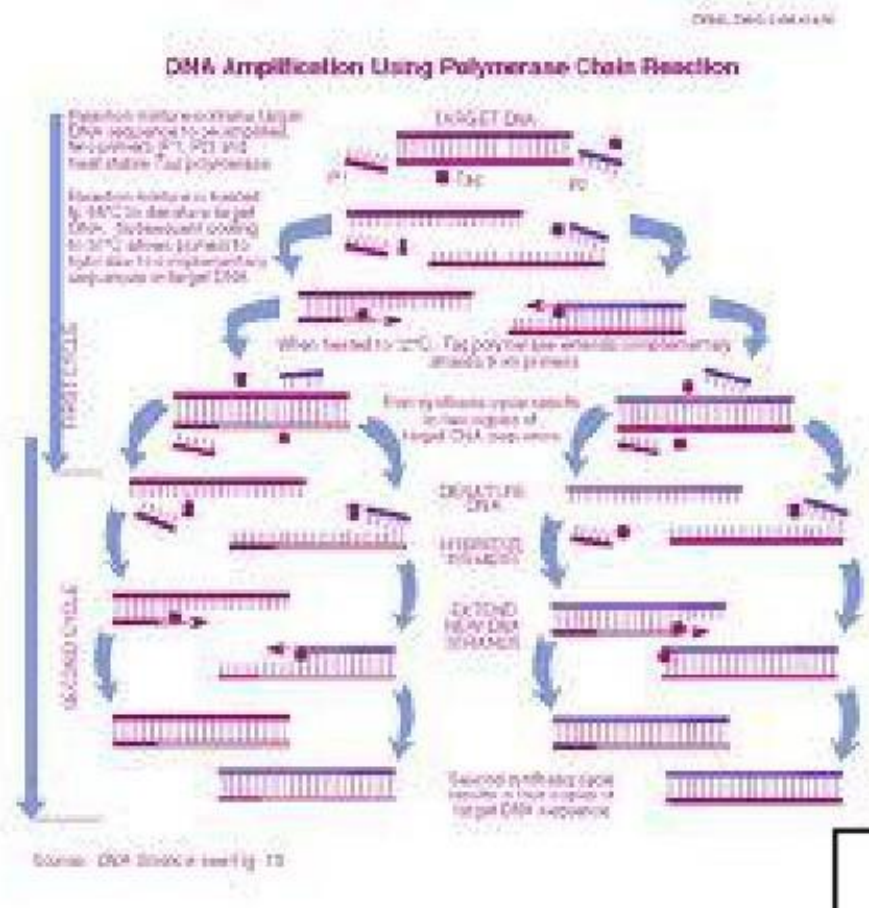
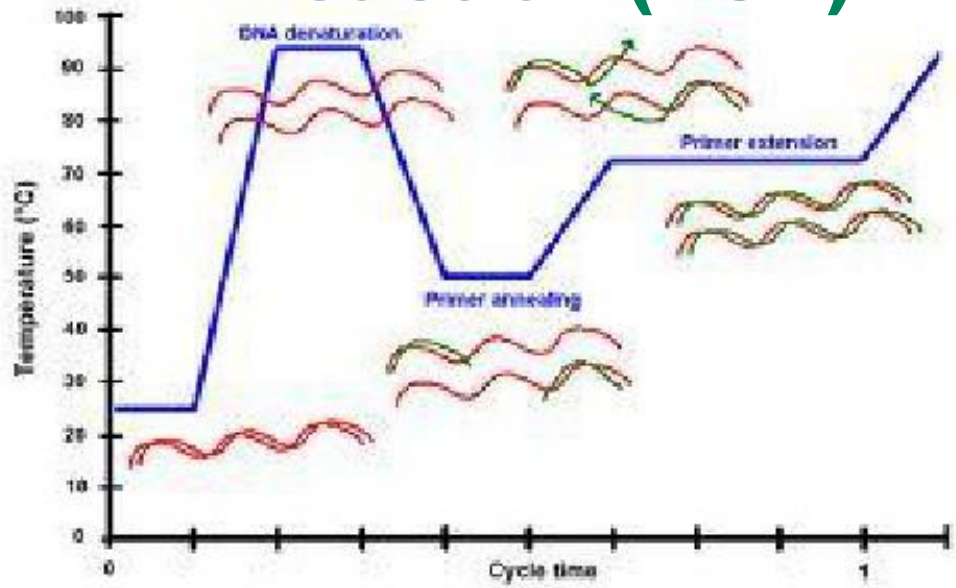
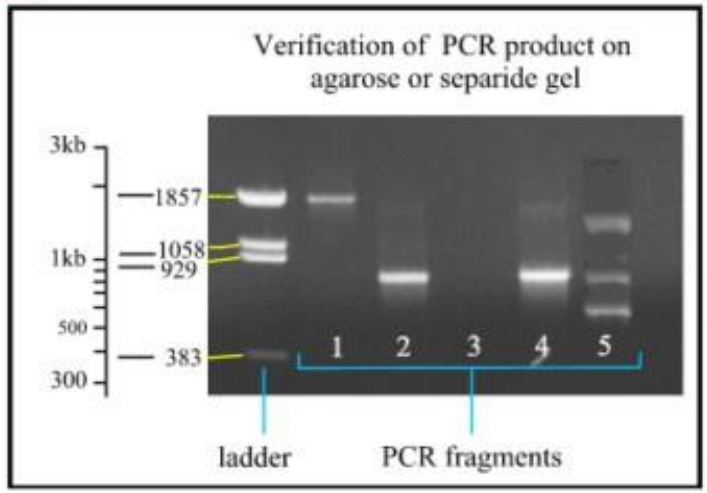
Polymerase Chain Reaction (PCR)







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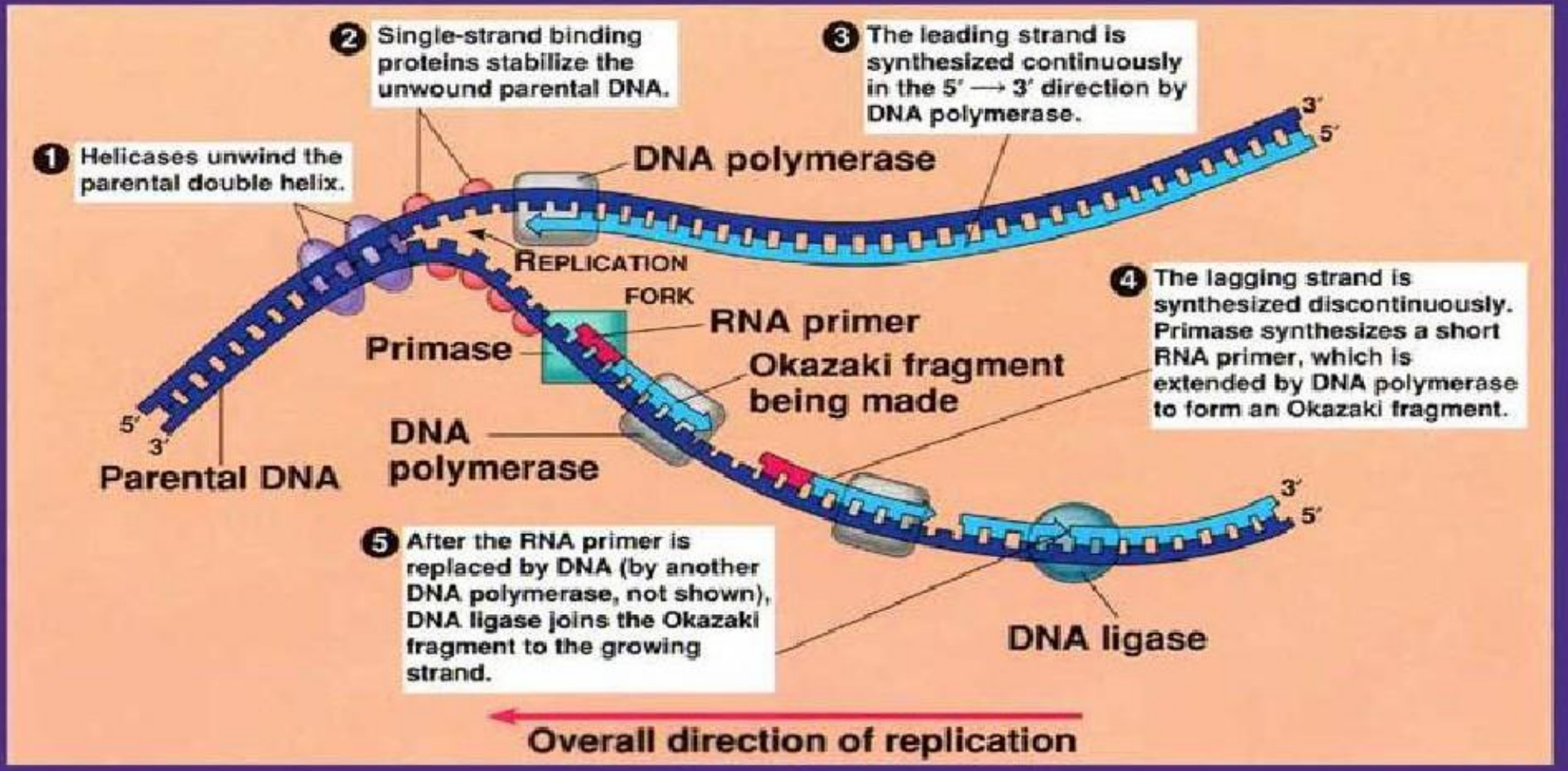
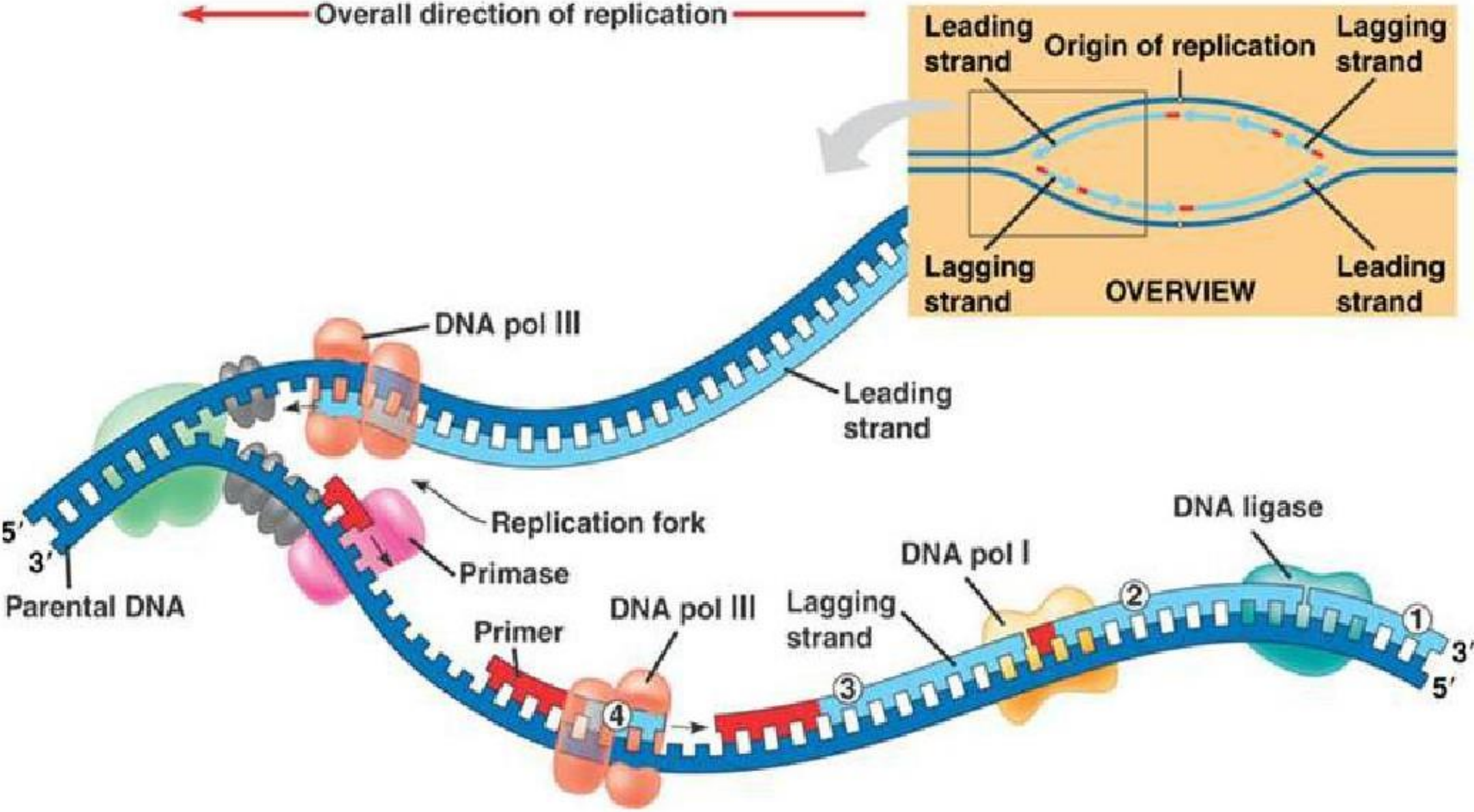
A SUMMARY OF DNA REPLICATION




Overall direction of replication
Leading strand
Origin of replication
Lagging strand
Lagging strand
OVERVIEW
Leading strand
DNA pol III
Leading strand
Replication fork
5′
3′
Parental DNA
Primase
Primer
DNA pol III
DNA pol I
DNA ligase
Lagging strand
1
2
3
4
3′
5′

What is PCR?

PCR is an exponentially progressing synthesis of the defined target DNA sequences in vitro.

It was invented in 1983 by Dr. Kary Mullis, for which he received the Nobel Prize in Chemistry in 1993.

Why “Polymerase”?

It is called “polymerase” because the only enzyme used in this reaction is DNA polymerase.

Why “Chain”?

It is called “chain” because the products of the first reaction become substrates of the following one, and so on.

“Reaction” Components

1) Target DNA - **contains the sequence to be amplified.**

2) Pair of Primers - **oligonucleotides that define the sequence to be amplified.**

3) dNTPs - **deoxynucleotidetriphosphates: DNA building blocks.**

4) Thermostable DNA Polymerase - **enzyme that catalyzes the reaction**

5) Mg^{++} ions - **cofactor of the enzyme**

6) Buffer solution - **maintains pH and ionic strength of the reaction solution suitable for the activity of the enzyme**

Comparative benefits of the PCR

1 **Sensitivity:** PCR can be used to amplify so tiny amount of the DNA that forms one genome.

2 **Rate:** very fast procedure (2 - 72 h).

3 **Safety:** no radioactivity used

4 **Molecular product:** the product is appropriate for further molecular analyses.

