Lars Paulin

New DNA sequencing technologies

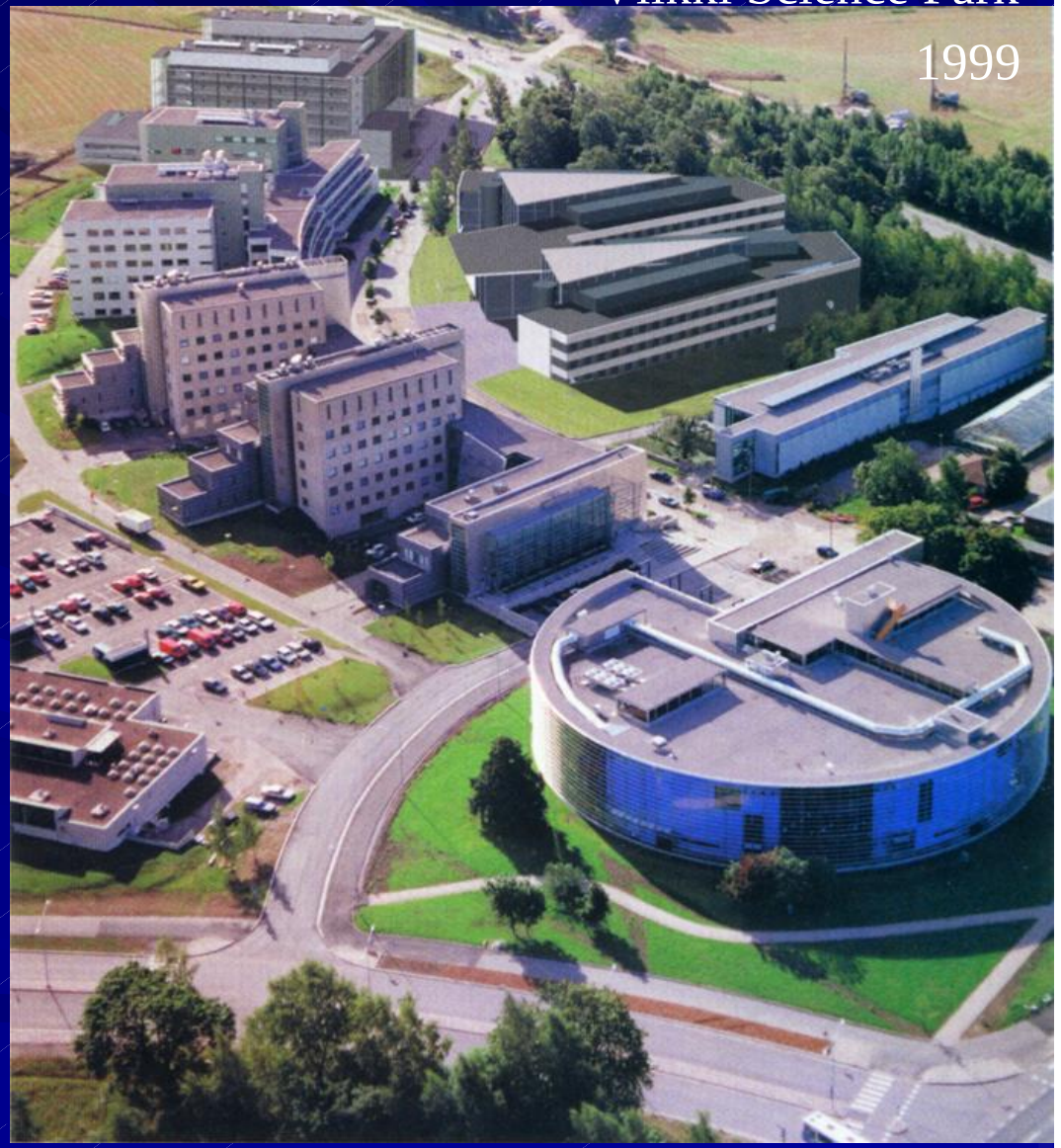
DNA Sequencing and Genomics Laboratory

Institute of Biotechnology
University of Helsinki

http://www.biocenter.helsinki.fi/bi/dnagen/

Viikki Science Park

1999



Institute of Biotechnology

- http://www.biocenter.helsinki.fi/bi/
- Independent Research Unit of the University of Helsinki
- About 300 people
- 30 Research groups

- Research Programs :
  - Developmental Biology
  - Cellular Biotechnology
  - Structural Biology and Biophysics
- Director's Laboratory

- Core Facilities :
  - NMR Laboratory
  - Electron Microscopy
  - Protein Chemistry
  - DNA Sequencing and Genomics Laboratory
  - Transgenic unit
  - Light Microscopy unit

DNA Sequencing and Genomics Laboratory

Cultivator 2, Viikinkaari 4

- Started in 1990 with DNA Synthesis
- 1991 DNA Sequencing
- 1994 EU Yeast Genome Project
- 1999 - 2000 High-throughput pipeline
- 1999 – 2002 Five EST Sequencing Projects
- 2000 Microarray Laboratory
- 2003 First Microbe Genome Project
  - Move together with Microarray Laboratory to Cultivator 2
- 2006 Genome Sequencer 20, 2007 FLX
- 2008 DNA Sequencing and Genomics Laboratory

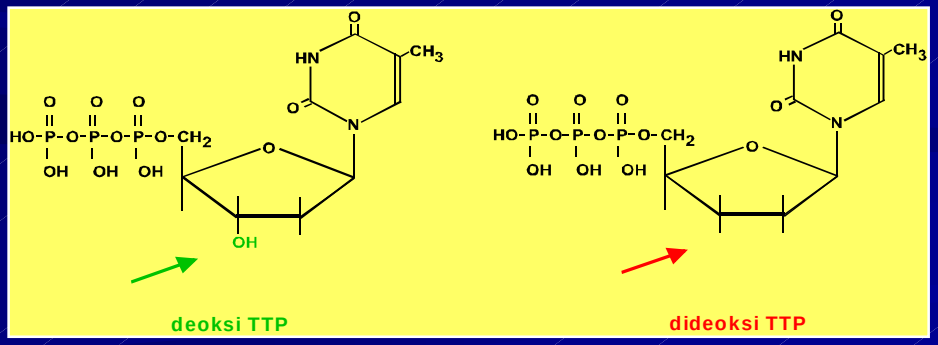
- Core Facility
  - Service DNA sequencing and whole projects
  - Collaborative projects
    - ”Research hotel”
  - Develope high-throughput methods
  - Consulting

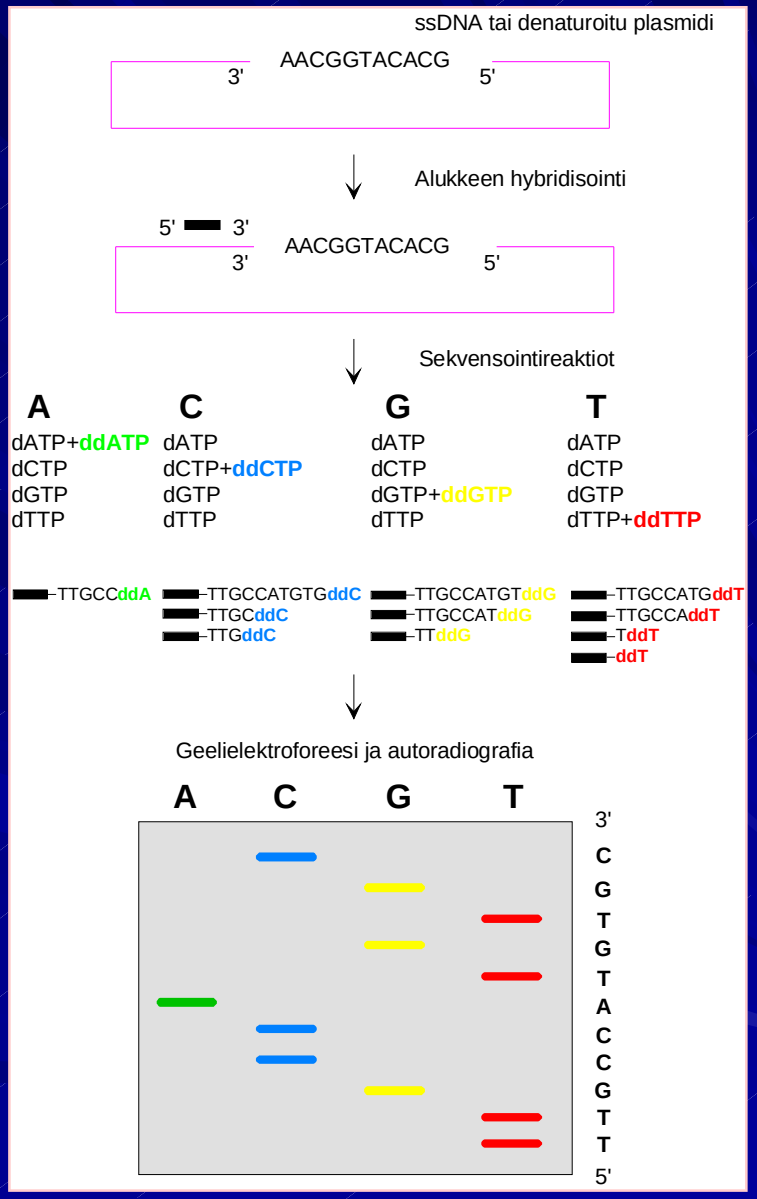
Short History of DNA Sequencing

- 1977
  - Maxam-Gilbert
  - Sanger
- 1986
  - First Automated DNA Sequencer ABI 370 (373)
- 1988
  - Pharmacia ALF
- 1995
  - ABI 377
    - Up to 96 lanes
- 1996
  - First Capillary DNA Sequencer ABI 310
- 1998
  - First 96 Capillary instruments MegaBace, ABI 3700
- 2000
  - ABI 3100, 16 Capillary
- 2002
  - ABI 3730, 48 or 96 Capillary
- 2005
  - Genome Sequencer GS20
- 2006
  - Solexa (Illumina)
- 2007
  - SOLiD

Sanger DNA Sequencing

1. Template
   - ssDNA or dsDNA
2. Primer annealing
   - Sequencing primer
3. Elongation
   - DNA polymerase
     - Steps 2 and 3 can be done repeatedly => cycle sequencing
4. Electrophoresis





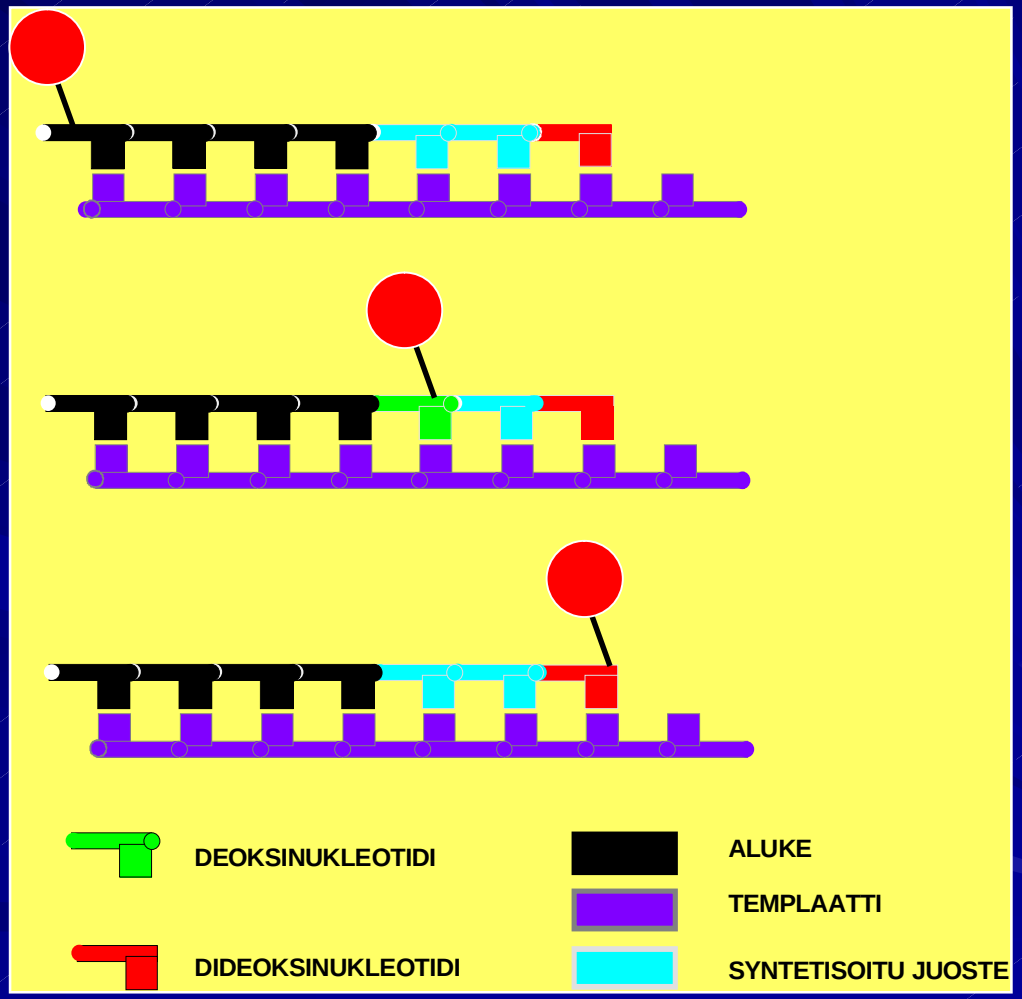
Lars Paulin Institute of Biotechnology University of Helsinki

