Sequence Assembly and Next Generation Sequencing

BIOL 7711
Computational Bioscience

Biochemistry and Molecular Genetics
Computational Bioscience Program
Consortium for Comparative Genomics
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Evolution
Genomics
Biodiversity

Computation on Nucleotides

- Alignment--generally more uncertain than amino acids
  - Occasionally provides a more correct answer
- Sequence Assembly
- Sequence Annotation
  - Genes, splice sites
  - Regulatory regions, TFBS
  - Chromatin binding
- Mutation processes
- Route to information much faster, cheaper

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- Sequence Annotation
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  - Chromatin binding
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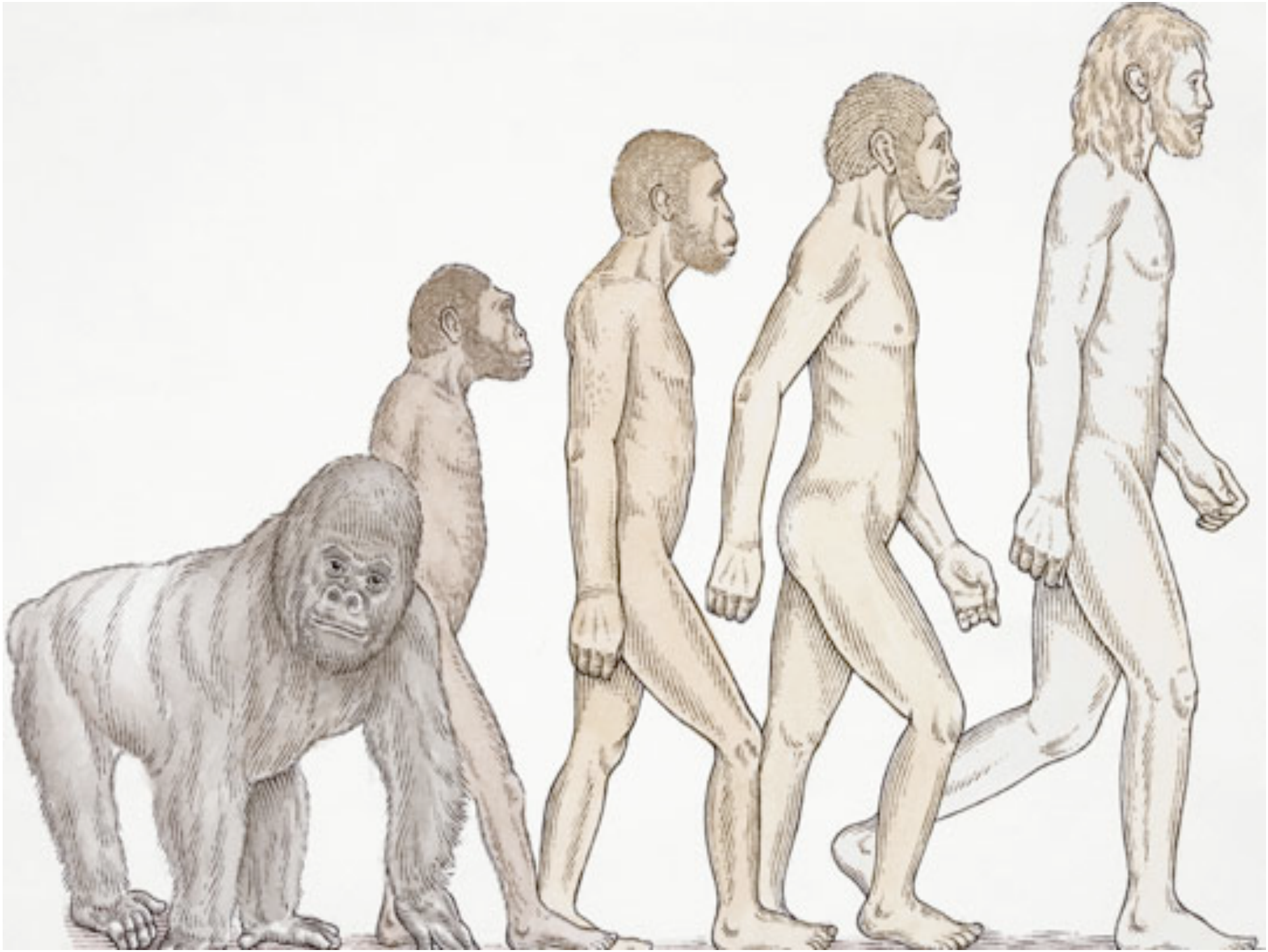
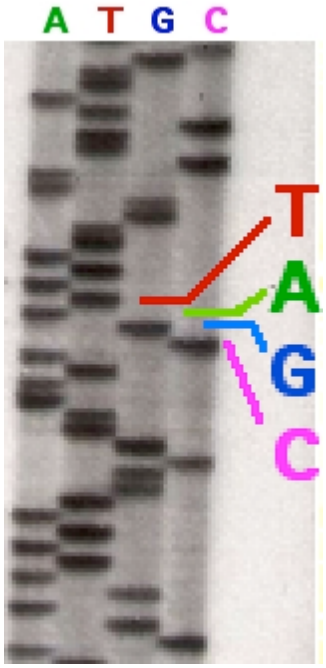
- Route to information much faster, cheaper
  - **High-throughput next-generation sequencing**

The Evolution of Sequencing

- Sanger sequencing
  - Gels
  - Cycle sequencing
  - Fluorescence
  - Capillary electrophoresis
- Sequencing, the “Next Generation”
  - “Sequencing by synthesis”
    - Pyrosequencing (Roche/454)
    - Cluster sequencing (Illumina/Solexa)
  - Sequencing by ligation (ABI/SOLiD)

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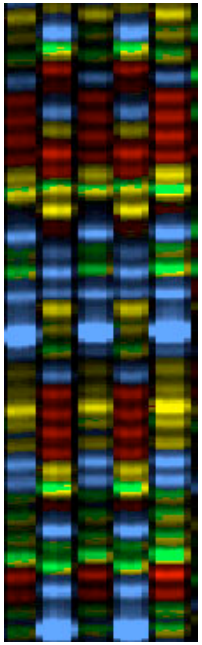

