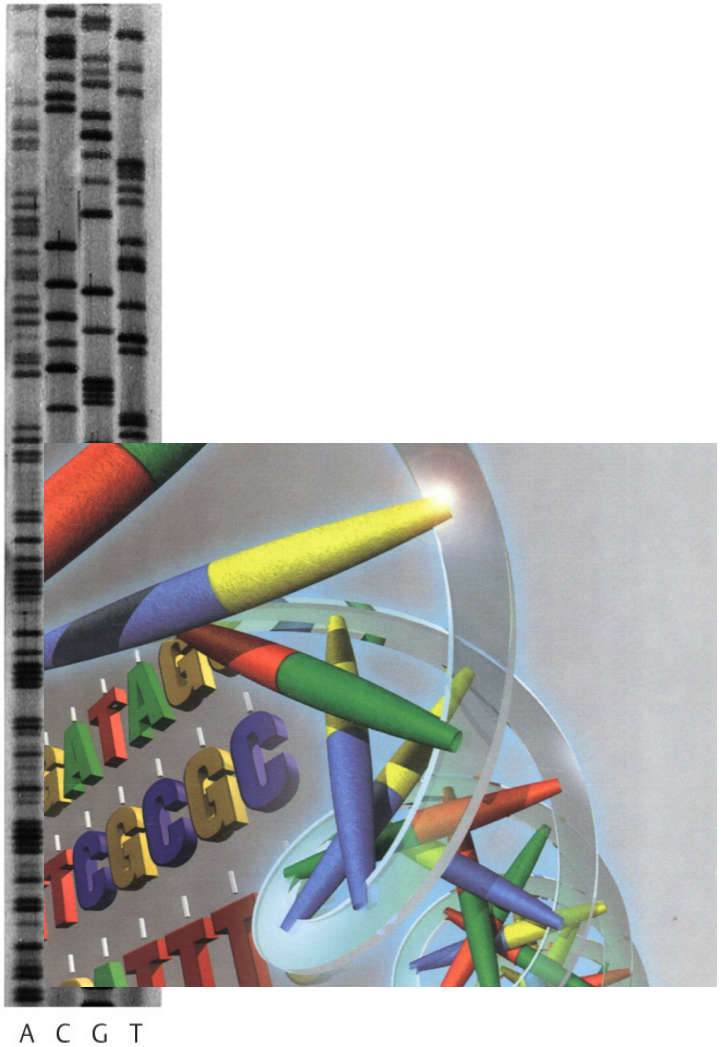
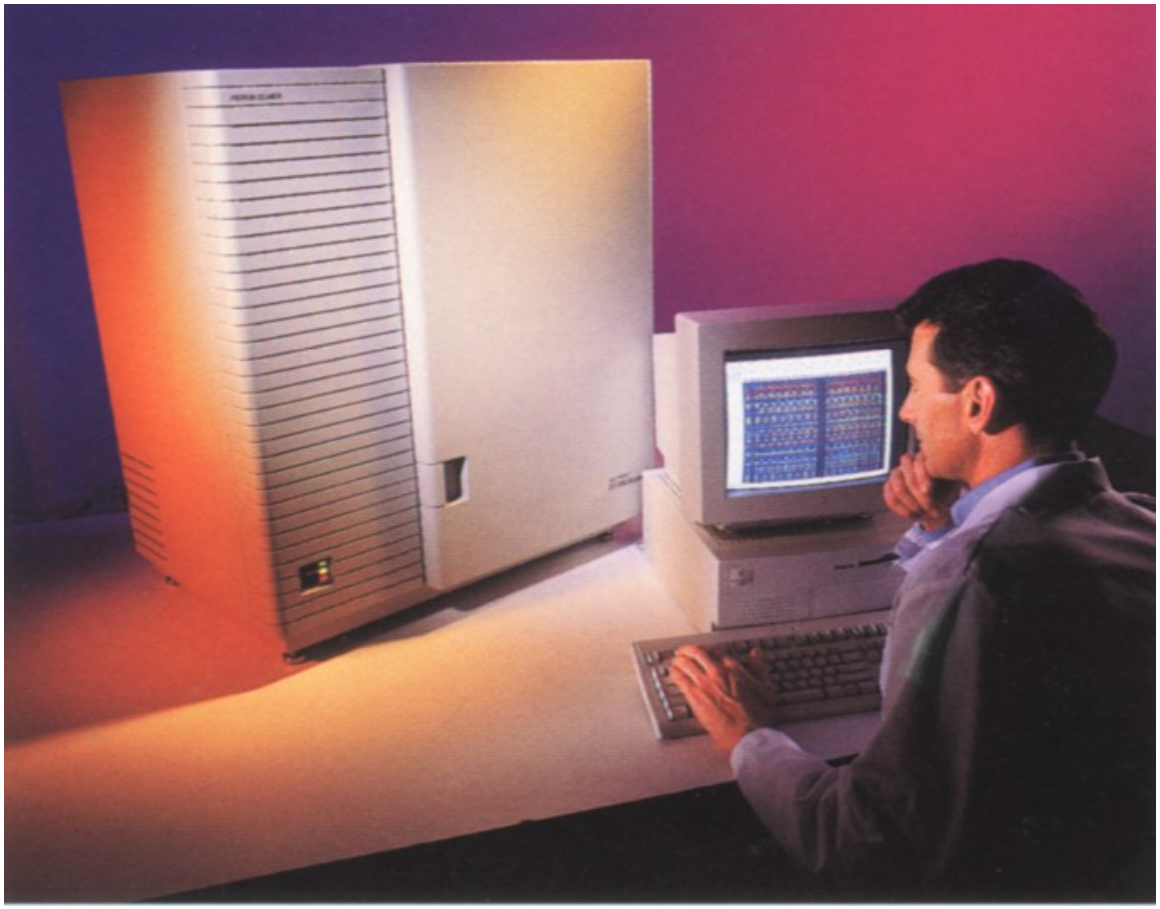


