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INTRUMENTASI BIOTEKNOLOGI

Program Studi Bioteknologi

Oleh: Seprianto, S.Pi, M.Si

Meeting 13

Peralatan Rekayasa Genetika dan Pembuatan Protein Rekombinan

Universitas Esa Unggul

Tujuan Perkuliahan

- Mahasiswa dapat mengidentifikasi dan mengetahui prinsip bekerjanya peralatan Rekayasa Genetika dan analisis protein: Electrophorator, DNA concentrator, Nanodrop, DNA Sequencer, Next Generation Sequencer, MALDI TOF dan SELDI TOF

Electroporator

Nanodrop

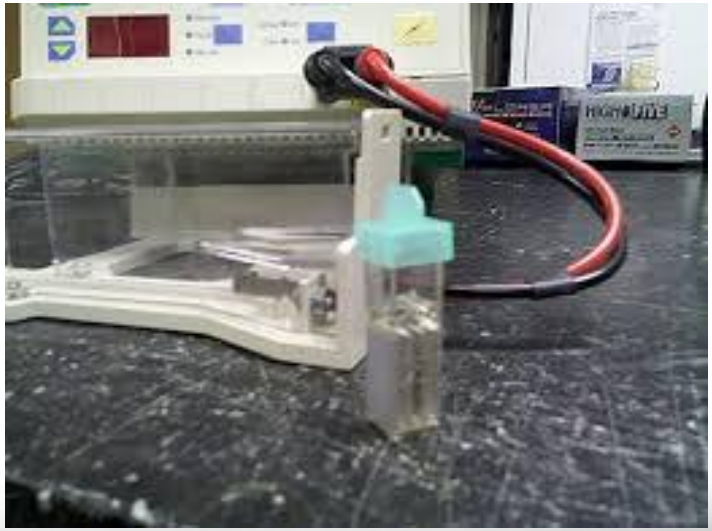
Next Generation Sequencer

Electroporator

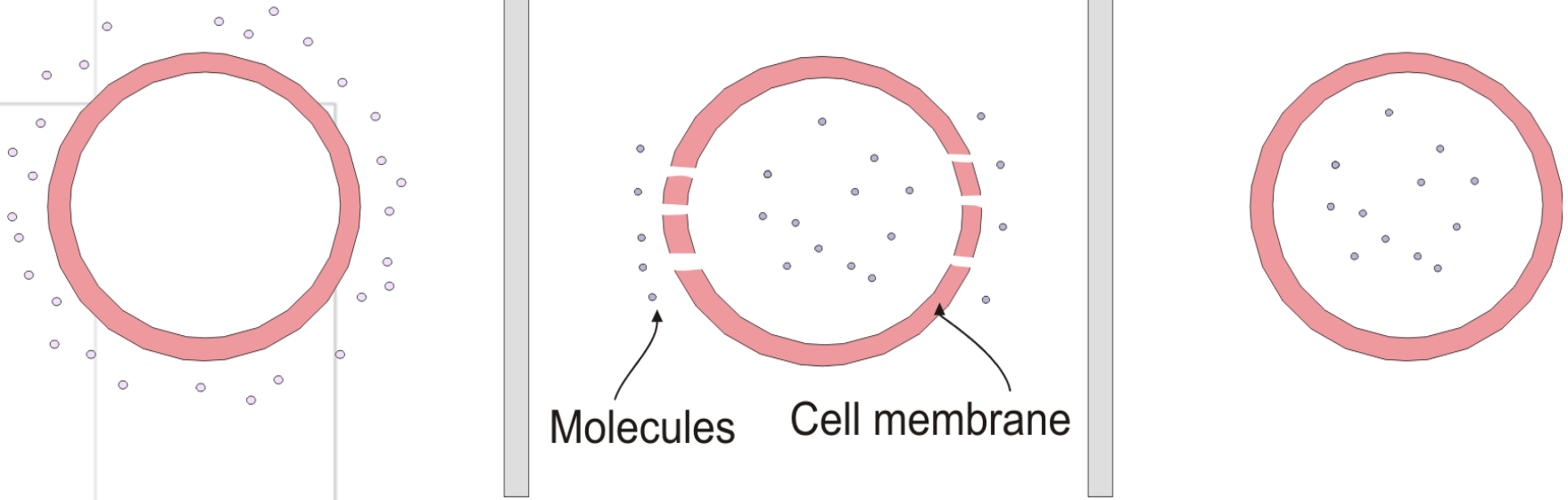
- Electroporator

Adalah alat untuk electroporation atau electropermeabilization

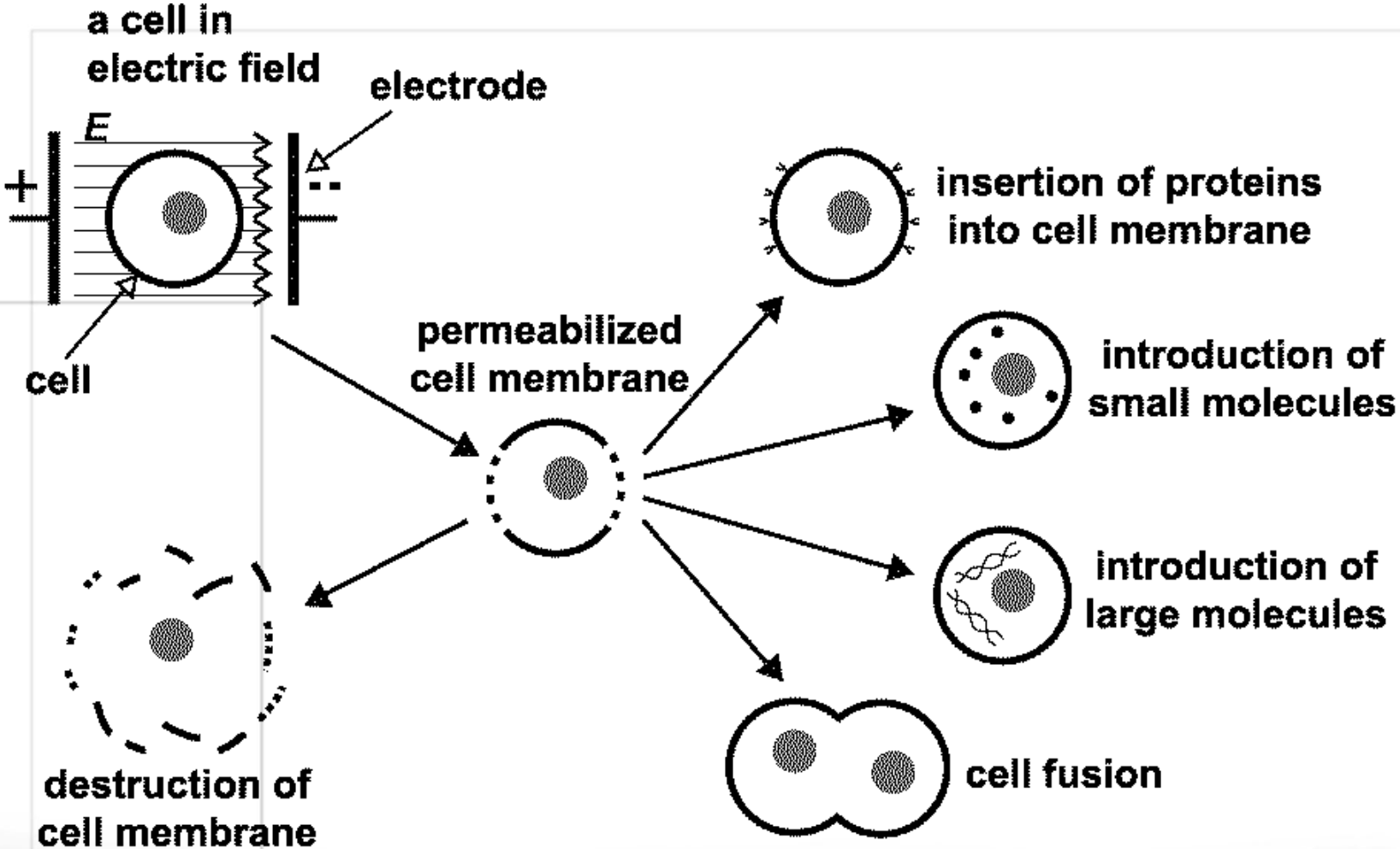
- Electroporation adalah suatu teknik mengunakan listrik untuk mengubah permeabilitas membrane sel sehingga memungkinkan suatu plasmid masuk ke dalam sel



Prinsip Elektroforator



The phenomena



Applications

Reversible electroporation:

Cell fusion

Gene transfection

Drug delivery

Cancer treatment

Ireversible electroporation:

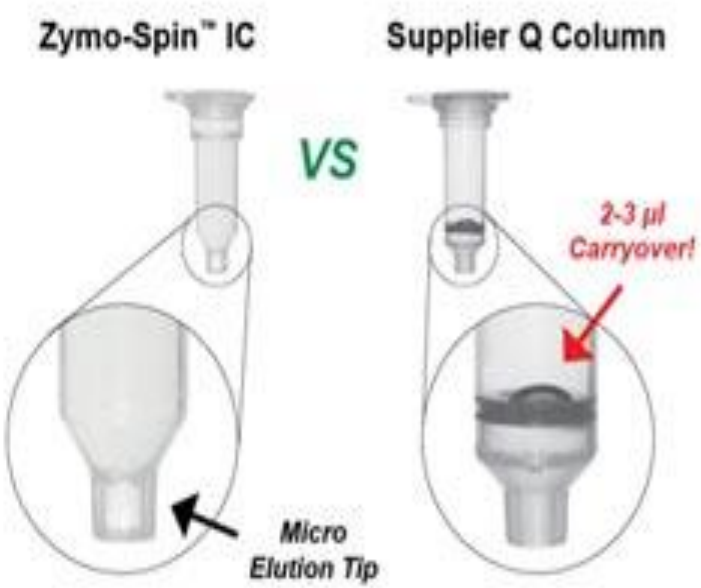
Food pasterization

Tissue ablation

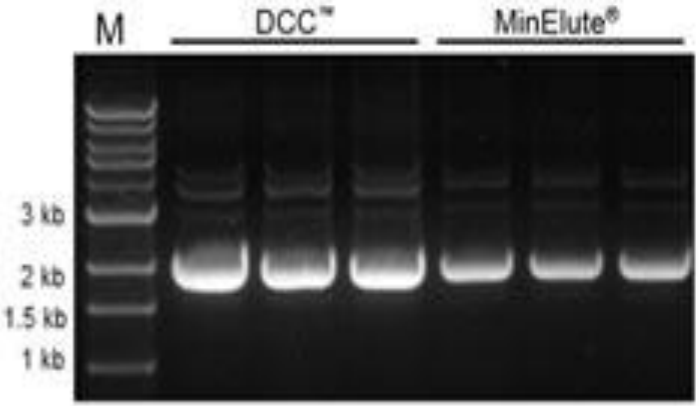
DNA Concentrator

DNA concentrator adalah alat untuk menaikkan konsentrasi DNA yang diperoleh

Innovative Column Technology with No Wash Residue Carryover!



