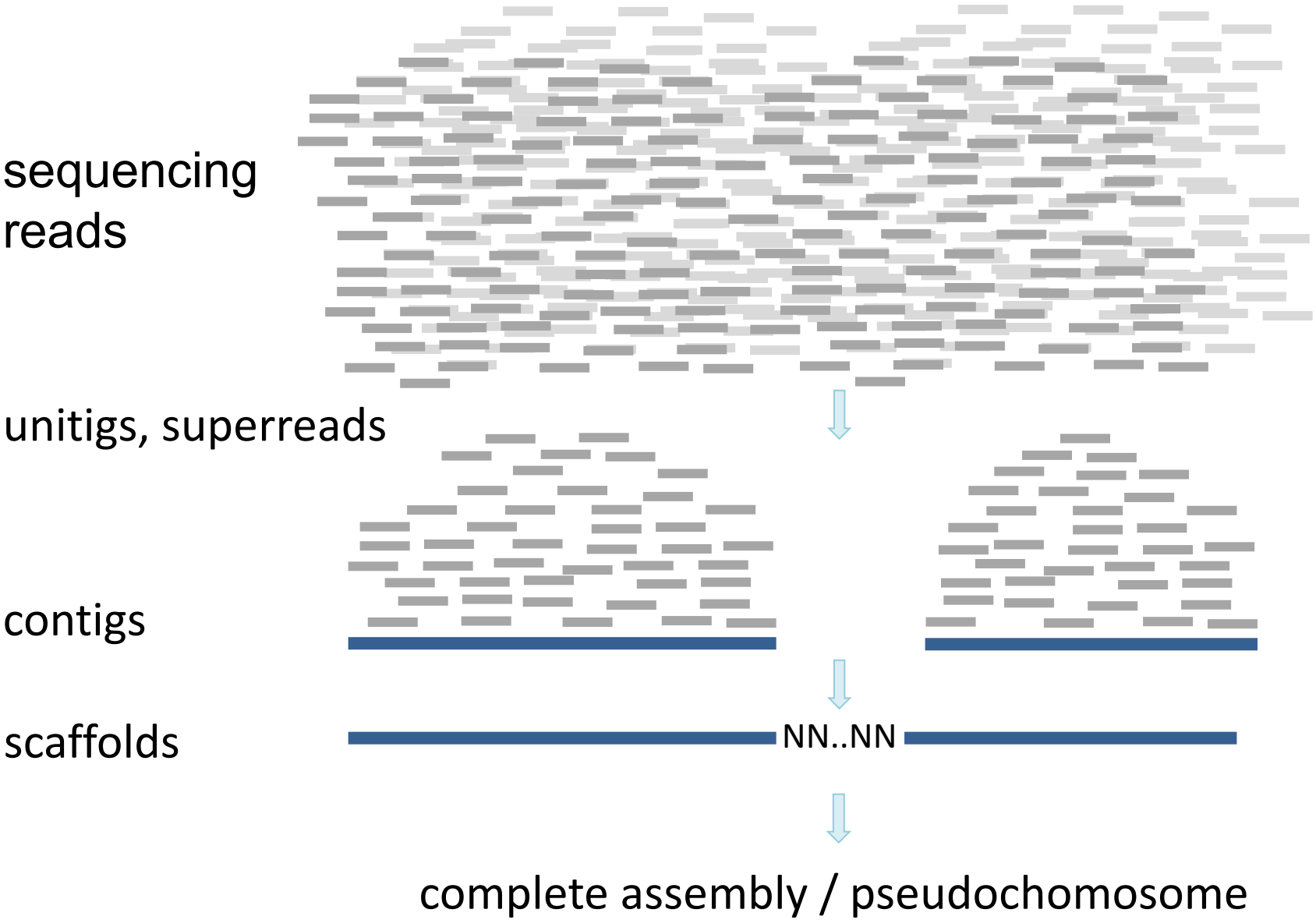
chromosome

sequencing reads

Complexity of genome assembly

Algorithm Can Solve 10, 000 Piece Jigsaw in 24 Hours

Genome assembly



Assembly algorithms

- **Overlap layout consensus (OLC)**

1. all-to-all pair-wise read alignments
2. Construct graph based on overlaps
3. Trace paths for the assembly

- **De Bruijn graph**

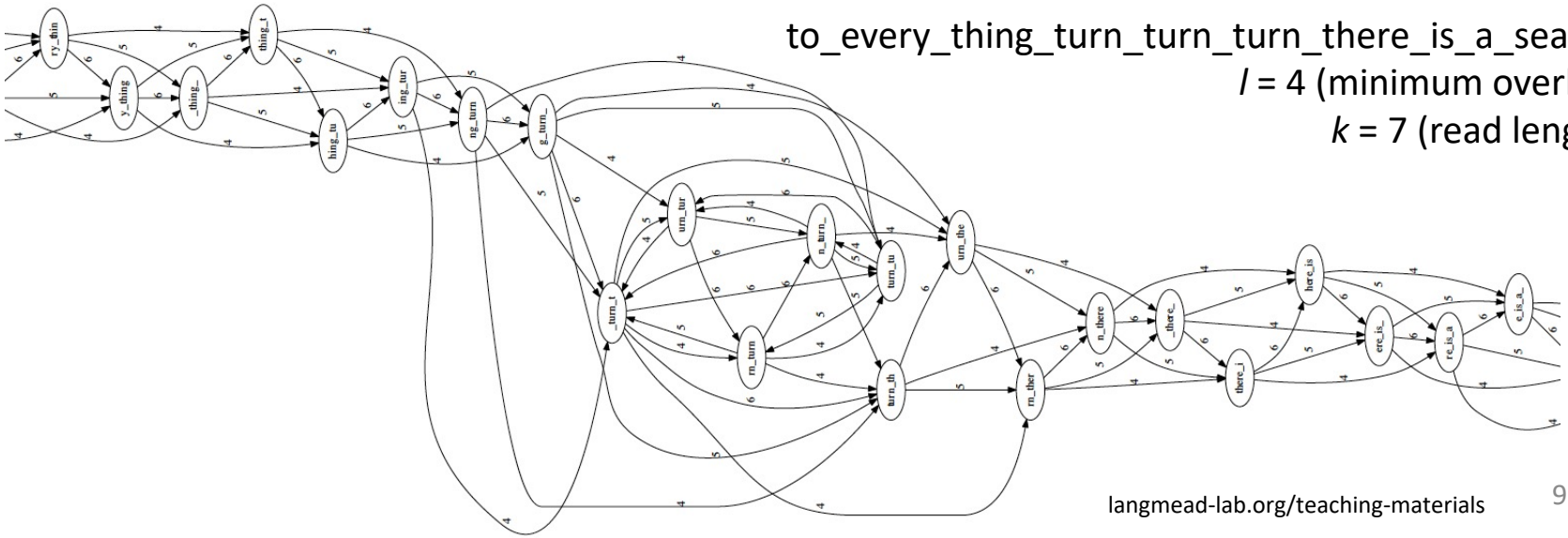
1. Determine k-mer from reads
2. Construct k-mer graph
3. Trace paths for the assembly

Overlap layout consensus (I)

Overlap → Layout → Consensus

- Overlap graph

1. to identify all pairs of reads that overlap **sufficiently well**
2. to organize the overlapping information into a graph containing a **node** for every read and an **edge** between any pair of reads that overlap each other.



to_every_thing_turn_turn_turn_there_is_a_season
l = 4 (minimum overlap)
k = 7 (read length)

Overlap layout consensus (II)

Overlap

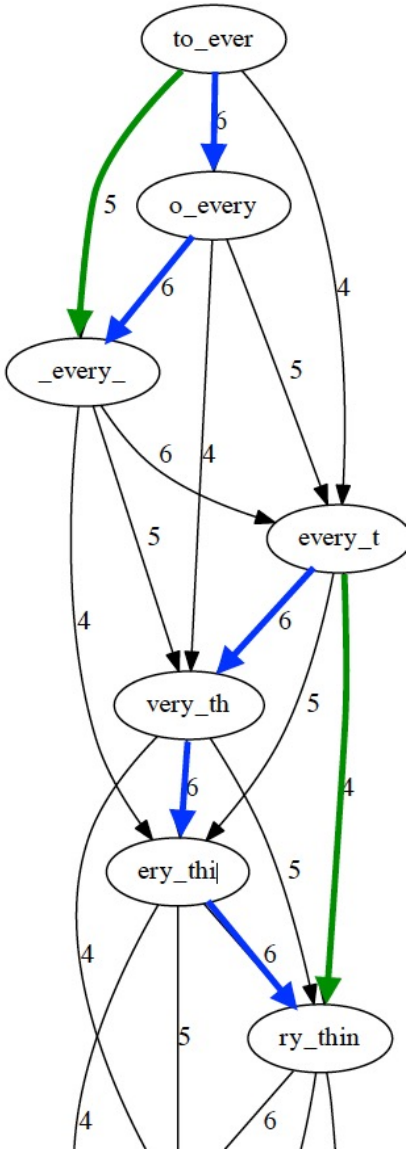
↓

Layout

↓

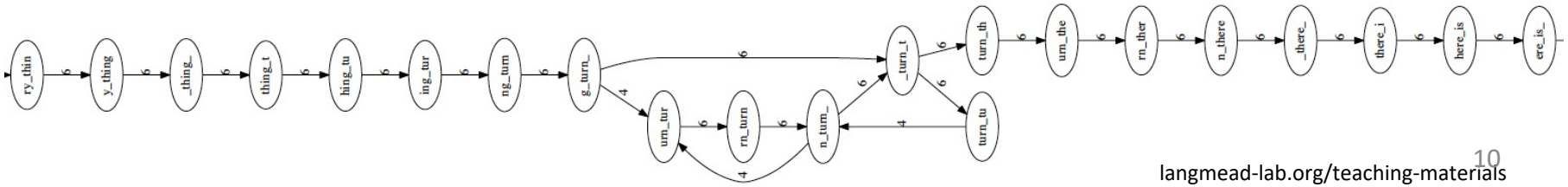
Consensus

transitive edges: edges that skip one or more nodes



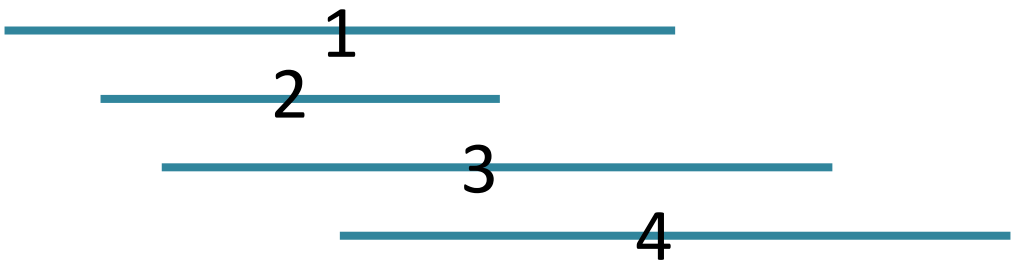
- Layout (**string graph**)

1. to simplify the global overlap graph by removing redundant information (transitive edges)

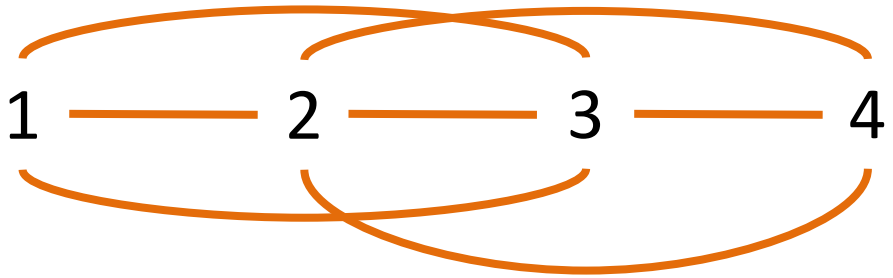


Simplification of the overlap graph

Read alignment

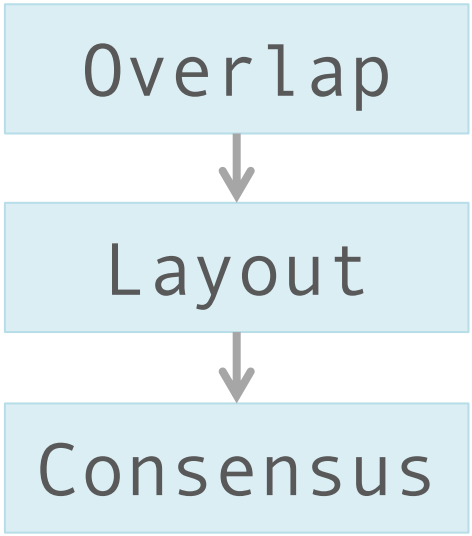


Overlap graph



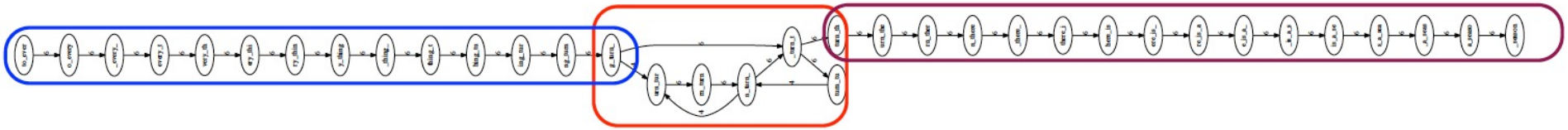
Simplify graph by removing transitive edges

Overlap layout consensus (III)



- Layout

1. Simplifies the global overlap graph by removing redundant information
2. Emits contigs corresponding to the non-branching paths



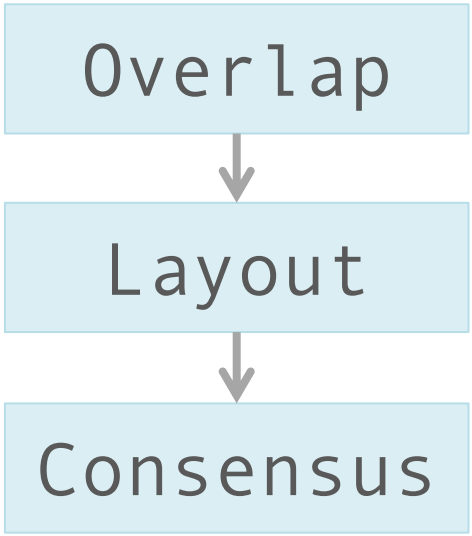
contig 1
to_every_thing_turn_

unresolved repeat
(gap)

contig 2
turn_there_is_a_season

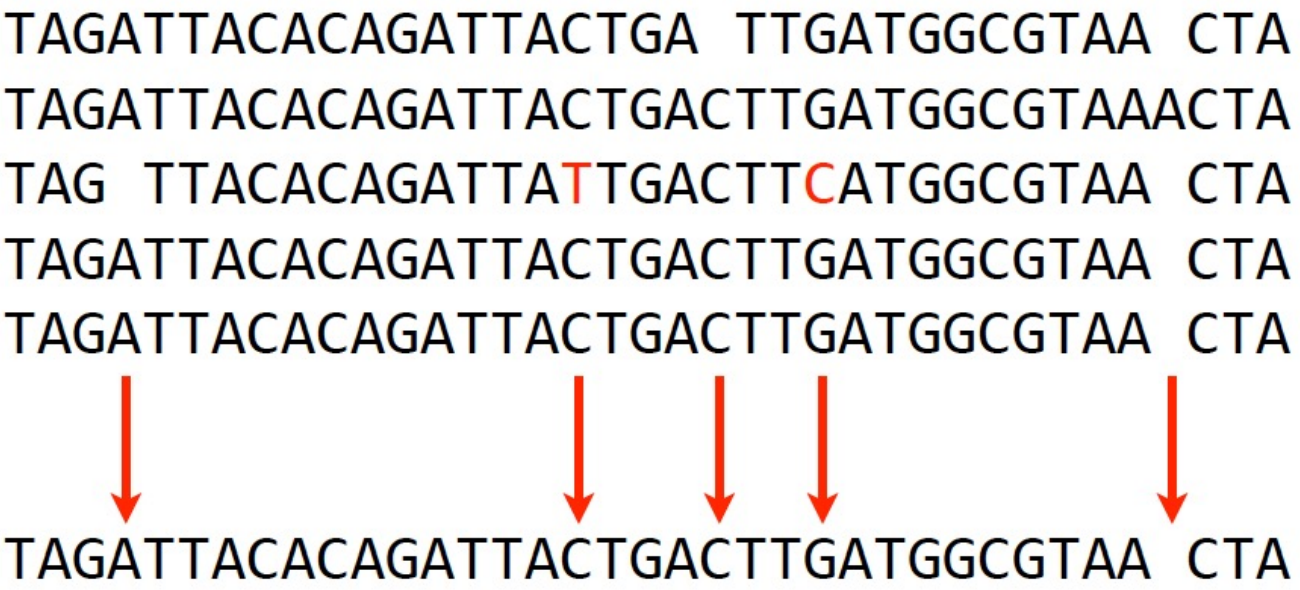
to_every_thing_turn_turn_turn_there_is_a_season

Overlap layout consensus (IV)



- Consensus

Extract consensus sequences (major votes) based on the alignment of reads that make up a contig



OLC drawbacks

- Identifying all-to-all overlaps is a slow procedure, especially when read number is millions or billions.

