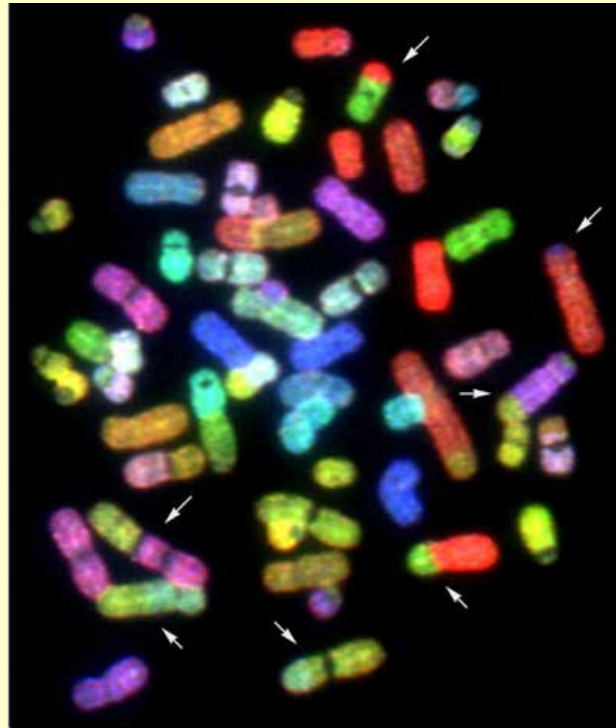


Next Generation Sequencing

Genomics & Medicine

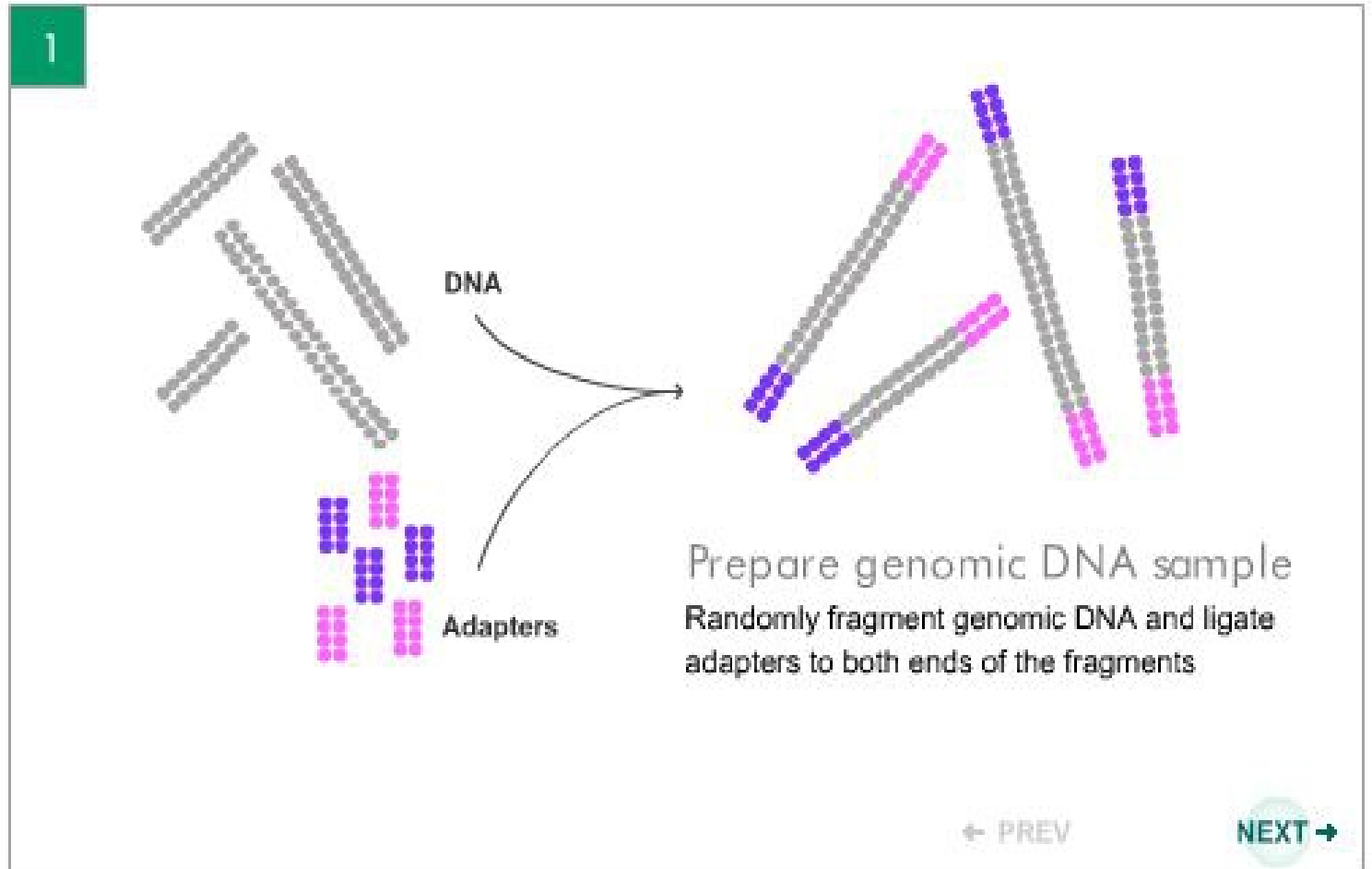
<http://biochem118.stanford.edu/>



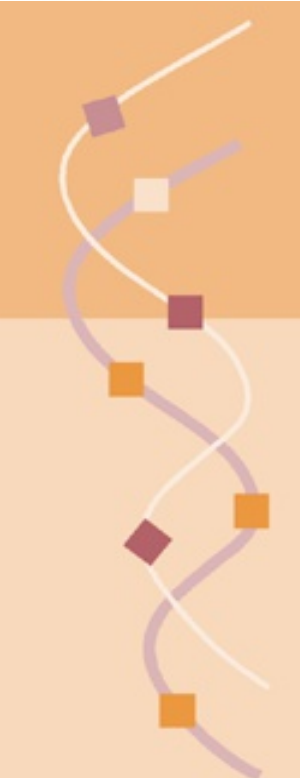
Doug Brutlag, Professor Emeritus
of Biochemistry & Medicine (by courtesy)
Stanford University School of Medicine