Incorporating Labels

Labelled primers
- 1 or 4 labels

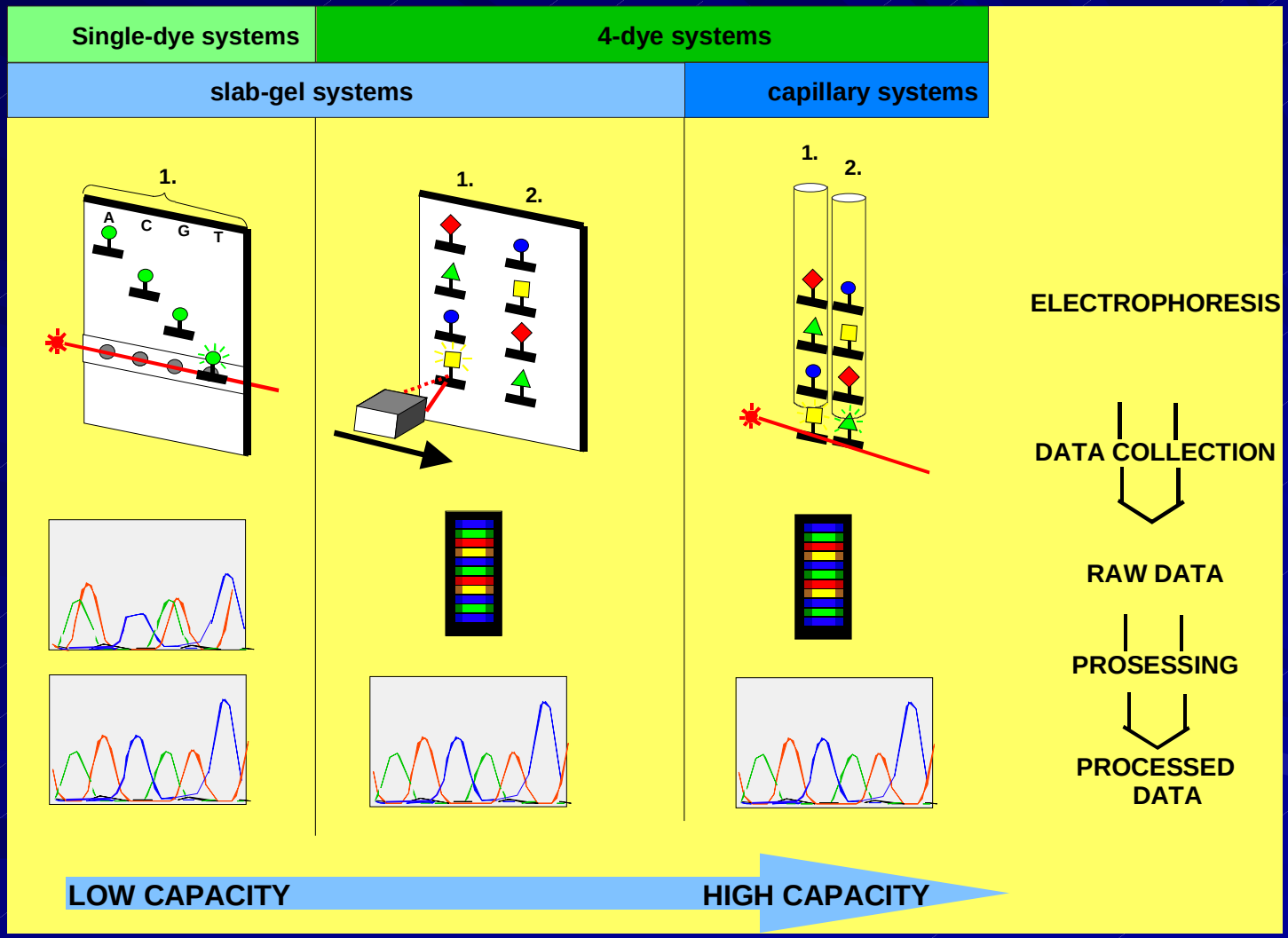
Labelled deoxynucleotides
- 1 label

Labelled dideoxynucleotides
- 1 or 4 labels
- BigDye, ET terminators

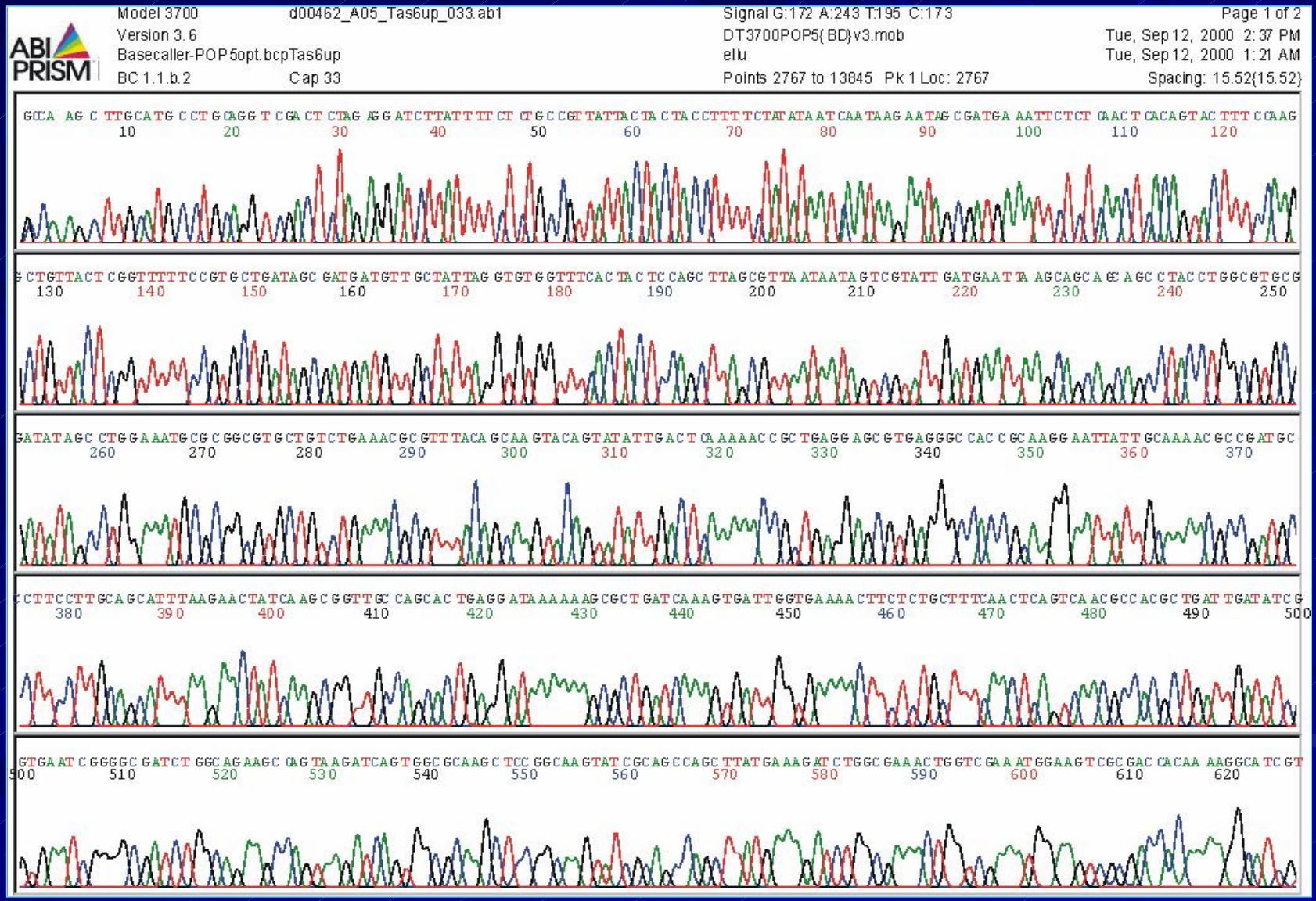


Sarén, A-M *et.al.* Kemia-Kemi 1996, 23, 724-727

Automated DNA Sequencing



Sarén, A-M *et.al.* Kemia-Kemi 1996, 23, 724-727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Version 3.6
Basecaller-POP5opt.bcpTas6up
BC 1.1.b.2
d00462_A05_Tas6up_033.ab1
Cap 33
Signal G:172 A:243 T:195 C:173
DT3700POP5{BD}v3.mob
elu
Points 2767 to 13845 Pk 1 Loc: 2767
Page 1 of 2
Tue, Sep 12, 2000 2:37 PM
Tue, Sep 12, 2000 1:21 AM
Spacing: 15.52{15.52}

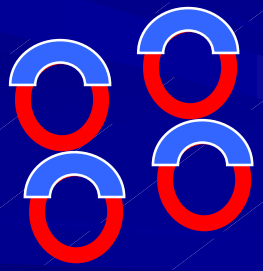
Strategies for Genome Sequencing

- Shotgun approach
  - random sequencing of different sized libraries
  - assembly using different software
  - closing of gaps using different methods
- Libraries
  - usually made by random shearing of genomic DNA
  - 2 kb, 4-6 kb, 10 kb plasmid libraries
  - fosmid or cosmid libraries with 30 - 50 kb inserts

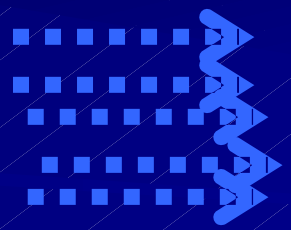
Whole Genome Shotgun Sequencing

Whole Genome:
~ 3 Mb

Sheared DNA:
~ 2 kb

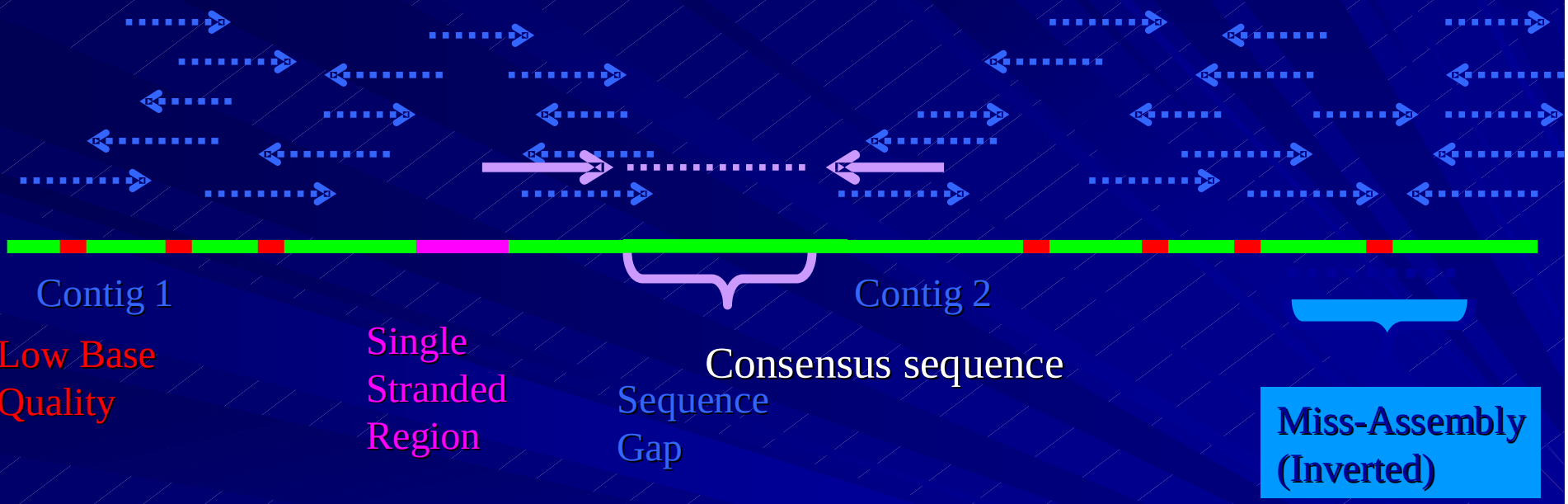


Sequencing
Templates



Random
Reads
Both ends

Shotgun Sequencing :ASSEMBLY



- 0.5 -1.0 X (2 reads/kb) - 'Skimming'
- 3.5 - 4.0 X (~9 reads/kb) -'half-shotgun'
- 6.5 - 8.0 X (~18 reads/kb) - 'pre-finished'
- 10 X (22-24 reads/kb) - 'deep shotgun'

Phred, Phrap and Staden Package Program

Phred and Phrap

- University of Washington
- Phil Green, http://www.phrap.org/

Phred quality score:

$QV = -10 * \log_{10}(P_e)$

where P_e is the probability that the base call is an error.

Phred score	P_e	Accuracy of the base call
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1,000	99.9%
40	1 in 10,000	99.99%
50	1 in 100,000	99.999%

Staden Program

- Cambridge, Sanger Center
- Roger Staden, http://staden.sourceforge.net/
- Trace editing
- Phrap assembly and Gap4 editing
  - display of traces from sequencers
  - translations, orfs, RE etc.
  - good capacity

New DNA Sequencing Technology

Parallel Sequencing Technology

- Massive throughput
- Fast sequencing
- No cloning step
- PCR

- Currently three systems ready
  - Genome Sequencer (http://www.454.com/,http://www.roche.com)
    - 454 Life Sciences, Roche
    - Launched in October 2005
  - Solexa (http://www.illumina.com)
    - Illumina
    - Launched 2006
  - SOLiD (http://www.appliedbiosystems.com)
    - Applied Biosystems
    - Launched in October 2007

nature Vol 437|15 September 2005|doi:10.1038/nature03959

ARTICLES

Genome sequencing in microfabricated high-density picolitre reactors

Marcel Margulies[1]*, Michael Egholm[1]*, William E. Altman[1], Said Attiya[1], Joel S. Bader[1], Lisa A. Bemben[1], Jan Berka[1], Michael S. Braverman[1], Yi-Ju Chen[1], Zhoutao Chen[1], Scott B. Dewell[1], Lei Du[1], Joseph M. Fierro[1], Xavier V. Gomes[1], Brian C. Godwin[1], Wen He[1], Scott Helgesen[1], Chun He Ho[1], Gerard P. Irzyk[1], Szilveszter C. Jando[1], Maria L. I. Alenquer[1], Thomas P. Jarvie[1], Kshama B. Jirage[1], Jong-Bum Kim[1], James R. Knight[1], Janna R. Lanza[1], John H. Leamon[1], Steven M. Lefkowitz[1], Ming Lei[1], Jing Li[1], Kenton L. Lohman[1], Hong Lu[1], Vinod B. Makhijani[1], Keith E. McDade[1], Michael P. McKenna[1], Eugene W. Myers[2], Elizabeth Nickerson[1], John R. Nobile[1], Ramona Plant[1], Bernard P. Puc[1], Michael T. Ronan[1], George T. Roth[1], Gary J. Sarkis[1], Jan Fredrik Simons[1], John W. Simpson[1], Maithreyan Srinivasan[1], Karrie R. Tartaro[1], Alexander Tomasz[3], Kari A. Vogt[1], Greg A. Volkmer[1], Shally H. Wang[1], Yong Wang[1], Michael P. Weiner[4], Pengguang Yu[1], Richard F. Begley[1] & Jonathan M. Rothberg[1]

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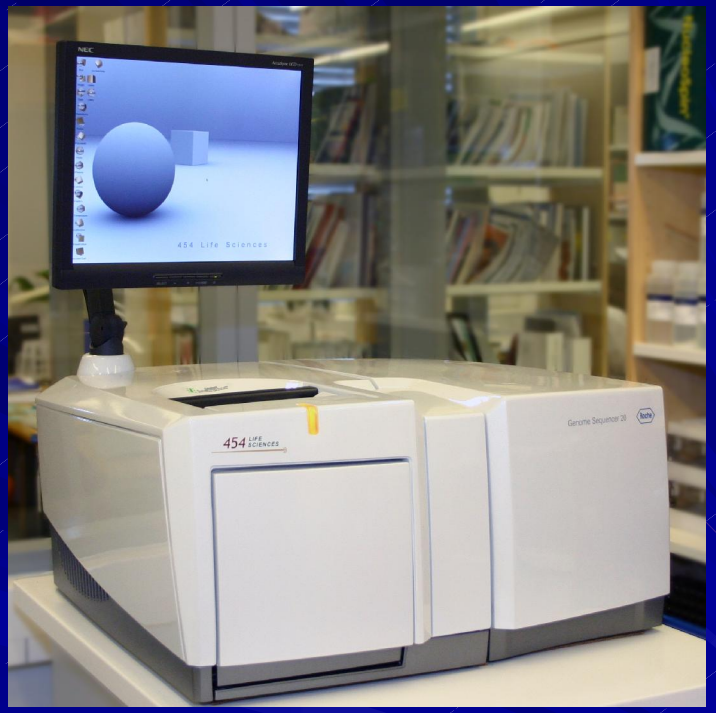
*These authors contributed equally to this work.

Genome Sequencer

(http://www.454.com/,http://www.roche.com)

- Genome Sequencer GS20;FLX
  - Manufacturer 454 Life Science
  - Marketing Roche
- Parallel Sequencing
  - Shotgun sequencing
    - No plasmid libraries
    - Linkers ligated to fragments
    - Emulsion PCR
    - Picotiter plate, 1 600 000 wells
  - Pyrosequencing

    (Nyren, P. et al Anal Biochem. 1993, 208,171-5)
    - Detection with sensitive CCD camera
    - Run time ca. 4,5 h; 7,5 h
    - Read lenght 100 -120 bp; 250 – 300 bp
    - Raw sequence ca. 25 – 35 Mb/run; 80 – 100 Mb/run



Genome Sequencer GS 20/FLX

DNA Library Preparation

1. DNA fragmentation
2. Fragment end polishing
3. Adaptor ligation
4. Library immobilisation
5. Fill-in reaction
6. Single-stranded template DNA (sstDNA) library isolation
7. sstDNA library quality assessment and quantitation

Emulsion PCR Amplification

1. Preparation of the live amplification mix
2. sstDNA library capture
3. Emulsification
4. Amplification
5. Bead recovery
6. sstDNA library bead enrichment
7. Sequencing primer annealing

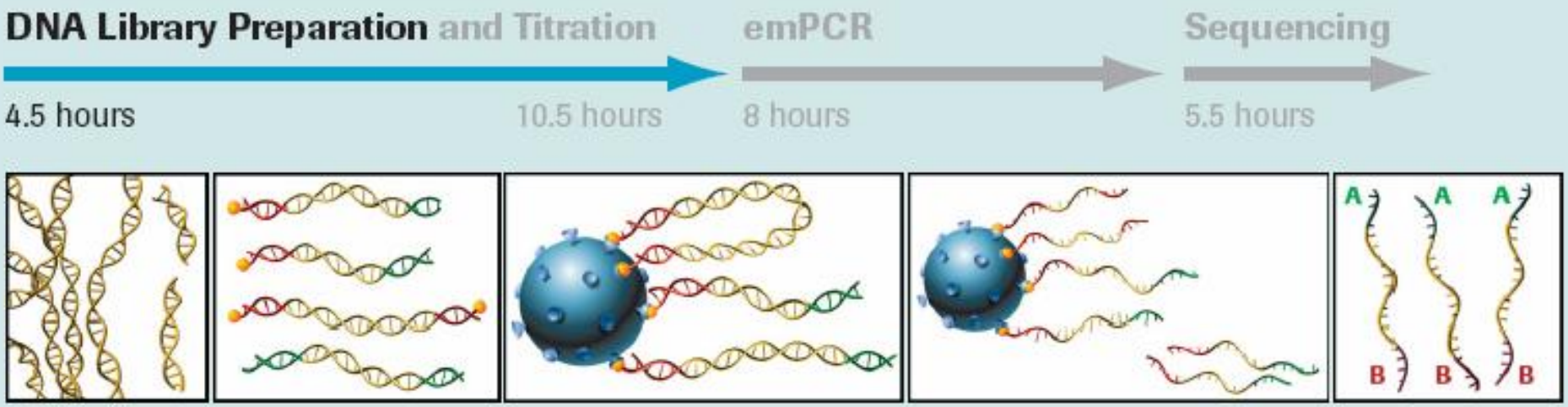
Sequencing/ Genome Sequencer 20 Operation