- Overlap graph is big when the read number is huge. One node per read, and in practice the number of edges grows superlinearly with the number of reads.

- The computational complexity limits its application.

De Bruijn graph – k-mer

De Bruijn graph assemblers model the relationship between **exact substrings** of length *k* (k-mer) extracted from input reads.

(k=3)

reads A T G G C G T

k-mer (k=3)

1.ATG
2.TGG
3.GGC
4.GCG
5.CGT

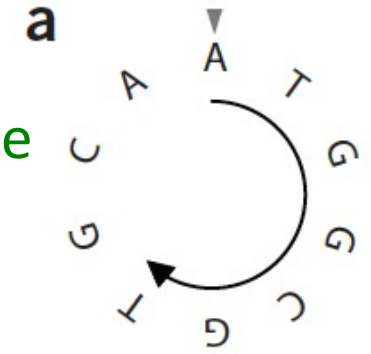
Compeau et al., 2011. Nature Biotechnology 29: 987-991.

Problem: list all K-mers (*k*=4)

CGATCGATCGATCGAT

De Bruijn graph - assembly

genome sequence



reads

b

1. ATGGCGT
2. GGCGTCG
3. CTTGCAA
4. TGCAATG
5. CAATGGC
6. ATGGCGT

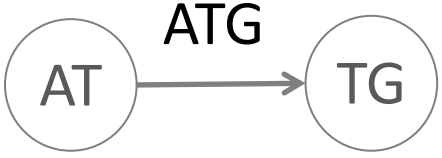
k-mer (k=3)

c

1. ATG
2. TGG
3. GGC
4. GCG
5. CGT
6. GTG
7. TGC
8. GCA
9. CAA
10. AAT

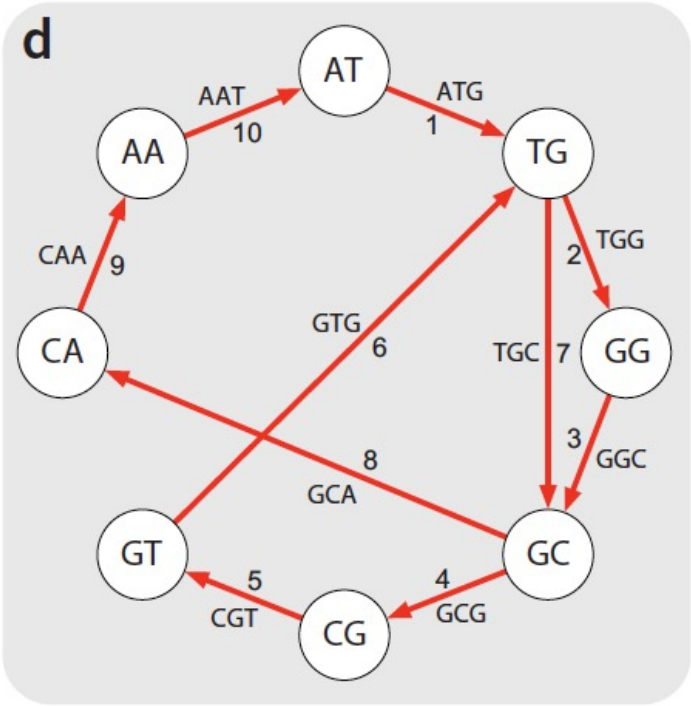
edge

ATG



Node

De Bruijn graph



visit each edge once

ATGGCGTGCAATG

Compeau et al., 2011. Nature Biotechnology 29: 987-991.

de Bruijn algorithm

- To find a path containing all k-mers but also is as short as possible, as it contains each k-mer exactly once.
- De Bruijn answered this question by borrowing Euler's solution of the Bridges of Königsberg problem. An Eulerian cycle represents a shortest path that contains each k-mer exactly once.

Compeau et al., 2011. Nature Biotechnology 29: 987-991.

Sequencing errors complicate assemblies

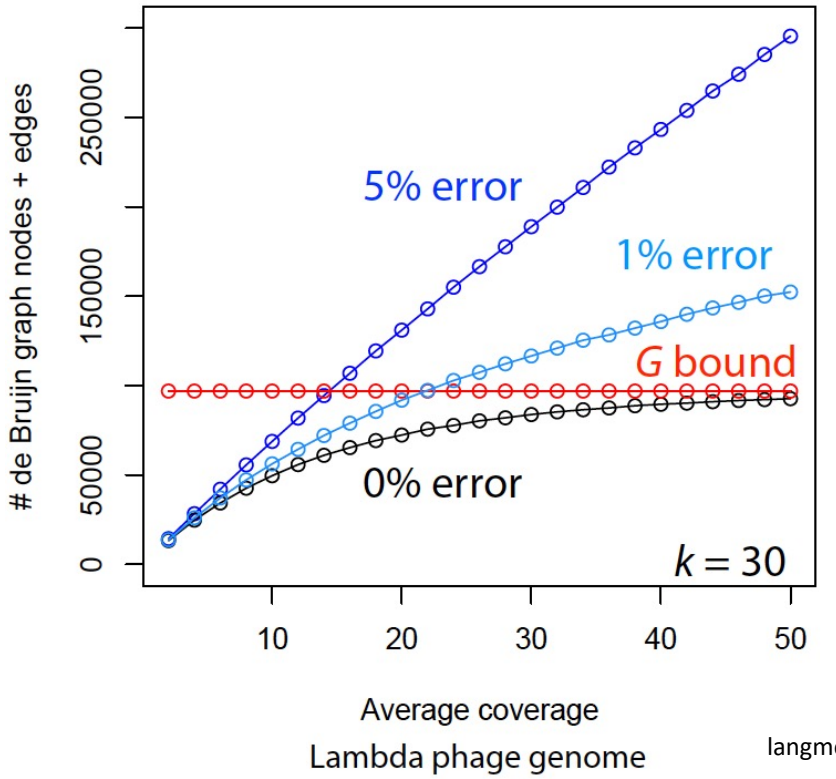
The complexity of De Bruijn graph grows when reads contain errors.

Reads

TGCGTGA
TGCGTGA
TGCGTGA
TGGGTGA

k-mer count profile (k=5)

TGCGT
GCGTG
CGTGA
TGGGT
GGGTG
GGTGA



G: genome

langmead-lab.org/teaching-materials

Sequence error correction before graph construction is a critical step for De Bruijn graph assembly.

Error identification

Reads (depth = 10)

```
TGCGTGATACG
TGCGTGATACG
TGGGTGATACG
TGCGTGATACG
TGCGTGATACG
TGCGTGATACG
TGCGTGATACG
TGCGTGATACG
TGCGTGATACG
TGCGTGATACG
```

k-mer count profile (k=5)

```
TGGGT 1
GGGTG 1
GGTGA 1
TGCGT 9
GCGTG 9
CGTGA 9
GTGAT 10
TGATA 10
GATAC 10
ATACG 10
```

Error identification

Reads (depth = 10)

```
TGCGTGATACG
TGCGTGATACG
TGGGTGATACG
TGCGTGATACG
TGCGTGATACG
TGCGTGATACG
TGCGTGATACG
TGCGTGATACG
TGCGTGATACG
TGCGTGATACG
```

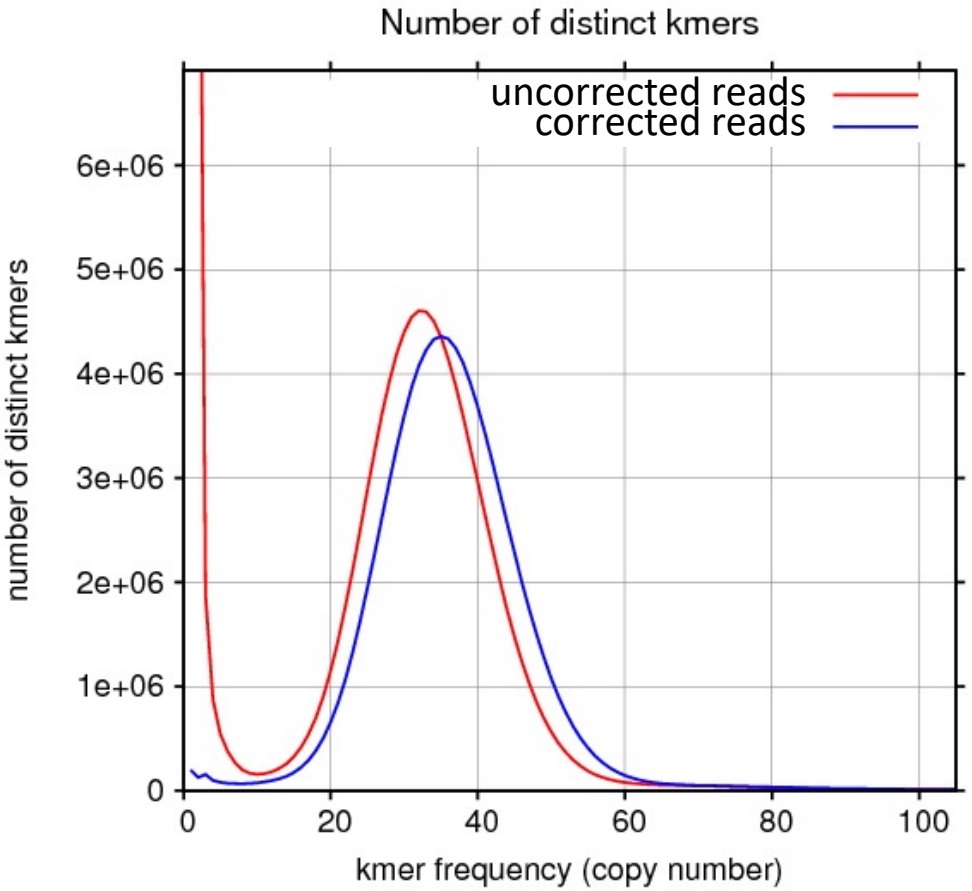
k-mer count profile (k=5)

```
TGGGT 1
GGGTG 1
GGTGA 1
TGCGT 9
GCGTG 9
CGTGA 9
GTGAT 10
TGATA 10
GATAC 10
ATACG 10
```

k-mer count profile indicates where errors are.

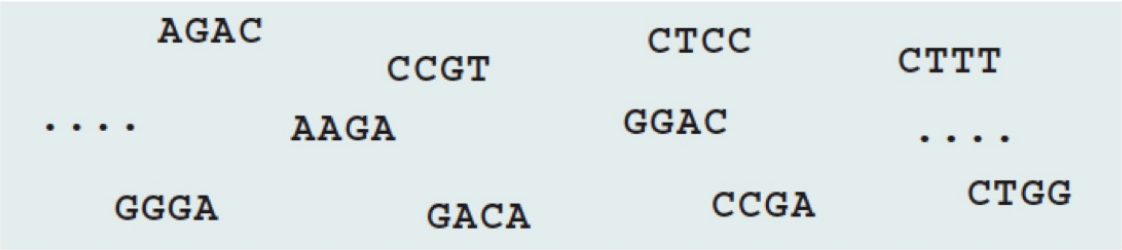
Error correction

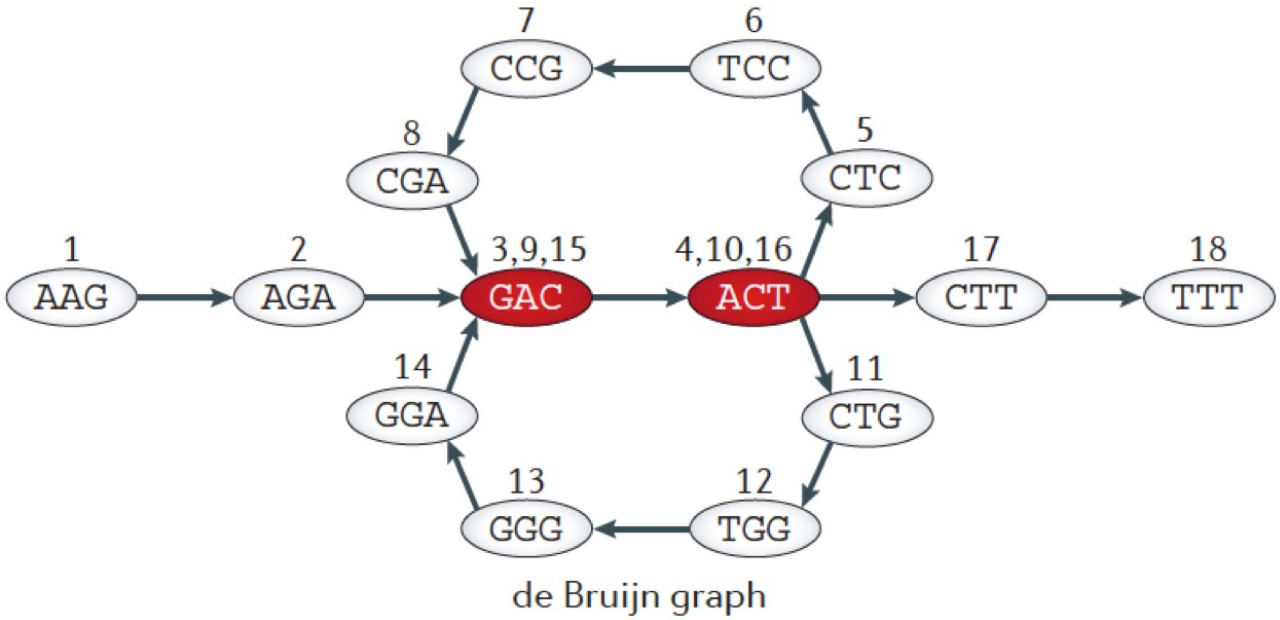
Essentially, the error correction algorithm removes k-mers with a few copies and correct reads carrying these errors.



from Allpaths-LG

Repeats





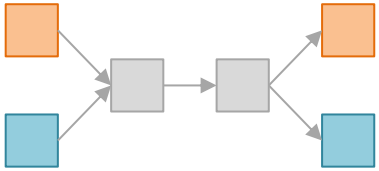
de Bruijn graph

AAGACTCCGACTGGGACTTT

Berger, B., et al., 2013 Nature Reviews Genetics 14: 333-346.

Unresolved repeats produce gaps

Long repeats, especially for those whose lengths are longer than read length, and high-copy repeats are challenging to resolve. If no other information can be used to assist in resolving repeats, gaps will be introduced.

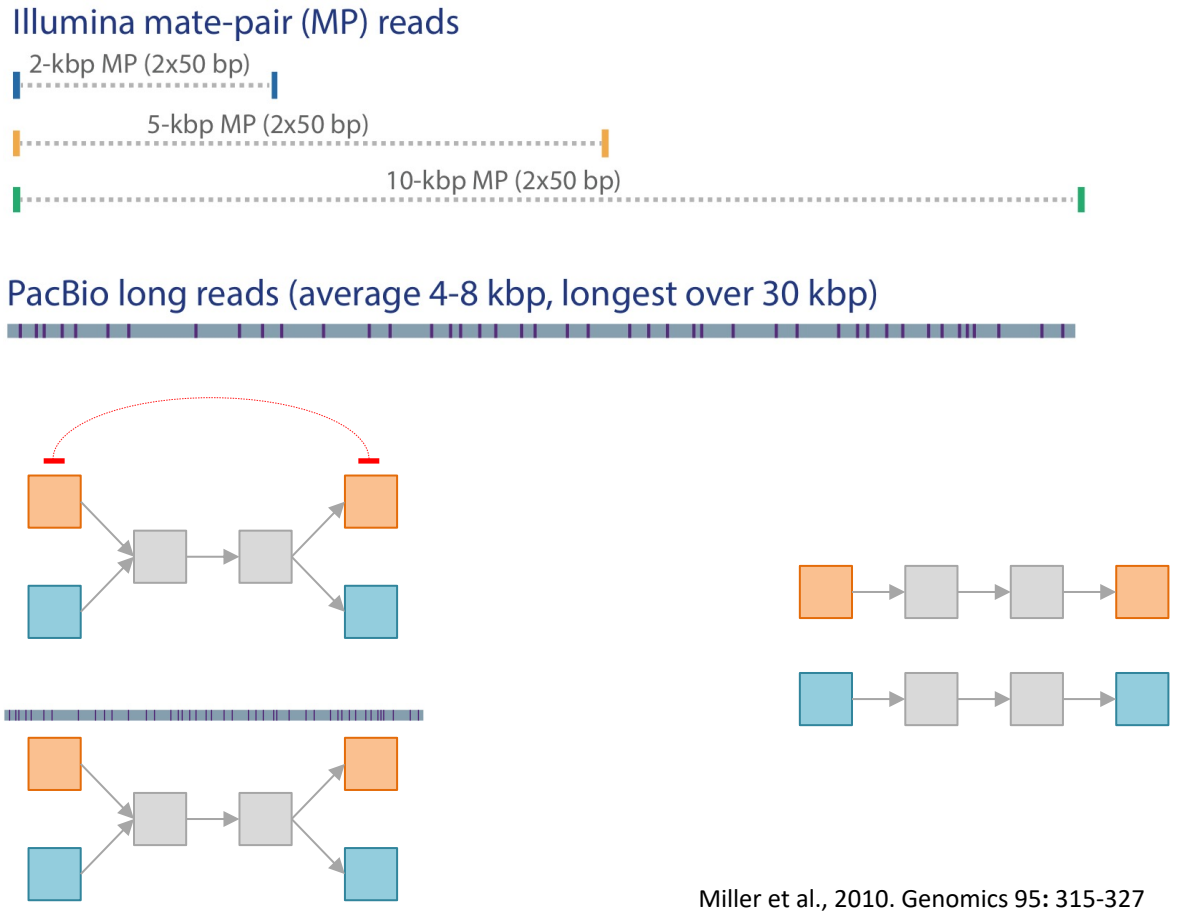


What strategies can be used to resolve repeats?

