From discovery to technology explosion

- 1868: Discovery of DNA
- 1953: Watson and Crick propose double helix structure
- 1977: Sanger sequencing
- 1985: PCR
- 2000: Working draft human genome announced (Sanger method)

- 2005: 454 sequencer launch (pyrosequencing)
- 2006: Genome Analyzer launched (Solexa sequencing)
- 2007: SOLiD launched (ligation sequencing)
- 2009: Whole human genome no longer merits Nature/Science paper
- 2010: “third-gen” systems

$ human Genome

$3 billion

$2-3 million

$250k

$50k

$20k

<$1k

Applied Biosystems
ABI 3730XL
1 Mb / day

Roche / 454
Genome Sequencer FLX
100 Mb / run

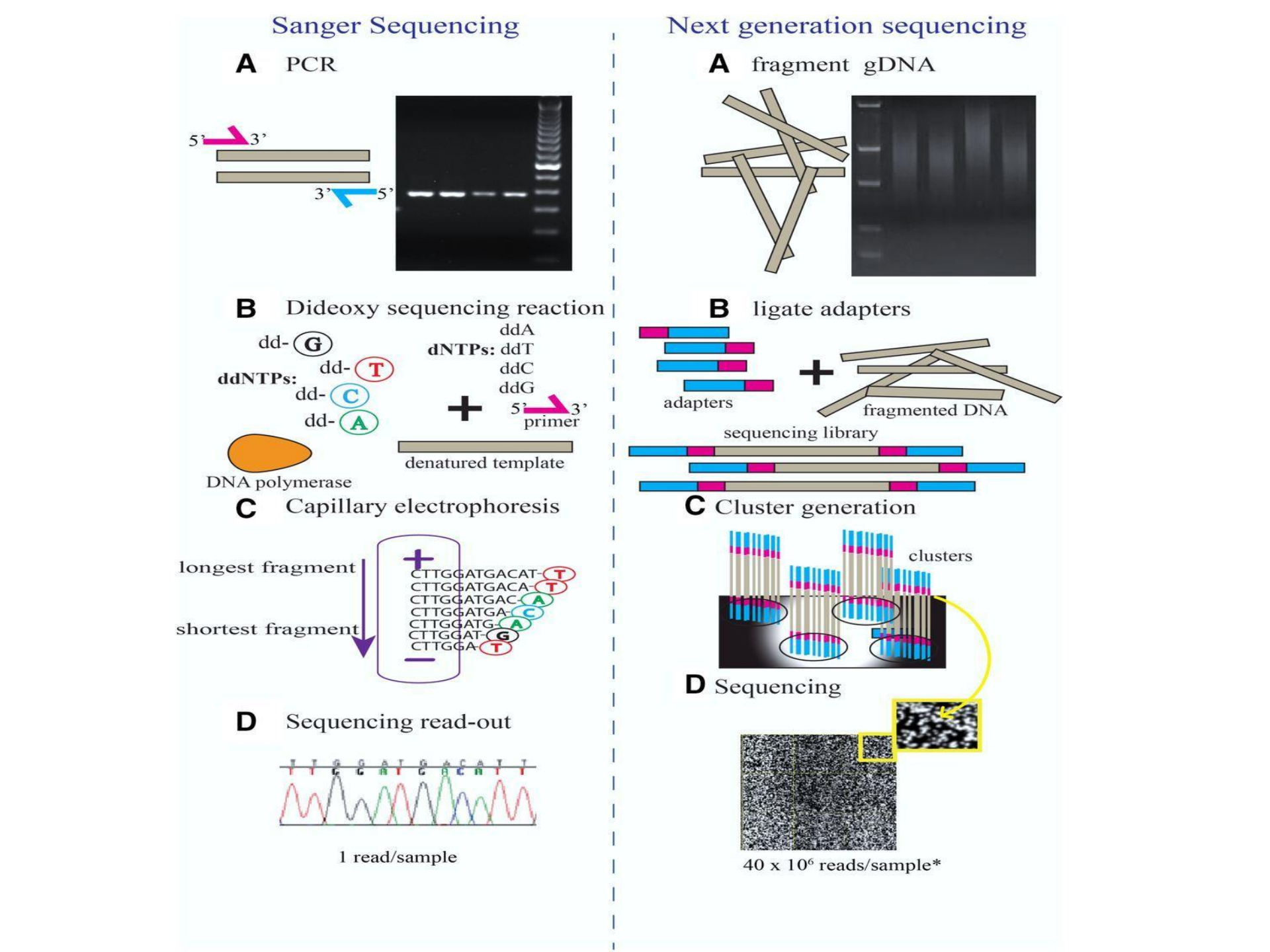
Illumina / Solexa
Genetic Analyzer
2000 Mb / run

Applied Biosystems
SOLiD
3000 Mb / run

PACIFIC BIOSCIENCES®

ion torrent

Oxford NANOPORE Technologies®


Sanger Sequencing
A PCR
5'
3'
3'
5'
B Dideoxy sequencing reaction
dd- G
dd- T
ddNTPs:
dd- C
dd- A
dNTPs: ddA
ddT
ddC
ddG
5'
3'
primer
DNA polymerase
denatured template
C Capillary electrophoresis
longest fragment
shortest fragment
CTTGGATGACAT T
CTTGGATGACA T
CTTGGATGAC A
CTTGGATGA C
CTTGGATG A
CTTGGAT G
CTTGGA T
D Sequencing read-out
1 read/sample
Next generation sequencing
A fragment gDNA
B ligate adapters
adapters
fragmented DNA
sequencing library
C Cluster generation
clusters
D Sequencing
40 x 10⁶ reads/sample*

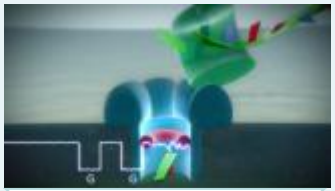
Sequencer	454 GS FLX	HiSeq 2000	SOLiDv4	Sanger 3730xl
Sequencing mechanism	Pyrosequencing	Sequencing by synthesis	Ligation and two-base coding	Dideoxy chain termination
Read length	700 bp	50SE, 50PE, 101PE	50 + 35 bp or 50 + 50 bp	400~900 bp
Accuracy	99.9%*	98%, (100PE)	99.94% *raw data	99.999%
Reads	1 M	3 G	1200~1400 M	—
Output data/run	0.7 Gb	600 Gb	120 Gb	1.9~84 Kb
Time/run	24 Hours	3~10 Days	7 Days for SE 14 Days for PE	20 Mins~3 Hours
Advantage	Read length, fast	High throughput	Accuracy	High quality, long read length
Disadvantage	Error rate with polybase more than 6, high cost, low throughput	Short read assembly	Short read assembly	High cost low throughput

Oxford Nanopore

Application Specific

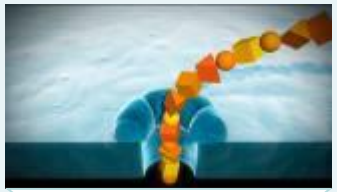
Adaptable protein nanopore:

DNA Sequencing

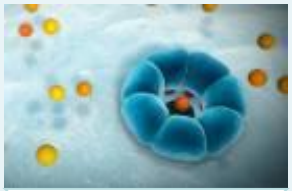


Proteins

Polymers

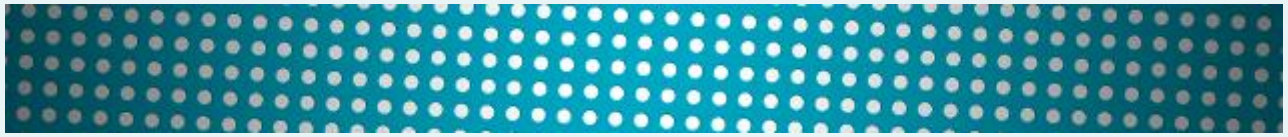


Small Molecules



Generic Platform

Sensor array chip: many nanopores in parallel



Electronic read-out system

Illumina

Illumina, Inc. (ILMN)
NasdaqGS - NasdaqGS Real Time Price. Currency in USD

Add to watchlist

240.09 +24.24 (+11.23%)
At close: November 10 04:00PM EST

239.48 -0.61 (-0.25%)
After hours: 07:29PM EST

Summary | Chart | Conversations | Statistics | Historical Data | Profile | Financials | Analysis | Options | Holders | Sustainabili

Previous Close	215.85	Market Cap	37.766B
Open	229.88	Beta (5Y Monthly)	1.17
Bid	240.04 x 800	PE Ratio (TTM)	54.07
Ask	239.98 x 1000	EPS (TTM)	4.44
Day's Range	229.88 - 240.75	Earnings Date	Feb 08, 2023 - Feb 13, 2023
52 Week Range	173.45 - 428.00	Forward Dividend & Yield	N/A (N/A)
Volume	1,789,955	Ex-Dividend Date	N/A
Avg. Volume	1,800,996	1y Target Est	248.94

DNA degradation

Mechanical damage during tissue homogenization.

Wrong pH and ionic strength of extraction buffer.

Incomplete removal / contamination with **nucleases**.

Phenol: too old, or inappropriately buffered (**pH 7.8 – 8.0**); incomplete removal.

Wrong pH of **DNA solvent** (acidic water).
Recommended: 1:10 TE for short-term storage, or 1xTE for long-term storage.

Vigorous pipetting (wide-bore pipet tips).

Vortexing of DNA in high concentrations.

Too many **freeze-thaw** cycles (*we tested 5, still Ok*).

Debatable: sequence-dependent

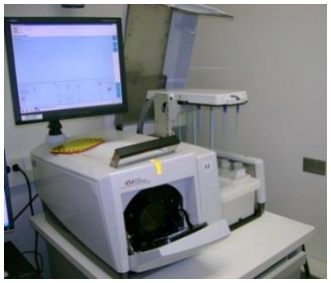
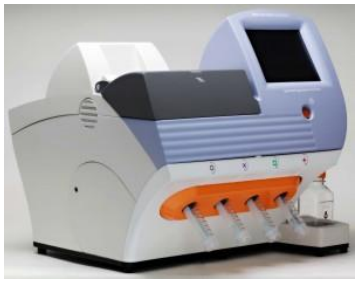
Genome sequencing

Two strategies

- **Whole genome shotgun (bottom-top)**
- **Clone-by-clone (top-bottom)**

Sequencing without a limit?

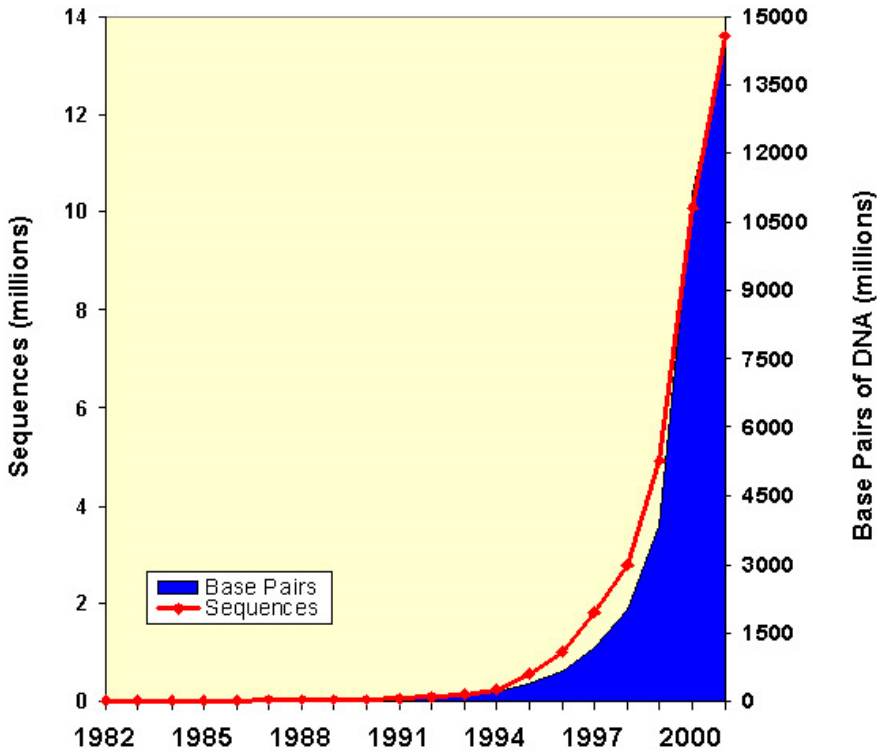
- A rapid progress in next generation sequencing technologies promises to provide complete (reference) DNA sequences


- **The bottleneck:**
  - NOT the sequencing capacity
  - BUT the ability to assemble many short reads with prevalence of repeated DNA (and polyploidy)

Genome sequencing

GenBank 1982 Los Alamos Sequence Database



Walter Goad

Frederick Sanger

1958 – Nobel prize – insuline structure

1975 - Dideoxy sequencing method

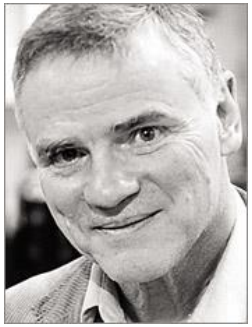
1977 – Φ-X174 (5,368 bp) sequence

1980 – second Nobel prize

λ phage sequence
shotgun method (48,502 bp)

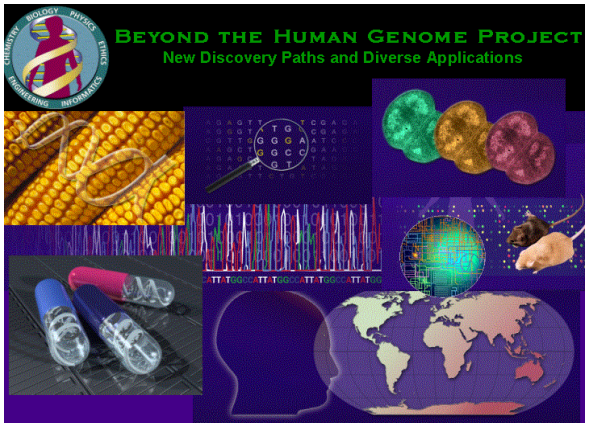
Genome sequencing

- **1986 Leroy Hood:**
  automatic sequencing machine



Leroy Hood

- **1986 Human Genome Initiative**



Genome sequencing

- **1995** John Craig Venter
  first bacterial genome

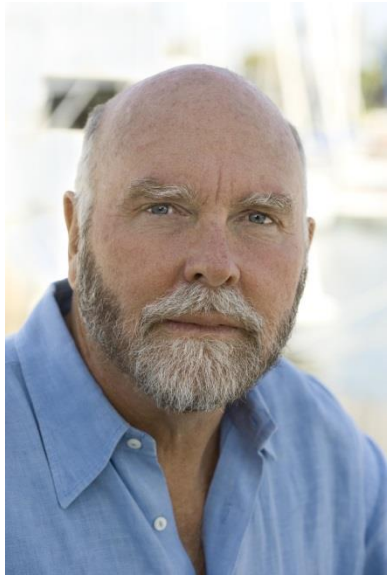
John Craig Venter

Craig Venter

Global Ocean Sampling Expedition

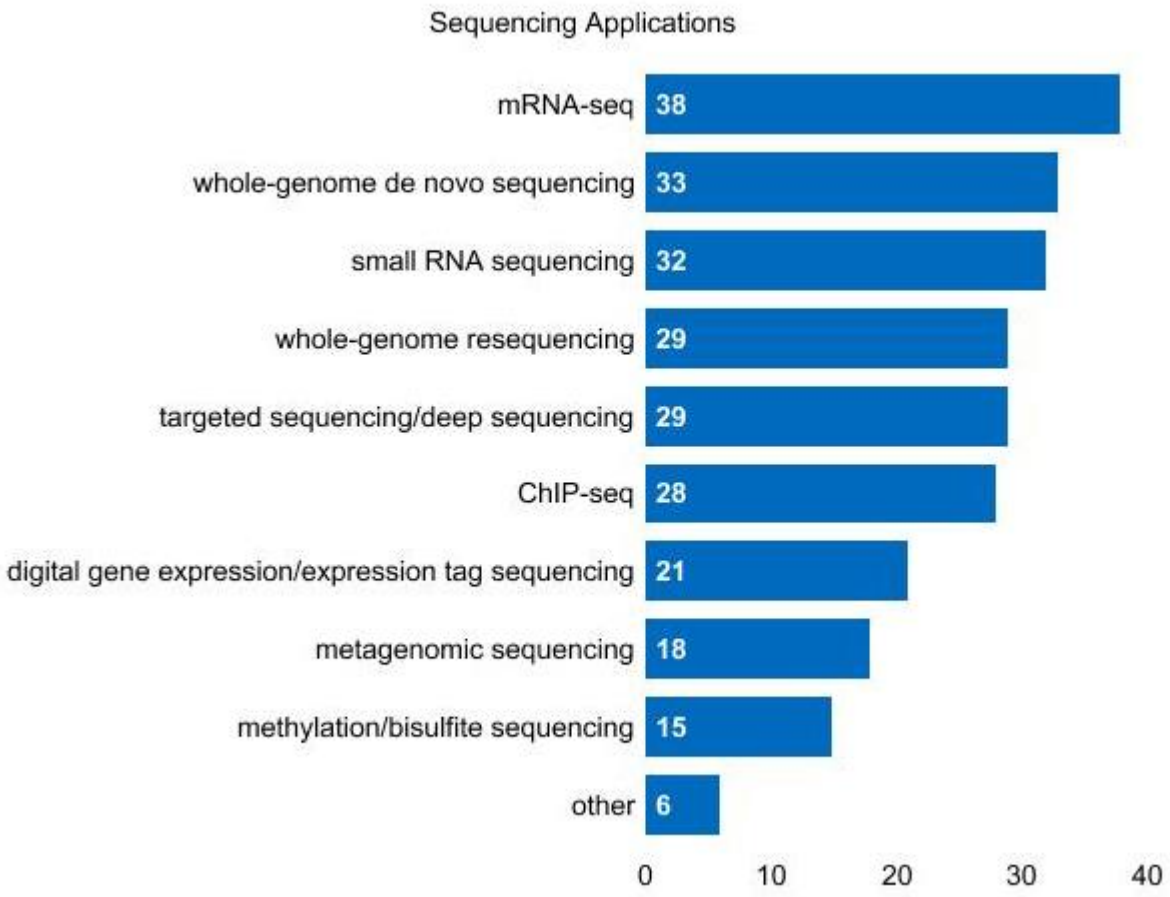
Synthetic genomics

Human Longevity Inc



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

Which applications are labs performing?

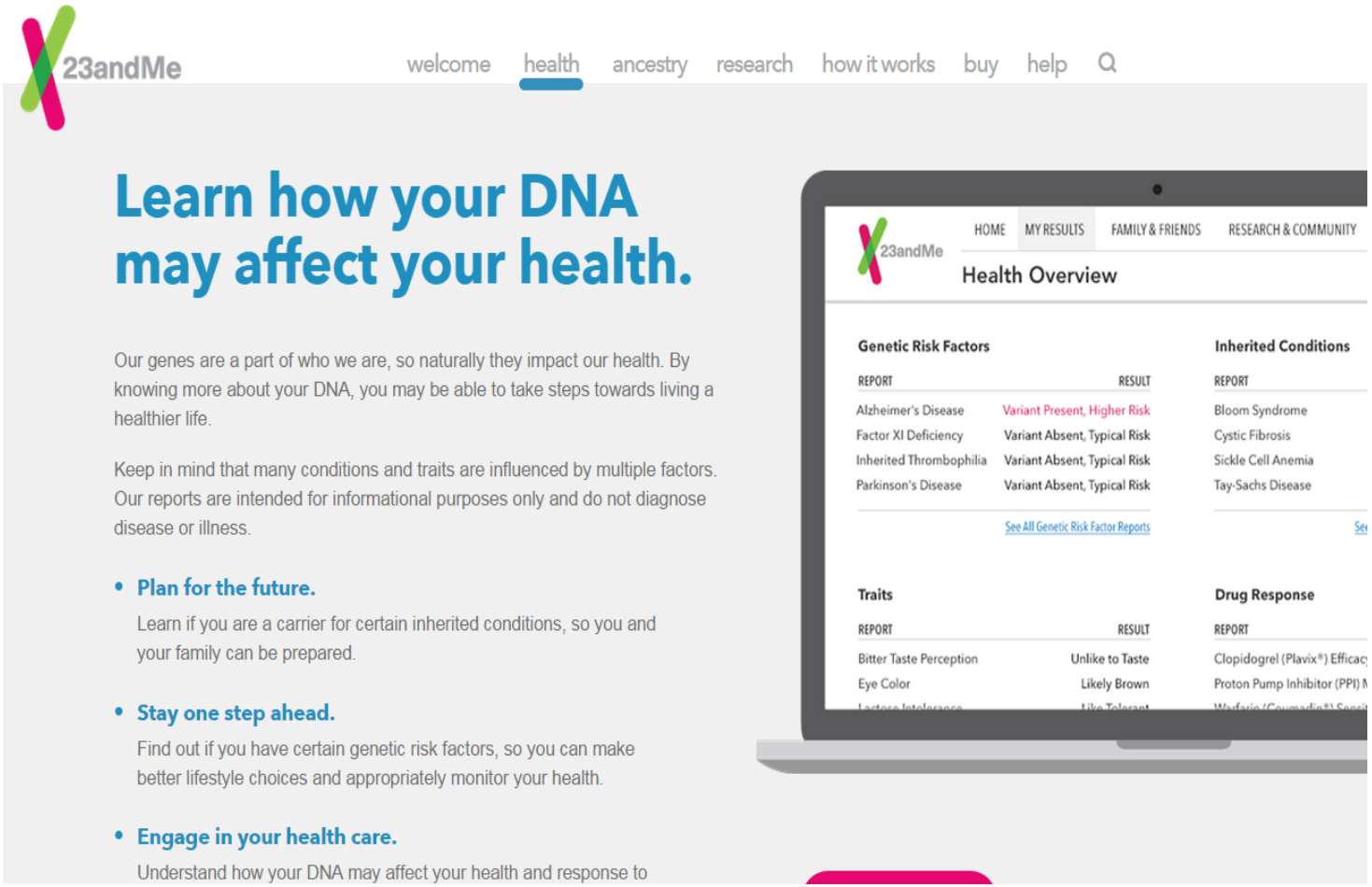


2010 Human genome reference

2010 Human genome reference

23andme (30% GSK)

Anne Wojcicki CEO - manželka spoluzakladatele Google
Sergey Mikhaylovich Brin



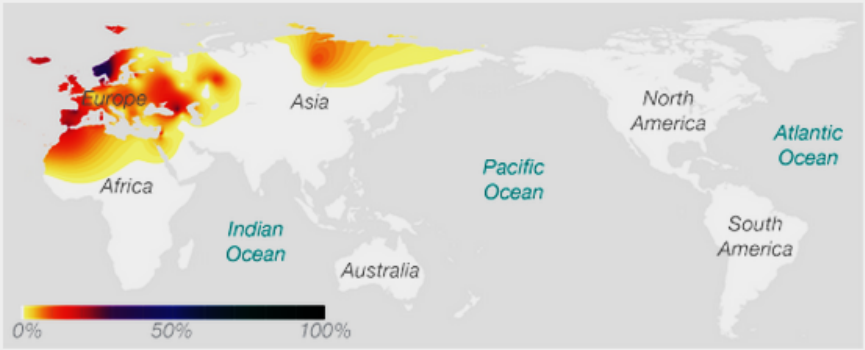

23andMe
DNA Genetic
BIO-SPECIMEN BAG

23andMe Chip Versions Comparison

- **v1**: November 2007
- **v2**: September 2008, ~555K SNPs (Illumina)
- **v3**: November 2010, >900K SNPs (Illumina OmniExpress)
- **v4**: November 2013, ~570K SNPs (Illumina OmniExpress)
- **v5**: August 2017, ~640K SNPs (Illumina Global Screening Array)

MATERNAL LINE: H1

Locations of haplogroup H1 before the widespread migrations of the past few hundred years.



Haplogroup H1 is widespread in Europe, especially the western part of the continent. It originated about 13,000 years ago, not long after the Ice Age ended.

Maternal haplogroups are families of mitochondrial DNA types that all trace back to a single mutation at a specific place and time. By looking at the geographic distribution of mtDNA types, we learn how our ancient female ancestors migrated throughout the world.

Haplogroup: H1, a subgroup of H
Age: 13,000 years
Region: Europe, Near East, Central Asia, Northwestern Africa
Example Populations: Spanish,Berbers,Lebanese
Highlight: H1 appears to have been common in Doggerland, an ancient land now flooded by the North Sea.

PATERNAL LINE: I1*

I1* is a subgroup of I1

Locations of haplogroup I1 before the widespread migrations of the past few hundred years.

Haplogroup I1 can be found at levels of 10% and higher in many parts of Europe, due to its expansion with men who migrated northward after the end of the Ice Age about 12,000 years ago. It reaches its highest levels in Denmark and the southern parts of Sweden and Norway.

Paternal haplogroups are families of Y chromosomes that all trace back to a single mutation at a specific place and time. By looking at the geographic distribution of these related lineages, we learn how our ancient male ancestors migrated throughout the world.

Haplogroup: I1, a subgroup of I
Age: 28,000 years
Region: Northern Europe
Example Populations: Finns, Norwegians, Swedes
Highlight: Haplogroup I1 reaches highest frequencies in Scandinavia.

