Next Generation Sequencing

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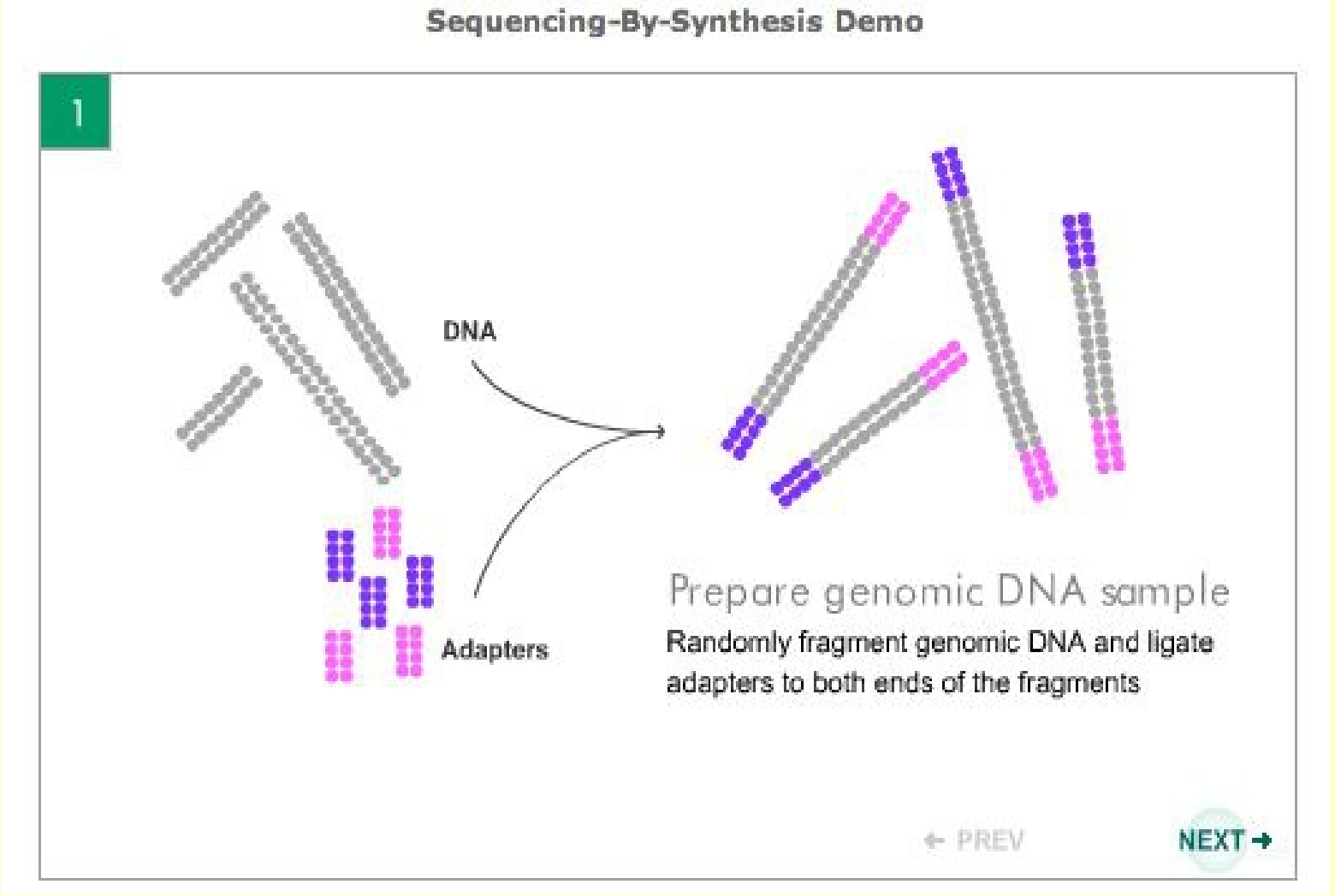
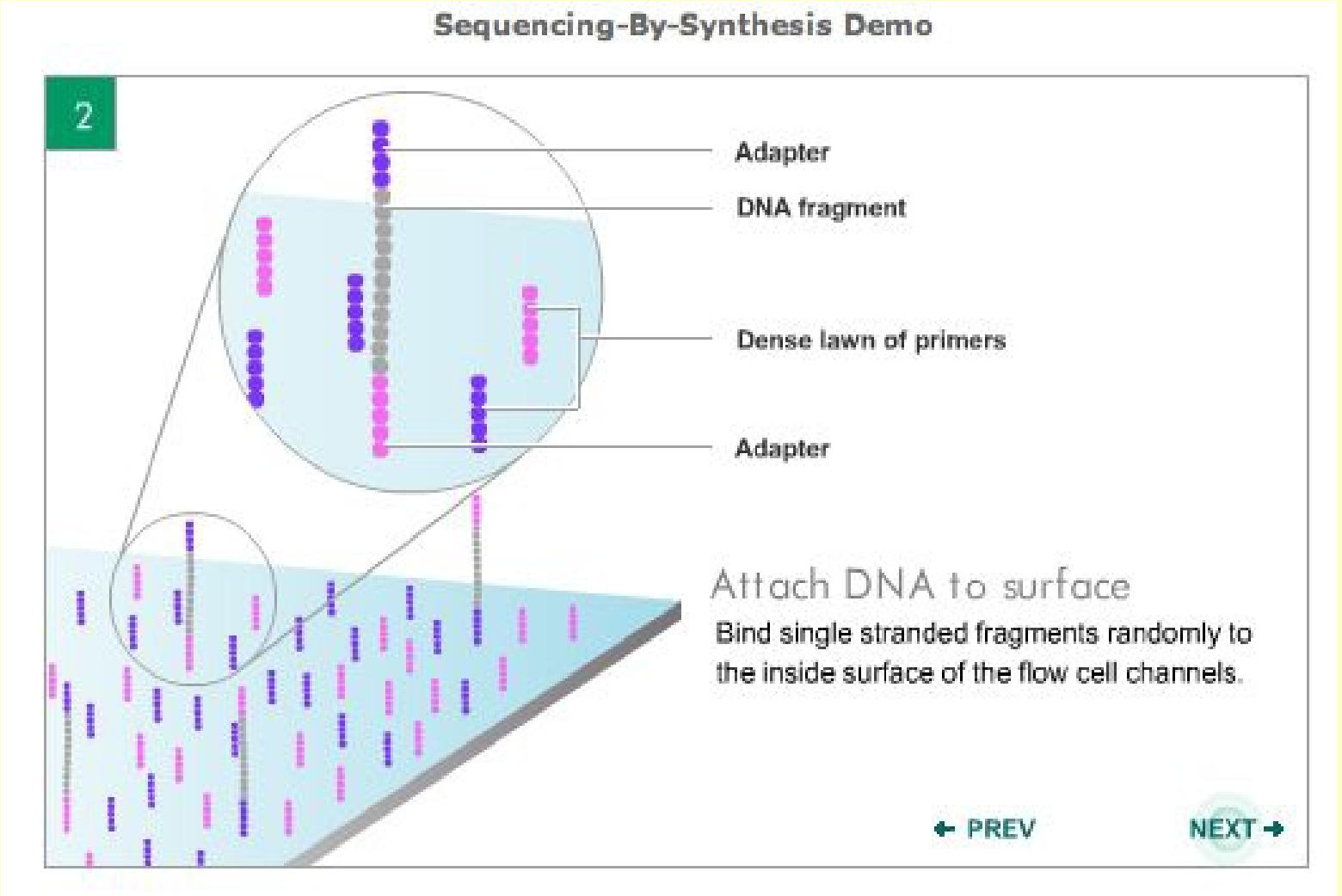
Doug Brutlag, Professor Emeritus
of Biochemistry & Medicine (by courtesy)
Stanford University School of Medicine