Miller et al., 2010. Genomics 95: 315-327

Some strategies to resolve repeats

Mate-pair reads, long reads (e.g., PacBio), genetic map, Hi-C data, and physical map (BioNano) can resolve some repeats



Miller et al., 2010. Genomics 95: 315-327

Gaps due to other reasons

- Low sequence coverage (depth)
- Sequencing biases (e.g., regions recalcitrant to sequencing)

```
CGGATCTGCGTGATACGGAATAGCCTAGCA
  GATCTGCGTGATACGGAAT
   ATCTGCGTGATACGGAATA
    TCTGCGTGATACGGAATAG
     CTGCGTGATACGGAATAGC
      TGCGTGATACGGAATAGCC
        CGTGATACGGAATAGCCT
           6 (coverage; depth)
```

Recalcitrant genomic regions:

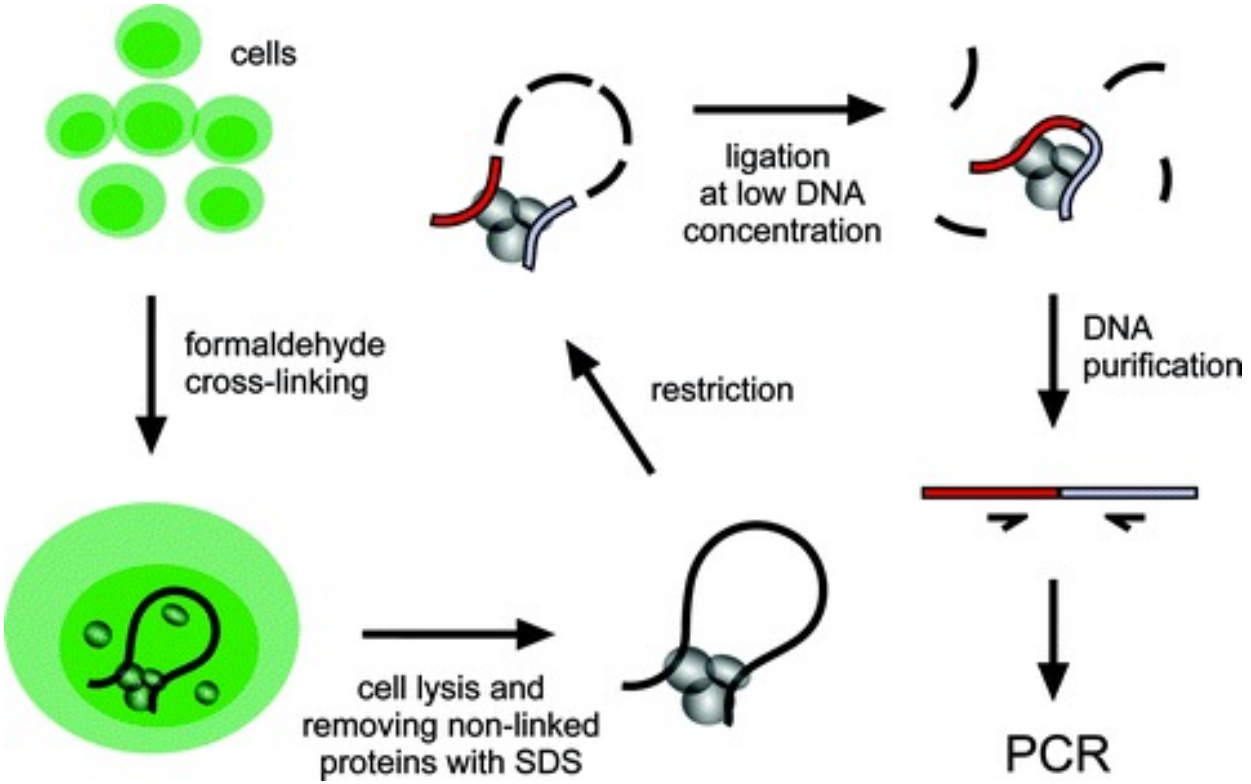
1. extremely high GC or AT
2. complicated secondary structure

Preferred genome sequencing reads:

1. Long reads
2. High-quality reads
3. High sequencing depth (at least 30x)
4. No sequencing biases

Hi-C: the method to quantify physical interactions between all possible pairs of genomic regions simultaneously

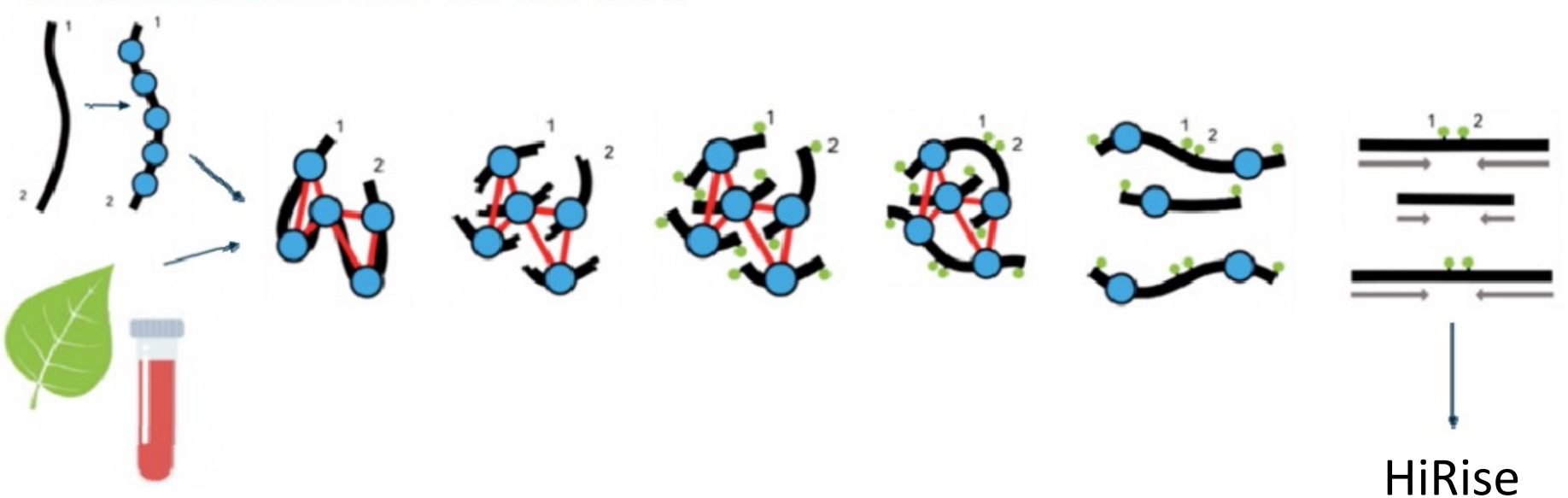
Inside a cell, genomic regions in a chromosome are physically connected to form chromosome conformation**. Closer genomic regions generally have higher frequent physical contacts than regions far apart in distance.**



MMB, 567:171-188

Chicago + Dovetail Hi-C

Chicago generated libraries starting from pure DNA that is reconstituted into chromatin



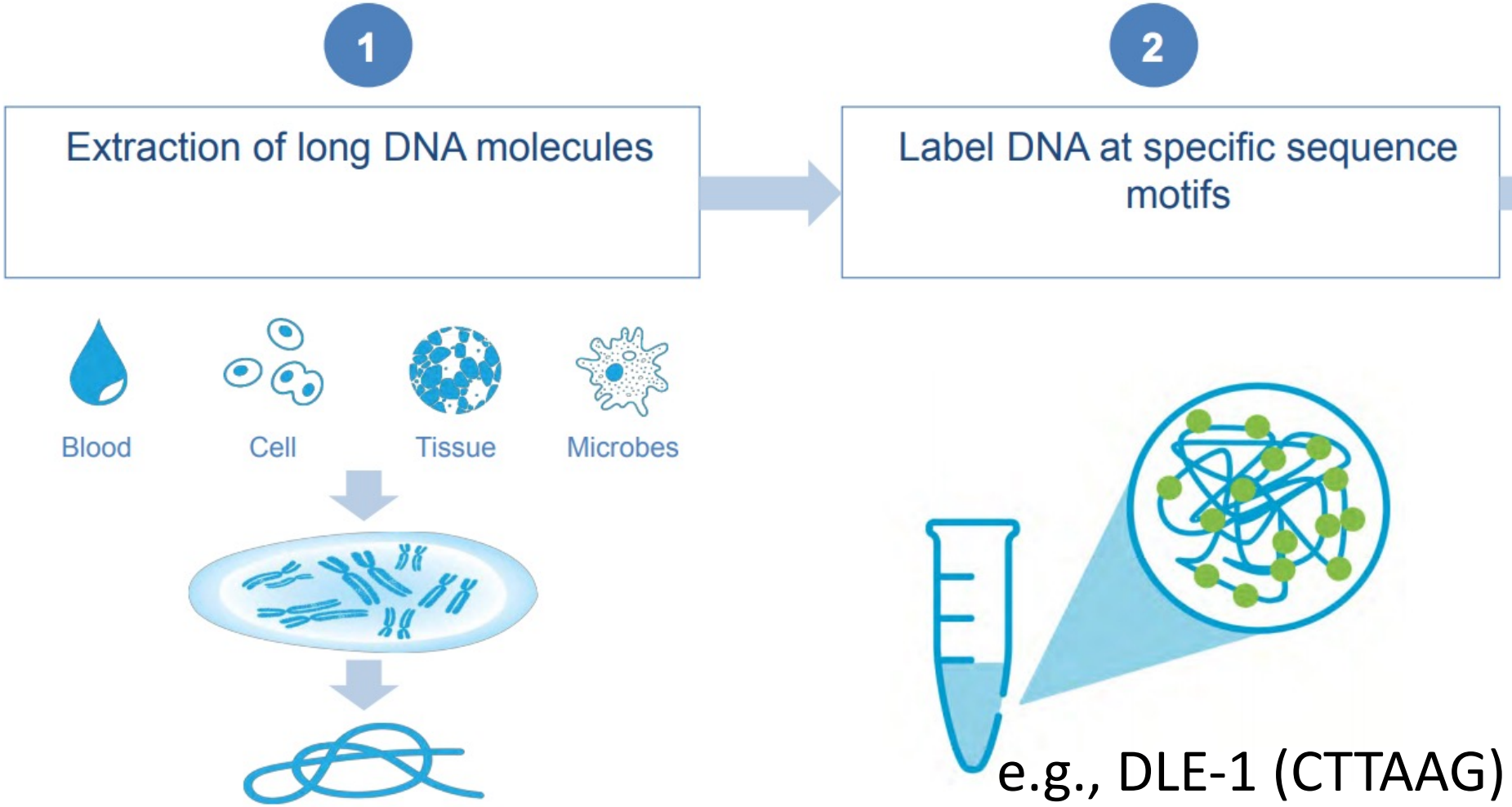
Dovetail Hi-C generated libraries starting from tissue or cell culture and endogenous chromatin

HiRise scaffolding pipeline

https://youtu.be/OIxFFB3daIM

https://www.biorxiv.org/content/biorxiv/early/2018/08/15/392126.full.pdf

BioNano – library preparation



e.g., DLE-1 (CTTAAG)
Direct label enzyme

https://bionanogenomics.com/wp-content/uploads/2019/03/Bionano-Genomics_Poster_PAG-2019_A-Lee_S-Bocklandt_Hybrid-Scaffold.pdf

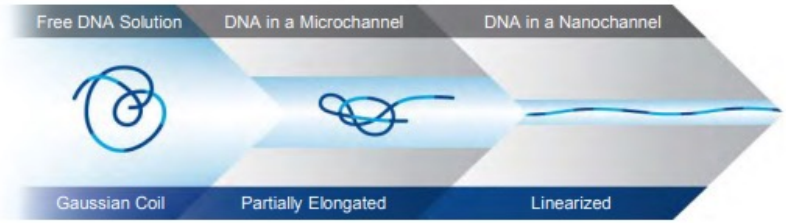
BioNano – running on Saphyr

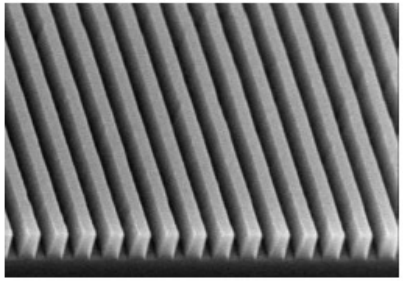
3

Saphyr Chip linearizes DNA in NanoChannel arrays

4

Saphyr automates imaging of single molecules in NanoChannel arrays





https://bionanogenomics.com/wp-content/uploads/2019/03/Bionano-Genomics_Poster_PAG-2019_A-Lee_S-Bocklandt_Hybrid-Scaffold.pdf

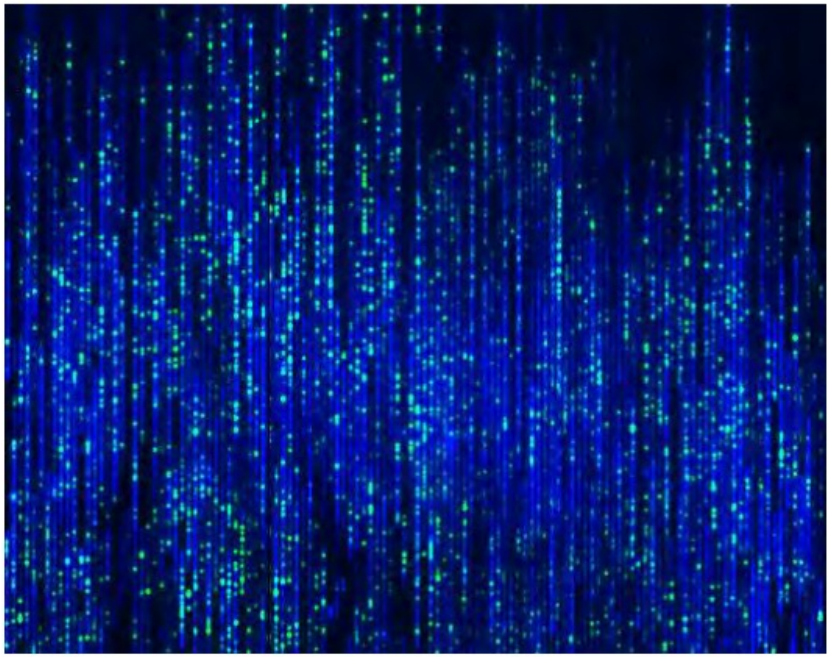
BioNano – image analysis and assembly of molecular data

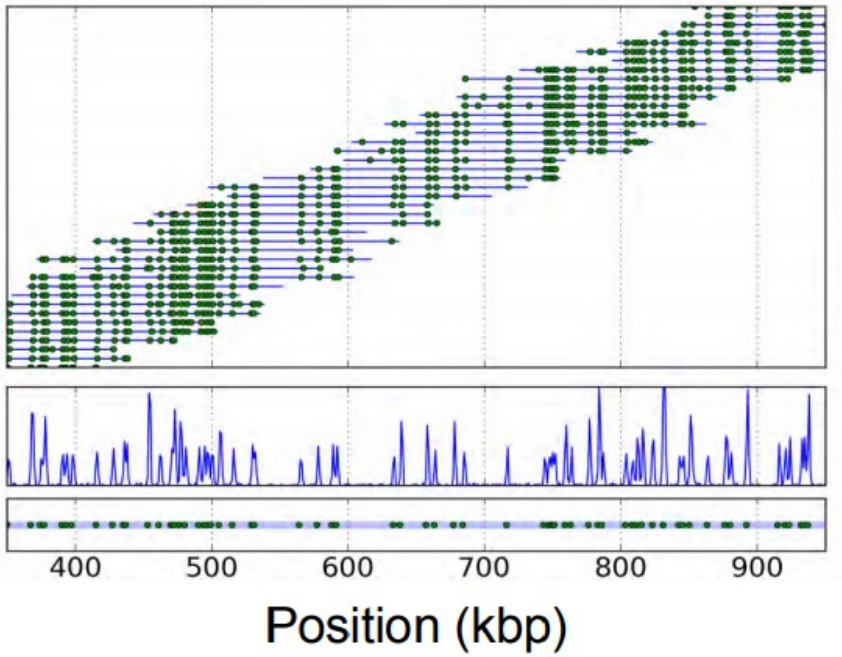
5

Molecules and labels detected in images

6

Bionano Access software assembles optical maps





https://bionanogenomics.com/wp-content/uploads/2019/03/Bionano-Genomics_Poster_PAG-2019_A-Lee_S-Bocklandt_Hybrid-Scaffold.pdf

