# Fragment Assembly
## (in whole-genome shotgun sequencing)

# Fragment Assembly

# Steps to Assemble a Genome

**Some Terminology**

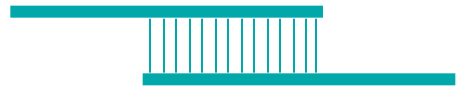
***read*** a 500-900 long word that comes out of sequencer

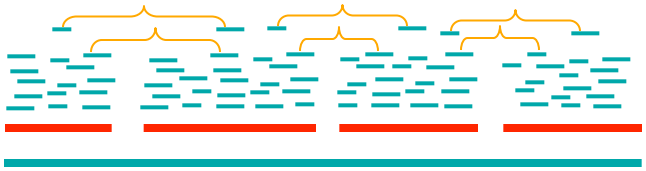
***mate pair*** a pair of reads from two ends of the same insert fragment

***contig*** a contiguous sequence formed by several overlapping reads with no gaps

***supercontig*** (scaffold) an ordered and oriented set of contigs, usually by mate pairs

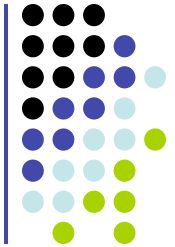
***consensus sequene*** sequence derived from the multiple alignment of reads in a contig





..ACGATTACAATAGGTT..

# 1. Find Overlapping Reads

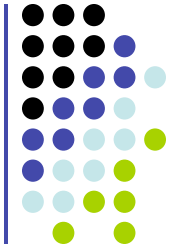


```
aaactgcagtacggatct
aaactgcag
 aactgcagt
...
         gtacggatct
          tacggatct
gggcccaaactgcagtac
gggcccaaa
 ggcccaaac
...
         actgcagta
          ctgcagtac
gtacggatctactacaca
gtacggatc
 tacggatct
...
         ctactacac
          tactacaca
```

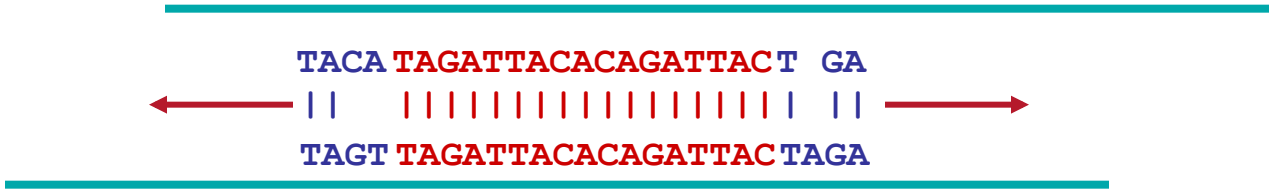
```
(read, pos., word, orient.)
aaactgcag
aactgcagt
actgcagta
...
gtacggatc
tacggatct
gggcccaaa
ggcccaaac
gcccaaact
...
actgcagta
ctgcagtac
gtacggatc
tacggatct
acggatcta
...
ctactacac
tactacaca
```

```
(word, read, orient., pos.)
aaactgcag
aactgcagt
acggatcta
actgcagta
actgcagta
cccaaactg
cggatctac
ctactacac
ctgcagtac
ctgcagtac
gcccaaact
ggcccaaac
gggcccaaa
gtacggatc
gtacggatc
tacggatct
tacggatct
tactacaca
```

# 1. Find Overlapping Reads

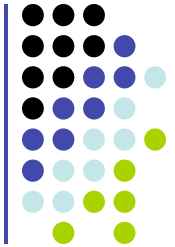


- Find pairs of reads sharing a k-mer, k ~ 24
- Extend to full alignment – throw away if not >98% similar



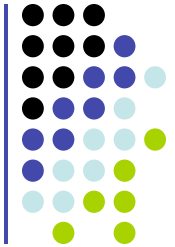

- Caveat: repeats
  - A k-mer that occurs N times, causes $O(N^2)$ read/read comparisons
  - ALU k-mers could cause up to $1{,}000{,}000^2$ comparisons
- Solution:
  - Discard all k-mers that occur “too often”
    - Set cutoff to balance sensitivity/speed tradeoff, according to genome at hand and computing resources available

# 1. Find Overlapping Reads



Create local multiple alignments from the overlapping reads

# 1. Find Overlapping Reads



- Correct errors using multiple alignment

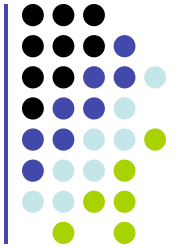

insert A

replace T with C

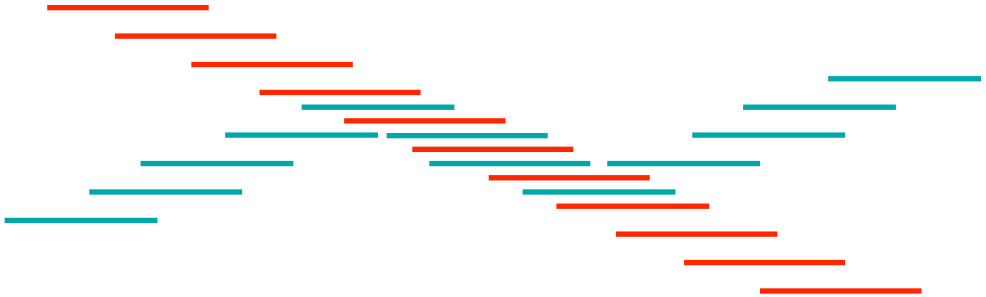

correlated errors—
probably caused by repeats
⇒ disentangle overlaps


In practice, error correction removes up to 98% of the errors

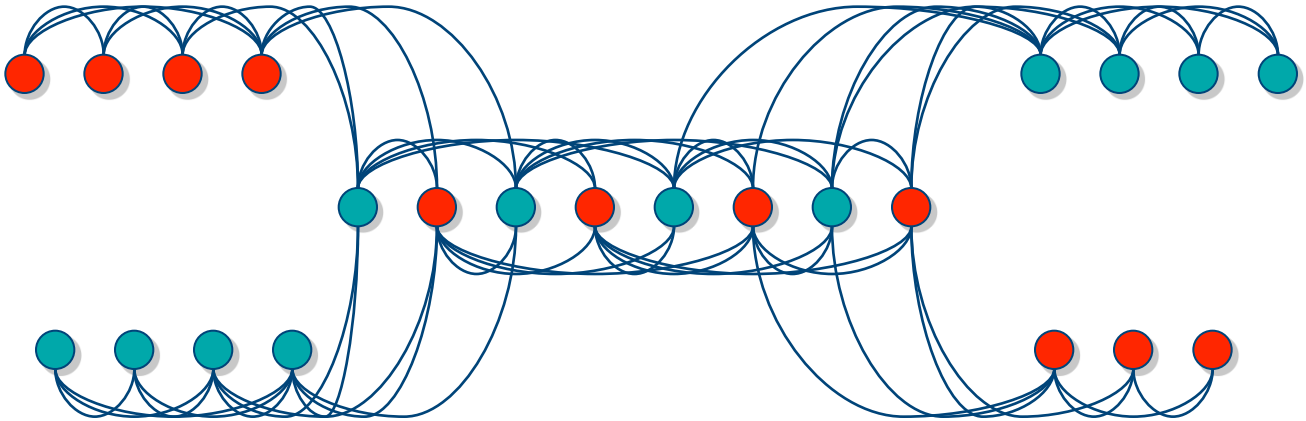
# 2. Merge Reads into Contigs



- Overlap graph:
  - Nodes: reads $r_1$…..$r_n$
  - Edges: overlaps ($r_i$, $r_j$, shift, orientation, score)