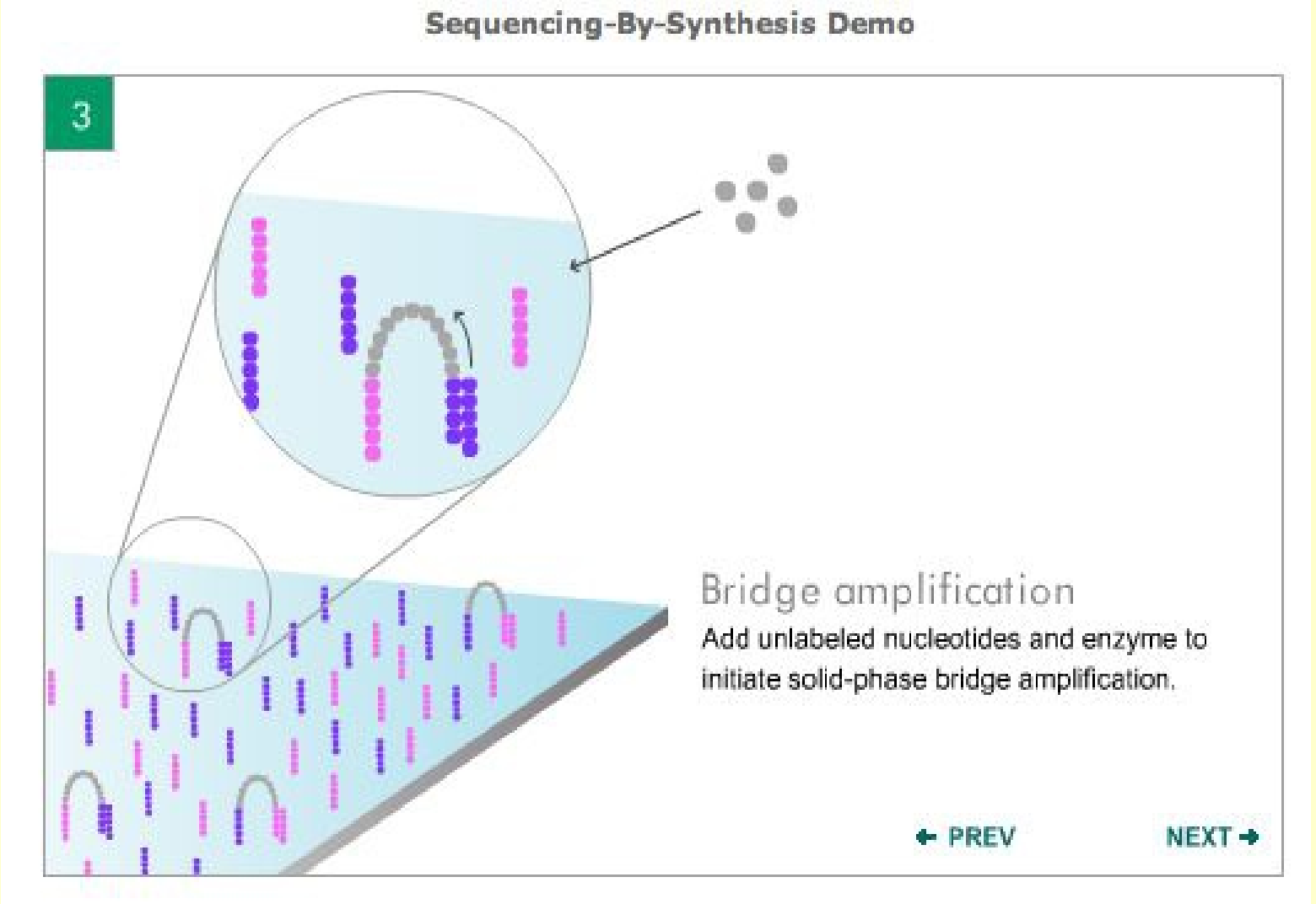
Illumina Solexa Sequencing Technology



Illumina Solexa Sequencing Technology



Illumina Solexa Sequencing Technology



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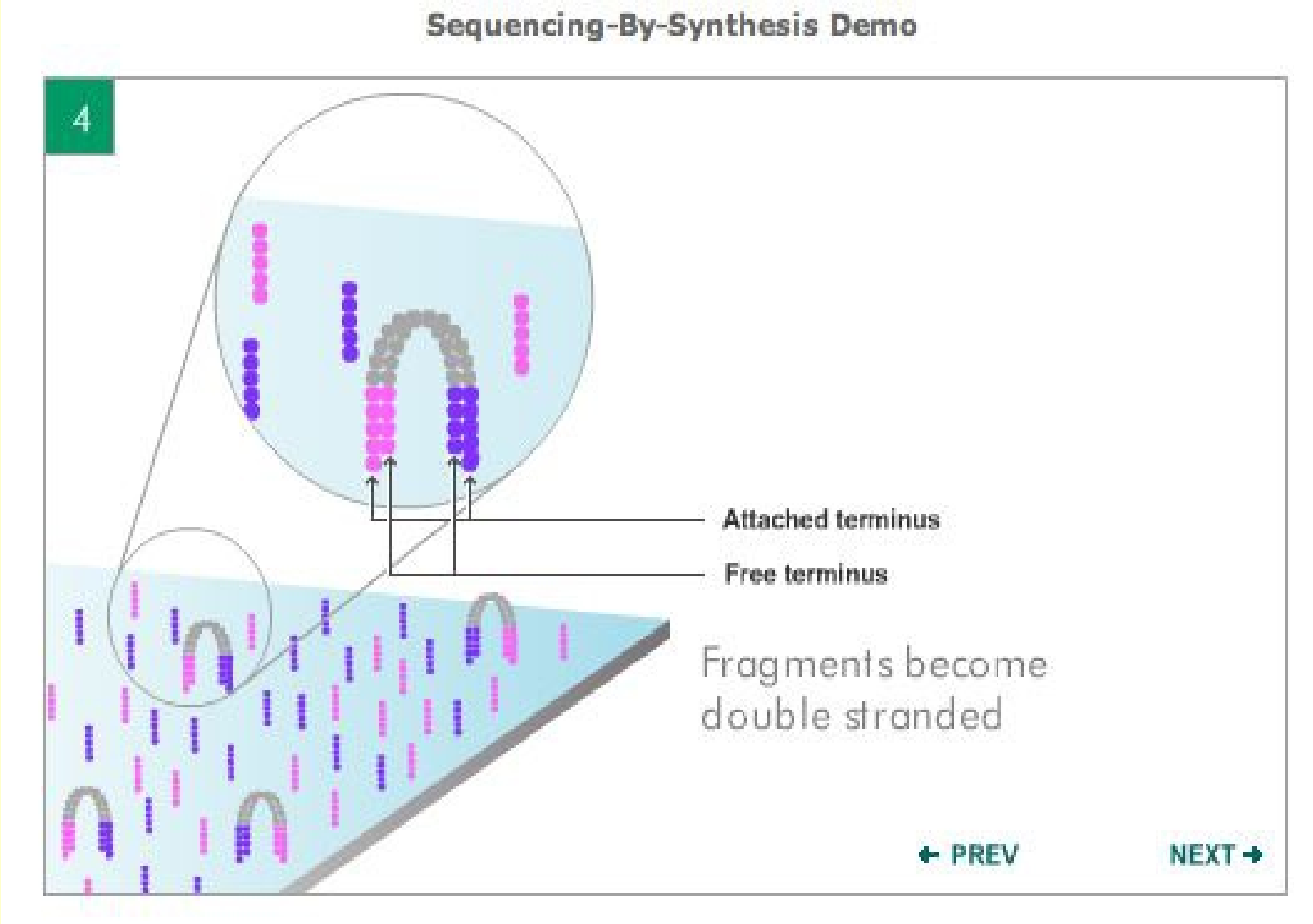

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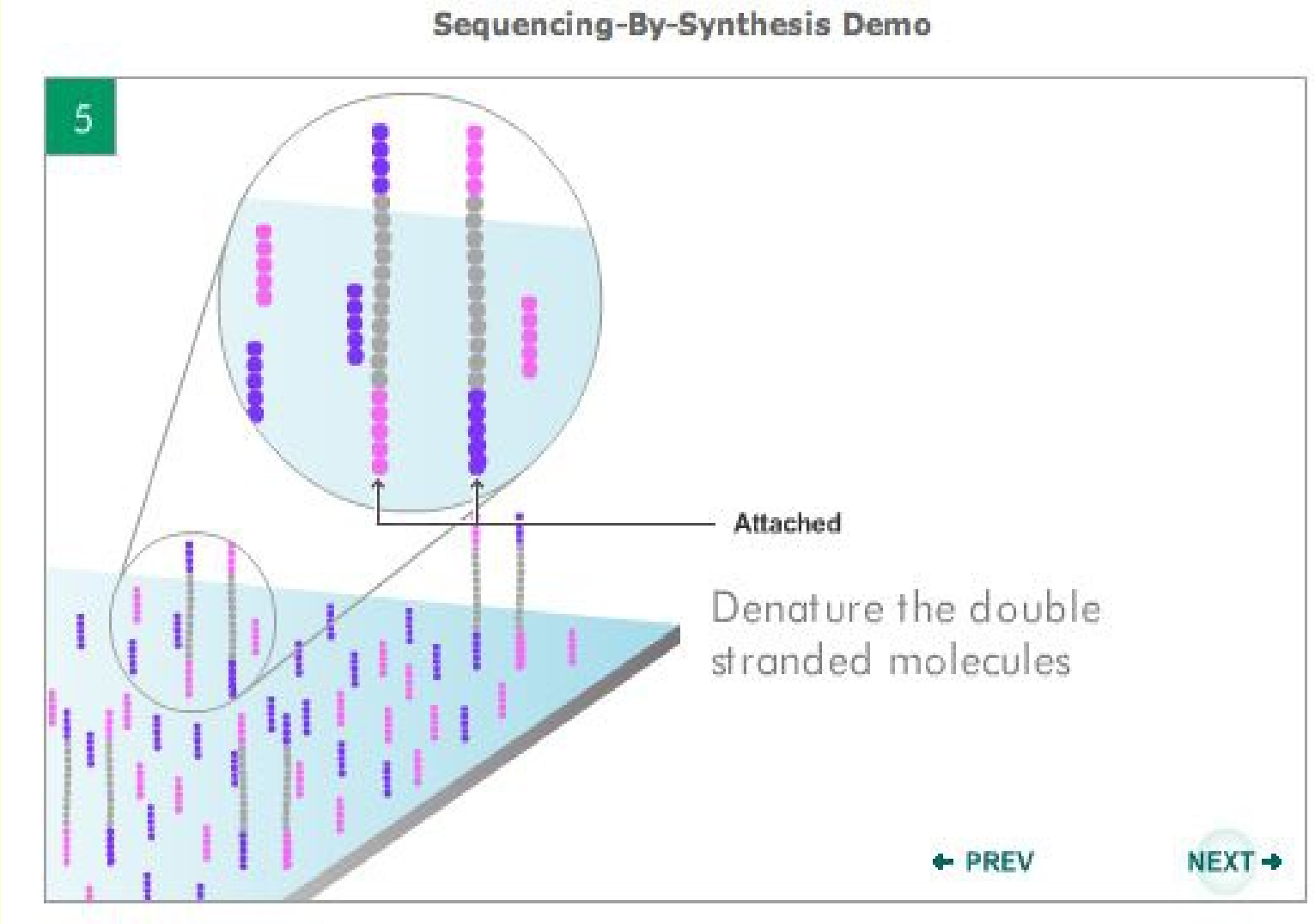
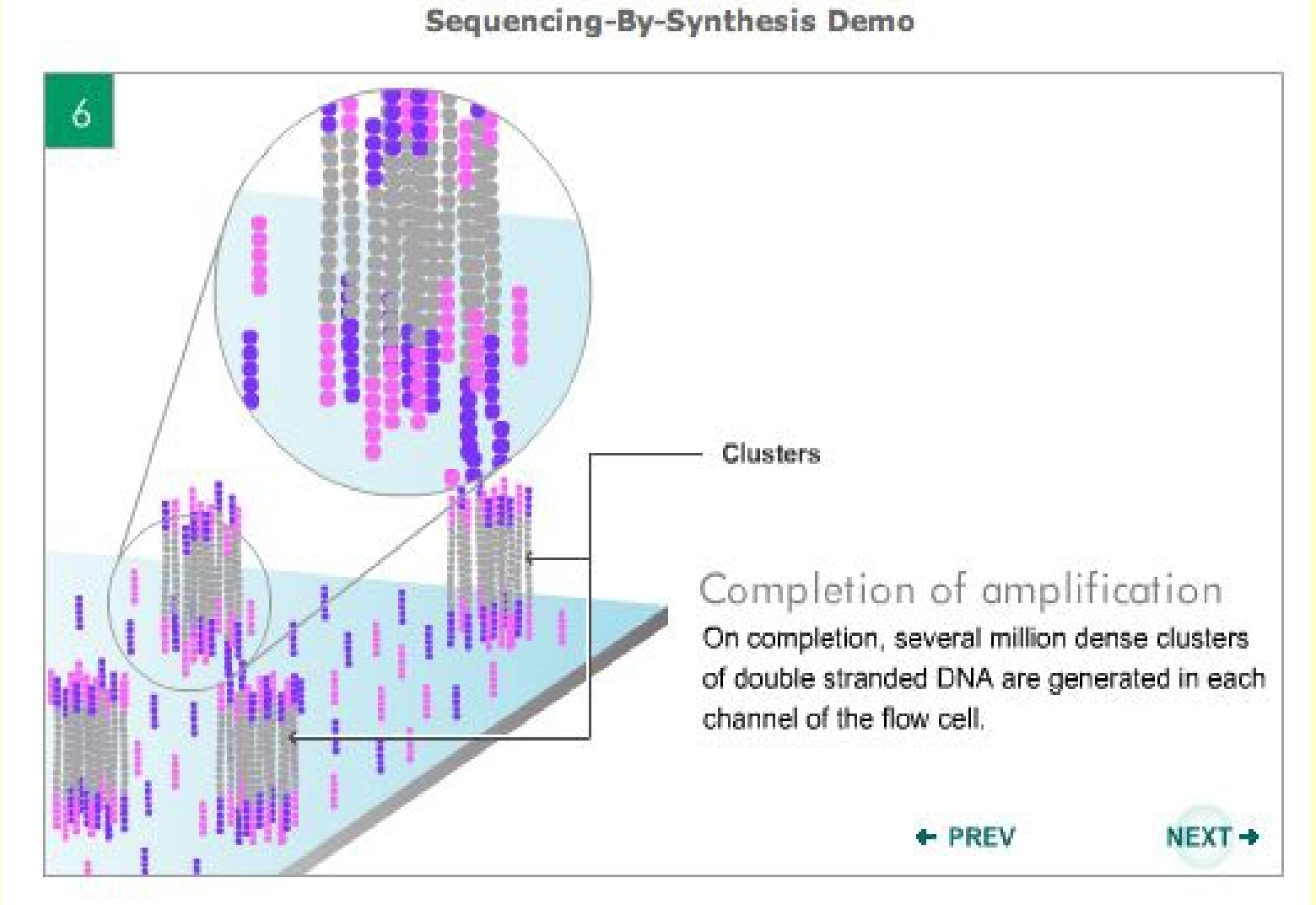
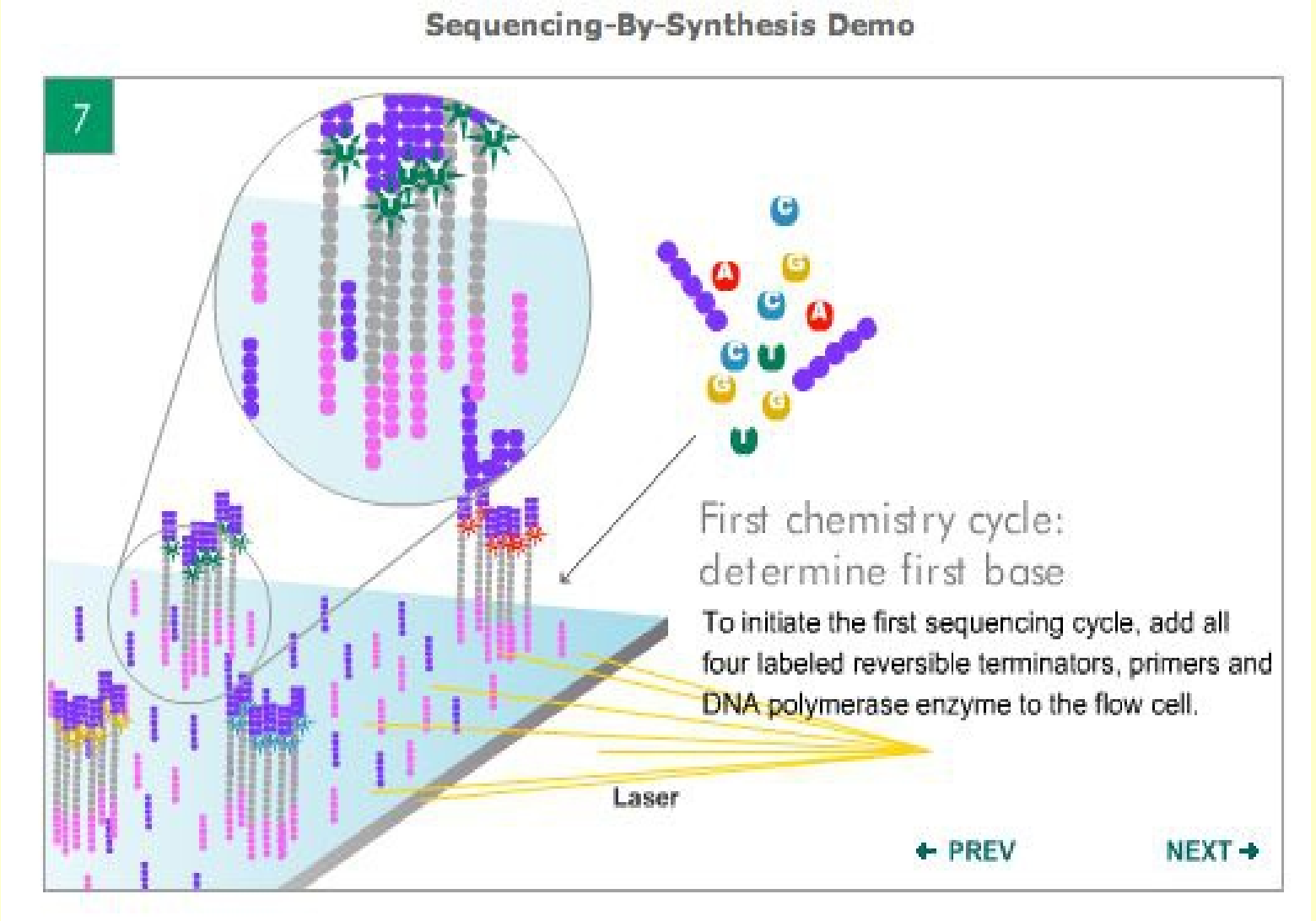