1. The pre-wash Run
2. PicoTiterPlate™ preparation
3. The PREP Run
4. The Sequencing Run

Output

1. FASTA file
2. Assembly
3. Mapping

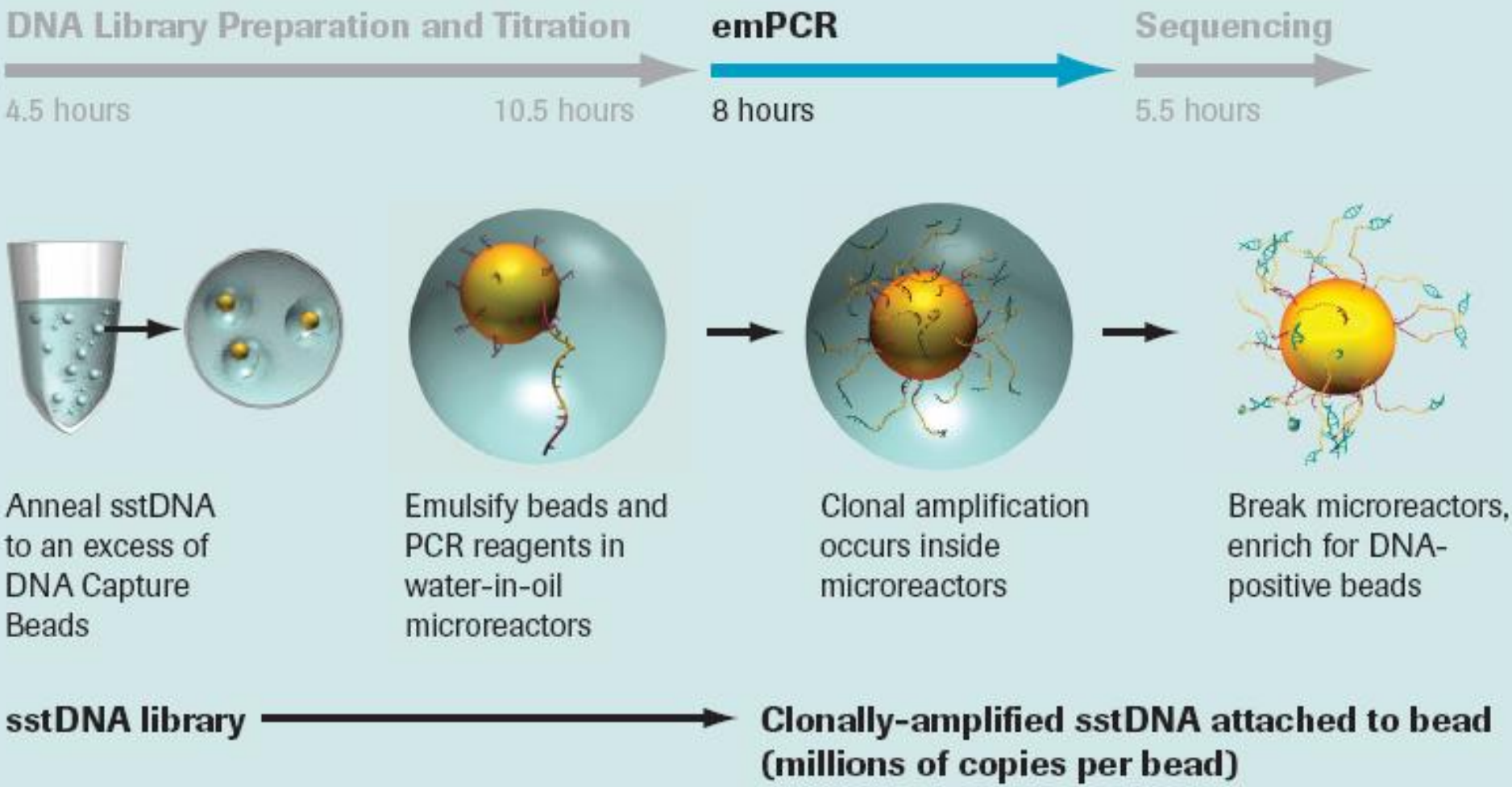
Library preparation



gDNA → **sstDNA library**

- Genome fragmented by nebulization
- No cloning; no colony picking
- sstDNA library created with adaptors. The adaptors are used as primers, and for binding to beads.
- A/B fragments selected using streptavidin-biotin purification

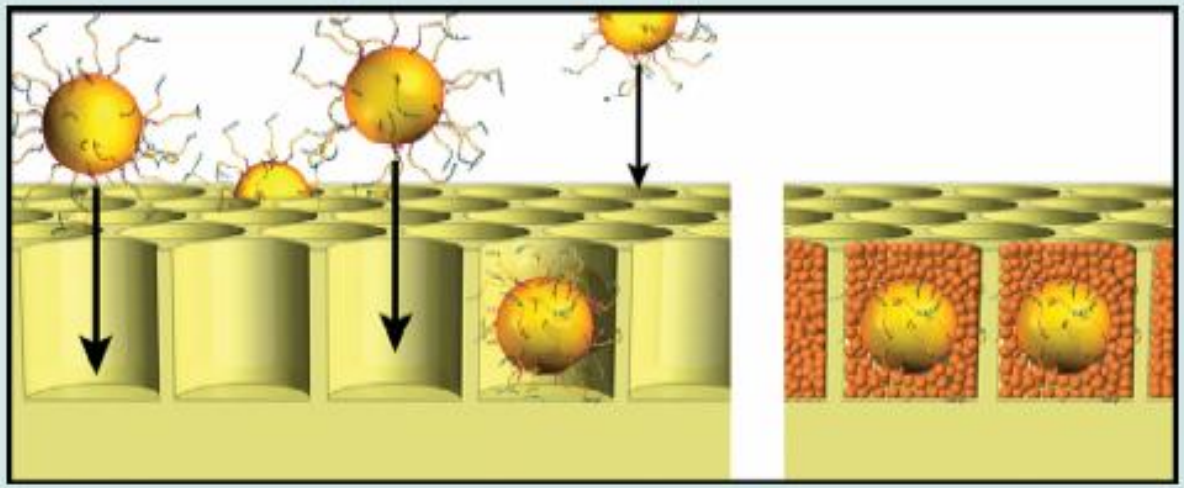
Emulsion PCR



PicoTiterPlate (PTP)

DNA Library Preparation and Titration → emPCR → **Sequencing**

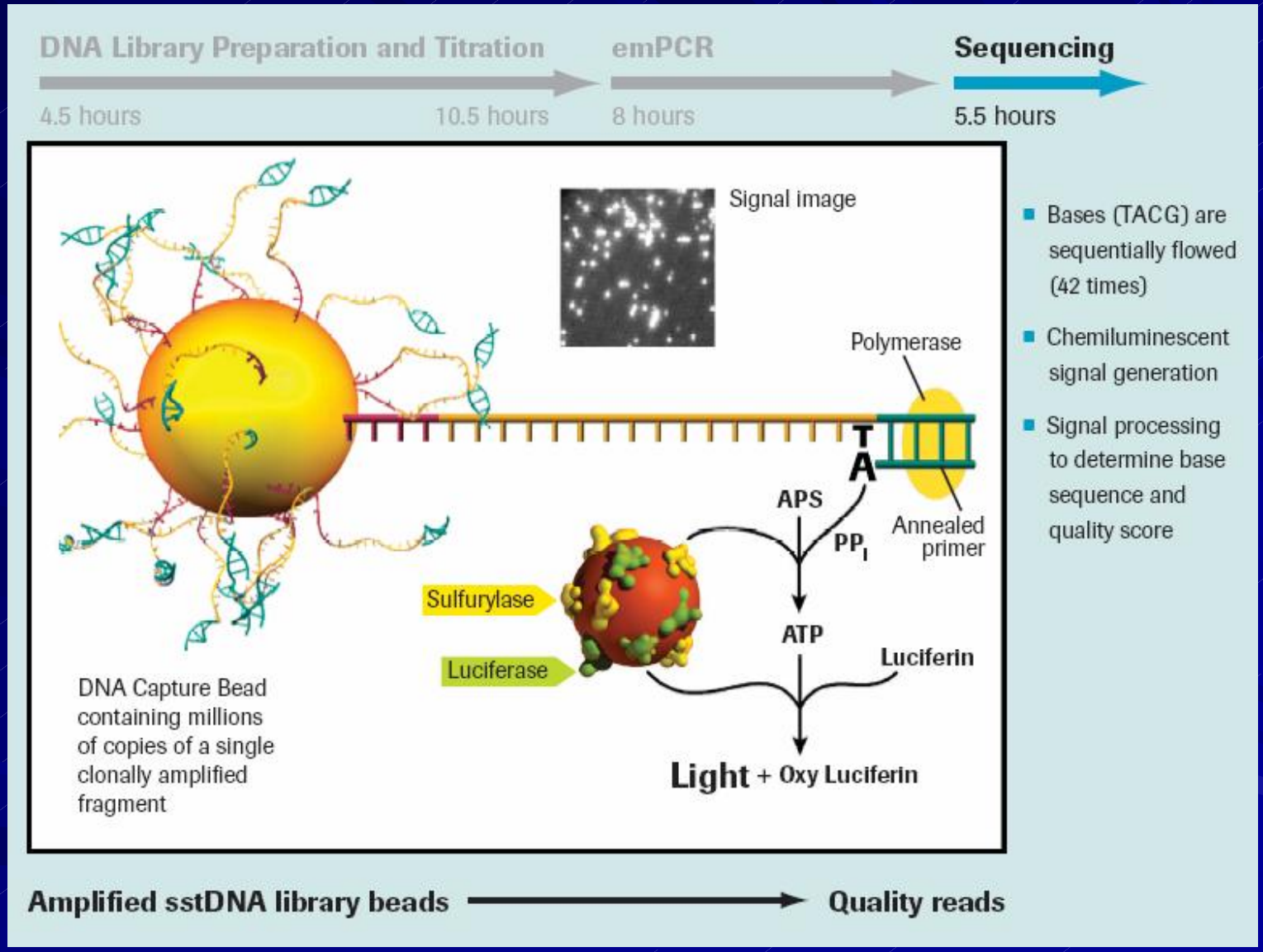
4.5 hours – 10.5 hours | 8 hours | 5.5 hours



- Well diameter: average of 44 µm
- A single clonally amplified sstDNA bead is deposited per well
- 200,000 reads obtained in parallel on large-format PicoTiterPlate device

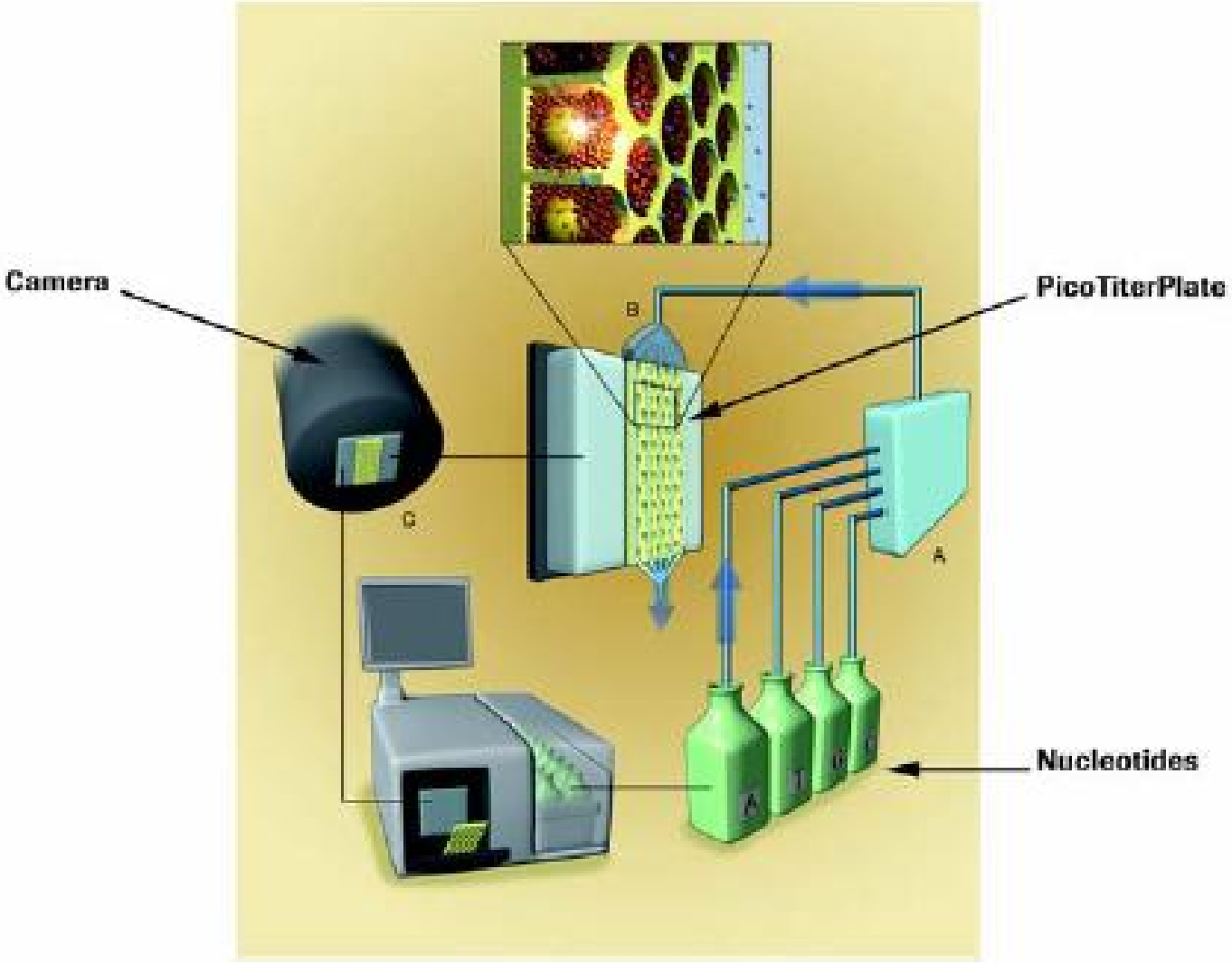
Amplified sstDNA library beads → **Quality reads**