DNA-Analytik II

Technologien der
DNA-Sequenzierung

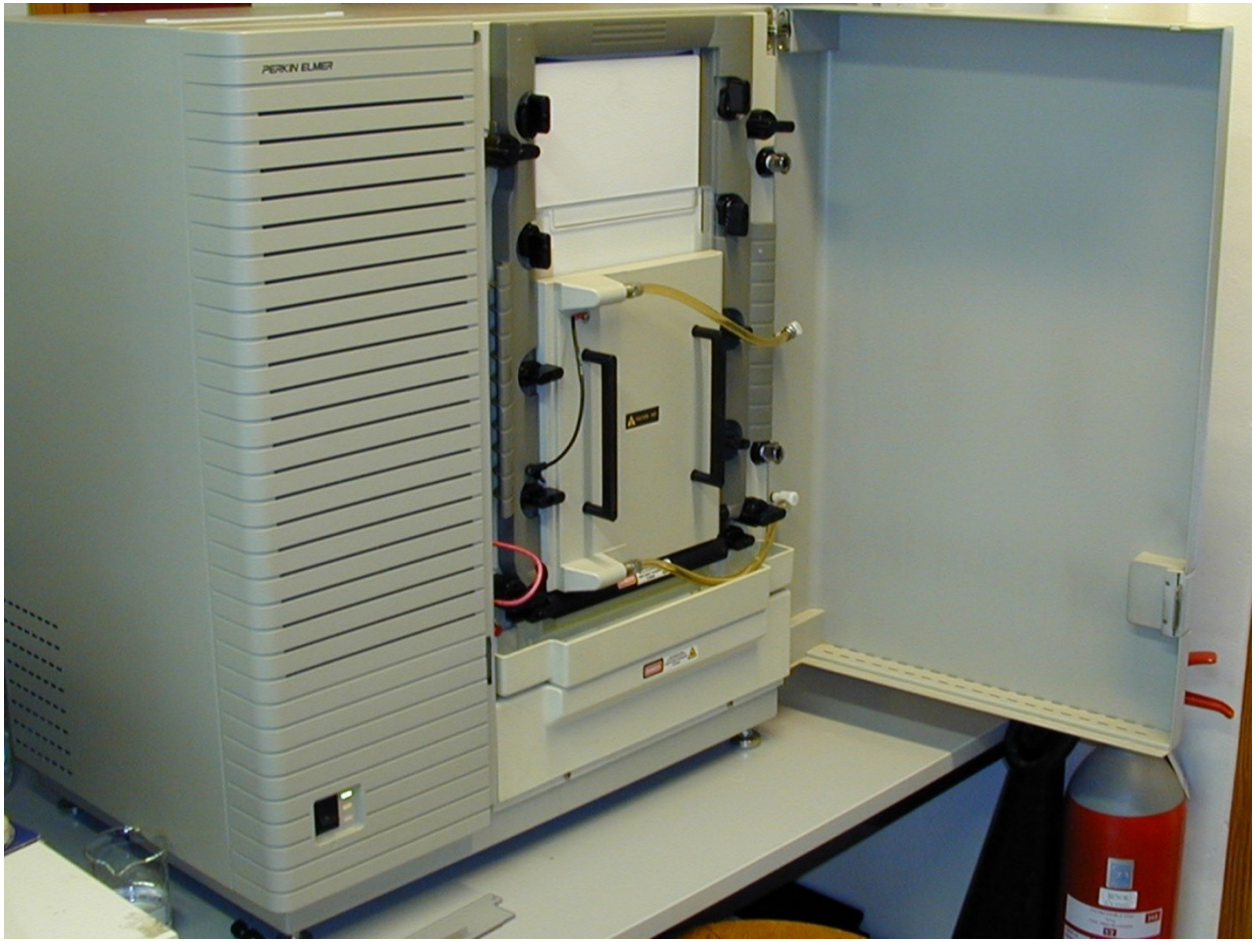
Platten-Sequenzierautomat 1990-2000

ABI 377



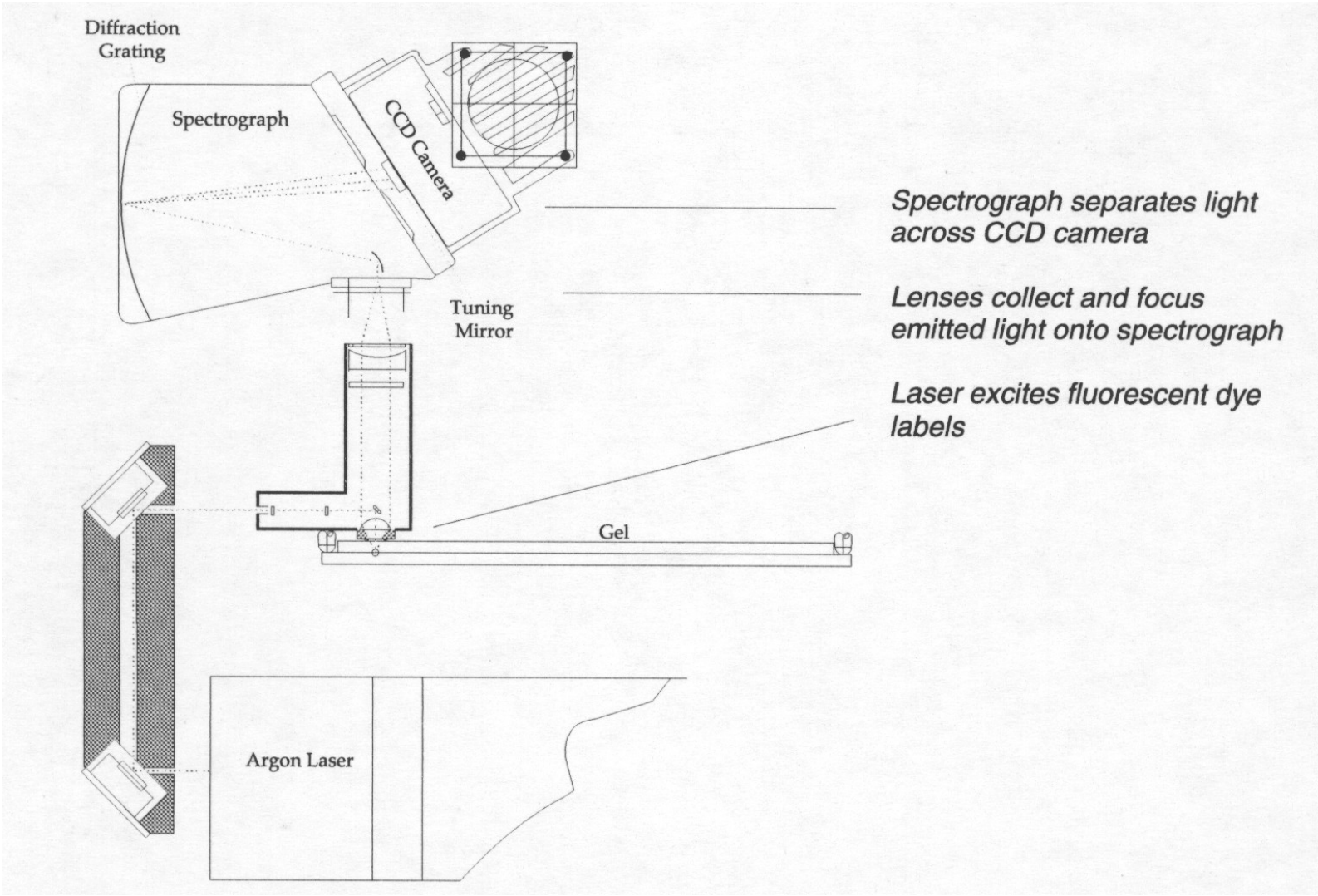
ABI 377

Aufbau



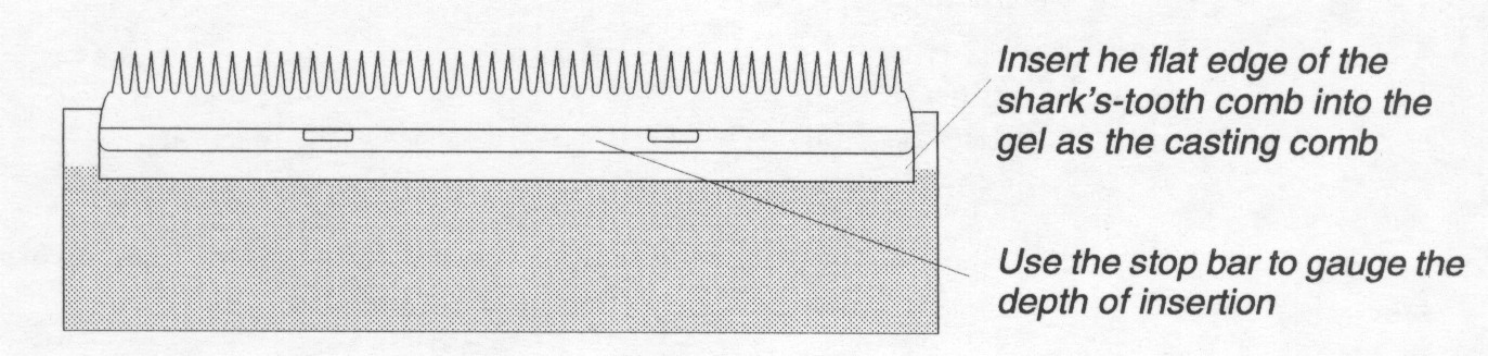
Platten-Sequenzierautomat ABI377

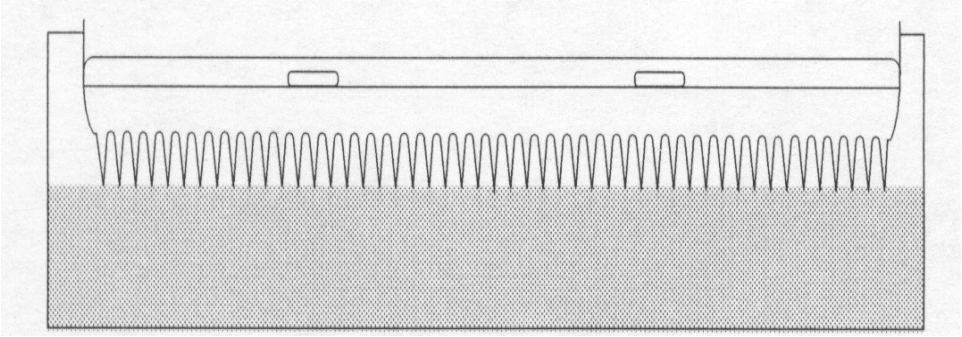
Scanning/Detektionssystem



Platten-Sequenzierautomat ABI377

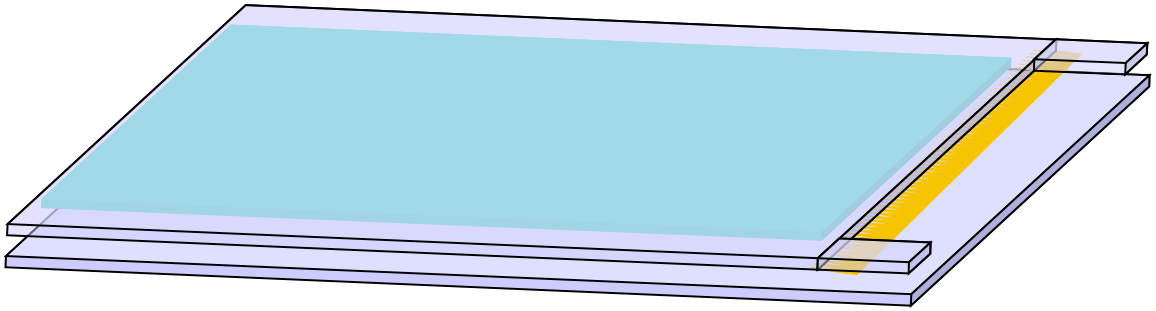
PA-Gel





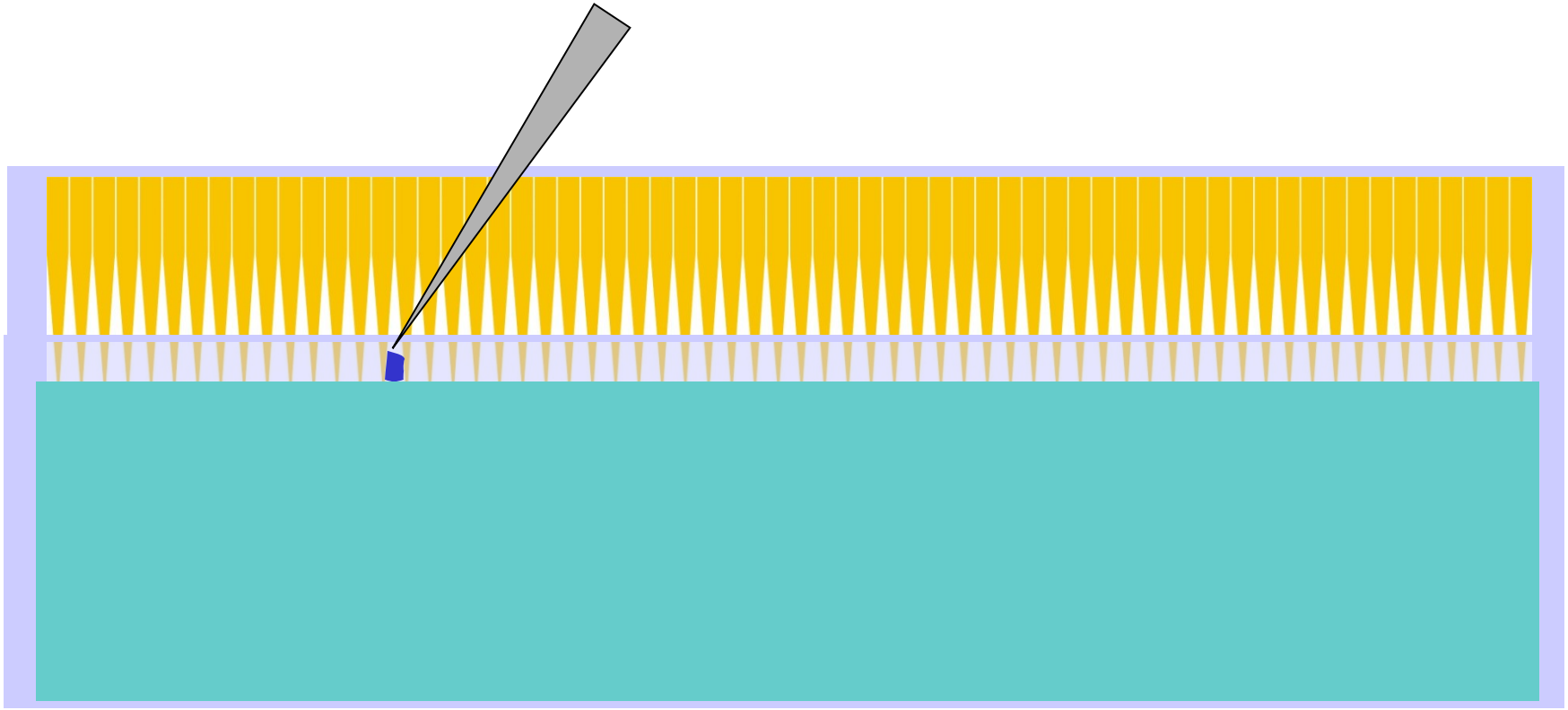
Platten-Sequenzierautomat ABI377

PA-Gel



Platten-Sequenzierautomat ABI377

PA-Gel

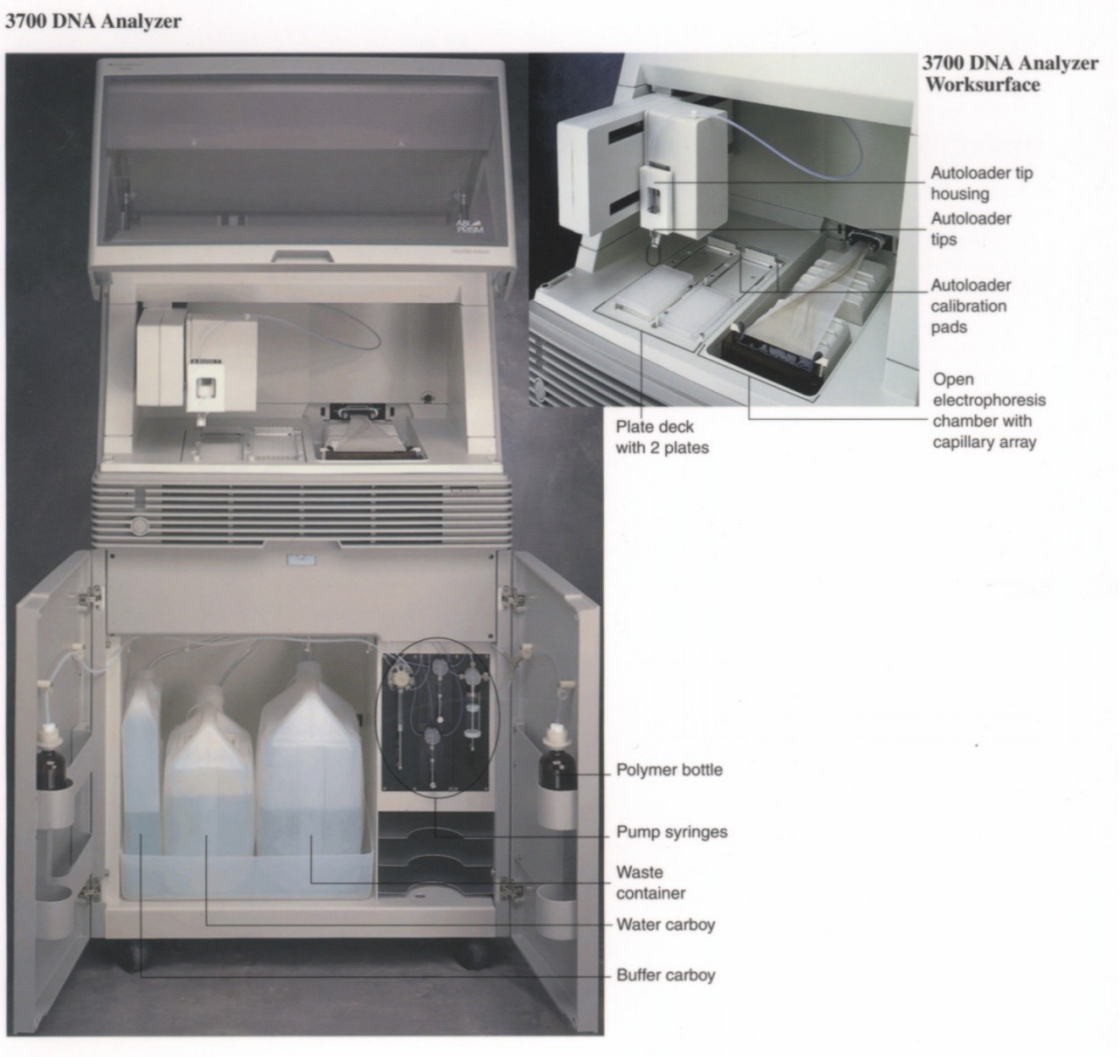


Kapillar-Sequenzierer

ABI 3700 1999-2002

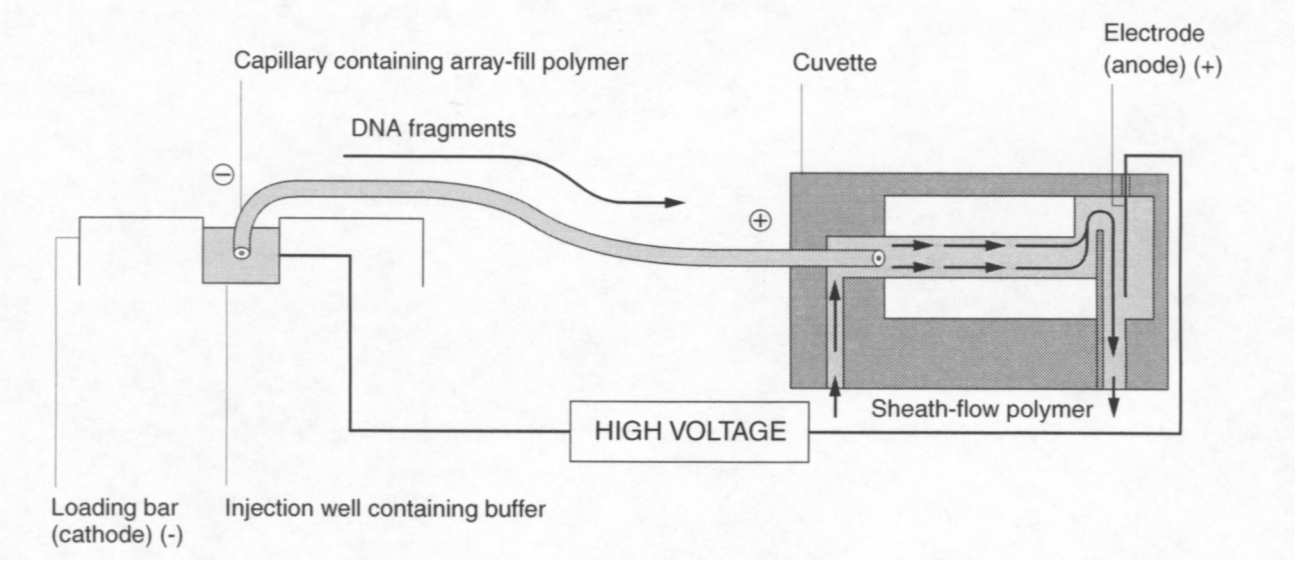
ABI 3700

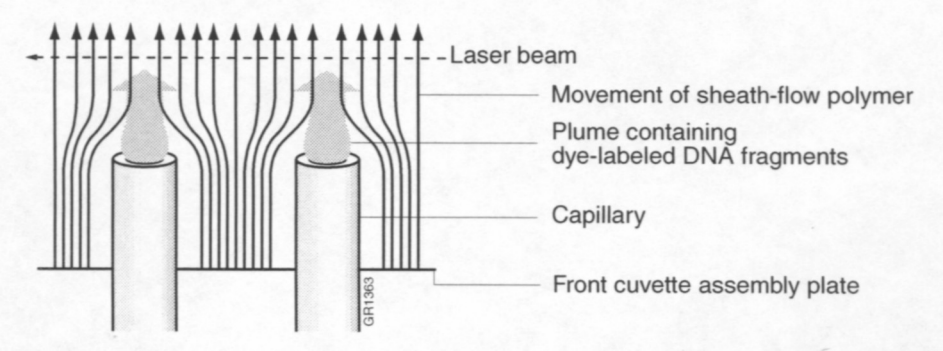
Aufbau



ABI 3700

Elektrophorese & Detektion



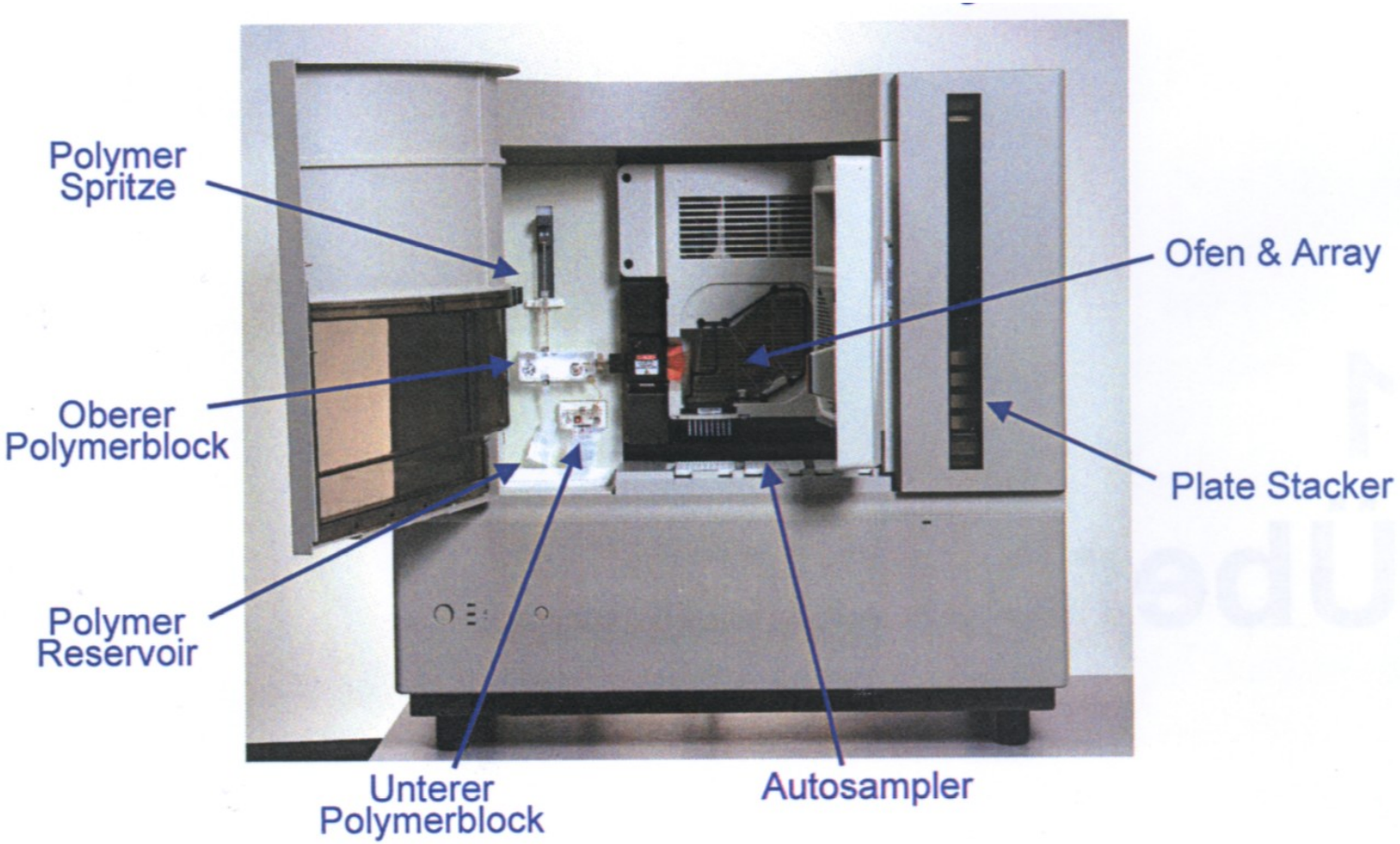


Kapillar-Sequenzierer

ABI 3730 2002-…

ABI 3730

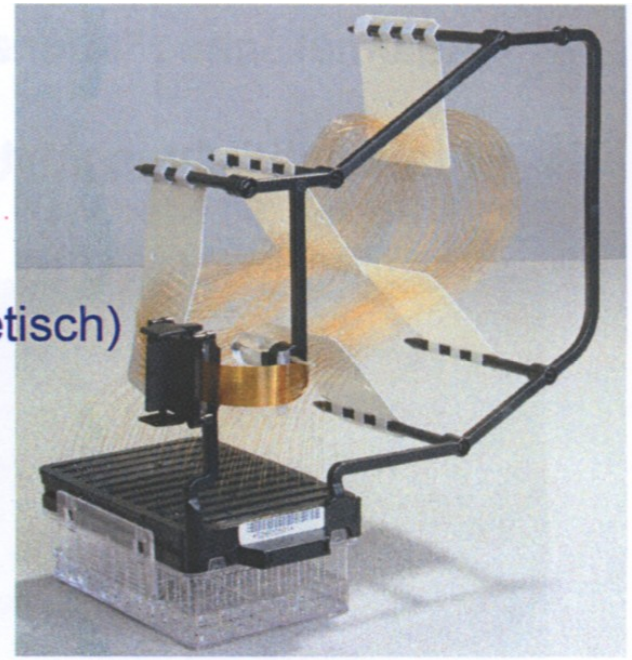
Aufbau



ABI 3730

Kapillar-Array

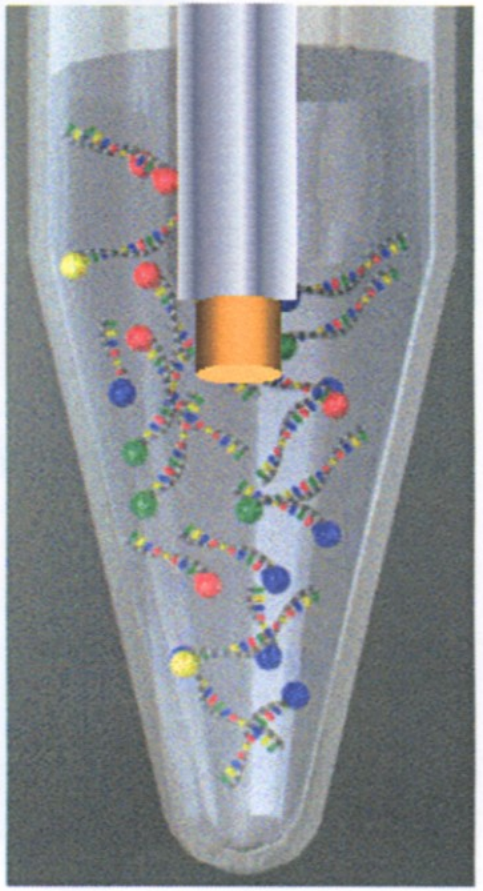
- Unbeschichtete Kapillaren
- 50µm Innendurchmesser
- Präzises Kapillar-Alignment
- Simultane Injektion (elektrokinetisch)
- 36cm und 50cm Trennstrecke
- 48 oder 96 Kapillaren
- 300 Runs/Array
- POP-7™ Polymer für alle Applikationen