DCC™ for Highly Concentrated DNA



High efficiency DNA recovery using the DCC™. Equivalent amounts of DNA was purified using the DCC™ or the MinElute® Kit, and eluted into minimal elution volumes. DNA recovered with the DCC™ consistently yielded higher concentrations than DNA isolated using the MinElute® Kit.

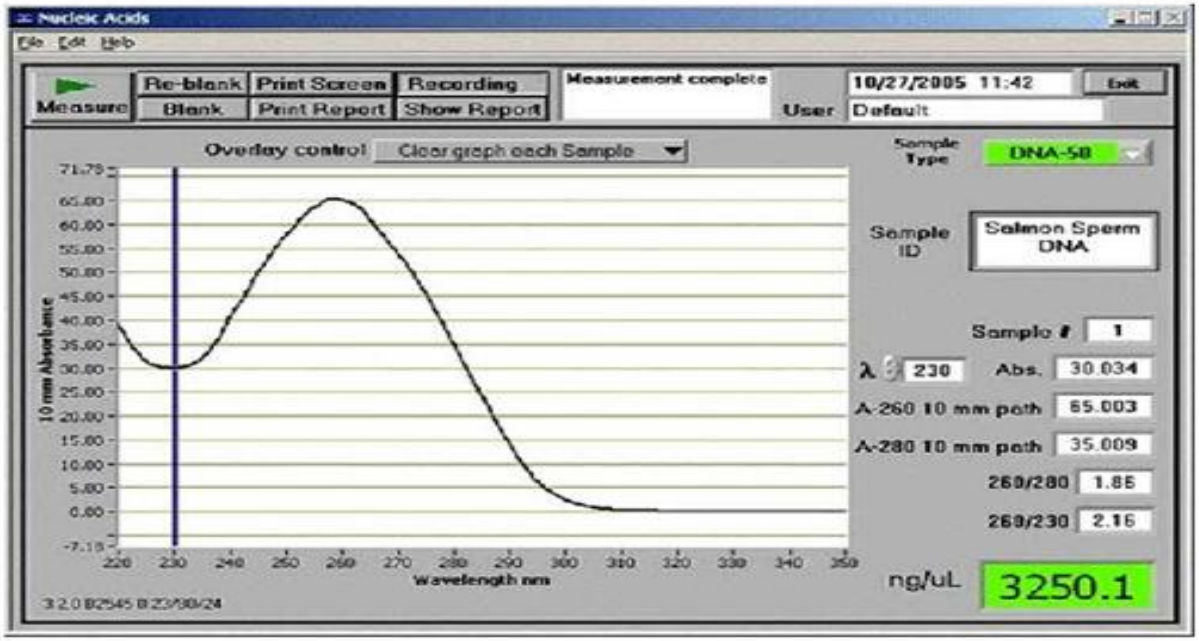
Nanodrop

- Alat untuk mengukur konsentrasi sampel RNA, DNA dan Protein berbasis UV-Vis Spektrofotometer yang hanya memerlukan jumlah sampel yang sangat sedikit (1-2 ul)

NanoDrop

- The NanoDrop® ND-1000 is a full-spectrum (220-750nm) spectrophotometer that measures 1 ul samples with high accuracy and reproducibility.
- Nucleic acid concentration and purity of nucleic acid samples up to 3700 ng/ul (dsDNA) without dilution
- Fluorescent dye labeling density of nucleic acid microarray samples
- General UV-Vis spectrophotometry

NANODROP: great for SMALL VOLUMES



Results from a DNA sample

Gives concentration of sample in ng/µl

260/230 ratio = Organic chemicals and solvent contamination

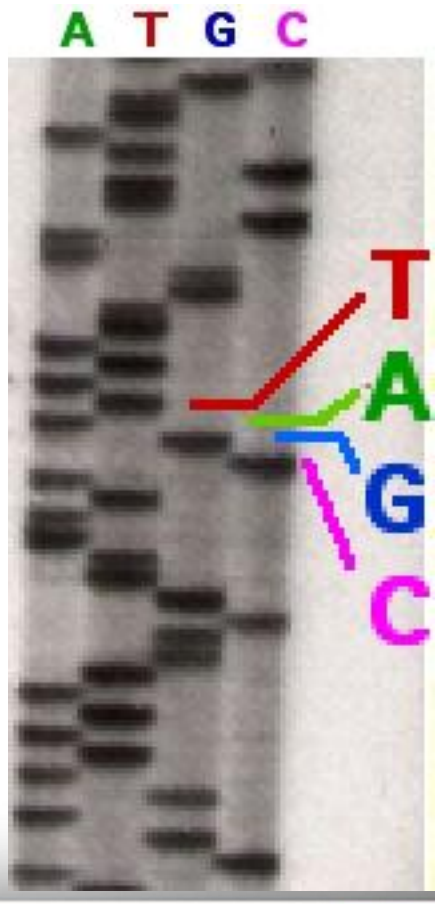
- Important to have this ratio above 1.6 for QPCR

260/280 ratio = Protein Contamination

- Ratios above 1.8 are considered pure

DNA Sequencer

Suatu proses atau teknik penentuan urutan basa nukleotida pada suatu molekul **DNA**. Urutan tersebut dikenal sebagai sekuens **DNA**, yang merupakan informasi paling mendasar suatu gen atau genom karena mengandung instruksi yang dibutuhkan untuk pembentukan tubuh makhluk hidup.



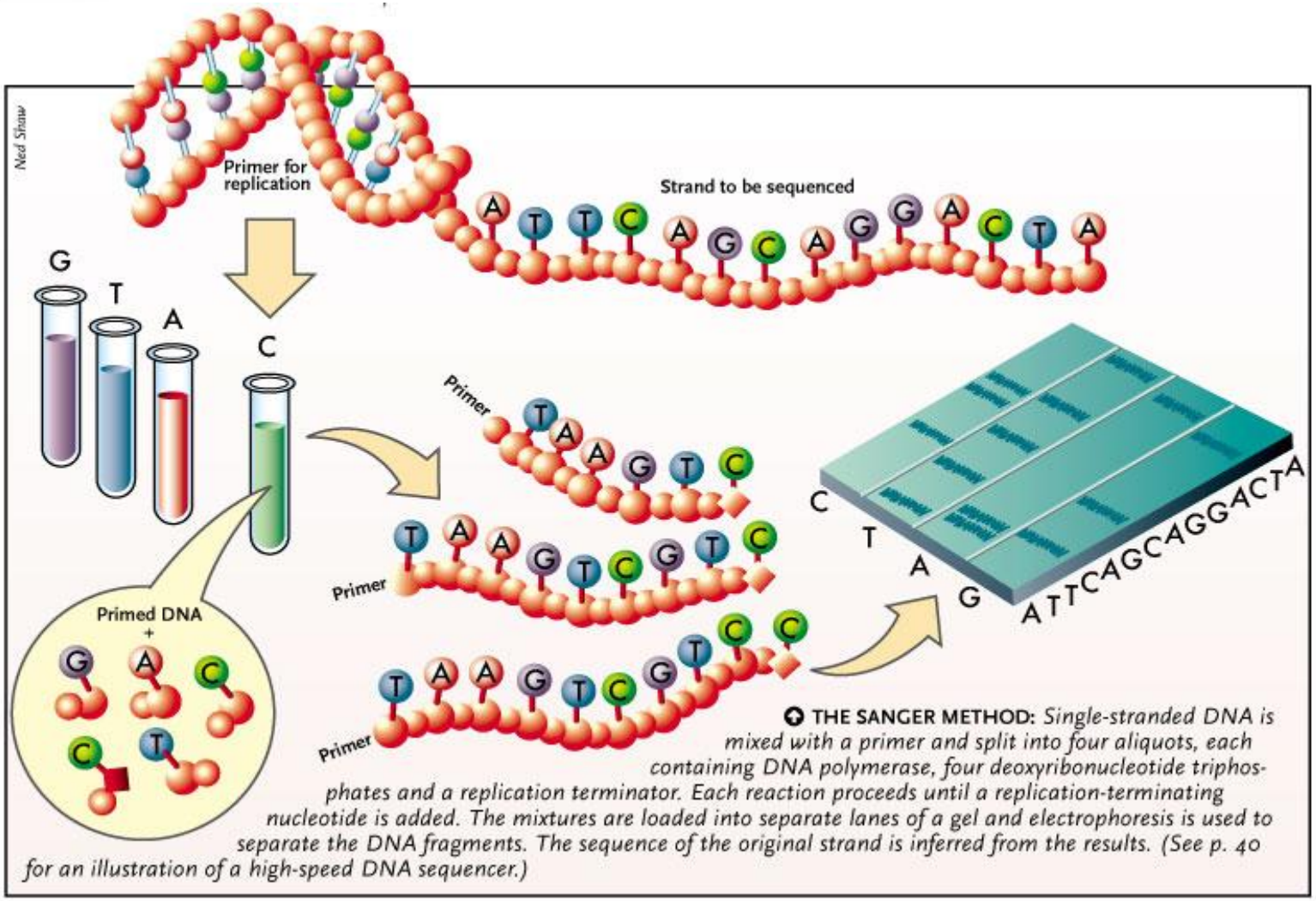
Metode :

1. Sanger
2. Dye Terminator
3. Pyrosequencing
4. Ilumina

- **What is DNA sequencing**

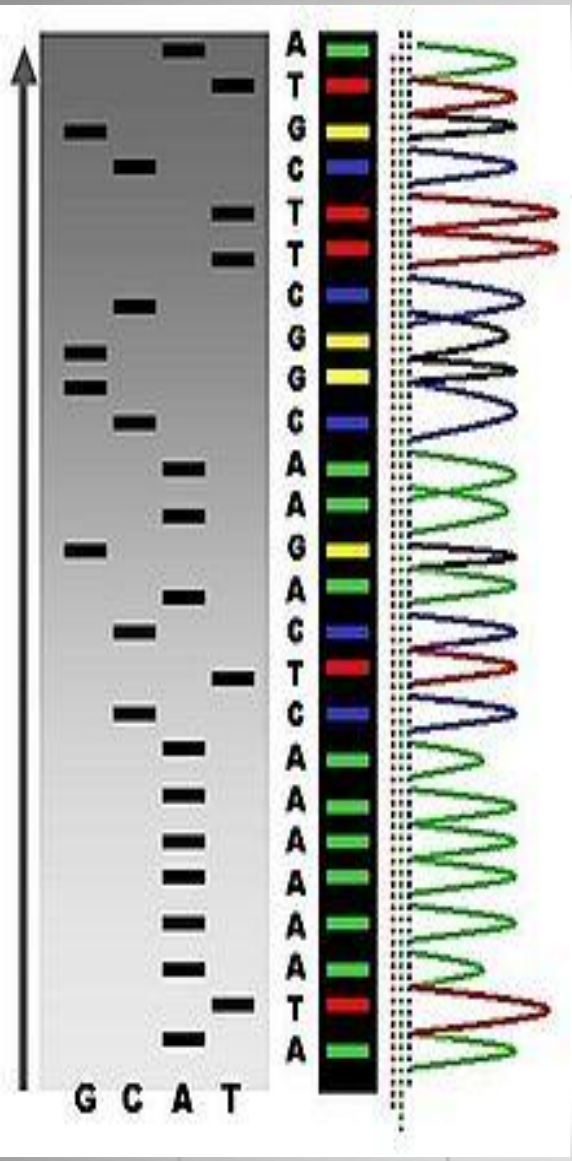
-Determining the precise order of nucleotides in a piece of DNA
-DNA sequence is useful in studying fundamental biological processes and in applied fields such as diagnostic or forensic research
-DNA sequencing methods have been around for 40 years, and since the mid-1970s