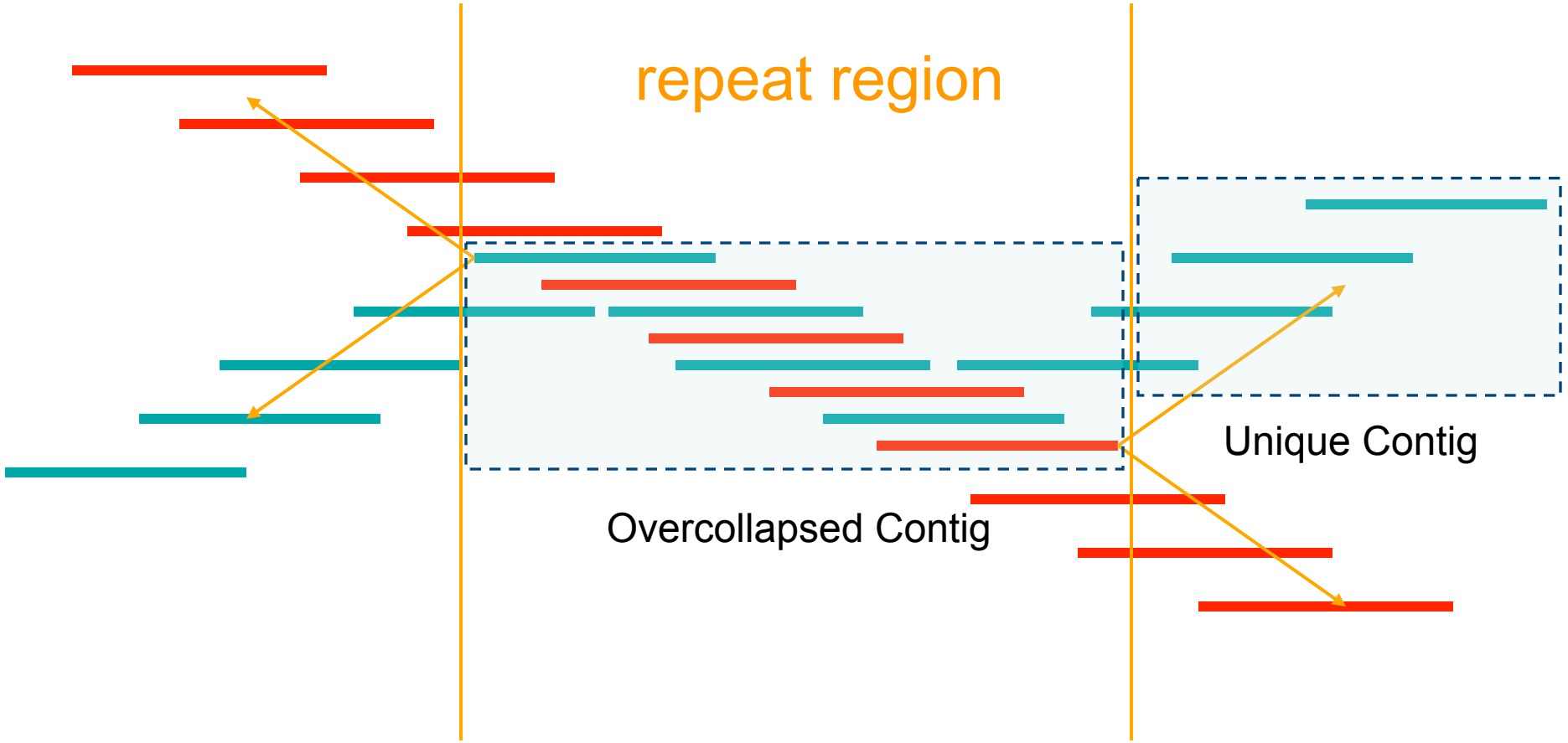


Reads that come from two regions of the genome (blue and red) that contain the same repeat



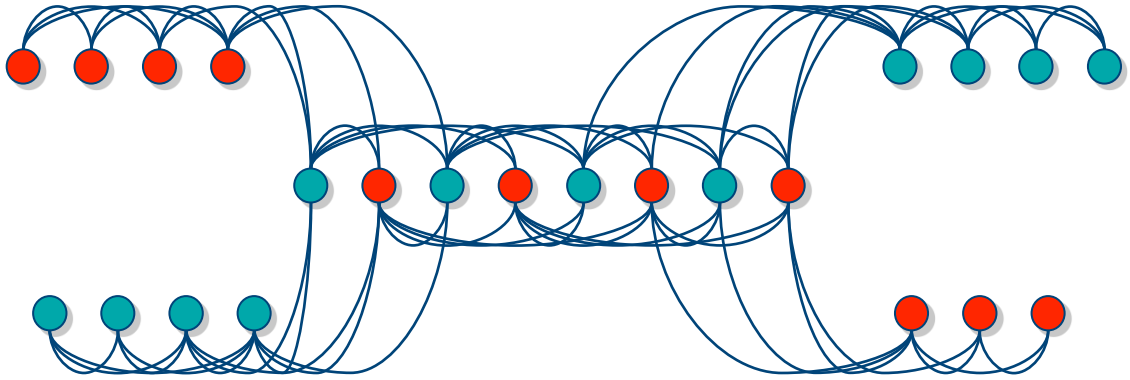
Note:
of course, we don't know the "color" of these nodes