Haplogroups of You and Your Connections

I1*	Roman Hobza

Haplogroups of Example Profiles

SHOW RESULTS FOR Roman Hobza ▾

SEE NEW AND RECENTLY UPDATED REPORTS »

These reports provide information about your possible risk for developing certain health conditions based on genetics. Environmental and lifestyle factors also often play a large role in your risk for developing these conditions.

Elevated Risk

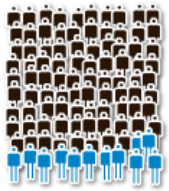
NAME	CONFIDENCE	YOUR RISK	AVG. RISK	COMPARED TO AVERAGE
Venous Thromboembolism	★★★★	41.8%	12.3%	3.39x
Gout	★★★★	35.7%	22.8%	1.57x
Melanoma	★★★★	4.0%	2.9%	1.38x
Restless Legs Syndrome	★★★★	2.5%	2.0%	1.25x
Exfoliation Glaucoma	★★★★	2.2%	0.7%	2.90x
Esophageal Squamous Cell Carcinoma (ESCC)	★★★★	0.43%	0.36%	1.21x
Stomach Cancer (Gastric Cardia Adenocarcinoma)	★★★★	0.28%	0.23%	1.22x
Primary Biliary Cirrhosis	★★★★	0.11%	0.08%	1.43x
Scleroderma (Limited Cutaneous Type)	★★★★	0.08%	0.07%	1.24x

Show information for Roman Hobza assuming European ethnicity and an age range of 0-79

Roman Hobza

41.8 out of 100

men of European ethnicity who share Roman Hobza's genotype will develop Venous Thromboembolism between the ages of 0 and 79.



Average

12.3 out of 100

men of European ethnicity will develop Venous Thromboembolism between the ages of 0 and 79.

What does the Odds Calculator show me?

Use the ethnicity and age range selectors above to see the estimated incidence of Venous Thromboembolism due to genetics for men with **Roman Hobza**'s genotype. The 23andMe Odds Calculator assumes that a person is free of the condition at the lower age in the range. You can use the name selector above to see the estimated incidence of Venous Thromboembolism for the genotypes of other people in your account.

The 23andMe Odds Calculator only takes into account effects of markers with known associations that are also on our genotyping chip. Keep in mind that aside from genetics, environment and lifestyle may also contribute to one's risk for Venous Thromboembolism.

55 %
Attributable to Genetics

Understanding Your Results

The heritability of venous thromboembolism is estimated to be 55%. This means that genetics (including unknown factors and known ones such as the SNPs we describe here) and environment play nearly equal roles in this condition. There are a number of environmental factors of various strengths that contribute to venous thromboembolism. Strong risk factors include hip or leg fractures, hip or knee replacement, major surgery or trauma, and spinal cord injury or surgery. Moderate risk factors include arthroscopic knee surgery, having central venous lines, congestive heart or respiratory failure, hormone replacement or oral contraceptive use, cancer, pregnancy, paralytic stroke, previous venous thromboembolism, and thrombophilia. Weak risk factors include bed rest for more than three days, immobility due to sitting (such as a long car or plane trip), specific types of chemotherapy, increasing age, laparoscopic surgery, obesity, and varicose veins. (sources)

What You Can Do

Assuming the ethnicity setting above is correct, your test results indicate you are at increased risk for venous thromboembolism based on genetics. Note that family history and non-genetic factors can also influence your risk for venous thromboembolism. Below are some steps you can take to reduce your risk.

Gene or region: F5
SNP: rs6025

	SNP used	Genotype	Adjusted Odds Ratio*
Roman Hobza	rs6025	CT	European: 4.69
** Odds ratios are reported for all available ethnicities.*			

Factor V is the last clotting factor in the pathway before the activation step that turns prothrombin into thrombin. Clotting is usually kept from spiraling out of control by a feedback loop, similar to the way a thermostat operates. Once enough thrombin has been activated, it binds to a protein called "protein C." Protein C then inactivates factor V, thus cutting off activation of prothrombin into thrombin.

The SNP in the F5 gene causes a change in the protein sequence of factor V that prevents protein C from inactivating it. Since this version of factor V can still participate in the activation of thrombin, a situation results in which thrombin can be turned on but cannot be turned off. Once the clotting cascade is set off (whether appropriately or not), the riskier version of the SNP makes it more difficult to shut it off.

The riskiness of the T version of this SNP is further increased for women who also take hormonal birth control.

(The riskier version of this gene is also sometimes called Factor V Leiden, after the city in the Netherlands where this SNP and its effects on factor V's role in clotting were first discovered.)

The studies whose data we report as applicable to those of "European" ancestry confirmed the association between this SNP and VTE in samples from the Netherlands, Sweden, the United Kingdom, Brazil, Italy, and France.

African and Asian populations appear to have only one version of the SNP, meaning that association studies are very difficult to perform.

Citations

Rosendaal et al. (1995) . "High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance)." *Blood* 85(6):1504-8.

Smith et al. (2007) . "Association of genetic variations with nonfatal venous thrombosis in postmenopausal women." *JAMA* 297(5):489-98.

Emmerich et al. (2001) . "Combined effect of factor V Leiden and prothrombin 20210A on the risk of venous thromboembolism--pooled analysis of 8 case-control studies including 2310 cases and 3204 controls. Study Group for Pooled-Analysis in Venous Thromboembolism." *Thromb Haemost* 86(3):809-16.

Bertina et al. (1994) . "Mutation in blood coagulation factor V associated with resistance to activated protein C." *Nature* 369(6475):64-7.

Lane et al. (2000) . "Role of hemostatic gene polymorphisms in venous and arterial thrombotic disease." *Blood* 95(5):1517-32.

Gene or region: F2
SNP: i3002432

Decreased Risk

NAME	CONFIDENCE	YOUR RISK	AVG. RISK	COMPARED TO AVERAGE
Type 2 Diabetes	★★★★	17.7%	25.7%	0.69x
Alzheimer's Disease	★★★★	4.3%	7.2%	0.60x
Rheumatoid Arthritis	★★★★	1.6%	2.4%	0.68x
Parkinson's Disease	★★★★	1.2%	1.6%	0.73x
Age-related Macular Degeneration	★★★★	0.92%	6.55%	0.14x
Crohn's Disease	★★★★	0.31%	0.53%	0.58x
Multiple Sclerosis	★★★★	0.24%	0.34%	0.69x
Type 1 Diabetes	★★★★	0.12%	1.02%	0.12x
Celiac Disease	★★★★	0.05%	0.12%	0.44x

BRCA Cancer Mutations (Selected)	★★★★	Variant Absent
Beta Thalassemia	★★★★	Variant Absent
Bloom's Syndrome	★★★★	Variant Absent
Canavan Disease	★★★★	Variant Absent
Congenital Disorder of Glycosylation Type 1a (PMM2-CDG)	★★★★	Variant Absent
Connexin 26-Related Sensorineural Hearing Loss	★★★★	Variant Absent
Cystic Fibrosis	★★★★	Variant Absent
D-Bifunctional Protein Deficiency	★★★★	Variant Absent
DPD Deficiency	★★★★	Variant Absent
Dihydrolipoamide Dehydrogenase Deficiency	★★★★	Variant Absent
Factor XI Deficiency	★★★★	Variant Absent
Familial Dysautonomia	★★★★	Variant Absent
Familial Hypercholesterolemia Type B	★★★★	Variant Absent
Familial Hyperinsulinism (ABCC8-related)	★★★★	Variant Absent
Familial Mediterranean Fever	★★★★	Variant Absent
Fanconi Anemia (FANCC-related)	★★★★	Variant Absent
G6PD Deficiency	★★★★	Variant Absent

Reading Ability	★★★	Typical Nonword Reading Score
Response to Diet	★★★	See Report
Response to Exercise	★★★	See Report
Sex Hormone Regulation	★★★	See Report
Sweet Taste Preference	★★★	See Report
Tooth Development	★★★	See Report
Tuberculosis Susceptibility	★★★	See Report
Breast Morphology	★★★	Not Applicable
Menarche	★★★	Not Applicable
Menopause	★★★	Not Applicable
Eating Behavior	★★	Greater tendency to overeat
HIV Progression	★★	See Report
Hair Thickness	★★	Typical, if European or African
Longevity	★★	See Report
Measures of Intelligence	★★	Lower Non-Verbal IQ
Memory	★★	Typical Episodic Memory
Odor Detection	★★	Typical Sensitivity to Sweaty Odor
Pain Sensitivity	★★	Increased
Avoidance of Errors	★	See Report

Genome Sequencer 20 System
454 pyrosequencing (2005)