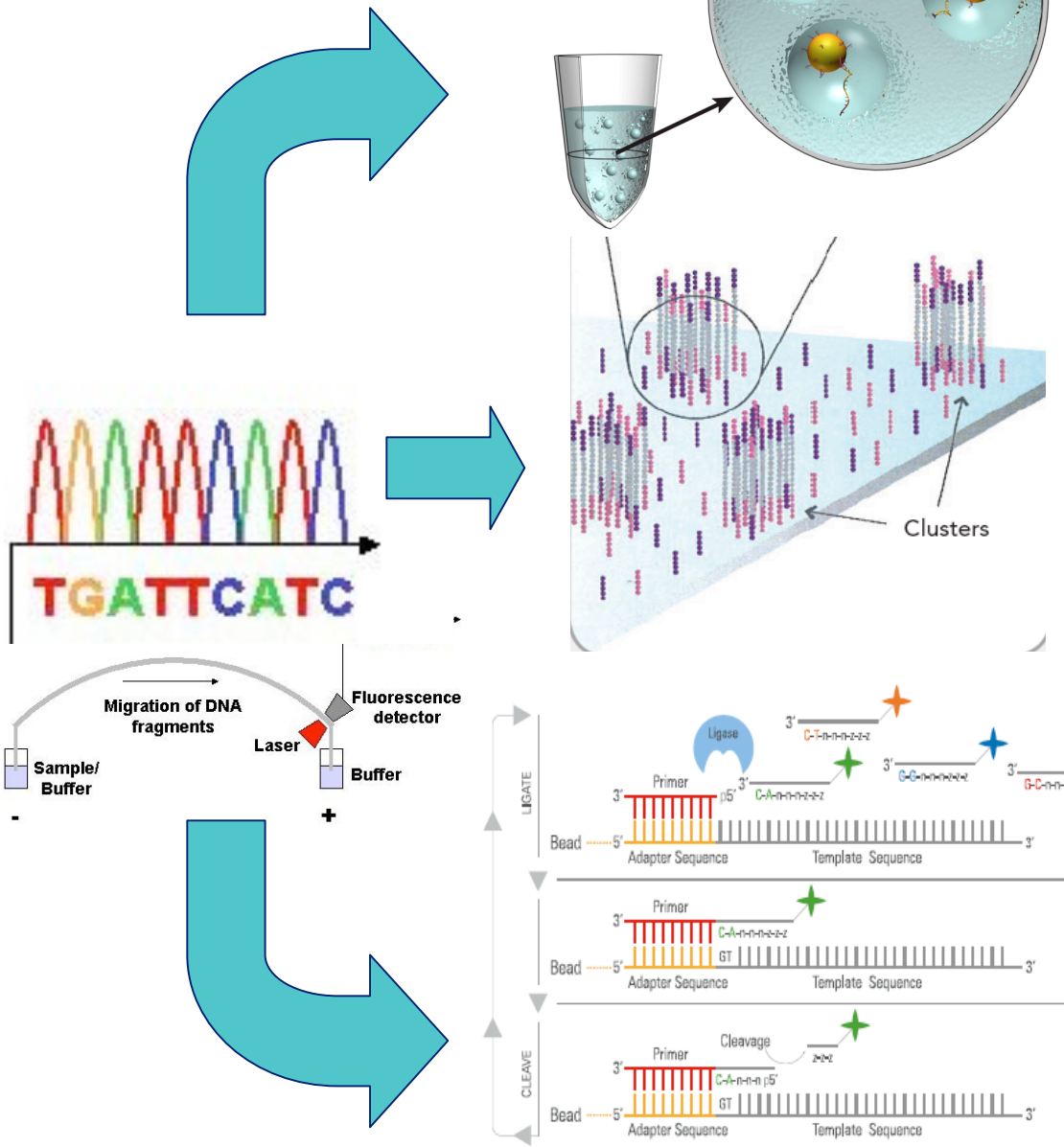
TGATTCATC
Migration of DNA fragments
Fluorescence detector
Laser
Sample/ Buffer
Buffer
-
+
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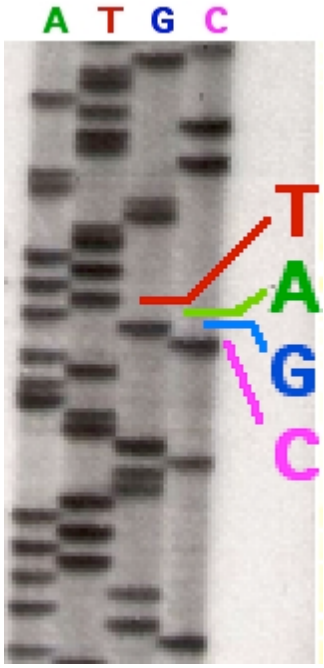
- Next “Next Generation”
  - 4th Generation?
  - Single molecule sequencing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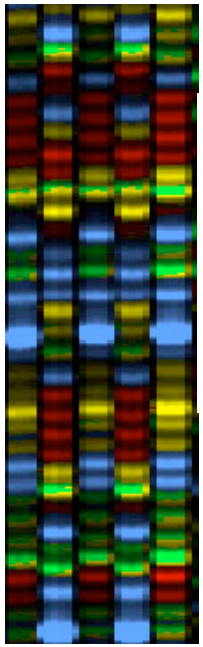

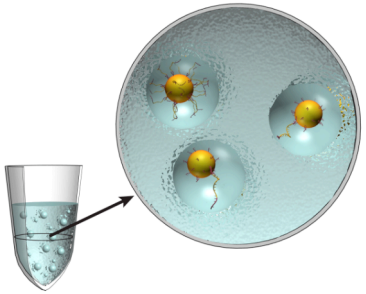

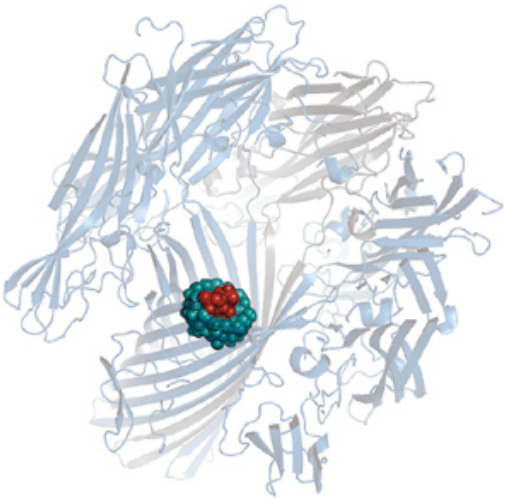

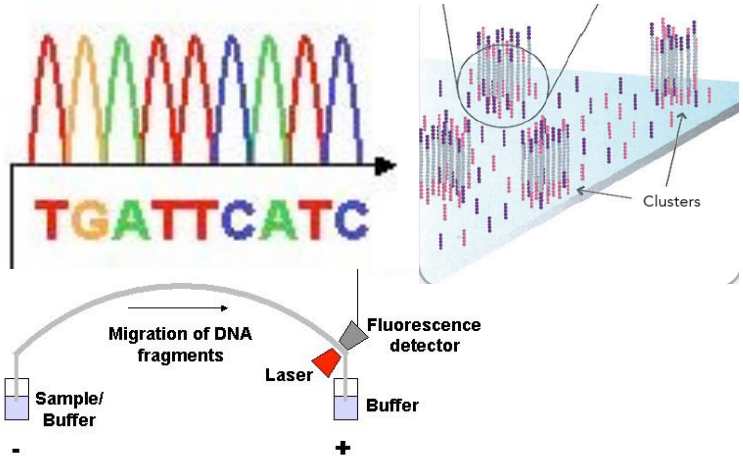
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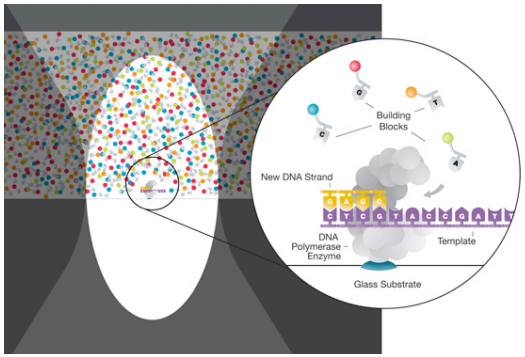
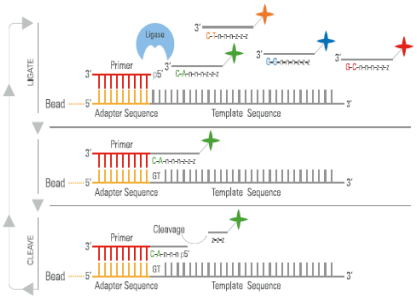

