Overview of Next Generation Sequencing (NGS) Technologies

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National Institute of Allergy and Infectious Diseases

NIAID Genomic Sequencing Centers for Infectious Diseases

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UNIVERSITY of MARYLAND SCHOOL OF MEDICINE INSTITUTE FOR GENOME SCIENCES

BROAD INSTITUTE

- Sample Processing Method Develop
- High Throughput Sequencing Pipelines
- Metagenomics Transcriptomics
- Bioinformatics Tools Data Analysis Pipelines
- Genomics Bioinformatics Training

What is ‘NextGen’ sequencing?

- Different chemistry from Sanger
- Sequences **everything** in a sample
  - Host, pathogen, cells, etc.
- Sequences clonally amplified molecule

- Sequencing occurs in parallel
  - Millions of sequences produced concurrently
- Gigabytes of sequences

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What is ‘NextGen’ sequencing?

- Less time than Sanger
- Large capacity
- Multiplexing, variation detection, gene expression, metagenomics
- Address various biological questions

Sanger vs Next-generation sequencing

100 of these….

ABI 3730x

= 1 of these….

GS-FLX Roche/454

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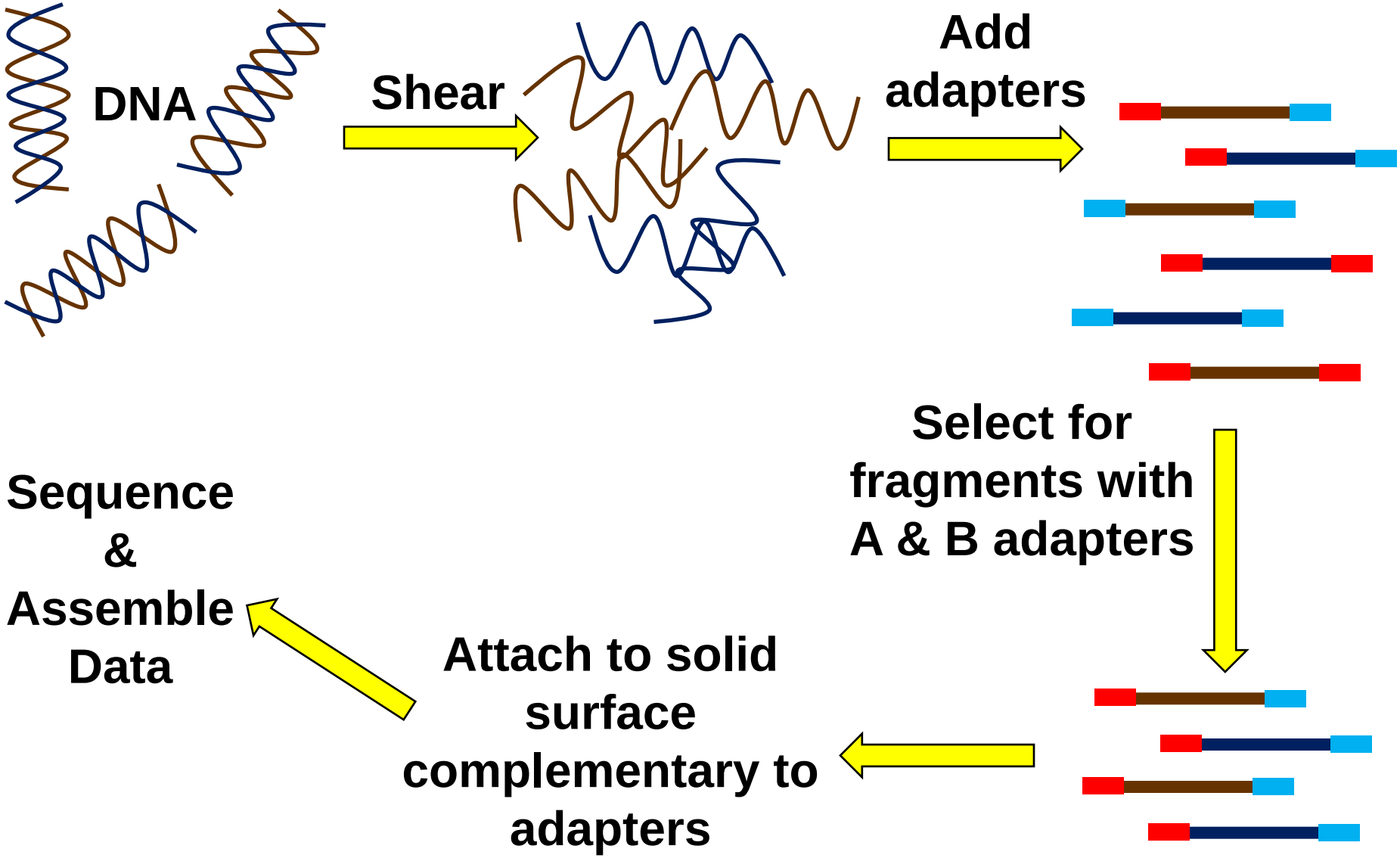
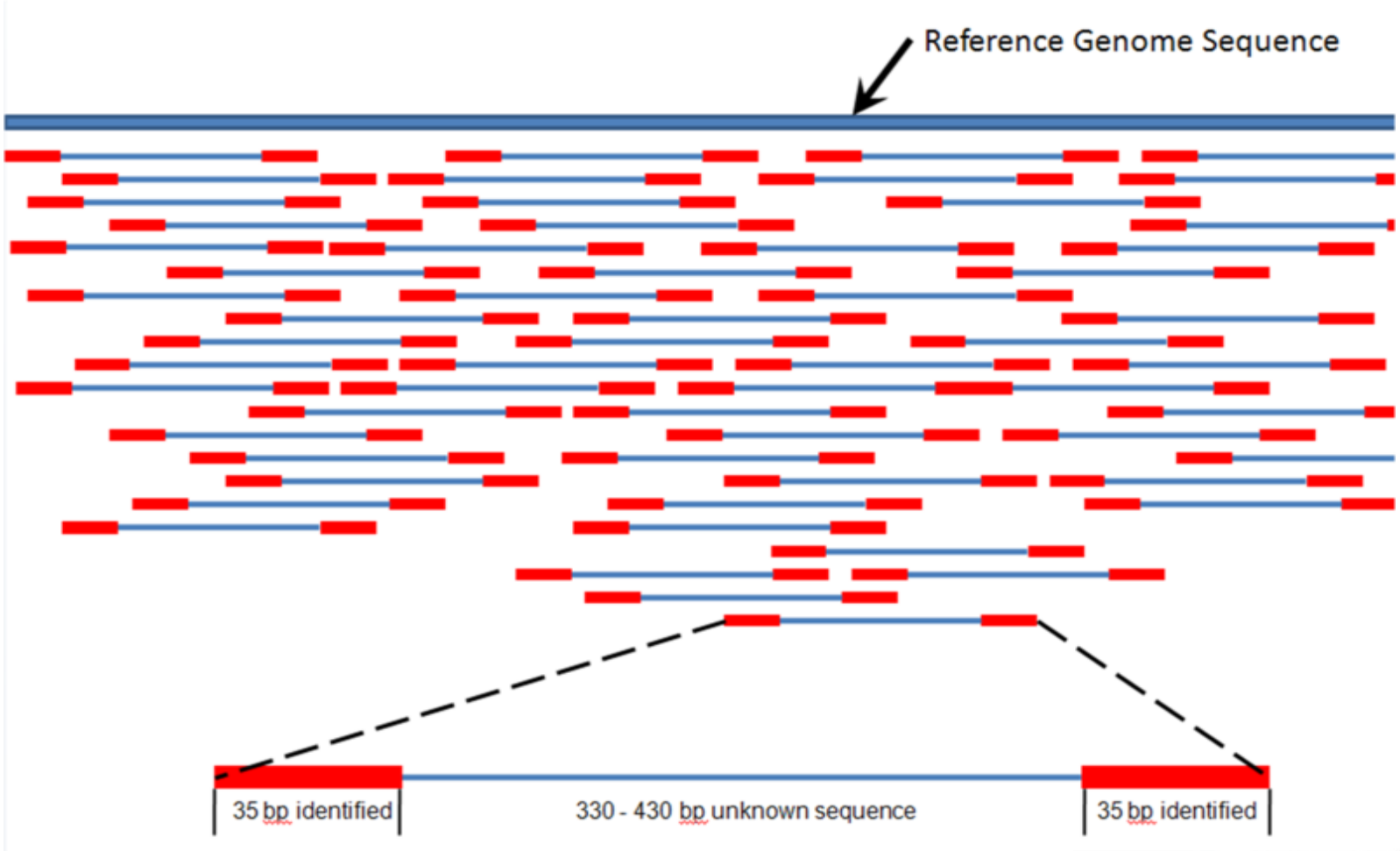
“Single Molecule Sequencing”