Illumina Solexa Sequencing Technology

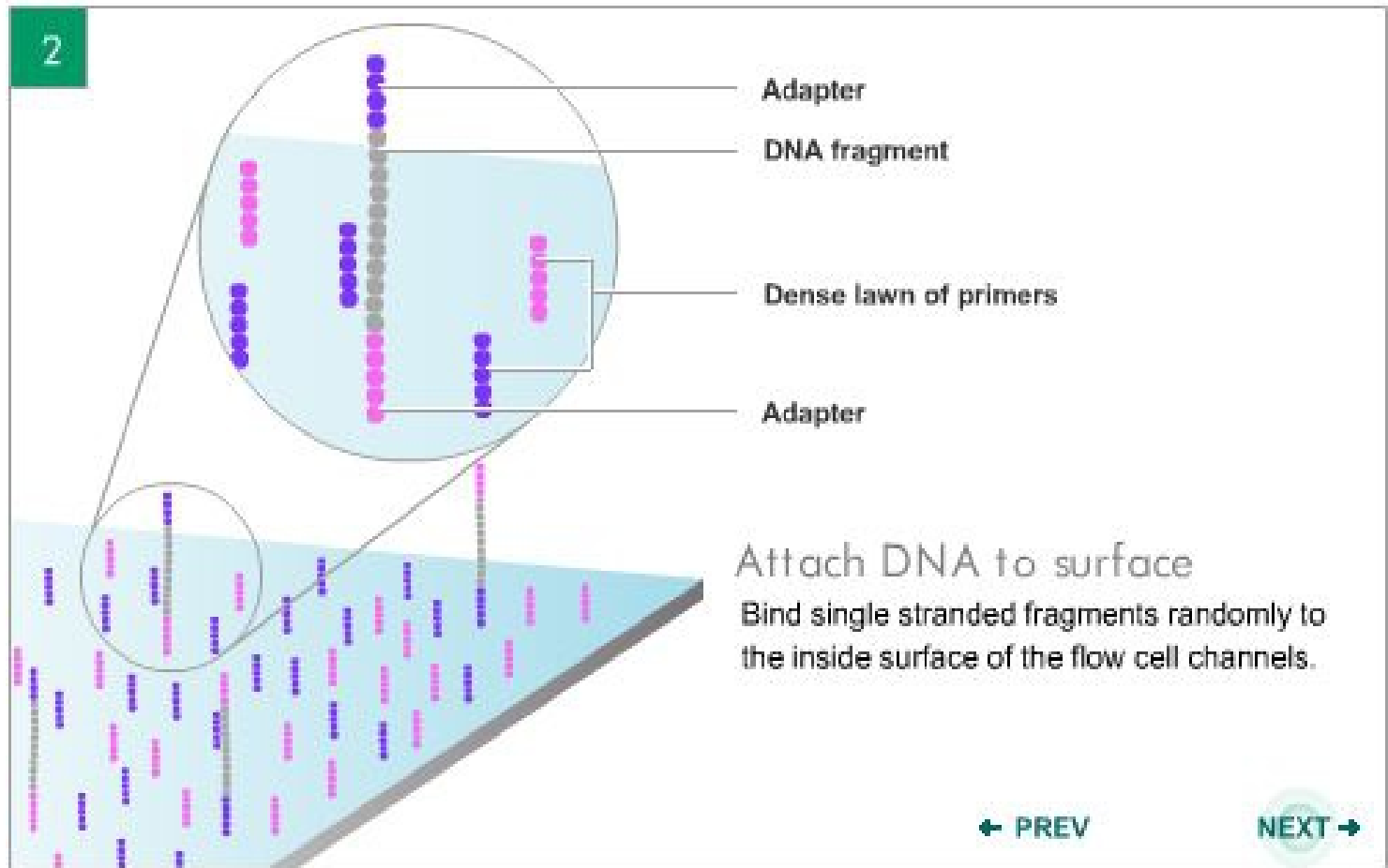
Sequencing-By-Synthesis Demo



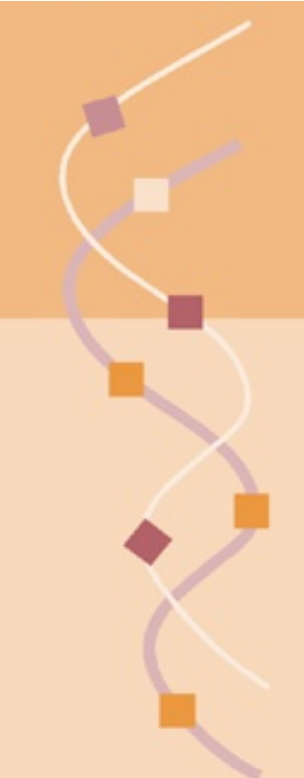
Illumina Solexa Sequencing Technology



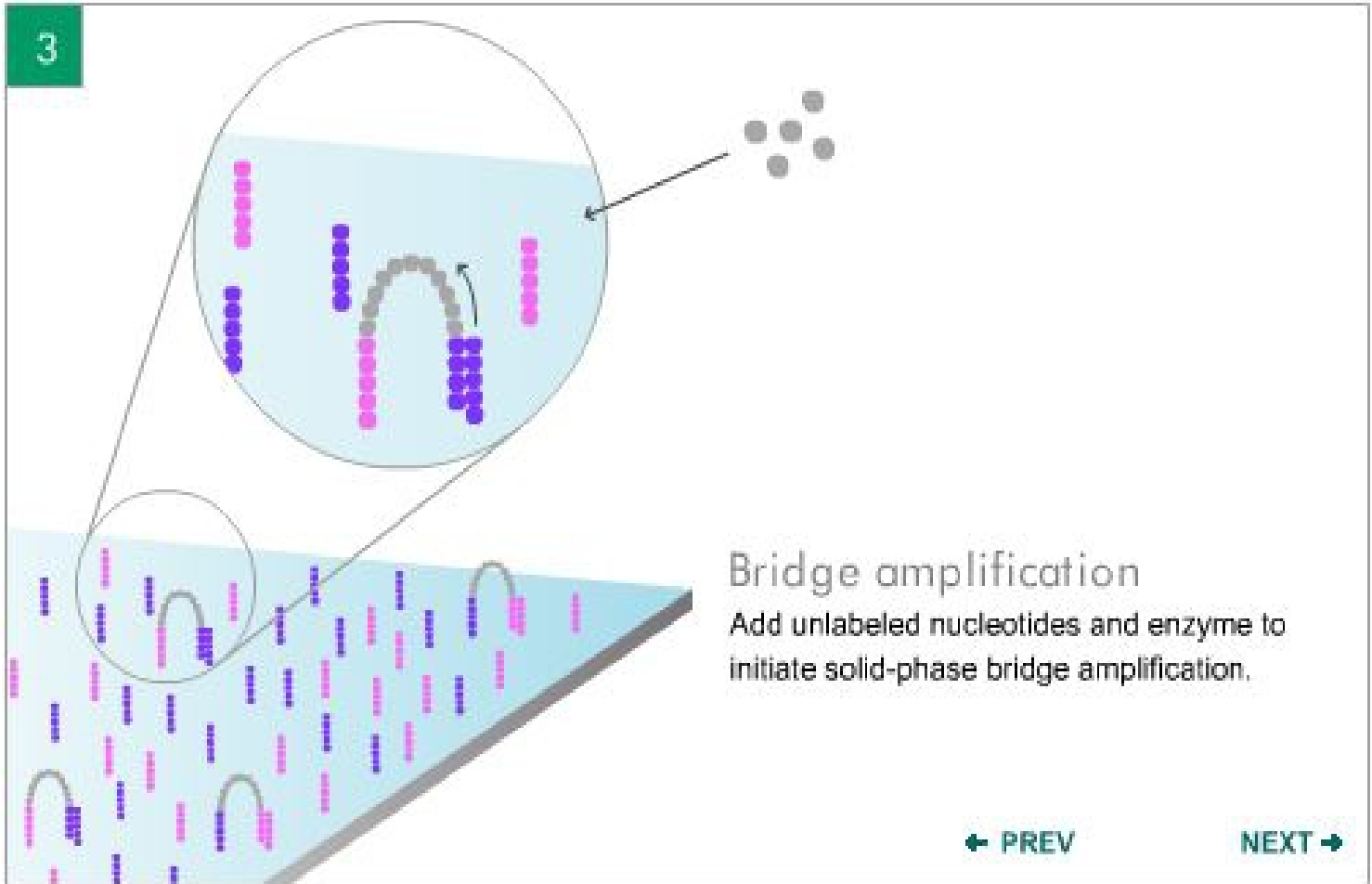
Sequencing-By-Synthesis Demo



Illumina Solexa Sequencing Technology

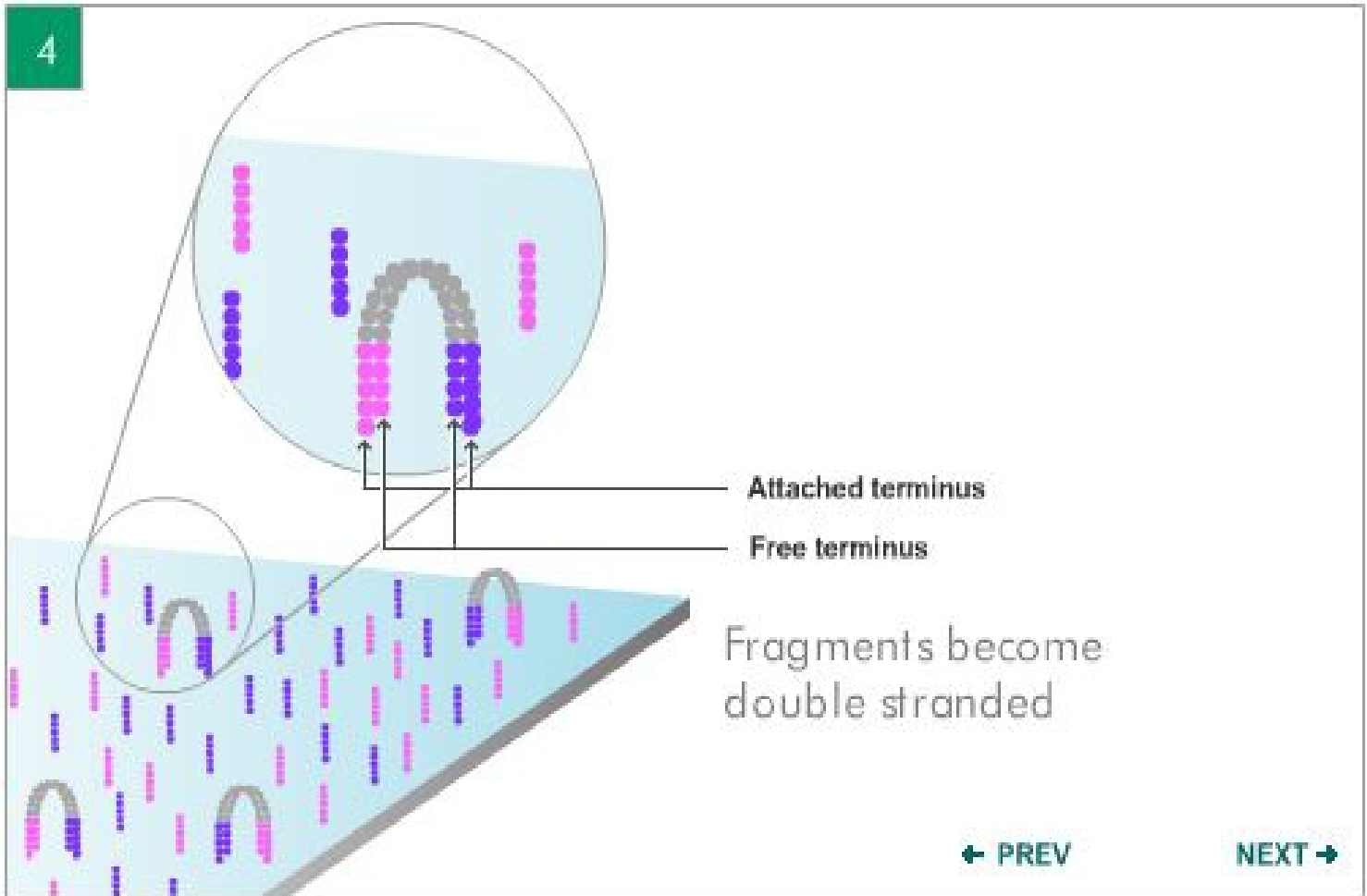


Sequencing-By-Synthesis Demo

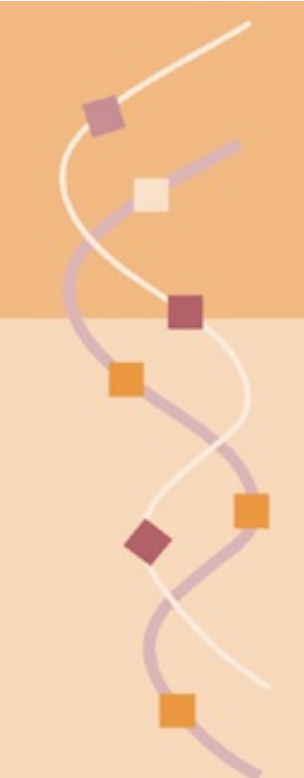


Illumina Solexa Sequencing Technology

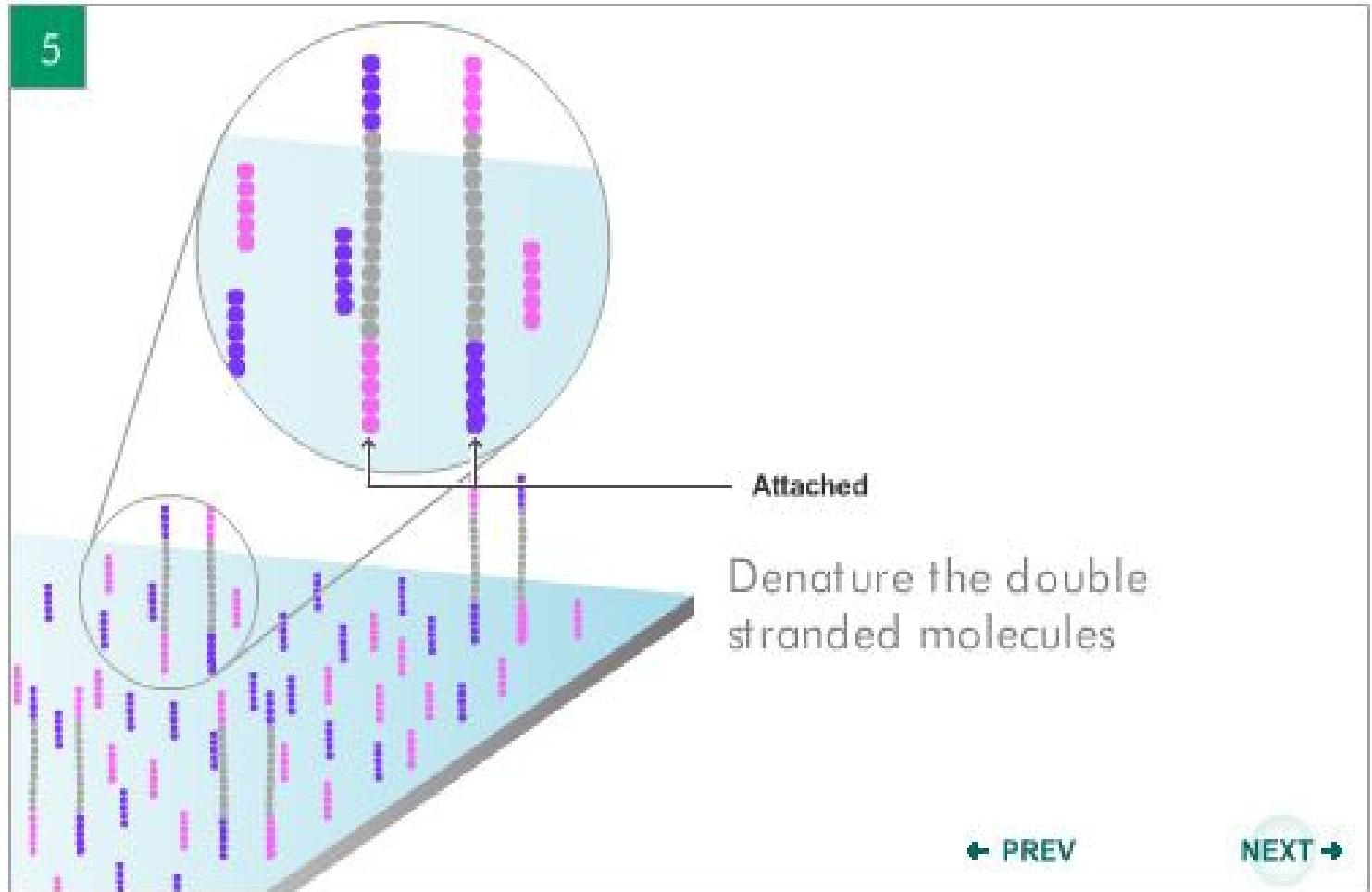
Sequencing-By-Synthesis Demo



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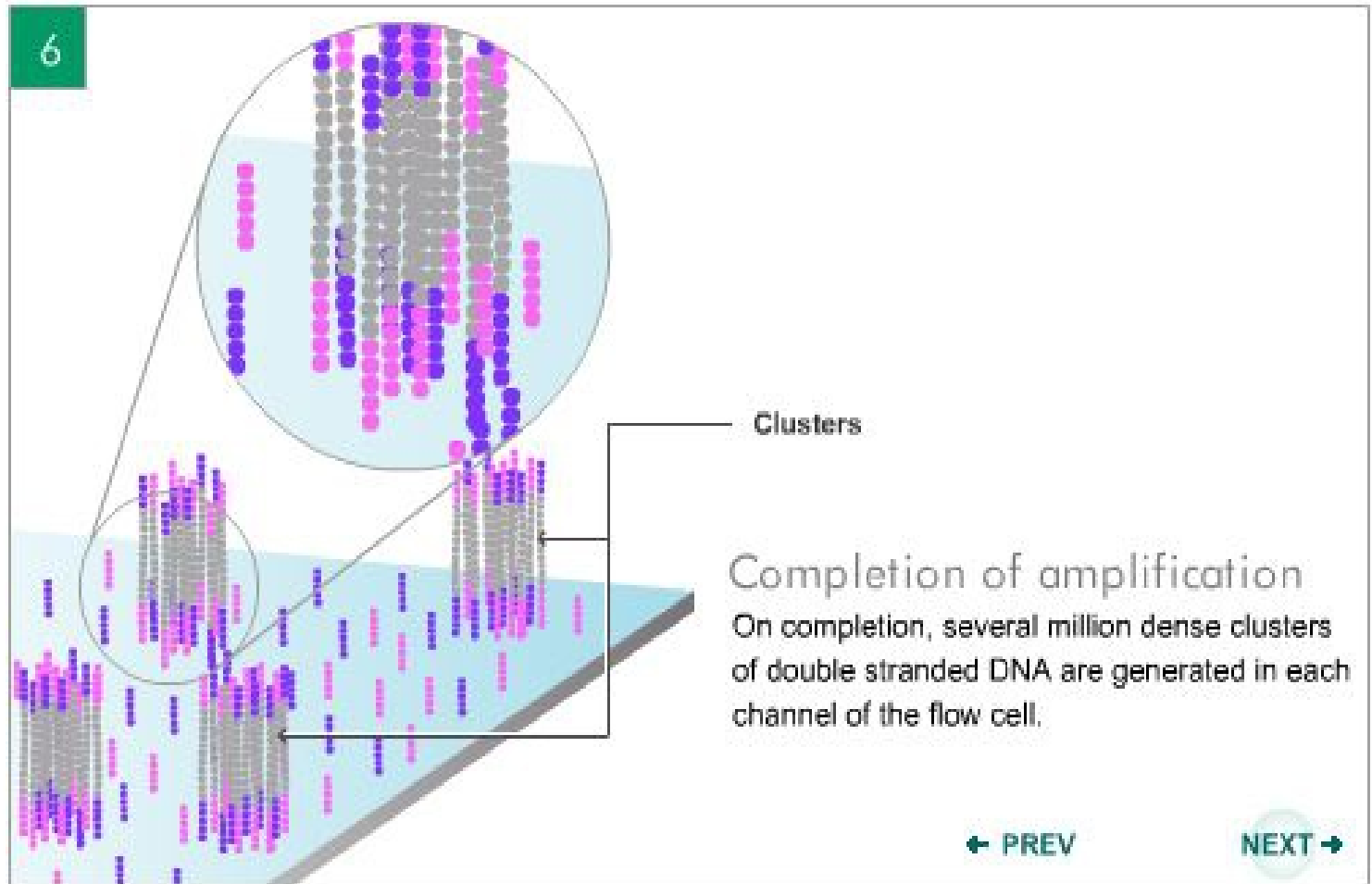