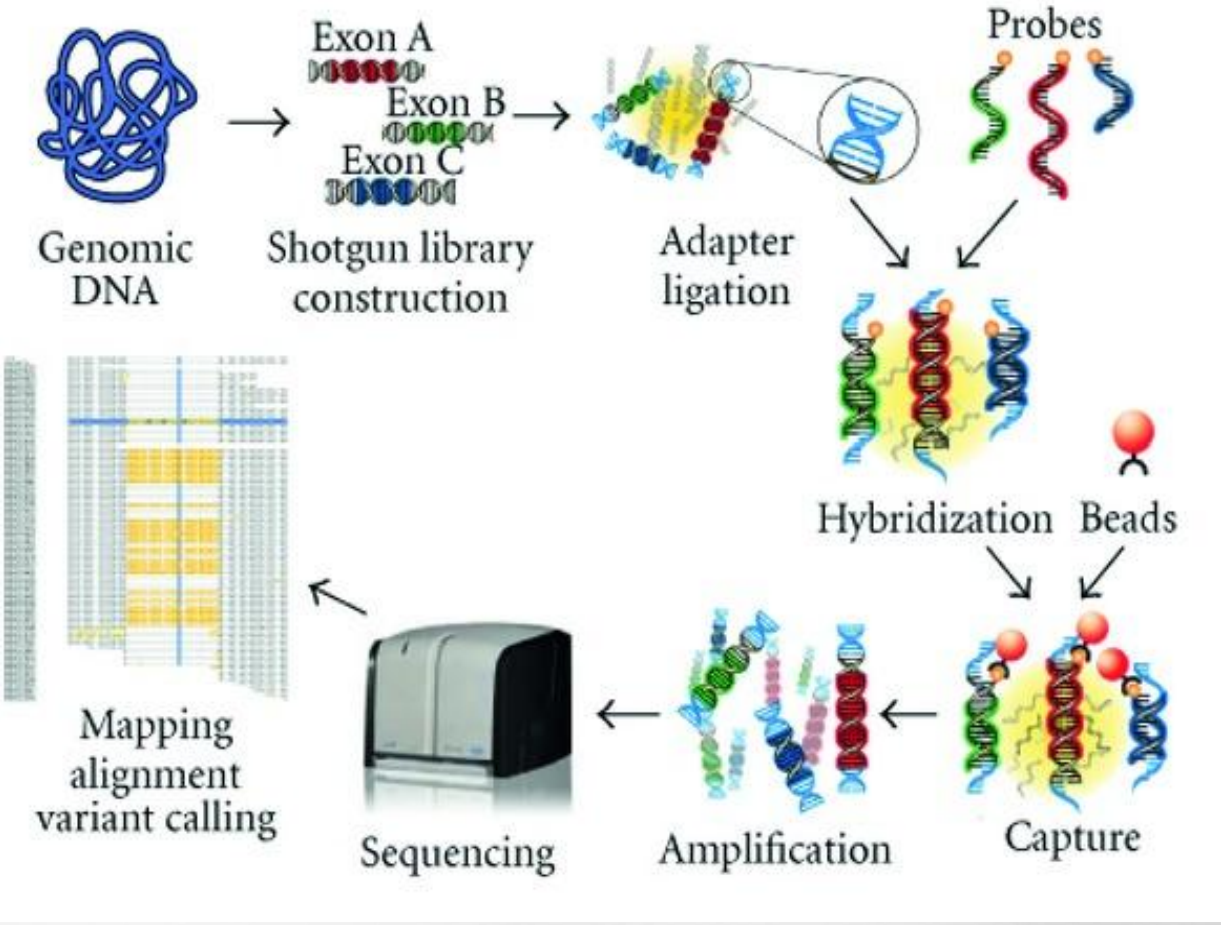
DNA Sequencer



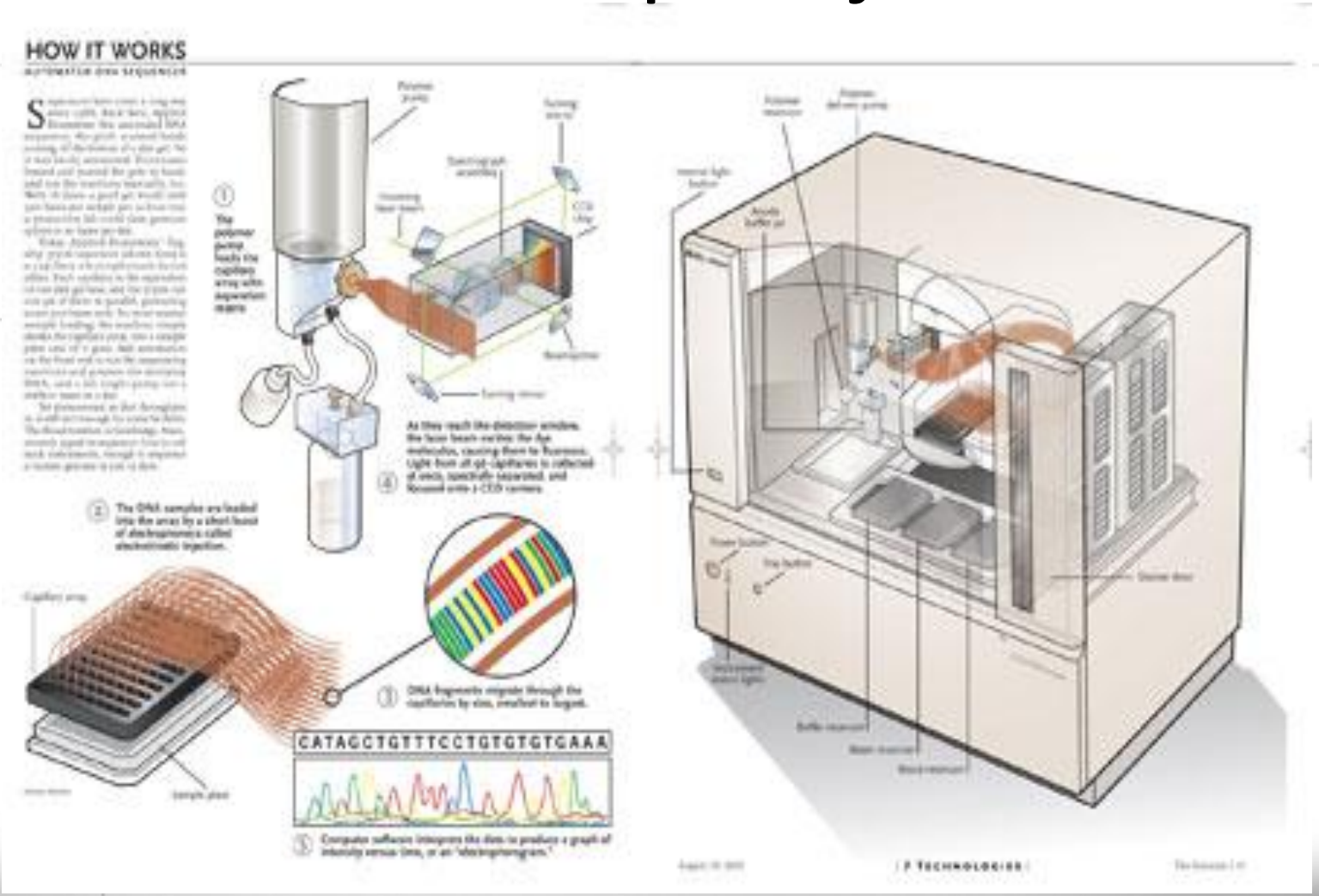
THE SANGER METHOD: *Single-stranded DNA is mixed with a primer and split into four aliquots, each containing DNA polymerase, four deoxyribonucleotide triphosphates and a replication terminator. Each reaction proceeds until a replication-terminating nucleotide is added. The mixtures are loaded into separate lanes of a gel and electrophoresis is used to separate the DNA fragments. The sequence of the original strand is inferred from the results. (See p. 40 for an illustration of a high-speed DNA sequencer.)*

Sequencing


A T G C T T C G G G C A A G A C T C A A A A A A T A
G C A T


Exon A
Exon B
Exon C
Probes
Genomic DNA
Shotgun library construction
Adapter ligation
Hybridization
Beads
Capture
Amplification
Sequencing
Mapping alignment variant calling