Mapping sequence reads to reference

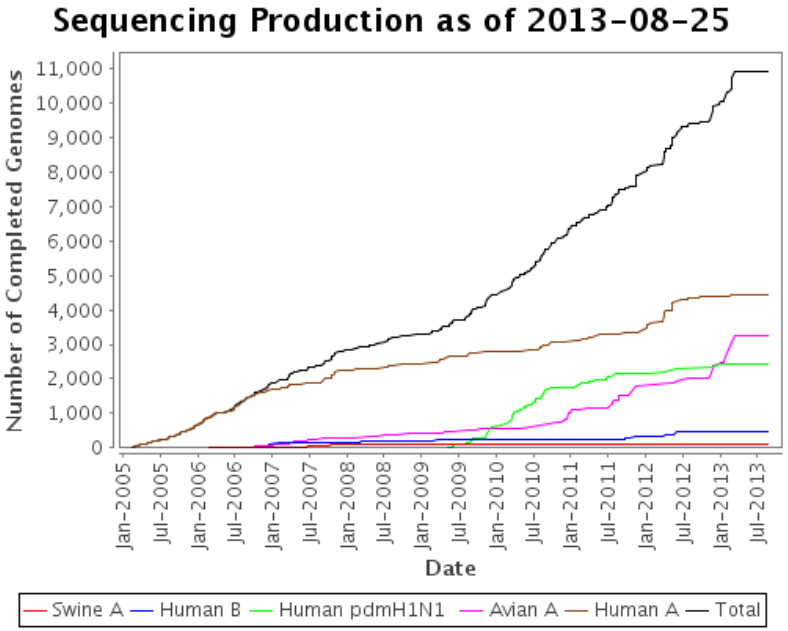


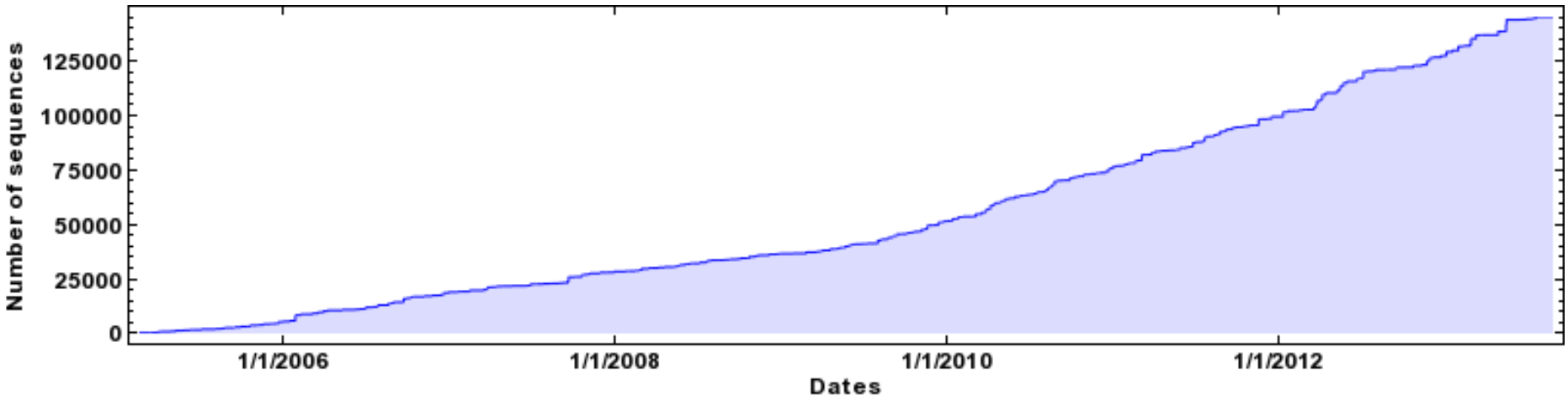
Why use NextGen?

- High rates of accuracy
- Many reads per sequencing run
- Faster time per sequencing run
- Multiplexing capabilities
- Decreased cost
- Useful for many different applications

Why use NextGen?

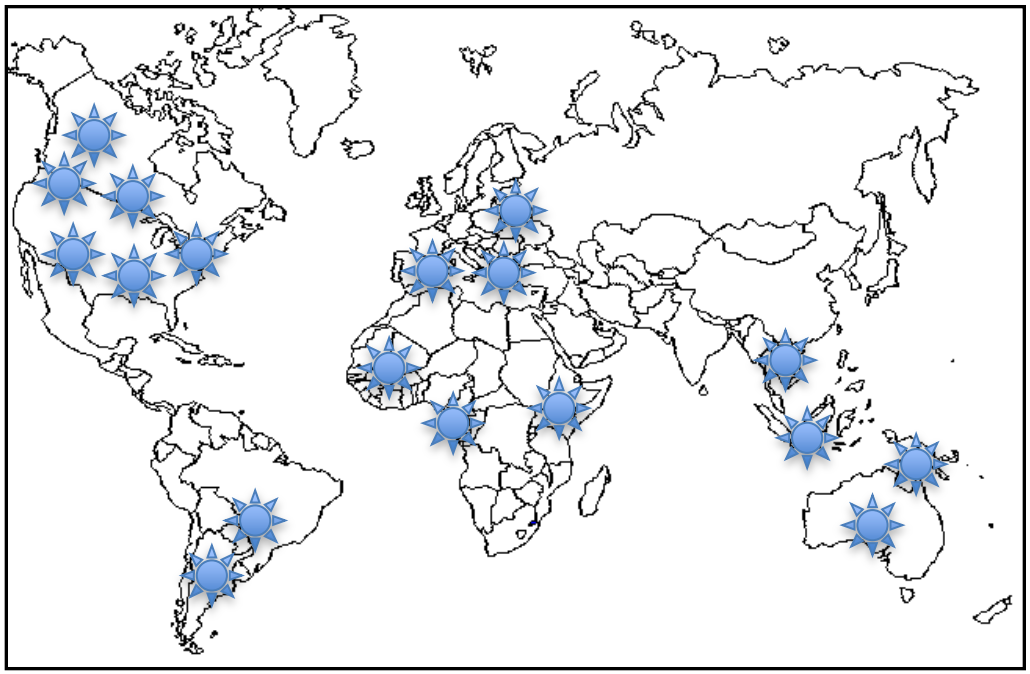
- 2004: < 100 influenza genomes in NCBI
- 2013: 14,000+ influenza genomes in NCBI



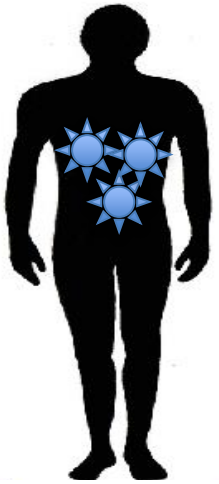


Genomics Analysis at the Population Level

Molecular Epidemiology

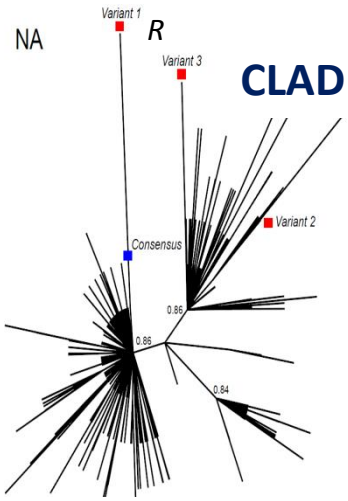


Consensus sequencing



Diversity

Deep sequencing



CLADE 2

CLADE 7

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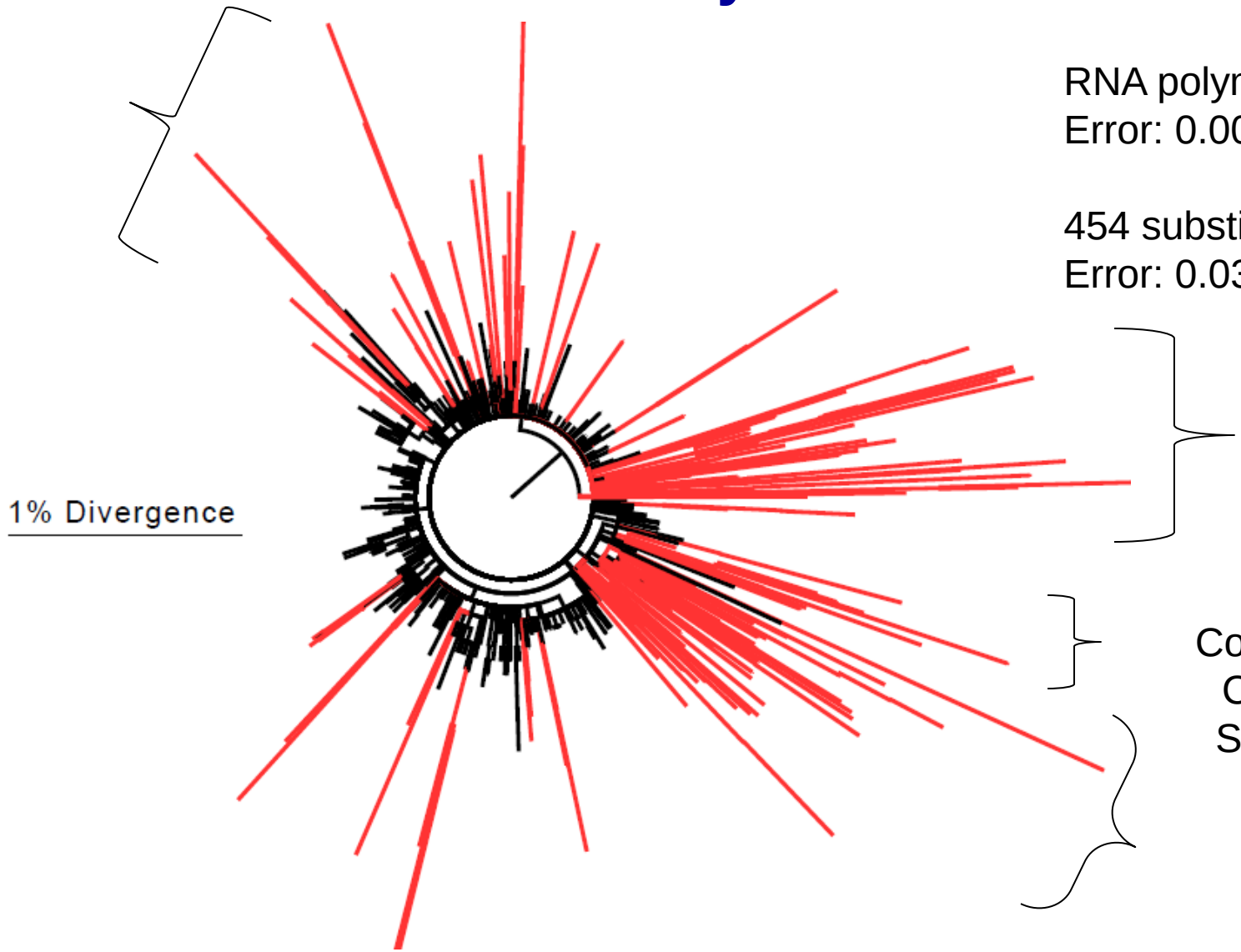
Dept. Computational & Systems Biology

NGS: Things to Consider

- Each platform has advantages & disadvantages
  - Read length, accuracy, reads per run, time, sequencing error rates
- Biology of the pathogen of interest
- What is your goal in sequencing?
  - Complete genome
  - Specific region or gene

True Diversity or Error?