Building Blocks
New DNA Strand
DNA Polymerase Enzyme
Template
Glass Substrate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Primer
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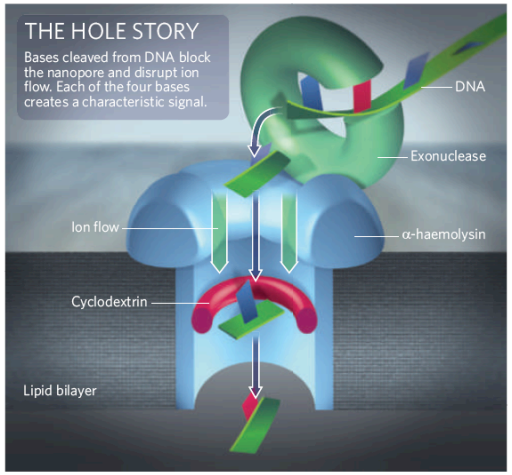
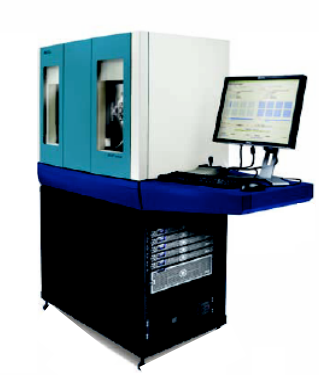

THE HOLE STORY
Bases cleaved from DNA block the nanopore and disrupt ion flow. Each of the four bases creates a characteristic signal.
DNA
Exonuclease
Ion flow
α-haemolysin
Cyclodextrin
Lipid bilayer

Macro versus Micro Reads



Applied Biosystems
SOLiD

Read Length
35 - 75bp **<=>** 250 - 450bp

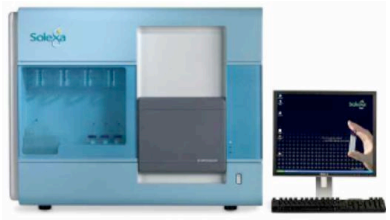
Base Pairs Per Run
3 - 10 Gb **<=>** 0.1 - 0.5 Gb

Base Pairs Per Day
1 - 1.5 Gb **<=>** 0.2 - 1.0 Gb

Number of Sequences
100 M **<=>** 1.2 M

Roche / 454 FLX

Run Time
3 - 7 days **<=>** 0.5 days



Illumina / Solexa
Genetic Analyzer

Reagent Cost per Run
~$4K - $12K **<=>** $6K

Error Rate
Varies, different characteristics

Technology and Informatics

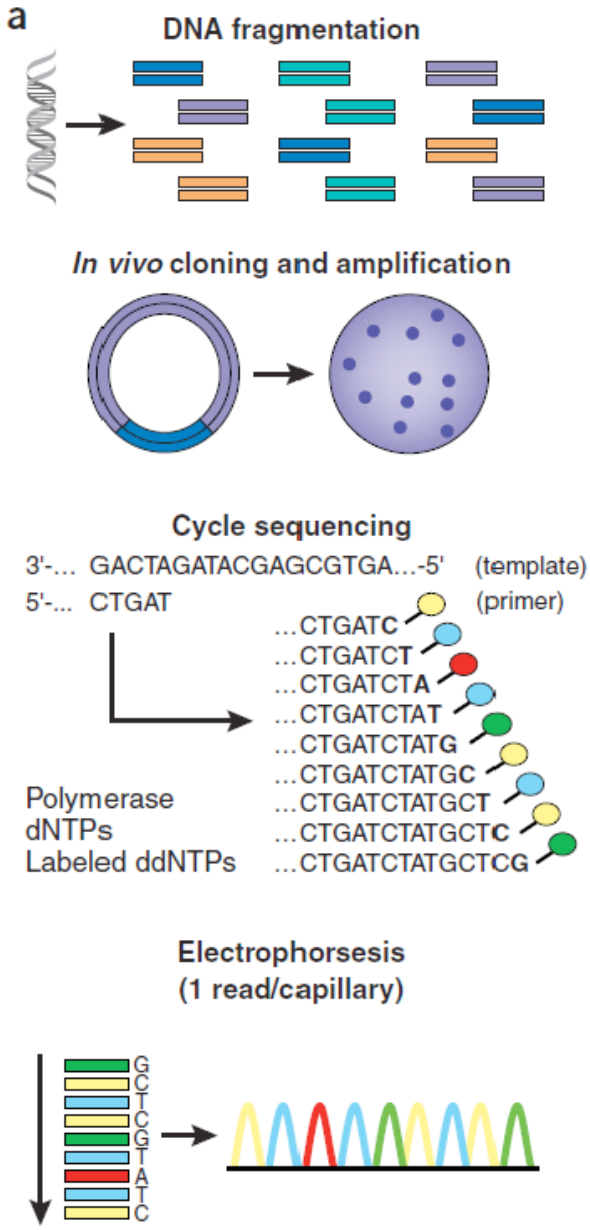
PR Space versus Science Space

- Flow and phasing
- Data quality and Error rate
  - Variation along sequence
  - Quality scores (Equivalence?)
- Length distribution versus average
- Raw versus recovered sequence
  - How much coverage with different methods?
- Tagging (barcodes) and multiplexing
  - Variation in coverage

Next-Gen Basics

- Library creation
  - Shearing, size selection
    - Size distribution
  - Specific primer sequences (adaptors) flank target sequence
    - Allows amplification
      - Opportunity for extra “mutation”
- Tagging (barcodes)
  - Proportion of sequence wasted
- Ligation or amplification (454)
- Paired ends

Sanger Sequencing



- DNA is fragmented
- **Cloned** to a vector
  - Plasmid, BAC
  - Linkage
- Cyclic sequencing
- Separation by electrophoresis
- Read fluorescent tags

