Molecular Tools of Medicine

or

The use of Molecular Biology to Diagnose, Study and Treat Diseases

Techniques

- DNA, RNA and protein analytical methods
- Cloning methods
- Diagnostic methods
- Potential treatment protocols

DNA, RNA and Protein Analysis

- Gel electrophoresis
- Blotting techniques
- Basic mapping techniques

- Video on gel electrophoresis

Blotting Techniques

- Three basic protocols dependent upon the macromolecule
  - Western

    variations include north- and southwesterns
  - Northern

    similar variations
  - Southern

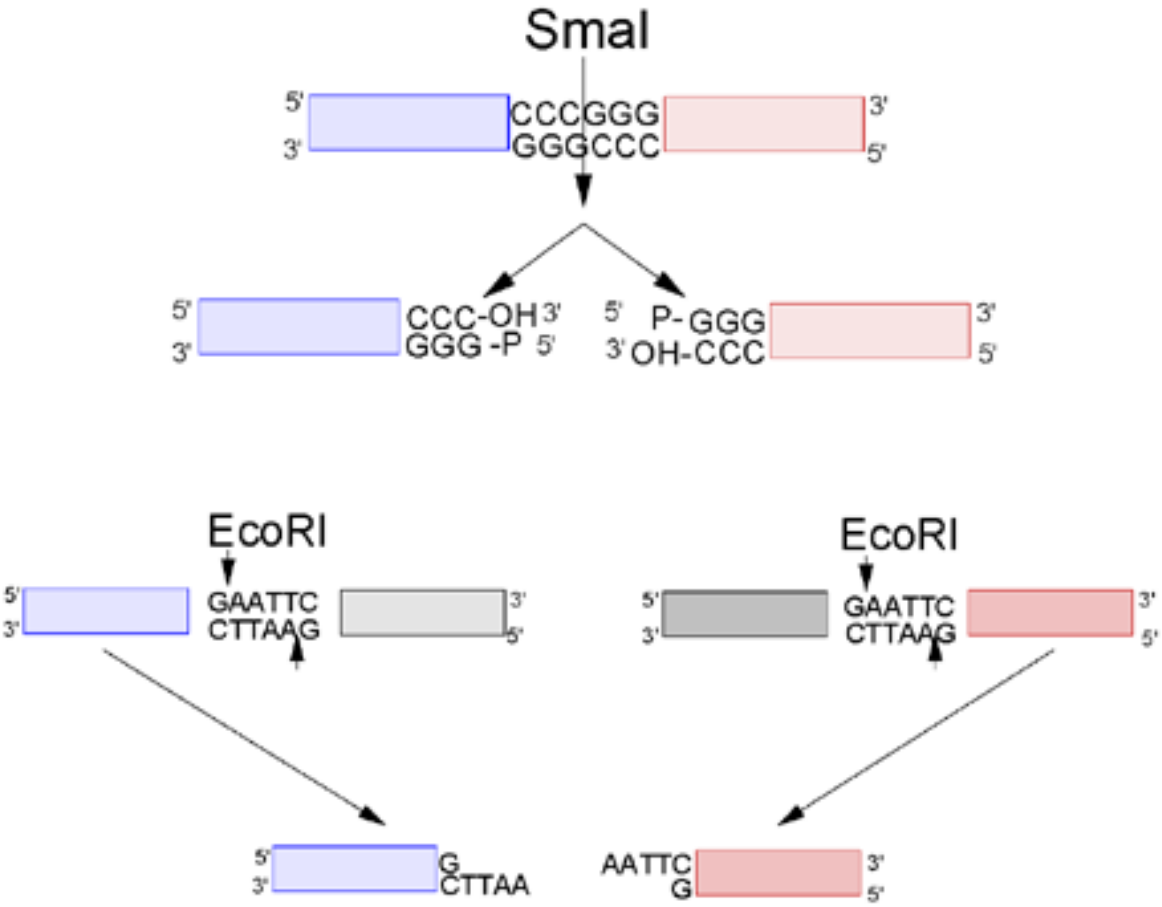
    similar variations

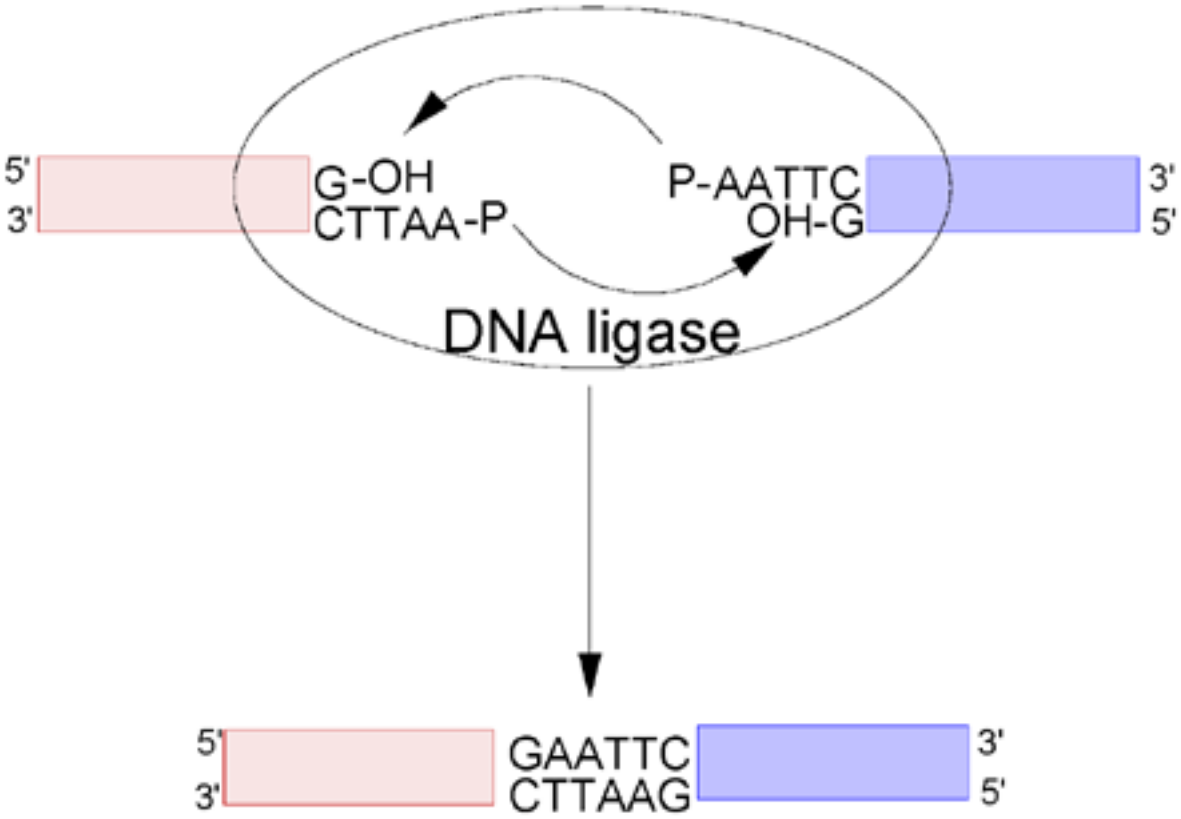
- Video on Southern blotting
- https://www.youtube.com/watch?v=3I9wzwj0b_A

