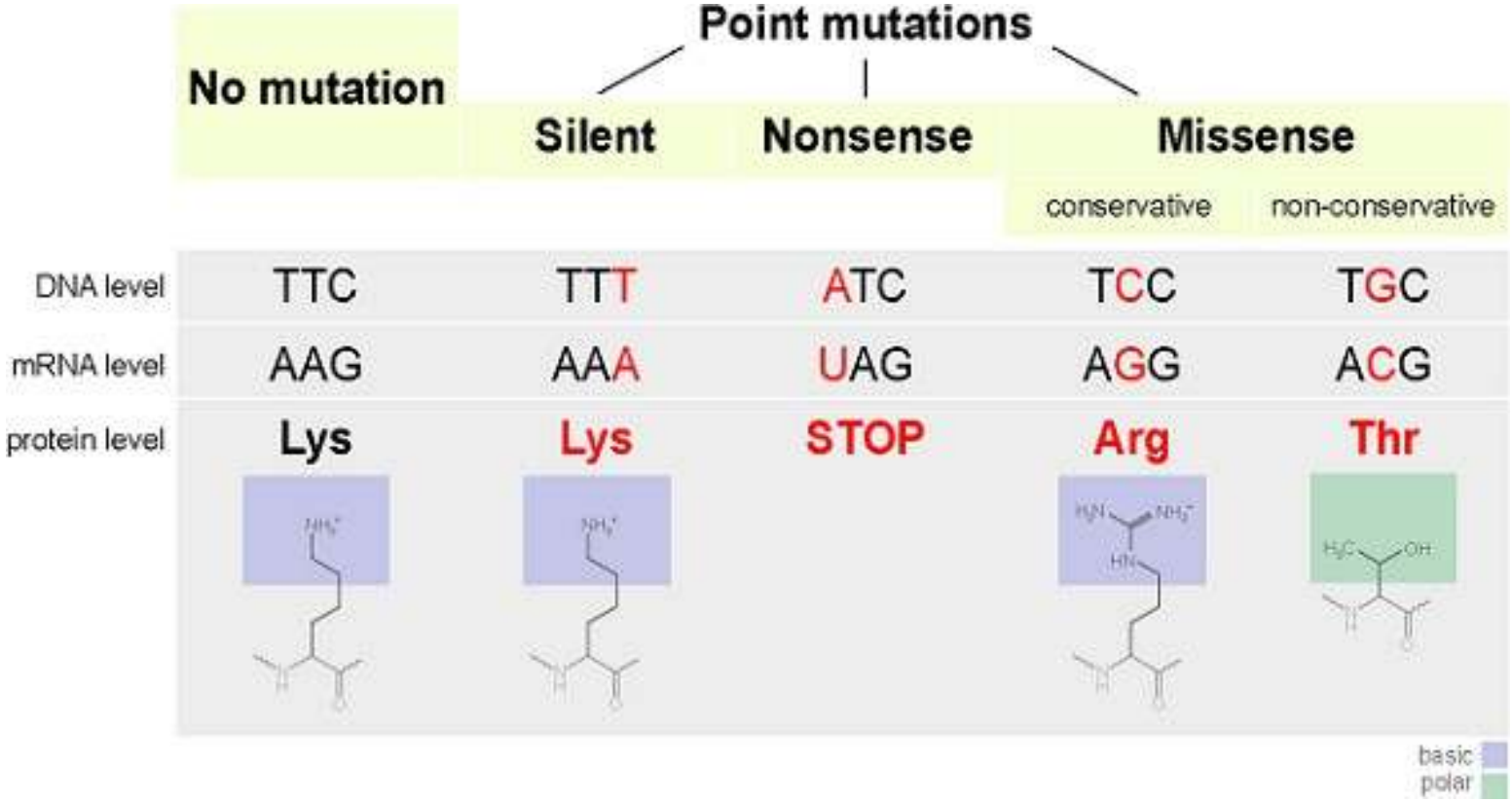
Research techniques in genetics

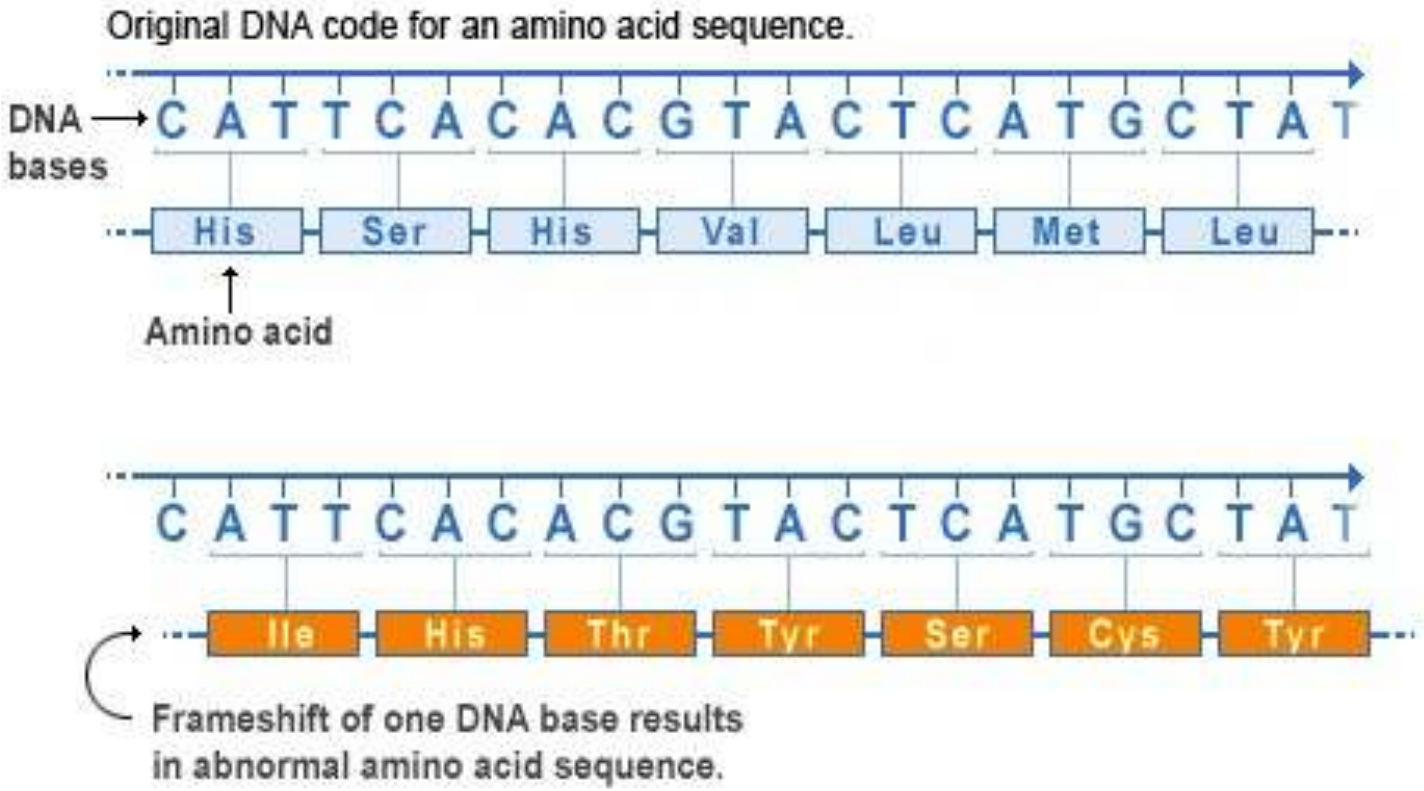
Medical studies in English, 2nd year, Medical genetics, 2019./20.

Prof. Ivana Novak Nakir

Mutations

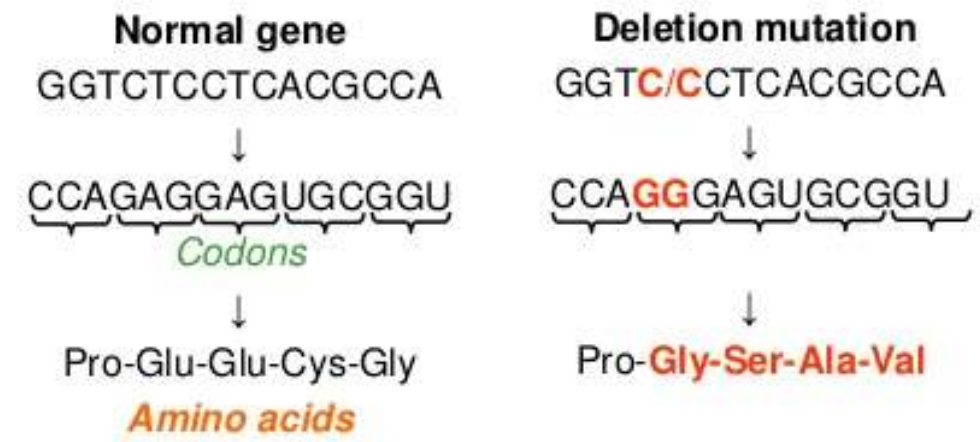

Point mutations
No mutation
Silent
Nonsense
Missense
conservative
non-conservative
DNA level
TTC
TTT
ATC
TCC
TGC
mRNA level
AAG
AAA
UAG
AGG
ACG
protein level
Lys
Lys
STOP
Arg
Thr
basic
polar