Micro Reads: SOLiD & Solexa

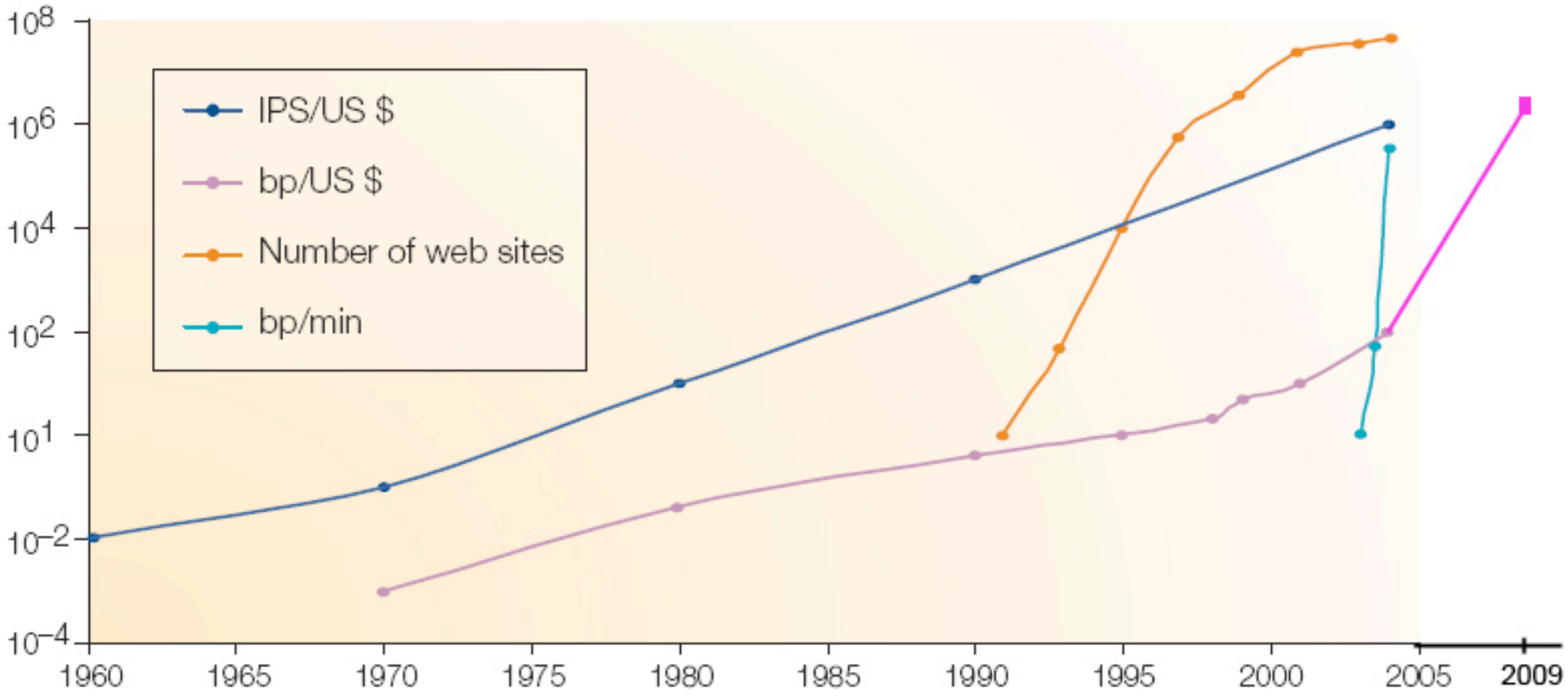
- Reseqeuncing
- SNP detection
- Micro RNA (23 bp)
- Counting (e.g., transcriptome profiling, ChIPSeq)
  - Adjustable dynamic range ($$)
- Hard to place near repetitive elements
- Harder to assemble *de novo*
- *75-100 bp reads intermediate*

Macro Reads: 454, PacBio

Sort of Solexa?

- Many fewer reads
- Much longer
- De novo sequencing
- Amplicons and tagging
- Repetitive regions

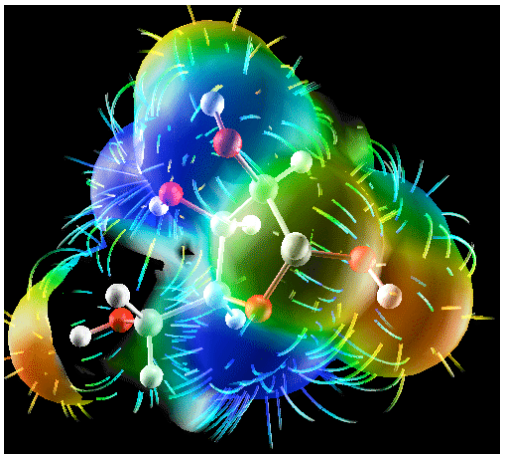
Getting Cheaper all the Time



Adapted from Shendure et al 2004

Informatics Challenges

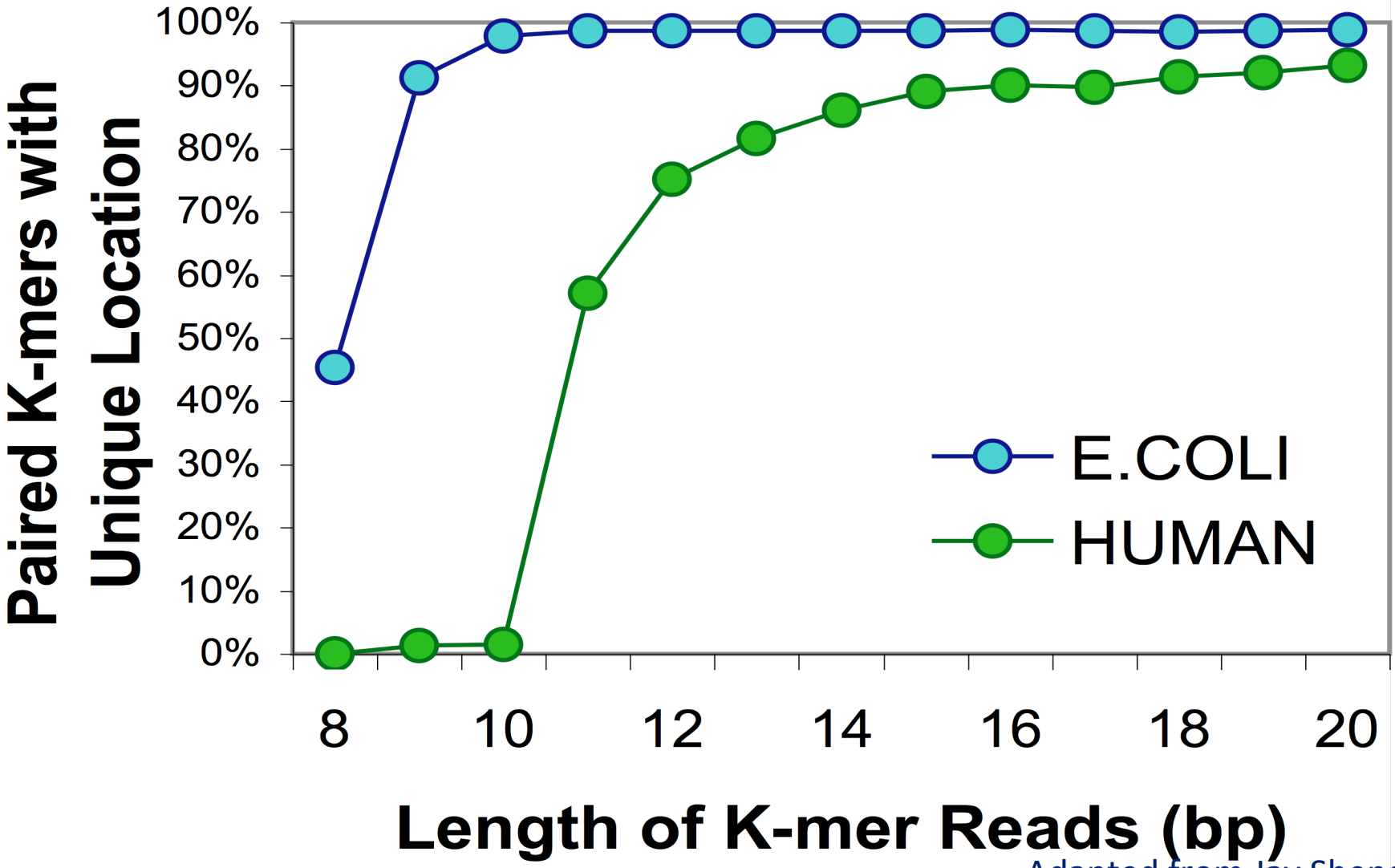
- Data storage
  - 6+ TB for microread raw image files
    - Toss them out: calculate on the fly
- Computation Speed
  - Faster to align long reads
    - Exponential with number of reads if comparing to each other
- Software
  - Getting better
  - Assembly, mapping
  - counting, variation



