Next-Generation Sequencing
De novo Assembly

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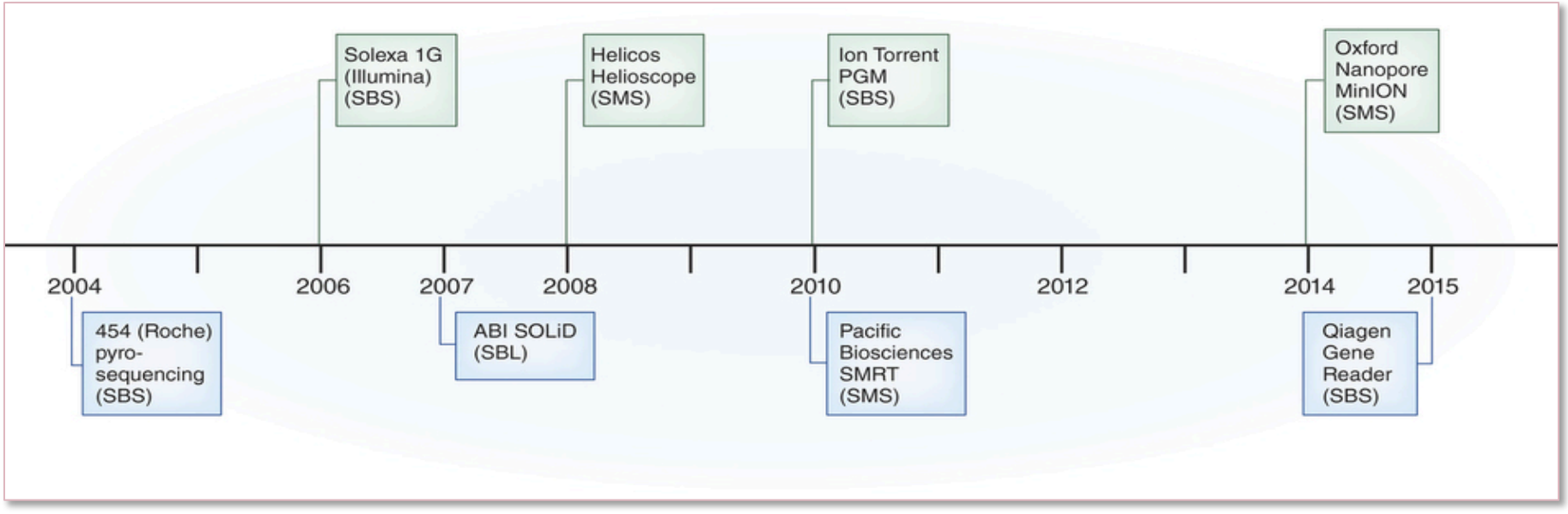
MOL.923 SS 2018

NGS platforms timeline



- Year of introduction of NGS platforms that successfully achieved commercial introduction during the past decade.
- SBS: sequencing by synthesis, SMS: single-molecule sequencing, SBL: sequencing by ligation.

Mardis, E.R. (2017) DNA sequencing technologies: 2006–2016, Nature Protocols 12:213–218

Comparison of NGS platforms

Company	Read length	Applications
454/Roche	400 bp (single end)	Bacterial and viral genomes, multiplex-PCR products, validation of point mutations, targeted somatic-mutation detection
Illumina	150–300 bp (paired end)	Complex genomes (human, mouse and plants) and genome-wide NGS applications, RNA-seq, hybrid capture or multiplex-PCR products, somatic-mutation detection, forensics, noninvasive prenatal testing
ABI SOLiD	75 bp (single end) or 50 bp (paired end)	Complex genomes (human, mouse, plants) and genome-wide NGS applications, RNA-seq, hybrid capture or multiplex-PCR products, somatic-mutation detection
Pacific Biosciences	Up to 40 kb (single end or circular consensus)	Complex genomes (human, mouse and plants), microbiology and infectious-disease genomes, transcript-fusion detection, methylation detection
Ion Torrent	200–400 bp (single end)	Multiplex-PCR products, microbiology and infectious diseases, somatic-mutation detection, validation of point mutations
Oxford Nanopore	Variable: depends on library preparation (1D or 2D reads)	Pathogen surveillance, targeted mutation detection, metagenomics, bacterial and viral genomes
Qiagen GeneReader	107 bp (single end)	Targeted mutation detection, liquid biopsy in cancer