# 2. Merge Reads into Contigs

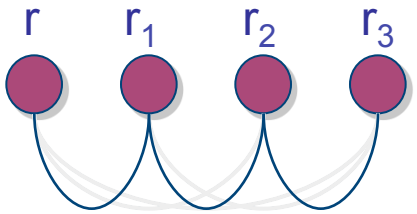


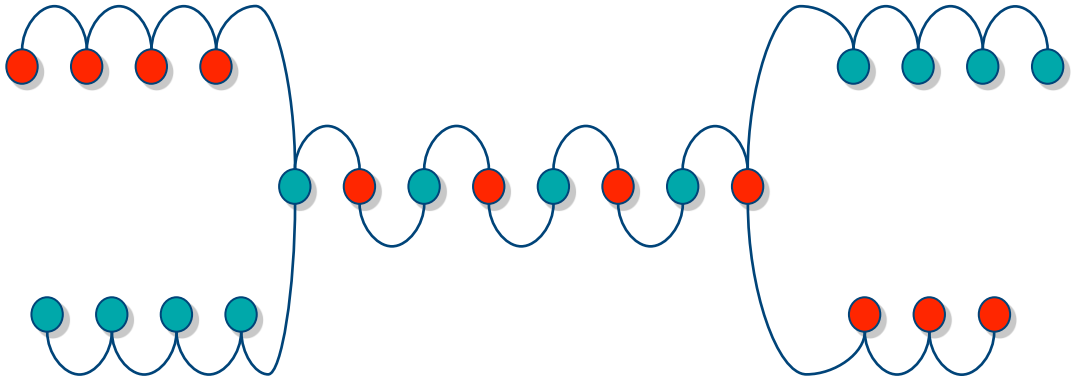

We want to merge reads up to potential repeat boundaries

# 2. Merge Reads into Contigs

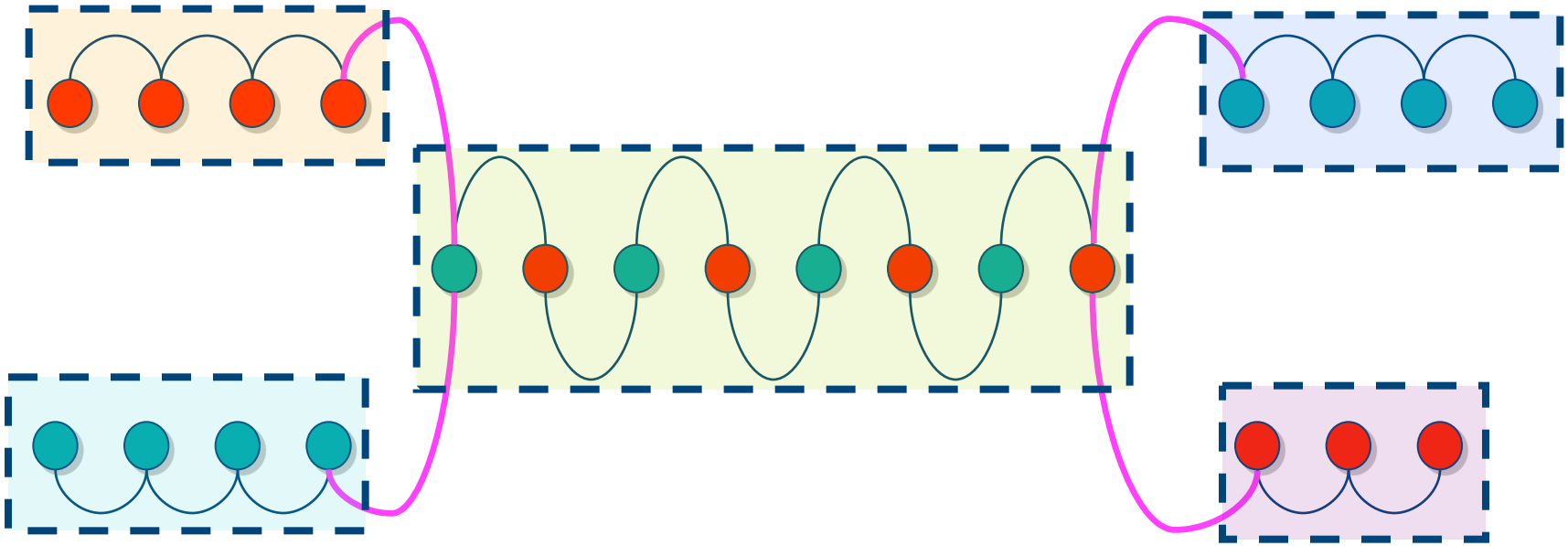


- Remove transitively inferable overlaps
  - If read r overlaps to the right reads $r_1$, $r_2$, and $r_1$ overlaps $r_2$, then (r, $r_2$) can be inferred by (r, $r_1$) and ($r_1$, $r_2$)

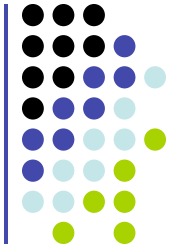


# 2. Merge Reads into Contigs



# Repeats, errors, and contig lengths



- Repeats shorter than read length are easily resolved
  - Read that spans across a repeat disambiguates order of flanking regions
- Repeats with more base pair diffs than sequencing error rate are OK
  - We throw overlaps between two reads in different copies of the repeat
- To make the genome **appear** less repetitive, try to:
  - Increase read length
  - Decrease sequencing error rate

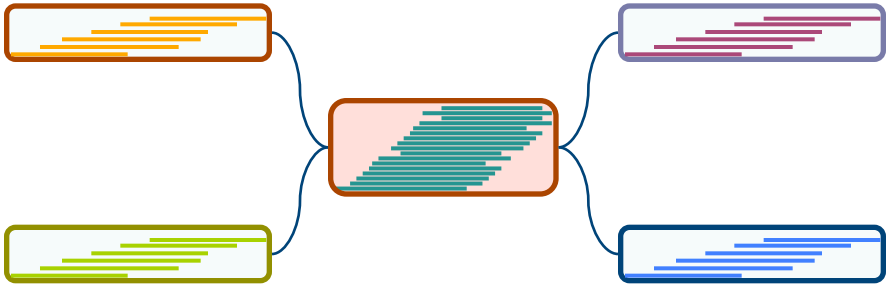
**Role of error correction:**

Discards up to 98% of single-letter sequencing errors
decreases error rate
⇒ decreases effective repeat content
⇒ increases contig length

# 3. Link Contigs into Supercontigs

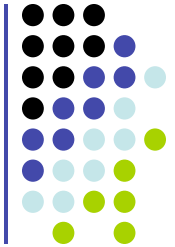
Normal density

Too dense
⇒ Overcollapsed



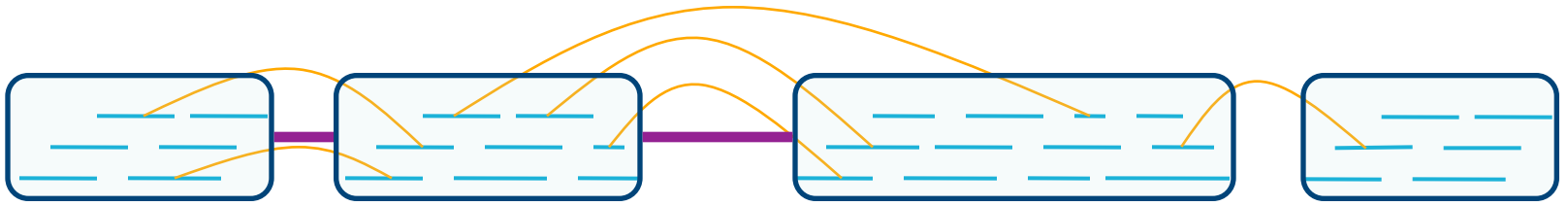
Inconsistent links
⇒ Overcollapsed?

# 3. Link Contigs into Supercontigs



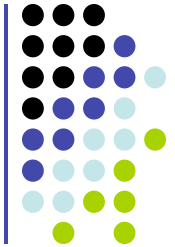
Find all links between unique contigs

Connect contigs incrementally, if ≥ 2 forward-reverse links



*supercontig*
(aka *scaffold*)

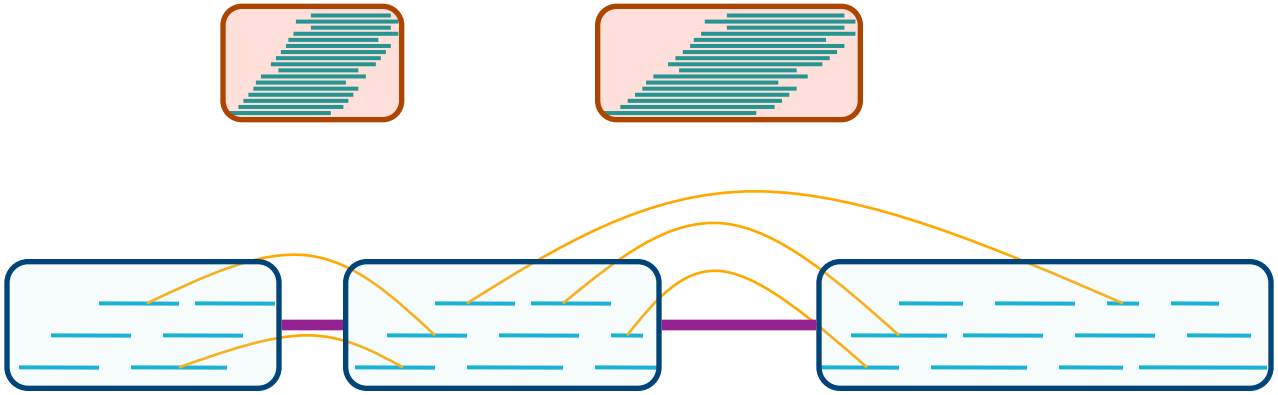
# 3. Link Contigs into Supercontigs



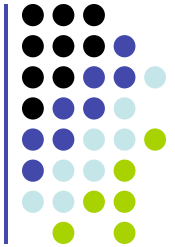
Fill gaps in supercontigs with paths of repeat contigs