Prinsip Kerja



Sequencer

Sequencing methods

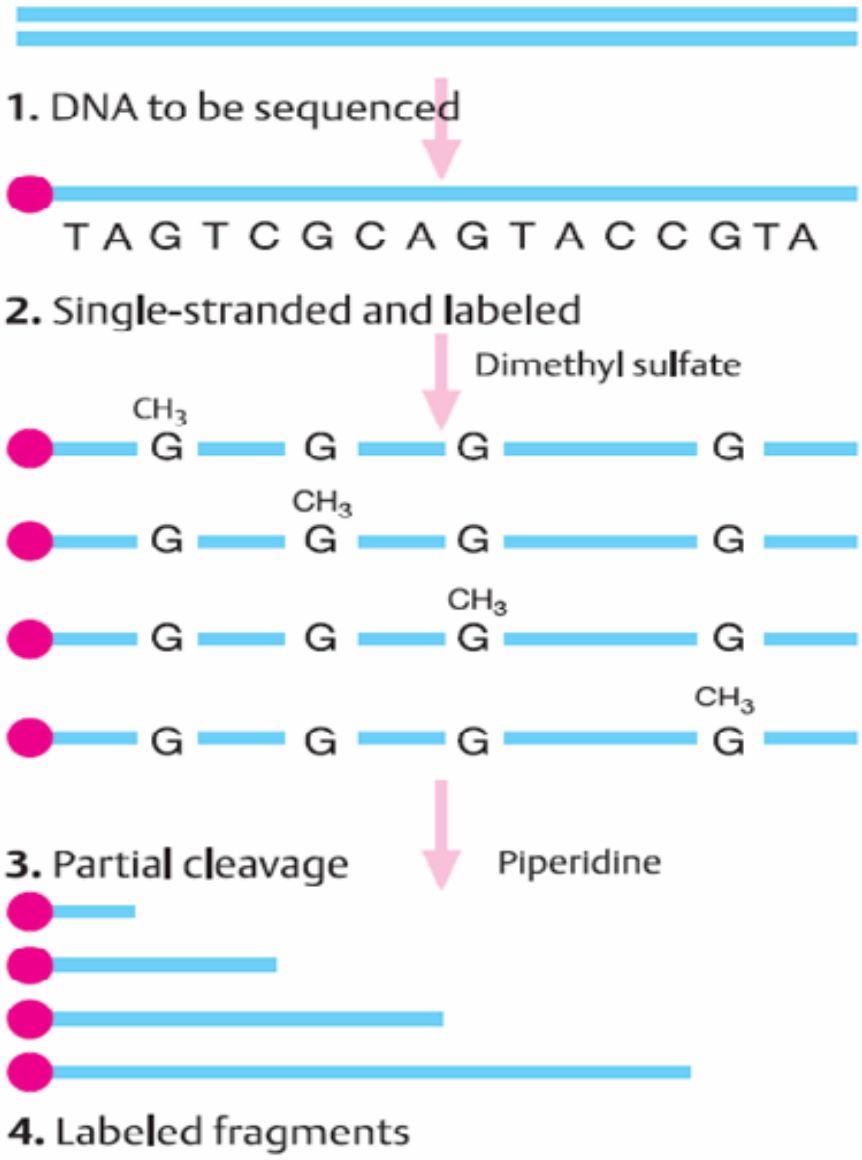
Three basic methods for DNA sequencing

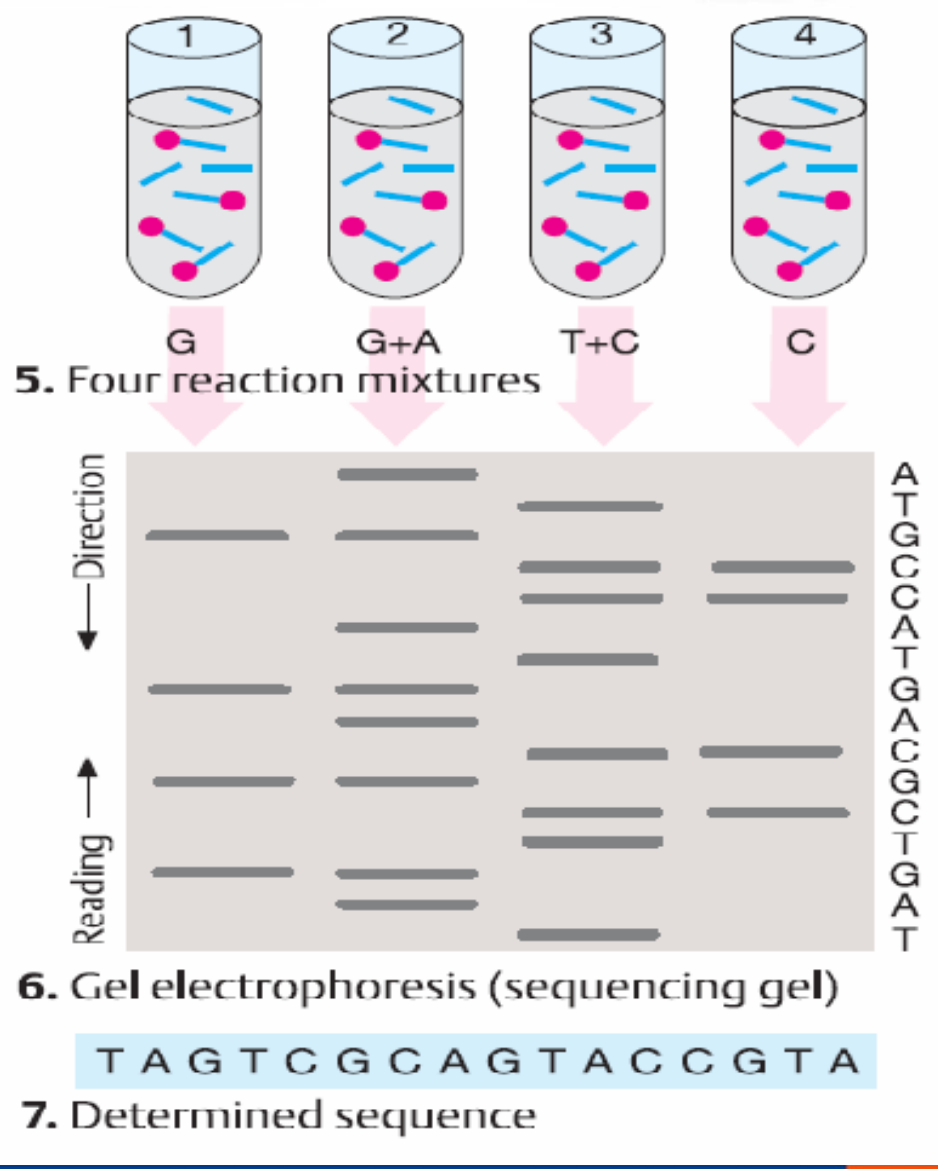
- **A- Chemical cleavage method (Maxam and Gilbert, 1977)**
  - ✓ pelabelan pada unjung 5’ dengan menggunakan γ-^{32}P
  - ✓ modifikasi dan pelepasan basa nitrogen
  - ✓ pemutusan rantai DNA
  - ✓ deteksi dengan Polyacrylamide gel electrophosesis
- **B- Enzymatic method (Sanger, 1981)**
- **NGS (Next-Generation Sequence)**

Prinsip kerja dari metode Gilbert

- DMS (dimethyl sulfate) akan memetilasi basa G dan C, dengan penambahan piperidin menghasilkan fragmen yang berbeda beda
- hidrazin akan menghidrolisis C dan T, tetapi garam yang tinggi akan menghalangi reaksi T sehingga hanya bekerja pada C
- dihasilkan empat macam fragmen, masing-masing dengan ujung G, ujung A atau G, ujung C atau T, dan ujung C

Chemical degradation method (Maxam–Gilbert method)





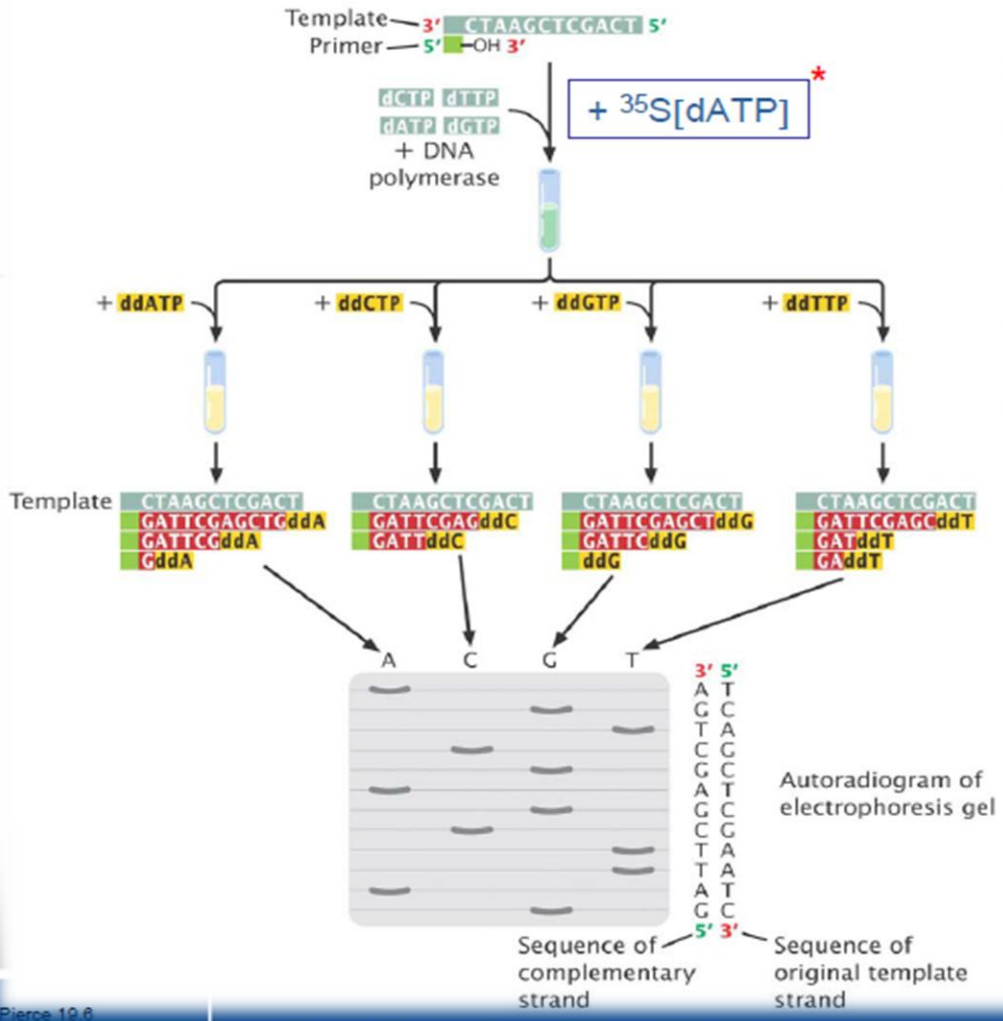
The Sanger DNA sequencing method

Uses dideoxy nucleotides to terminate DNA synthesis.

- •- DNA synthesis reactions in four separate tubes
- •- Radioactive dATP is also included in all the tubes so the DNA products will be radioactive.
- •-Yielding a series of DNA fragments whose sizes can be measured by electrophoresis.
- •- The last base in each of these fragments is known.