4th Gen PR Space
The 2nd Coming

- 1 Kb sequences, highly accurate
- Fast, cheap
  - $300 genome (10x) in 30 minutes (??)
- Less front-end preparation and labor
- What is required for personal genomics?
- 10,000 vertebrate genomes project

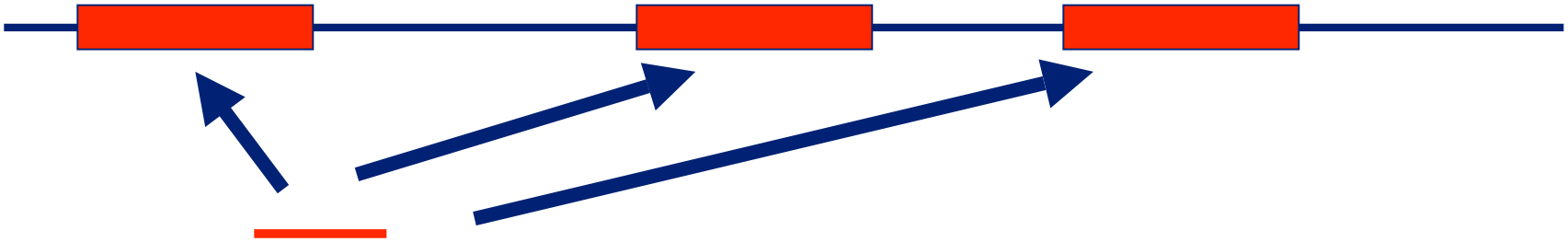
Read Length & Resequencing



Adapted from Jay Shendure

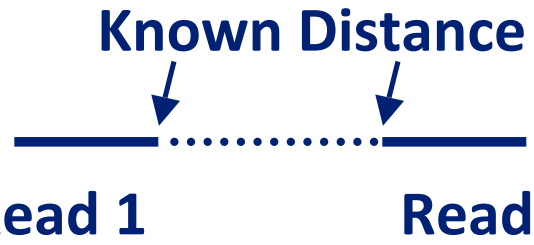
Mapping Unique Reads

Repetitive DNA **Unique DNA**



Single reads map to multiple positions if they hit repetitive DNA

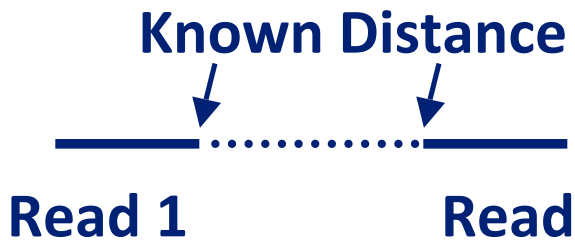
Paired End Reads

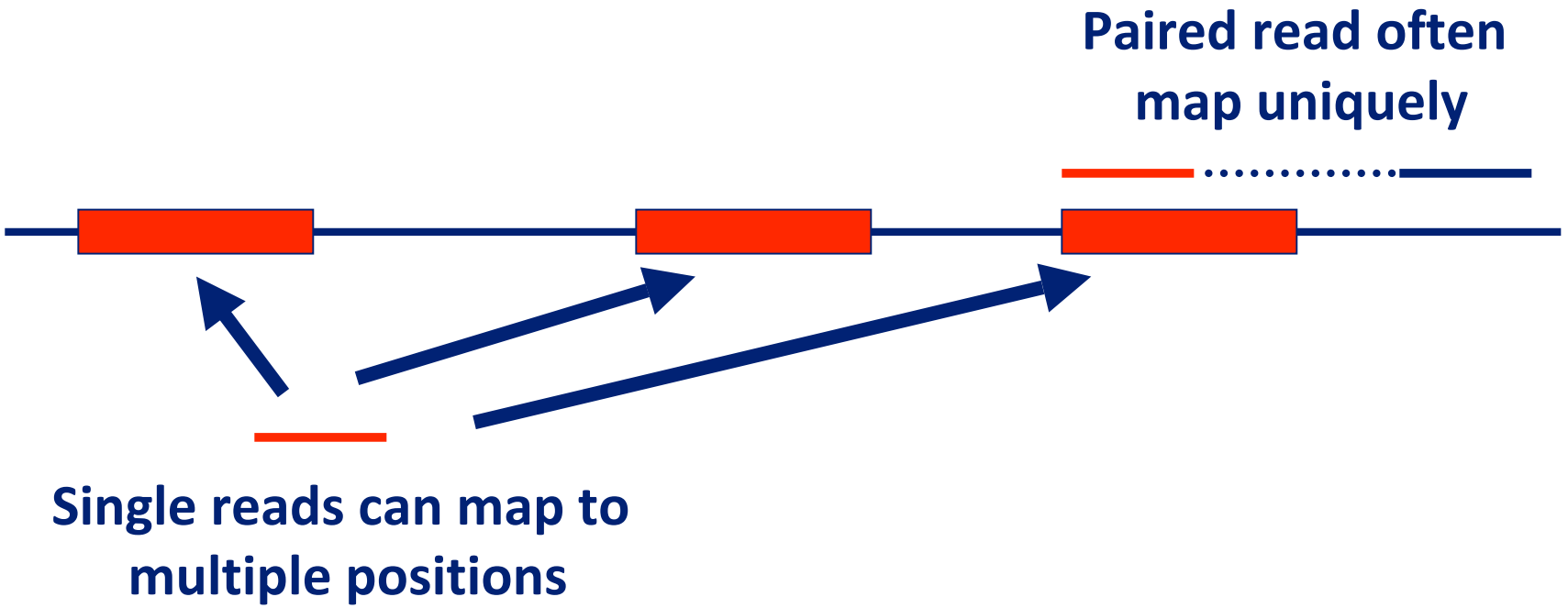


Solexa: paired end is both ends of ~300 bp fragment (shorter than a 454 read, shorter than most TEs)

