

New developments in DNA sequencing

Method of the Year

There are events of the year, persons of the year, images of the year.... We could not resist: why not a Method of the Year?

Methods are a driving force of scientific progress. We think they should be celebrated as such. So the editors set out, a couple of months ago, to select the most notable method of 2007—not a method just off the inventor's bench but rather one that came into its own in 2007 and had a wide-ranging impact. But our discussion was quick: we soon had a clear winner in next-generation sequencing.

It is actually not one single method but several, developed in parallel. The first two next-generation sequencing methods made their official entry on the scene in 2005, with publications by the groups of Jonathan Rothberg and George Church, and two related platforms became available within two years. A feature on page 11 recounts the development of these methods and some of the events in 2007 that contributed to firmly establish them in the community.

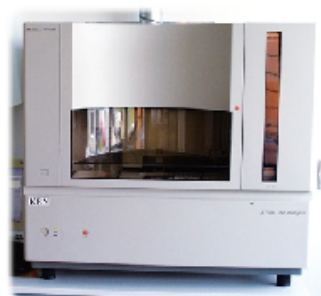
PUBLISHED ONLINE 19 DECEMBER 2007; DOI:10.1038/NMETH1153

NATURE METHODS | VOL.5 NO.1 | JANUARY 2008 | 1

“old” way of genome sequencing

Cloning and clone handling are very labor intensive

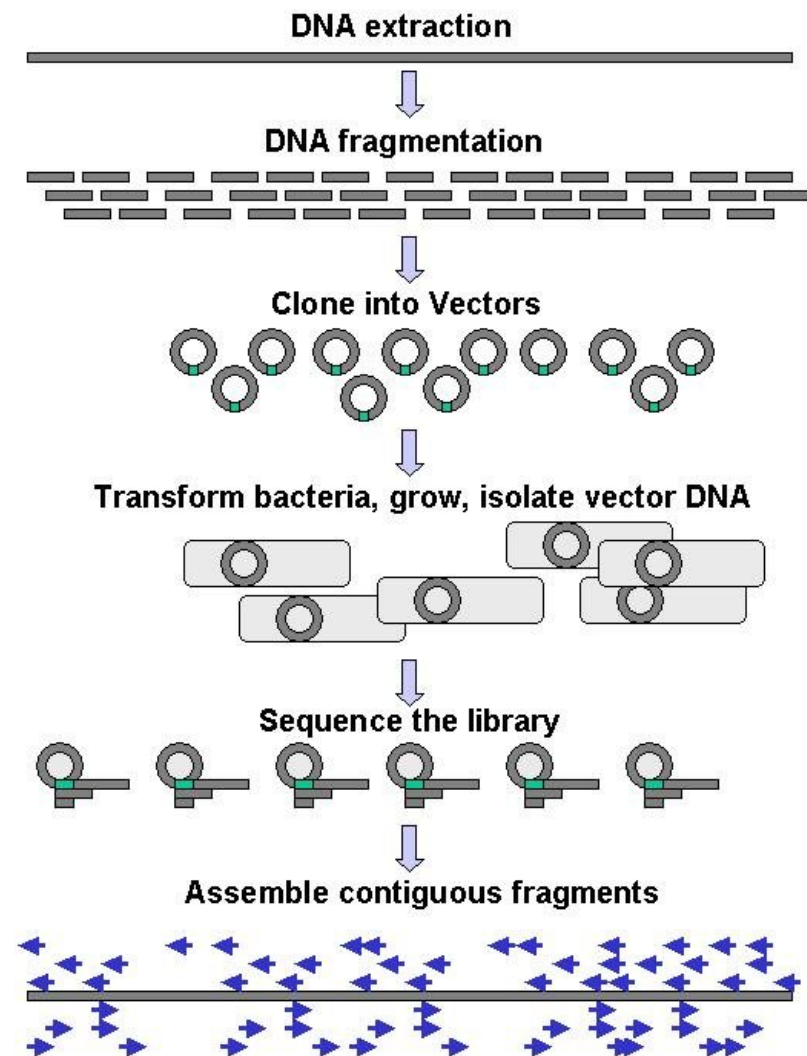
Throughput of capillary sequencing machines is limited



ABI 3730XL
(Applied Biosystems/Sanger)

up to 1,100 bases/read
96 reads/run
approx. 1 MB/day and machine

First choice for finishing projects; full length cDNA sequencing; single sample sequencing.



The human genome 2001

A 2.91-billion base pair (bp) consensus sequence of the euchromatic portion of the human genome was generated by the whole-genome shotgun sequencing method. The 14.8-billion bp DNA sequence was generated over 9 months from 27,271,853 high-quality sequence reads (5.11-fold coverage of the genome) from both ends of plasmid clones made from the DNA of five individuals.

Science 16 February 2001:

Vol. 291. no. 5507, pp. 1304 - 1351

DOI: 10.1126/science.1058040

J. Craig Venter,1* Mark D. Adams,1 Eugene W. Myers,1 Peter W. Li,1 Richard J. Mural,1 Granger G. Sutton,1 Hamilton O. Smith,1 Mark Yandell,1 Cheryl A. Evans,1 Robert A. Holt,1 Jeannine D. Gocayne,1 Peter Amanatides,1 Richard M. Ballew,1 Daniel H. Huson,1 Jennifer Russo Wortman,1 Qing Zhang,1 Chinnappa D. Kodira,1 Xiangqun H. Zheng,1 Lin Chen,1 Marian Skupski,1 Gangadharan Subramanian,1 Paul D. Thomas,1 Jinghui Zhang,1 George L. Gabor Miklos,2 Catherine Nelson,3 Samuel Broder,1 Andrew G. Clark,4 Joe Nadeau,5 Victor A. McKusick,6 Norton Zinder,7 Arnold J. Levine,7 Richard J. Roberts,8 Mel Simon,9 Carolyn Slayman,10 Michael Hunkapiller,11 Randall Bolanos,1 Arthur Delcher,1 Ian Dew,1 Daniel Fasulo,1 Michael Flanigan,1 Liliانا Florea,1 Aaron Halpern,1 Sridhar Hannenhalli,1 Saul Kravitz,1 Samuel Levy,1 Clark Mobarry,1 Knut Reinert,1 Karin Remington,1 Jane Abu-Threideh,1 Ellen Beasley,1 Kendra Biddick,1 Vivien Bonazzi,1 Rhonda Brandon,1 Michele Cargill,1 Ishwar Chandramouliswaran,1 Rosane Charlab,1 Kabir Chaturvedi,1 Zuoming Deng,1 Valentina Di Francesco,1 Patrick Dunn,1 Karen Eilbeck,1 Carlos Evangelista,1 Andrei E. Gabrielian,1 Weiniu Gan,1 Wangmao Ge,1 Fangcheng Gong,1 Zhiping Gu,1 Ping Guan,1 Thomas J. Heiman,1 Maureen E. Higgins,1 Rui-Ru Ji,1 Zhaoxi Ke,1 Karen A. Ketchum,1 Zhongwu Lai,1 Yiding Lei,1 Zhenya Li,1 Jiayin Li,1 Yong Liang,1 Xiaoying Lin,1 Fu Lu,1 Gennady V. Merkulov,1 Natalia Milshina,1 Helen M. Moore,1 Ashwinikumar K Naik,1 Vaibhav A. Narayan,1 Beena Neelam,1 Deborah Nusskern,1 Douglas B. Rusch,1 Steven Salzberg,12 Wei Shao,1 Bixiong Shue,1 Jingtao Sun,1 Zhen Yuan Wang,1 Aihui Wang,1 Xin Wang,1 Jian Wang,1 Ming-Hui Wei,1 Ron Wides,13 Chunlin Xiao,1 Chunhua Yan,1 Alison Yao,1 Jane Ye,1 Ming Zhan,1 Weiqing Zhang,1 Hongyu Zhang,1 Qi Zhao,1 Liansheng Zheng,1 Fei Zhong,1 Wenyang Zhong,1 Shiaooping C. Zhu,1 Shaying Zhao,12 Dennis Gilbert,1 Suzanna Baumhueter,1 Gene Spier,1 Christine Carter,1 Anibal Cravchik,1 Trevor Woodage,1 Feroze Ali,1 Huijin An,1 Aderonke Awe,1 Danita Baldwin,1 Holly Baden,1 Mary Barnstead,1 Ian Barrow,1 Karen Beeson,1 Dana Busam,1 Amy Carver,1 Angela Center,1 Ming Lai Cheng,1 Liz Curry,1 Steve Danaher,1 Lionel Davenport,1 Raymond Desilets,1 Susanne Dietz,1 Kristina Dodson,1 Lisa Doup,1 Steven Ferriera,1 Neha Garg,1 Andres Gluecksmann,1 Brit Hart,1 Jason Haynes,1 Charles Haynes,1 Cheryl Heiner,1 Suzanne Hladun,1 Damon Hostin,1 Jarrett Houck,1 Timothy Howland,1 Chinyere Ibegwam,1 Jeffery Johnson,1 Francis Kalush,1 Lesley Kline,1 Shashi Koduru,1 Amy Love,1 Felecia Mann,1 David May,1 Steven McCawley,1 Tina McIntosh,1 Ivy McMullen,1 Mee Moy,1 Linda Moy,1 Brian Murphy,1 Keith Nelson,1 Cynthia Pfannkoch,1 Eric Pratts,1 Vinita Puri,1 Hina Qureshi,1 Matthew Reardon,1 Robert Rodriguez,1 Yu-Hui Rogers,1 Deanna Romblad,1 Bob Ruhfel,1 Richard Scott,1 Cynthia Sitter,1 Michelle Smallwood,1 Erin Stewart,1 Renee Strong,1 Ellen Suh,1 Reginald Thomas,1 Ni Ni Tint,1 Sukyee Tse,1 Claire Vech,1 Gary Wang,1 Jeremy Wetter,1 Sherita Williams,1 Monica Williams,1 Sandra Windsor,1 Emily Winn-Deen,1 Keriellen Wolfe,1 Jaysree Zaveri,1 Karena Zaveri,1 Josep F. Abril,14 Roderic Guigó,14 Michael J. Campbell,1 Kimmen V. Sjolander,1 Brian Karlak,1 Anish Kejariwal,1 Huaiyu Mi,1 Betty Lazareva,1 Thomas Hatton,1 Apurva Narechania,1 Karen Diemer,1 Anushya Muruganujan,1 Nan Guo,1 Shinji Sato,1 Vineet Bafna,1 Sorin Istrail,1 Ross Lippert,1 Russell Schwartz,1 Brian Walenz,1 Shibu Yooseph,1 David Allen,1 Anand Basu,1 James Baxendale,1 Louis Blick,1 Marcelo Caminha,1 John Carnes-Stine,1 Parris Caulk,1 Yen-Hui Chiang,1 My Coyne,1 Carl Dahlke,1 Anne Deslattes Mays,1 Maria Dombroski,1 Michael Donnelly,1 Dale Ely,1 Shiva Esparham,1 Carl Foster,1 Harold Gire,1 Stephen Glanowski,1 Kenneth Glasser,1 Anna Glodek,1 Mark Gorokhov,1 Ken Graham,1 Barry Gropman,1 Michael Harris,1 Jeremy Heil,1 Scott Henderson,1 Jeffrey Hoover,1 Donald Jennings,1 Catherine Jordan,1 James Jordan,1 John Kasha,1 Leonid Kagan,1 Cheryl Kraft,1 Alexander Levitsky,1 Mark Lewis,1 Xiangjun Liu,1 John Lopez,1 Daniel Ma,1 William Majoros,1 Joe McDaniel,1 Sean Murphy,1 Matthew Newman,1 Trung Nguyen,1 Ngoc Nguyen,1 Marc Nodell,1 Sue Pan,1 Jim Peck,1 Marshall Peterson,1 William Rowe,1 Robert Sanders,1 John Scott,1 Michael Simpson,1 Thomas Smith,1 Arlan Sprague,1 Timothy Stockwell,1 Russell Turner,1 Eli Venter,1 Mei Wang,1 Meiyuan Wen,1 David Wu,1 Mitchell Wu,1 Ashley Xia,1 Ali Zandieh,1 Xiaohong Zhu1