BioNano scaffolding

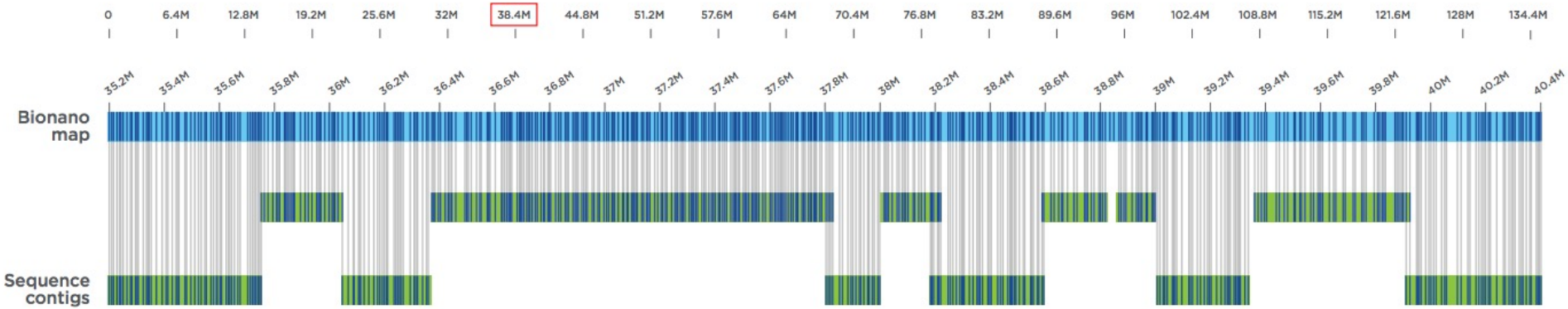


Figure 1:
Combining Bionano maps with sequence assemblies. Sequence contigs are anchored and oriented using the de novo generated Bionano maps.

https://bionanogenomics.com/wp-content/uploads/2017/02/Bionano_HumanPAG_Hybrid-Scaffolding-White-Paper.pdf

Assembly statistics – N50 and L50

- N50: A statistic used for assessing the contiguity of a genome assembly.
- L50: The number of contigs whose summed length is N50.
- The contigs in an assembly are sorted by size and added, starting with the largest. The contig N50 is the size of the contig that makes the total greater than or equal to 50% of the total contig size.
- OR: The contig N50 is the length of the smallest contig in the set that contains the fewest (largest) contigs whose combined length represents at least 50% of the assembly.

An example to determine N50 and NG50

assembly: 20 contigs

contig N50? 360 kbp

ctg_ID	ctg_size (kbp)	Accumulated (kbp)	% ASM
1	615	615	13%
2	515	1,130	25%
3	512	1,642	36%
4	450	2,092	46%
5	360	2,452	53%
6	356	2,808	61%
7	310	3,118	68%
8	201	3,319	72%
9	189	3,508	76%
10	186	3,694	80%
11	160	3,854	84%
12	150	4,004	87%
13	120	4,124	90%
14	102	4,226	92%
15	95	4,321	94%
16	86	4,407	96%
17	82	4,489	98%
18	54	4,543	99%
19	32	4,575	100%
20	21	4,596	100%
Total	4,596		

Assembly statistics – NG50

Problem of N50:

N50 values of the assemblies with significantly different total assembly space (lengths) are usually not comparable. Even if the same data set is used for the assembly.

50,000	50,000
30,000	**40,000**
10,000	30,000
	20,000
	10,000

NG50: The NG50 statistic is the same as N50 except that it is 50% of the known or estimated genome size. This allows for meaningful comparisons between different assemblies.

An example to determine N50 and NG50

- Genome size: 5 Mbp
- 20 contigs

contig N50 360 bp

contig NG50? 356 kbp

ctg_ID	ctg_size (kbp)	Accumulated (kbp)	% ASM	% genome
1	615	615	13%	12%
2	515	1,130	25%	23%
3	512	1,642	36%	33%
4	450	2,092	46%	42%
5	360	2,452	53%	49%
6	356	2,808	61%	56%
7	310	3,118	68%	62%
8	201	3,319	72%	66%
9	189	3,508	76%	70%
10	186	3,694	80%	74%
11	160	3,854	84%	77%
12	150	4,004	87%	80%
13	120	4,124	90%	82%
14	102	4,226	92%	85%
15	95	4,321	94%	86%
16	86	4,407	96%	88%
17	82	4,489	98%	90%
18	54	4,543	99%	91%
19	32	4,575	100%	92%
20	21	4,596	100%	92%
Total	4,596			

Software for short-read assemblies

Software	Description	URLandreference
Velvet	Original de Bruijn graph assembler	http://github.com/dzerbino/velvet
SOAPdenovo	De Bruijn graph assembler with error-correction step	http://soap.genomics.org.cn/
Meraculous	Hybrid k-mer/read-based	https://jgi.doe.gov/data-and-tools/meraculous/
ALLPATHS-LG	Uses unipath graph to collapse repeats	http://software.broadinstitute.org/allpaths-lg/blog/
SGA	Uses string graphs	https://github.com/jts/sga
ABySS	Represents de Bruijn graph with a Bloom filter	https://github.com/bcgsc/abyss
DISCOVAR de novo	Requires 250-bp PCR-free reads	https://software.broadinstitute.org/software/discovar/blog/
Supernova	Assembles 10× linked reads	https://github.com/10XGenomics/supernova

Long-read assemblies and polishing

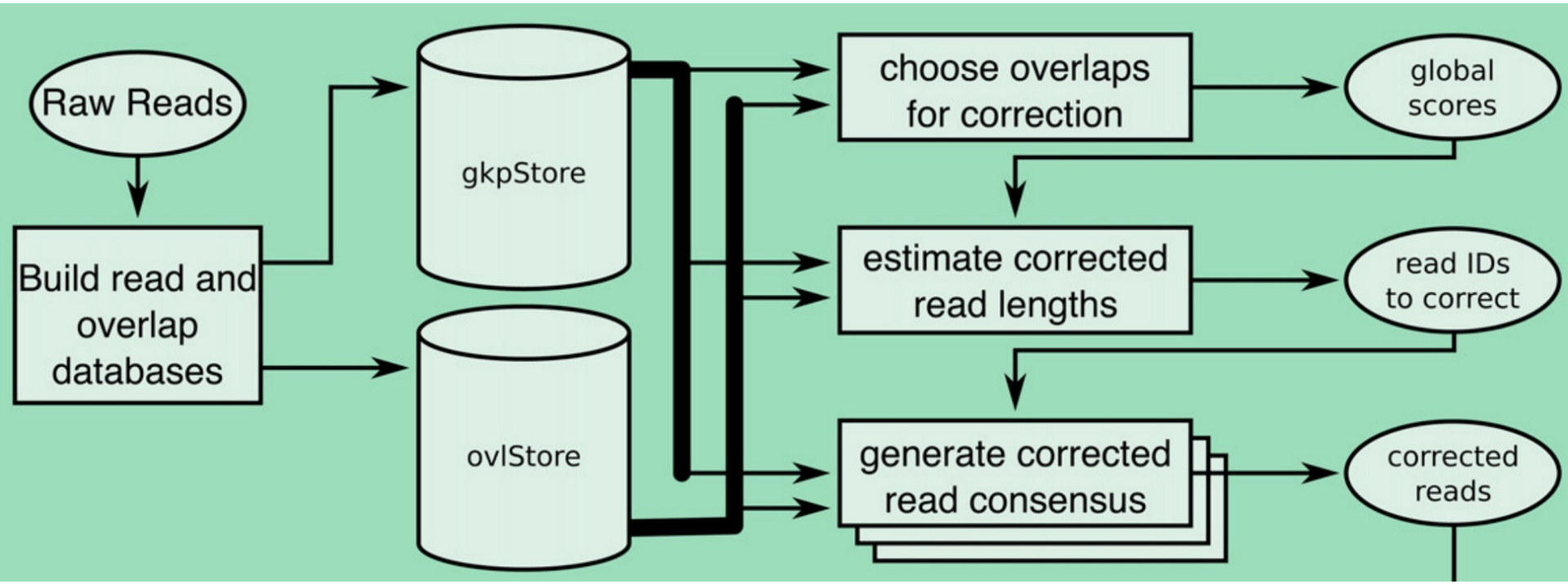
Long-read assemblies

Software	Description	URLandreference
HGAP	Error correction, OLC assembly, polishing	https://github.com/PacificBiosciences/Bioinformatics-Training/wiki/HGAP
Canu	K-mer-based overlap computation	https://github.com/marbl/canu
FALCON	Assembles phased diploid genomes	https://github.com/PacificBiosciences/FALCON
Flye	Uses A-Bruijn graph	https://github.com/fenderglass/Flye
Miniasm	Fast, but no error correction	https://github.com/lh3/miniasm

Polishing

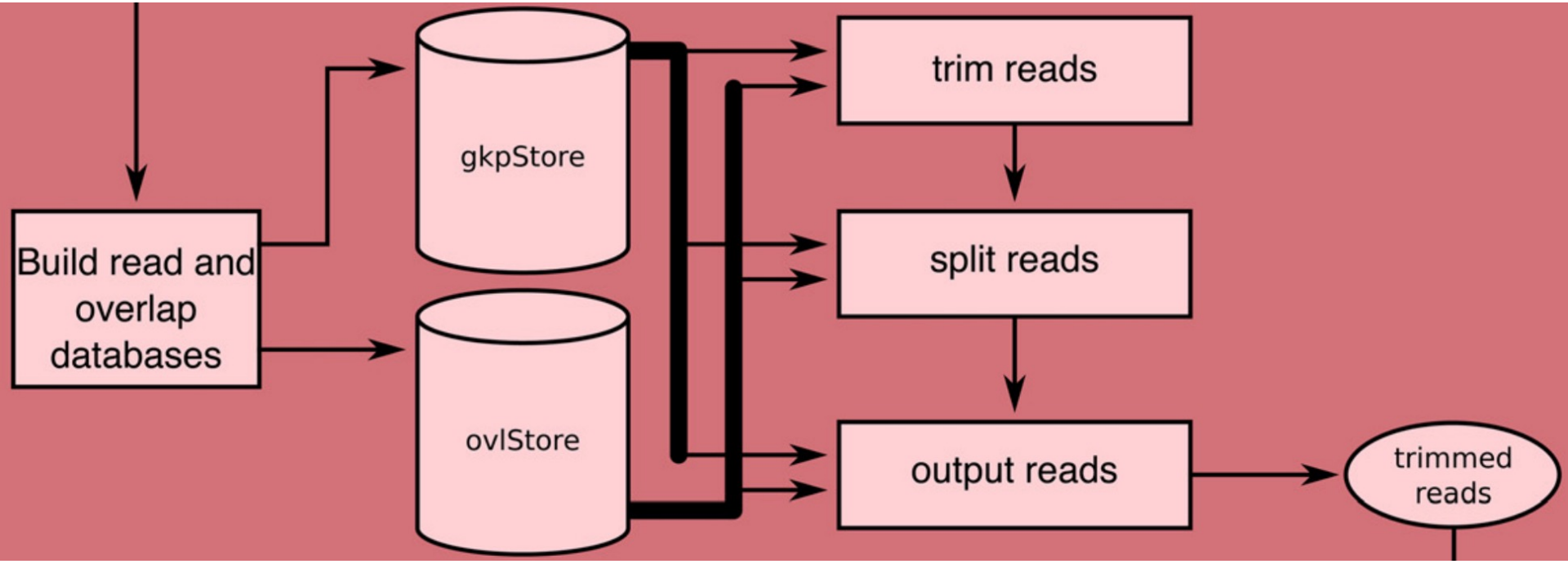
Software	Description	URLandreference
Pilon	Uses short-read alignments to correct errors	https://github.com/broadinstitute/pilon
Arrow	Hidden Markov model and long-read alignments	https://github.com/PacificBiosciences/GenomicConsensus
Nanopolish	uses voltage data to correct errors	https://github.com/jts/nanopolish

Canu - read correction

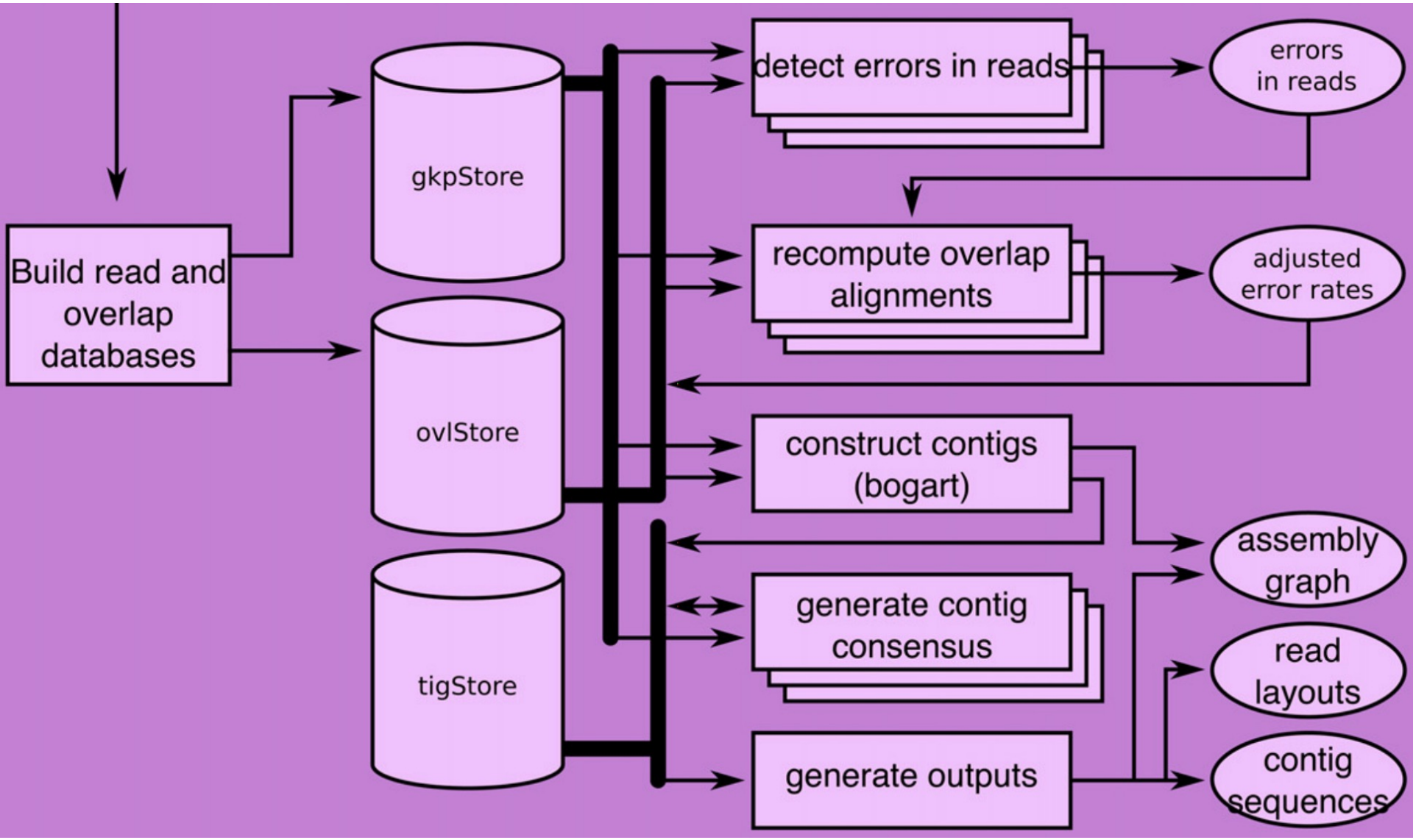


Koren et al., Genome Research, 27:722–736 (2017)

Canu - read trimming



Canu - assembly (contigs and consensus)