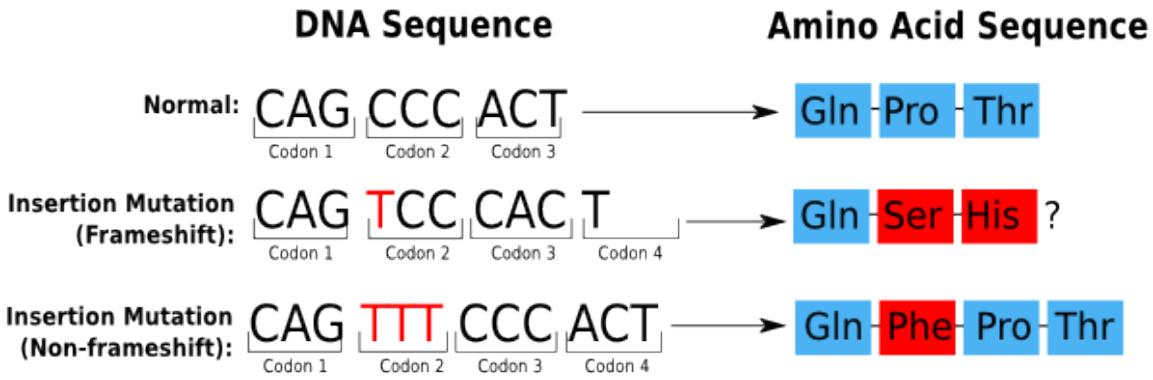
Frameshift mutation



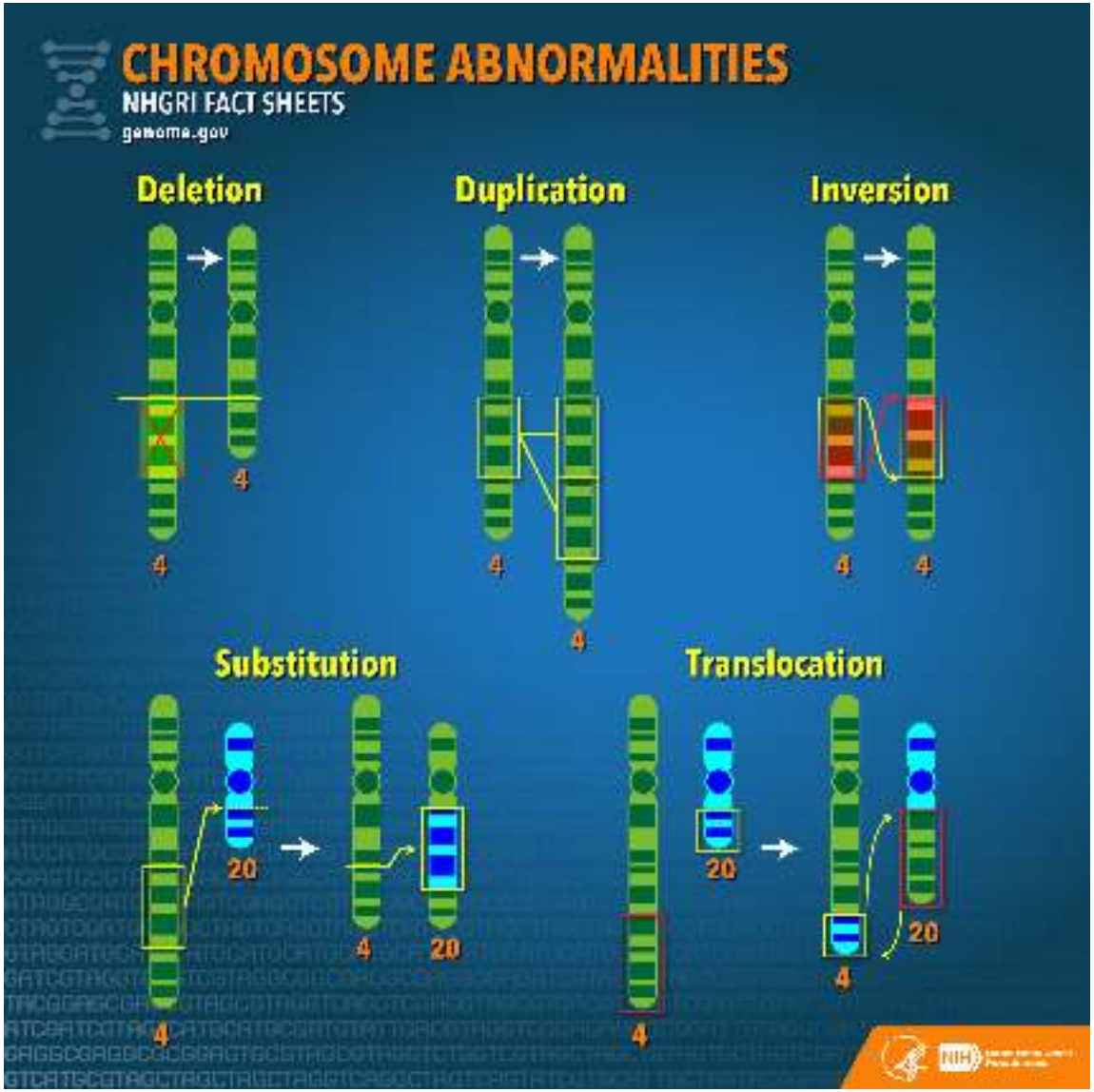
U.S. National Library of Medicine

Deletions, insertions





Chromosomal aberrations



DNA based analysis techniques

Used for SNP detection, mutation analysis, submicroscopic deletions, duplications, …

- PCR, RT-PCR
- Restriction digestion (RFLP)
- Southern blot
- Sequencing:
  - Sanger method (dideoxy)
  - next generation sequencing
- Quantitative fluorescent PCR
- Multiple ligation dependant probe amplification (MLPA)

Chromosome based analysis techniques

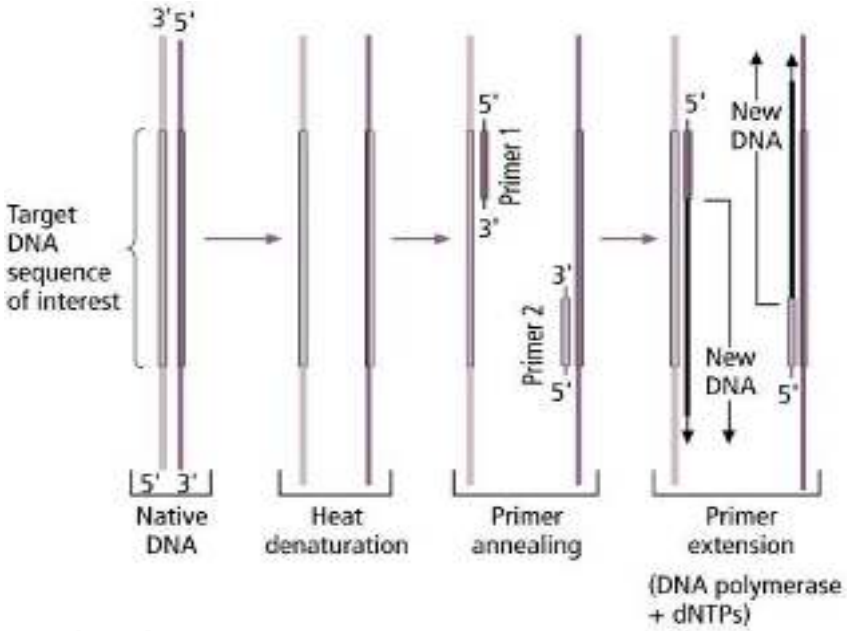
Used for deletions, aneuploidy, translocations, satelite polymorphism, fragile sites, copy number variations, …

- Karyotype (G, R, C, etc. banding)
- Flow cytometry (EB stain)
- FISH
- Array comparative genomic hybridization (Array CGH)

PCR

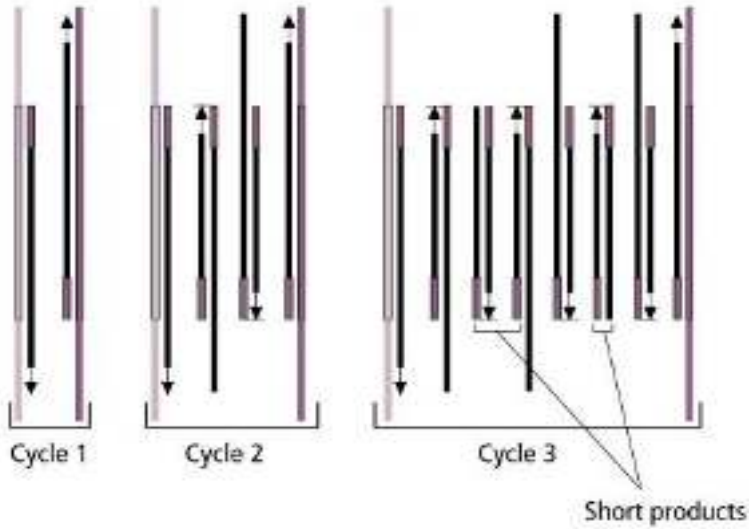
Kary Mullis (Nobel-chem:1993.)