- http://www.454.com

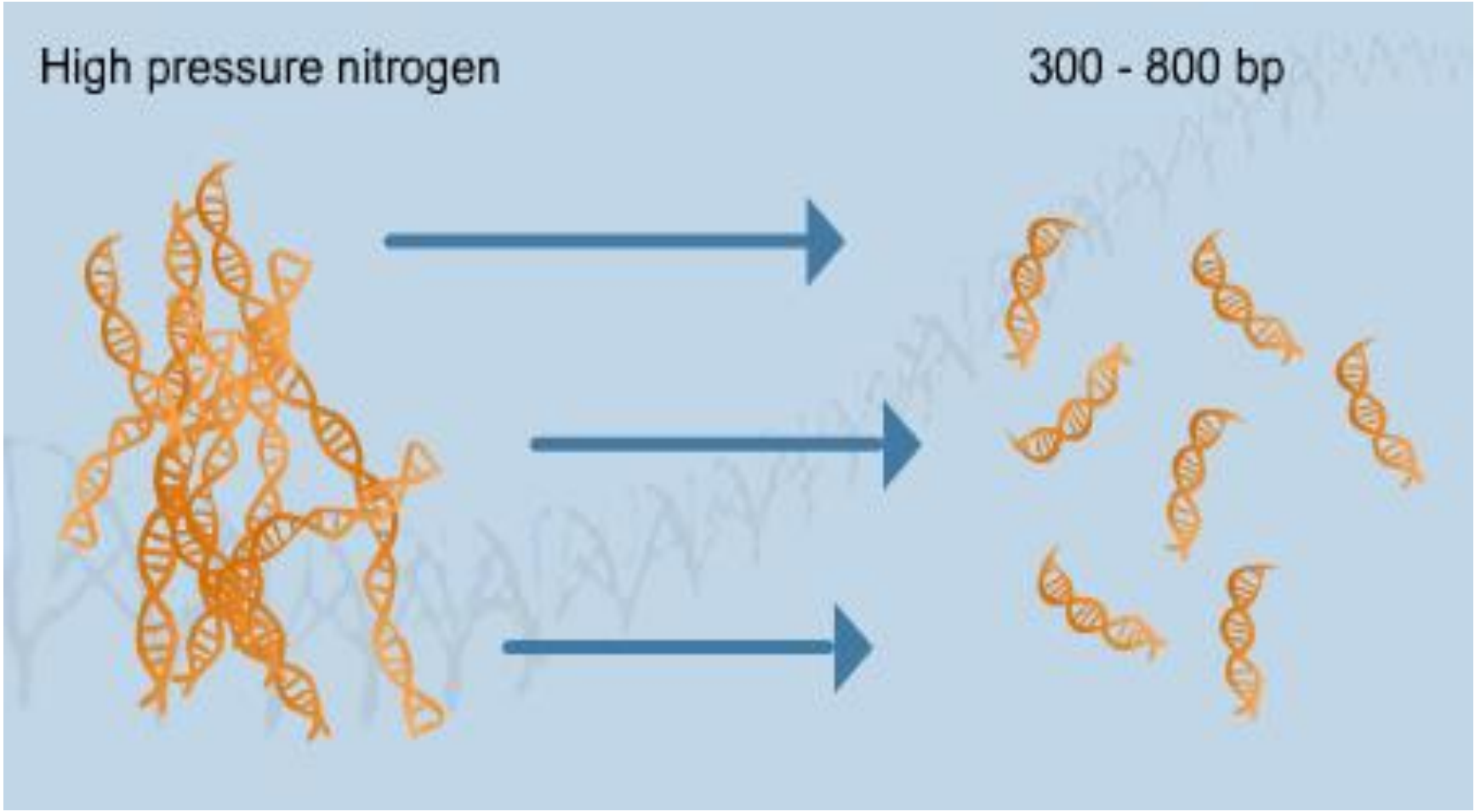
DNA library preparation

One sample preparation per genome

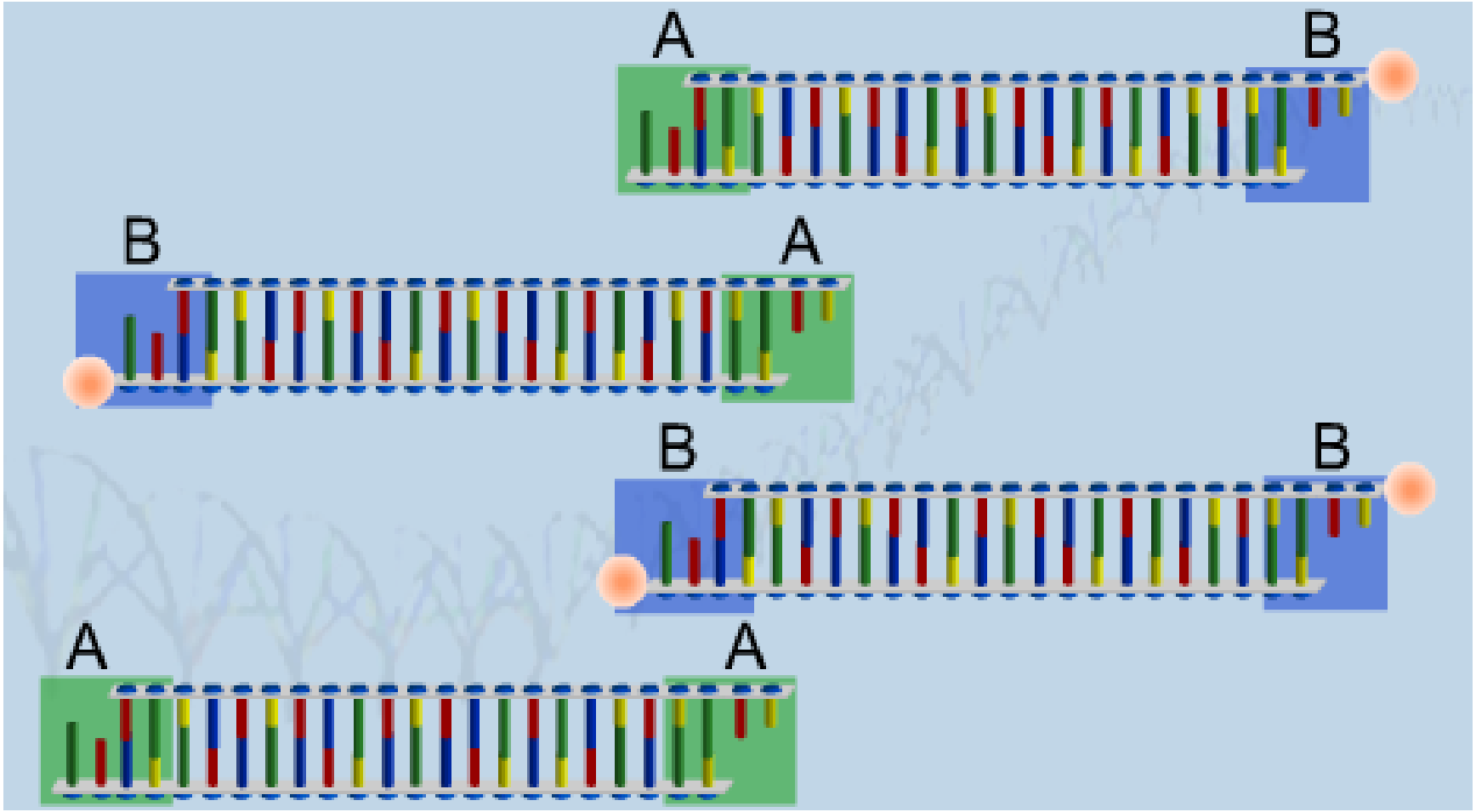
No Cloning

No Colony Picking

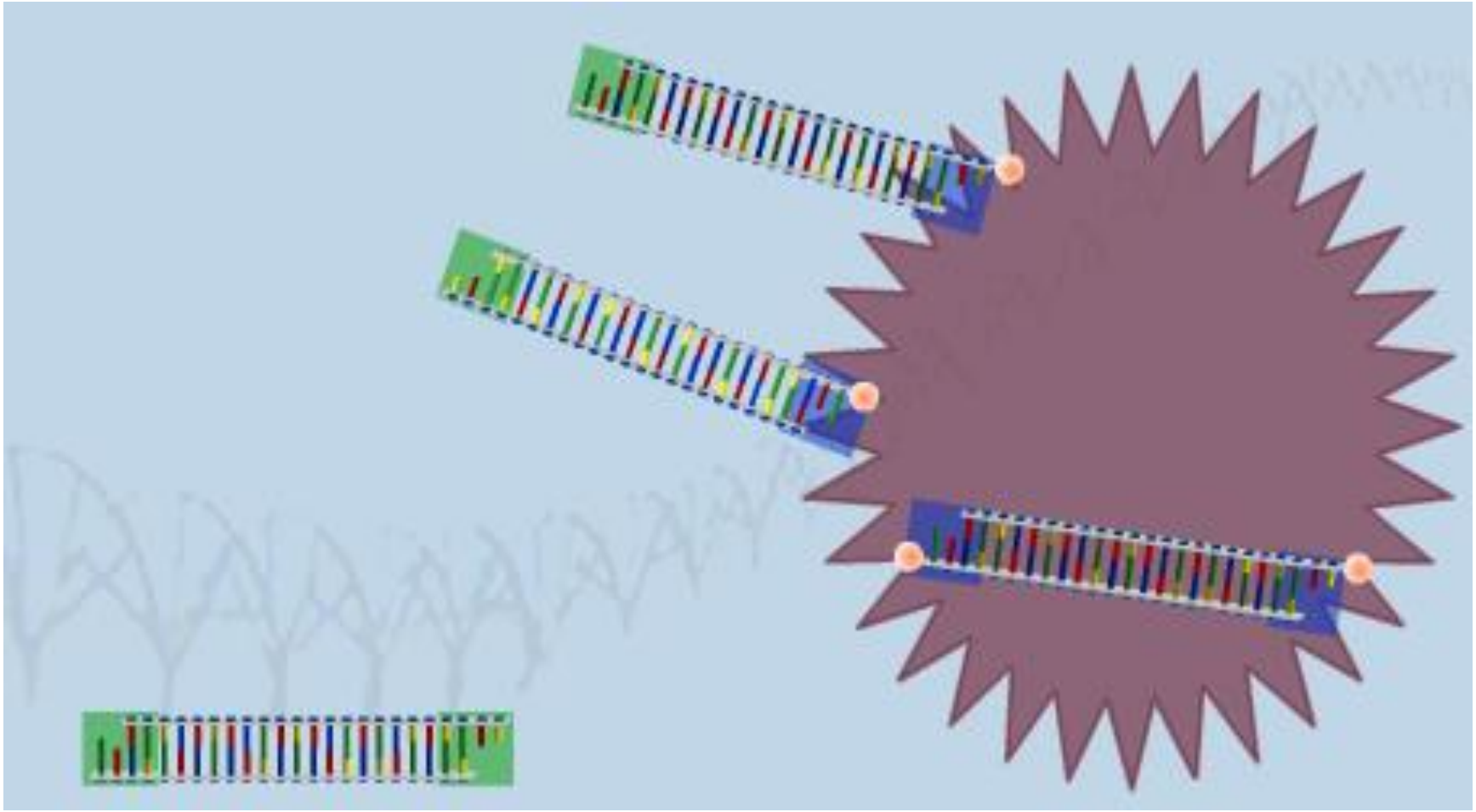
Fragmentace DNA



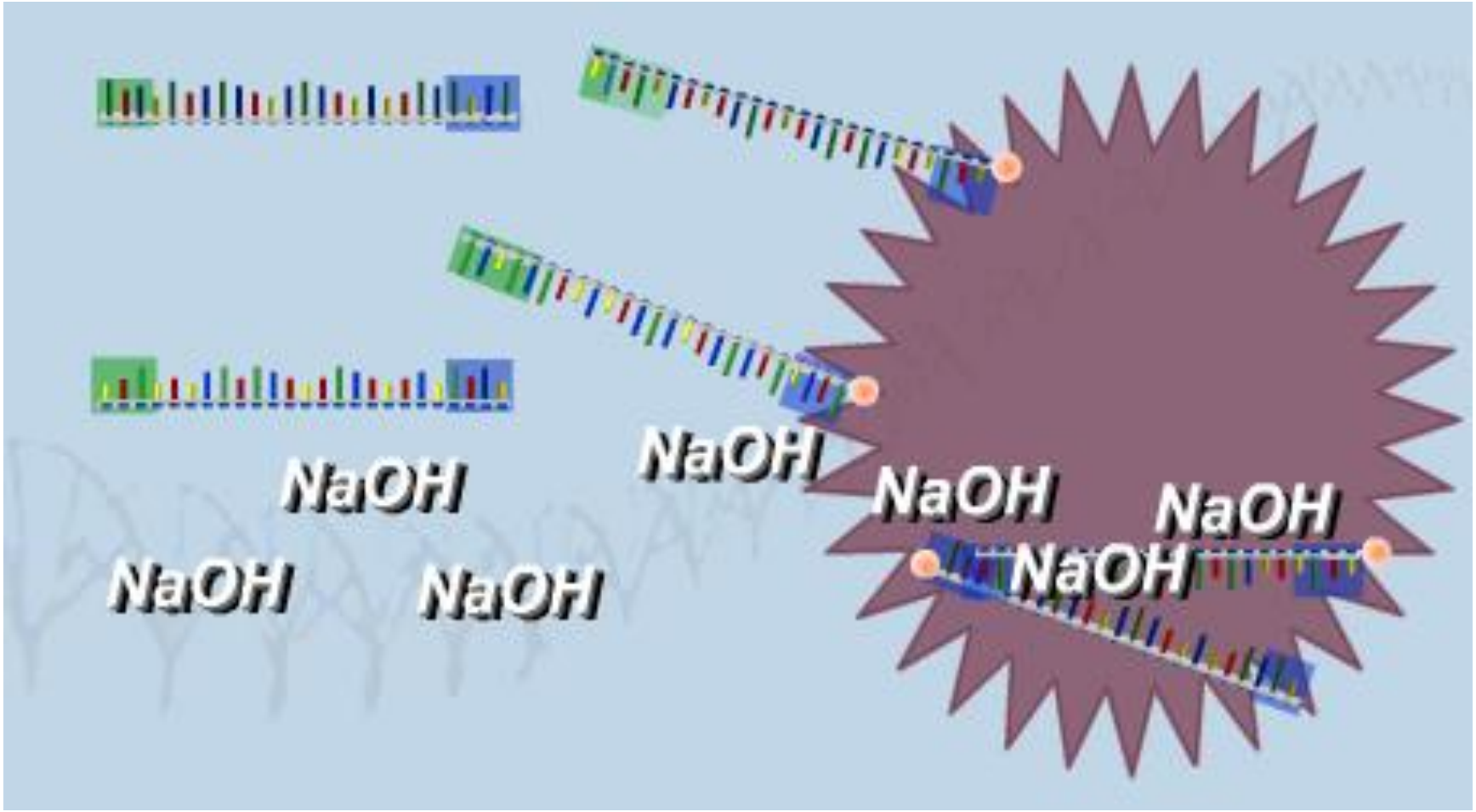
Ligace adaptoru

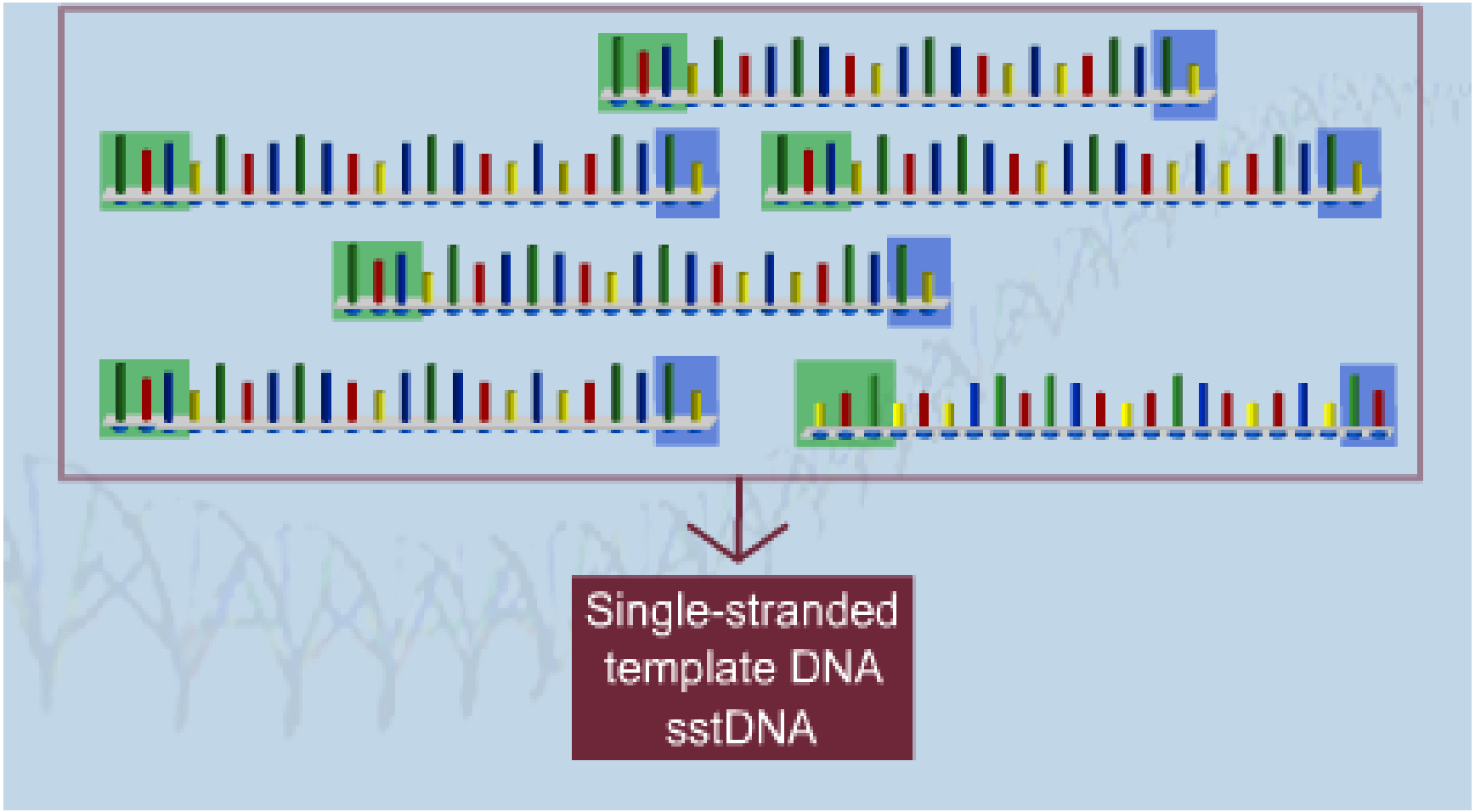


Vychytání DNA molekul

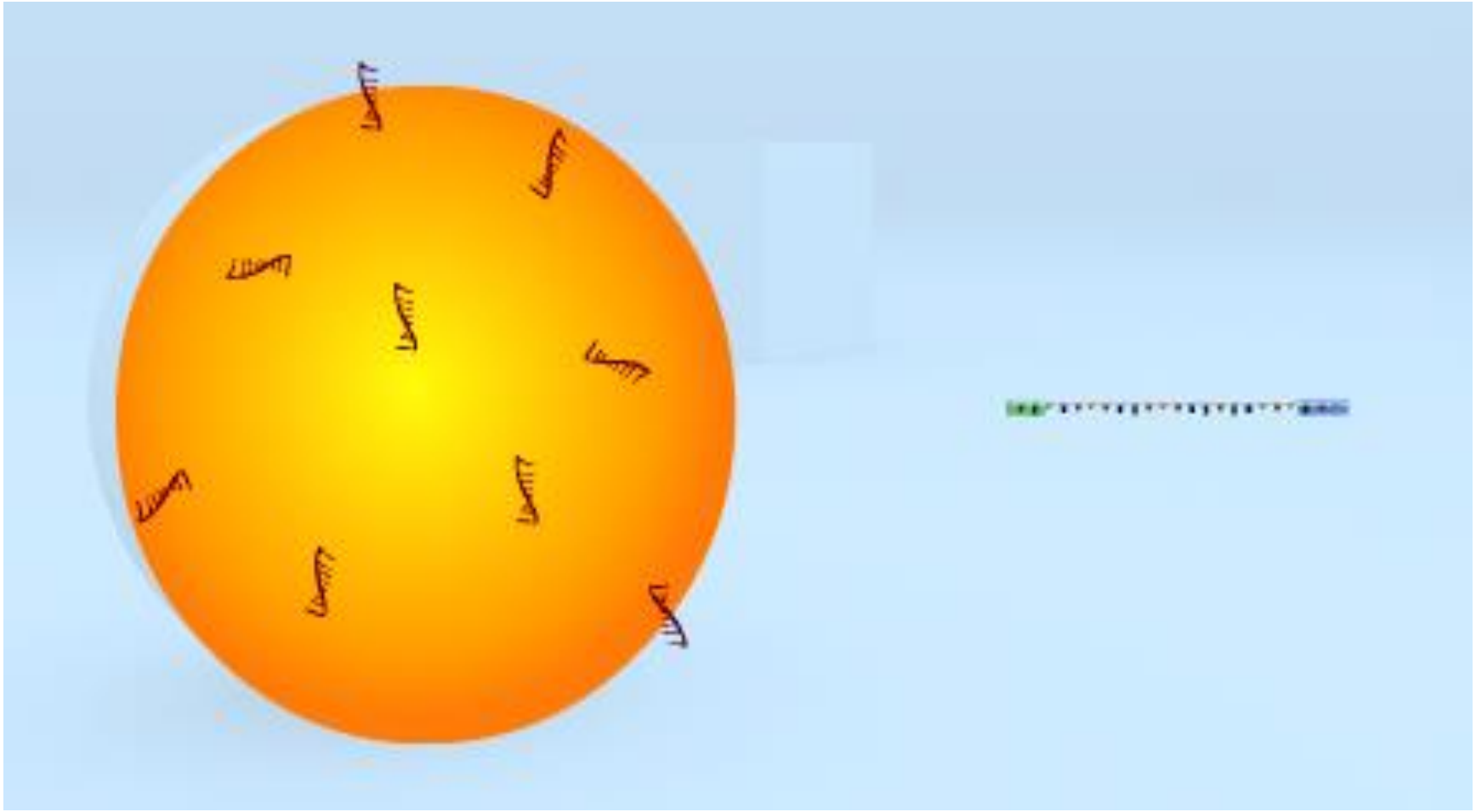


denaturace

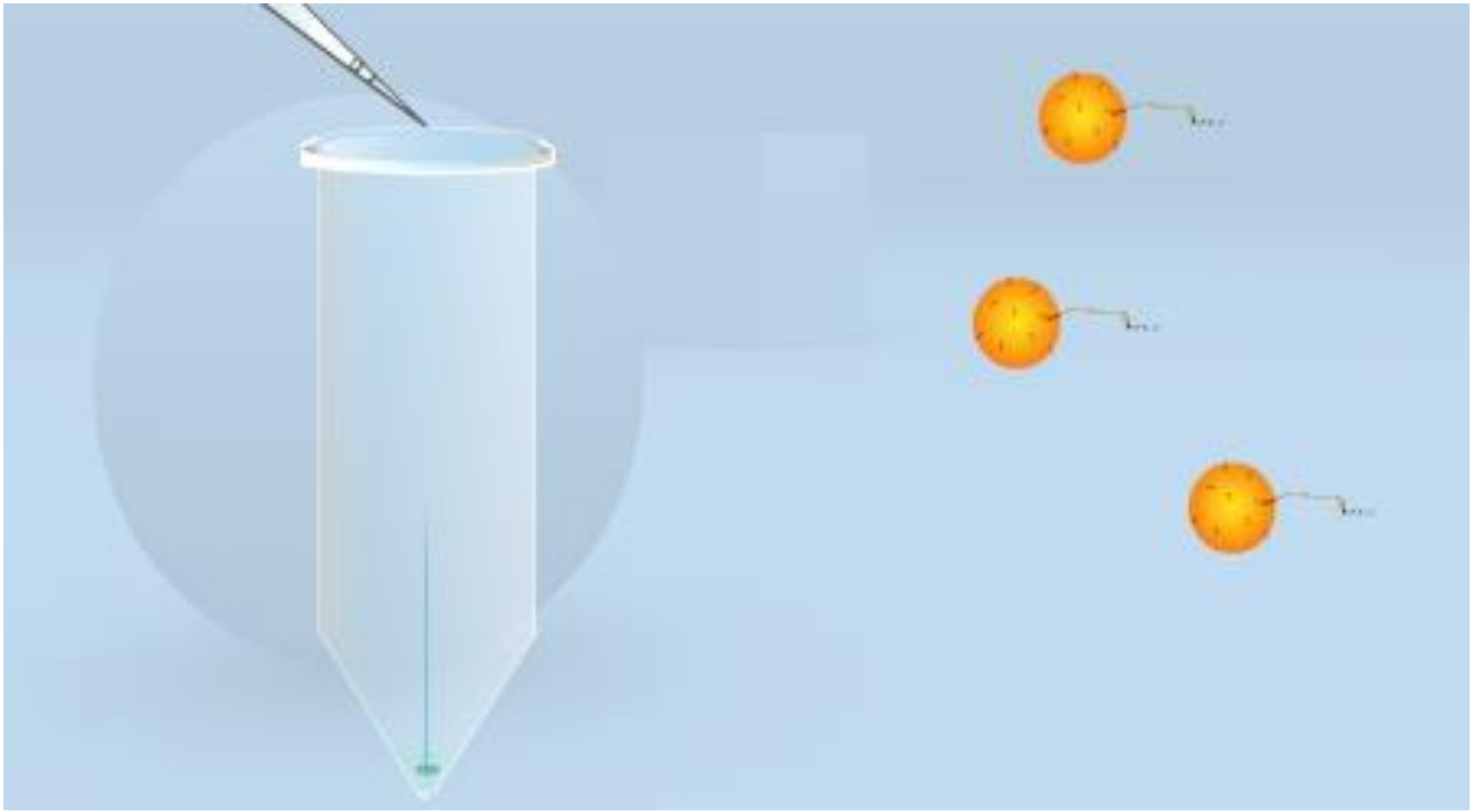



Single-stranded
template DNA
sstDNA

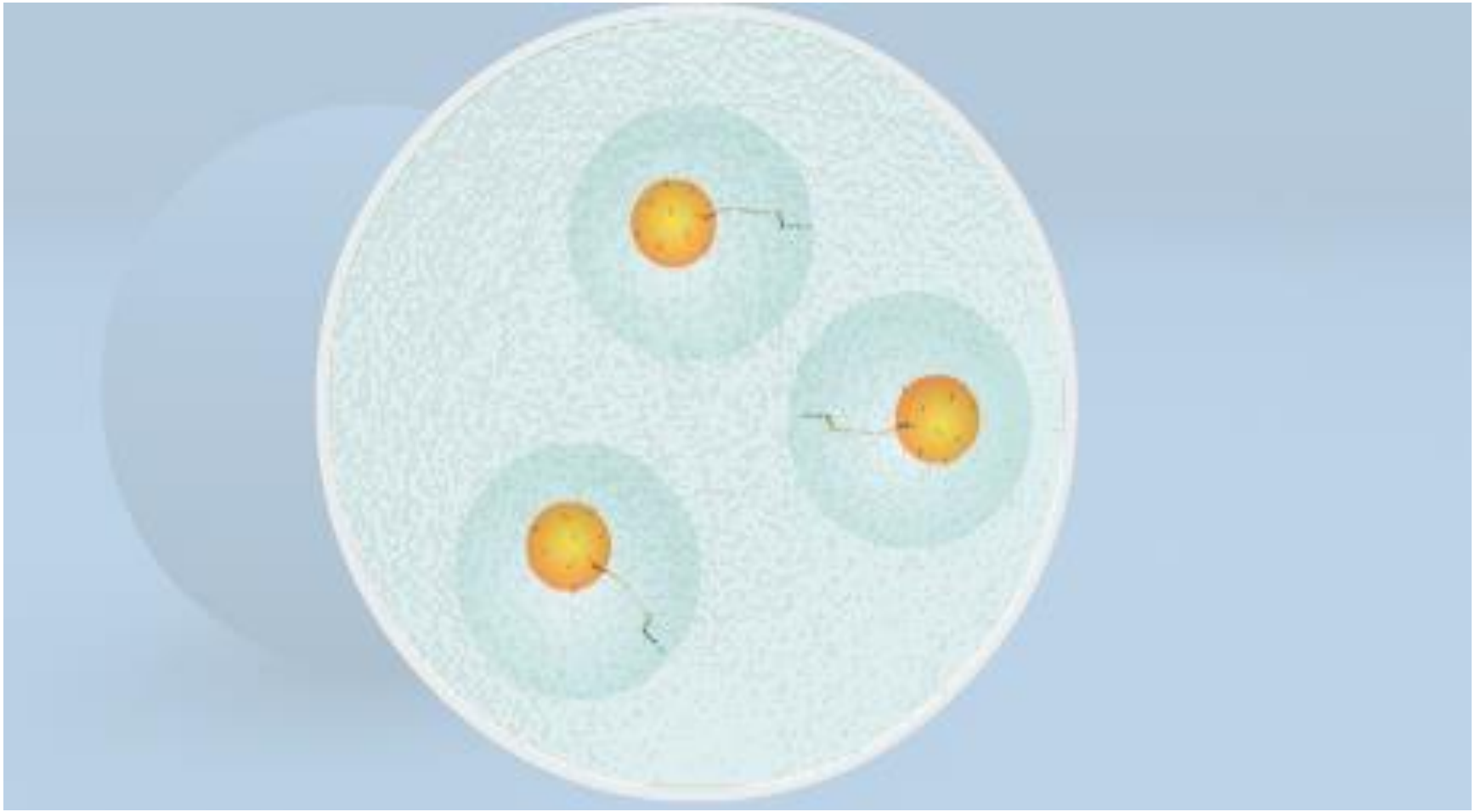
emPCR



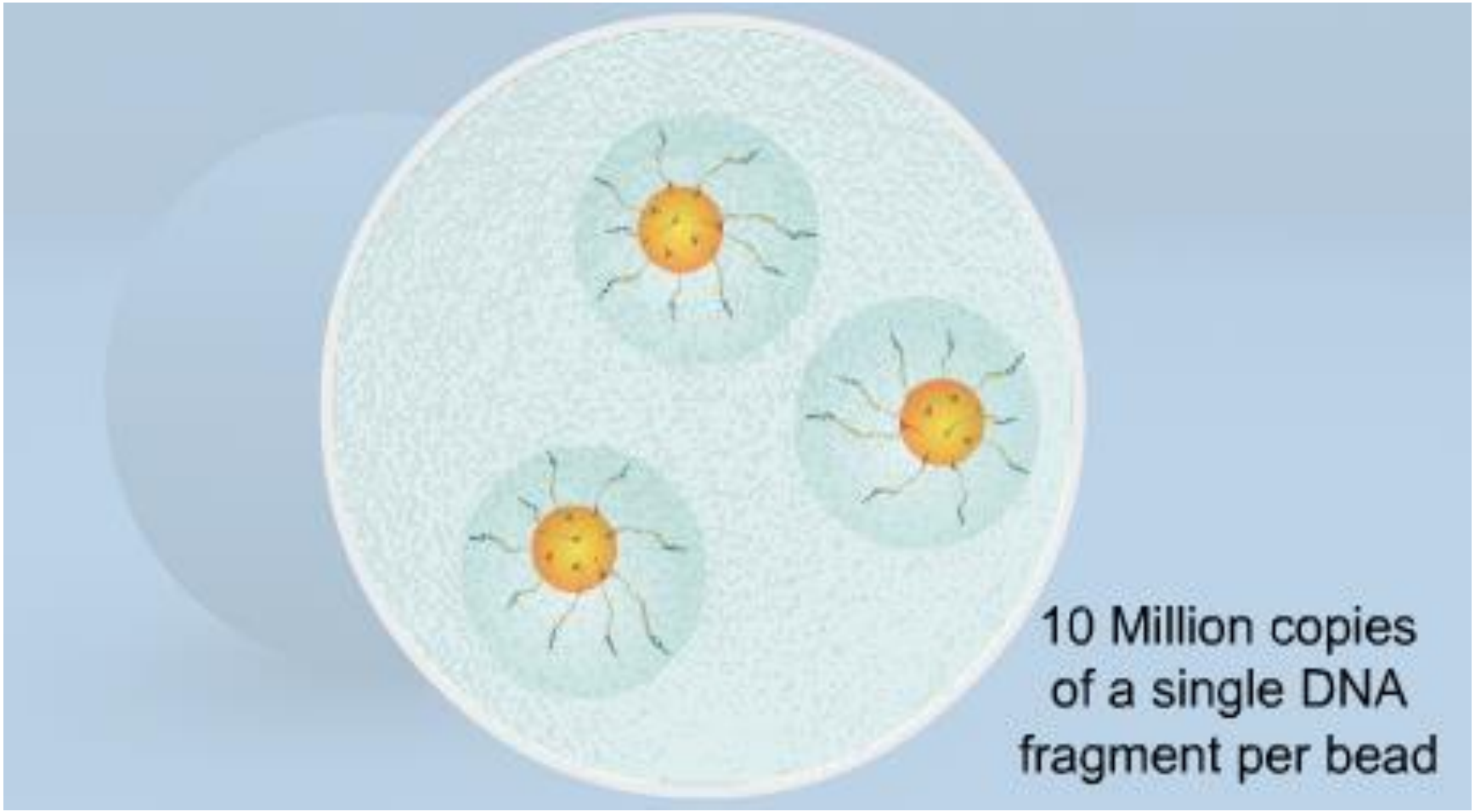
Vznik emulze (olej)