The dideoxy sequencing method (Sanger method)

Dideoxy method of DNA sequencing



Annealing

Polymerization and labeling

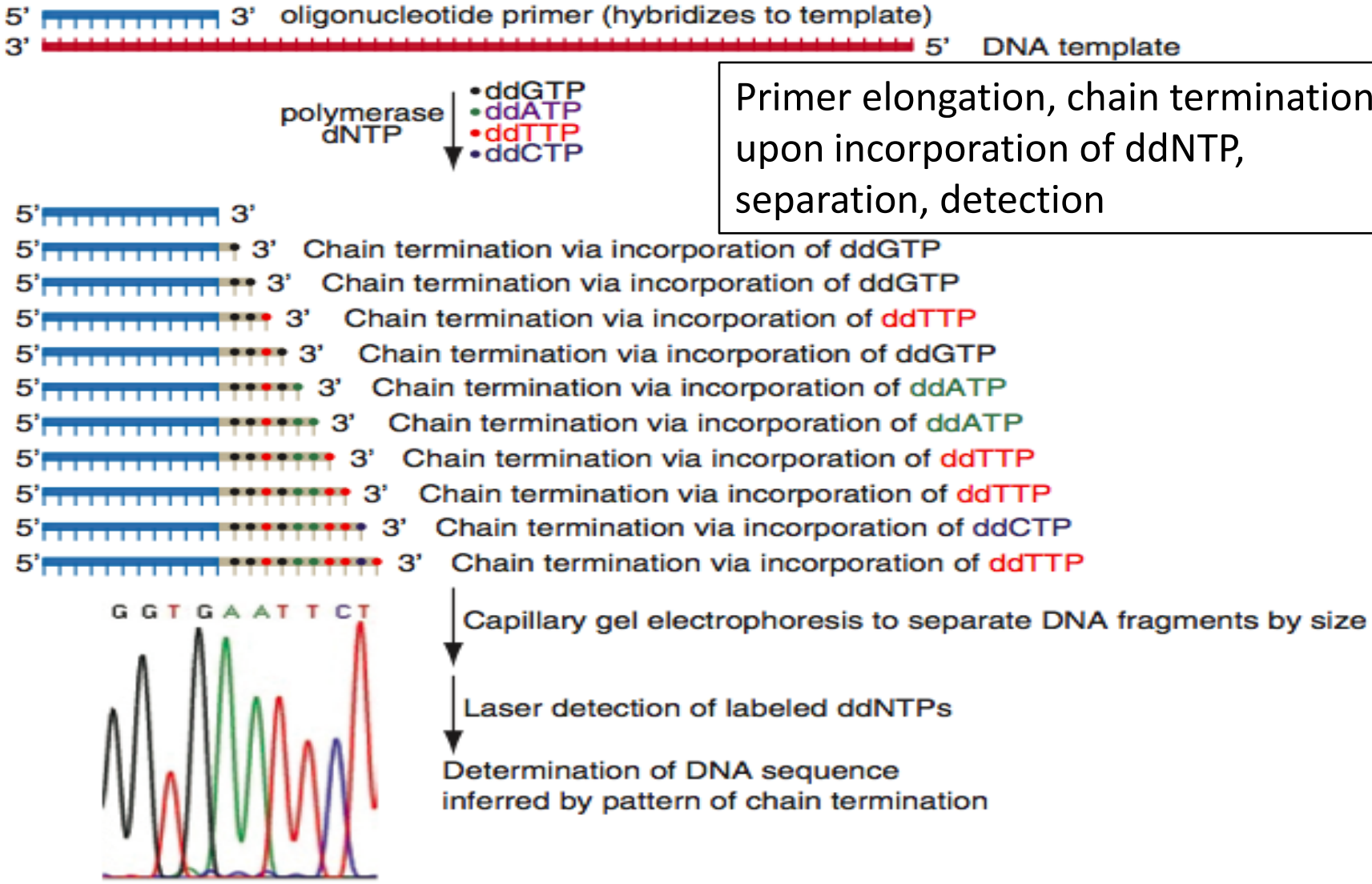
A labeled primer is used to initiate DNA synthesis. The addition of four different dideoxy nucleotides randomly arrests synthesis.

Termination

Polyacrylamide/urea gel electrophoresis

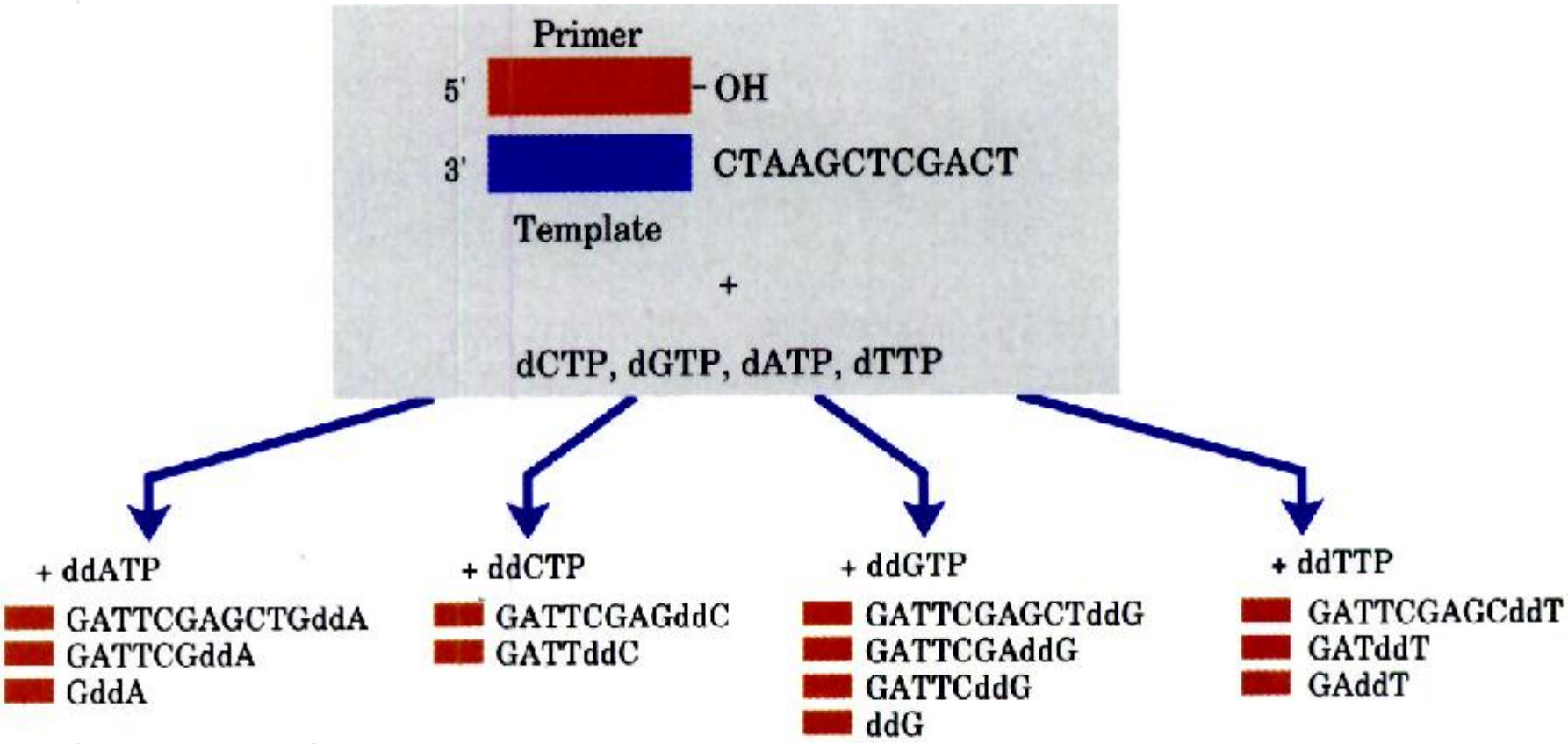
*- substituded automated DNA sequncing methods

DNA sequencing by the Sanger method

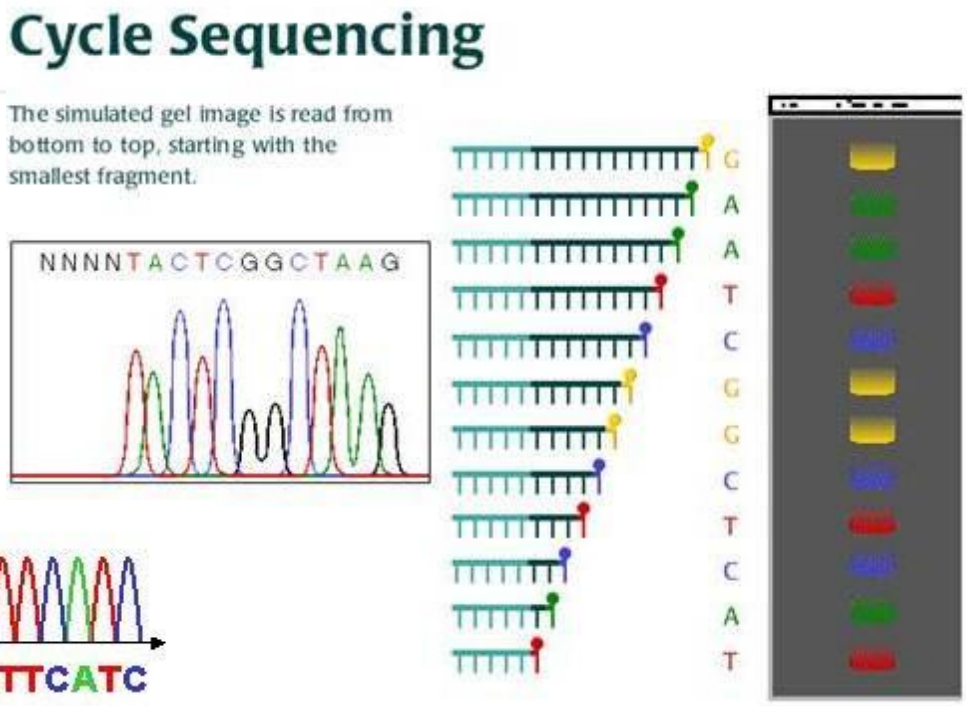


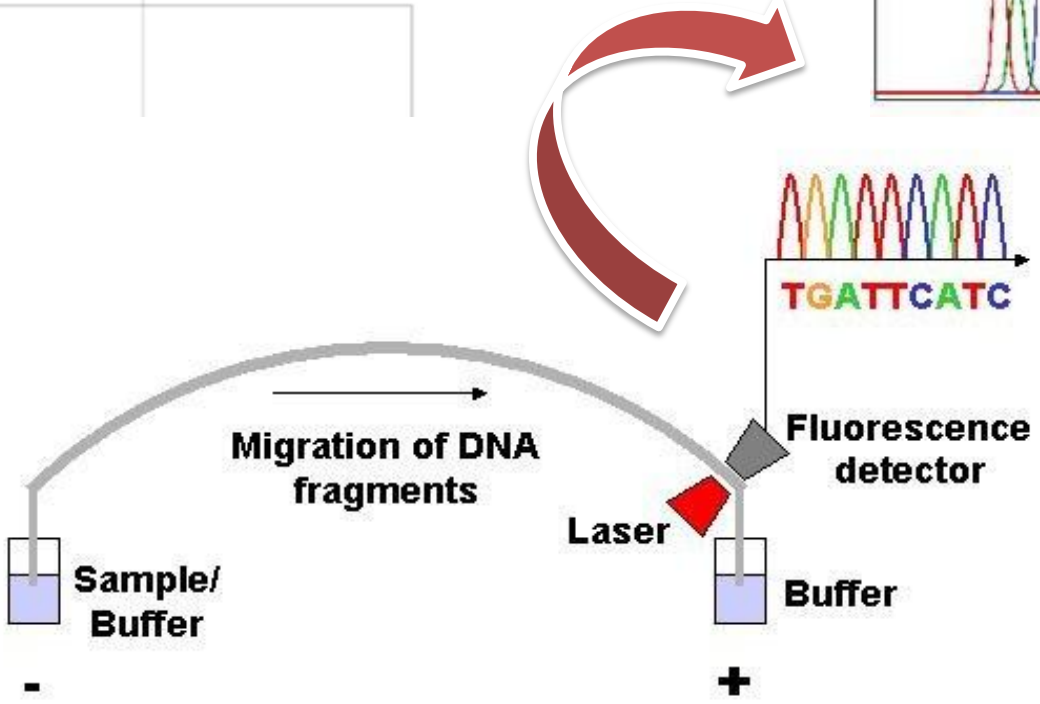
Primer elongation, chain termination upon incorporation of ddNTP, separation, detection

DNA Sequencing Reactions



Gel electrophoretic Fractionation





View genomic DNA (here from the beta globin locus) from the Trace Archive at NCBI: FASTA format

NEXT GENERATION SEQUENCING

Also known as

- High throughput sequencing or
- ultra-deep sequencing or
- massively parallel sequencing.