PCR



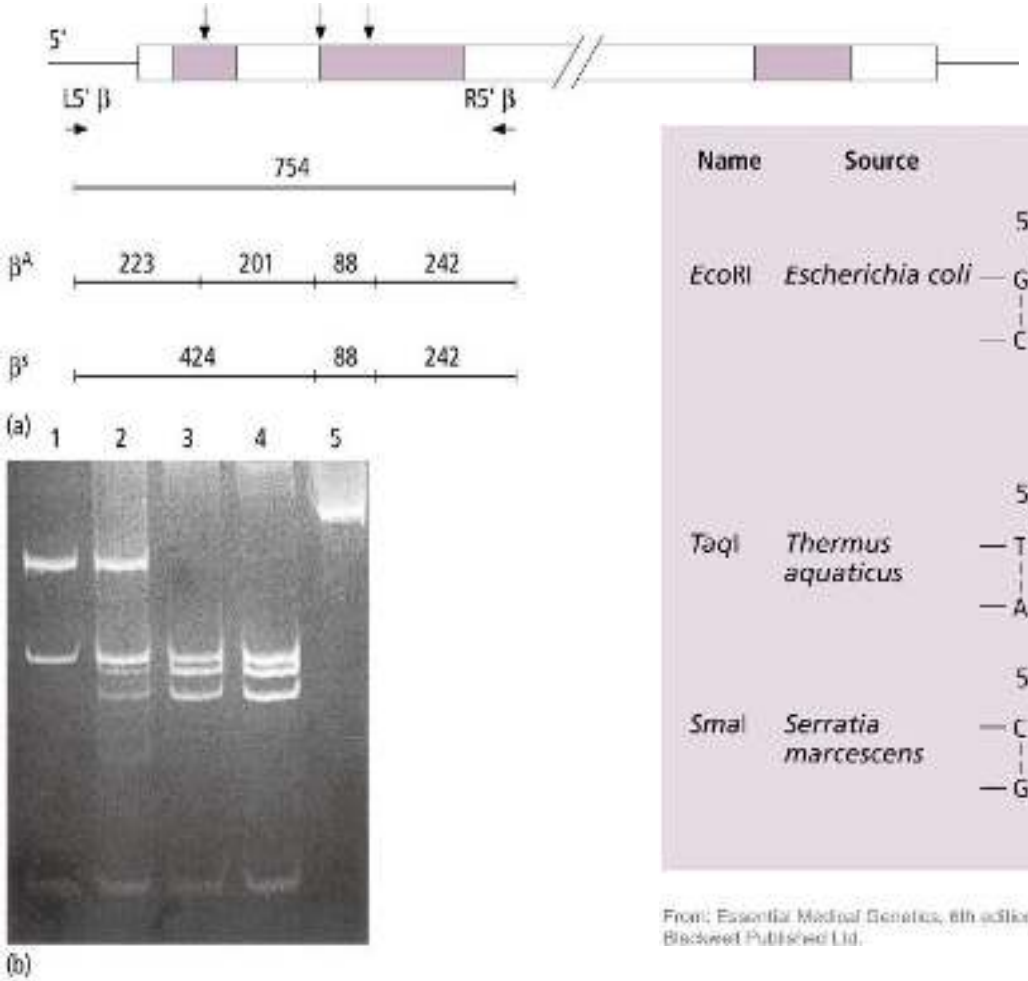
First round of the PCR

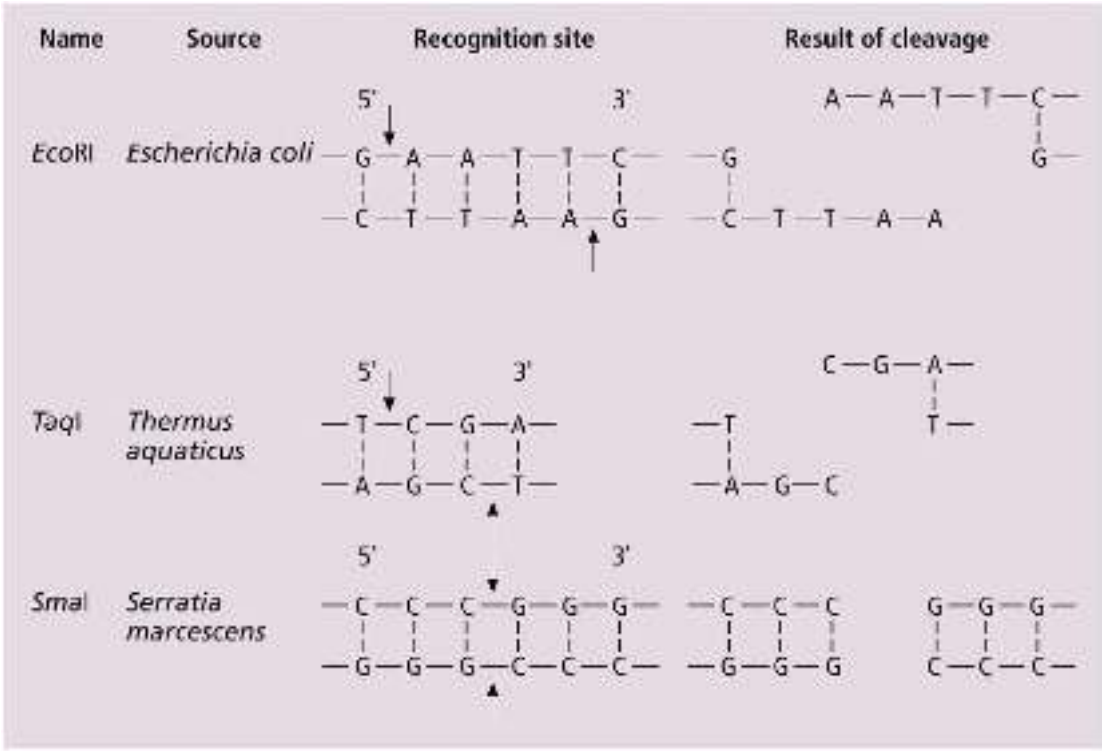
40 cycle → a trillion copies !
(10^{12})



Products at the ends of early PCR cycles

RFLP (Restriction fragment length polymorphism)





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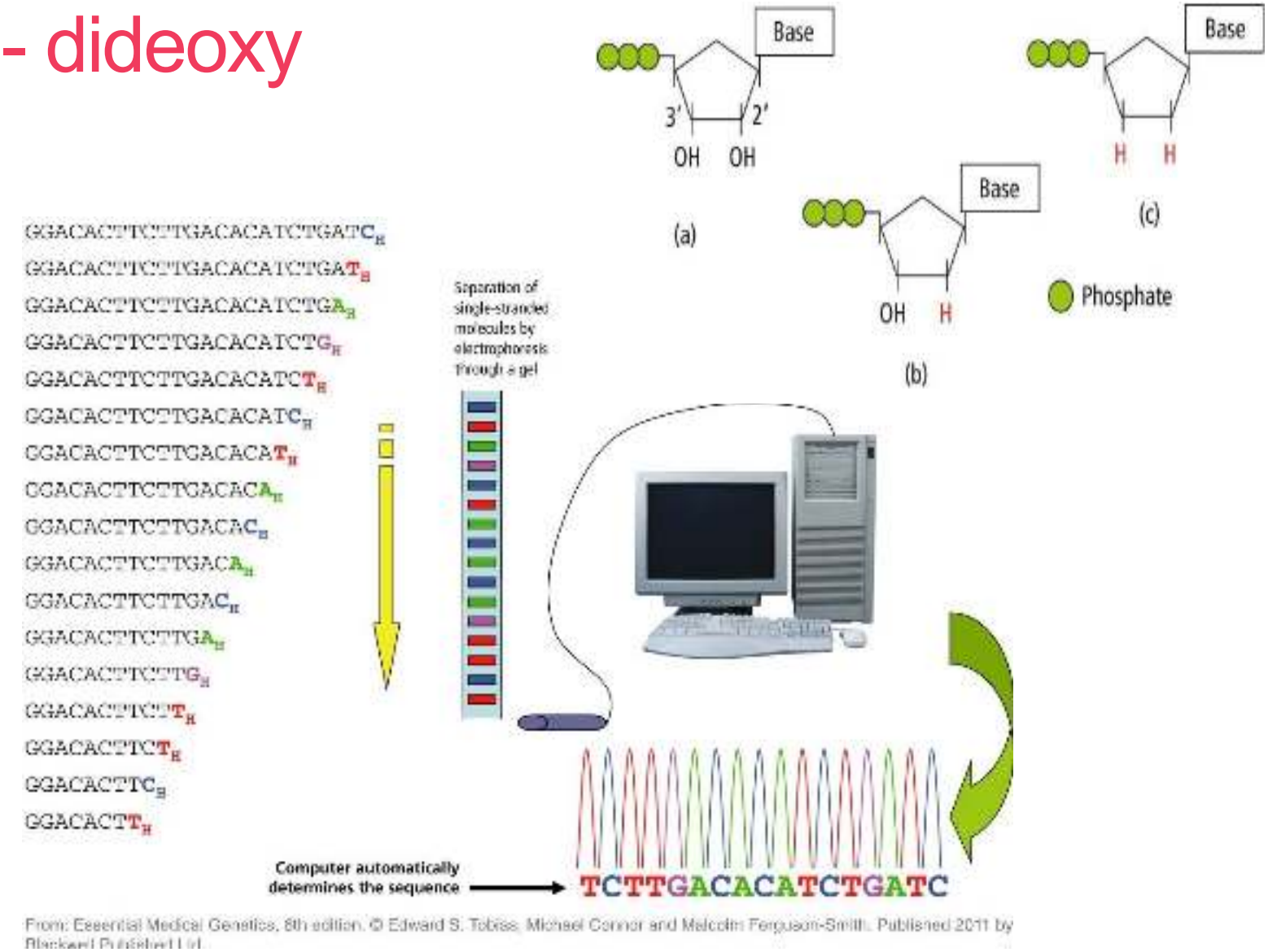
Sanger sequencing method - dideoxy