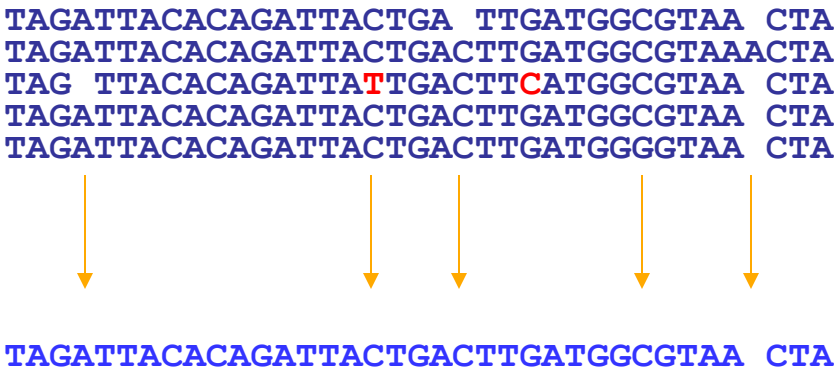
Complex algorithmic step

- Exponential number of paths
- Forward-reverse links



# 4. Derive Consensus Sequence






Derive multiple alignment from pairwise read alignments

Derive each consensus base by weighted voting

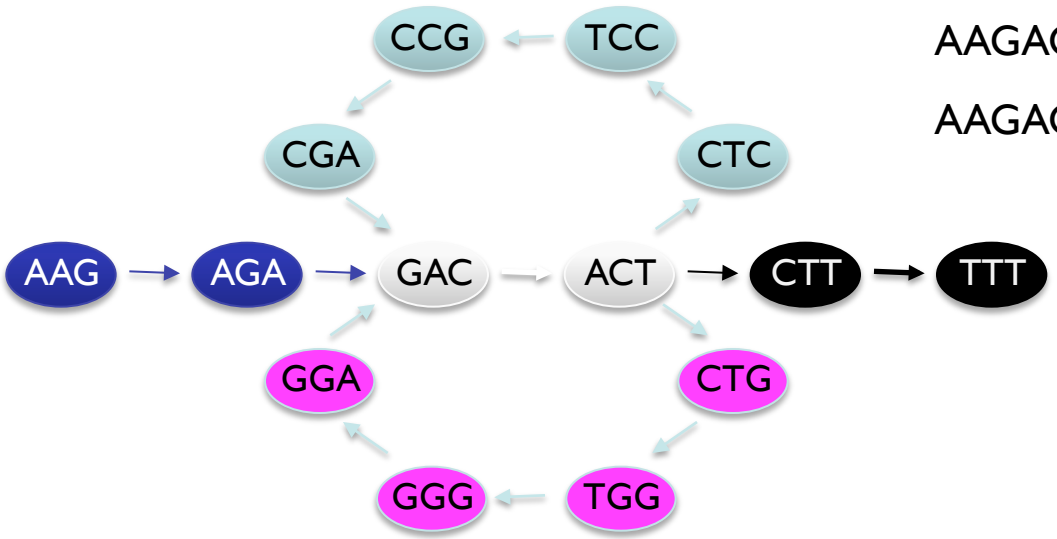
(**Alternative:** take maximum-quality letter)

# De Brujin Graph formulation

- Given sequence $x_1 \ldots x_N$, k-mer length k,
  Graph of $4^k$ vertices,
  Edges between words with (k-1)-long overlap

Reads

AAGA
ACTT
ACTC
ACTG
AGAG
CCGA
CGAC
CTCC
CTGG
CTTT
…



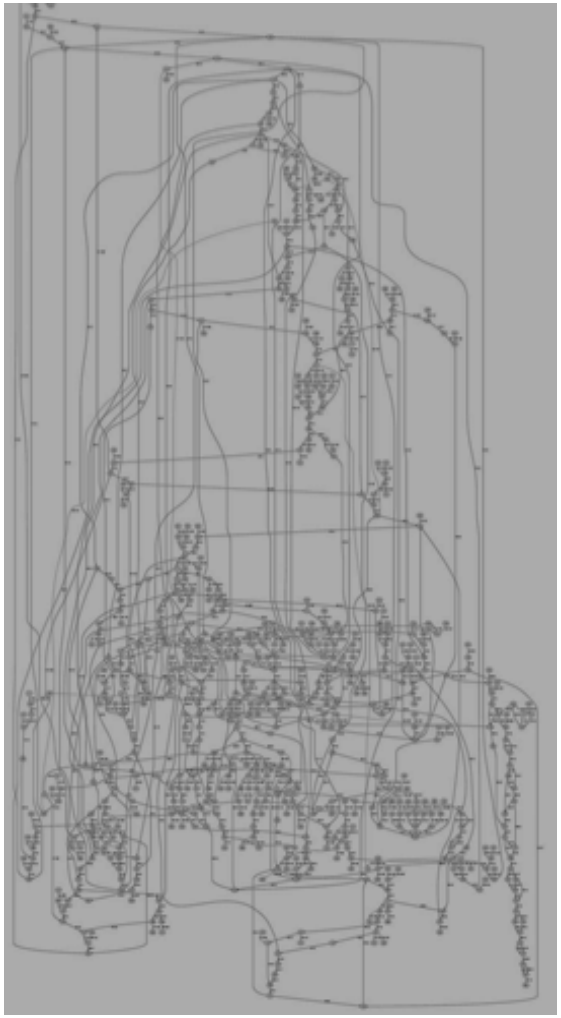

Potential Genomes

AAGACTCCGACTGGGACTTT

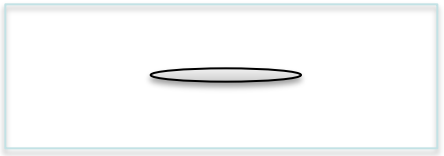
AAGACTGGGACTCCGACTTT

Slide by Michael Schatz

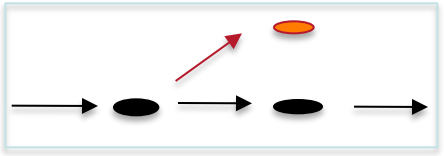
# Node Types



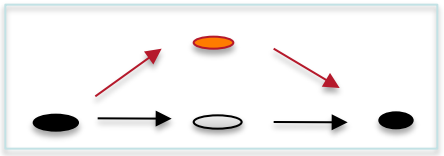
(Chaisson, 2009)



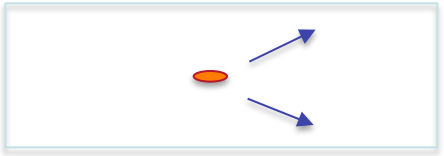
Isolated nodes (10%)



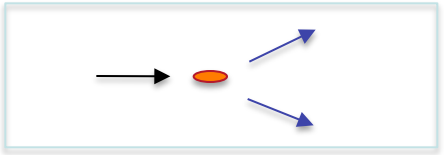
Tips (46%)



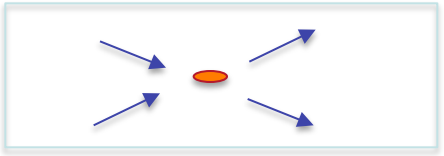
Bubbles/Non-branch (9%)



Dead Ends (.2%)



Half Branch (25%)

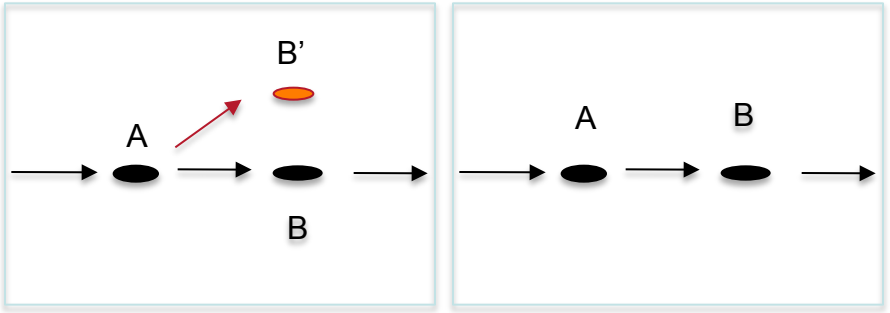


Full Branch (10%)