ABI 3730

Elektrokinetische Injektion

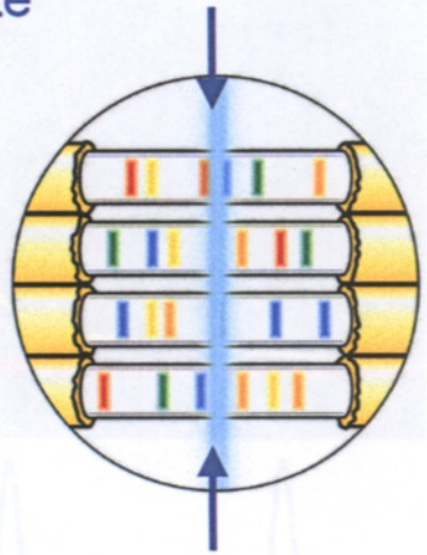
- Kapillare und Elektrode tauchen in die Probe ein
- Spannung wird angelegt
- Negativ geladene (DNA -) Moleküle wandern in die Kapillare
- Elektrophorese zur Anode



ABI 3730

Detektion

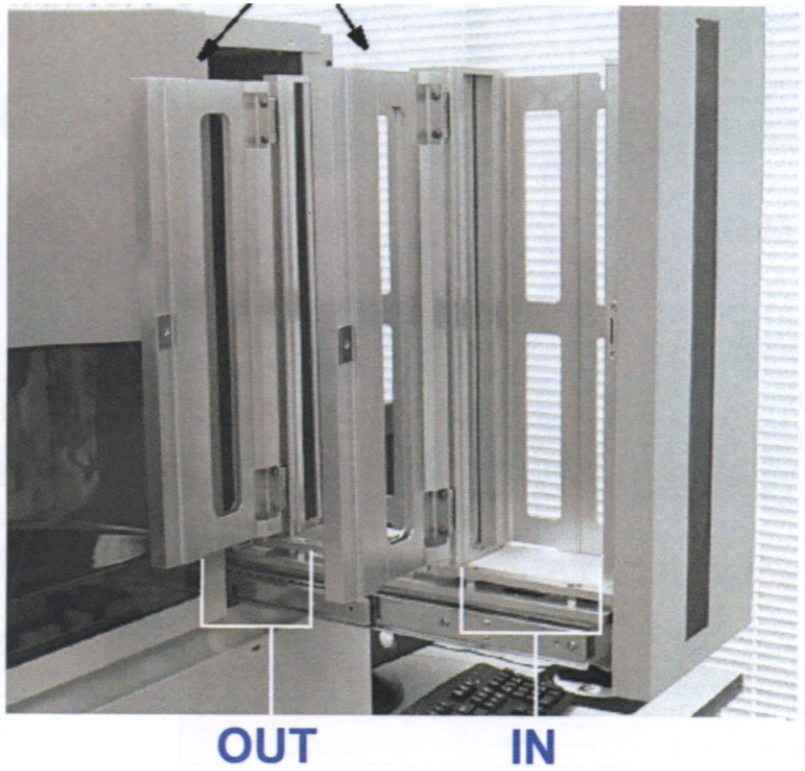
- Laser Beam Splitter
  - gleichmäßige Anregung über die gesamte Detektionszelle
  - Eliminierung von Signalvariationen
  - Maximale Sensitivität



- Mit Flüssigkeit gefüllte Detektionszelle
  - Vermeidung optischer Verzerrungen
  - Gleiche optische Dichte wie POP-7™ Polymer
  - Kapillar-'Position' auf CCD-Kamera ermittelt durch Chemie-freie Spatial-Kalibrierung

ABI 3730

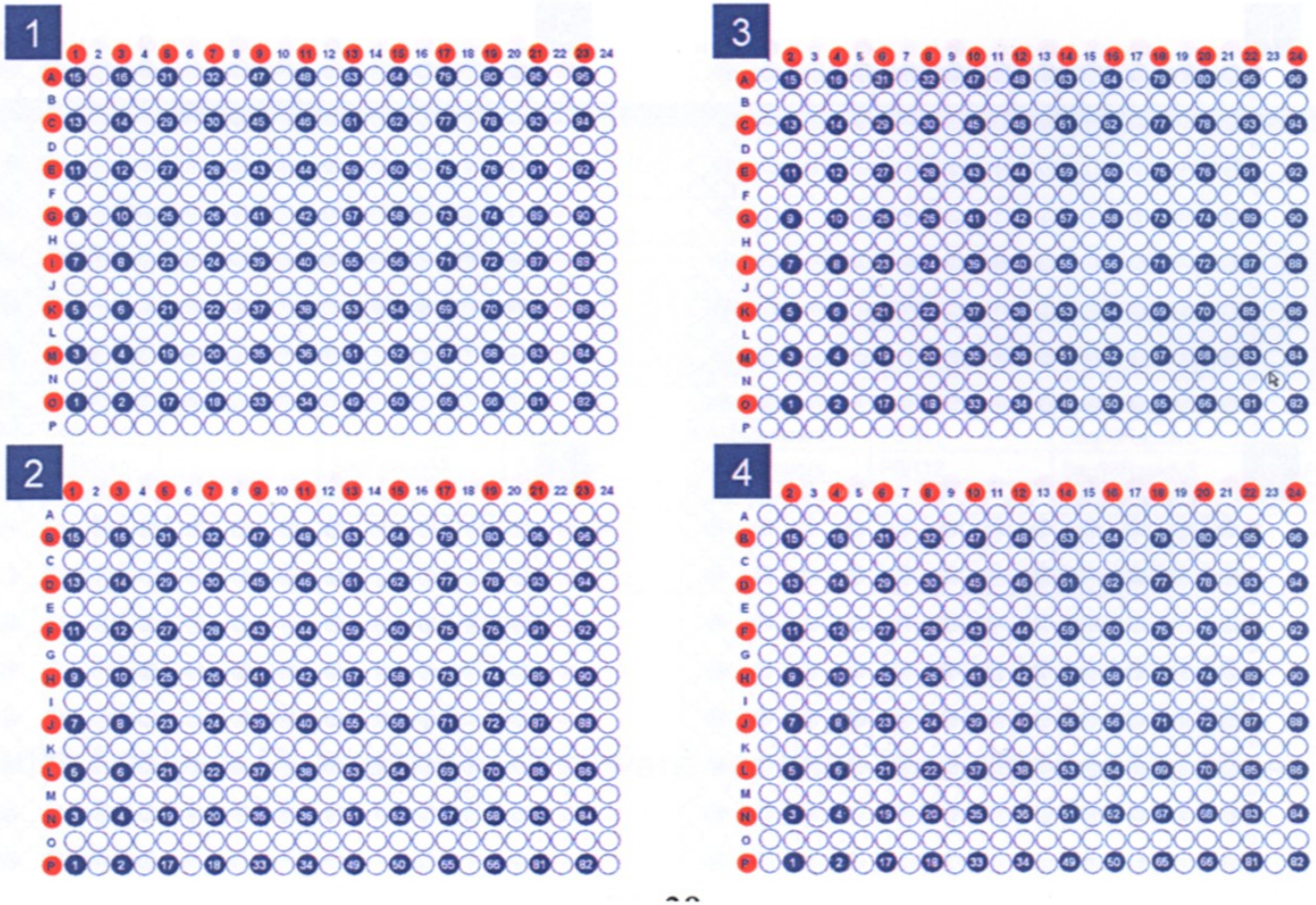
Beschickung



- In-put und Out-put Stacker
- bis zu 16 Platten
  - 96 und/oder 384 well
- Automatische Plattenzufuhr
  - Manual Mode
  - Auto Mode
- Interner Barcode Reader

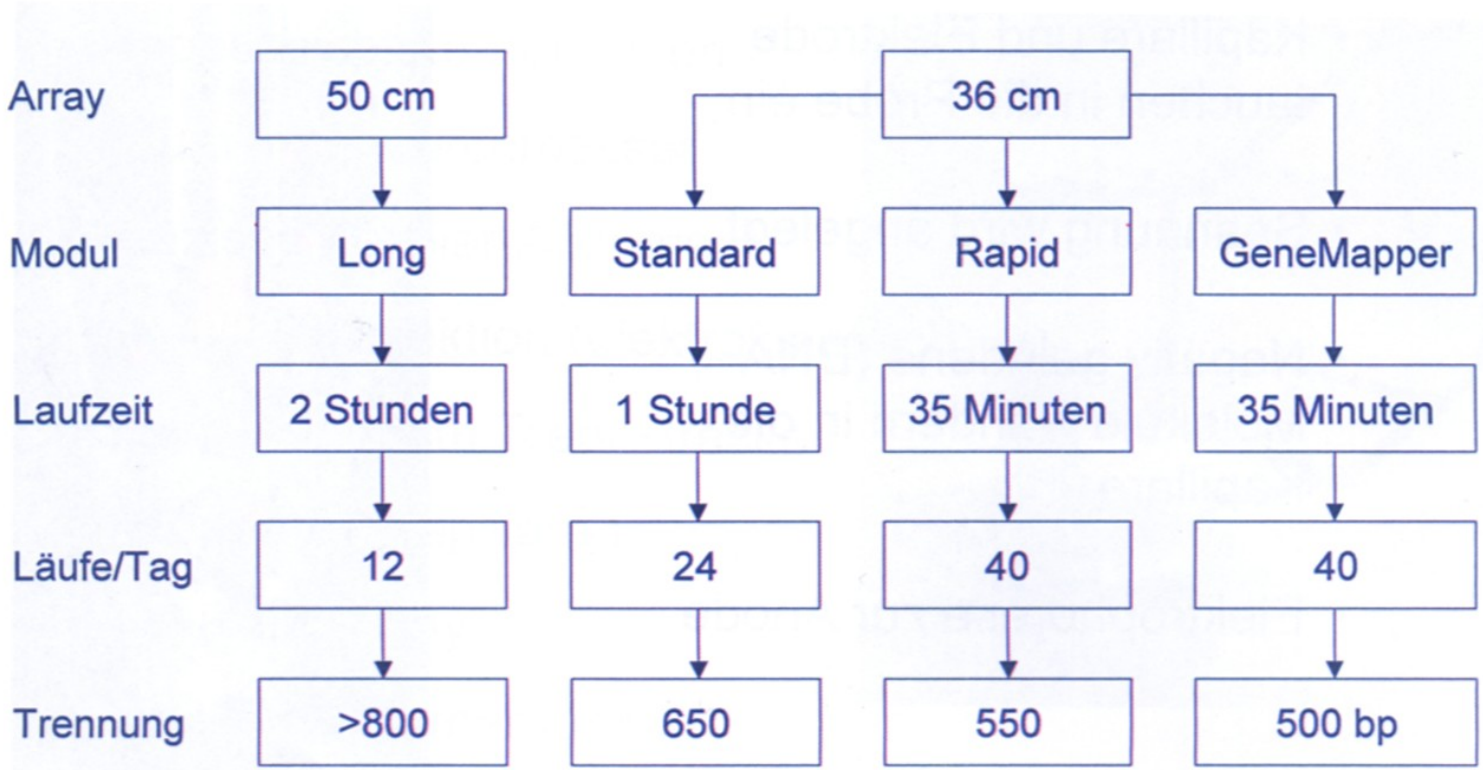
ABI 3730

96 Kapillaren – 384 Proben



ABI 3730

Probendurchsatz



1.0 Mb/d **1.5** Mb/d **2.1** Mb/d

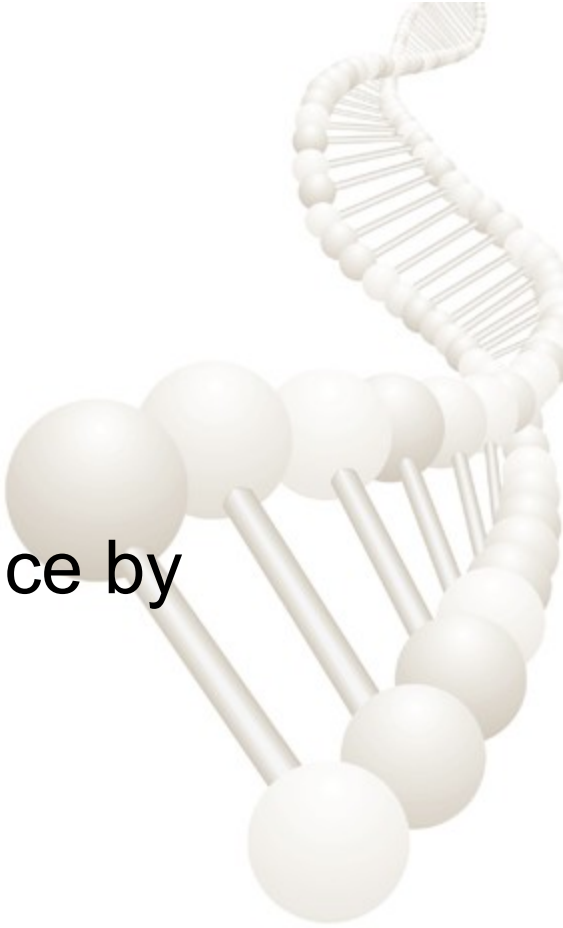
ABI 3730

Demo

Pyro-Sequenzierung

PSQ™ 96

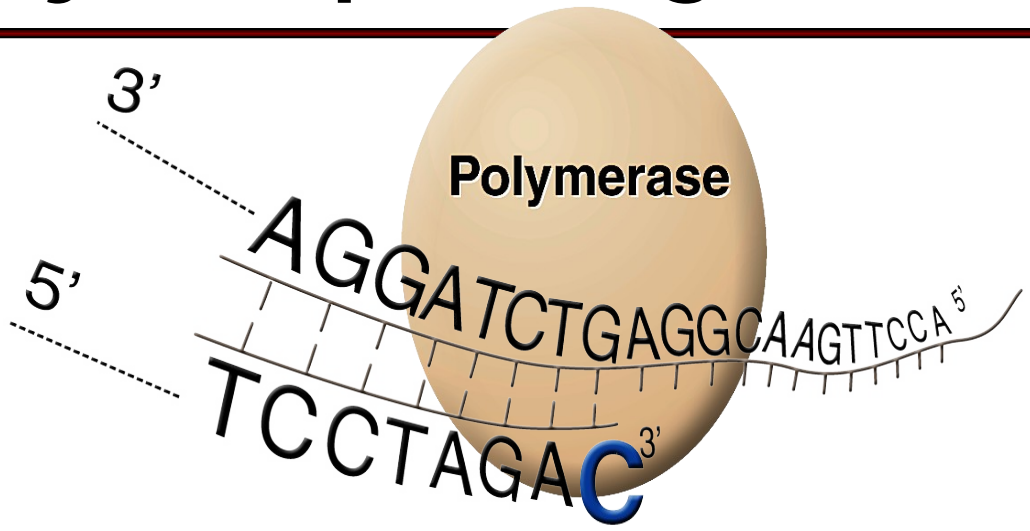
Pyrosequencing

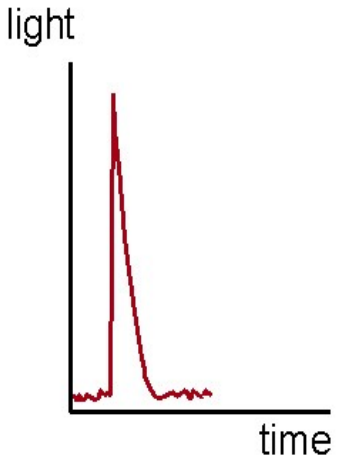


- Real time sequencing (Sequence by synthesis)
- Four-enzyme mixture

PYROSEQUENCING

Pyrosequencing





Sulfurylase

Apyrase

Luciferase

Pyrosequencing

Step 1

- Sequencing primer hybridized to ssDNA , PCR amplified template
- Primer and template incubated with