5 **Differentiation potential:** the procedure can be used for analysis of heavily damaged DNA

Reaction conditions and components I

- **Template** to be tested or amplified (DNA), 100 - 35 000 bp
- **Primers** (pair of synthetic oligonucleotides complementary to **5´and 3´ ends** of amplified template, the most important constituents, a vast molar excess of primers)
- **Deoxyribonucleotide triphosphates** (dATP, dGTP, dCTP a dTTP) (construction stones)
- **DNA-polymerase (Taq-polymerase)**, produces daughter fragments based on complementarity of bases to template sequence (thermostable enzyme - Taq-poly)

Template

Different size of template

- **Location of each primer sequence**
- **Extension beyond the end point of the sequence complementary to the second primer - long templates - linear function**
- **From the third cycle - short templates - exponential function**

“Reaction” Components

Primers

- Short segments of DNA 20-30 bp long which “bracket” the desired DNA segment
- One primer is a complement “forward” primer to produce DNA strand from left to right while one is a “reverse” primer that is for right to left strand

dNTP's

- **Deoxyribonucleoside triphosphates**
  - **Nitrogen bases: adenine, thymine, cytosine, and guanine**
  - **These dNTP's attach to the exposed complementary bases of the original DNA**

Heat stable DNA polymerase

- **Most commonly use *Taq* polymerase -**

  Thermus aquaticus **(a bacteria found around hot springs)**

  Is an enzyme that helps form new bonds between the nucleotides in new strands of bacteria

Reaction conditions and components II

- Easy thermal denaturation of the template
- Presence of divalent cations Mg^{2+} (activators of Taq-polymerase)
- Presence of solvents (they enable the enzyme stabilization, they raise their specificity and stabilize T_m

Steps of the Process

3 phases for each cycle (these vary slightly from one protocol to another)

- **Denaturing:** (94-95°C) DNA strands separate into single strands

- **Annealing:** (58°C) Primers anneal (attach) to the separated DNA strands

- **Extending:** (72°C) New complementary strands are made as the Taq enzyme helps to form bonds with the DNTP's

Steps of the Process

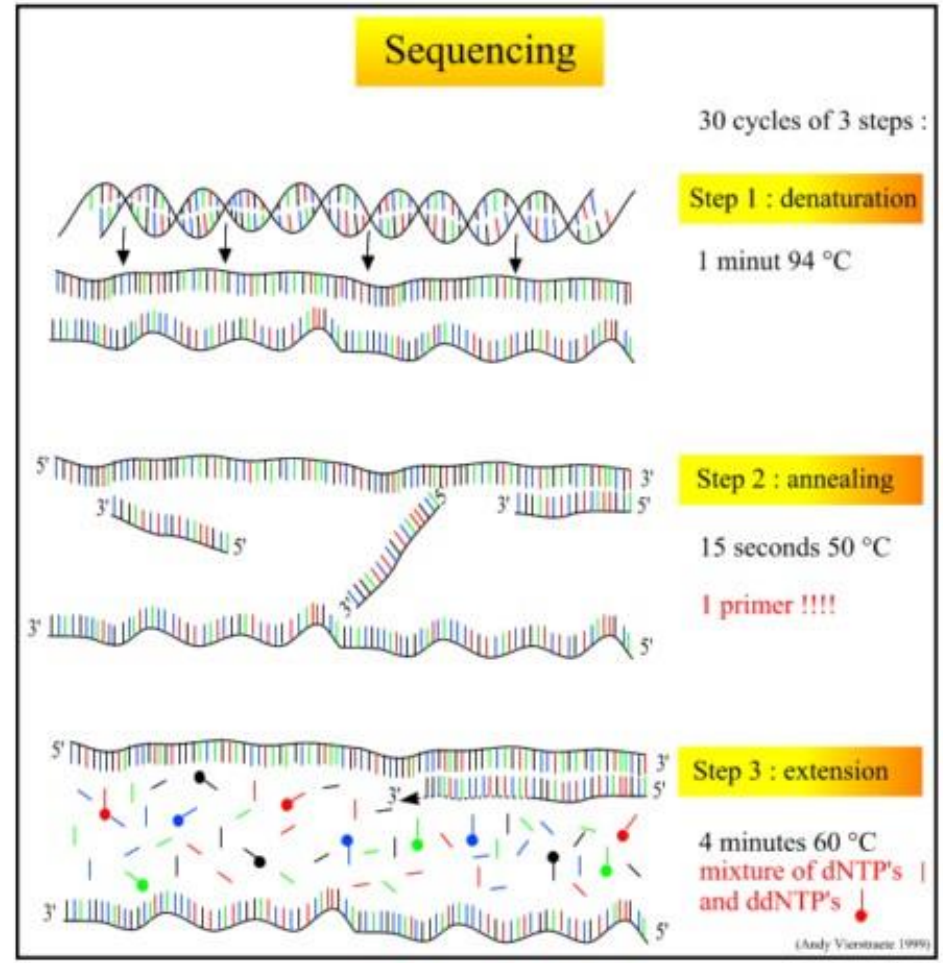
- Approximately 30 cycles of these 3 phases are used
- Each cycle produces twice as many targeted DNA segments as existed before
- After 30 cycles approximately 1 billion copies are produced
- Takes approximately 2-3 hours

1 cycle = 3 steps

Steps are thermally differentiated

- **1st step:** DNA denaturation - thermal denaturation of the template duplex: 92-96 °C
- **2nd step:** DNA renaturation, hybridization, annealing of primers to complementary sequences of template: 45-70 °C
- **3rd step:** DNA synthesis, primer extension) temperature is raised to 72 °C extension of daughter chain from hybridized primers using dNTP as a construction stones and the Taq-polymerase: 72 °C

Different steps in cycle



Thermal mode of the PCR and its timing

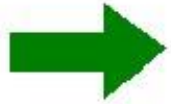
Thermal regimen of the PCR

(common protocol)

- Step 1 93-95 °C several minutes
- Step 2 94 °C e.g. 1 minute
- Step 3 T_a °C e.g. 1 minute
- Step 4 72 °C e.g. 1 minute

  29 times to step 2
- Step 5 72 °C e.g. 10 minutes
- Step 6 4 °C .....

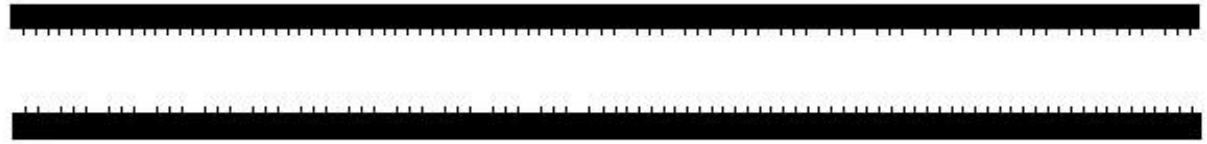
Let us start
The Reaction



PCR tube

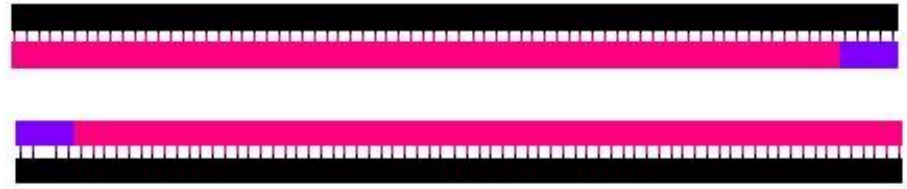
THERMOCYCLER

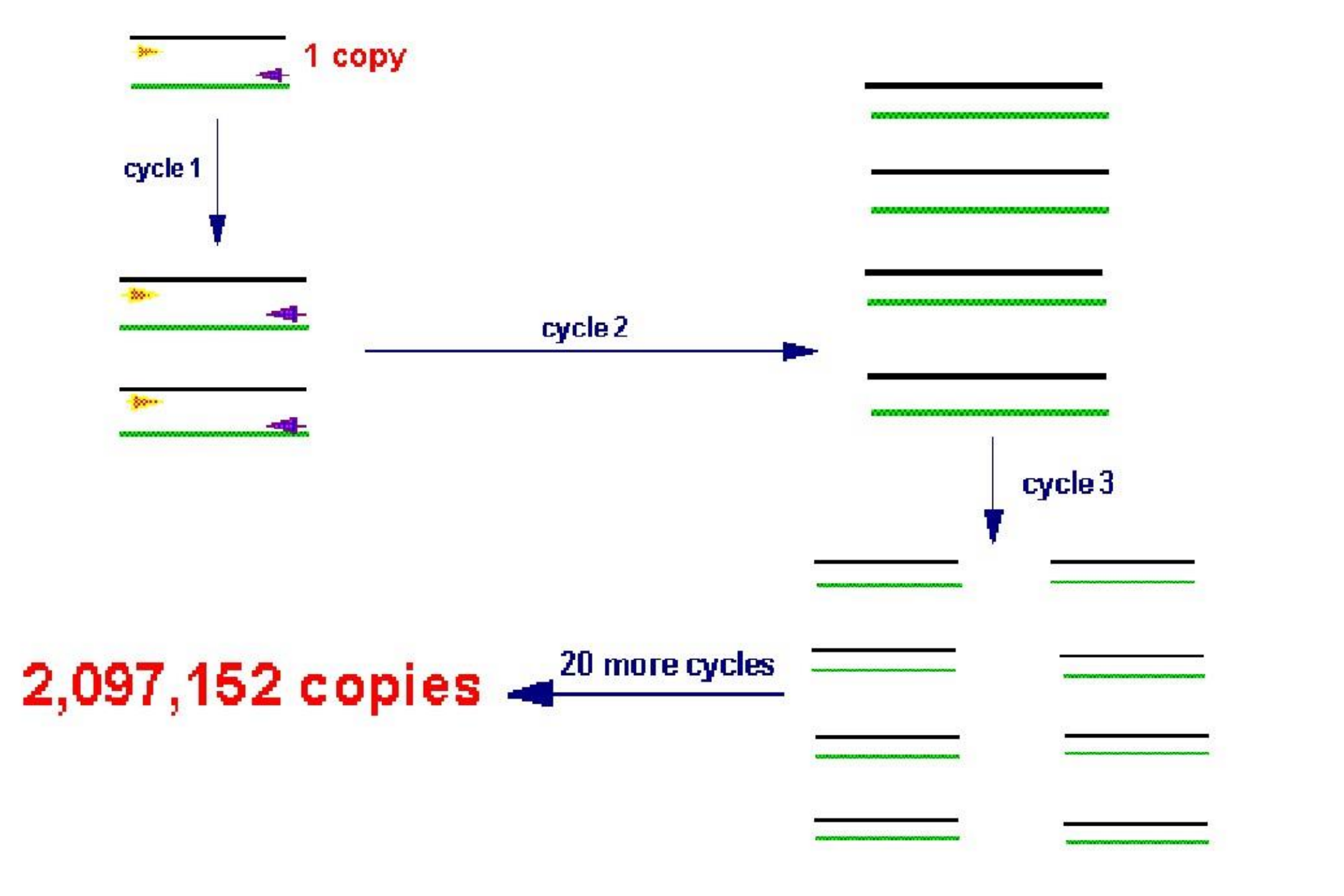
Denature (heat to 95oC)



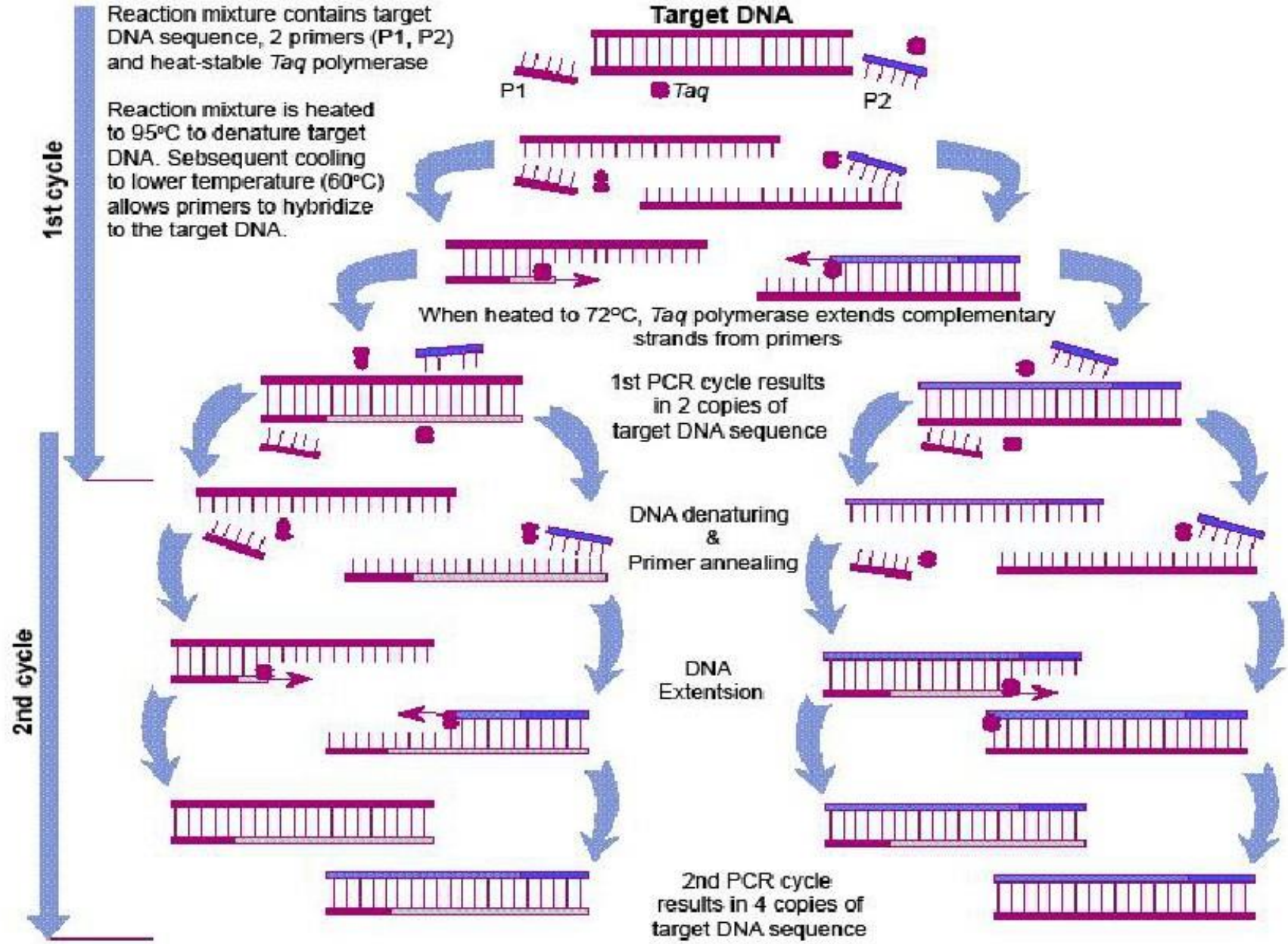
Lower temperature to 56oC
Anneal with primers

Increase temperature to 72oC
DNA polymerase + dNTPs



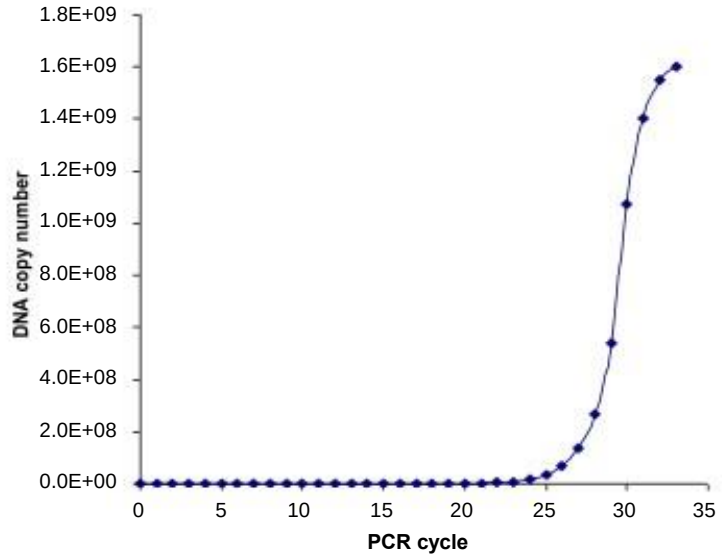

1 copy
cycle 1
cycle 2
cycle 3
20 more cycles
2,097,152 copies

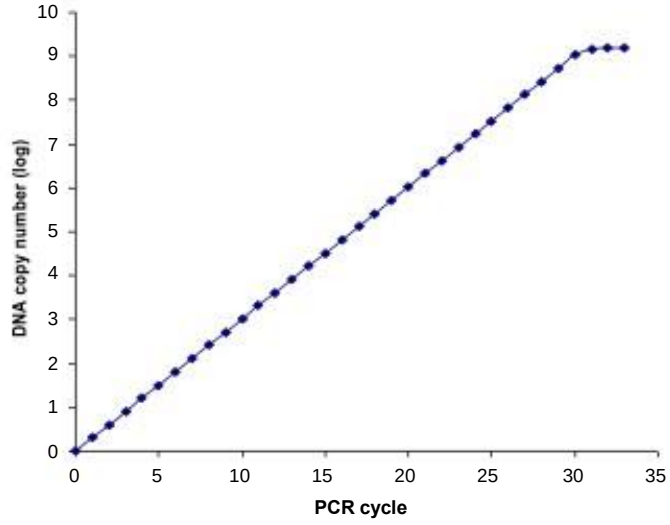
Polymerase Chain Reaction (PCR)



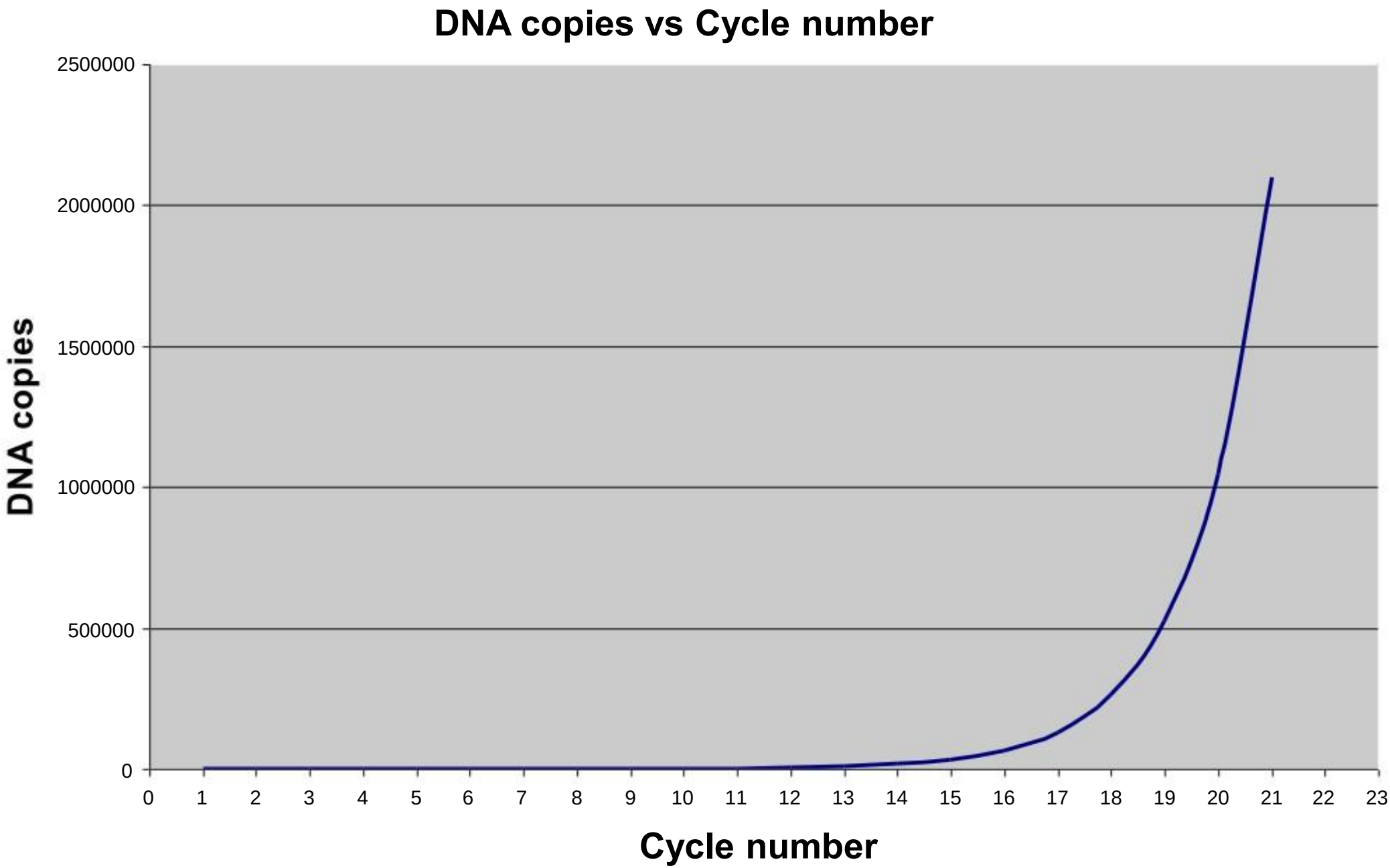
Copies of DNA=2^N

CYCLE NUMBER	DNA copy number
0	1
1	2
2	4
3	8
4	16
5	32
6	64
7	128
8	256
9	512
10	1,024
11	2,048
12	4,096
13	8,192
14	16,384
15	32,768
16	65,536
17	131,072
18	262,144
19	524,288
20	1,048,576
21	2,097,152
22	4,194,304
23	8,388,608
24	16,777,216
25	33,554,432
26	67,108,864
27	134,217,728
28	268,435,456
29	536,870,912
30	1,073,741,824