Important Enzymes

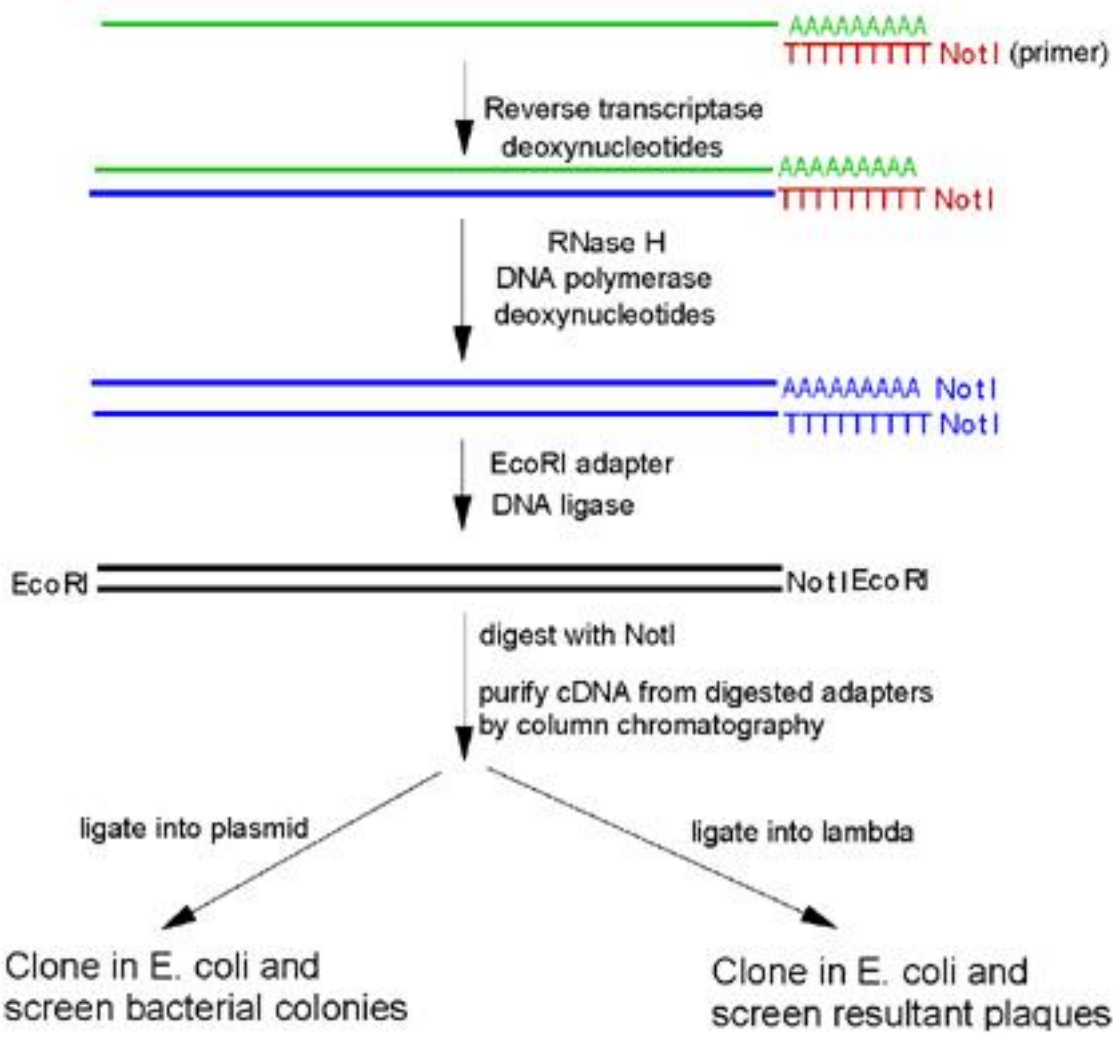
Restriction endonucleases	recognize specific nucleotide sequences and cleaves the DNA within or near to the recognition sequences	
Reverse transcriptase (RT)	retrovirally encoded RNA-dependent DNA polymerase	used to convert mRNA into a complimentary DNA(cDNA) copy for the purpose of cloning cDNAs
RNAse H	recognizes RNA-DNA duplexes and randomly cleaves the phosphodiester backbone of the RNA	used primarily to cleave themRNA strand that is annealed to the first strand of cDNA generated by reverse transcription
DNA polymerase	synthesis of DNA	used during most procedures where DNA synthesis is required, also used in *in vitro* mutagenesis
Klenow DNA polymerase	proteolytic fragment of DNA polymerase that lacks the 5' > 3' exonuclease activity	used to incorporate radioactive nucleotides into restriction enzyme generated ends of DNA, also can be used in place of DNA polymerase
DNA ligase	covalently attaches a free 5' phosphate to a 3' hydroxyl	used in all procedures where to molecules of DNA need to be covalently attached
Alkaline phosphatase	removes phosphates from 5' ends of DNA molecules	used to allow 5' ends to be subsequently radiolabeled with the γ-phosphate ofATP in the presence of polynucleotide kinase, also used to prevent self-ligation of restriction enzyme digested plasmids and lambda vectors

Polynucleotide kinase	introduces γ-phosphate of ATP to 5' ends of DNA	see above for alkaline phosphatase
DNAse I	randomly hydrolyzes the phosphodiester bonds of double-stranded DNA	is used in the identification of regions of DNA that are bound by protein and thereby protected fromDNase I digestion, also used to identify transcriptionally active regions of chromatin since they are more susceptible to DNase I digestion
S1 Nuclease	exonuclease that recognizes single stranded regions of DNA	used to remove regions of single strandedness in DNA or RNA-DNA duplexes
Exonuclease III	exonuclease that removes nucleotides from the 3' end of DNAs	used to generate deletions in DNA for sequencing, or to map functional domains of DNA duplexes
Terminal transferase	DNA polymerase that requires only a 3'-OH, lengthens 3' ends with any dNTP	used to introduce homopolymeric (same dNTP) "tails" onto the 3' ends of DNA duplexes, also used to introduce radiolabeled nucleotides on the 3' ends of DNA
T3, T7, and SP6 RNA polymerases	bacterial virus encoded RNA polymerase, recognize specific nucleotide sequences for initiation of transcription	used to synthesize RNA *in vitro*
Taq and Vent DNA polymerase	thermostable DNA polymerase	polymerases used in PCR
Taq and Vent DNA ligases	thermostable DNA ligases	used in LCR