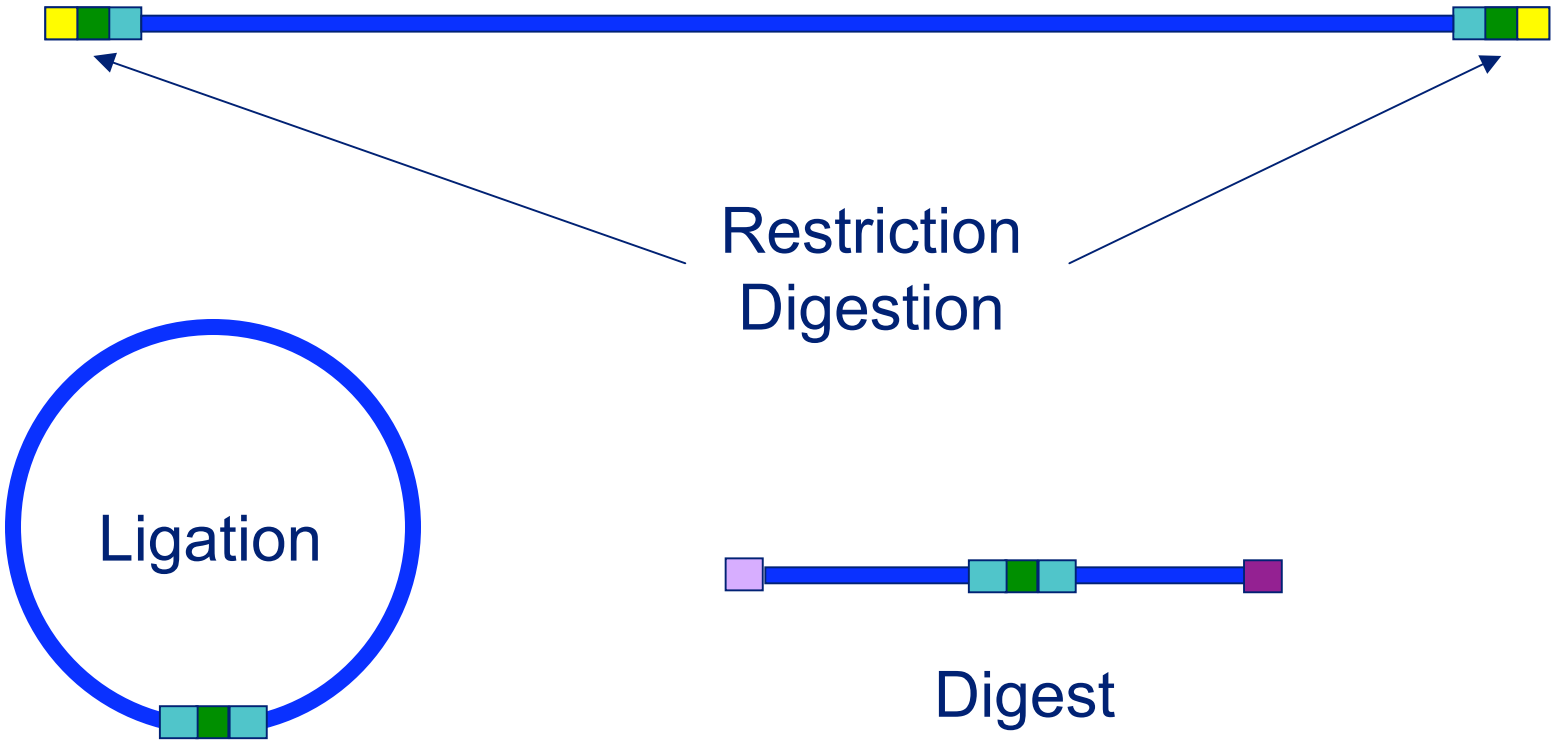
454 paired ends are:
~3Kb
~8Kb
~20Kb

Paired End Reads





454 Paired-End Library Construction



Other Order Information

- FISH mapping
- Recombination map
- BAC paired ends
- Verification by PCR
  - Quite expensive; usually long-term follow-up, only samples

Contig Assembly

- Significant overlap at ends of fragments
  - **IF** overlap fragment is unique in genome, then perfect assembly of contigs (with gaps in between)
  - So, want long enough to be likely unique
  - Want to identify repeat sequences
  - “Shortest Common Superstring” Problem
    - But, tend to delete duplicate regions

Oligo Frequency Model

- $$P(oligo) = \left(\prod_{nuc} freq_{nuc} \right)^{L}$$
- Expected occurrences in genome?
  - Genome length $N=3x10^9$
  - Nucleotide frequencies equal
- What length expected to occur <1 time?
- For that length, what is probability of 2, 3, 5, 10?
  - Use Poisson