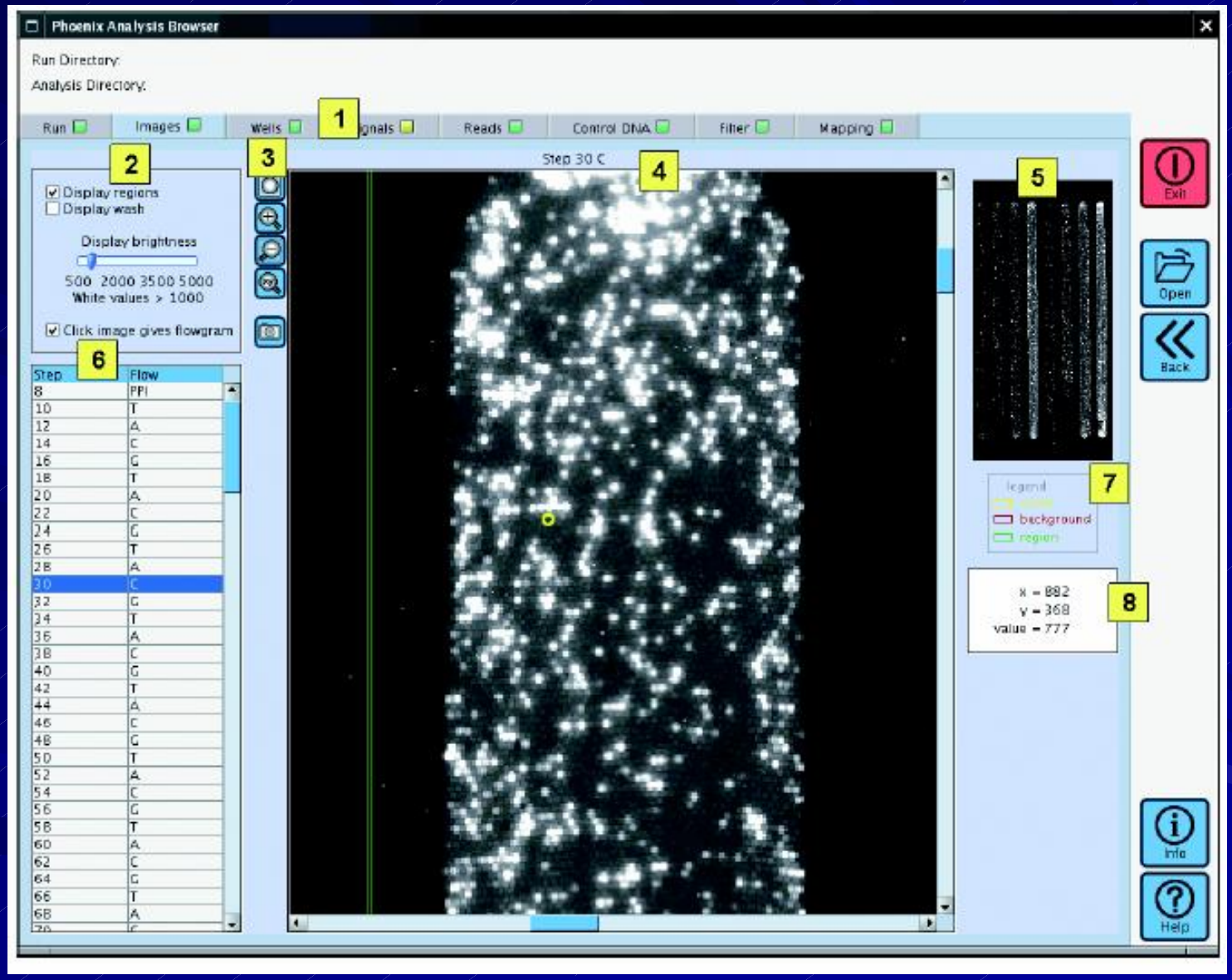
Pyrosequencing



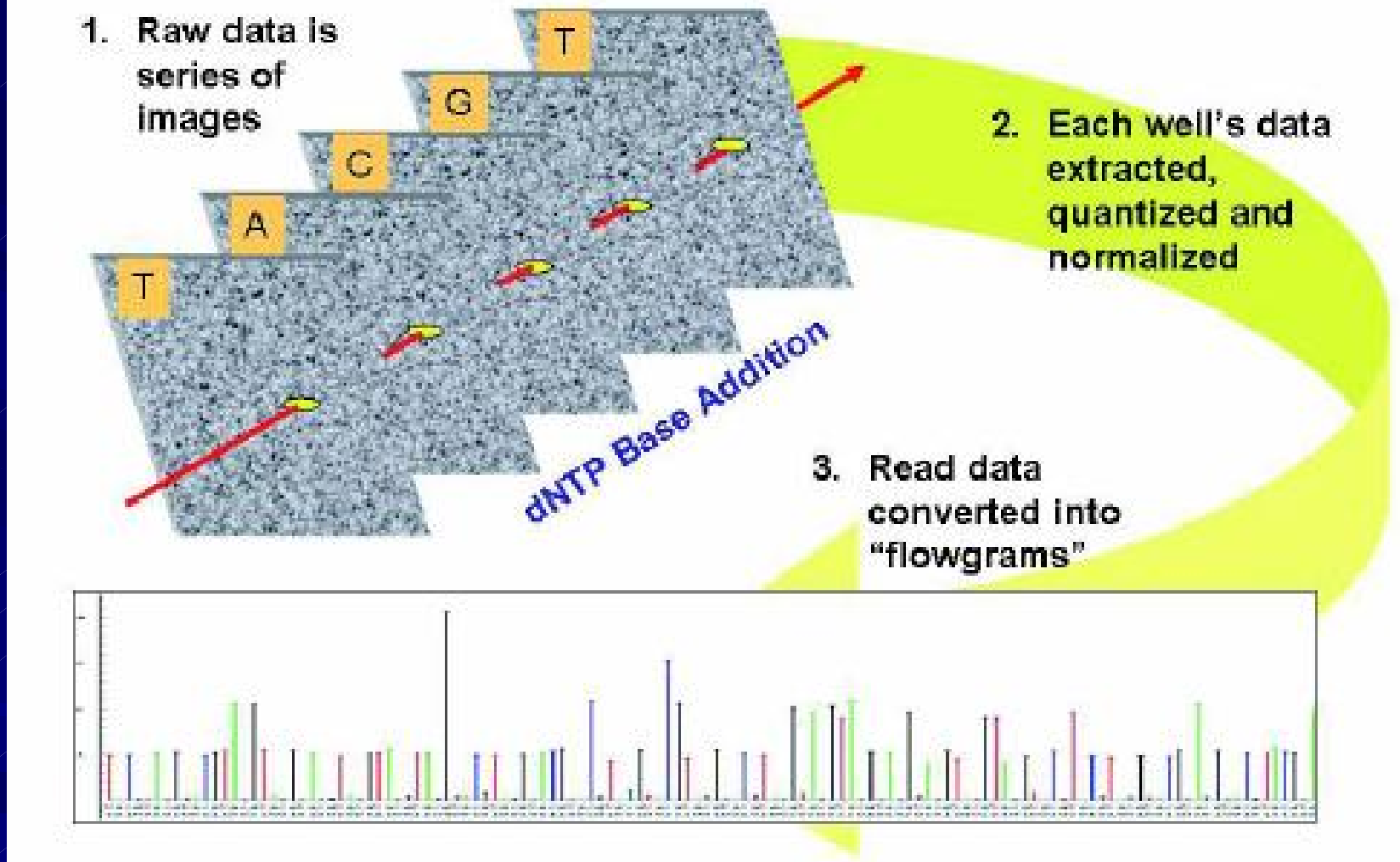
Adaptor Taq TCAG -- CTGA

Genome Sequencer GS20/FLX



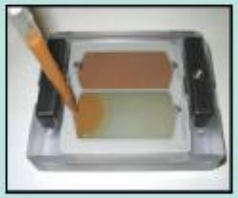

Phoenix Analysis Browser
Run Directory:
Analysis Directory:
Run
Images
Wells
1
Reads
Control DNA
Filter
Mapping
2
3
Step 30 C
4
5
Display regions
Display wash
Display brightness
500 2000 3500 5000
White values > 1000
Click image gives flowgram
6
Step
Flow
8 PPi
10 T
12 A
14 C
16 G
18 T
20 A
22 C
24 G
26 T
28 A
30 C
32 G
34 T
36 A
38 C
40 G
42 T
44 A
46 C
48 G
50 T
52 A
54 C
56 G
58 T
60 A
62 C
64 G
66 T
68 A
7
background
region
x = 882
y = 368
value = 777
8
Exit
Open
Back
Info
Help

Flowgram



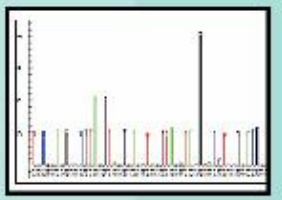
Adaptor Taq TCAG -- CTGA

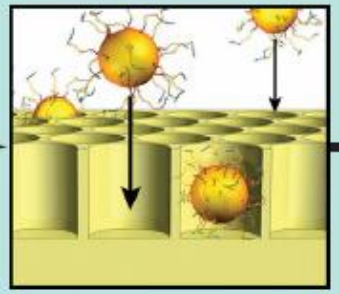
PicoTiterPlate device



Genome Sequencer 20 Instrument

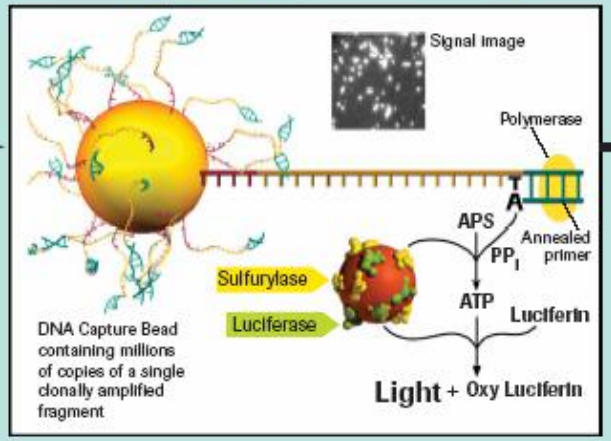
Flowgram





Sequencing

- Loading PicoTiterPlate device
- One bead per well

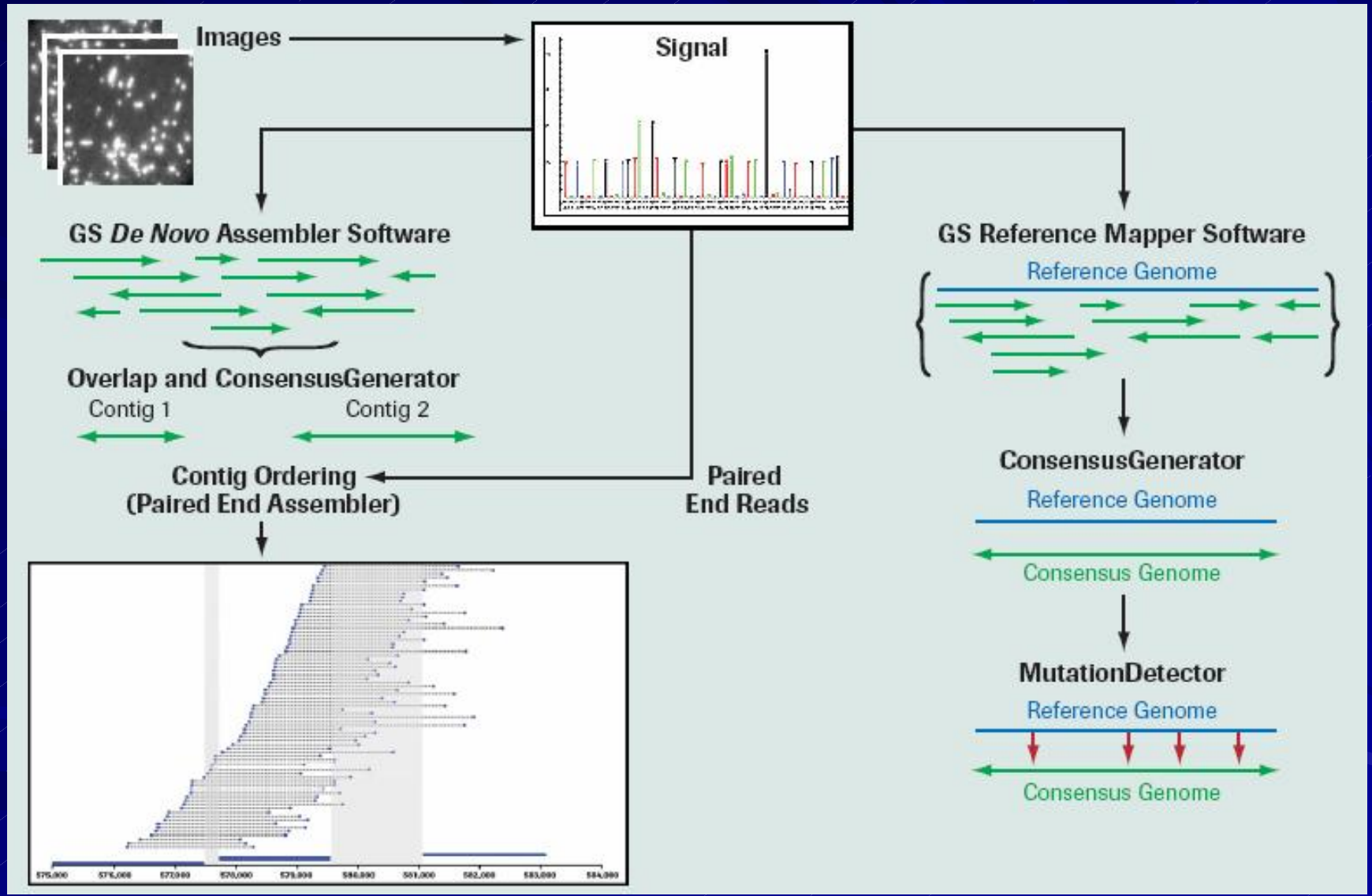


- Sequencing-by-synthesis
- Light signal proportional to incorporated nucleotide is captured by CCD camera
- Image and signal processing to determine sequence and quality score