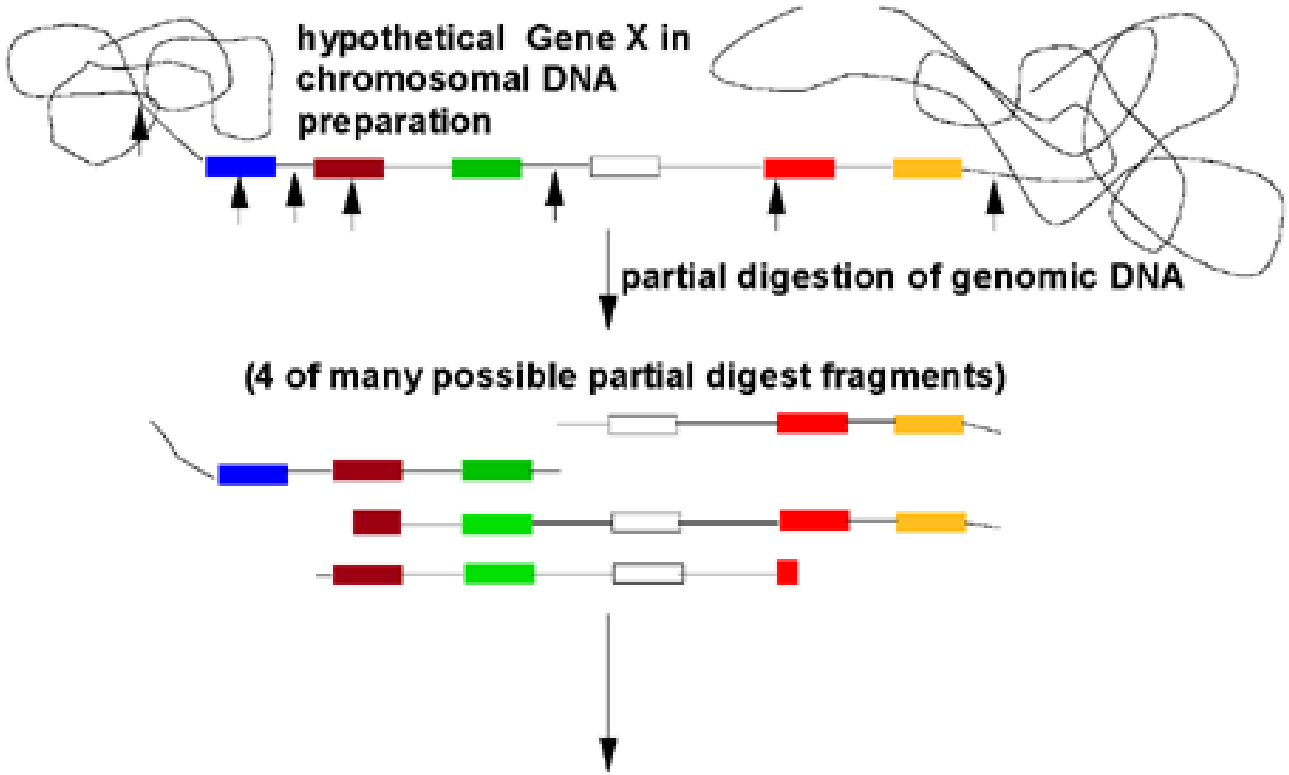

SmaI
5'
3'
CCCGGG
GGGCCC
3'
5'
5'
3'
CCC-OH 3'
GGG -P 5'
5'
3'
P-GGG
OH-CCC
3'
5'
EcoRI
5'
3'
GAATTC
CTTAAG
3'
5'
EcoRI
5'
3'
GAATTC
CTTAAG
3'
5'
5'
3'
G
CTTAA
AATTC
G
3'
5'


5'
3'
G-OH
CTTAA-P
P-AATTC
OH-G
3'
5'
DNA ligase
5'
3'
GAATTC
CTTAAG
3'
5'

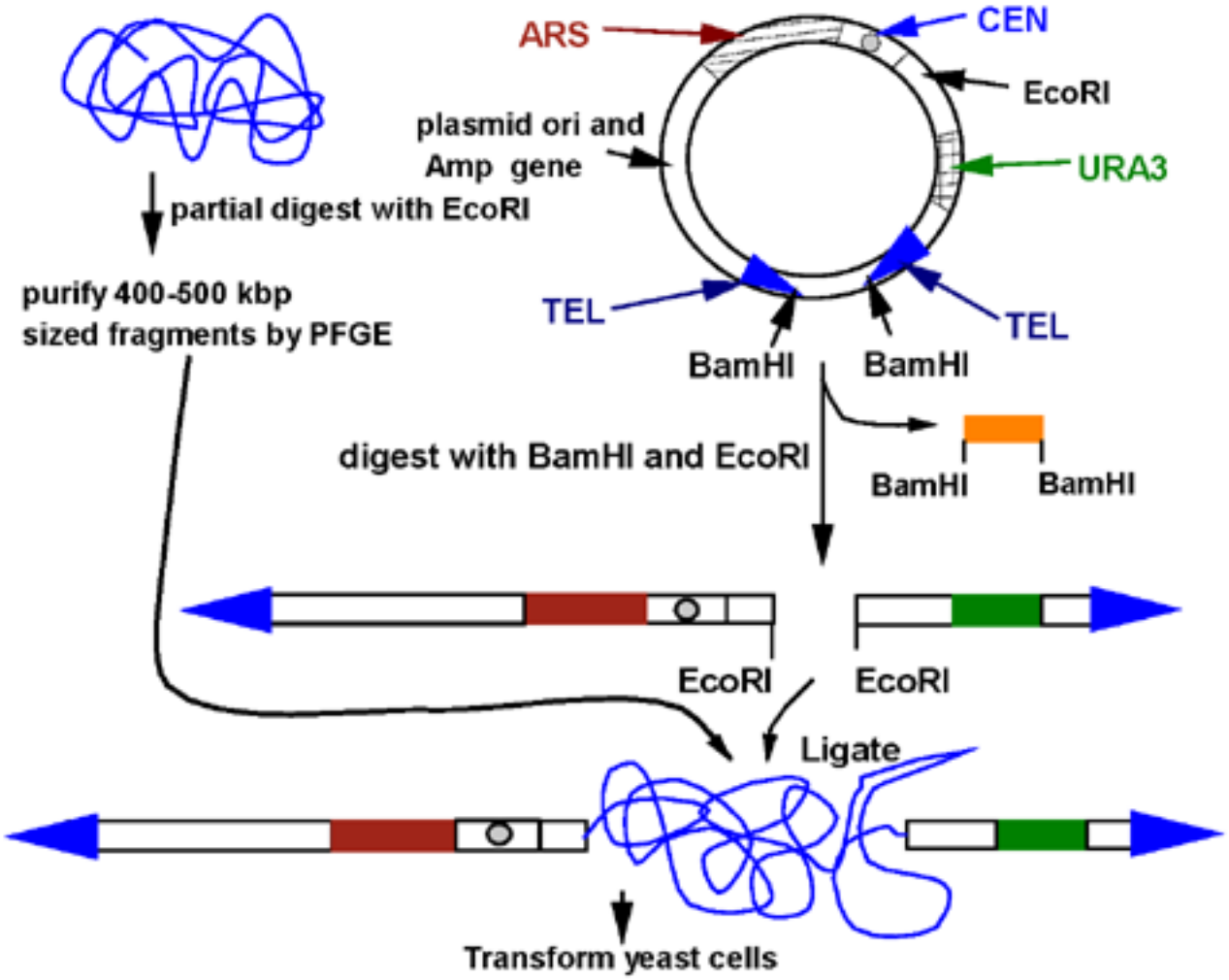
Cloning cDNAs


AAAAAAAAA
TTTTTTTTT NotI (primer)
Reverse transcriptase
deoxynucleotides
AAAAAAAAA
TTTTTTTTT NotI
RNase H
DNA polymerase
deoxynucleotides
AAAAAAAAA NotI
TTTTTTTTT NotI
EcoRI adapter
DNA ligase
EcoRI
NotI EcoRI
digest with NotI
purify cDNA from digested adapters
by column chromatography
ligate into plasmid
ligate into lambda
Clone in E. coli and
screen bacterial colonies
Clone in E. coli and
screen resultant plaques