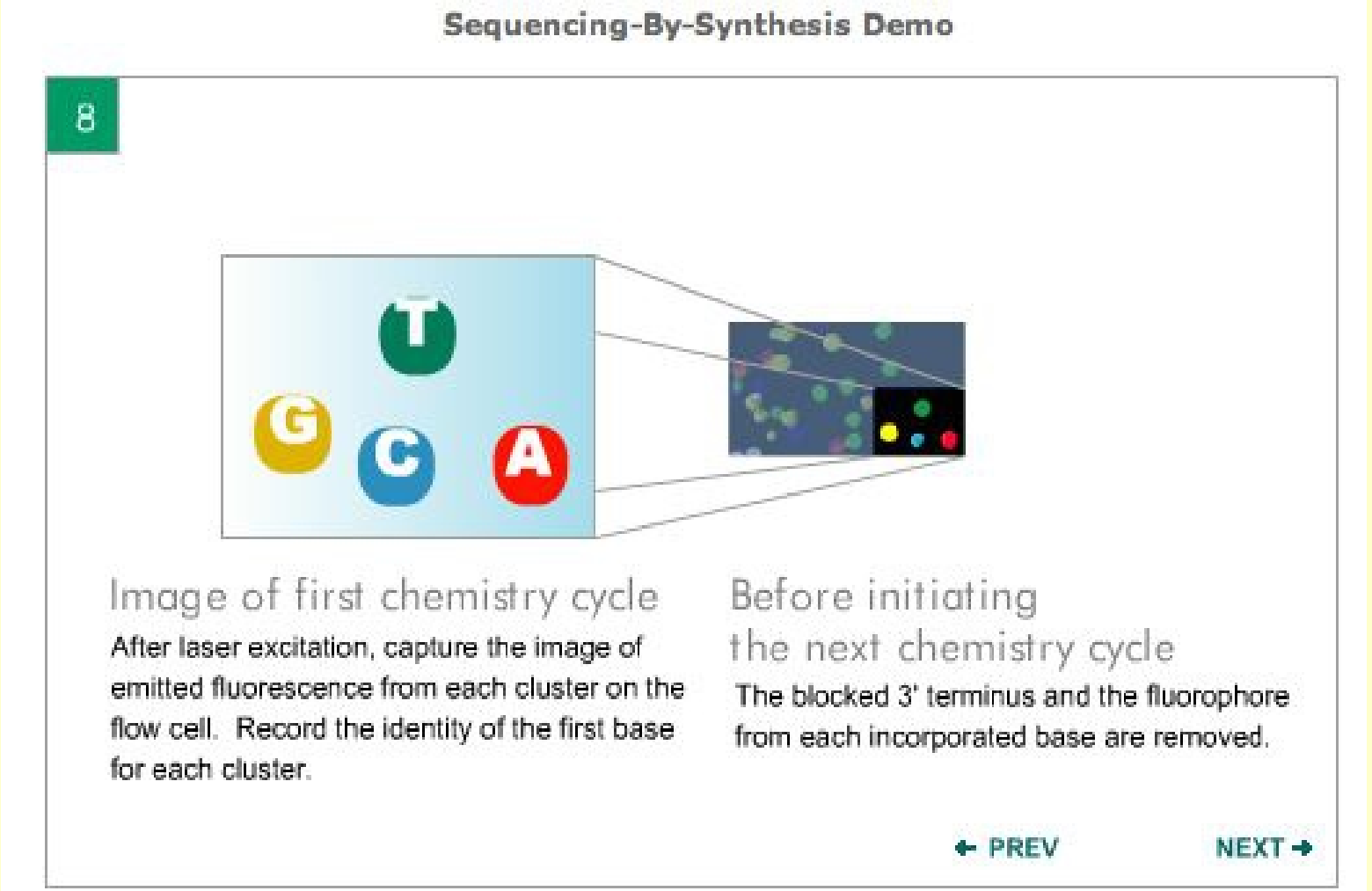
Illumina Solexa Sequencing Technology



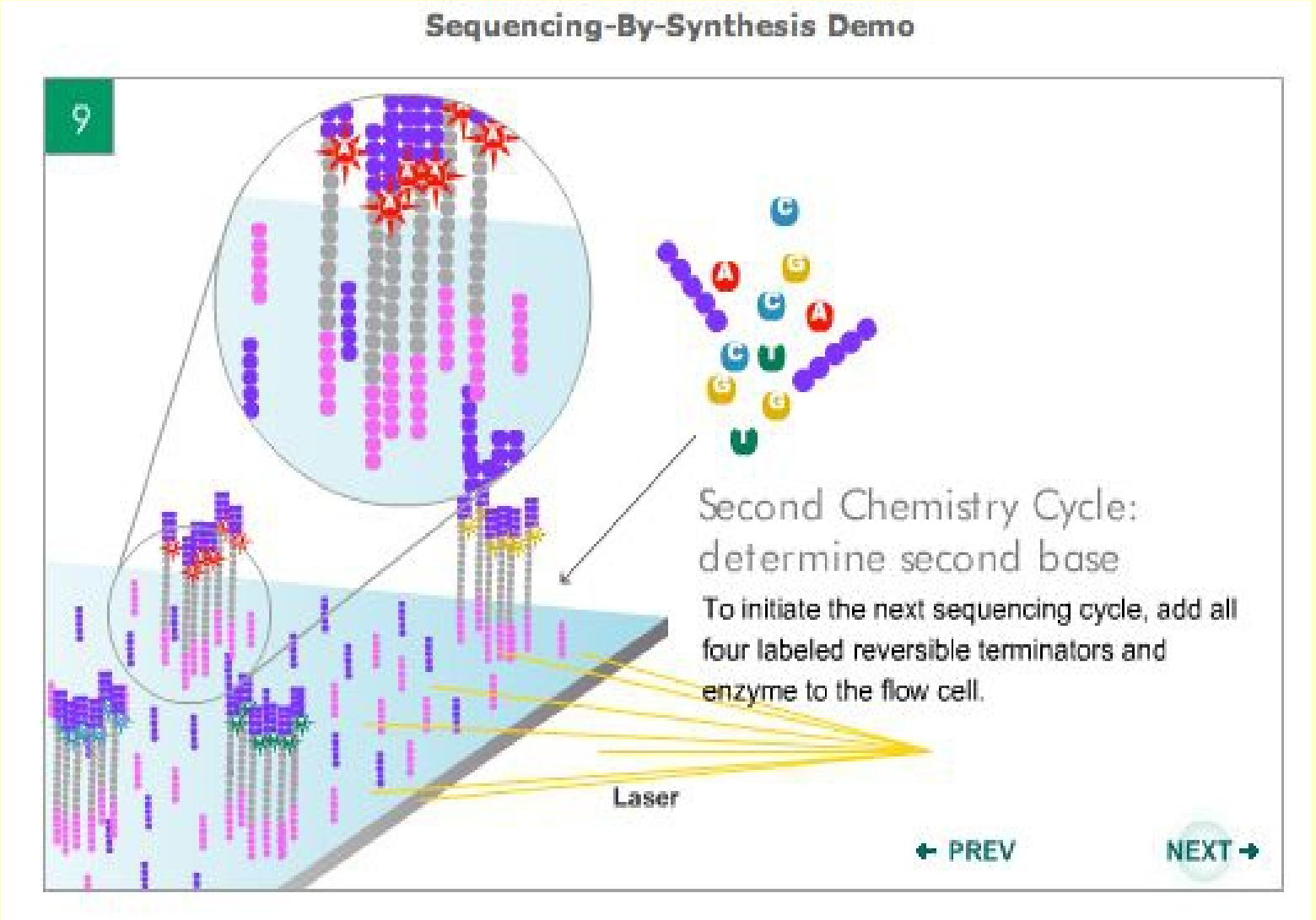
Illumina Solexa Sequencing Technology



Illumina Solexa Sequencing Technology



Illumina Solexa Sequencing Technology



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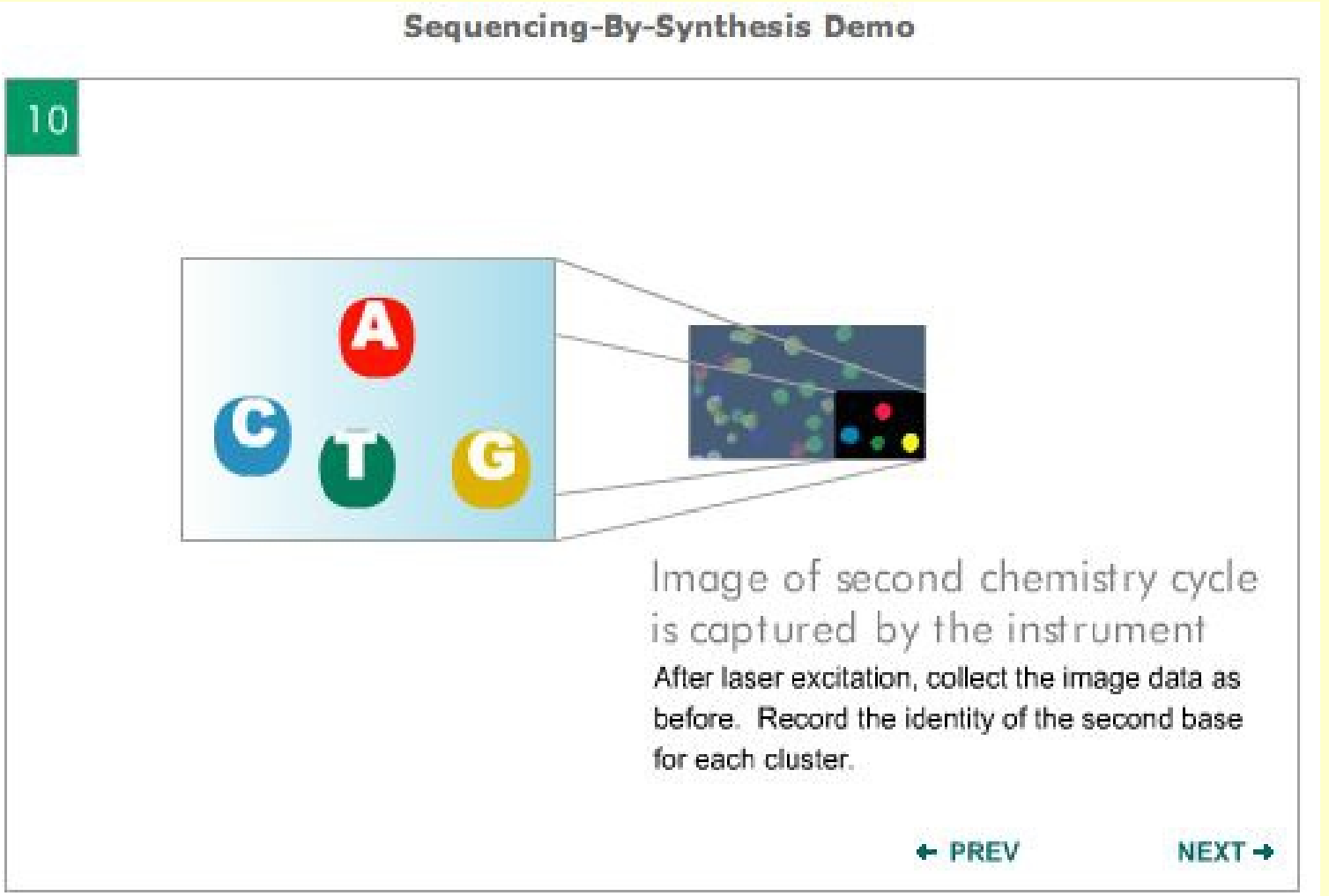
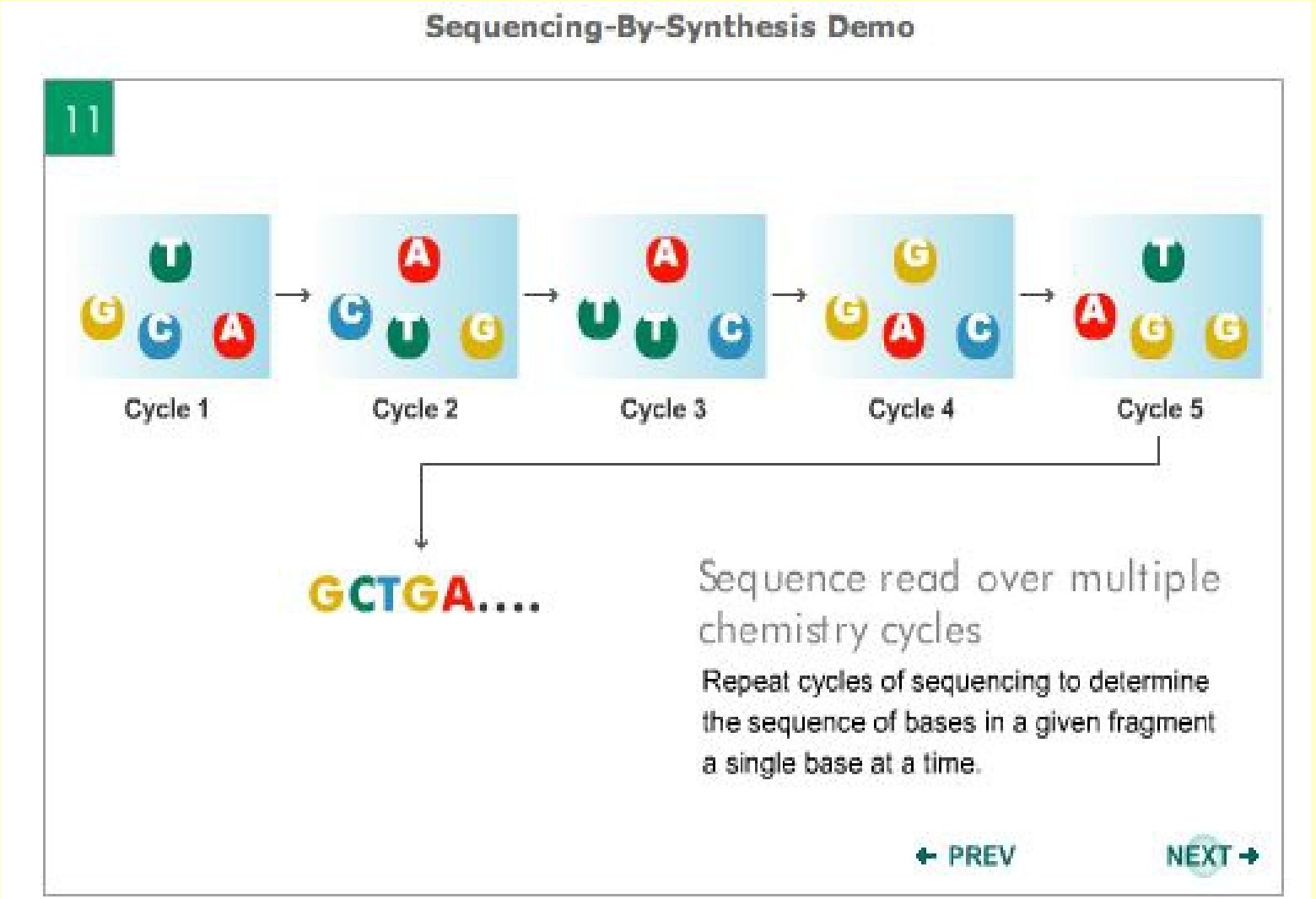