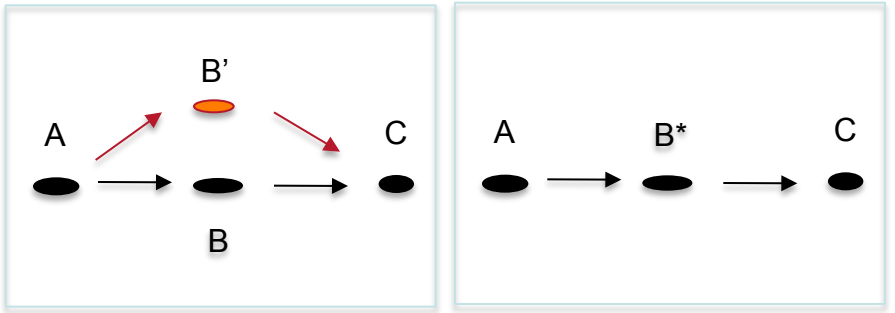
Slide by Michael Schatz

# Error Correction

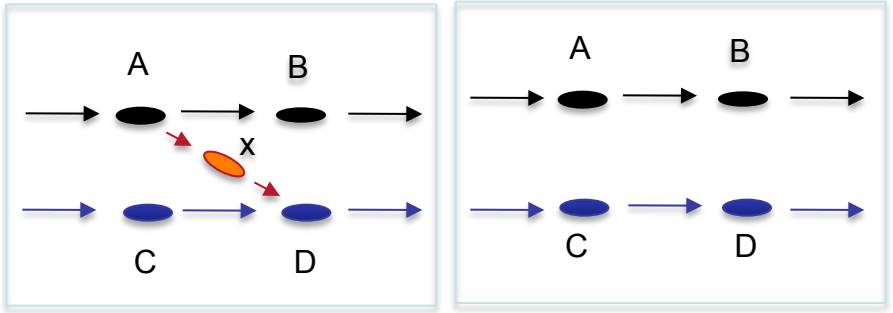
- Errors at end of read
  - Trim off ‘dead-end’ tips




- Errors in middle of read
  - Pop Bubbles

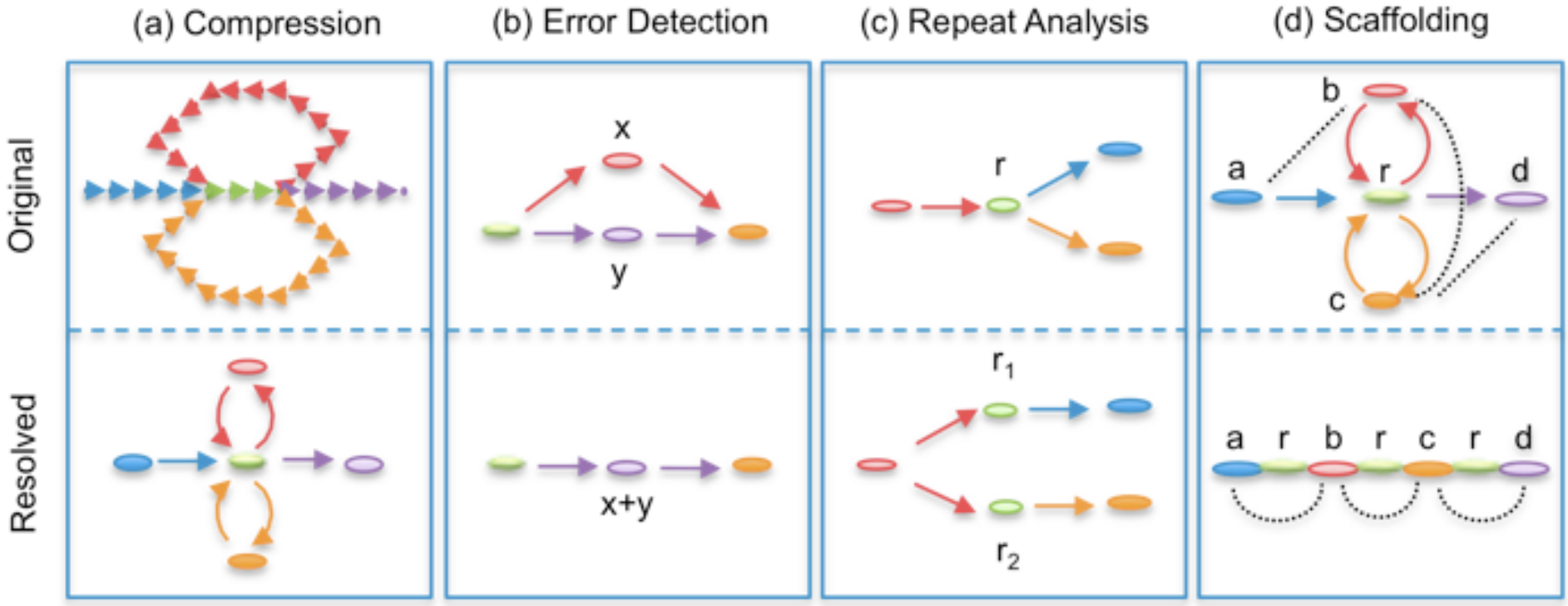



- Chimeric Edges
  - Clip short, low coverage nodes




Slide by Michael Schatz

# De Brujin Graph formulation



# Quality of assemblies—mouse

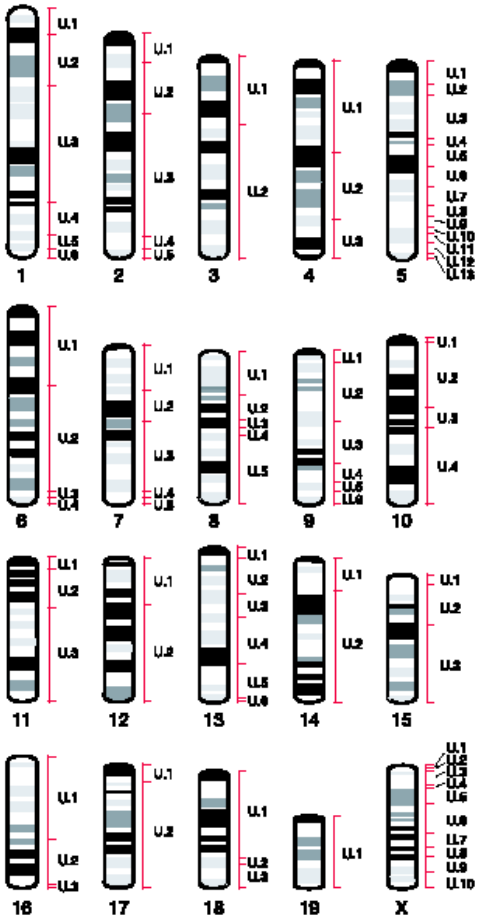



**Figure 1** The mouse genome in 88 sequence-based ultracontigs. The position and extent of the 88 ultracontigs of the MGSCv3 assembly are shown adjacent to ideograms of the mouse chromosomes. All mouse chromosomes are acrocentric, with the centromeric end at the top of each chromosome. The supercontigs of the sequence assembly were anchored to the mouse chromosomes using the MIT genetic map. Neighbouring supercontigs were linked together into ultracontigs using information from single BAC links and the fingerprint and radiation-hybrid maps, resulting in 88 ultracontigs containing 95% of the bases in the euchromatic genome.