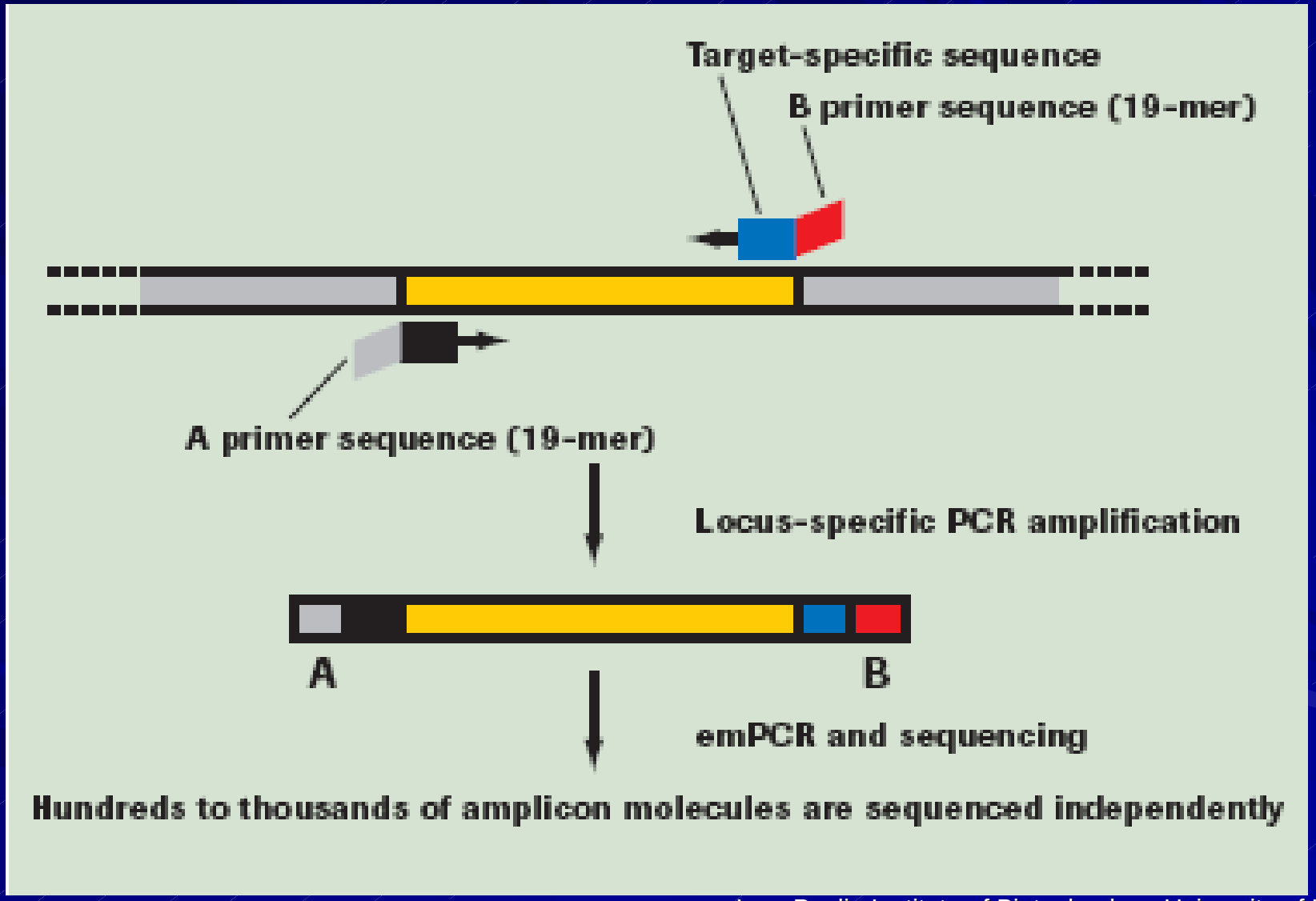
Bioinformatics Tools

- Resequencing
- *De novo* sequencing
- Amplicon Sequencing

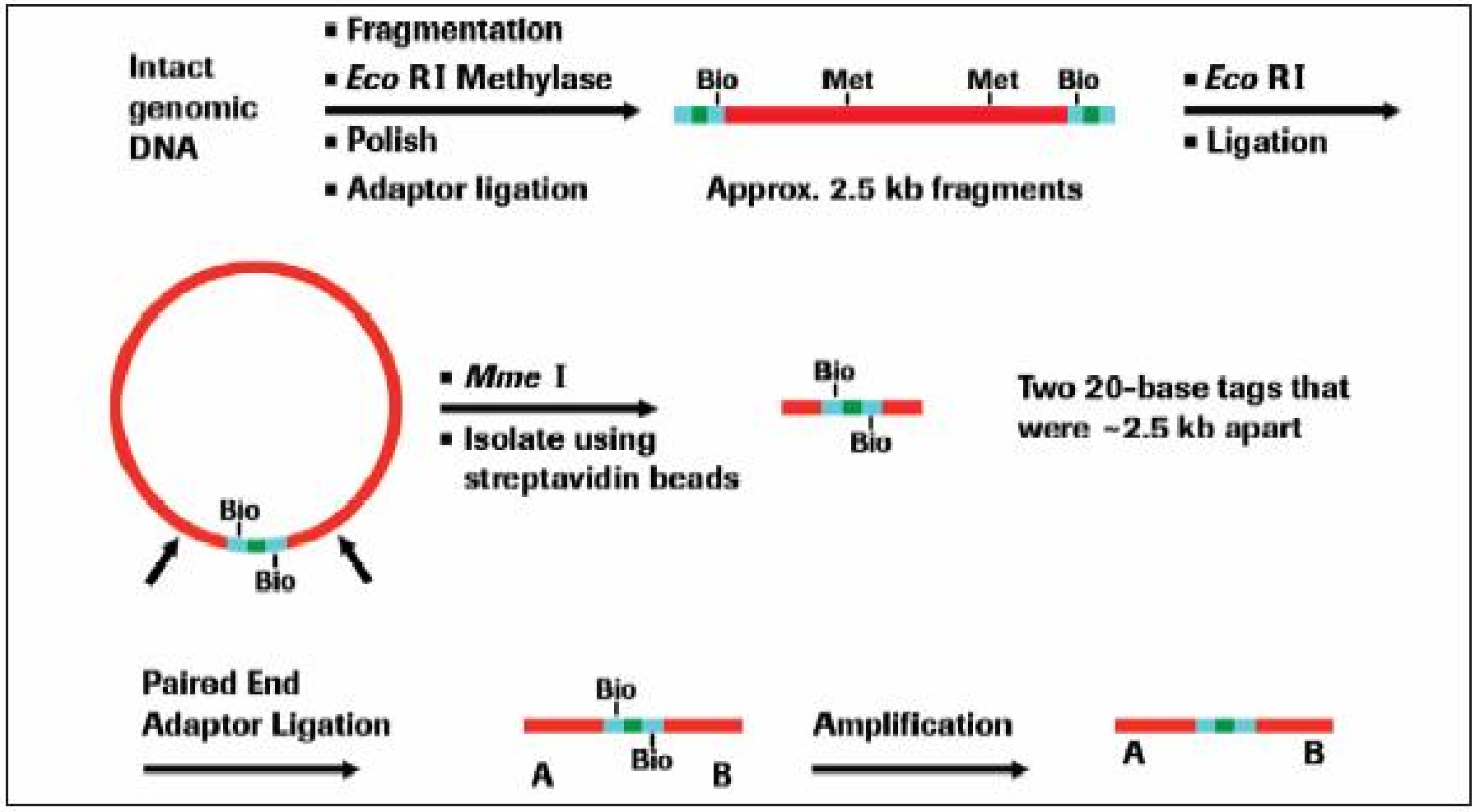
Publicly available tools


Images
Signal
GS De Novo Assembler Software
Overlap and ConsensusGenerator
Contig 1
Contig 2
Contig Ordering
(Paired End Assembler)
Paired
End Reads
GS Reference Mapper Software
Reference Genome
ConsensusGenerator
Reference Genome
Consensus Genome
MutationDetector
Reference Genome
Consensus Genome

Amplicon sequencing



Paired-end Sequencing



Illumina/Solexa Genome Analyzer

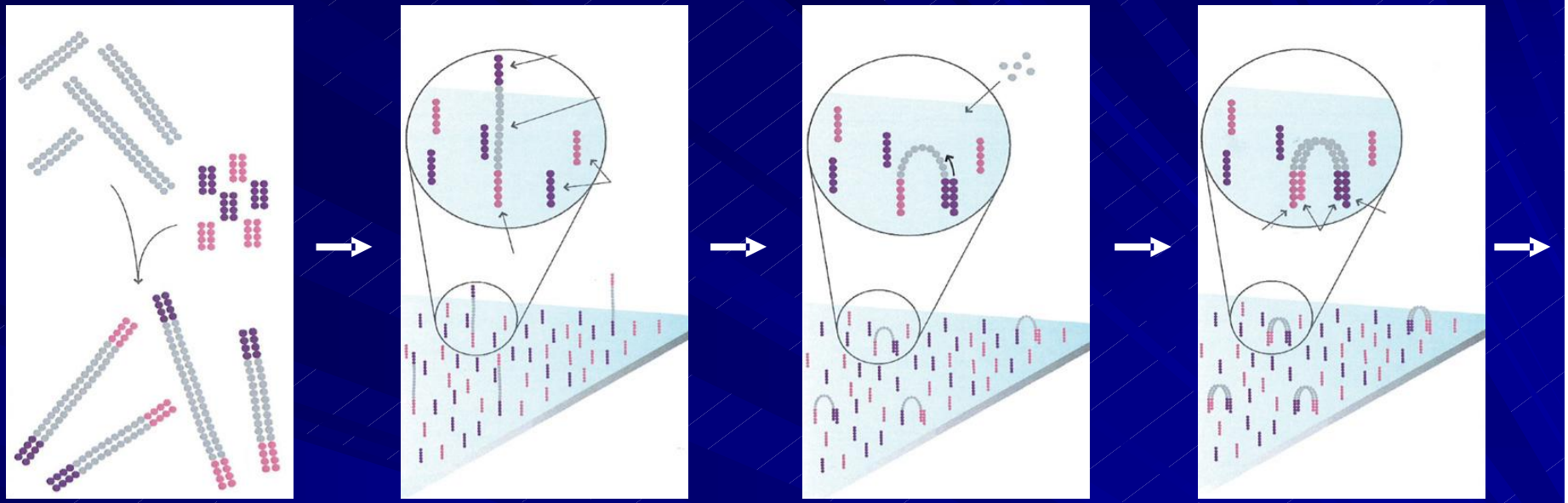
(http://www.illumina.com)

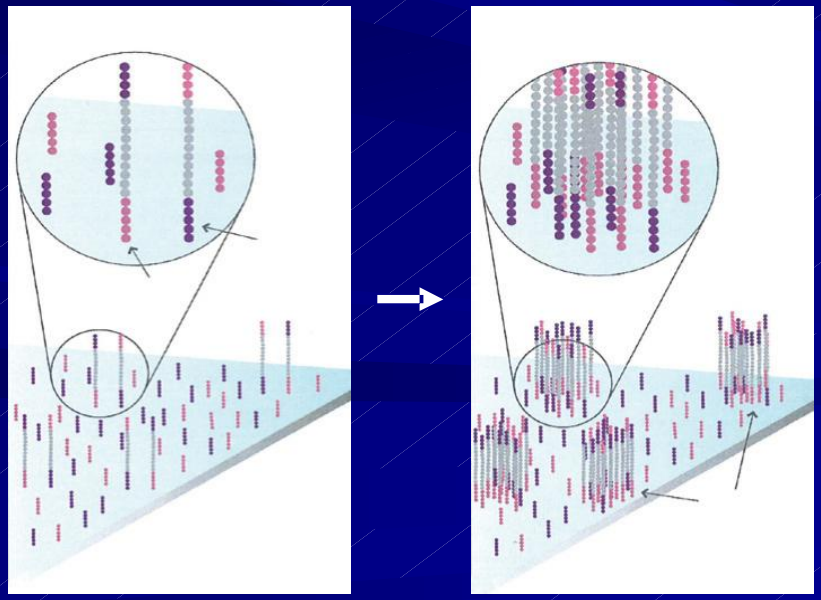
- Clonal Single Molecule Array technology
  - Sequencing-by-synthesis technology
  - Reversible terminator-based sequencing
    - removable fluorescence
  - Flow cell with > 10 million clusters
    - Each cluster ~1,000 copies of template /cm^2
  - 1–8 samples / run
  - 3 laser system (660, 635, and 532 nm)
  - Read length 35 - 50 bp, 1- 2 Gb / run
    - Run time 3 – 6 days,

Cluster Station

Flow cell

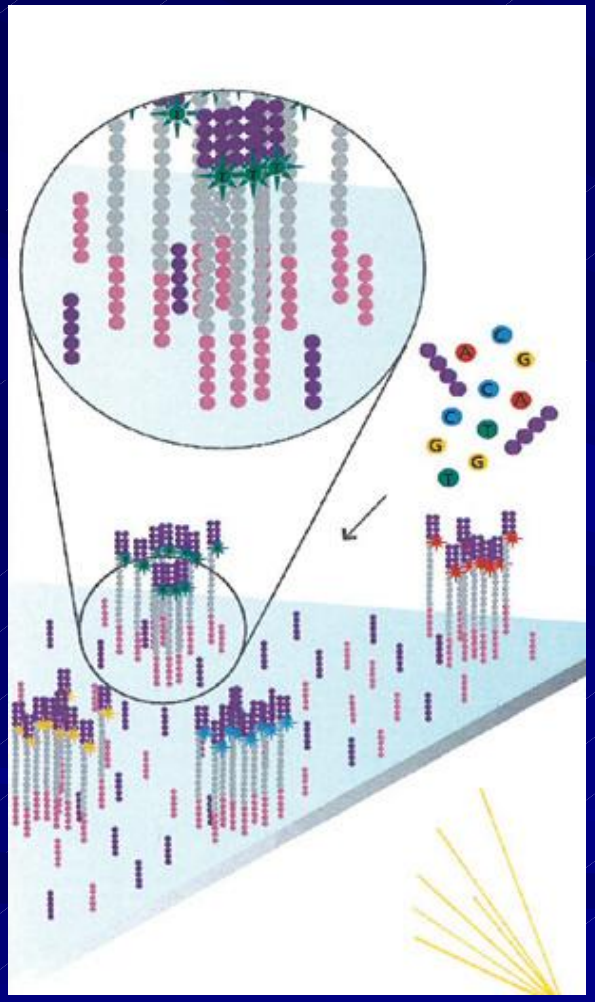
Illumina/Solexa

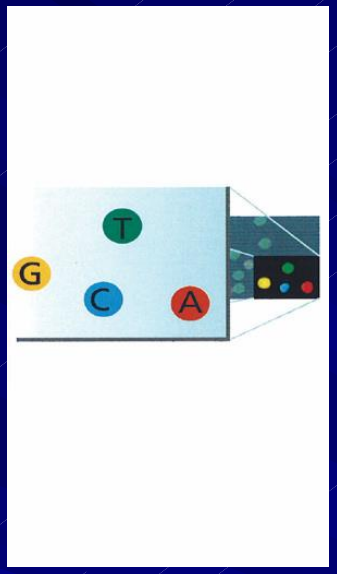




- Sample preparation
  - 100ng–1µg
  - Attaching to Flow cell
  - Bridging
  - PCR
    - Elongation
    - Denaturation
    - Clonal amplification

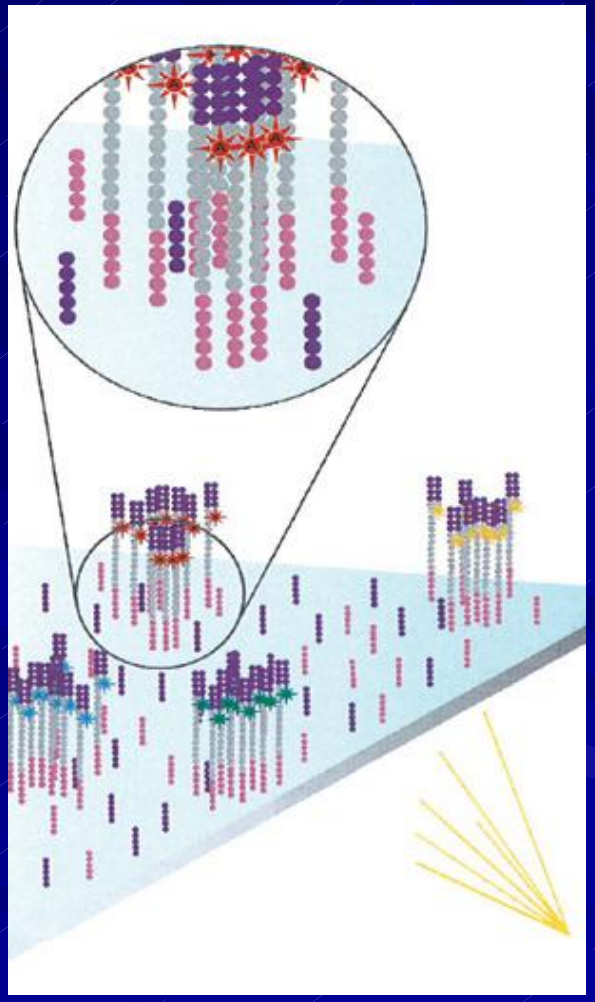
Illumina/Solexa sequencing





Sequencing

- First bases
- Fluorescent reversible terminators
- Detection with laser and CCD camera



Sequencing

- Second bases detected after removal of label and blocking

SOLiD, Applied Biosystems

(http://www.appliedbiosystems.com)

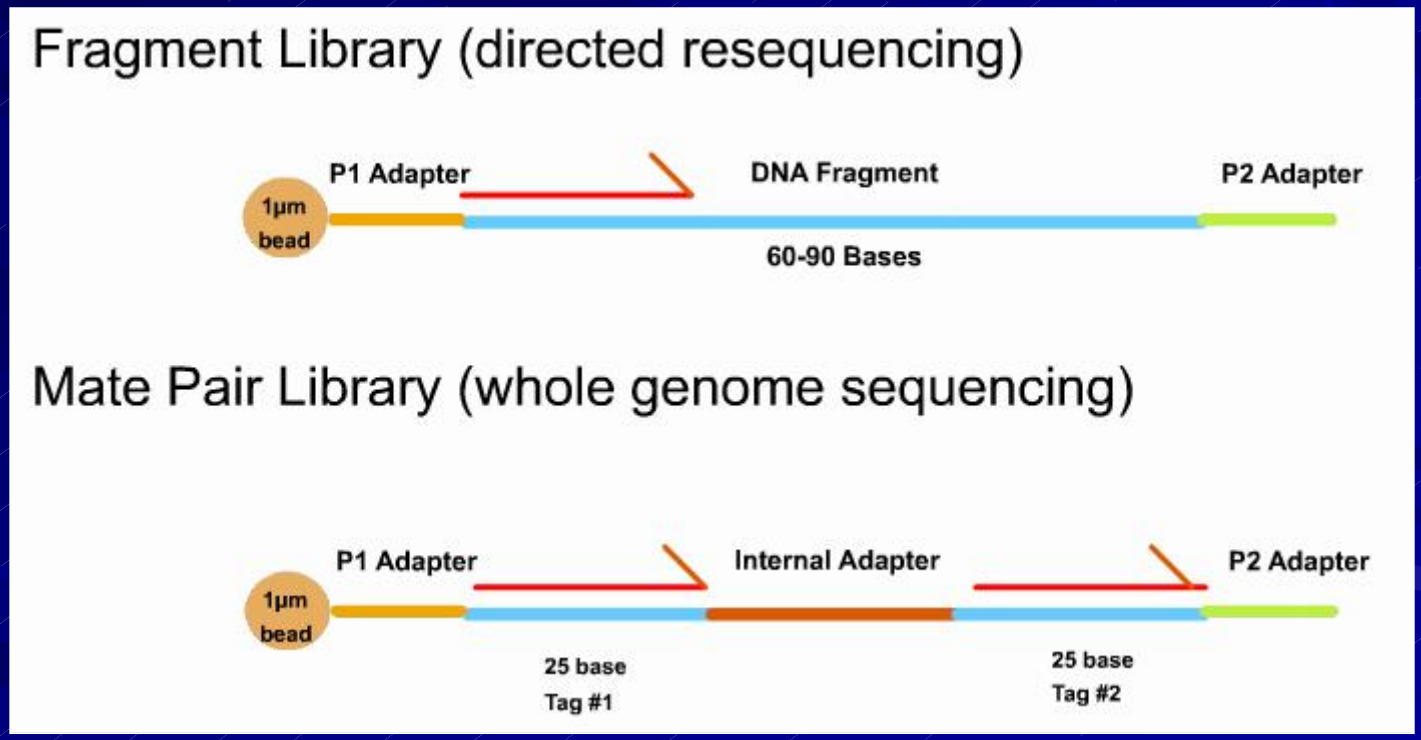
- Sequencing by Ligation
  - emPCR
    - Small beads, 1µm
  - Attaching to glass slides
  - Labelled probes
    - Fuor colours
    - 2 base encoding system
  - Repeated ligation steps
  - Detection with 4 Mpixel camera
  - Read lenght 25-30 bp
  - 1-2 slides / run
  - 1-2 Gb / run
  - Run time 5 -10 days