What is next generation sequencing ??

- Automated Sanger method (1st generation) •
- Technologies developed after that are known as next generation sequencing.
- NGS enables the sequencing of biological codes at a very rapid pace with low cost per operation.
- This is the primary advantage over conventional methods.
- For example Billions of short reads can be sequenced in one operation

Sangers vs. NGS

	Sanger	NGS
Sequencing samples	Clones, PCR	DNA Libraries
Sample Tracking	Many samples in 96, 384 well plates	Few
Preparation steps	Few, Sequencing reactions clean up	Many, Complex procedures
Data Collection	Samples in plates 96, 384	Samples on slides 1 – 16+
Data	One read/ sample	Thousands and Millions of reads/Samples.

DNA Sequence

NCBI Resources How To

Nucleotide Nucleotide Advanced

FASTA

Homo sapiens genomic DNA, chromosome 21q

GenBank: BA000005.3

GenBank Graphics

```
>BA000005.3 Homo sapiens genomic DNA, chromosome 21q
CATGTTTCCACTTACAGATCCTTCAAAAAGAGTGTTTCAAAACTGCTCTATGAAAAGGAATGTTCAACTC
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TCCAACGAAAGCCTCGAAGCTGTCCAAATATCCACTTGTAAGTGCTGCAAAAAGAGTGTTTCAAAACTGC
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TAGAAAGTTTCACCTCTGTGAGCTGAATGCACACAGAAGTTTCTGAGAATGCTTCTGTCTGGTTTTTATG
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```

Protein Sequence