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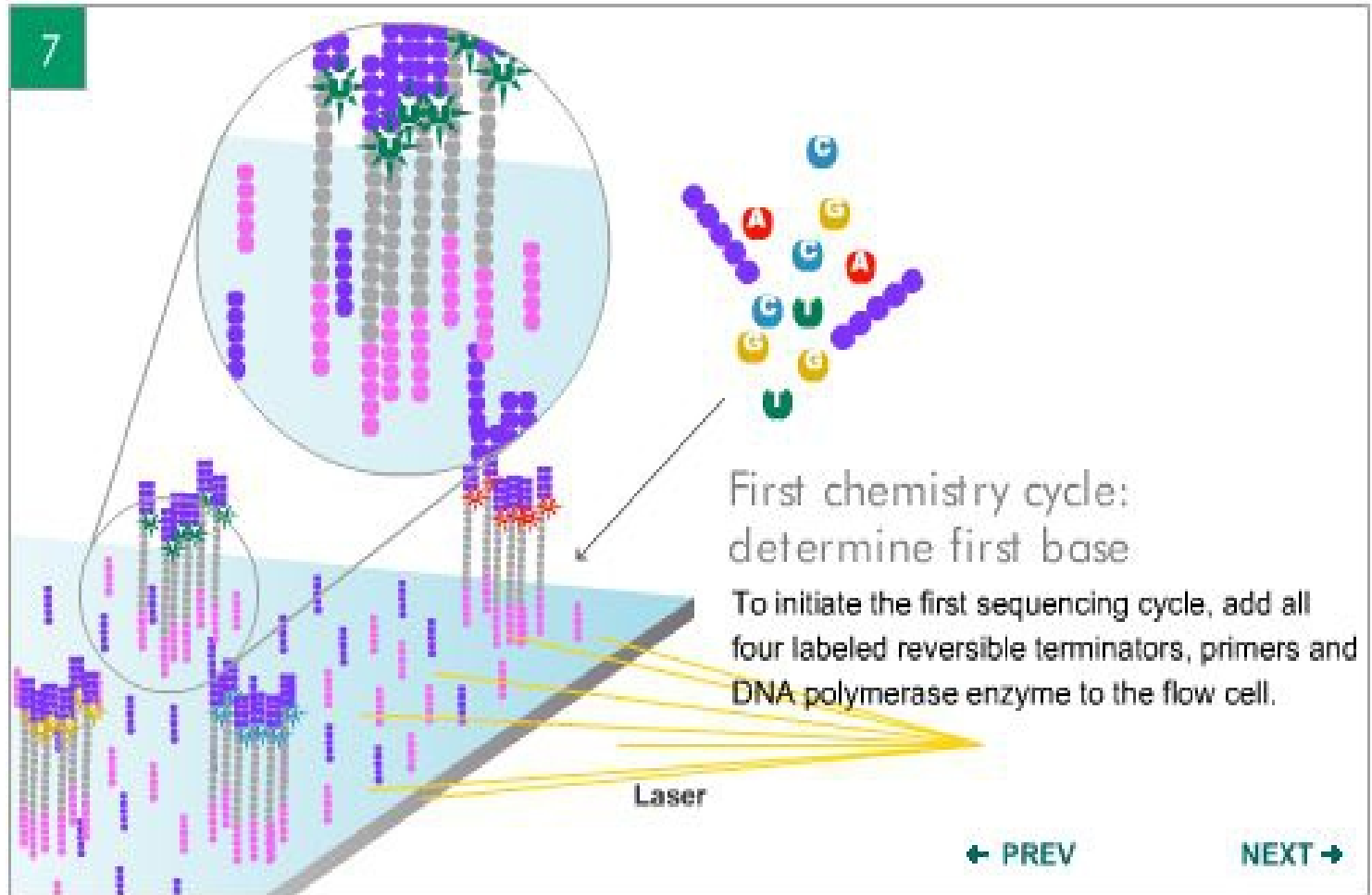
Sequencing-By-Synthesis Demo



Illumina Solexa Sequencing Technology



Sequencing-By-Synthesis Demo



Illumina Solexa Sequencing Technology



Sequencing-By-Synthesis Demo

8

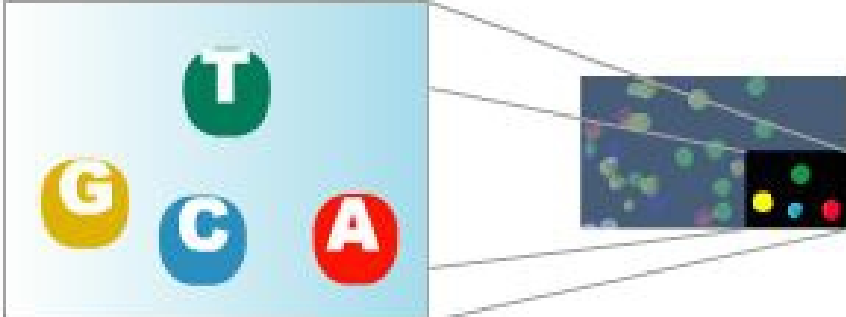


Image of first chemistry cycle

After laser excitation, capture the image of emitted fluorescence from each cluster on the flow cell. Record the identity of the first base for each cluster.

Before initiating the next chemistry cycle

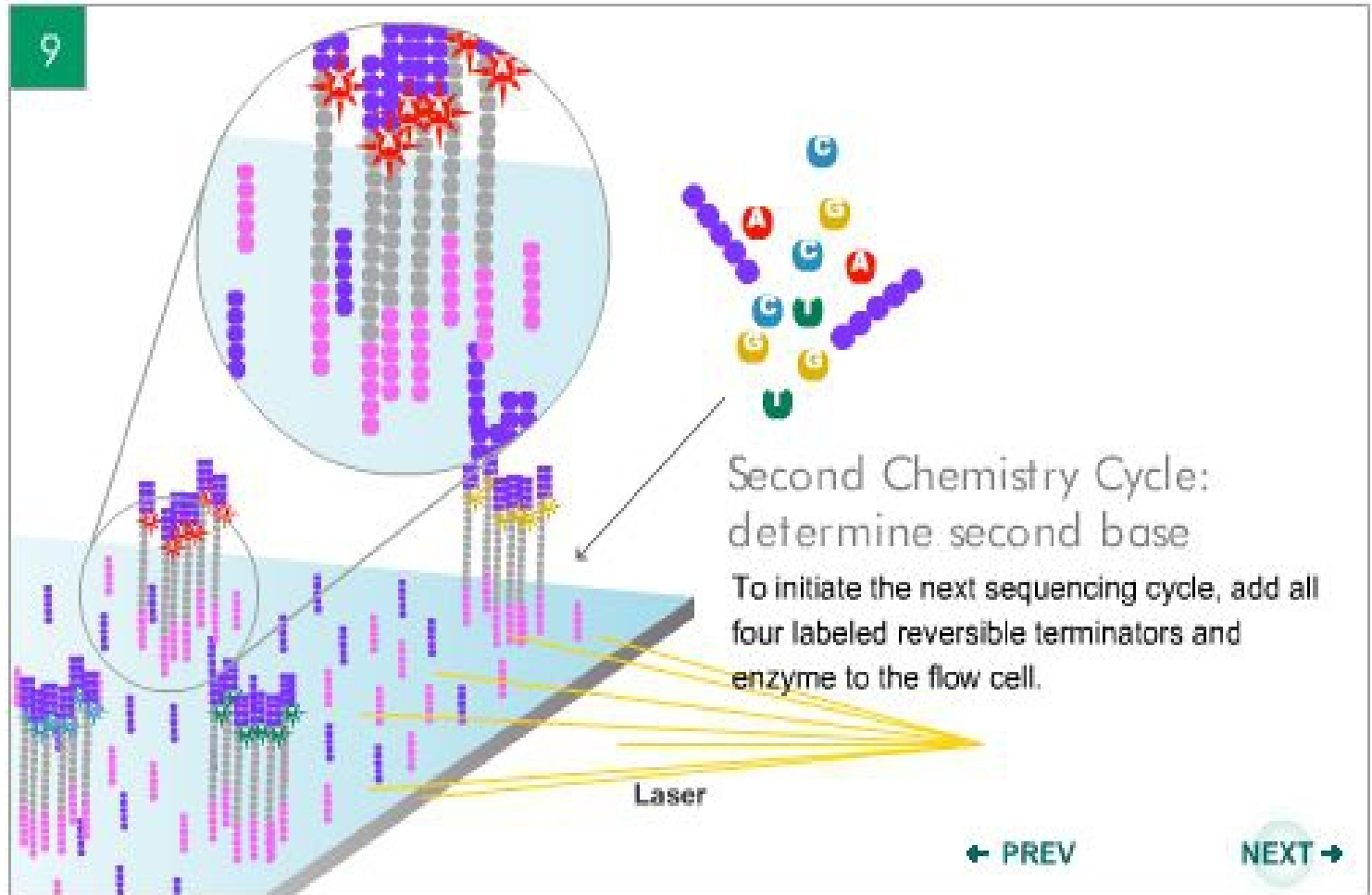
The blocked 3' terminus and the fluorophore from each incorporated base are removed.