Illumina Solexa Sequencing Technology



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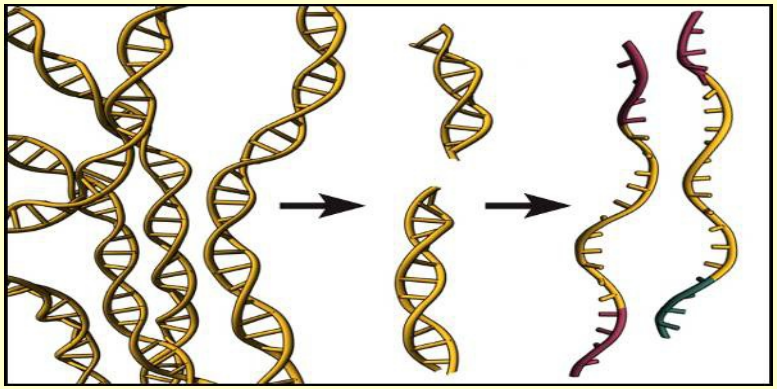
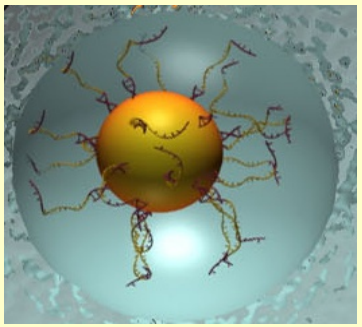
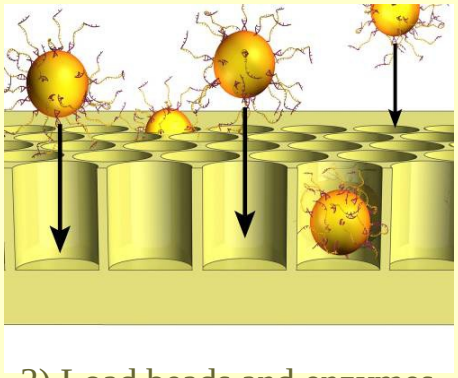
Life Sciences 454 Process Overview



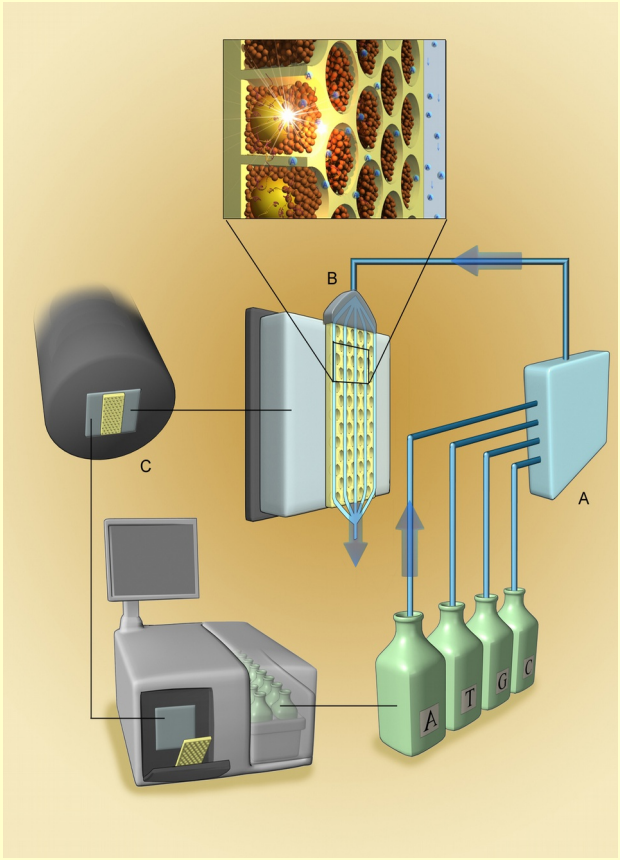
1) Prepare Adapter Ligated ssDNA Library



2) Clonal Amplification on 28 µ beads



3) Load beads and enzymes in PicoTiter Plate™



4) Perform Sequencing by synthesis on the 454 Instrument

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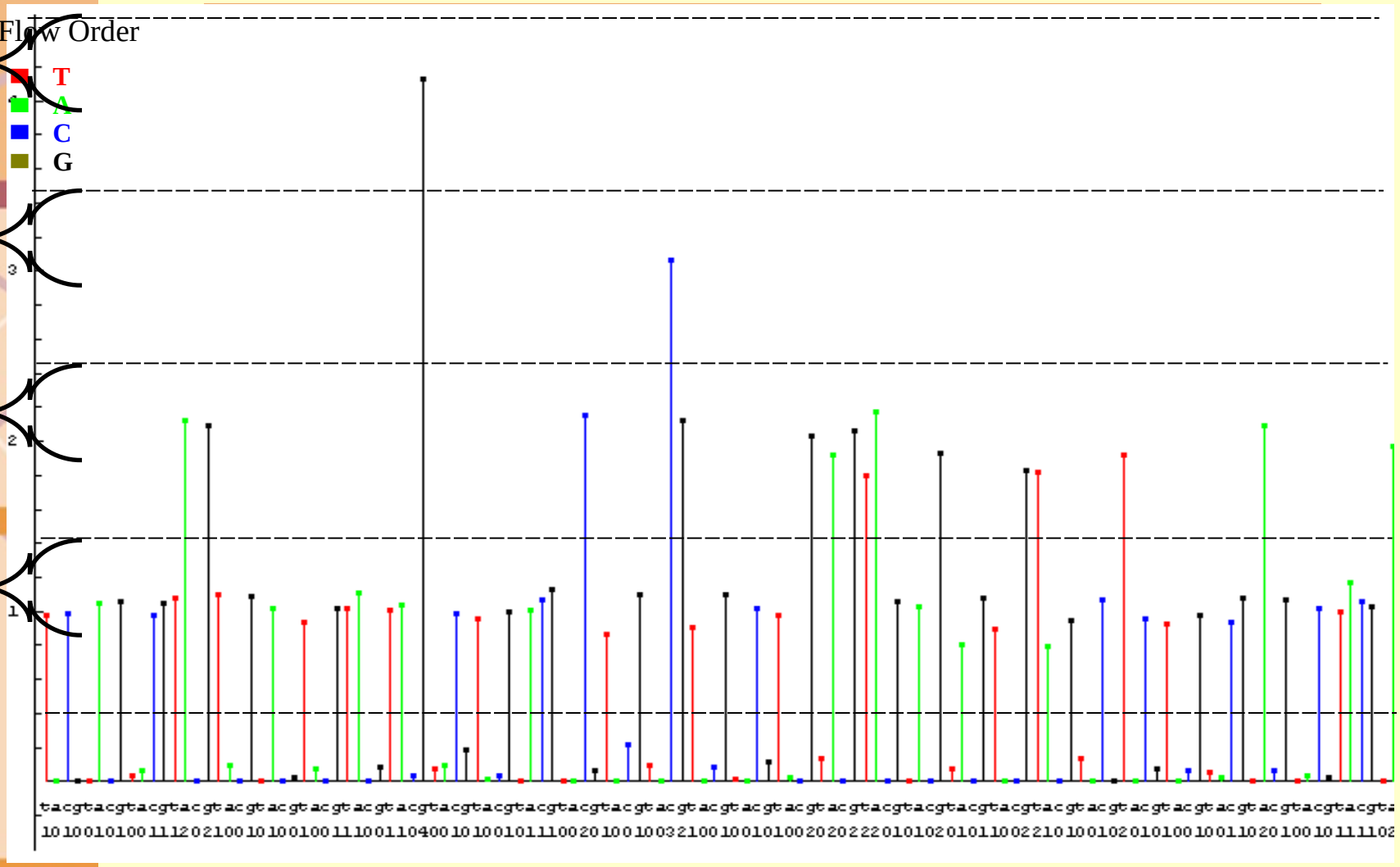
Flowgrams and BaseCalling



Ion Torrent Sequencing

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Semiconductor Sequencing for Life™

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fastest-selling sequencer
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Ion 316™ -- Everything moves faster when The Chip is the Machine™

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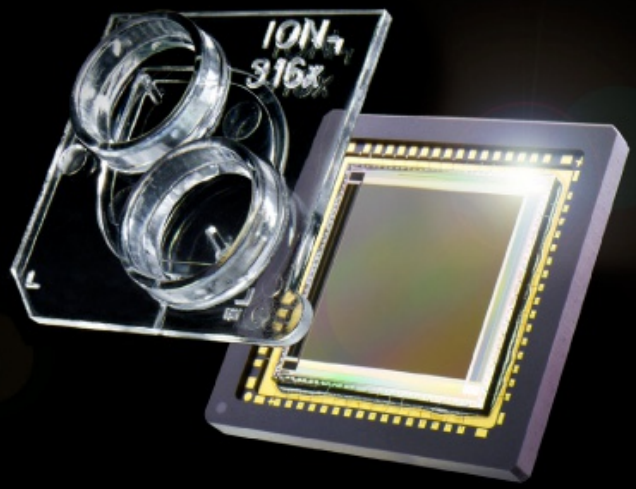