```
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ORIGIN
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       61 gpqgftgstg lsglkgergf pgllgpygpk gdkgpmgvpg flgingipgh pgqpgprgpp
      121 gldgcngtqg avgfpgpdgy pgllgppglp gqkgskgdpv lapgsfkgmk gdpglpgldg
      181 itgpqgapgf pgavgpagpp glqgppgppg plgpdgnmgl gfqgekgvkg dvglpgpagp
      241 ppstgelefm gfpkgkkgsk gepgpkgfpg isgppgfpgl gttgekgekg ekgipglpgp
      301 rgpmgsegvq gppgqqgkkg tlgfpglngf qgiegqkgdi glpgpdvfid idgavisgnp
      361 gdpgvpglpg lkgdegiqgl rgpsgvpglp alsgvpgalg pqgfpglkgd qgnpgrttig
      421 aaglpgrdgl pgppgppgpp spefetetlh nkesgfpglr geqgpkgnlg lkgikgdsgf
      481 cacdggvpnt gppgepgppg pwgliglpgl kgargdrgsg gaqgpagapg lvgplgpsgp
      541 kgkkgepils tiqgmpgdrg dsgsqgfrgv igepgkdgvp glpglpglpg dggqgfpgek
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      661 sygpsgfpgt pgfpgpkgsr glpgtpgqpg ssgskgepgs pglvhlpelp gfpgprgekg
      721 lpgfpglpgk dglpgmigsp glpgskgatg difgaengap geqglqgltg hkgflgdsgl
      781 pglkgvhgkp gllgpkgerg spgtpgqvgq pgtpgssgpy gikgksglpg apgfpgisgh
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      901 vgfvgfpgip glpgisgtrg lkgipgstgk mgpsgragtp gekgdrgnpg pvgipsprrp
      961 msnlwlkgdk gsqgsagsng fpgprgdkge agrpgppglp gapglpgiik gvsgkpgppg
     1021 fmgirglpgl kgssgitgfp gmpgesgsqg irgspglpga sglpglkgdn gqtveisgsp
     1081 gpkgqpgesg fkgtkgrdgl ignigfpgnk gedgkvgvsg dvglpgapgf pgvagmrgep
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     1201 lpgpkgekgy pgigigapgk pglrgqkgdr gfpglqgpag lpgapgislp sliagqpgdp
     1261 grpgldgerg rpgpagppgp pgpssnqgdt gdpgfpgipg fsglpgelgl kgmrgepgfm
     1321 gtpgkvgppg dpgfpgmkgk agargssglq gdpgqtptae avqvppgplg lpgidgipgl
     1381 tgdpgaqgpv glqgskglpg ipgkdgpsgl pgppgalgdp glpglqgppg fegapgqqgp
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     1561 psqaiavhsq ditipqcplg wrslwigysf lmhtaagaeg ggqslvspgs cledfratpf
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//
```
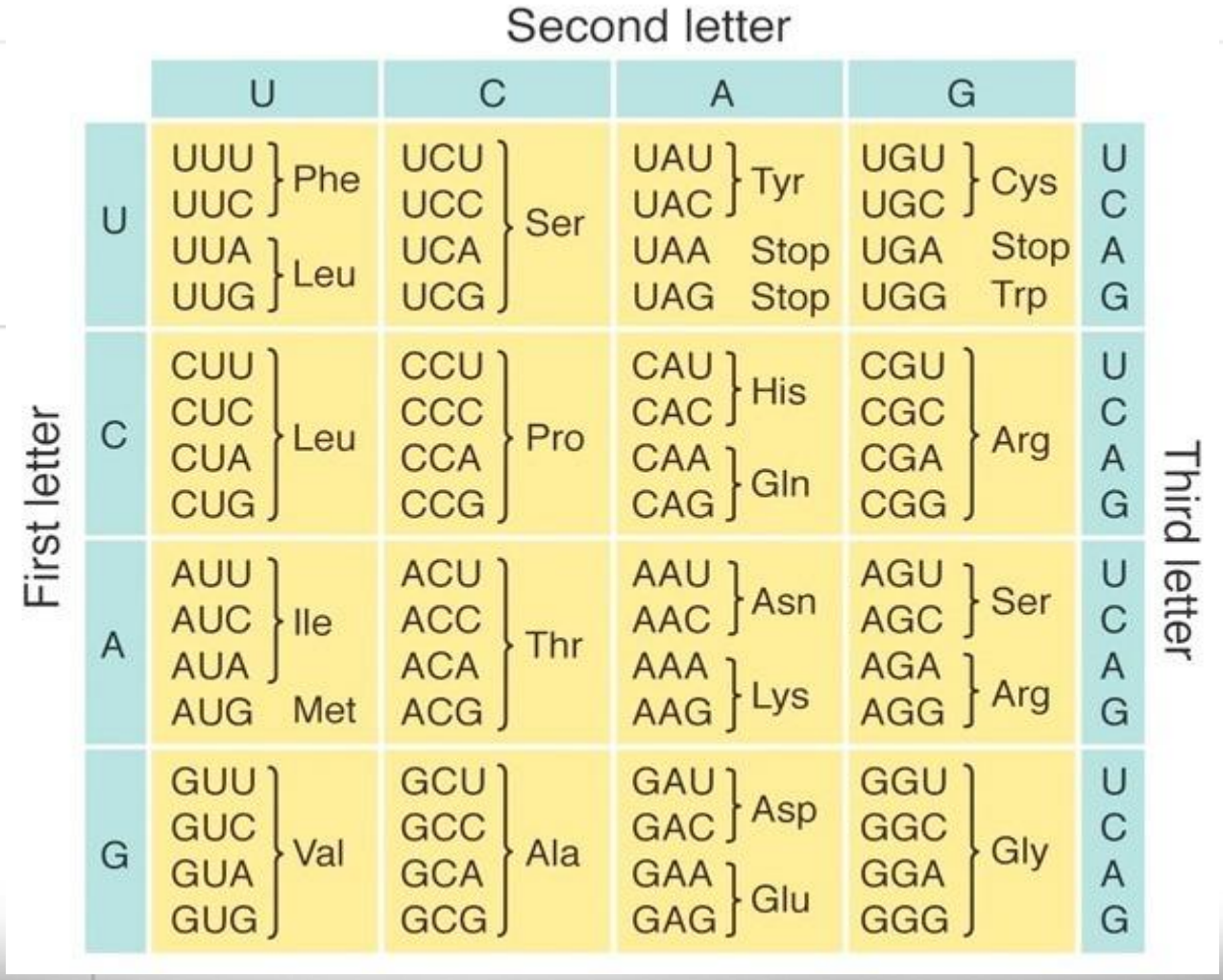

SELDI MALDI


Second letter
U
C
A
G
First letter
Third letter
U
C
A
G
UUU Phe
UUC
UUA Leu
UUG
UCU
UCC
UCA Ser
UCG
UAU Tyr
UAC
UAA Stop
UAG Stop
UGU Cys
UGC
UGA Stop
UGG Trp
CUU
CUC
CUA Leu
CUG
CCU
CCC
CCA Pro
CCG
CAU His
CAC
CAA Gln
CAG
CGU
CGC
CGA Arg
CGG
AUU
AUC Ile
AUA
AUG Met
ACU
ACC
ACA Thr
ACG
AAU Asn
AAC
AAA Lys
AAG
AGU Ser
AGC
AGA Arg
AGG
GUU
GUC
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GCG
GAU Asp
GAC
GAA Glu
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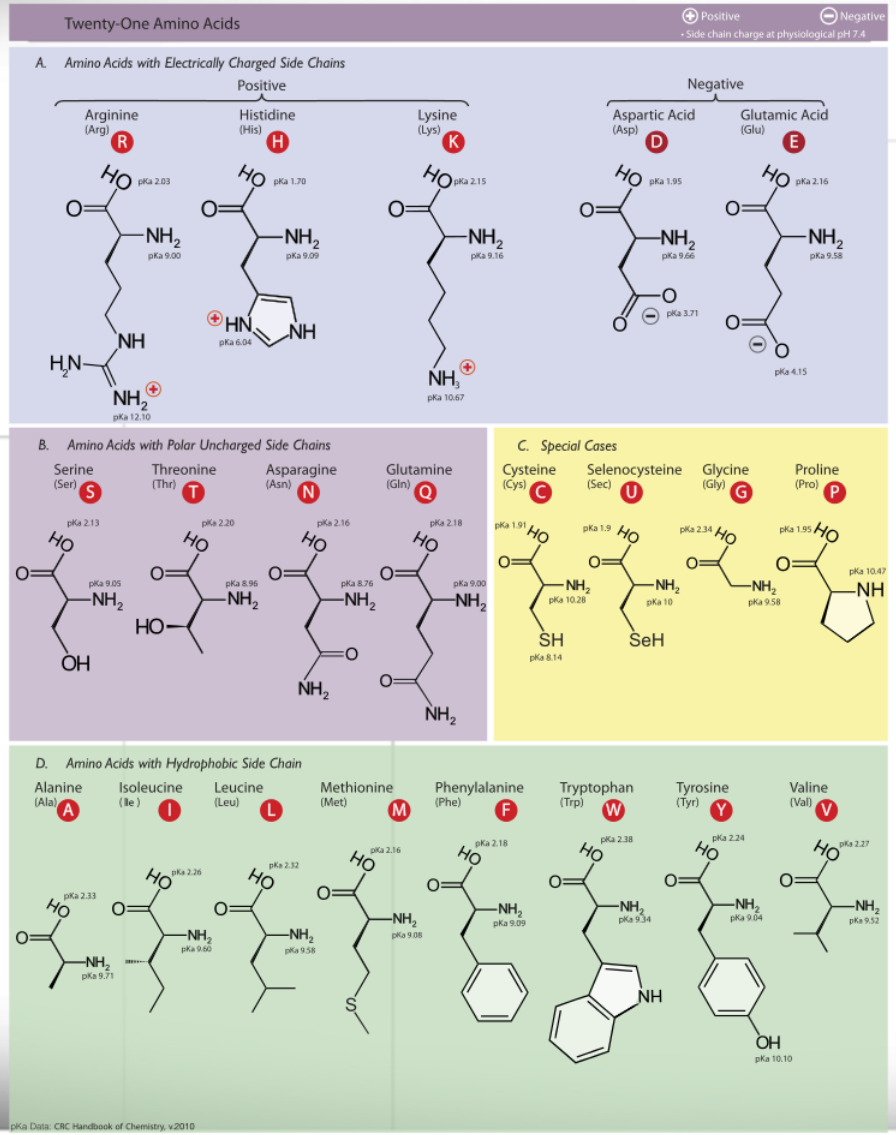