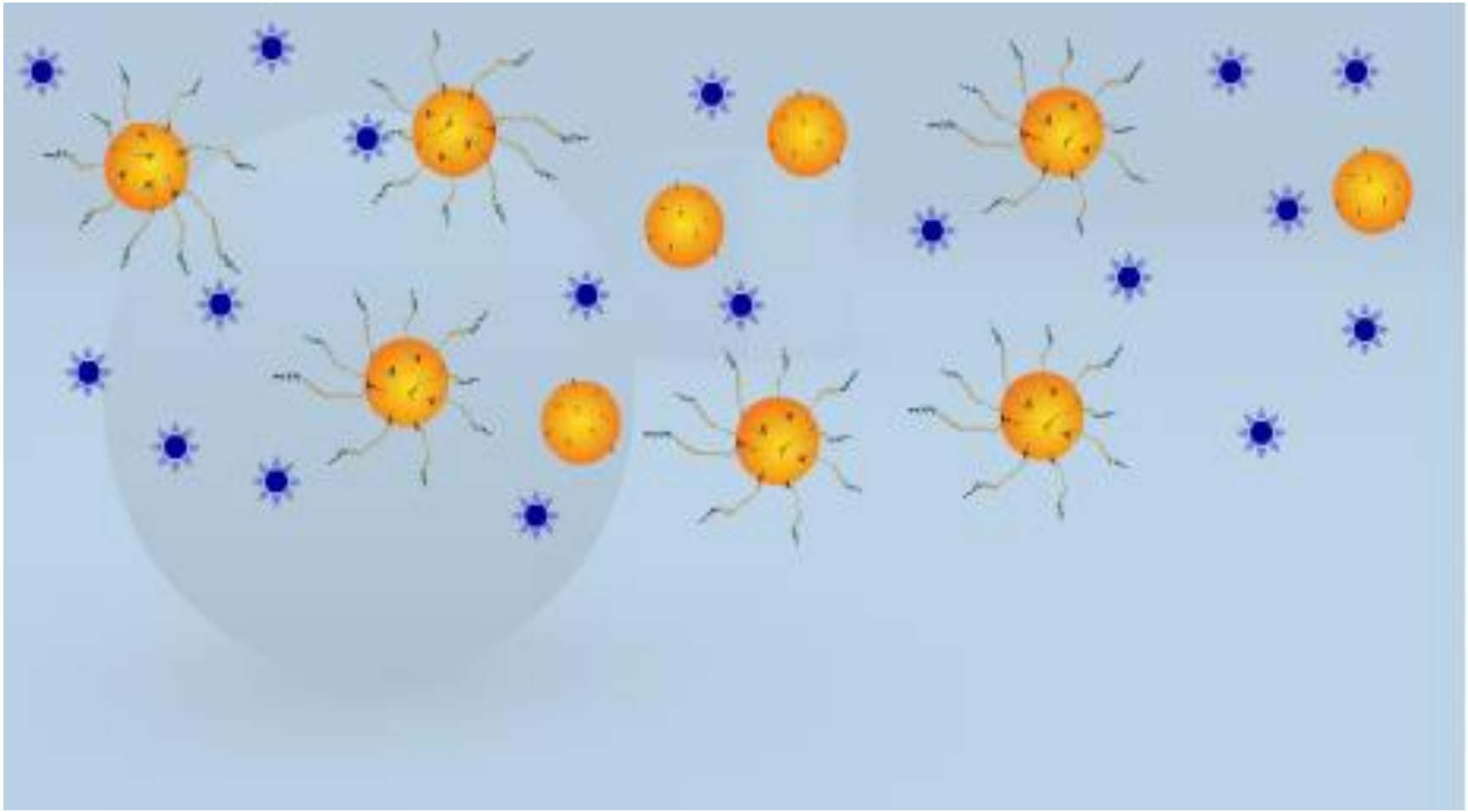
emPCR



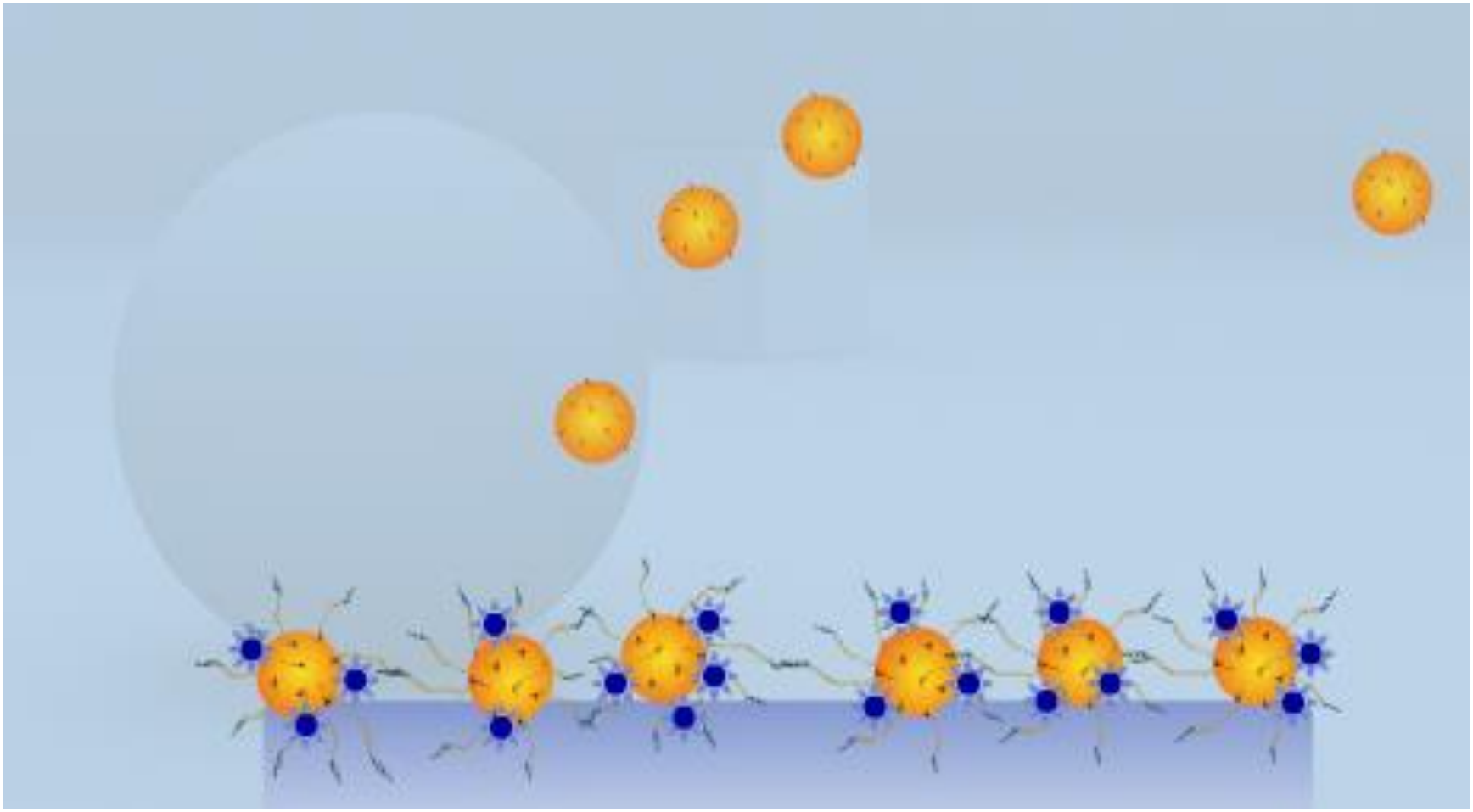
emPCR



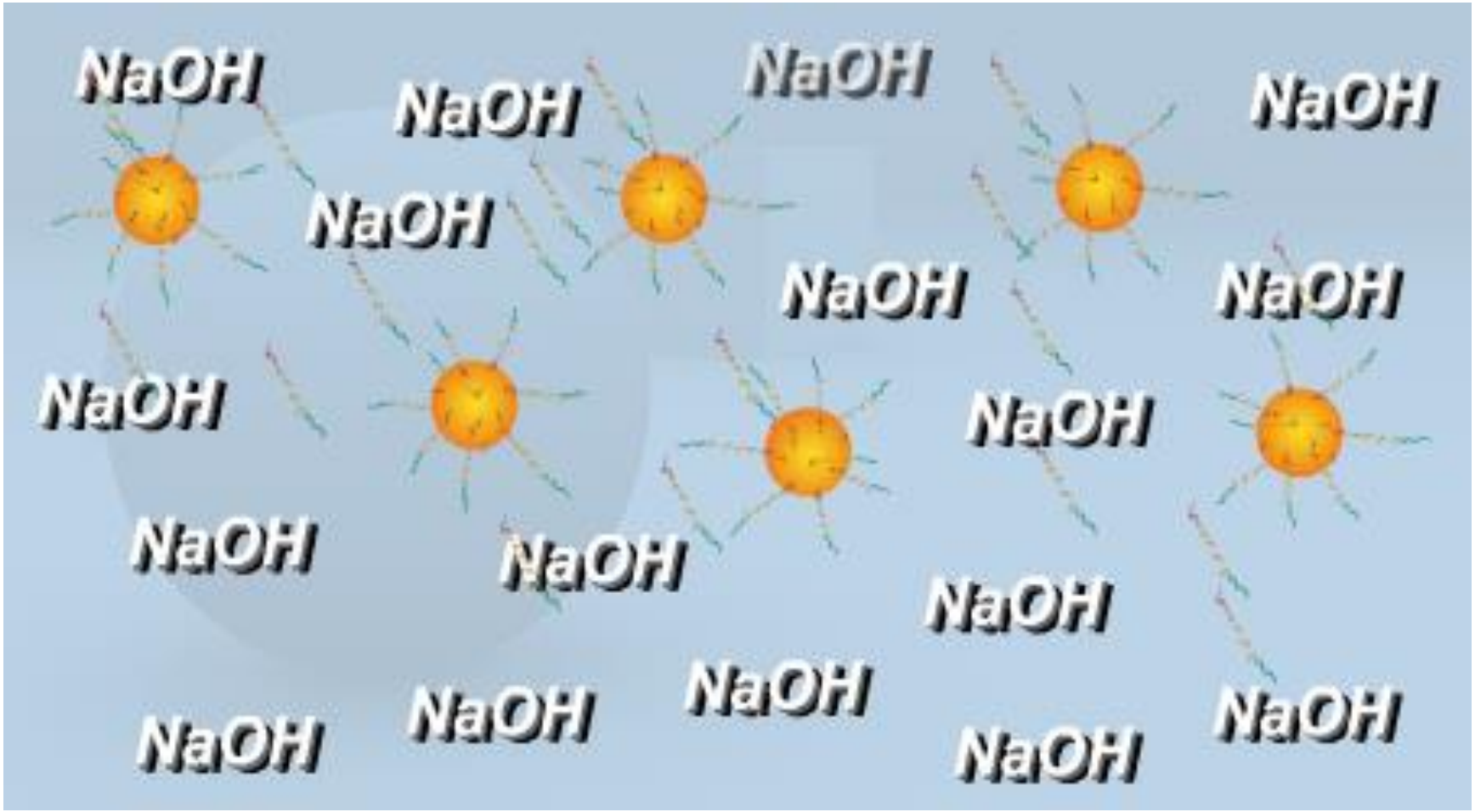
Vychytání kuliček



Vychytání kuliček



denaturace


NaOH
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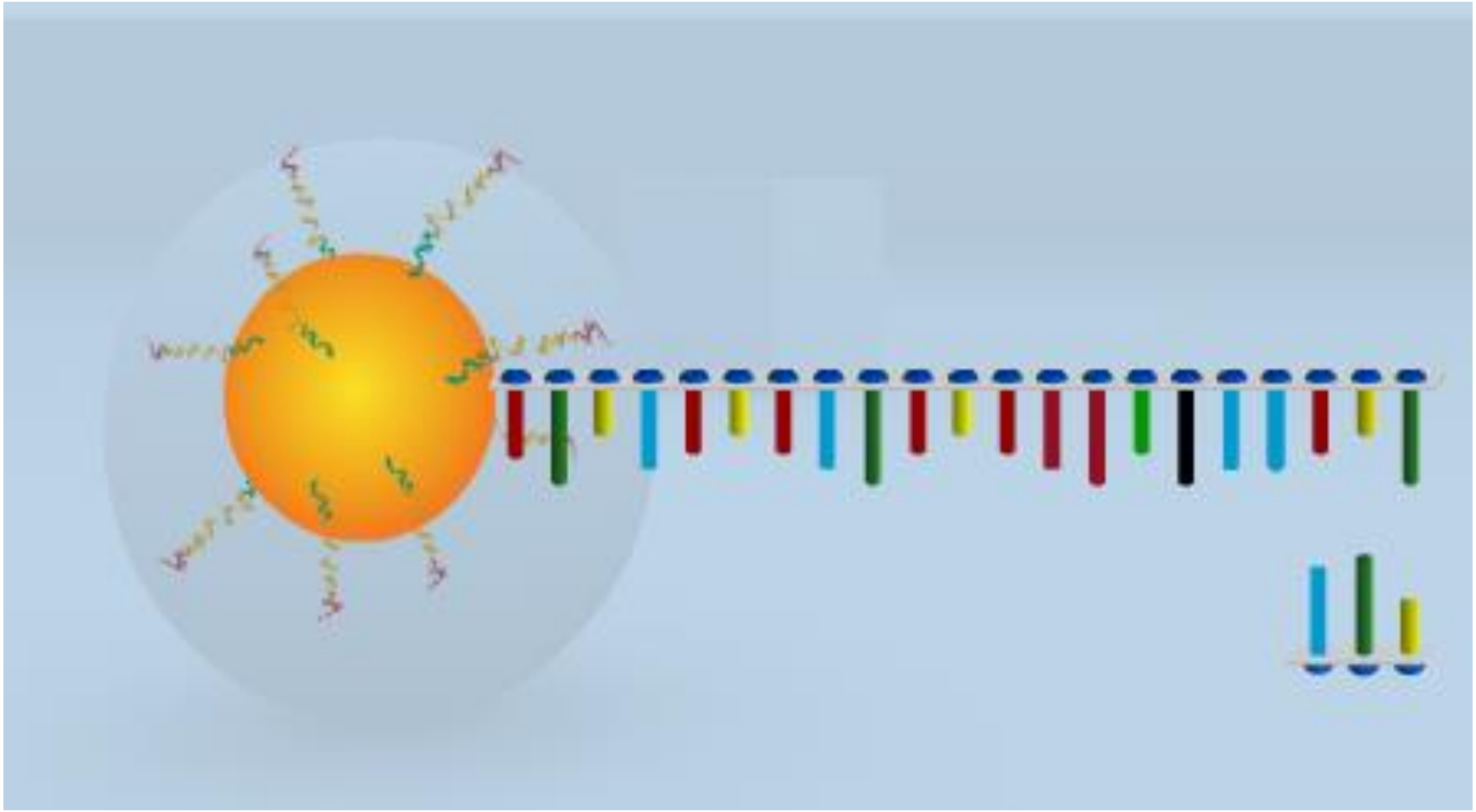
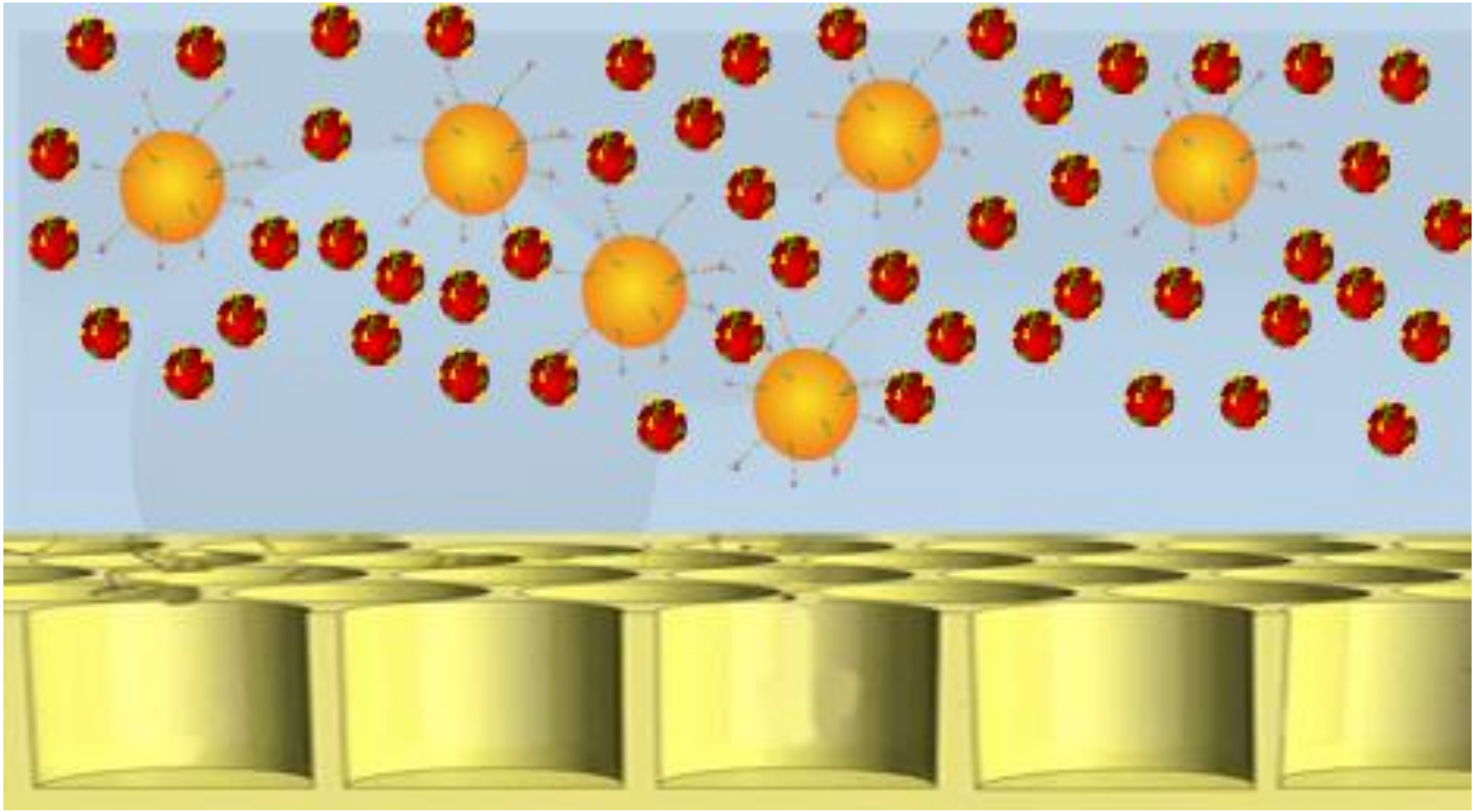
NaOH