Mardis, E.R. (2017) DNA sequencing technologies: 2006–2016, Nature Protocols 12:213–218

NGS platform trade-off

- Number of reads
- Read lengths
- Cost
- Accuracy
- Application

http://www.allseq.com/knowledgebank/sequencing-platforms

Platform considerations

- "Second generation"
  - 454: longer read length (defunct)
  - Illumina HiSeq: shorter read length, low cost
  - SOLiD: even shorter read length, more reads
- "Third generation"
  - PacBio RS: much longer read length, high error rate
  - Ion Torrent PGM: low machine cost
  - 454 GS Junior (defunct), Illumina MiSeq, Illumina NextSeq

NGS reads

- Short subsequences of the genome
  - No idea on the original position in the genome
  - Orientation (strand) unknown
- Oversampled (high coverage)
  - Reads overlap: only clue for assembly
- Contain base errors
  - Indels, substitutions, characteristic biases
- Supposed to cover entire genome
  - Not always true
  - Coverage not uniform

Genome assembly metaphor

DNA clones

Reads

Reconstructed genome

The Call—Chronicle—Examiner

SAN FRANCISCO, THURSDAY, APRIL 19, 1906.

EARTHQUAKE AND FIRE: SAN FRANCISCO IN RUINS

NO HOPE LEFT FOR SAFETY OF ANY BUILDINGS

BLOW BUILDINGS UP TO CHECK FLAMES

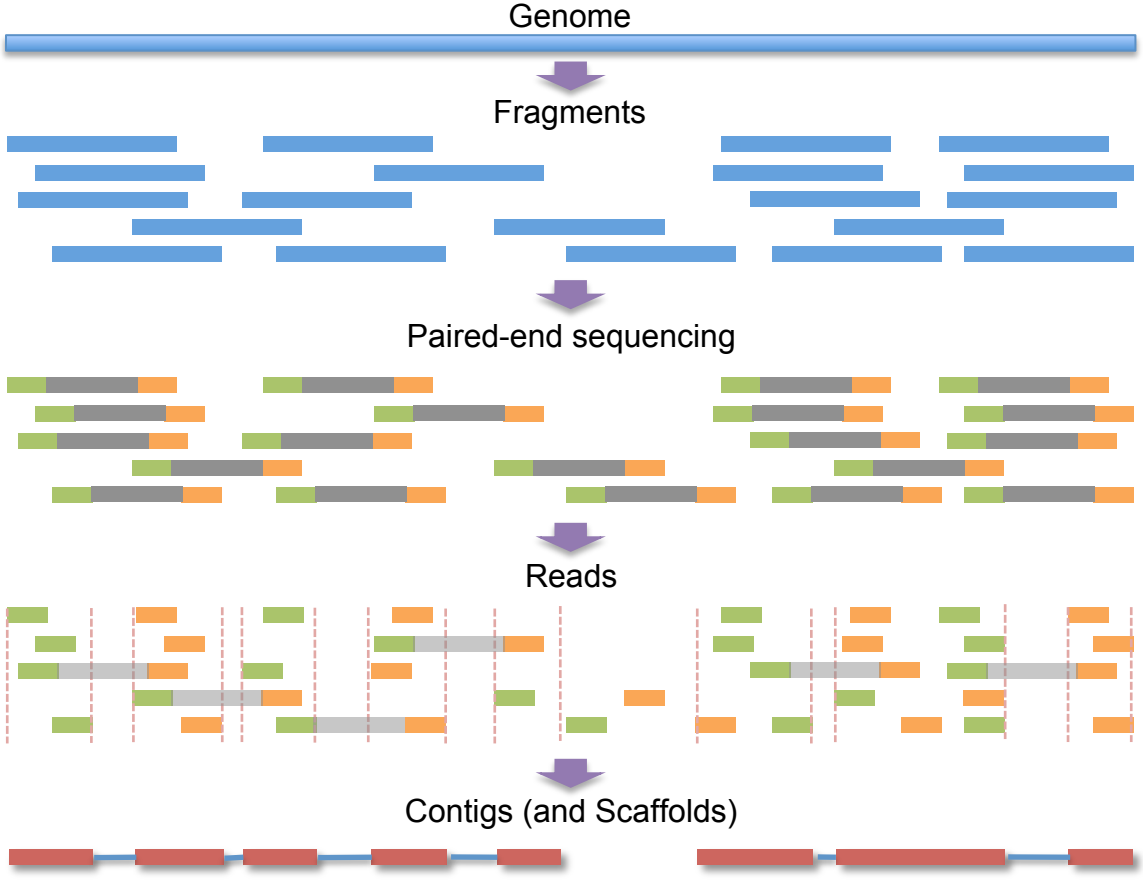
WHOLE CITY IS ABLAZE

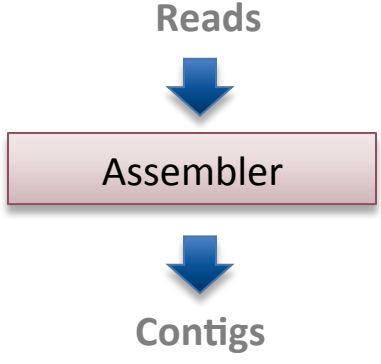
CHURCH OF SAINT IGNATIUS IS DESTROYED

MAYOR CONFERS WITH MILITARY AND CITIZENS

http://www.vicbioinformatics.com/documents/Genome%20Assembly%20Strategies%20-%20Torsten%20Seemann%20-%20IMB%20-%205%20Jul%202010.pdf