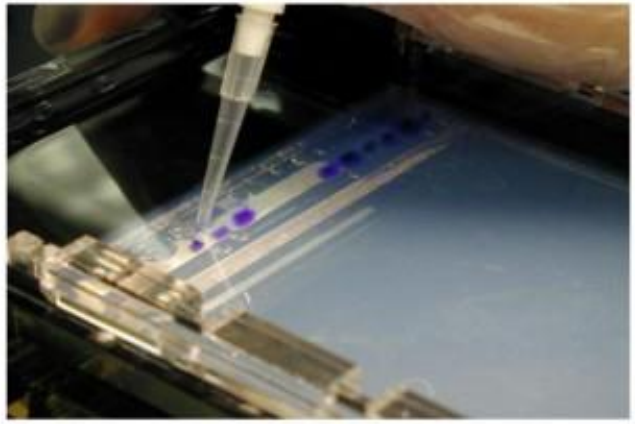


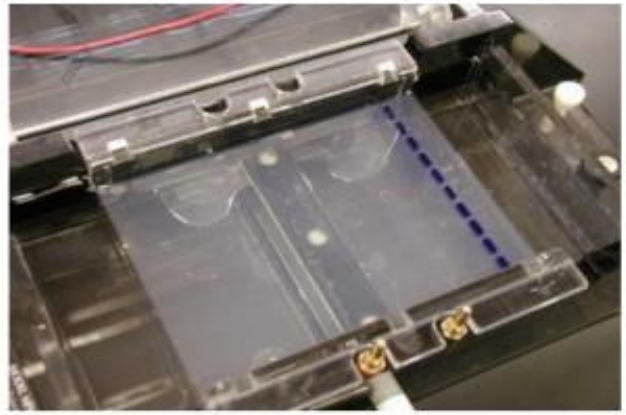


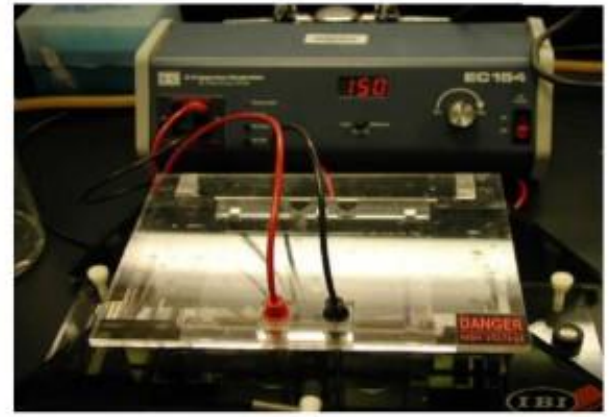
← **PCR reagent is the limiting factor!!**

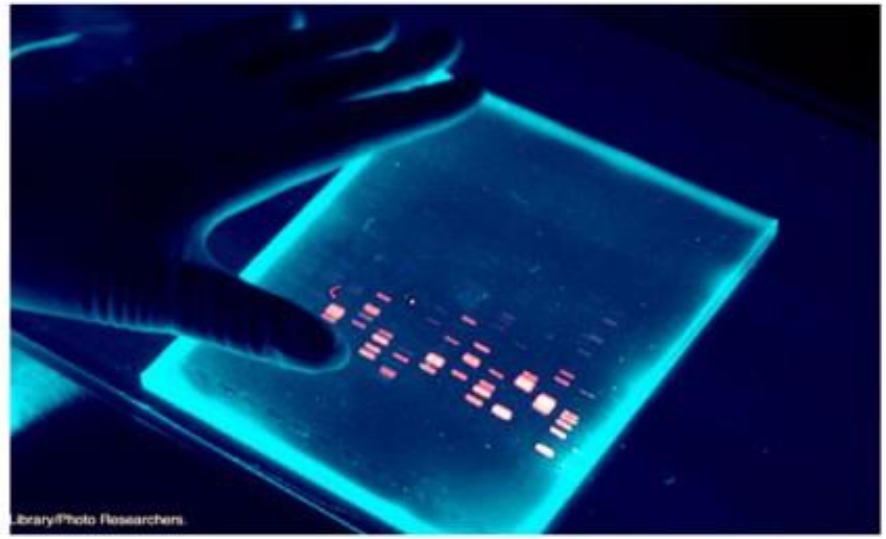

DNA copies vs Cycle number
DNA copies
2500000
2000000
1500000
1000000
500000
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Cycle number

Gel Electrophoresis

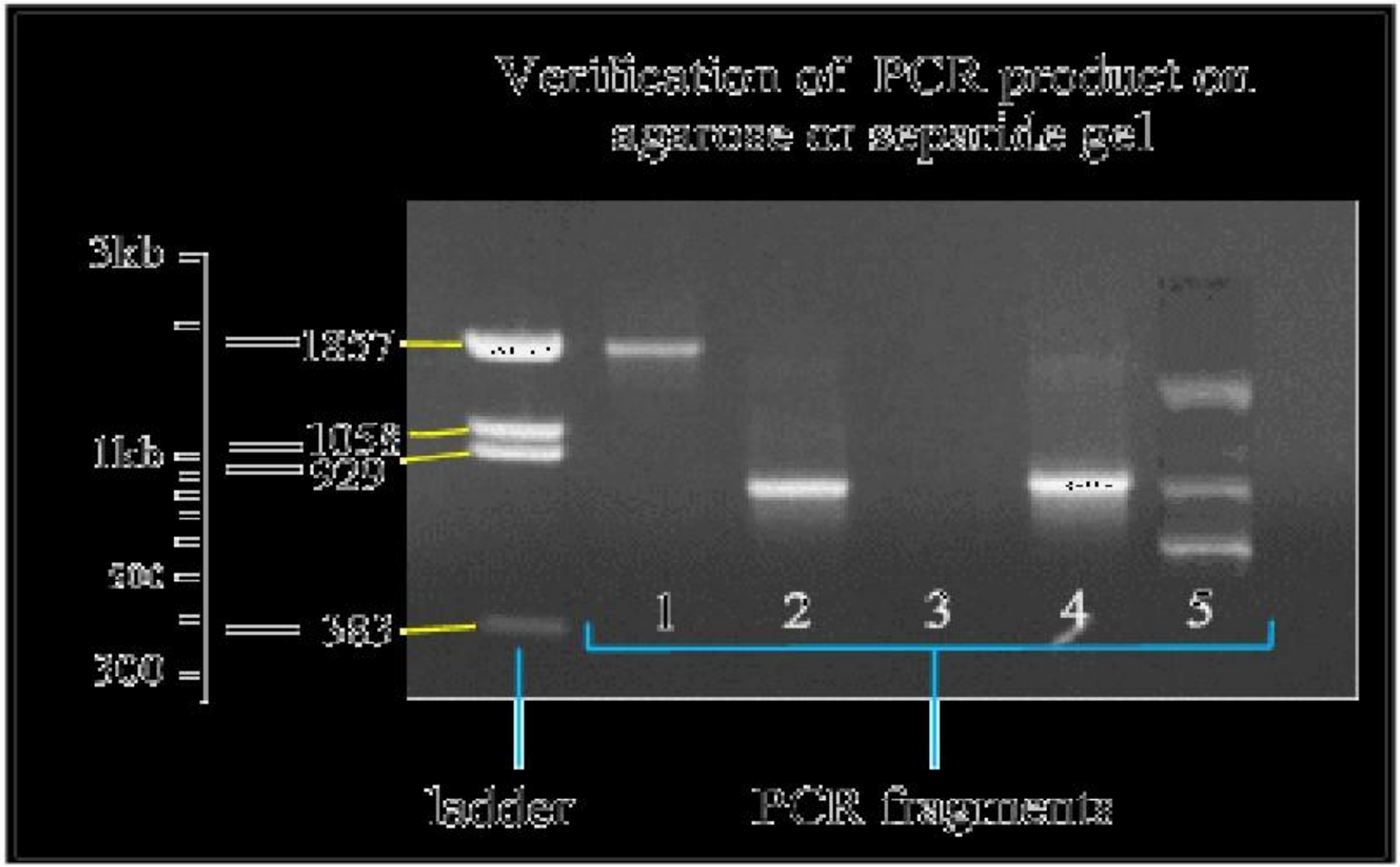








Verification of PCR product



Optimizing PCR protocols

While PCR is a very powerful technique, often enough it is not possible to achieve optimum results without optimizing the protocol

Critical PCR parameters:

- Concentration of DNA template, nucleotides, divalent cations (especially Mg^{2+}) and polymerase

-Error rate of the polymerase (Taq, Vent exo, Pfu)

- Primer design

Primer design

General notes on primer design in PCR

Perhaps the most critical parameter for successful PCR is the design of primers

Primer selection

Critical variables are:

1. - primer length
2. - melting temperature (T_m)
3. - specificity
4. - complementary primer sequences
5. - G/C content
6. - 3'-end sequence

Primer design

Primer length

i. - Specificity and the temperature of annealing are at least partly dependent on primer length

ii. - Oligonucleotides between 20 and 30bases are highly sequence specific

iii. - Primer length is proportional to annealing efficiency: in general, the longer the primer, the more inefficient the annealing

iv. - The primers should not be too short as specificity decreases

Primer design

Specificity

Primer specificity is at least partly dependent on primer length: there are many more unique 24 base oligos than there are 15 base pair oligos

Probability that a sequence of length n will occur randomly in a sequence of length m is:

$$P = (m - n + 1) \times (¼)^n$$

Example: the mtDNA genome has about 20,000 bases, the probability of randomly finding sequences of length n is:

n	P_n
5	19.52
10	1.91×10^{-2}
15	1.86×10^{-5}

Primer design

Complementary primer sequences

- Primers need to be designed with absolutely no intra-primer homology beyond 3 base pairs.

-If a primer has such a region of self-homology, "snap back" can occur

-Another related danger is inter-primer homology: partial homology in the middle regions of two primers can interfere with hybridization.

-If the homology should occur at the 3' end of either primer, primer dimer formation will occur

Primer design

G/C content

- ideally a primer should have a near random mix of nucleotides, a 50% GC content

- there should be no PolyG or PolyC stretches that can promote non-specific annealing

3’-end sequence

- the 3' terminal position in PCR primers is essential for the control of mis-priming

-inclusion of a G or C residue at the 3' end of primers helps to ensure correct binding (stronger hydrogen bonding of G/C residues)

Primer design

Melting temperature (T_m)

- the goal should be to design a primer with an annealing temperature of at least 50°C
- the relationship between annealing temperature and melting temperature is one of the "Black Boxes" of PCR
- a general rule-of-thumb is to use an annealing temperature that is 5°C lower than the melting temperature

Primer design

- The melting temperatures of oligos are most accurately calculated using nearest neighbor thermodynamic calculations with the formula:

$$T_m = H\,[S + R \ln (c/4)] - 273.15\ ^\circ C + 16.6 \log_{10} [K^+]$$

(H is the enthalpy, S is the entropy for helix formation, R is the molar gas constant and c is the concentration of primer)

A good working approximation of this value can be calculated using the Wallace formula:

$$T_m = 4x\ (\#C + \#G) + 2x\ (\#A + \#T)\ ^\circ C$$

- Both of the primers should be designed such that they have similar melting temperatures.
- If primers are mismatched in terms of Tm, amplification will be less efficient or may not work: the primer with the higher Tm will mis-prime at lower temperatures; the primer with the lower Tm may not work at higher temperatures.

Applications of PCR

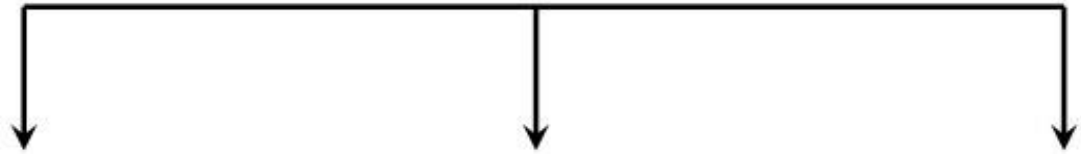
Basic Research

- Mutation screening
- Drug discovery
- Classification of organisms
- Genotyping
- Molecular Archaeology
- Molecular Epidemiology
- Molecular Ecology
- Bioinformatics
- Genomic cloning
- Site-directed mutagenesis
- Gene expression studies

Applied Research

- Genetic matching
- Detection of pathogens
- Pre-natal diagnosis
- DNA fingerprinting
- Gene therapy

Applications of PCR



Molecular Identification

- Molecular Archaeology
- Molecular Epidemiology
- Molecular Ecology
- DNA fingerprinting
- Classification of organisms
- Genotyping
- Pre-natal diagnosis
- Mutation screening
- Drug discovery
- Genetic matching
- Detection of pathogens

Sequencing

- Bioinformatics
- Genomic cloning
- Human Genome Project

Genetic Engineering

- Site-directed mutagenesis
- Gene expression studies

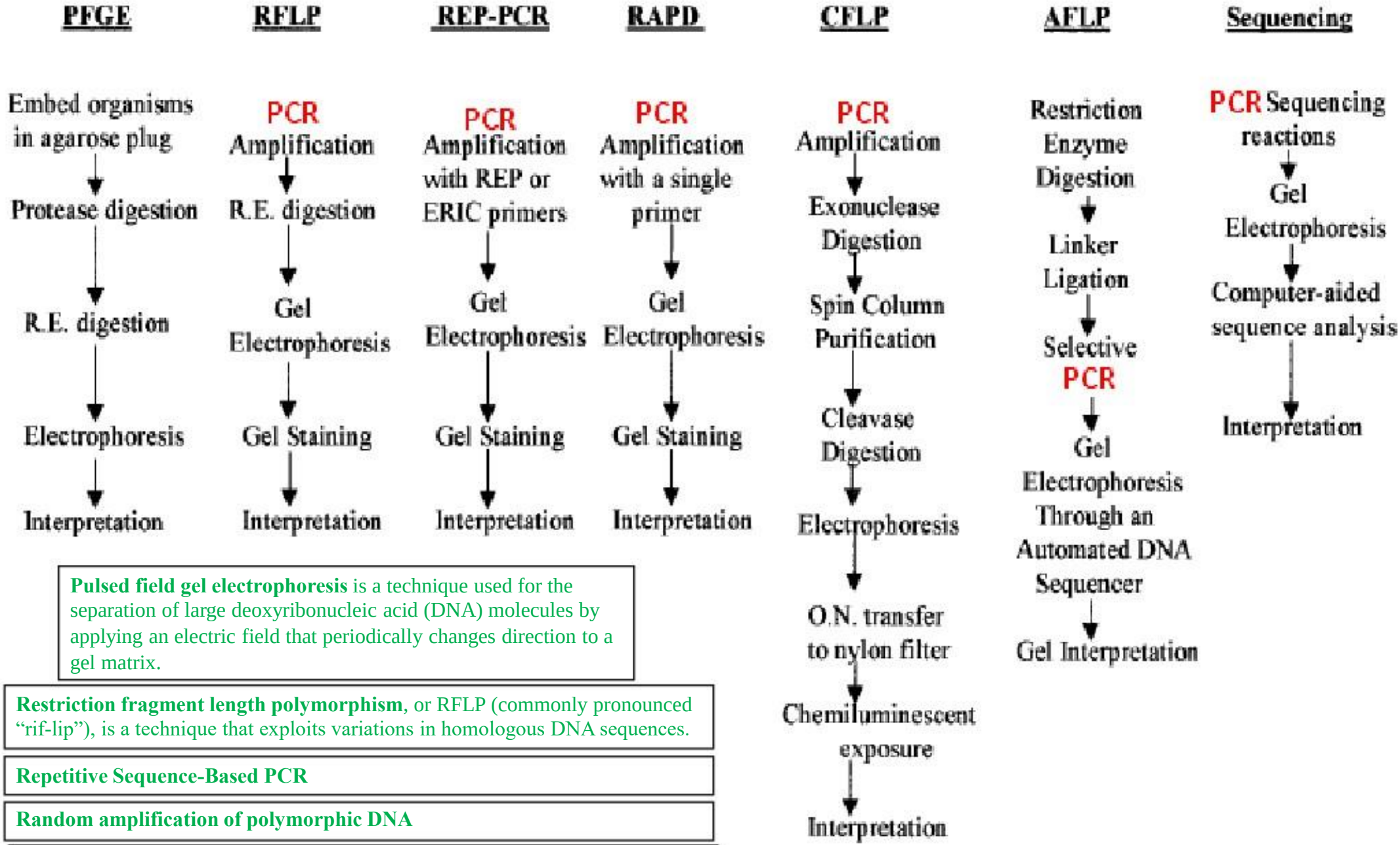
MOLECULAR IDENTIFICATION

DNA is unique for each single type of organism.