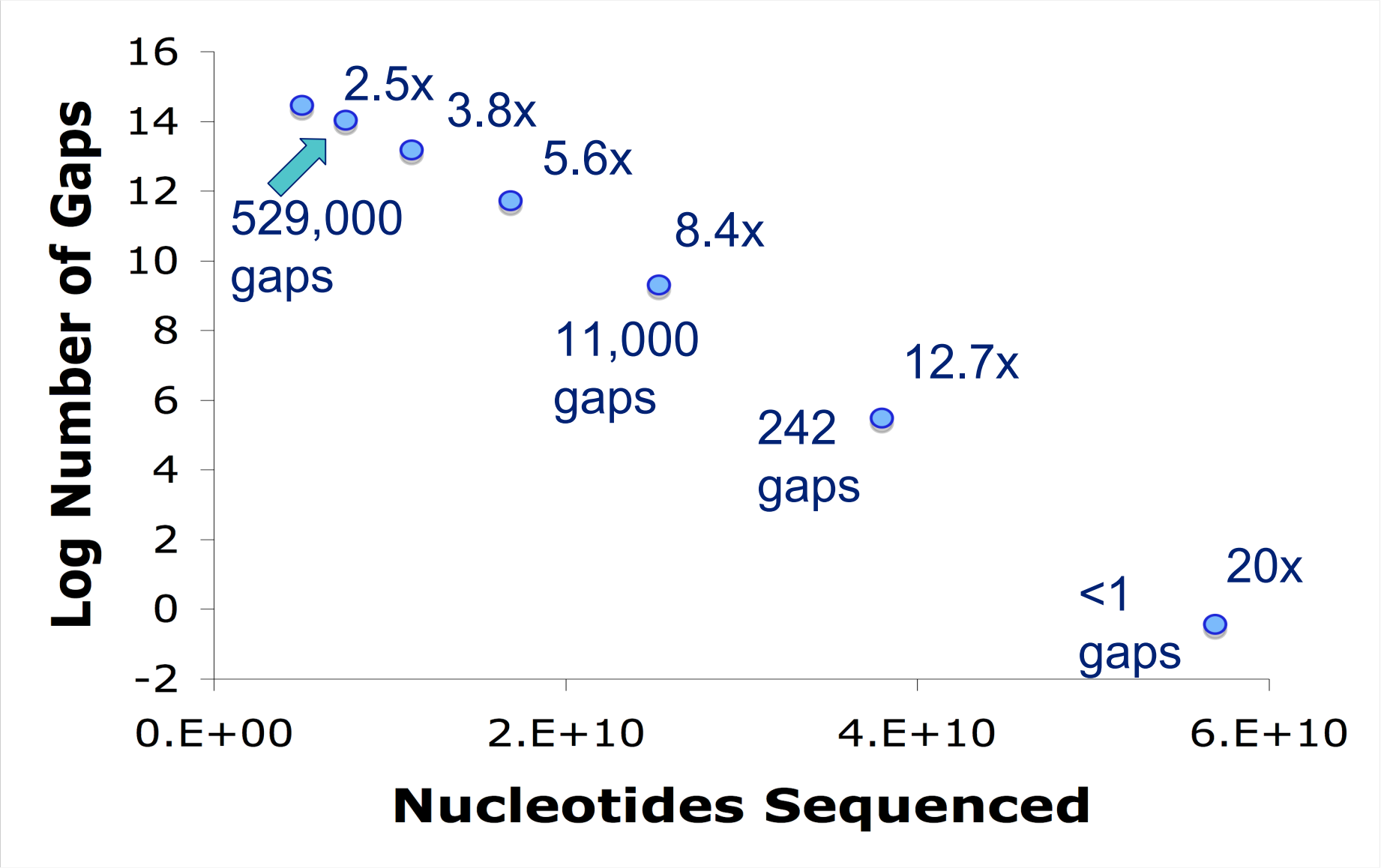
Shotgun Sequencing

- Random fragments
- Coverage (C), or redundancy, is average number of times a nucleotide should be sequenced
  - C=NL/G
    - Number reads sequenced
    - Length of read (average)
    - Genome size
- How many nucleotides covered at least once?
  - Poisson approximation: $1 - e^{-c}$

More Shotgun Rough Expectations

- Average contig length: $(L/c)e^{c}$
- Number of gaps: Ne^{-c}
- Average gap length: L/c

A Quick Visual



But It's Not That Simple

- Calculations assume you know where the reads go
- Sequencing errors
  - Quality scores, low error in the first place
- Sampling bias
  - Cloning bias is particularly bad
    - Some sequences are poison
- Repetitive sequence
  - TEs, mini-satellites, microsatellites, low complexity, tandem repeats
  - Gene paralogs (really want to get these right!)
- The more free unplaced ends, the more likely to have spurious overlap (orientation, revcomp)

More Concerns

- Over-collapsing
  - Leaves extra unplaceable fragments
  - More reads with no place to go
  - Shortest common superstring => biased
- BAC ends, paired end info
  - Drastically reduce the possibilities of where a contig can go
  - Supercontigs
- Polymorphisms

Screening Reads

- Prefer accurate reads: 98%+
  - Clip reads if predicted accuracy drops
- Remove vector and other contaminants
  - Alignment & identification
  - Especially other recent genome projects
- Screen for known repeats

Evaluate Overlap

- Compare each fragment with each other
  - This is why Illumina seqs so hard! N^2
- Example
  - >= 40 bp overlap, <= 6 mismatches
  - Either a true overlap, or a repeat
  - Figure this out asap!
  - Fragments with excessive numbers of overlaps are probably repeats

Unambiguous Contigs

- Combine fragments with only one possible assembly into longer sequences
  - Perfect matches
  - Match no other: no conflicting overlaps
- Drosophila
  - 3.158M reads => 54K unitigs
  - Still might be wrong
- Can extend unitigs up to one read length into repeat regions

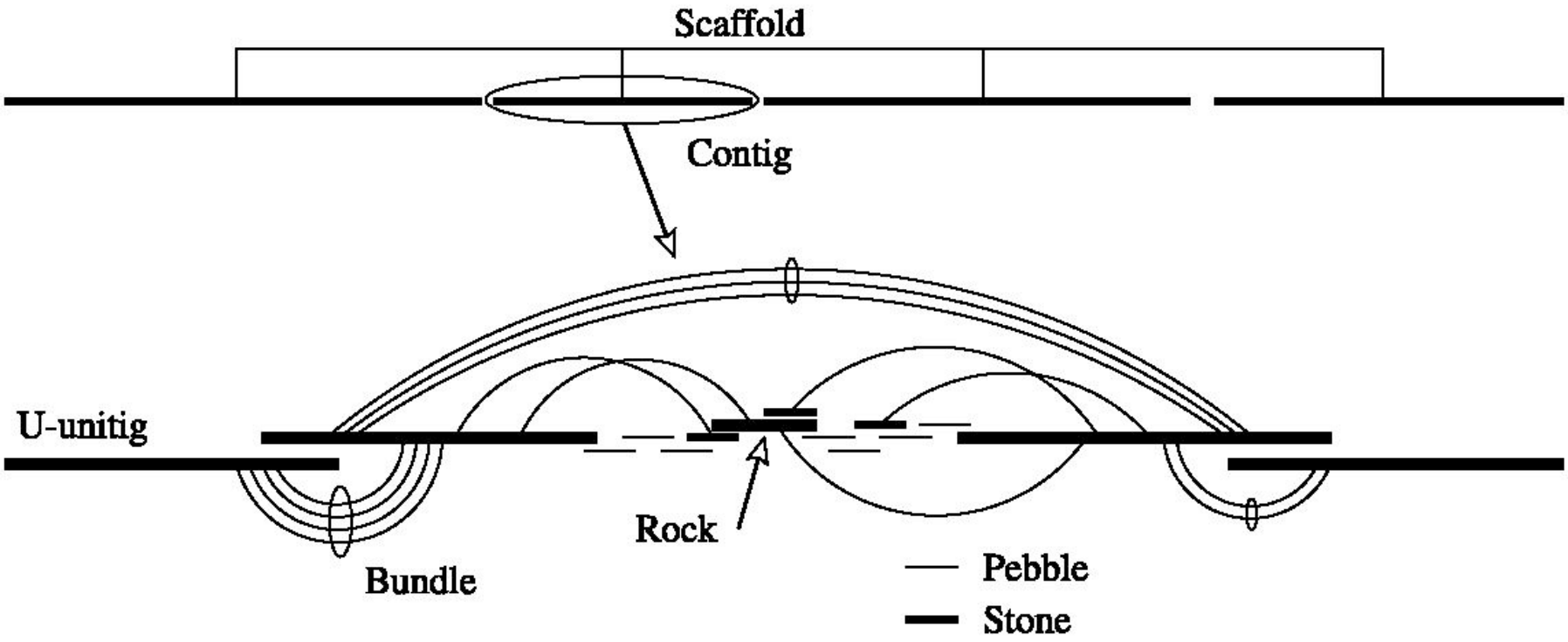
Scaffolds

- Set of ordered, oriented contigs
- Gaps of approximately known size
  - BAC ends in two different contigs
  - BAC library of tight known size range
  - Same concept for other paired end reads
- “Bundle” if more than one placement
  - The more mate pairs, the more reliable
- Map scaffolds with FISH, recombination

Place Repeats

- Placement evidence from mate pairs
  - Multiple = rocks
  - Single = stones
  - None = pebbles
    - Basically just guessing. Statistical