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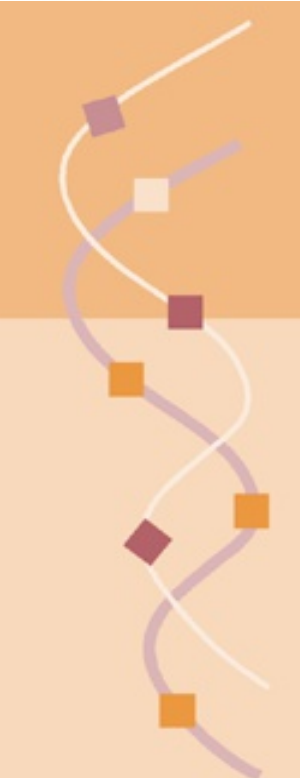
Illumina Solexa Sequencing Technology



Sequencing-By-Synthesis Demo



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Sequencing-By-Synthesis Demo

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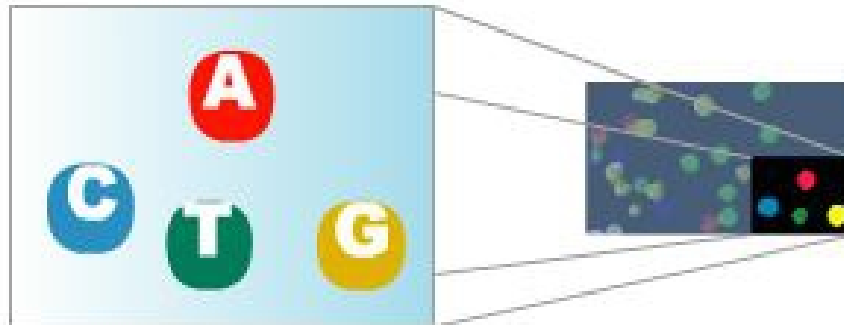
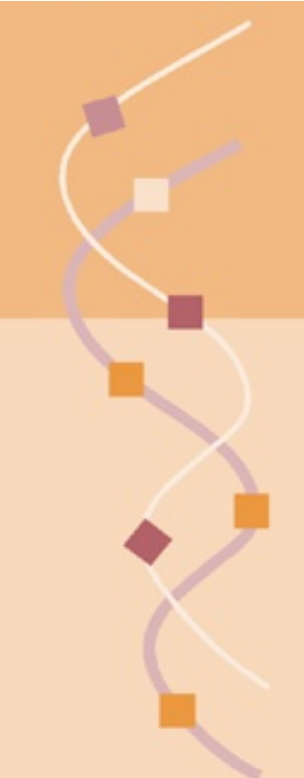


Image of second chemistry cycle is captured by the instrument
 After laser excitation, collect the image data as before. Record the identity of the second base for each cluster.

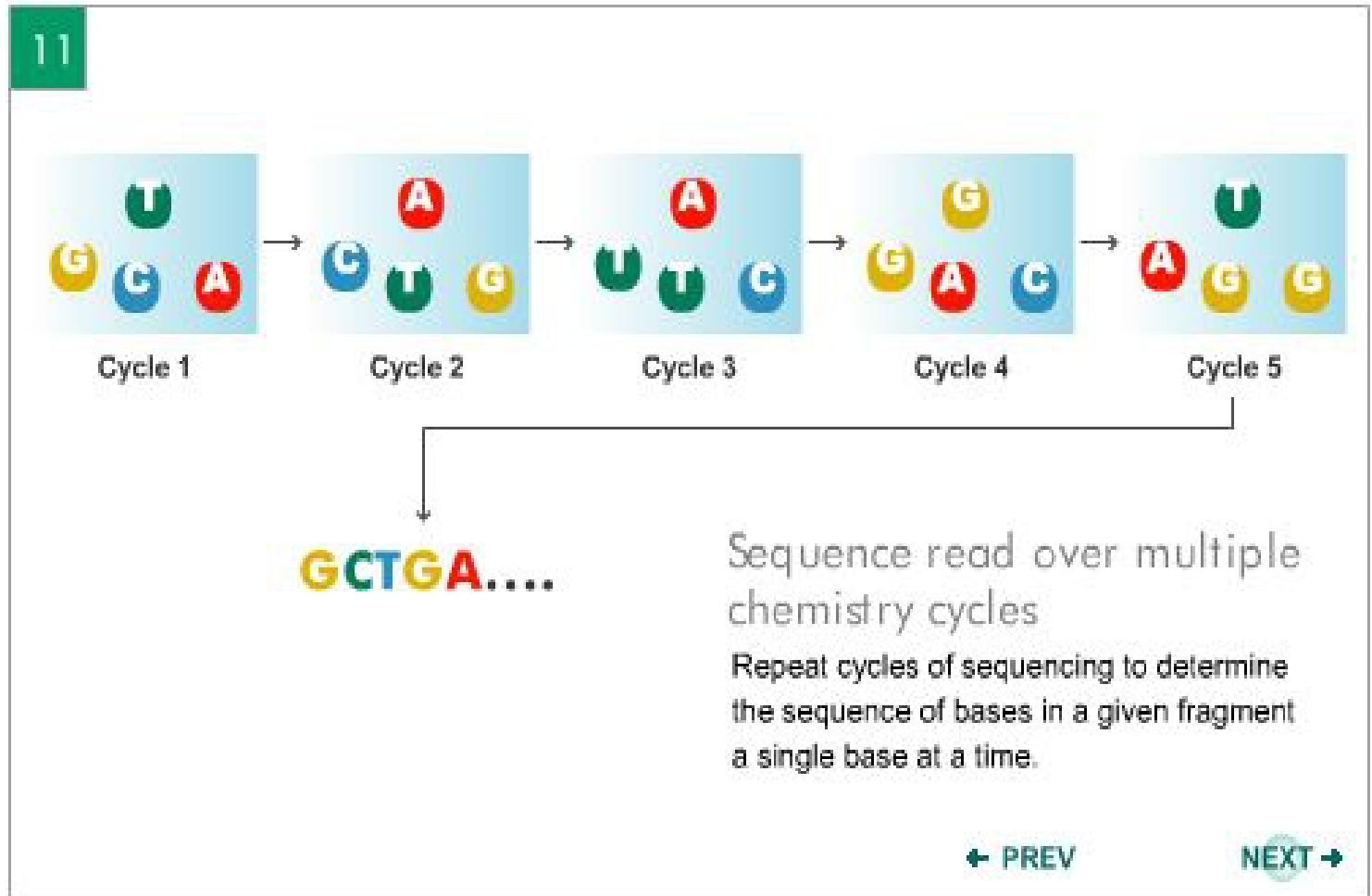
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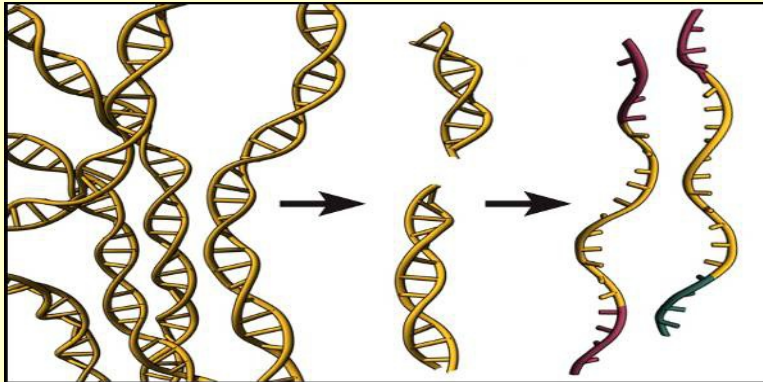
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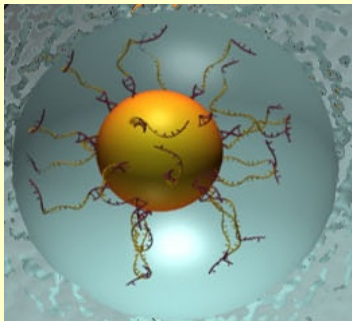
Sequencing-By-Synthesis Demo



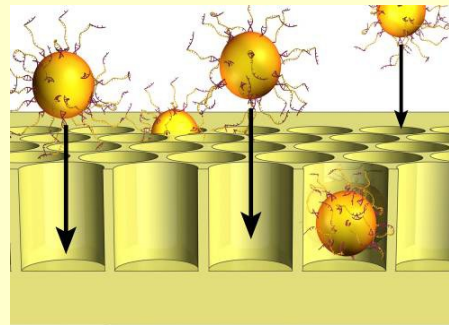
Life Sciences 454 Process Overview



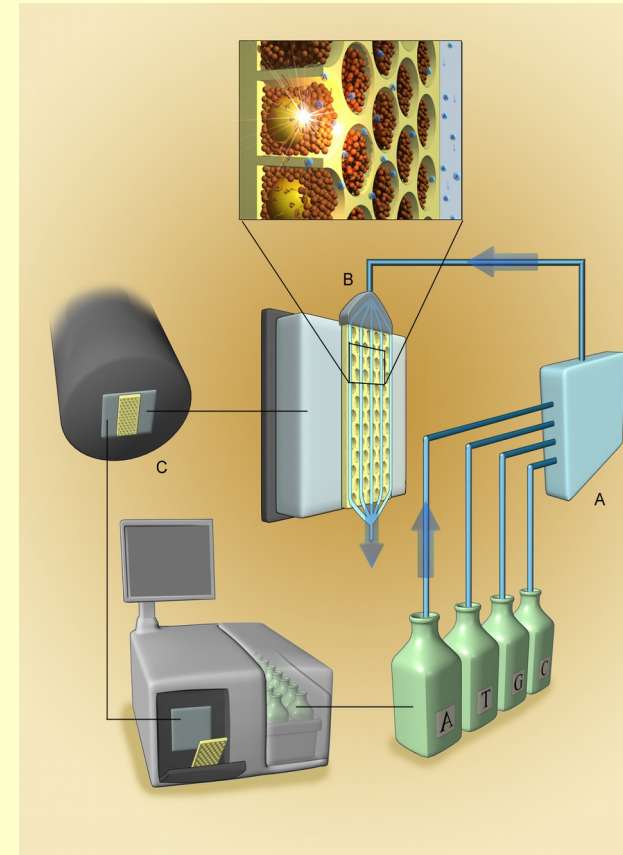
1) Prepare Adapter Ligated ssDNA Library



2) Clonal Amplification on 28 μ beads



3) Load beads and enzymes in PicoTiter Plate™



4) Perform Sequencing by synthesis on the 454 Instrument

Ion Torrent Sequencing

<http://www.iontorrent.com/>

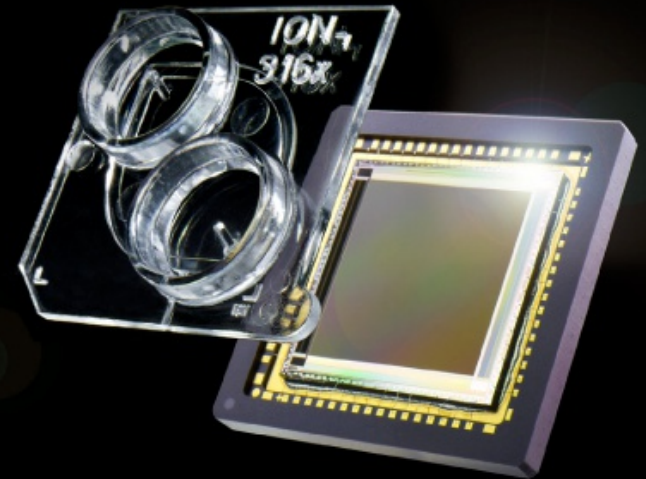


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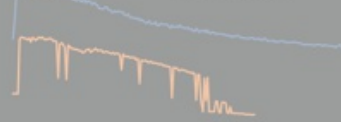
nature

An integrated semiconductor device enabling non-optical genome sequencing

Application Note

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New PacBio Sequencing Technology Video

<http://www.pacificbiosciences.com/products/smrt-technology/>



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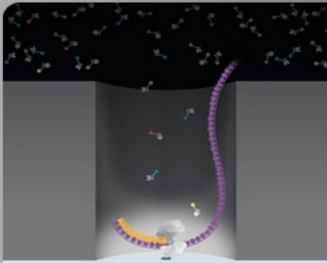
SMRT TECHNOLOGY

- SMRT Sequencing Advantage ›


Our SMRT technology harnesses the natural process of DNA replication, which is a highly efficient and accurate process. The enzyme responsible for replicating DNA in nature is called the DNA polymerase. The DNA polymerase attaches itself to a strand of DNA to be replicated, examines the individual base at the point it is attached, and then determines which of four building blocks, or nucleotides, is required to replicate that individual base. After determining which nucleotide is required, the polymerase incorporates that nucleotide into the growing strand that is being produced. After incorporation, the enzyme advances to the next base to be replicated and the process is repeated. Our SMRT technology enables the observation of DNA synthesis as it occurs in real time. To overcome the challenges inherent in observing an enzyme that is 15 nanometers, or nm, in diameter running in real time, we developed three key innovations:

- The SMRT Cell
- Phospholinked nucleotides
- The PacBio RS II

Watch an overview of SMRT Technology below.