DNA can be used to identify an organism.

Organisms can be identified by using PCR.

PCR allows easy manipulation of DNA.



Pulsed field gel electrophoresis is a technique used for the separation of large deoxyribonucleic acid (DNA) molecules by applying an electric field that periodically changes direction to a gel matrix.

Restriction fragment length polymorphism, or RFLP (commonly pronounced "rif-lip"), is a technique that exploits variations in homologous DNA sequences.

Repetitive Sequence-Based PCR

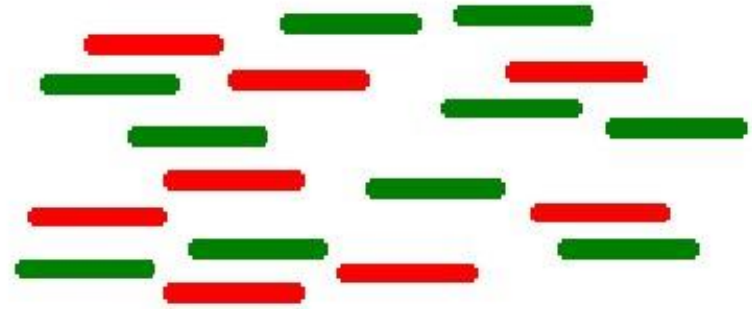
Random amplification of polymorphic DNA

Amplified fragment length polymorphism

Detection of Unknown Mutations

Whole Genomic DNA

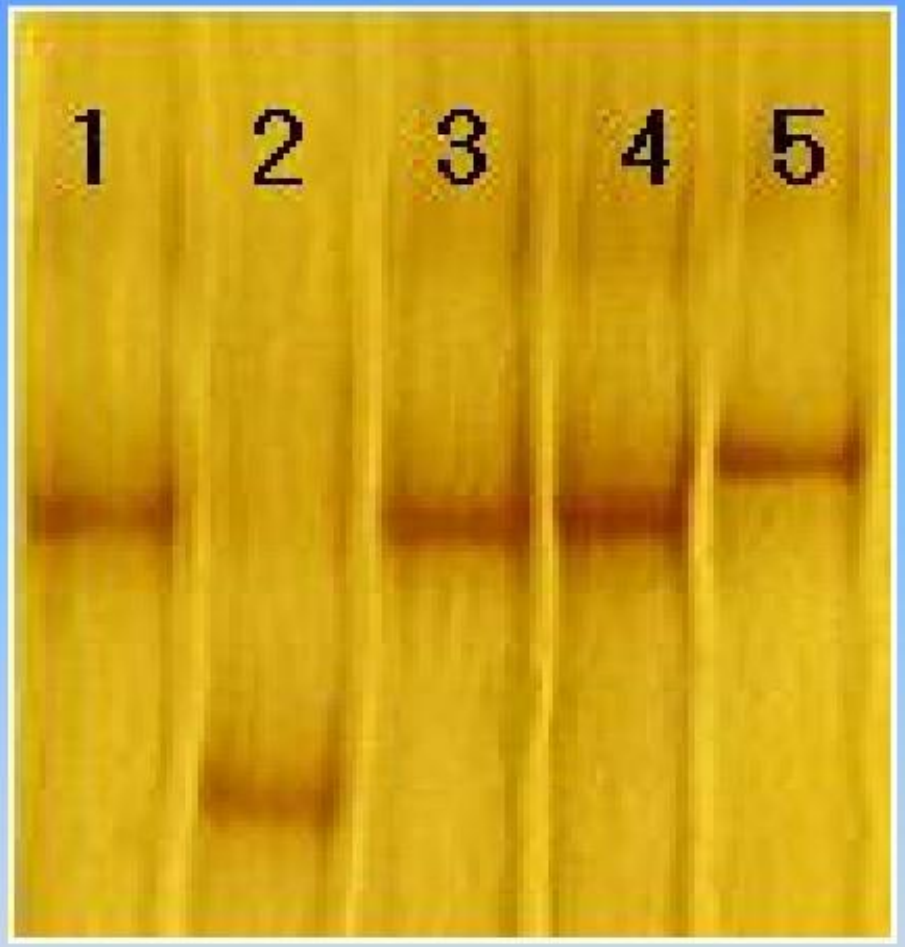
PCR

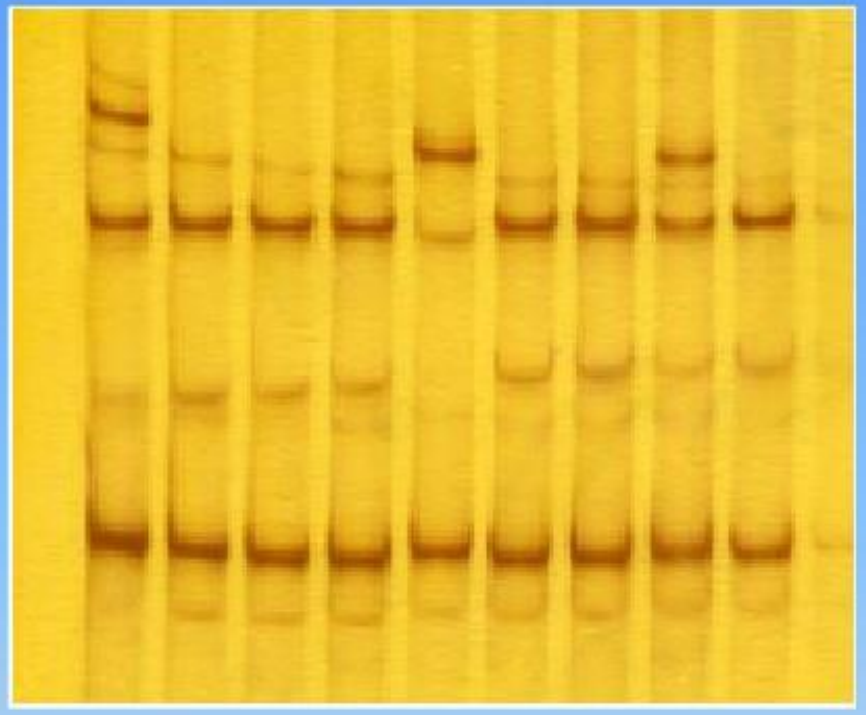


Desired DNA fragments that may contain a mutation in huge numbers.

SSCP

PCR-based Single-strand Conformation Polymorphism





SSCP (Single-Strand Conformational Polymorphism **) gels: "shifts" representing a mutation in the amplified DNA fragment**

Classification of Organisms

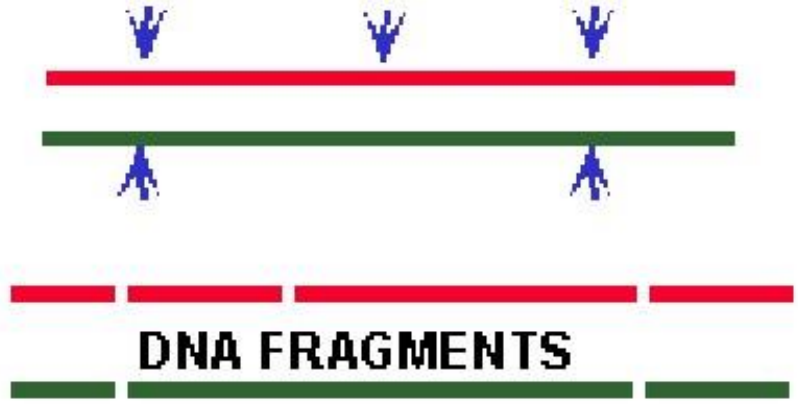
1) Relating to each other
2) Similarities
3) Differences

* Fossils
* Trace amounts
* Small organisms

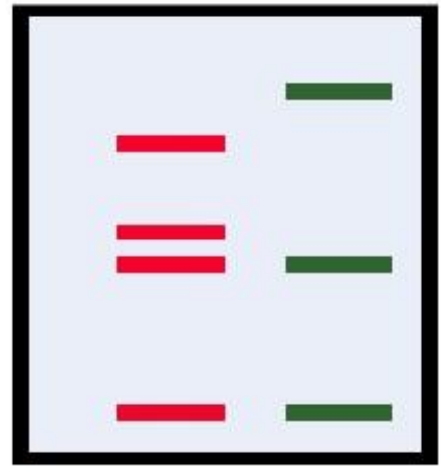
} Insufficient data

! DNA !

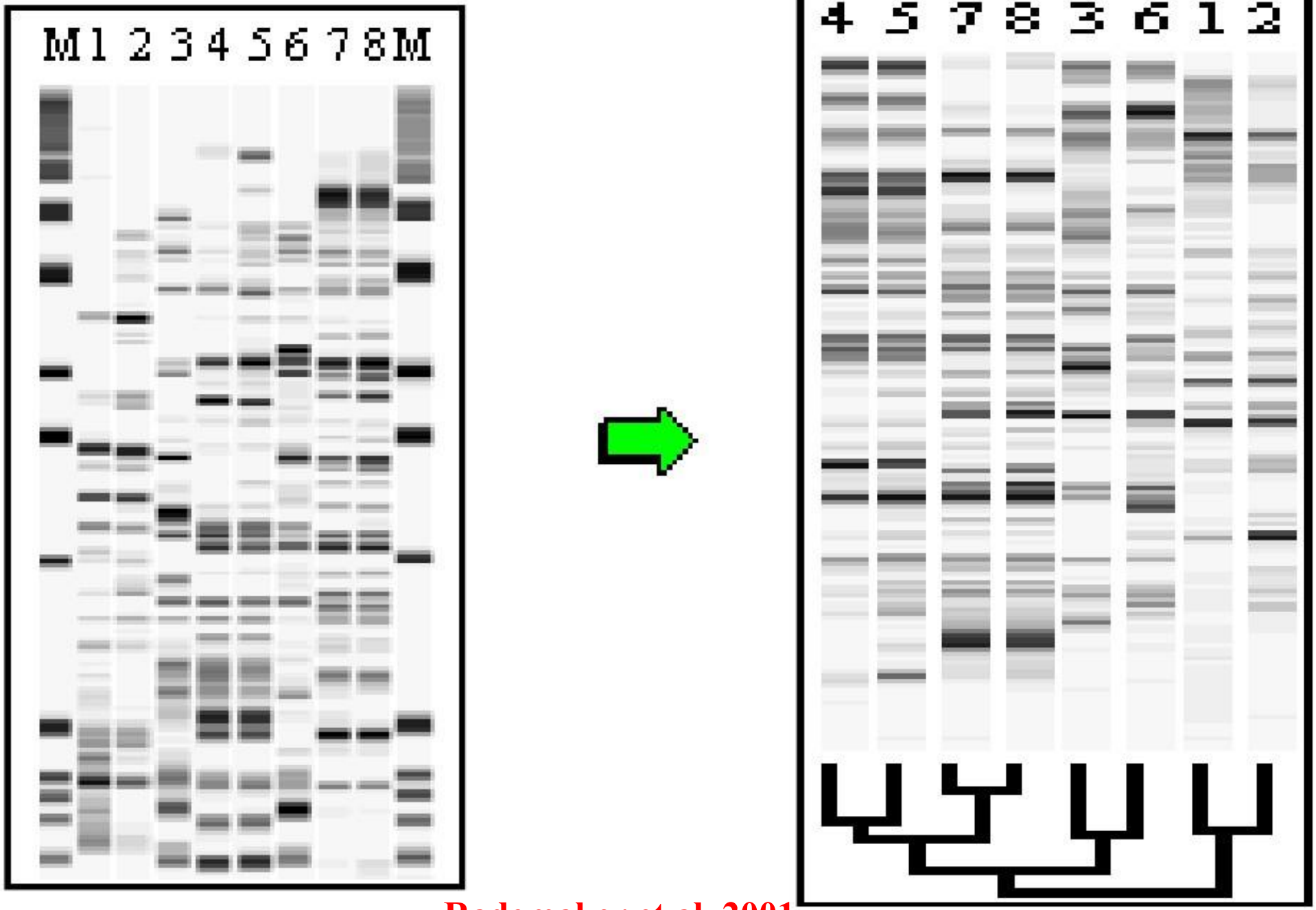
PCR



Specific PCR products are cut with restriction enzymes.



SEPARATE FRAGMENTS ON THE BASIS OF THEIR SIZE



Rademaker et al. 2001

Detection Of Pathogens

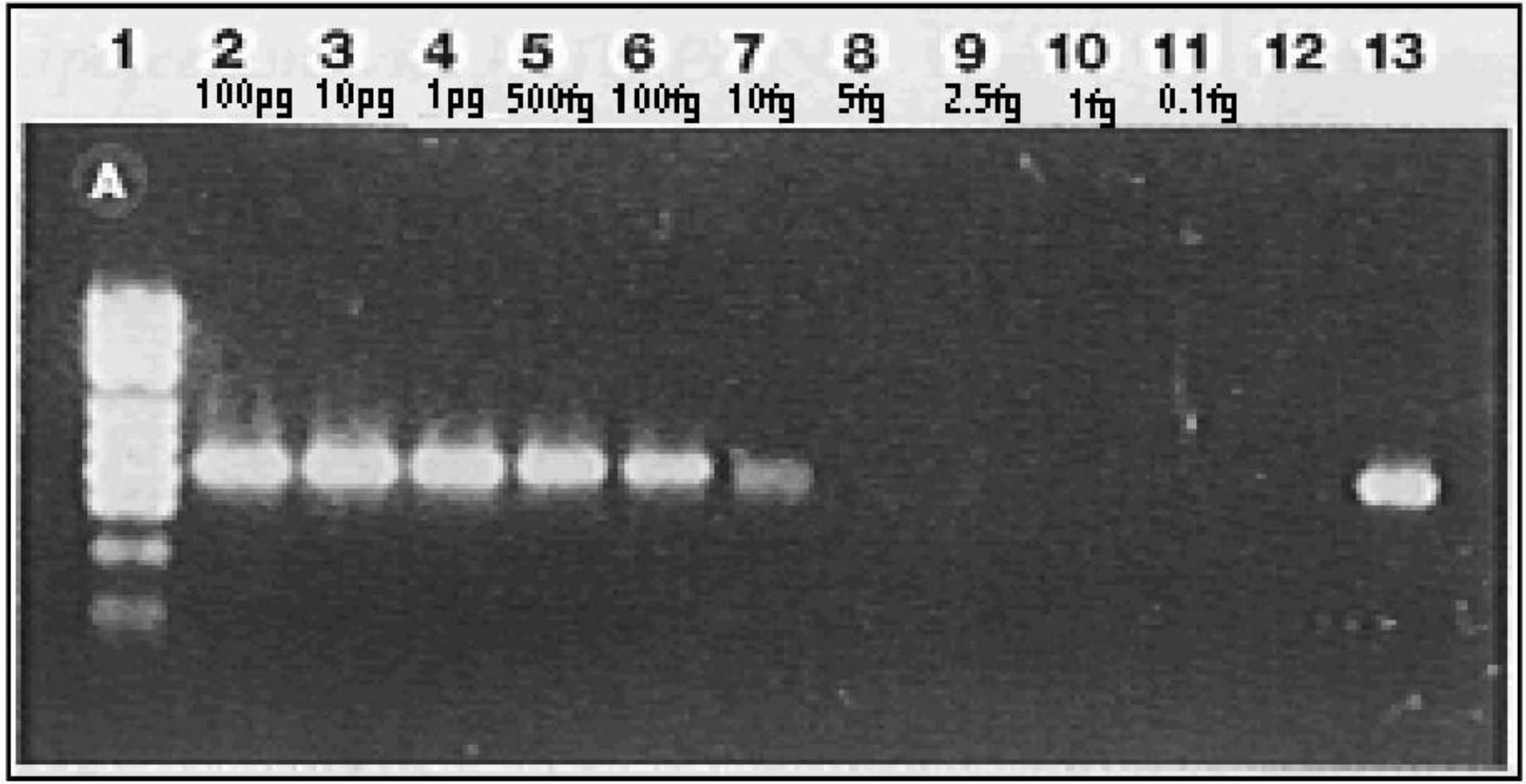
A sample that may contain pathogenic DNA.