DNA polymerase
ATP sulphurylase
Luciferase
Apyrase } enzymes

Adenosine 5' phosphosulphate
Luciferin } substrates

PYROSEQUENCING

Pyrosequencing

Step 2

- Addition of deoxynucleotide
- Incorporation by *DNA polymerase*
  - ➡ Release of pyrophosphate (PPi)
  - ➡ Equimolar quantity to incorporated nucleotide

PYROSEQUENCING

Pyrosequencing

Step 3

- PPi + APS $\xrightarrow{\textit{ATP sulphurylase}}$ ATP

- luciferin + ATP + O_2 $\xrightarrow{\textit{Luciferase}}$

oxyluciferin + AMP + PPi + CO_2 + Light

- Detection of light with CCD-camera

→ Peak in pyrogram

PYROSEQUENCING

Pyrosequencing

Step 4

- Degradation of unincorporated nucleotides and ATP by *apyrase*

  → Light is switched off

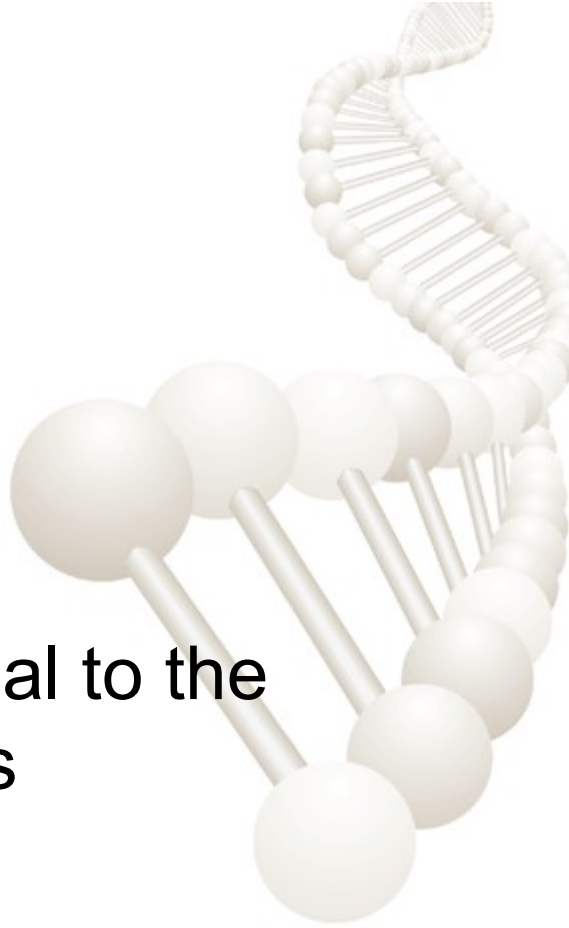
  → Solution conditioned for a new nucleotide addition

PYROSEQUENCING

Pyrosequencing

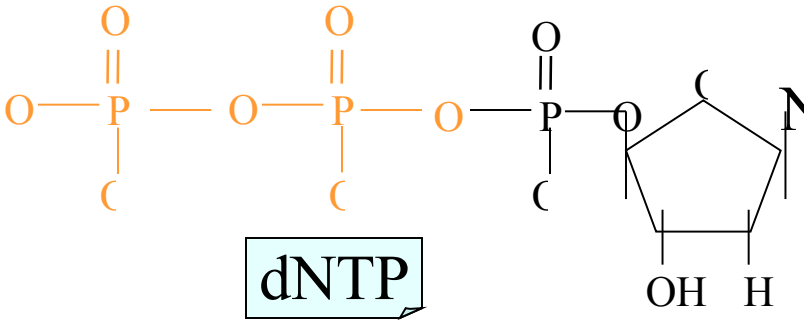
- Pyrogram

  the height of the peak is proportional to the amount of incorporated nucleotides

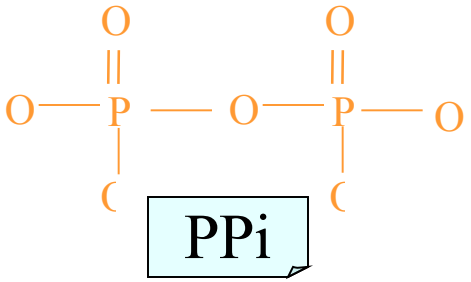


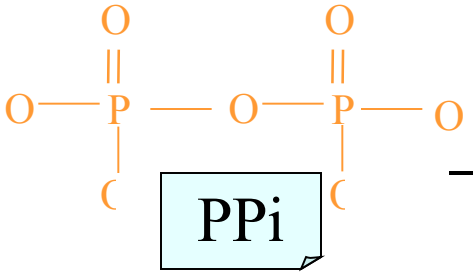
PYROSEQUENCING

Pyrosequencing

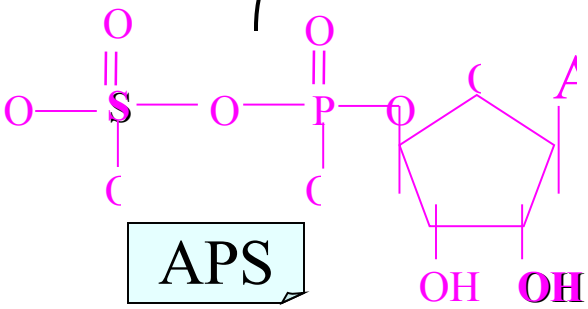


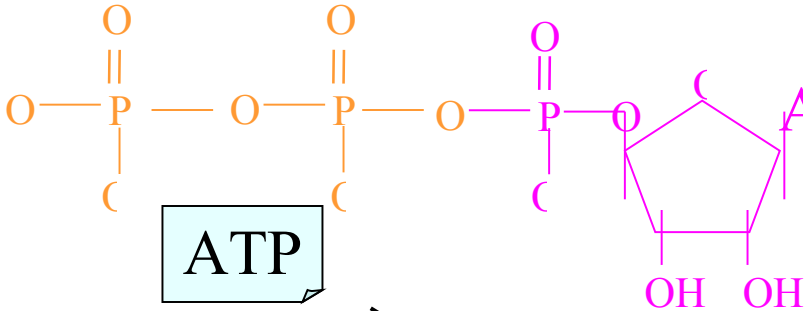
Polymerase





ATP-sulfurylase





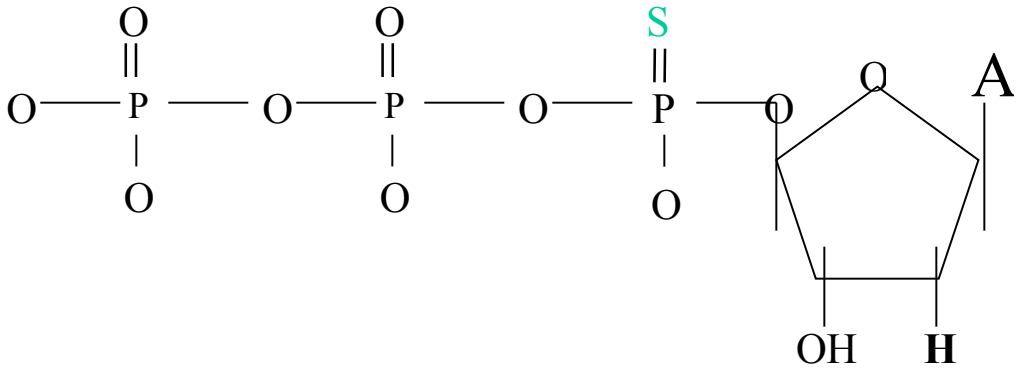
Luciferase

light

Pyrosequencing

- dATPαS is used in the pyrosequencing reaction

➡ Luciferase <u>does not</u> use dATPαS as substrate



dATPαS

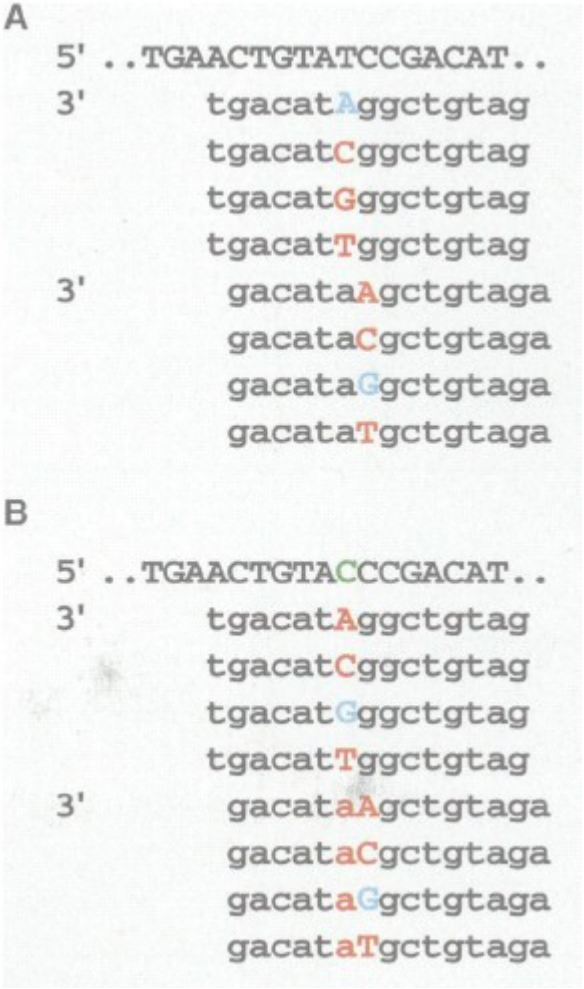
PYROSEQUENCING

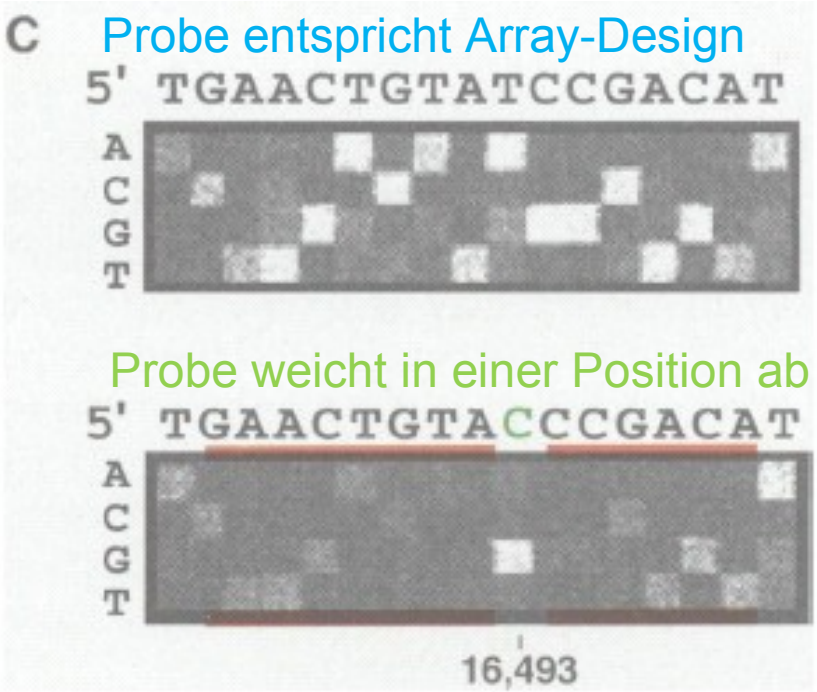
Pyro-Sequenzierung

Demo

Sequenzierung durch Hybridisierung

DNA-Chip



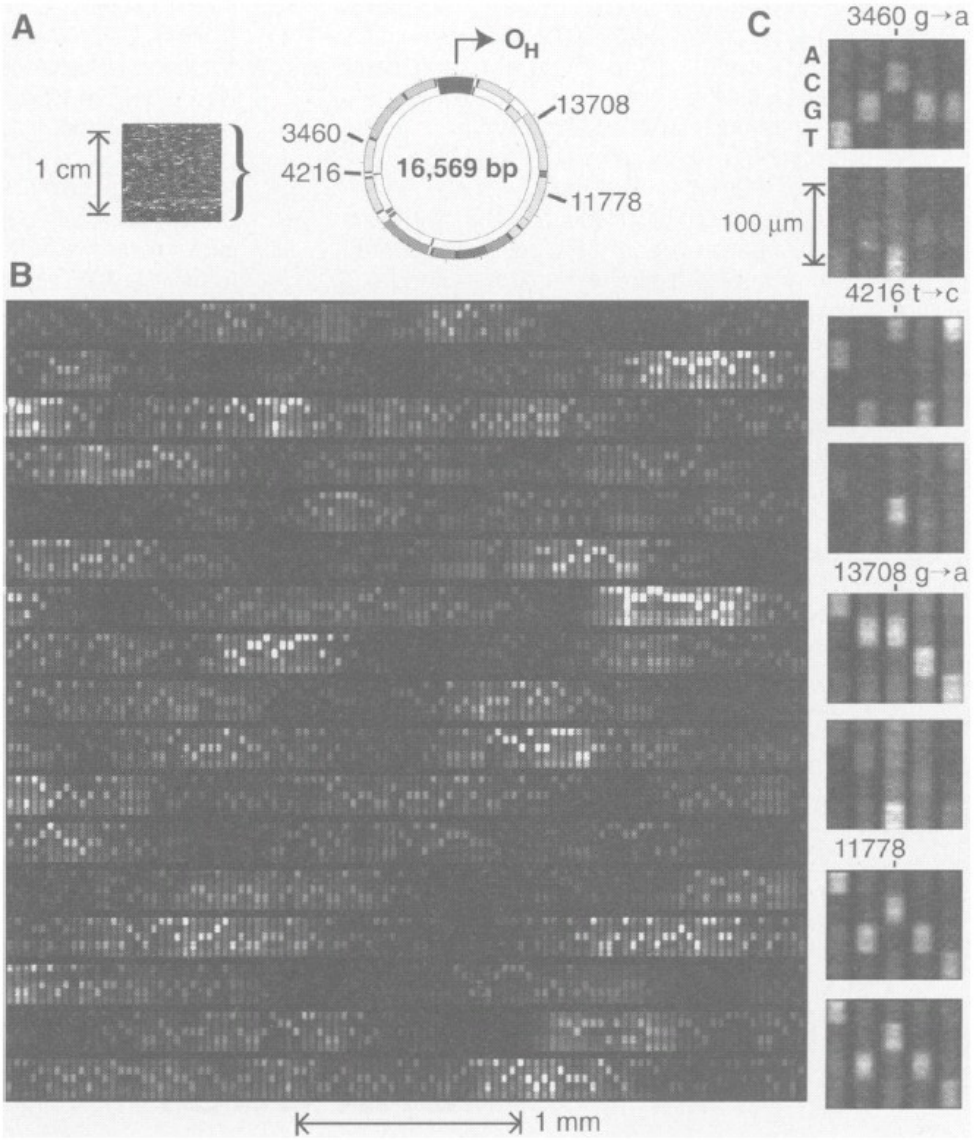


match missmatch variation

M. Chee et al. Science 274:610 (1996)

Sequenzierung durch Hybridisierung

Chip für das menschliche Mitochondrium



M. Chee et al. Science 274:610 (1996)

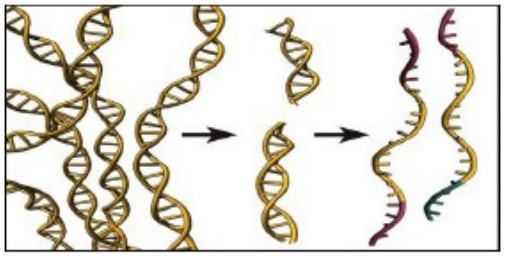
454

Genome Sequencer GS20/FLX

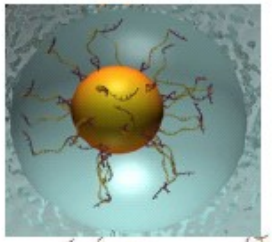
454

Workflow

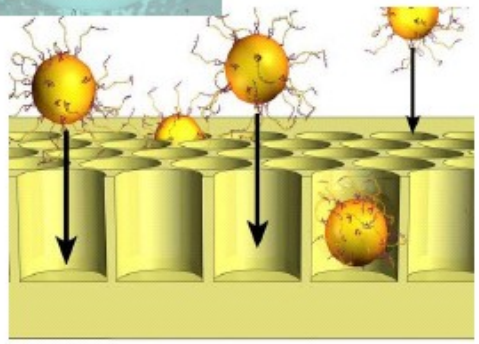
- DNA Isolation
- DNA Fragmentation
- DNA Amplification
- DNA Sequencing
- Bioinformatics
  - Contigs are constructed