Publications

nature
An integrated semiconductor device enabling non-optical genome sequencing

Application Note

The Ion PGM™ sequencer exhibits superior long-read accuracy
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Life Grand Challenges

life grand challenges

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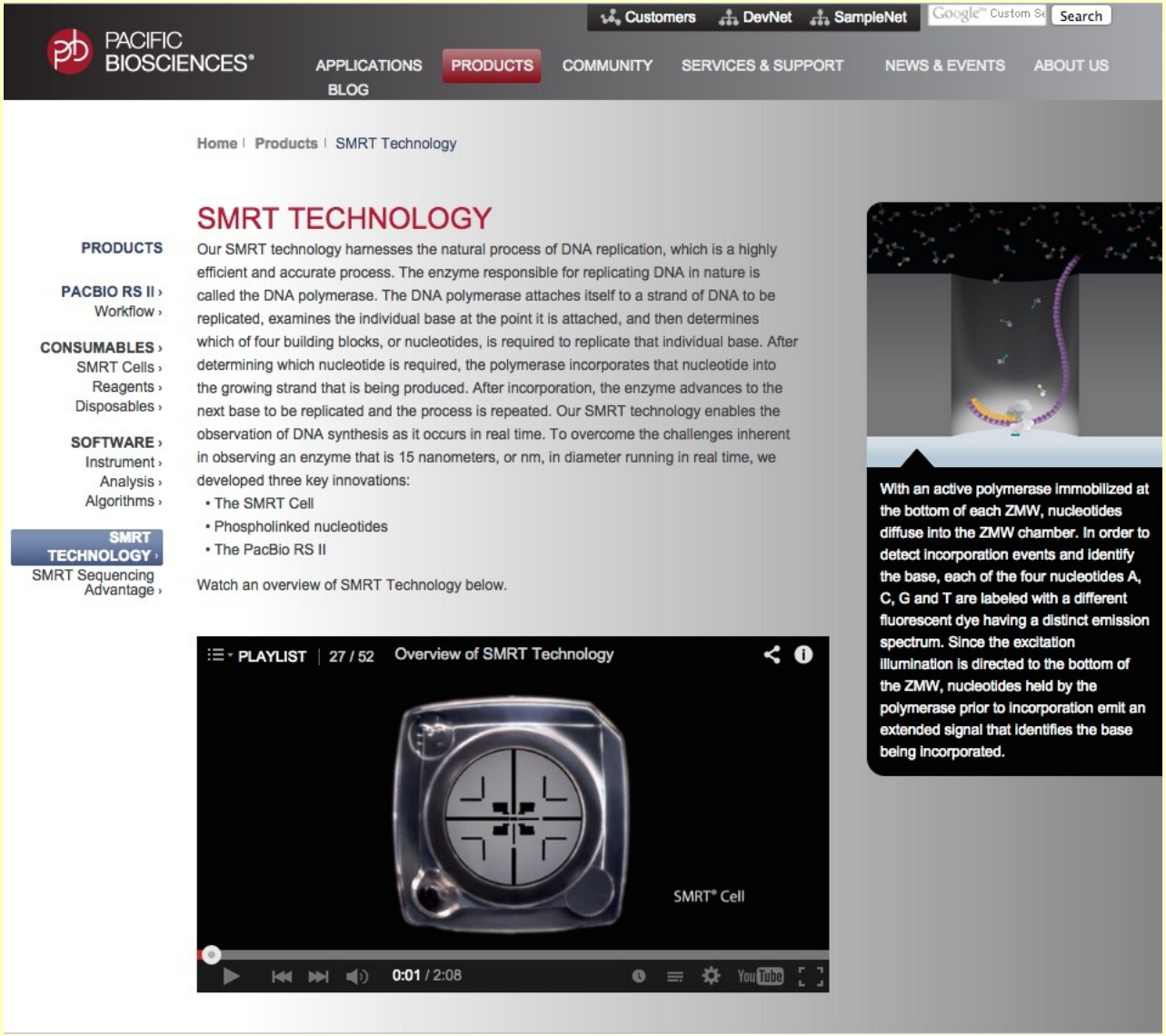
http://www.pacificbiosciences.com/products/smrt-technology/



