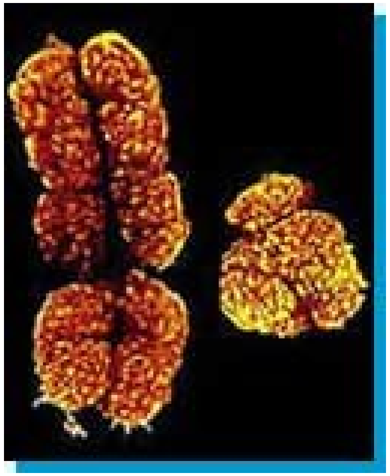
Historie a metody genomiky

Eduard Kejnovský a Roman Hobza

Objev pohlavních chromosomů



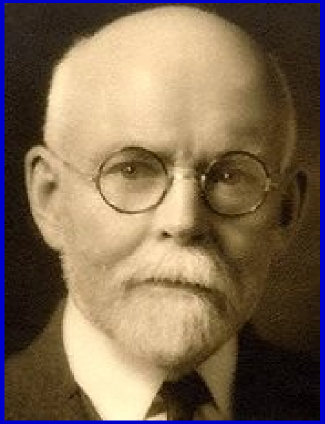
1891 Henking pracoval na spermatogenezi u Pyrrhocoris apterus

Polovina dceřiných buněk měla jeden element (chromosom) navíc

X bylo označení neurčitosti pozorovaného

1902 McClung označil tento element jako „accessory“ chromosom (X chromosome)

Chromosomální determinace pohlaví (1905)



Nettie Stevens

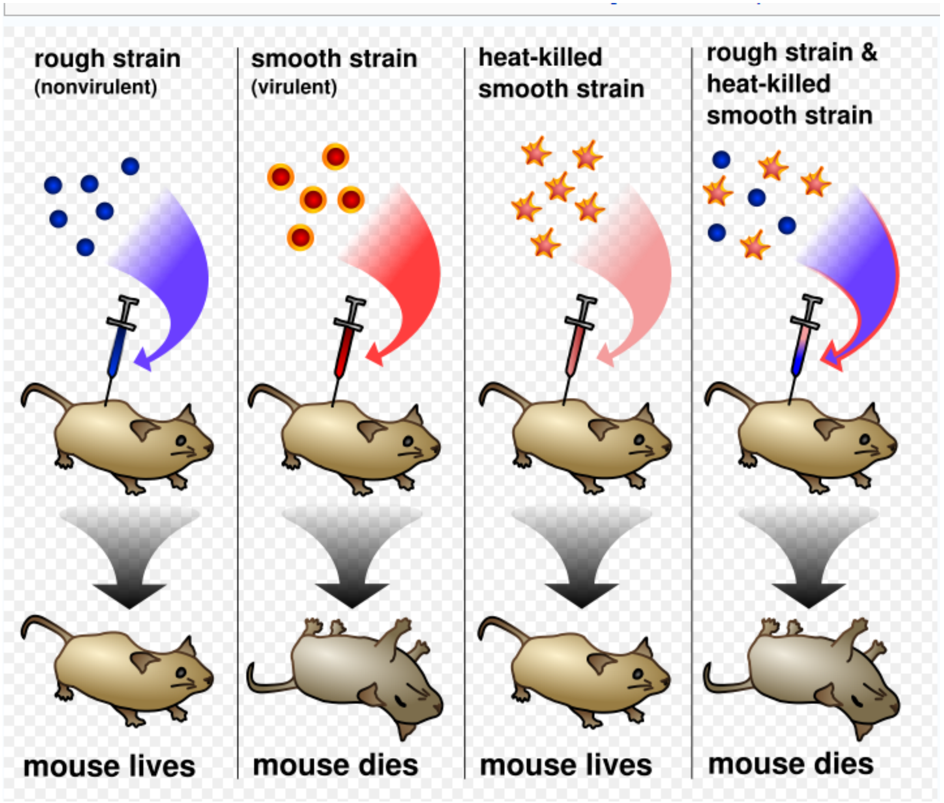
Edmund Wilson

Tenebrio molitor, Coleoptera

(F = AAXX, M = AAXY)

mealworm

Přeměna neškodných bakterií ve virulentní

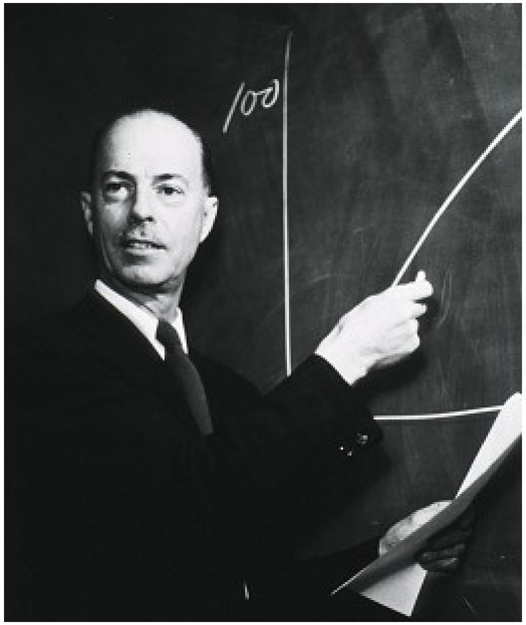


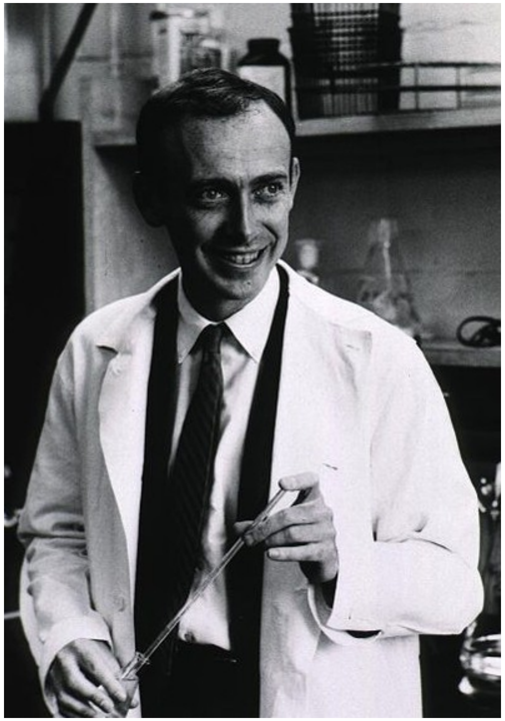
1928 - Fred Griffith: experimenty na *Streptococcus pneumonia*

Dva kmeny:
Smooth (S) - virulentní

Rough (R) - nevirulentní

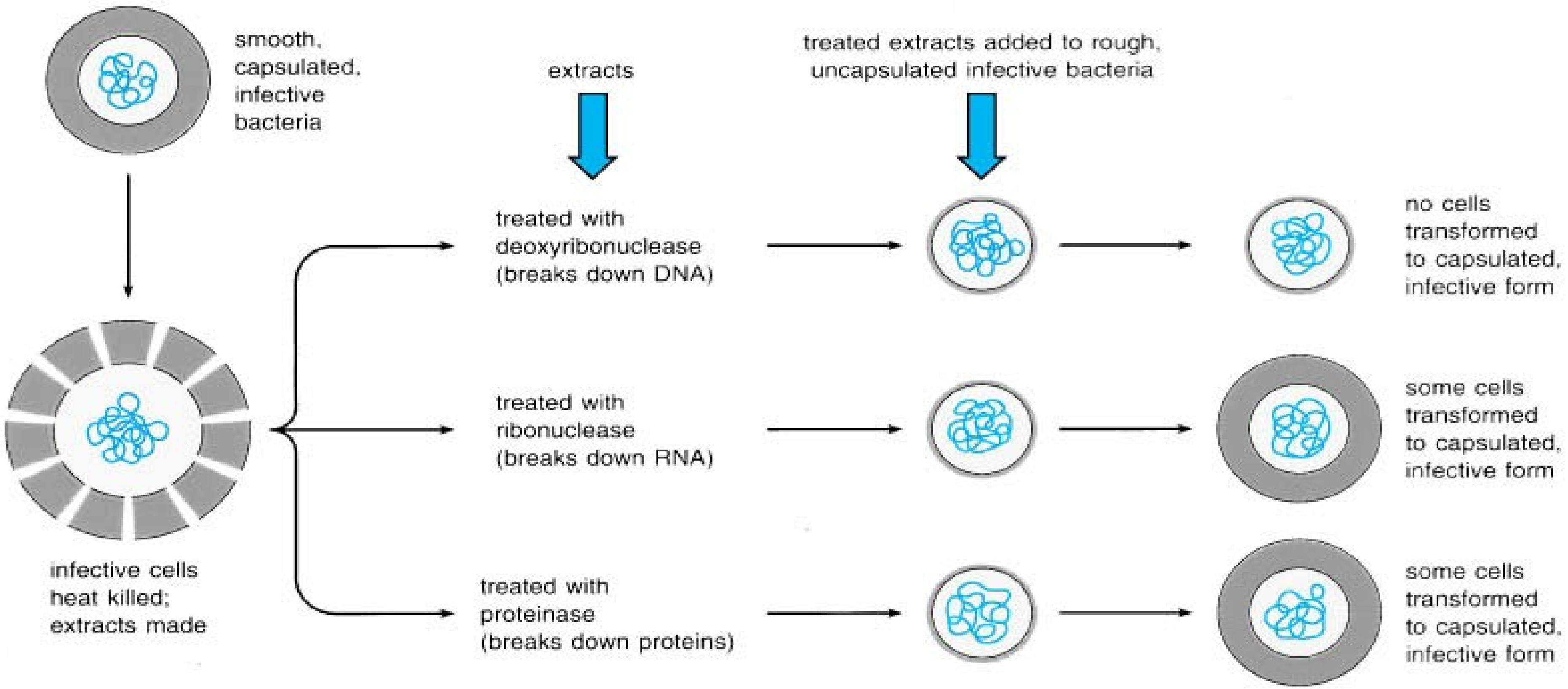
Injeckce R a teplotně inaktivovaných S bakterií - myši zemřely a obsahovaly S bakterie


100



1944 - Avery, MacLeod, and McCarty

- **Smíchali R kmen s DNA z S kmene a izolovali S bakterie**
- **Přidali DNase - inhibice transformace**
- **Přidali proteasy - transformace proběhla**
- **DNA určena jako “transformační princip”**



Genome: 30.000 genes

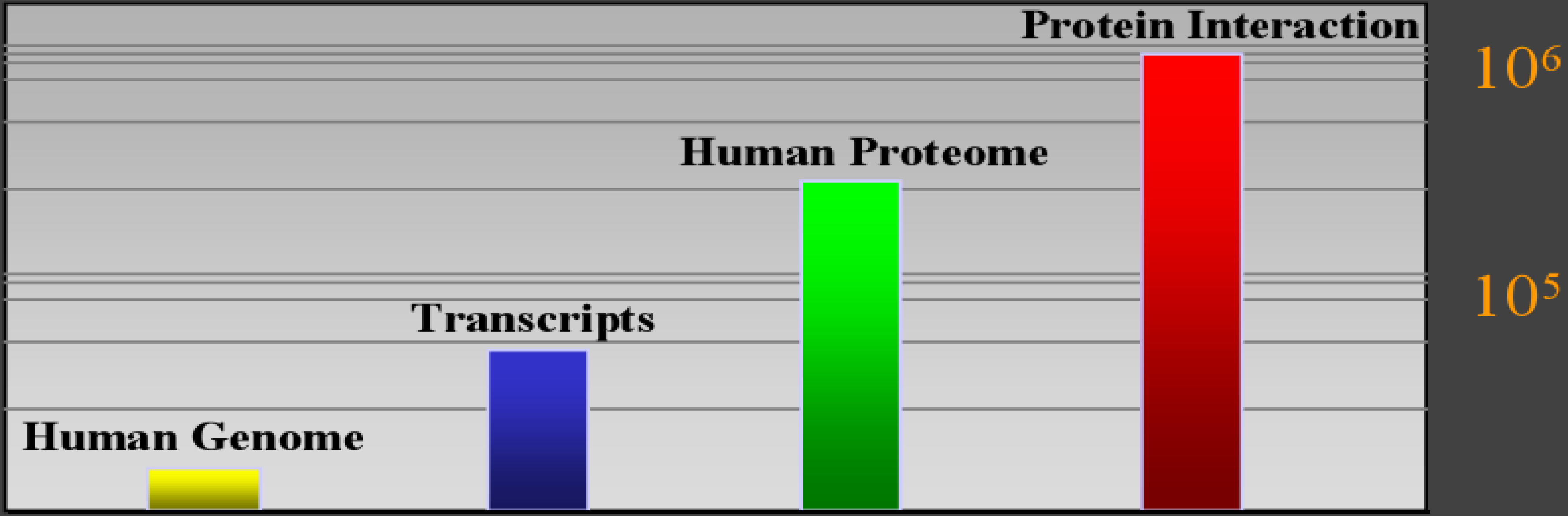
↓

Transcriptome: 40-100.000 mRNAs

↓

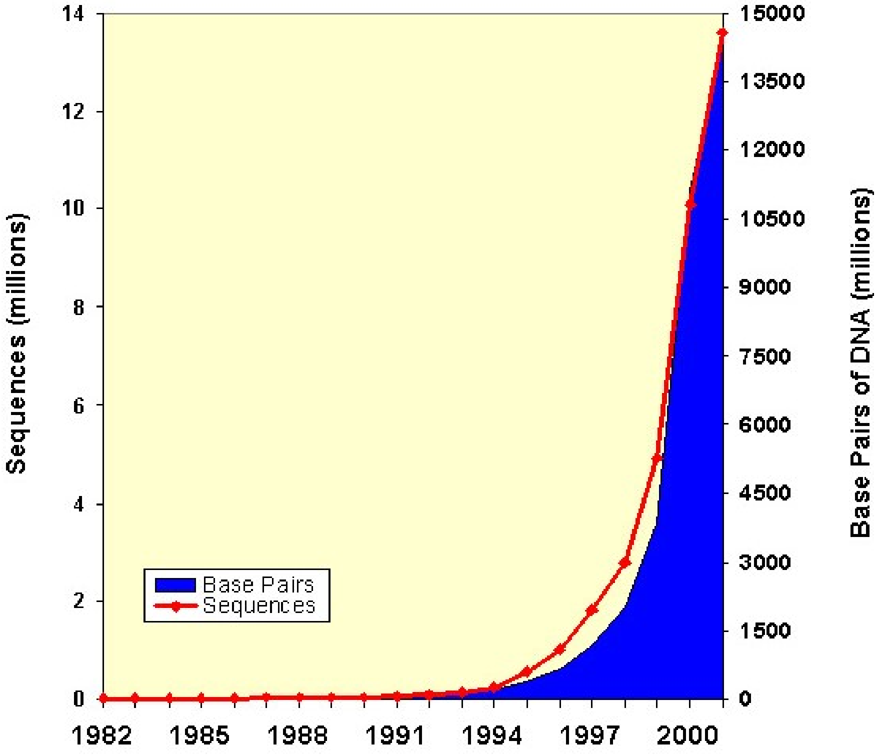
Proteome: 100-400.000 proteins

\>1.000.000 interactions



Sekvenování genomů

- 1988 americký kongres stál u zrodu Genbank



Applied Biosystems
ABI 3730XL
1 Mb / day

Roche / 454
Genome Sequencer FLX
100 Mb / run

Illumina / Solexa
Genetic Analyzer
2000 Mb / run

Applied Biosystems
SOLiD
3000 Mb / run

PACIFIC BIOSCIENCES®

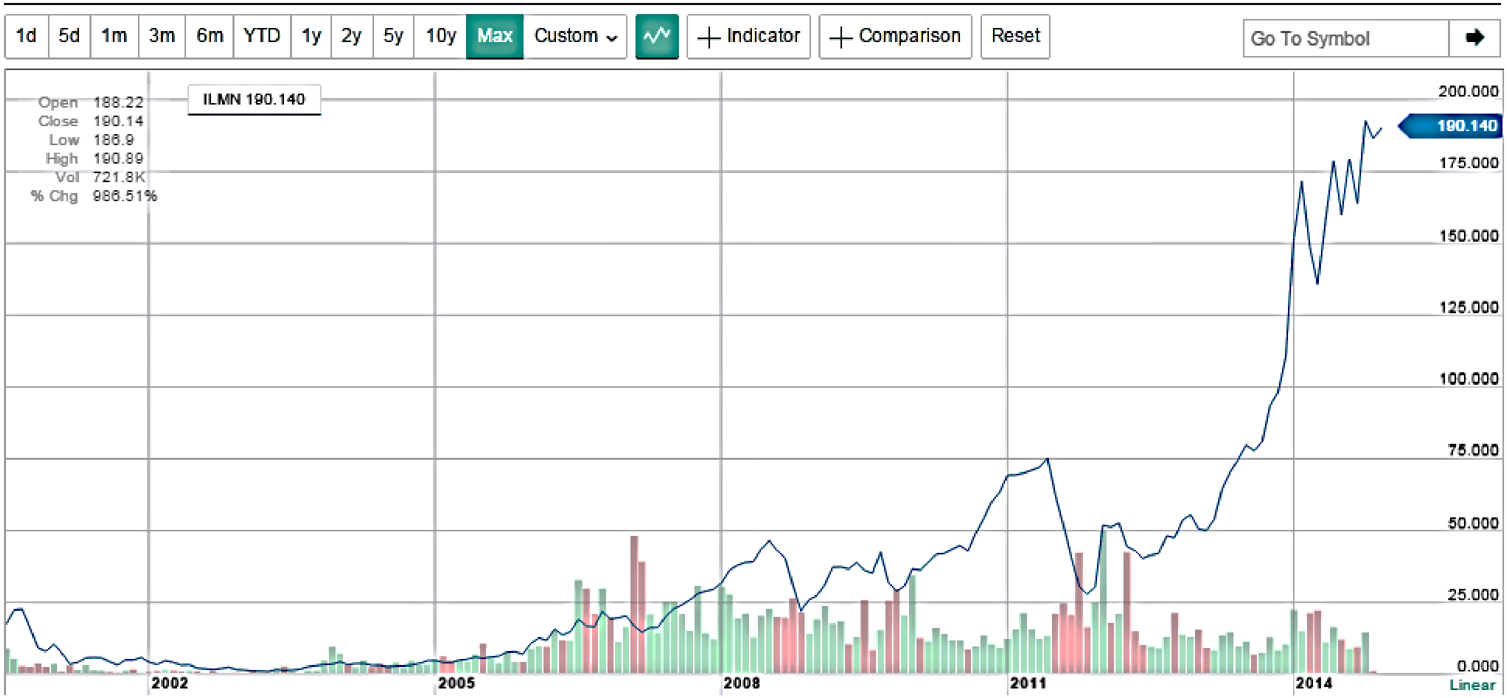
ion torrent

Oxford NANOPORE Technologies®

Illumina Inc. (ILMN) - NasdaqGS

48.51 ↑0.07 (0.14%) 11:41AM EST - Nasdaq Real Time Price

Pacific Biosciences of Californ

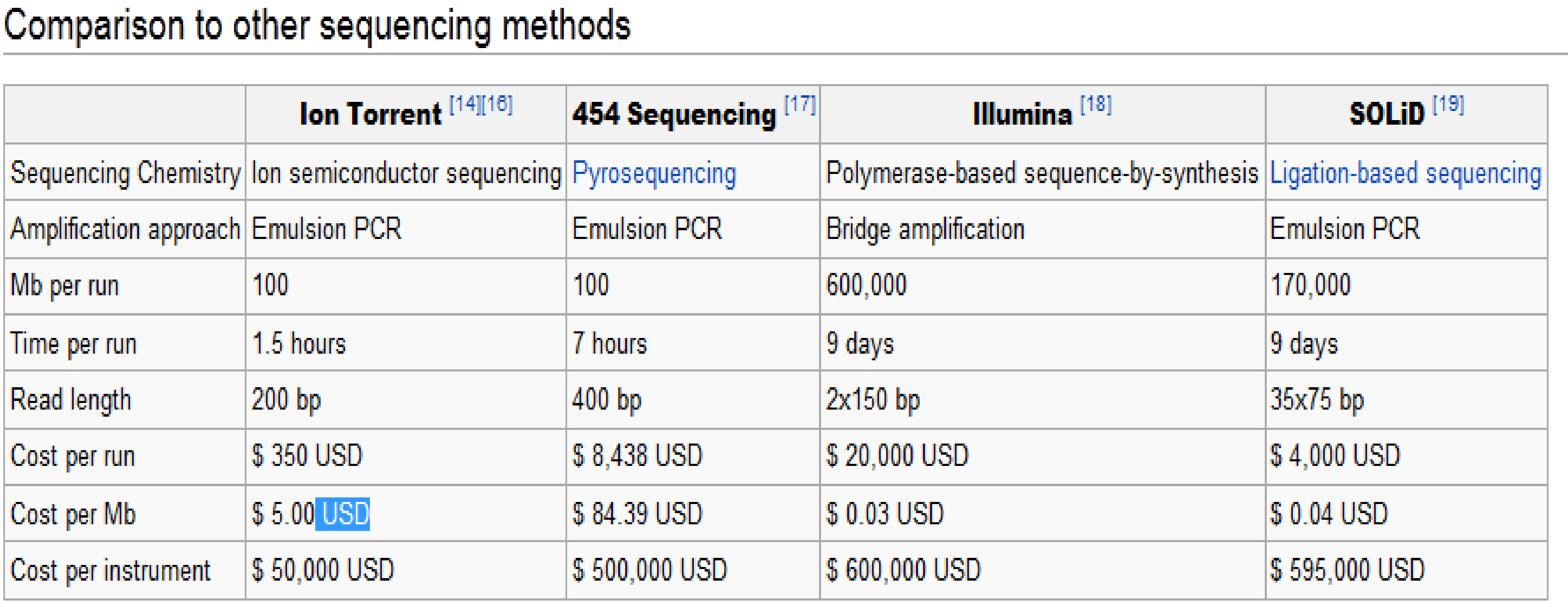


ILMN 190.140

Open 188.22
Close 190.14
Low 186.9
High 190.89
Vol 721.8K
% Chg 986.51%

PACB 2.00

Open 1.88
Close 2
Low 1.77
High 2.21
Vol 913.2K
% Chg -88.1%



Comparison to other sequencing methods

	Ion Torrent [14][16]	**454 Sequencing** [17]	**Illumina** [18]	**SOLiD** [19]
Sequencing Chemistry	Ion semiconductor sequencing	Pyrosequencing	Polymerase-based sequence-by-synthesis	Ligation-based sequencing
Amplification approach	Emulsion PCR	Emulsion PCR	Bridge amplification	Emulsion PCR
Mb per run	100	100	600,000	170,000
Time per run	1.5 hours	7 hours	9 days	9 days
Read length	200 bp	400 bp	2x150 bp	35x75 bp
Cost per run	\$ 350 USD	\$ 8,438 USD	\$ 20,000 USD	\$ 4,000 USD
Cost per Mb	\$ 5.00 USD	\$ 84.39 USD	\$ 0.03 USD	\$ 0.04 USD
Cost per instrument	\$ 50,000 USD	\$ 500,000 USD	\$ 600,000 USD	\$ 595,000 USD

Proč sekvenovat dál?

- Komparativní genomika
- Biomedicínský výzkum
- Osobní genom

Frederick Sanger

1975 - Dideoxy sekvenační metoda

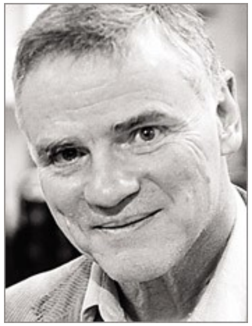
1977 – osekvenoval Φ-X174 (5,368 bp)

1980 – dostal druhou Nobelovu cenu za chemii

Později (polovina 80-tých let) osekvenoval bakteriofága λ pomocí shotgun metody (48,502 bp)

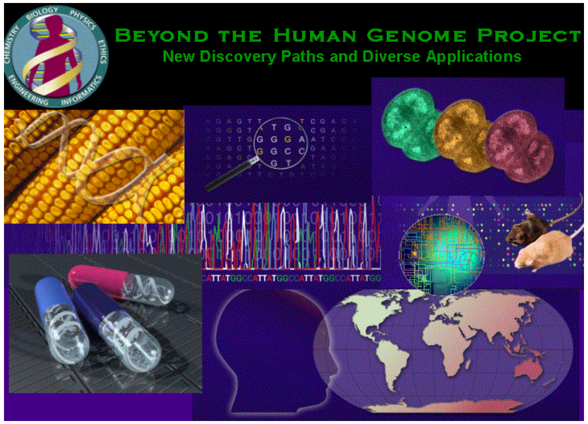
Sekvenování genomů

- **1986 Leroy Hood: první automatický sekvenátor**



Leroy Hood

- **1986 Human Genome Initiative**
- **1990 započat projekt sekvenování lidského genomu (předpokládaná doba 15 let)**



Sekvenování genomů

- **1995** John Craig Venter sekvenoval první bakteriální genom
- **1996** první eukaryotický genom (kvasinka) sekvenován

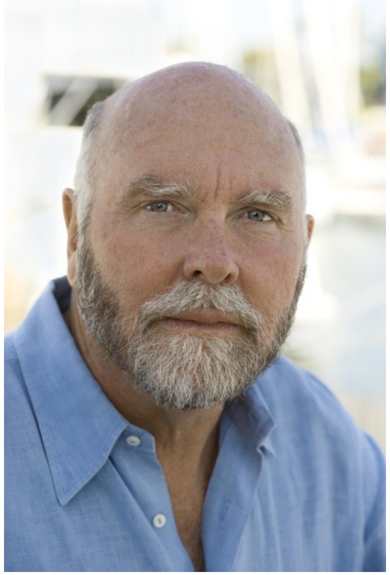
John Craig Venter

Craig Venter

Global Ocean Sampling Expedition

Synthetic genomics

Human Longevity Inc



http://www.youtube.com/watch?v=J0rDFbrhjtI

Sekvenování genomů

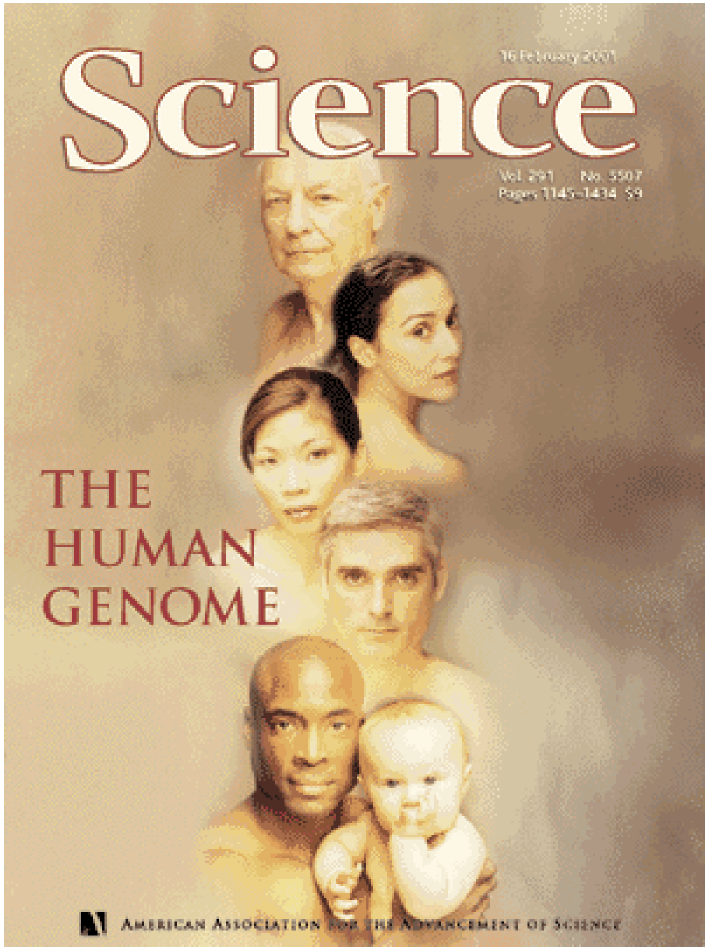
- **1997** sekvence *E. coli*
- **1998** *Caenorhabditis elegans* genom (první multicelulární genom)
- **1999** lidský chromozom 22 sekvenován

2010 Ideální lidský genom sekvenován

2010 Ideální lidský genom sekvenován

Sekvenování genomů

- **2000** *Drosophila melanogaster* genom
- **2001** Human Genome Sequencing: předběžná sekvence lidského genomu

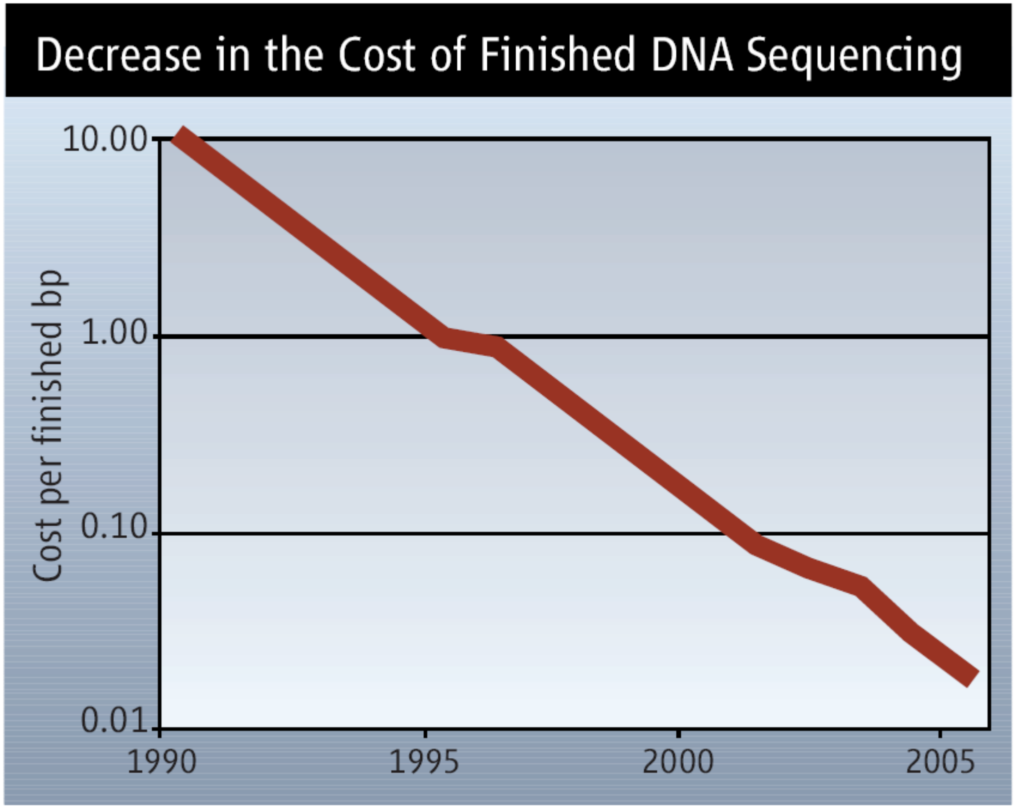


Sekvenování genomů

- **duben 2003** Lidský genom. Sekvence myšího genomu.
- **duben 2004**

  Sekvence krysího genomu

Honba za sekvencemi


Decrease in the Cost of Finished DNA Sequencing

Free fall. As with computer technology, the plunging cost of DNA sequencing has opened new applications in science and medicine.