Shendure, J. *et.al.* Science 2005, 309, 1728-1732

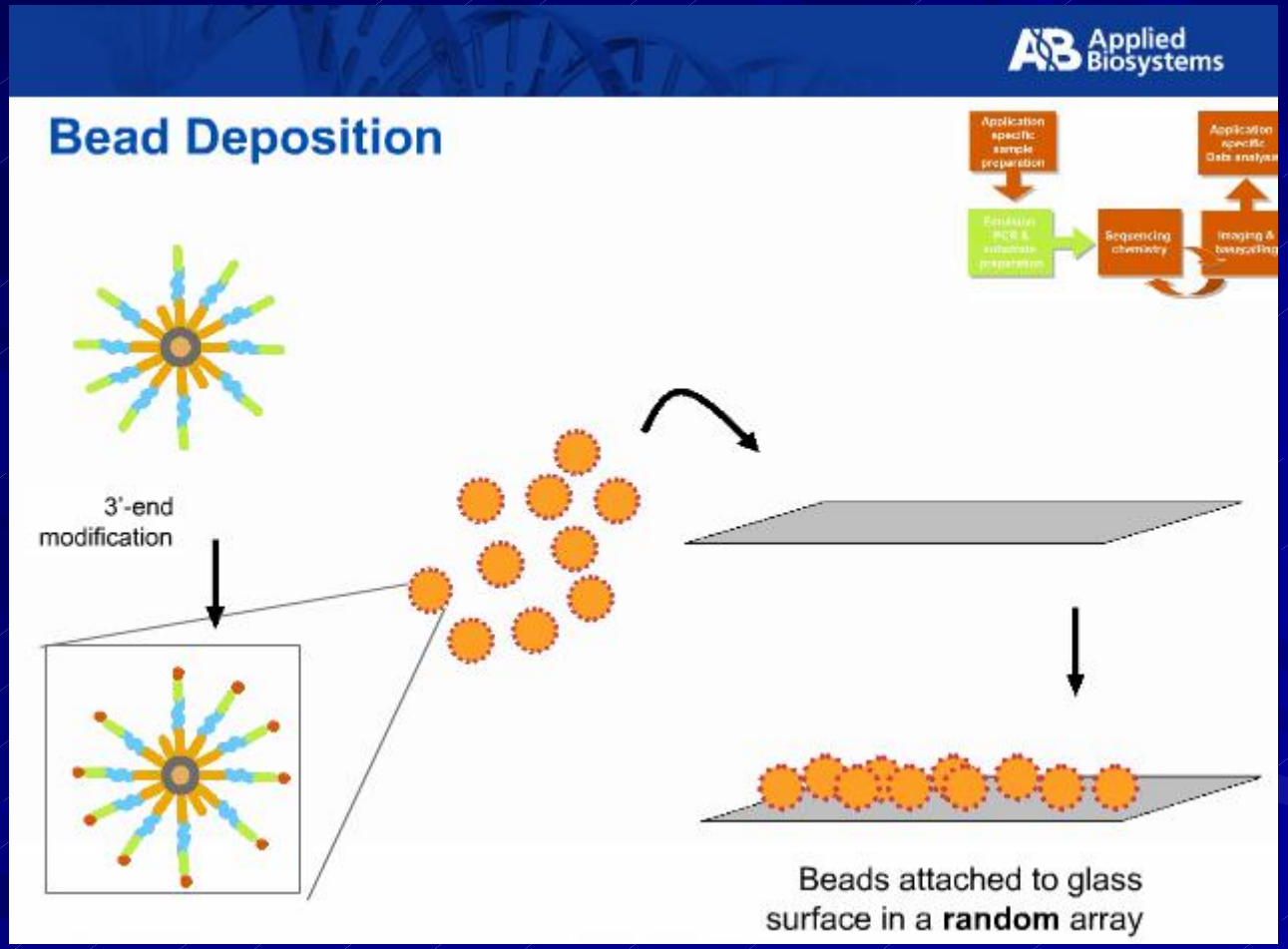
SOLiD

SOLiD

- Library preparation



SOLiD

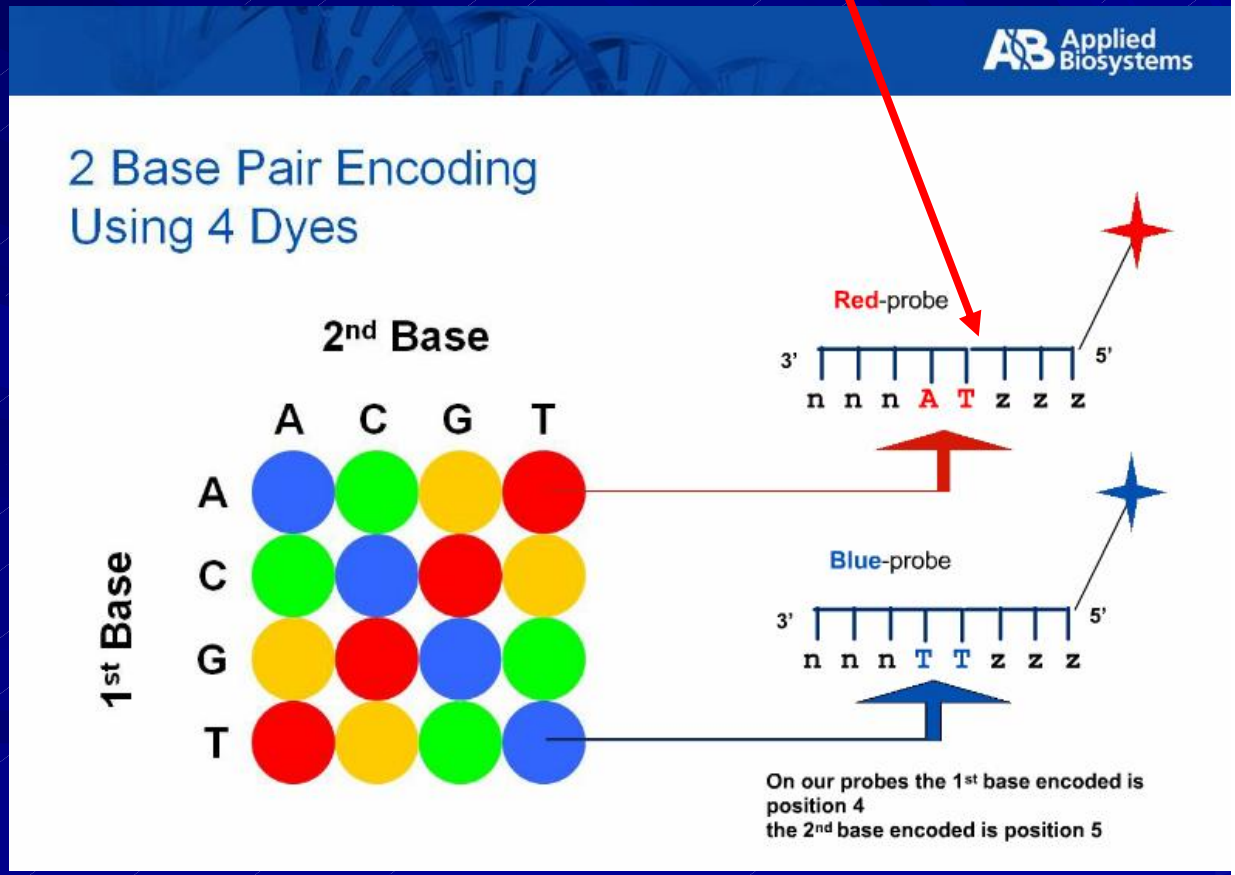

Applied Biosystems
Bead Deposition
Application specific sample preparation
Sequencing chemistry
3'-end modification
Beads attached to glass surface in a **random** array

SOLiD

- Probes
  - 1 024 Octamer Probes
  - 4 Dyes
  - 4 dinucleotides
  - 256 probes / dye

N = degenerate bases

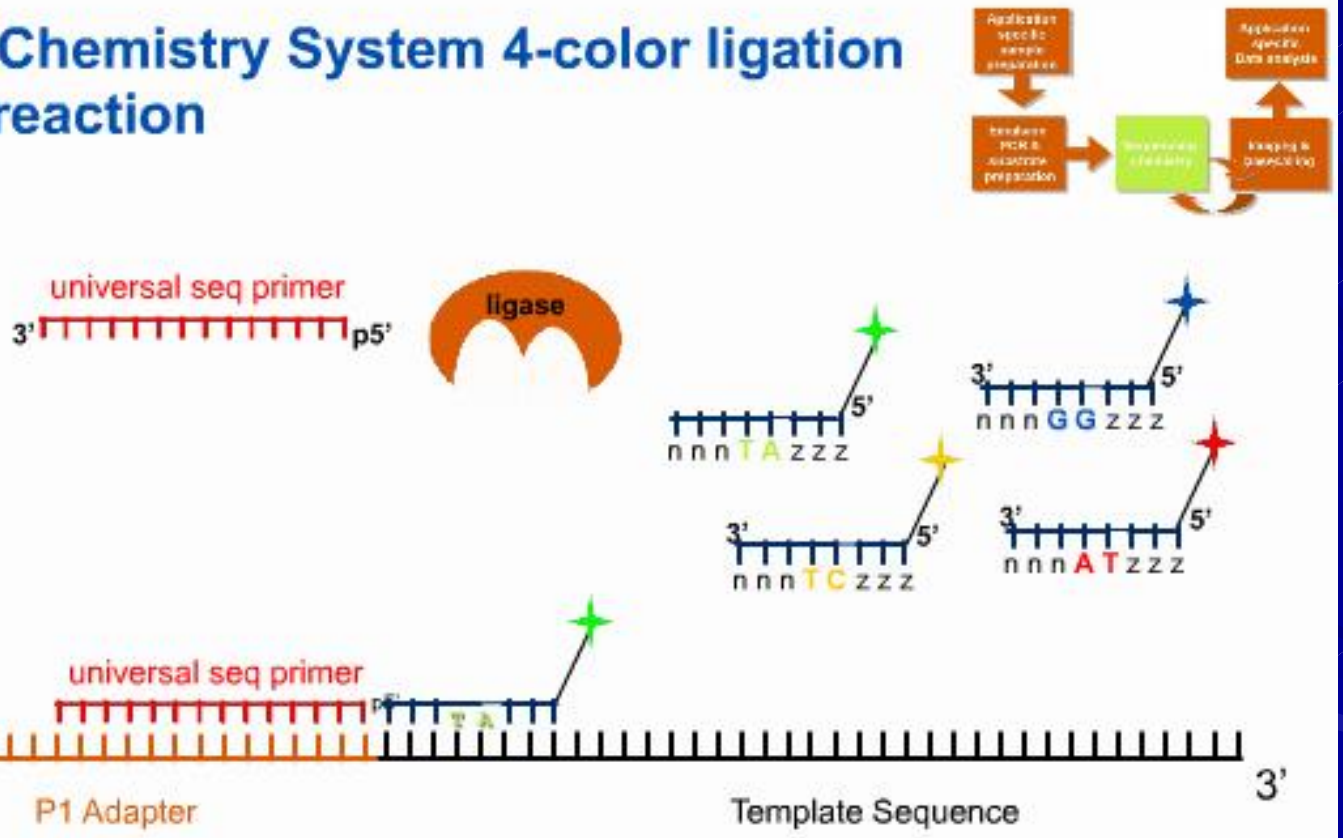
Z = universal base



SOLiD

Applied Biosystems

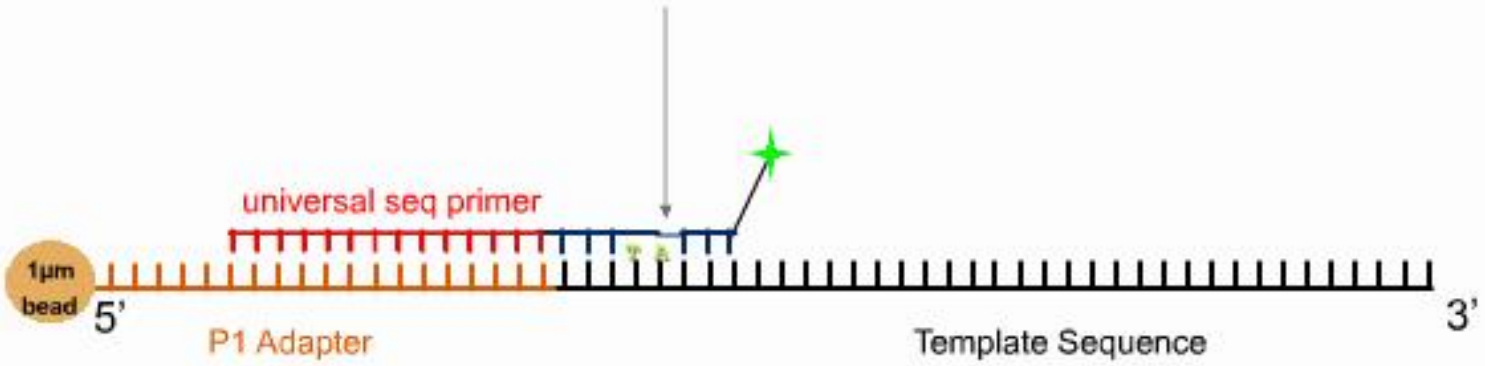
SOLiD™ Chemistry System 4-color ligation Ligation reaction



SOLiD

Applied Biosystems

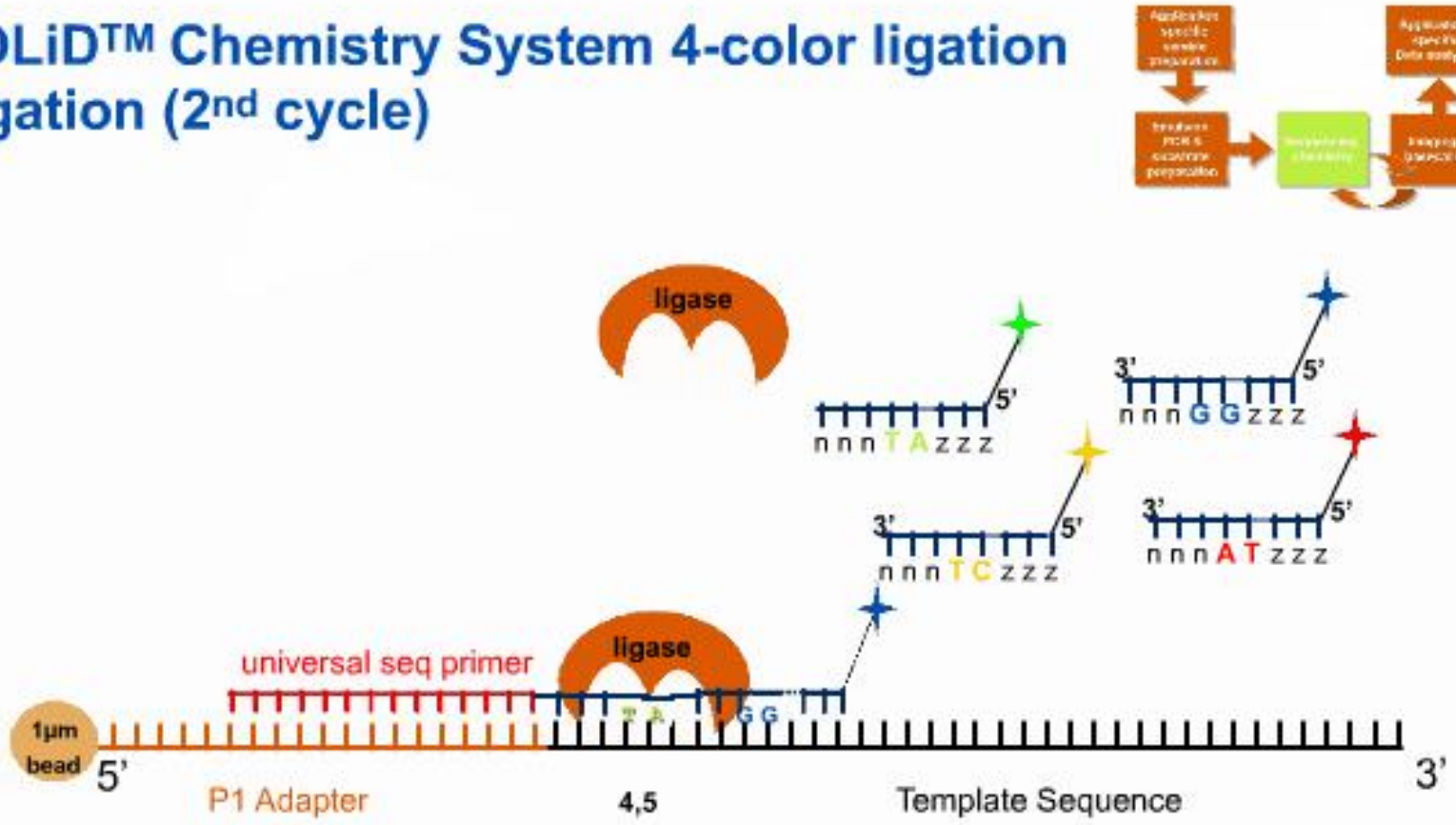
SOLiD™ Chemistry System 4-color ligation Cleavage