PCR

YES PCR → YES Pathogen

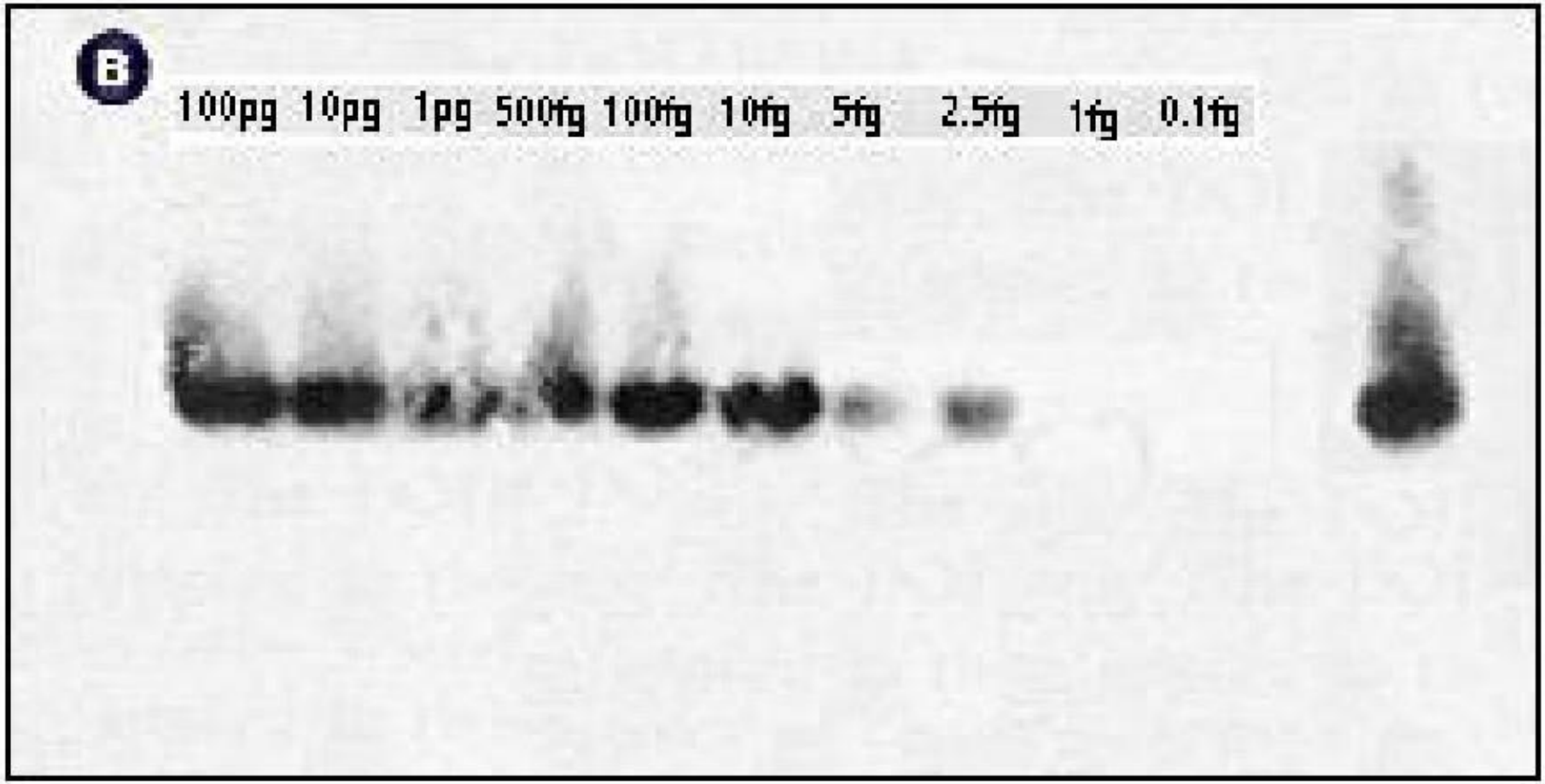
NO PCR → NO Pathogen

Detection Of Pathogens



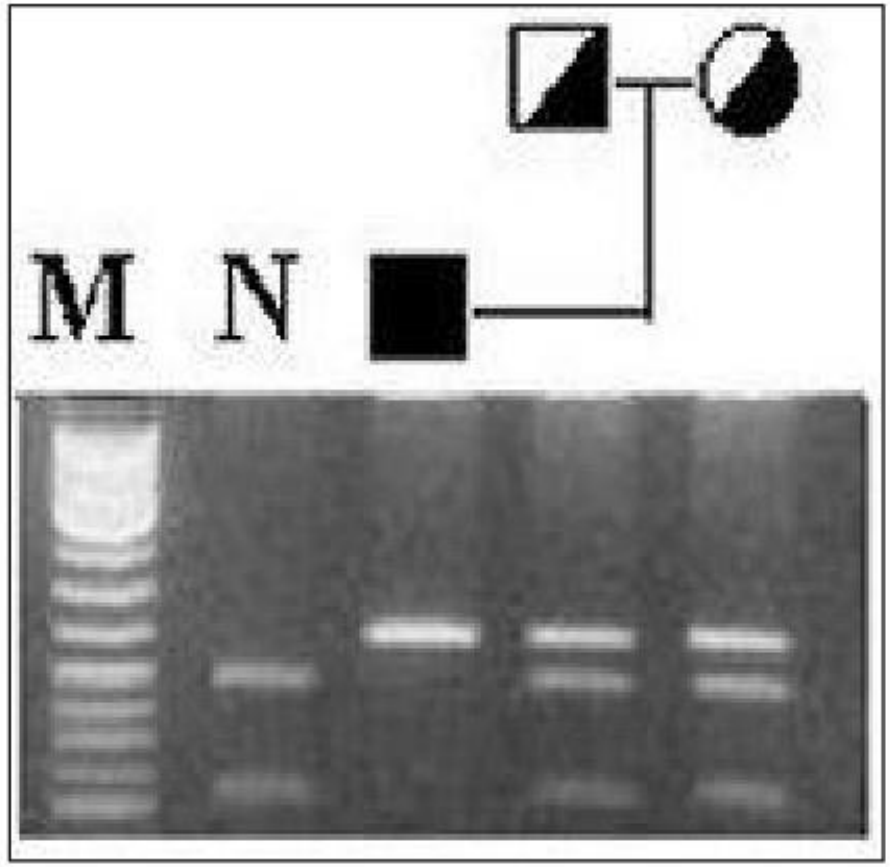
Sensitivity of detection of PCR-amplified *M. tuberculosis* DNA. (Kaul *et al*.1994)

Detection Of Pathogens



Sensitivity of detection of PCR-amplified *M. tuberculosis* DNA. (Kaul *et al*.1994)

Prenatal Diagnosis



- Chorionic Villus
- Amniotic Fluid

Molecular analysis of a family with an autosomal recessive disease

Applications of PCR

- Neisseria gonorrhea
- Chlamydia trachomatis
- HIV-1
- Factor V Leiden
- Forensic testing and many others

Applications of PCR

- Neisseria gonorrhea and Chlamydia trachomatis are two of the most common sexually transmitted diseases

- The infections are asymptomatic and can lead to pelvic inflammatory disease, salpingitis in women, epididymitis in men, infertility, and ectopic pregnancy

Applications of PCR

- Specimens include endocervical swabs, urethral swabs, and urine samples

- The swabs are placed in a vial with transport buffer containing · 50mM $MgCl_2$ and sodium azide as a preservative

Applications of PCR

- The swab specimens can be stored 2-30°C for 4 days or frozen at -20°C
- The urine samples are refrigerated at 2-8°C or stored at -20°C
- A target sequence is chosen for both, amplified with polymerase, and then evaluated with an enzyme immunoassay

Applications of PCR

HIV-1 and Factor V Leiden also have a specific target sequence amplified, and then quantitated by using a microwell probe, horse-radish peroxidase enzyme, and chromogen substrate

Applications of PCR

The HIV-1 test is used as a monitor of the severity of the virus

The HIV-1 causes a depletion of CD4+ T lymphocytes, causing immunodeficiency, multiple opportunistic infections, malignancies, and death

Applications of PCR

The HIV-1 specimen is plasma collected in EDTA that must be separated from the cells within 6 hours

Heparin cannot be used as an anticoagulant because it inhibits PCR

Applications of PCR

A 142 base target sequence in the HIV-1 gag gene is converted from RNA to complementary DNA, and to double stranded DNA using Thermus thermophilus DNA polymerase in the presence of manganese and buffers, which performs the reverse transcription and the amplification steps simultaneously

The standard specimen procedure can quantitate HIV-1 RNA in a range of 400-75,000 copies/mL

Applications of PCR

Factor V Leiden is the Factor V in the coagulation cascade

Factor V is a genetic point mutation that causes increased risk of life-threatening blood clots

The mutation causes the Factor V molecule to be unresponsive to the natural anti-coagulant protein C

Applications of PCR

Factor V Leiden shifts the patient's hemostatic balance to thrombosis

Factor V mutation gives an increase risk of venous thrombosis in a homozygous person, during pregnancy, surgery, or while using oral contraceptives

Applications of PCR

Thrombosis - is the development of a blood clot that occurs in 20-40% of patients with venous thrombosis

Thrombophilia - a tendency towards clotting that occurs in 40-65% of adults with unexplained thrombophilia

Protein C - a naturally occurring anti-coagulant that occurs in 95-100% of people with activated protein C resistance

Application of PCR

Treatment for patients with Factor V Leiden mutations are to give lifelong coumadin

Women with the mutation should not take oral contraceptives, and they have increased risk of thrombosis during pregnancy

Coumadin (warfarin) is an anticoagulant. Coumadin reduces the formation of blood clots by blocking the formation of certain clotting factors. Coumadin is used to prevent heart attacks, strokes, and blood clots in veins and arteries.

Applications of PCR

PCR can also be used in forensic testing

The DNA sequences used are of short repeating patterns called **VNTR** (variable number of tandem repeat), which can range from **4 to 40 nucleotides in different individuals**

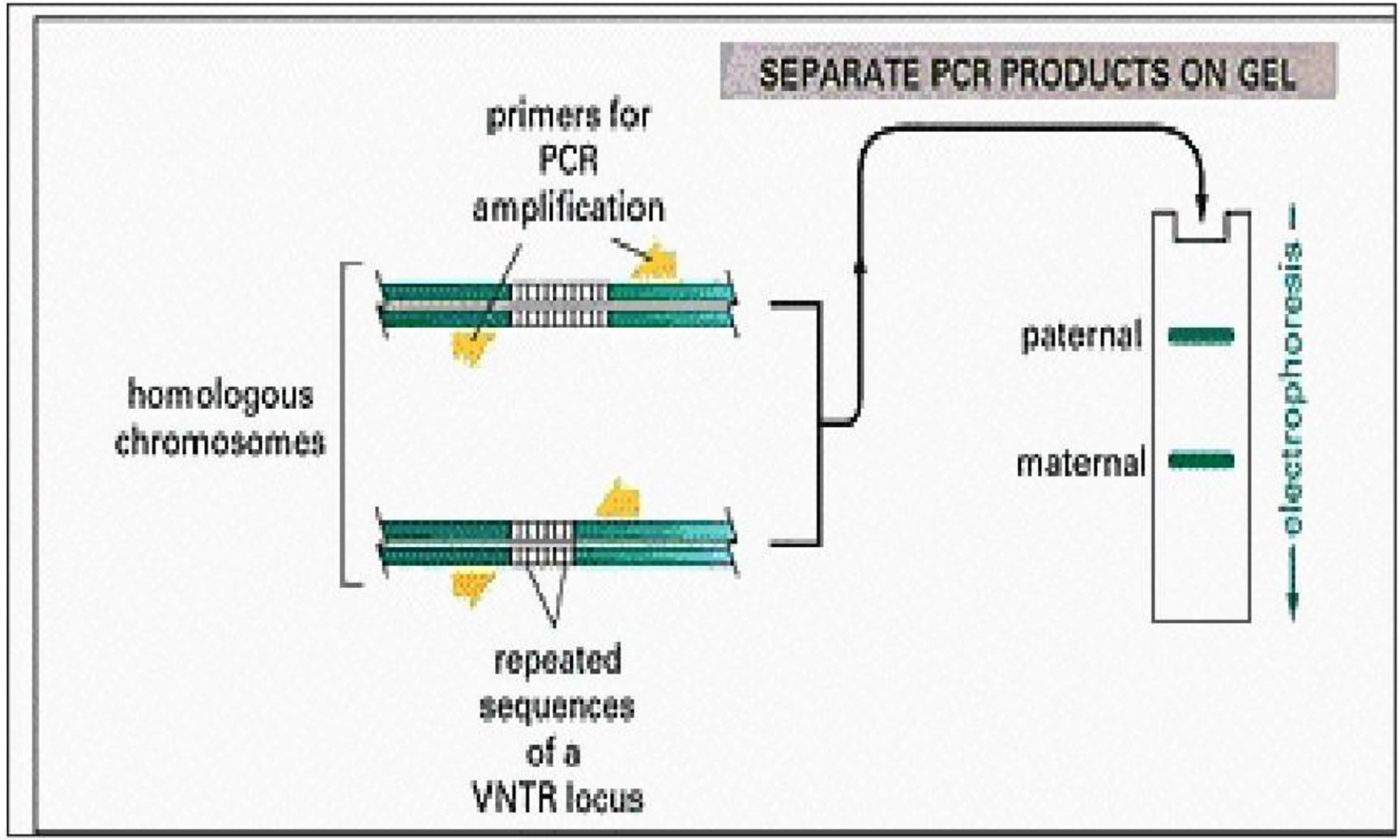
Applications of PCR

One set of VNTR locus are inherited from the mother and one set from the father

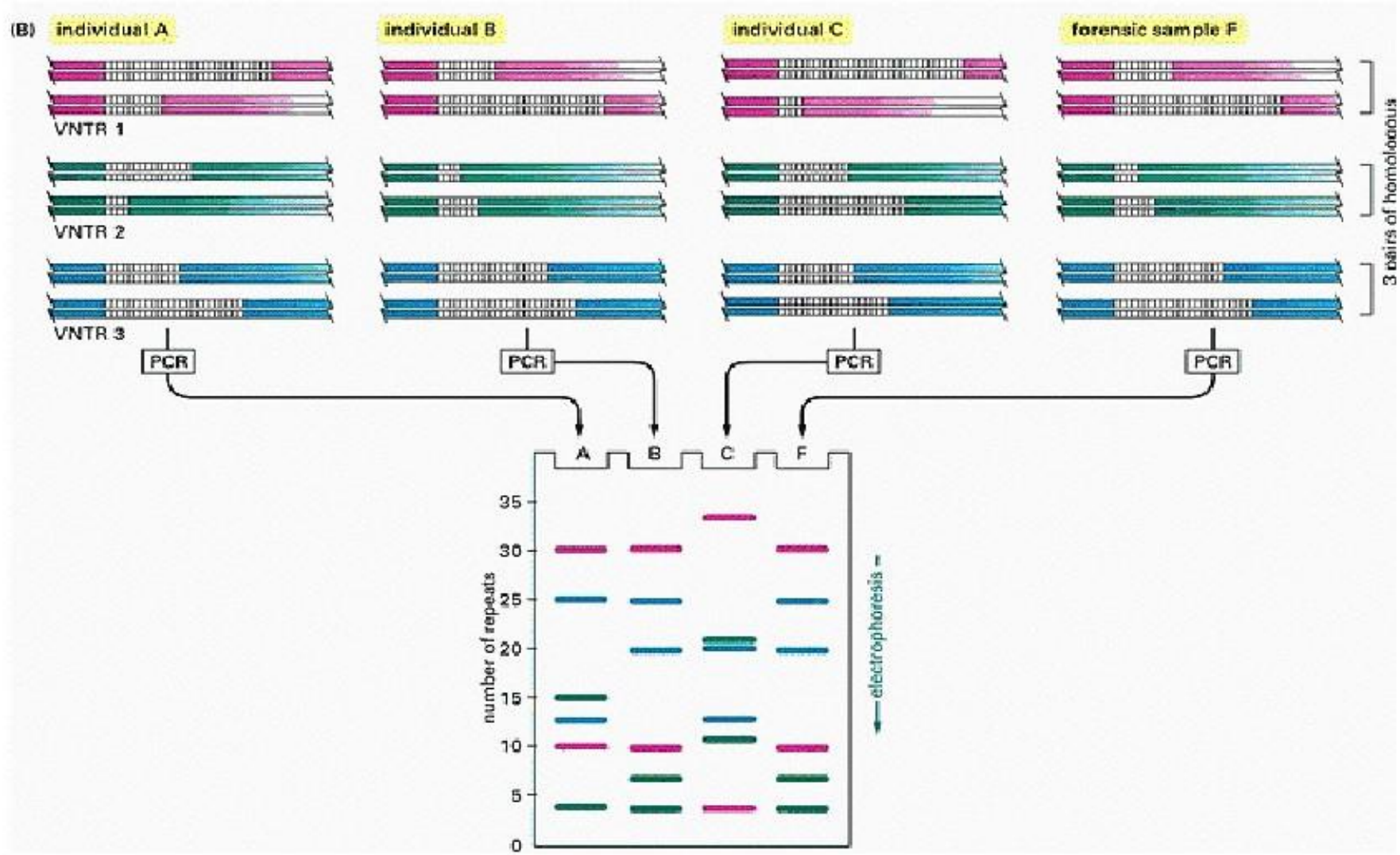
The genes are amplified using PCR, and then run through electrophoresis