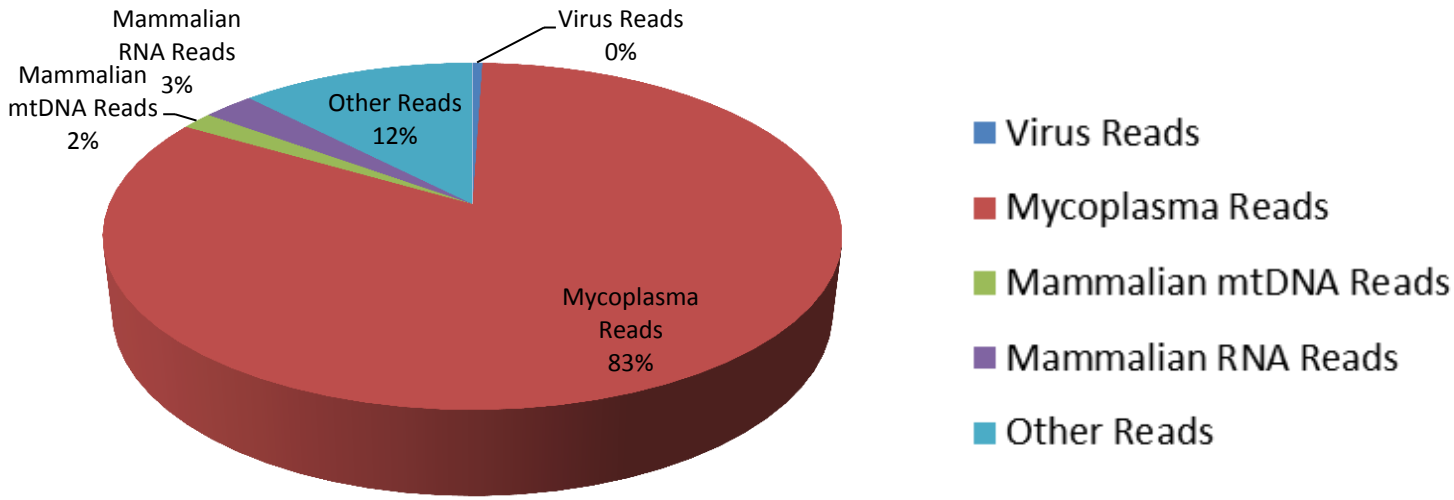
RNA polymerase
Error: 0.001%

454 substitution
Error: 0.03%

Consensus of Clusters to Smooth out errors

NGS: Things to Consider

- Sample preparation is important
  - Sequencing everything in the sample



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Summary

- Next Generation sequencing provides increasingly vital information not previously available
- NGS technologies becoming more commonly used in the field of infectious disease research
- Sequencing technologies, assembly and analyses tools rapidly improving

NGS Criteria to Consider

- Ultimate goal
- Sequencing platform(s)
  - Coverage level/depth
  - Read length
  - Error rates
- Sample preparation
- Confirmatory sequencing

Overview of Next Generation Sequencing (NGS) Technologies

Timothy Stockwell (JCVI)
Vivien Dugan (NIAID/NIH)

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Outline

- Some history of DNA sequencing
- Overview of NextGen Sequencing Technologies at JCVI
- Roche/454 Pyrosequencing
- LifeTechnologies/IonTorrent Semiconductor Sequencing
- Illumina/Solexa Sequencing By Synthesis (SBS)
- Other technologies

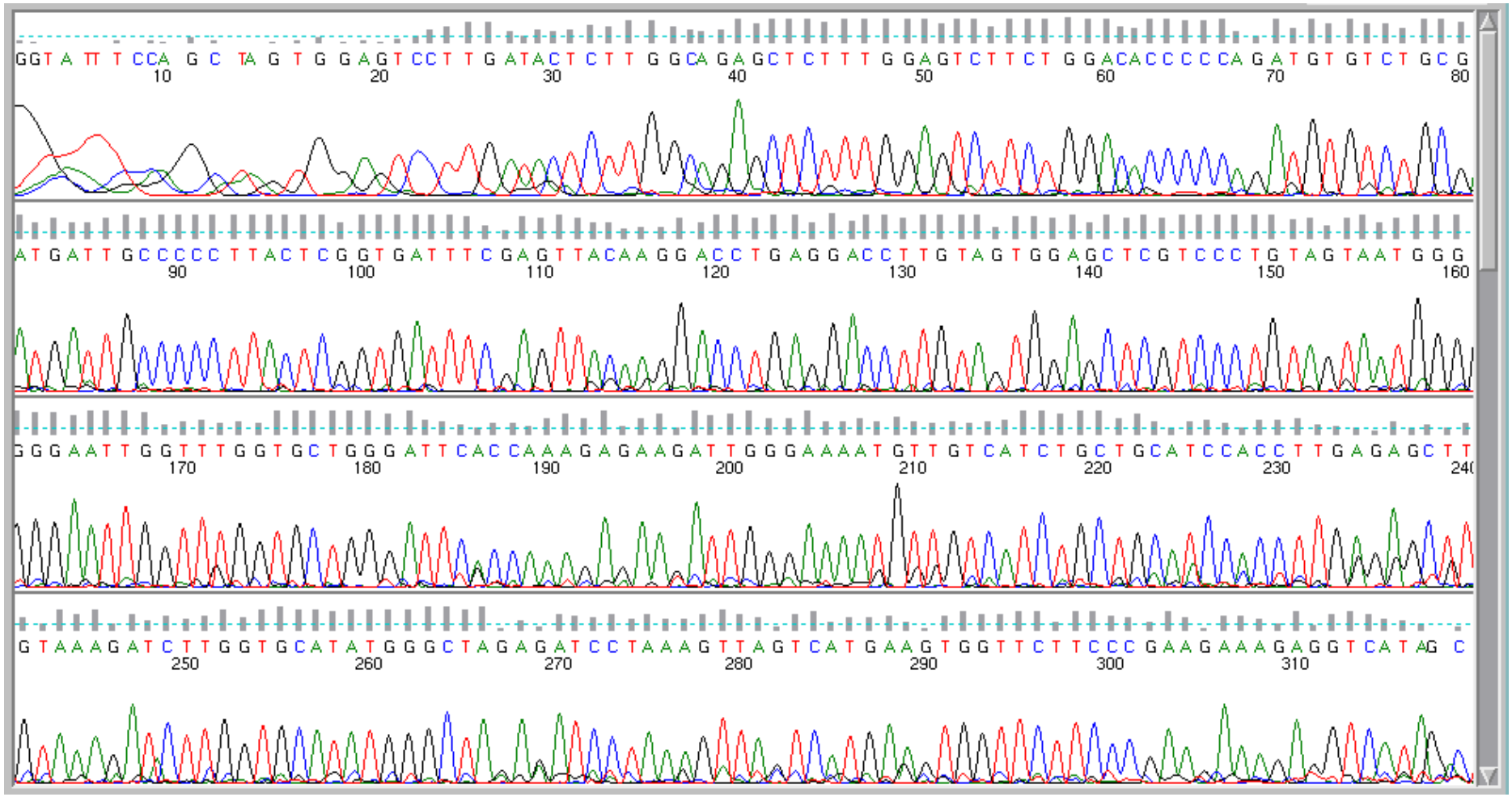
Review - Sanger Sequencing

- Randomly shear DNA, put it in a vector, and amplify with E. coli, or PCR amplify a region of a genome
- The Sanger sequencing reaction is like PCR, except there is only one primer, and in addition to regular nucleotides, there are also a small amount of dye labelled dideoxy nucleotides, with a distinct dye for each base
- As polymerase makes new ssDNA fragments, when a dye labelled dideoxy nucleotide is added, extension stops, and the fragment is labelled with a dye corresponding to the last base added.

Review - Sanger Sequencing

- Over many cycles, fragments of all the different lengths are formed, with each length fragment ending with the dye corresponding to the base at that position
- Capillary electrophoresis in polyacrylamide gel is used to separate the fragments by length and pass them by a laser and reader to interrogate the base at each position
- The result is a chromatogram, that is then “base called” using algorithms to output the most likely base at each position, usually with an indication of accuracy of the base call.

A chromatogram



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Sanger Sequencing

- Think about the issues of scaling Sanger sequencing to obtain 1 million reads
- The E. coli clones or PCR reactions need separated wells – 2600 384-well plates
- To read the DNA from both ends, need double the number of wells, and have to keep track of mate pairs – 5200 384-well plates
- Also think about storage, pipet tips, labor required, etc.
- So then came along Next Generation Sequencing (NGS) Technologies

NextGen Sequencing Technologies

454 GS FLX

Illumina HiSeq 2000

Illumina MiSeq

Ion PGM

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Sequencing Technologies in Use at JCVI

	Read length bp	Throughput /machine run	Run time	Throughput /day	Accuracy
ABI 3730xl	600-800	75,000bp	30-60 min	1-2 Mb	> QV 30
454	400-600	400 Mb	7 hr	800 Mb	QV 20
Illumina HiSeq	up to 100	up to 600 Gb	up to 12 days	50 Gb	~80% bases > QV30
Illumina MiSeq	up to 250	up to 8.5 Gb	up to 39 hours	5.2 Gb	~75% bases ➢QV30
Ion Torrent	~150	900 Mb	up to 4.5 hours		80%bases > QV20

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Roche 454 Sequencing

- Library Construction
- Sequencing Process Overview

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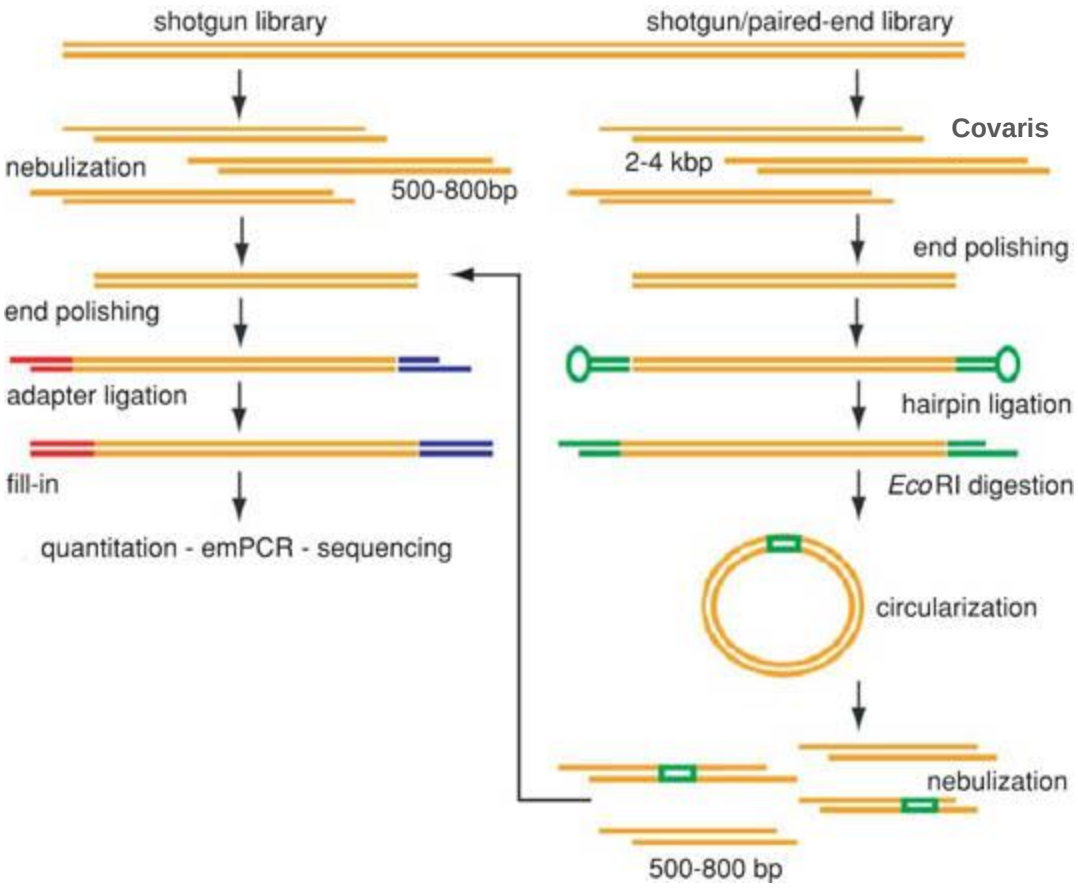
454 Library construction