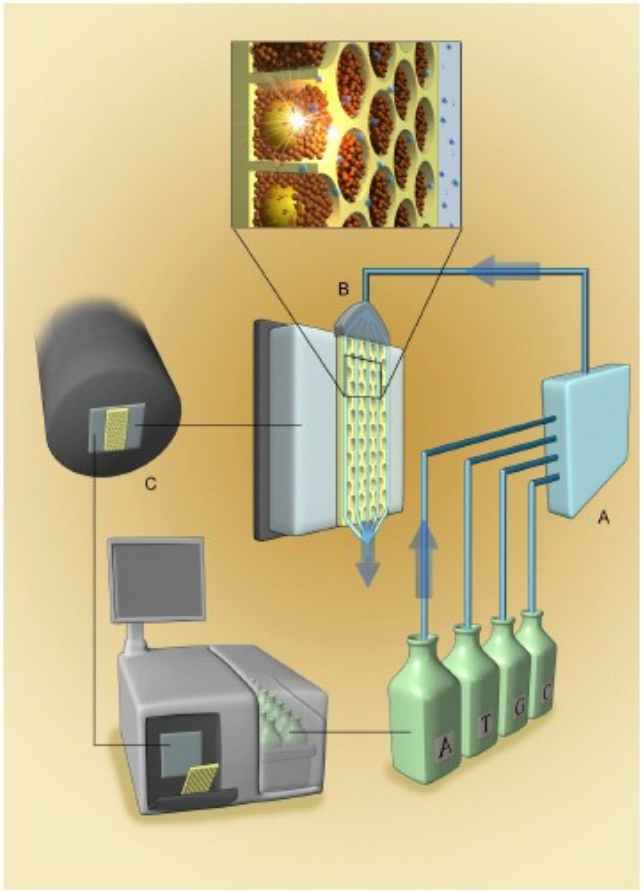
Adapter Ligated ssDNA Library



Clonal Amplification

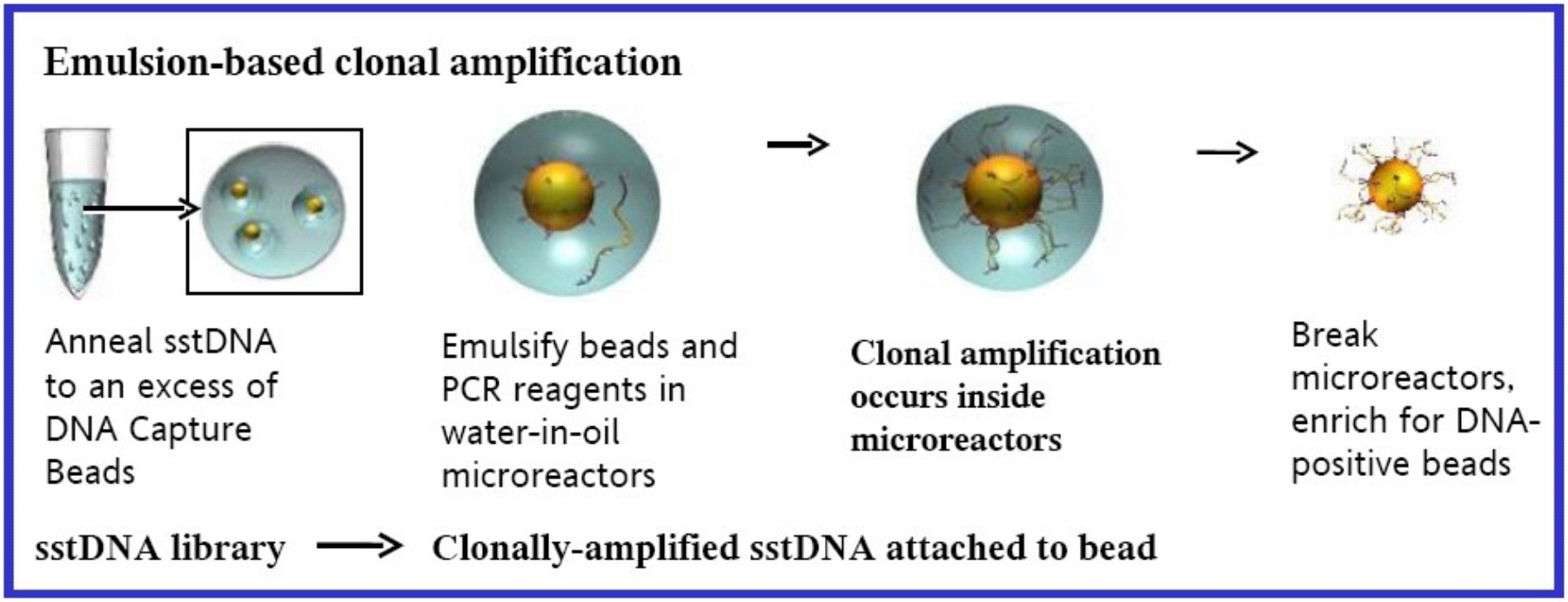


Beads enzymes in PicoTiter Plate™



454

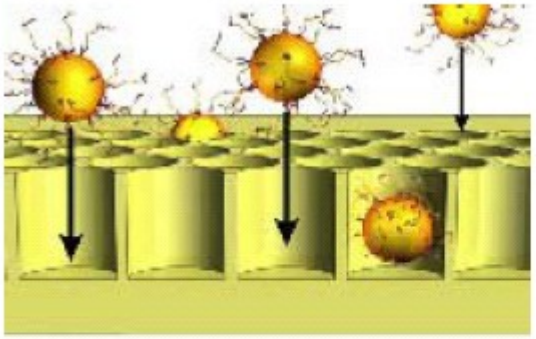
emPCR

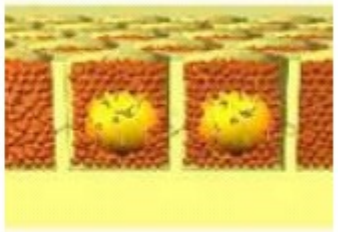

Emulsion-based clonal amplification
Anneal sstDNA to an excess of DNA Capture Beads
Emulsify beads and PCR reagents in water-in-oil microreactors
Clonal amplification occurs inside microreactors
Break microreactors, enrich for DNA-positive beads
sstDNA library
Clonally-amplified sstDNA attached to bead

454

Sample loading

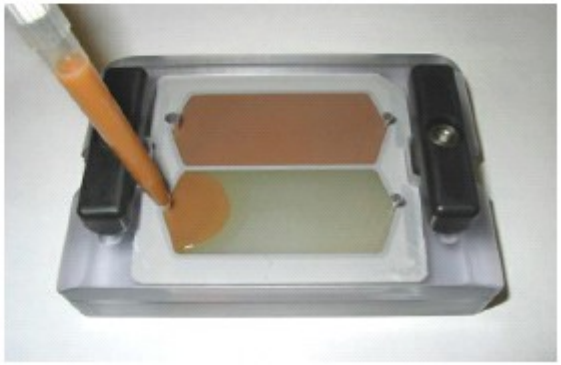
Depositing DNA beads into the PicoTiterPlate device

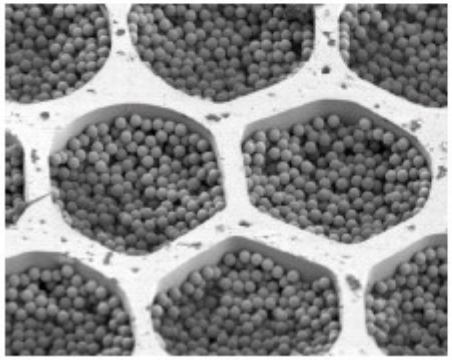




- Well diameter: average of 44 μm
- 200,000 reads obtained in parallel
- A single clonally amplified sstDNA bead is deposited per well

Amplified sstDNA library beads → **Quality filtered bases**





454

Parameters

GS 20

- 25 Mb
- 100 nt read length
- 4h

GS FLX

	Titanium Chemistry 10/2009
• 100 Mb	500 Mb
• 250 nt read length	400 nt
• 7h	10 h

- 100/400/2.000x currant Sanger technology

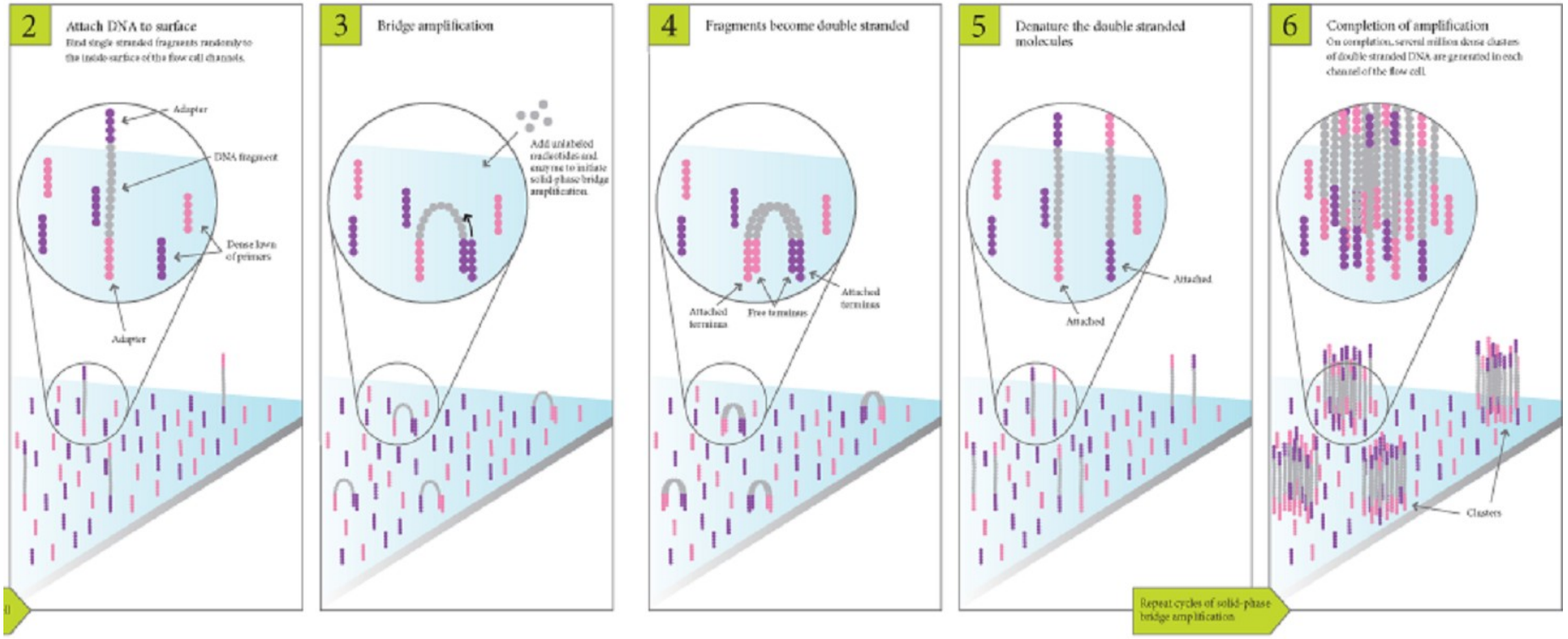
Solexa

Advanced genetic analysis

one billion bases at a time

Solexa

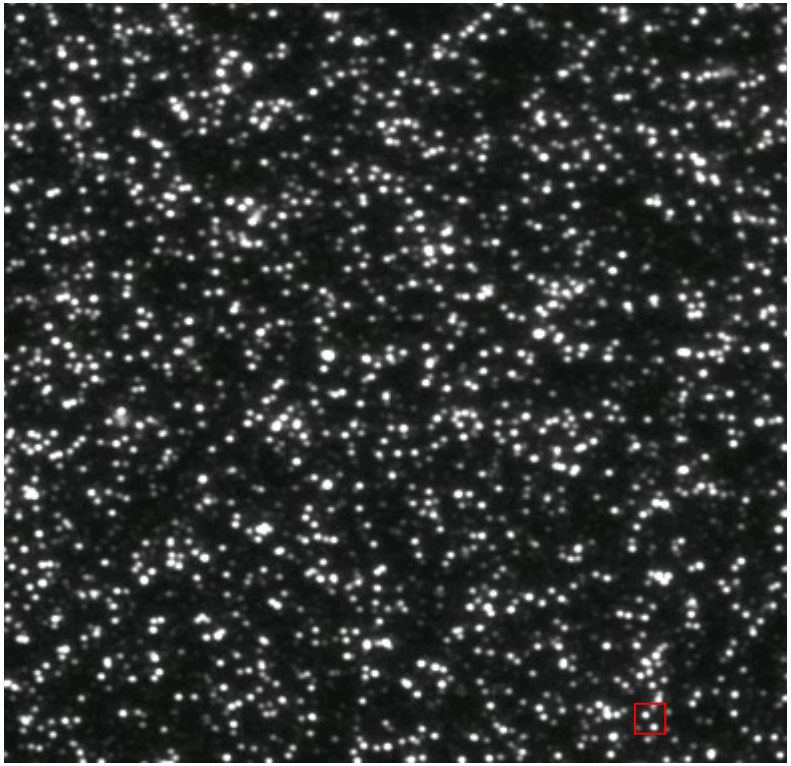
Solid-phase clonal single molecule PCR



"Bridge amplification"

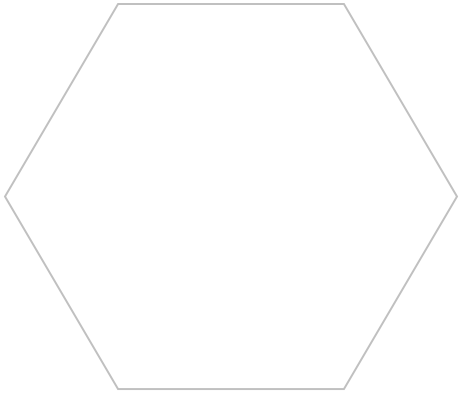
Solexa

Solid-phase clonal single molecule PCR



100µm

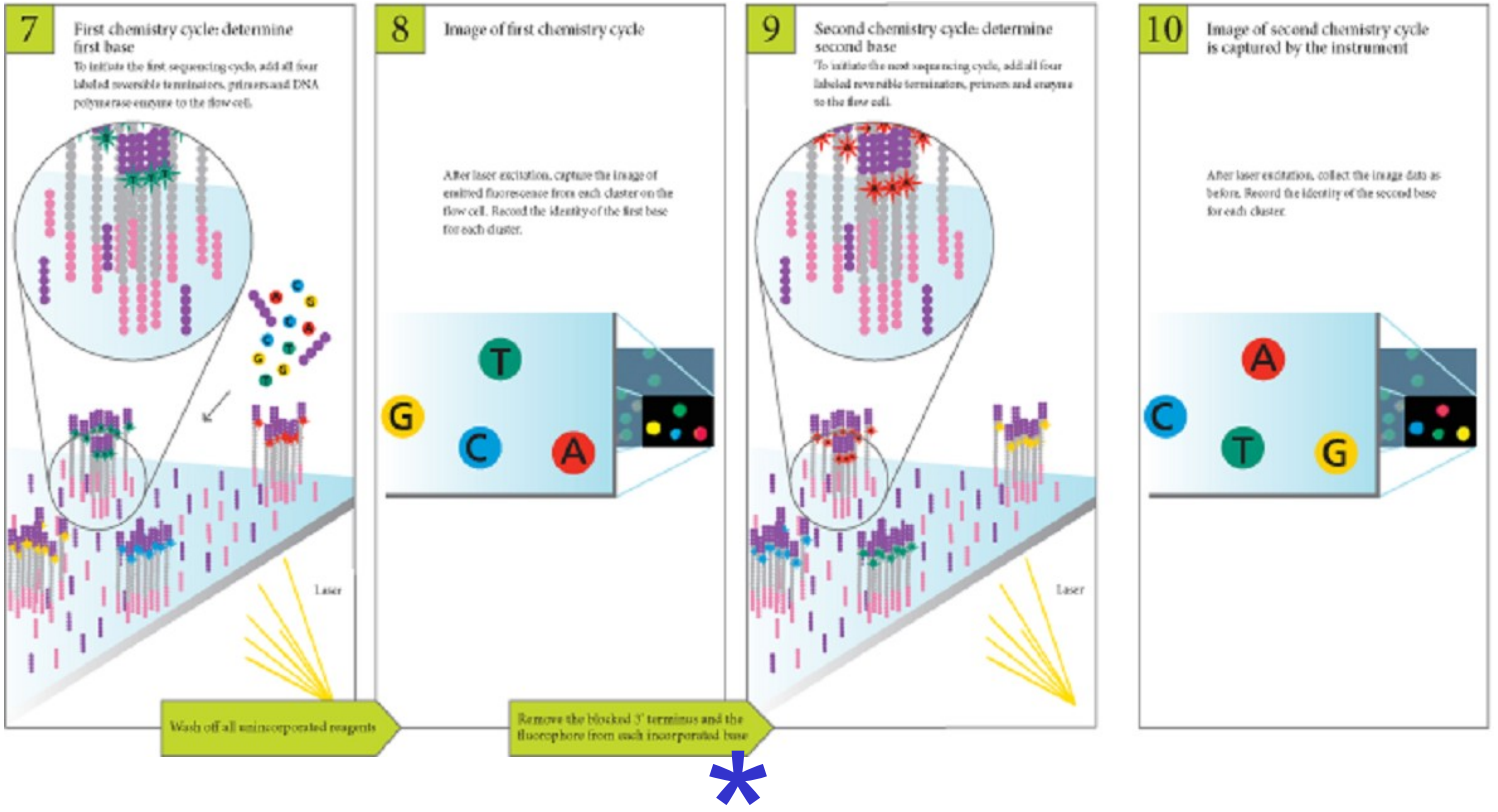
colony of ≈1000 single-stranded DNA templates