Cloning Genomic DNA

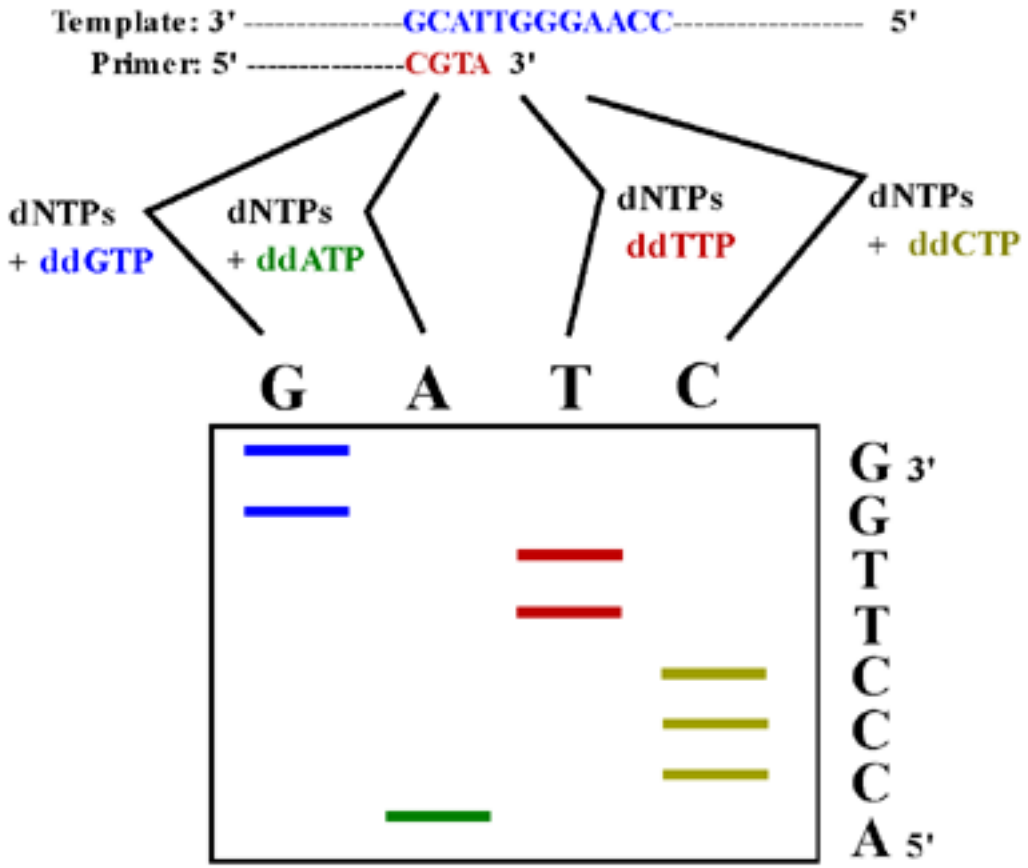


size select and ligate fragments into lambda vector, clone in *E. coli* and screen resultant plaques


ARS
CEN
EcoRI
plasmid ori and
Amp gene
URA3
partial digest with EcoRI
purify 400-500 kbp
sized fragments by PFGE
TEL
TEL
BamHI
BamHI
digest with BamHI and EcoRI
BamHI
BamHI
EcoRI
EcoRI
Ligate
Transform yeast cells

DNA Sequencing

Sanger ddNTP Chain Termination Sequencing



Mapping Techniques for Diagnosis

- Limit to DNA mapping since most pertinent for clinical diagnosis
  - linkage mapping

    used to demonstrate linked genes
  - cytogenetic mapping

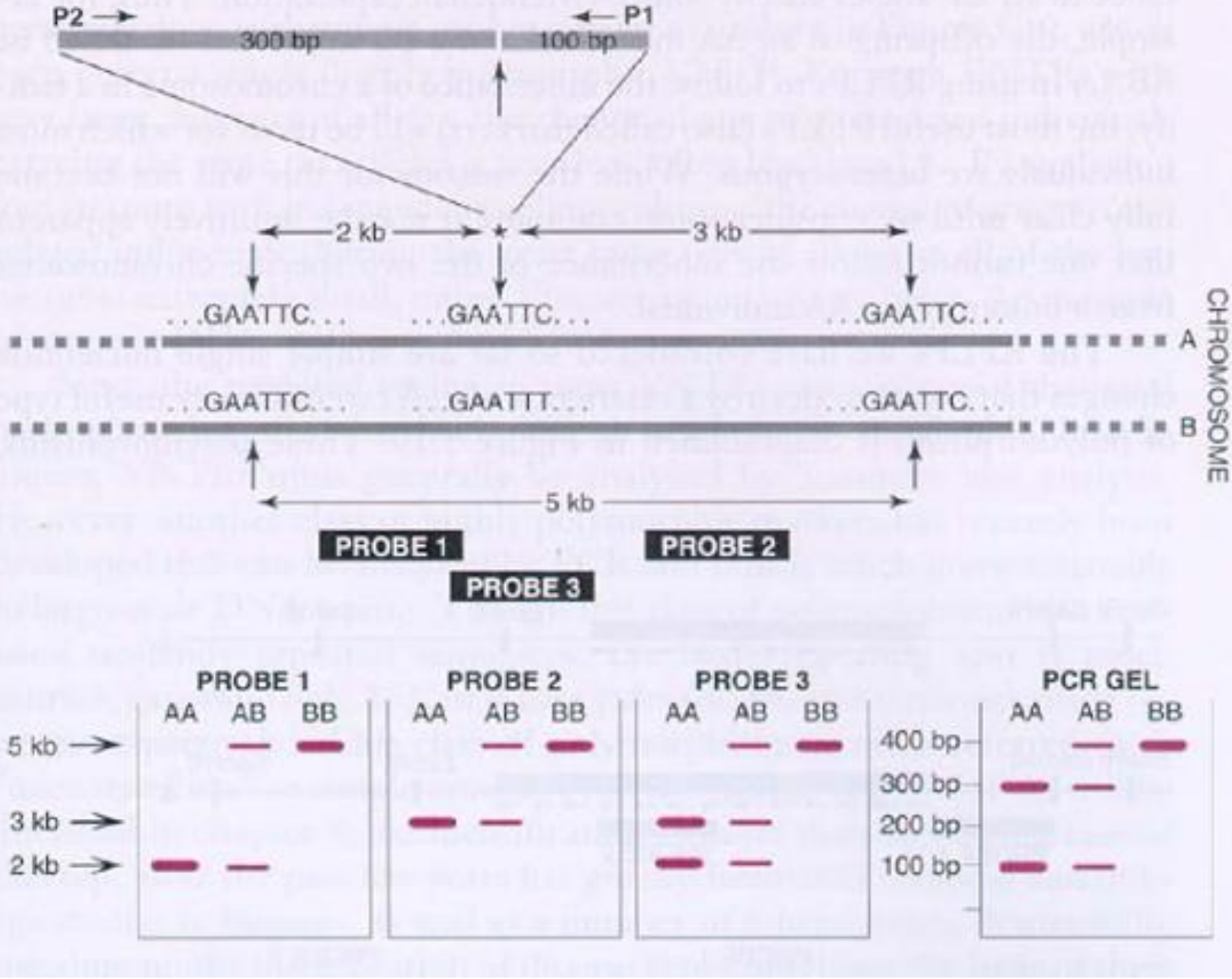
    used to show relative chromosomal location
  - physical maps

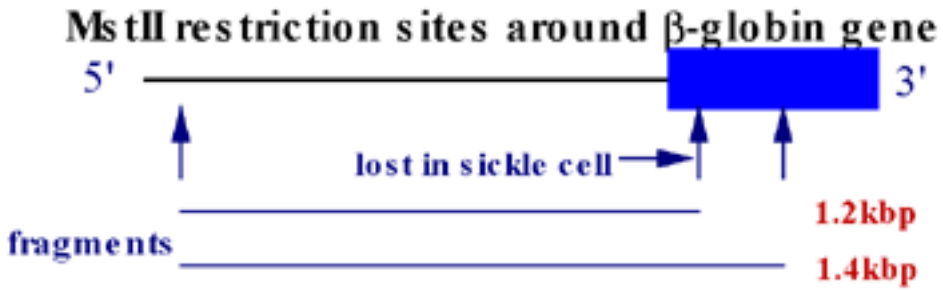
    demonstrate order and spacing of genes