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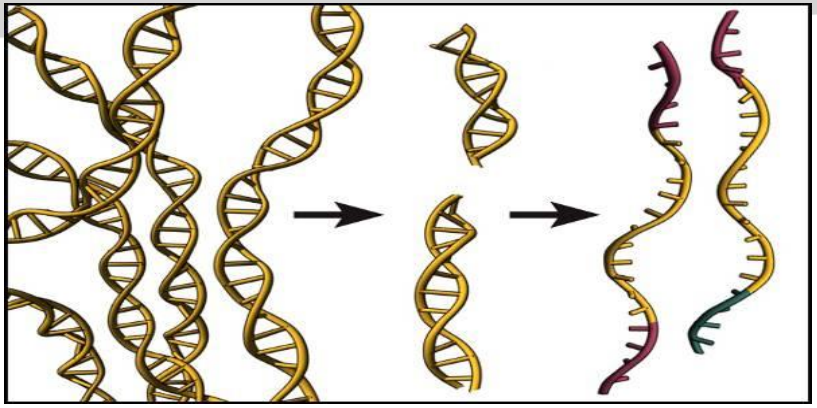
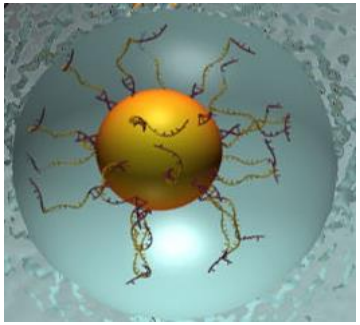
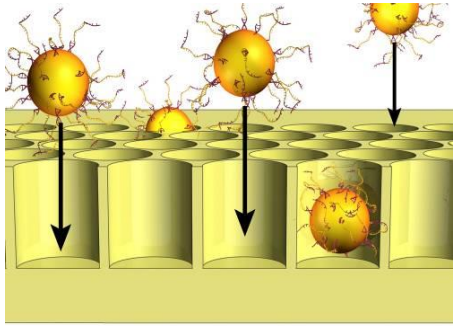
454 Massively Parallel Pyrosequencing
Process Overview



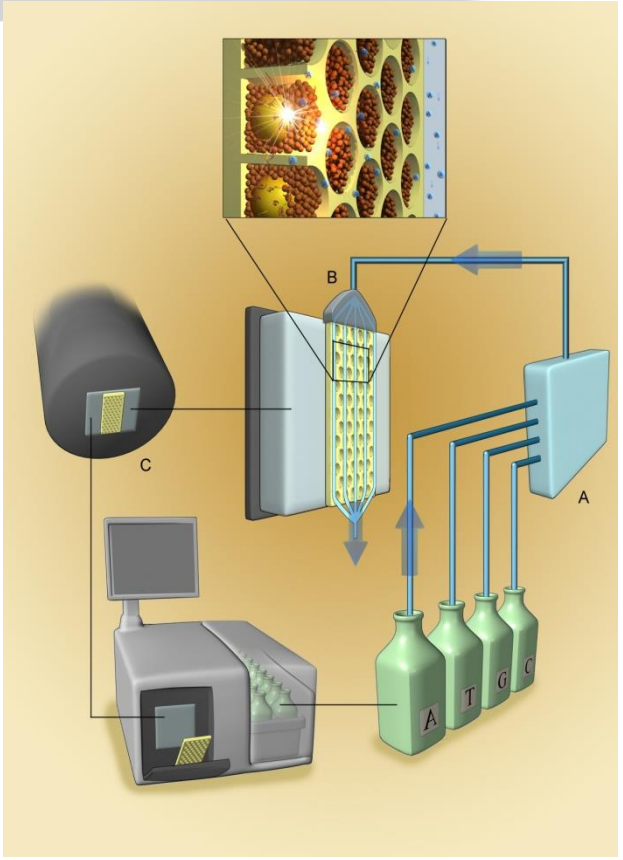
1) ssDNA library preparation



2) emPCR amplification



3) Load beads & enzymes in PicoTiter Plate™



4) Perform sequencing by synthesis on the 454 instrument

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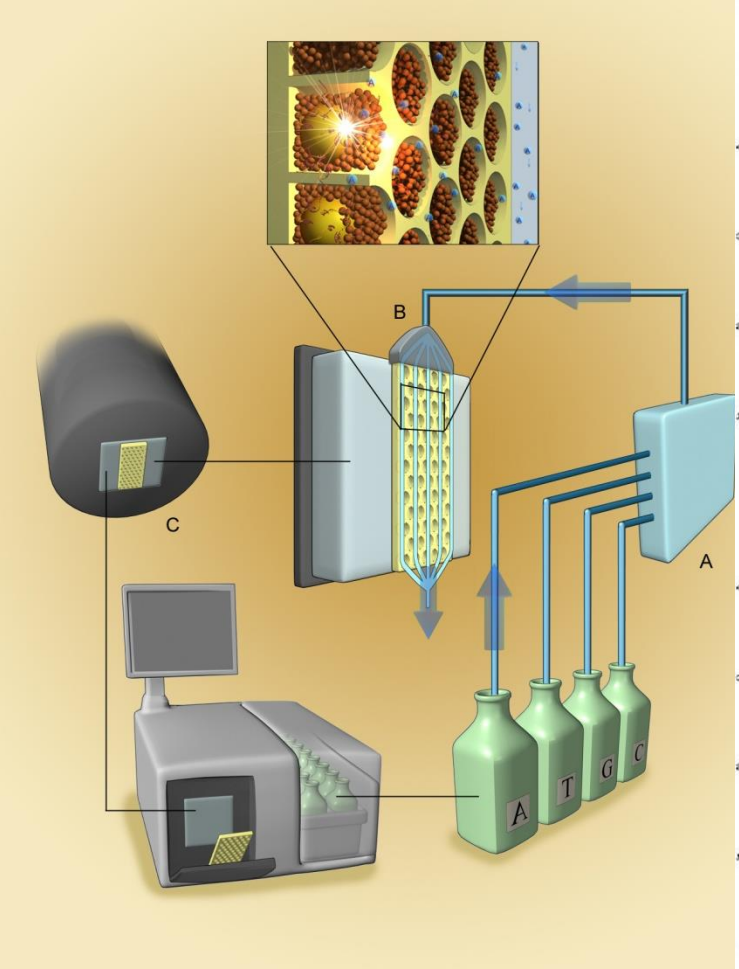
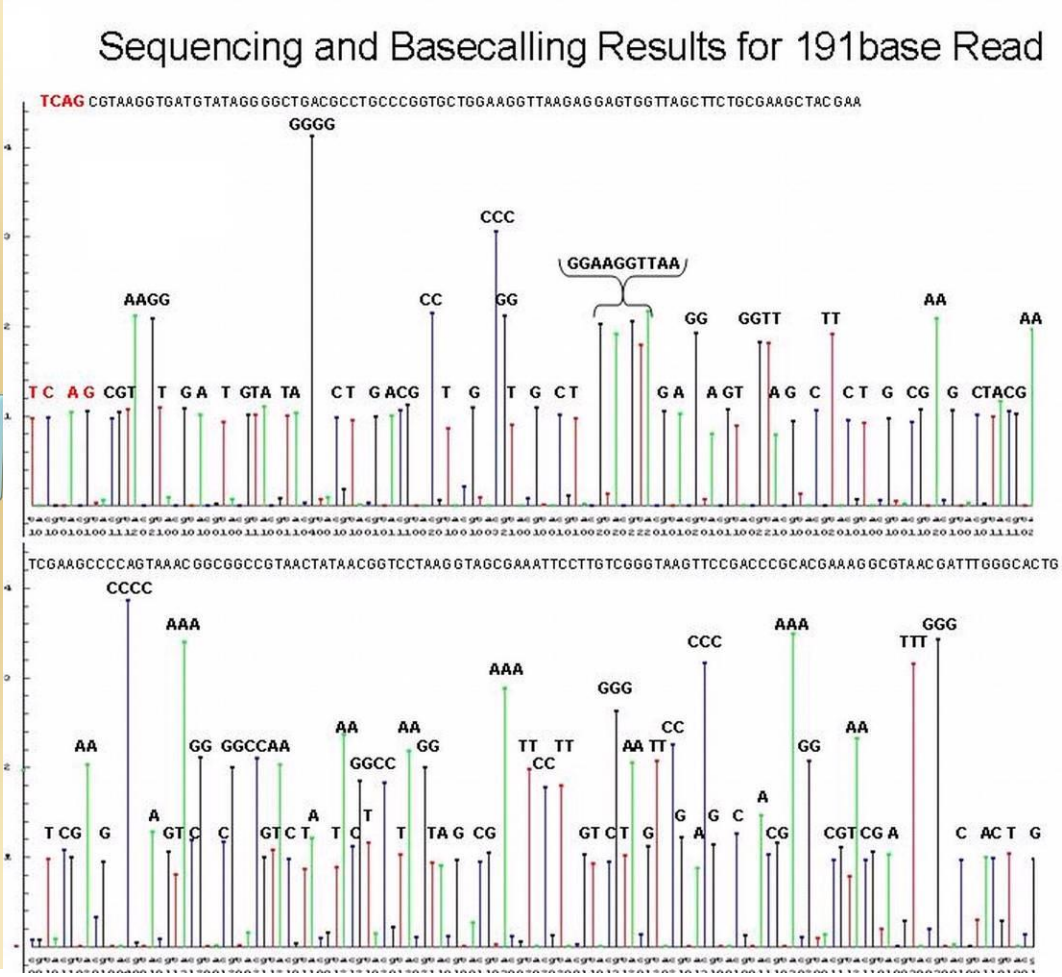
454 Instrument and Data Output