The position of the two bands on the electrophoresis gel depends on the exact number of repeats at the locus

Applications of PCR



Applications of PCR



SEQUENCING

Nucleotides (dNTP) are modified (dideoxynucleotides = ddNTP)

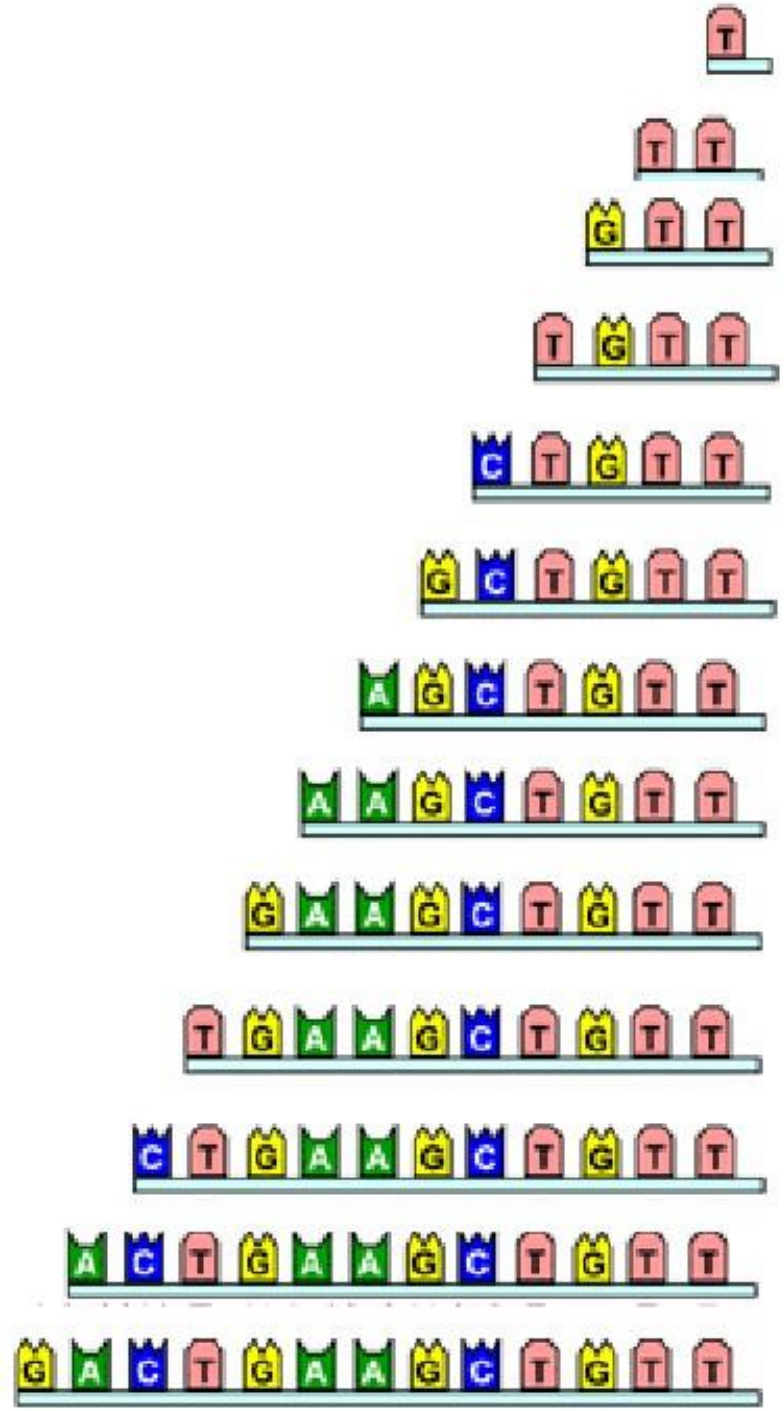
NO polymerisation after a dideoxynucleotide!

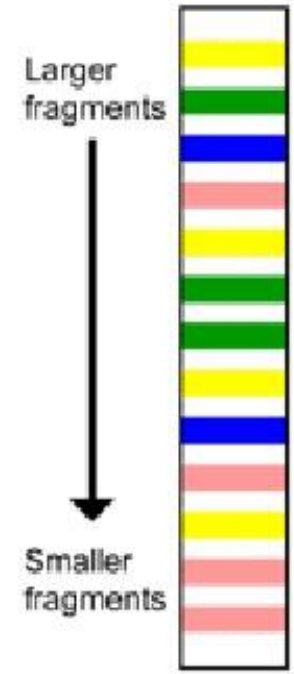
Fragments of DNA differing only by one nucleotide are generated

Nucleotides are either radioactive or fluroscent

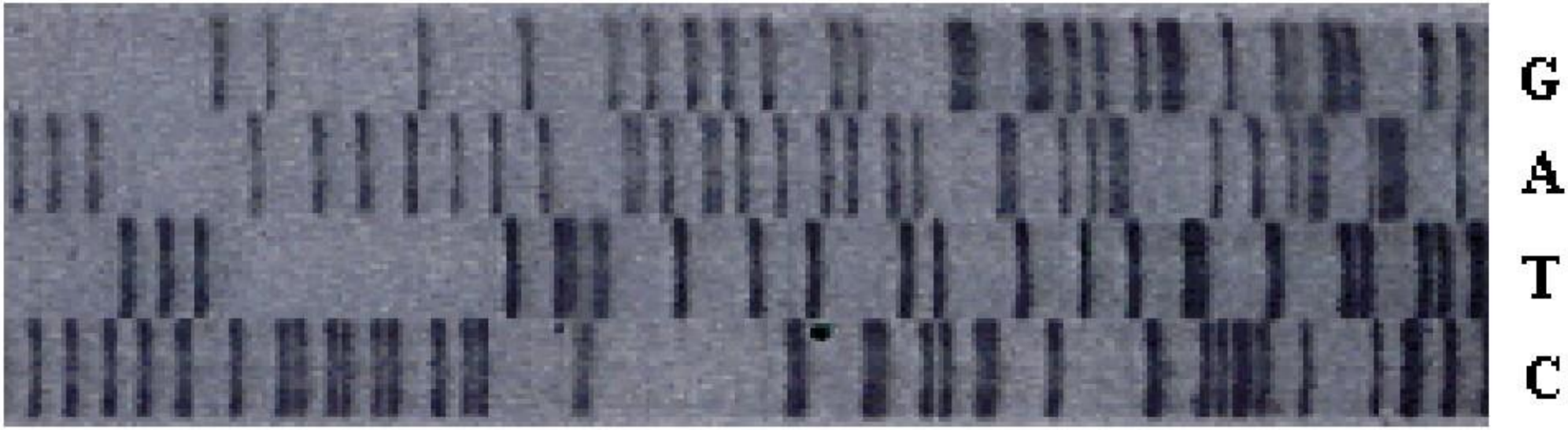
DNA Polymerase I
dATP
dGTP
dCTP
dTTP
plus limiting amounts of
fluorescently labelled

ddATP
ddGTP
ddCTP
ddTTP

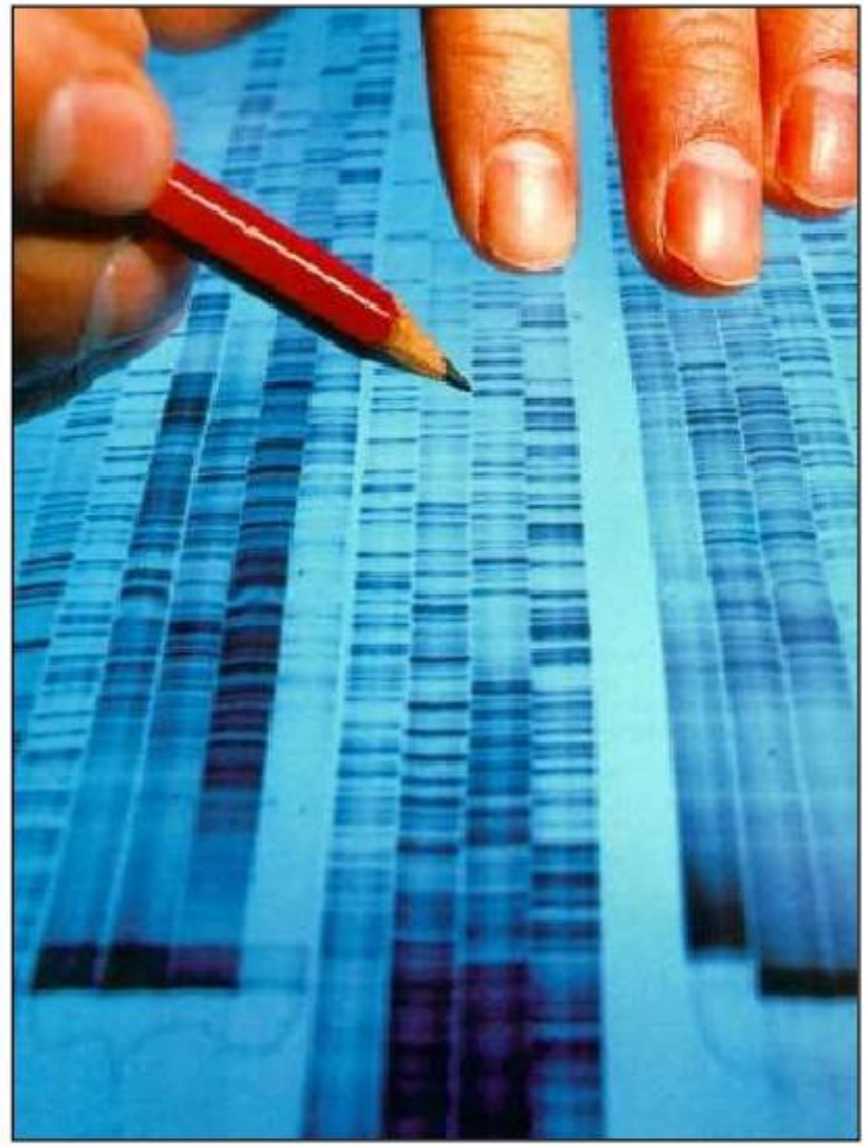


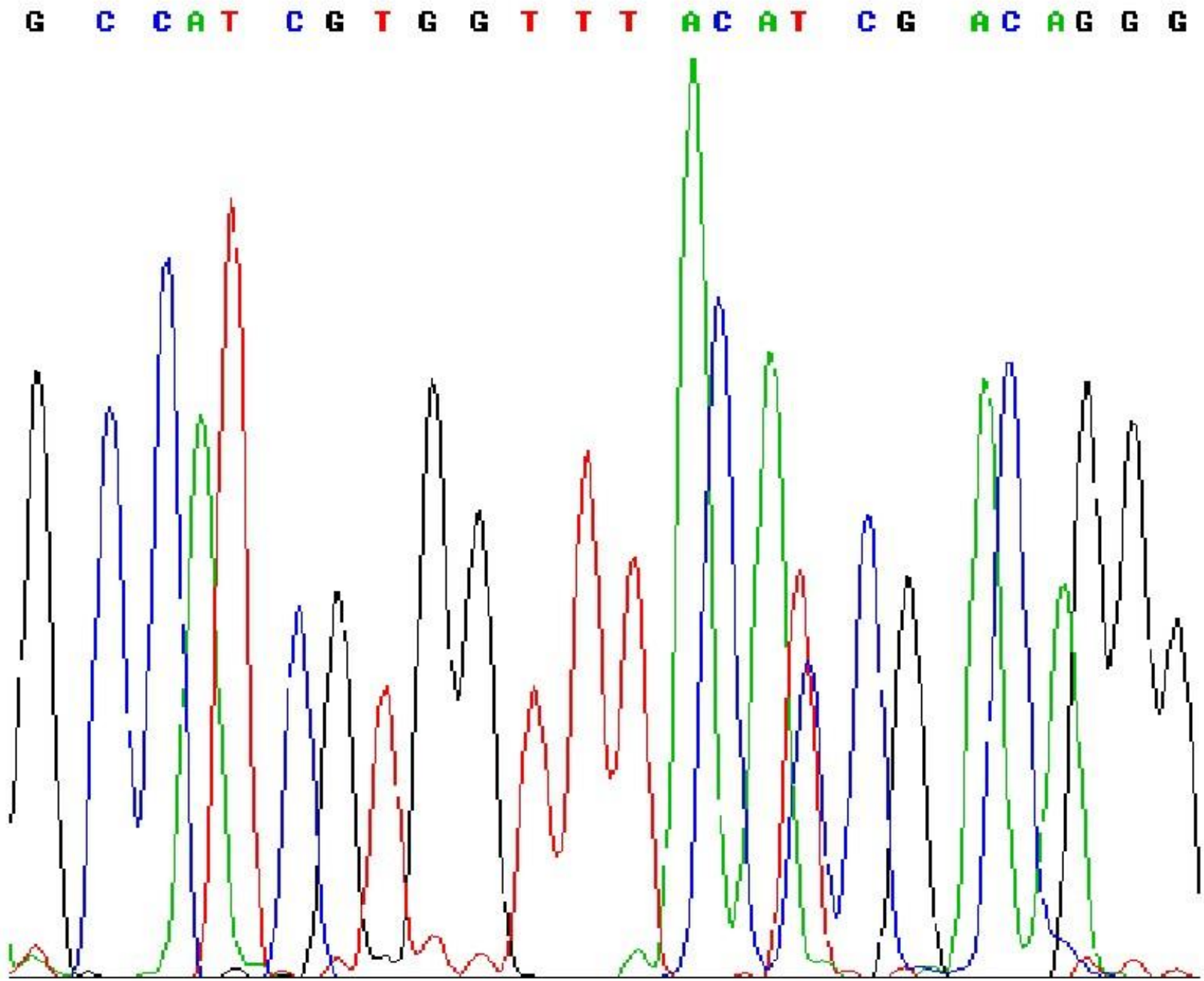


Classical Sequencing Gel



Reading Classical Sequencing Gels




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

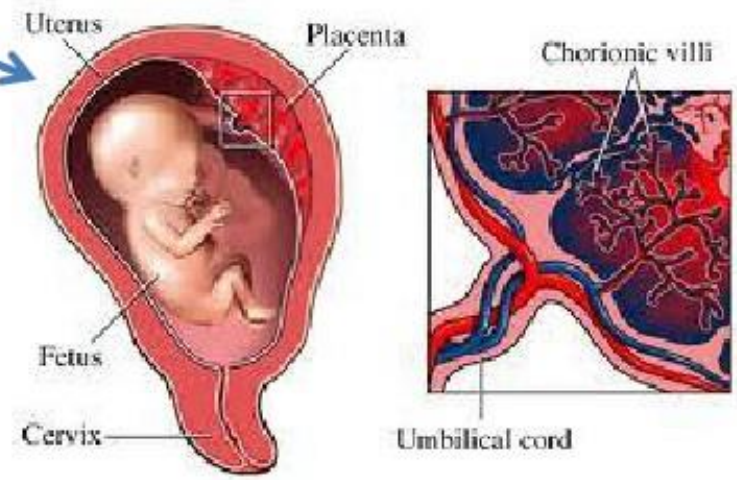
Summary

DNA source → PCR → Desired DNA fragments in huge amounts. → Further manipulation of the amplififed DNA fragments