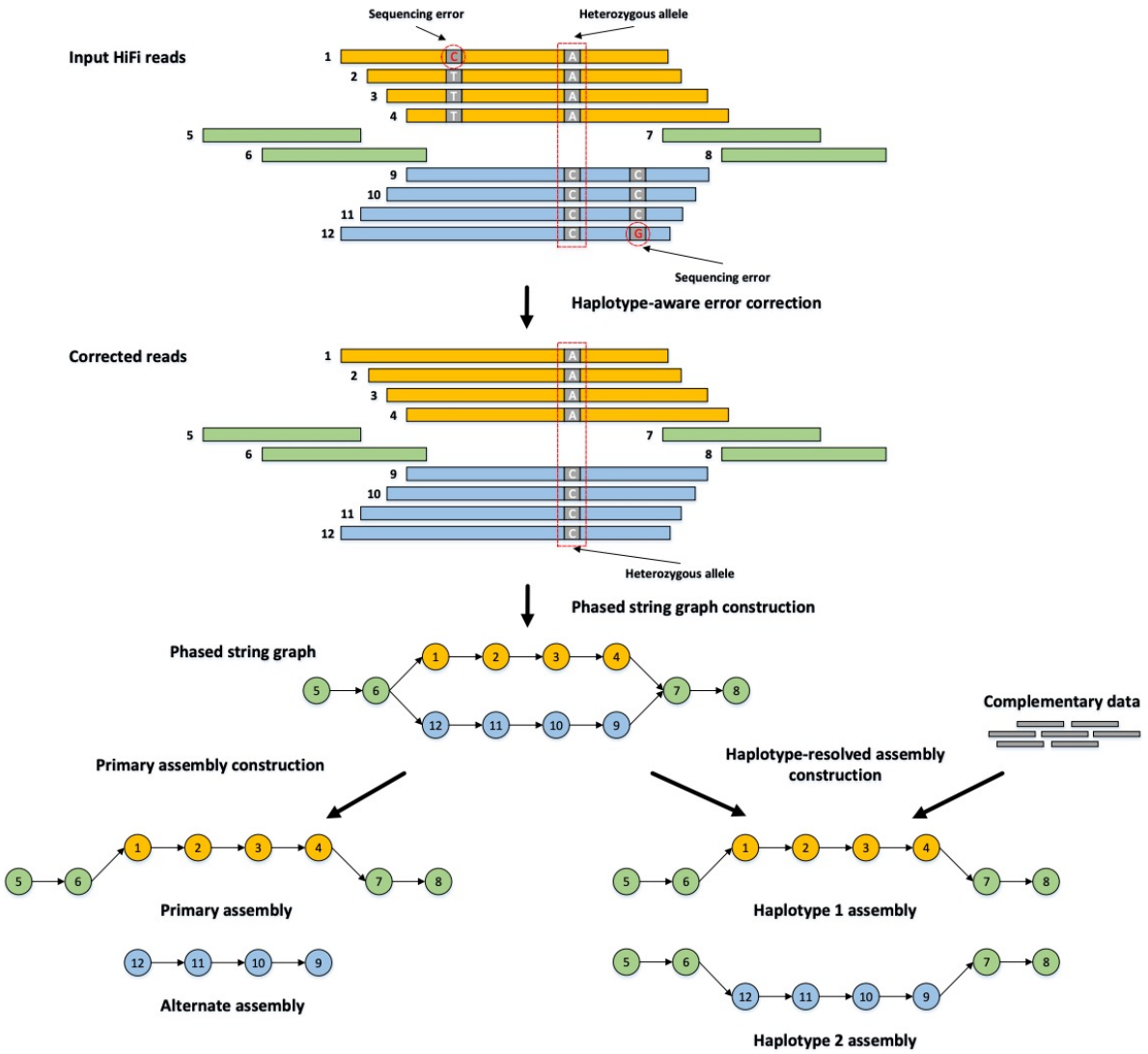
Assembly meets long HiFi PacBio reads

Assembly with hifiasm

Dataset	Size	Coverage	CPU time	RAM	N50
Maize (B73)	2.2Gb	×22	203.2h	68G	36.7Mb
Human (CHM13)	3.1Gb	×32	310.7h	114G	88.9Mb

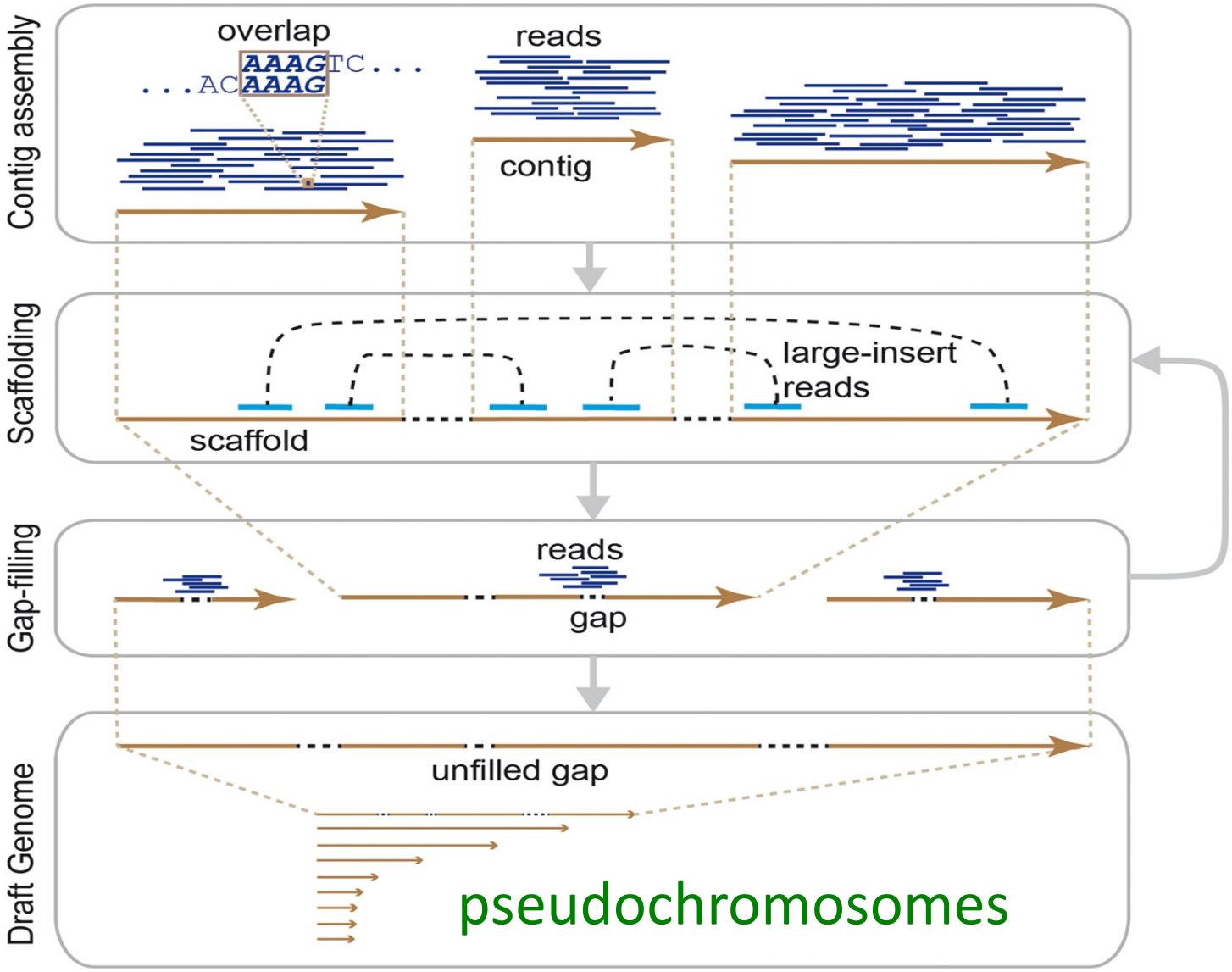
https://github.com/chhylp123/hifiasm

Haplotype-resolved *de novo* assembly (Phased assembly using hifiasm)



Cheng et al., Nature Methods, 18, 170–175 (2021)

General workflow of *de novo* assemblies



Assemblies are hypotheses

... there will always be ***some degree of error*** in the characterized genome sequence, both on the level of individual nucleotides and in the ordering of sequence blocks. ..., every genome assembly is the result of a series of assembly heuristics and should accordingly be treated as **a working hypothesis**.

Evolutionary Conservation, 7:1026-1042, 2014

Assembly evaluation

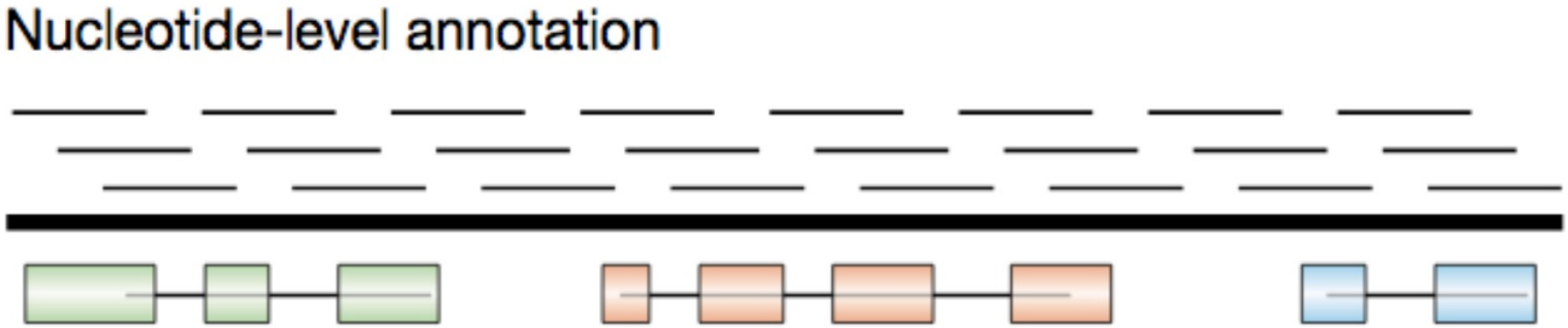
1. No. of contigs: the total number of contigs in the assembly.
2. Largest contig: the length of the largest contig in the assembly.
3. Total length: total assembly length
4. Nxx (N50), NGxx (NG50)
5. Using a reference genome, No. of misassemblies, No. INDEL per 100 kbp, and No. of mismatches per 100 kbp are reported
6. Evaluation with BUSCO

Gurevich et al., 2013 Bioinformatics 29: 1072-1075.

Outline

- Genome assembly: concept
- Assembly algorithms: OLC/De Bruijn graph
- Error correction (Kmer counts)
- Assembly strategy to cope with the repeat problem (mate-pair reads, long reads, others)
- Assembly evaluation (N50, comparison to a reference genome)
- Genome annotation

Gene annotation of eukaryotic genomes



Gene prediction from genomic DNA sequences:

to predict the ***exon-intron*** structures of the protein-encoding portions of transcripts (open reading frames or ORFs) and ***untranslated regions*** (UTR).

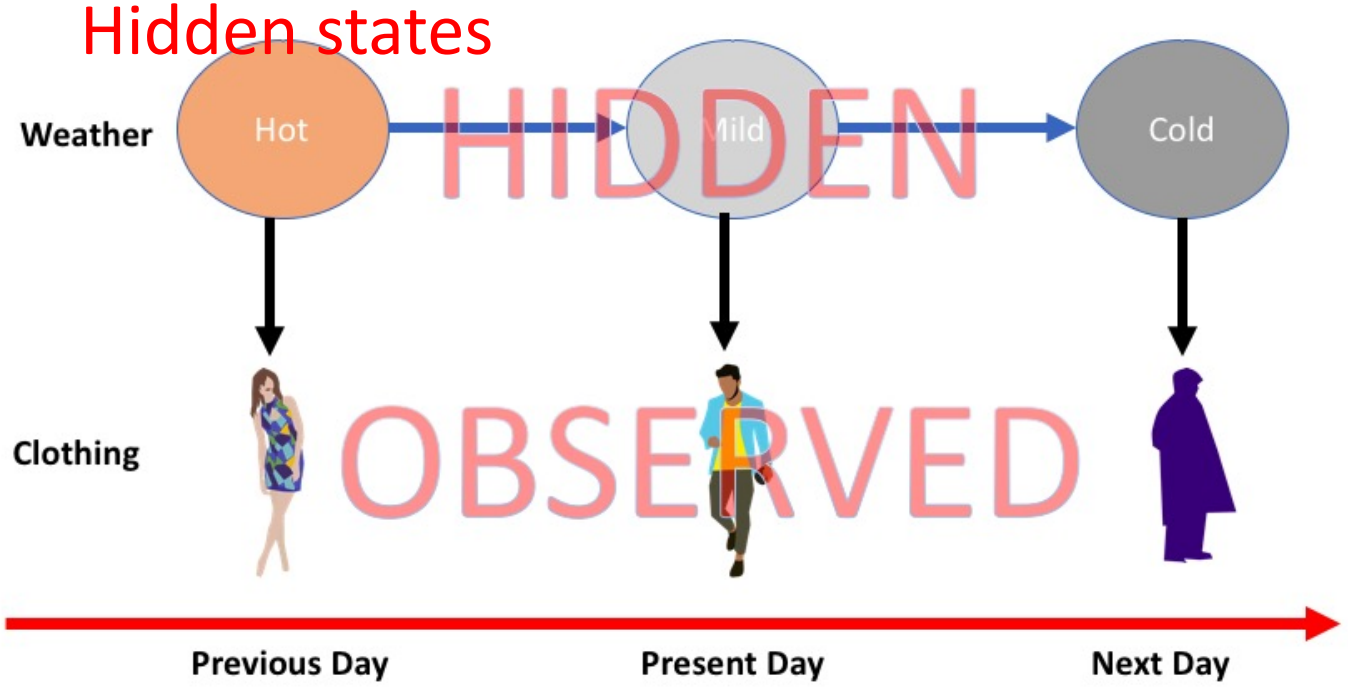
Gene prediction approaches

- ***ab initio* gene prediction** (Hidden Markov Model)

to assign probability scores to many potential coding exons, and then join consecutive high-scoring exons with consistent reading frames to form high-scoring *exon–intron structures*

- **Evidence-driven gene prediction** (e.g., RNA sequencing data)

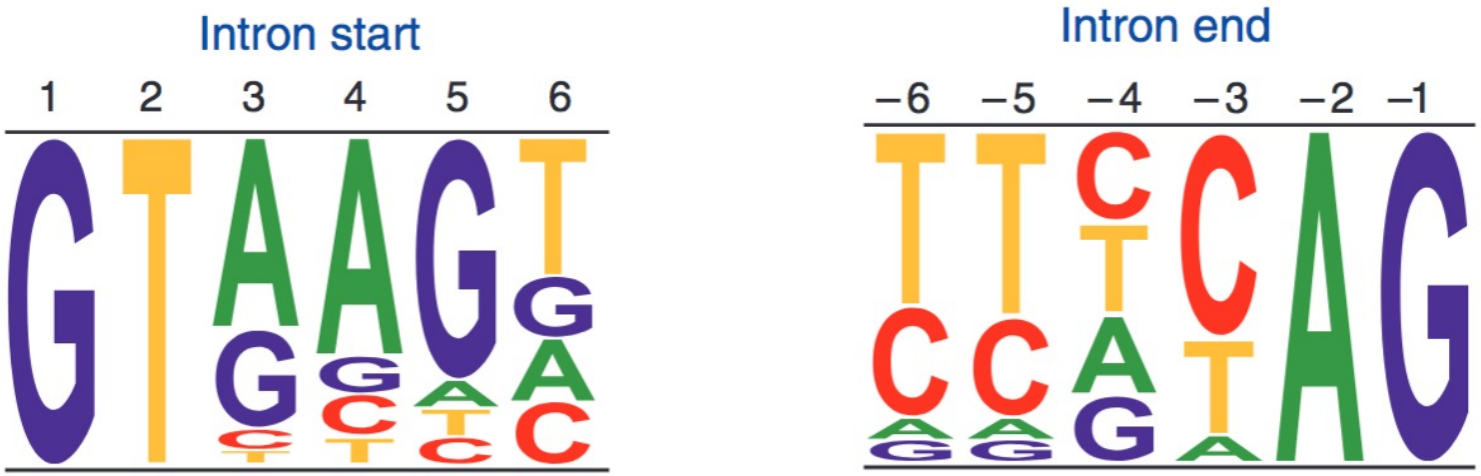
Hidden Markov Model (HMM)



HMM is a class of probabilistic graphical model that is used to predict a sequence of ***unknown*** (hidden) variables from ***observed*** variables.