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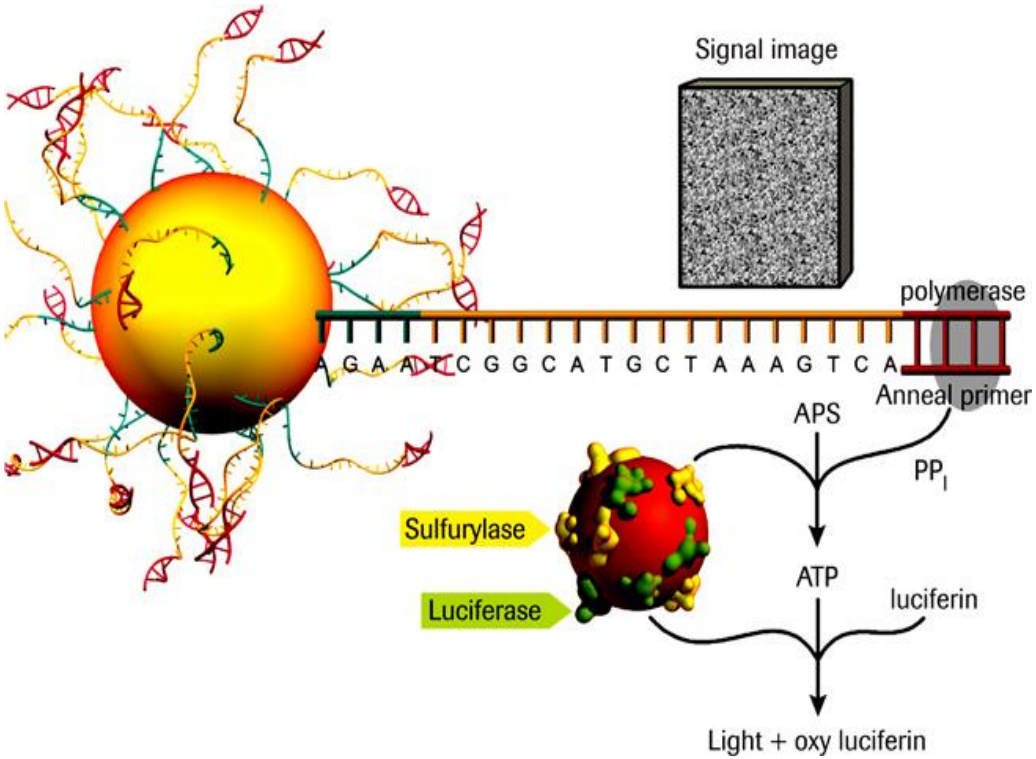
454 Sequencing Workflow
Sequencing by Synthesis



- Bases (TACG) are flowed sequentially and always in the same order (100 times for a large GS FLX run) across the PicoTiterPlate device during a sequencing run.
- A nucleotide complementary to the template strand generates a light signal.
- The light signal is recorded by the CCD camera.
- The signal strength is proportional to the number of nucleotides incorporated.

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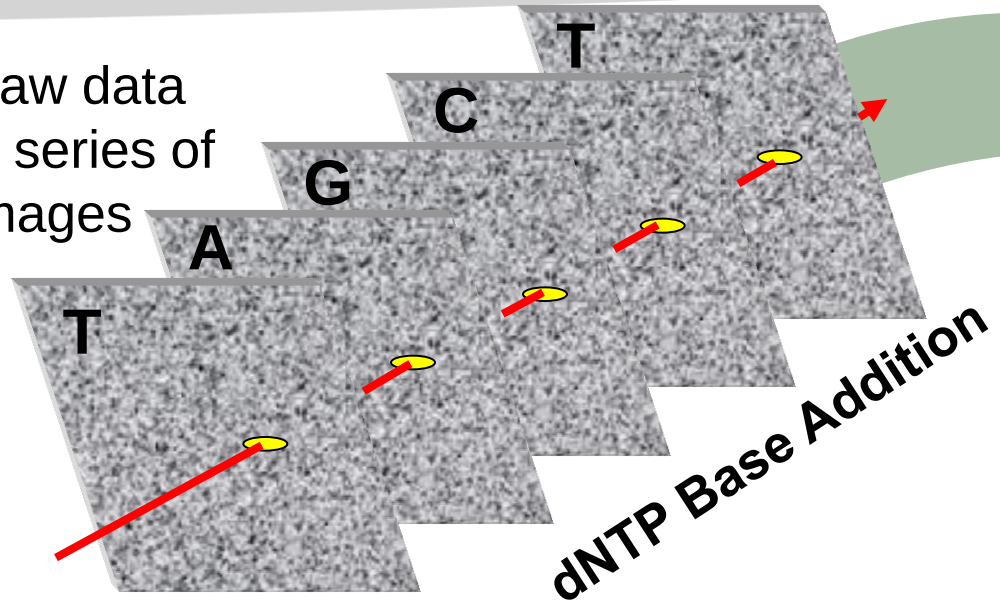
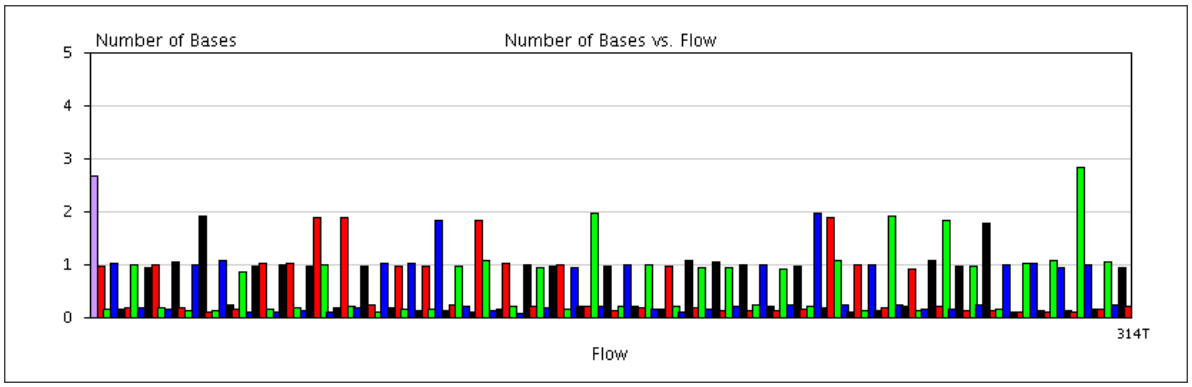
454 GS FLX Data
Image Processing Overview

1. Raw data is series of images



1. Each well's data extracted, quantified and normalized



1. Read data converted into "flowgrams"

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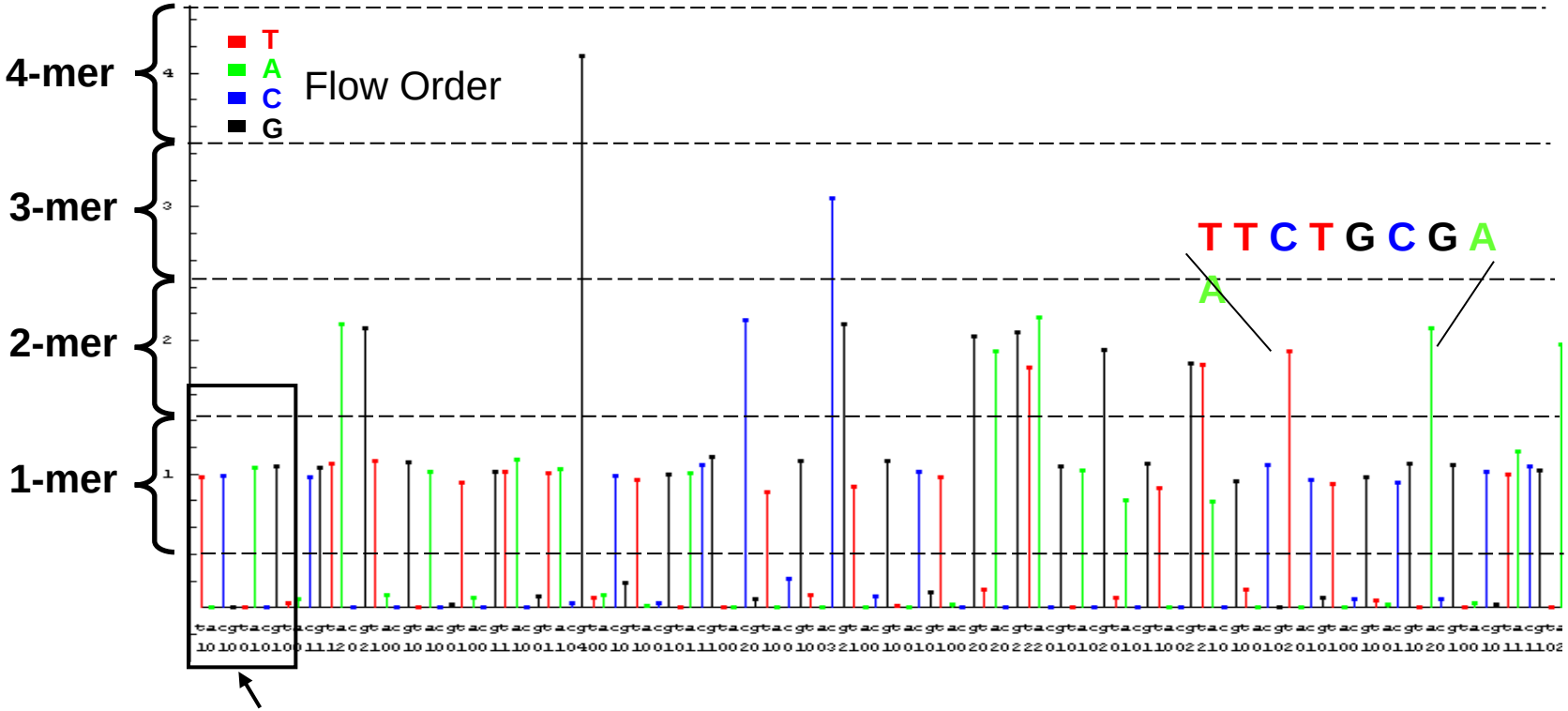
454 GS FLX Data
Flowgram Generation