The human genome in the future

Massive high-throughput is needed

Avoiding manual manipulations as much as possible

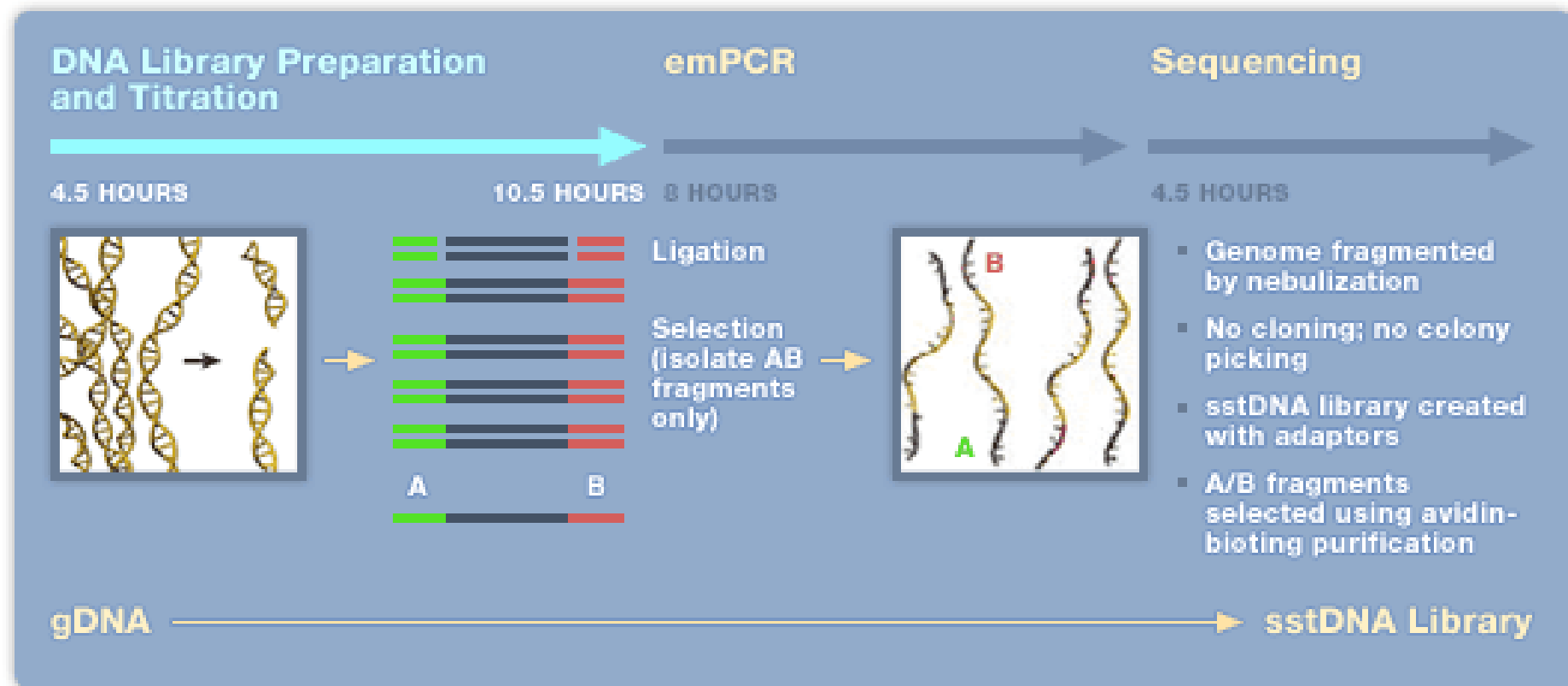
Such as

- Clone picking
- Clone administration
- Plate handling

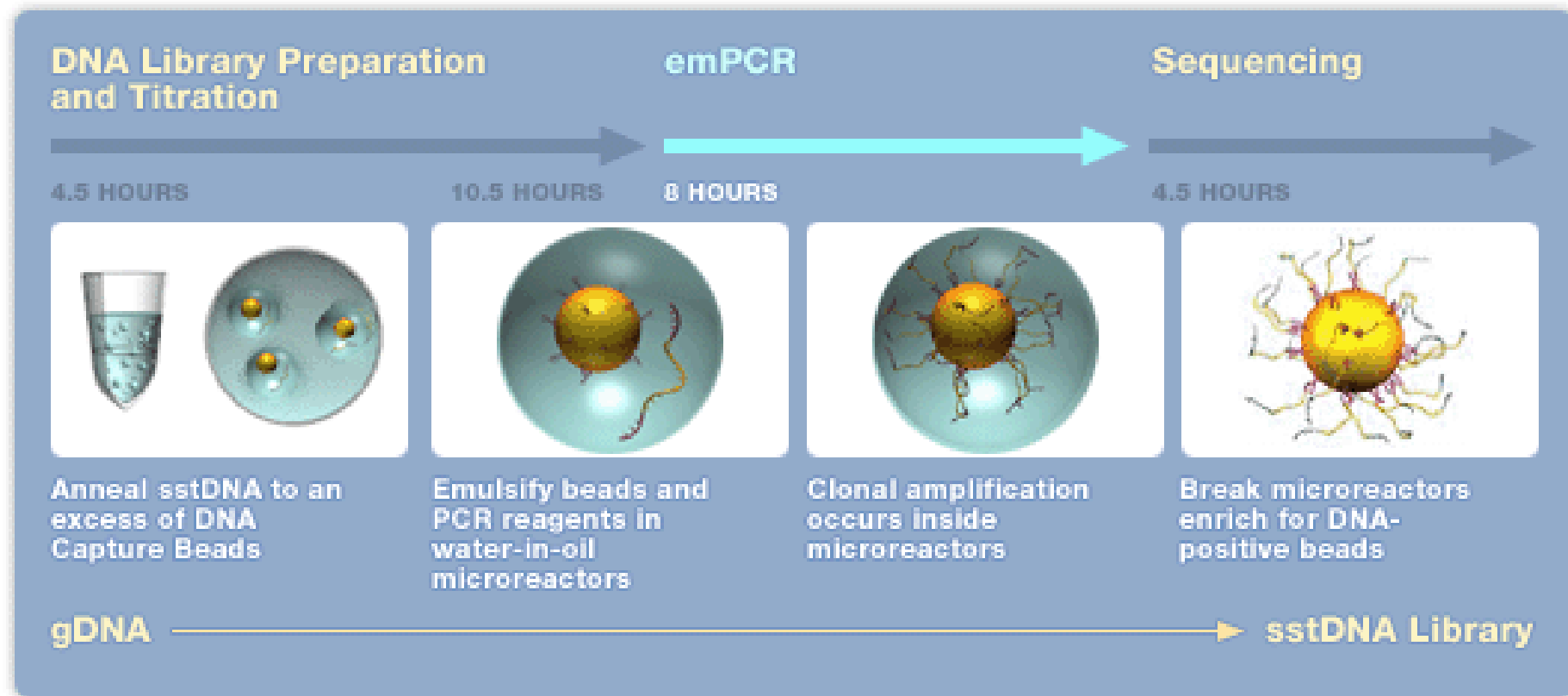
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Bias introduced in genome sequencing by the use of bacteria for cloning and producing the DNA → not all sequences are well tolerated and reproduced in *E. coli*