https://medium.com/@postsanjay/hidden-markov-models-simplified-c3f58728caab

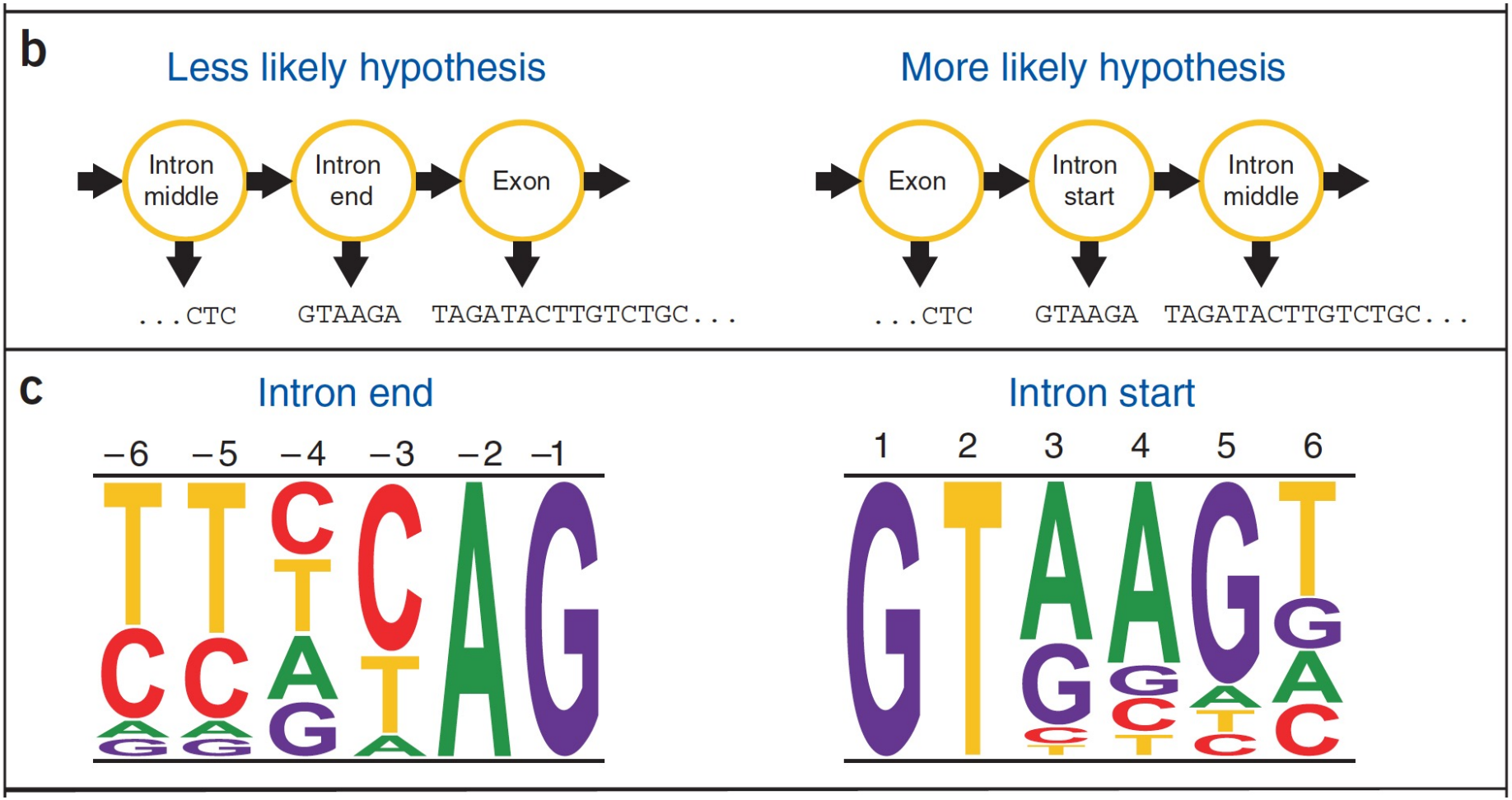
Exon-intron structure



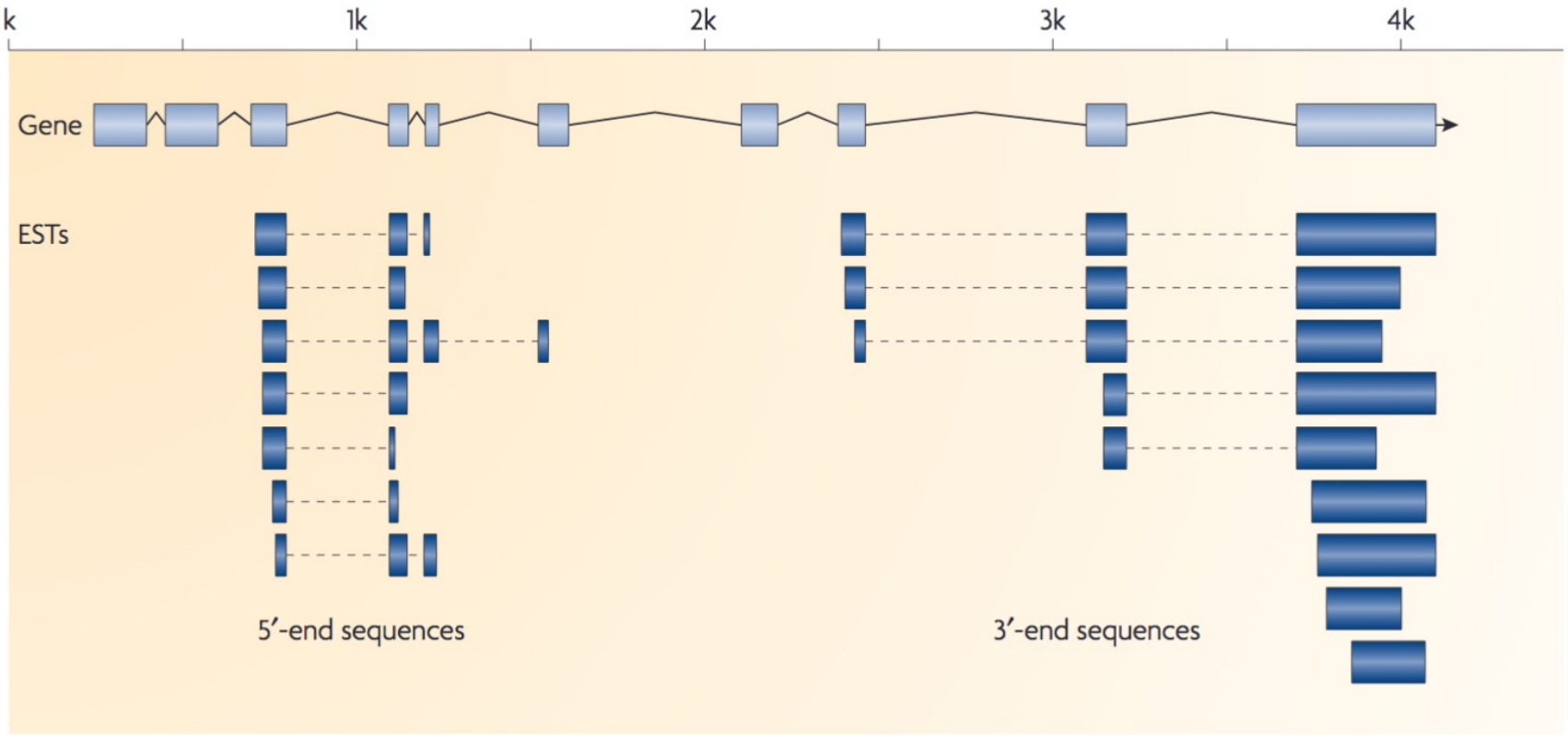
Sequence logos represent weight matrices for the first six bases of an intron (left) and the last six bases of an intron (right). In plants and animals, ~99% of introns begin with GT.

Other genomic sequence patterns: translation initiation and termination sites.

Generalized Hidden Markov Model (GHMM)



Evidence-driven approaches



Ideally, full-length cDNA sequences of all genes are collected for evidence-based genome annotation.

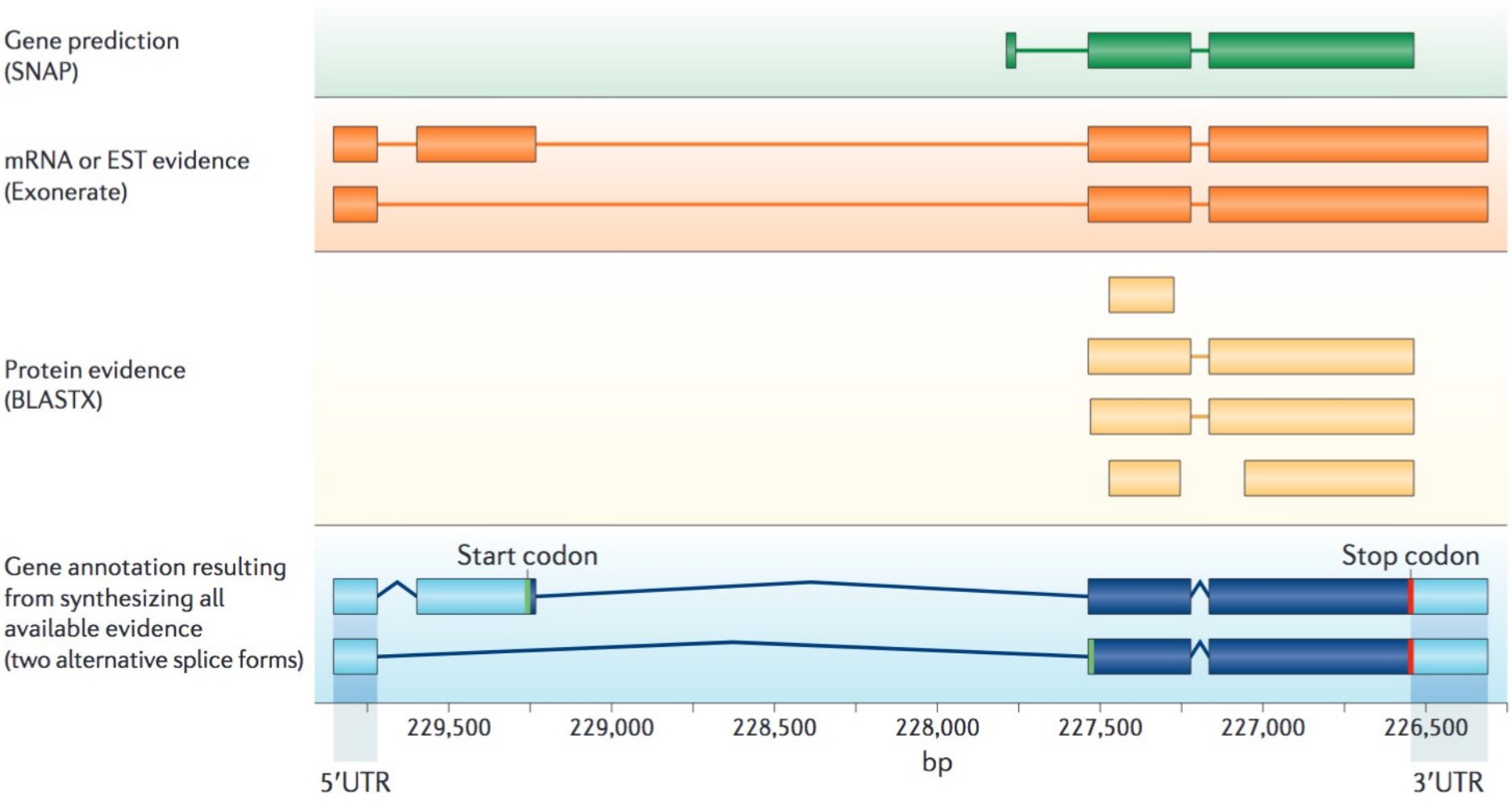
evidence and limitations

- Full-length cDNAs
- Protein sequences
- Expression sequence tags (ESTs)
- RNA-Seq (de novo assembly and alignment-based)

Limitations:

- Full-length cDNA databases are not available
- Not all genes are transcribed at sufficient levels at certain conditions
- Alignments produce errors

annotation: *ab initio* prediction + evidence

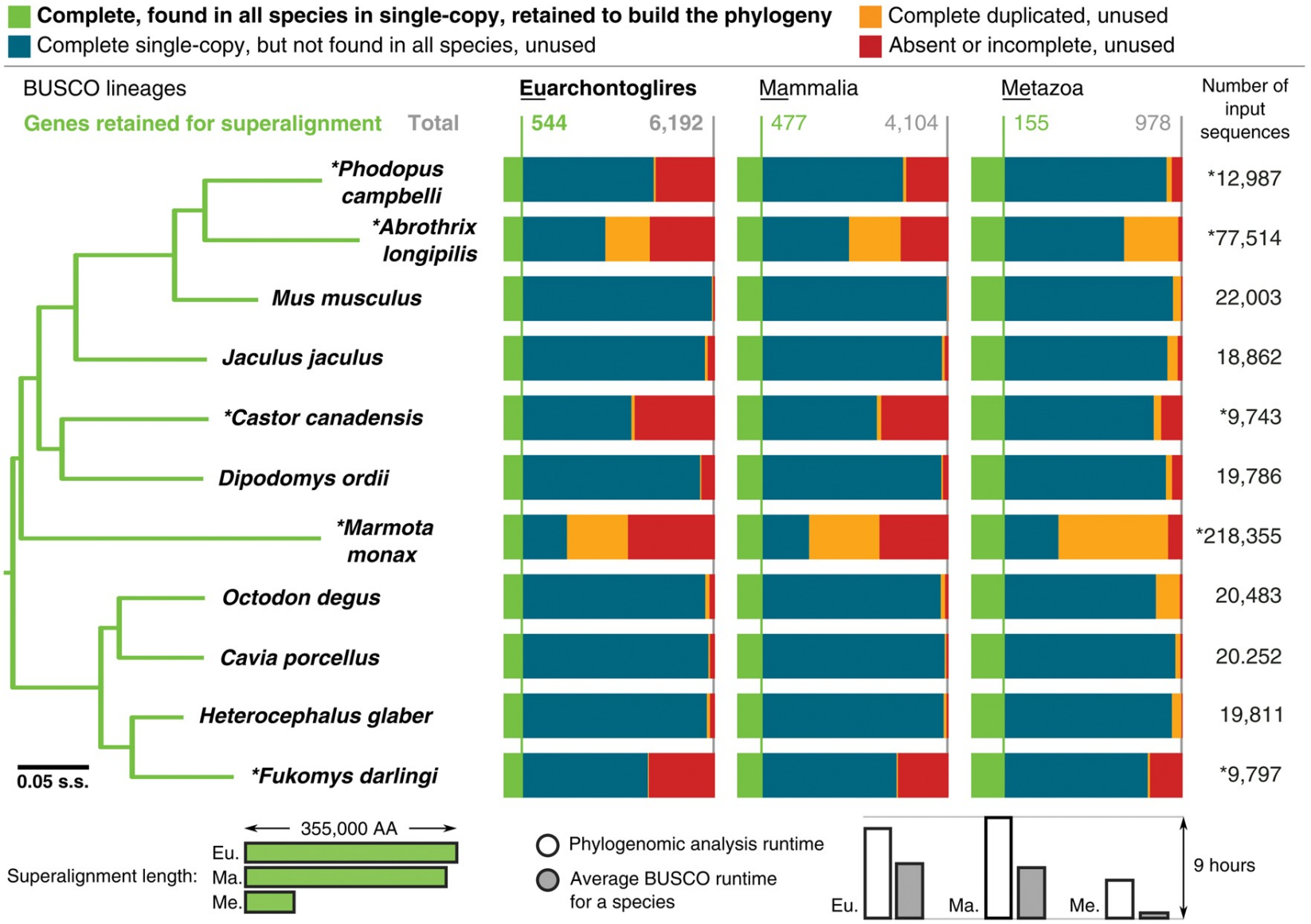


Nature Reviews Genetics 13:329–342 (2012)

Question?