68,719,476,736 copies

Gel Analysis, Restriction Digestion, Sequencing

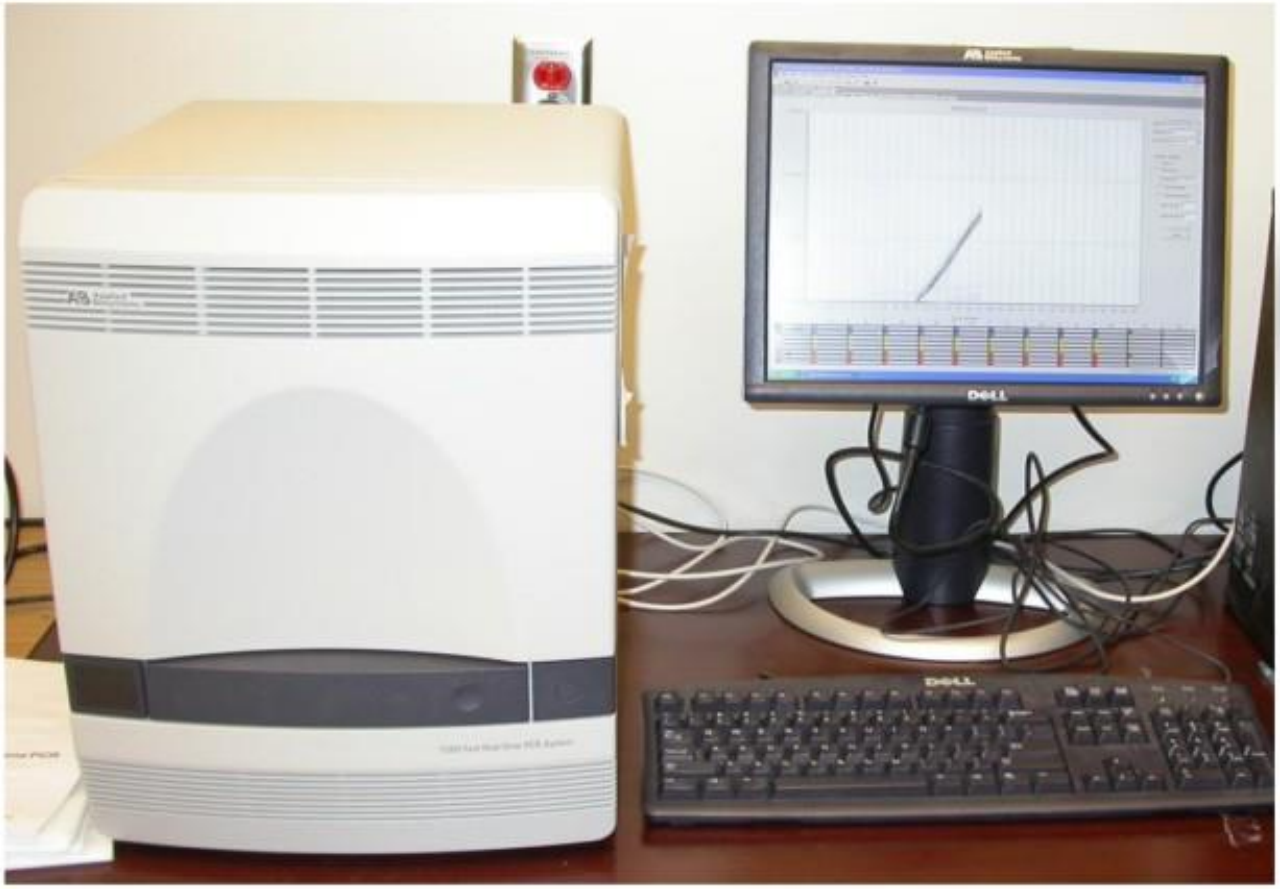
blood, chorionic villus (**Chorionic villi are villi that sprout from the chorion in order to give a maximum area of contact with the maternal blood**), amniotic fluid, semen, hair root, saliva

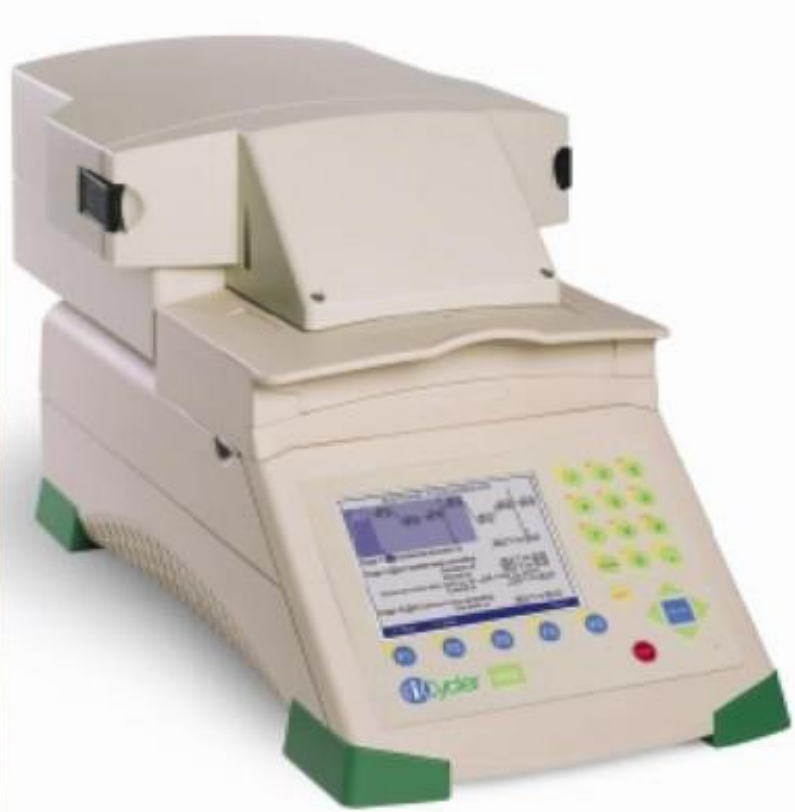


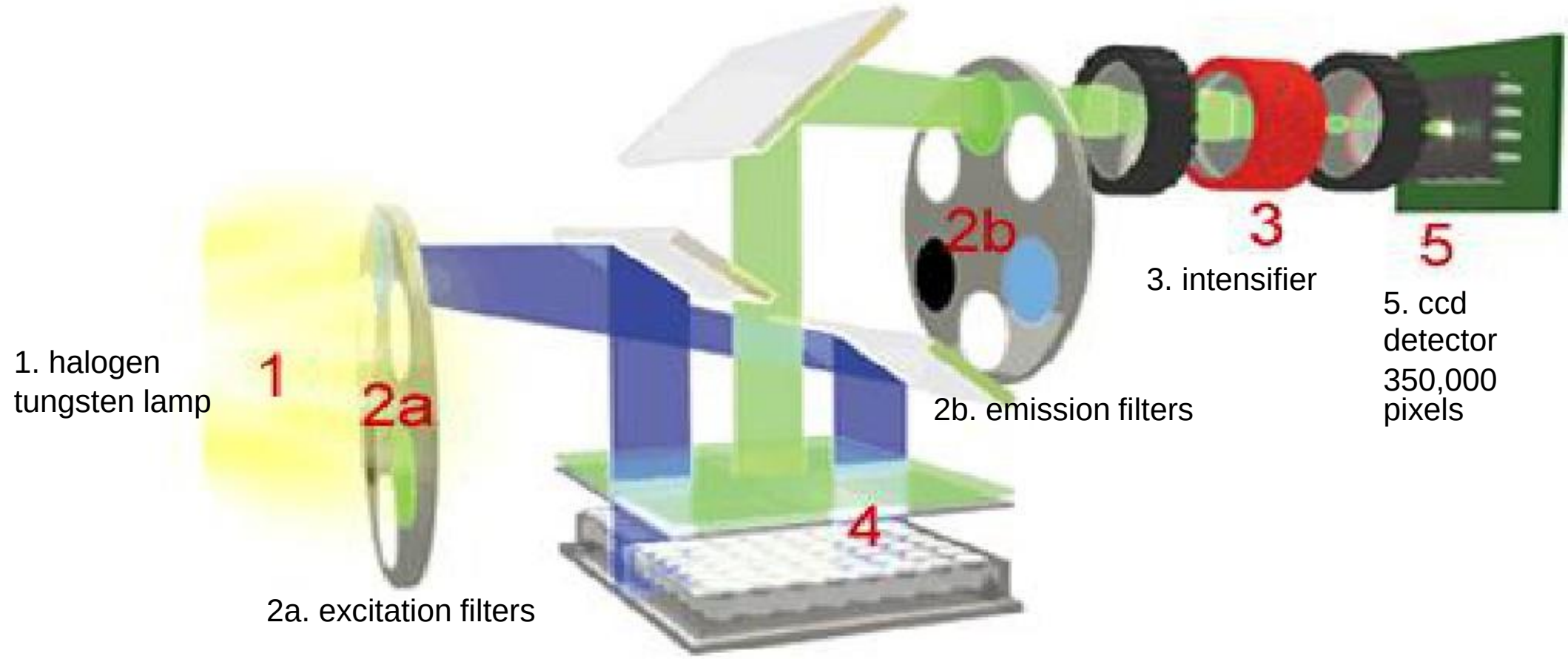
How do we accurately quantify the amount of DNA?

Real-time PCR

Real Time PCR

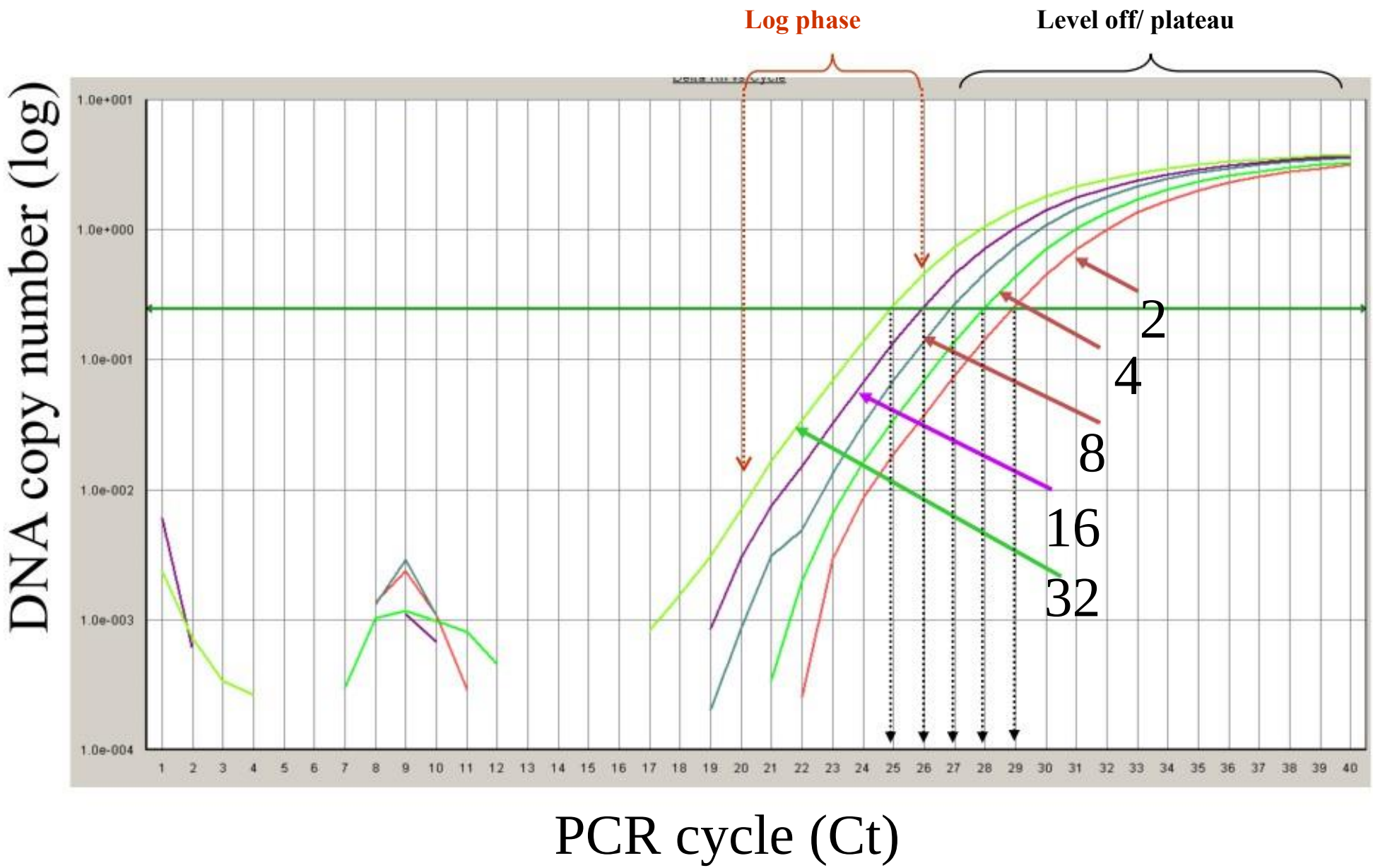






Representation of Optical Detection System layout.

Amplification Plot of real-time PCR



Application

Journal of Virological Methods 102:119-128, 2002

Real-time RT-PCR for quantitation of hepatitis C virus RNA

Ji-Hong Yang [a], Jian-Ping Lai [a], Steven D. Douglas [a], David Metzger [b], Xian-Hua Zhu [c], Wen-Zhe Ho [a,*]

[a] *Division of Immunologic and Infectious Diseases, Joseph Stokes Jr. Research Institute, Children's Hospital of Philadelphia, 34th & Civic Center Blvd., Philadelphia, PA 19104, USA*

[b] *Department of Pediatrics and Medicine, The Treatment Center, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA*

[c] *Department of Pathology, Thomas Jefferson University, Philadelphia, PA 19107, USA*

Table 5
Correlation of HCV real-time RT-PCR and HCV antibody ELISA

Plasma		Real-time RT-PCR	ELISA
Normal donor	14	−[a]	−
Drug abuser	15	+[b]	+
	2	−	−

[a] Negative.
[b] Positive.

Journal of Clinical Virology 28:233-238, 2003

Early diagnosis of SARS Coronavirus infection by real time RT-PCR

Leo L.M. Poon [a,*], Kwok Hung Chan [b], On Kei Wong [a], Wing Cheong Yam [a], Kwok Yung Yuen [a], Yi Guan [a], Y.M. Dennis Lo [c], Joseph S.M. Peiris [a]

Table 1
Detection of SARS CoV in clinical specimens by different conventional and real-time RT-PCR protocols in relation to time after onset of disease

Day of onset	Sample Size	Number of positives		
		Conventional RT-PCR assay	Conventional RT-PCR assay with a modified RNA extraction protocol*	Real-time RT-PCR assay with a modified RNA extraction protocol*,**
1	8	0 (0%)	2 (25%)	5 (63%)
2	16	3 (19%)	8 (50%)	14 (88%)
3	26	8 (31%)	12 (46%)	21 (81%)

* The overall detection rate of the assay is statistically different from that of the conventional RT-PCR assay (McNemar's test, $P < 0.001$).

** The overall detection rate of the assay is statistically different from that of the conventional RT-PCR assay with a modified RNA extraction protocol (McNemar's test, $P < 0.0001$).

What if 10,000 genes are to be quantitated by PCR??

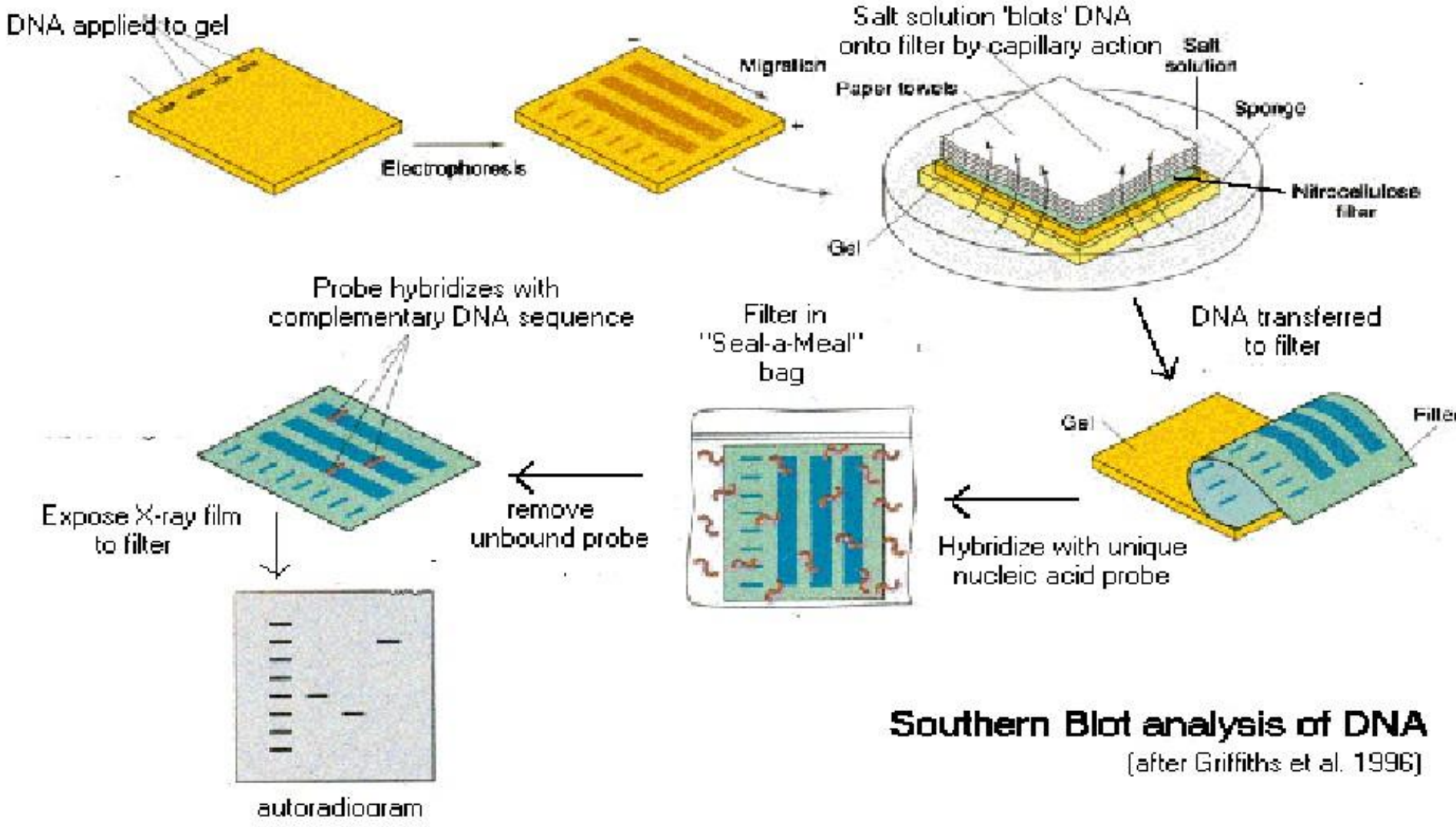
Expression Microarray

- High throughput technology that allows detection of thousands of gene simultaneously