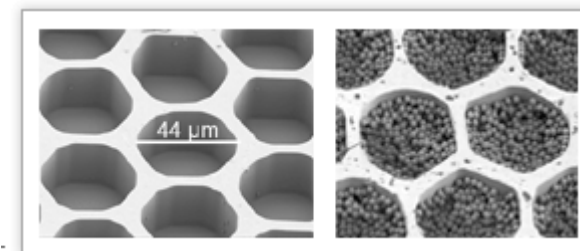
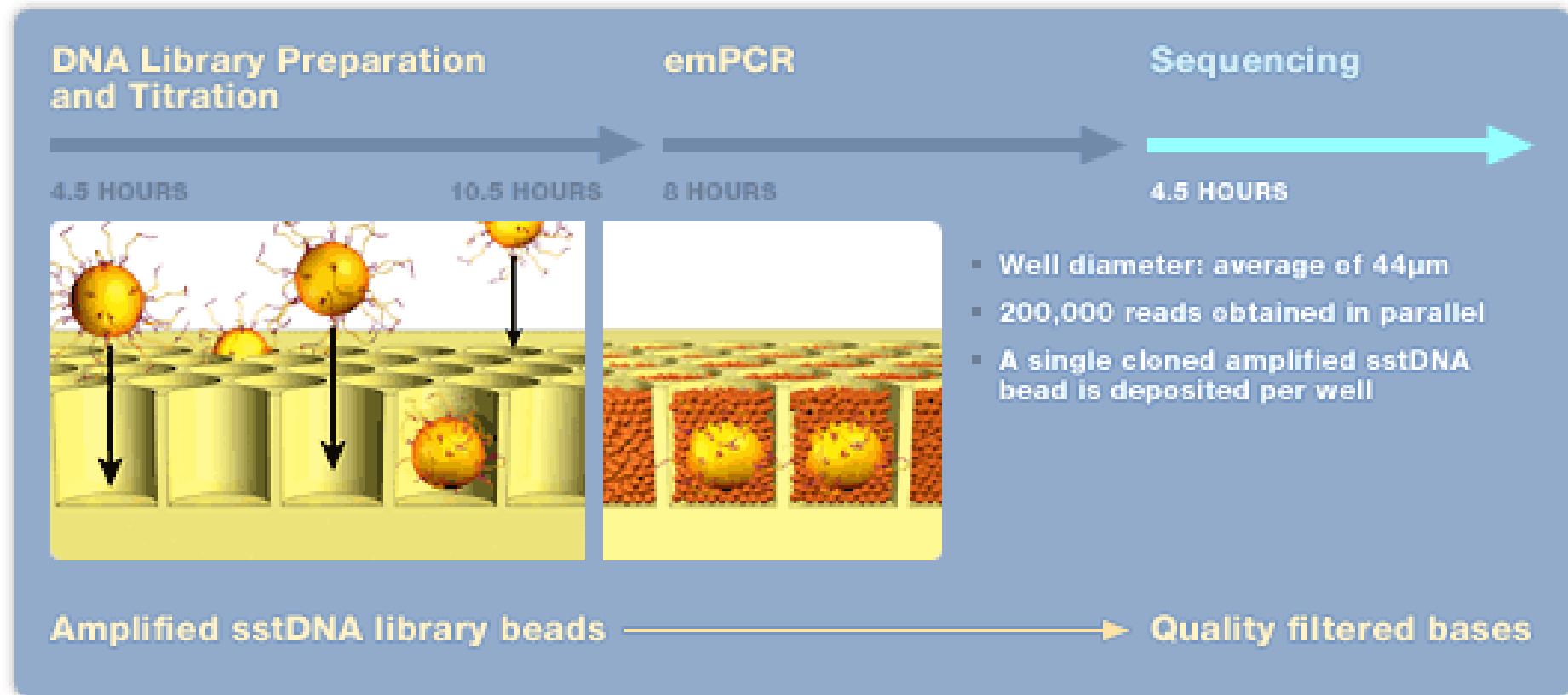
454 DNA sequencing



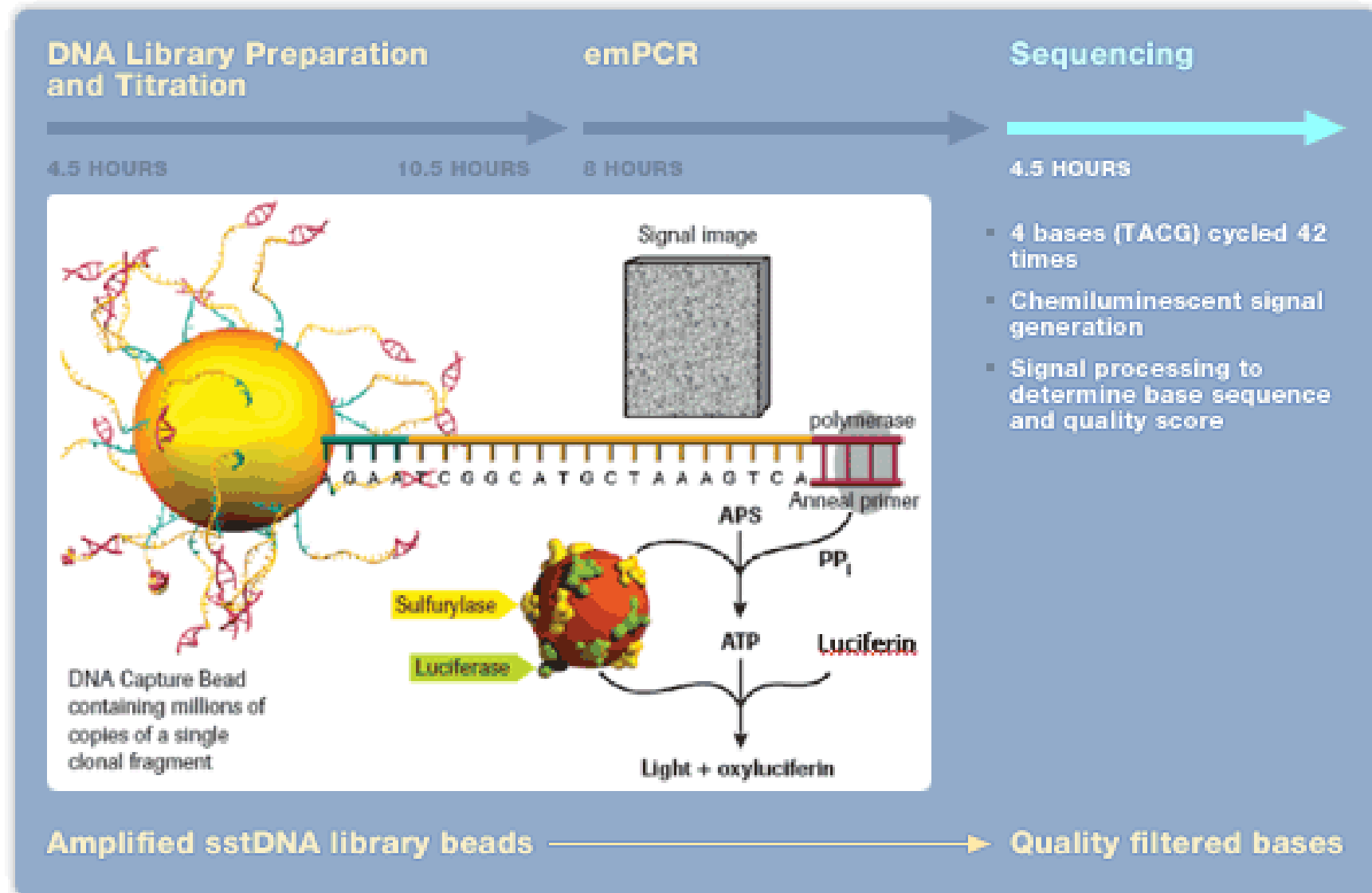
Genome sequencing in microfabricated high-density picolitre reactors