New PacBio Sequencing Technology Video

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

PACIFIC BIOSCIENCES

Pacific Biosciences Sequencing

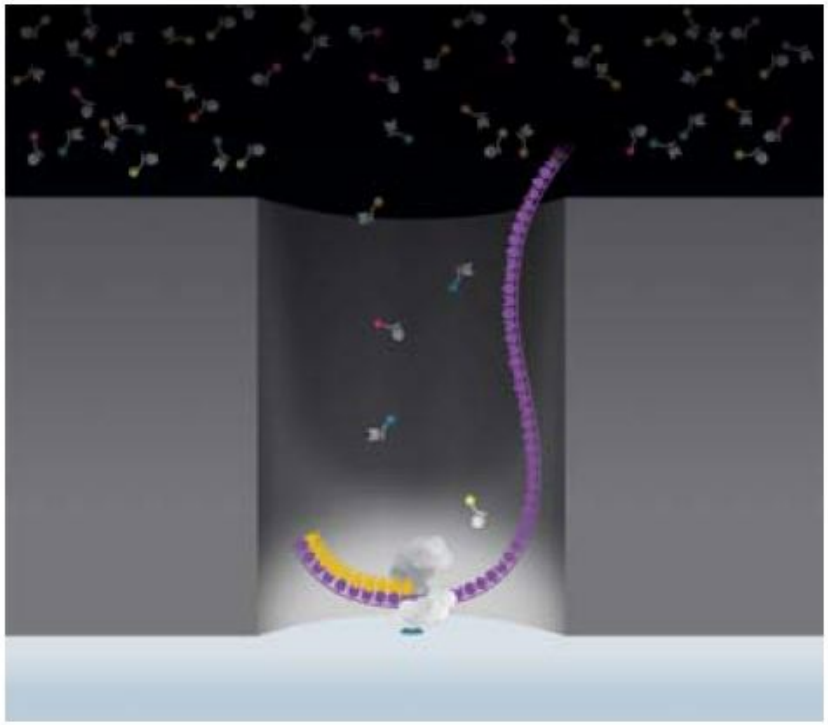


Figure 6. ZMW with DNA polymerase and phospholinked nucleotides

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

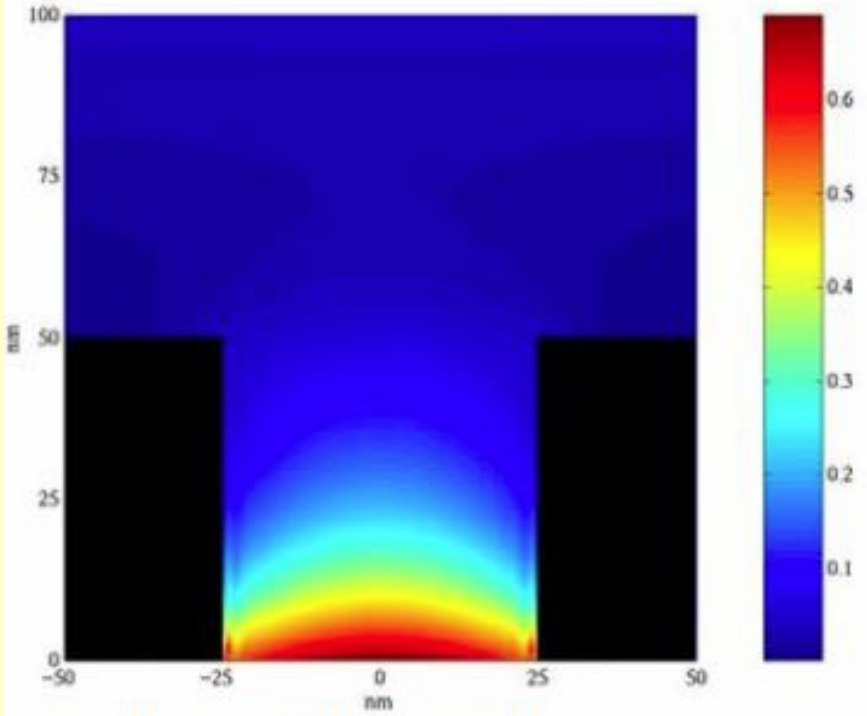


Figure 4. Detection volume

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

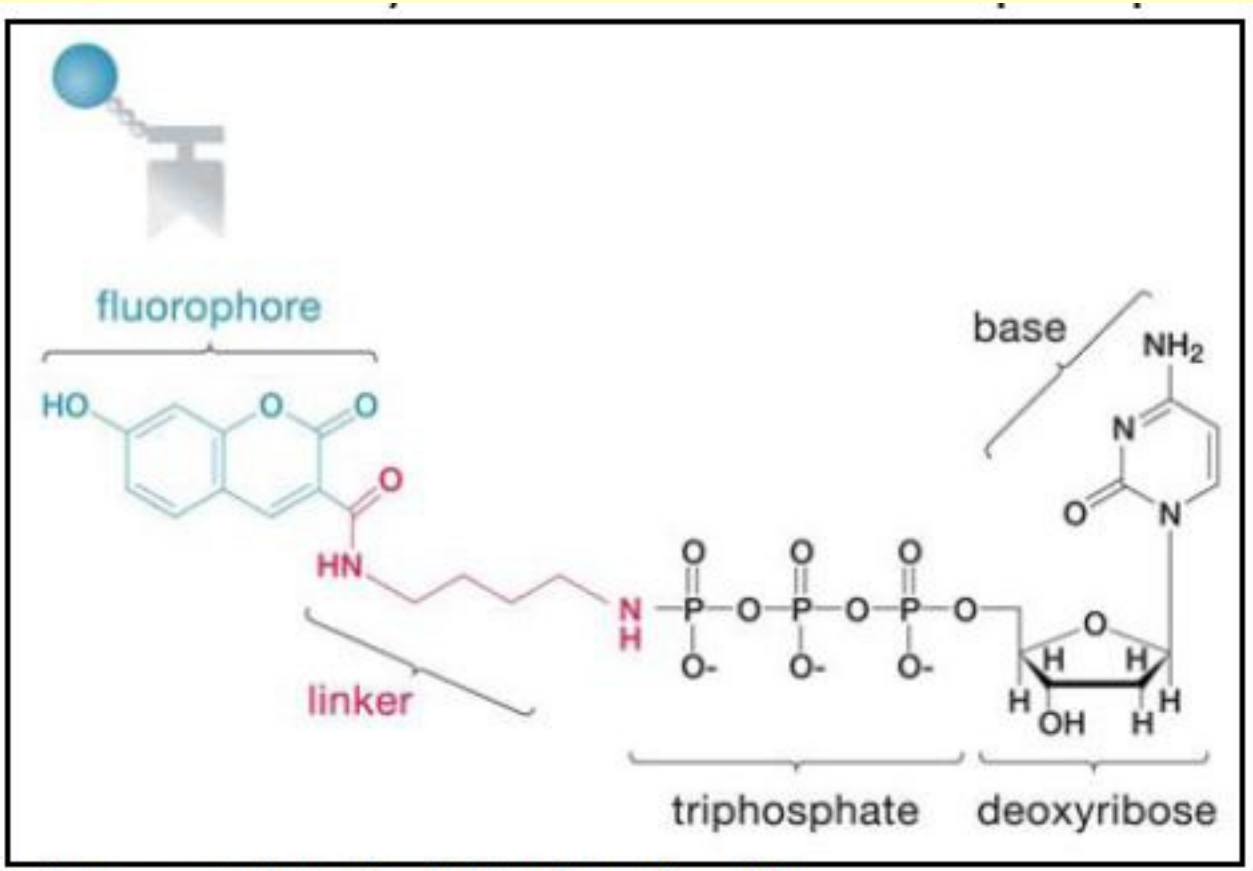


Figure 9. Phospholinked nucleotides

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

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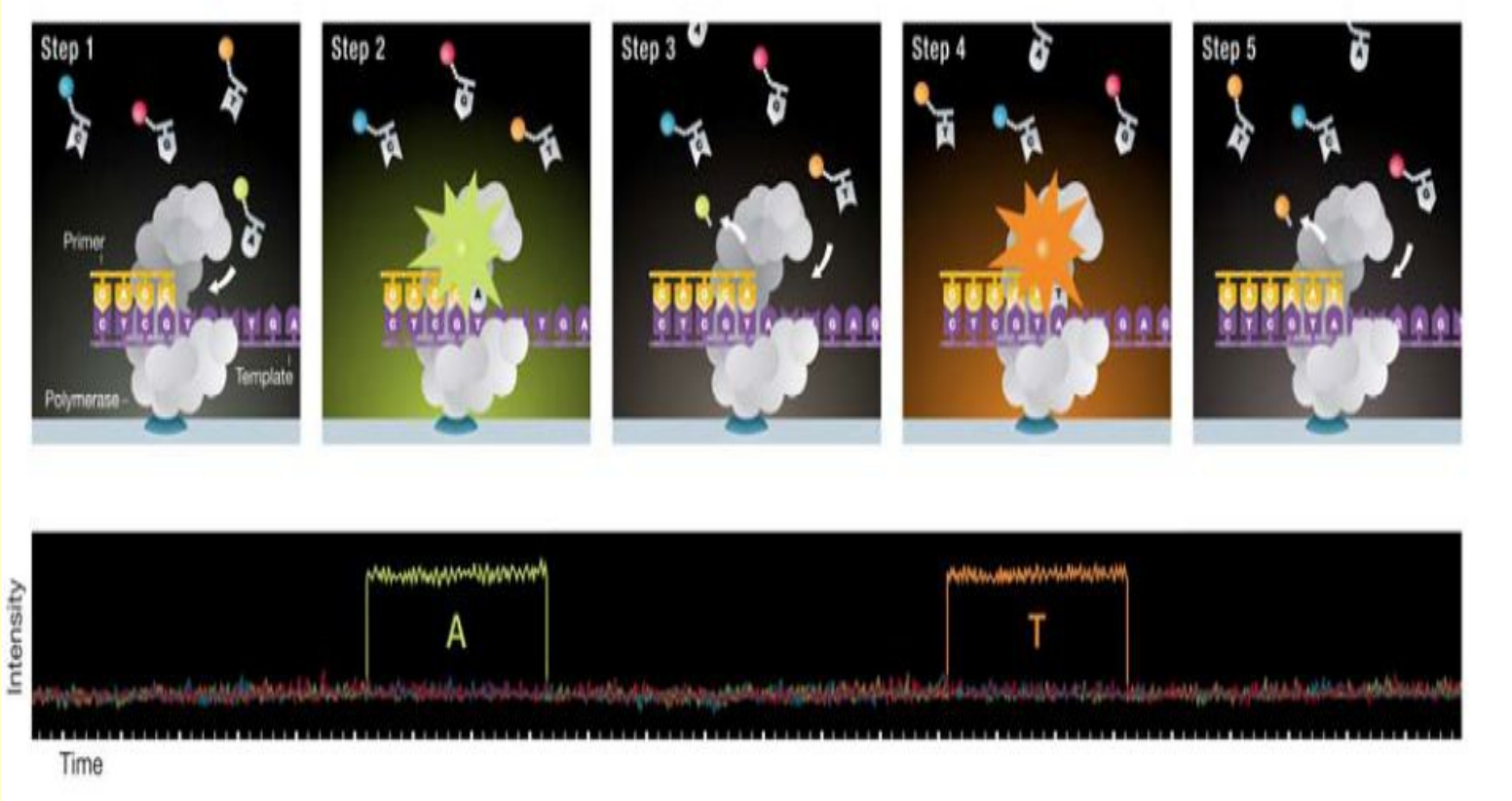
Processive Synthesis



Synthesis of Long Duplex DNA

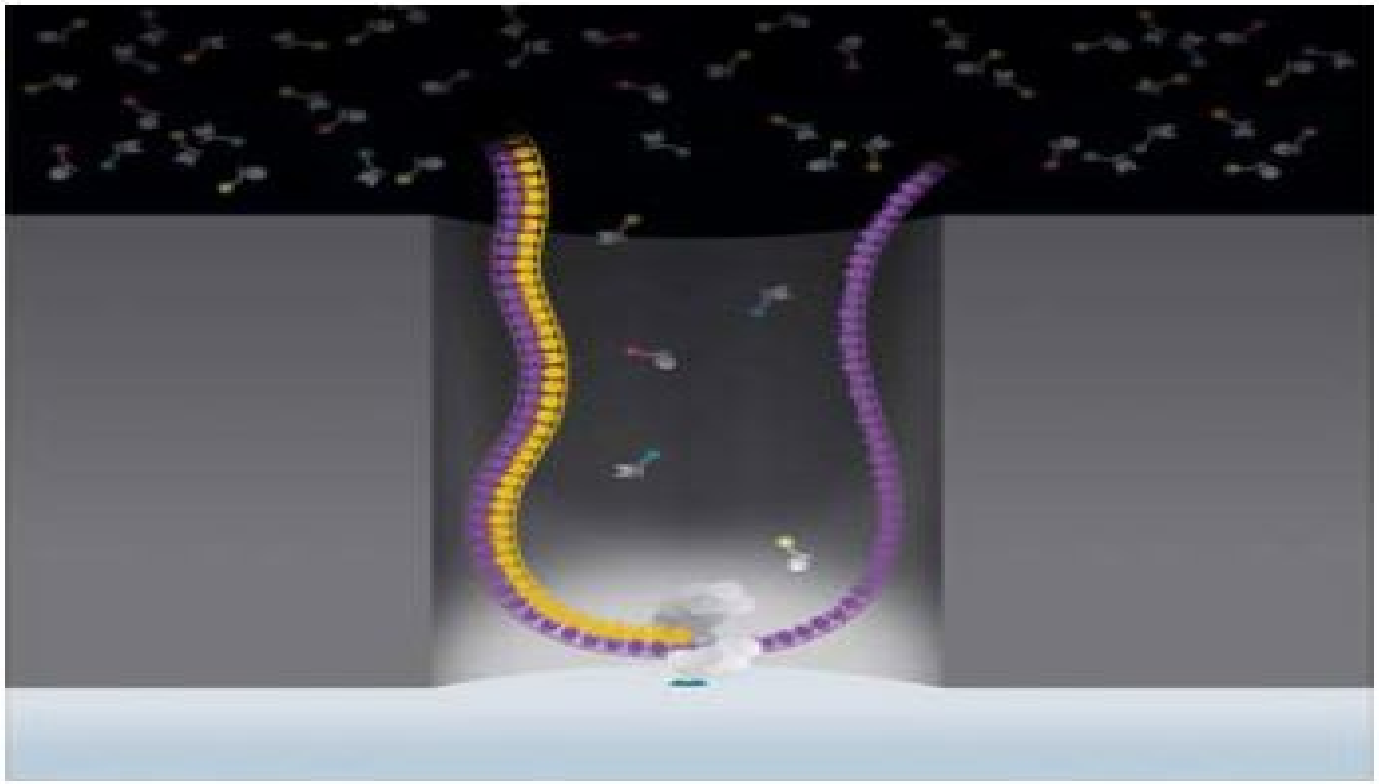


Figure 11. Synthesis of long DNA.

DNA polymerase processively incorporates nucleotides producing long, natural DNA.

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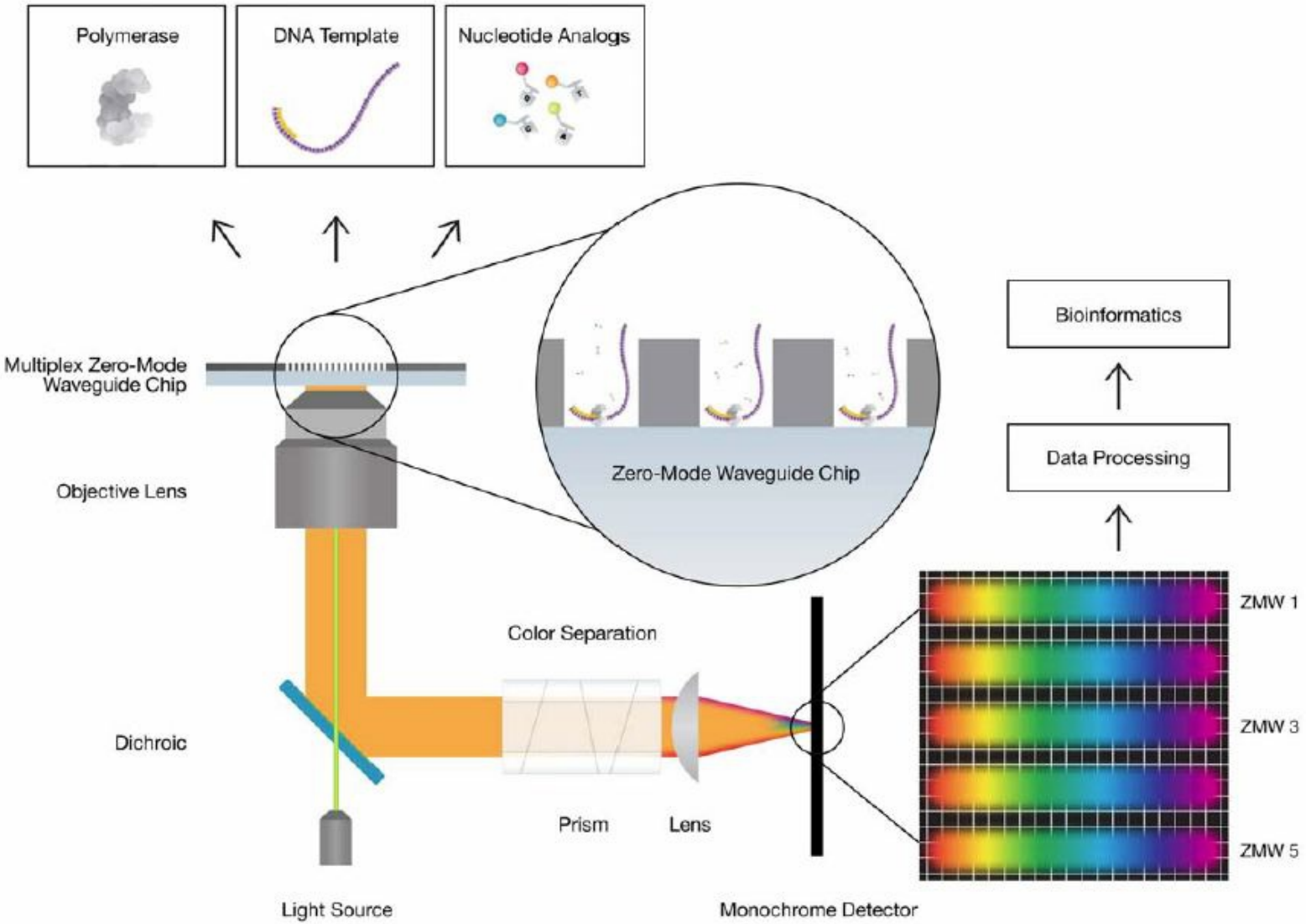
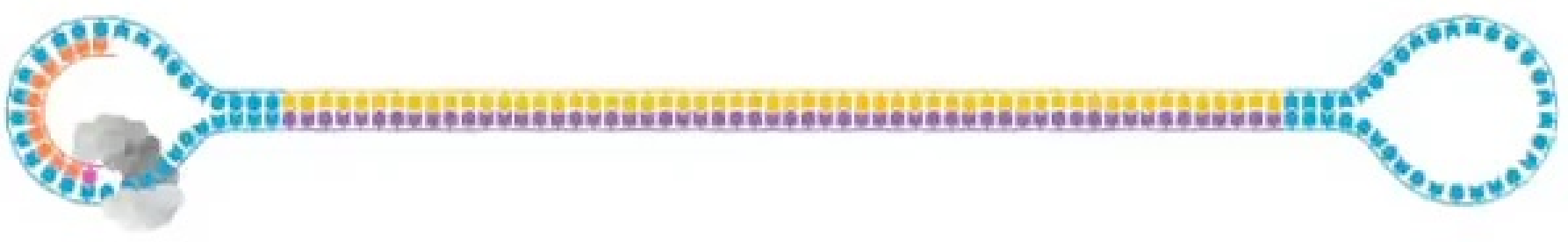
Highly Parallel Optics System