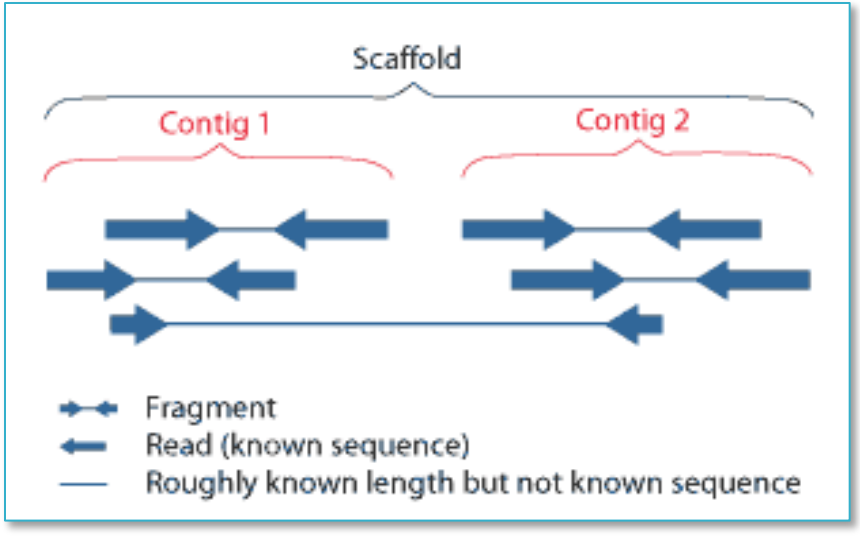
Paired-end sequencing and assembly





Scaffolding

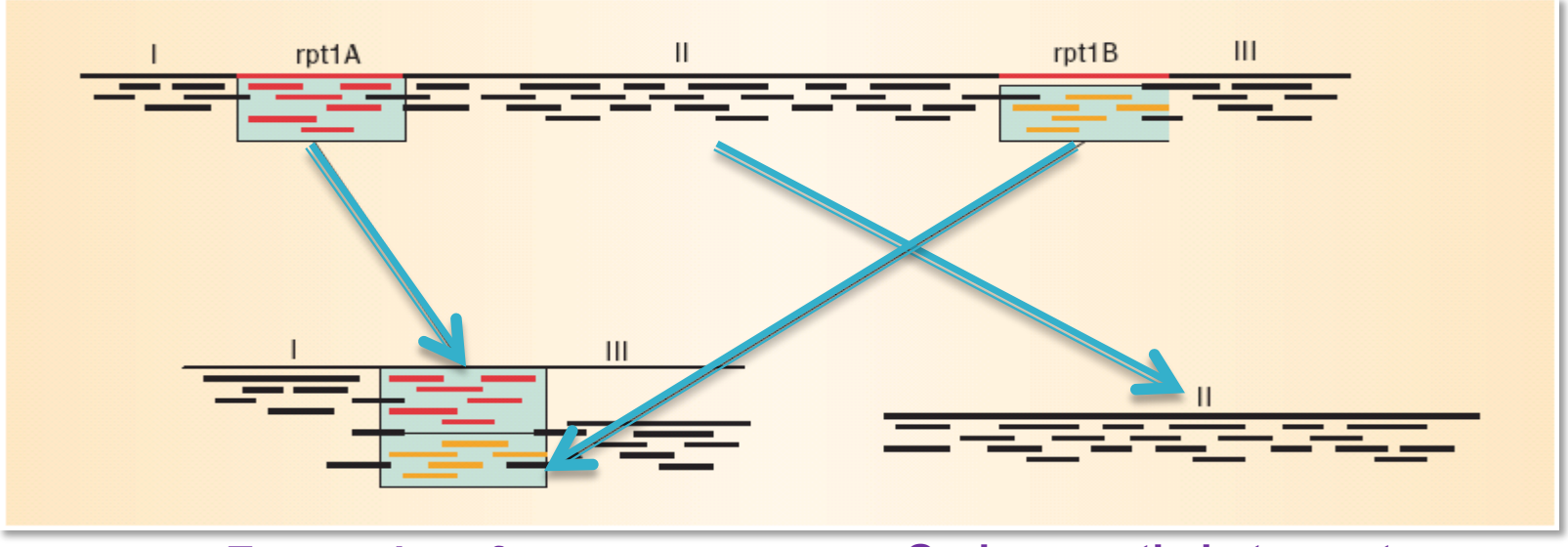
- Joining a set of contigs
  - Scaffolds have gaps (N's)
- Paired-end reads
  - Known sequences at both ends of a fragment
  - Between ends: distance roughly known, sequence unknown
- Ends can belong to
  - 1 contig: not useful for scaffolding (most pairs)
  - 2 contigs: used to link the contigs



http://en.wikipedia.org/wiki/File:PET_contig_scaffold.png

Assembly challenge: repeats

Correct layout of three DNA sequences: I, II and III



Two copies of a repeat collapsed in a misassembled contig

Orphan contig between two repeat copies

Pop M, Salzberg, SL, Shumway M (2002) Genome sequence assembly: Algorithms and issues. Computer *35*(7), 47-54.

Two types of assembly

- *De novo* assembly
  - No reference genome, only reads available
  - Needed for novel genomes
- Reference alignment/mapping
  - Aligning reads to an existing reference genome
  - Sequencing errors not major concern
  - Useful for reassembly or variation detection

Two classes of assembly algorithms

- Overlap-Layout-Consensus (OLC)
  - Better suited for low-coverage long reads
- De Bruijn graph-based
  - Better suited for deep-sequenced short reads, especially for huge genome assembly

Overlap-layout-consensus (OLC)

- Three steps:
  - Overlaps among all reads are calculated
  - Layout the overlaps onto a graph
    - Overlapping reads (nodes) are connected (edge)
  - Consensus sequence is generated from the graph
- Huge computing resources required
  - Each read compared against every other read
- Examples:
  - Newbler, MIRA, Celera Assembler, CABOG, ARACHNE, Edena