GGU
GGC
GGA Gly
GGG

Protein Sequence



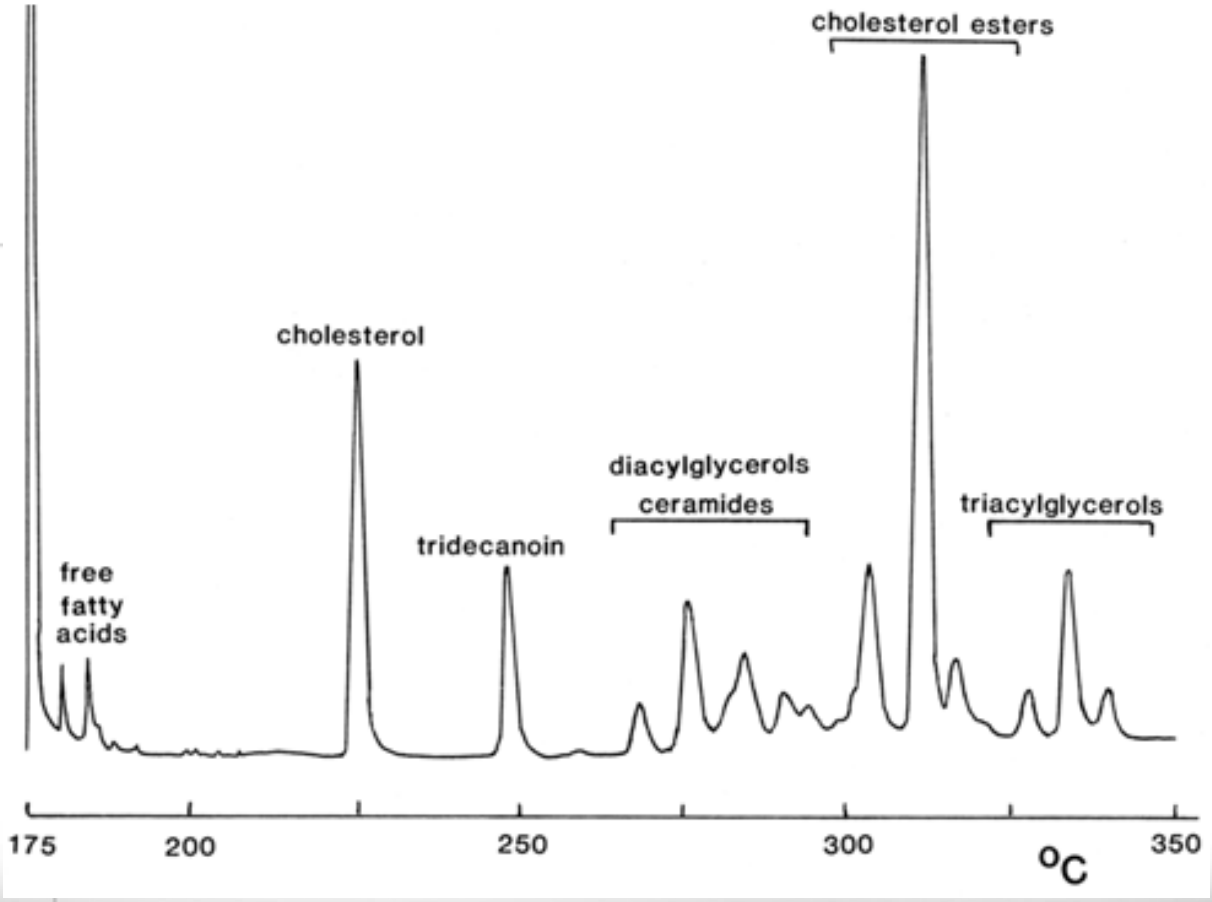
single-letter code	*abbreviation*	*full name*
A	Ala	Alanine
R	Arg	Arginine
N	Asn	Asparagine
D	Asp	Aspartic acid
C	Cys	Cysteine
Q	Gln	Glutamine
E	Glu	Glutamic acid
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
L	Leu	Leucine
K	Lys	Lysine
M	Met	Methionine
F	Phe	Phenylalanine
P	Pro	Proline
S	Ser	Serine
T	Thr	Threonine
W	Trp	Tryptophan
Y	Tyr	Tyrosine
V	Val	Valine

Mass Spectrofotometry (Spektrofometri massa)

- suatu teknik analisis dengan prinsip dasar membuat suatu molekul netral menjadi bermuatan sehingga bisa dideteksi. Tujuan utama dari spektroskopi massa adalah mengetahui berat molekul.

- Informasi yang diperoleh dari spektrum MS adalah berat ion, yakni massa molekul isolat ditambah atau dikurangi sumber ion. Berat ion biasanya disajikan dalam $[M+H]^+$ atau $[M+OH]^-$ atau dalam bentuk radikal $[M^*]^+$. Berat molekul sesungguhnya diperkirakan bertambah satu atau berkurang satu angka yang mendekati.
- Adakalanya ionisasi melalui penambahan berat molekul air (Saifudin, 2014).

Spektrofotometri Massa



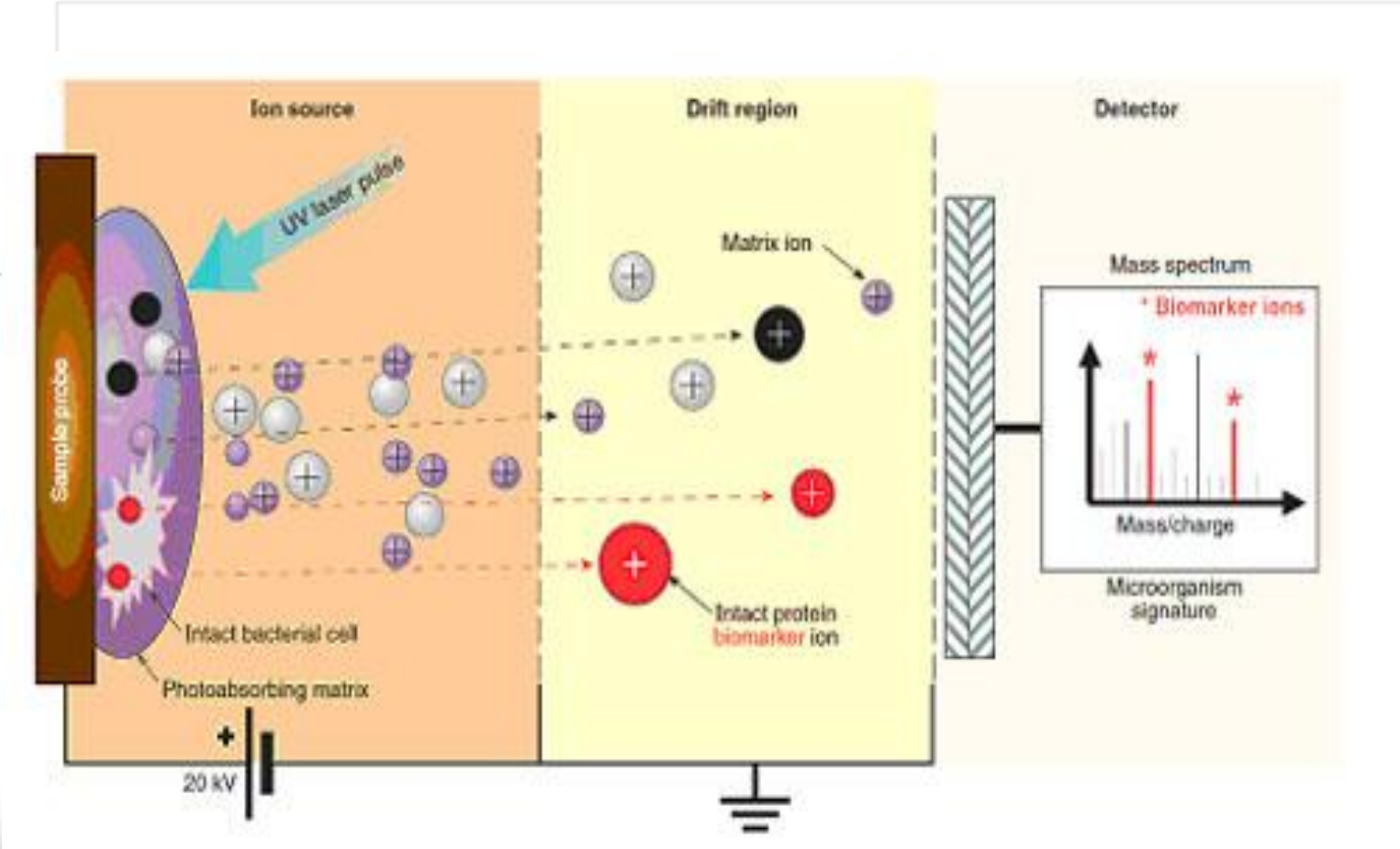
SELDI MALDI

mass spectrometry, matrix-assisted laser desorption/ionization (MALDI)

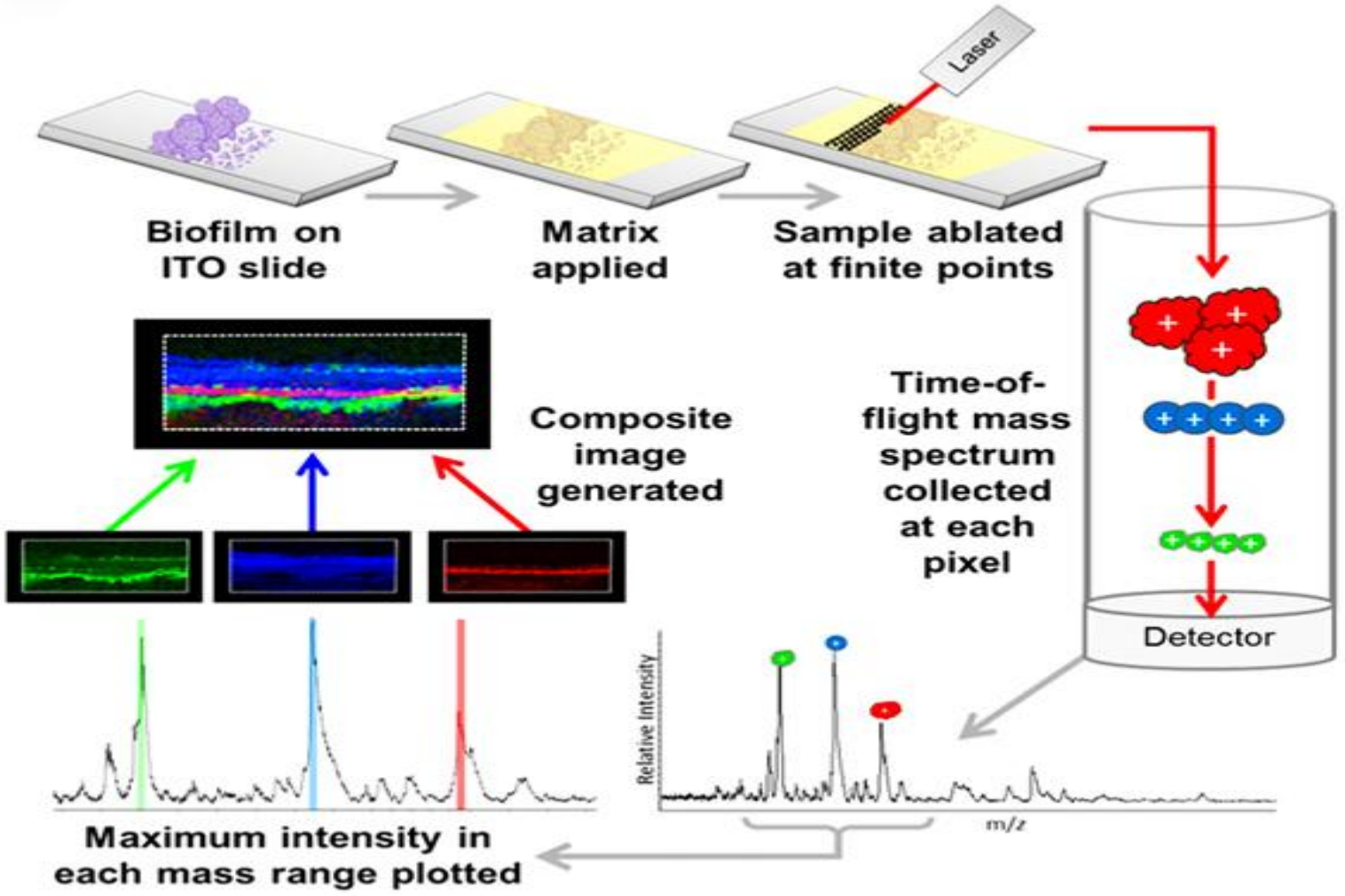
Surface-enhanced laser desorption/ionization (*SELDI*)

ionization technique that uses a laser energy absorbing matrix to create ions from large molecules with minimal fragmentation.

SELDI MALDI


Ion source
Drift region
Detector
UV laser pulse
Sample probe
Matrix ion
Mass spectrum
Biomarker ions
Mass/charge
Microorganism signature
Intact bacterial cell
Photoabsorbing matrix
Intact protein biomarker ion
20 kV

SELDI MALDI

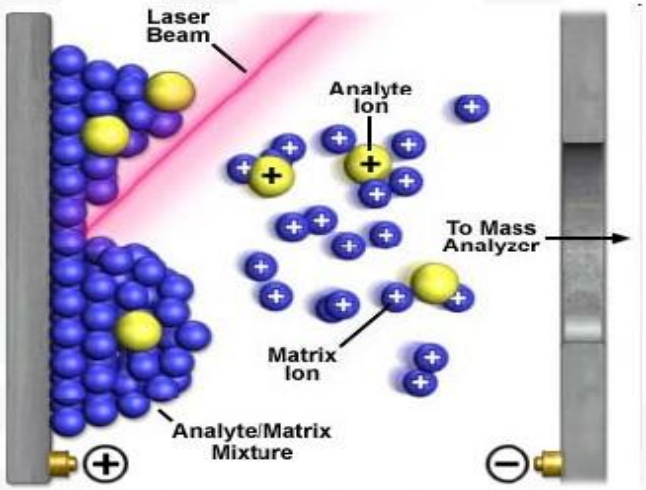

Laser
Biofilm on ITO slide
Matrix applied
Sample ablated at finite points
Time-of-flight mass spectrum collected at each pixel
Detector
Composite image generated
Relative Intensity
m/z
Maximum intensity in each mass range plotted

UNC SCHOOL OF MEDICINE

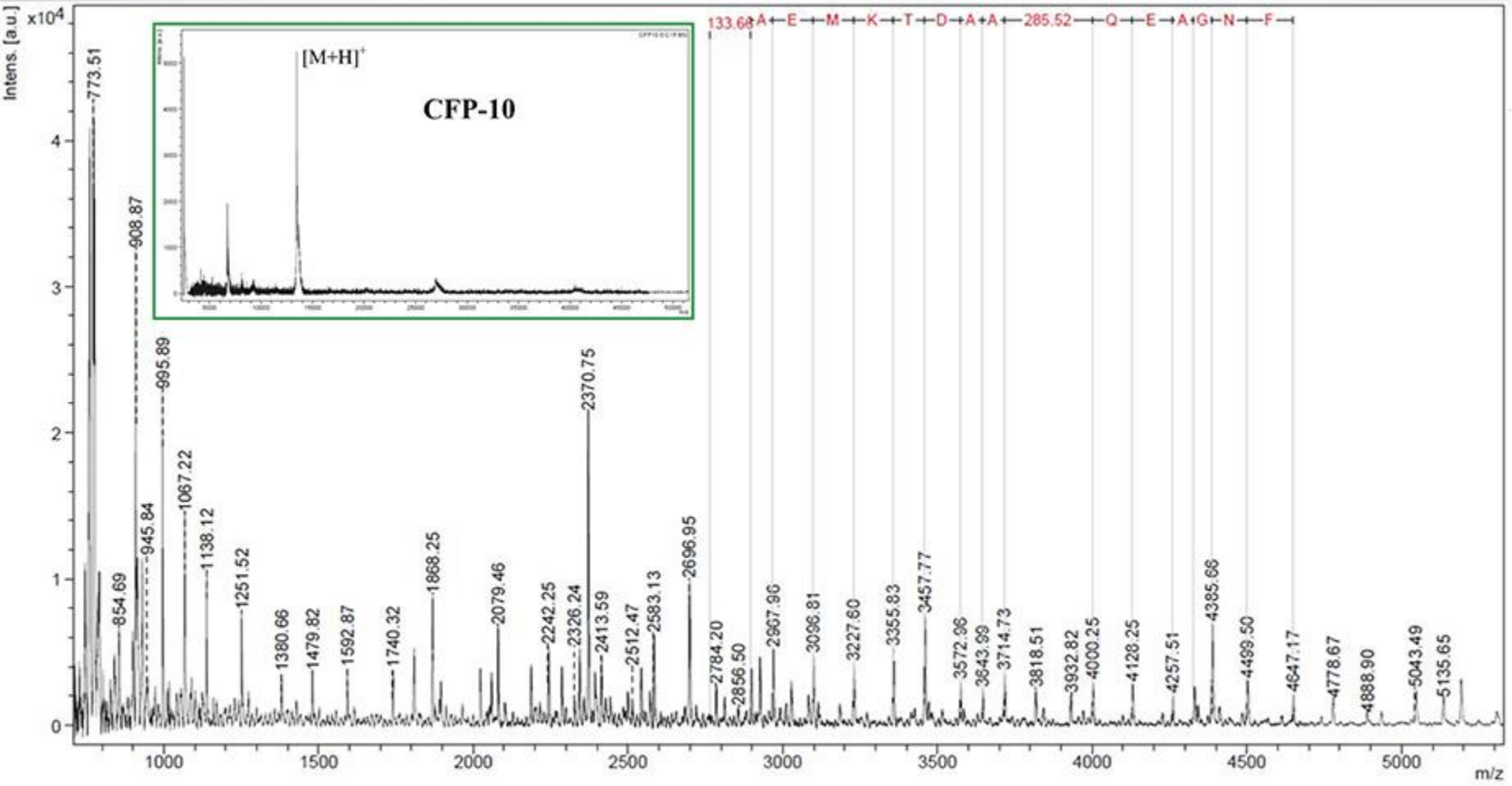
MALDI-TOF

- Matrix Assisted Laser Desorption Ionization (MALDI)-Time of Flight (TOF)
  - Bruker Daltonics MALDI BioTyper (TM)
  - BD and bioMerieux also have MALDI in the pipeline

- Sample mixed with UV-absorbing acid matrix and spotted on a MALDI plate
- Laser Irradiation forms an excited plume
- Proton transfer from the matrix forms ions



SELDI MALDI



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Thank You!!!