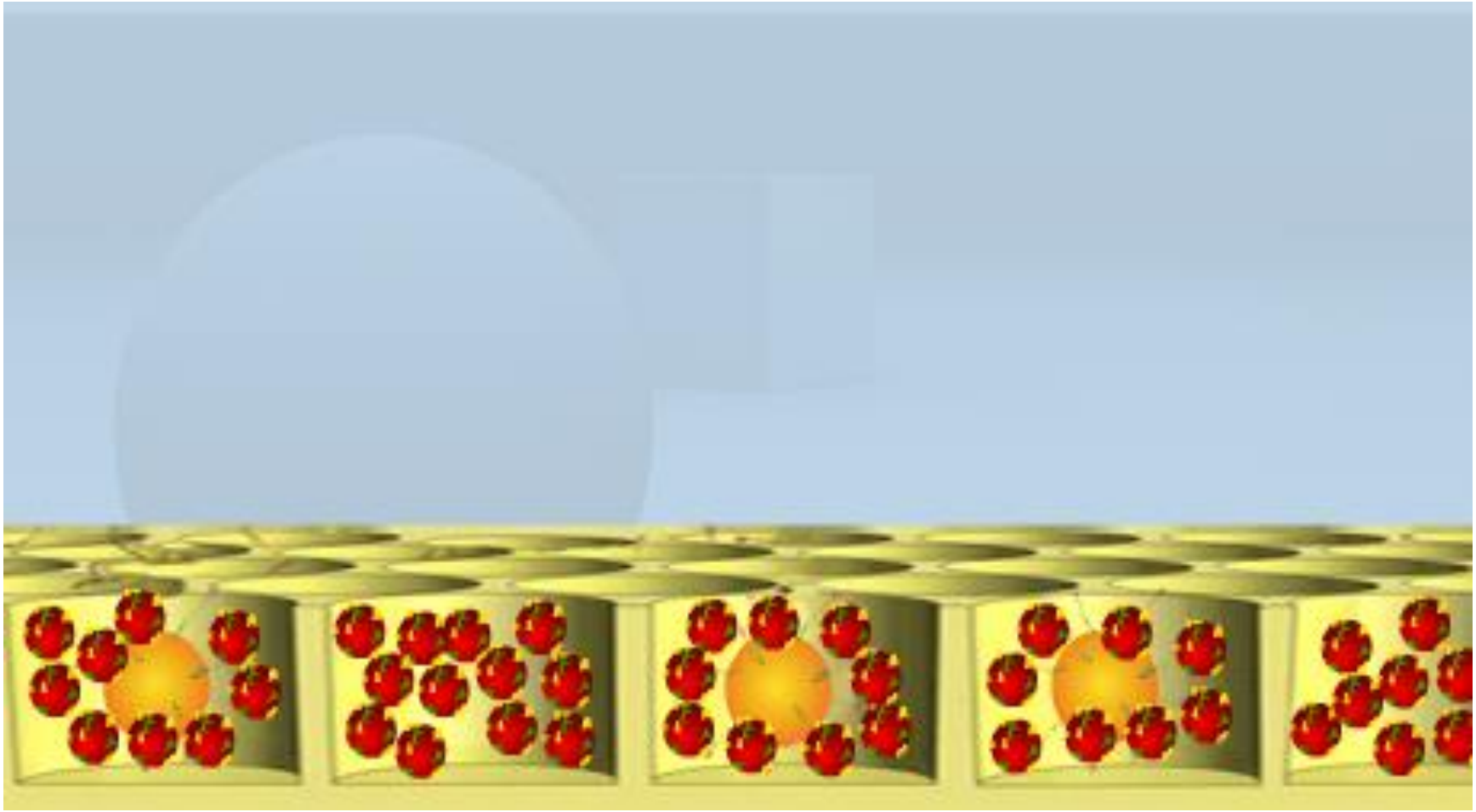
Sekvenační primer



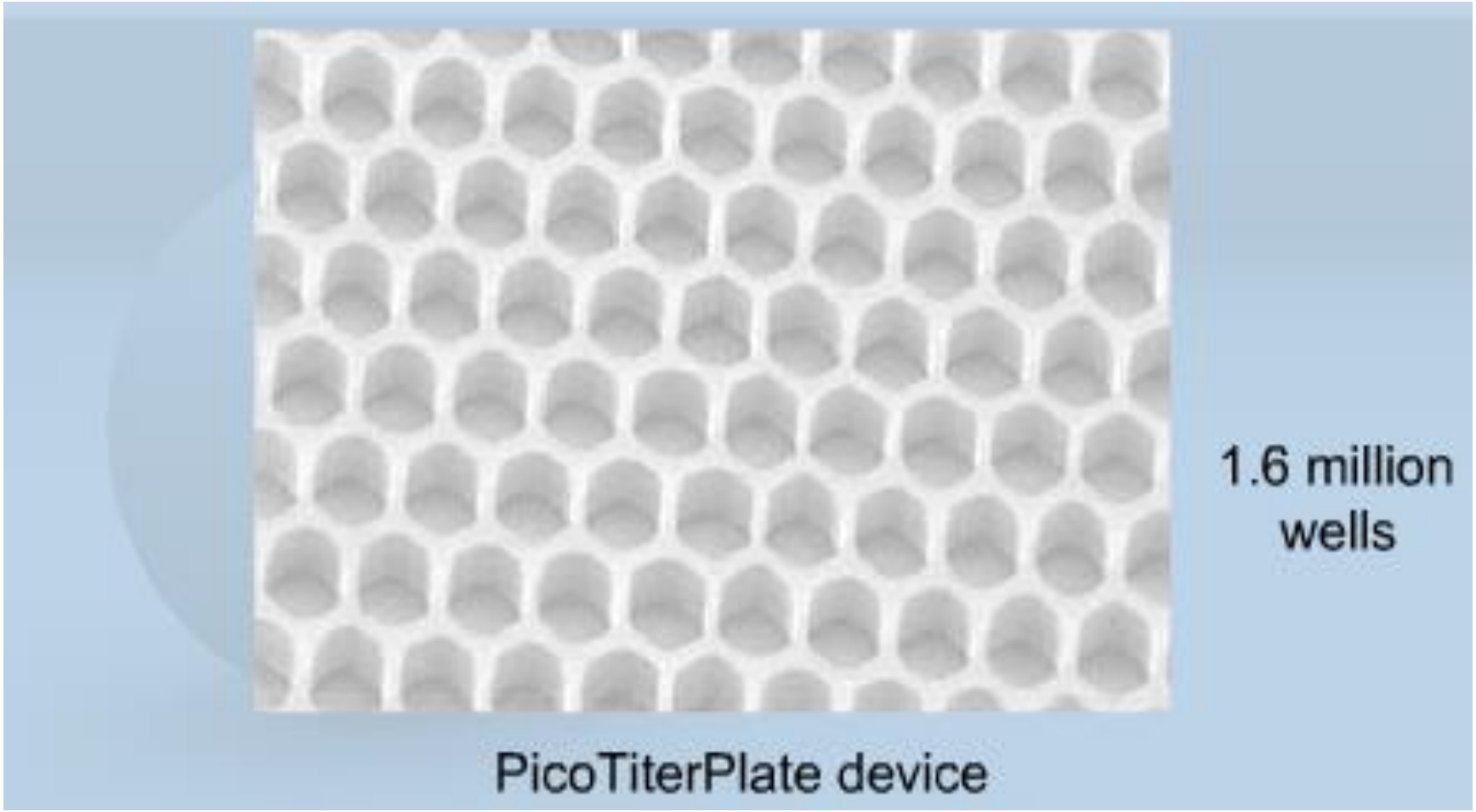
Disperze na sklíčko



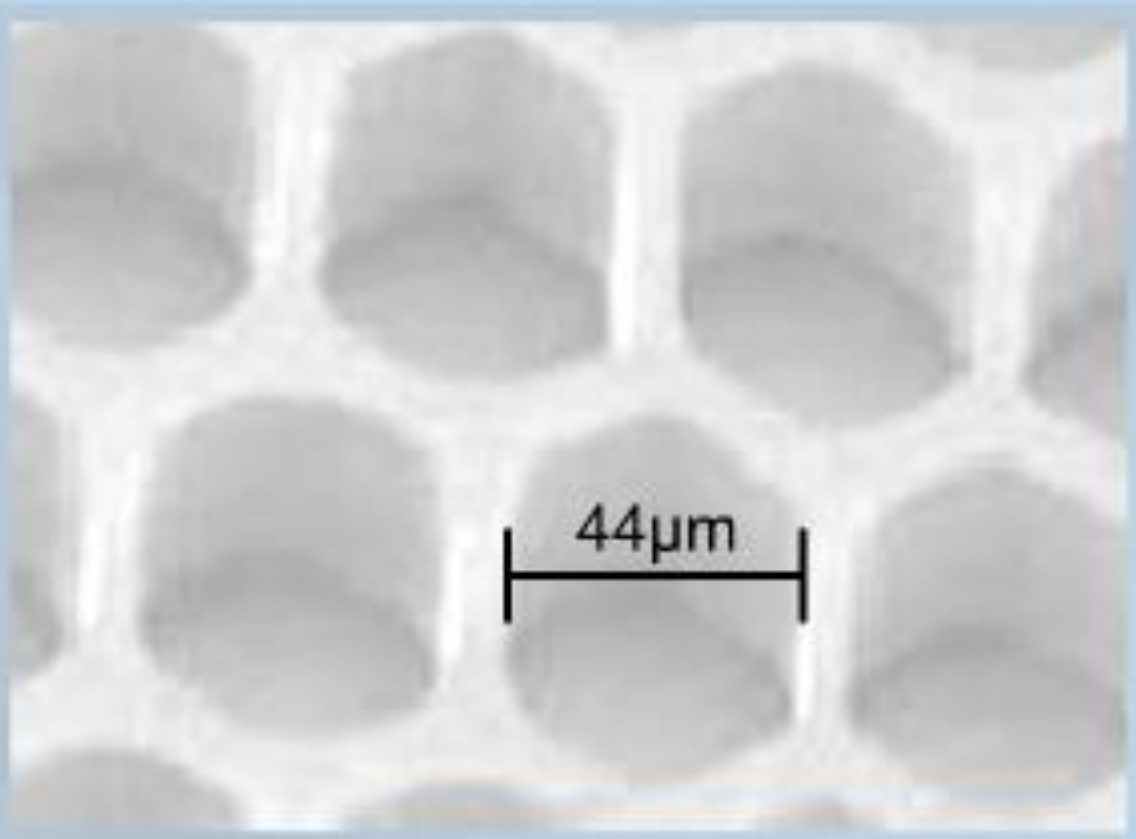
Disperze na sklíčko



Parametry mikroreaktorů

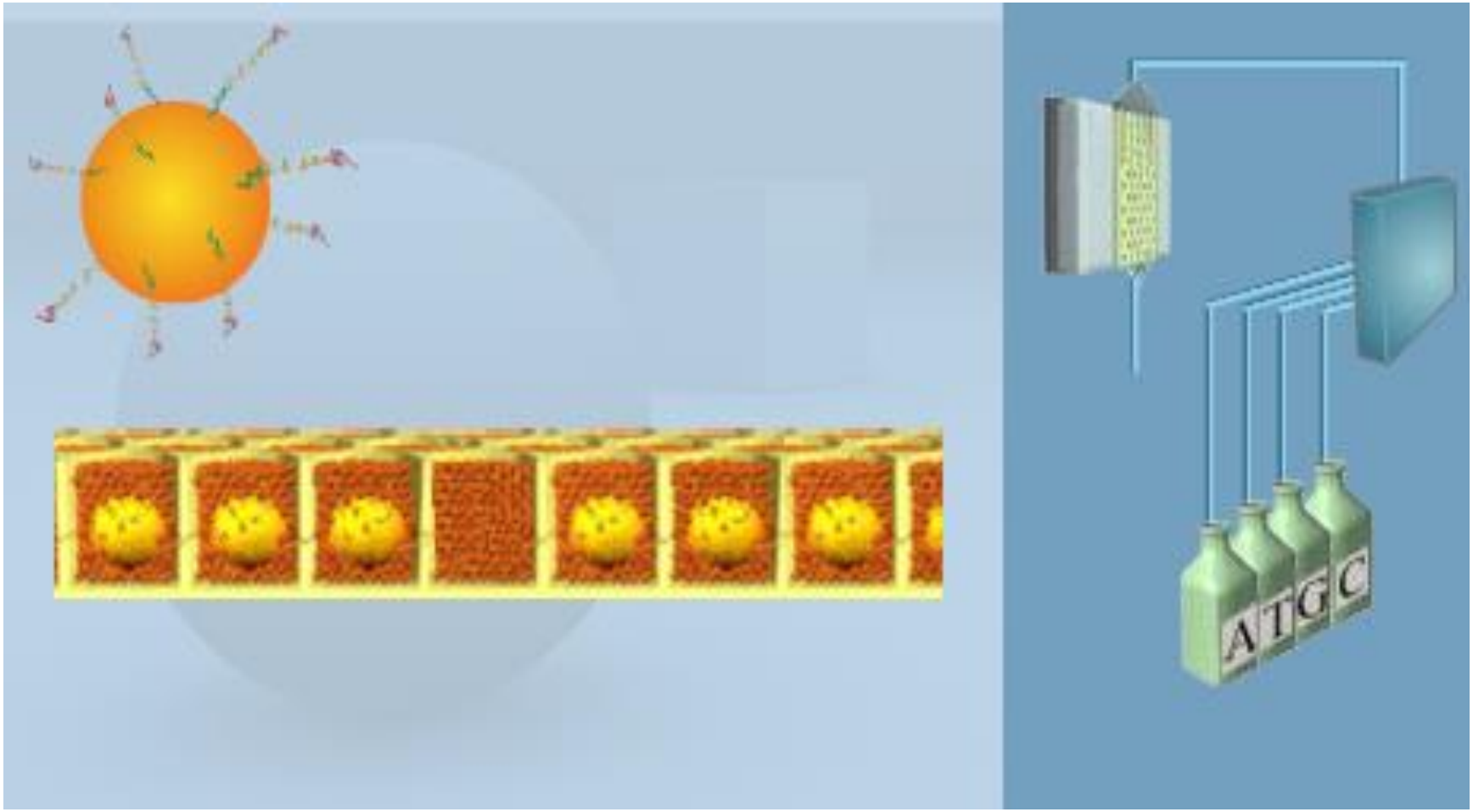


Parametry mikroreaktorů

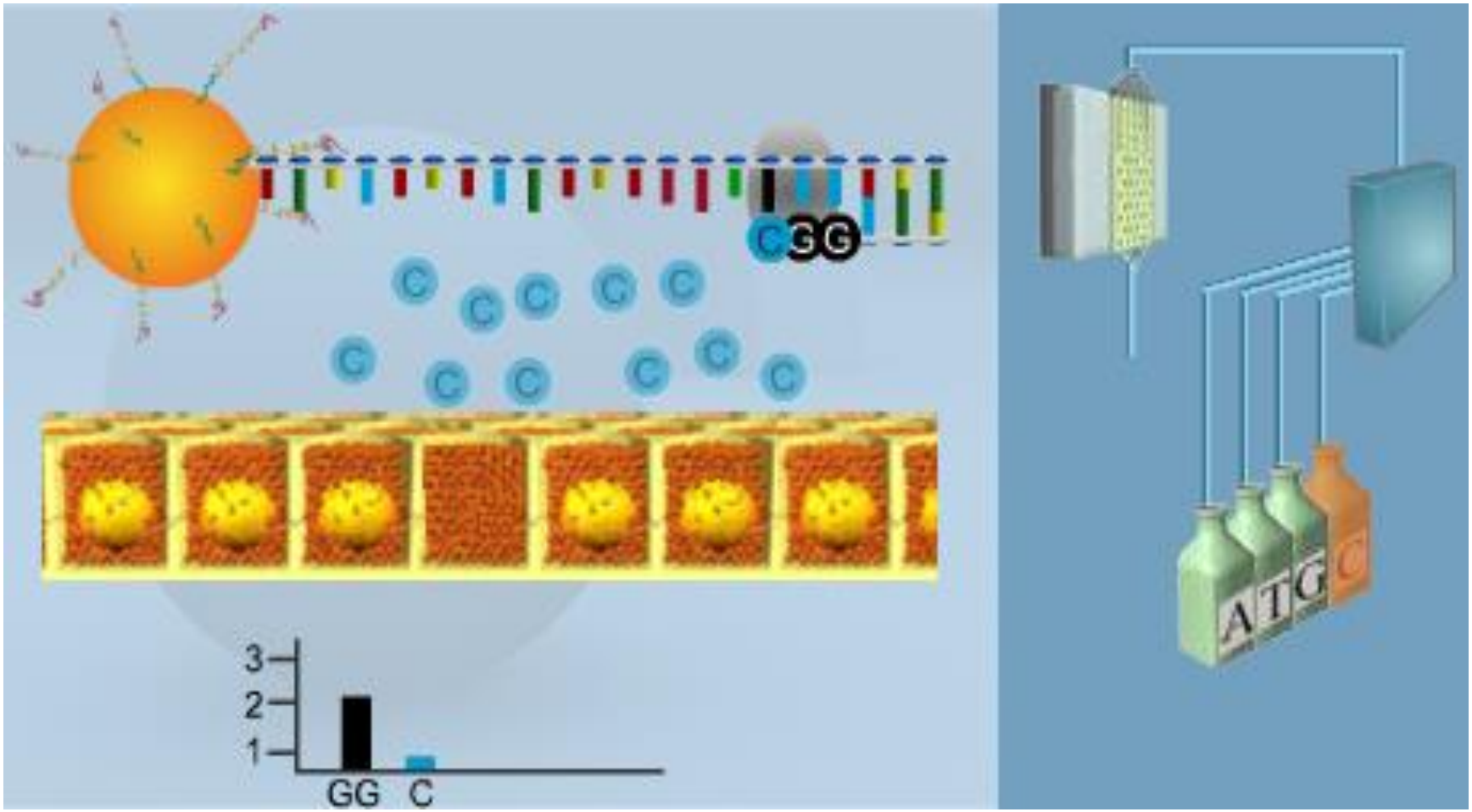


PicoTiterPlate device

sekvenace



sekvenace



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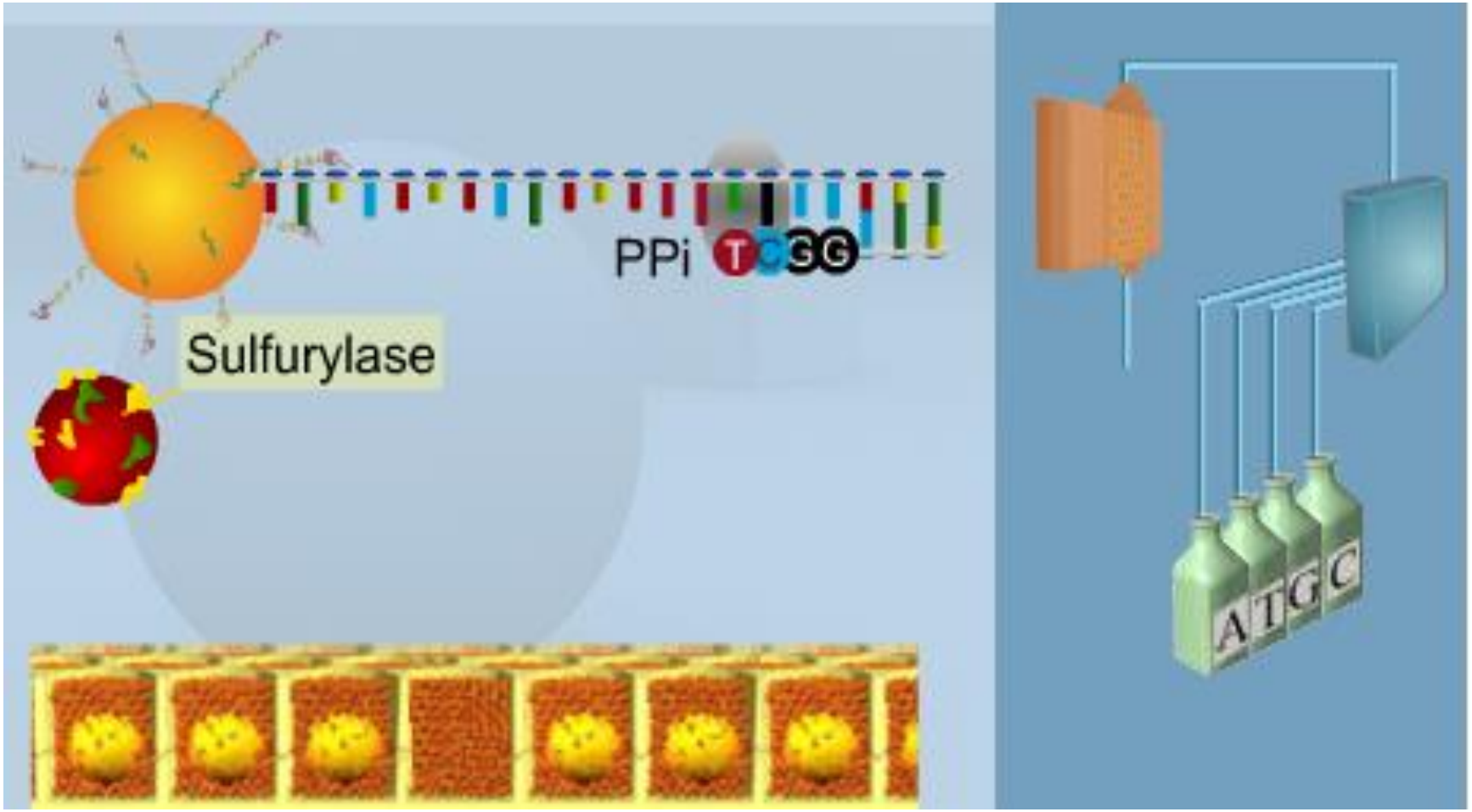
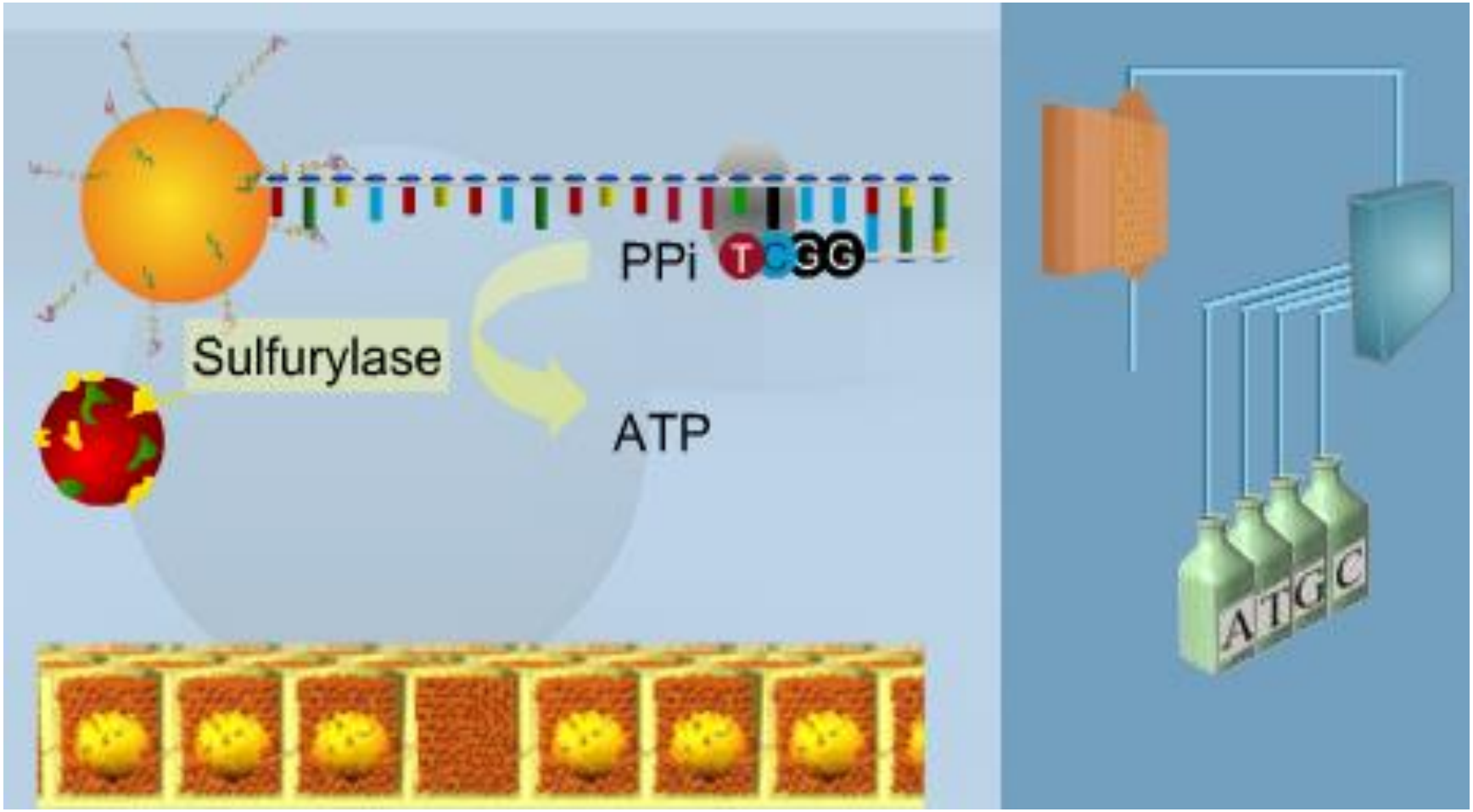
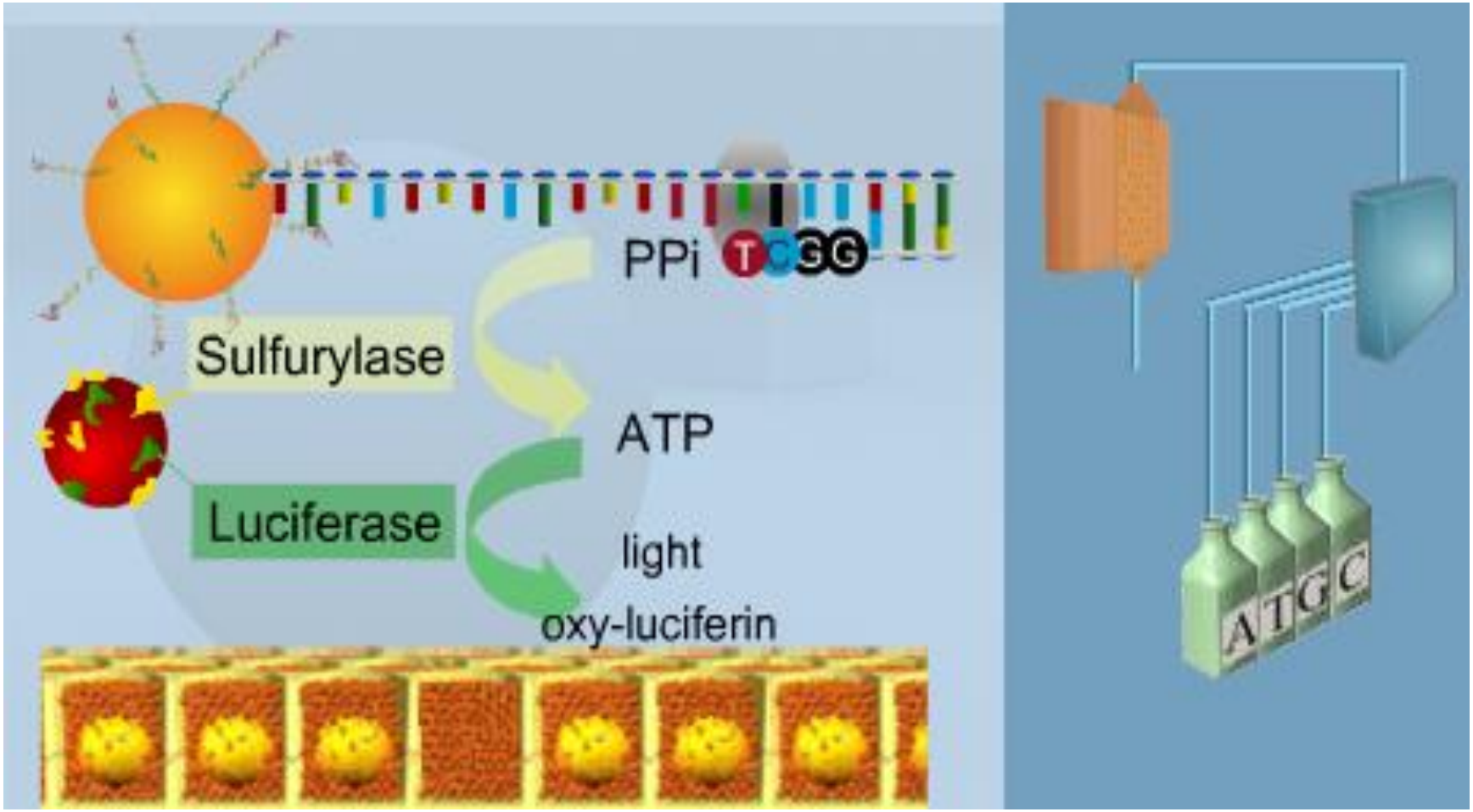


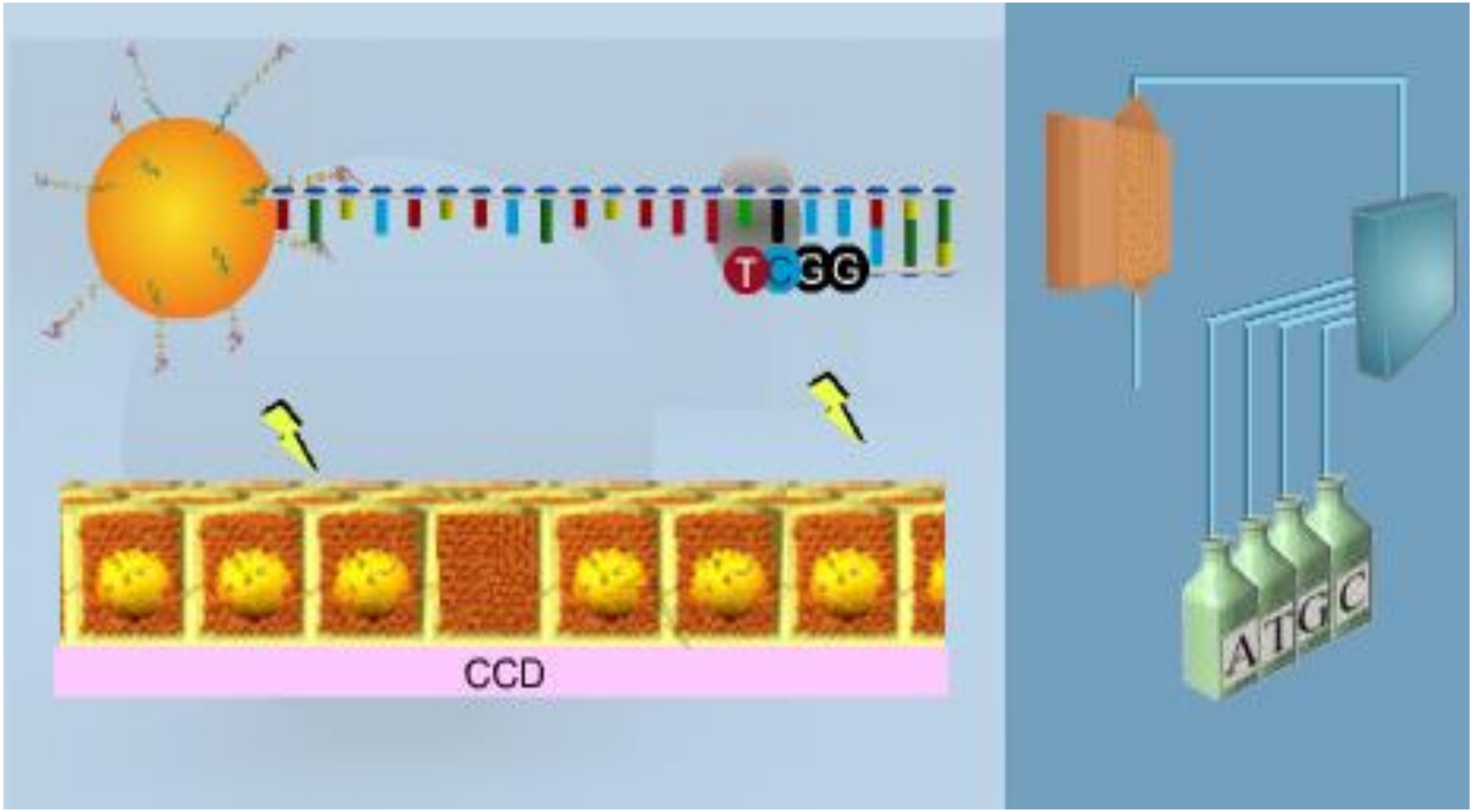
sekvenace



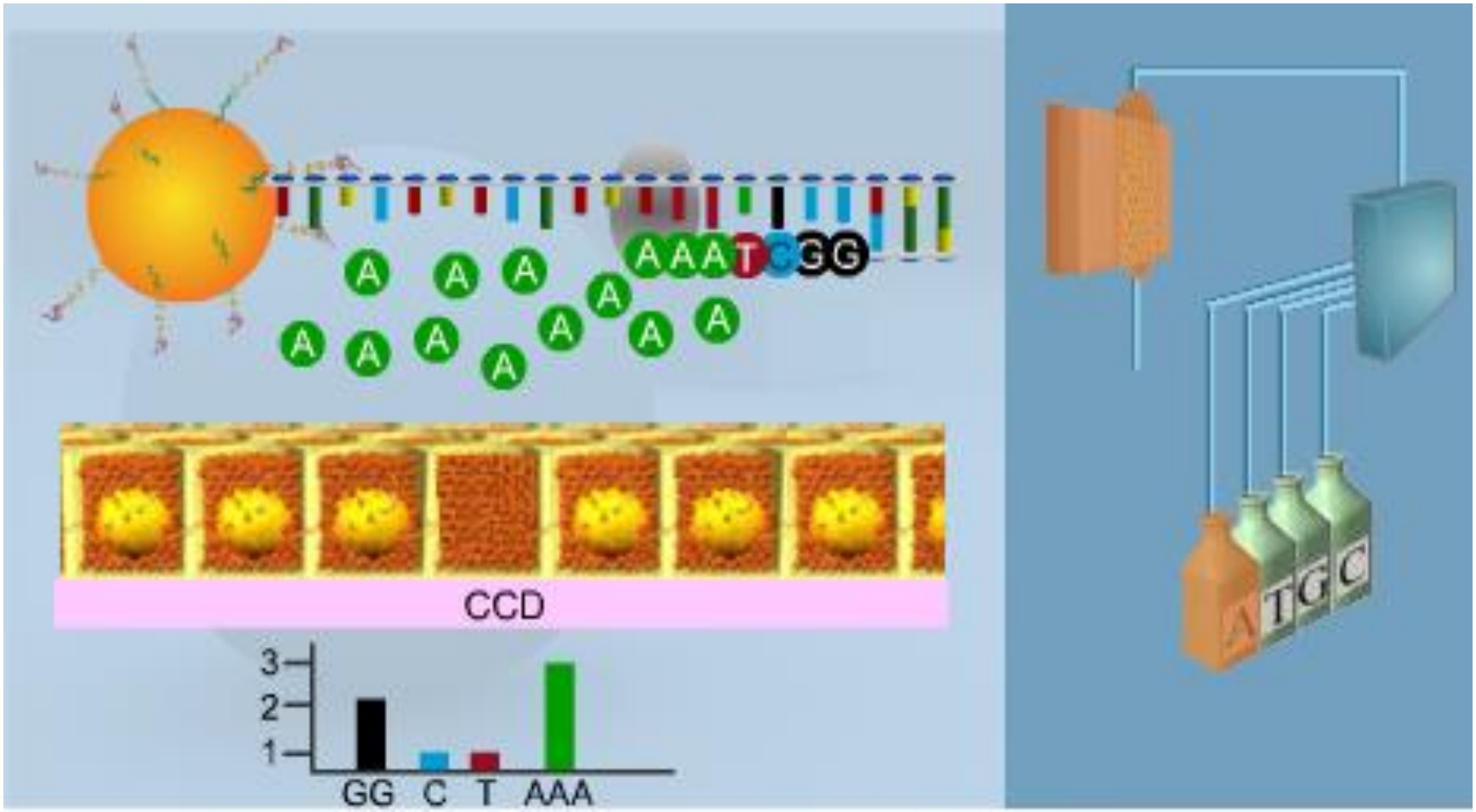
sekvenace


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

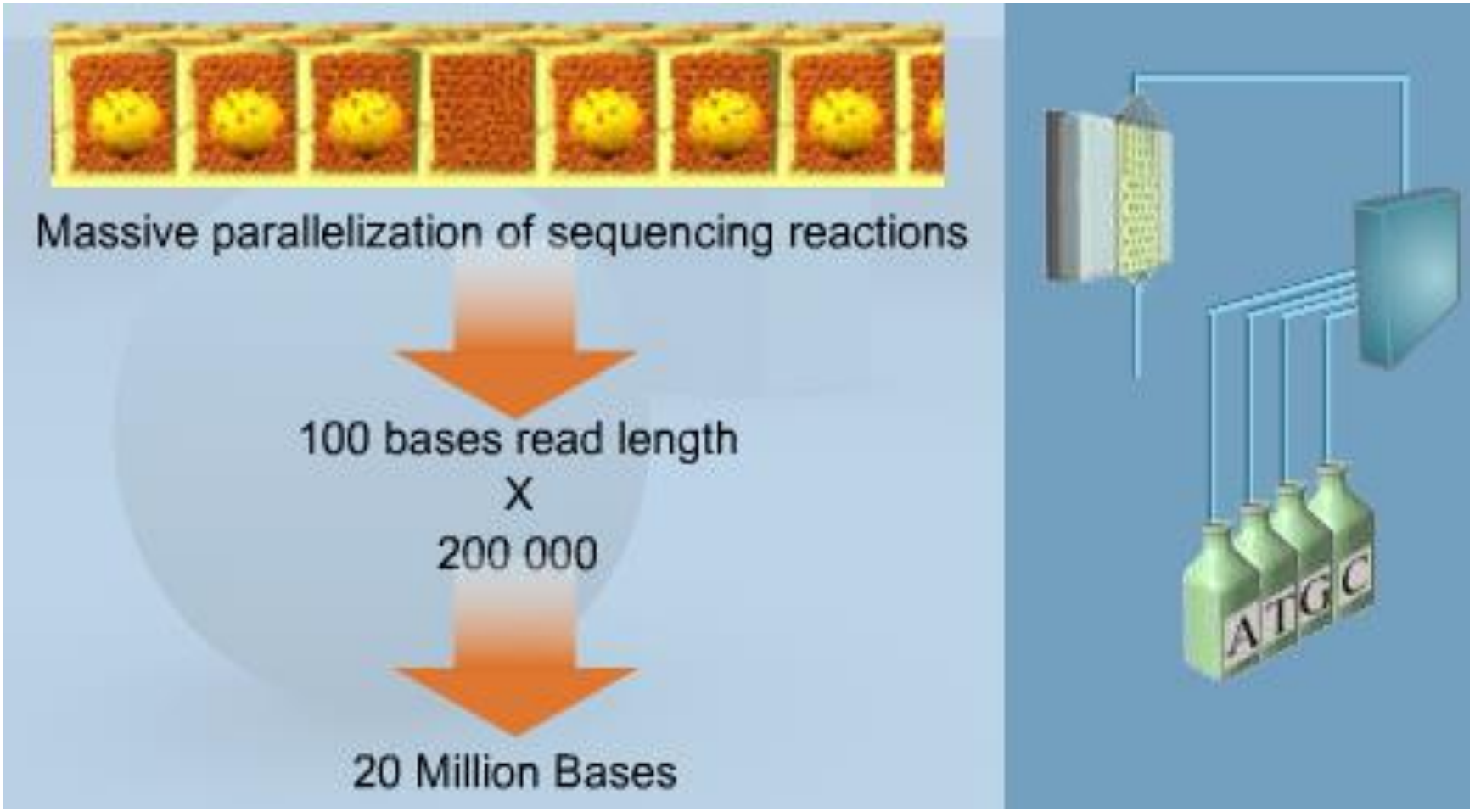
sekvenace



sekvenace

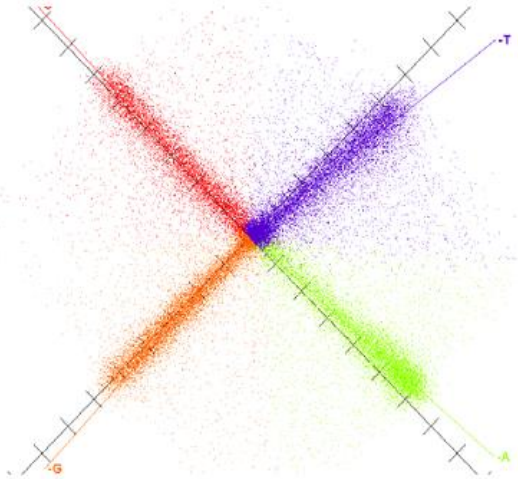


sekvenace


Massive parallelization of sequencing reactions
100 bases read length
X
200 000
20 Million Bases
ATGC