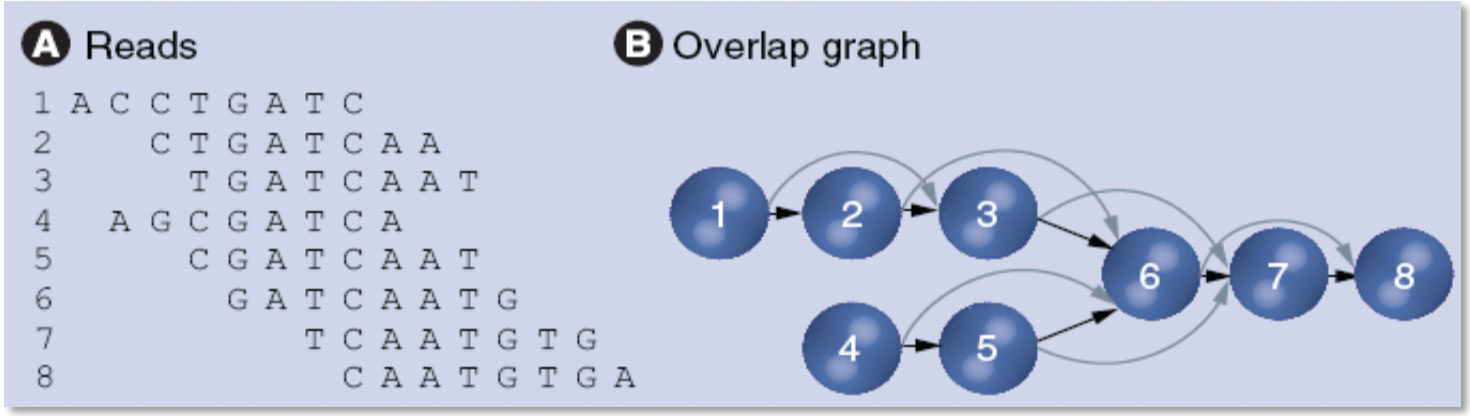
k-mer

- A substring of length *k*
- A string of length *L* has (L–*k*+1) *k*-mers
- Example:
  - If we have a read (string) "**ATGCTCGA**" (length *L*=8) and are looking for 5-mers (*k*=5), there are 4 (L–*k*+1 = 8–5+1) 5-mers :
    - **ATGCT**
    - **TGCTC**
    - **GCTCG**
    - **CTCGA**

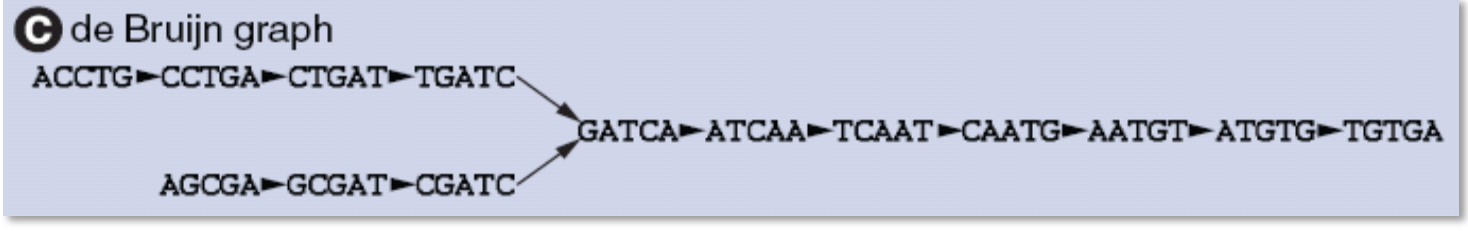
de Bruijn graph (DBG)

- Three steps:
  - Chop reads into *k*-mers
  - Form DBG using all the *k*-mers
    - Create a node for each unique *k*-mer
    - Connect two nodes if two *k*-mers are adjacent in a read with *k*–1 base overlap
  - Infer genome sequence from the DBG
- Examples:
  - ULER-USR, Velvet, ABySS, ALLPATHS-LG, SOAPdenovo, SPAdes, Ray

OLC and DBG



Nodes: reads, edges: overlaps



Nodes: *k*-mers, edges: adjacent *k*-1 base overlap (*k* = 5)

Henson J, Tischler G, Ning Z (2012) Next-generation sequencing and large genome assemblies. Pharmacogenomics 13(8), 901-915

Assembly output: contigs

- Contigs
  - Ideally one contig (genome), normally many contigs
  - Number of contigs: smaller is better?
- Contig size metrics
  - Larger is better?
  - Min, max, median, N50, N90, …
- Assembly size
  - Sum of all contig sizes (total number of bases in assembly)
  - Ideally genome size
- Number of ambiguous bases (N's)
  - Smaller is better