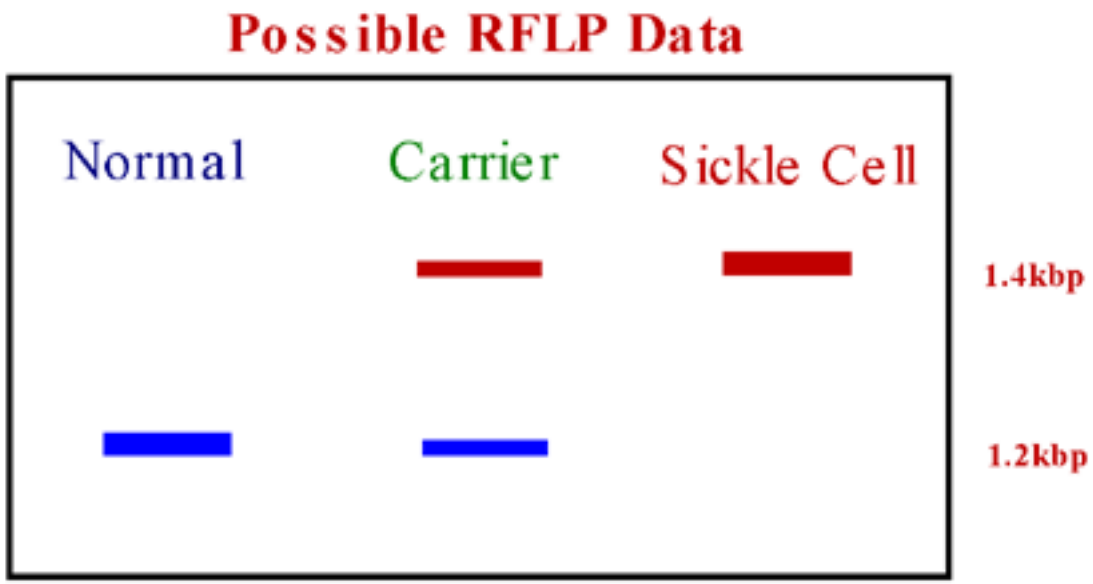
Genetic Analysis

- RFLP Mapping
- VNTR Analysis
  - both techniques utilize Southern blots
- PCR
- SS Polymorfisms
- ASO Probing
- PCR-SSCP

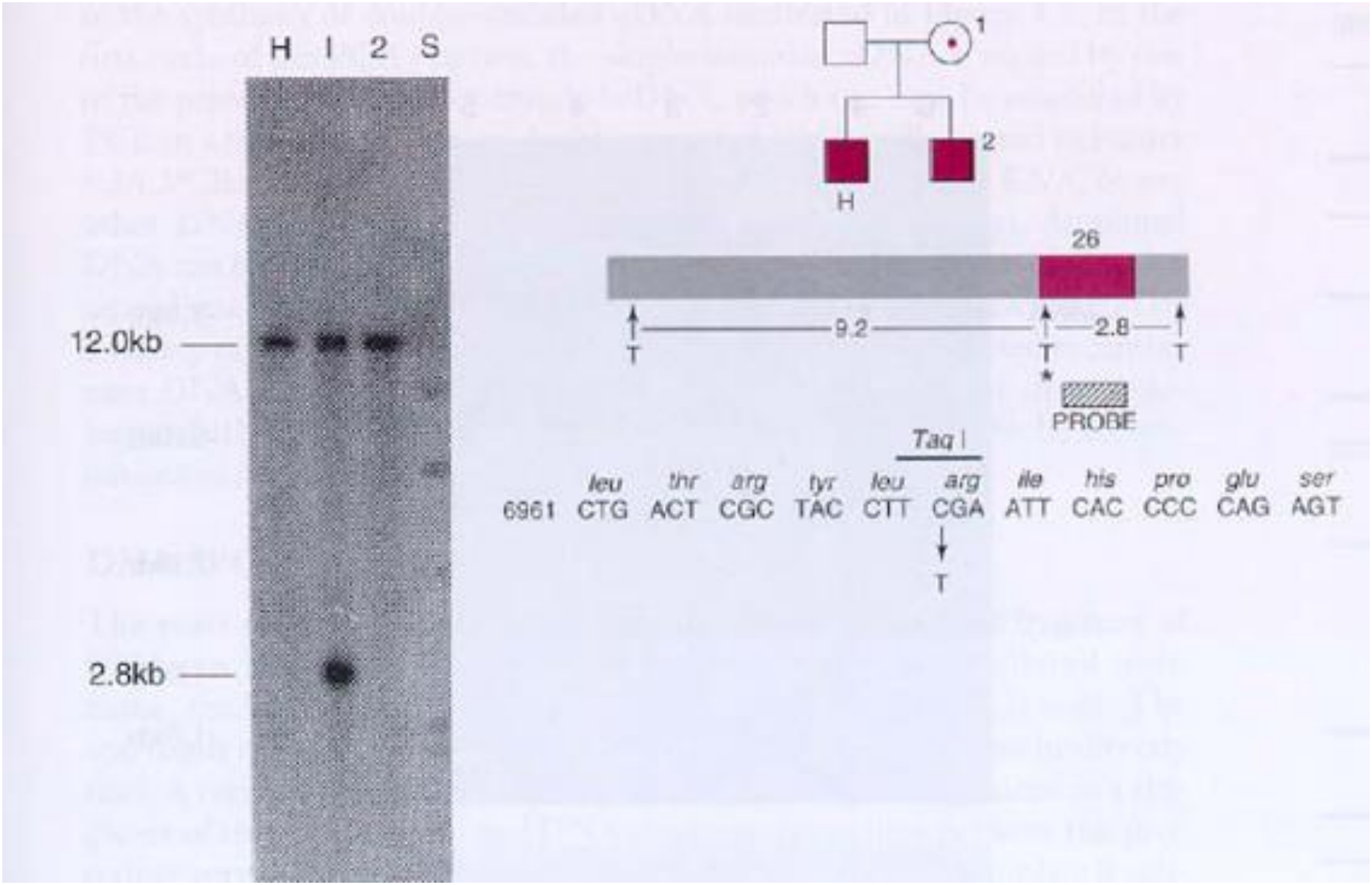
Restriction Fragment Length Polymorphism {RFLP} Mapping


P2
P1
300 bp
100 bp
2 kb
3 kb
...GAATTC...
...GAATTC...
...GAATTC...
A
CHROMOSOME
...GAATTC...
...GAATTT...
...GAATTC...
B
5 kb
PROBE 1
PROBE 2
PROBE 3
PROBE 1
PROBE 2
PROBE 3
PCR GEL
AA AB BB
5 kb
3 kb
2 kb
400 bp
300 bp
200 bp
100 bp