Genome sequencing in microfabricated high-density picolitre reactors

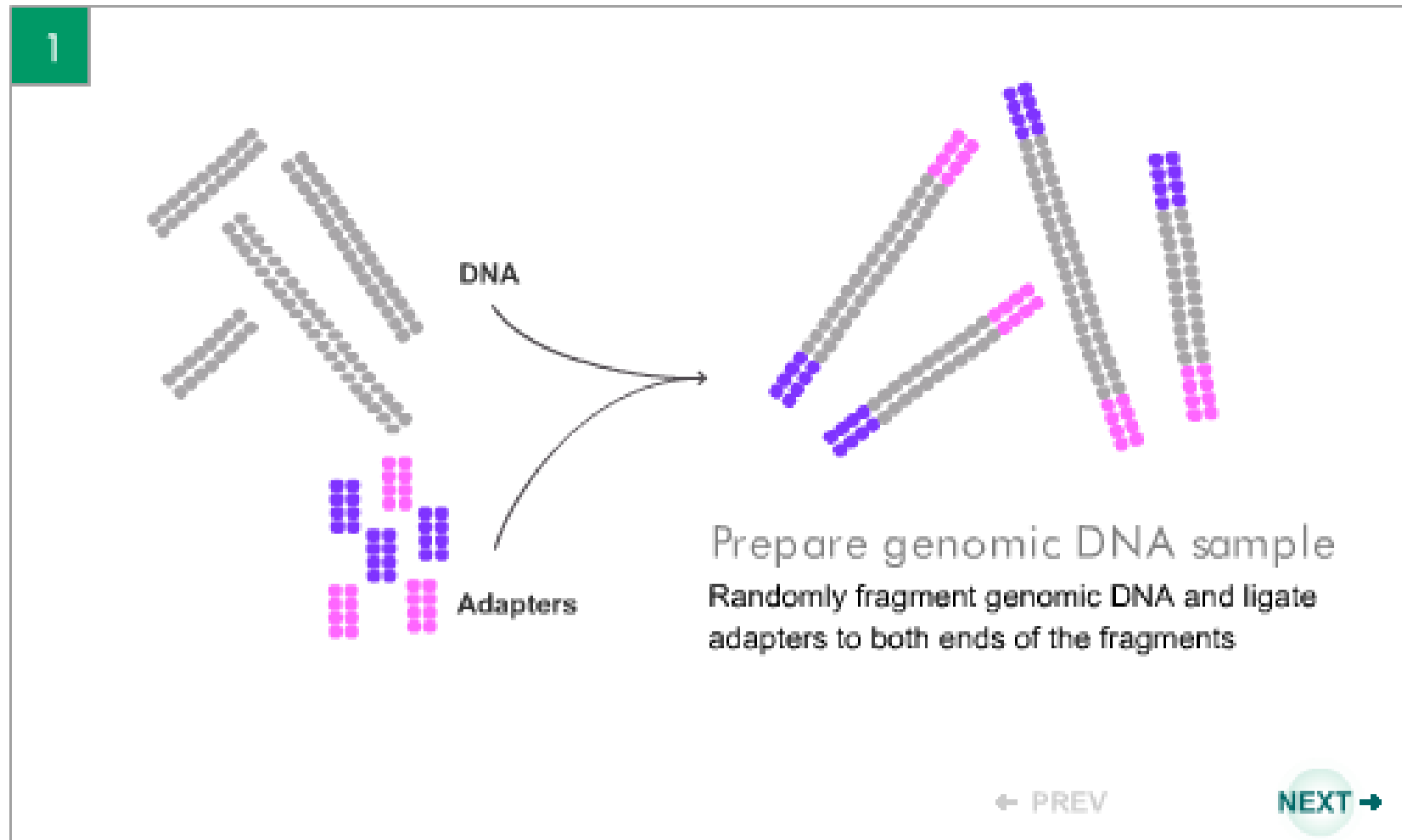


Genome sequencing in microfabricated high-density picolitre reactors

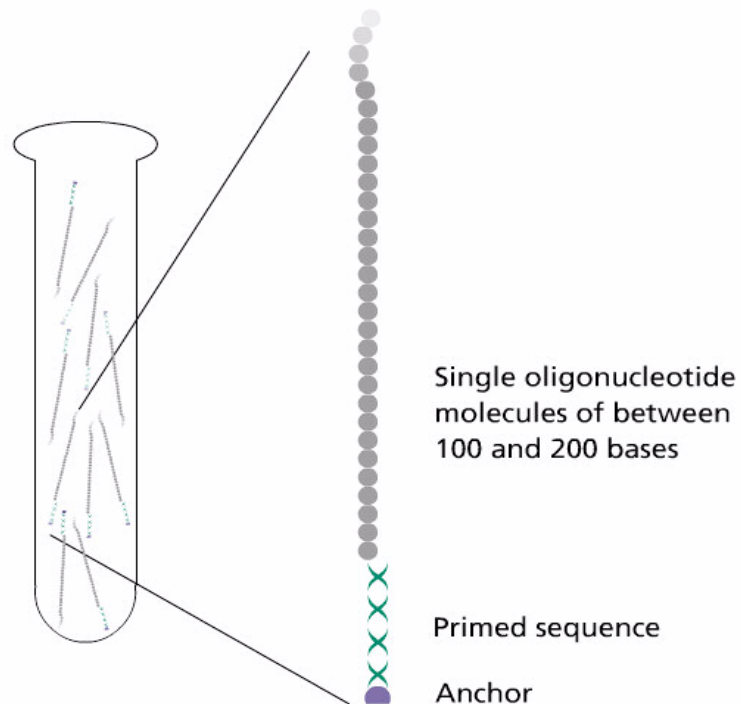


solexa sequencing technology

Sequencing-By-Synthesis Demo



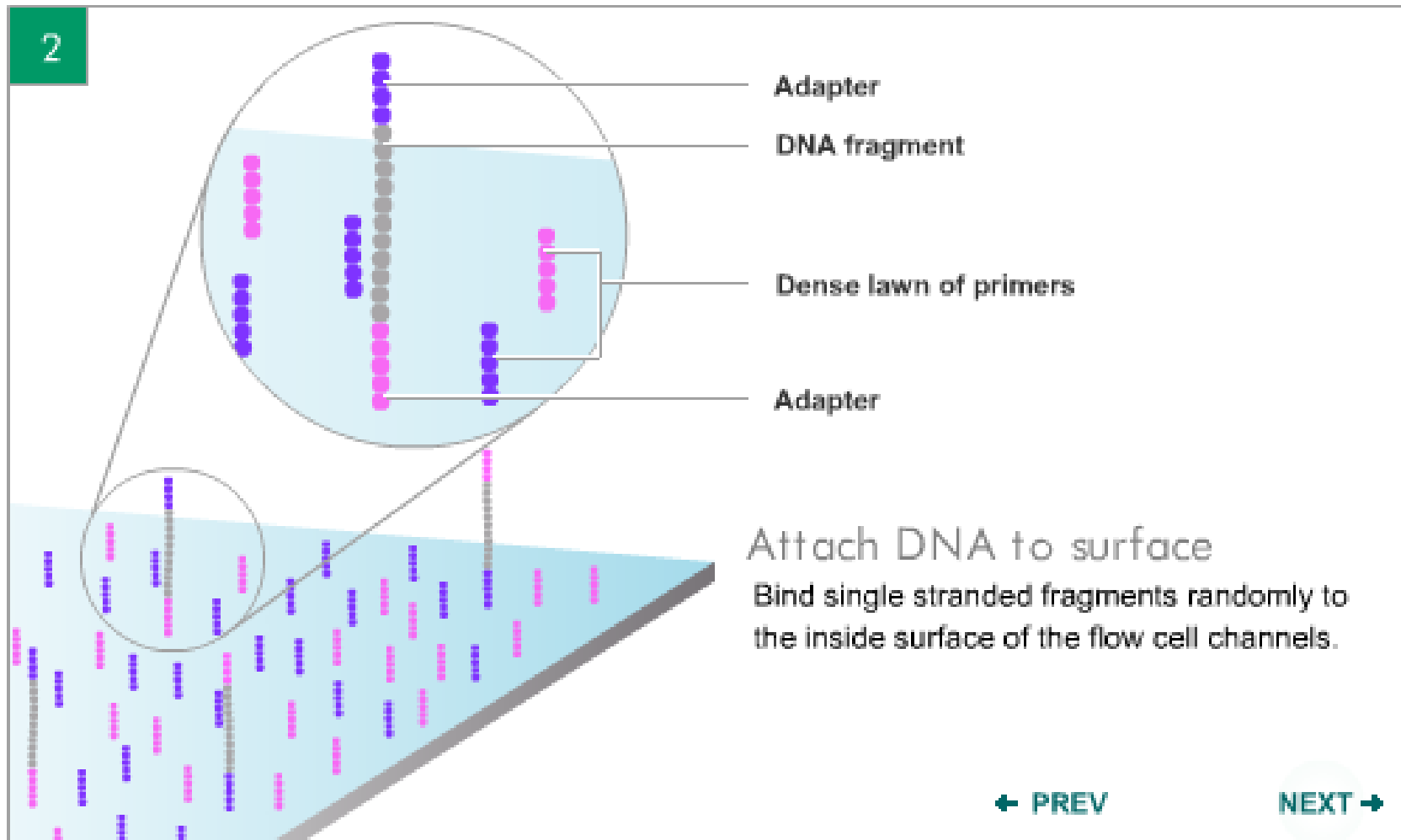
Sequencing by Synthesis



- Single “tube” reaction
- Fragmented DNA molecules
- Proprietary primer and anchor molecules

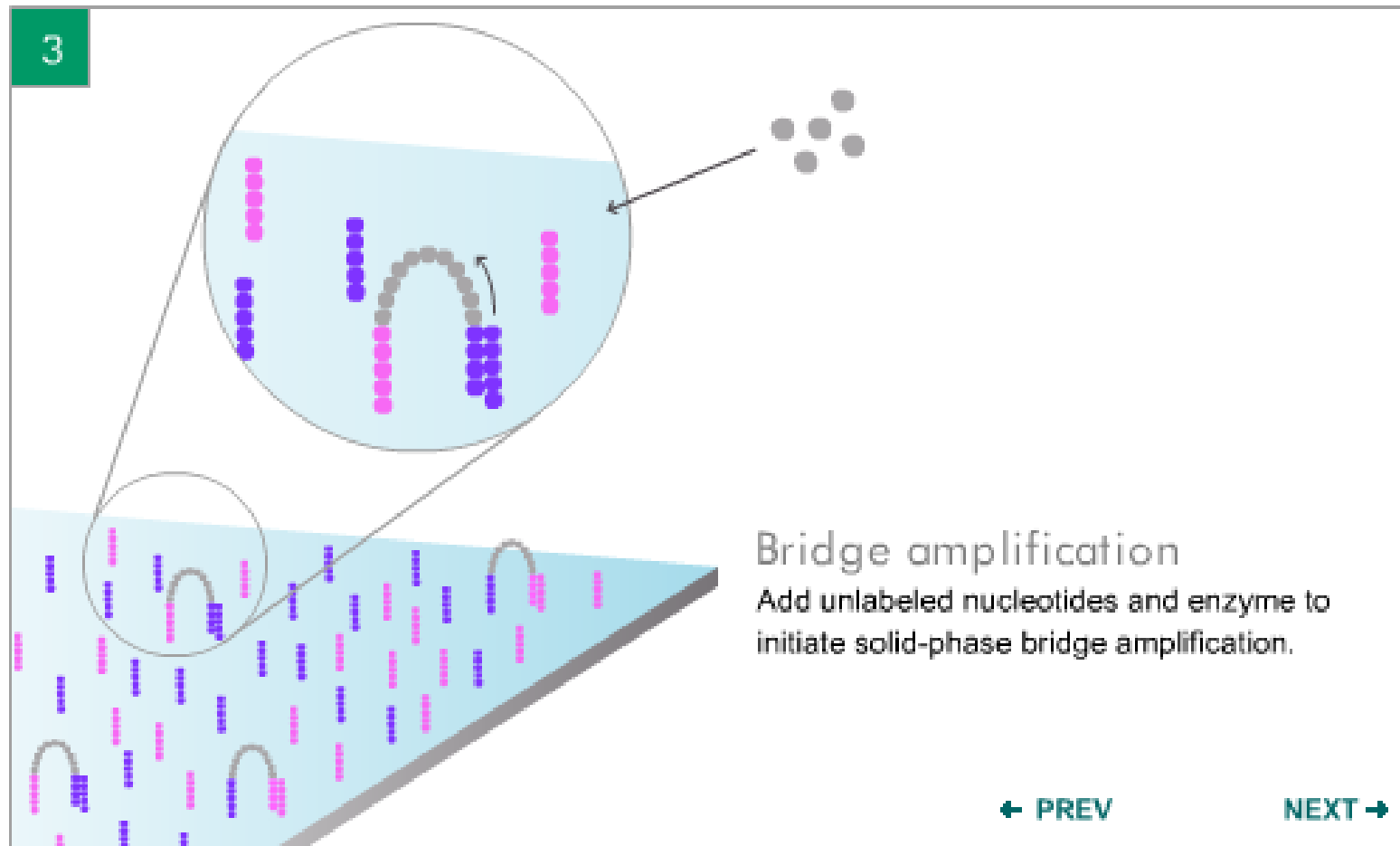
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Sequencing-By-Synthesis Demo