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454 GS FLX Plate

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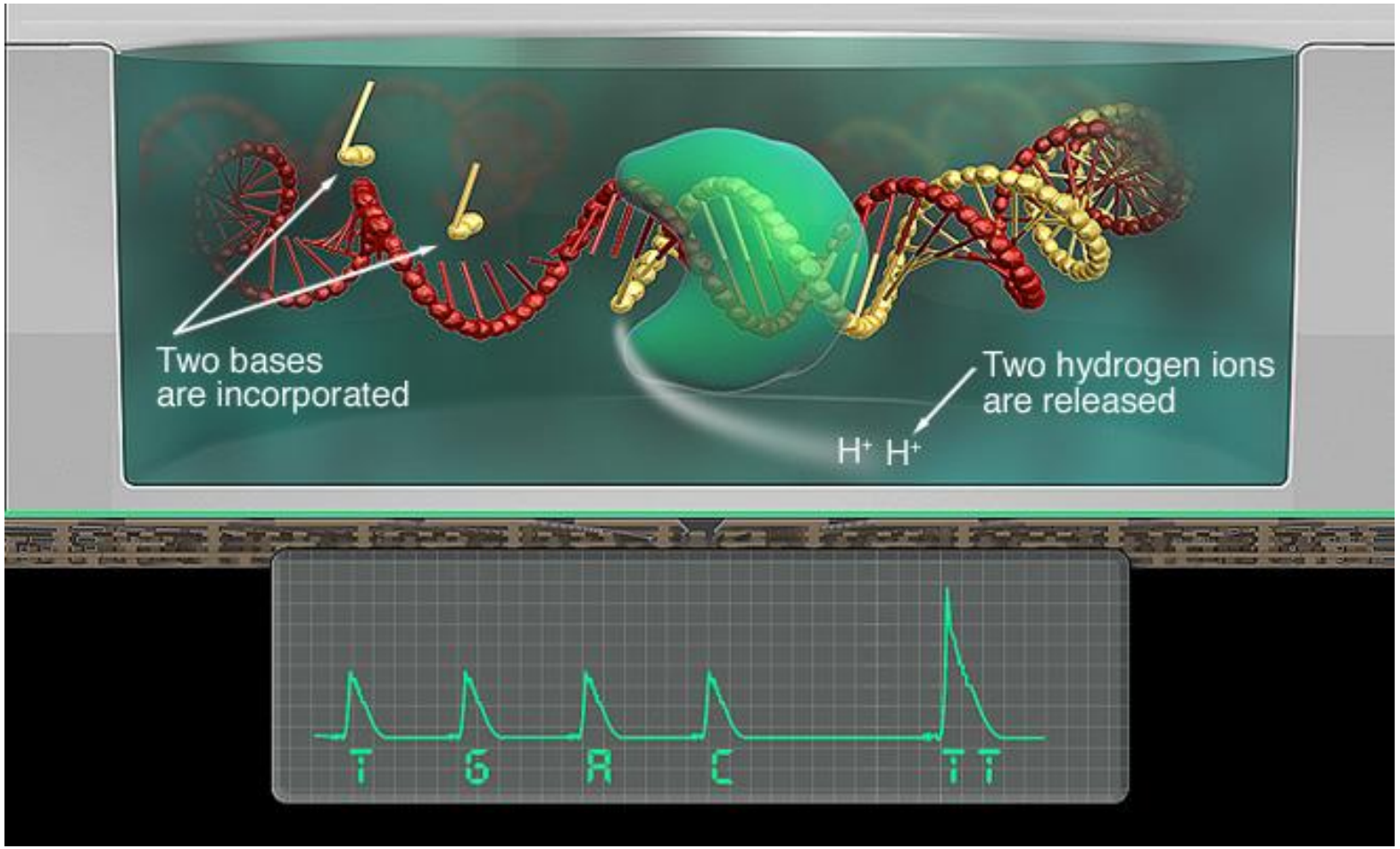
454 GS FLX Sequencer

Ion Torrent Sequencing

- Similar to 454, but rather than creating fluorescence and measuring light, Ion Torrent instead measures pH changes due to protons released during base incorporation
- The Ion Torrent chips are a massively parallel array of the world's smallest pH meters
- As a semiconductor device, Ion Torrent has been able to make there chips denser and denser (more and more wells), following the trend of the electronics industry

Ion Torrent Sequencing



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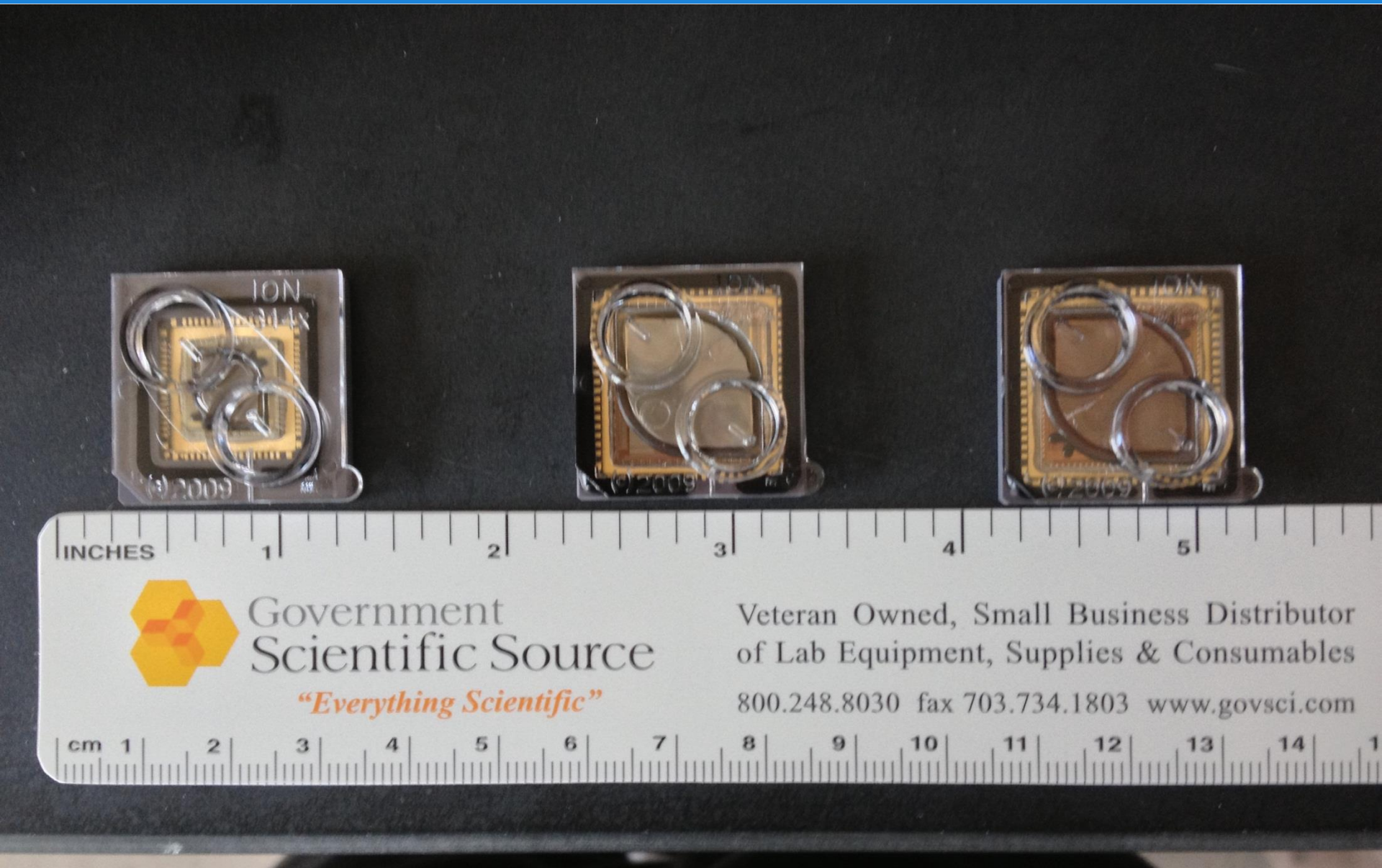
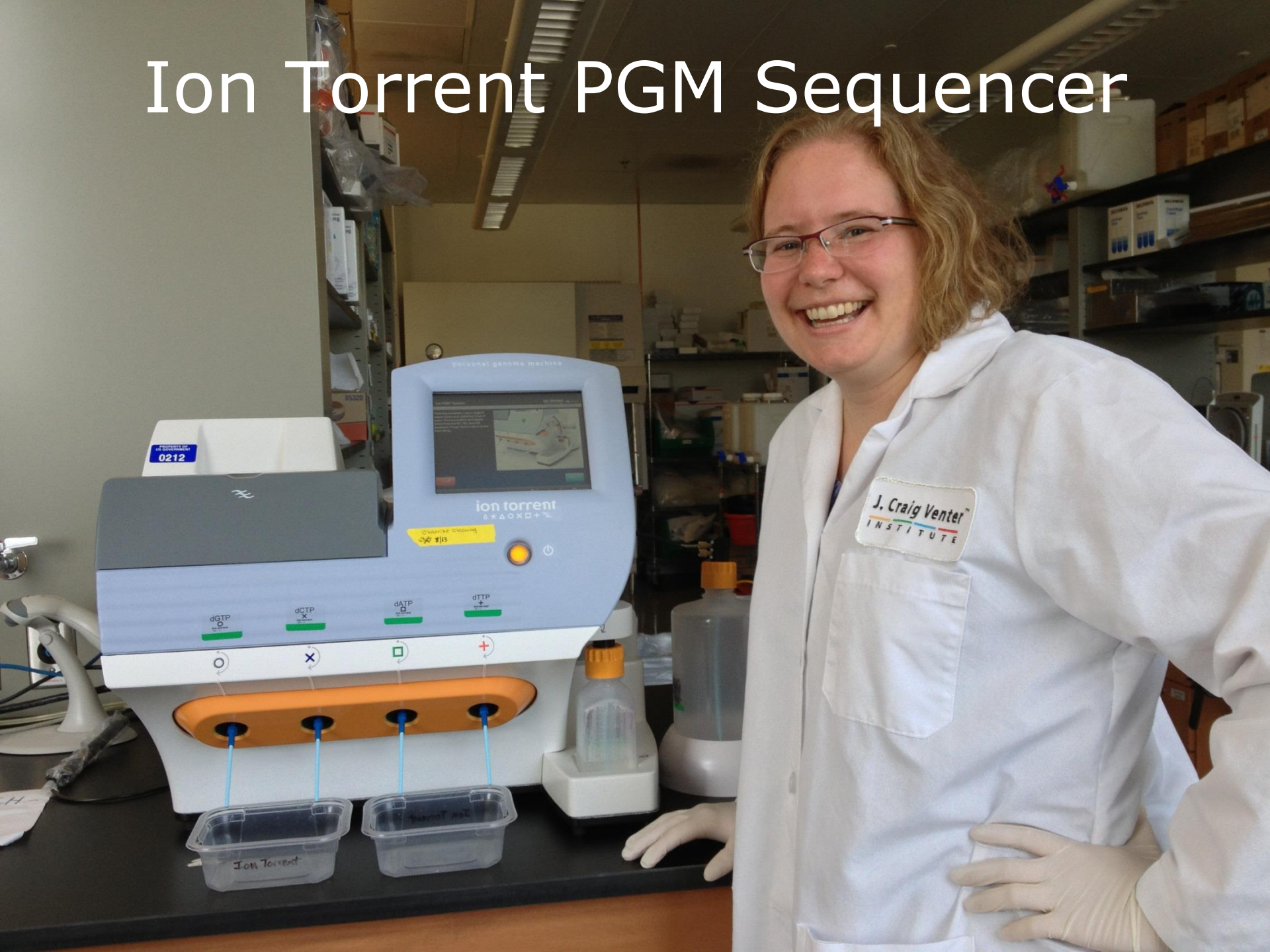
Ion Torrent Chips