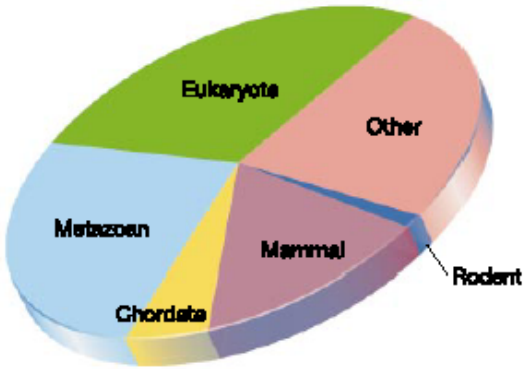
| N50 length (kb)* | Bases (Gb) | Bases plus gaps (Gb) | Percentage of genome |
|---|---|---|---|
| 25.9 | 2.372 | 2.372 | 94.9 |
| 18,600 | 2.372 | 2.477 | 99.1 |
| 50,600 | 2.372 | 2.493 | 99.7 |
| 2.3 | 0.106 | 0.106 | – |
| 18,700 | 2.352 | 2.455 | 98.2 |
| 22,900 | 1.955 | 2.039 | 81.6 |

des spanned gaps.
ercontigs with an N50 value of 3.4 kb. The N50 value for all contigs is 24.8 kb, and for all supercontigs is 16,900 kb (excluding gaps to gaps in the ultracontigs and are thus accounted for in the 'bases plus gaps' estimate.

**Terminology: *N50 contig length***

If we sort contigs from largest to smallest, and start Covering the genome in that order, N50 is the length Of the contig that just covers the 50th percentile.

**7.7X sequence coverage**



# Panda Genome

**Table 1 | Summary of the panda genome sequencing and assembly**

| Step | Paired-end insert size (bp)* | Sequence coverage (×)† | Physical coverage (×)† | N50 (bp) ‡ | N90 (bp) ‡ | Total length (bp) |
|---|---|---|---|---|---|---|
| Initial contig | 110–230; 380–570 | 38.5 | 96 | 1,483 | 224 | 2,021,639,596 |
| Scaffold 1 | | | | 32,648 | 7,780 | 2,213,848,409 |
| Scaffold 2 | Add 1,700–2,800 | 8.4 | 151 | 229,150 | 45,240 | 2,250,442,210 |
| Scaffold 3 | Add 3,700–7,500 | 6.5 | 450 | 581,933 | 127,336 | 2,297,100,301 |
| Scaffold 4 | Add 9,200–12,300 | 2.6 | 373 | 1,281,781 | 312,670 | 2,299,498,912 |
| Final contig | All | 56.0 | 1,070 | 39,886 | 9,848 | 2,245,302,481 |

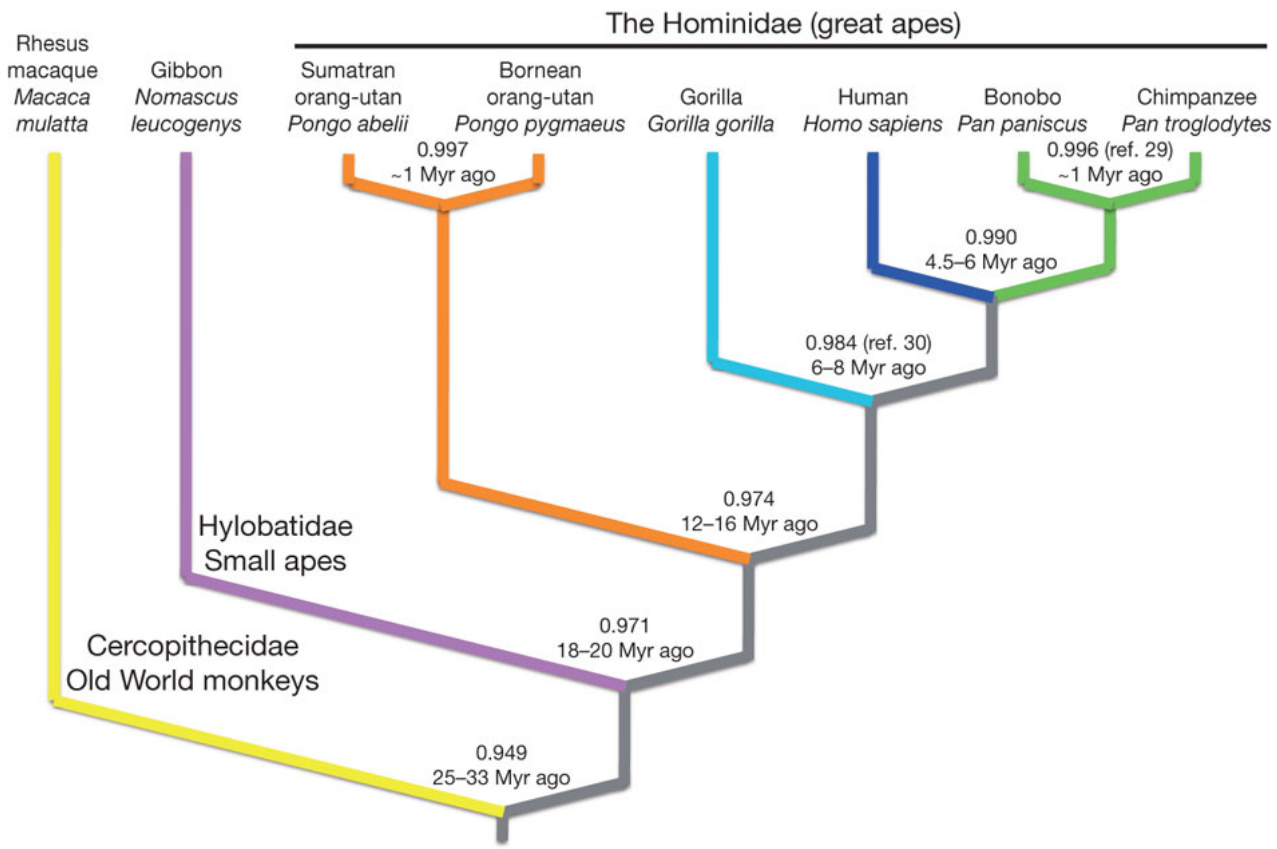
Add denotes accumulative; for example, scaffold 2 uses data of 110–230, 380–570 and 1,700–2,800.

* Approximate average insert size of Illumina Genome Analyser sequencing libraries. The sizes were estimated by mapping the reads onto the assembled genome sequences.

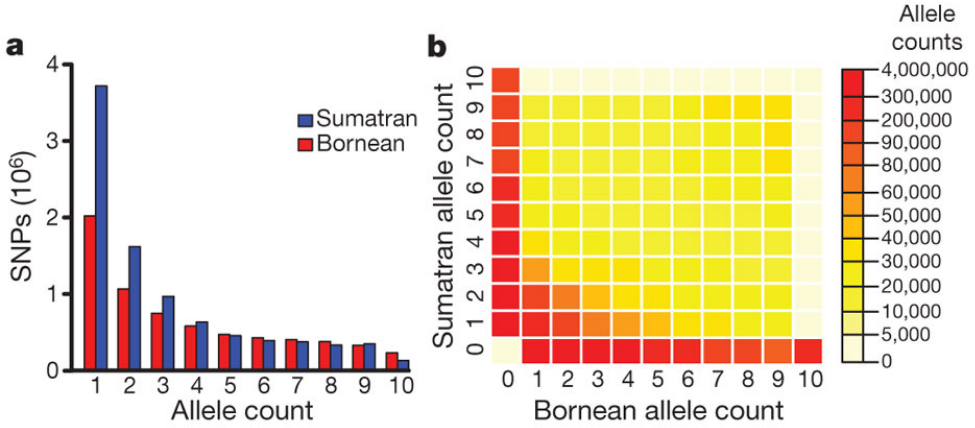
† High-quality read sequences that were used in assembly. Coverage was estimated assuming a genome size of 2.4 Gb. Sequence coverage refers to the total length of generated reads, and physical coverage refers to the total length of sequenced clones of the libraries.