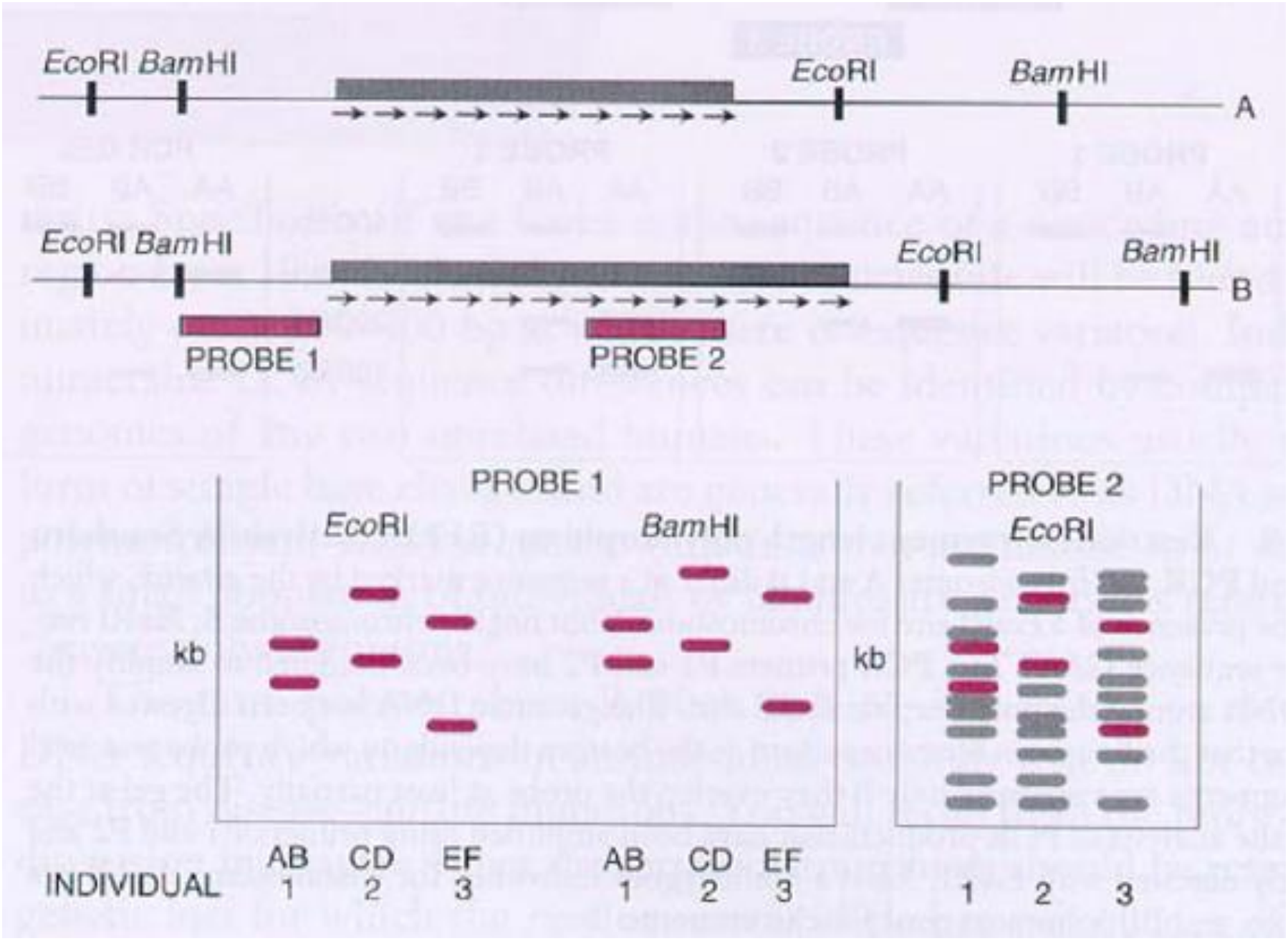

MstII restriction sites around β-globin gene
5'
3'
lost in sickle cell
fragments
1.2kbp
1.4kbp


Possible RFLP Data
Normal
Carrier
Sickle Cell
1.4kbp
1.2kbp

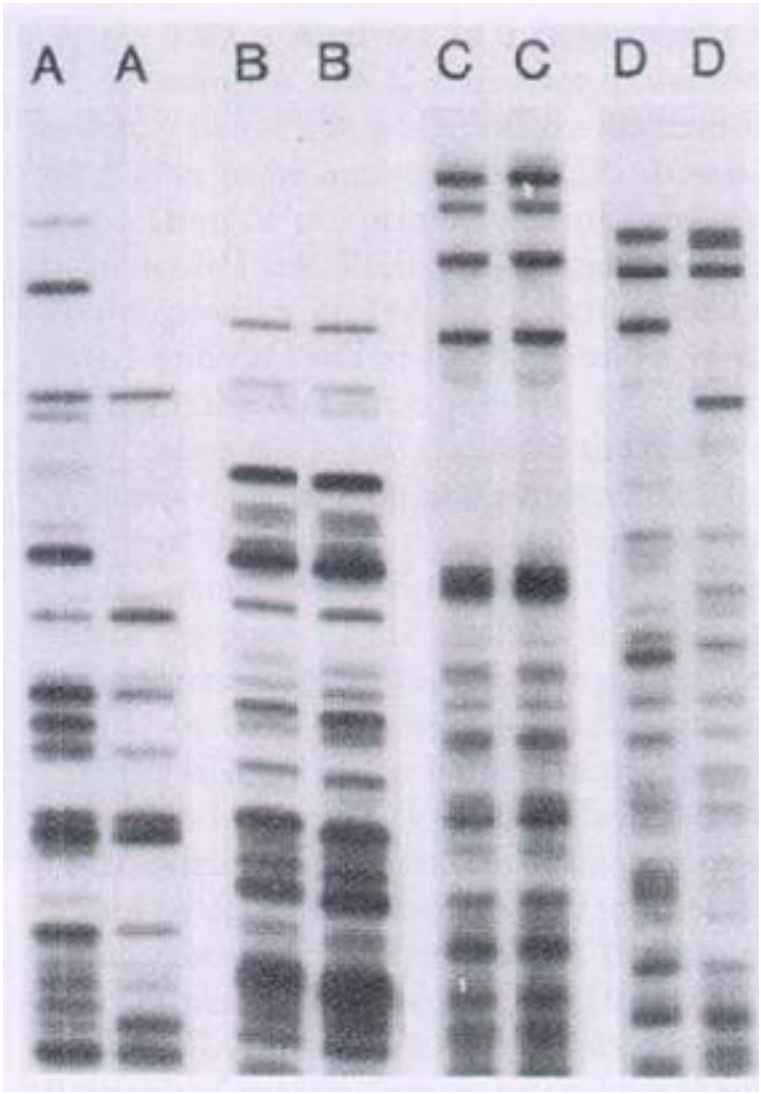
Detection of Hemophilia A Mutation by Southern Blotting