Ion Torrent PGM Sequencer



Illumina Sequencing

- Technology Overview
- Mate Pair Library Construction

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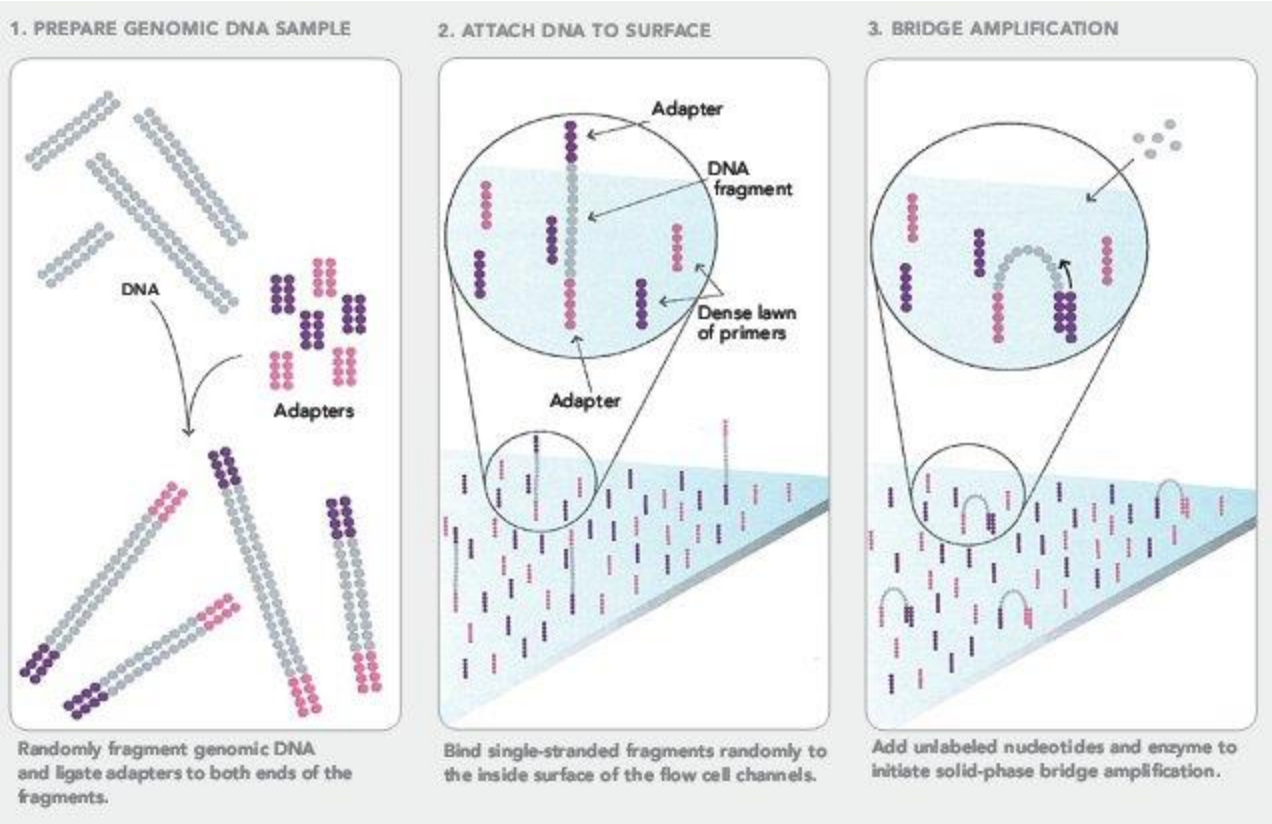
Illumina Technology Overview (1)



http://seqanswers.com/forums/showthread.php?t=21v

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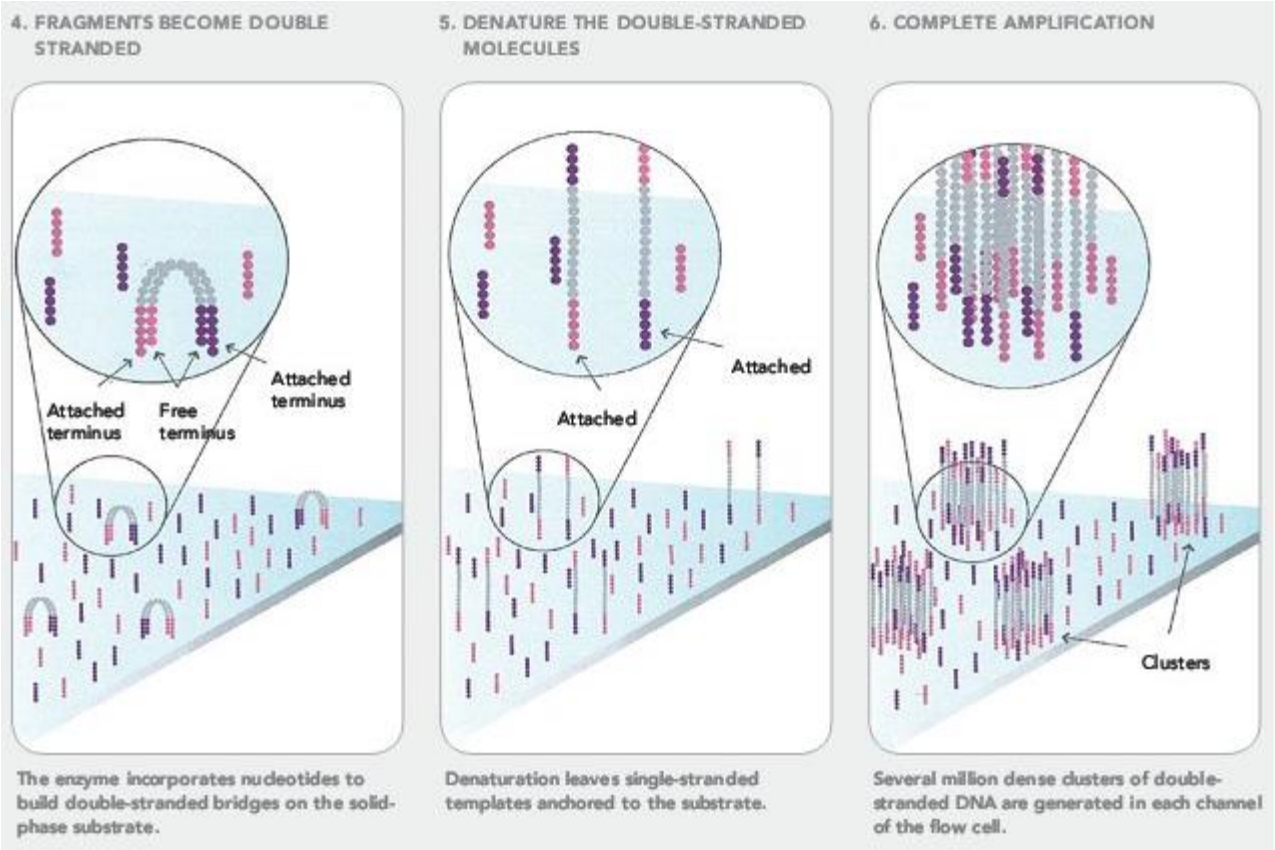
Illumina Technology Overview (2)