Single well of 454 Life Sciences PicoTiterPlate™ (to same scale)

Solexa

Sequencing by synthesis (SBS)

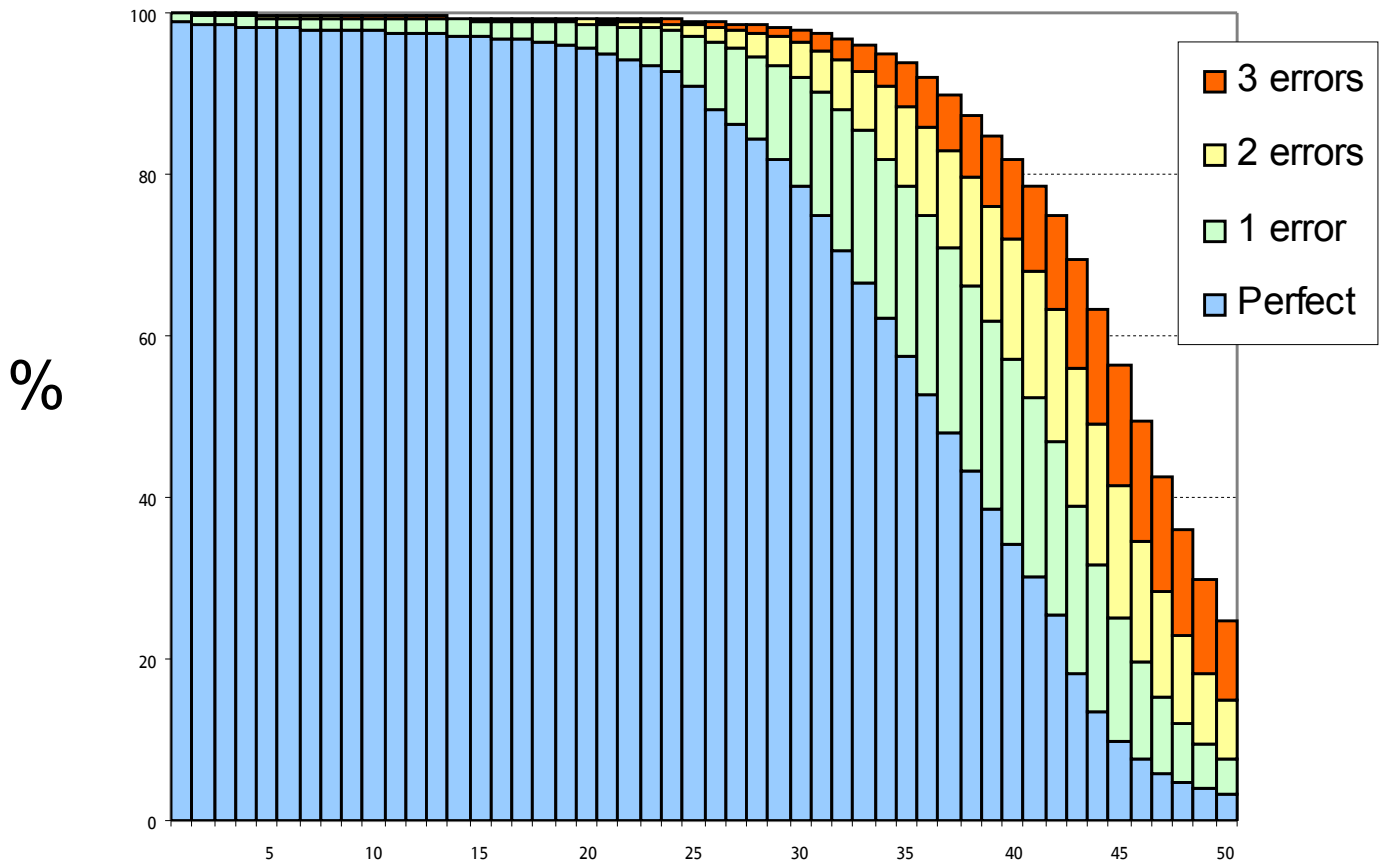


*

removal of fluorescent labels & 3'OH blocking

Solexa

Read length

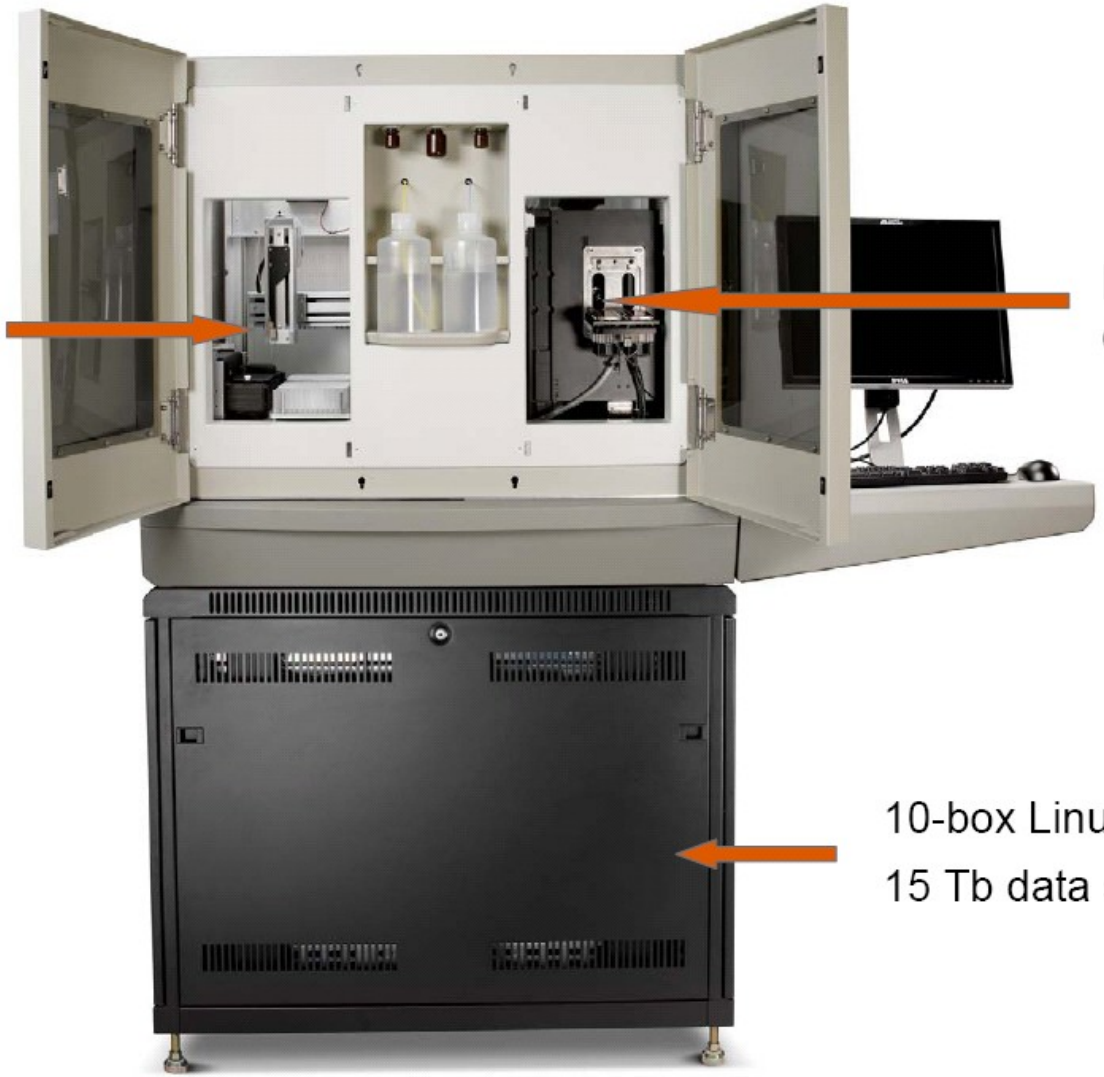


ABI Agencourt

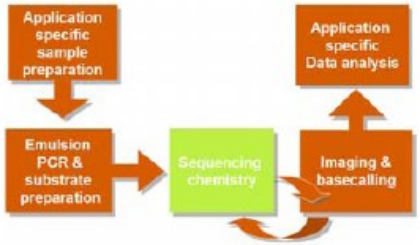
SOLID

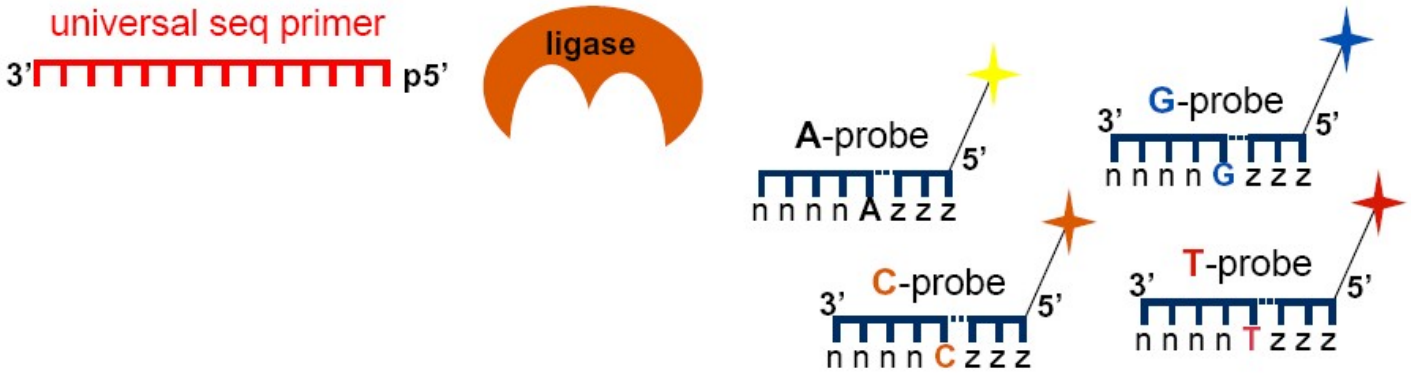
SOLID

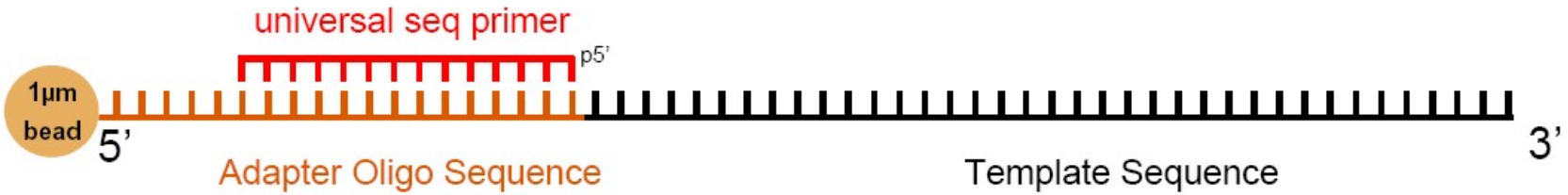
Hardware



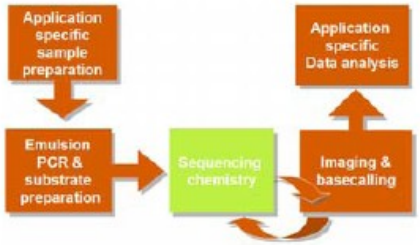
SOLiD™ system 4-color ligation Ligation reaction

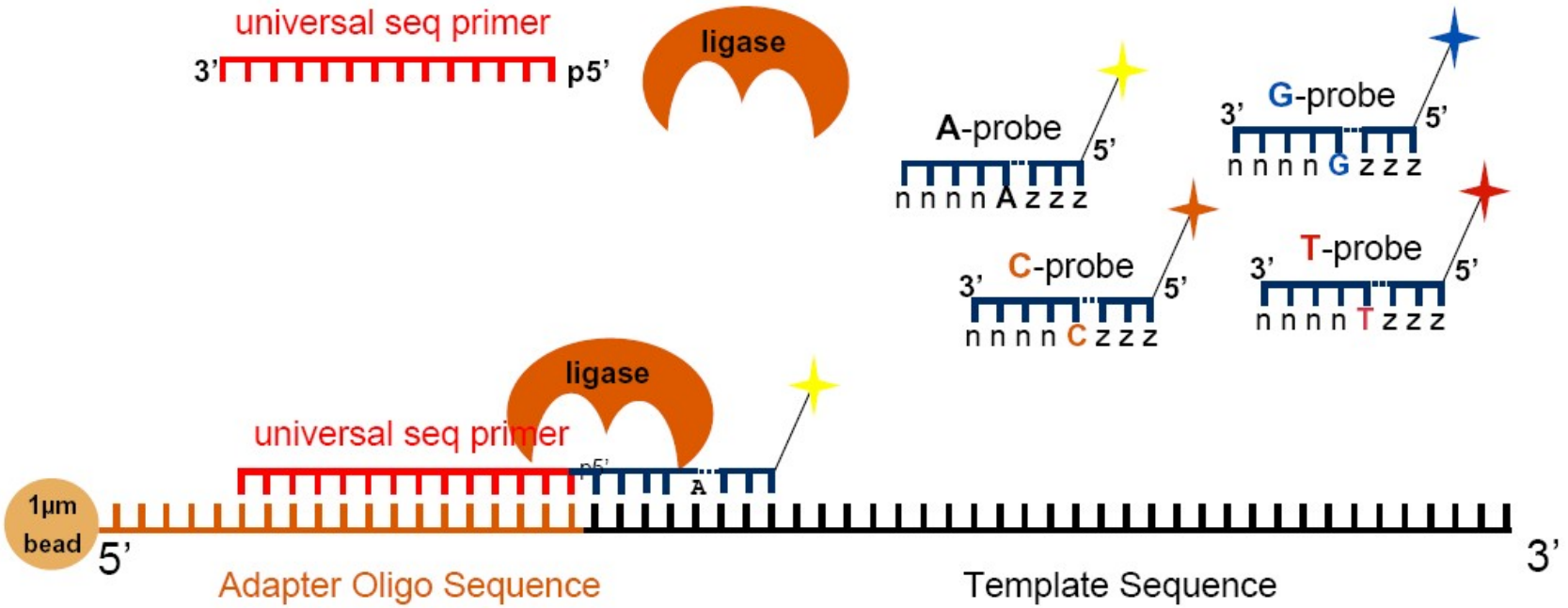




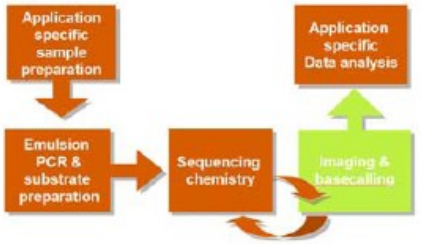


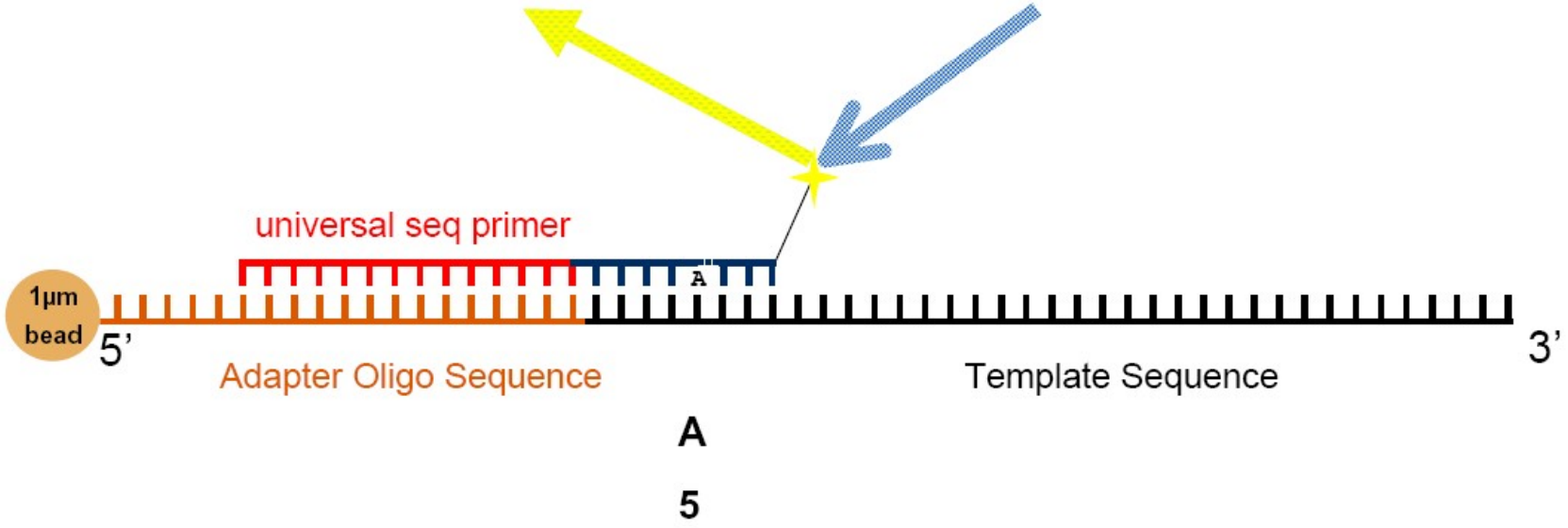
SOLiD™ system 4-color ligation
Ligation reaction