ØHuman genome (first draft) –
$300 million (2001)

ØRhesus macaque –
$22 million (2006)

ØMammalian genome –
$100,000 (by the end of 2006)

The Race for the $1000 Genome. ***Science*** 311: 1544 – 1546, **2006**

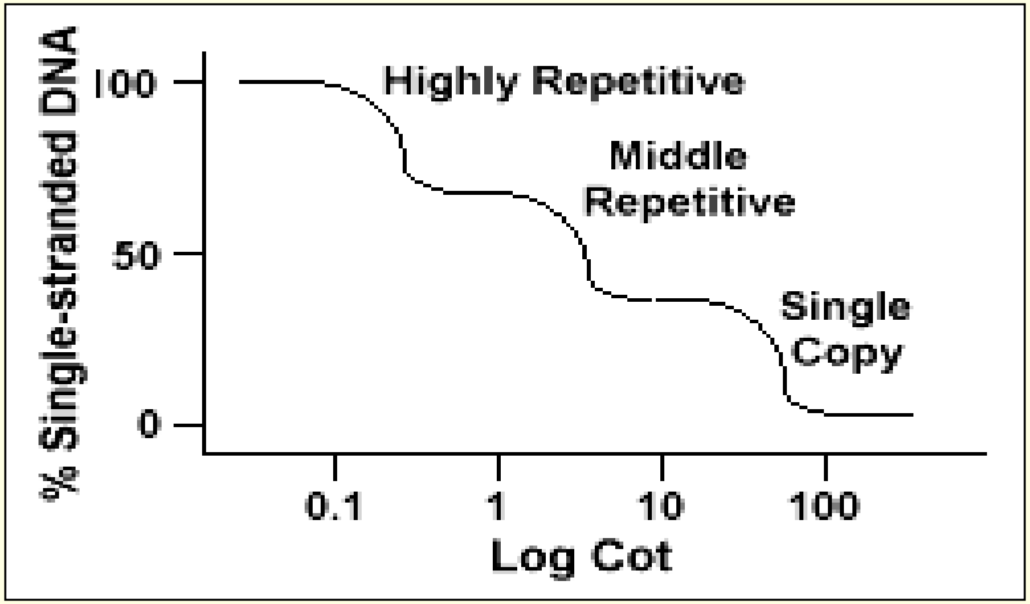
Honba za sekvencemi

- X prize foundation

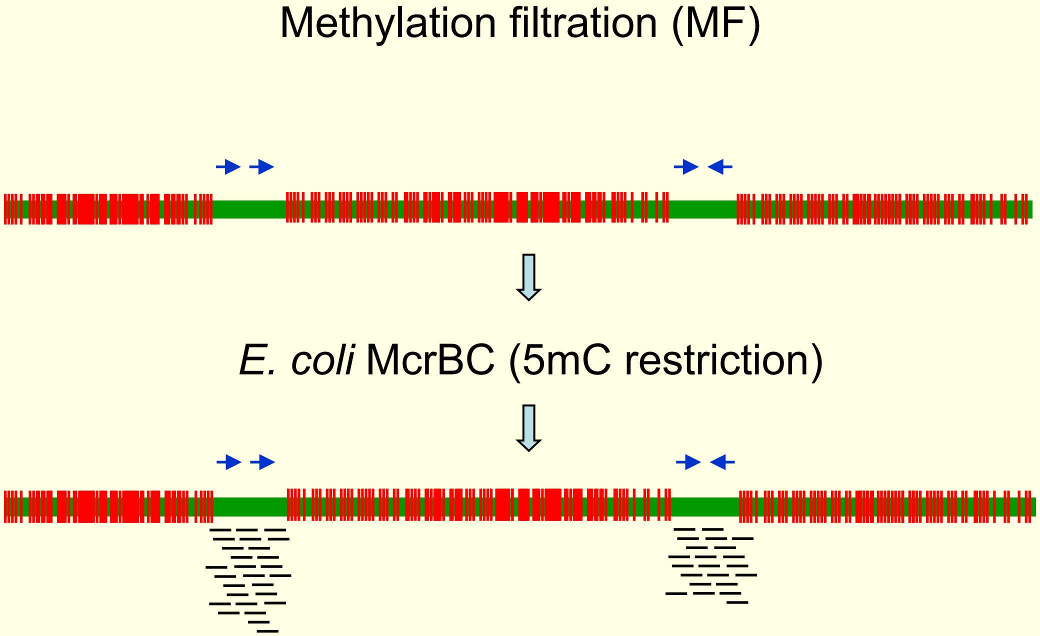
10,000,000$ tomu, kdo osekvenuje během 10 dnů sto lidských genomů (Hawking…..)

Redukce kopmlexity

Hi-Cot selection



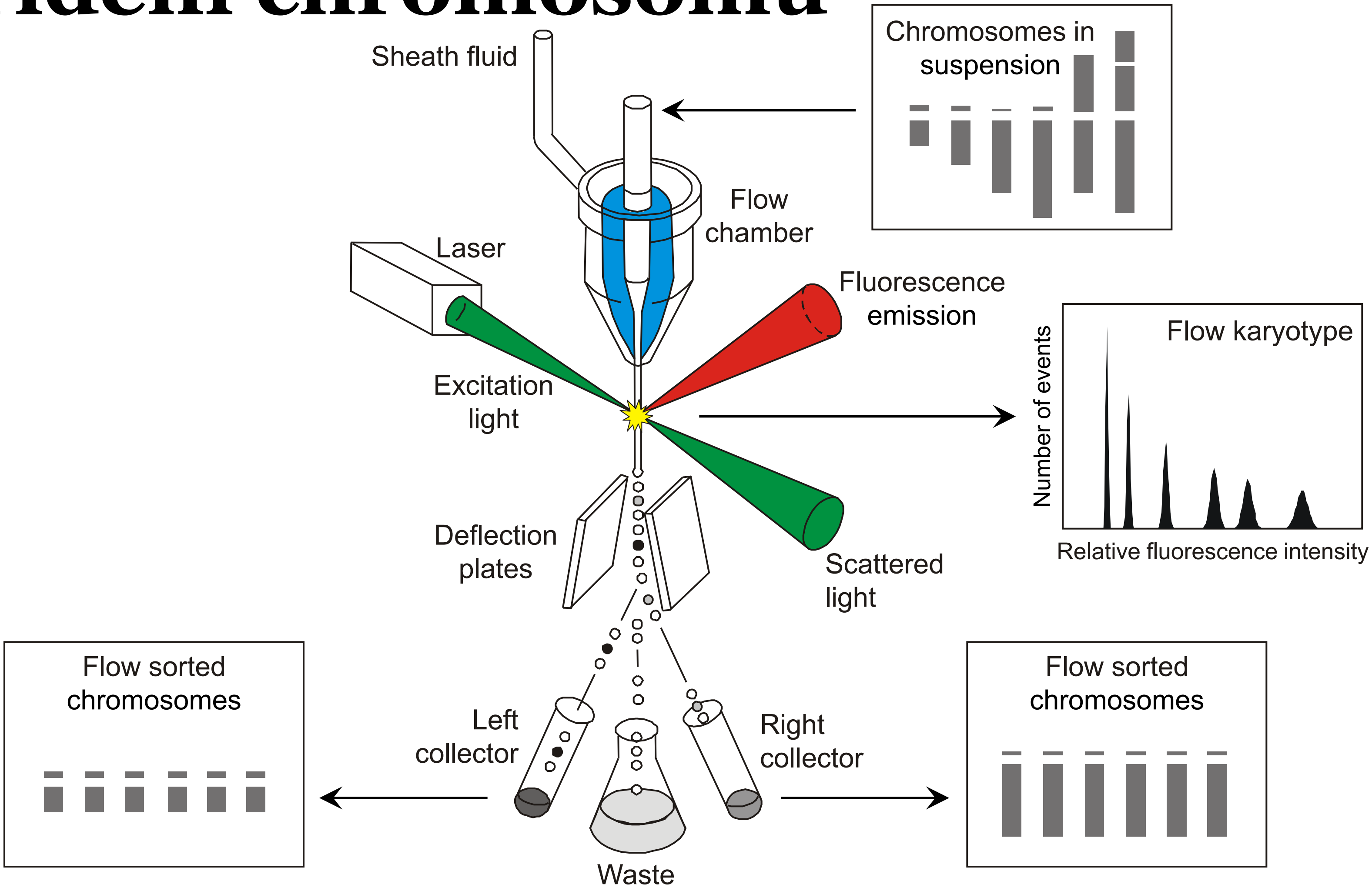
Redukce kopmlexity



Methylation filtration (MF)

E. coli McrBC (5mC restriction)

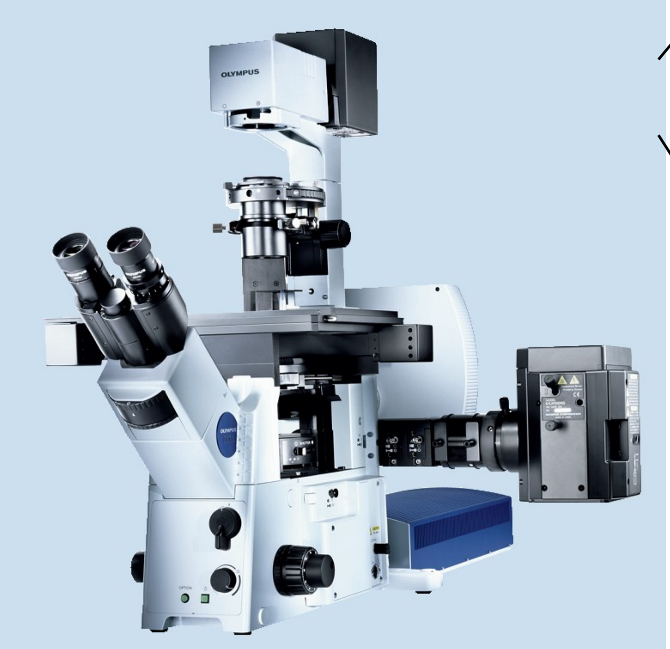
Redukce kopmlexity – třídění chromosomů

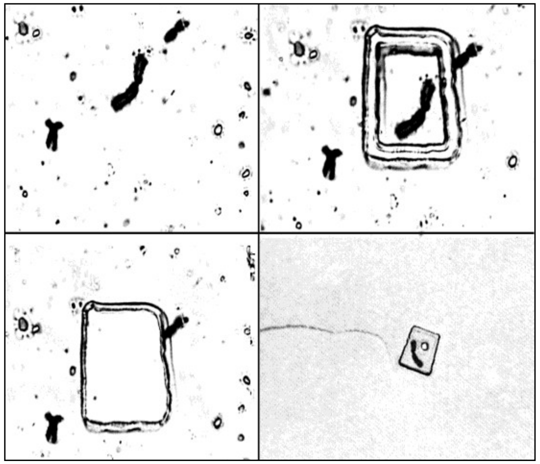


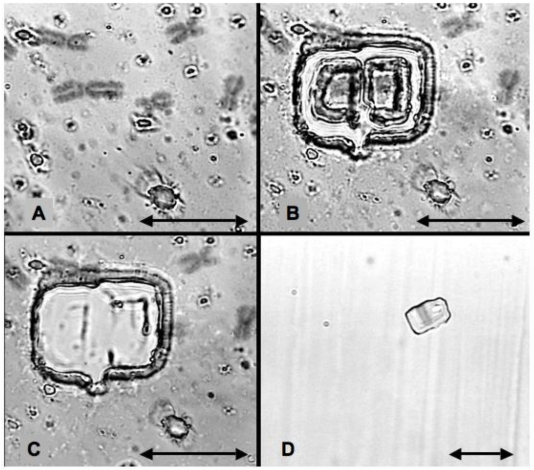
Laserová mikrodisekce

Výhody: vysoká čistota

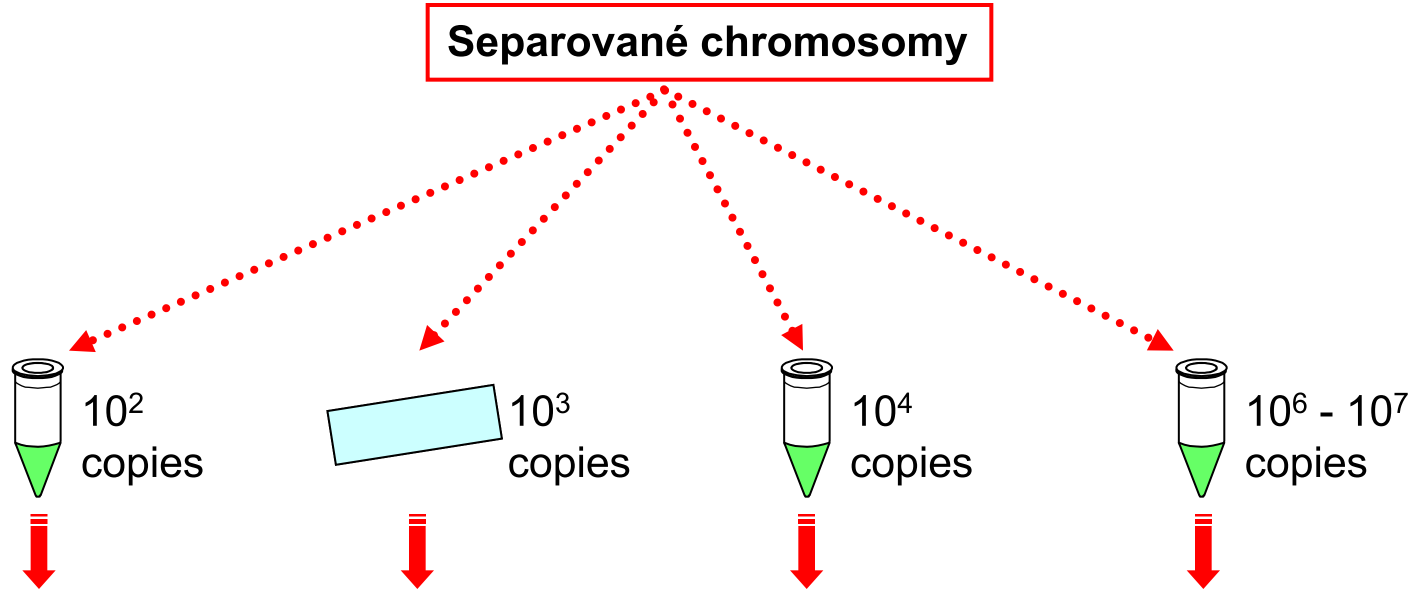
Nevýhody: malý počet chromozomů, pracnost







Aplikace



Separované chromosomy

10^2 copies

- Physical mapping using PCR

10^3 copies

- Cytogenetic mapping

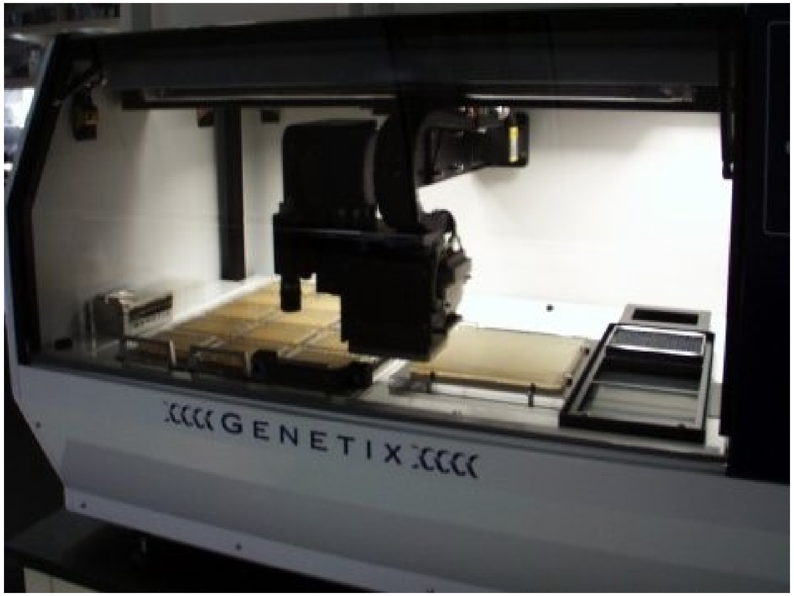
10^4 copies

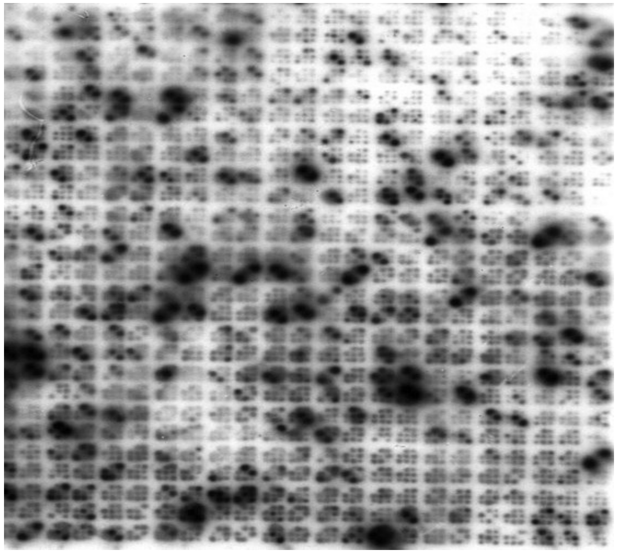
Linear amplification

10^6 - 10^7 copies

- BAC libraries
- 454-sequencing

Chromosomálně specifické genomové knihovny





Malování chromosomů – chromosome painting

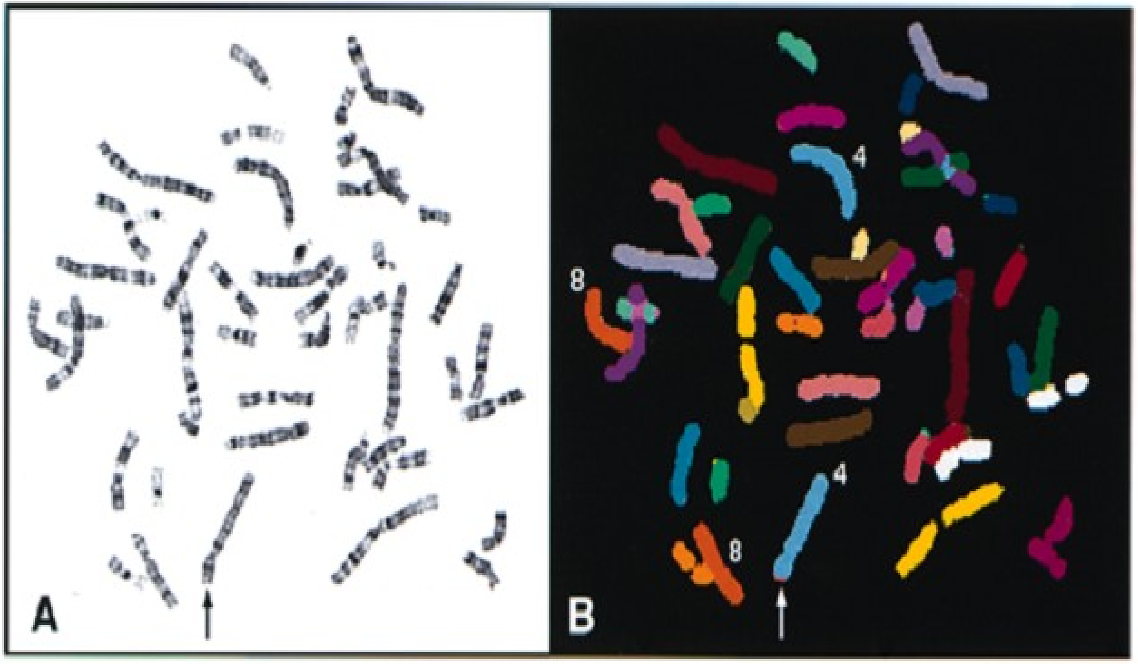


Figure 1. SKY analysis of a patient with Wolf–Hirschhorn syndrome. (**A**) G-banded metaphase. The telomeric region of one chromosome 4 (arrow) shows a slightly abnormal banding pattern. (**B**) The same metaphase as shown in (A) after SKY analysis. Chromosome classification revealed an unbalanced translocation between chromosomes 4 and 8 [46,XY,der(4)t(4;8)].

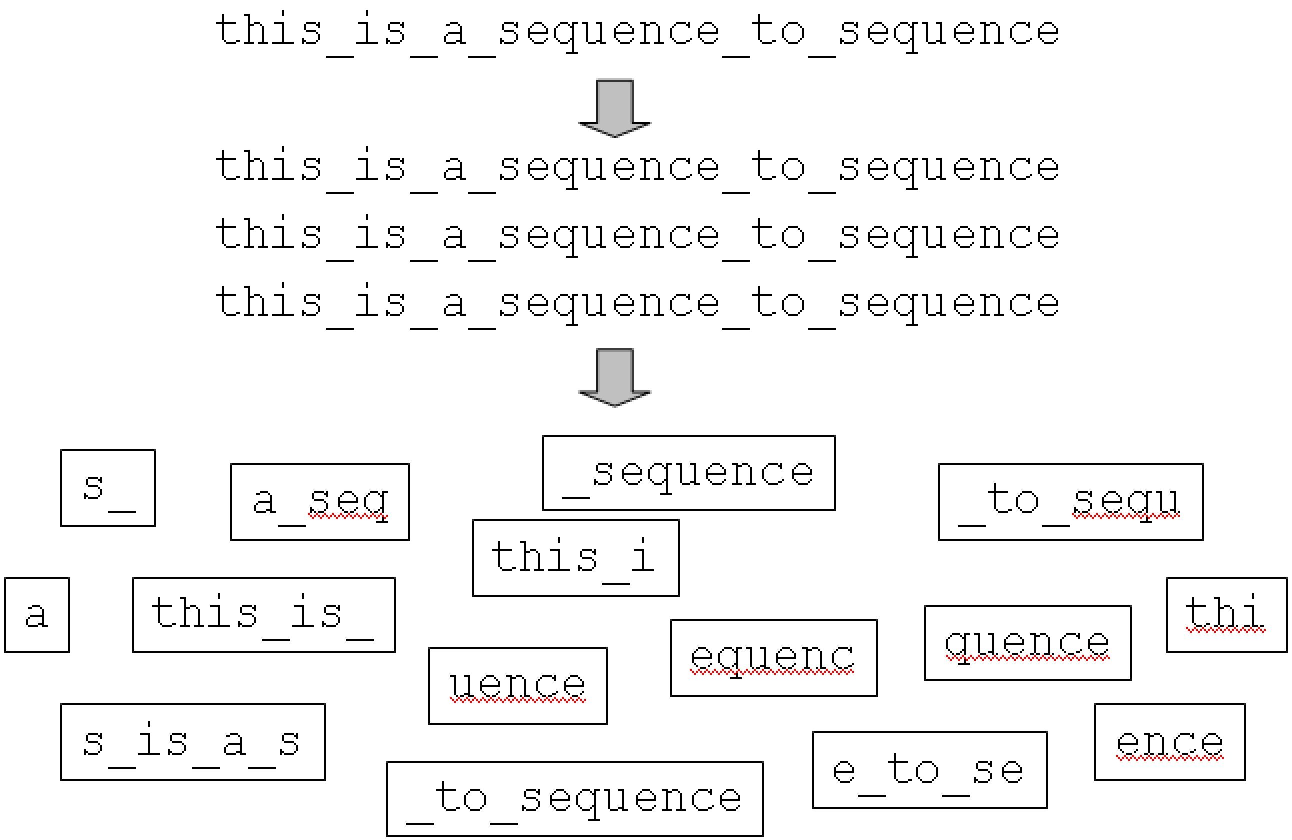
Applied Biosystems
ABI 3730XL
1 Mb / day

Roche / 454
Genome Sequencer FLX
100 Mb / run

Illumina / Solexa
Genetic Analyzer
2000 Mb / run

Applied Biosystems
SOLiD
3000 Mb / run

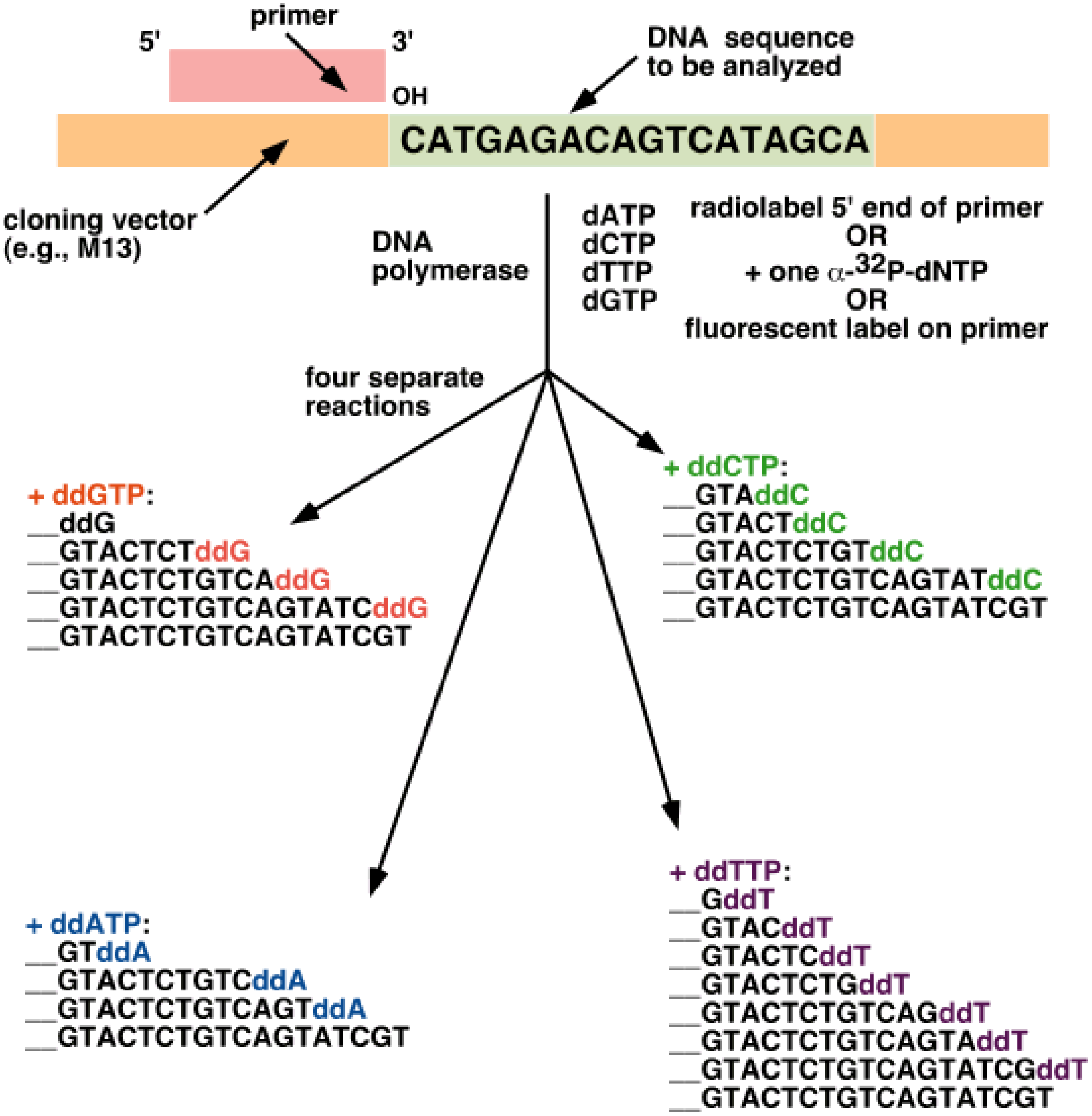
Metody sekvenování



`this_is_a_sequence_to_sequence`

`this_is_a_sequence_to_sequence`
`this_is_a_sequence_to_sequence`
`this_is_a_sequence_to_sequence`

`s_` `a_seq` `_sequence` `_to_sequ` `this_i` `a` `this_is_` `thi` `equenc` `quence` `uence` `s_is_a_s` `ence` `e_to_se` `_to_sequence`



primer

5'

3'

OH

DNA sequence
to be analyzed

CATGAGACAGTCATAGCA

cloning vector
(e.g., M13)

DNA
polymerase

dATP
dCTP
dTTP
dGTP

radiolabel 5' end of primer
OR
+ one α-^{32}P-dNTP
OR
fluorescent label on primer

four separate
reactions

+ ddGTP:
__ddG
__GTACTCTddG
__GTACTCTGTCAddG
__GTACTCTGTCAGTATCddG
__GTACTCTGTCAGTATCGT

+ ddCTP:
__GTAddC
__GTACTddC
__GTACTCTGTddC
__GTACTCTGTCAGTATddC
__GTACTCTGTCAGTATCGT

+ ddATP:
__GTddA
__GTACTCTGTCddA
__GTACTCTGTCAGTddA
__GTACTCTGTCAGTATCGT

+ ddTTP:
__GddT
__GTACddT
__GTACTCddT
__GTACTCTGddT
__GTACTCTGTCAGddT
__GTACTCTGTCAGTAddT
__GTACTCTGTCAGTATCGddT
__GTACTCTGTCAGTATCGT

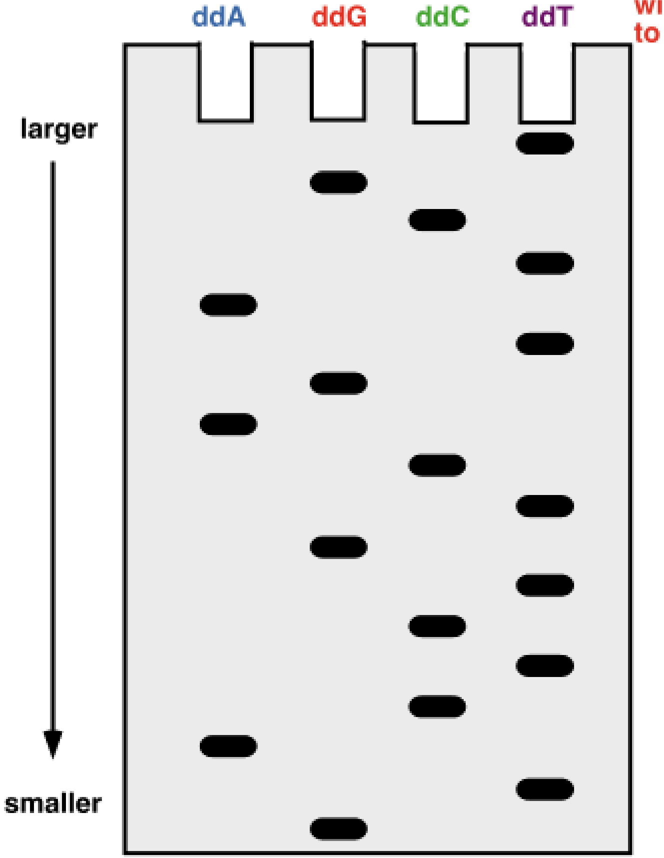
+ ddGTP:
__ddG
__GTACTCTddG
__GTACTCTGTCAddG
__GTACTCTGTCAGTATCddG
__GTACTCTGTCAGTATCGT

+ ddCTP:
__GTAddC
__GTACTddC
__GTACTCTGTddC
__GTACTCTGTCAGTATddC
__GTACTCTGTCAGTATCGT

+ ddATP:
__GTddA
__GTACTCTGTCddA
__GTACTCTGTCAGTddA
__GTACTCTGTCAGTATCGT

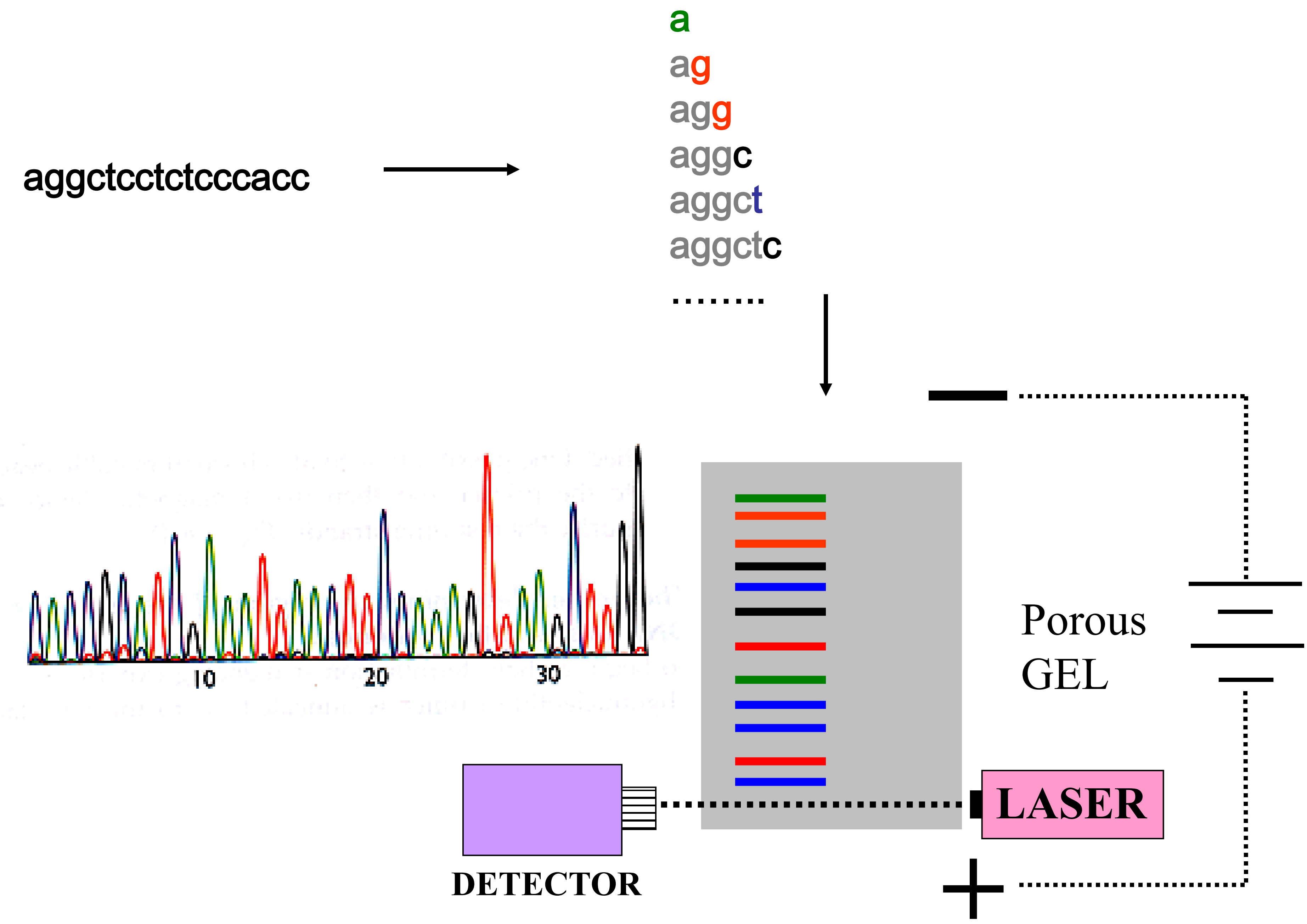
+ ddTTP:
__GddT
__GTACddT
__GTACTCddT
__GTACTCTGddT
__GTACTCTGTCAGddT
__GTACTCTGTCAGTAddT
__GTACTCTGTCAGTATCGddT
__GTACTCTGTCAGTATCGT

gel electrophoresis
autoradiography (if radiolabeled)



sequence read from gel
is the complementary strand
with respect to the sequence
to be analyzed

3'	5'
T	A
G	C
C	G
T	A
A	T
T	A
G	C
A	T
C	G
T	A
G	C
T	A
C	G
T	A
C	G
A	T
T	A
G	C
5'	3'



aggctcctctcccacc

a
ag
agg
aggc
aggct
aggctc
....