SOLID (Sequencing by Oligonucleotide Ligation and Detection)

2-base encoding sequencing (2007)



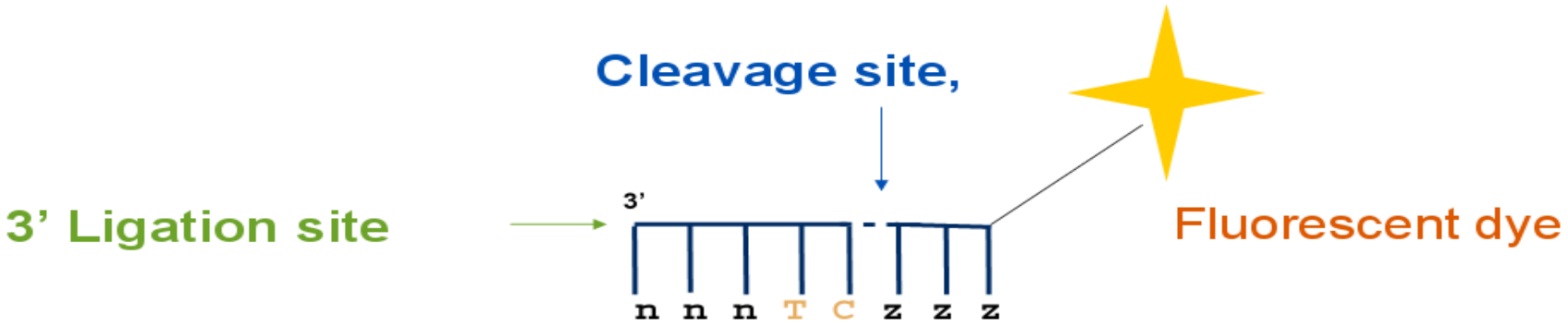
Applied Biosystems

SOLiD™ System

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

Properties of the Probes

Spatial separation among dye, ligation & cleavage sites

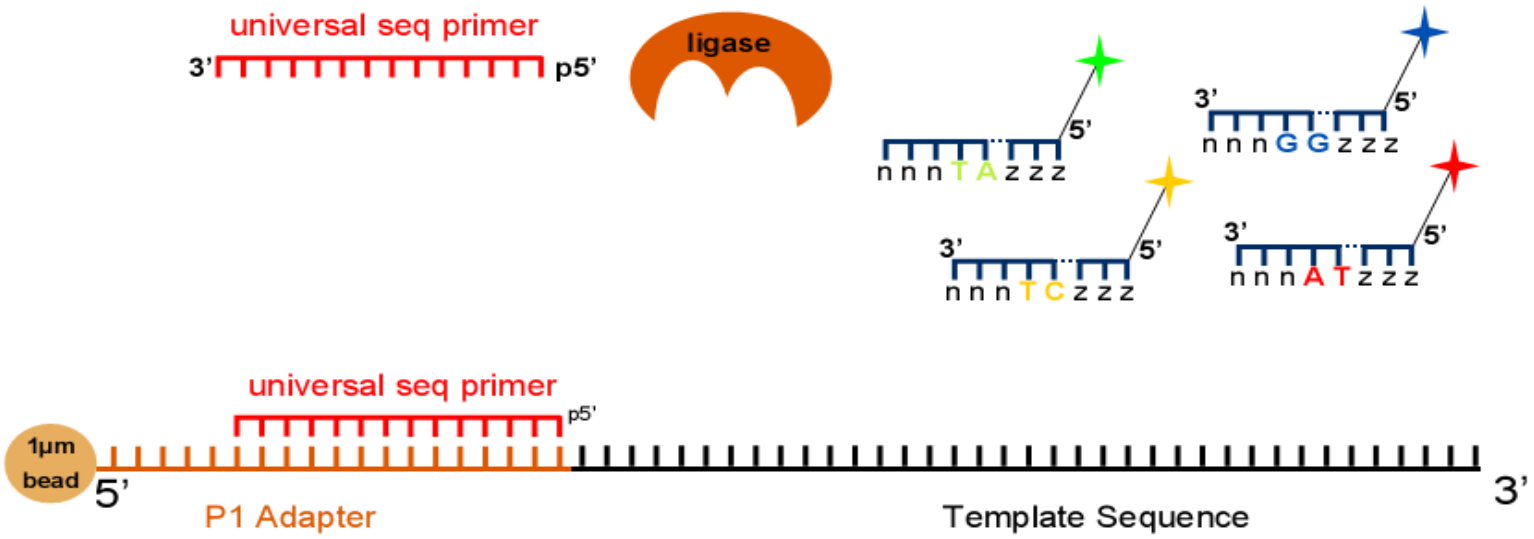


1,024 Octamer Probes (4^5)

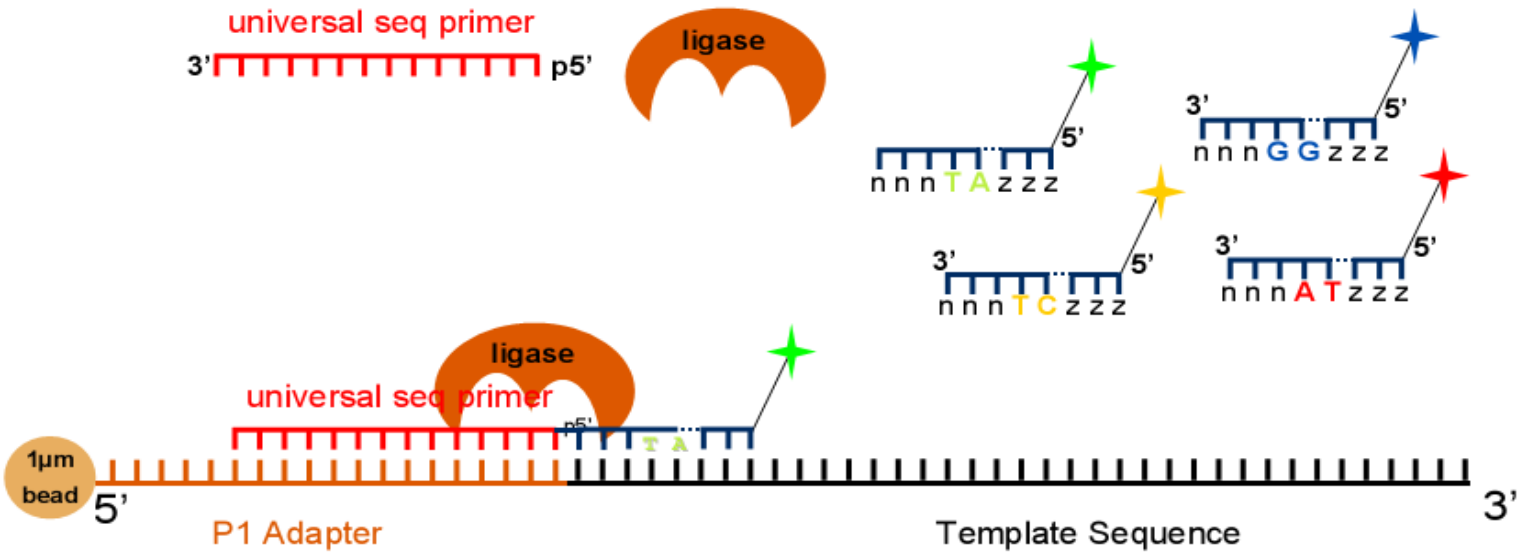
4 Dyes, 4 dinucleotides, 256 probes per dye

N= degenerate bases Z= Universal bases

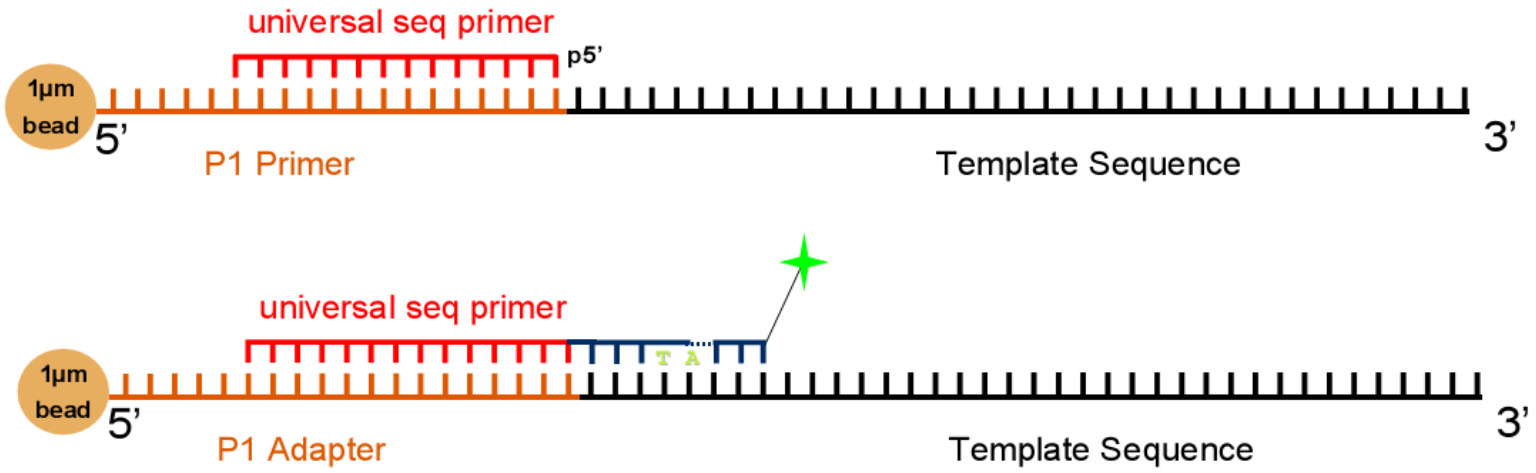
SOLiD Chemistry System 4-color ligation
Ligation reaction



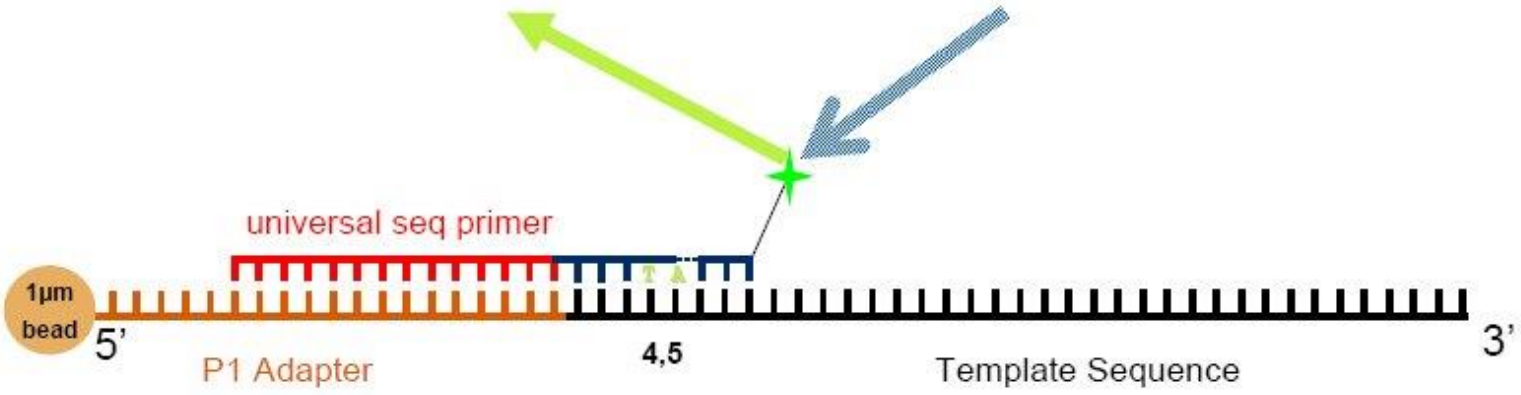
SOLiD Chemistry System 4-color ligation
Ligation reaction



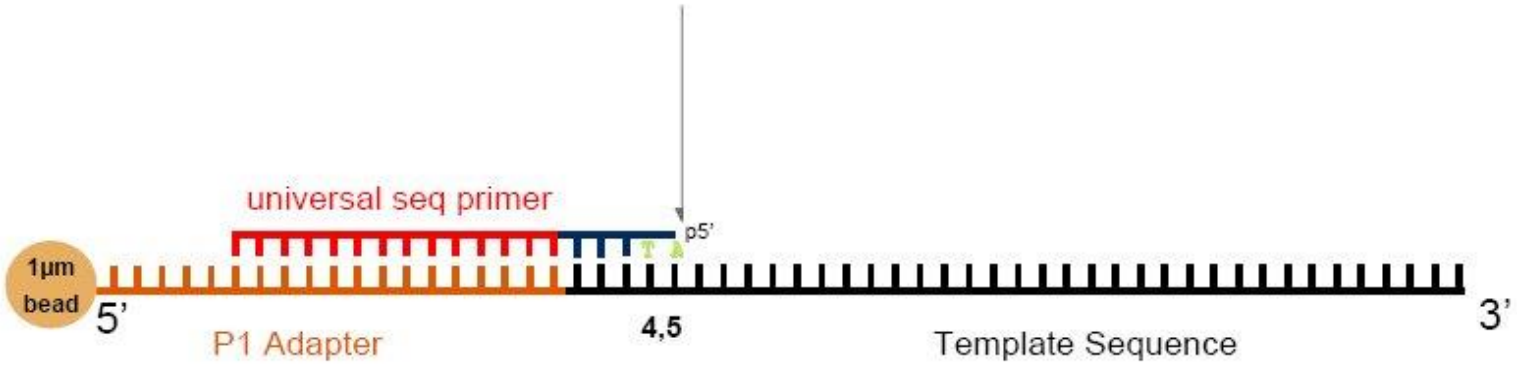
SOLiD Chemistry System 4-color ligation De-Phosphorylation



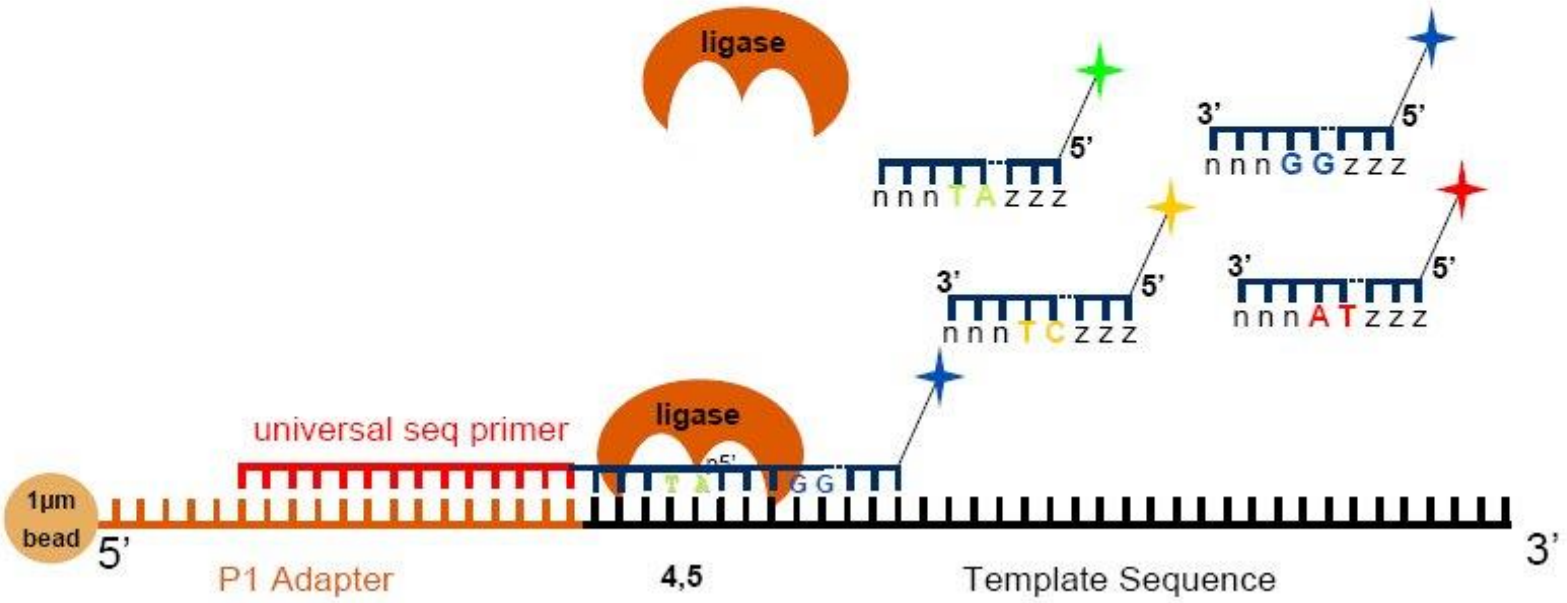
SOLiD Chemistry System 4-color ligation Visualization



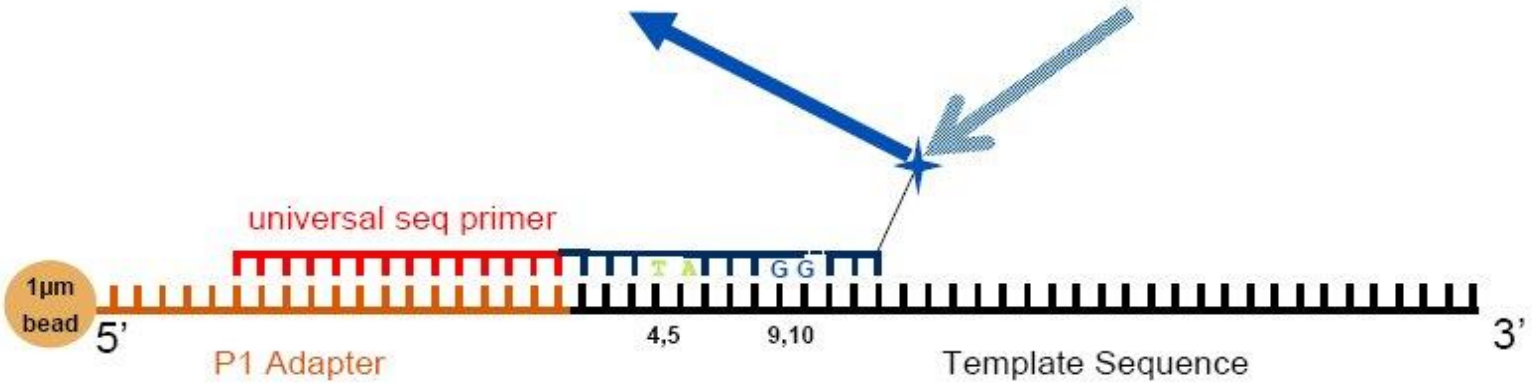
SOLiD Chemistry System 4-color ligation
Cleavage



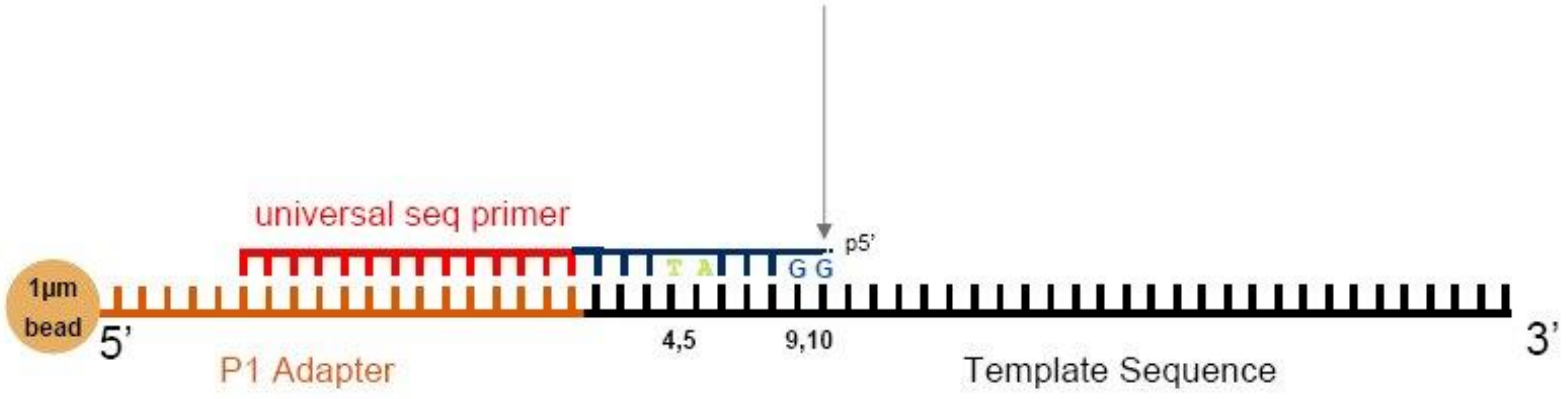
SOLiD Chemistry System 4-color ligation
Ligation (2nd cycle)



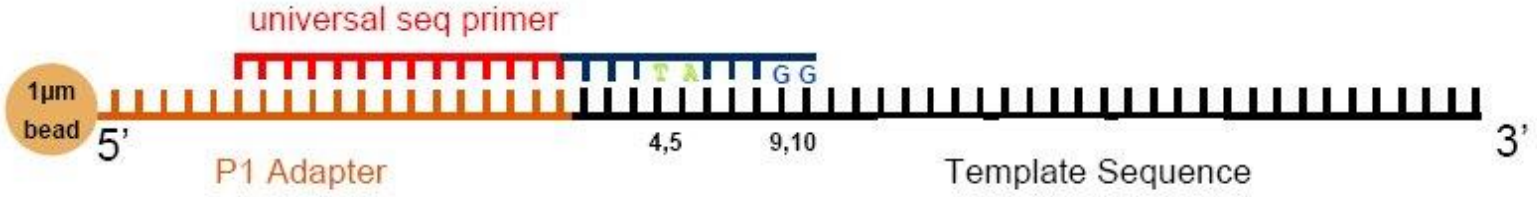
SOLiD Chemistry System 4-color ligation Visualization (2nd cycle)



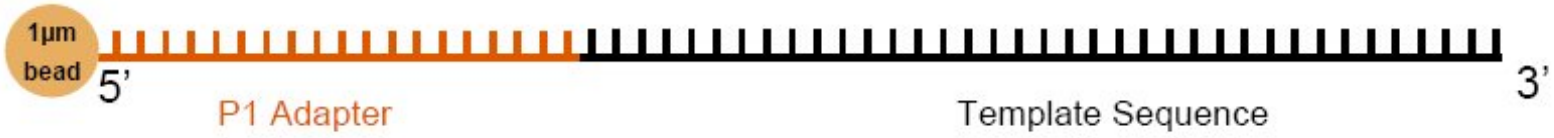
SOLiD Chemistry System 4-color ligation Cleavage (2nd cycle)