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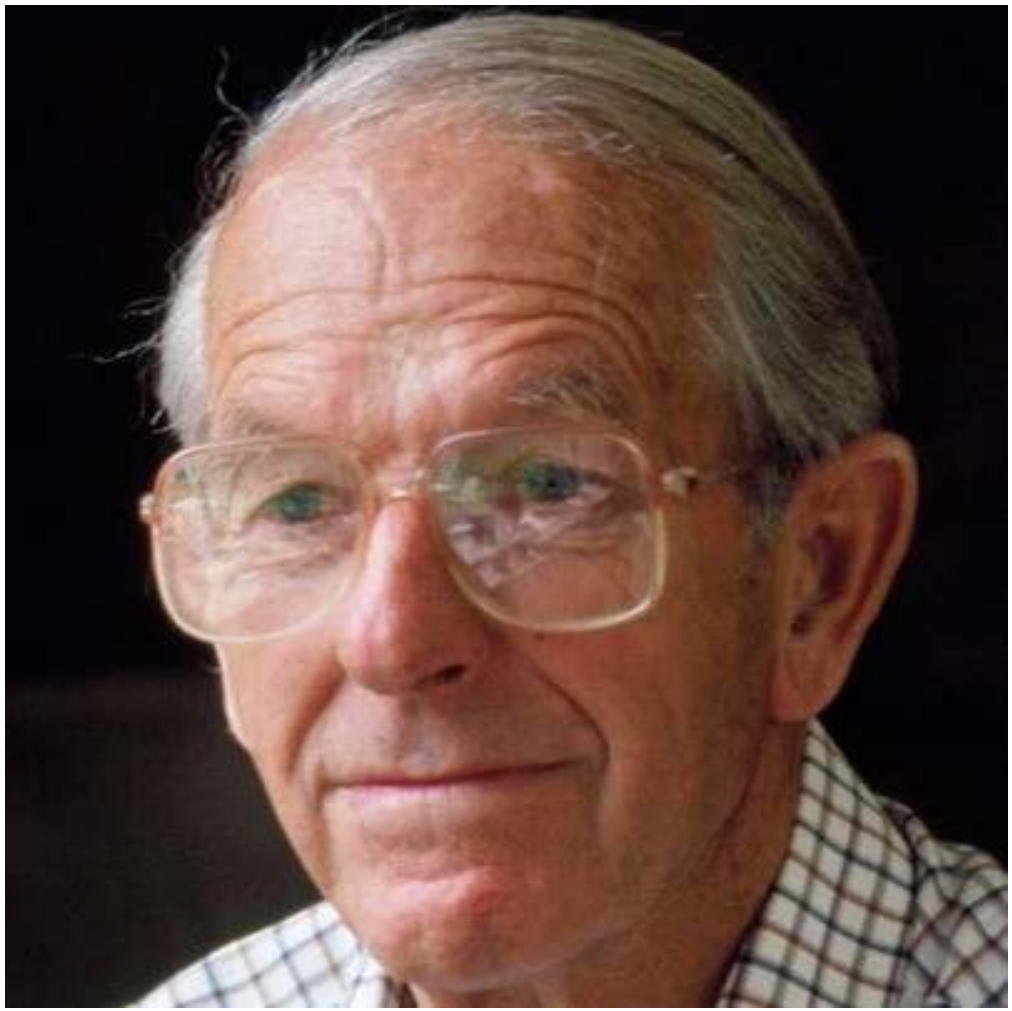
Frederic Sanger – DNA Sequencing

(Nobel – chem: 1958 and 1980.)



Next-Generation Sequencing

- Generation of millions of sequences at once – high-throughput
- Sequence reads are short (100-250 bp), need to be aligned to the reference sequence
- Useful for genetic diagnostics of rare diseases

Sanger Sequencing	Next-Generation 'Clonal' Sequencing
One sequence read per sample	Massively parallel sequencing
500–1000 bases per read	100–400 bases per read
Approx. 1 million bases per day per machine	Approx. 2 billion bases per day per machine
Approx. $1 per 1000 bases	Approx. $1 per 5,000,000 bases

MiSeq®

NextSeq® 500

HiSeq® 2500

HiSeq® 3000

Next Generation Sequencing
platforms from trusted names

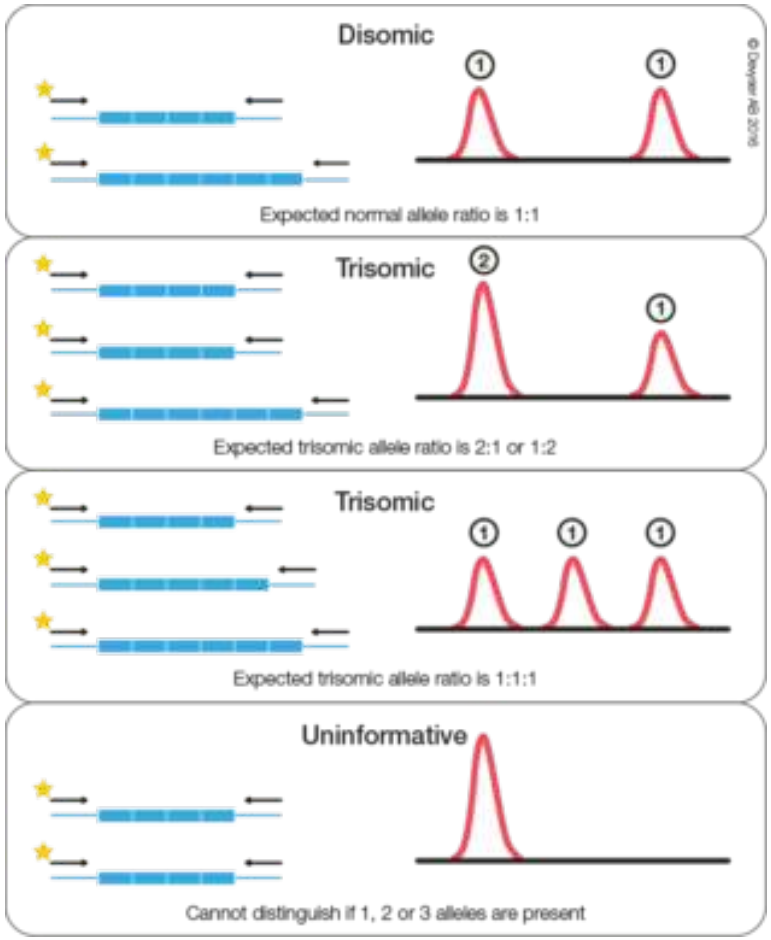
Ion Torrent™

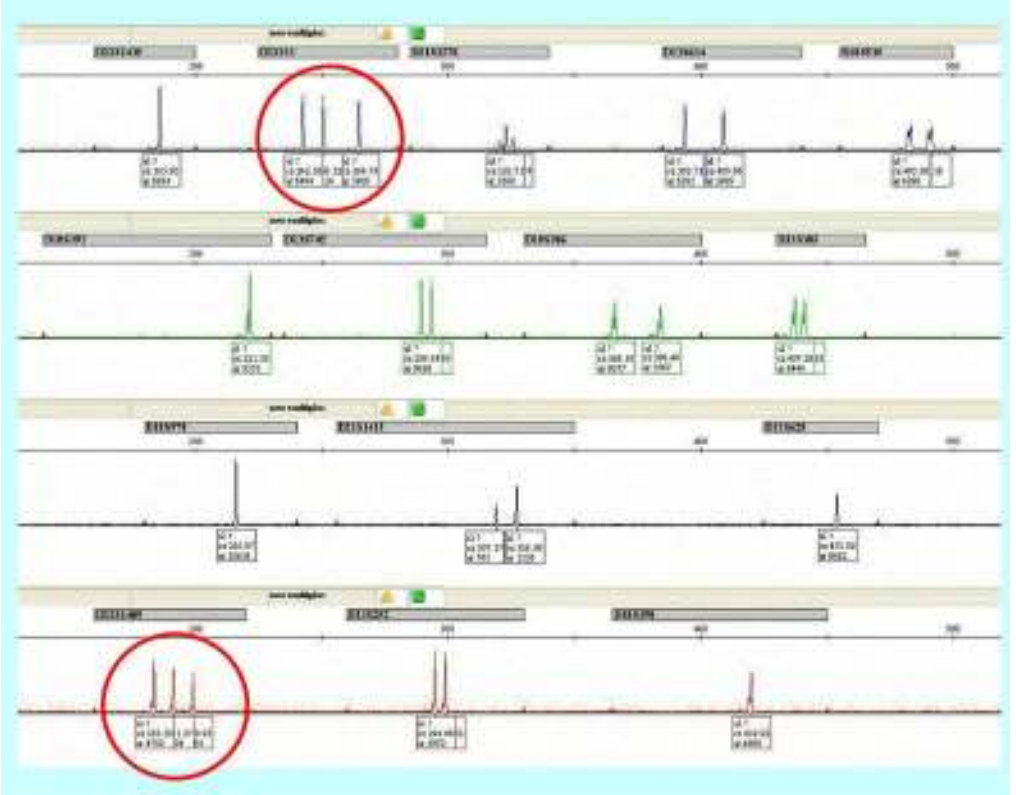
PacBio RS II System

HiSeq® 4000

Quantitative fluorescent PCR

(For the detection of **aneuploidy** (trisomy or monosomy).