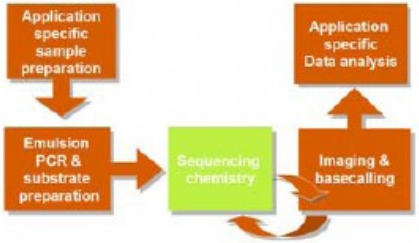


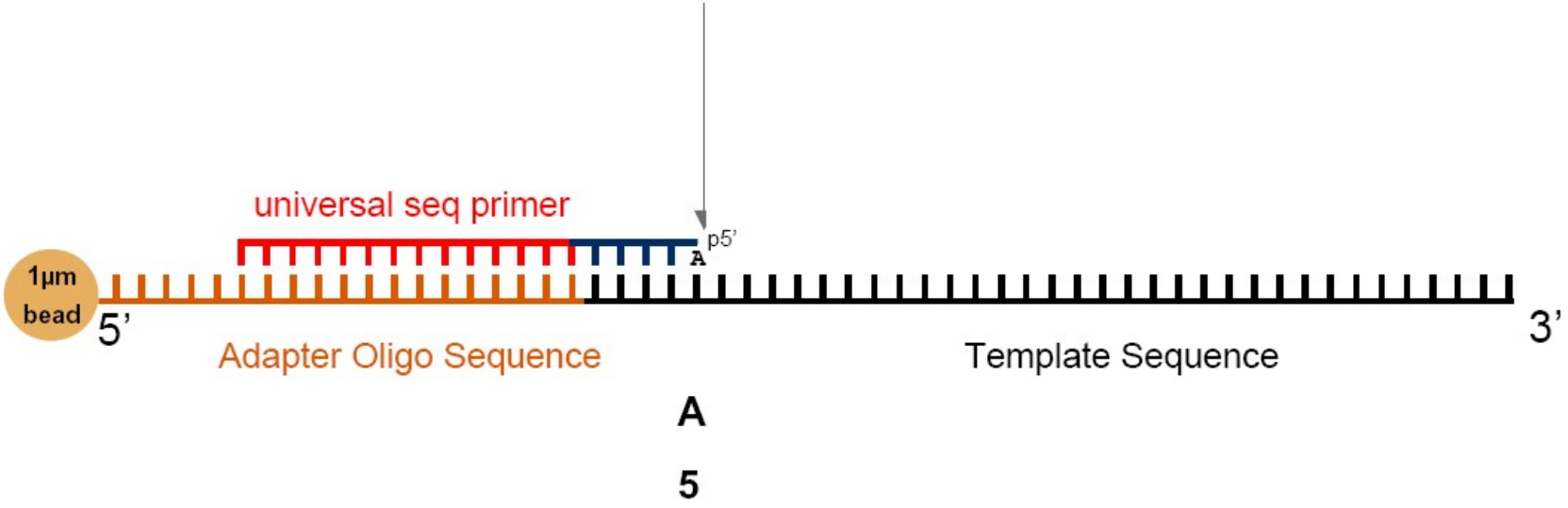
SOLiD™ system 4-color ligation Visualization



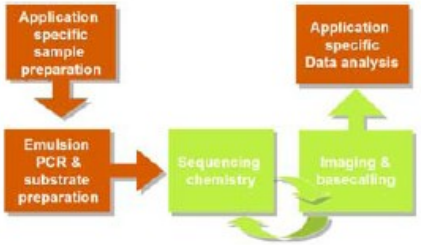


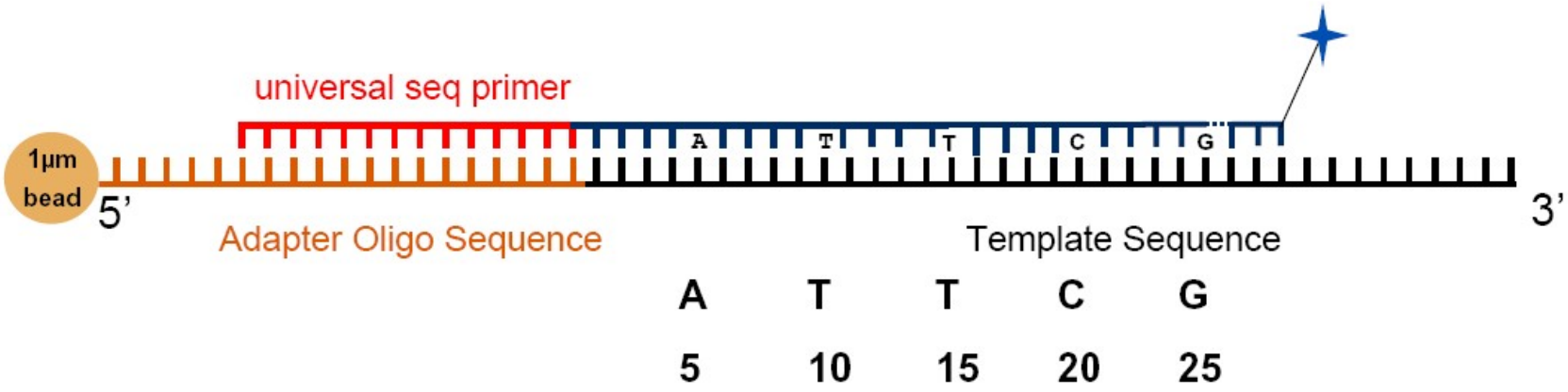
SOLiD™ system 4-color ligation Cleavage



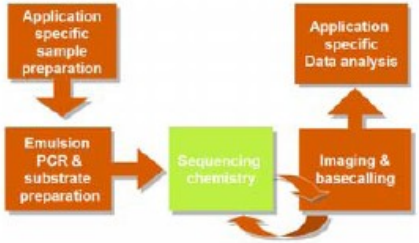


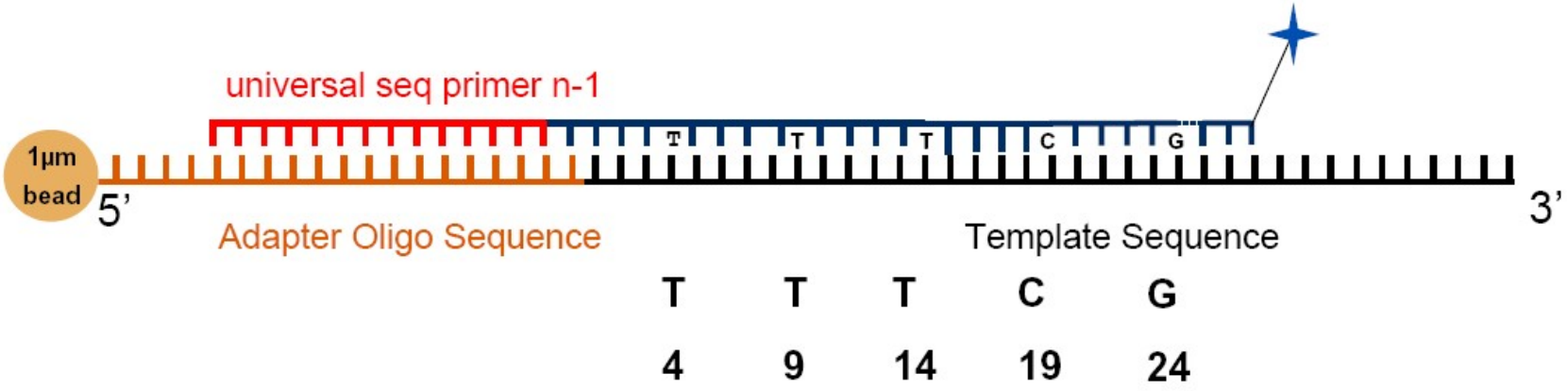
SOLiD™ system 4-color ligation interrogates every 5th base



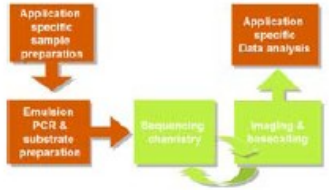


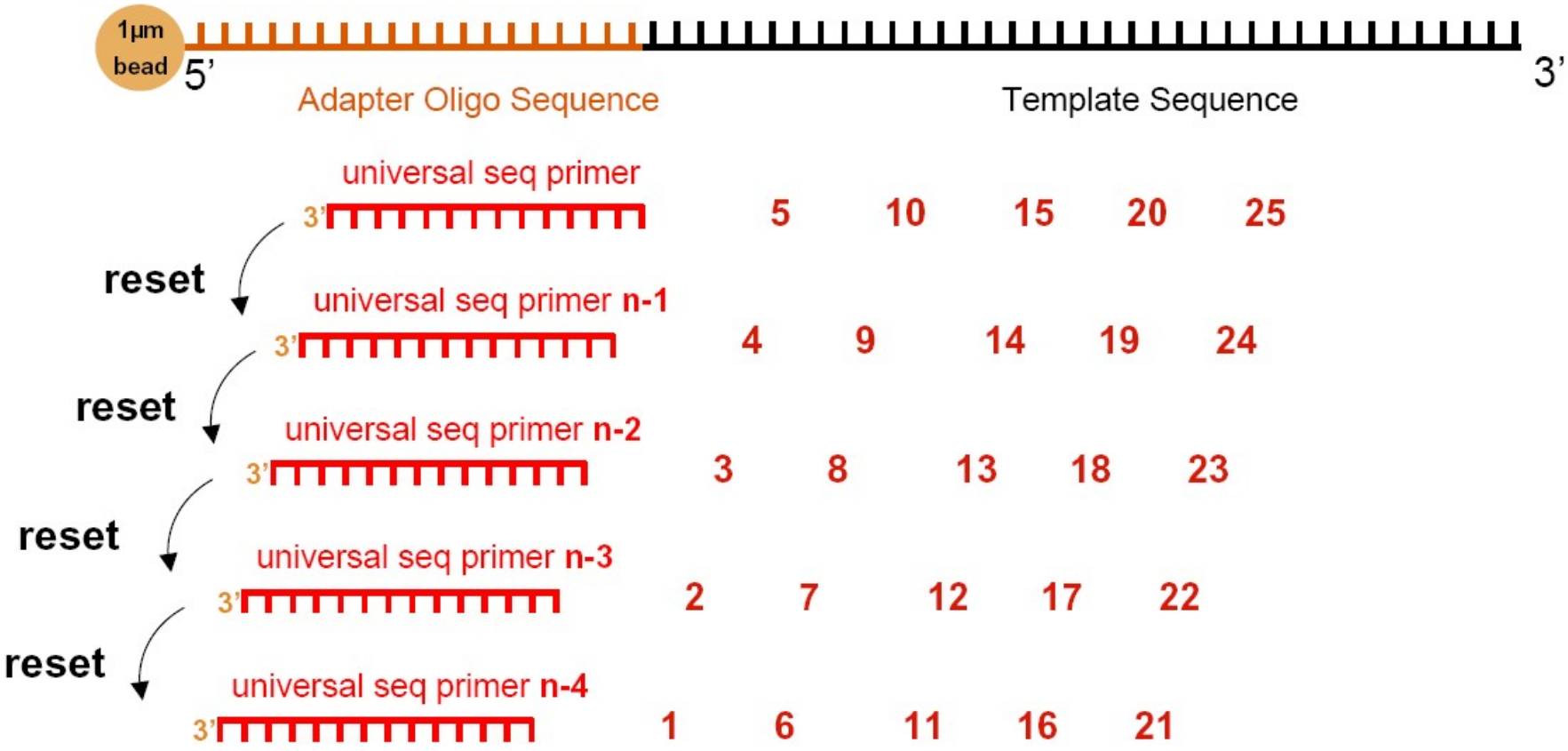
SOLiD™ system 4-color ligation (2nd Round)





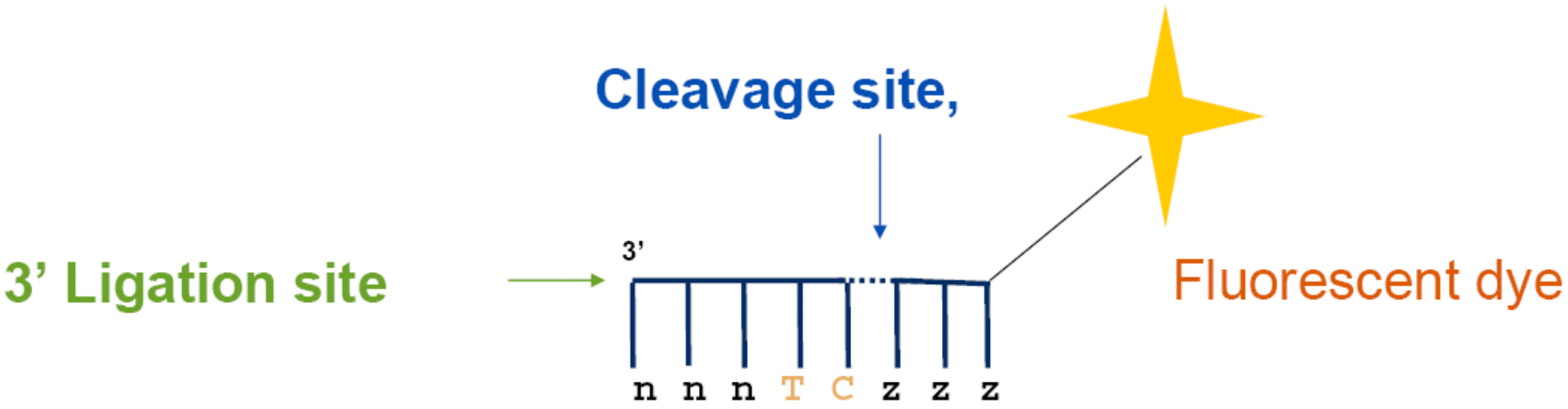
Sequential rounds of sequencing
Multiple cycles per round





SOLID

2-base encoding



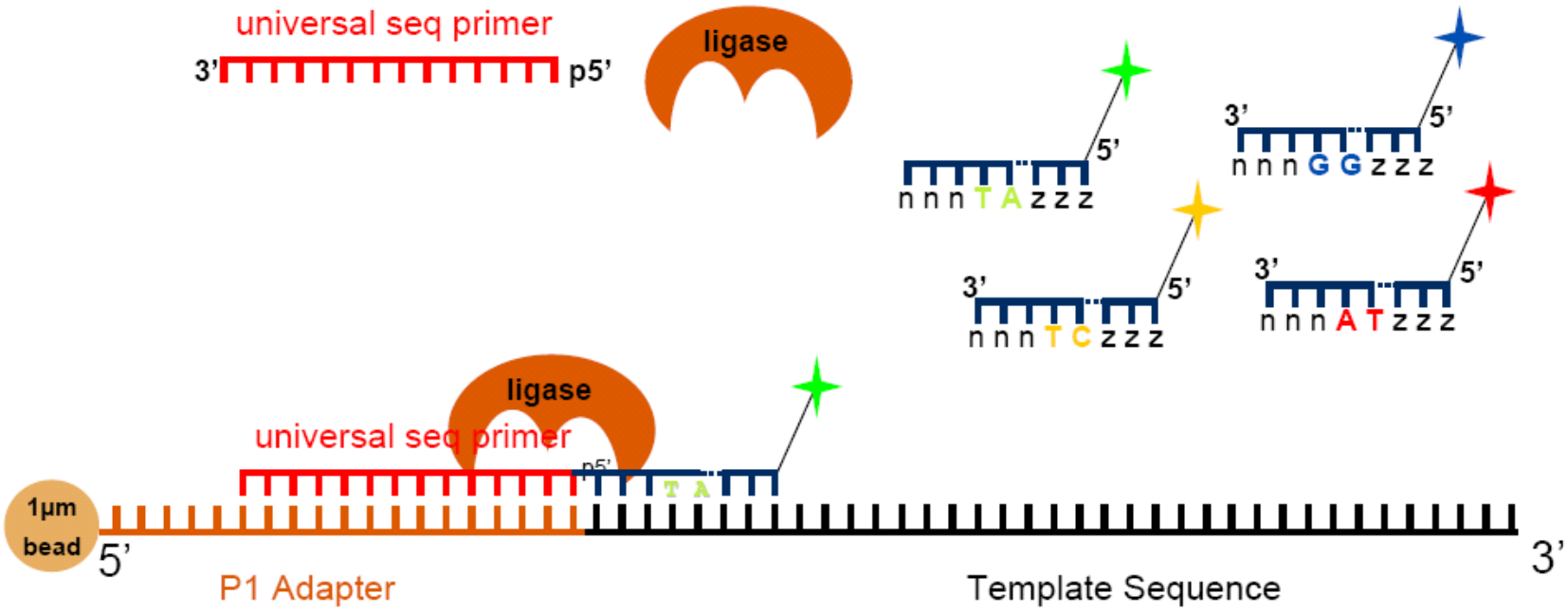
1,024 Octamer Probes (4^5)