http://seqanswers.com/forums/showthread.php?t=21v

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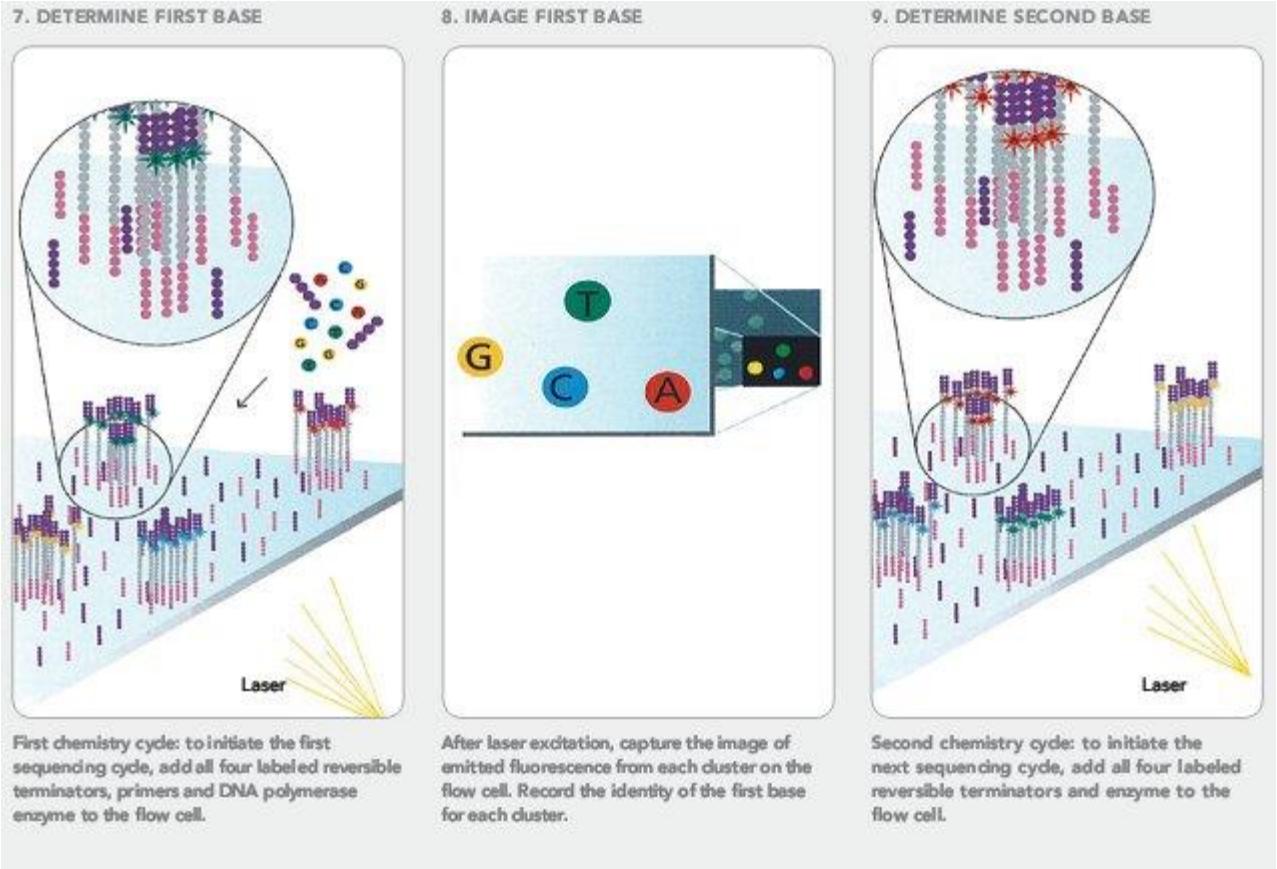
Illumina Technology Overview (3)



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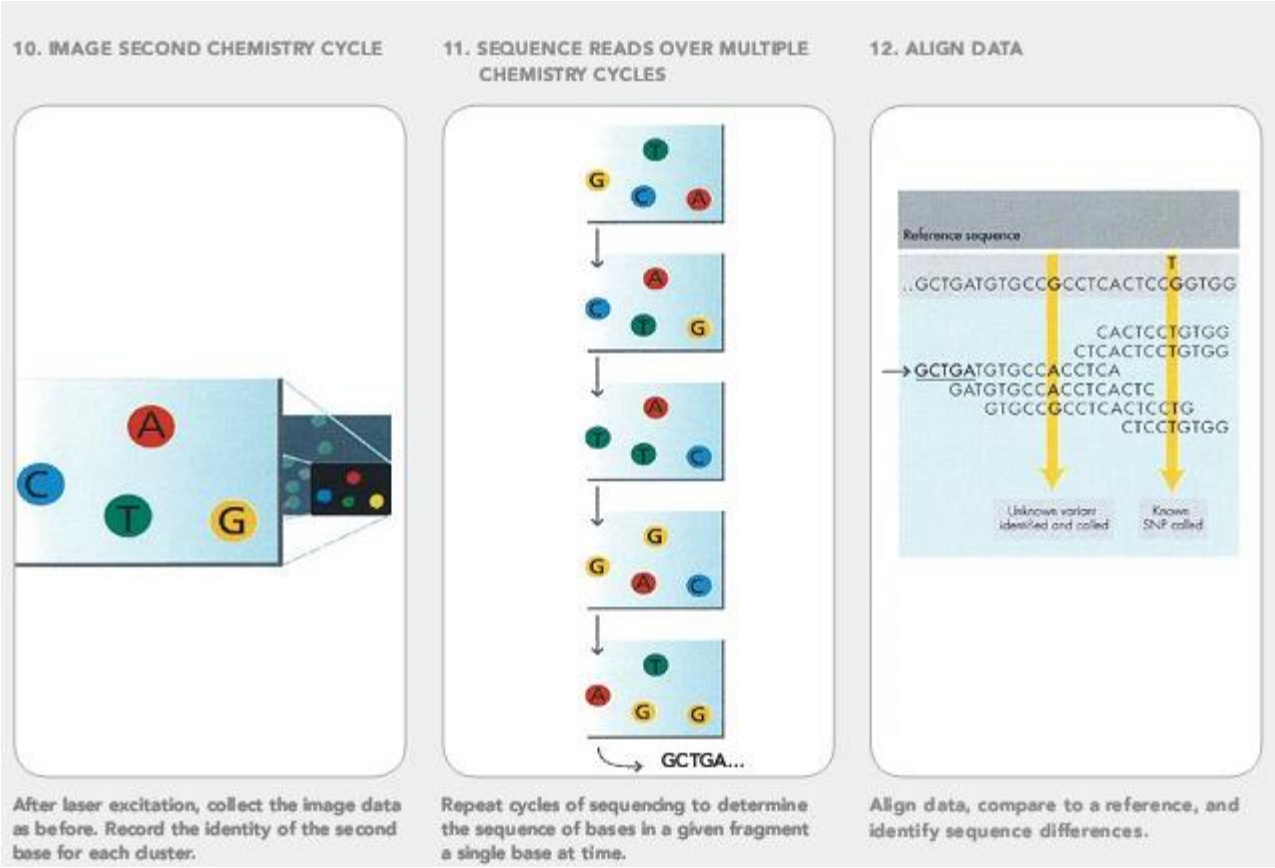
Illumina Technology Overview (4)



http://seqanswers.com/forums/showthread.php?t=21v

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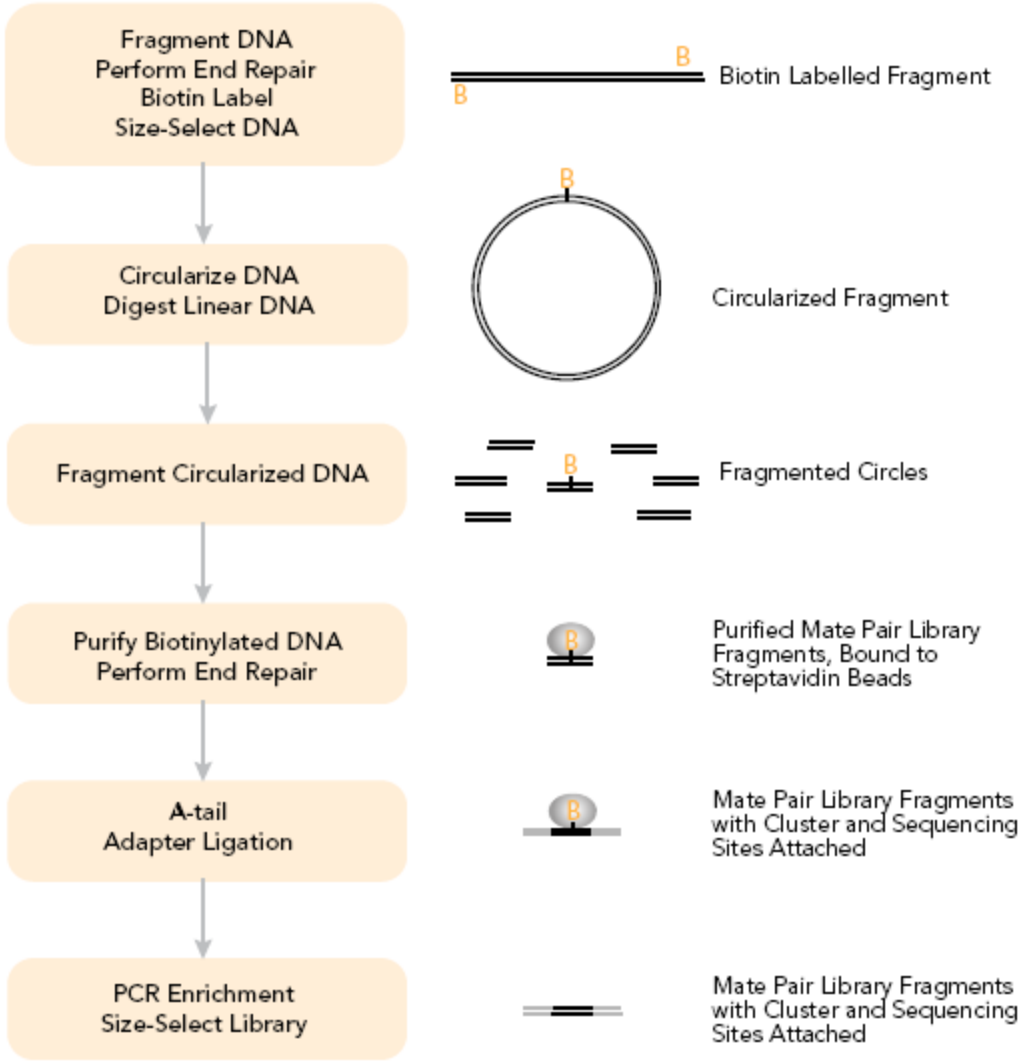
Illumina Mate Pair Library Construction



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Illumina Flow Cells

Illumina MiSeq Sequencer

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Illumina HiSeq Sequencer

Other Technologies

- **Pacific Biosciences** – single molecule sequencing, measures the incorporation of a single dye labelled base at a time, by laser-excitation of an *extremely* small volume that contains the polymerase and the DNA
- ***Oxford Nanopore*** – single molecule sequencing, measures the electrical changes in a pore that arise when bases enter and exit the pore.

Readings

- Zagori et al. (2012) **Read length versus depth of coverage for viral quasispecies reconstruction**. *PloS One 7(10):e47046*
- Quail et al. (2012) **A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers** *BMC Genomics 13:341*
- Liu et al. (2012) **Comparison of Next-Generation Sequencing Systems** *J. Biomed Biotechnol July 5.*
- Metzker (2010) **Sequencing technologies – The next generation**. *Nature Reviews Genetics 11:31*
- Harismendy et al. (2009) **Evaluation of next generation sequencing platforms for population targeted sequencing studies.** *Genome Biology 10:R32*
- Nagarajan and Pop (2010) **Sequencing and genome assembly using next-generation technologies.** *Computational Biology, Methods in Molecular Biology Vol. 673*