Porous
GEL

LASER

DETECTOR

Genome Sequencer 20 System
454 pyrosequencing (2005)

- http://www.454.com

Neandertal sequenced *now*!

Max Planck Institut uses 454 Sequencing™ technology.

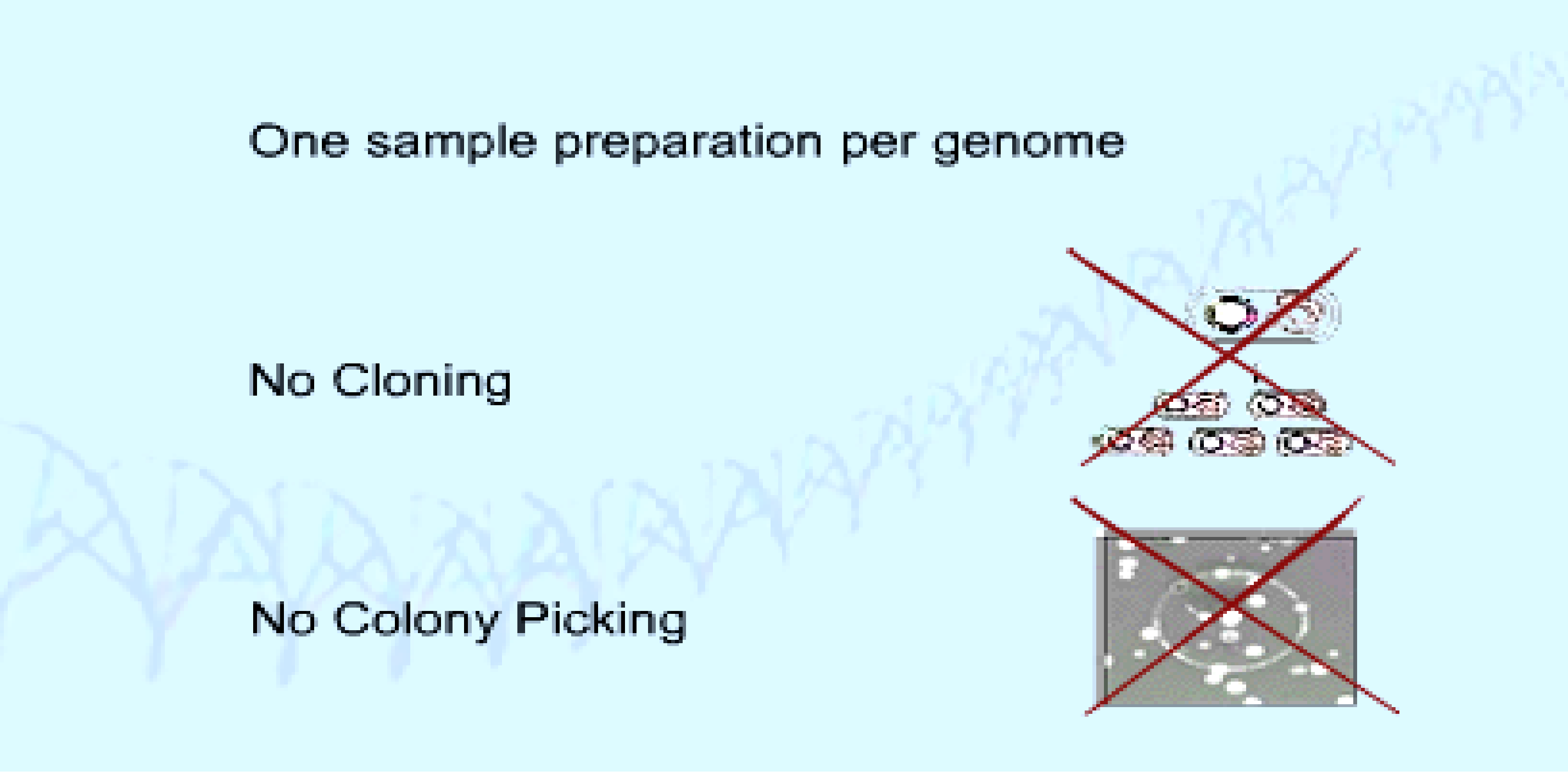
Read about it ➡

Watch the CNN video ➡

Hear the NPR broadcast ➡

Image credit:
Ken Mowbray and Blaine Maley,
American Museum of Natural History

DNA library preparation

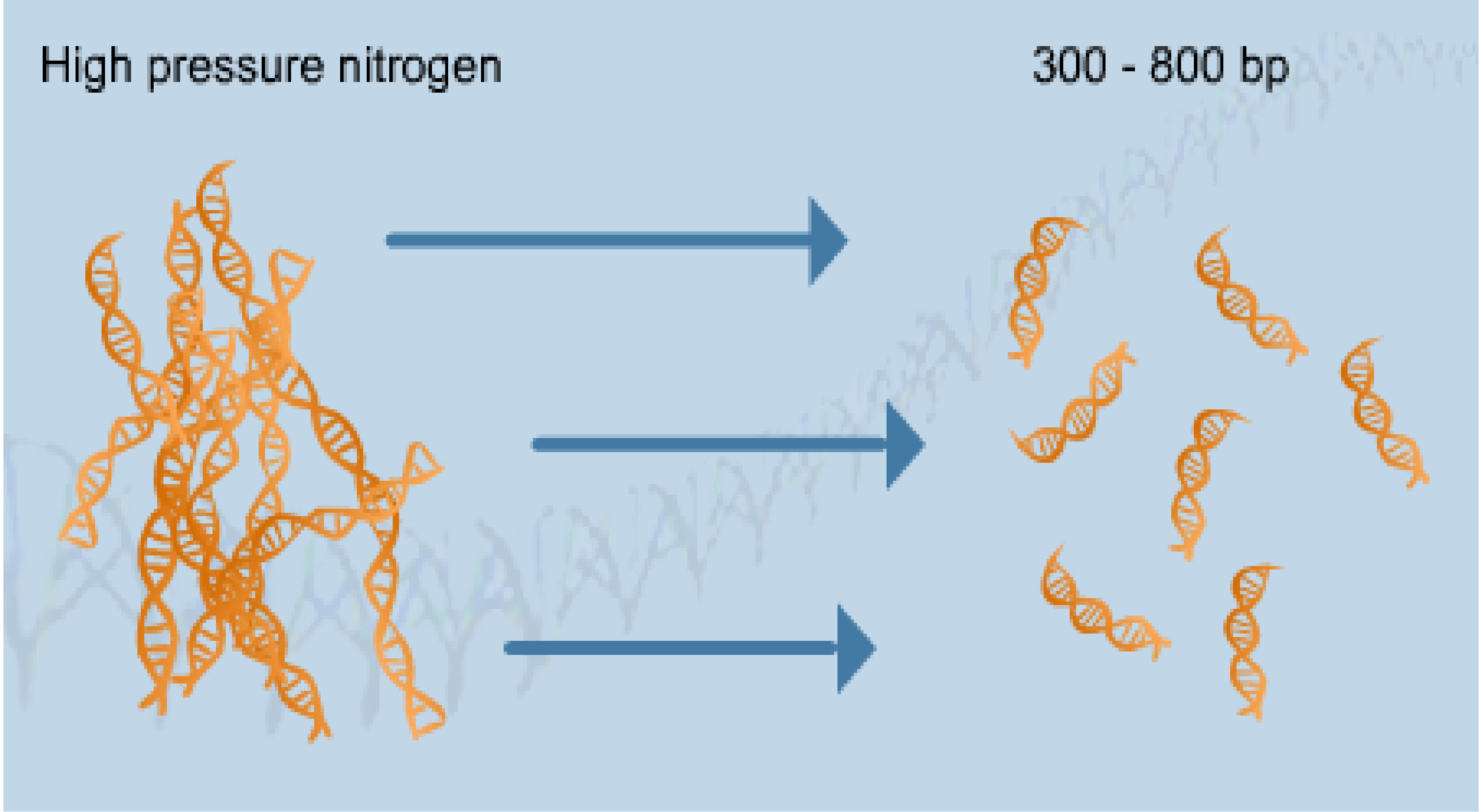


One sample preparation per genome

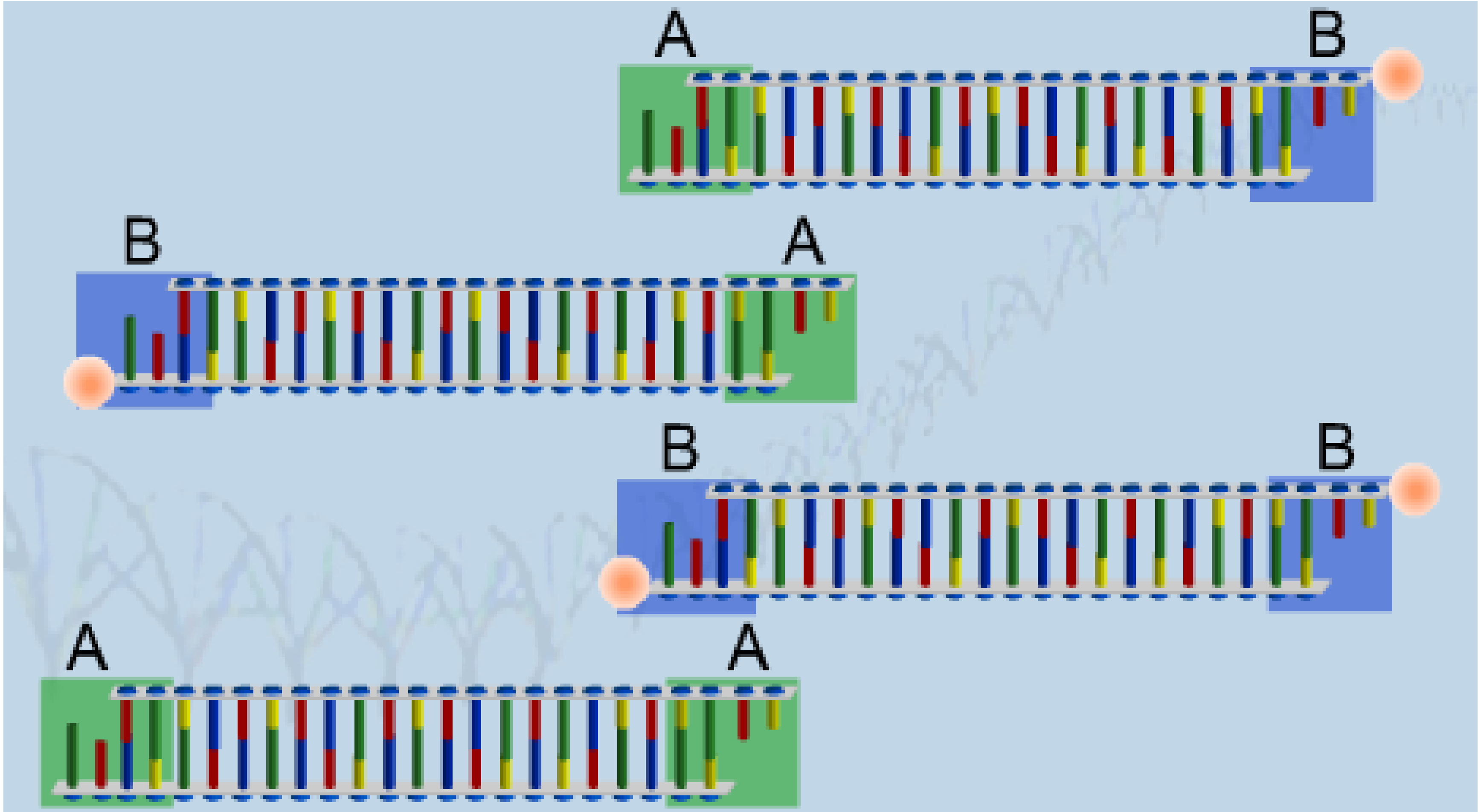
No Cloning

No Colony Picking

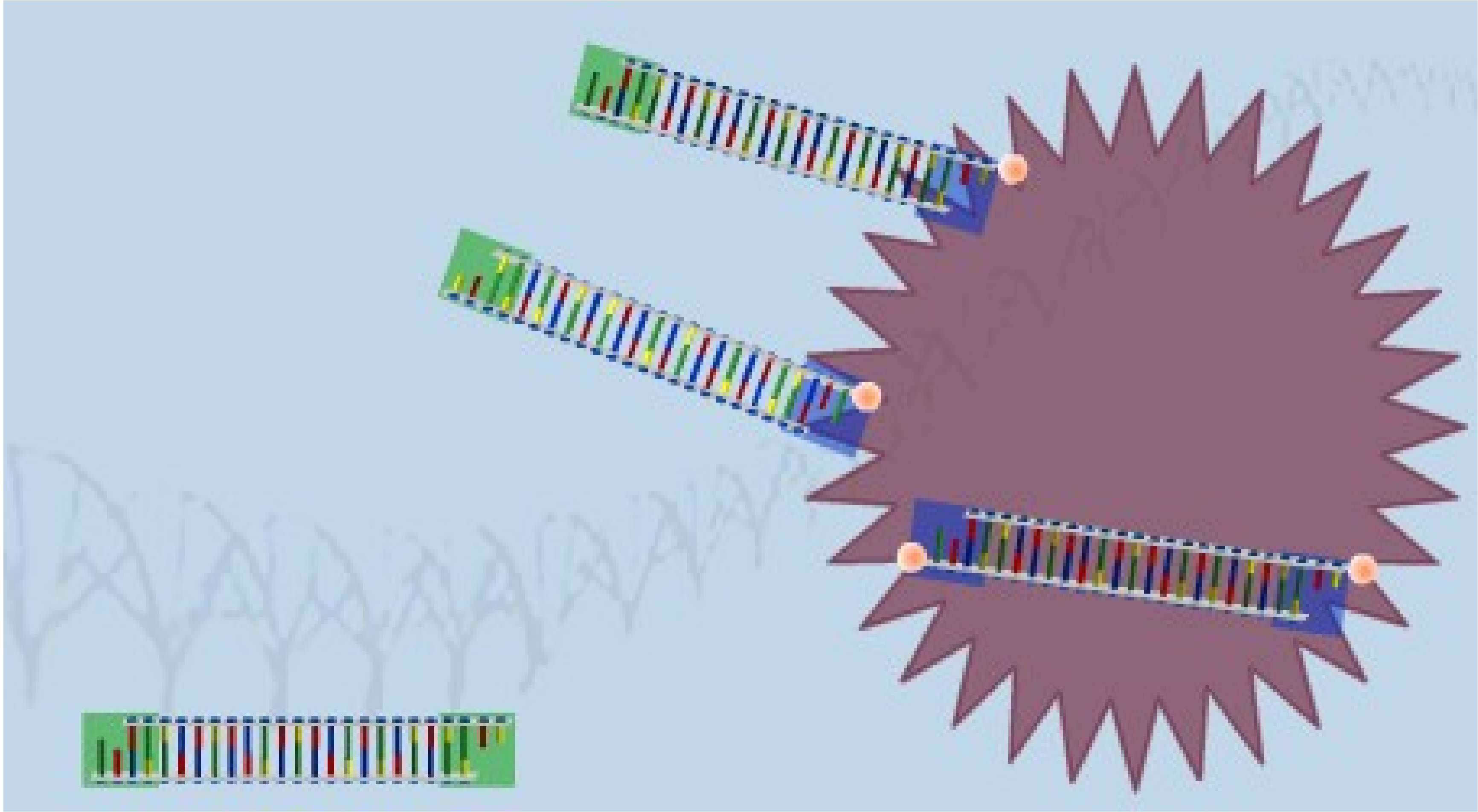
Fragmentace DNA



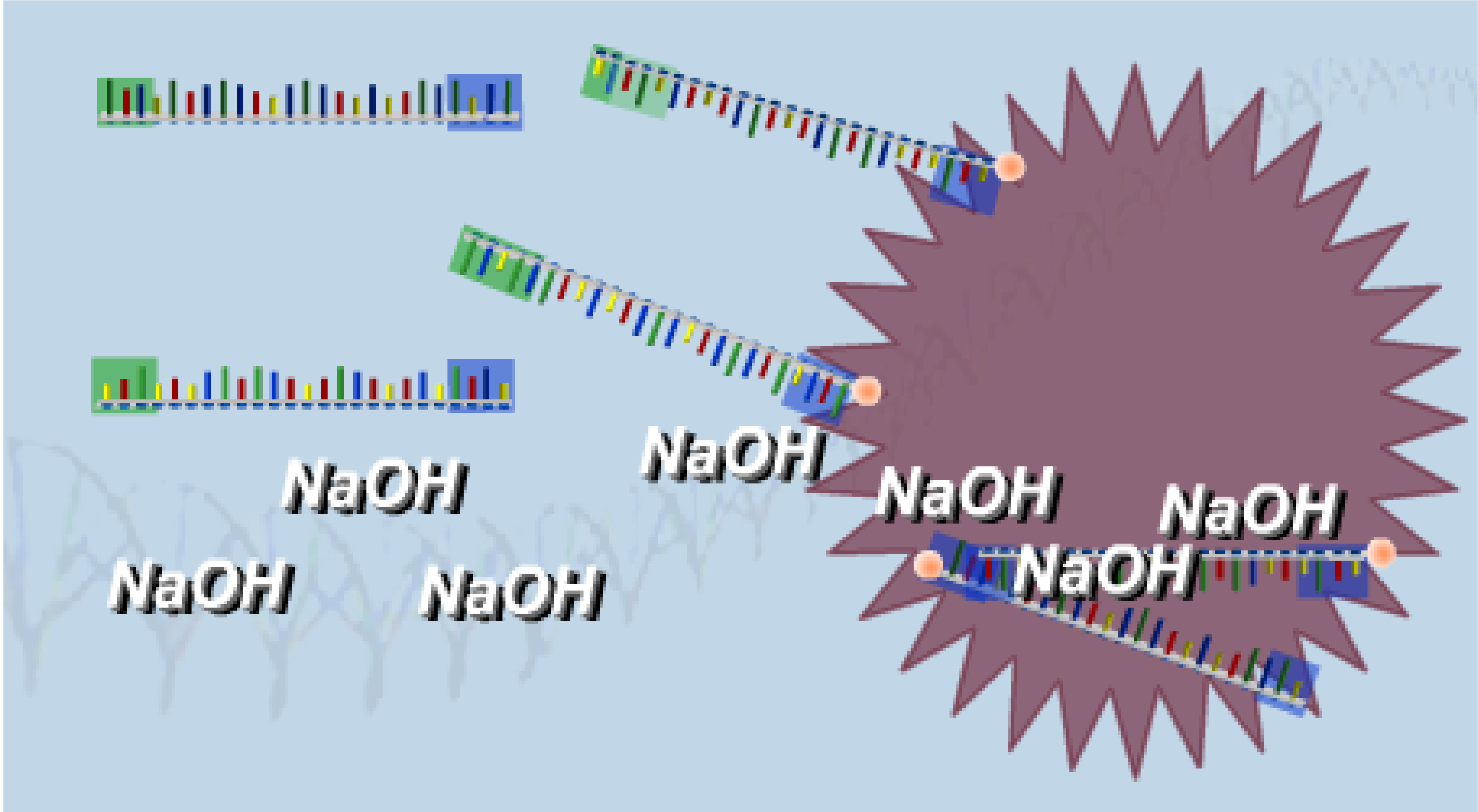
Ligace adaptoru

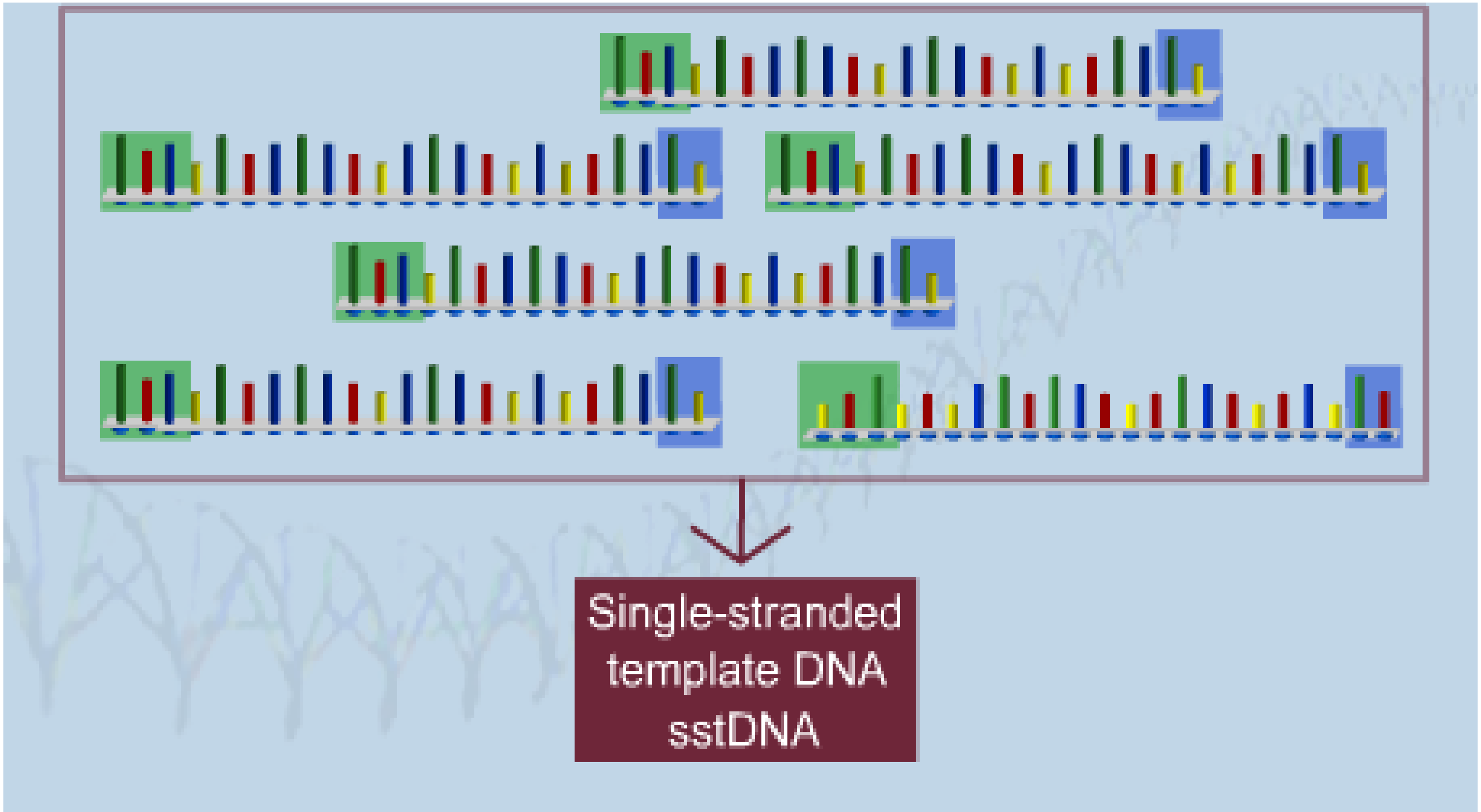