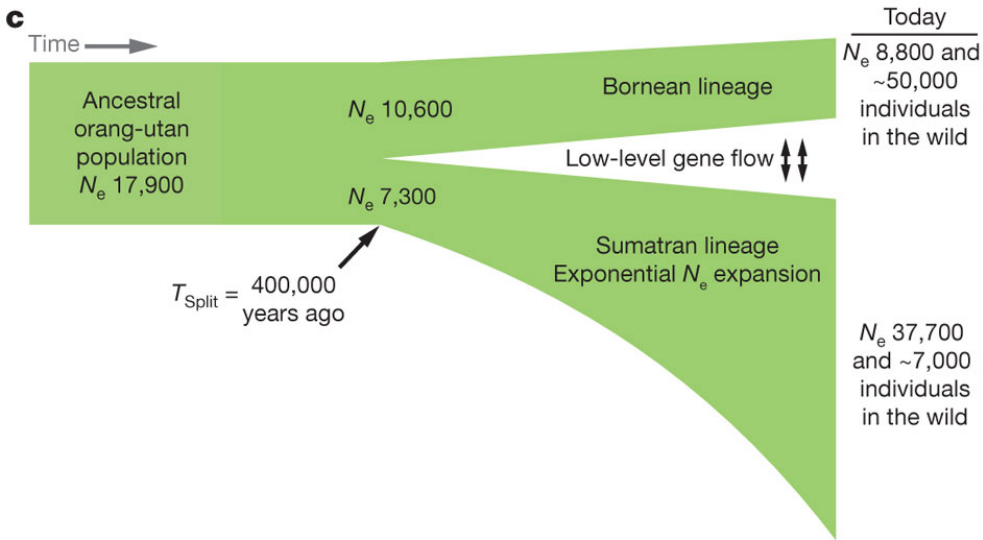
‡ N50 size of contigs or scaffolds was calculated by ordering all sequences then adding the lengths from longest to shortest until the summed length exceeded 50% of the total length of all sequences. N90 is similarly defined.

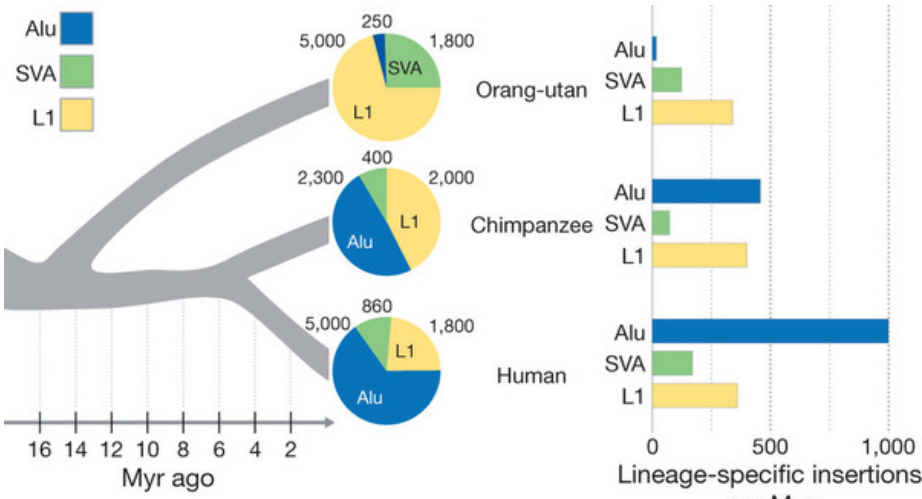
# Hominid lineage



# Orangutan genome



# Assemblathon

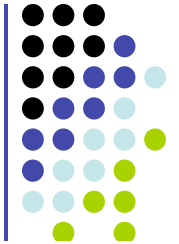


**Table 2 Overview of sequencing data provided for Assemblathon 2 participants**

| Species | Estimated genome size | Illumina | Roche 454 | Pacific biosciences |
|---|---|---|---|---|
| Bird (*Melopsittacus undulatus*) | 1.2 Gbp | 285x coverage from 14 libraries (mate pair and paired-end) | 16x coverage from 3 library types (single end and paired-end) | 10x coverage from 2 libraries |
| Fish (*Maylandia zebra*)* | 1.0 Gbp | 192x coverage from 8 libraries (mate pair and paired-end) | NA | NA |
| Snake (*Boa constrictor constrictor*) | 1.6 Gbp | 125x coverage from 4 libraries (mate pair and paired-end) | NA | NA |

**Table 1 Assemblathon 2 participating team details**

| Team name | Team identifier | Number of assemblies submitted | | | Sequence data used for bird assembly | Institutional affiliations | Principal assembly software used |
|---|---|---|---|---|---|---|---|
| | | Bird | Fish | Snake | | | |
| ABL | ABL | 1 | 0 | 0 | 4 + I | Wayne State University | HyDA |
| ABySS | ABYSS | 0 | 1 | 1 | | Genome Sciences Centre, British Columbia Cancer Agency | ABySS and Anchor |
| Allpaths | ALLP | 1 | 1 | 0 | I | Broad Institute | ALLPATHS-LG |
| BCM-HGSC | BCM | 2 | 1 | 1 | 4 + I + P[1] | Baylor College of Medicine Human Genome Sequencing Center | SeqPrep, KmerFreq, Quake, BWA, Newbler, ALLPATHS-LG, Atlas-Link, Atlas-GapFill, Phrap, CrossMatch, Velvet, BLAST, and BLASR |
| CBCB | CBCB | 1 | 0 | 0 | 4 + I + P | University of Maryland, National Biodefense Analysis and Countermeasures Center | Celera assembler and PacBio Corrected Reads (PBcR) |
| CoBiG[2] | COBIG | 1 | 0 | 0 | 4 | University of Lisbon | 4Pipe4 pipeline, Seqclean, Mira, Bambus2 |
| CRACS | CRACS | 0 | 0 | 1 | | Institute for Systems and Computer Engineering of Porto TEC, European Bioinformatics Institute | ABySS, SSPACE, Bowtie, and FASTX |
| CSHL | CSHL | 0 | 3 | 0 | | Cold Spring Harbor Laboratory, Yale University, University of Notre Dame | Metassembler, ALLPATHS, SOAPdenovo |
| CTD | CTD | 0 | 3 | 0 | | National Research University of Information Technologies, Mechanics, and Optics | Unspecified |
| Curtain | CURT | 0 | 0 | 1 | | European Bioinformatics Institute | SOAPdenovo, fastx_toolkit, bwa, samtools, velvet, and curtain |
| GAM | GAM | 0 | 0 | 1 | | Institute of Applied Genomics, University of Udine, KTH Royal Institute of Technology | GAM, CLC and ABySS |
| IOBUGA | IOB | 0 | 2 | 0 | | University of Georgia, Institute of Aging Research | ALLPATHS-LG and SOAPdenovo |
| MLK Group | MLK | 1 | 0 | 0 | I | UC Berkeley | ABySS |
| Meraculous | MERAC | 1 | 1 | 1 | I | DOE Joint Genome Institute, UC Berkeley | meraculous |
| Newbler-454 | NEWB | 1 | 0 | 0 | 4 | 454 Life Sciences | Newbler |
| Phusion | PHUS | 1 | 0 | 1 | I | Wellcome Trust Sanger Institute | Phusion2, SOAPdenovo, SSPACE |
| PRICE | PRICE | 0 | 0 | 1 | | UC San Francisco | PRICE |
| Ray | RAY | 1 | 1 | 1 | I | CHUQ Research Center, Laval University | Ray |
| SGA | SGA | 1 | 1 | 1 | I | Wellcome Trust Sanger Institute | SGA |
| SOAPdenovo | SOAP | 3 | 1 | 1 | I[2] | BGI-Shenzhen, HKU-BGI | SOAPdenovo |
| Symbiose | SYMB | 0 | 1 | 1 | | ENS Cachan/IRISA, INRIA, CNRS/ Symbiose | Monument, SSPACE, SuperScaffolder, and GapCloser |