With an active polymerase immobilized at the bottom of each ZMW, nucleotides diffuse into the ZMW chamber. In order to detect incorporation events and identify the base, each of the four nucleotides A, C, G and T are labeled with a different fluorescent dye having a distinct emission spectrum. Since the excitation illumination is directed to the bottom of the ZMW, nucleotides held by the polymerase prior to incorporation emit an extended signal that identifies the base being incorporated.



PLAYLIST | 27 / 52 Overview of SMRT Technology

SMRT® Cell

0:01 / 2:08

New PacBio Sequencing Technology Video

<http://www.pacificbiosciences.com/products/smrt-technology/>



SMRT® Cell

Pacific Biosciences Sequencing

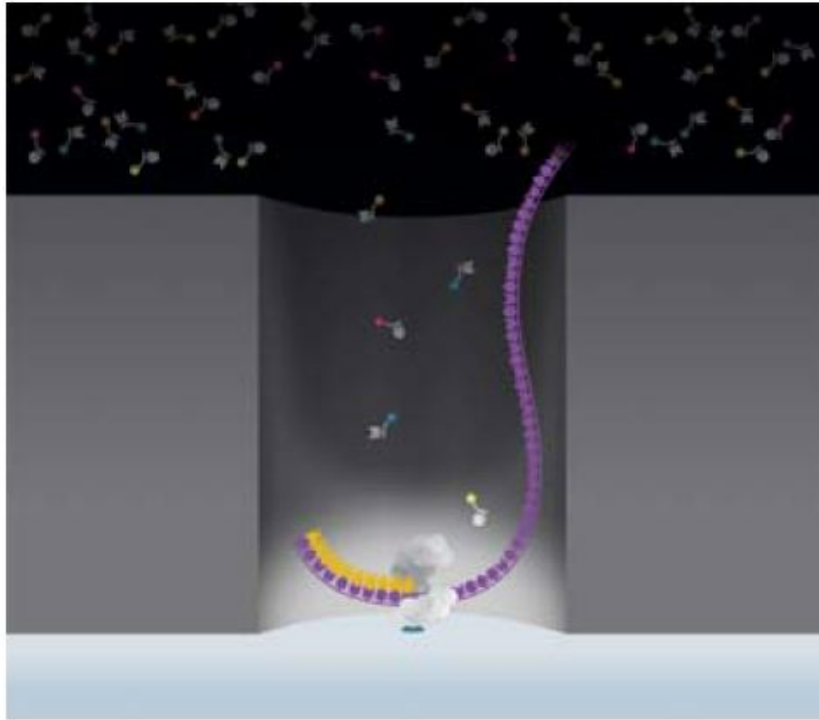


Figure 6. ZMW with DNA polymerase and phospholinked nucleotides

Phospholinked nucleotides are added into the ZMW at the high concentrations required for proper enzyme functioning.

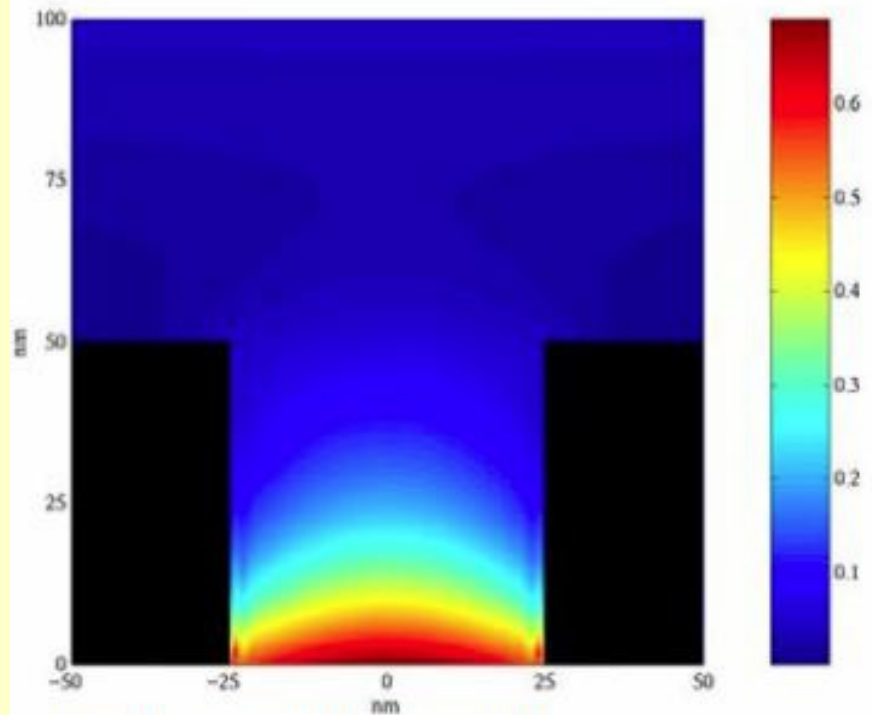


Figure 4. Detection volume

Attenuated light from the excitation beam penetrates only the lower 20-30 nm of each waveguide, creating a detection volume of 20 zeptoliters (10^{-21} liters).

Phospholinked Fluorophores

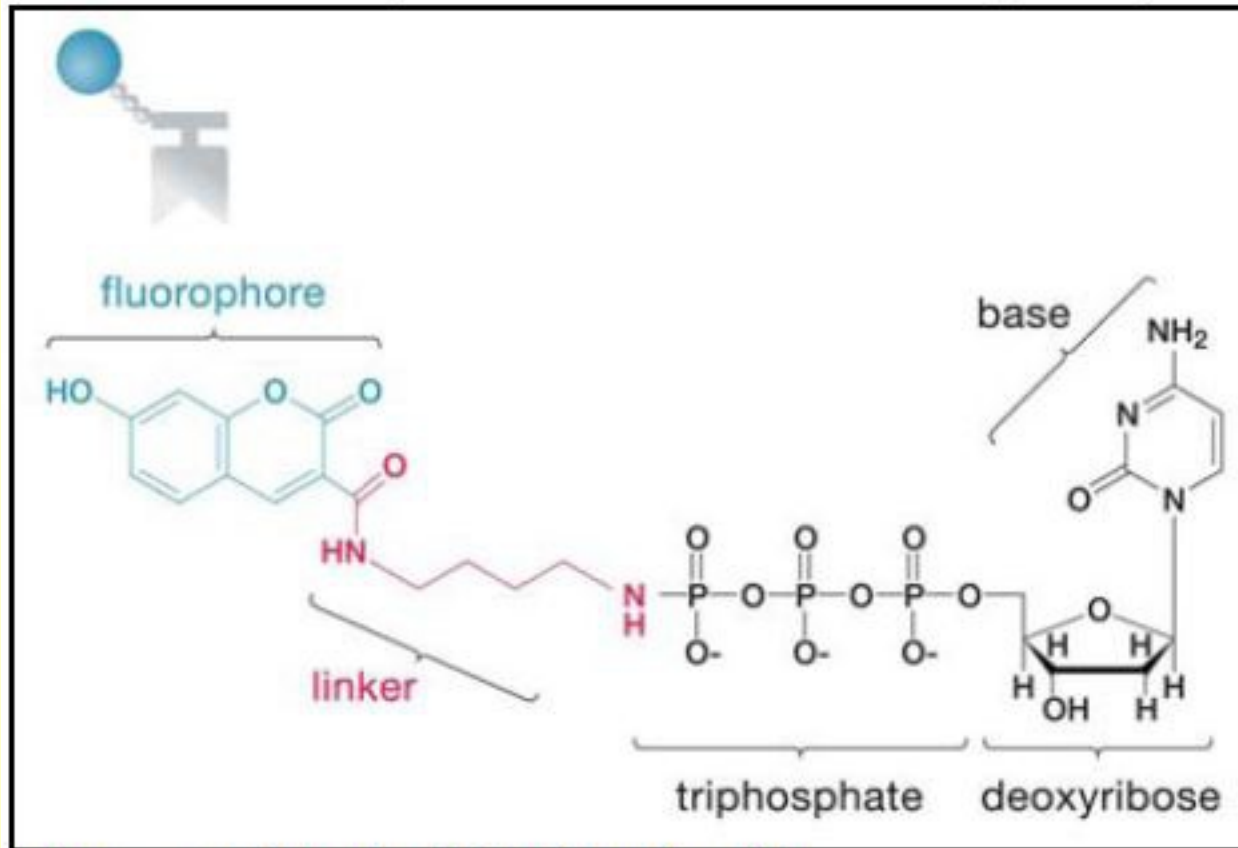
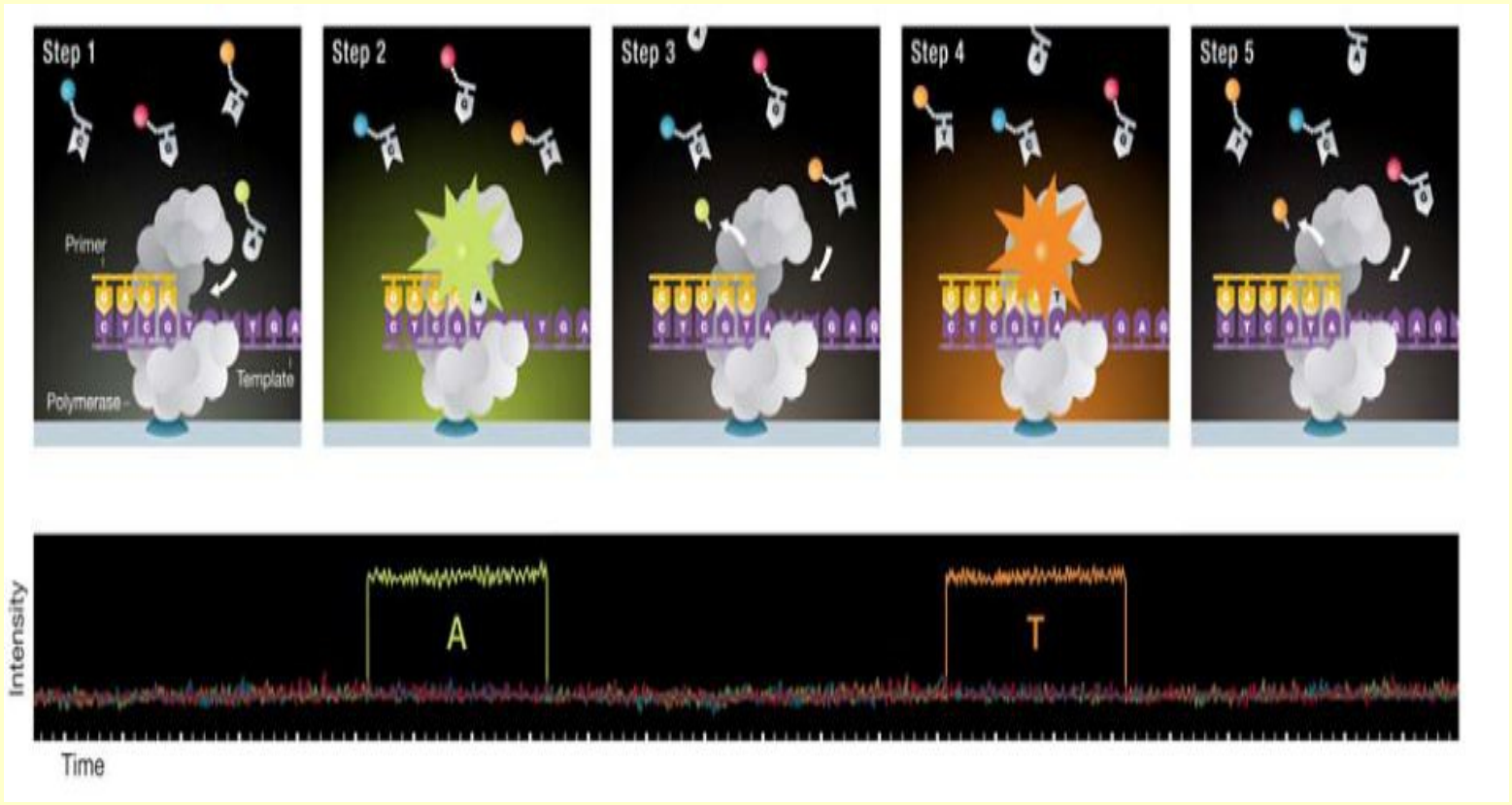


Figure 9. Phospholinked nucleotides

Phospholinked nucleotides have fluorophores attached to the triphosphate chain, which is naturally cleaved when the nucleotide is incorporated.