Vychytání DNA molekul



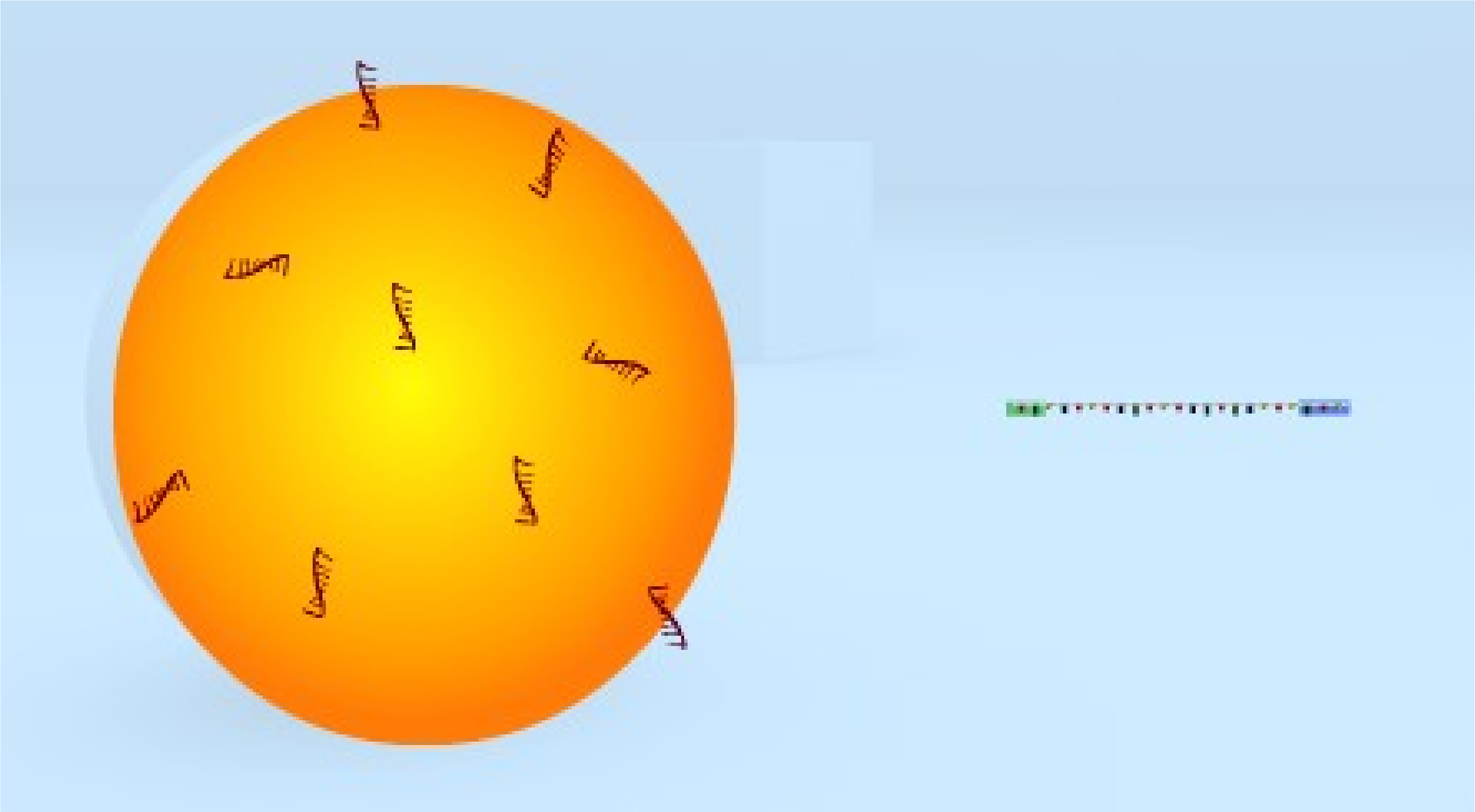
denaturace



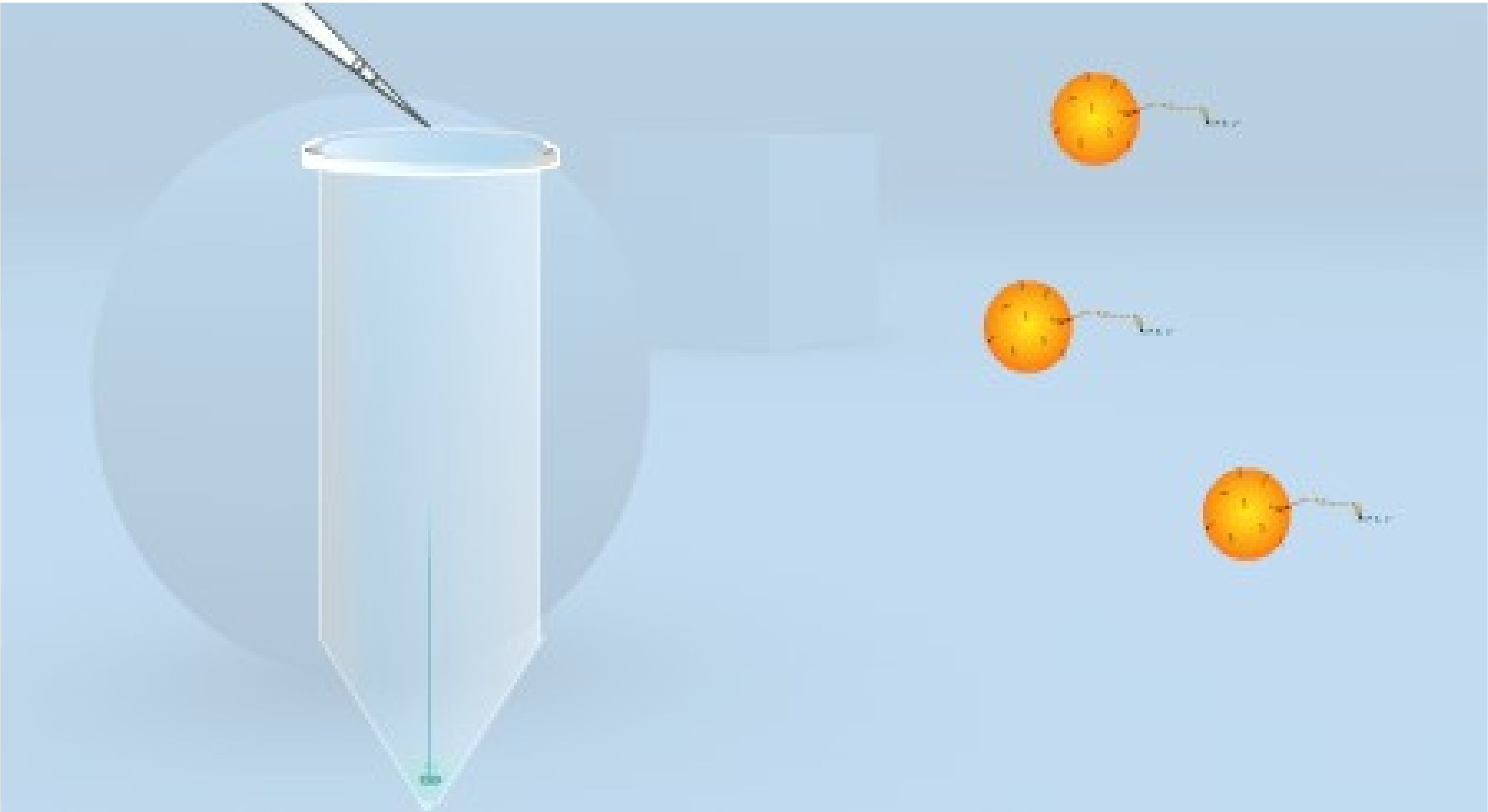


Single-stranded template DNA sstDNA

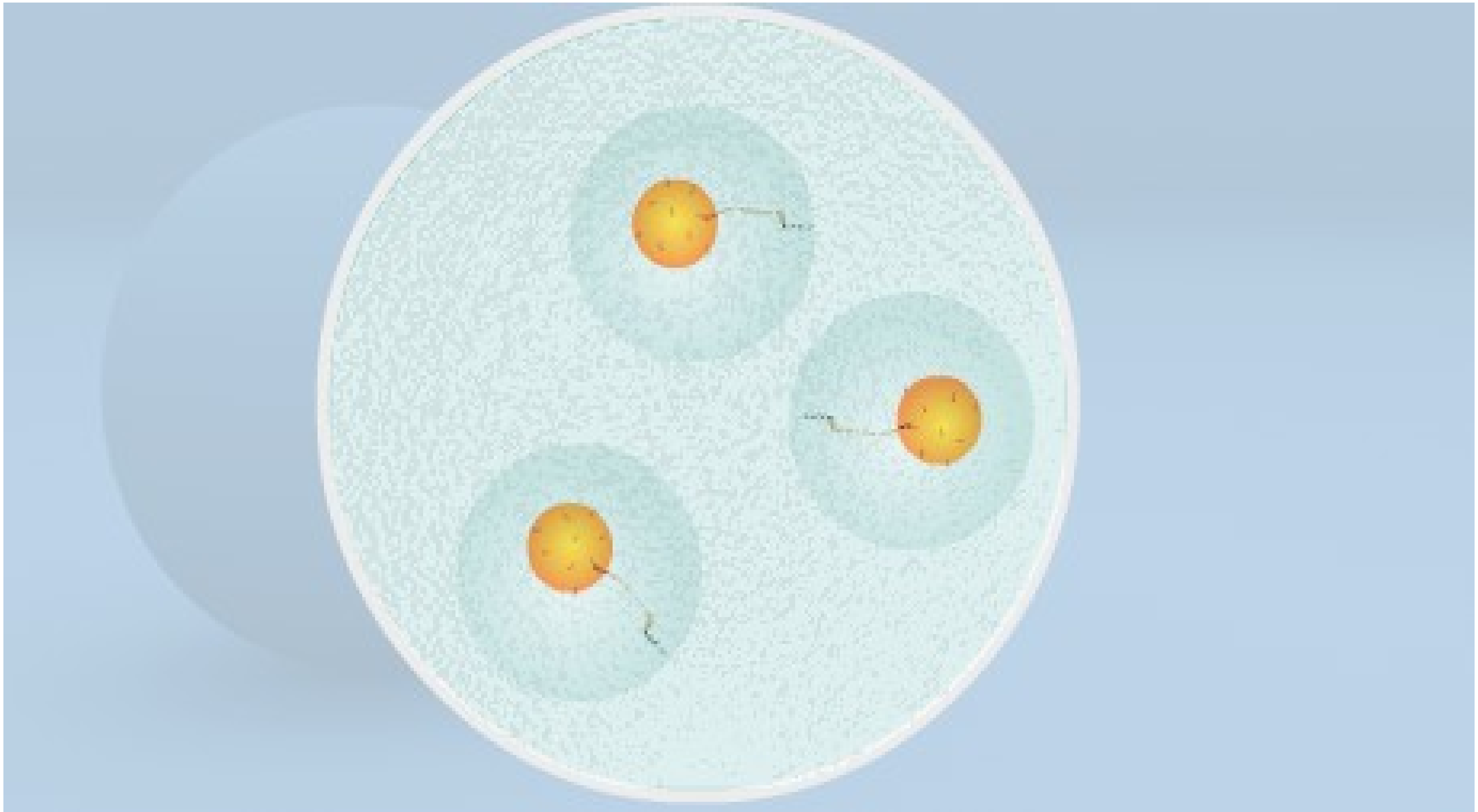
emPCR



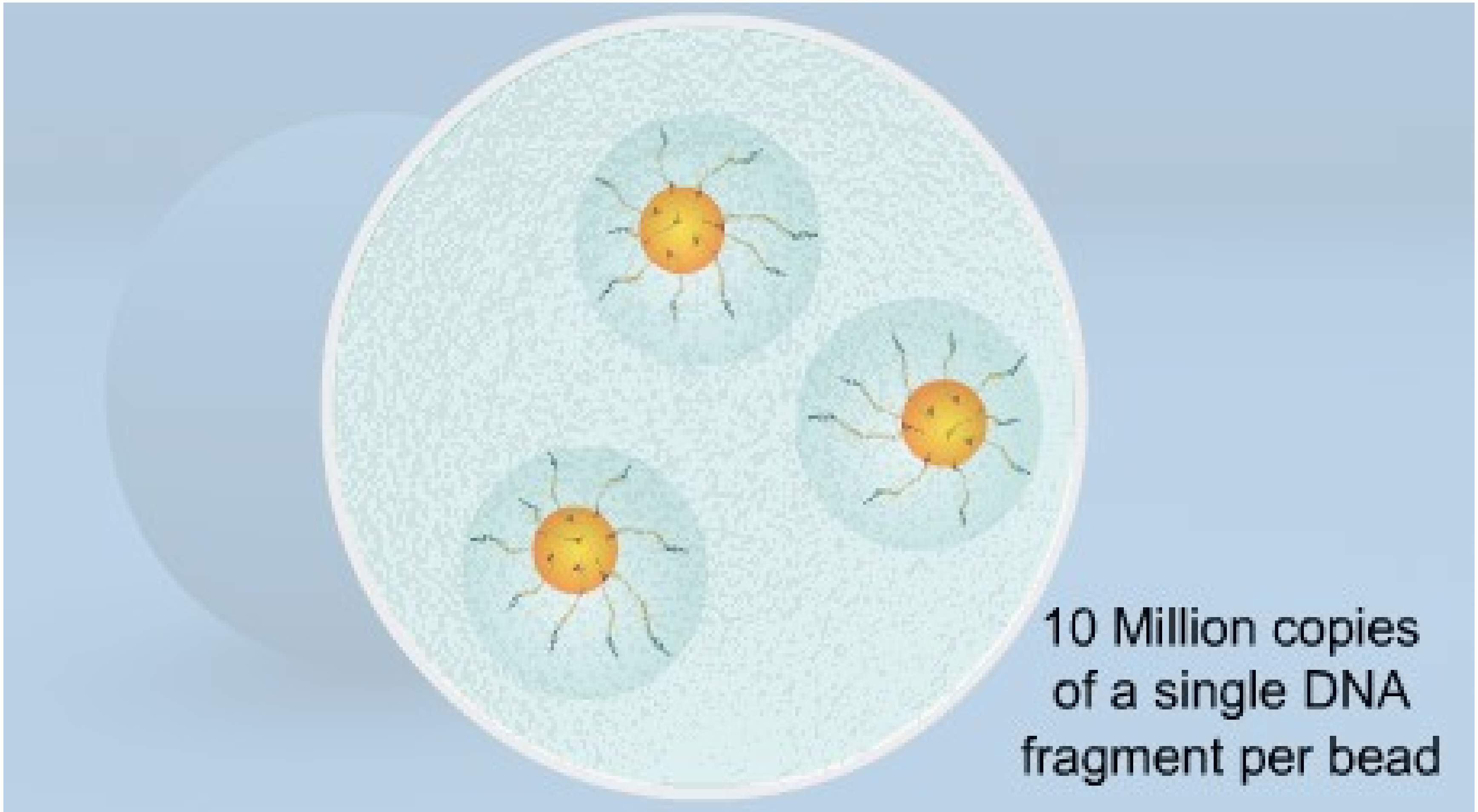
Vznik emulze (olej)



emPCR

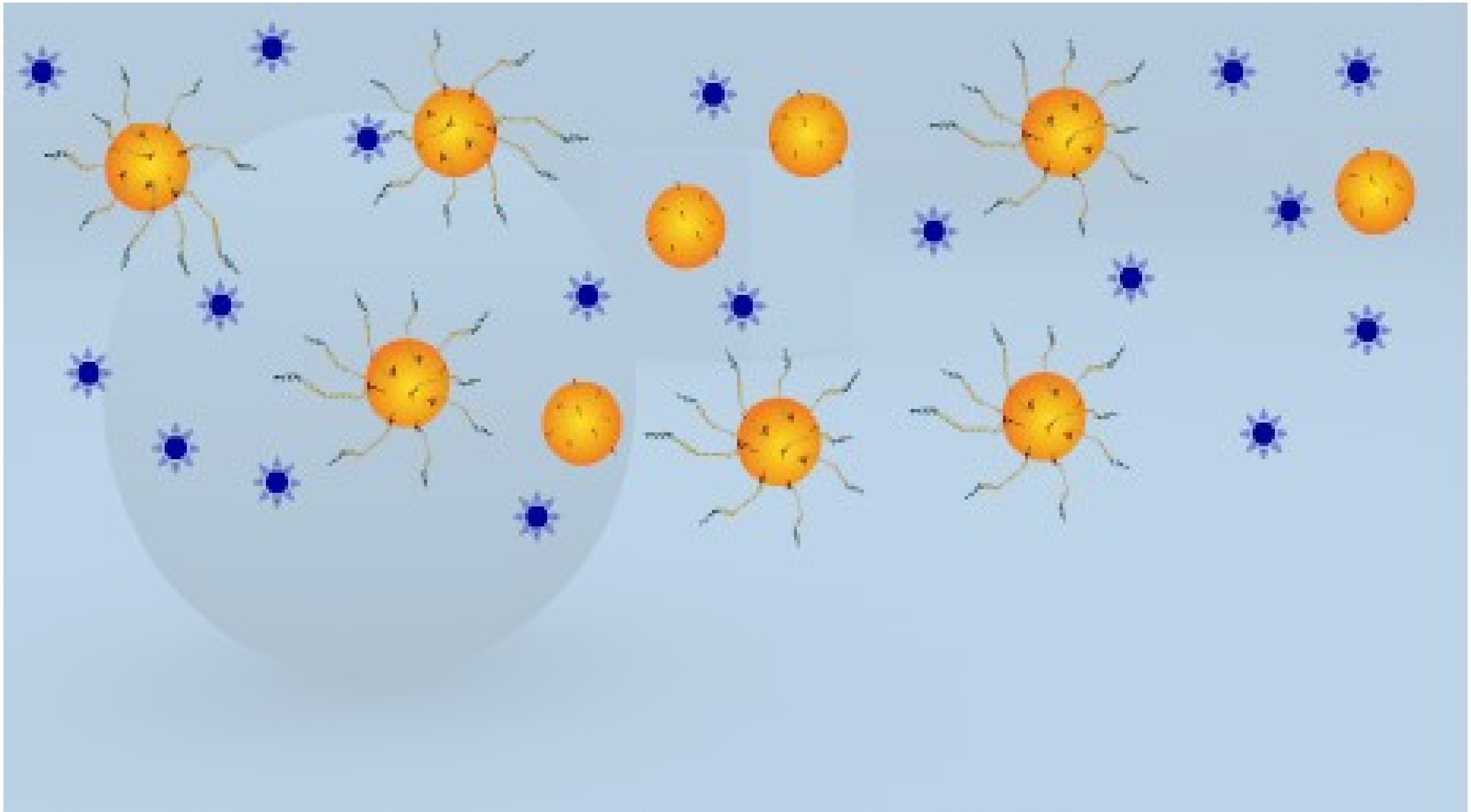


emPCR

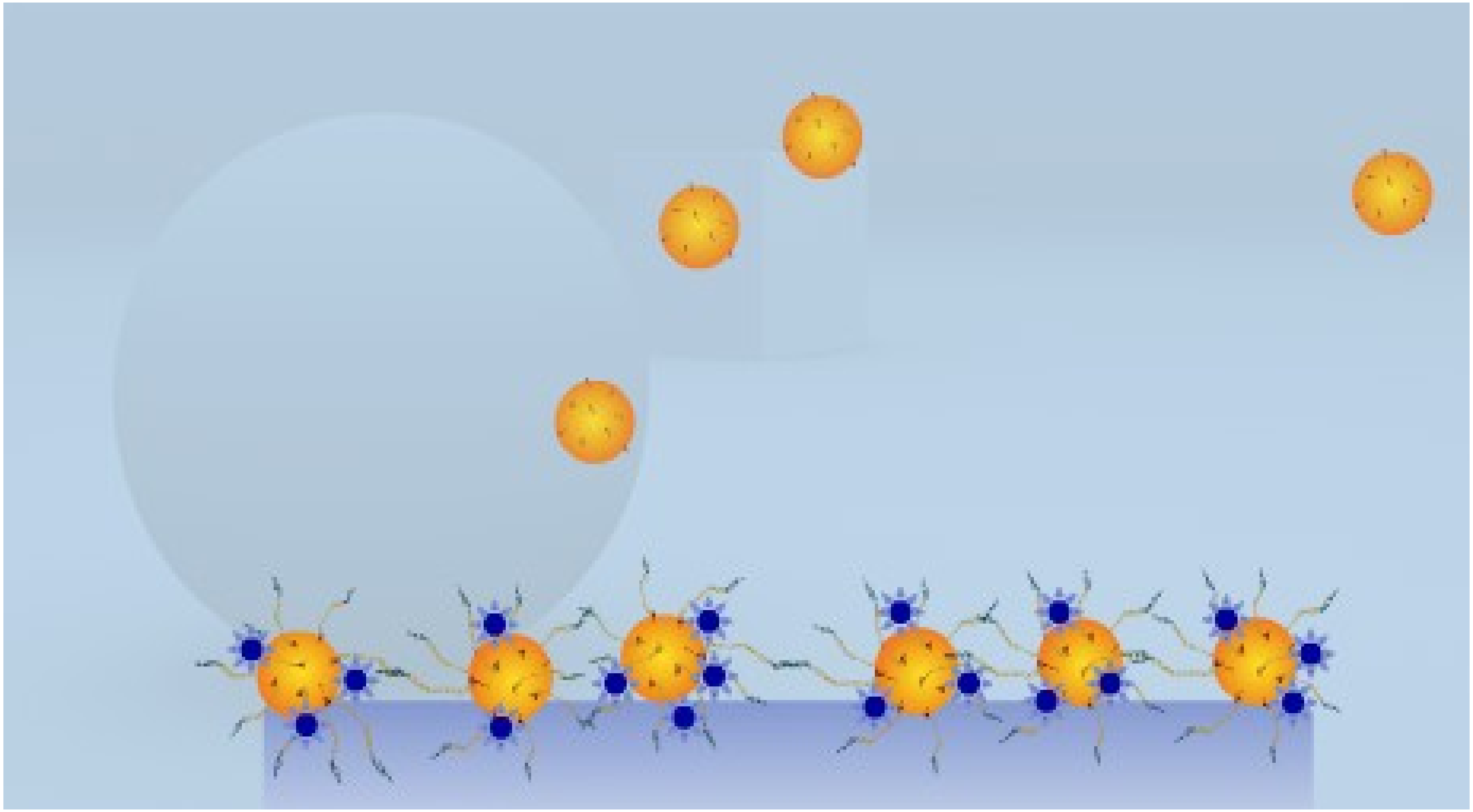


10 Million copies
of a single DNA
fragment per bead

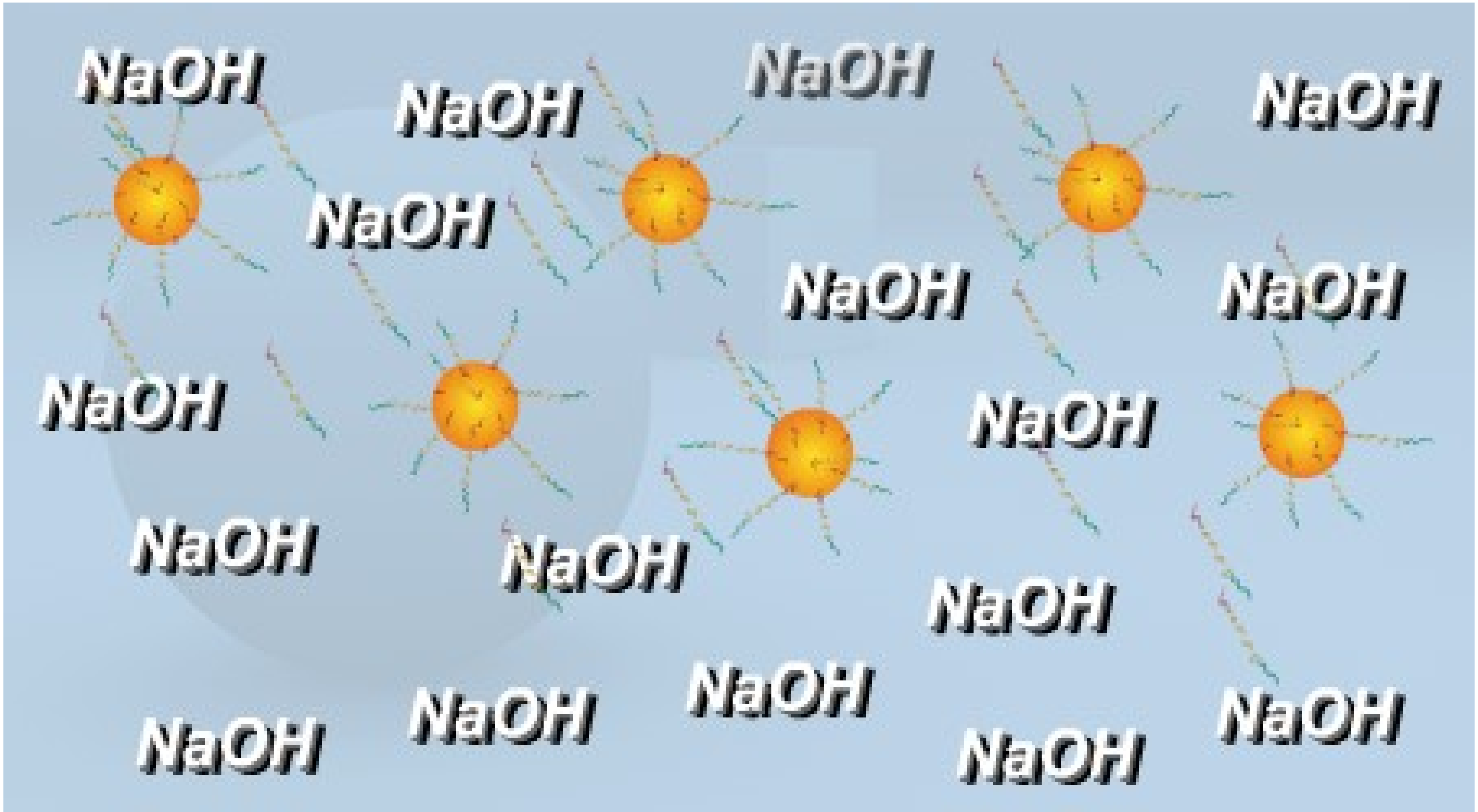
Vychytání kuliček



Vychytání kuliček



denaturace


NaOH
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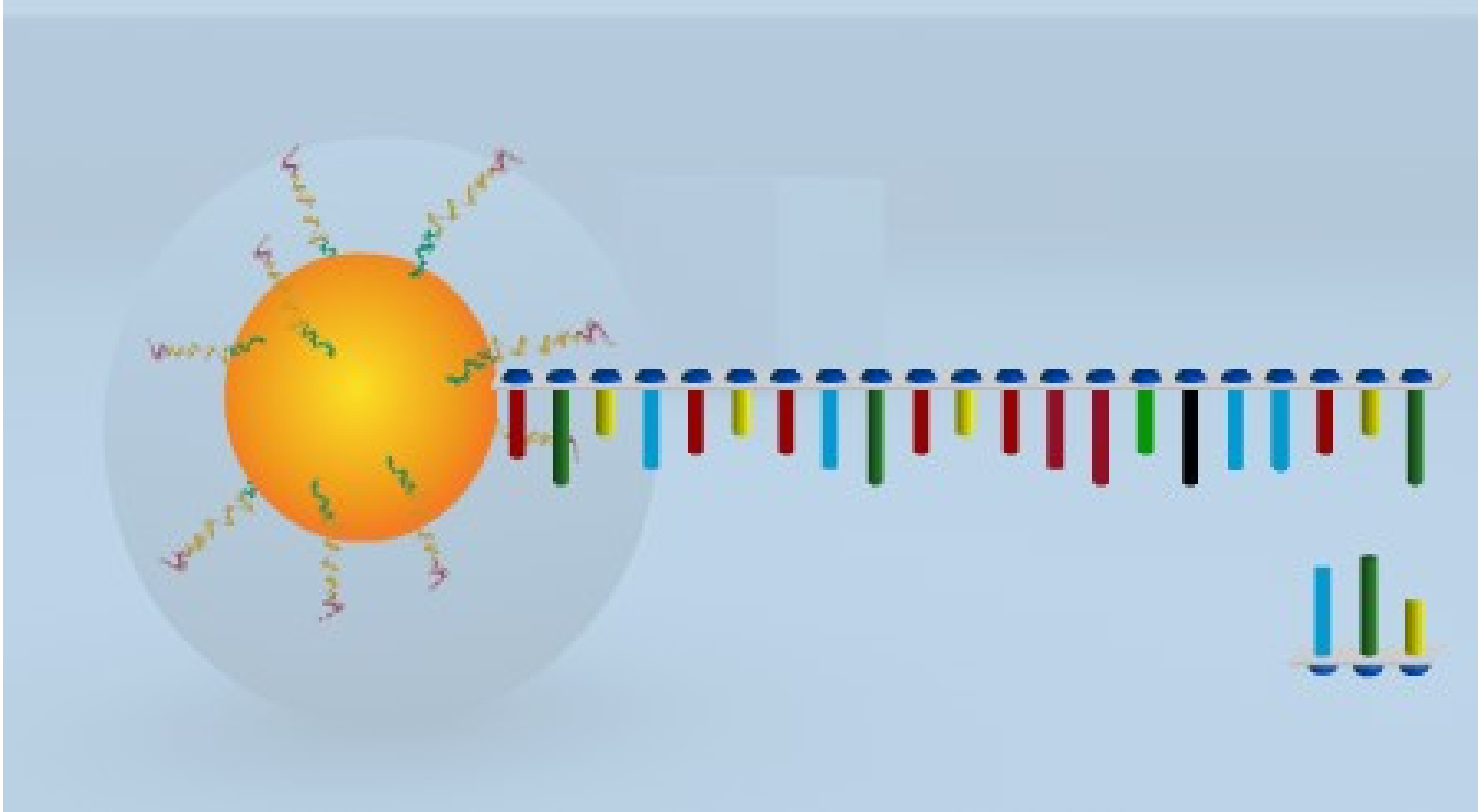
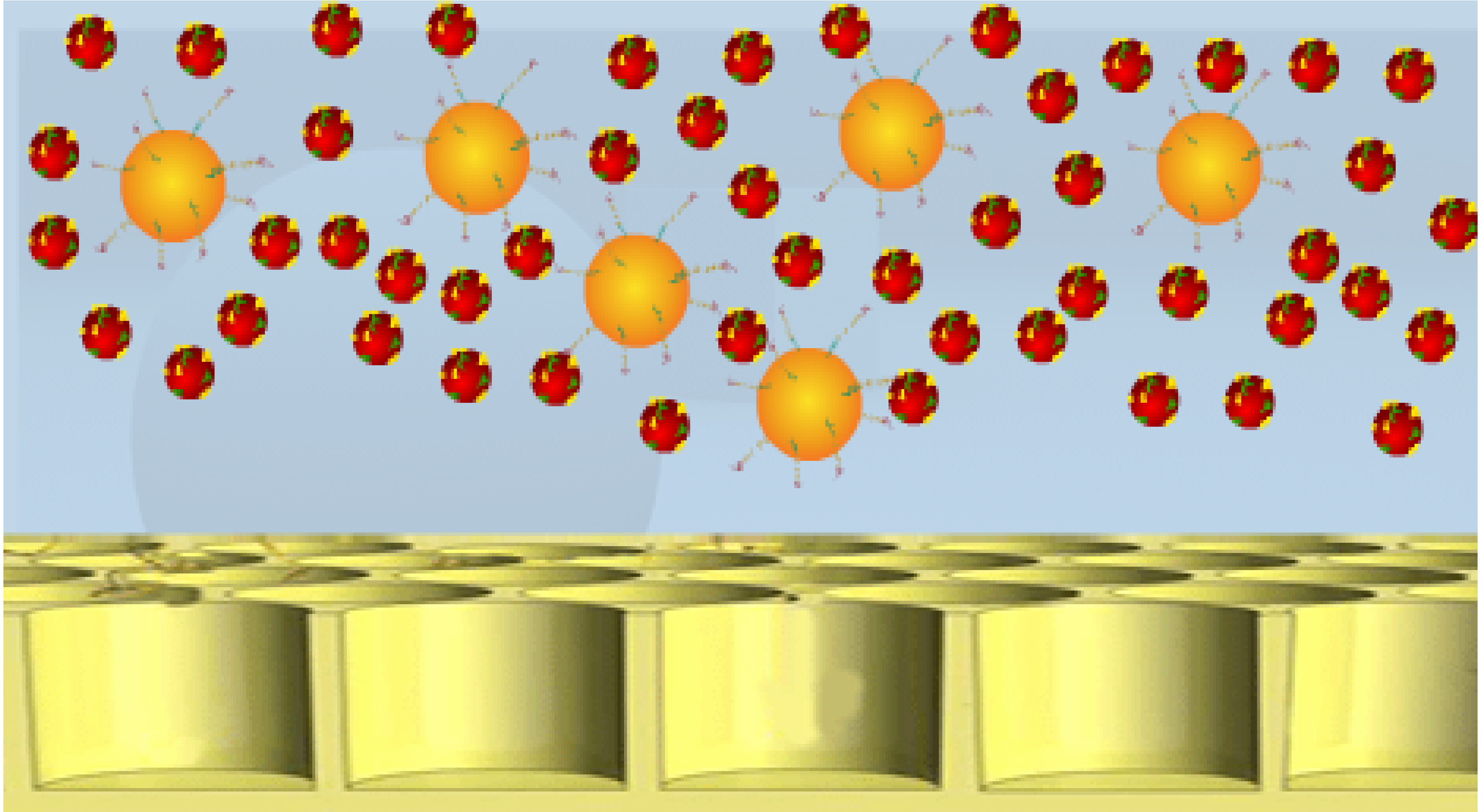
NaOH