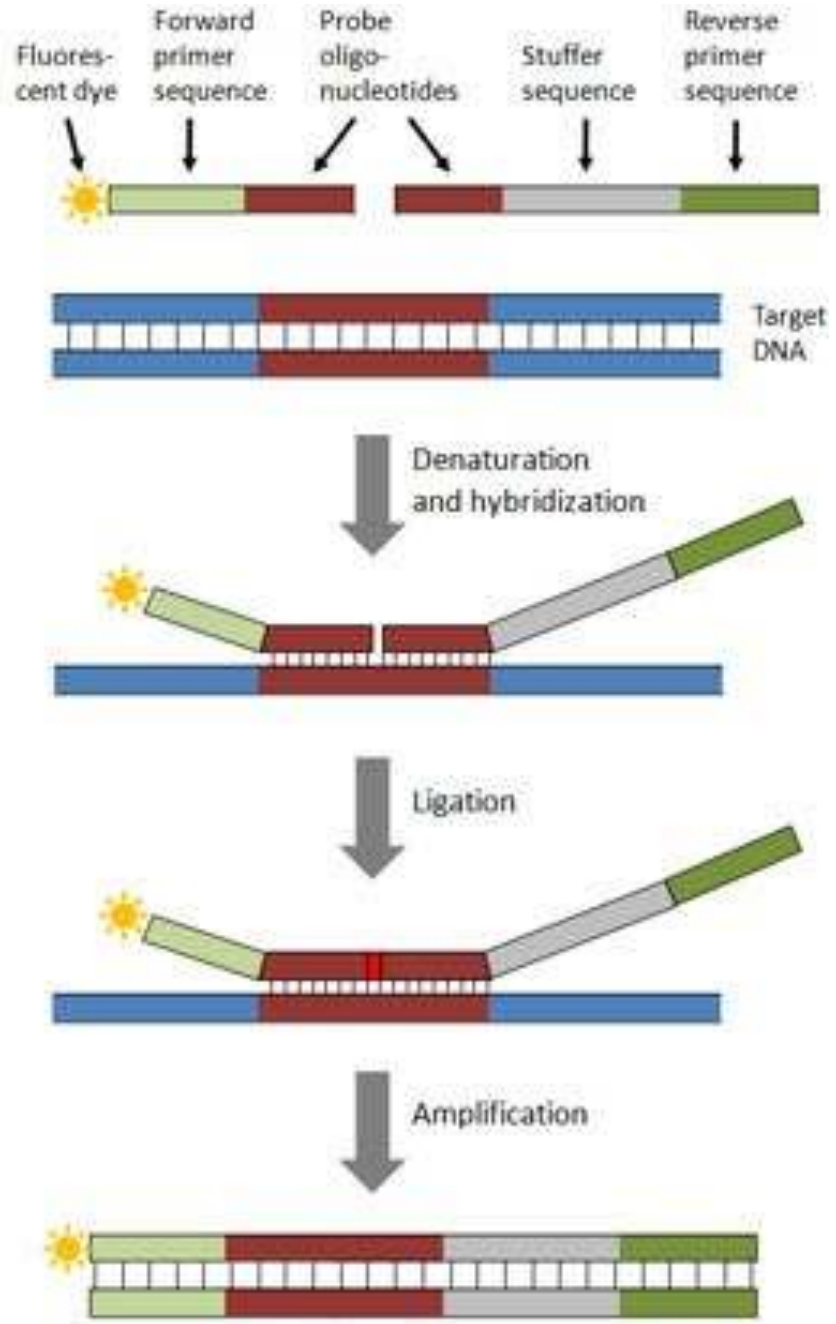




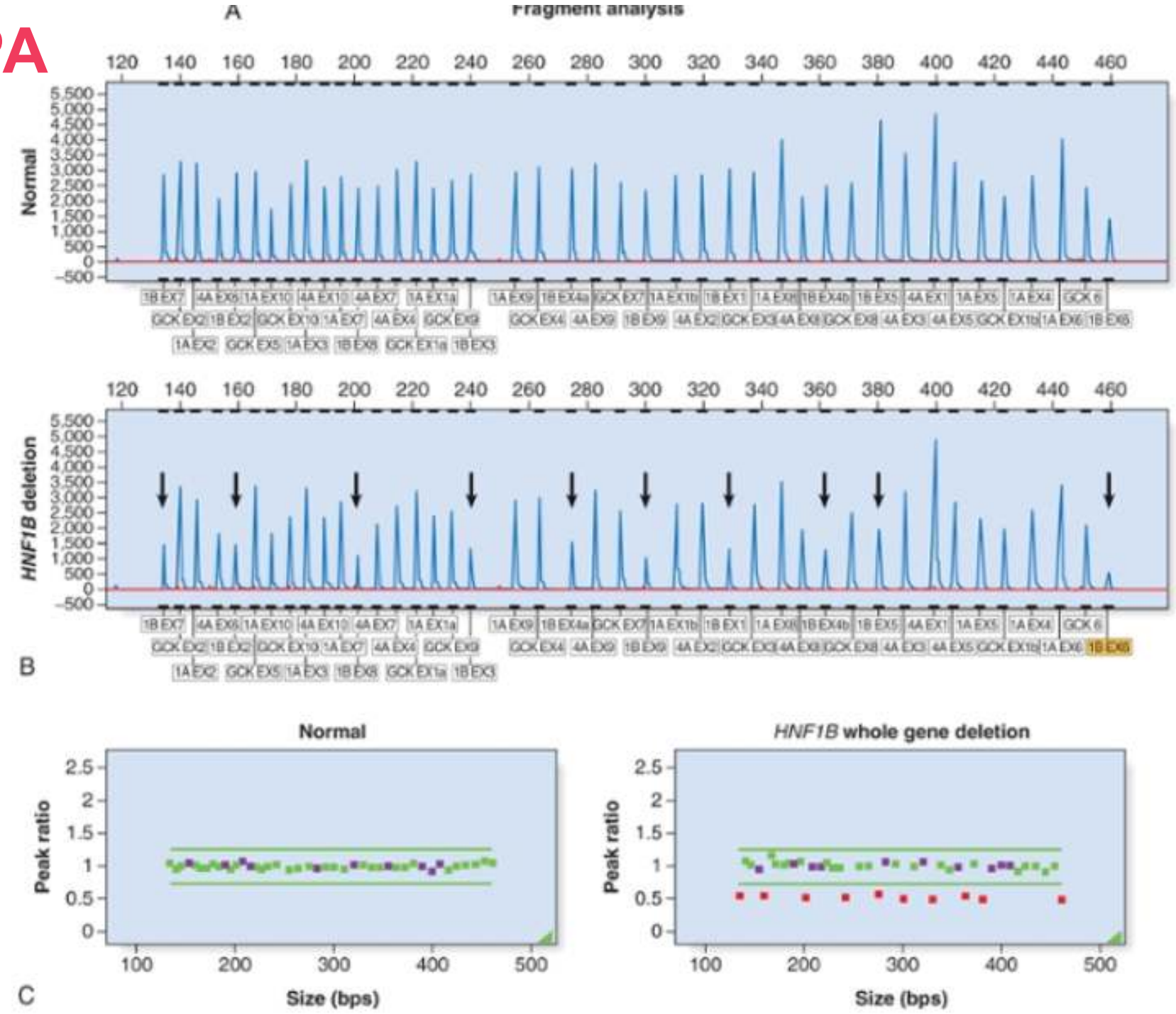
Multiplex ligation-dependent probe amplification - **MLPA**

Used for a detection of **deletions and duplications** on a specific location of chromosome (usually for cancer mutations, e.g. HER2)