- Apply hybridization principle (imagine thousand of Southern blots)

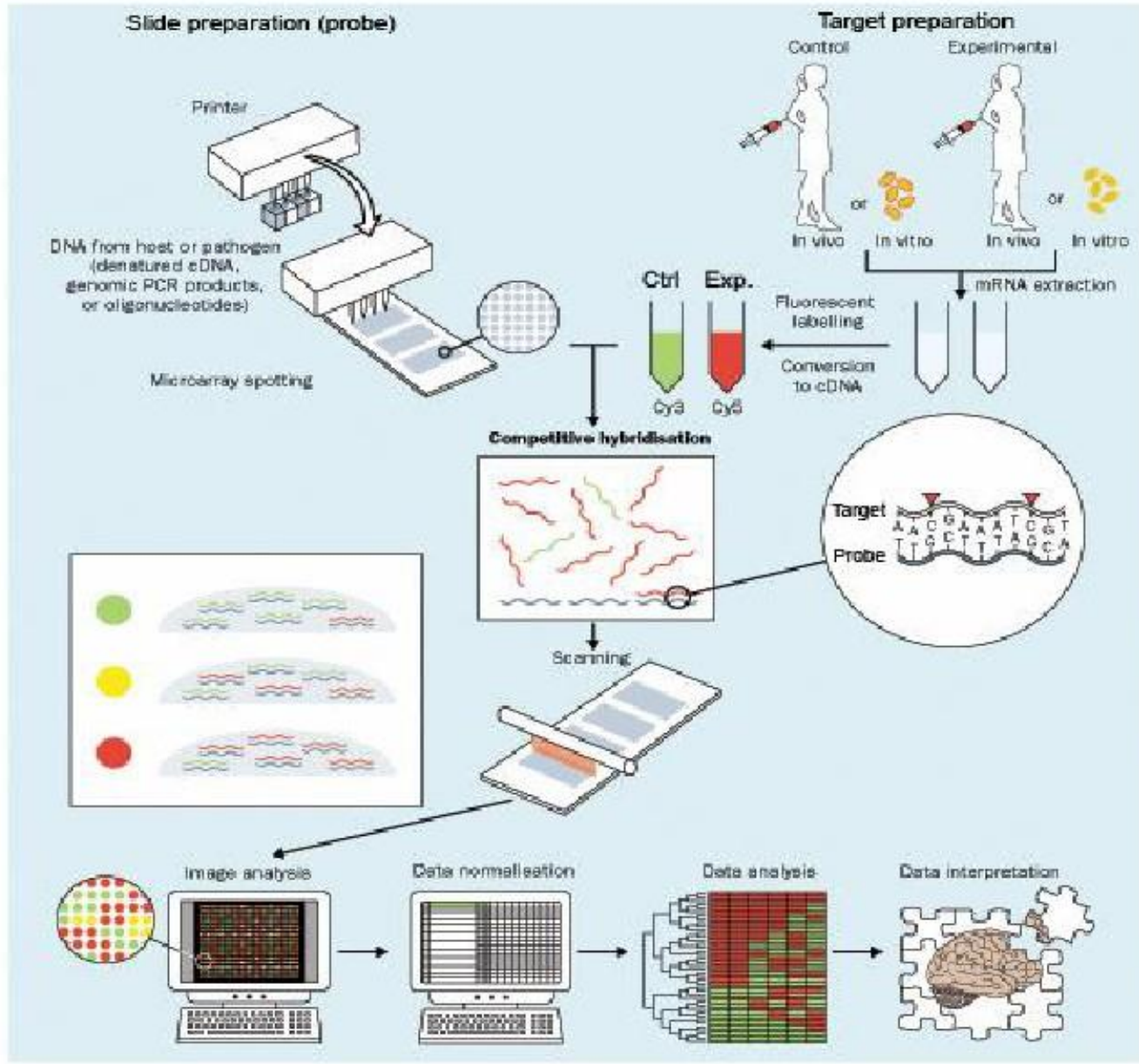
- Data require further statistical analysis (bioinformatics)

Southern Blot Analysis

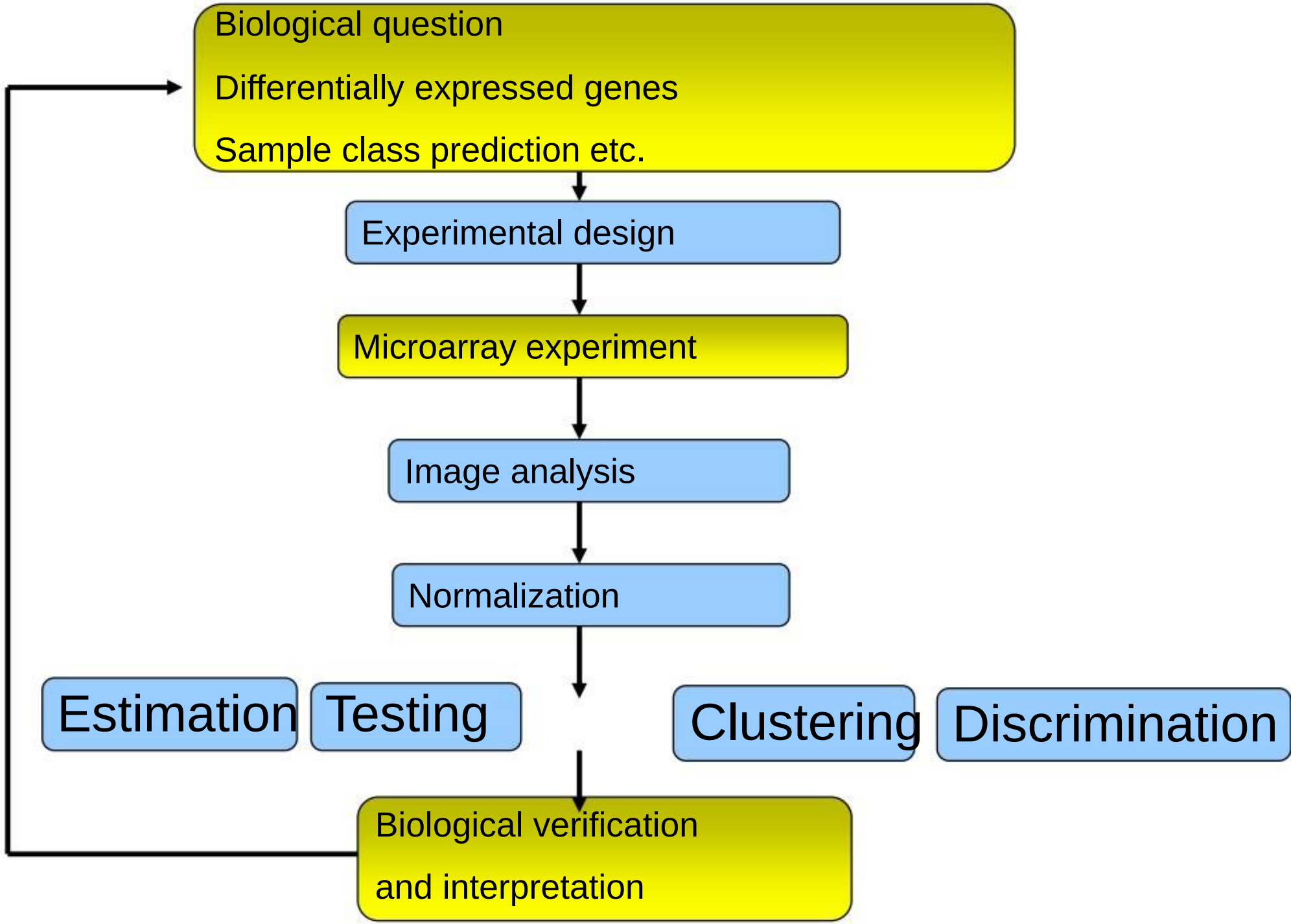


Southern Blot analysis of DNA
(after Griffiths et al. 1996)

cDNA Microarray analysis



Adapted from Bryant *et al*., 2004, Lancet infectious disease


Biological question
Differentially expressed genes
Sample class prediction etc.
Experimental design
Microarray experiment
Image analysis
Normalization
Estimation
Testing
Clustering
Discrimination
Biological verification
and interpretation

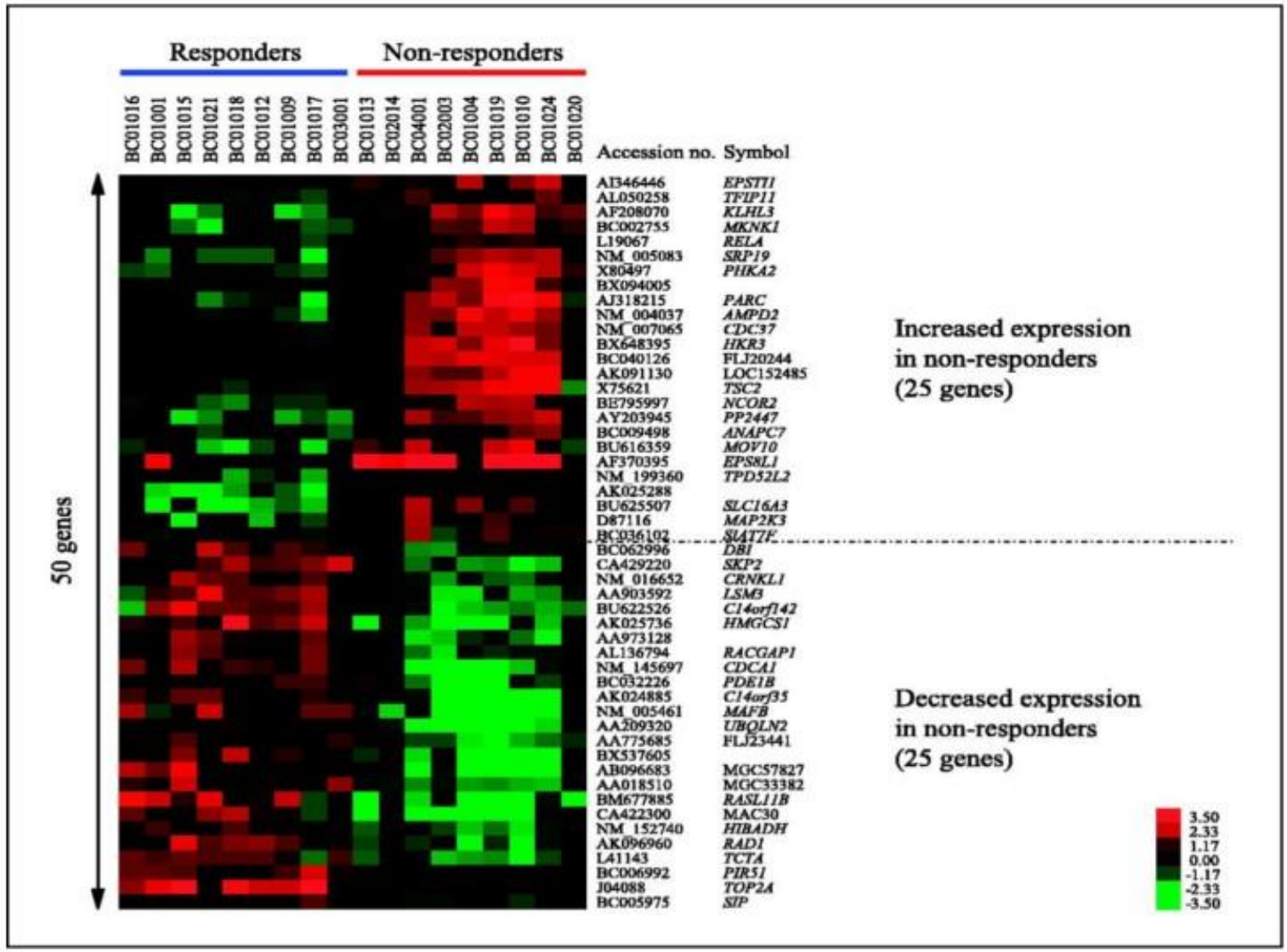
Principal Uses of Microarrays

Genome-scale gene expression analysis

- **Differential gene expression between two (or more) sample types**
- **Responses to environmental factors**
- **Disease processes (e.g. cancer, diabetes, etc...)**
- **Effects of drugs**
- **Identification of genes associated with clinical outcomes (e.g. survival)**

Predicting Response to Methotrexate, Vinblastine, Doxorubicin, and Cisplatin Neoadjuvant Chemotherapy for Bladder Cancers through Genome-Wide Gene Expression Profiling

Ryo Takata,[1,7] Toyomasa Katagiri,[1] Mitsugu Kanehira,[1,7] Tatsuhiko Tsunoda,[2] Taro Shuin,[3] Tsuneharu Miki,[4] Mikio Namiki,[5] Kenjiro Kohri,[6] Yasushi Matsushita,[7] Tomoaki Fujioka,[7] and Yusuke Nakamura[1]



Clinical Cancer Research 11:2625-2636, 2005

Limitation of expressing Microarray

- Detecting mRNA level not protein level
- Many factors can affect microarray result:
  - Chip type
  - sample preparation
  - data analysis
- Require bioinformatics for data analysis

Other types of microarray platform

- **ChIP (Chromatin Immunoprecipitation) microarray**

- **Methylation microarray (Differential Methylation Hybridization)**

- **CGH (comparative genomic hybridization) microarray**

Variations on the basic PCR technique

Allele-specific PCR

- A diagnostic or cloning technique based on single-nucleotide polymorphisms (SNPs) (single-base differences in DNA).

- It requires prior knowledge of a DNA sequence, including differences between alleles, and uses primers whose 3' ends encompass the SNP.

Assembly PCR or Polymerase Cycling Assembly (PCA)

Artificial synthesis of long DNA sequences by performing PCR on a pool of long oligonucleotides with short overlapping segments.

Oligonucleotides alternate between sense and antisense directions, and the overlapping segments determine the order of the PCR fragments, thereby selectively producing the final long DNA product.

Asymmetric PCR

- Preferentially amplifies one DNA strand in a double-stranded DNA template.
- It is used in sequencing and hybridization probing where amplification of only one of the two complementary strands is required.
- PCR is carried out as usual, but with a great excess of the primer for the strand targeted for amplification.

Intersequence-specific PCR (ISSR)

PCR method for DNA fingerprinting that amplifies regions between simple sequence repeats to produce a unique fingerprint of amplified fragment lengths.

Inverse PCR

Commonly used to identify the flanking sequences around genomic inserts.

It involves a series of DNA digestions and self ligation, resulting in known sequences at either end of the unknown sequence.

Ligation-mediated PCR

Uses small DNA linkers ligated to the DNA of interest and multiple primers annealing to the DNA linkers; it has been used for DNA sequencing, genome walking, and DNA footprinting.

Miniprimer PCR

- Uses a thermostable polymerase that can extend from short primers ("small oligos") as short as 9 or 10 nucleotides.

- This method permits PCR targeting to smaller primer binding regions, and is used to amplify conserved DNA sequences, such as the 16S (or eukaryotic 18S) rRNA gene.

Multiplex-PCR

- Consists of multiple primer sets within a single PCR mixture to produce amplicons of varying sizes that are specific to different DNA sequences.

- By targeting multiple genes at once, additional information may be gained from a single test-run that otherwise would require several times the reagents and more time to perform.

- Annealing temperatures for each of the primer sets must be optimized to work correctly within a single reaction, and amplicon sizes.

Reverse Transcription PCR (RT-PCR)

- For amplifying DNA from RNA. Reverse transcriptase reverse transcribes RNA into cDNA, which is then amplified by PCR.

- RT-PCR is widely used in expression profiling, to determine the expression of a gene or to identify the sequence of an RNA transcript, including transcription start and termination sites.

- If the genomic DNA sequence of a gene is known, RT-PCR can be used to map the location of exons and introns in the gene.

- The 5' end of a gene (corresponding to the transcription start site) is typically identified by RACE-PCR(Rapid Amplification of cDNA Ends).

Quantitative PCR (Q-PCR)

- Used to measure the quantity of a PCR product (commonly in real-time). It quantitatively measures starting amounts of DNA, cDNA, or RNA.

- Q-PCR is commonly used to determine whether a DNA sequence is present in a sample and the number of its copies in the sample.

- QRT-PCR methods use fluorescent dyes, such as Sybr Green, EvaGreen or fluorophore-containing DNA probes to measure the amount of amplified product in real time.

- It is also sometimes abbreviated to RT-PCR (Real Time PCR) or RQ-PCR. QRT-PCR or RTQ-PCR are more appropriate contractions, since RT-PCR commonly refers to reverse transcription PCR, often used in conjunction with Q-PCR.

Conclusion

The speed and ease of use, sensitivity, specificity and robustness of PCR has revolutionised molecular biology and made PCR the most widely used and powerful technique with great spectrum of research and diagnostic applications