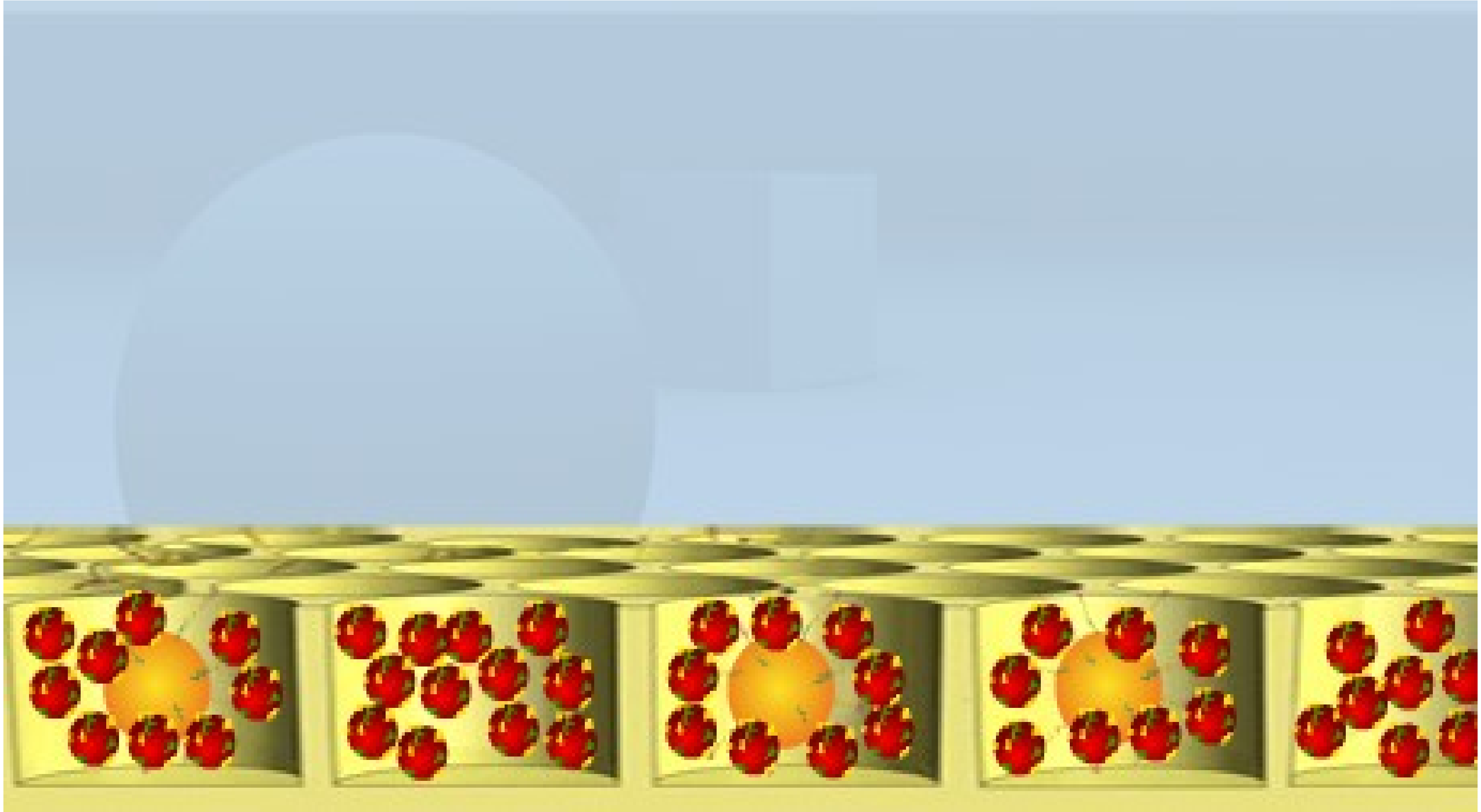
Sekvenační primer



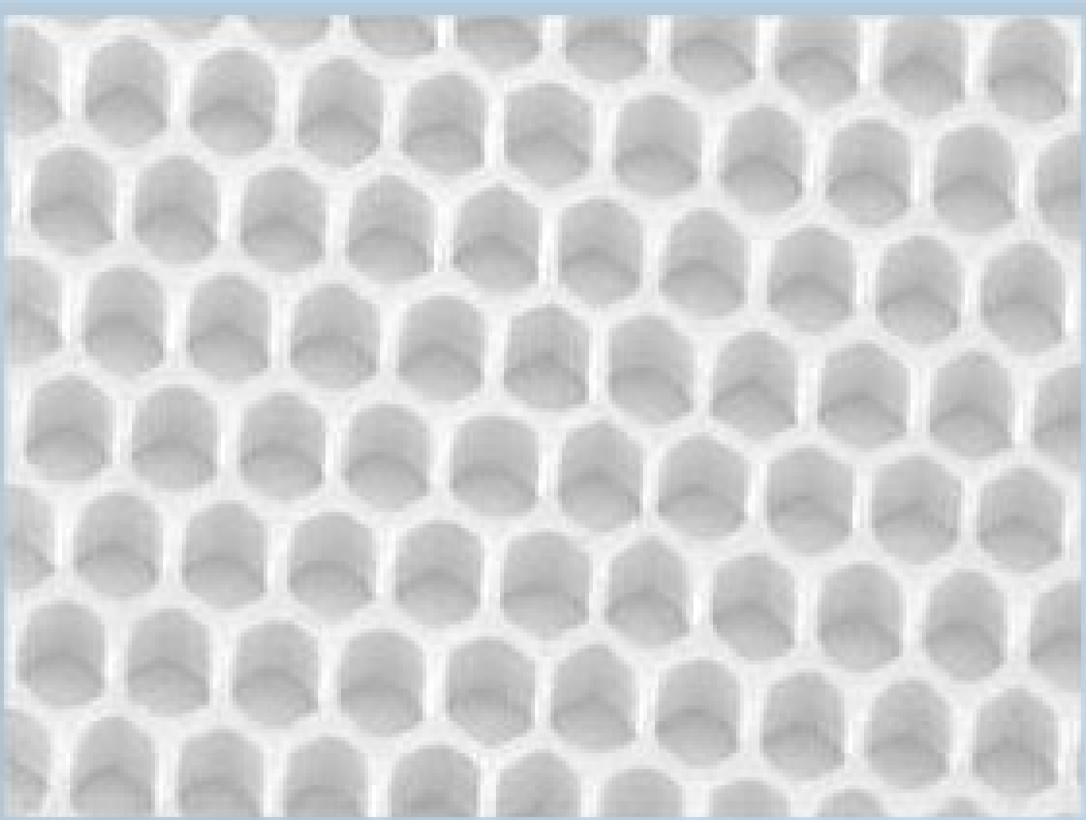
Disperze na sklíčko



Disperze na sklíčko



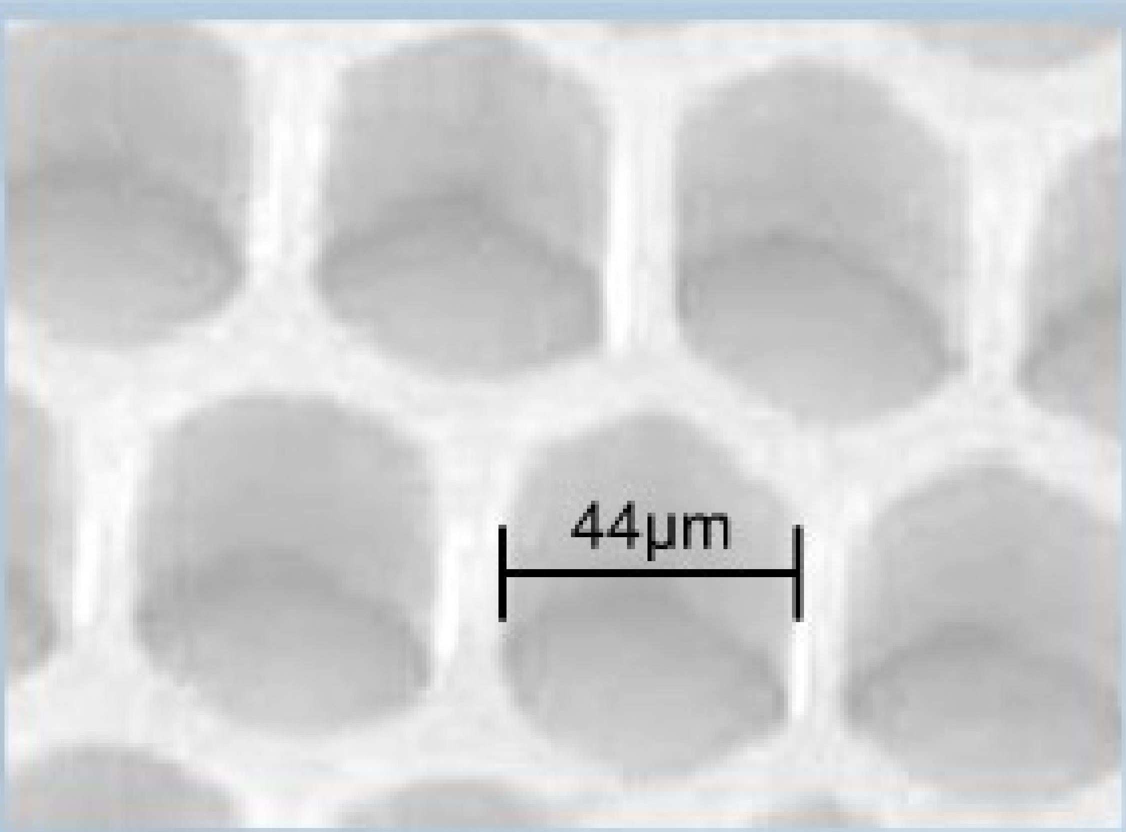
Parametry mikroreaktorů



1.6 million wells

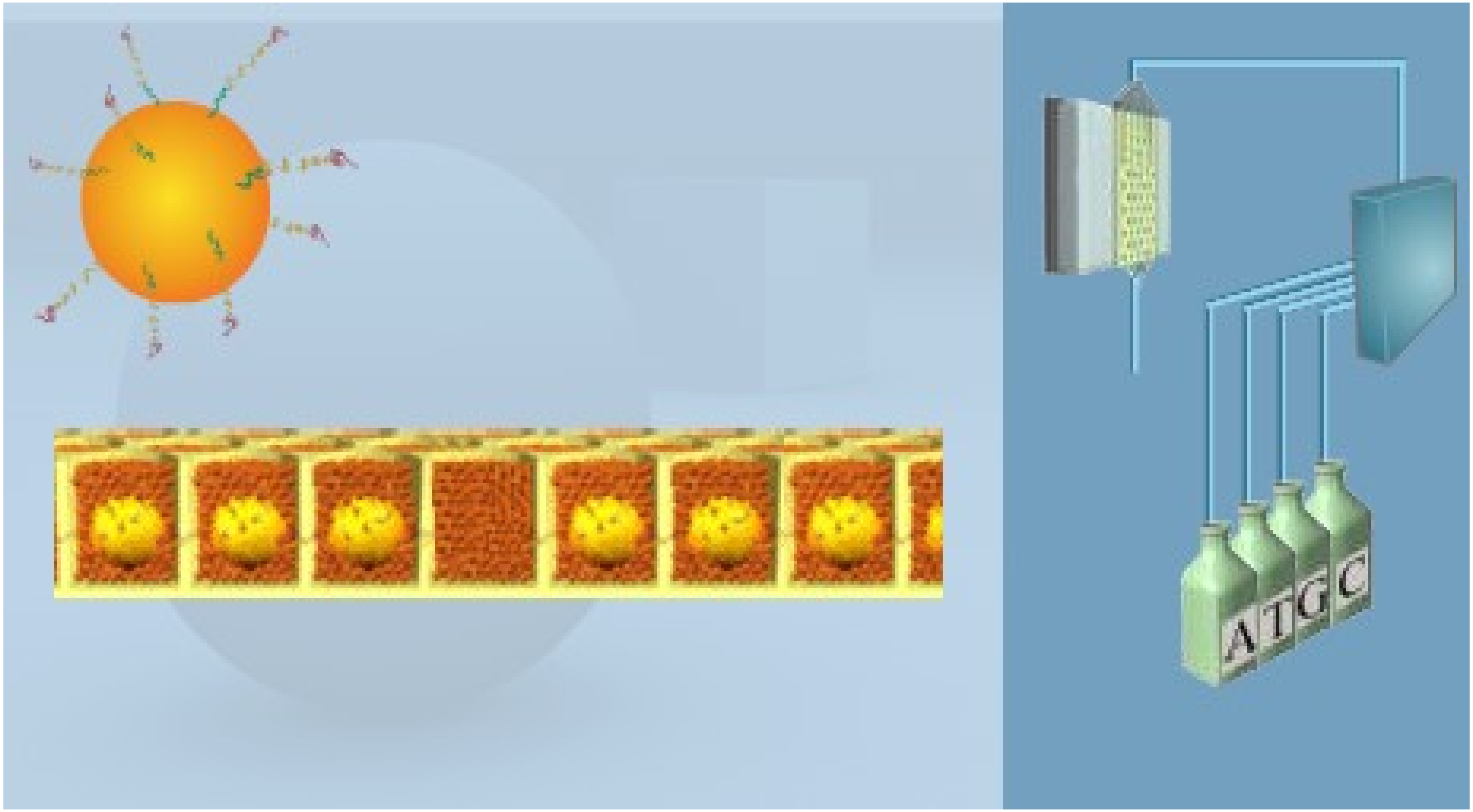
PicoTiterPlate device

Parametry mikroreaktorů

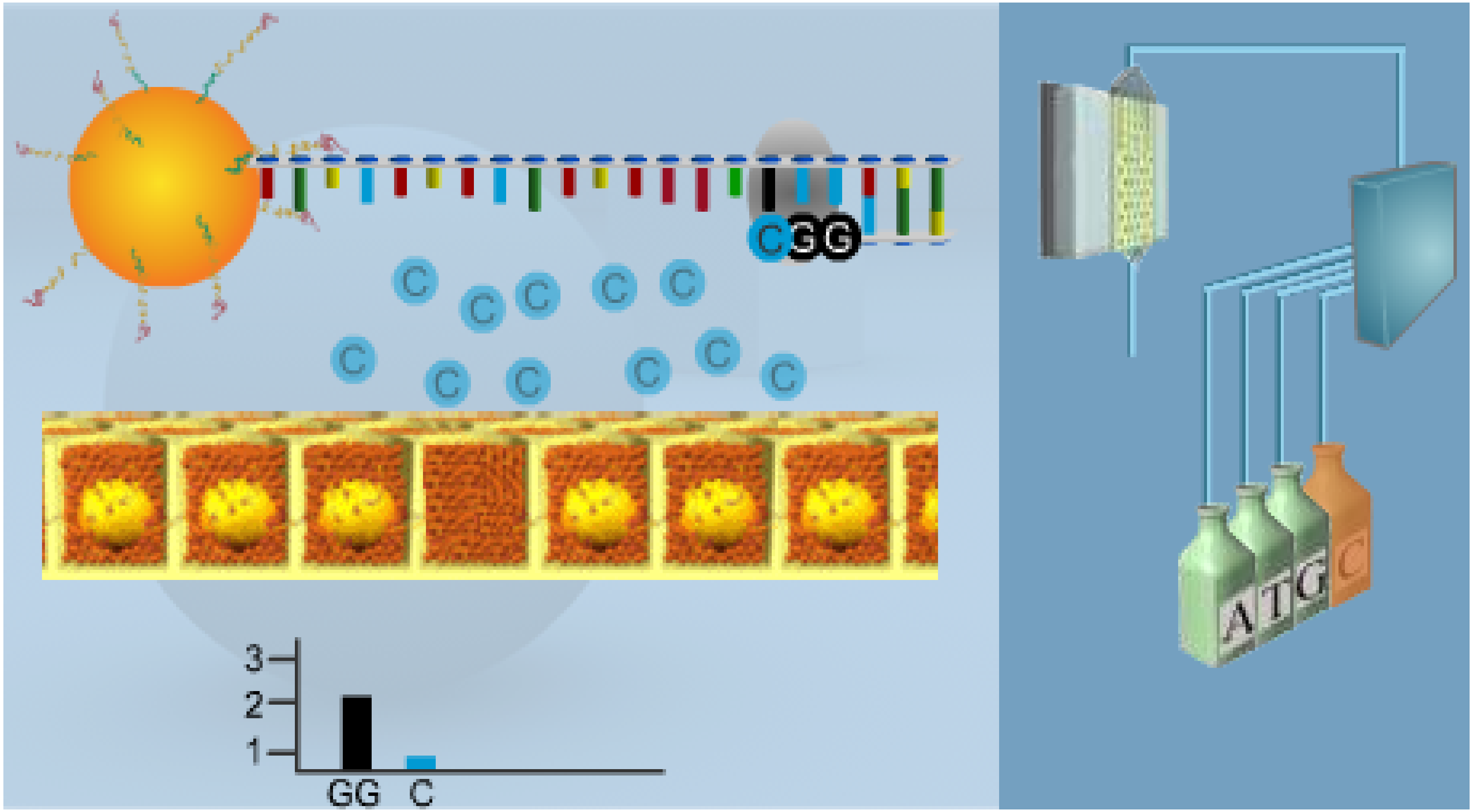


PicoTiterPlate device

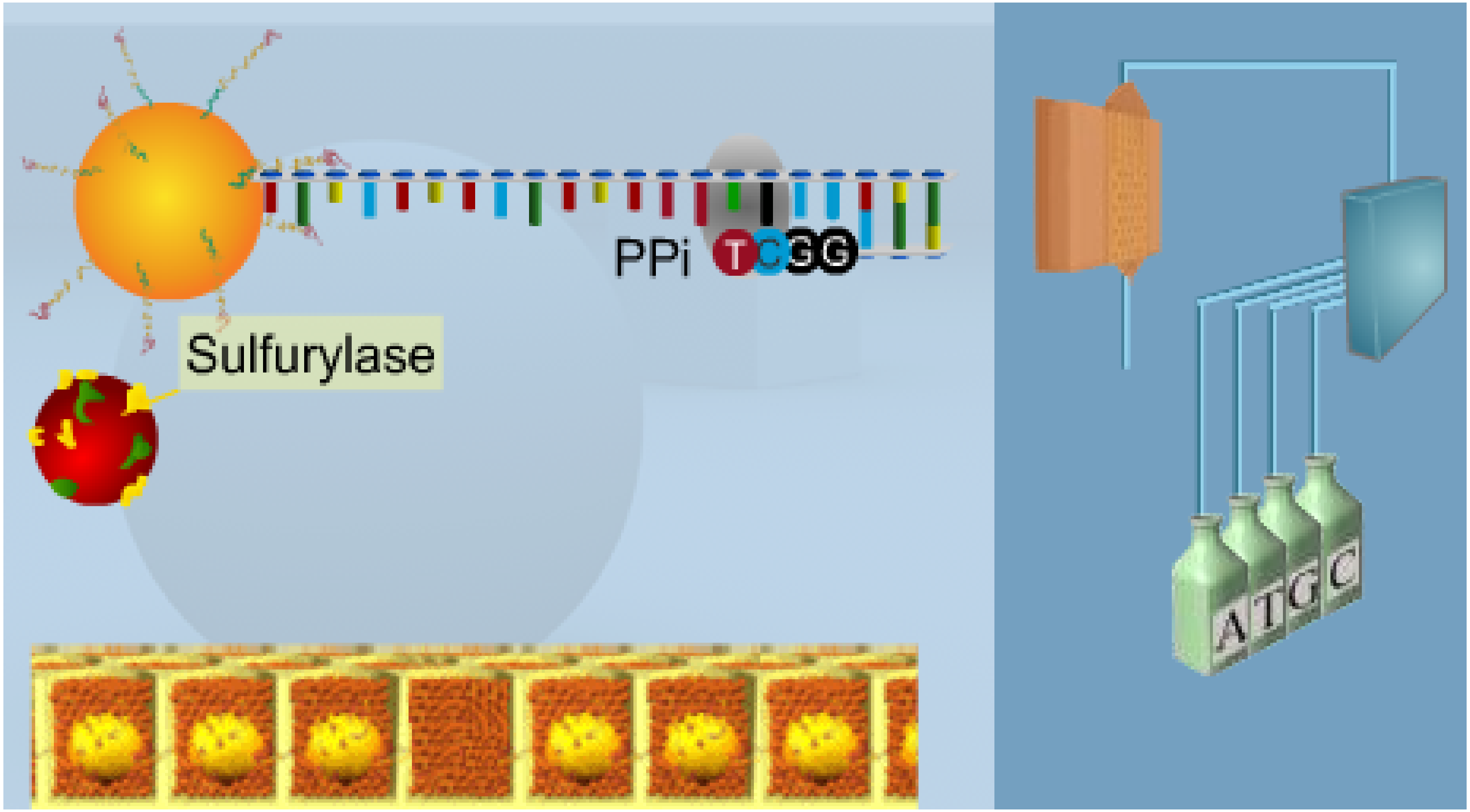
sekvenace



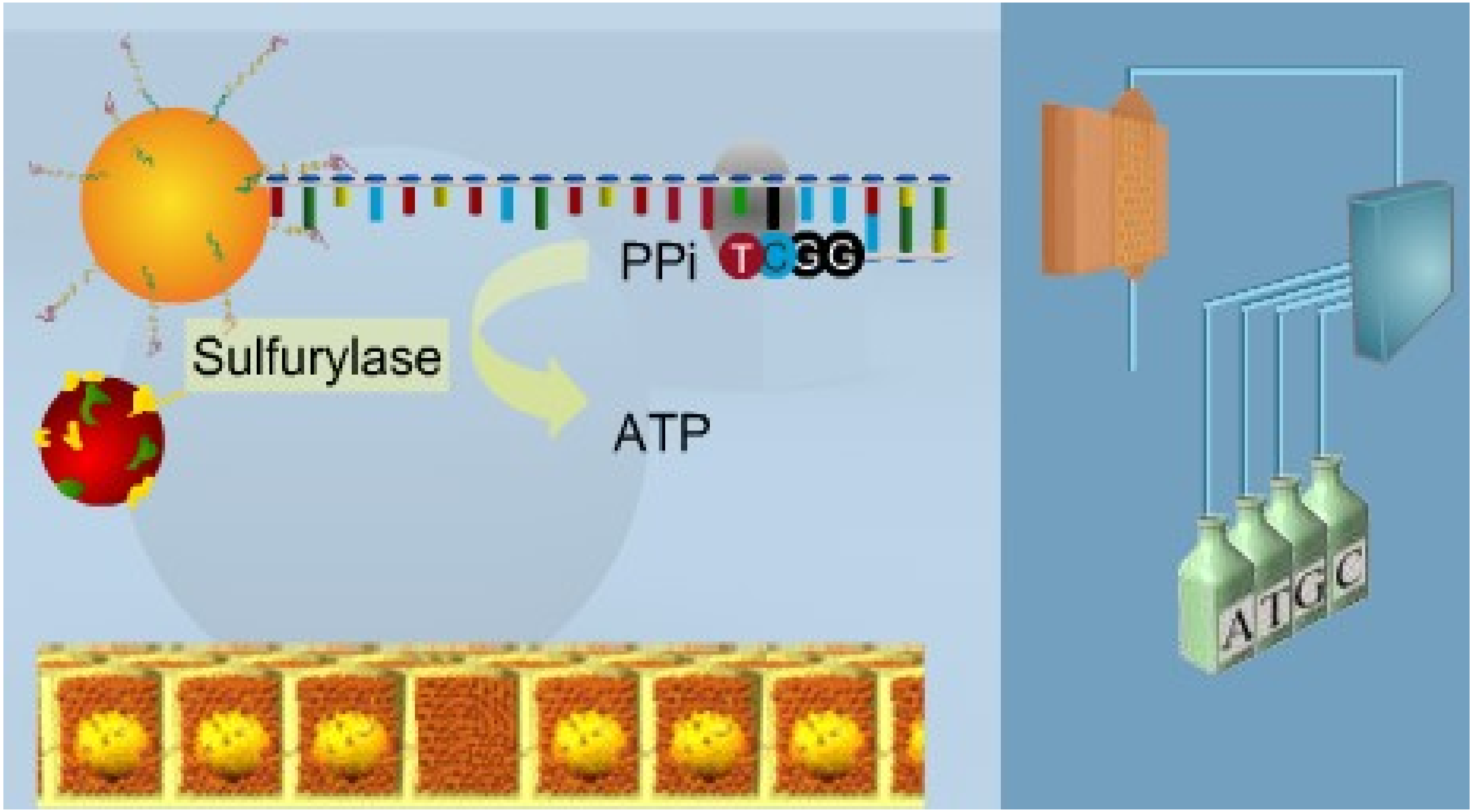
sekvenace



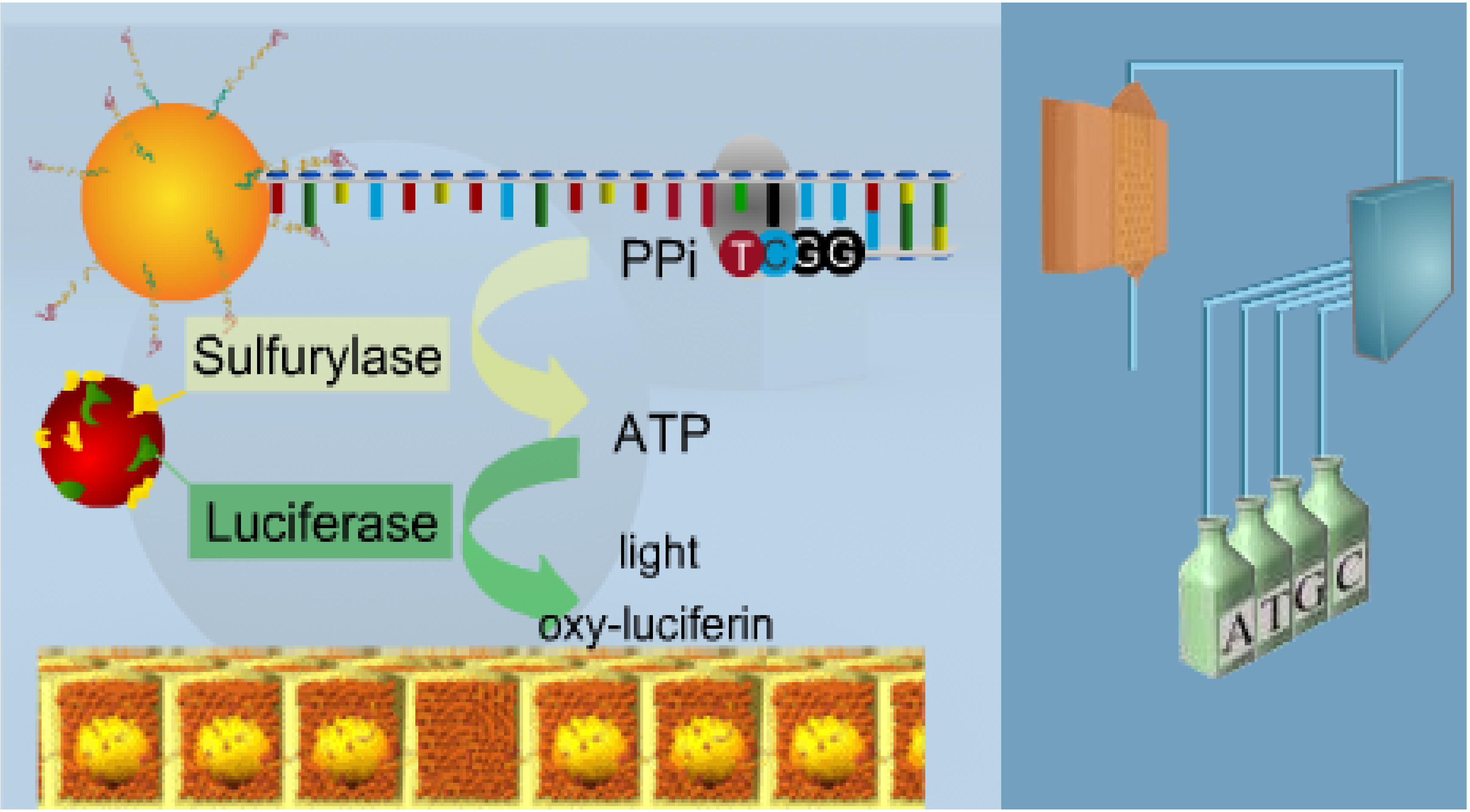
sekvenace



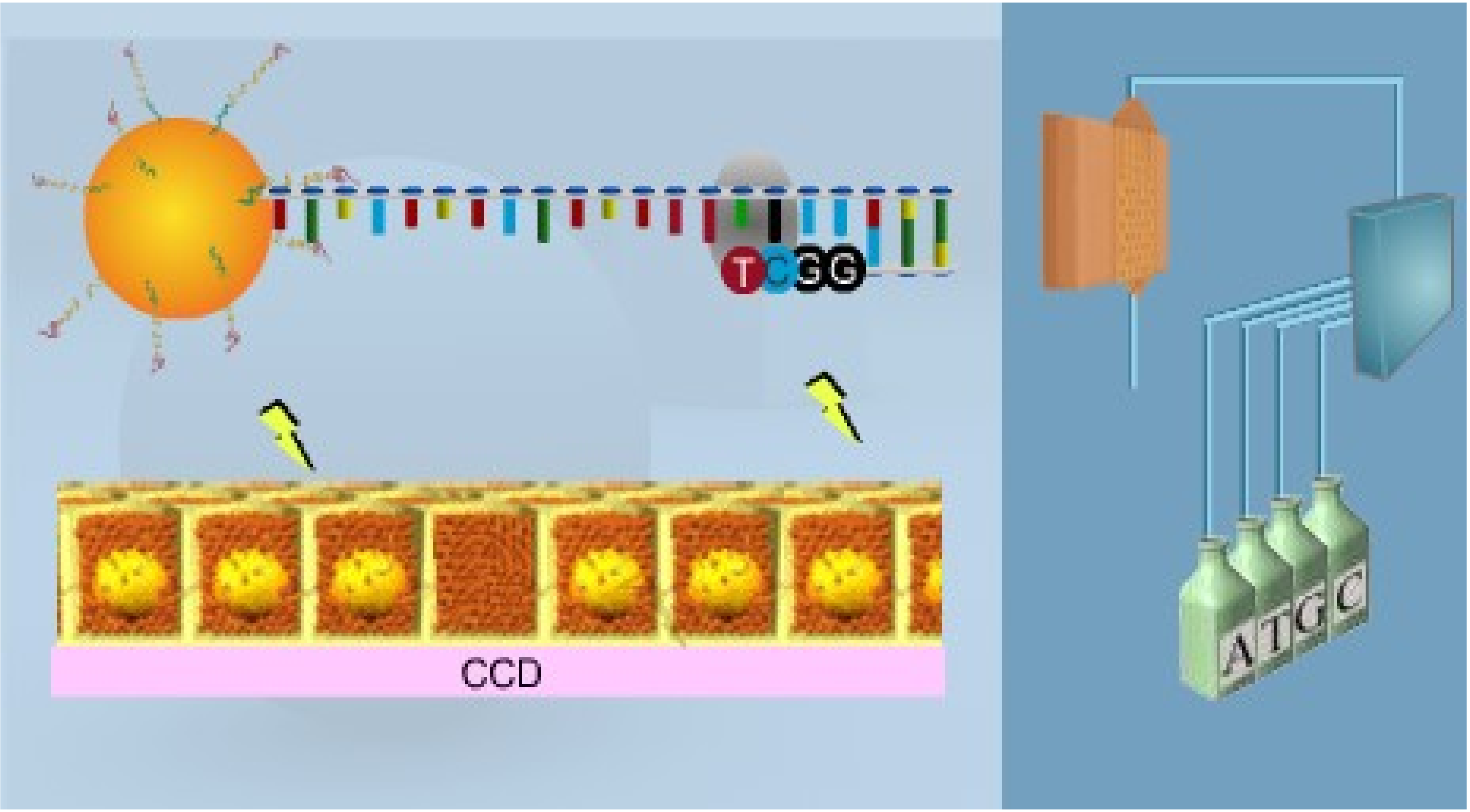
sekvenace



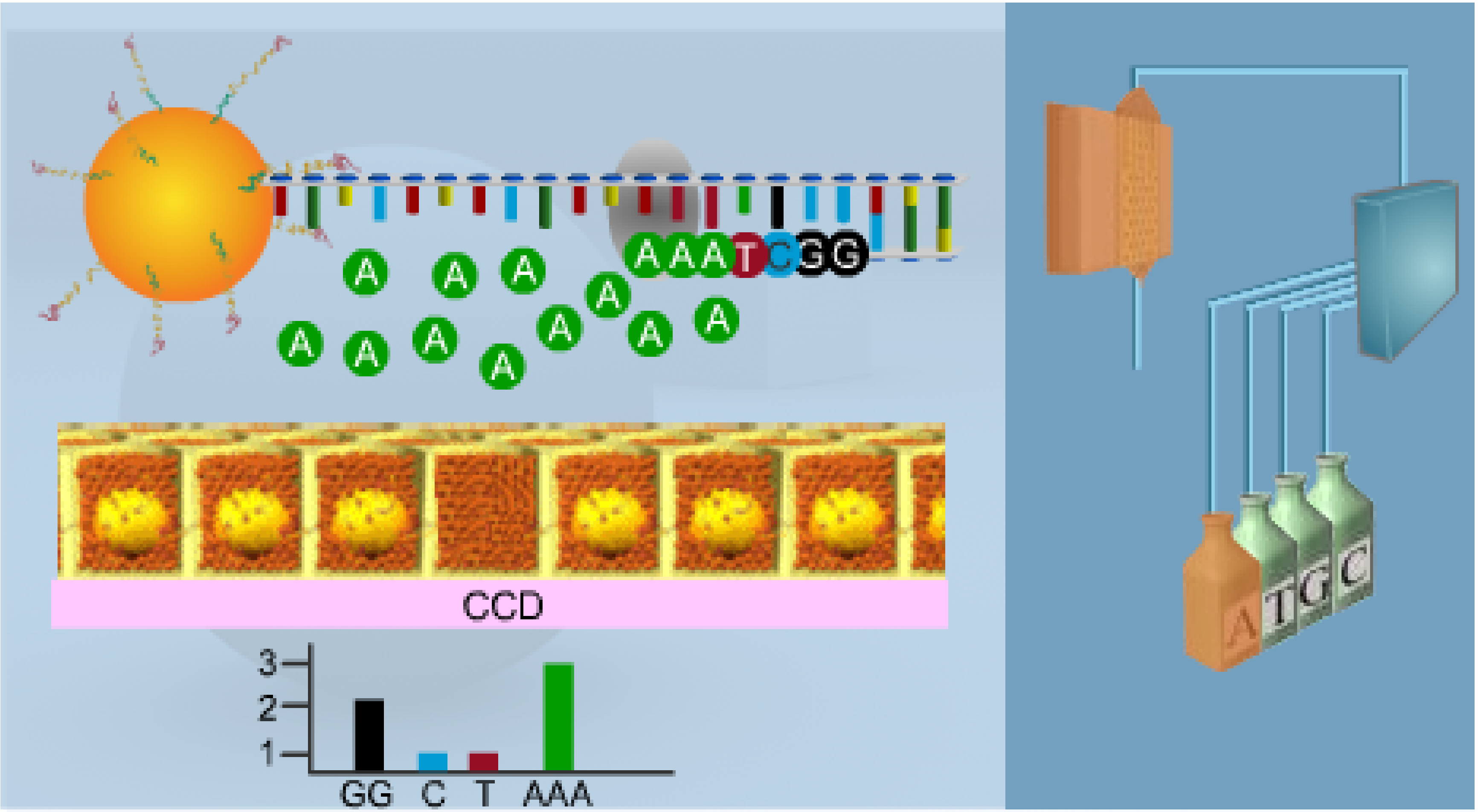
sekvenace


PPi
T
C
G
G
Sulfurylase
ATP
Luciferase
light
oxy-luciferin
A
T
G
C

sekvenace



sekvenace



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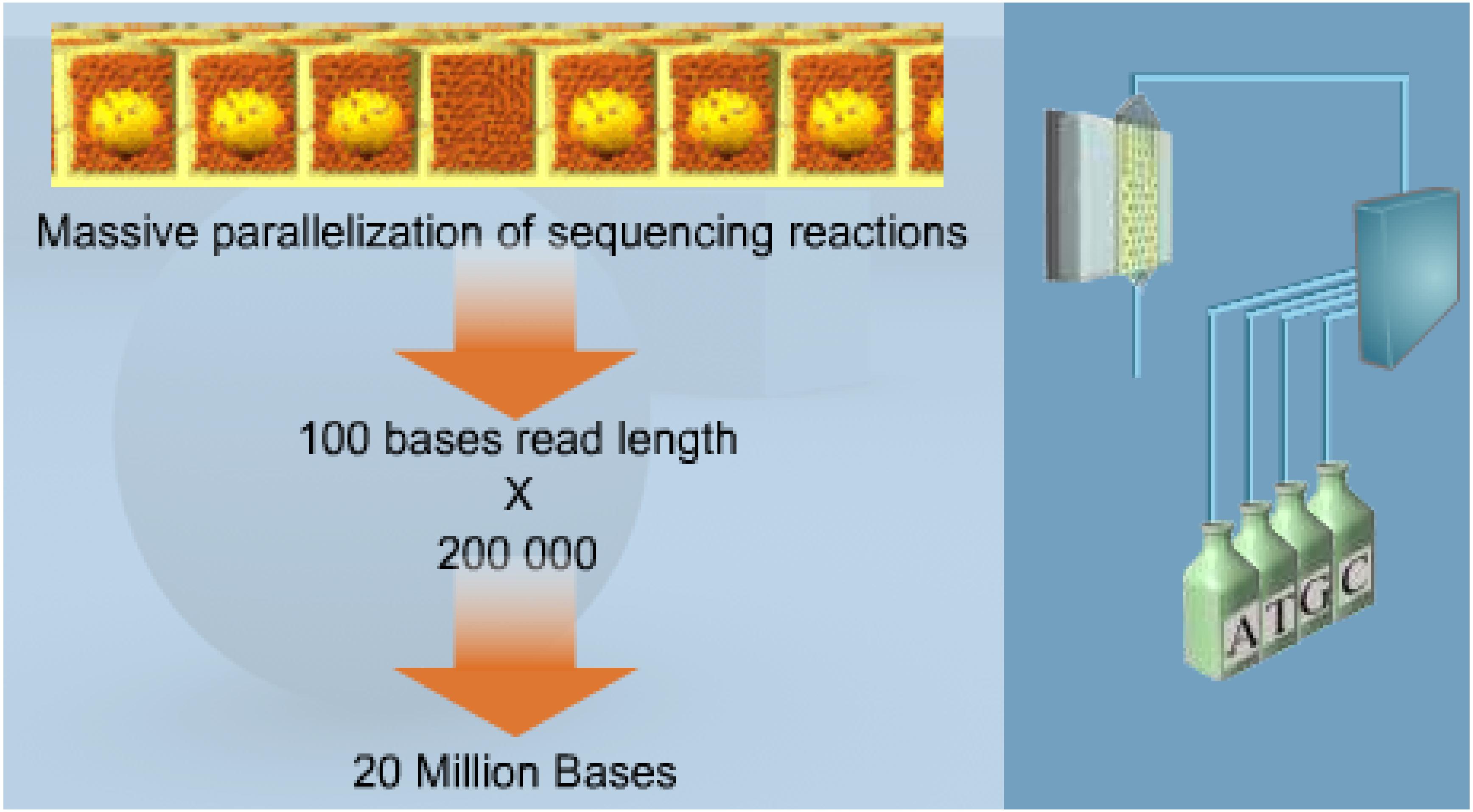