Processive Synthesis



Synthesis of Long Duplex DNA

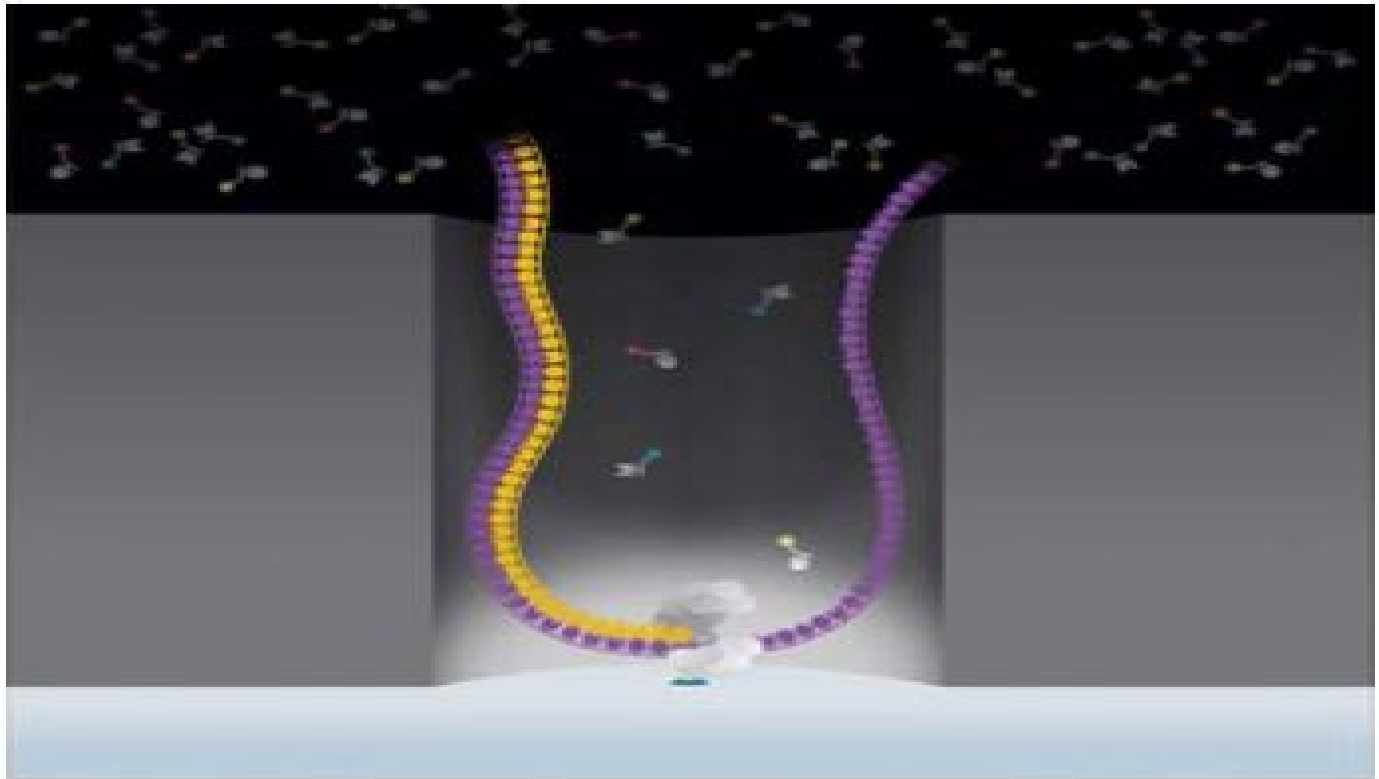
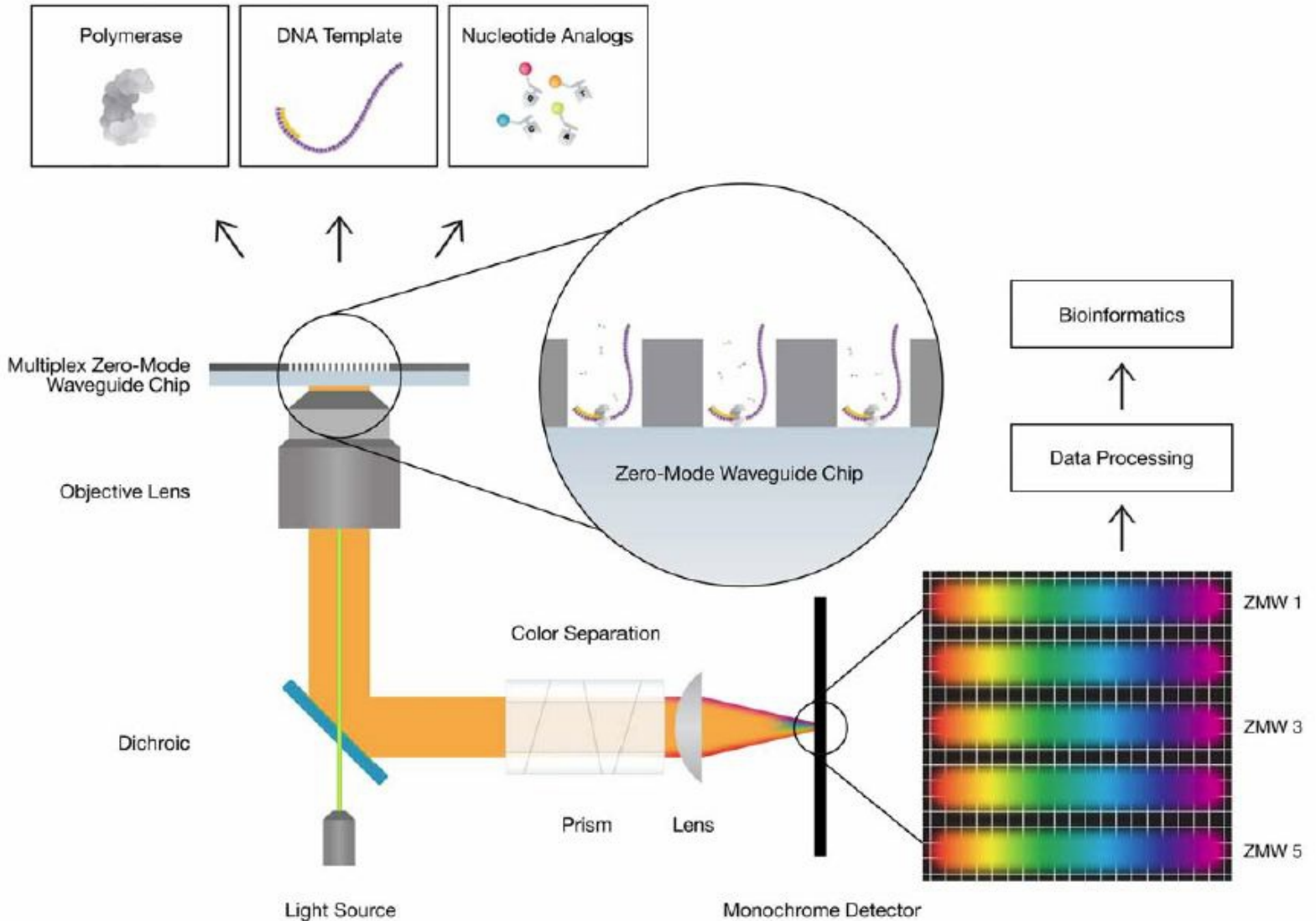


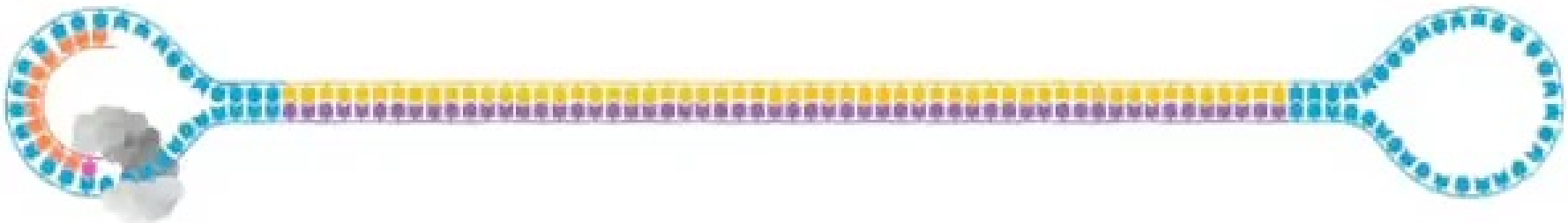
Figure 11. Synthesis of long DNA.

DNA polymerase processively incorporates nucleotides producing long, natural DNA.