# Assemblathon

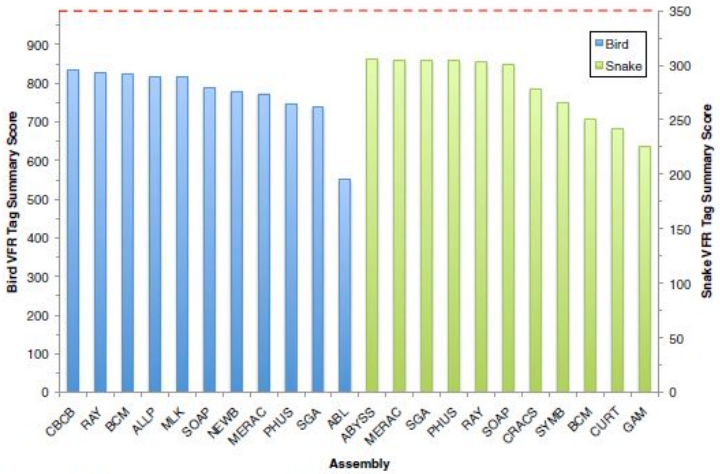



**Figure 12 Short-range scaffold accuracy assessment via Validated Fosmid Regions.** First, validated Fosmid regions (VFRs) were identified (86 in bird and 56 in snake, see text). Then VFRs were divided into non-overlapping 1,000 nt fragments and pairs of 100 nt 'tags' were extracted from ends of each fragment and searched (using BLAST) against all scaffolds from each assembly. A summary score for each assembly was calculated as the product of a) the number of pairs of tags that both matched the same scaffold in an assembly (at any distance apart) and b) the percentage of only the uniquely matching tag pairs that matched at the expected distance (± 2 nt). Theoretical maximum scores, which assume that all tag-pairs would map uniquely to a single scaffold, are indicated by red dashed line (988 for bird and 350 for snake).

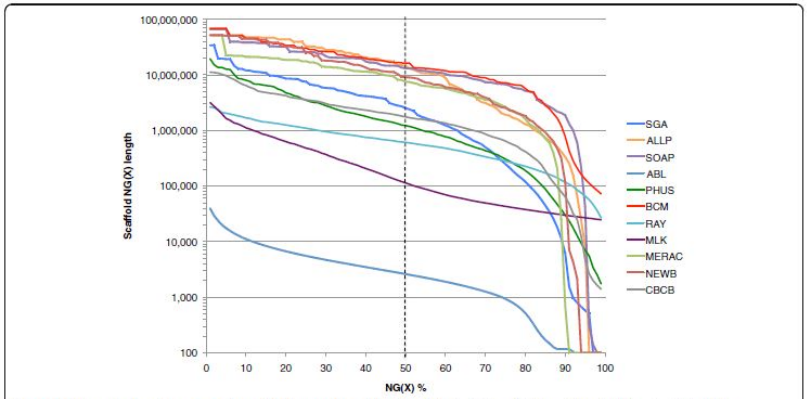



**Figure 1 NG graph showing an overview of bird assembly scaffold lengths.** The NG scaffold length (see text) is calculated at integer thresholds (1% to 100%) and the scaffold length (in bp) for that particular threshold is shown on the y-axis. The dotted vertical line indicates the NG50 scaffold length: if all scaffold lengths are summed from longest to the shortest, this is the length at which the sum length accounts for 50% of the estimated genome size. Y-axis is plotted on a log scale. Bird estimated genome size = ~1.2 Gbp.

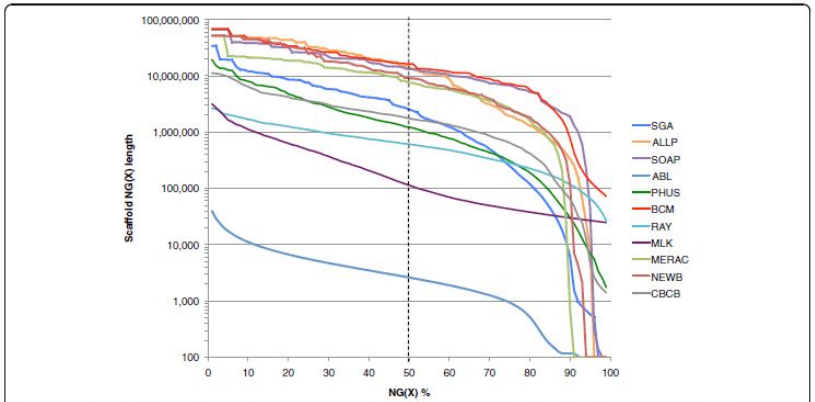



**Figure 1 NG graph showing an overview of bird assembly scaffold lengths.** The NG scaffold length (see text) is calculated at integer thresholds (1% to 100%) and the scaffold length (in bp) for that particular threshold is shown on the y-axis. The dotted vertical line indicates the NG50 scaffold length: if all scaffold lengths are summed from longest to the shortest, this is the length at which the sum length accounts for 50% of the estimated genome size. Y-axis is plotted on a log scale. Bird estimated genome size = ~1.2 Gbp.

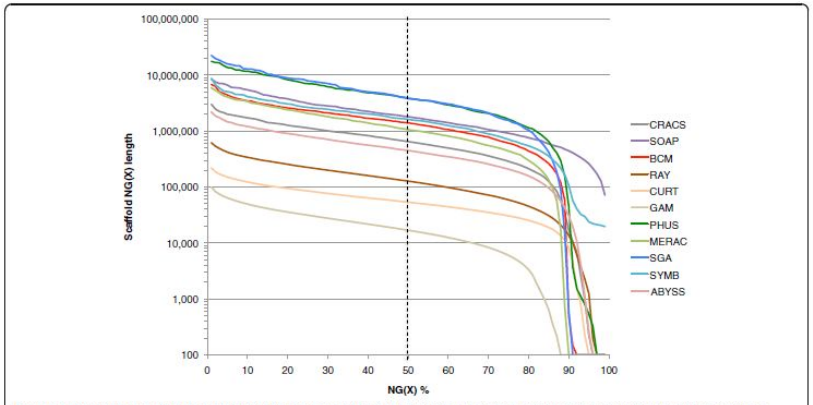
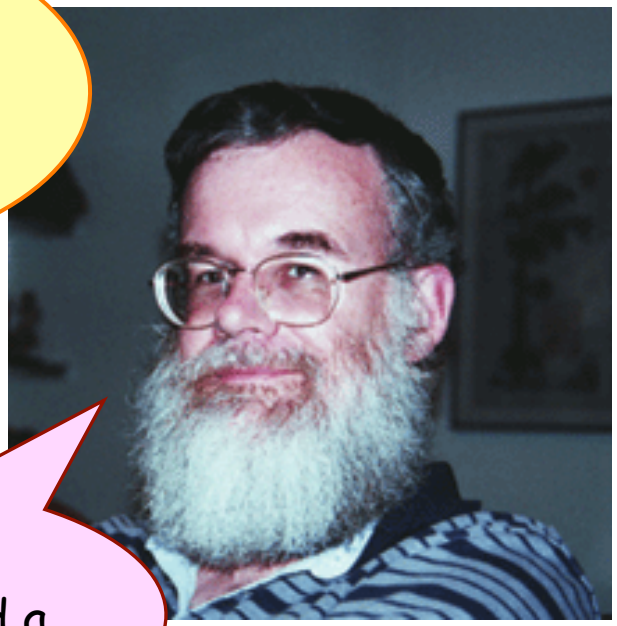



**Figure 3 NG graph showing an overview of snake assembly scaffold lengths.** The NG scaffold length (see text) is calculated at integer thresholds (1% to 100%) and the scaffold length (in bp) for that particular threshold is shown on the y-axis. The dotted vertical line indicates the NG50 scaffold length: if all scaffold lengths are summed from longest to the shortest, this is the length at which the sum length accounts for 50% of the estimated genome size. Y-axis is plotted on a log scale. Snake estimated genome size = ~1.0 Gbp.

# History of WGA

1997

Let's sequence the human genome with the shotgun strategy



That is impossible, and a bad idea anyway

Gene Myers

Phil Green