https://www.youtube.com/watch?v=gfLJxKuqleY

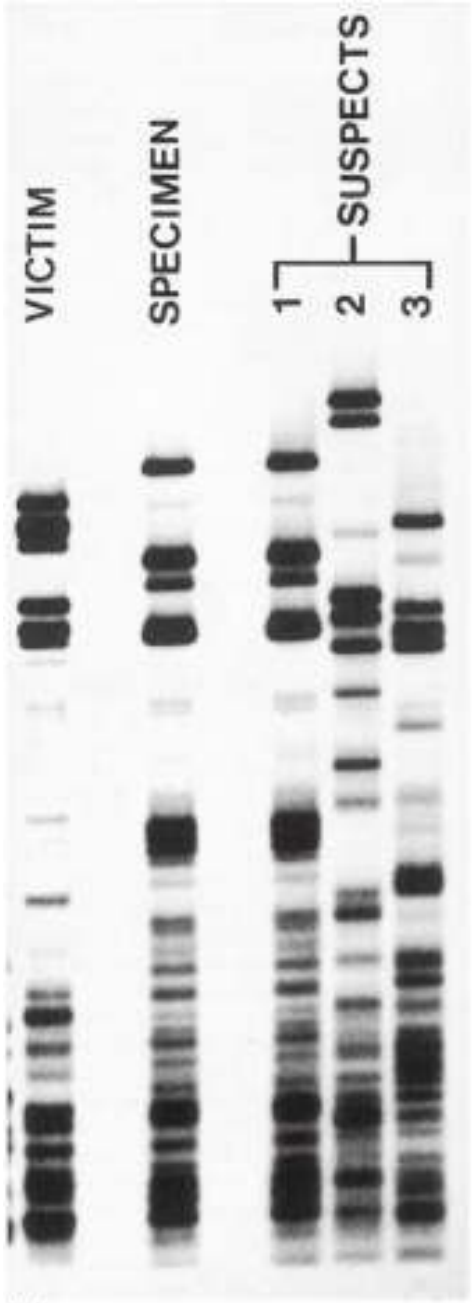


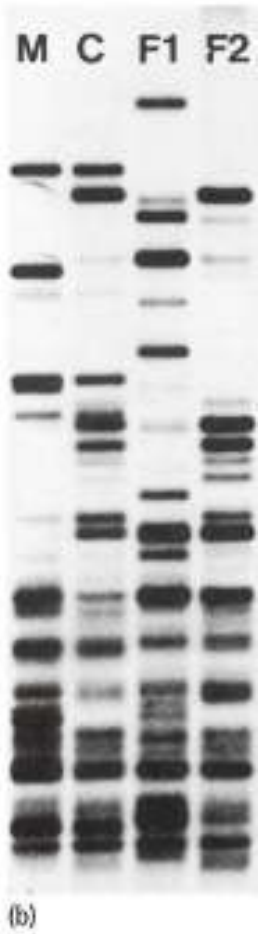
MLPA


A
Fragment analysis
Normal
HNF1B deletion
B
C
Normal
HNF1B whole gene deletion
Peak ratio
Size (bps)

DNA "fingerprinting"
analysis of minisatellite repeats

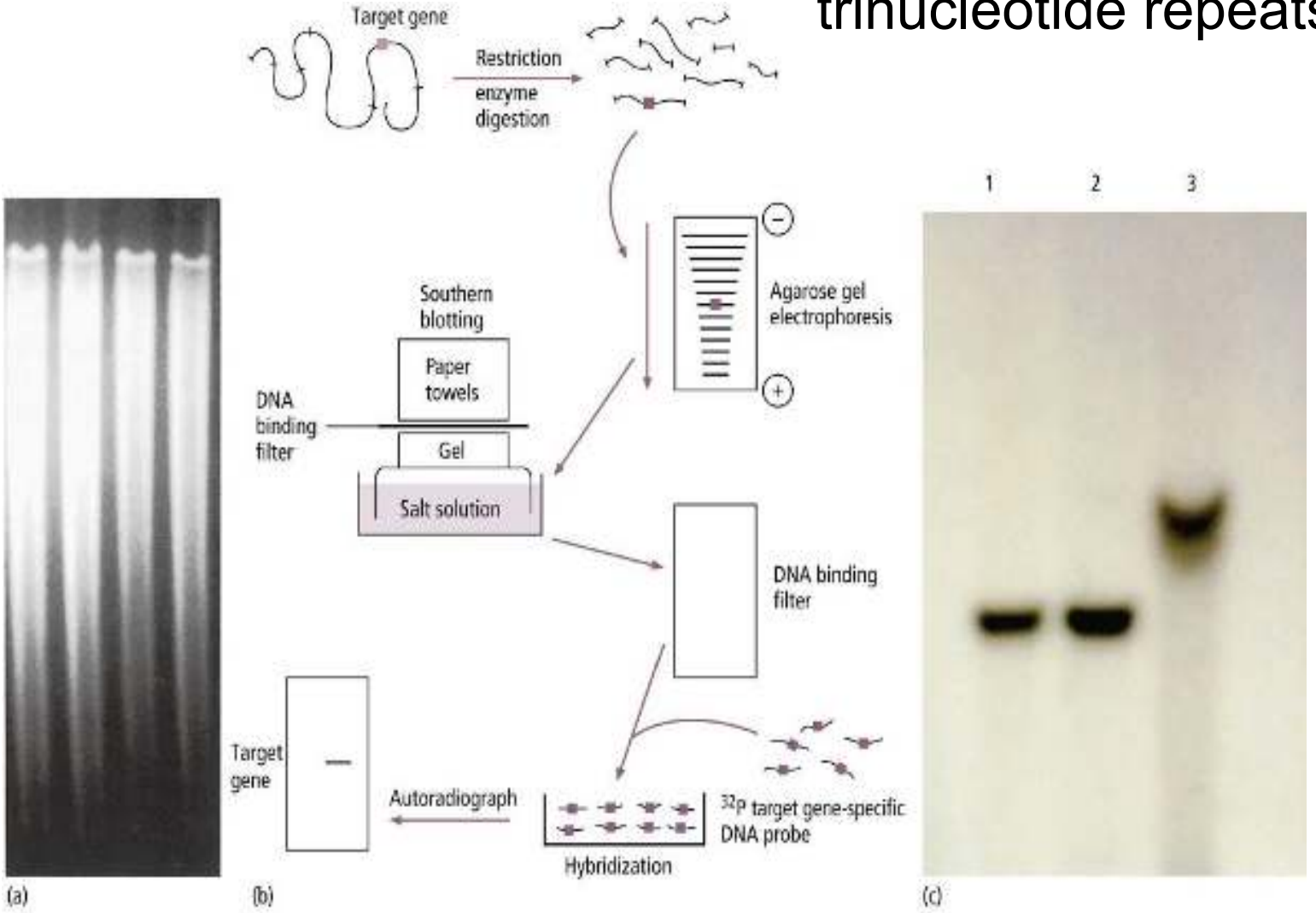
- Minisatellite: variable number of tandem repeats of 10-60 bp, repeated 5-50 times, on thousands of locations in the human genome
- Microsatellite: short tandem repeats of 2-5 bp, repeated 5-50 times, on thousands of locations in the human genome