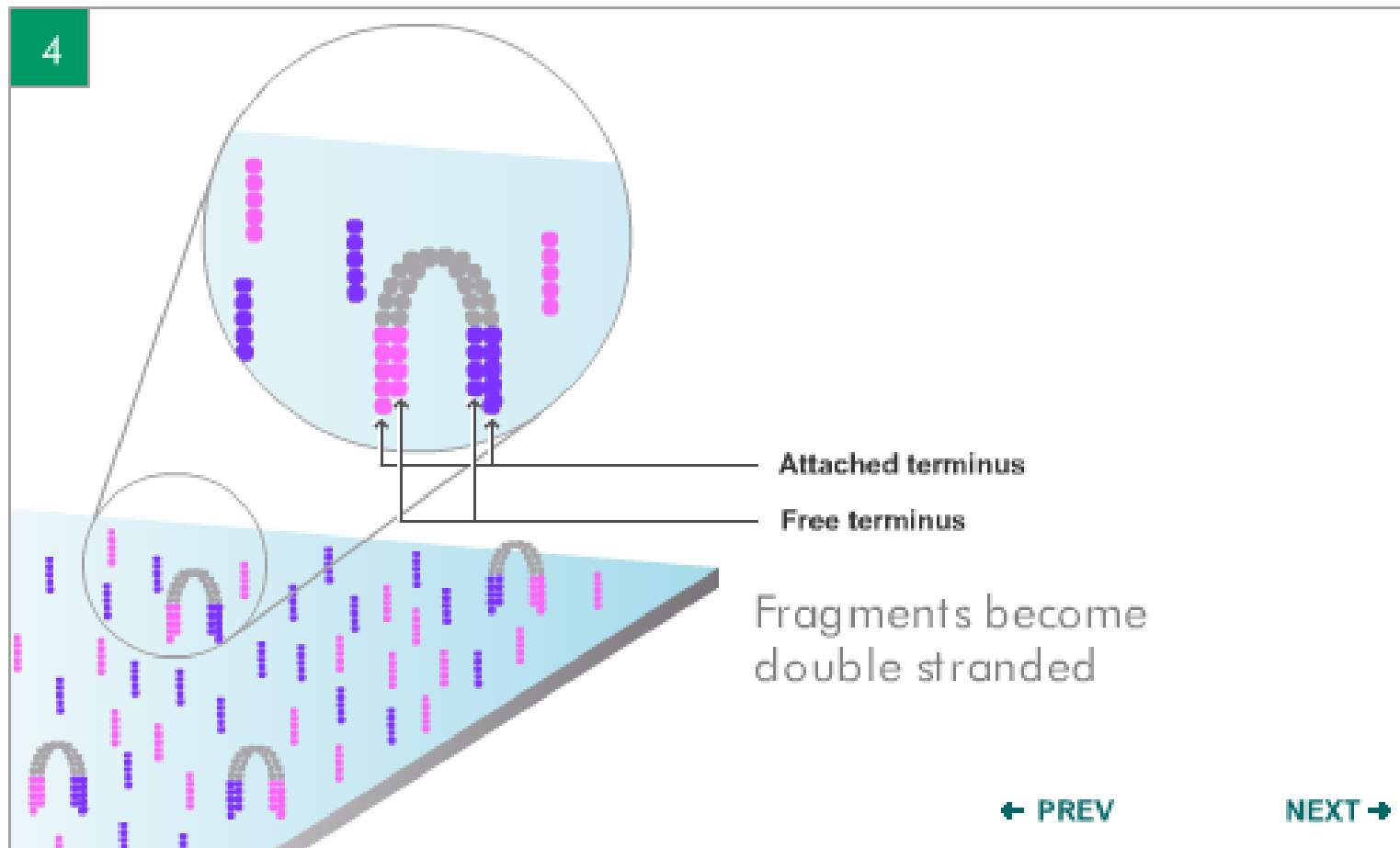
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Sequencing-By-Synthesis Demo



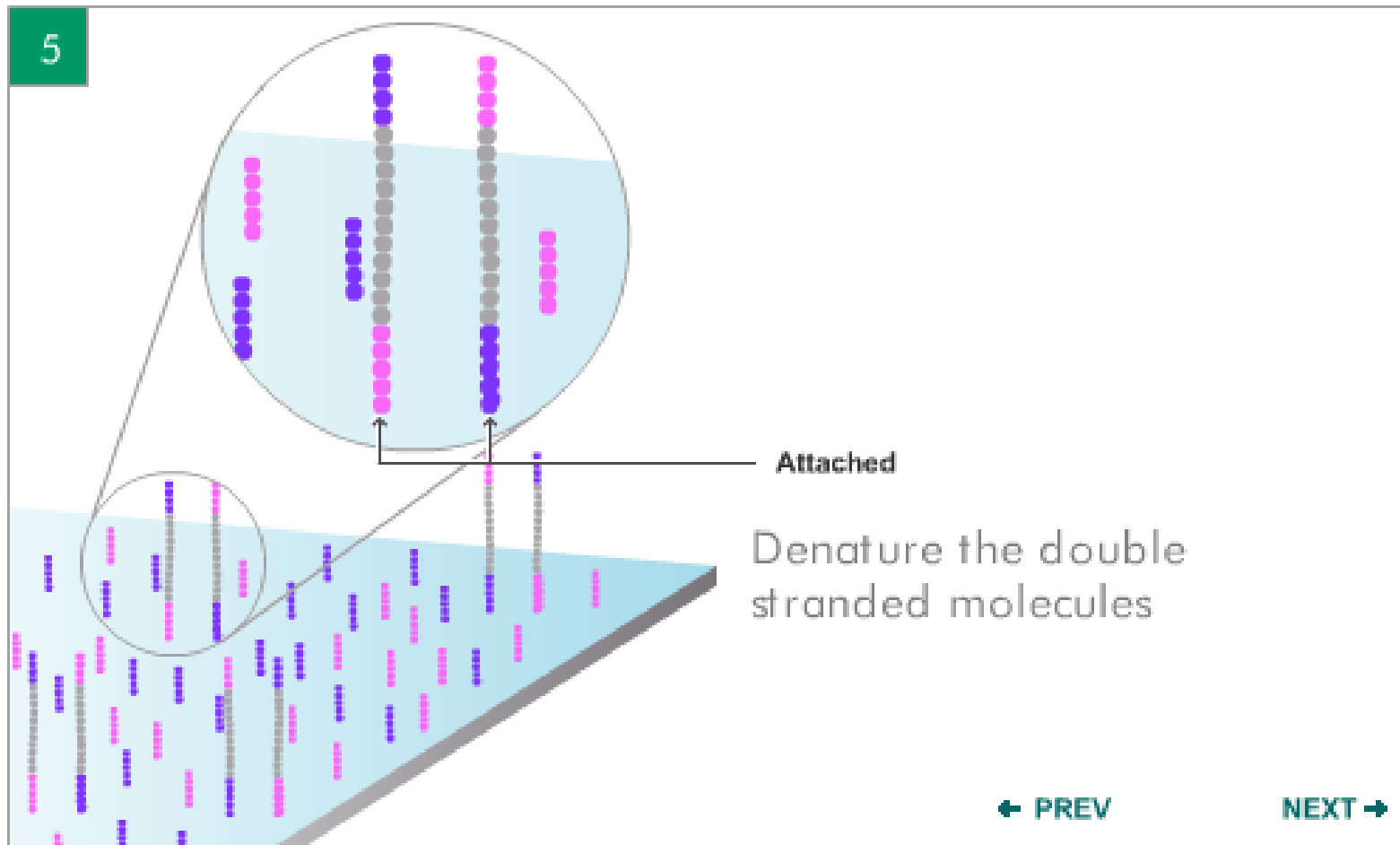
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Sequencing-By-Synthesis Demo