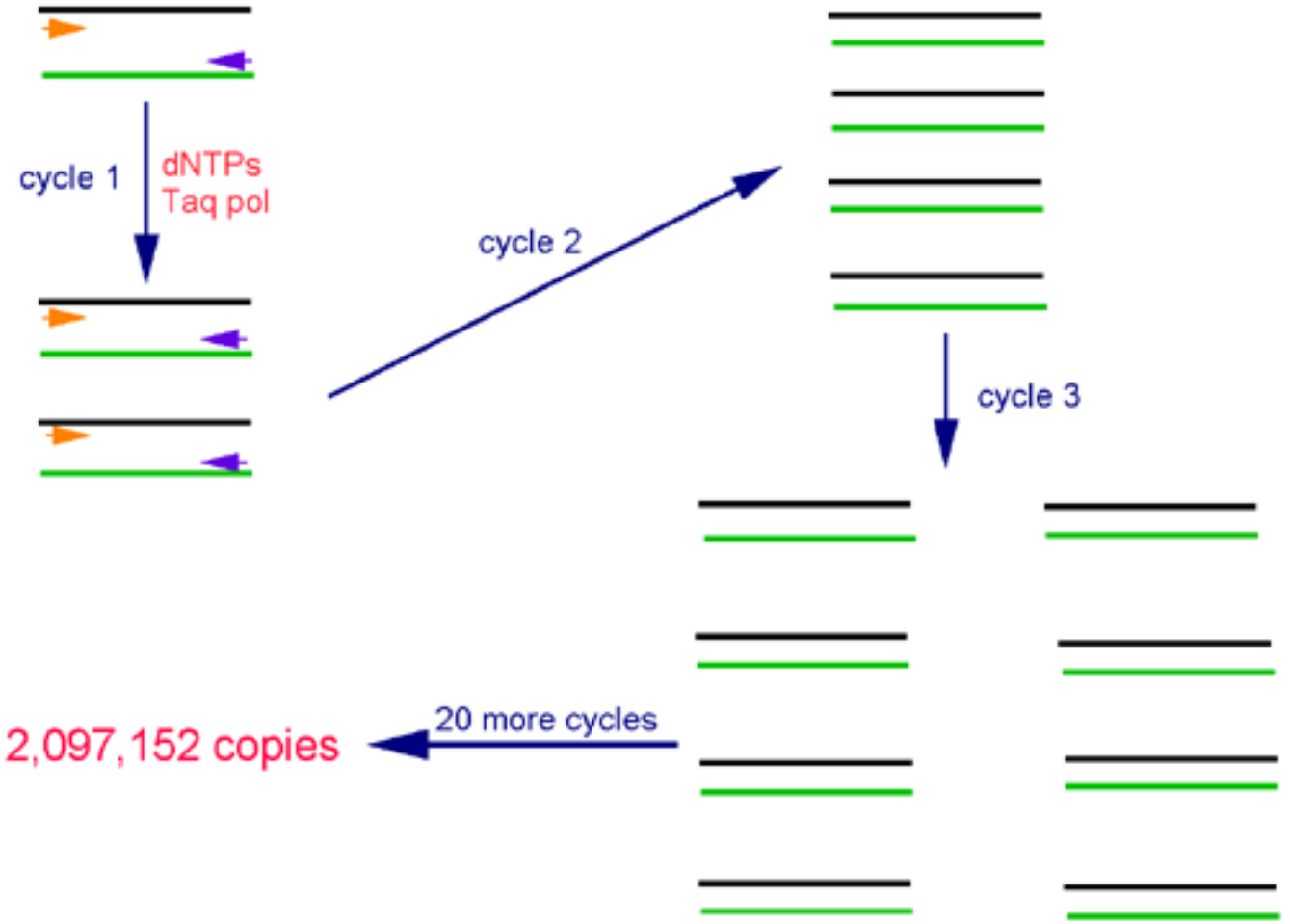
Variable Number Tandem Repeat {VNTR} Polymorphism Detection

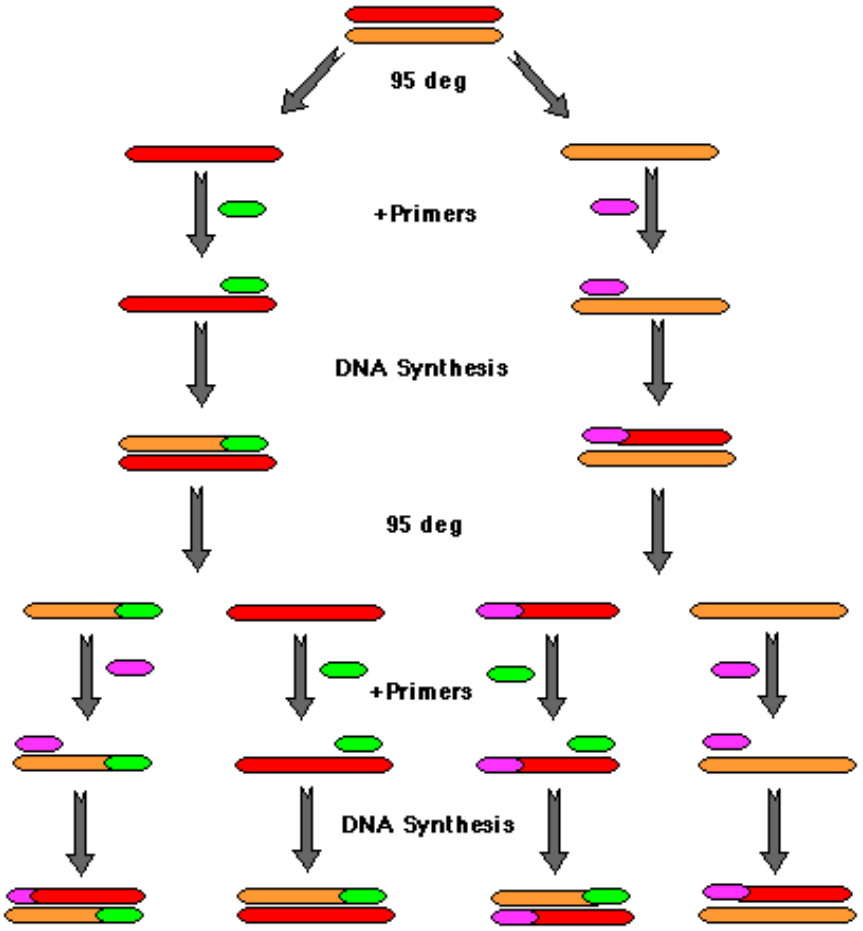

EcoRI BamHI
EcoRI
BamHI
A
EcoRI BamHI
EcoRI
BamHI
B
PROBE 1
PROBE 2
PROBE 1
EcoRI
BamHI
kb
AB
CD
EF
AB
CD
EF
INDIVIDUAL
1
2
3
1
2
3
PROBE 2
EcoRI
kb
1
2
3

VNTR Fingerprinting with a Single Probe

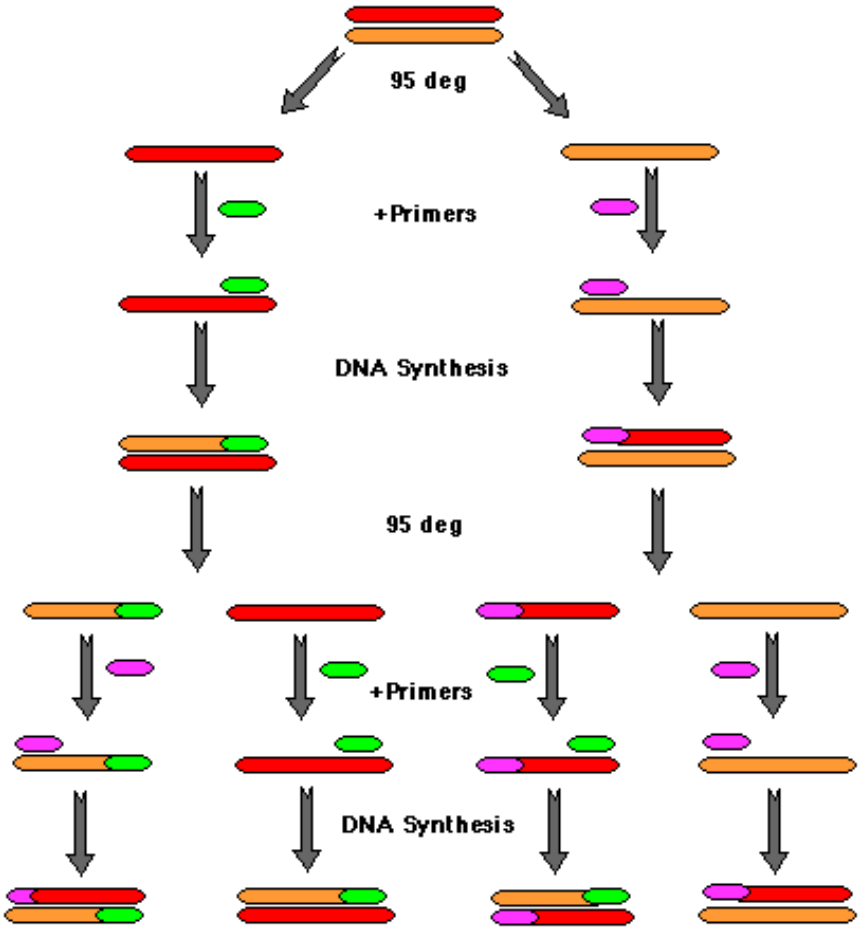


The PCR and Related Techniques


cycle 1
dNTPs
Taq pol
cycle 2
cycle 3
20 more cycles
2,097,152 copies



- Απαιτείται ένα προϋπάρχον DNA για να αντιγραφεί
  - Δεν μπορεί να συναρμολογήσει έναν νέο κλώνο από τα συστατικά του
  - Λέγεται πρότυπο DNA
- Μπορεί μόνο να επεκτείνει προϋπάρχοντα τμήματα DNA
  - Λέγονται εκκινητές