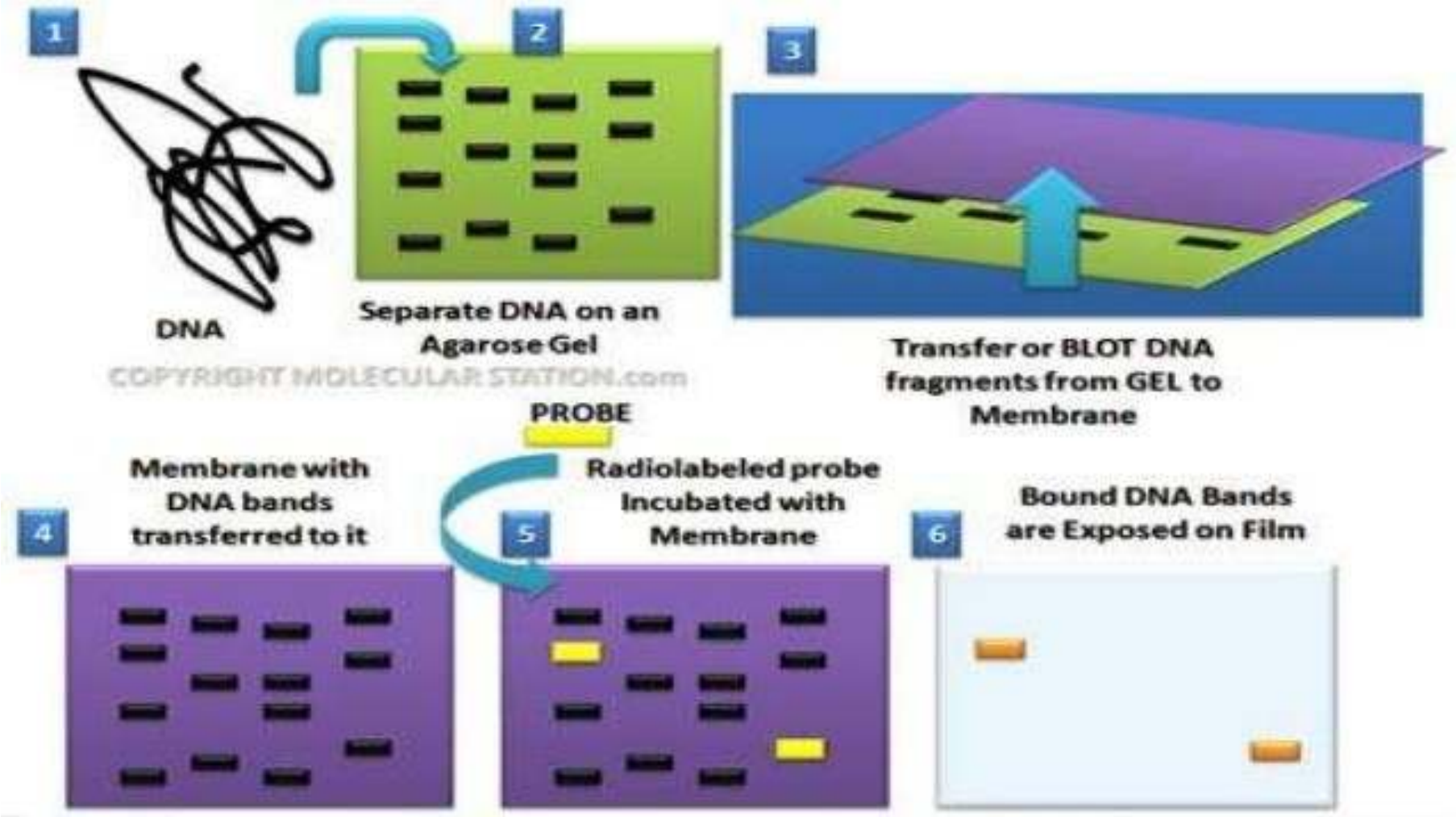


Southern blot

-THE method to detect trinucleotide repeats



Southern blotting

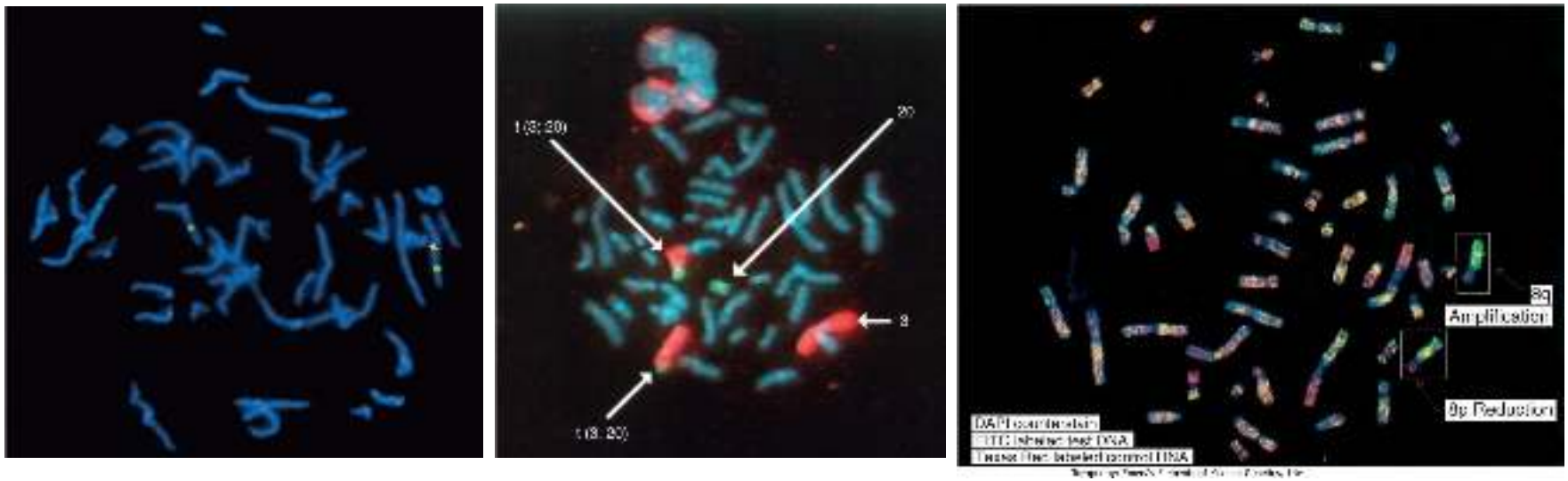


Fluorescente“In Situ” hybridization- **FISH**

Fluorescencently labelled oligonucleotides (“probes”) specific for a chromosomal region (centromere, telomere, microdeletion specific, translocation specific,…)

Hybridization of probes to chromosome during renaturation (cooling)

Comparative genomic hybridization – classical or microarray



https://www.youtube.com/watch?v=b81DcJC1jAs

Array CGH – comparative genomic hybridization (Microarray analysis)

A great number of oligonucleotides (probes) imobilised on a microchip as tiny dots (up to several thousands), in predesigned order

Tested DNA (or cDNA) is coloured green, and a control DNA is coloured red (egz: tumor vs. healthy control)

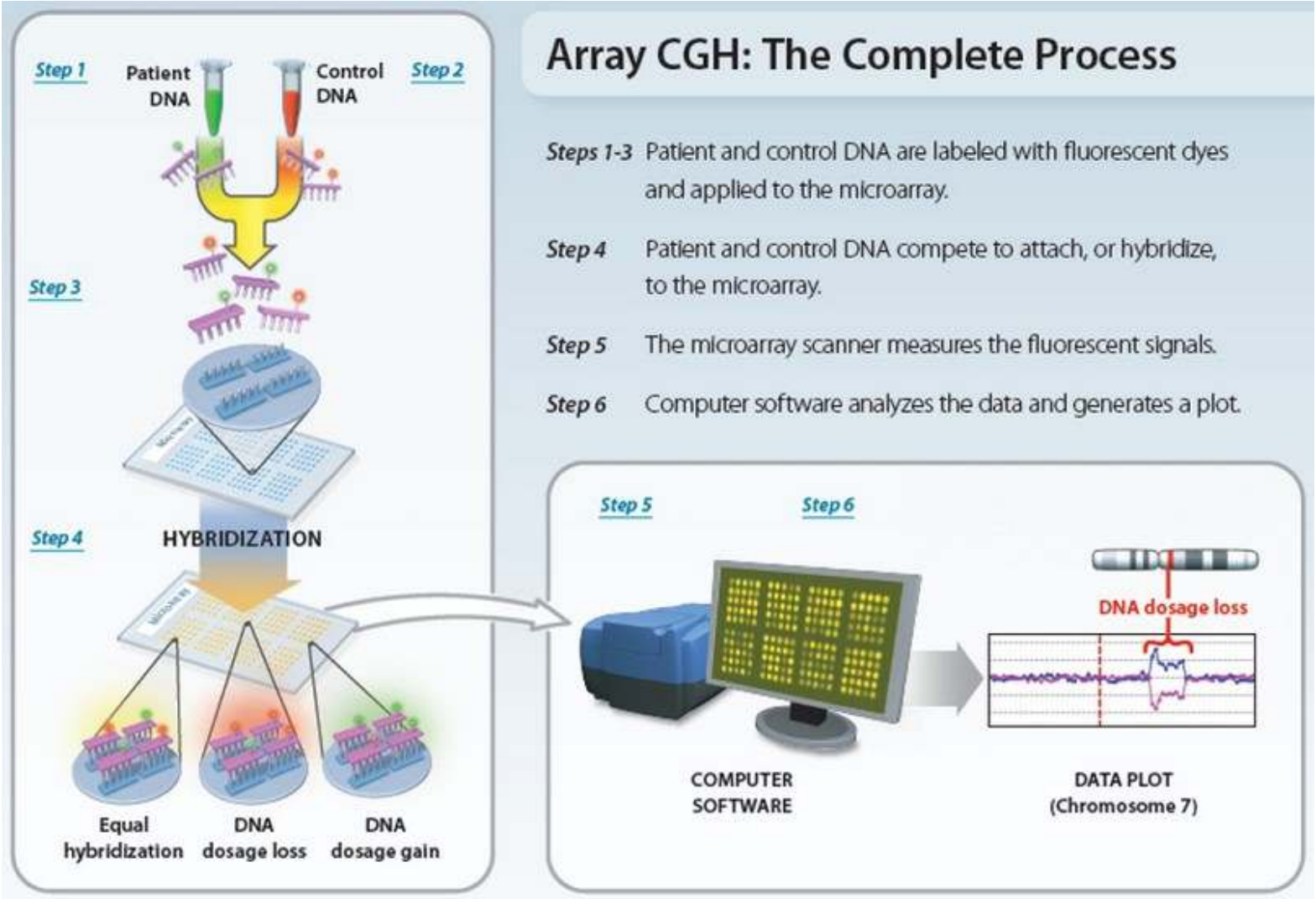
After mixing, DNA is applied on a chip and hybridized with probes

Detection of colour and intensity is used for DNA genotyping (copy number variation, mutations) or detection of difference in RNA expression (by software analysis)

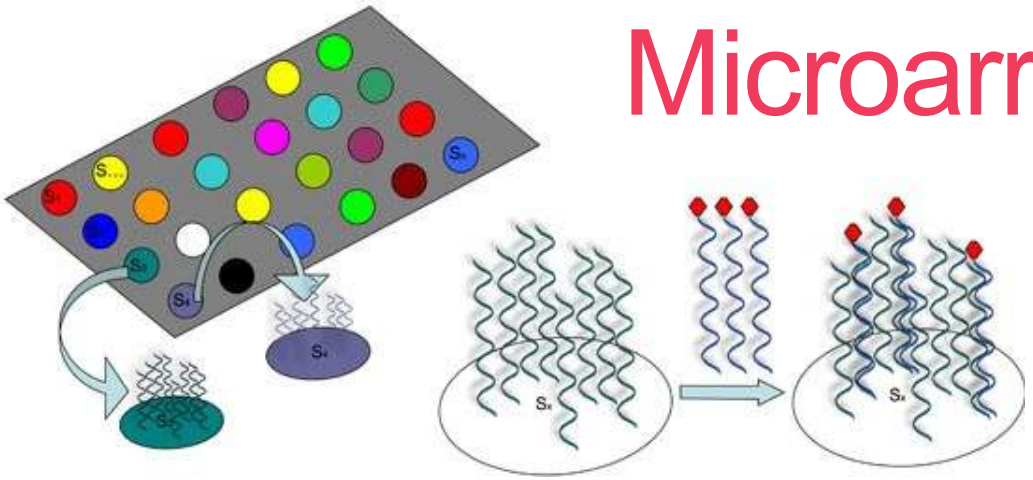
Copy number change at the level of 5-10 kb