Highly Parallel Optics System



Circular Templates Gives Redundant Sequencing and Accuracy



Circular Templates Gives Redundant Sequencing and Accuracy

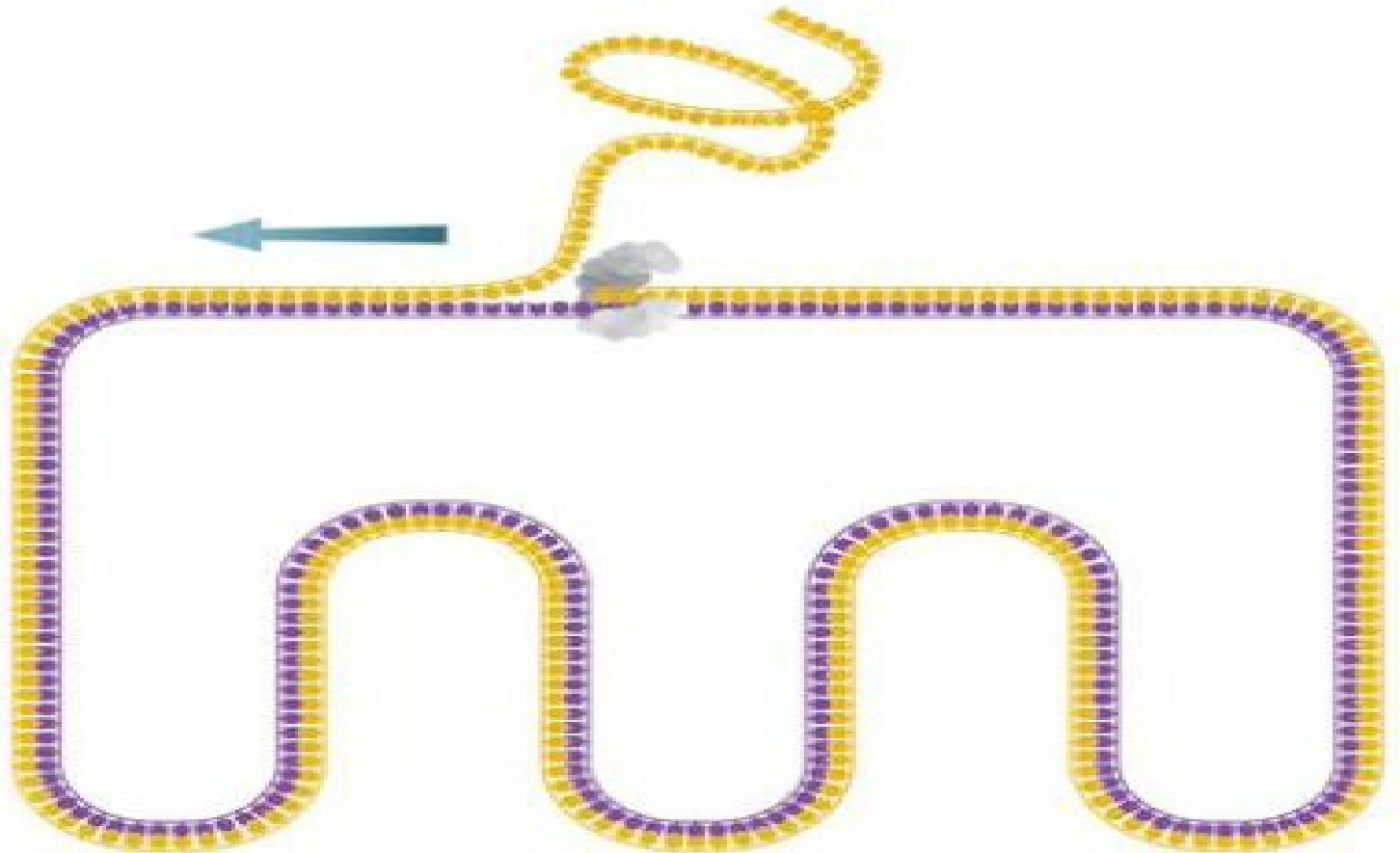
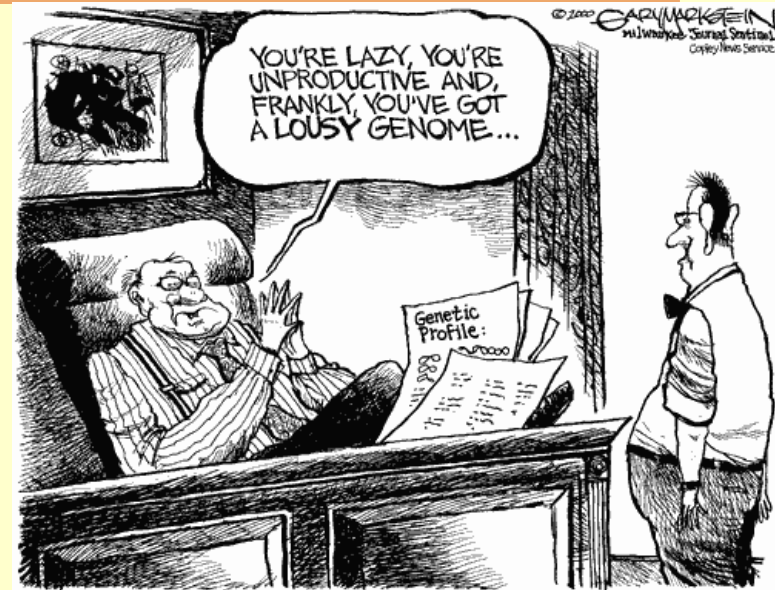


Figure 16. Molecular redundant sequencing

The Human Genome

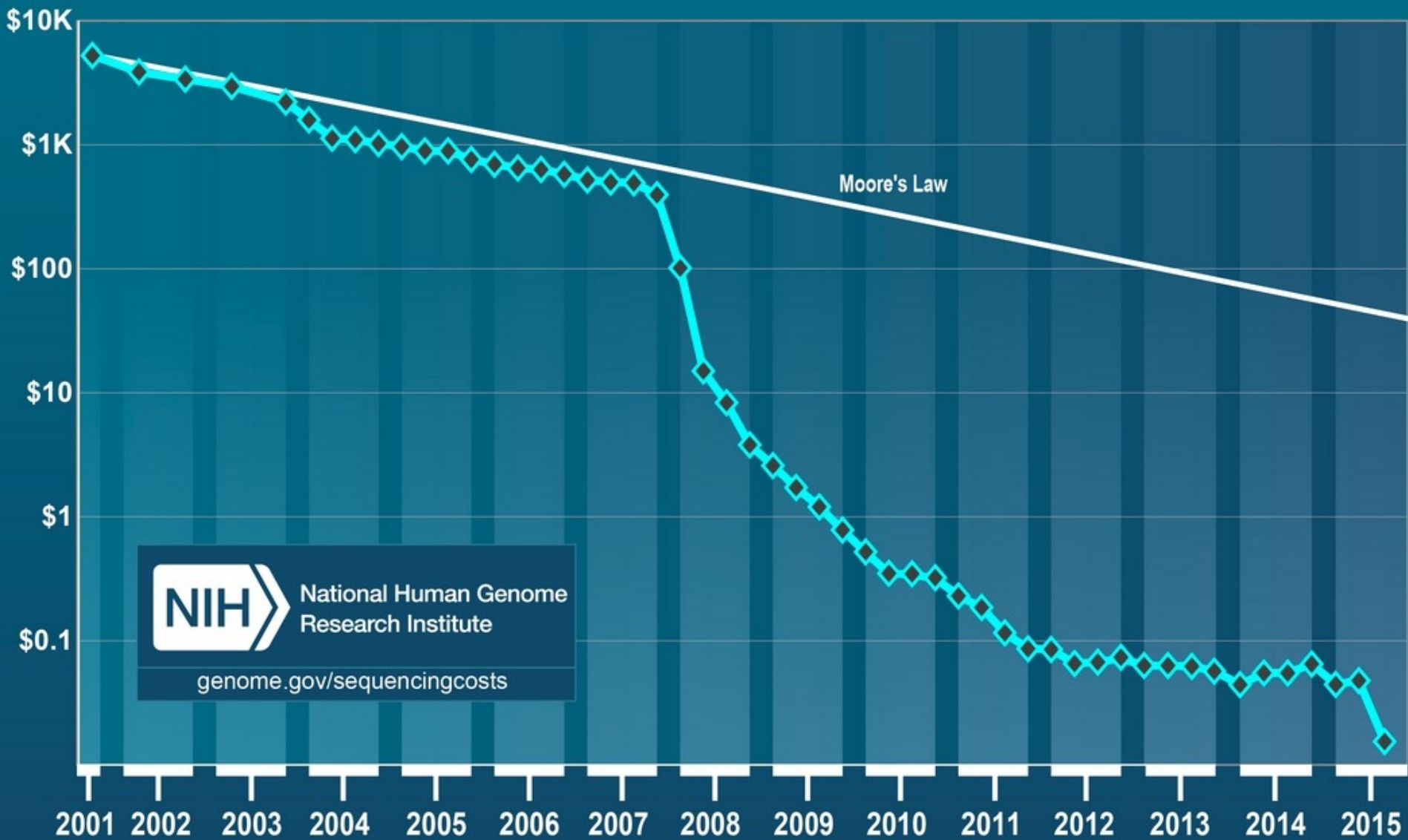
How fast is the cost going down?

- 2006: \$ 50 million
- 2008: \$500,000
- 2009: \$50,000
- 2010: \$20,000
- 2011: \$5,000
- 2012: \$4,000
- 2013: \$3,000
- 2014 \$1,400
- 2015 \$1,000

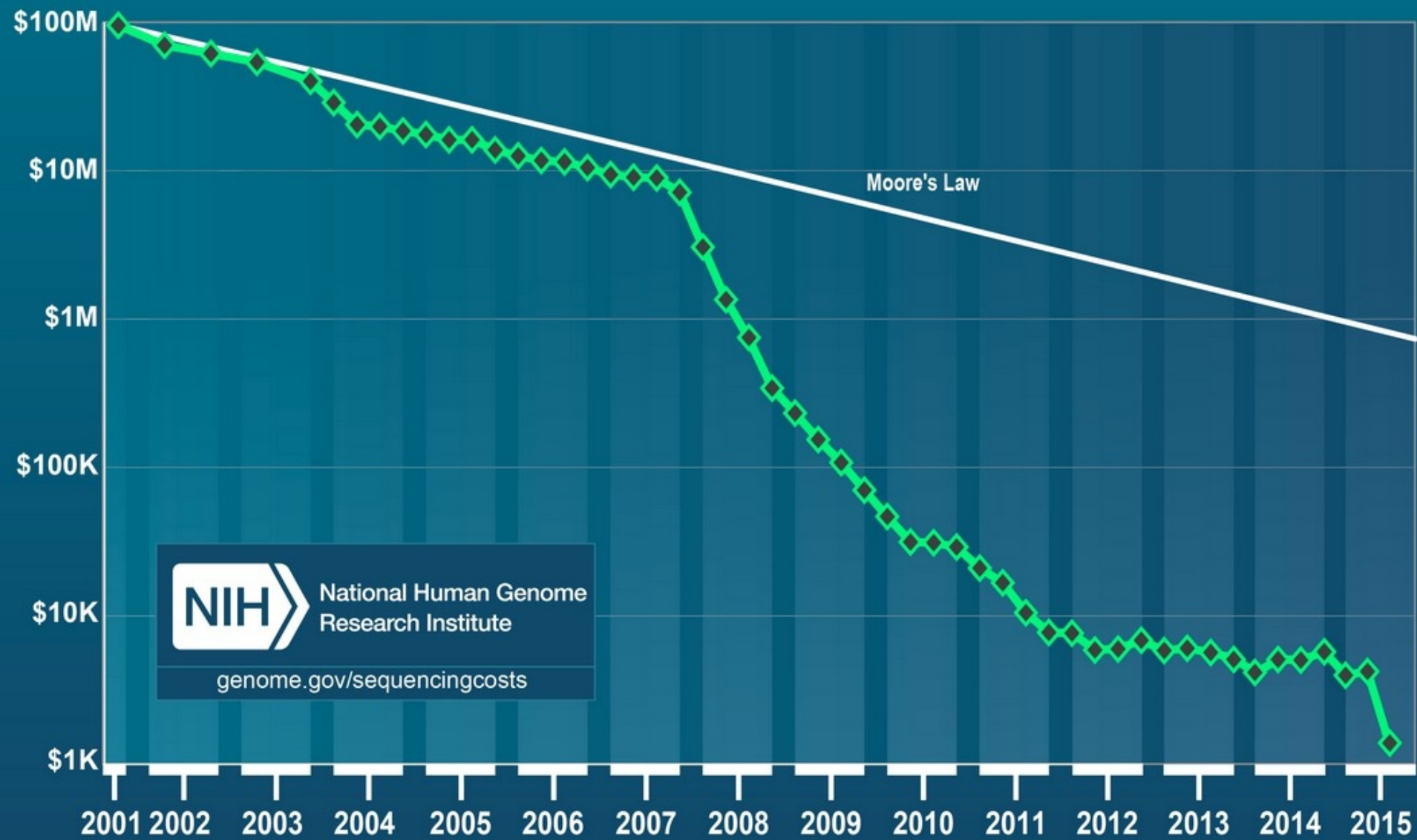


Thanks to Seraf in Batzoglou

Cost per Raw Megabase of DNA Sequence



Cost per Genome



Centers for Mendelian Genomics

<http://mendelian.org/>

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ONE GOAL
MANY PEOPLE
INFINITE POSSIBILITIES

Understanding the genetic basis of Mendelian conditions.

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The Annual ASHG conference is quickly approaching. This year the CMG will be live tweeting from sunny San Diego.

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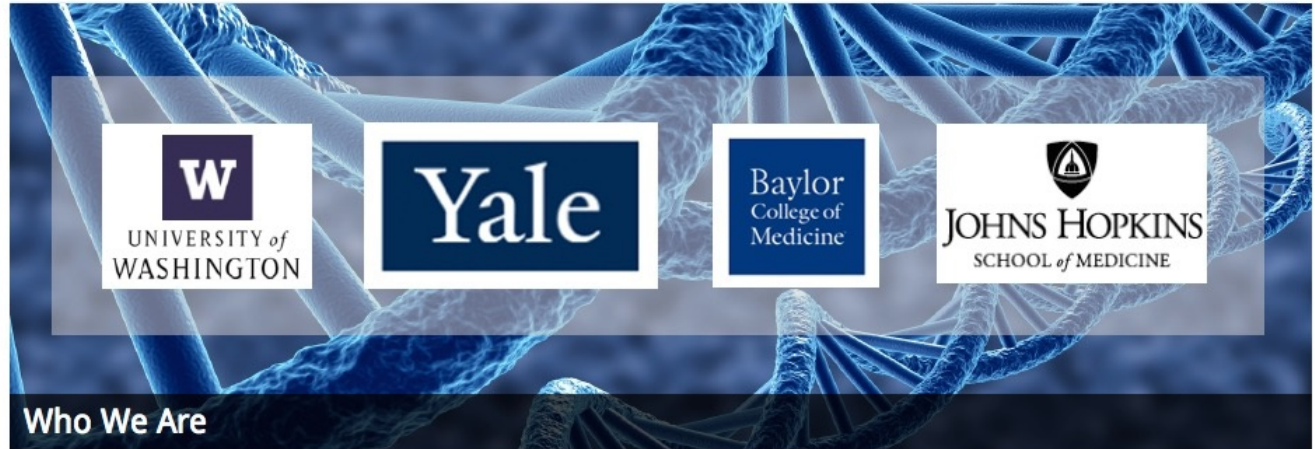
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Genomics England

<http://www.genomicsengland.co.uk/>



Genomics England is delivering the **100,000 Genomes Project**.