Circular Templates Gives Redundant Sequencing and Accuracy



Circular Templates Gives Redundant Sequencing and Accuracy

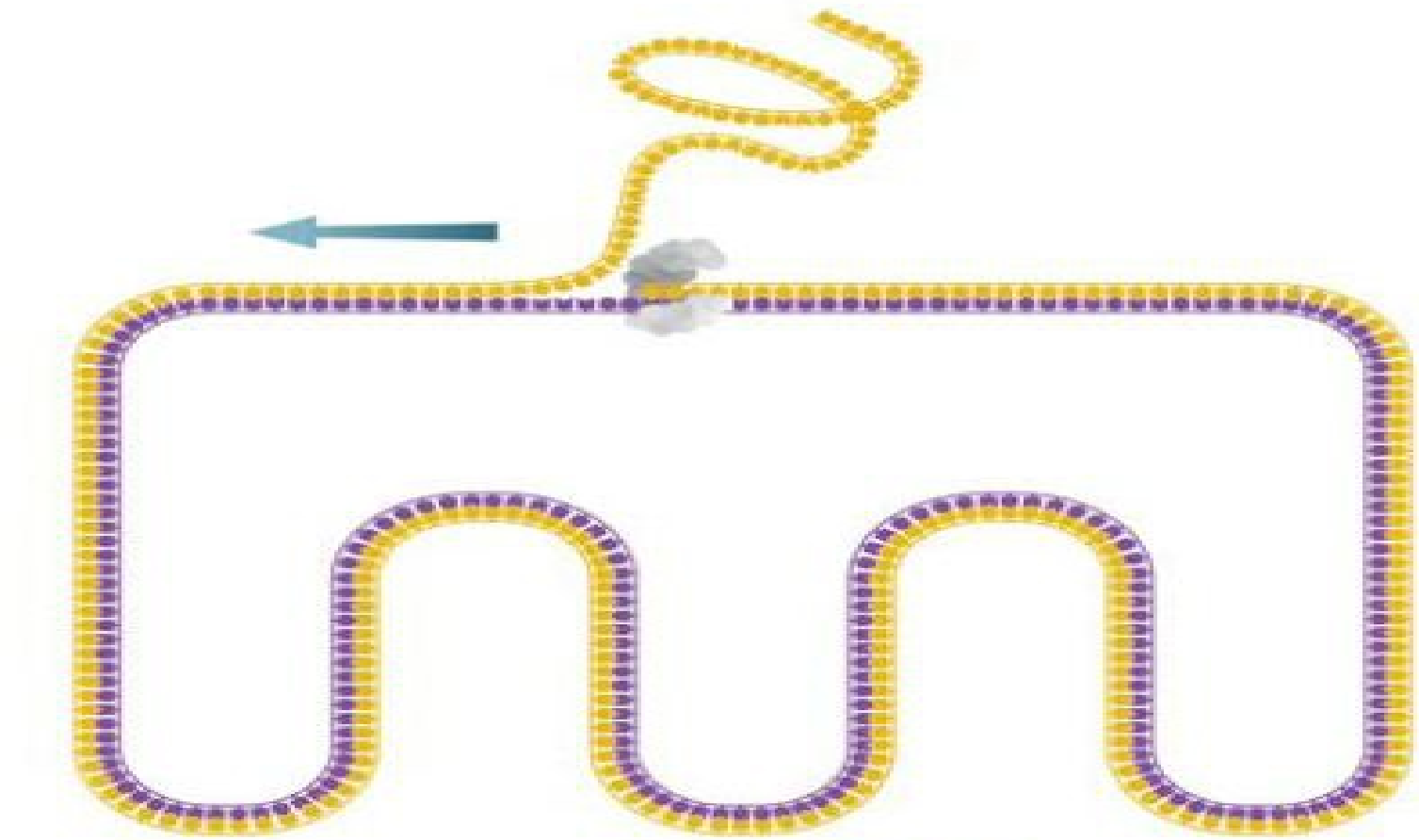
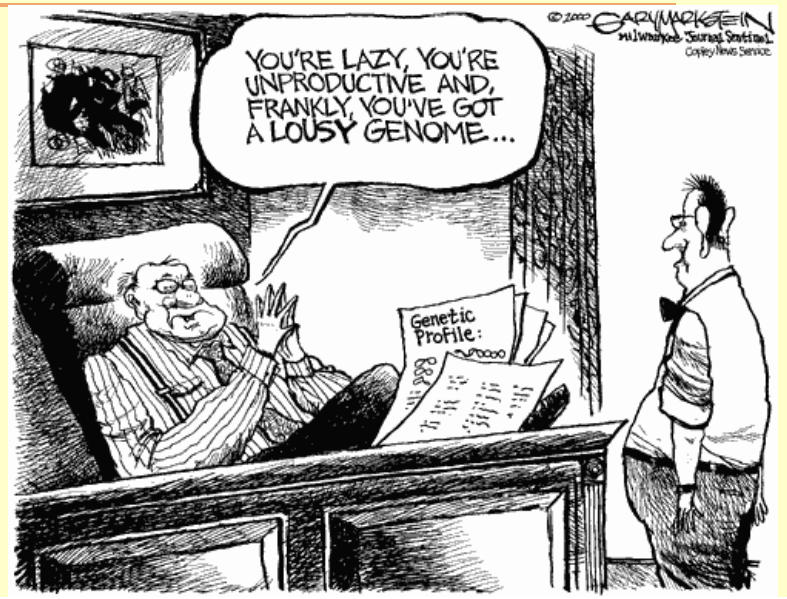
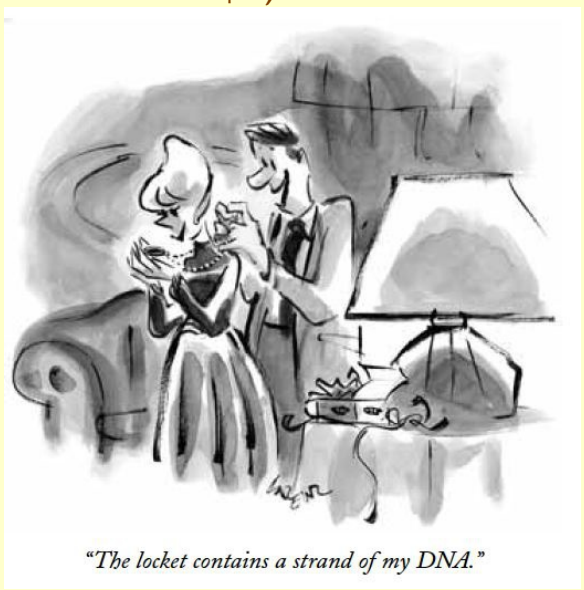


Figure 16. Molecular redundant sequencing

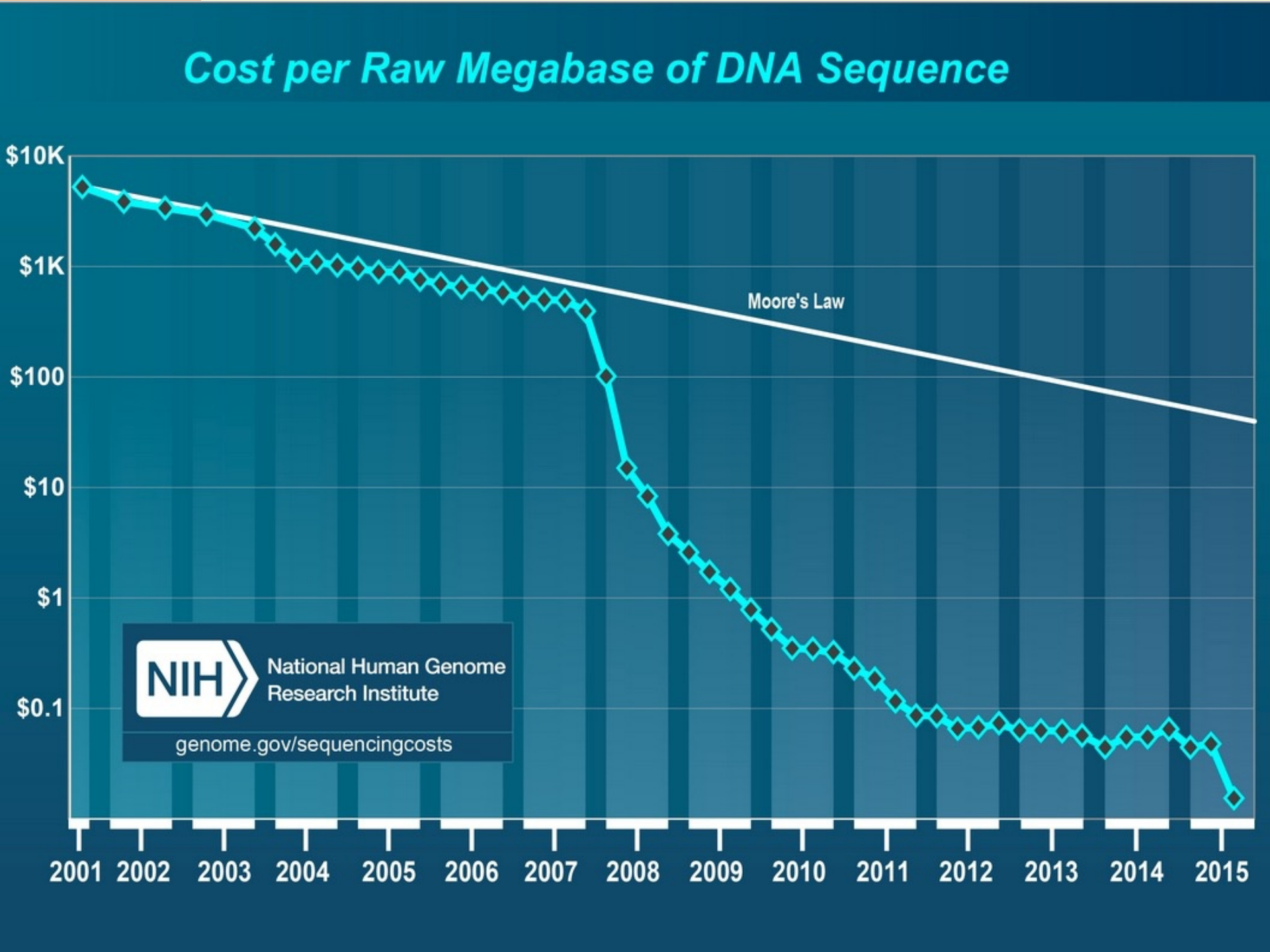
The Human Genome
How fast is the cost going down?

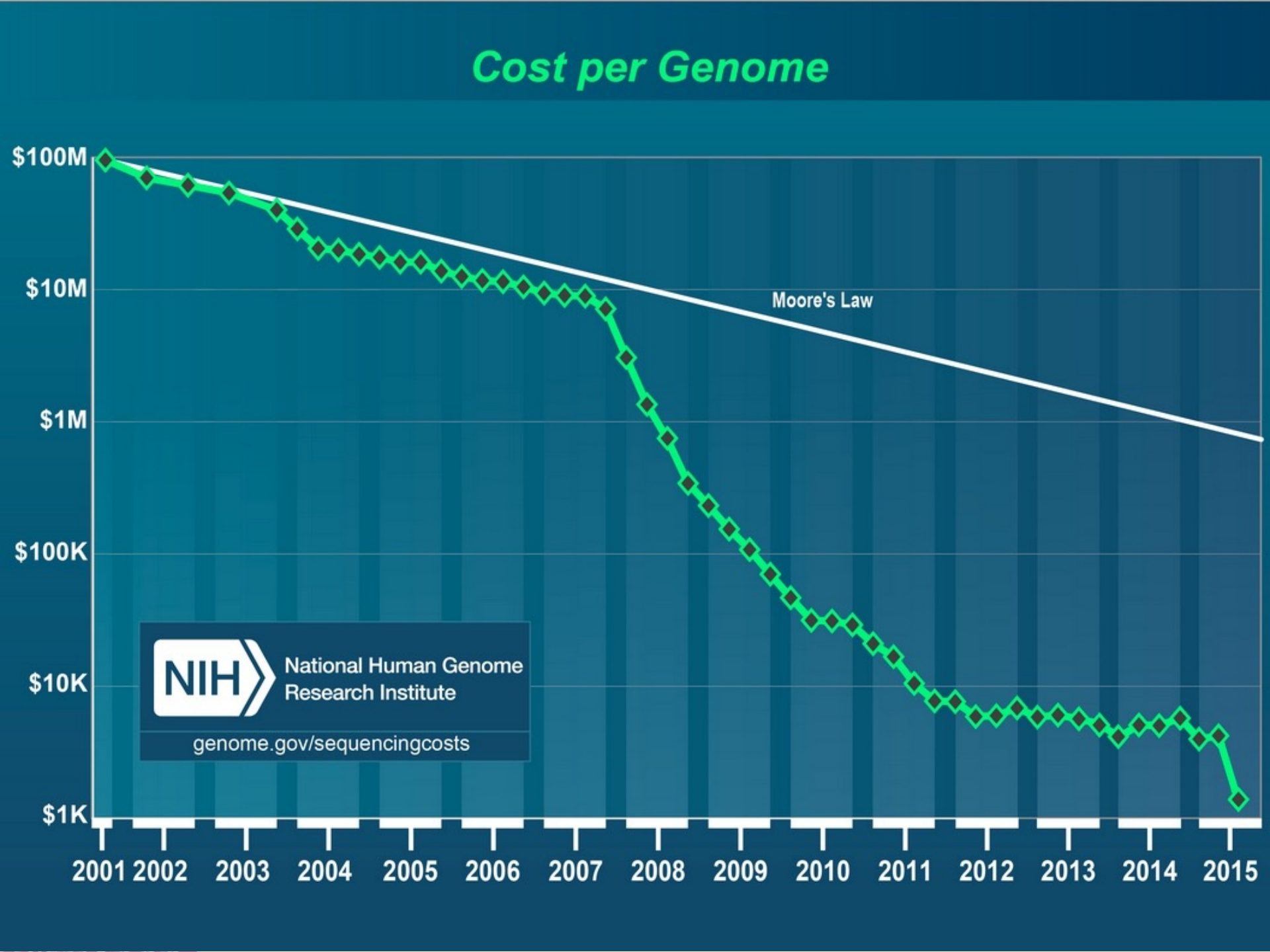
- 2006: $ 50 million
- 2008: $500,000
- 2009: $50,000
- 2010: $20,000
- 2011: $5,000
- 2012: $4,000
- 2013: $3,000
- 2014 $1,400
- 2015 $1,000




"The locket contains a strand of my DNA."