Scaffold Visual



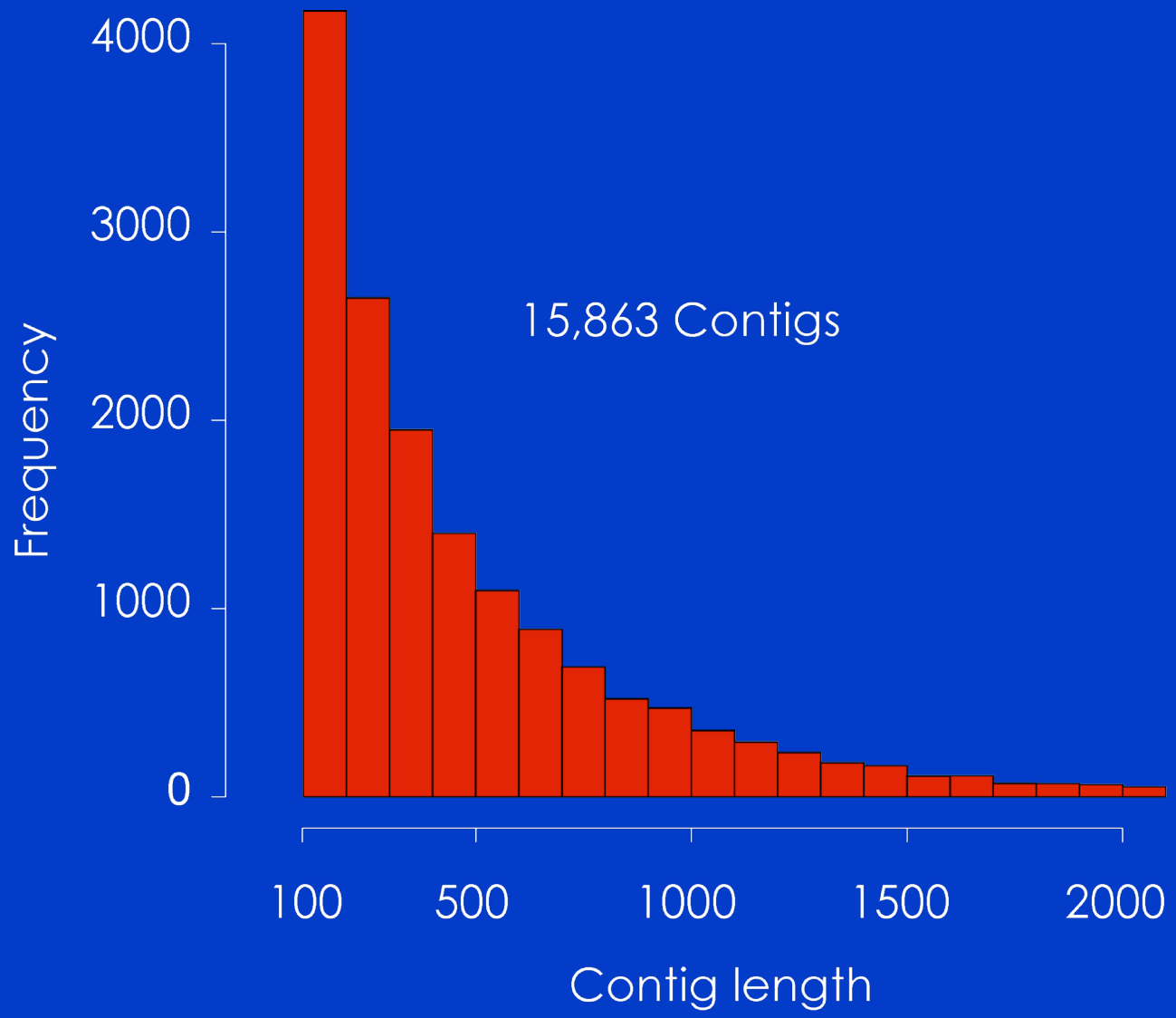
Finishing and Validating

- Manual review and adjustment
- Quality control
  - PCR
- Overlaps, mis-assembly, etc.
- Gaps can reflect unresolvable repeats or low coverage in a region
- More intense sequencing in a region
  - Compare to other sequencing efforts (Celera)

NextGen Assembly

- More faster cheaper shorter error-prone
- Bacterial example: Mycobacterium spp.
  - ~4 Mb genome
- Solexa/Illumina, de novo assembly with VELVET assembler
- 50x coverage = 2Gb, 36 bp reads

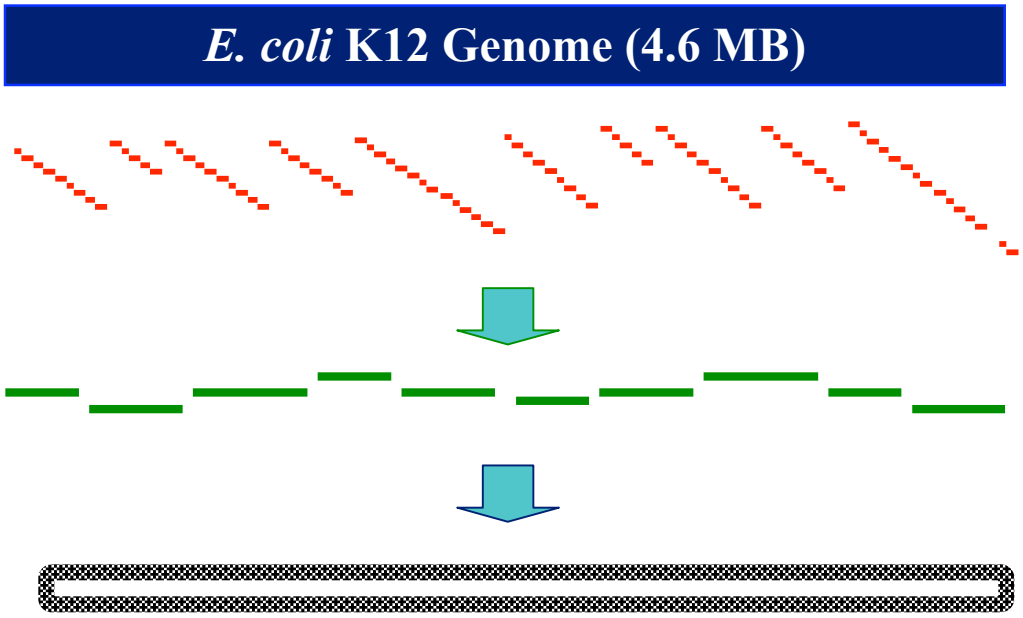
Assembly Result



454 E. coli Assembly

old 250 bp reads

454 Read Type	Genome Coverage	Number of Contigs/ Scaffolds
Shotgun	15×	98
+		
3 Kb Jump	18×	7
+		
20 Kb Jump	20×	1

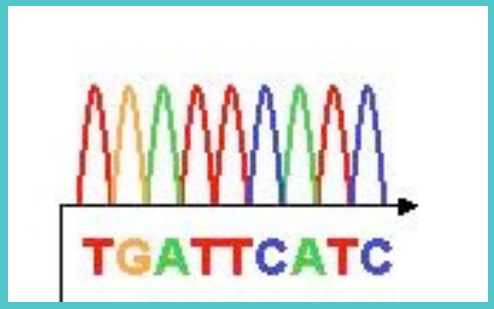


So, this is 20x total coverage

Consensus Accuracy: ~ 99.999%

Open Questions

- What is the most efficient way to combine various sequencing methods?
  - Solexa paired ends versus 454 single reads
    - SOLiD for its accuracy?
  - 454 paired end 3Kb, 8Kb, 20Kb mix
  - Sample repeat regions ahead of time
- Do you have to have BACs for a eukaryote genome?
- Any tricks to finish off gaps efficiently?
- Priors: low heterozygosity, few repeats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