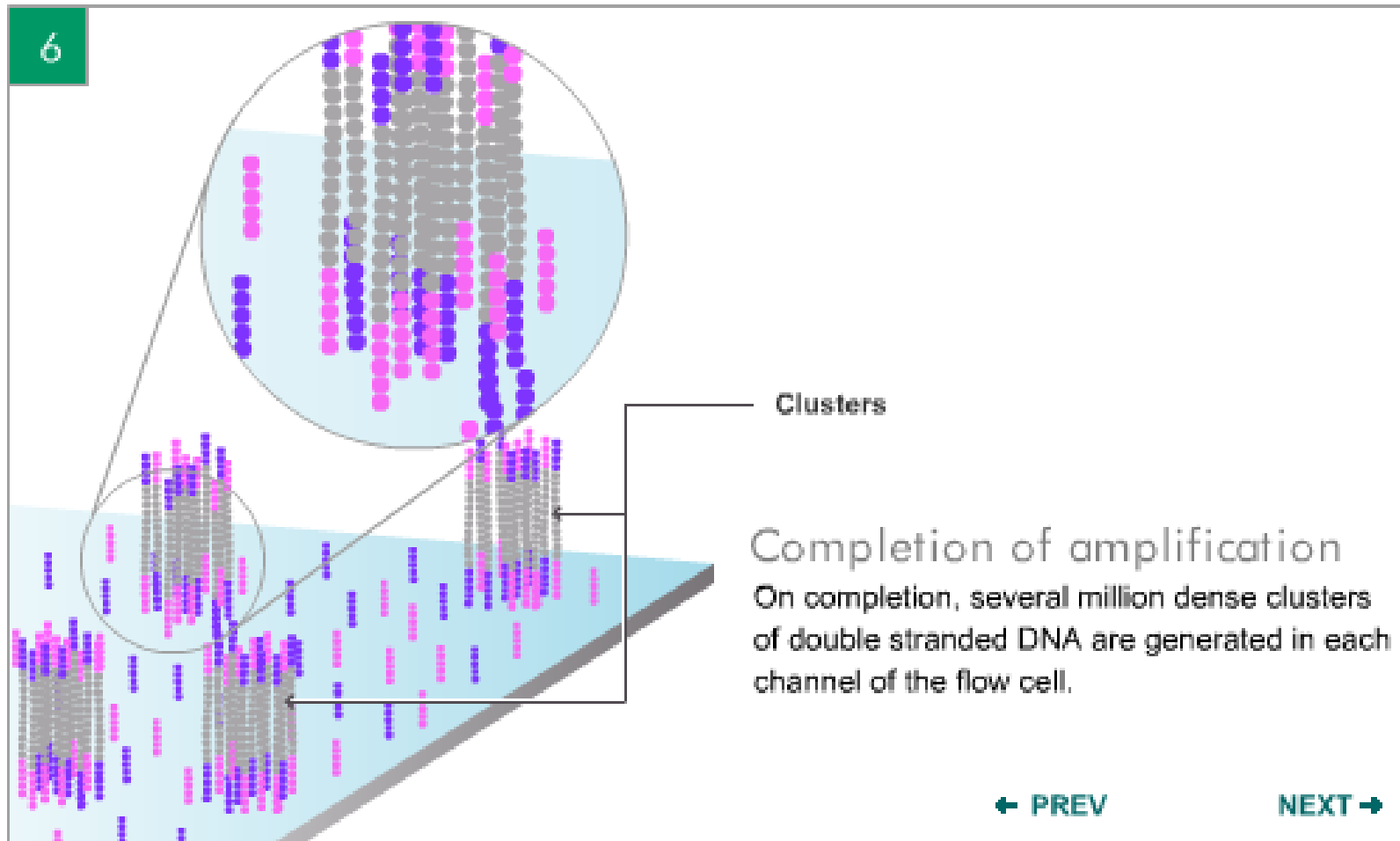
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Sequencing-By-Synthesis Demo



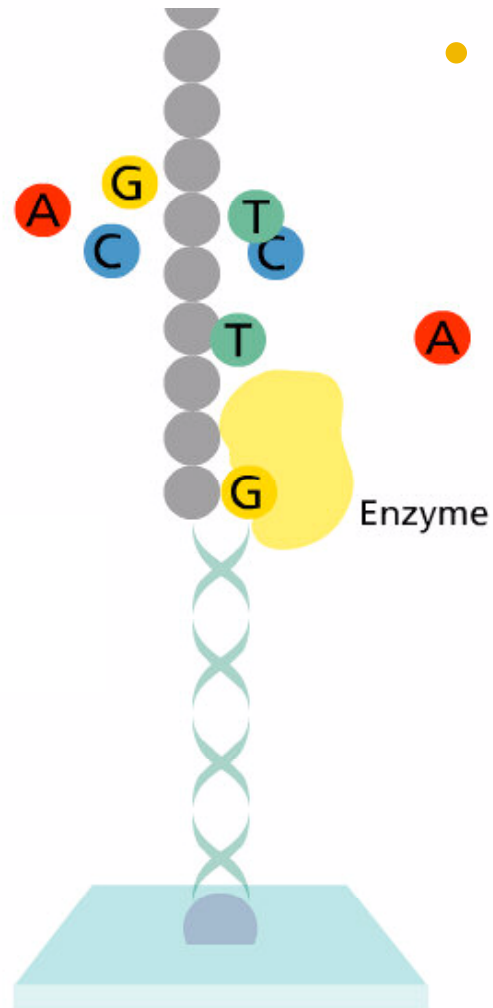
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Sequencing-By-Synthesis Demo



Sequencing by Synthesis

Cycle 1

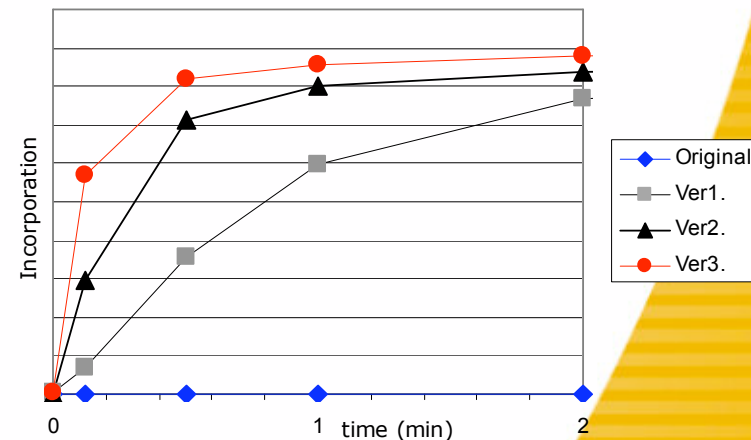


- Proprietary enzymes ensure nucleotide-specific incorporation

We Have Developed a New Sequencing Chemistry

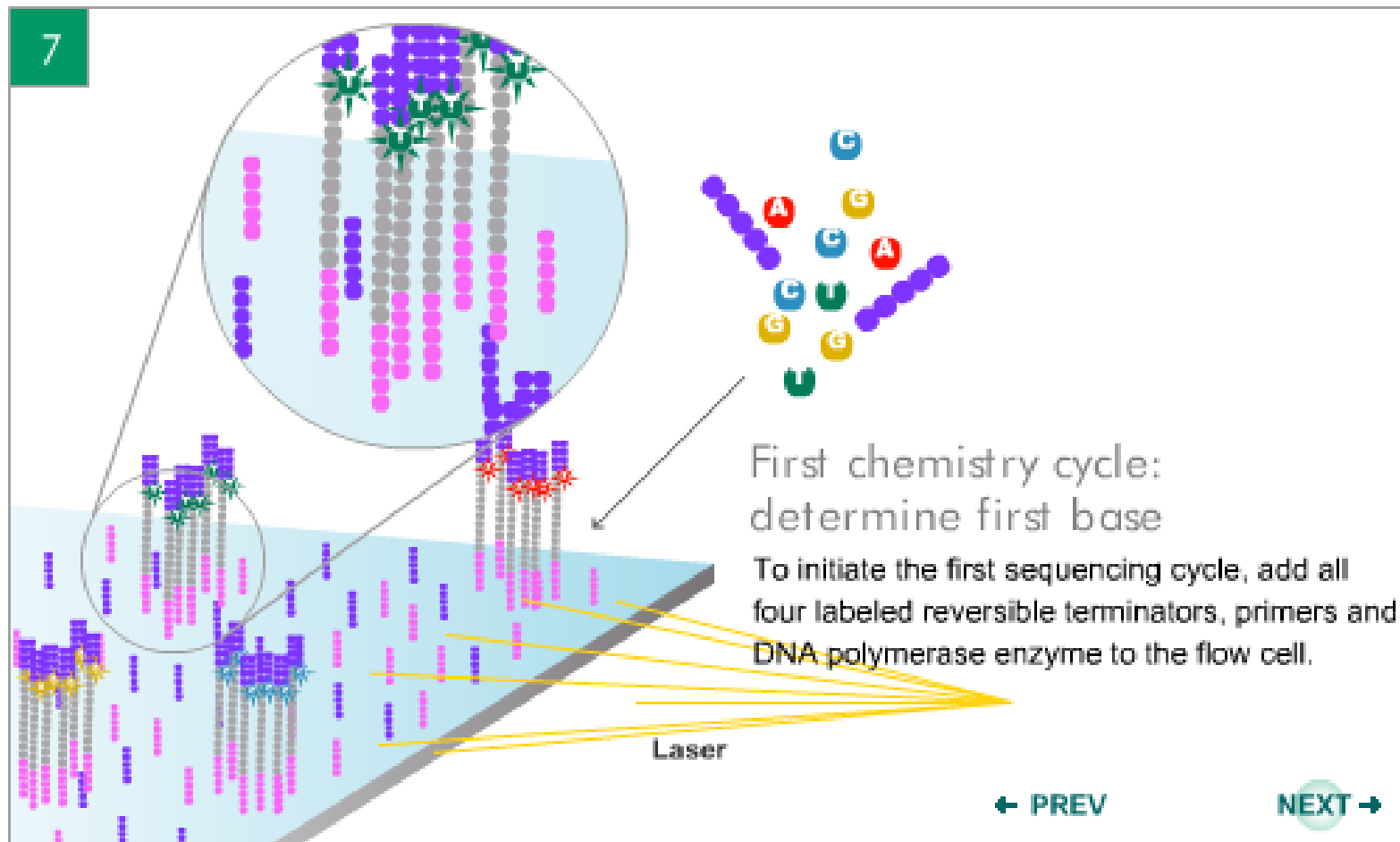


- Reversible fluorescent terminators
 - all 4 labelled nucleotides in 1 reaction
 - higher accuracy
 - no problems with homopolymer repeats
- Novel polymerases tolerant of nucleotide modifications
 - high fidelity
 - efficient incorporation