4 Dyes, (4 dinucleotides, 256 probes) per dye

N= degenerate bases Z= Universal bases

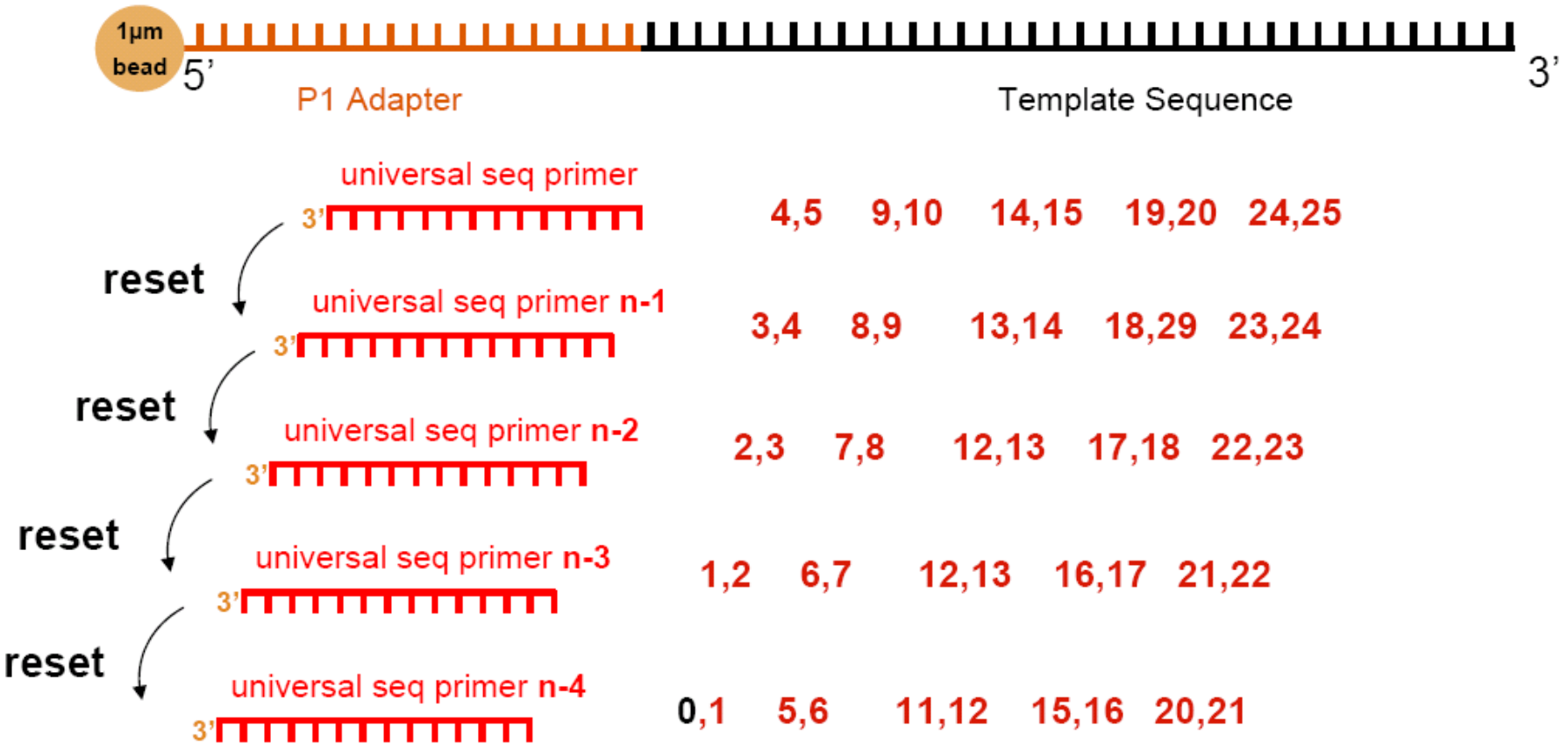
SOLID

2-base encoding



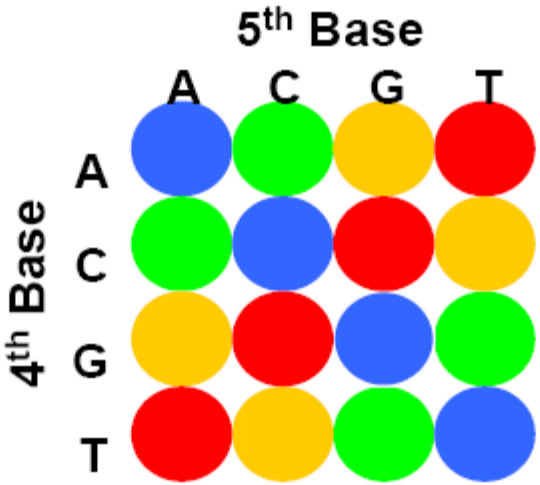
SOLID

2-base encoding



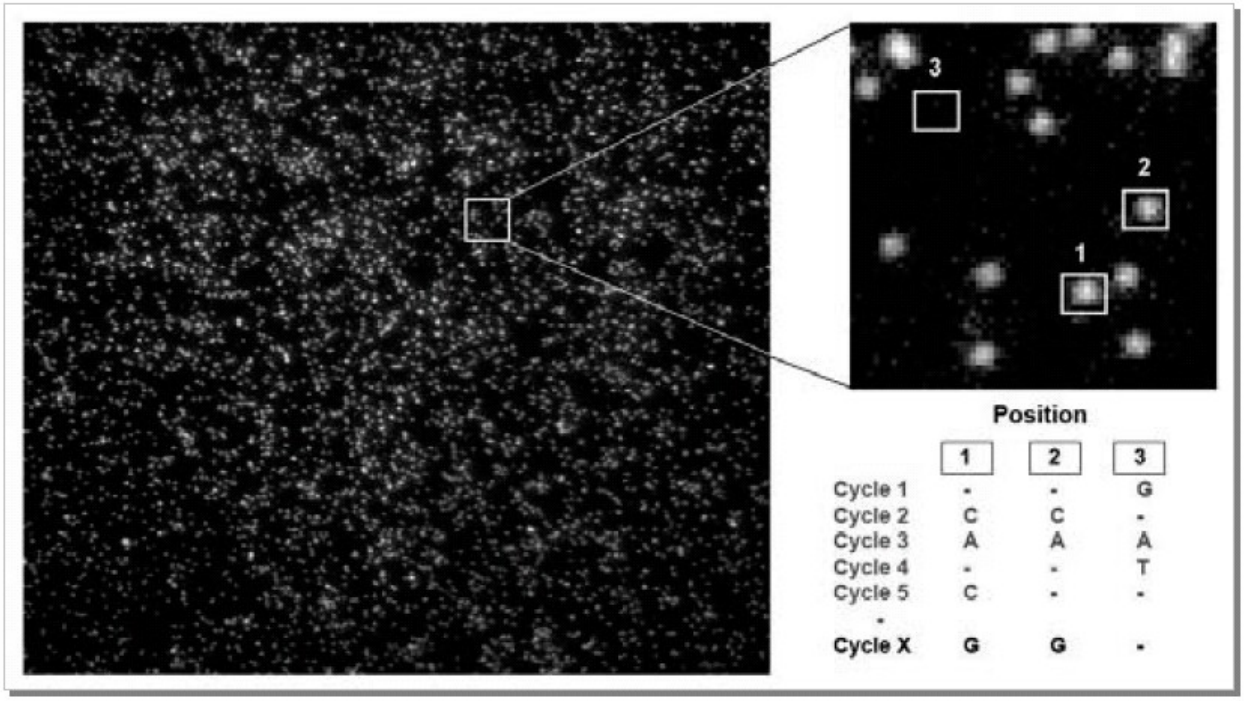
SOLID

2-base encoding



Futuristische Technologie

Einzel-Molekül-Sequenzierung (tSMS)

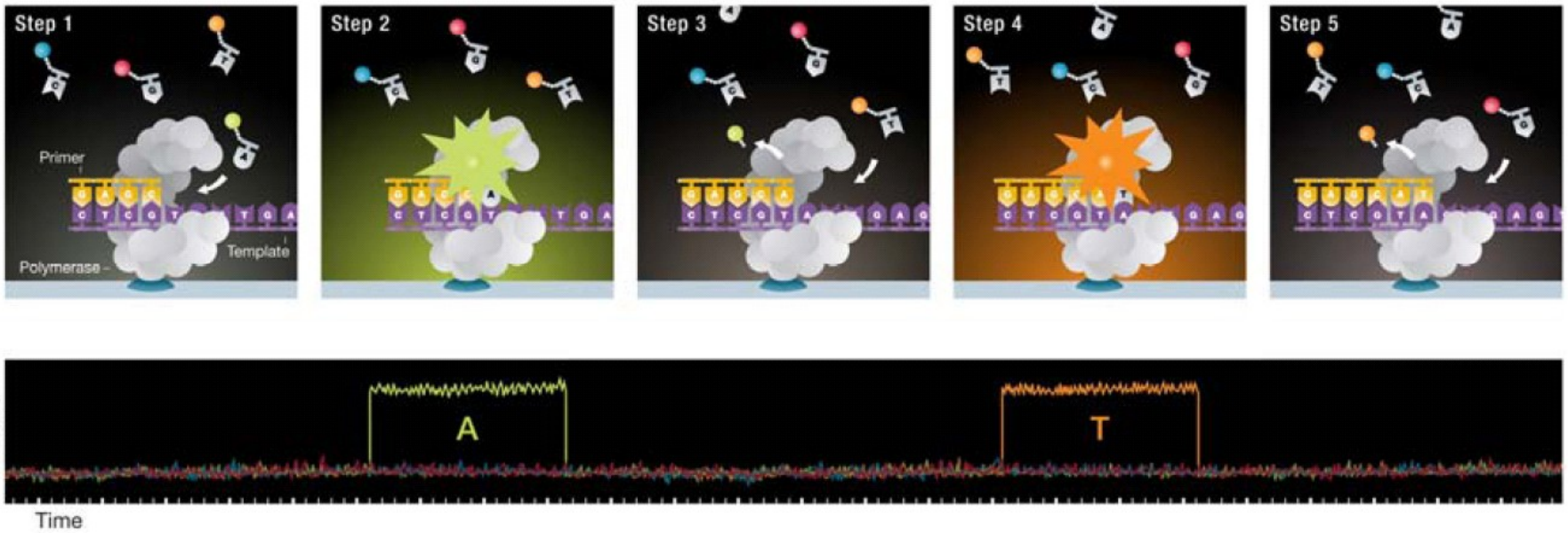


Helicos BioSciences Corporation

www.helicosbio.com

Futuristische Technologie

Immobilisierte Polymerase



lange Sequenzen

PACIFIC BIOSCIENCES

www.pacificbiosciences.com

Futuristische Technologie

Fluoreszens-Resonanz-Energie-Transfer (FRET)

F-Donor : Polymerase

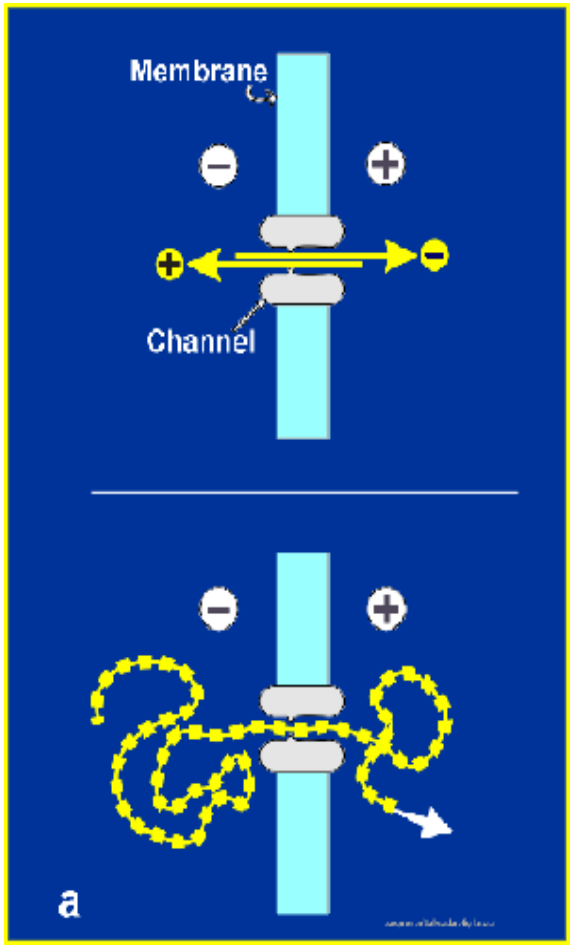
F-Akzeptor: γ-Phosphat des dNTP

1Mb/s

>1 humanes Genom/Tag

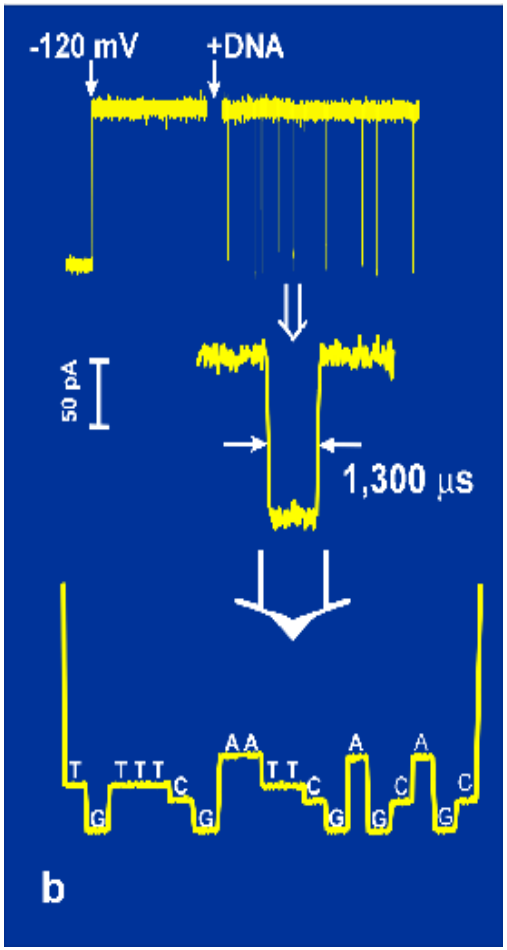
Futuristische Technologie

Nanopore



Messwert

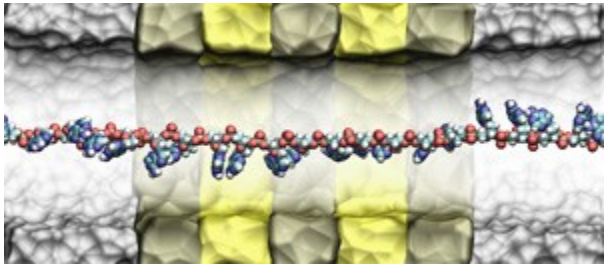
Strom von K^+-Ionen, die der DNA beim Porendurchtritt entgegen fließen

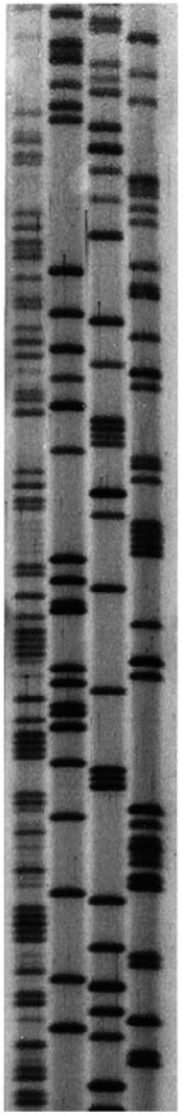


www.mcb.harvard.edu/branton/nanopore.html

IBM

Waver pore





genome.fli-leibniz.de
Teaching