SOLiD

Applied Biosystems

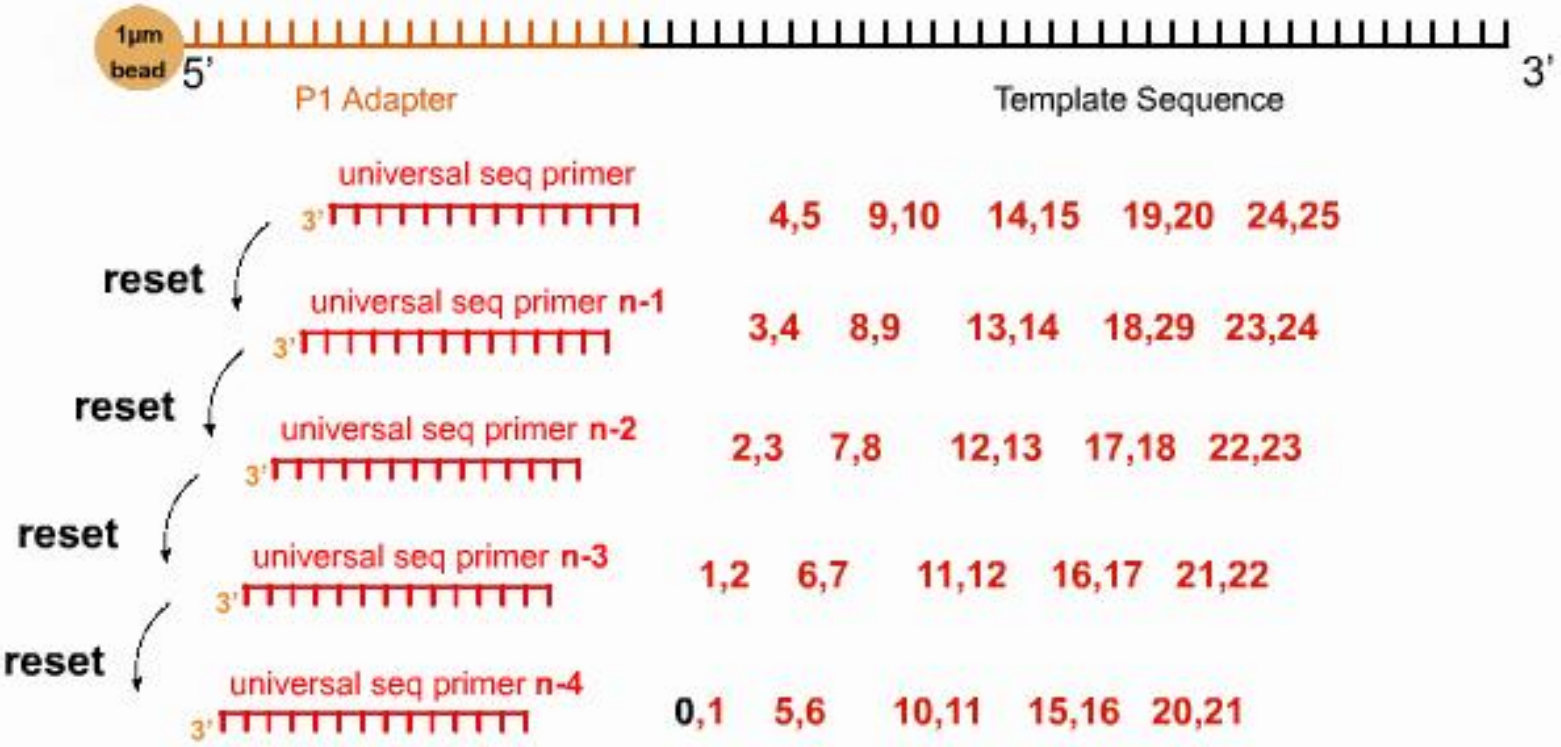
SOLiD™ Chemistry System 4-color ligation
Ligation (2nd cycle)



SOLiD

Applied Biosystems

Sequential rounds of sequencing
Multiple cycles per round



SOLiD

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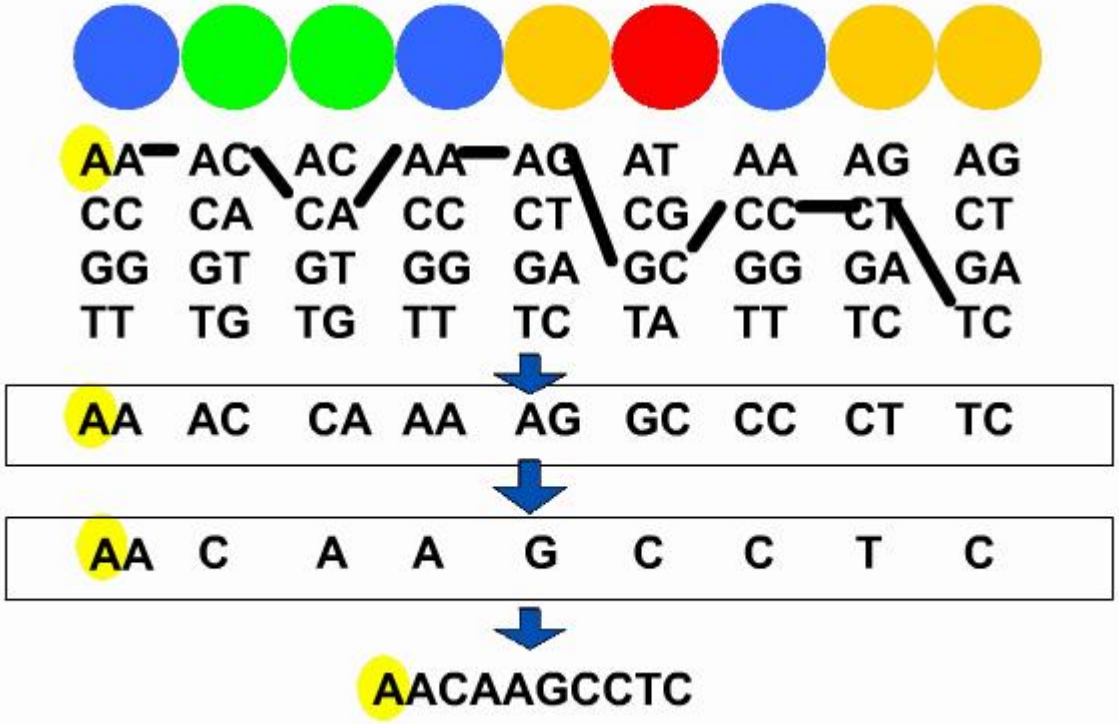
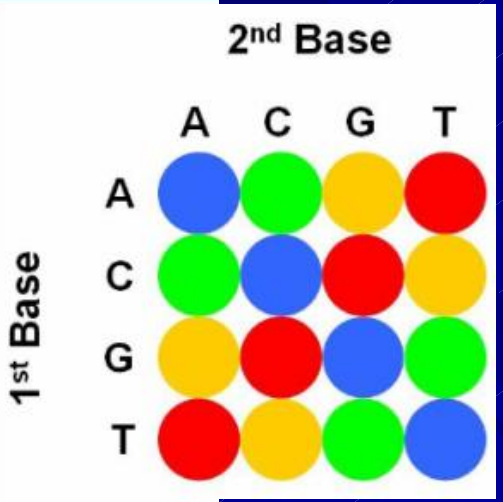
Summary of decoding





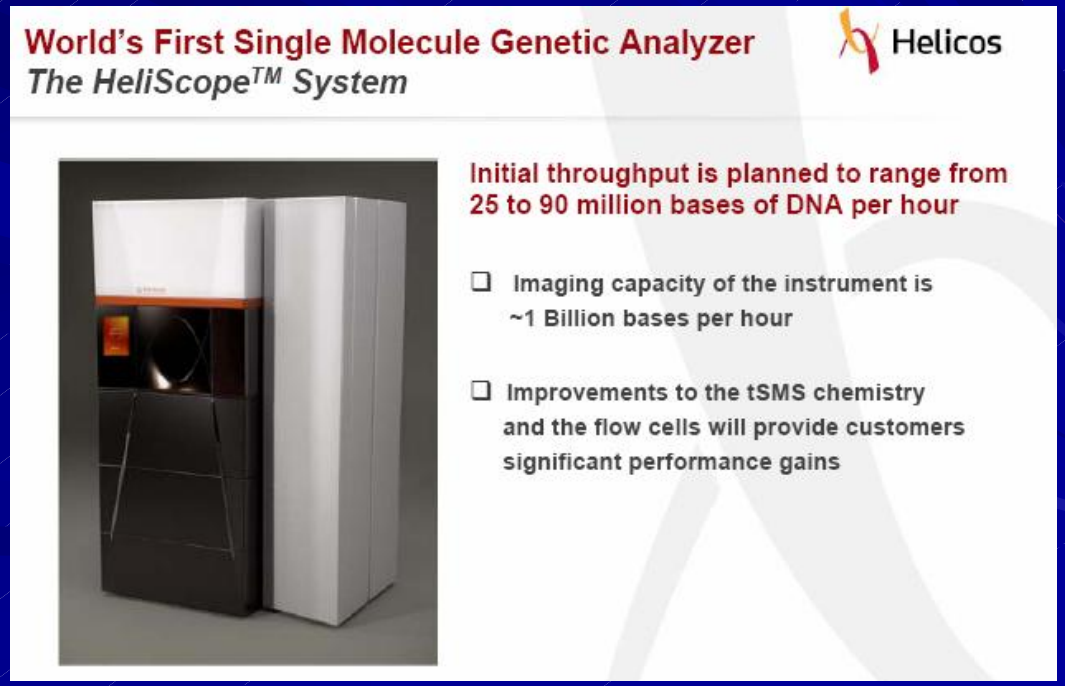
Applications

- Whole genome sequencing
  - *de novo* sequencing
    - Genome Sequencer FLX
- Comparative sequencing
  - All three systems
- Metagenomics
  - Genome Sequencer FLX
- Amplicon sequencing
  - Mutations / SNP
  - All three systems
- Transcriptome sequencing
  - cDNA
    - All three systems
  - Small RNA
    - All three systems
- ChIP sequencing
  - All three systems
- Methylation sequencing
  - All three systems

Helicos

(www.helicosbio.com)

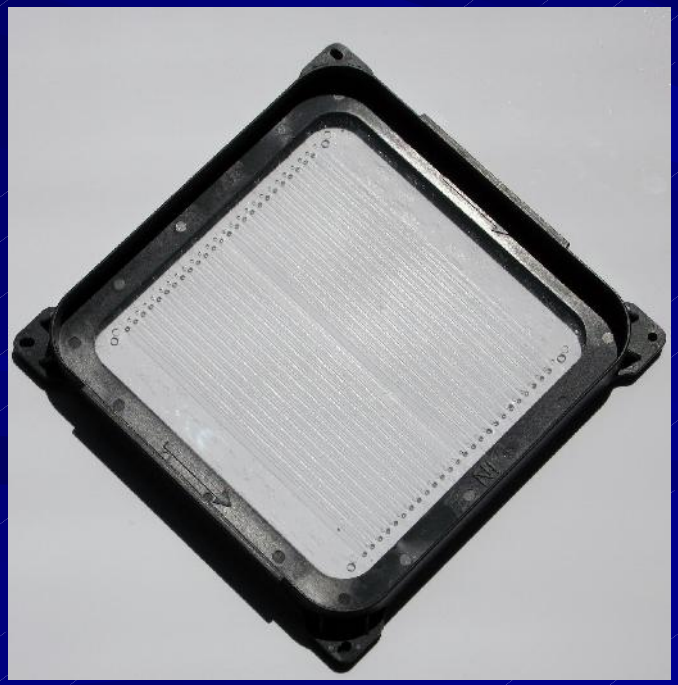
- HeliScope™ Single Molecule Sequencer
  - True Single Molecule Sequencing (tSMS)™
  - Sequencing-by-synthesis
  - Template 100 – 200 bp
    - Addition of polyA
  - No PCR amplification
  - 1 000 000 000 reads / experiment
  - 25-90 Mb / h
  - 2 + Gb / day



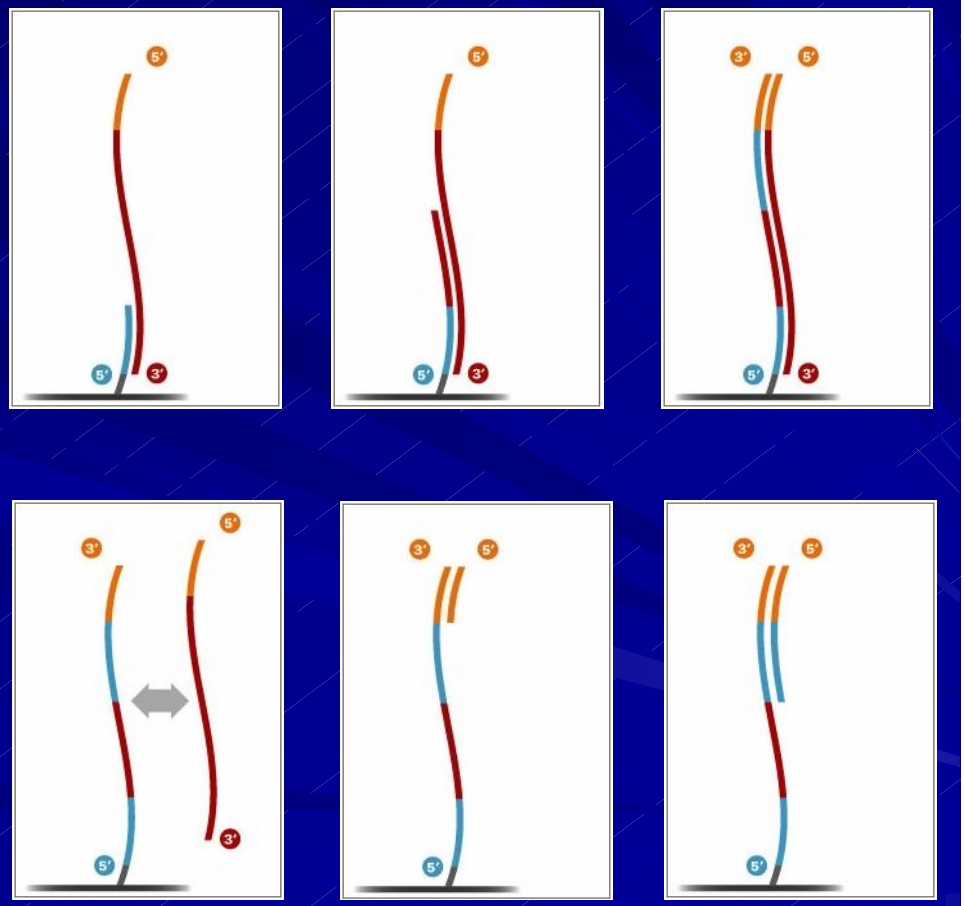
Helicos

Flow cell

- 25 discrete channels per flow cell
- Single molecule capture by hybridization, allowing densities of 100 million strands of DNA per square centimeter or higher



Paired-end Sequencing
(100 – 200 bp)