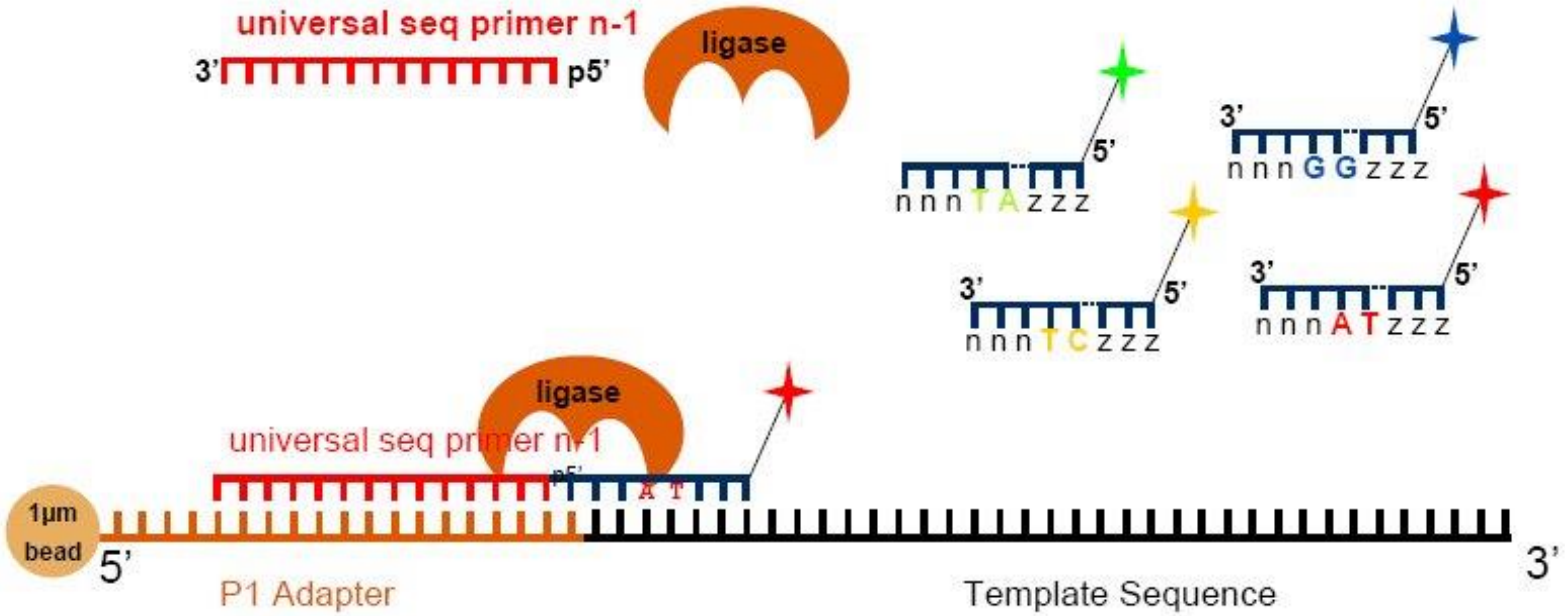
SOLiD Chemistry System 4-color ligation interrogates every 5th base



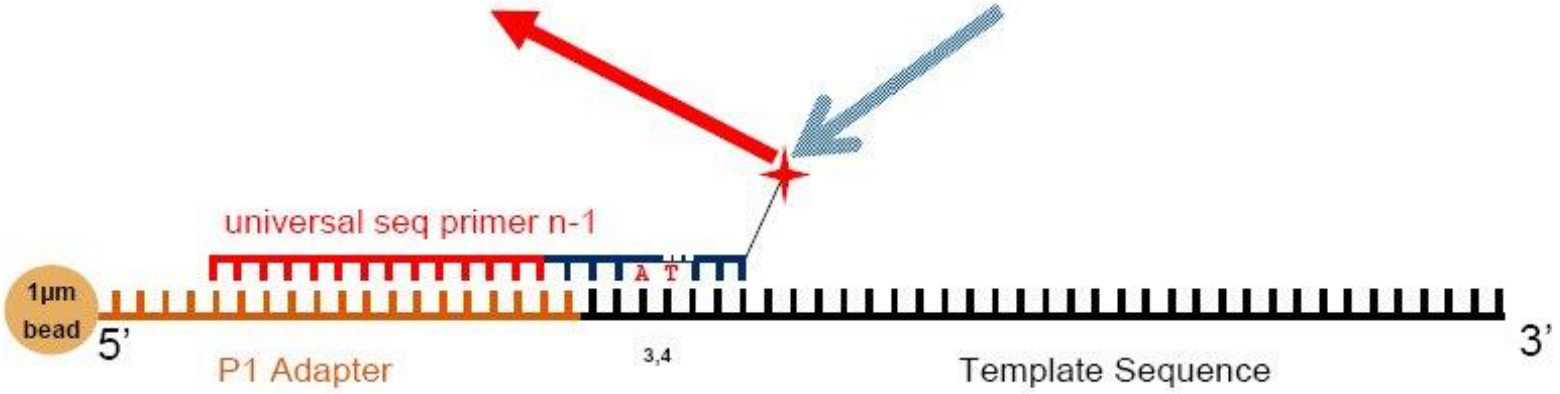
SOLiD Chemistry System 4-color ligation Reset



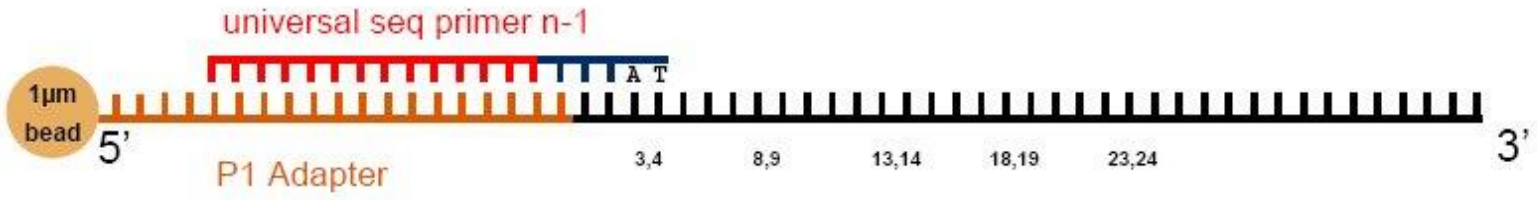
SOLiD Chemistry System 4-color ligation (1st cycle after reset)



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



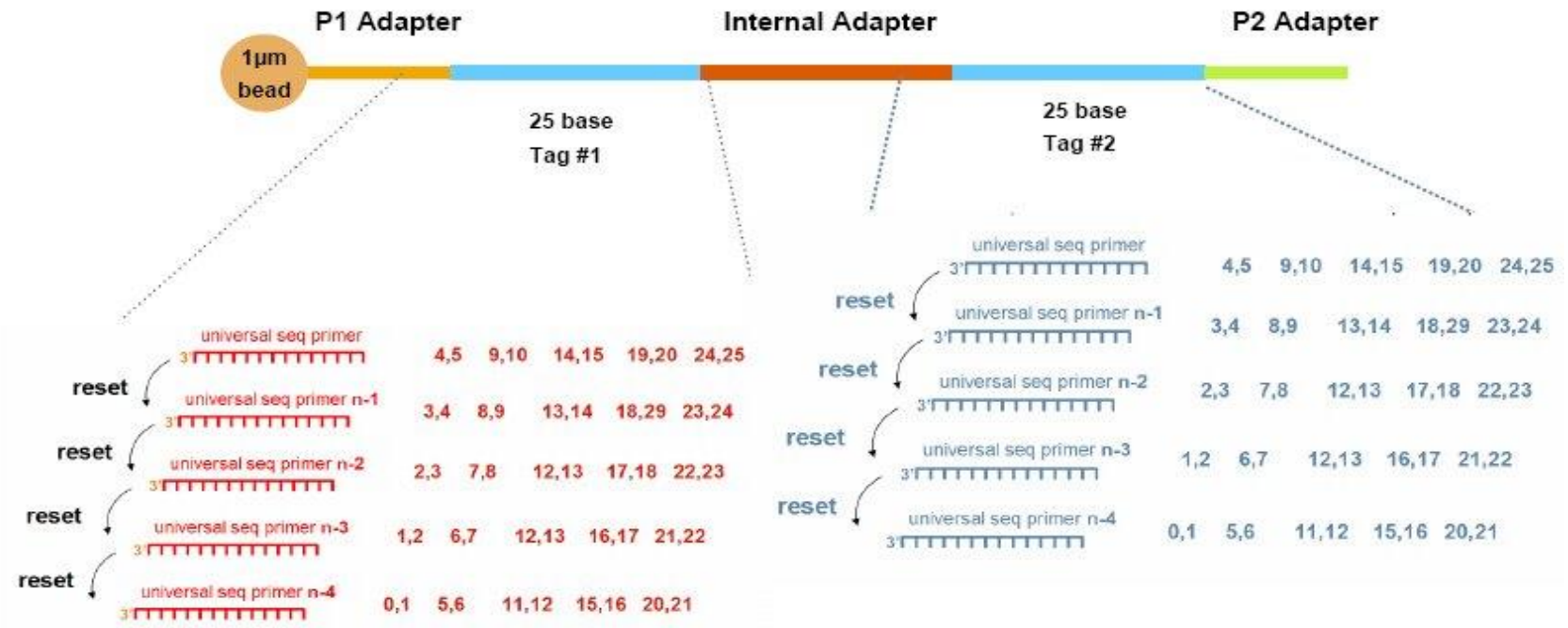
SOLiD Chemistry System 4-color ligation (2nd Round)

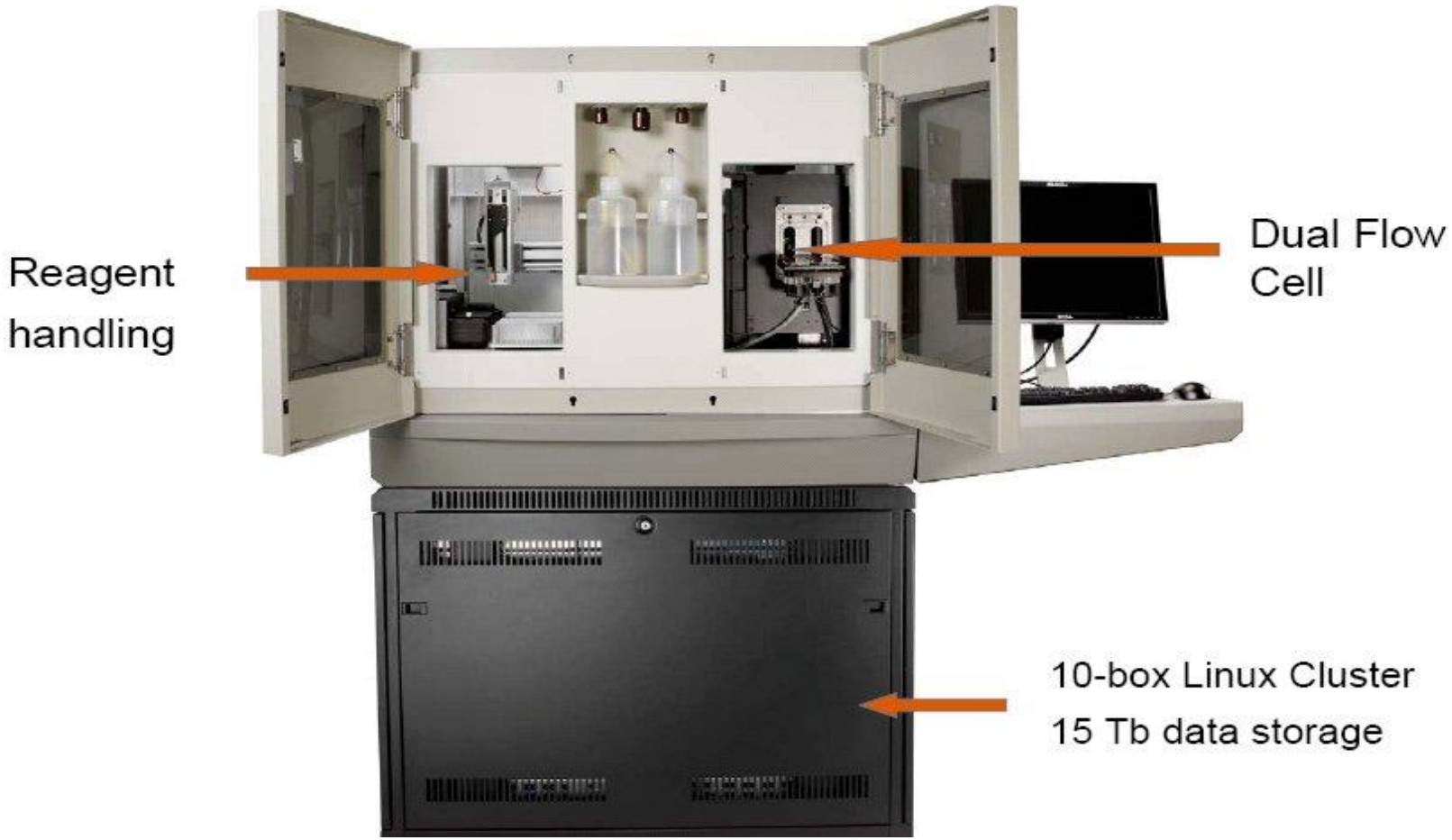


Sequential rounds of sequencing
Multiple cycles per round

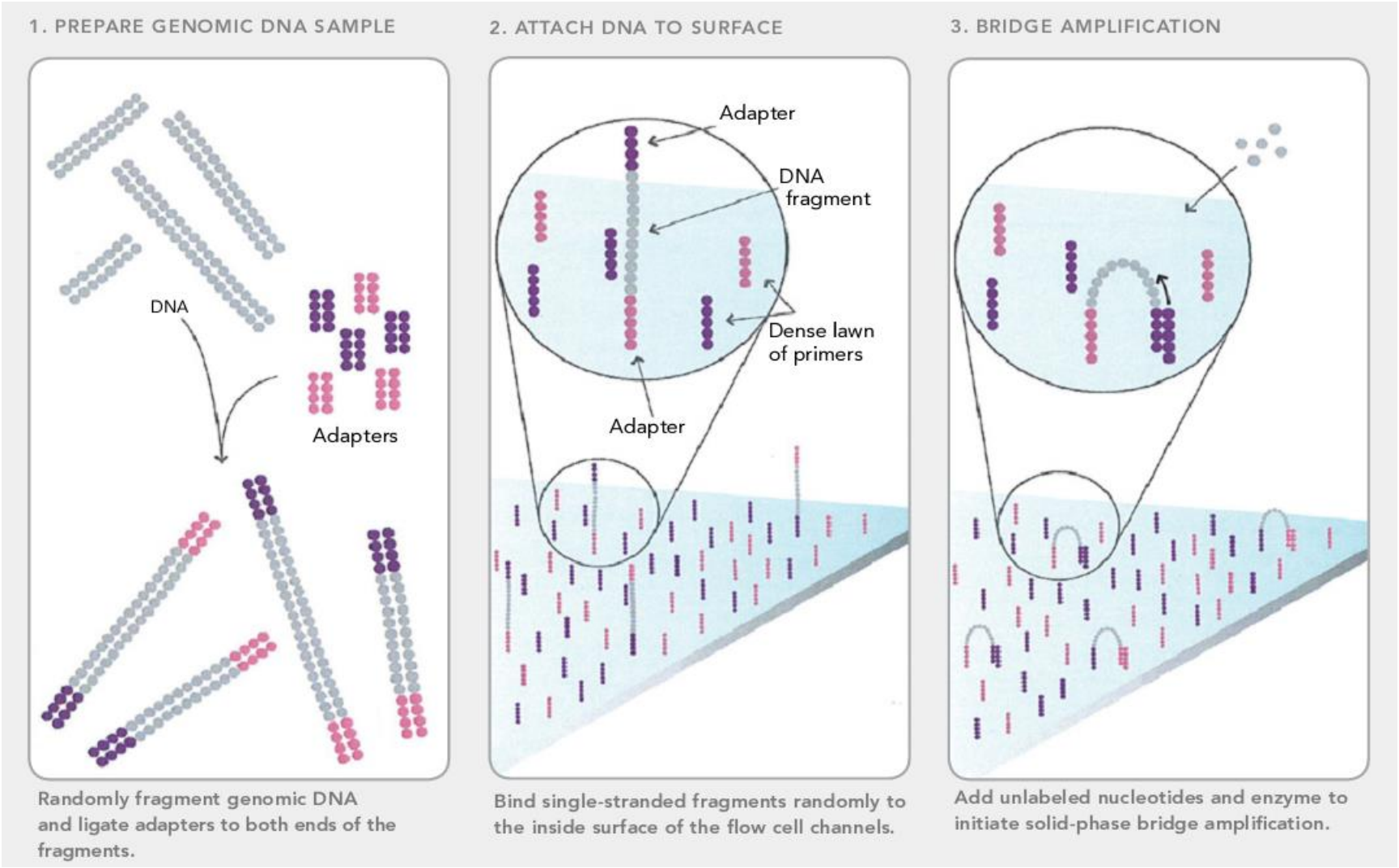
Paired End two sequences generated

Sequential rounds of sequencing
Multiple cycles per round

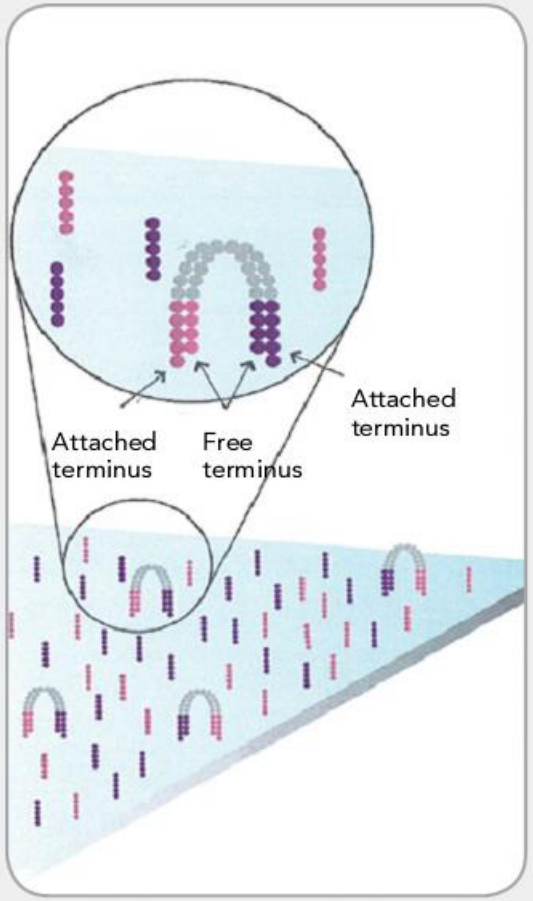



Reagent handling
Dual Flow Cell
10-box Linux Cluster
15 Tb data storage

Solexa (2007)

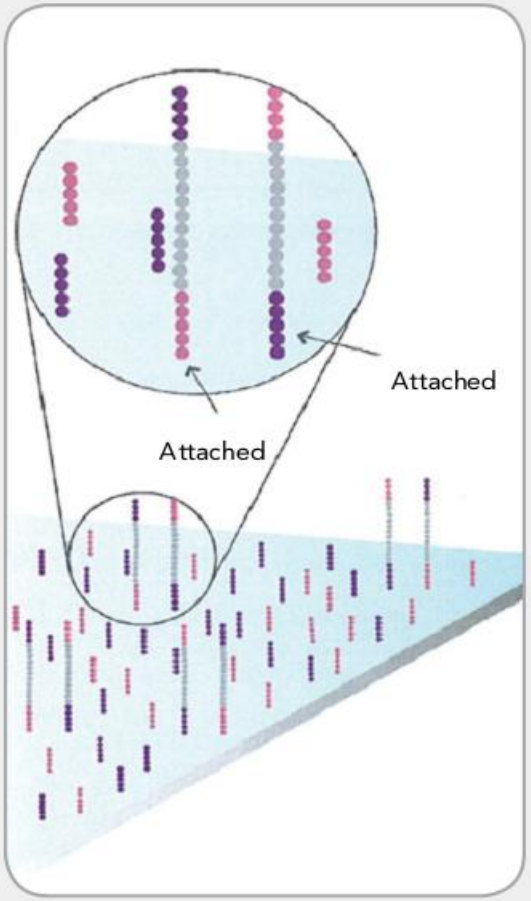

1. PREPARE GENOMIC DNA SAMPLE
DNA
Adapters
Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.
2. ATTACH DNA TO SURFACE
Adapter
DNA fragment
Dense lawn of primers
Adapter
Bind single-stranded fragments randomly to the inside surface of the flow cell channels.
3. BRIDGE AMPLIFICATION
Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification.

4. FRAGMENTS BECOME DOUBLE STRANDED



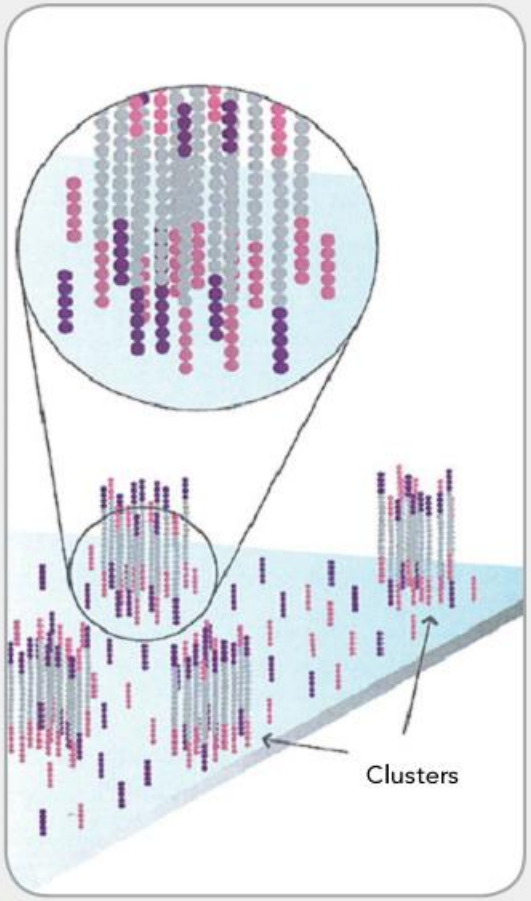
The enzyme incorporates nucleotides to build double-stranded bridges on the solid-phase substrate.

5. DENATURE THE DOUBLE-STRANDED MOLECULES



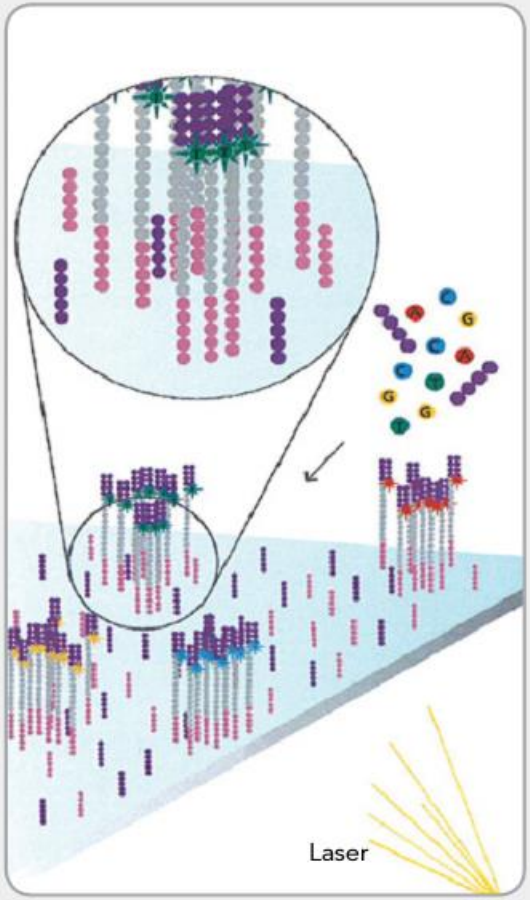
Denaturation leaves single-stranded templates anchored to the substrate.

6. COMPLETE AMPLIFICATION



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

7. DETERMINE FIRST BASE

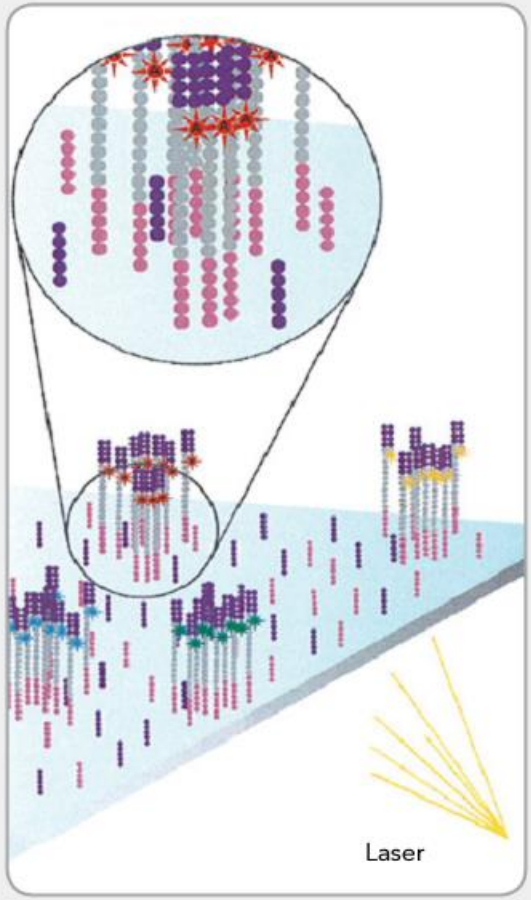


First chemistry cycle: to initiate the first sequencing cycle, add all four labeled reversible terminators, primers and DNA polymerase enzyme to the flow cell.