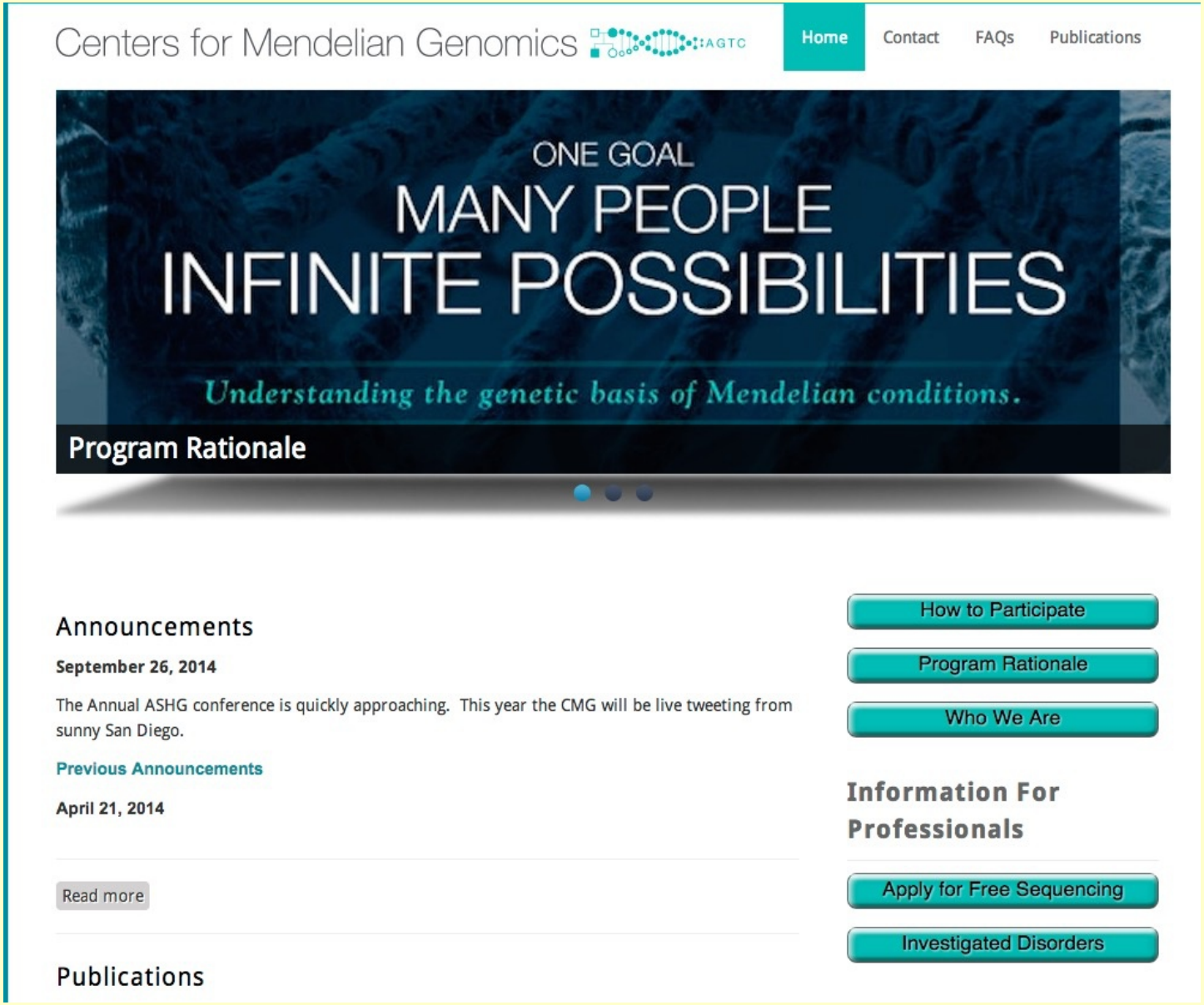
Thanks to Seraf in Batzoglou


Cost per Raw Megabase of DNA Sequence
$10K
$1K
$100
$10
$1
$0.1
Moore's Law
NIH
National Human Genome Research Institute
genome.gov/sequencingcosts
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015


Cost per Genome
$100M
$10M
$1M
$100K
$10K
$1K
Moore's Law
NIH
National Human Genome Research Institute
genome.gov/sequencingcosts
2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015

Centers for Mendelian Genomics

http://mendelian.org/



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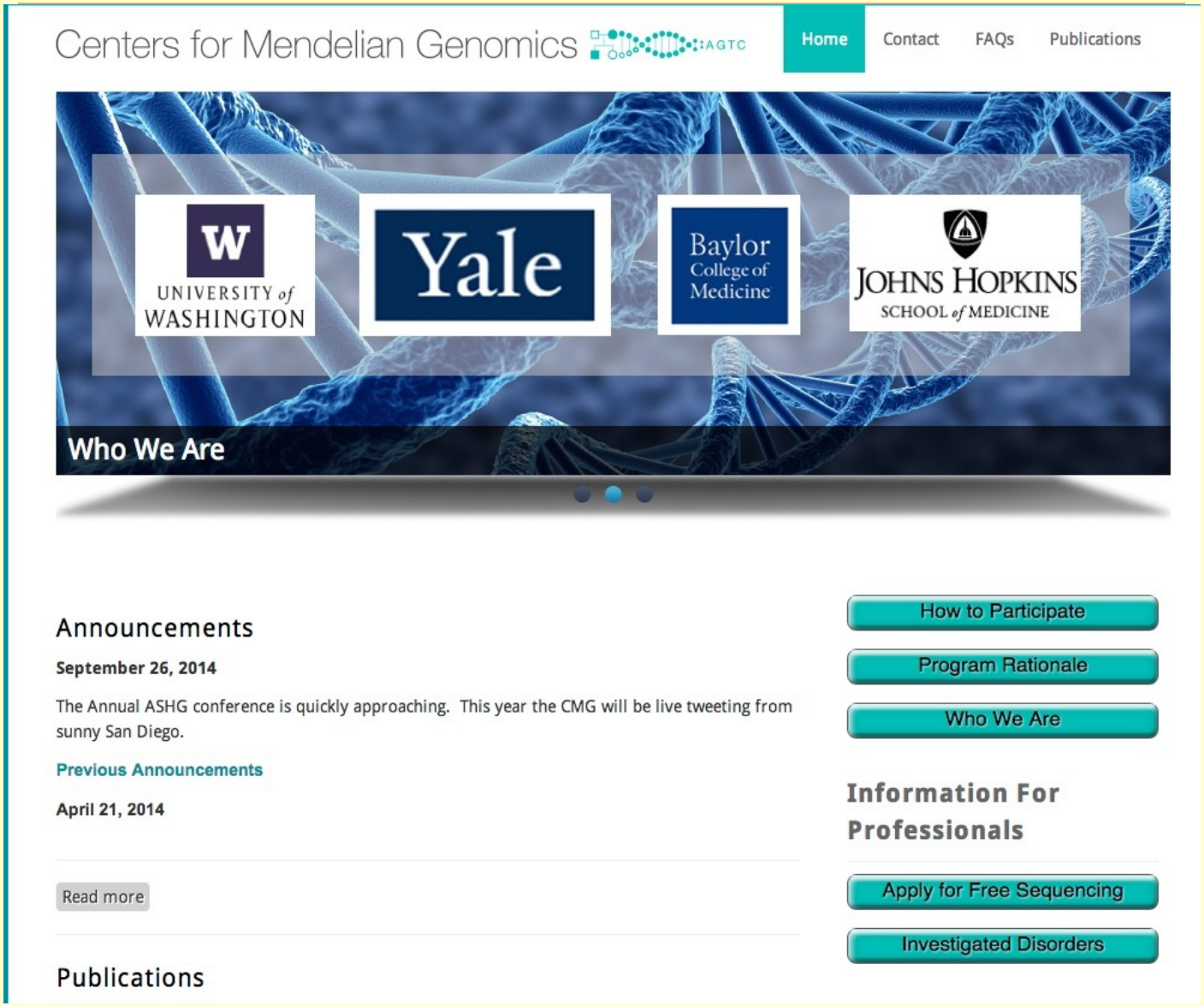

Genomics England

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

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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

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Retweeted by Genomics England

NIH Precision Medicine Initiative

http://www.nih.gov/precisionmedicine/

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 Punktate Keratosis

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

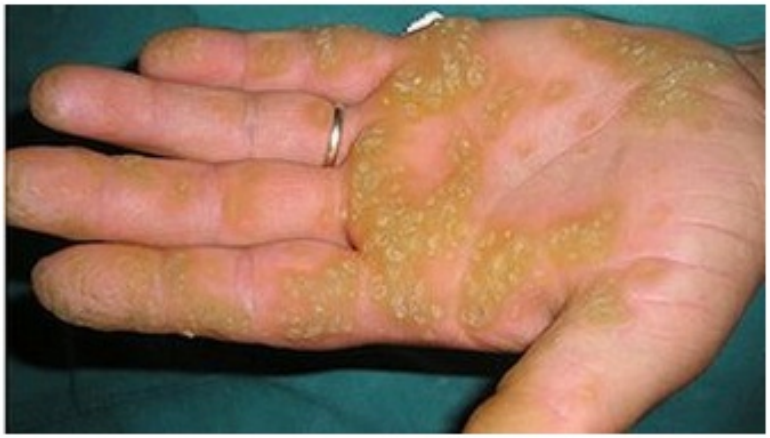
14 October 2012 Last updated at 20:02 ET

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.



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

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

Related Stories

Journals Publishing Genomic Medicine Results

- Nature Genetics
- Science Translational Medicine
- Genome Research
- New England Journal of Medicine
- Journal of the American Medical Association

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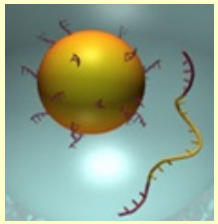
Emulsion Based Clonal Amplification

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

Adapter carrying library DNA



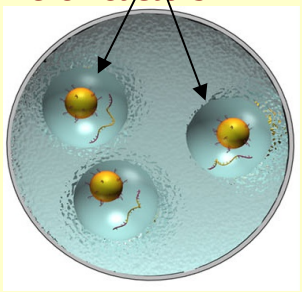
Anneal DNA template to capture beads

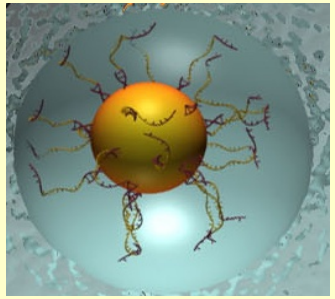
+ PCR Reagents

+ Emulsion Oil

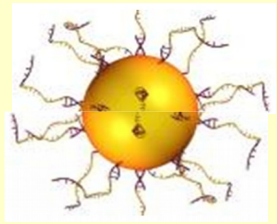
"Water-in-oil" emulsion

Micro-reactors





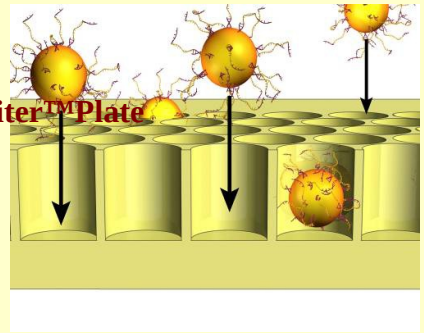
Perform emulsion PCR



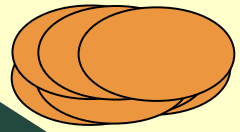
Break micro-reactors
Isolate DNA containing beads

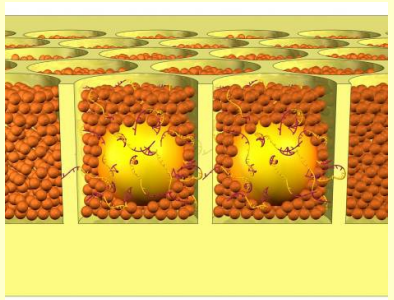
Depositing DNA Beads into the PicoTiter Plate

Load beads into PicoTiter™ Plate

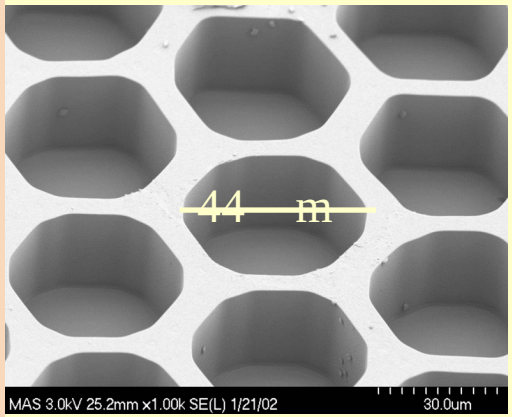


Load Enzyme Beads





Centrifuge Step



- 70x75mm array of fused optical f bers with etched wells
- 1.6 million wells monitored optically through f ber