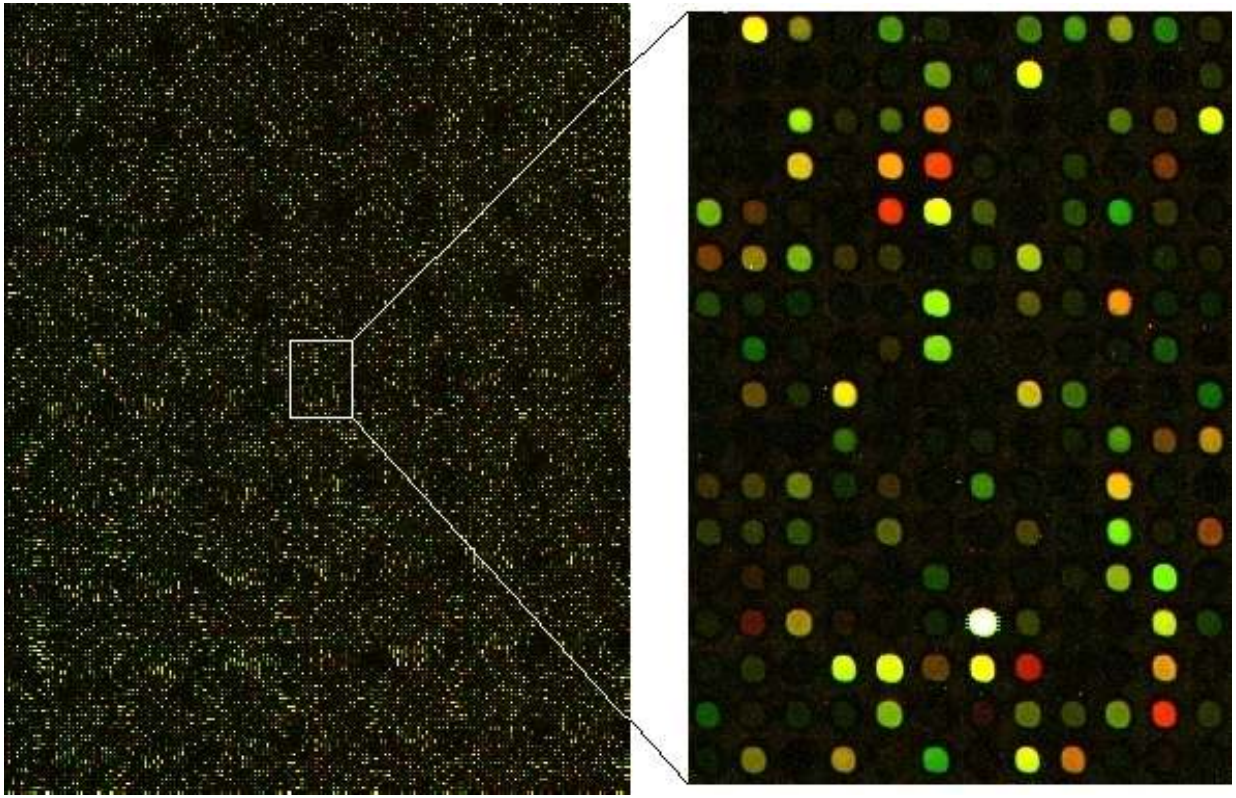
Cannot detect balanced translocations and inversions

Array CGH (Microarray analysis)

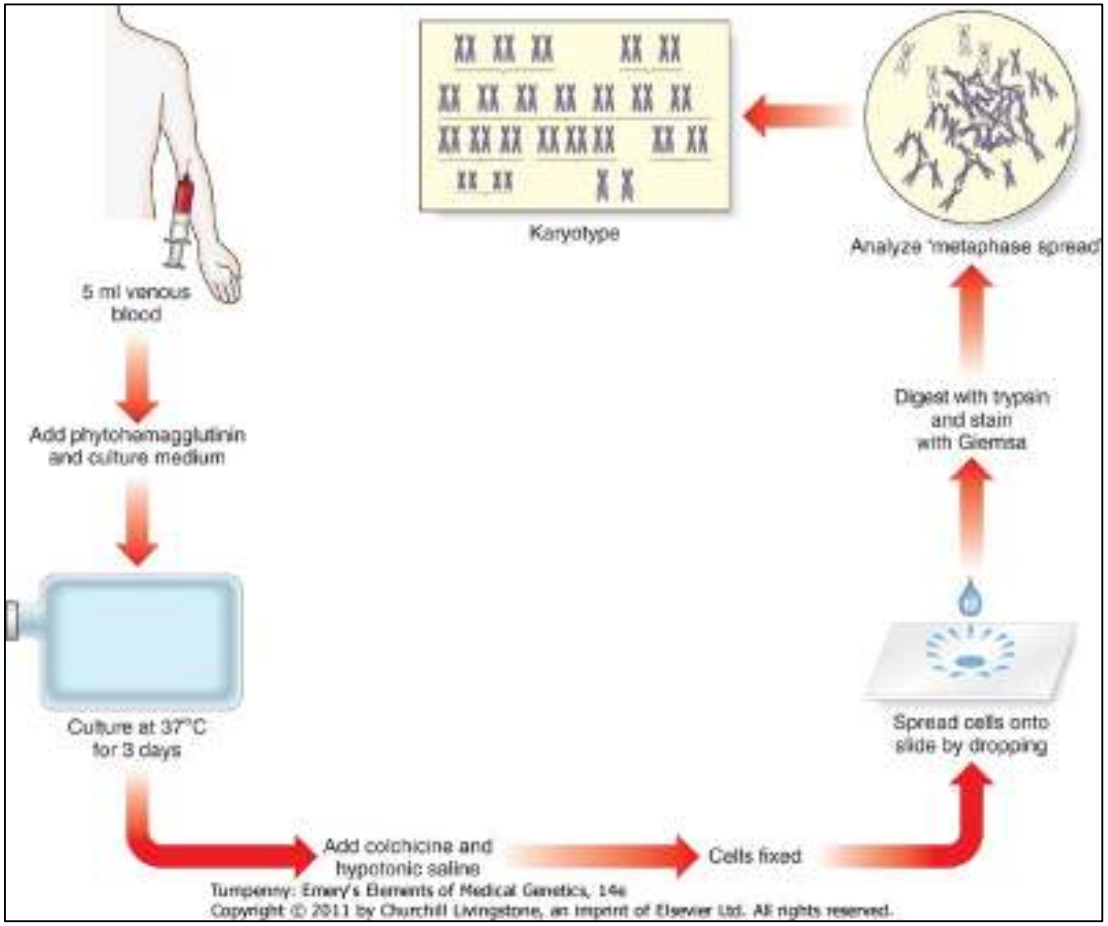


Microarray



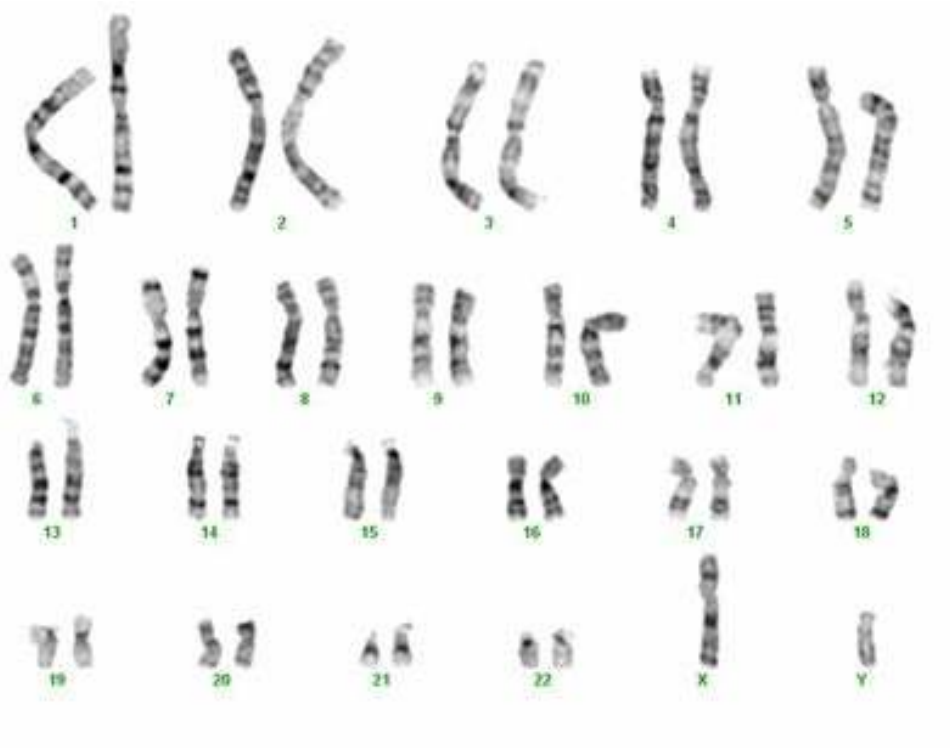


Karyogram (karyotype)



Any tissue with living nucleated cells that undergo division. i.e. lymphocytes - added to a nutrient medium with phytohemaglutinin, which stimulates cell division (3 days). Colchicine is then added, drug prevents formation of spindle, arresting cell division during metaphase (chromosomes are maximally condensed and most visible). Chromosomes are treated with trypsin, and then stained with a DNA binding dye- Giemsa.

Cytogenetics – karyotype analysis



400-500 bands per haploid set.
Each of these bands corresponds to app. 6000-8000kb
Analysis involves counting the number of chromosomes, followed by analysis of the banding pattern.
Ideal karyotype is known as an idiogram.

Cytogenetics – karyotype analysis

idiogram