We are creating a new genomic medicine service with the NHS – to support **better diagnosis and better treatments** for patients. We are also enabling medical research.

[Find out more...](#)

News

Genomics England announces interpretation partners and expands industry engagement

Genomics England has contracted with Congenica and Omicia to work with them on the interpretation of the genomes from 8,000 patients. Two new companies, Berg Health

Latest Videos

Data in the 100,000 Genomes Project

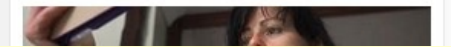
Find out what happens to data in the Project.



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NIH Precision Medicine Initiative

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NIH announces framework for national, large-scale research cohort.

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Get answers to frequently asked questions.

ABOUT THE PRECISION MEDICINE INITIATIVE

Far too many diseases do not have a proven means of prevention or effective treatments. We must gain better insights into the biology of these diseases to make a difference for the millions of Americans who suffer from them. Precision medicine is an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person. While significant advances in


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[News release: NIH framework points the way forward for building national, large scale research cohort](#)

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 (PDF - 1.05MB)

[NEJM Perspective: A New Initiative on Precision Medicine](#)

[White House Precision Medicine Web Page](#)

Rapid Disease Diagnosis in New Borns

Rapid Whole-Genome Sequencing for Genetic Disease Diagnosis in Neonatal Intensive Care Units

Carol Jean Saunders,^{1,2,3,4,5*} Neil Andrew Miller,^{1,2,4*} Sarah Elizabeth Soden,^{1,2,4*} Darrell Lee Dinwiddie,^{1,2,3,4,5*} Aaron Noll,¹ Noor Abu Alnadi,⁴ Nevene Andraws,³ Melanie LeAnn Patterson,^{1,3} Lisa Ann Krivohlavek,^{1,3} Joel Fellis,⁶ Sean Humphray,⁶ Peter Saffrey,⁶ Zoya Kingsbury,⁶ Jacqueline Claire Weir,⁶ Jason Betley,⁶ Russell James Grocock,⁶ Elliott Harrison Margulies,⁶ Emily Gwendolyn Farrow,¹ Michael Artman,^{2,4} Nicole Pauline Safina,^{1,4} Joshua Erin Petrikin,^{2,3} Kevin Peter Hall,⁶ Stephen Francis Kingsmore^{1,2,3,4,5†}

Monogenic diseases are frequent causes of neonatal morbidity and mortality, and disease presentations are often undifferentiated at birth. More than 3500 monogenic diseases have been characterized, but clinical testing is available for only some of them and many feature clinical and genetic heterogeneity. Hence, an immense unmet need exists for improved molecular diagnosis in infants. Because disease progression is extremely rapid, albeit heterogeneous, in newborns, molecular diagnoses must occur quickly to be relevant for clinical decision-making. We describe 50-hour differential diagnosis of genetic disorders by whole-genome sequencing (WGS) that features automated bioinformatic analysis and is intended to be a prototype for use in neonatal intensive care units. Retrospective 50-hour WGS identified known molecular diagnoses in two children. Prospective WGS disclosed potential molecular diagnosis of a severe *GJB2*-related skin disease in one neonate; *BRAT1*-related lethal neonatal rigidity and multifocal seizure syndrome in another infant; identified *BCL9L* as a novel, recessive visceral heterotaxy gene (*HTX6*) in a pedigree; and ruled out known candidate genes in one infant. Sequencing of parents or affected siblings expedited the identification of disease genes in prospective cases. Thus, rapid WGS can potentially broaden and foreshorten differential diagnosis, resulting in fewer empirical treatments and faster progression to genetic and prognostic counseling.

Science Translational Medicine 4, 154ra135 (2012);
<http://stm.sciencemag.org/content/4/154/154ra135.full.html>

New Gene for Palmoplantar Punctate Keratosis

<http://www.nature.com/ng/>

14 October 2012 Last updated at 20:02 ET



Dundee University uncover gene behind skin disease

A team led by the University of Dundee believes it has made a significant step in understanding a skin disease which affects thousands in the UK.

Researchers have identified how the "p34 gene" plays a key role in causing the disease punctate PPK.

The condition causes dots of hard, thickened skin which are painful and uncomfortable.

It is believed the discovery will allow for easier diagnosis of punctate PPK and help developing new therapies.



Punctate PPK causes dots of hard, thickened skin which cause pain and discomfort

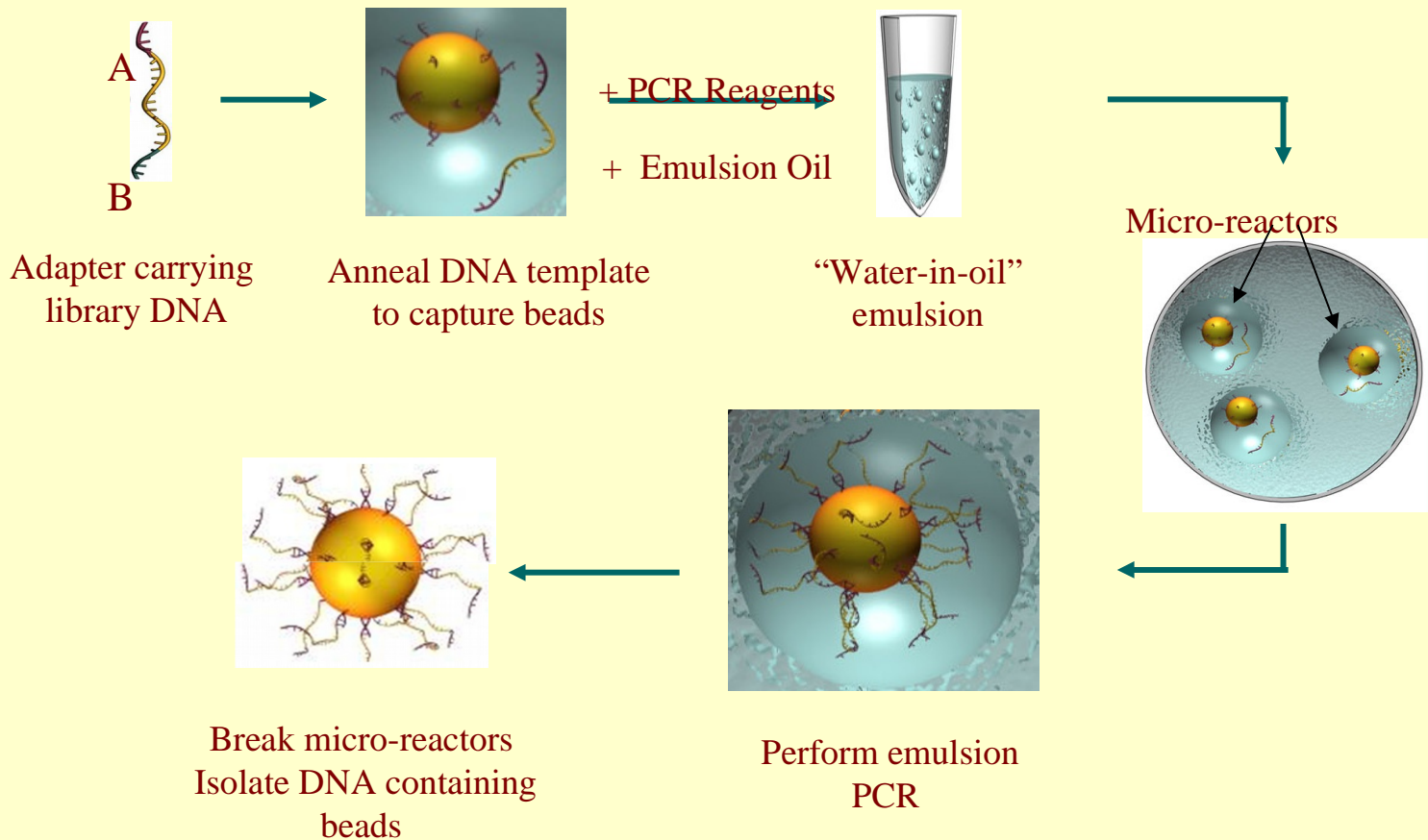
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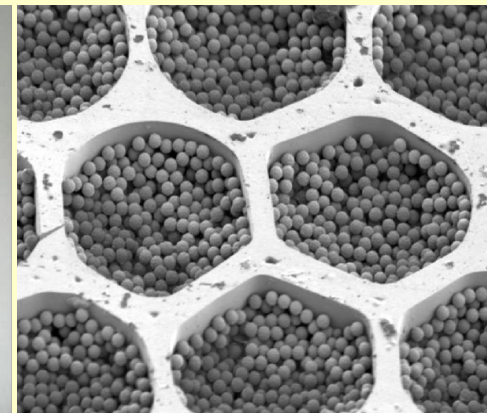
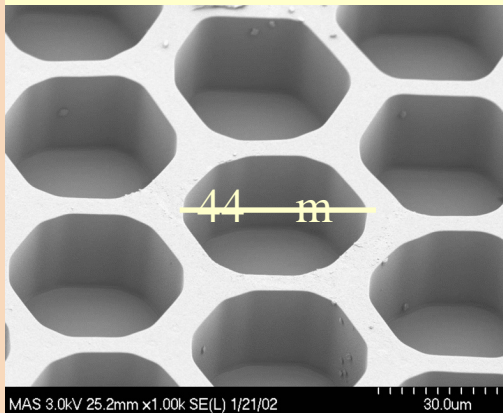
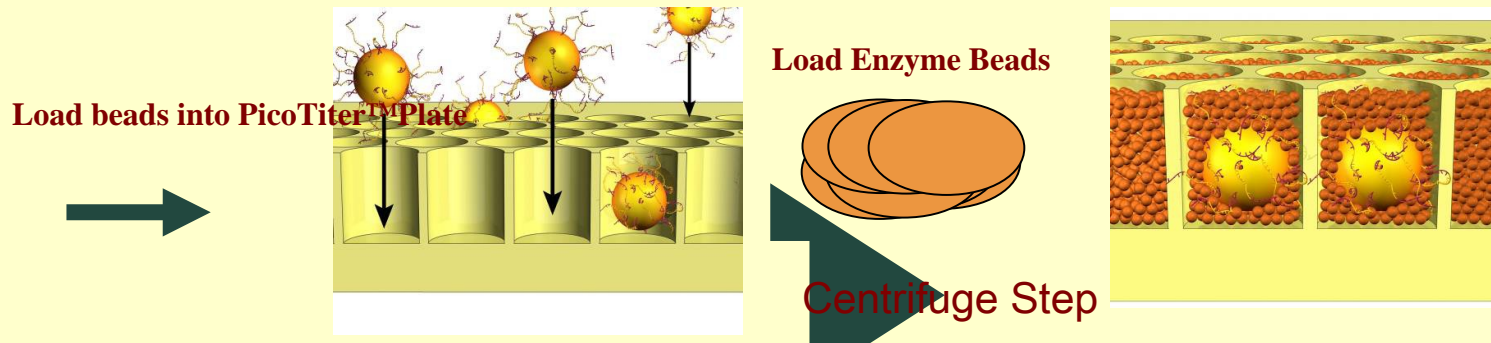
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- Science Translational Medicine
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- New England Journal of Medicine
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Emulsion Based Clonal Amplification

Single test tube generation of millions of clonally amplified sequencing templates
No cloning and colony picking



Depositing DNA Beads into the PicoTiter Plate



- 70x75mm array of fused optical fibers with etched wells
- 1.6 million wells monitored optically through fiber