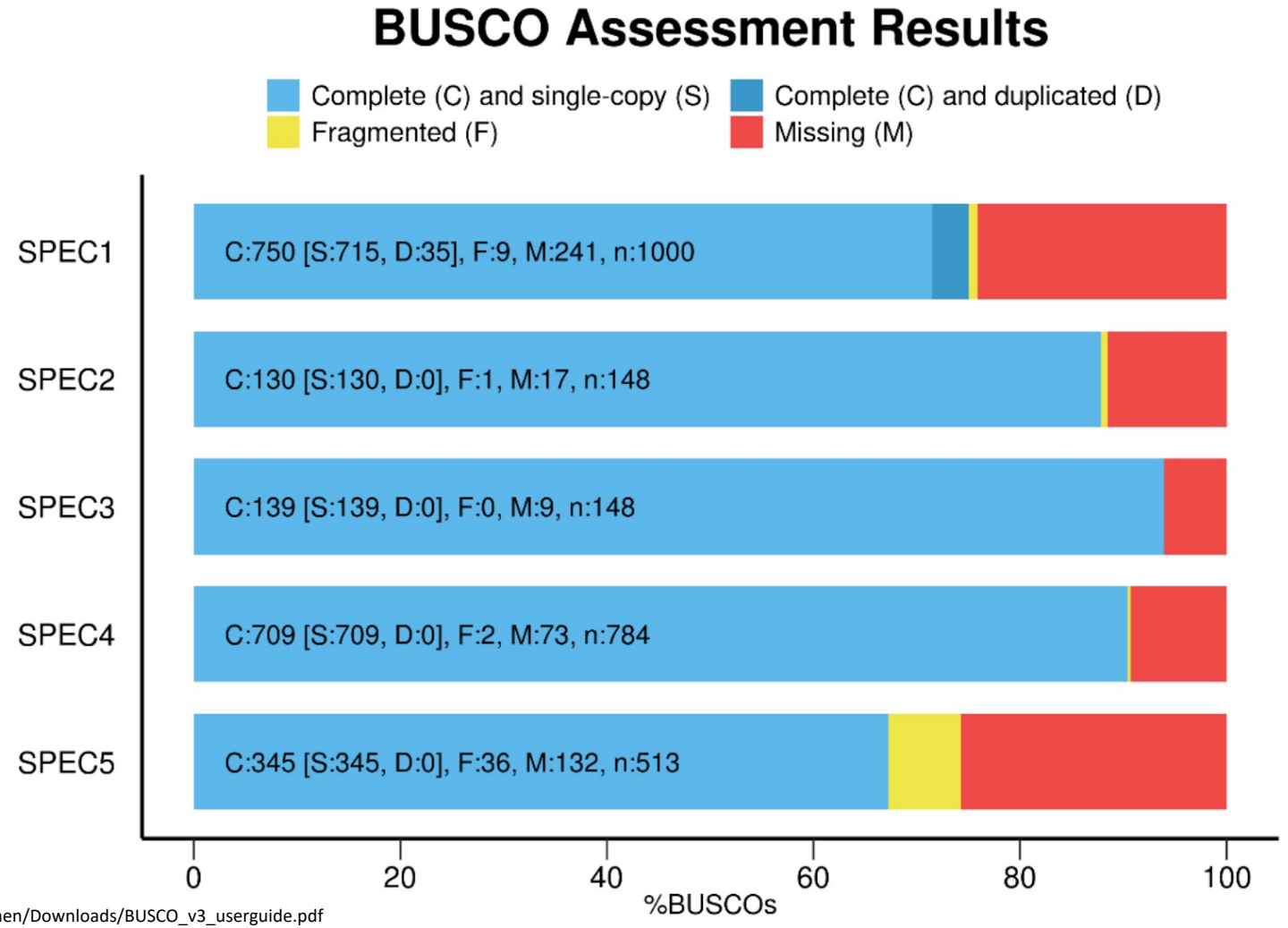
What method can you figure out to assess the completeness of a genome annotation?

Genome and transcriptome BUSCO assessments to identify universal single-copy genes



Evaluation with BUSCO

Assessing genome assembly and annotation completeness with Benchmarking Universal Single-Copy Orthologs.



file:///Users/liu3zhen/Downloads/BUSCO_v3_userguide.pdf

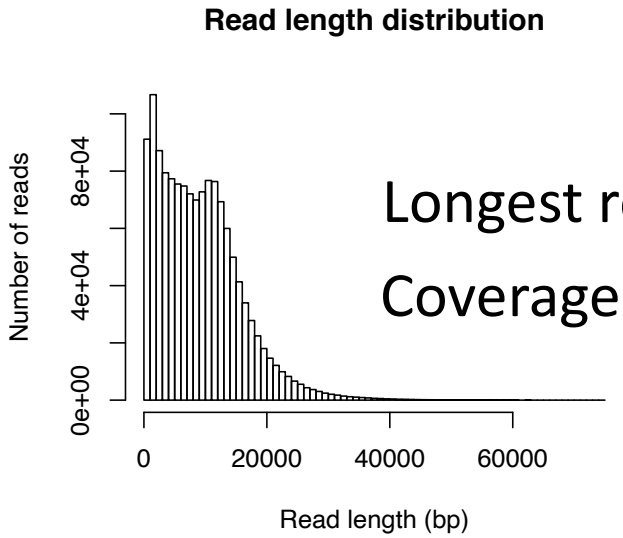
Outline

- Genome assembly: concept
- Assembly algorithms: OLC/De Bruijn graph
- Error correction (Kmer counts)
- Assembly strategy to cope with the repeat problem (mate-pair reads, long reads, others)
- Assembly evaluation (N50, comparison to a reference genome)
- Genome annotation
- Case study

Case 1: genome assembly of a wheat blast fungal strain

- 10 SMRTCell PacBio data (P6-C4)

PacBio RS II



Longest reads: 74,474 bp

Coverage*: 276x

- PCR-free Illumina TruSeq data

HiSeq 2500

>2x10^7 pairs of 2x250 paired-end reads

Coverage*: 222x

* assuming the genome size is 45 Mb

Summary of the assembly

Statistics of the assembly*

Item	Statistics
total contig number	**31**
total contig length	44,522,920
longest contig	**7,902,655**
shortest contig	12,906
N50	**5,402,116**
overall GC	0.50
min contig GC	0.28

* *Canu* for PacBio assembly
Quiver polishing
Further error correction using Illumina data

mitochondial sequence

List of contigs (N=31)

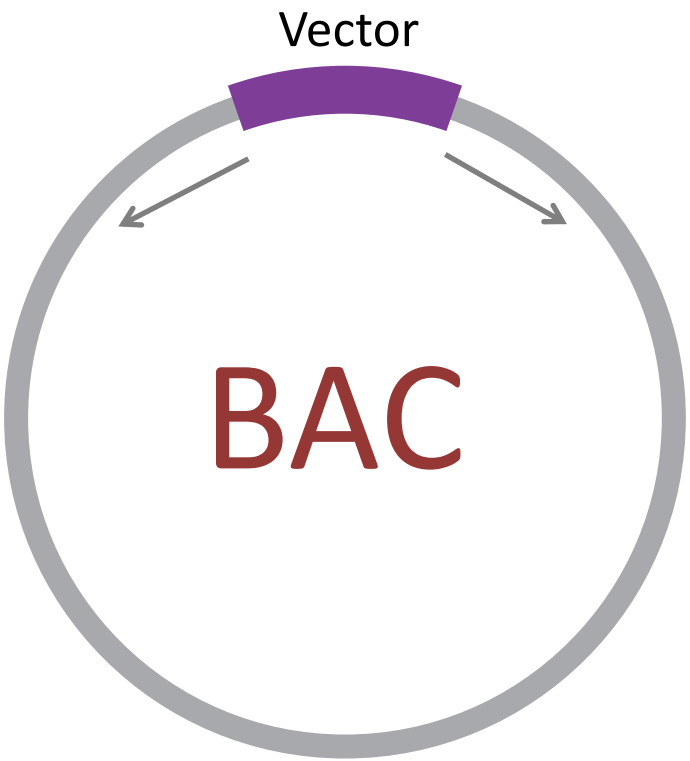
Contig	Length (bp)	GC%
tig00000000	7,902,655	51.2
tig00000024	7,531,155	49.1
tig00000030	6,090,985	50.2
tig00000003	5,402,116	51.2
tig00000011	4,657,194	49.2
tig00000004	4,442,877	51.2
tig00000005	4,042,640	49.2
tig00000001	1,778,515	46.4
tig00000014	461,599	49.4
tig00000025	398,015	44.3
tig00000039	253,502	45.1
tig00000026	240,676	43.8
tig00000018	237,459	47.4
tig00000016	184,678	48
tig00000007	138,010	49.9
tig00000022	110,918	46
tig00000020	78,737	49.6
tig00000006	69,533	51.1
tig00000033	69,131	49.2
tig00000043	57,199	28.4
tig00000015	51,862	48.5
tig00000008	47,134	47.6
tig00000031	42,261	48.6
tig00000041	36,641	52.5
tig00000023	36,455	44.2
tig00000037	35,037	48.9
tig00000032	32,608	49.7
tig00000017	28,099	51.7
tig00000038	27,162	49.5
tig00000019	25,161	51.2
tig00000027	12,906	47.3

BAC-end sequencing for assembly improvement?

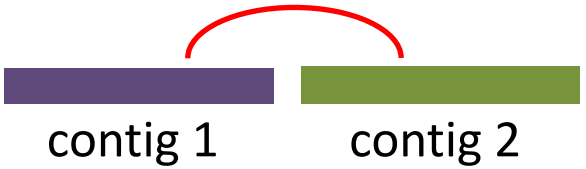
Vector

+ insert

long genomic DNA fragment (e.g., 200 kb)



scaffolding



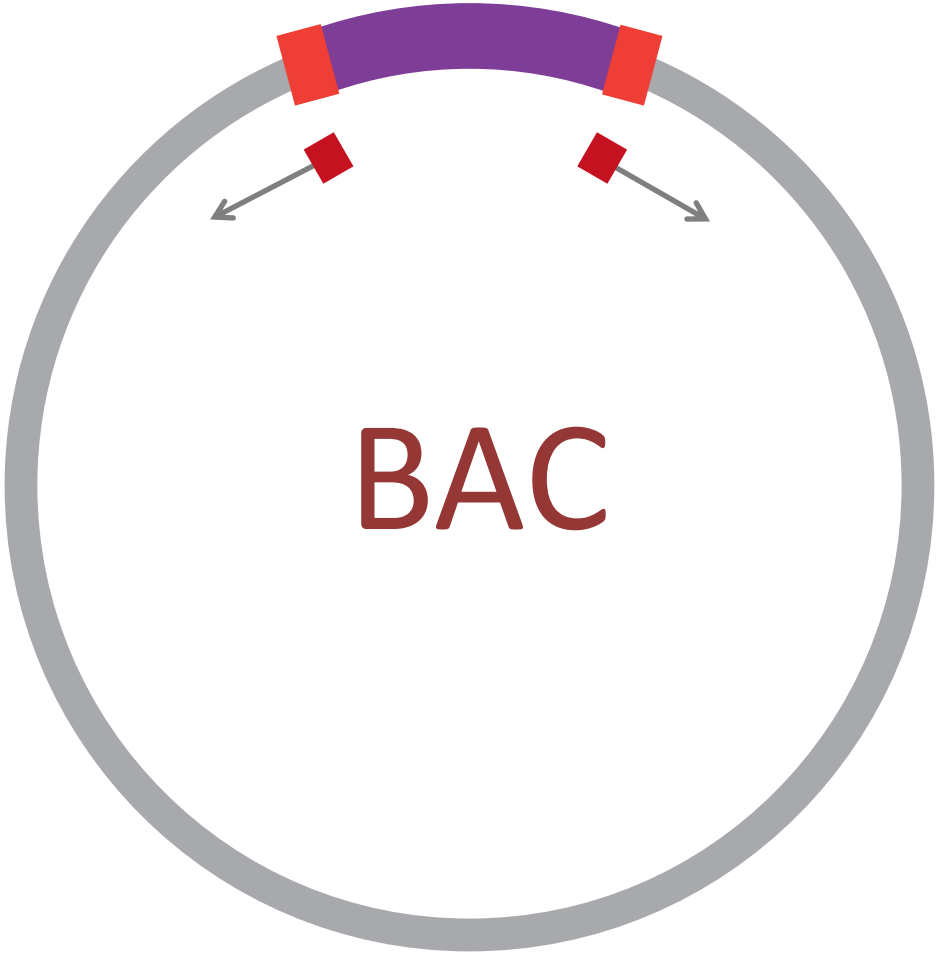
Sequence BAC ends of clones ***one by one***, which is very low-throughput and cost-inefficient.

Idea: build random barcodes in vectors to enable a high throughput (I)

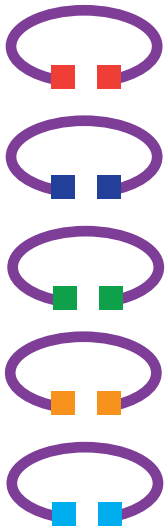
code (Red)

Vector with barcode (Red)

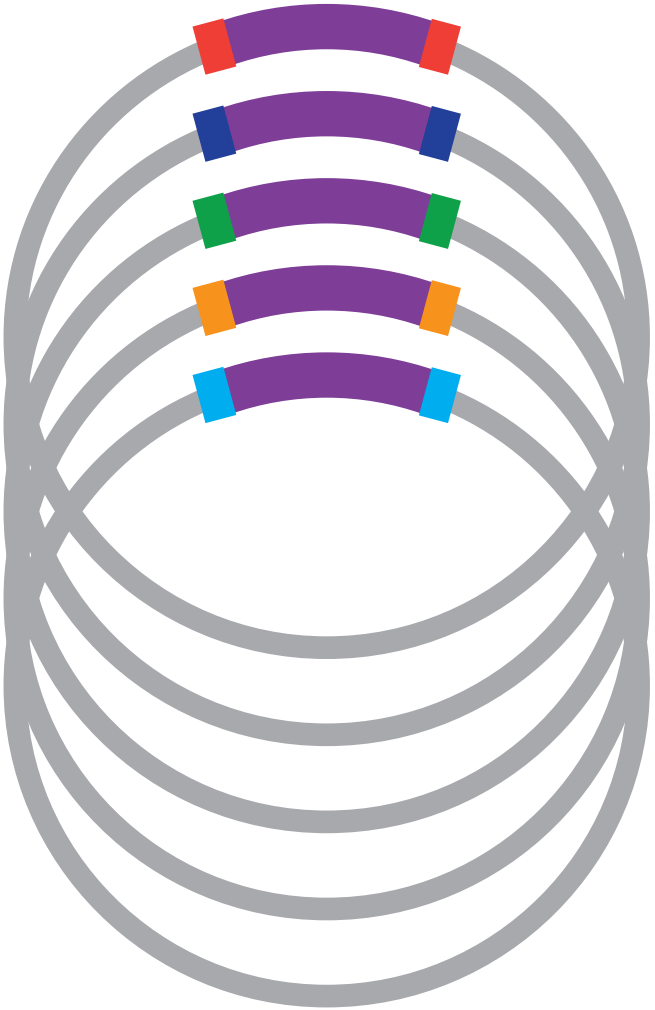
Sequences with barcode



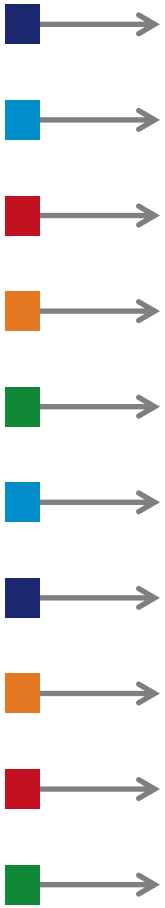
Idea: build random barcodes in vectors to enable a high throughput (II)



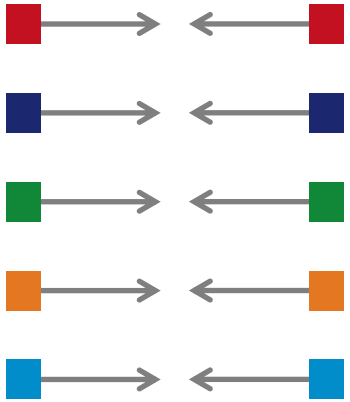
millions of barcoded vectors in a pool



tens of thousands of clones



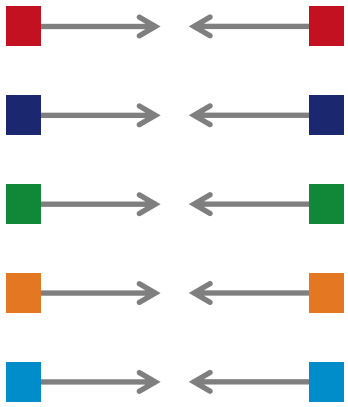
High-throughput end sequencing



Paired ends based on barcodes

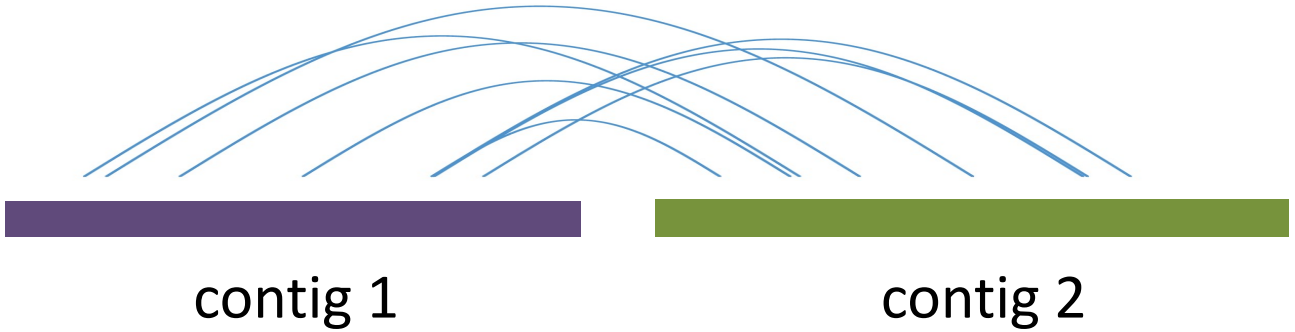
LIEP Scaffolding to improve the B71 assembly

Long Distance End Pairs (LIEP)