Massive parallelization of sequencing reactions

100 bases read length

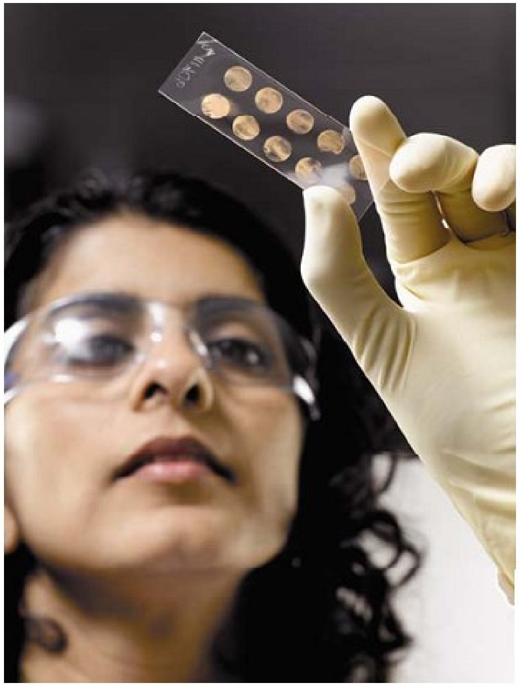
X

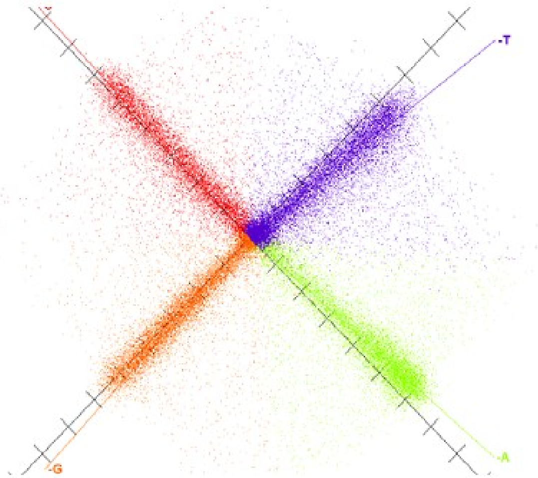
200 000

20 Million Bases

SOLID (Sequencing by Oligonucleotide Ligation and Detection)

2-base encoding sequencing (2007)



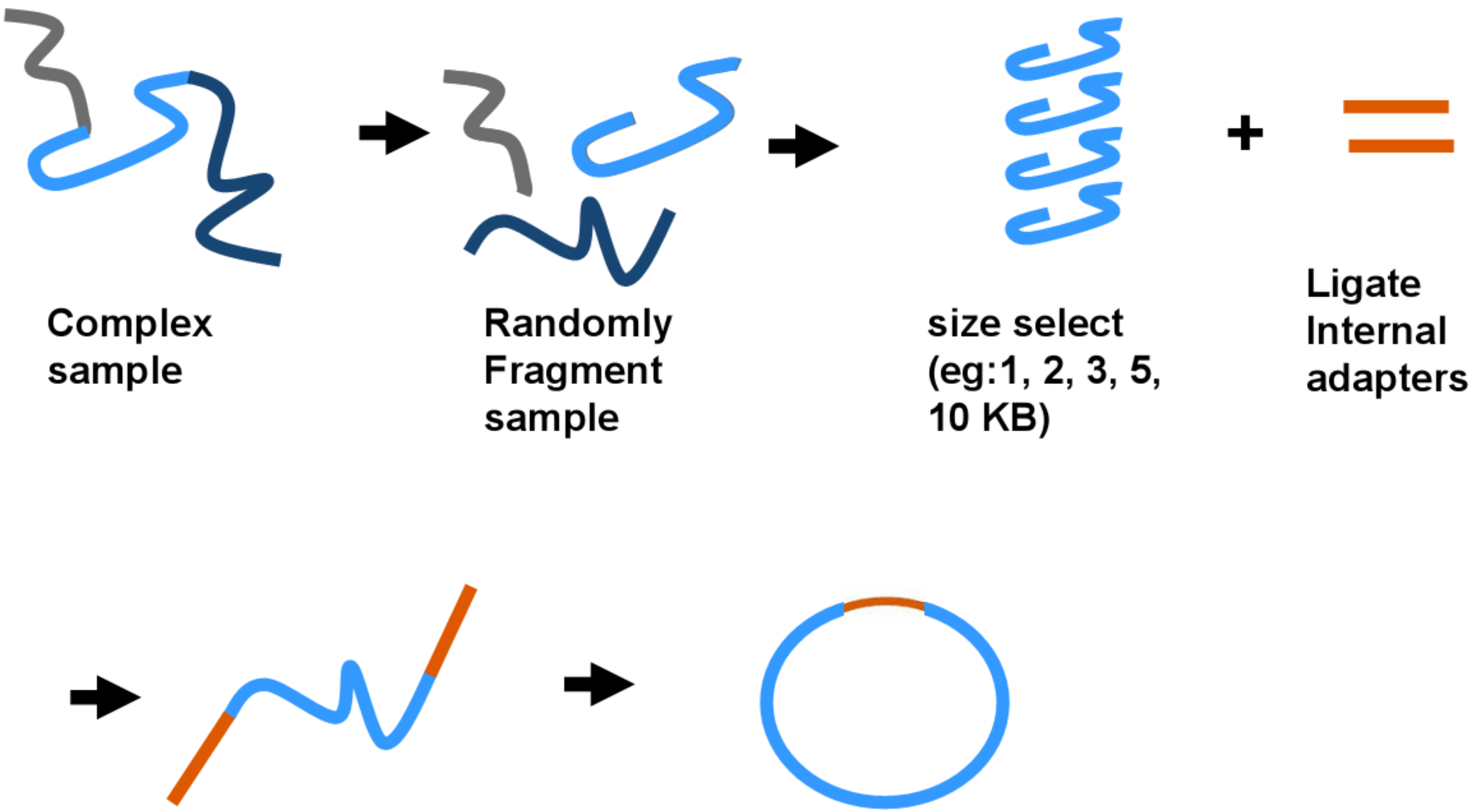


Applied Biosystems

SOLiD™ System

Sequencing by **O**ligonucleotide **Li**gation and **D**etection

Creating a mate-paired library (i)



Complex sample

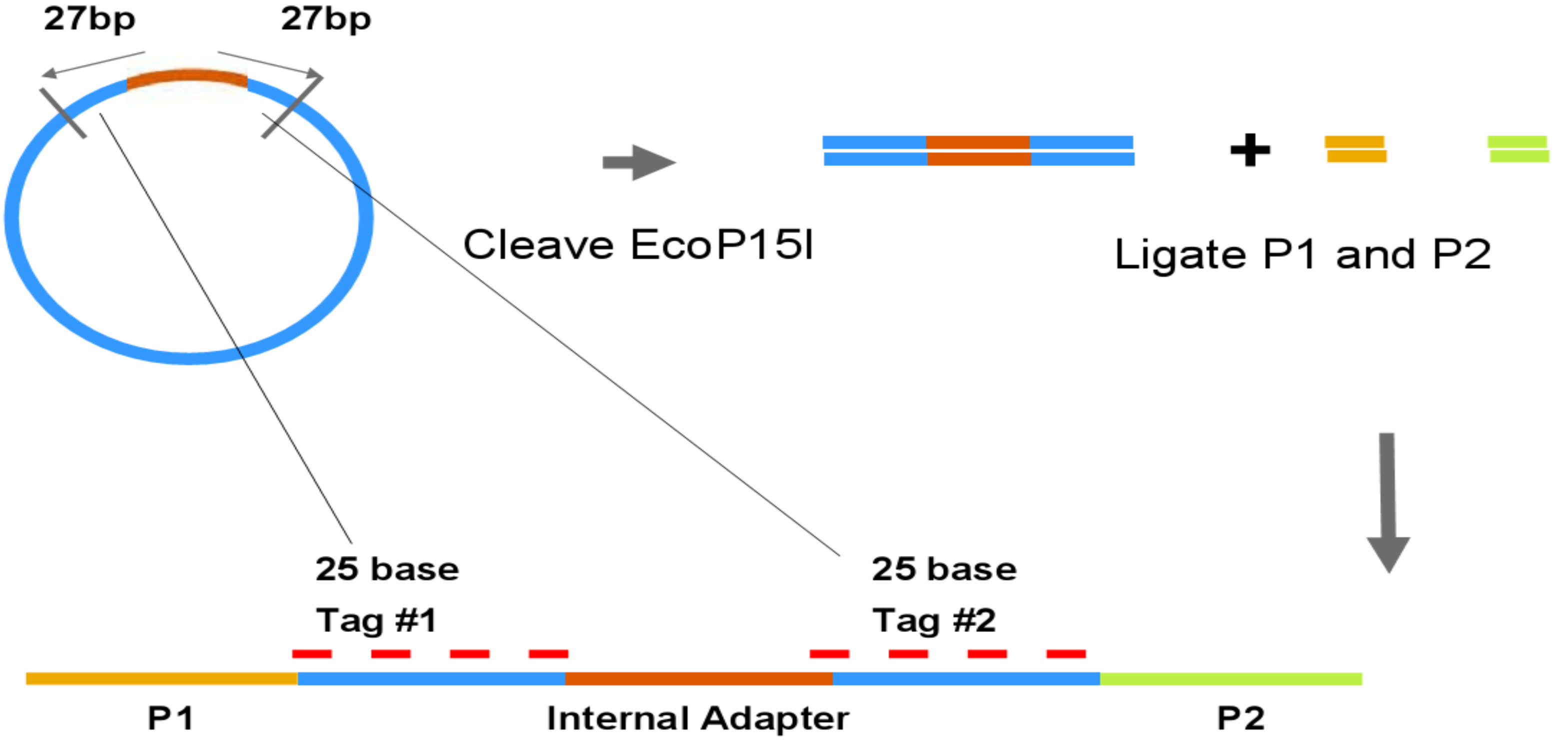
Randomly Fragment sample

size select (eg:1, 2, 3, 5, 10 KB)

\+ Ligate Internal adapters

Circularize

Creating a mate-paired library (ii)



EcoP15I

Nomenclature Update

Catalog #	Size	Concentration	Price	Qty	
R0646S	25 units	2,000 units/ml	$61.00	1	ADD TO CART
R0646L	125 units	2,000 units/ml	$244.00	1	ADD TO CART

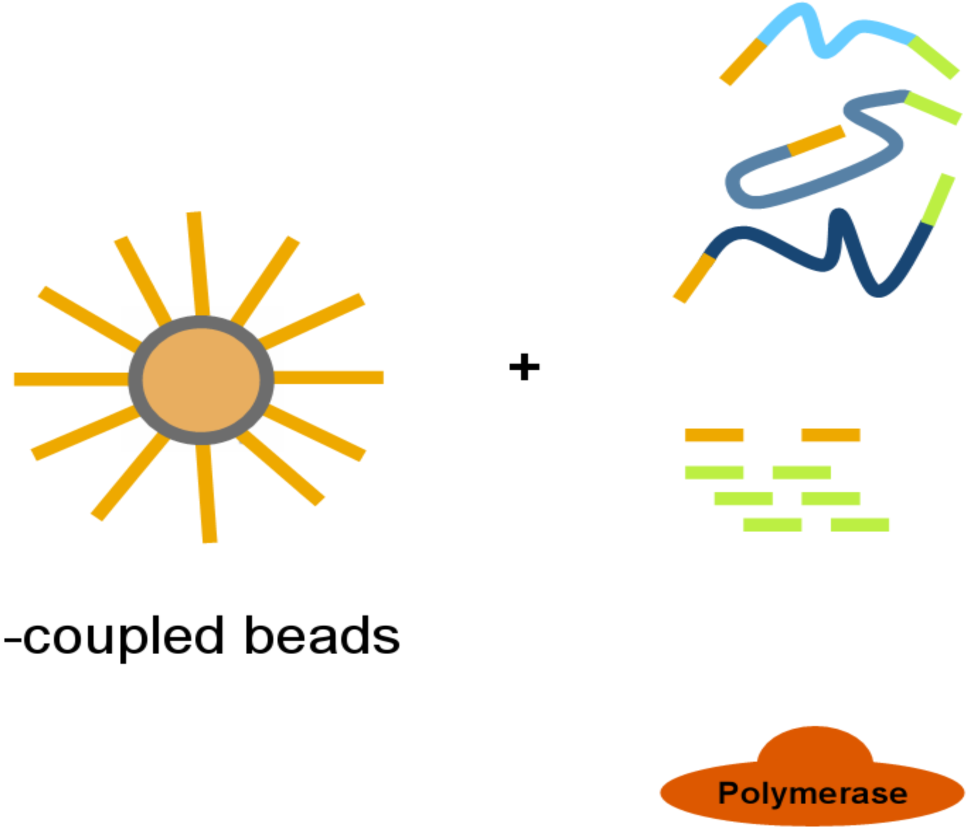
Prices are in US dollars and valid only for US orders.

Download: MSDS PDF

Recognition Site:

$5'...\text{CAGCAG}(N)_{25}{}^{\blacktriangledown}...3'$
$3'...\text{GTCGTC}(N)_{27}{}_{\blacktriangle}...5'$

Emulsion PCR



P1-coupled beads

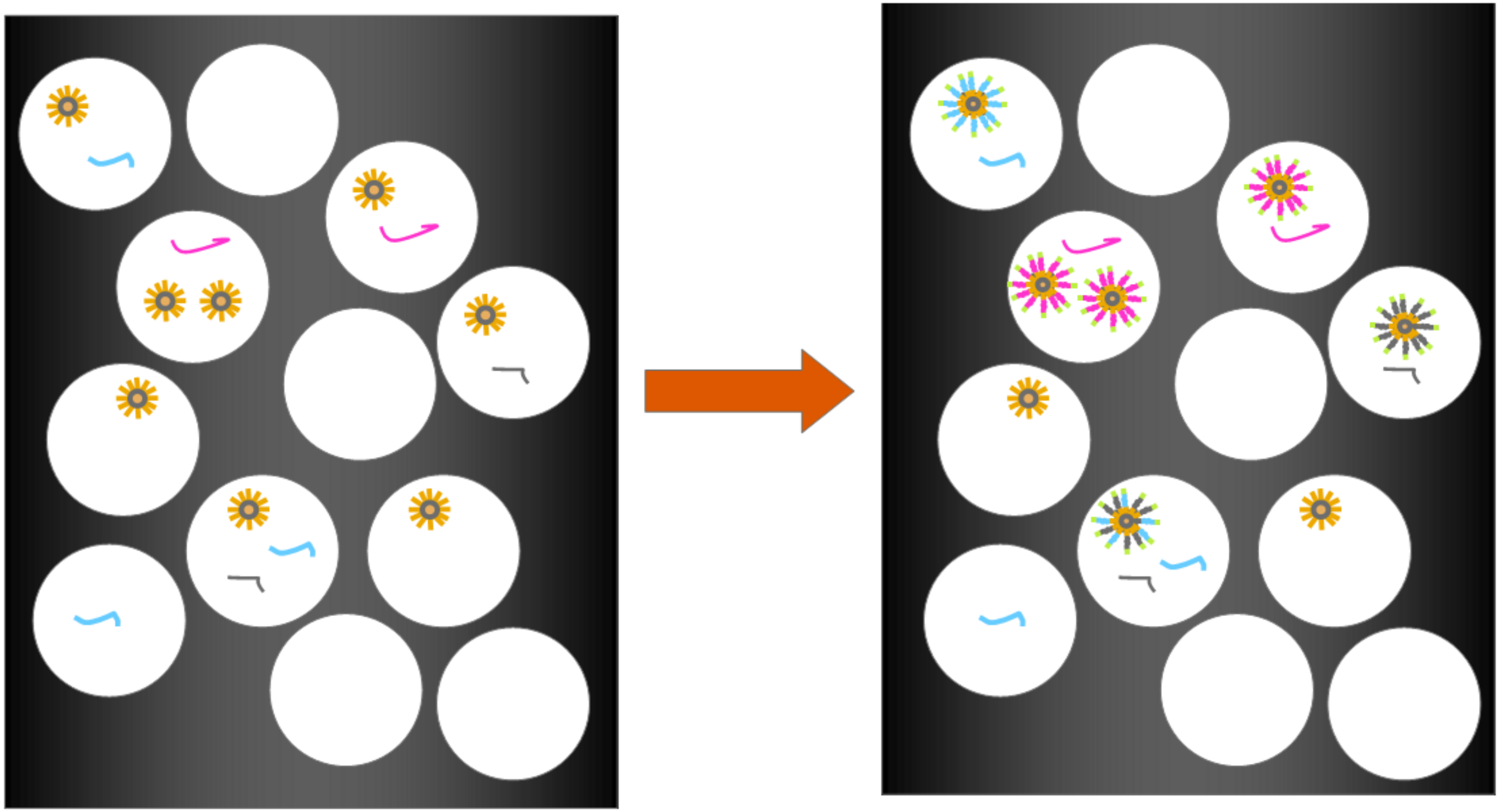
Templates

Primers P1<<P2

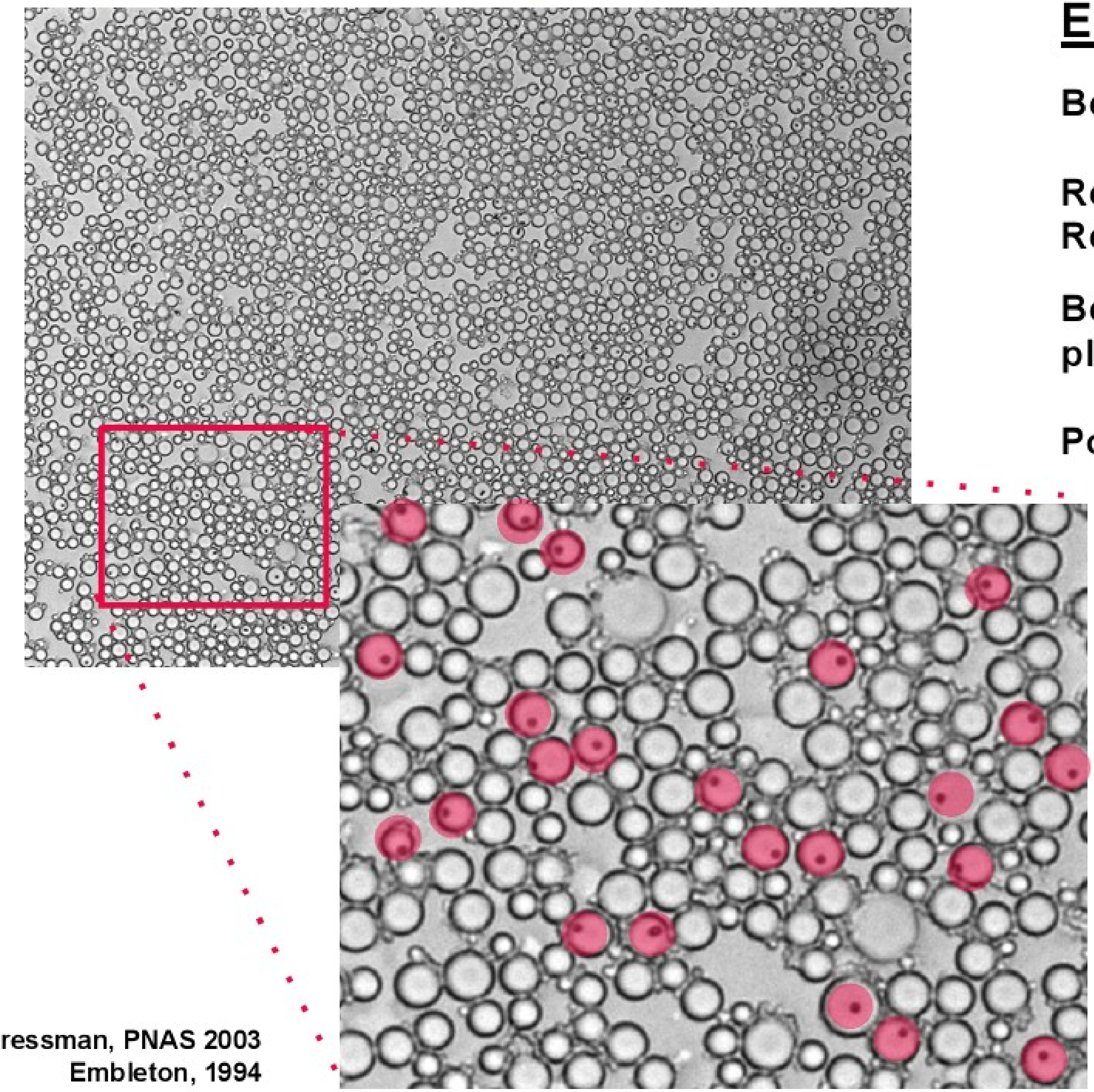
Enzyme

Emulsion PCR

Mix PCR aqueous phase into a water-in-oil (w/o) emulsion and carry out emulsion PCR



SOLiD system: Clonal Amplification

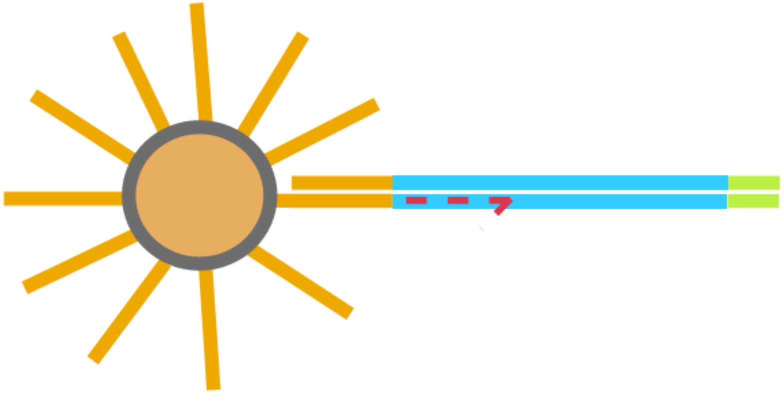


Dressman, PNAS 2003
Embleton, 1994

Emulsion Metrics

Bead size:	1 µm
Reactor size:	4 µm
Reactor volume:	34 fL
Beads / emulsion plate (96-well):	2-4 x 10^9
Post Enrichment:	~500M / plate

emulsion PCR Individual Bead



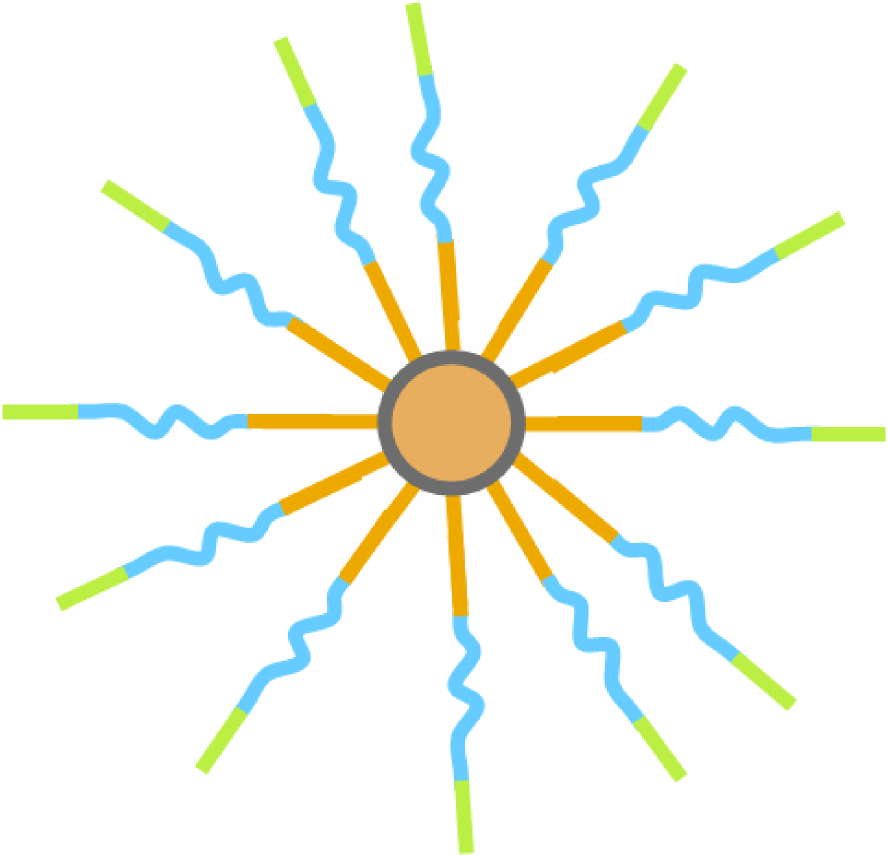
1) Template Anneals to P1

2) Polymerase extends from P1

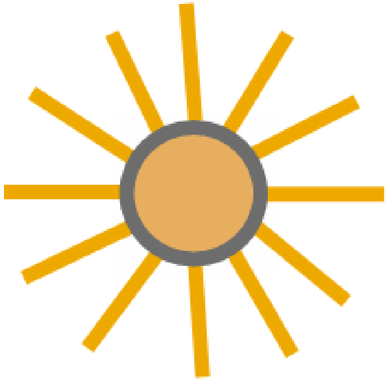
3) Complementary sequence is extended off bead surface