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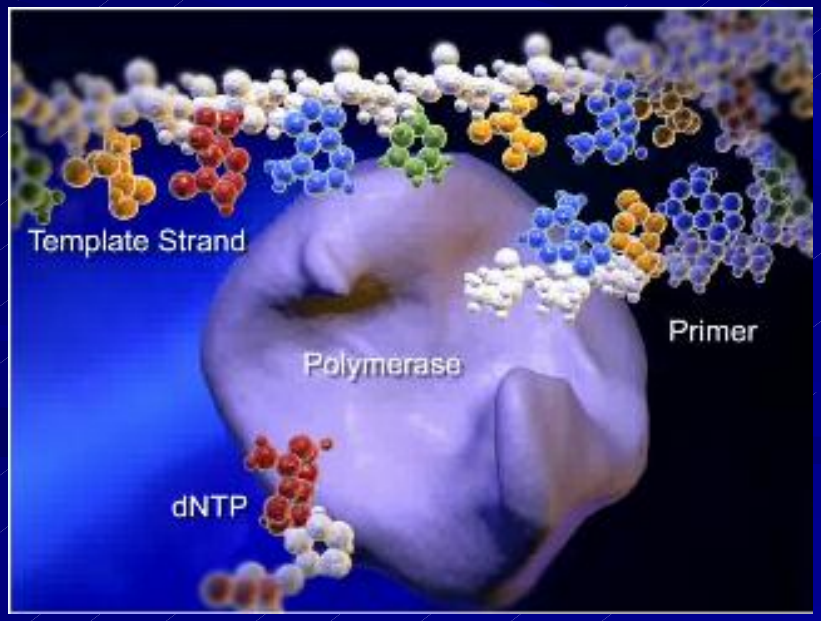
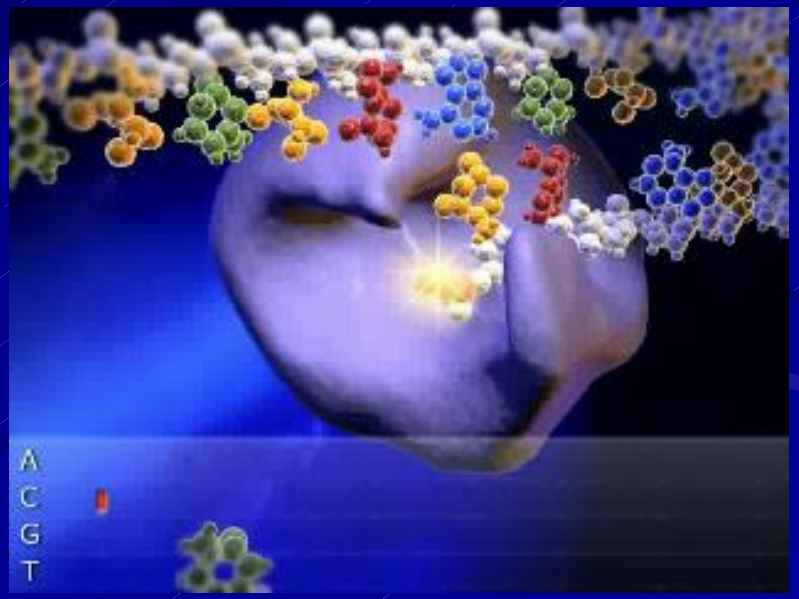
VisiGen

(www.visigenbio.com)

- Technology
  - No cloning or amplification
  - Intact DNA fragments
  - Real-time detection of DNA synthesis, FRET
  - Fluorescent donor on tip of the Polymerase attached on a glass slide
  - Acceptor fluorescent moiety on the nucleotides
    - On the gamma-phosphate
  - 1Mb/sec/machine





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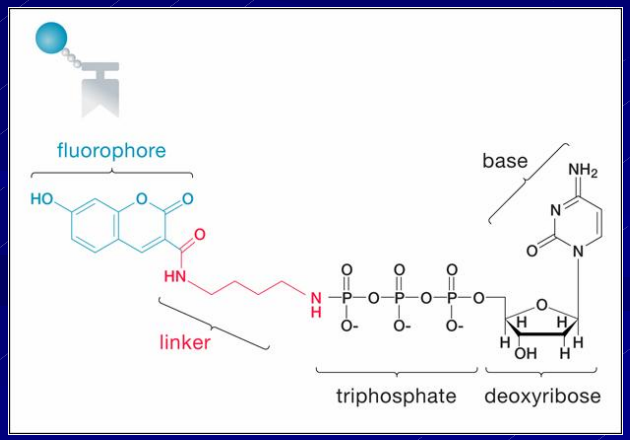
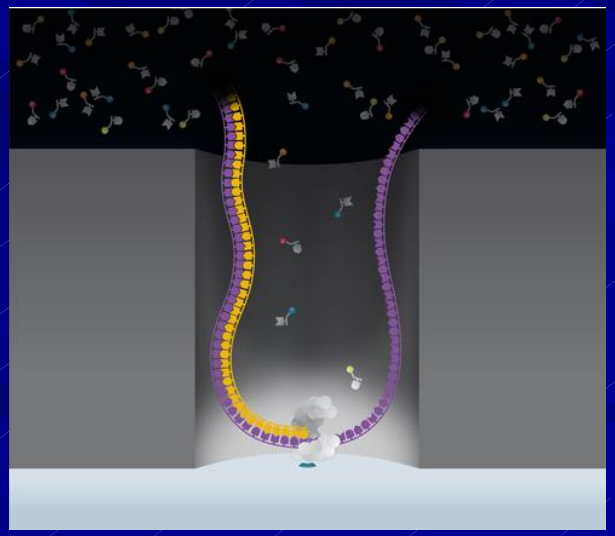
Pacific Biosciences

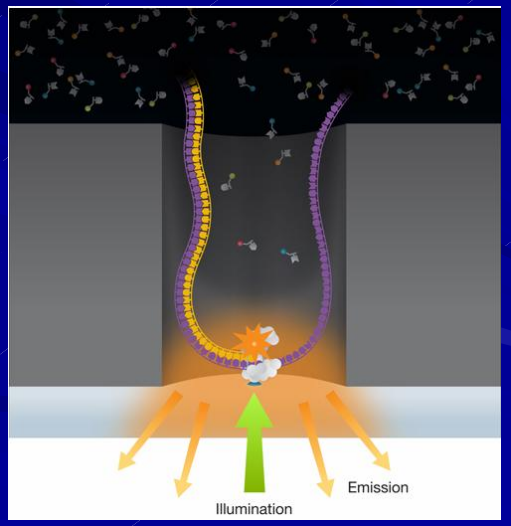
(www.pacificbiosciences.com)

(Korlach, J. *et.al.* PNAS 2008, 105, 1176-81, Levene, MJ. *et.al.* Science 2003, 299, 682-86)

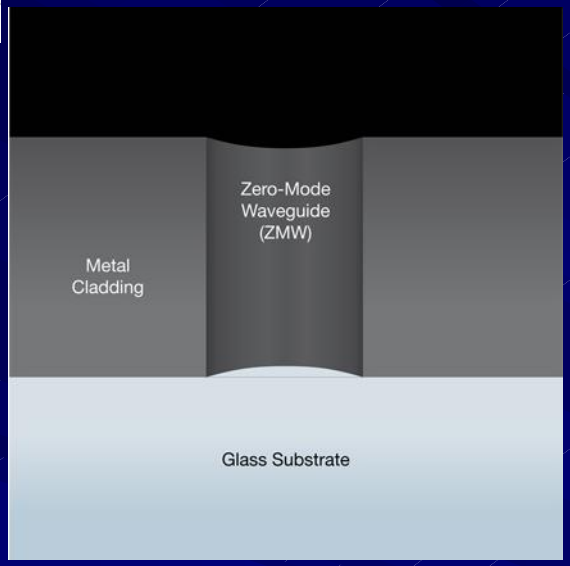
- Technology
  - Single-Molecule Real-Time (SMRT) DNA sequencing technology
  - SMRT chip
    - Thousands of zero-mode waveguides (ZMWs)
    - Holes 100 nm metal film, 20 zeptoliters (10^{-21} liters)
  - Real-time detection of DNA synthesis
    - Fluorescent dNTPs

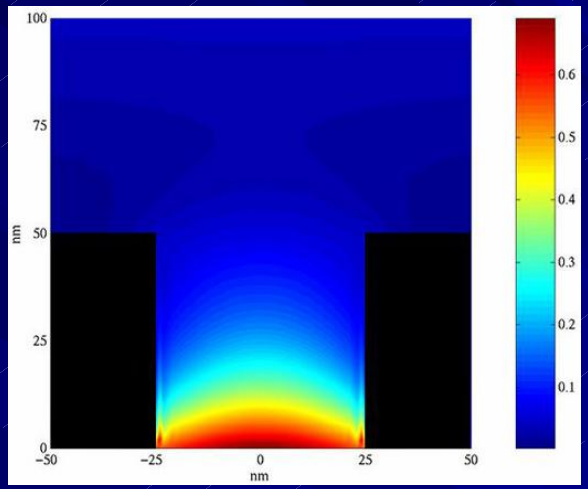


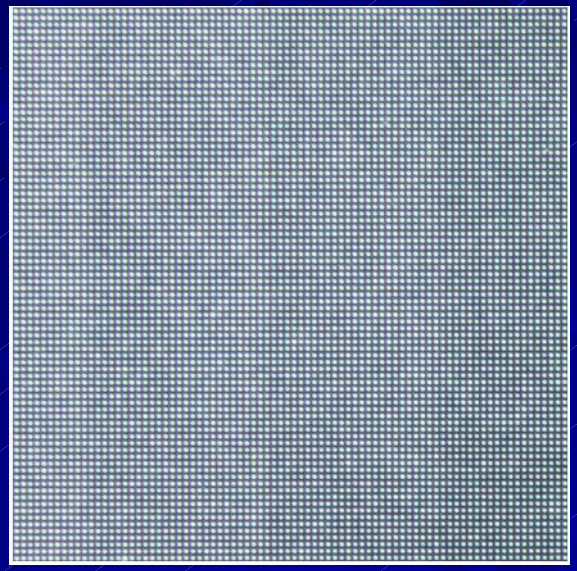


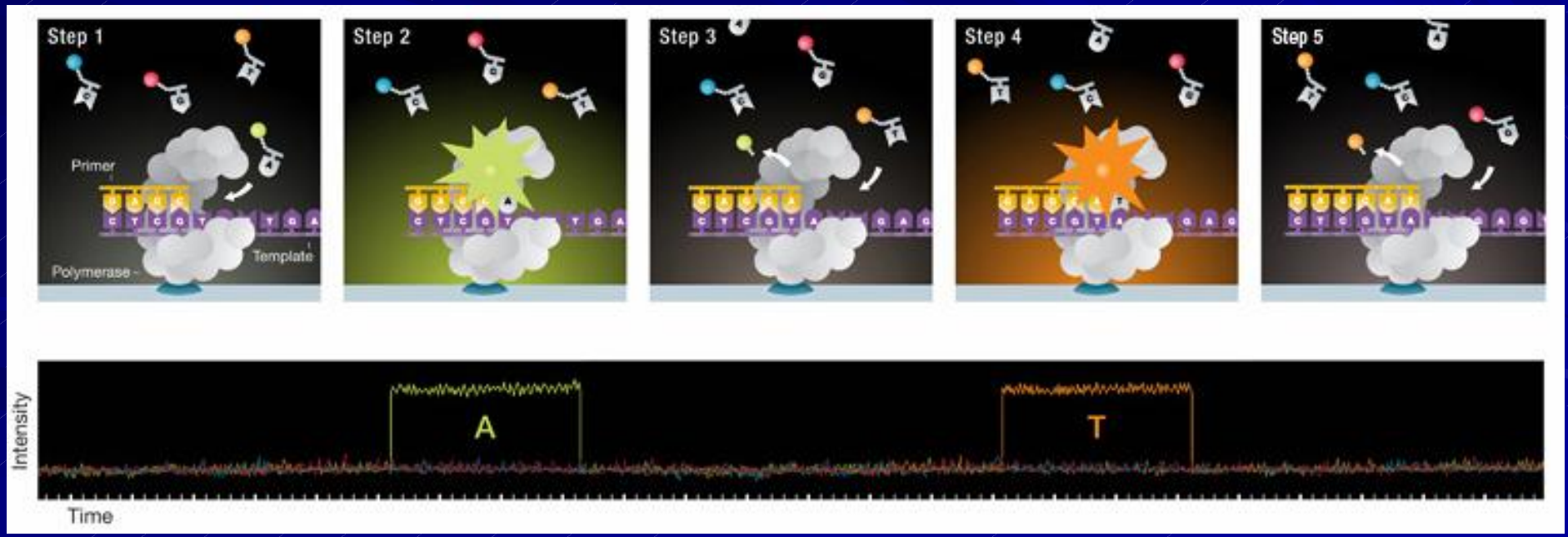


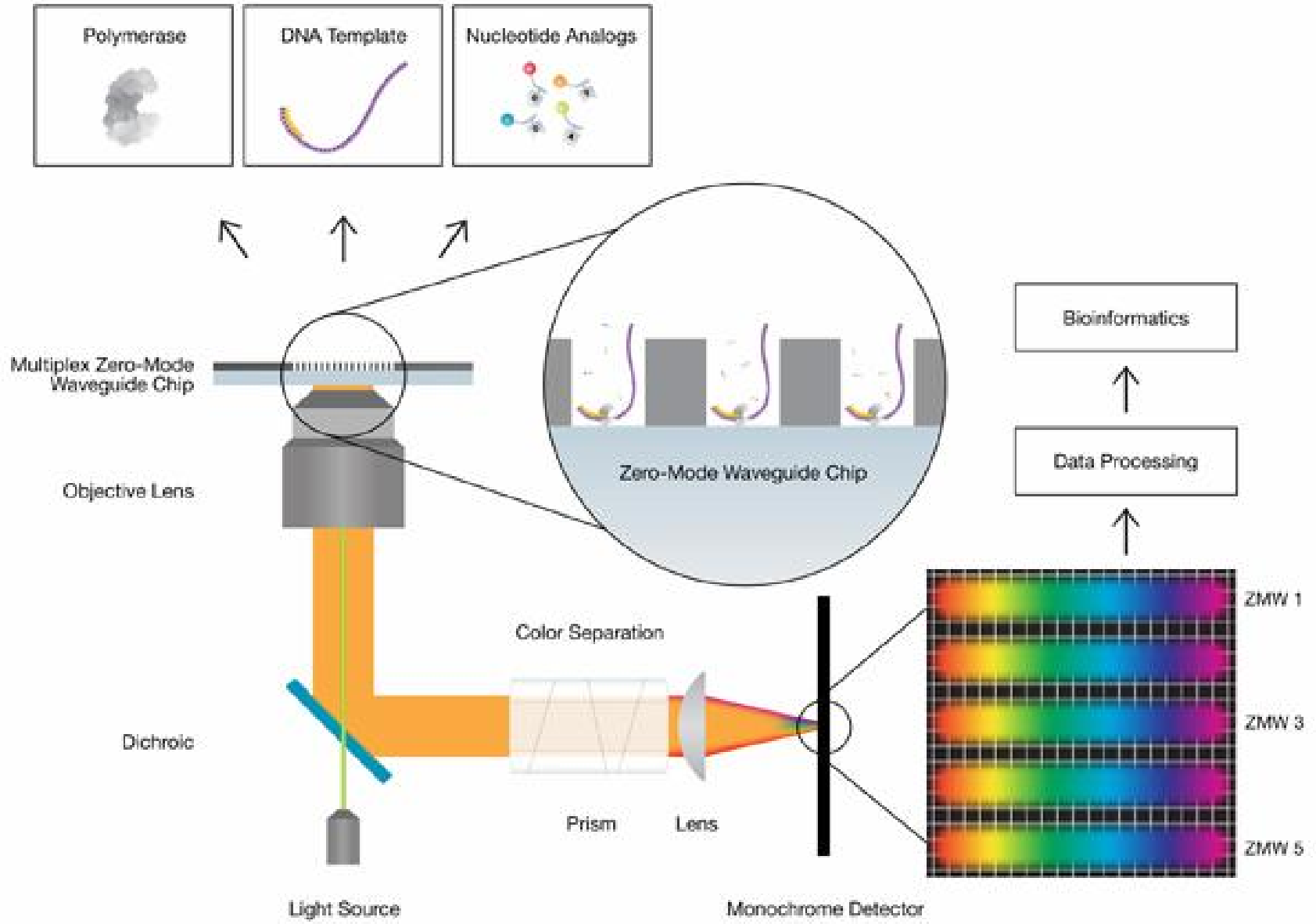
SMRT chip


Zero-Mode
Waveguide
(ZMW)
Metal
Cladding
Glass Substrate


100
75
50
25
0
nm
−50
−25
0
25
50
nm
0.6
0.5
0.4
0.3
0.2
0.1




Step 1
Primer
Polymerase
Template
Step 2
Step 3
Step 4
Step 5
Intensity
A
T
Time


Polymerase
DNA Template
Nucleotide Analogs
Multiplex Zero-Mode Waveguide Chip
Objective Lens
Zero-Mode Waveguide Chip
Bioinformatics
Data Processing
Color Separation
Dichroic
Prism
Lens
ZMW 1
ZMW 3
ZMW 5
Light Source
Monochrome Detector

ARCHON GENOMICS XPRIZE

(www.genomics.xprize.org/genomics)

$10M to the First Team to Sequence 100 Human Genomes in 10 Days

RegisteredTeams

- 454 Life Sciences (Roche) (www.454.com)
- VisiGen (www.visigenbio.com)
- FfAME (www.ffame.org)
- Reveo (www.reveo.com)
- Base4innovation (www.base4innovation.co.uk_)
- Personal Genome X-Team (PGx) (www.personalgenomes.org)
- ZS Genetics, Inc. (www.zsgenetics.com)