~95% are long-distance read pairs

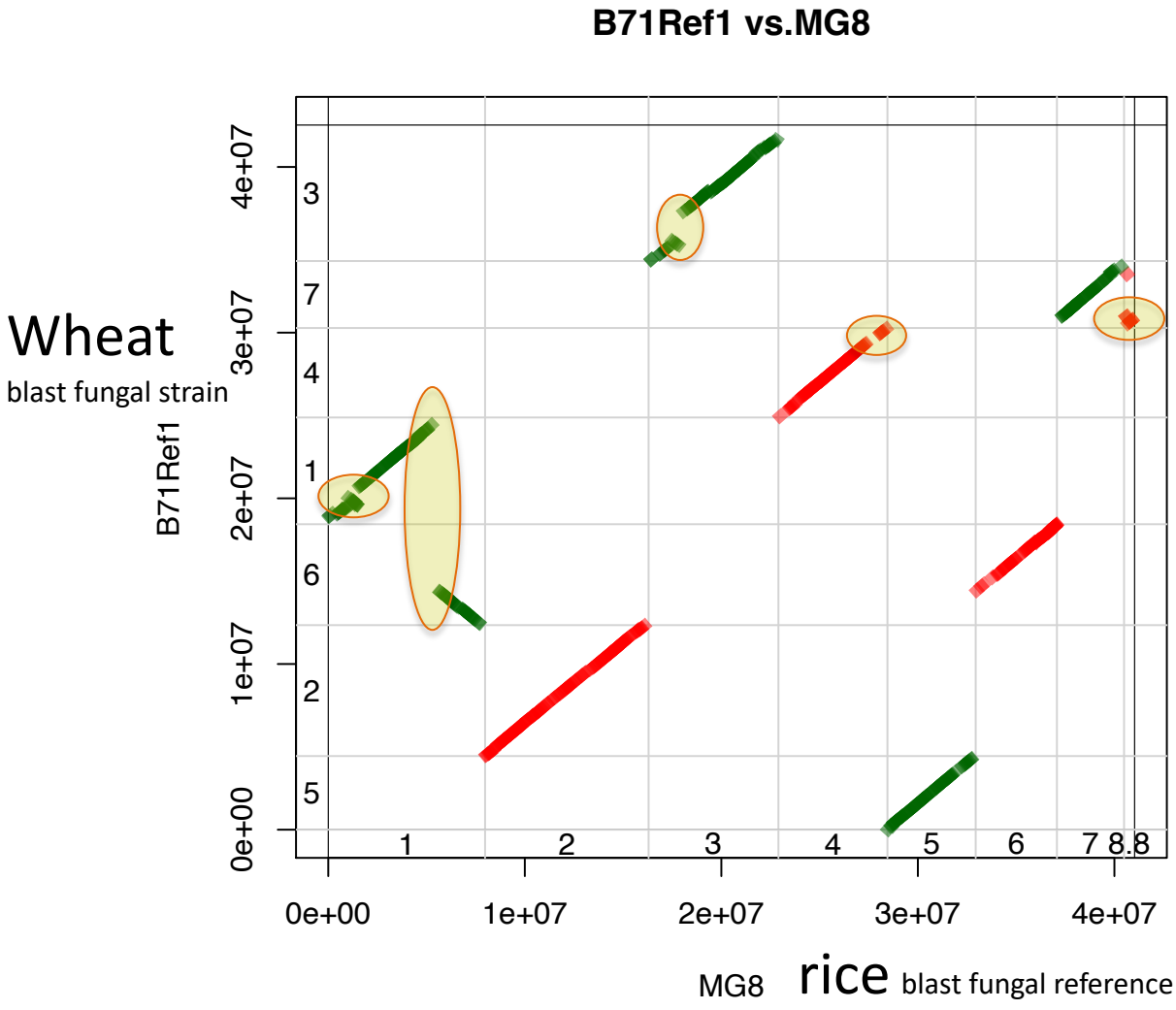
LIEP scaffolding



scaffolding two contigs to a sequence

MoT B71 final assembly vs. MG8 (70-15, *rice* strain)

Chr	Length (bp)
chr1	6,442,091
chr2	7,902,655
chr3	8,206,304
chr4	5,402,116
chr5	4,442,877
chr6	6,090,985
chr7	4,042,640
scaf1	941,816
scaf2	739,928
scaf3	104,670
scaf4	74,396
scaf5	69,131

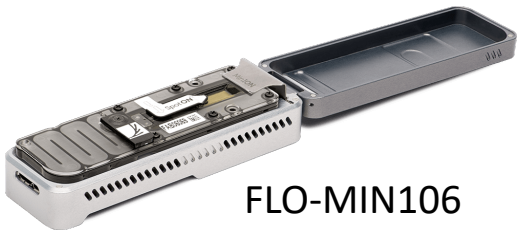


>10 kb overlap and >95% identity

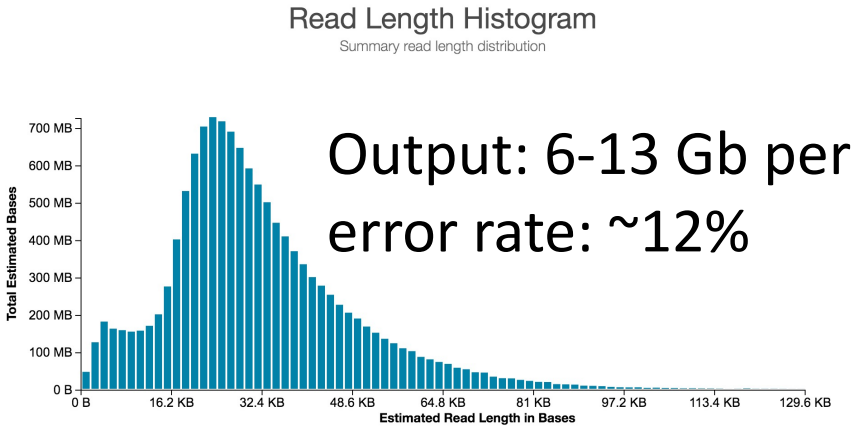
Case 2: Maize genome assembly with Nanopore MinION sequencing

Nanopore

MinION (N=31)

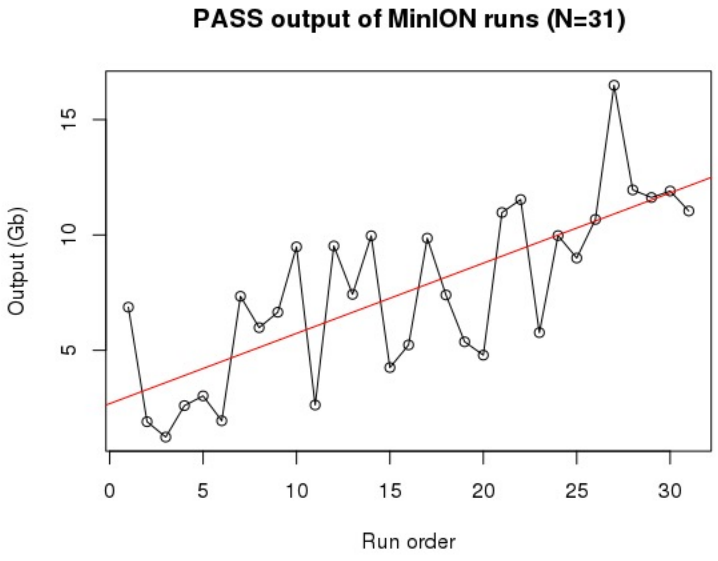


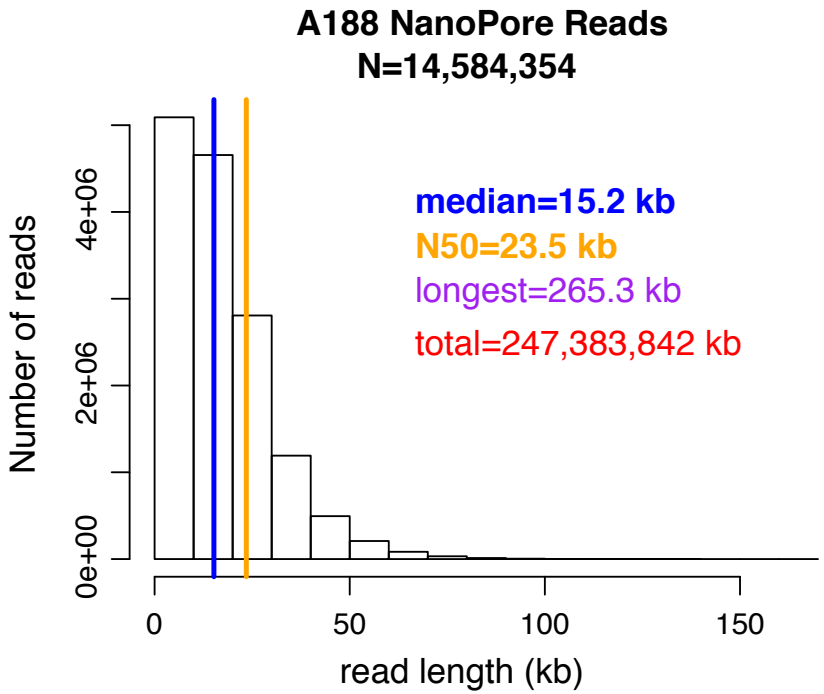
FLO-MIN106
SQK-LSK109



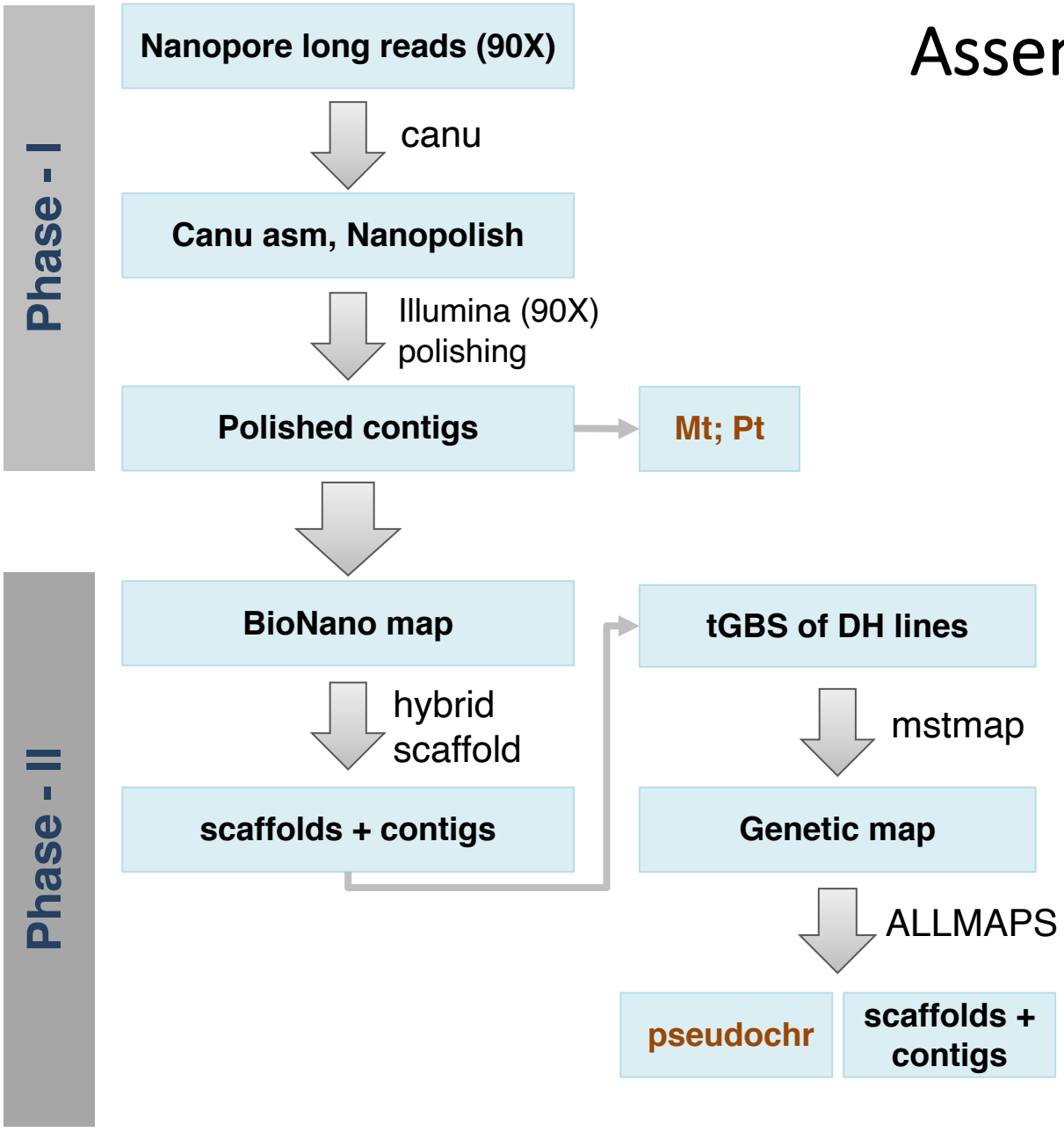
Output: 6-13 Gb per flowcell

error rate: ~12%



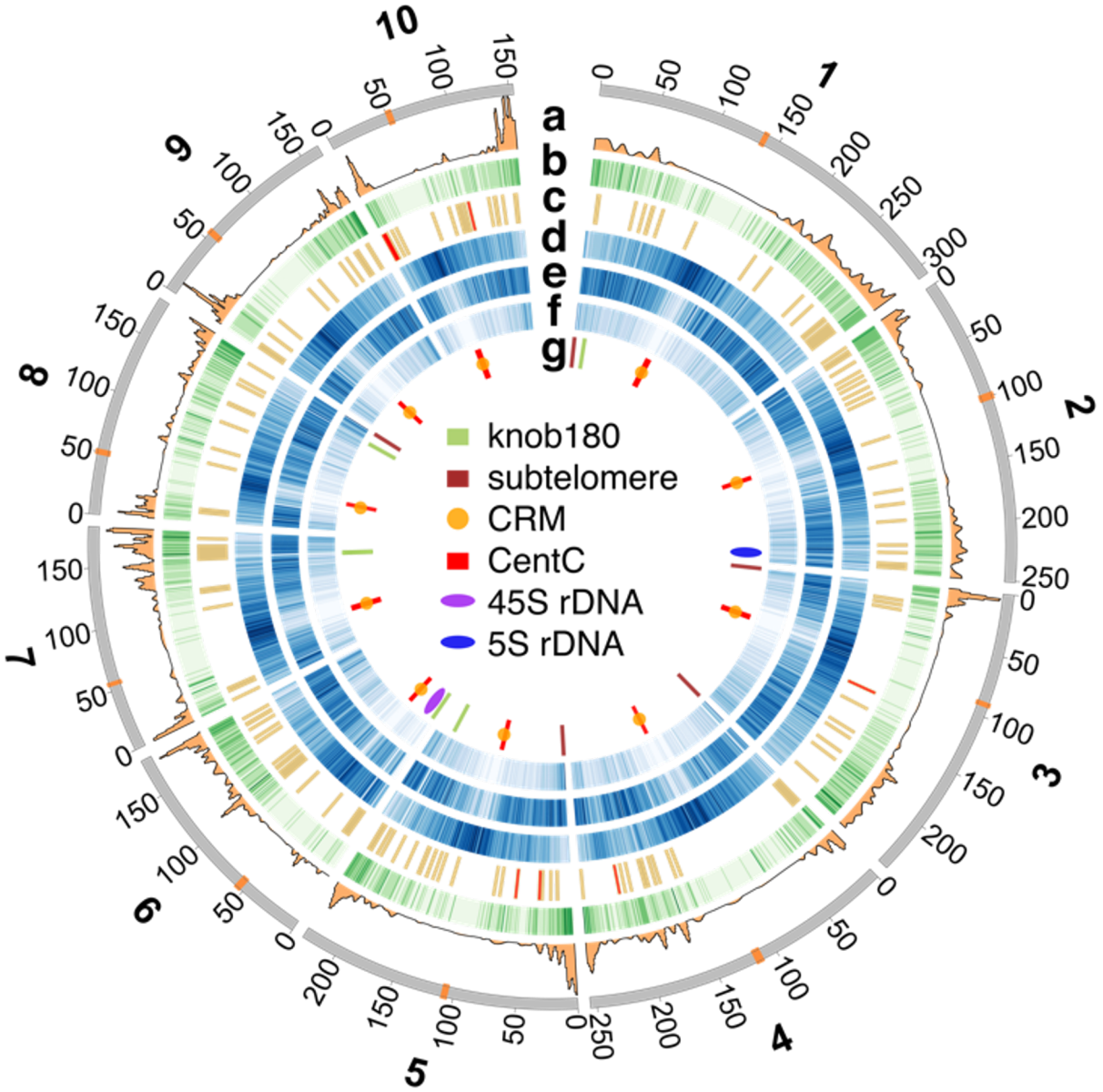


Assembly strategy



Chr	Length (bp)	# genes
1	307,989,483	6,034
2	251,027,758	4,873
3	243,219,806	4,313
4	255,421,021	4,315
5	229,324,730	4,613
6	181,596,323	3,412
7	183,343,242	3,208
8	182,018,909	3,653
9	165,494,689	3,082
10	153,829,095	2,824
mt	525,405	40
pt	140,437	39
scaffolds (986)	16,204-2,700,288	341
sum	**2,246,851,412**	**40,747**

Circos plot to display genomic contents



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