4) Template disassociates

Results of emulsion PCR, after breaking emulsion

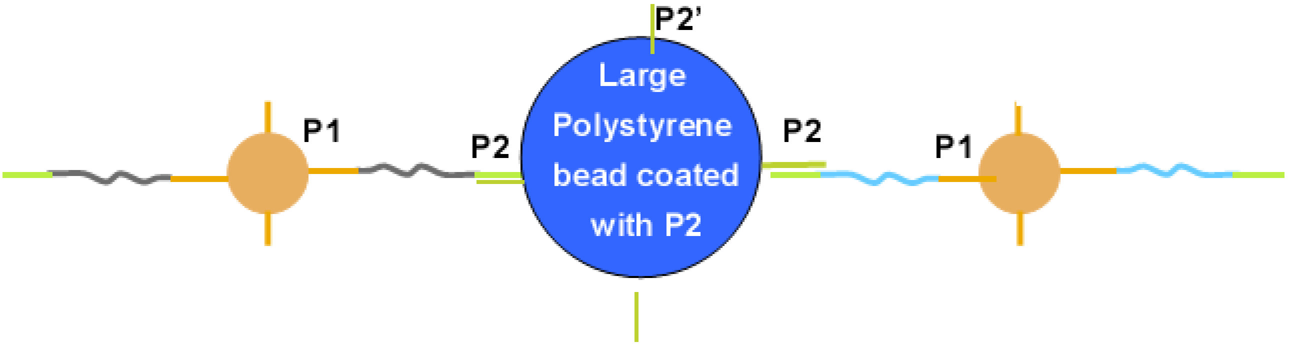


Bead contains ~20K amplified products from original single strand molecule

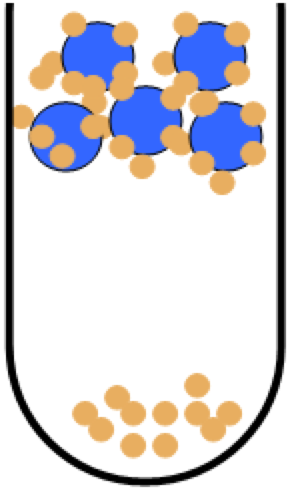


Beads with no product

Enrichment

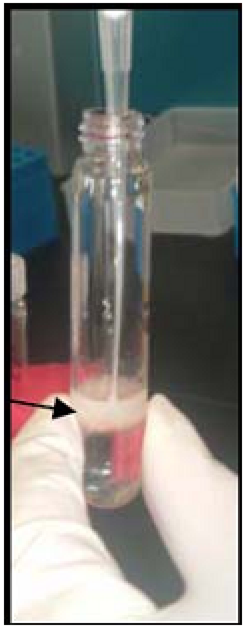


Centrifuge in glycerol gradient

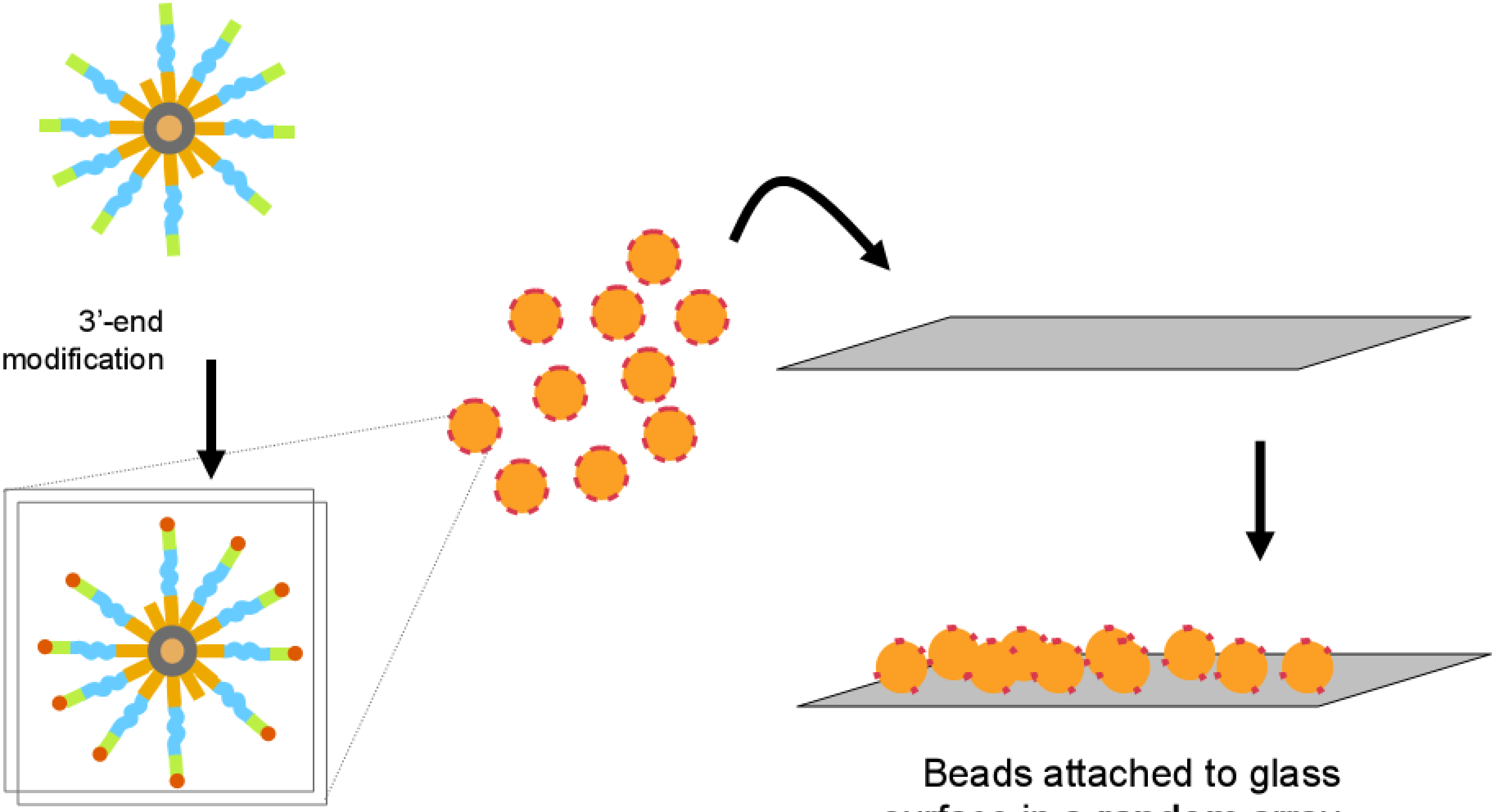


Supernatant
Captured beads with templates

Pellet
Beads with no template



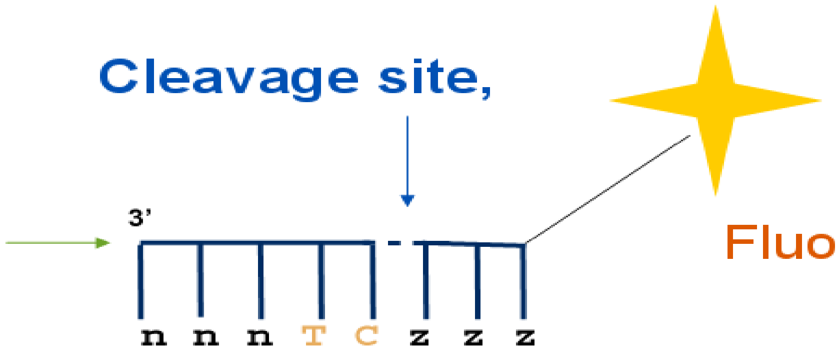
Bead Deposition



Beads attached to glass
surface in a **random** array

Properties of the Probes

Spatial separation among dye, ligation & cleavage sites



Cleavage site,

3' Ligation site

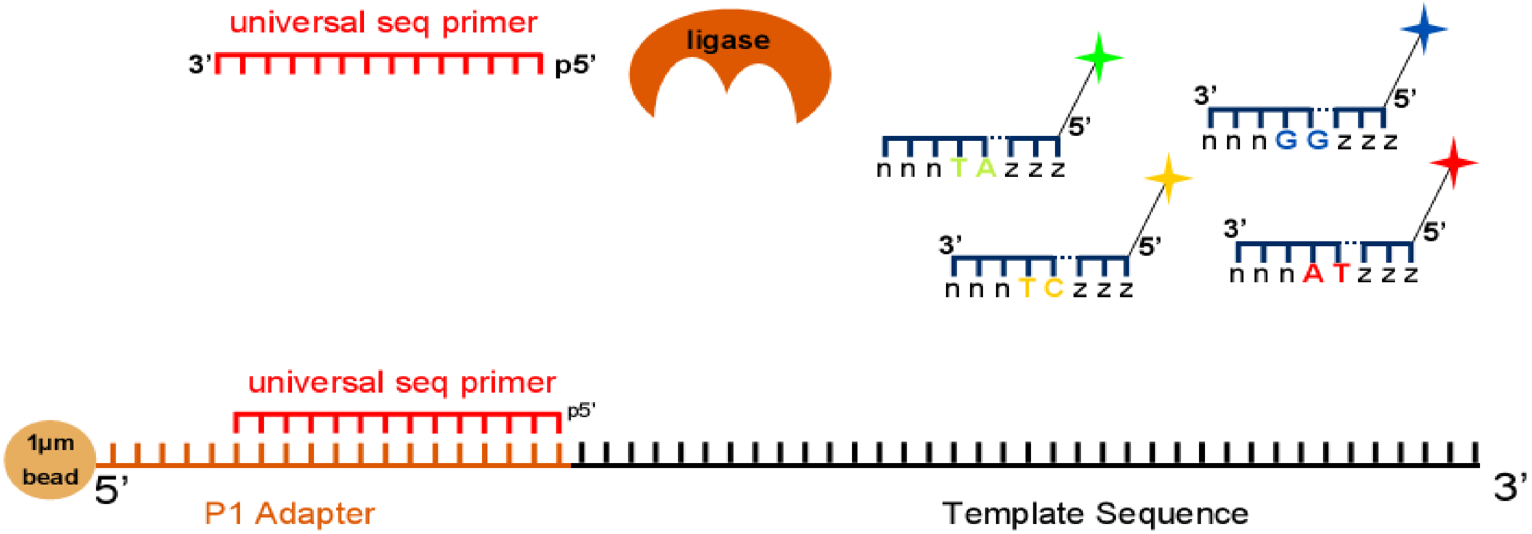
Fluorescent dye

1,024 Octamer Probes (4^5)

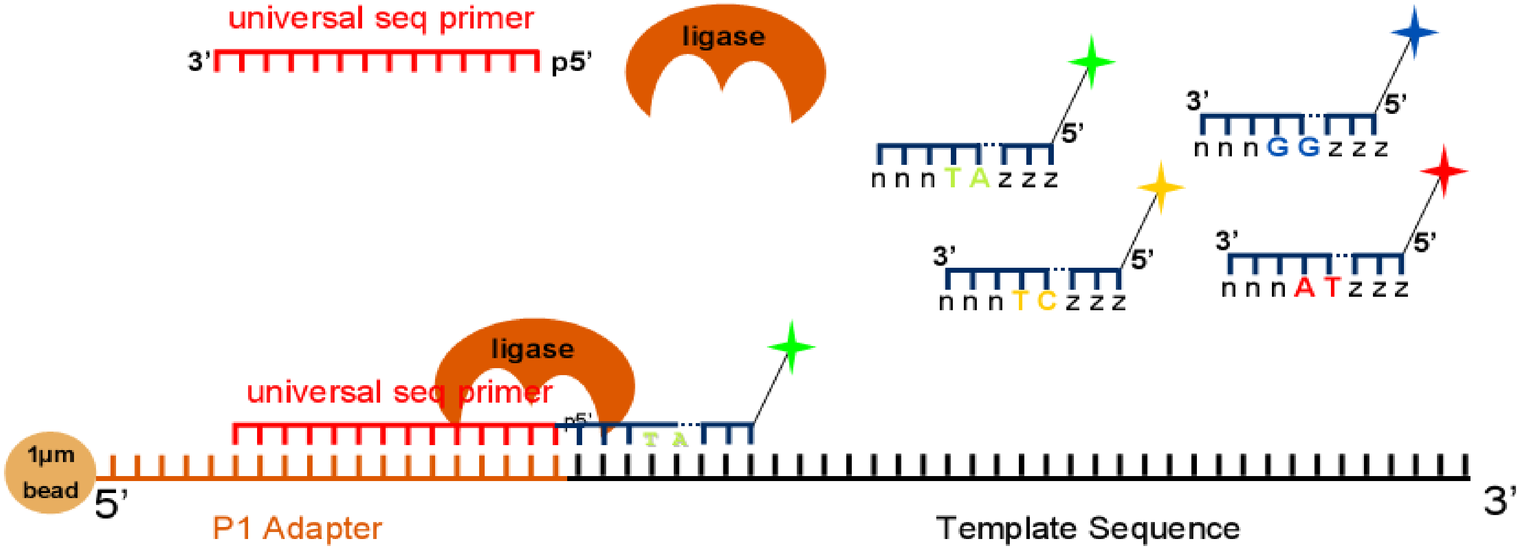
4 Dyes, 4 dinucleotides, 256 probes per dye

N= degenerate bases Z= Universal bases

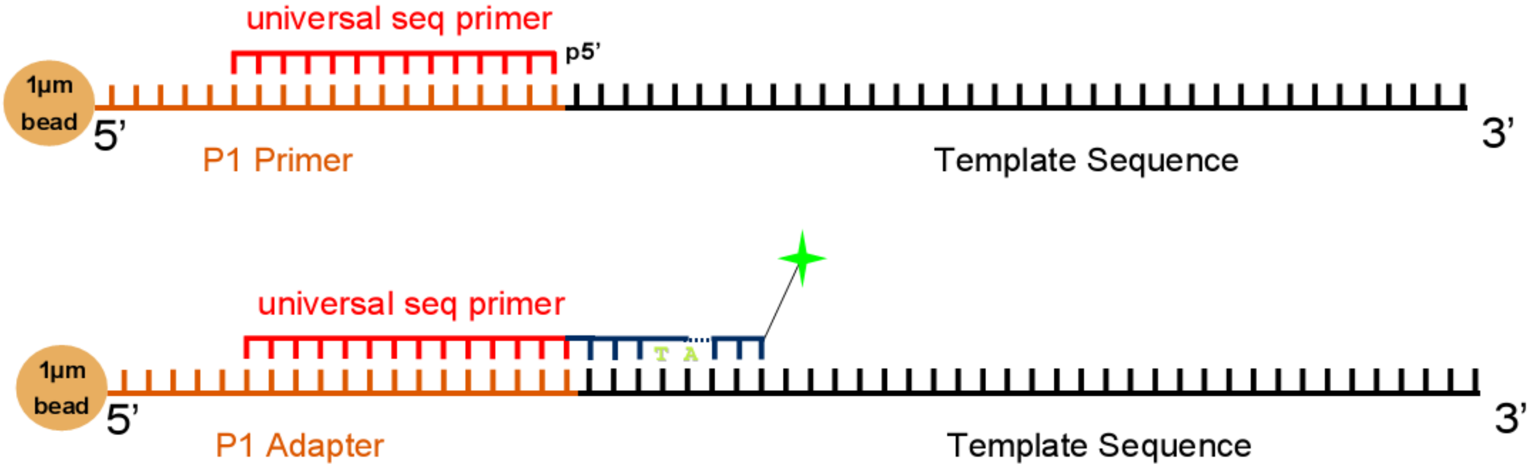
SOLiD Chemistry System 4-color ligation
Ligation reaction



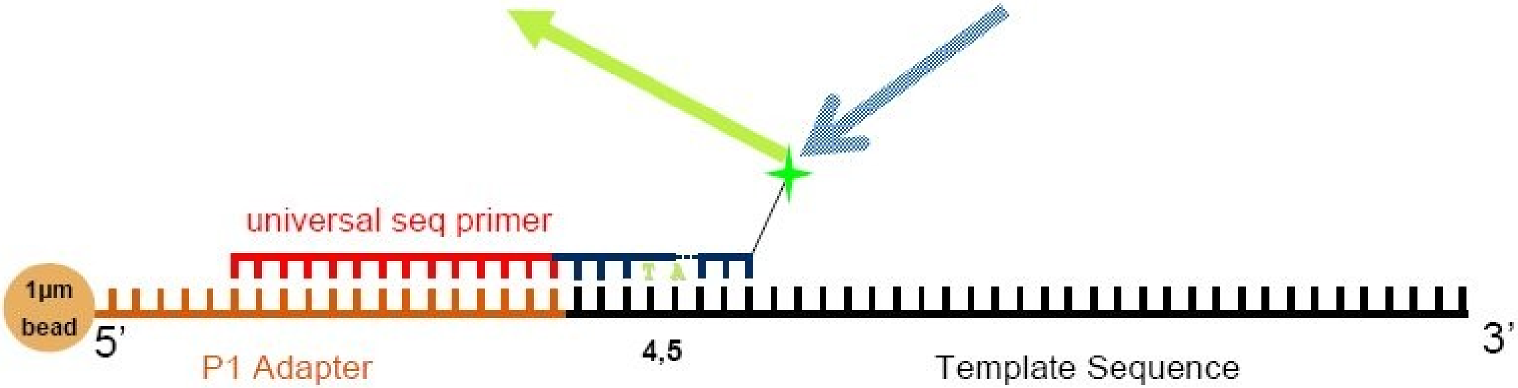
SOLiD Chemistry System 4-color ligation
Ligation reaction



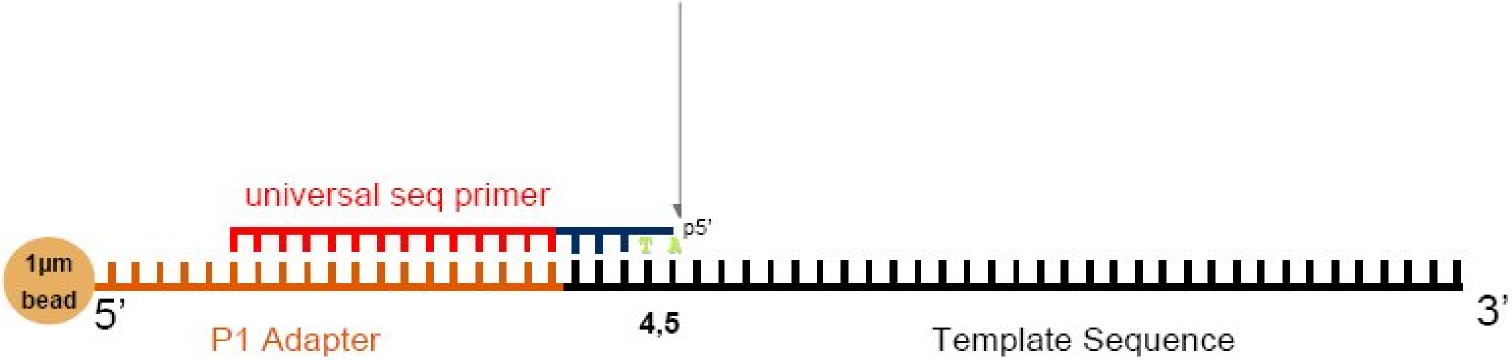
SOLiD Chemistry System 4-color ligation
De-Phosphorylation



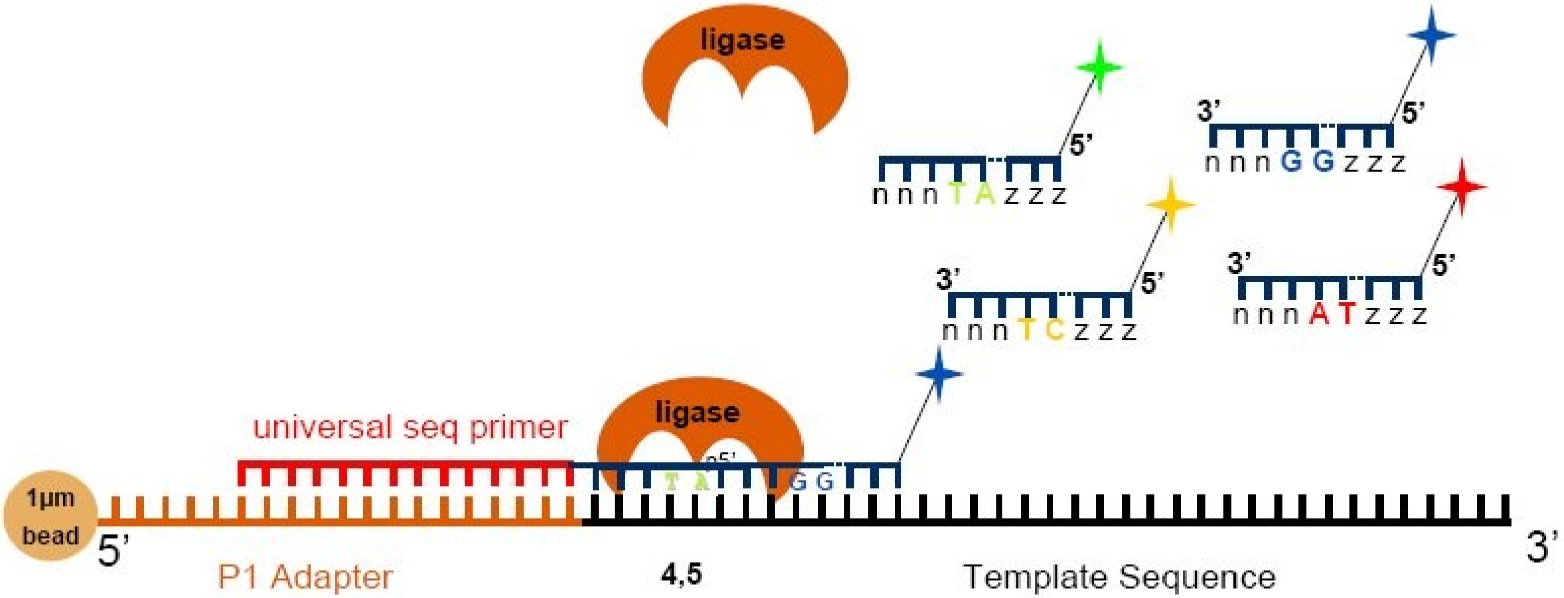
SOLiD Chemistry System 4-color ligation Visualization



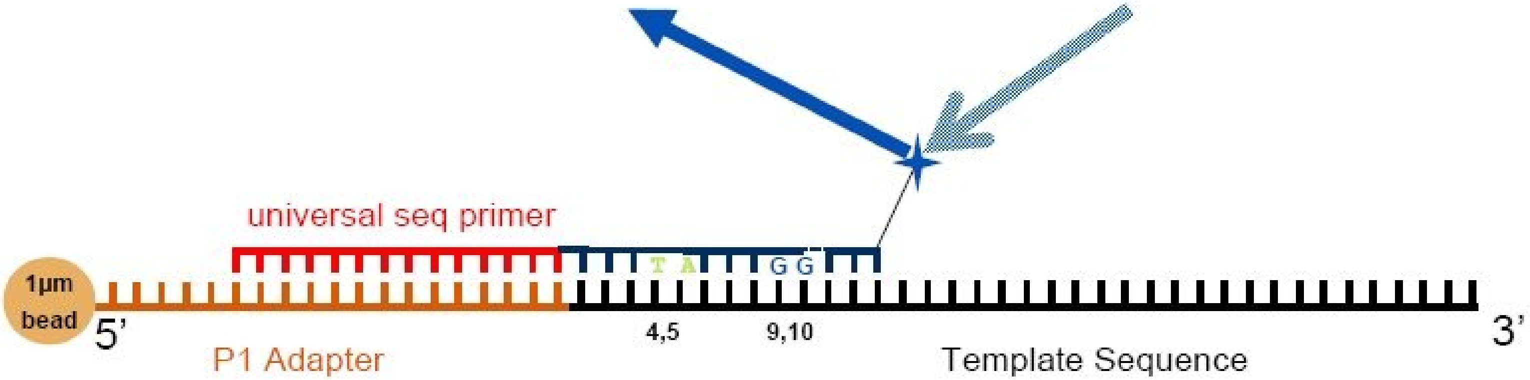
SOLiD Chemistry System 4-color ligation
Cleavage



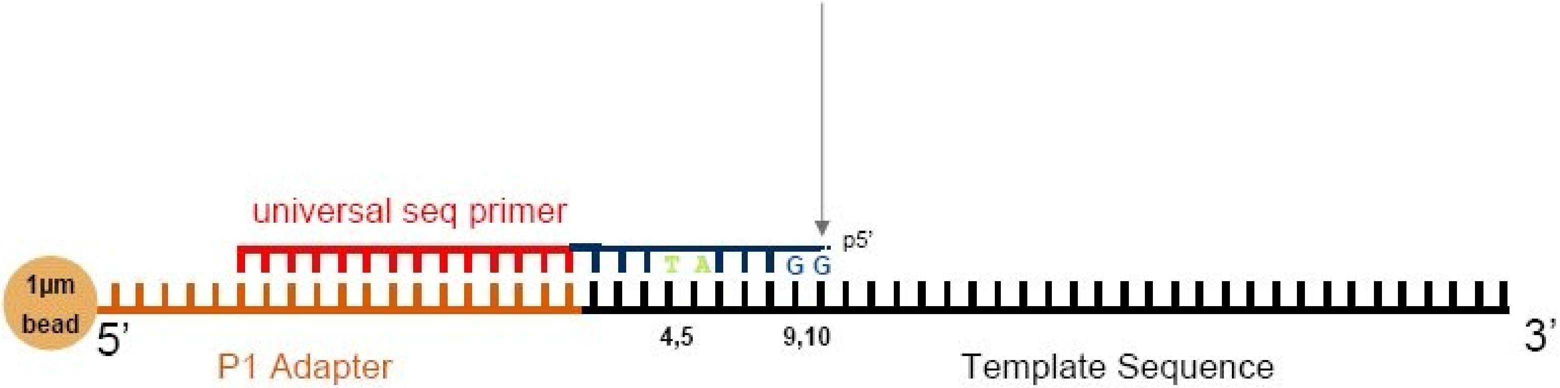
SOLiD Chemistry System 4-color ligation
Ligation (2nd cycle)



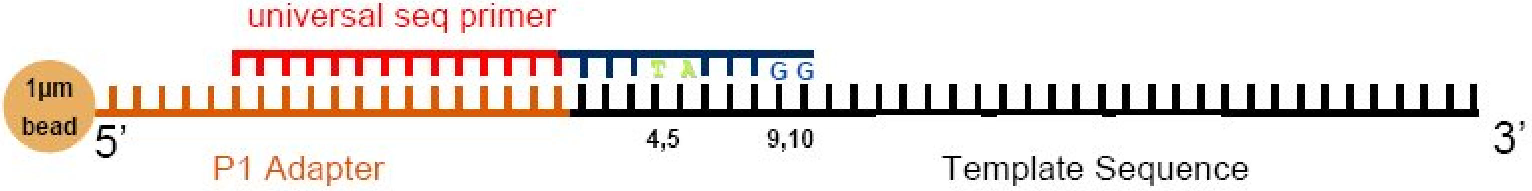
SOLiD Chemistry System 4-color ligation Visualization (2nd cycle)



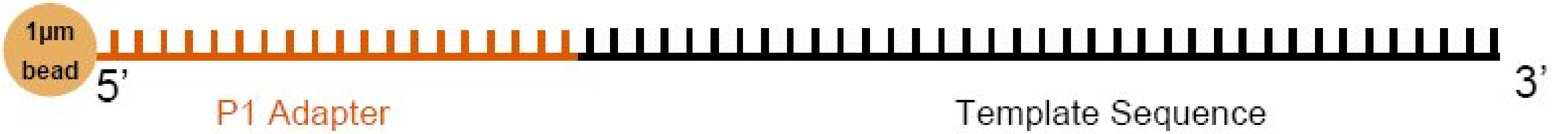
SOLiD Chemistry System 4-color ligation Cleavage (2nd cycle)



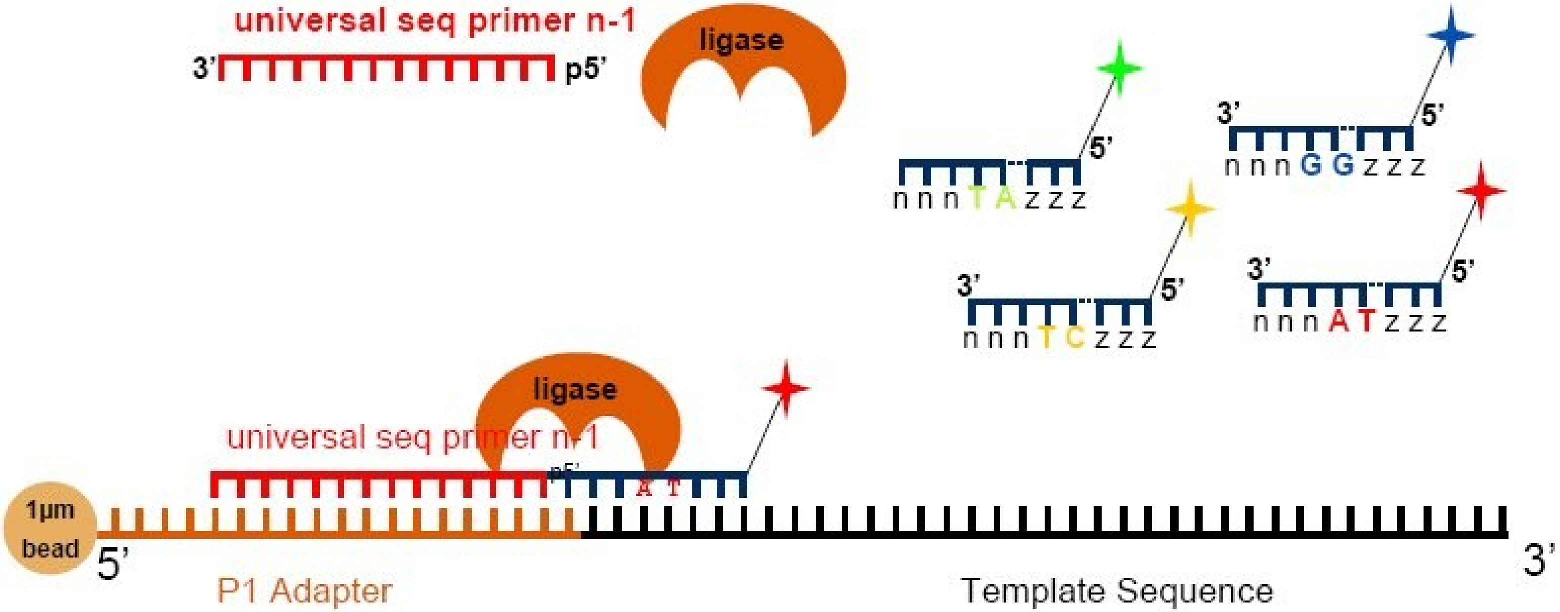
SOLiD Chemistry System 4-color ligation interrogates every 5th base



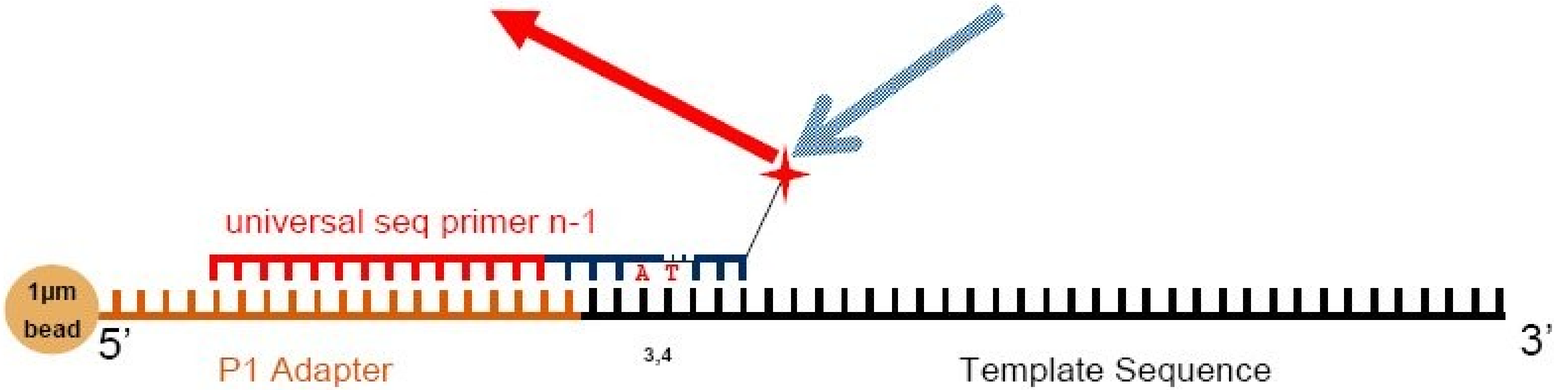
SOLiD Chemistry System 4-color ligation
Reset



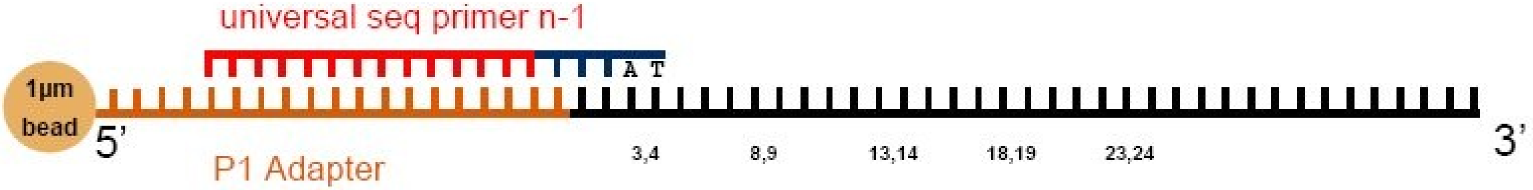
SOLiD Chemistry System 4-color ligation (1st cycle after reset)



SOLiD Chemistry System 4-color ligation
(1st cycle after reset)



SOLiD Chemistry System 4-color ligation (2nd Round)



Sequential rounds of sequencing
Multiple cycles per round

1µm bead

5'

3'

P1 Adapter

Template Sequence

universal seq primer

3'

4,5 9,10 14,15 19,20 24,25

reset

universal seq primer **n-1**

3'

3,4 8,9 13,14 18,29 23,24

reset

universal seq primer **n-2**

3'