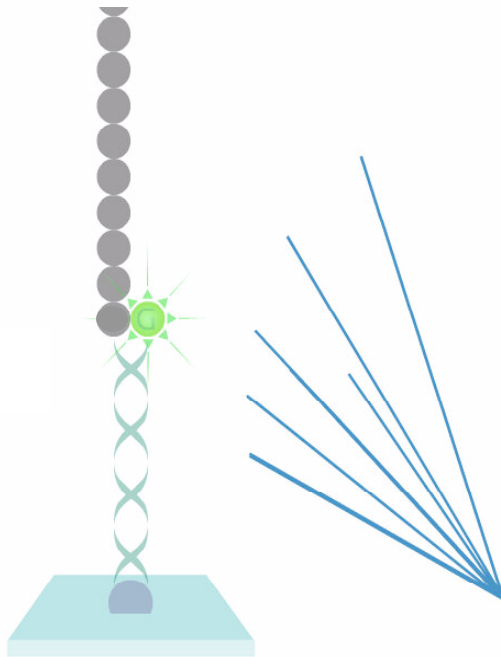
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Sequencing-By-Synthesis Demo



Sequencing by Synthesis

Cycle 1



- Incorporated nucleotides detected by laser-excited fluorescence
- Detected by a CCD camera that rapidly scans the entire array
- Fluorescence is then removed

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Sequencing-By-Synthesis Demo

8

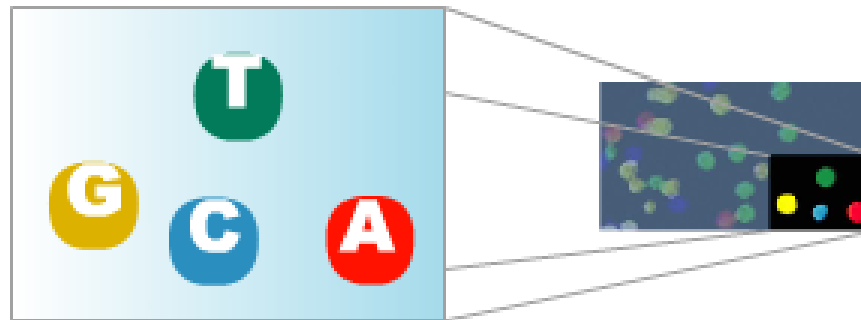


Image of first chemistry cycle

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.

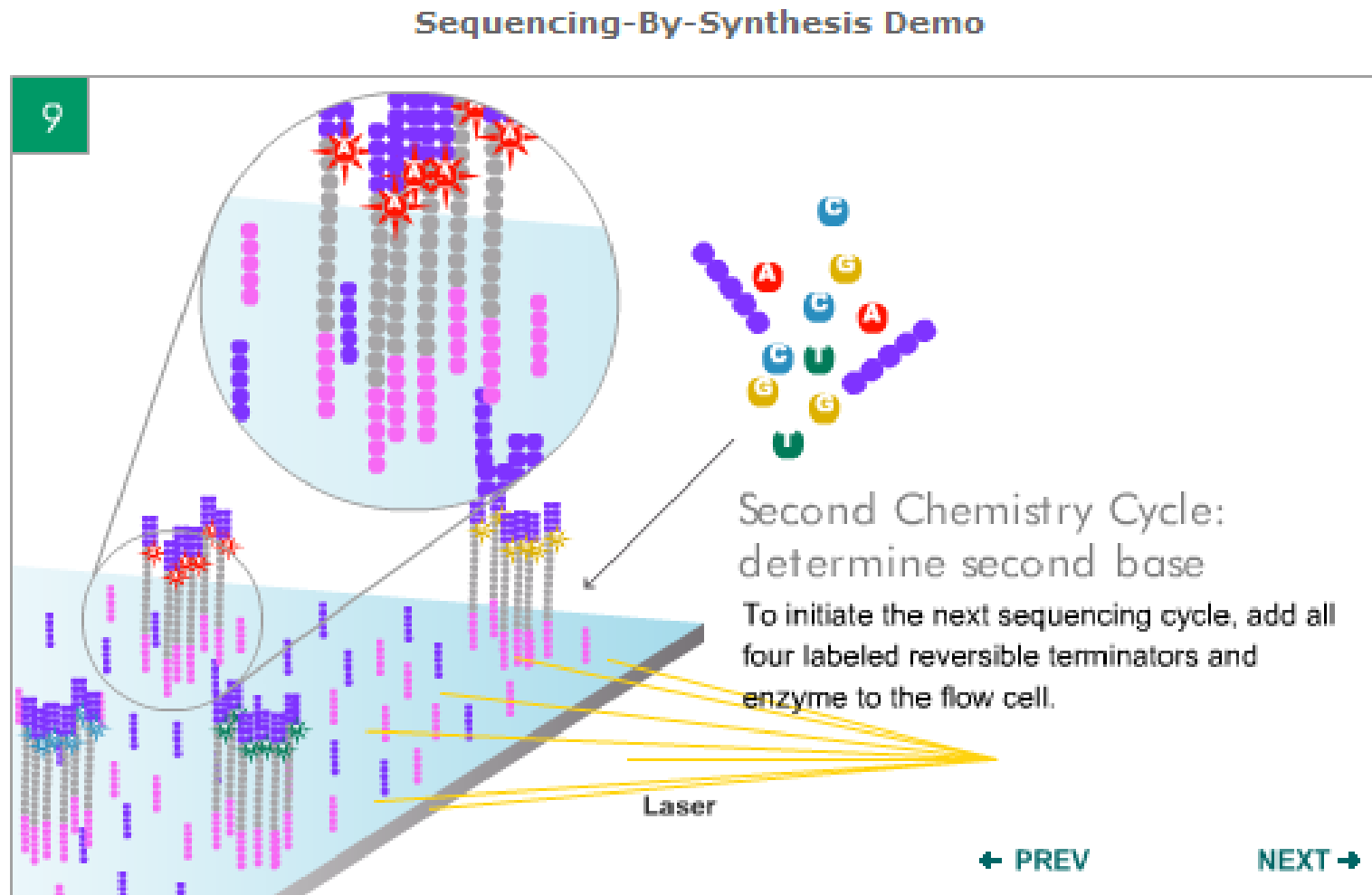
Before initiating
the next chemistry cycle