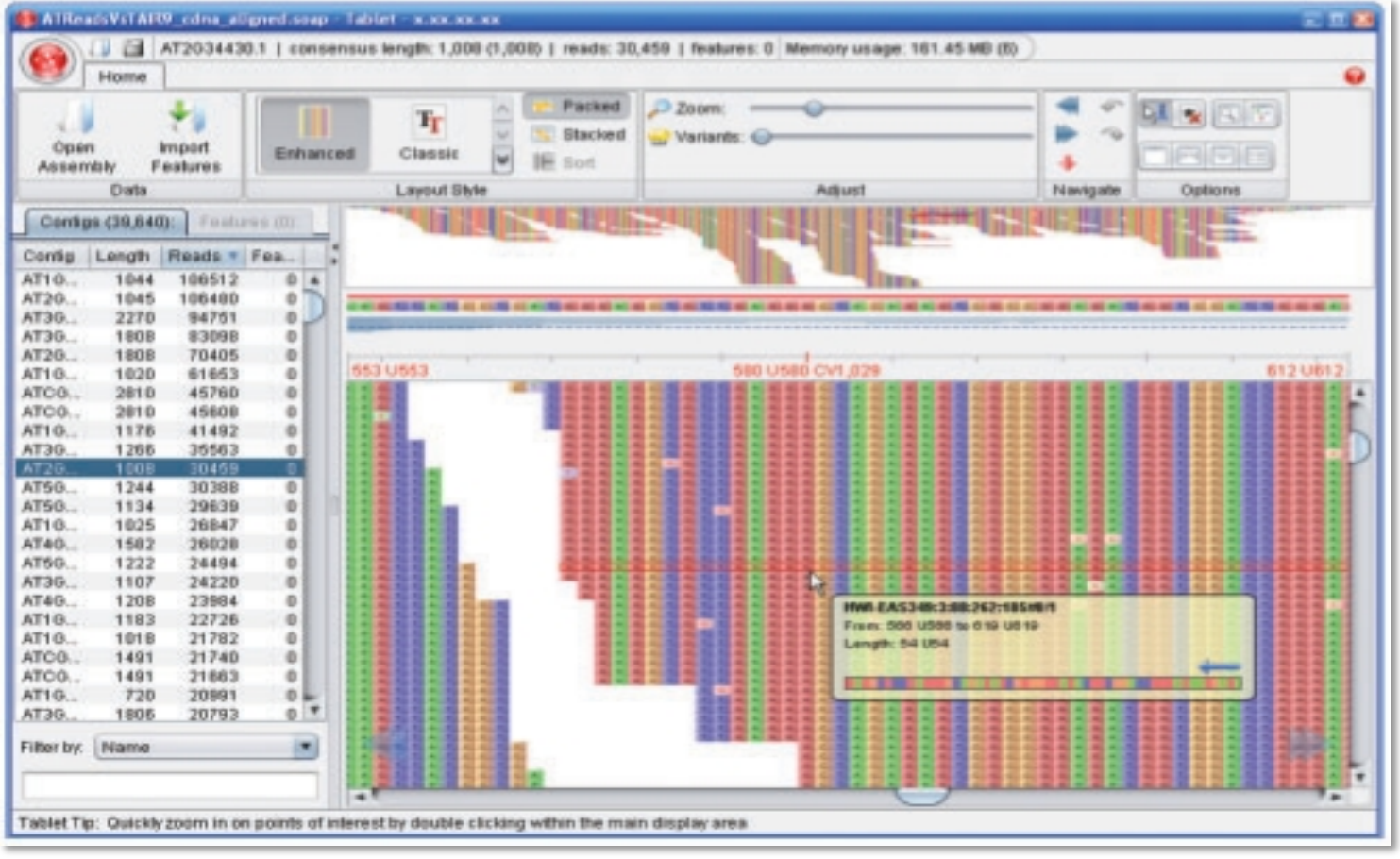
N50

- Most commonly used assembly metric
- Defined over a set of contigs as:
  - The size of the largest contig such that at least half the total size is contained in the contigs of that size or larger
- Example: contigs of sizes 10, 9, 8, 7, 6, 5, 4, 3, 2
  - Total size = 54, half size = 27
  - 10 + 9 + 8 = 27
  - N50 = 8, because 27 $\geq$ 27
- Not always a reliable metric
  - Can be made large by misassembled contigs

Genome assembly visualization

- Visualization is useful
  - View which reads map to which contigs
  - Find/view paired-end reads
  - View coverage depth profiles
  - Coloration to highlight different aspects of assembly
  - More assembly metrics
- Tablet is a widely used assembly visualization tool

Tablet visualization example



Milne I, Bayer M, Cardle L, et al. Tablet—next generation sequence assembly visualization. Bioinformatics. 2010;26(3):401-402.

Lab overview