2,3 7,8 12,13 17,18 22,23

reset

universal seq primer **n-3**

3'

1,2 6,7 12,13 16,17 21,22

reset

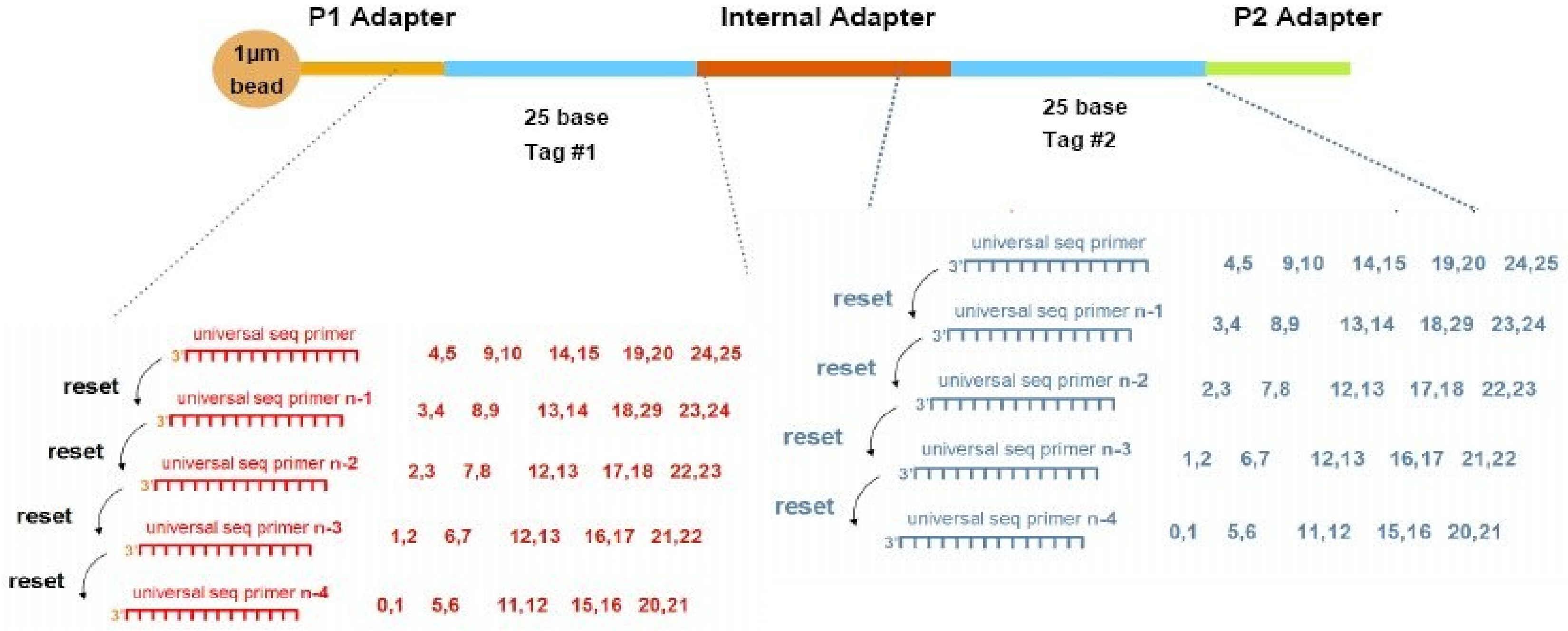
universal seq primer **n-4**

3'

0,1 5,6 11,12 15,16 20,21

Paired End two sequences generated

Sequential rounds of sequencing
Multiple cycles per round



1µm bead

P1 Adapter

25 base Tag #1

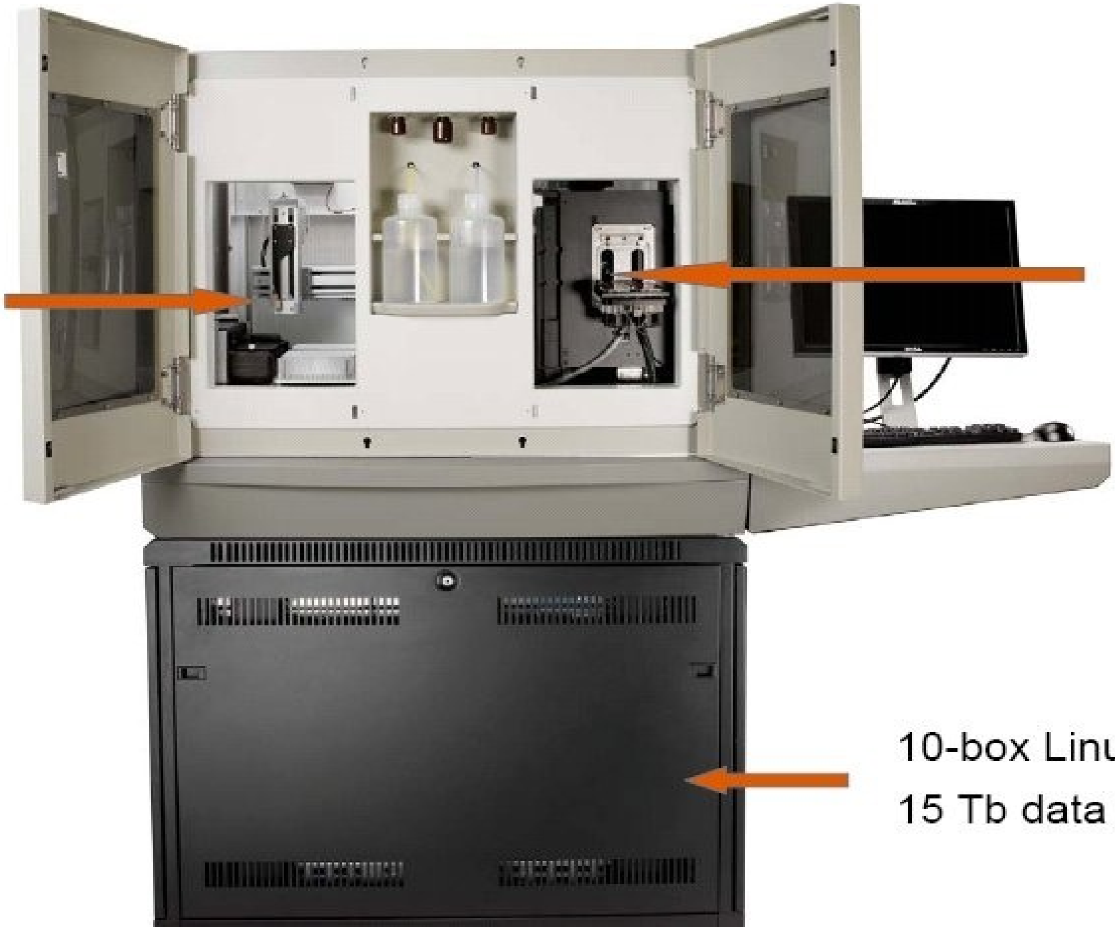
Internal Adapter

25 base Tag #2

P2 Adapter

universal seq primer 3' 4,5 9,10 14,15 19,20 24,25
reset
universal seq primer n-1 3' 3,4 8,9 13,14 18,29 23,24
reset
universal seq primer n-2 3' 2,3 7,8 12,13 17,18 22,23
reset
universal seq primer n-3 3' 1,2 6,7 12,13 16,17 21,22
reset
universal seq primer n-4 3' 0,1 5,6 11,12 15,16 20,21

universal seq primer 3' 4,5 9,10 14,15 19,20 24,25
reset
universal seq primer n-1 3' 3,4 8,9 13,14 18,29 23,24
reset
universal seq primer n-2 3' 2,3 7,8 12,13 17,18 22,23
reset
universal seq primer n-3 3' 1,2 6,7 12,13 16,17 21,22
reset
universal seq primer n-4 3' 0,1 5,6 11,12 15,16 20,21

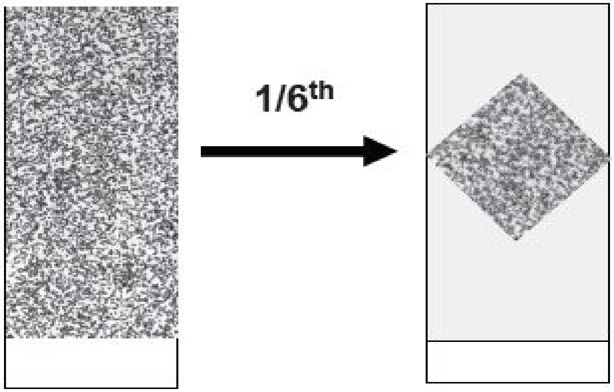


Reagent handling

Dual Flow Cell

10-box Linux Cluster
15 Tb data storage

SOLiD System Arrays: 4 Mega pixel Camera

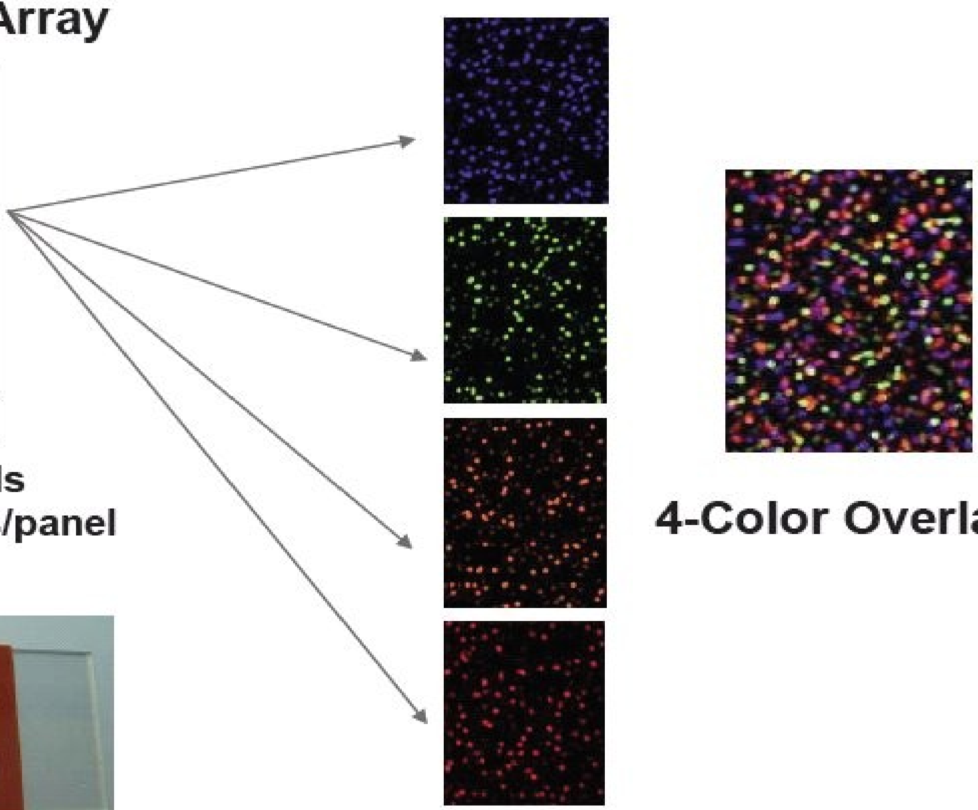


Full Array

Small Array

1800 panels

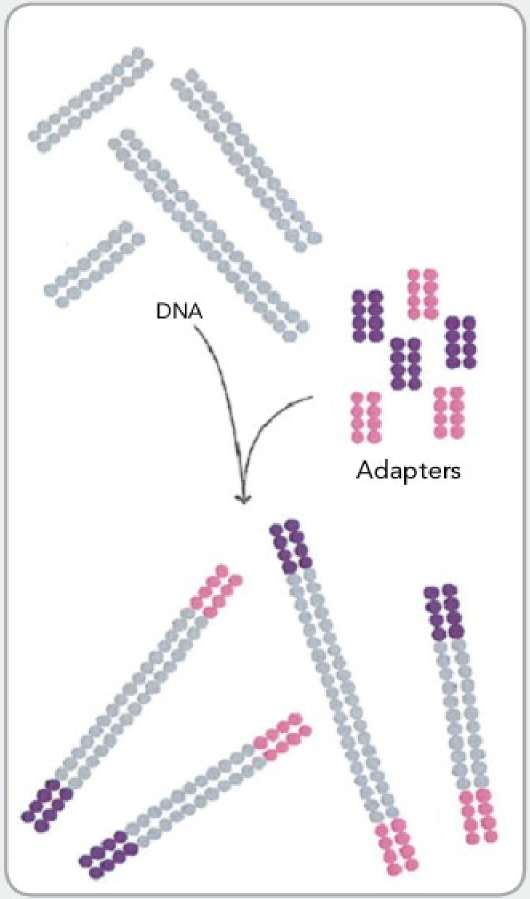
300 panels
~20,000 beads/panel



4-Color Overlay

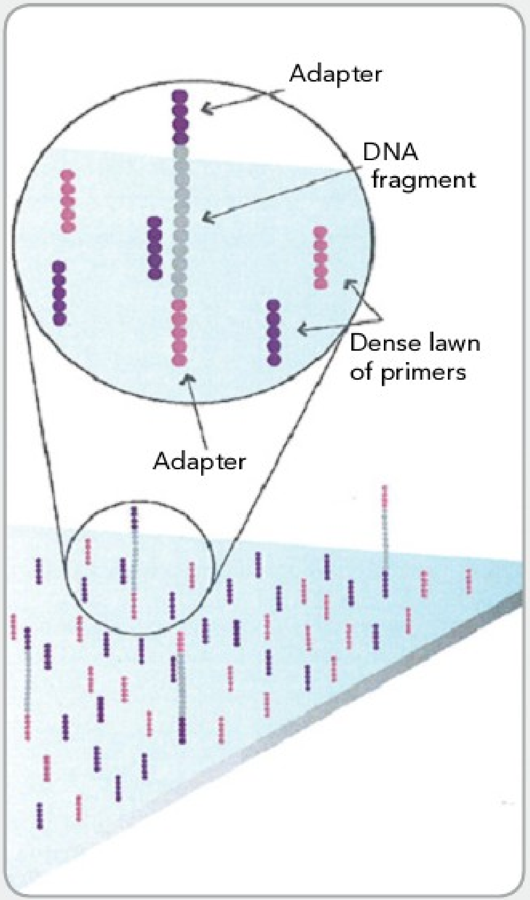
Solexa (2007)

1. PREPARE GENOMIC DNA SAMPLE



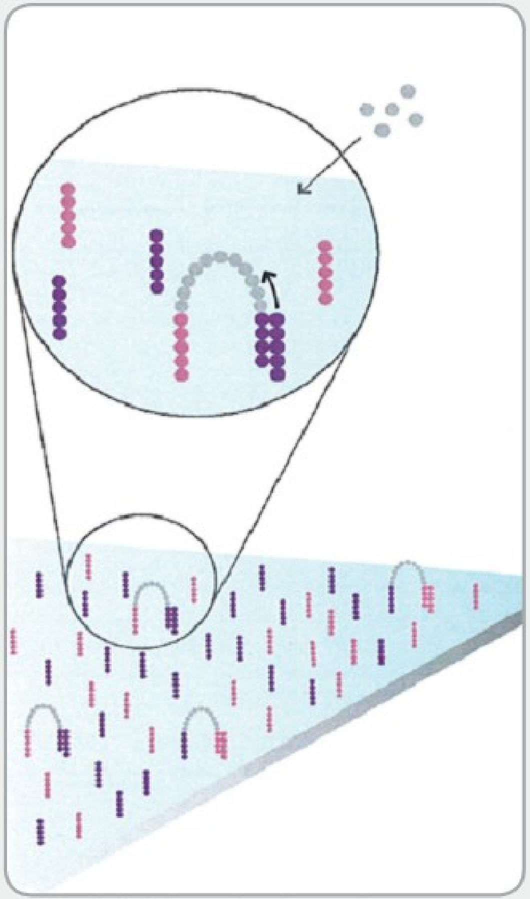
Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.

2. ATTACH DNA TO SURFACE



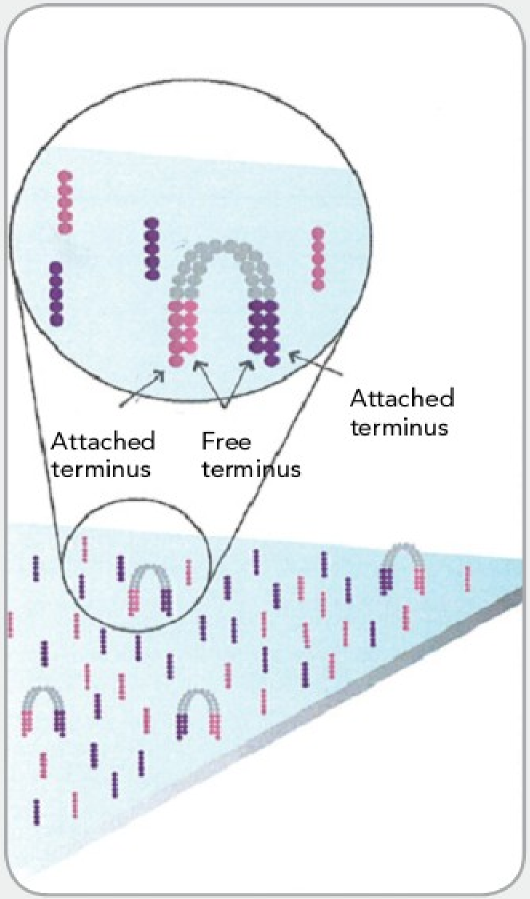
Bind single-stranded fragments randomly to the inside surface of the flow cell channels.

3. BRIDGE AMPLIFICATION



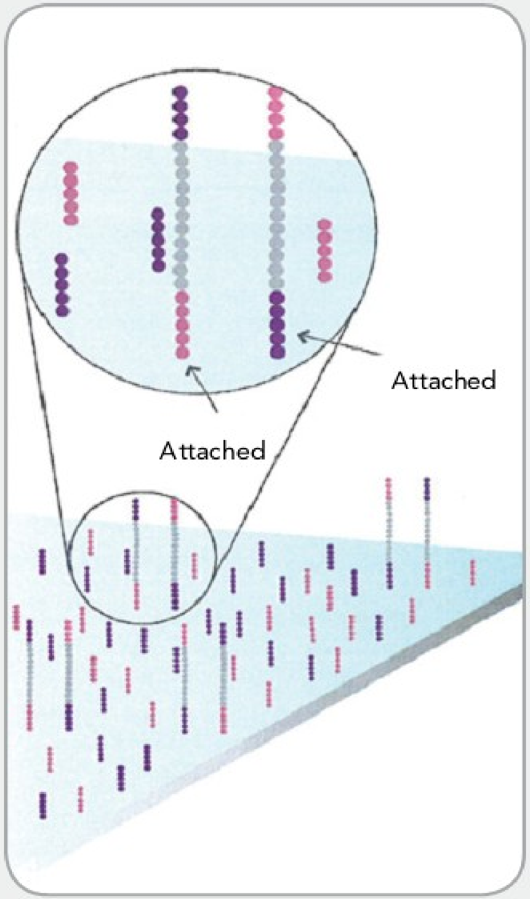
Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification.

4. FRAGMENTS BECOME DOUBLE STRANDED



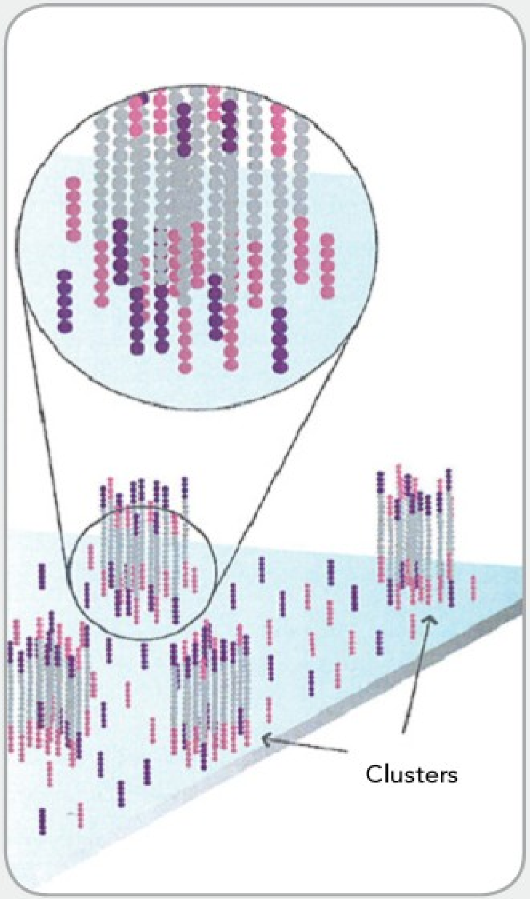
The enzyme incorporates nucleotides to build double-stranded bridges on the solid-phase substrate.

5. DENATURE THE DOUBLE-STRANDED MOLECULES



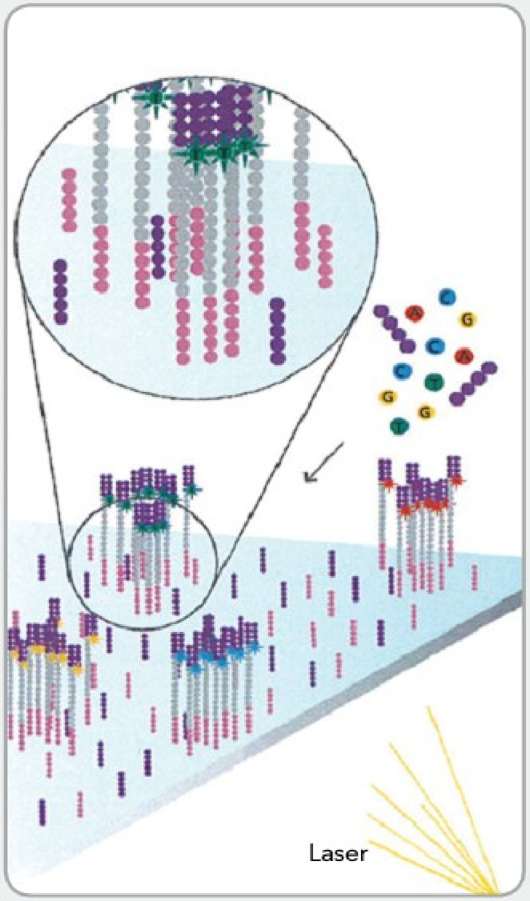
Denaturation leaves single-stranded templates anchored to the substrate.

6. COMPLETE AMPLIFICATION



Several million dense clusters of double-stranded DNA are generated in each channel of the flow cell.

7. DETERMINE FIRST BASE

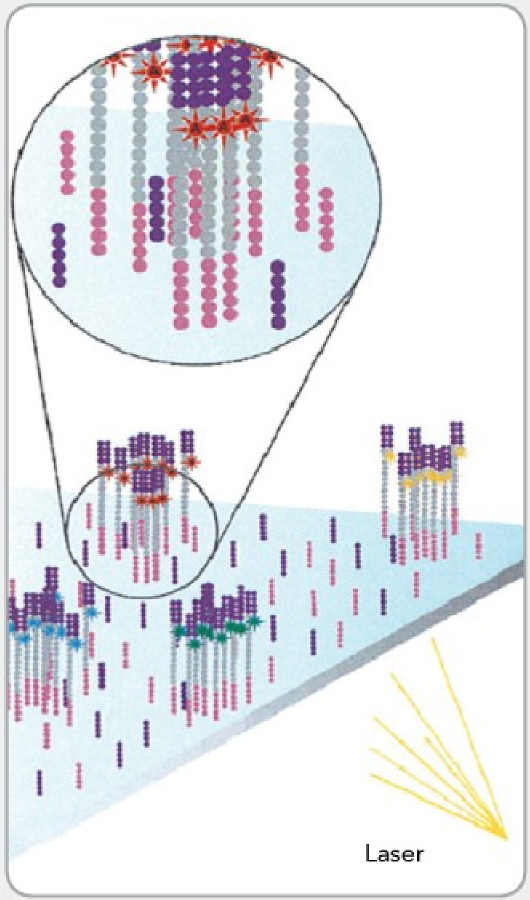


First chemistry cycle: to initiate the first sequencing cycle, add all four labeled reversible terminators, primers and DNA polymerase enzyme to the flow cell.

8. IMAGE FIRST BASE

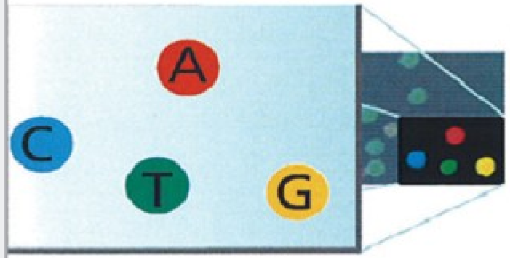
After laser excitation, capture the image of emitted fluorescence from each cluster on the flow cell. Record the identity of the first base for each cluster.

9. DETERMINE SECOND BASE



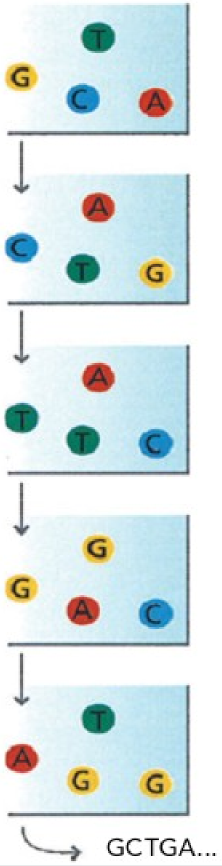
Second chemistry cycle: to initiate the next sequencing cycle, add all four labeled reversible terminators and enzyme to the flow cell.

10. IMAGE SECOND CHEMISTRY CYCLE



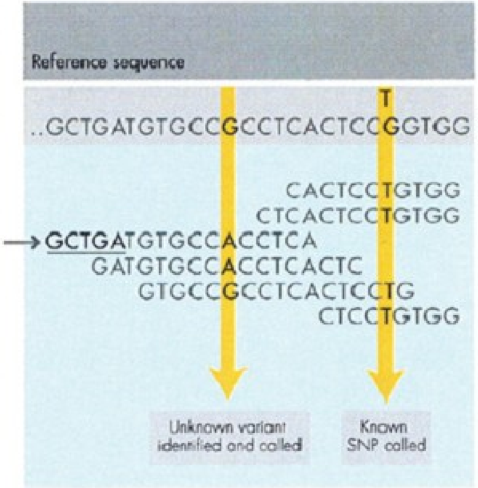
After laser excitation, collect the image data as before. Record the identity of the second base for each cluster.

11. SEQUENCE READS OVER MULTIPLE CHEMISTRY CYCLES



Repeat cycles of sequencing to determine the sequence of bases in a given fragment a single base at time.

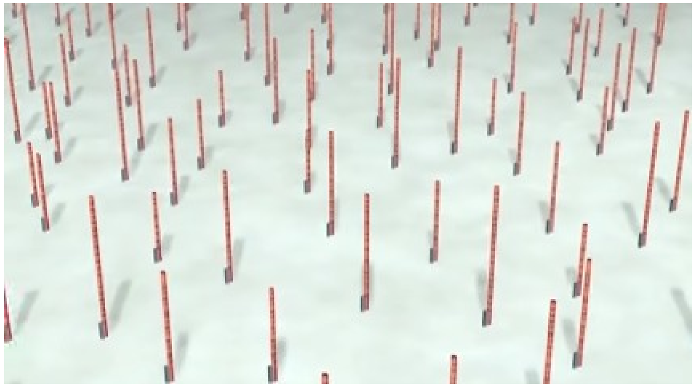
12. ALIGN DATA

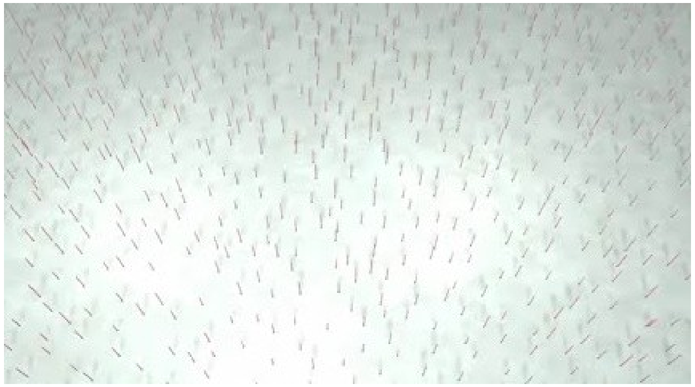


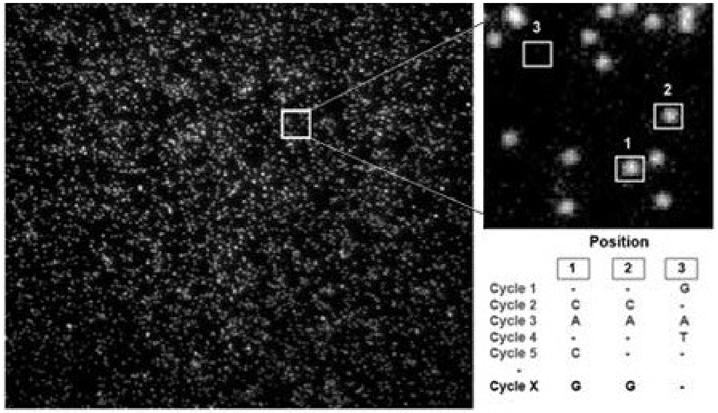
Align data, compare to a reference, and identify sequence differences.

HELICOS (2008)

True Single Molecule Sequencing (tSMS)







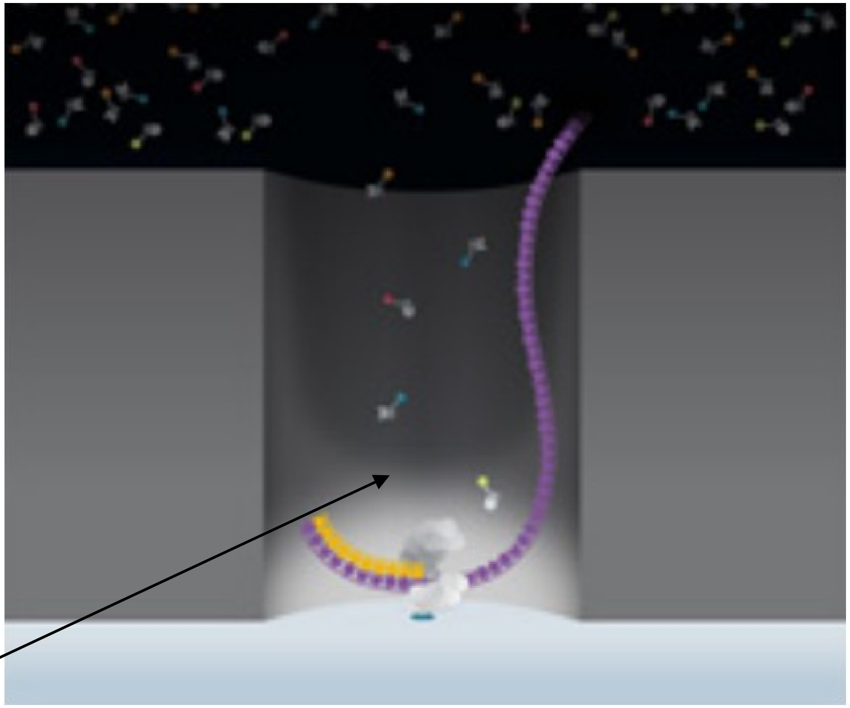
Position

	1	2	3
Cycle 1	-	-	G
Cycle 2	C	C	-
Cycle 3	A	A	A
Cycle 4	-	-	T
Cycle 5	C	-	-
-			
Cycle X	G	G	-

Coming soon: 15-minute, $100 human genome sequencing

Single Molecule Real-Time (SMRT)

Pacific Biosciences



20 zeptolitrů

Další technologie

- Nanopore sekvenování
- Mikroelektroforéza
- Sekvenování na bázi microarray