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

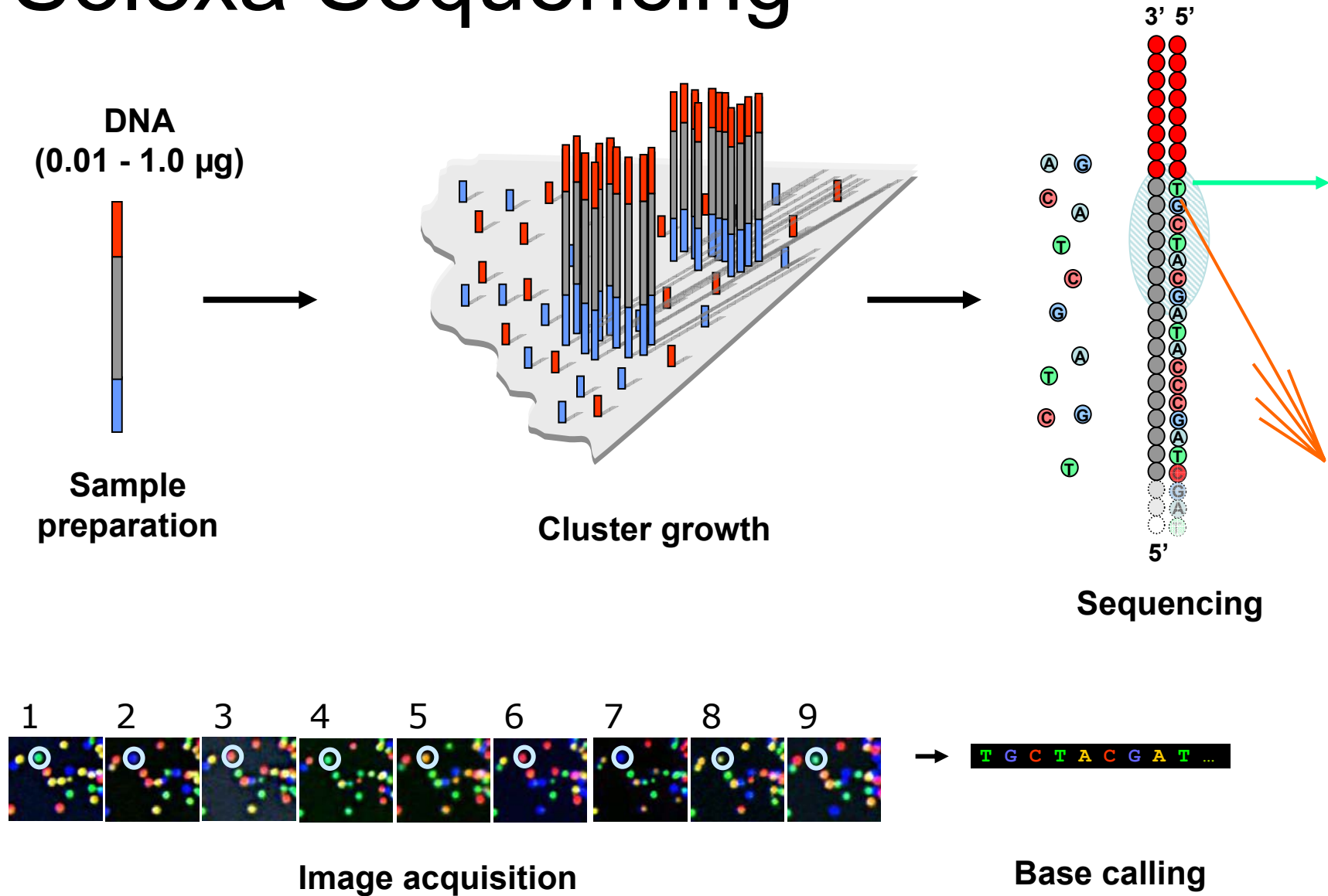
← PREV

NEXT →

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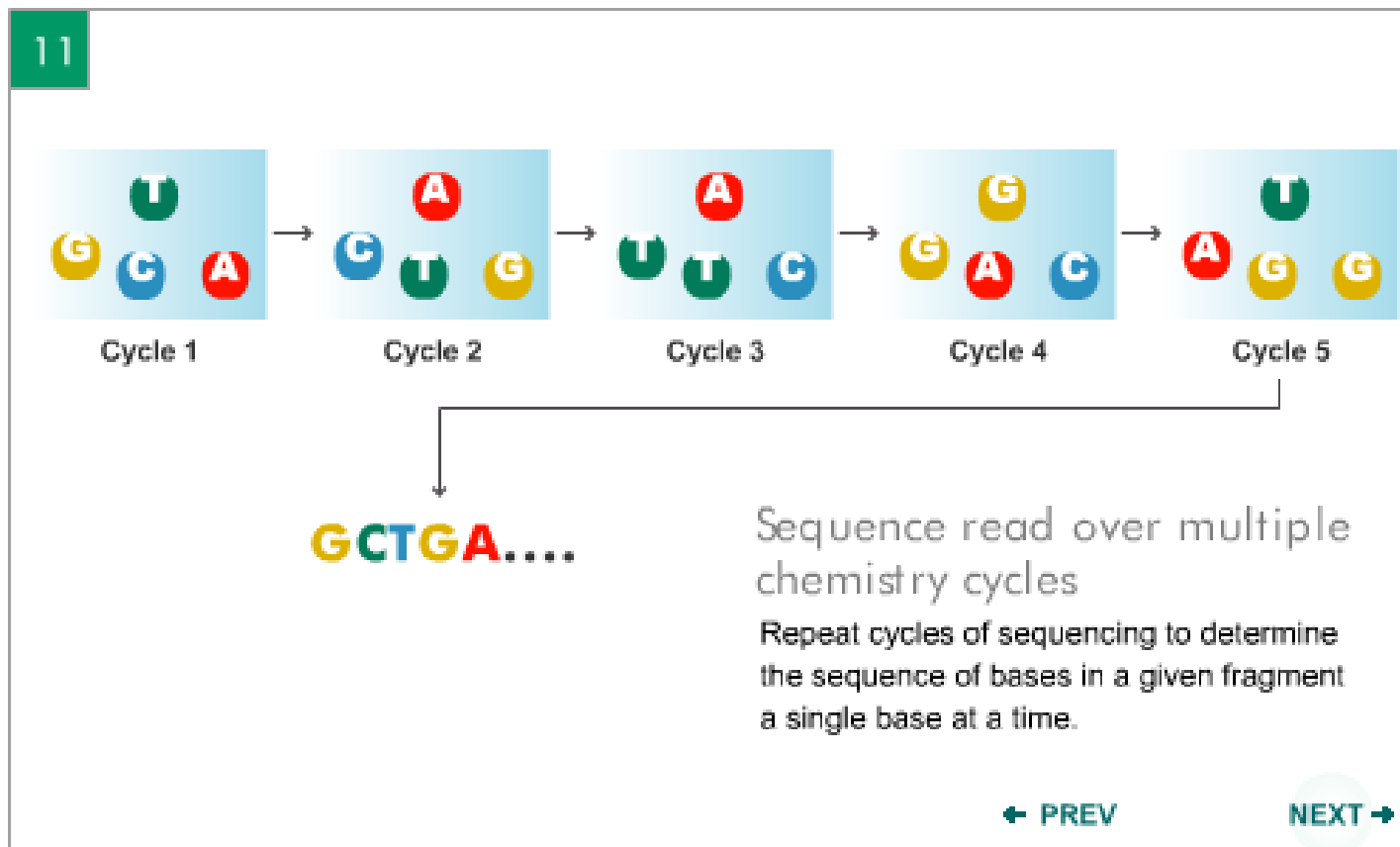


Solexa Sequencing



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Sequencing-By-Synthesis Demo



solexa sequencing technology

Solexa System Workflow

1. Sample Preparation



Duration: 7 hours

- Shear genomic DNA
- Repair ends
- Ligate adapters

2. Cluster Generation



Duration: < 4 hours

- Place reagents onto Cluster Station
- Place flow cell onto Cluster Station
- Add samples
- Press "start" button

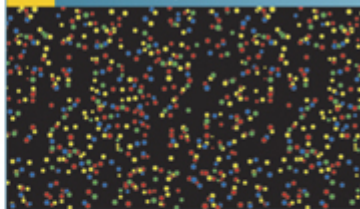
3. Sequencing-by-Synthesis



Duration: 48-72 hours*

- Place reagents onto the Solexa 1G Genetic Analyzer
- Place flow cell onto the Solexa 1G Genetic Analyzer
- Press "start" button

4. Data Analysis



Duration: ~8 hours

- Start automated analysis:
 - Image processing
 - Base calling
- Transfer data to automated analysis pipeline for secondary analysis

<http://www.illumina.com/pages.ilmn?ID=203>




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Willkommen
Sequenzierung
Software
...und mehr
Unternehmen
myGATC

Sie sind hier: > Willkommen



GATC plant die Sequenzierung von bis zu 100 Humangenomen bis Ende 2010!



Sehr geehrter Leser,

Vergangene öffentlich geförderte Humangenom-Projekte haben ein beispielloses Wissen über das menschliche Genom generiert. Jetzt geht es darum, Aufschluss über genetische Zusammenhänge für eine verbesserte Diagnose und für die Medikamentenentwicklung zu erlangen. Es geht auch um personalisierte Medizin – weg vom jetzigen Ansatz „one-drug-suits-all“.

Doch niemand kommt nun auf die Idee, sein komplettes Genom im nächsten Labor analysieren zu lassen. Die Sequenzierung des ersten Genoms benötigte 12 Jahre und kostete über 3 Milliarden US\$. Heute belaufen sich die Kosten auf etwa 5 Millionen US\$ und die Zeit für eine Analyse beträgt nur noch ein paar Jahre.

GATC hat es sich zur Aufgabe gemacht, die Kosten für die Genomsequenzierung weiter drastisch zu senken und gleichzeitig mitzuwirken, die Wissensbasis rund um das Humangenom zu vergrößern.

Die Sequenzierung vieler individueller Humangenome ist der erste Schritt um diese Vision des "500-Euro-Humangenoms" innerhalb der kommenden 10 Jahre zu realisieren.

Mit fast zwei Jahrzehnten Erfahrung in DNA-Sequenzierung und Bioinformatik in nahezu 100 mikrobiellen Genomprojekten ist GATC hervorragend positioniert, um die Sequenzierung von Humangenomen noch weiter zu optimieren.

Daher bieten wir unsere langjährige Expertise und Sequenzierkapazität jenen strategischen

myGATC Login:

Ihre E-Mail Adresse

Ihr Passwort

Login

Noch nicht registriert

Passwort zusenden

GATC News

Januar 08
GATC Biotech gründet web-basierten Informationsservice LifeCode

GATC eNewsletter

JETZT anmelden!

GATC Events

Wir nehmen an vielen Messen & Symposien teil. Bestimmt auch in Ihrer Nähe!

GATC & Mehr

BioLago
CONCERT

Wichtige Shortcuts

Sequenzierung

RUN24Barcode

Gute Gründe für GATC

GATC Viewer

Software

GRATIS Demo Software

Schulung & Support

Lasergene V7 (DNASTAR)

Softgenetics Mutation Surveyor

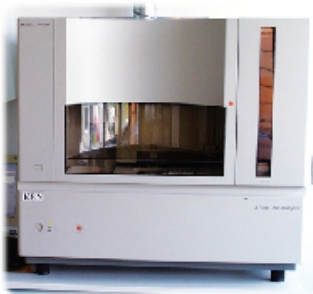
Softgenetics GeneMarker

Unternehmen

Forschung & Entwicklung

Comparison of sequencing technologies

Choose the technology that fits your project! Benefit from our experience to tailor complete sequencing solutions and programmes to meet your individual requirements!



ABI 3730XL
(Applied Biosystems/Sanger)

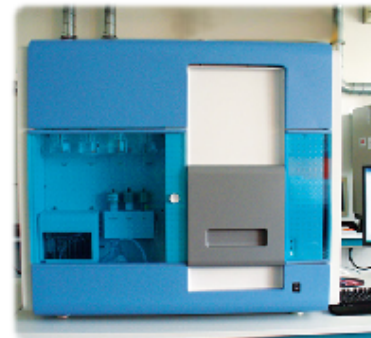
up to 1,100 bases/read
96 reads/run
approx. 1 MB/day and machine

First choice for finishing projects; full length cDNA sequencing; single sample sequencing.

GS FLX/454
(Roche Diagnostics)

up to 250 bases/read
up to 400,000 reads/run
up to 100 MB/run/7.5 hours

Optimal for in-depth analysis of whole transcriptomes; bacterial genomes; small eukaryotic genomes.



Genetic Analyzer/Solexa
(Illumina)

up to 50 bases/read
up to 60 Mio reads/run (paired-end)
up to 2,000 MB/run/6.5 days
Sequencing by synthesis

Highly attractive for resequencing projects of e.g. production strains; small RNA, SAGE and ChIP; ultra-deep sequencing of SNPs or mutations.

SOLiD DNA Sequencer
(Applied Biosystems)

up to 35 bases/read
up to 85 Mio reads/run (paired-end)
up to 3000MB/run/6 days
Sequencing by ligation

Highly attractive for resequencing projects of e.g. production strains; small RNA, SAGE and ChIP; ultra-deep sequencing of SNPs or mutations.

**To be installed
in autumn 2007!**

Sequence-ability: Human Genome 3,000 Mbp



Percentage of fragments which match back uniquely to the human genome