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

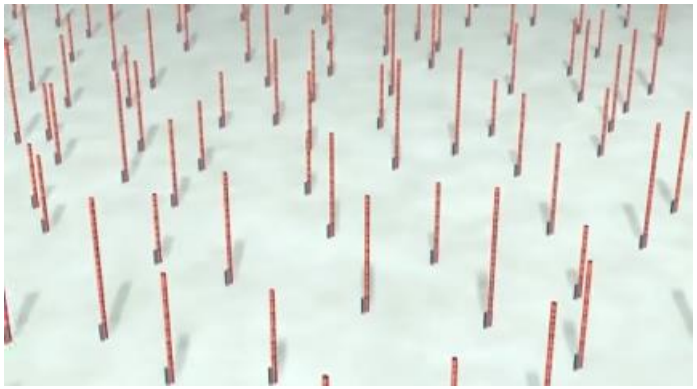


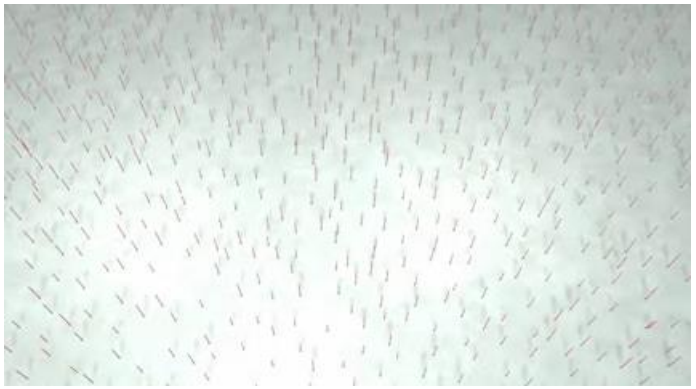
Second chemistry cycle: to initiate the next sequencing cycle, add all four labeled reversible terminators and enzyme to the flow cell.

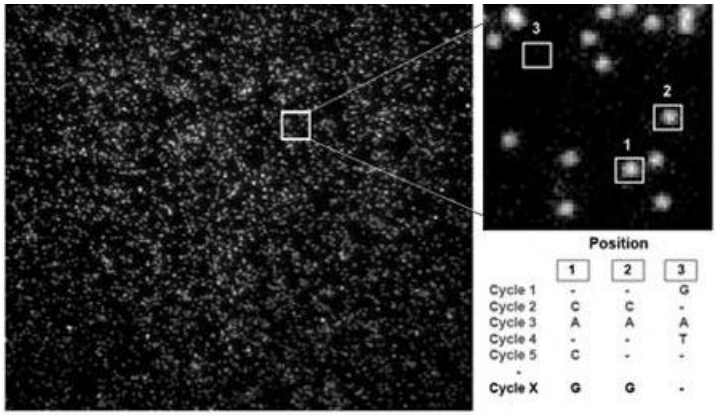
HELICOS (2008)

Helicos BioSciences Corporation

True Single Molecule Sequencing (tSMS)

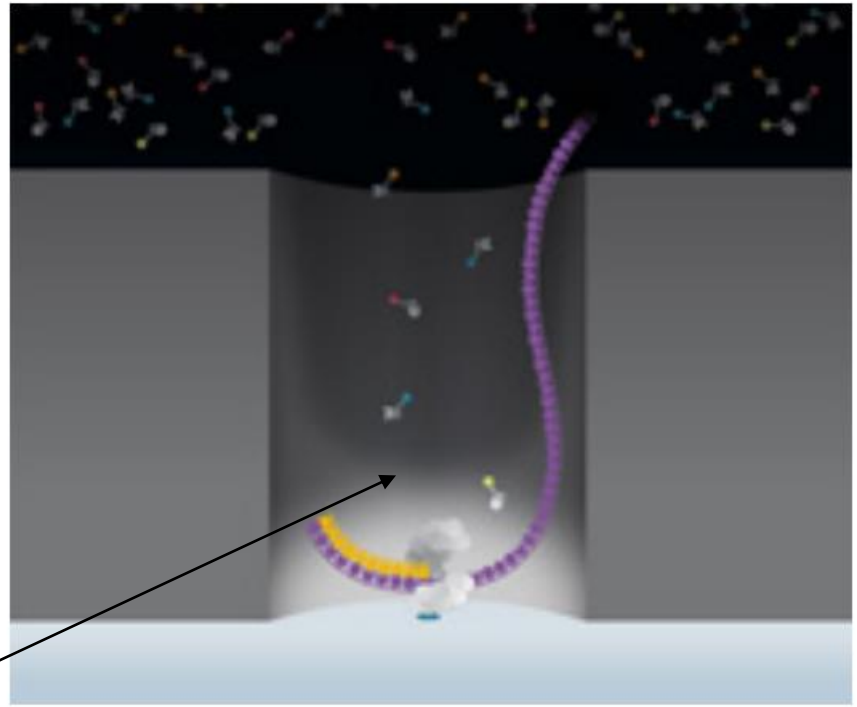






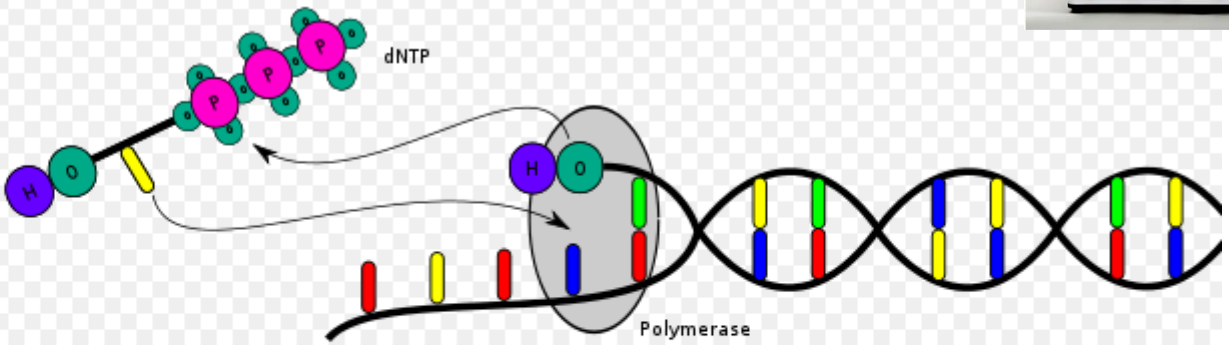
Single Molecule Real-Time (SMRT)

Pacific Biosciences

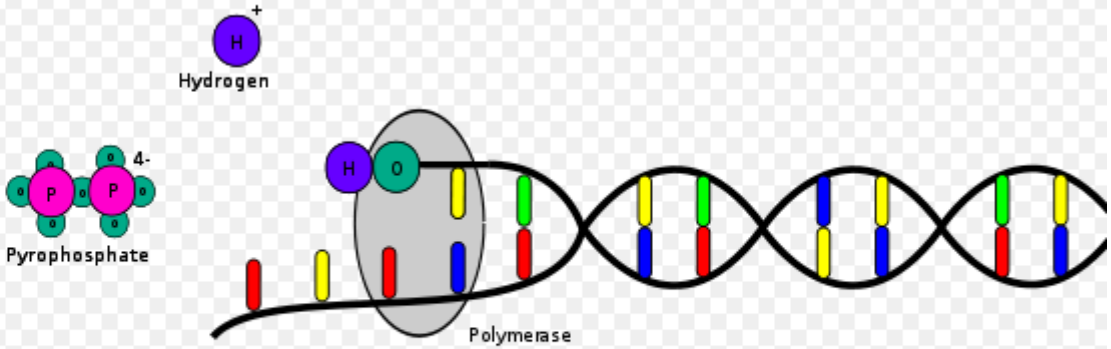


20 zeptolitrů

Ion Torrent

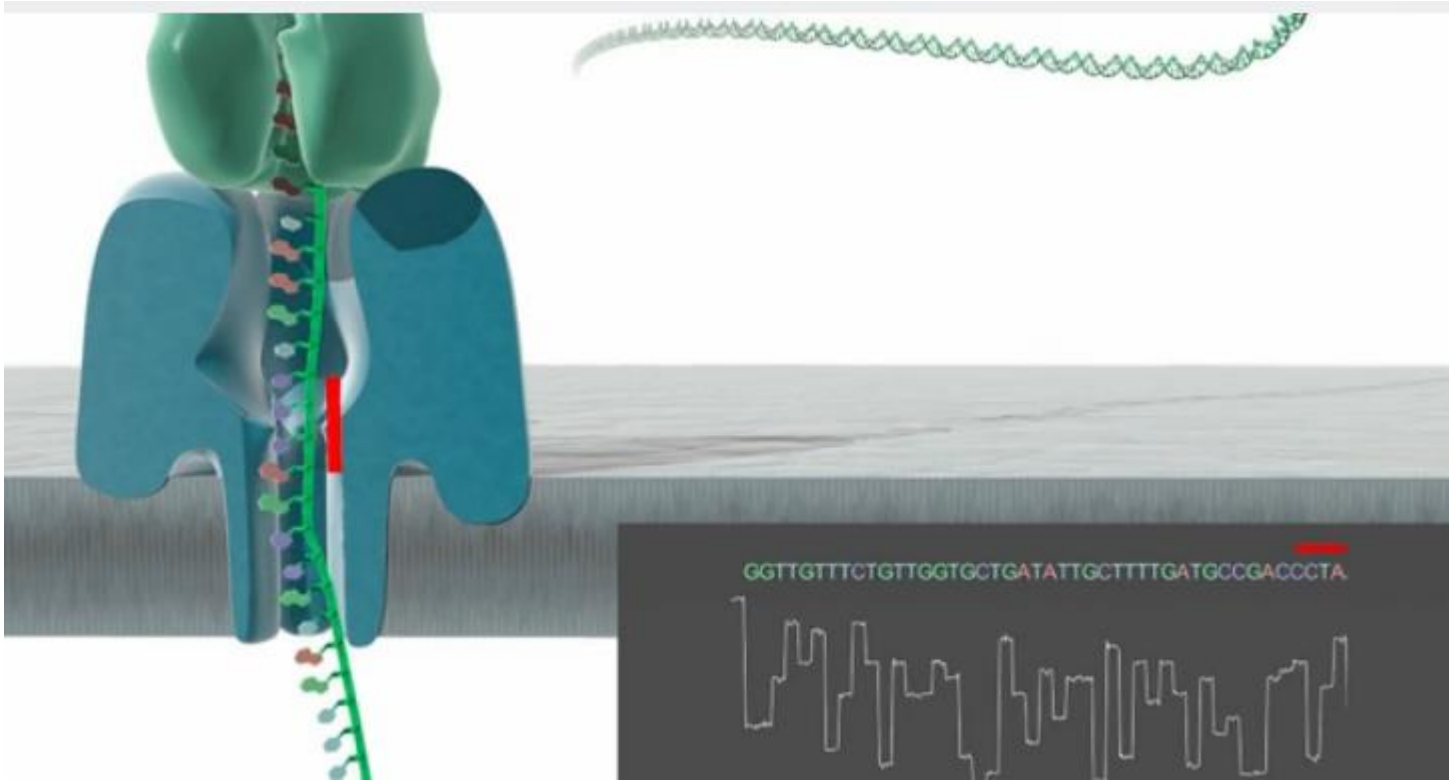


Polymerase integrates a nucleotide.



Hydrogen and pyrophosphate are released.

Oxford nanopore



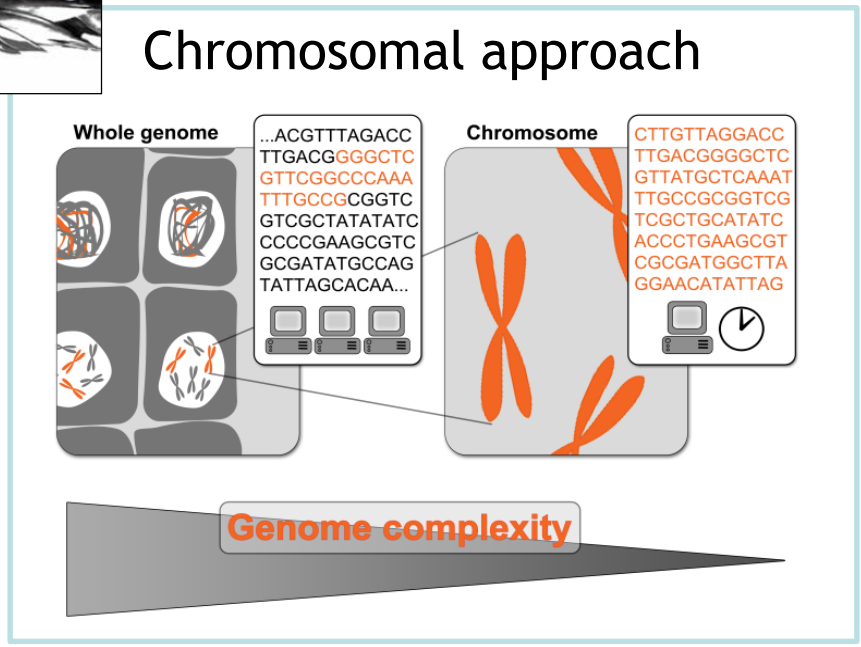
Další technologie

- Mikroelektroforéza
- Sekvenování na bázi microarray

Challenges in Genome Sequencing

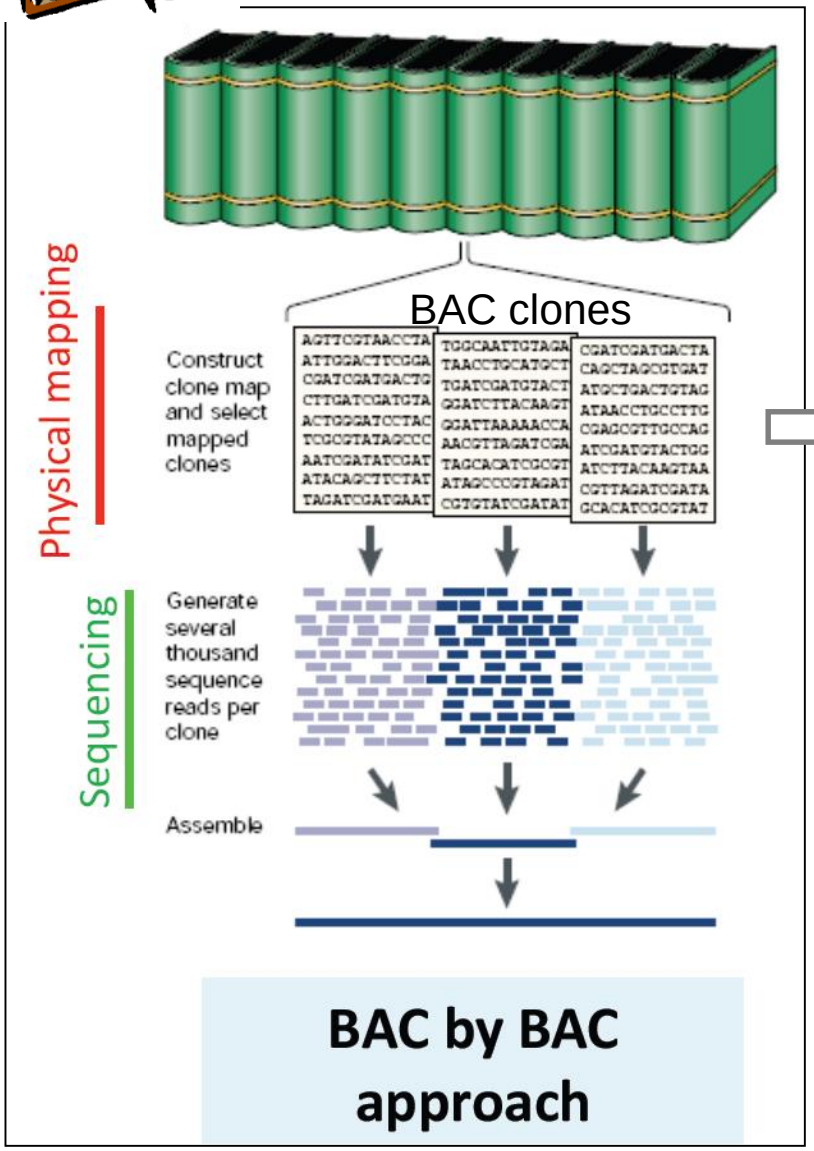
De novo genome assemblies using only short read data of NGS technologies are generally incomplete and highly fragmented due to

- Large duplications - chromosomal approach, BAC-by-BAC sequencing
- High proportion of repetitive DNA - challenge!

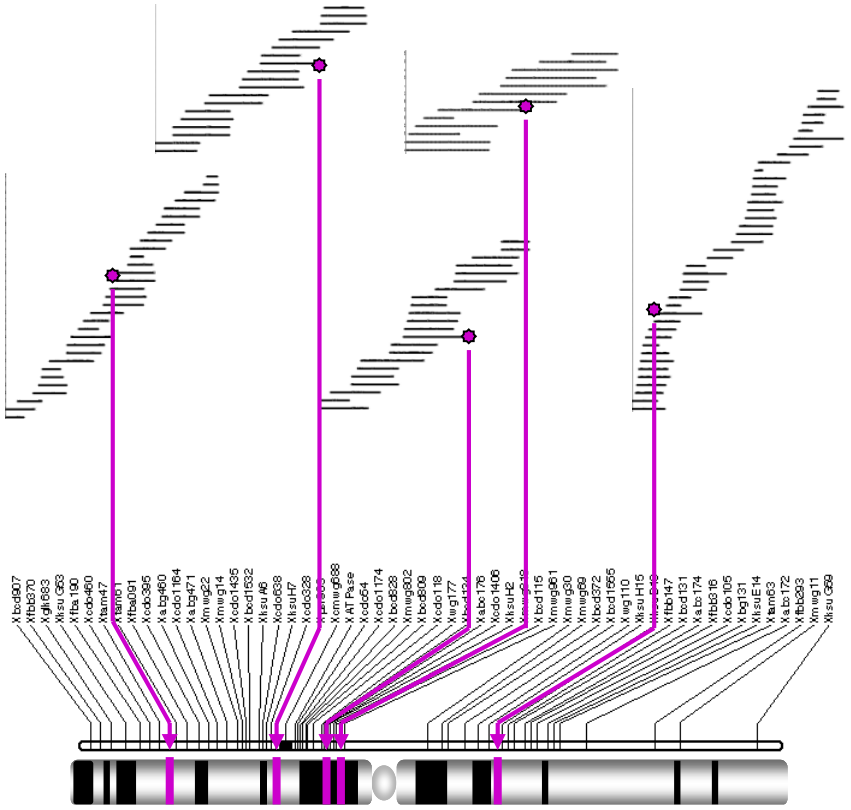


- Large genome size (~17 Gb)
- Polyploidy (3 subgenomes)

BAC-BY-BAC SEQUENCING



- **Physical map** is composed of contigs of overlapping BAC clones
- BAC contigs are landed on the chromosome through markers comprised in the contigs



SOLUTIONS FOR THE REPEATS

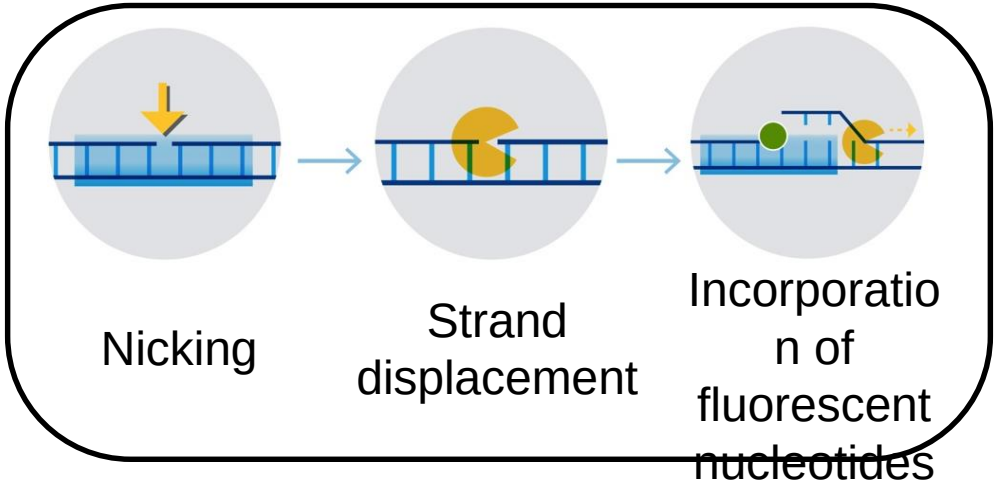
- **Long mate-pair reads** > 10 kb
- **Long read technologies** – PacBio, Oxford Nanopore
- **Optical mapping**
  - Single-molecule mapping of genomic DNA **hundreds of kilobases to several megabases in size**
  - Creates **sequence-motif maps**, which provide long-range template for ordering genomic sequences
  - **Visualisation of reality** "Seeing is Believing"

Optical Mapping

Three enzymatic approaches

- restriction enzymes:
  sequence-specifically cleave DNA immobilized on a surface

- **nicking enzymes:**
  fluorescent labelling of the nicking site in solution (BioNano Genomics - Irys)

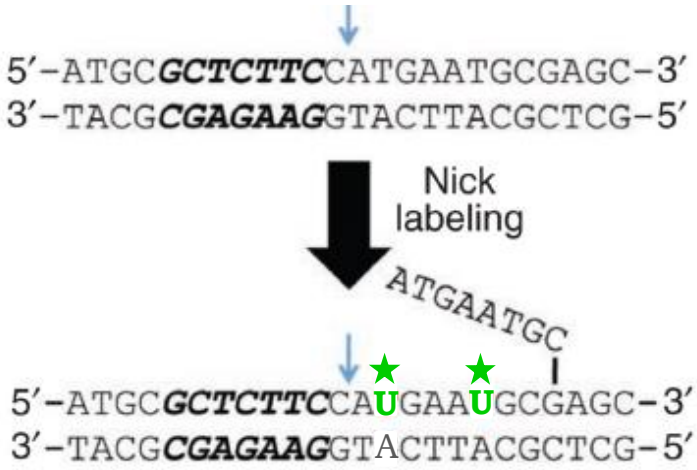


- methyltransferase enzymes:
  labelling with ultra-high density

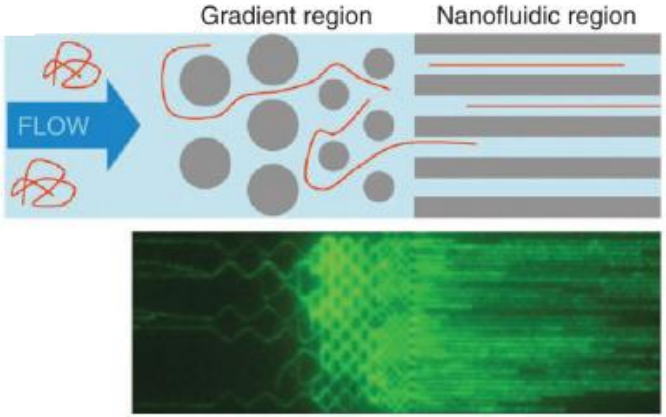
BioNano Genome mapping on Nanochanel Arrays

1 Sequence-specific labeling

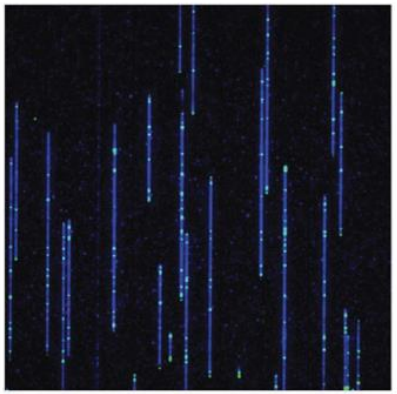
Nickase (Nt.BspQI)



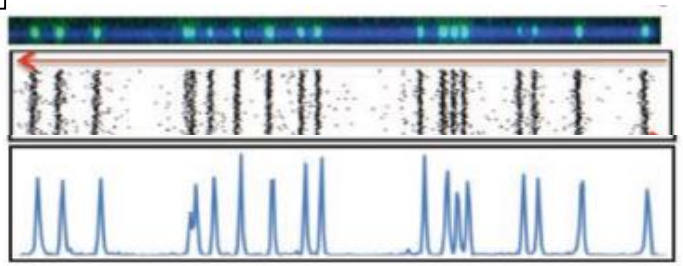
2 DNA linearization



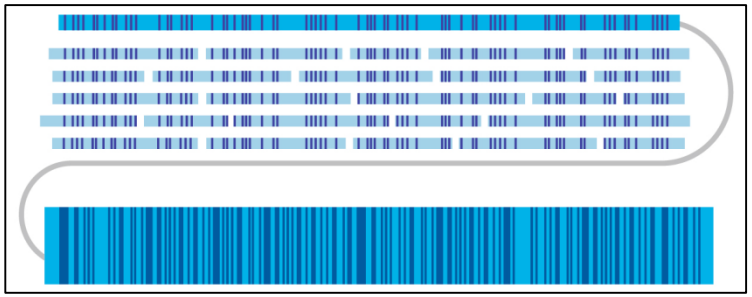
3 Fluorescence imaging



4 Map construction



5 Building consensus map



Lam et al., Nat. Biotechnol. 30(8) 2012