- A pre-existing DNA is required to replicate
  - It cannot assemble a new clone from its components
  - It's called template DNA
- It can only extend pre-existing DNA segments
  - They are called primers

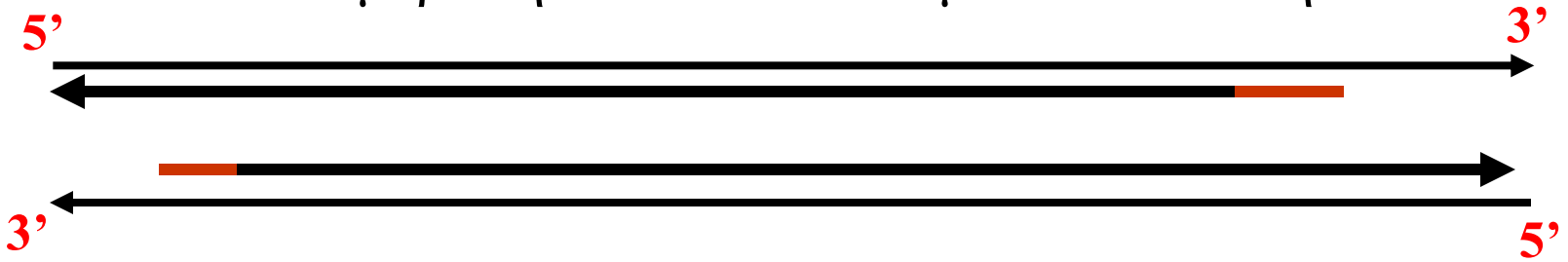
Κριτήρια επιλογής εκκινητών

- Βέλτιστο μήκος 20-26 βάσεις (bp)
- περιεκτικότητα σε βάσεις G, C 40-60%
- αποφυγή συμπληρωματικών αλληλουχιών εντός του κλώνου των εκκινητών, ειδικά στο 3’ άκρο
- αποφυγή συμπληρωματικών αλληλουχιών των εκκινητών με μη επιθυμητές αλληλουχίες DNA
- απόρριψη των εκκινητών που έχουν ομολογία με ανεπιθύμητες περιοχές άνω του 70%
- αποφυγή επανάληψης των G και C στο 3’ άκρο των εκκινητών (πχ GCCCC, GGGG)

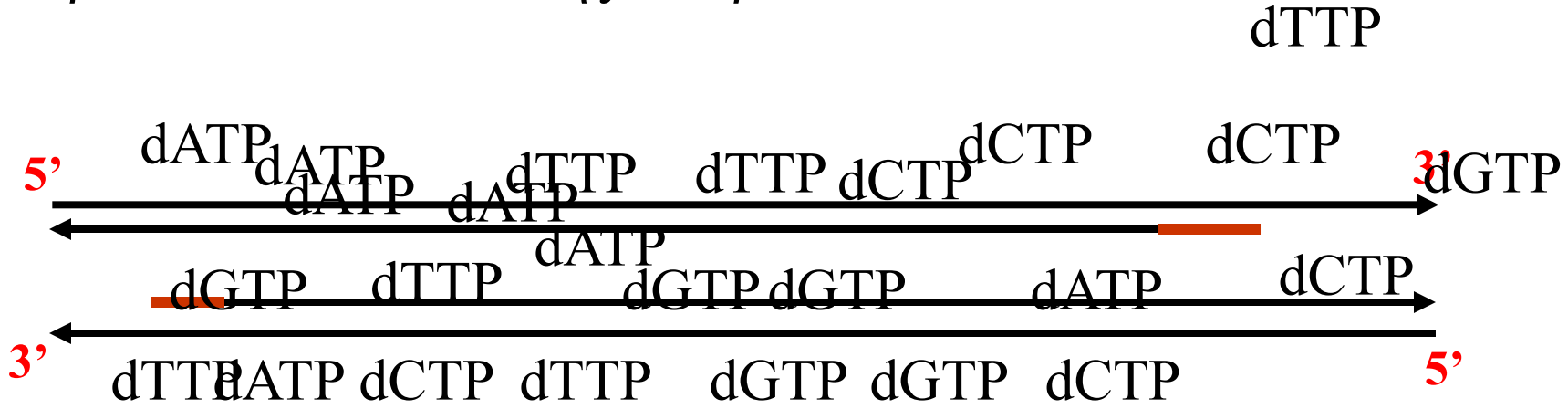
Criteria for the primers

- Optimal length 20-26 bases (bp)
- G, C content 40-60%
- avoidance of complementary sequences within the primer strand, especially at the 3' end
- avoiding complementary sequences of primers with undesired DNA sequences
- discard primers that have homology with unwanted regions above 70%
- avoiding repetition of G and C at the 3' end of primers (eg GCCCC, GGGG)

Η DNA πολυμεράση πάντα κινείται με κατεύθυνση 5’ → 3’

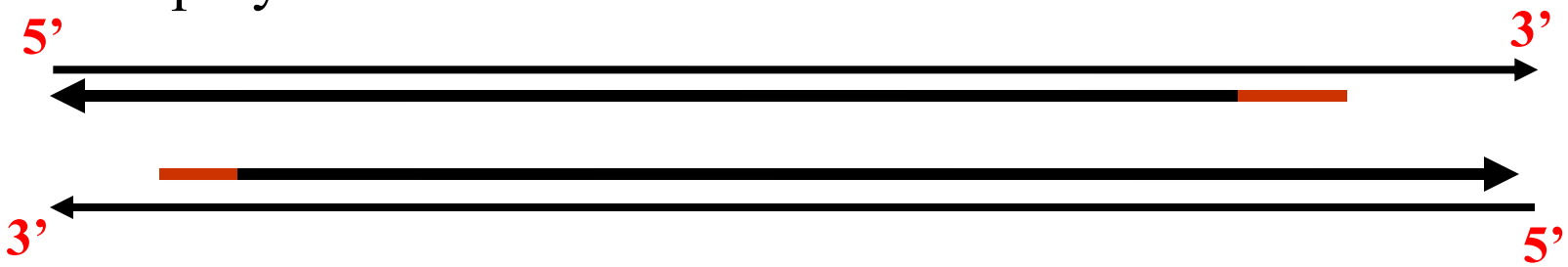


Η DNA πολυμεράση ενσωματώνει τα 4 δεοξυνουκλεοτίδια (A, T, G, C) στην αναπτυσσόμενη αλυσίδα σύμφωνα με τον πρότυπο κανόνα ένωσης των βάσεων



- Οι νεοπαραγόμενοι DNA κλώνοι λειτουργούν σαν πρότυπο DNA για τον επόμενο κύκλο

DNA polymerase uses the direction 5’ → 3’

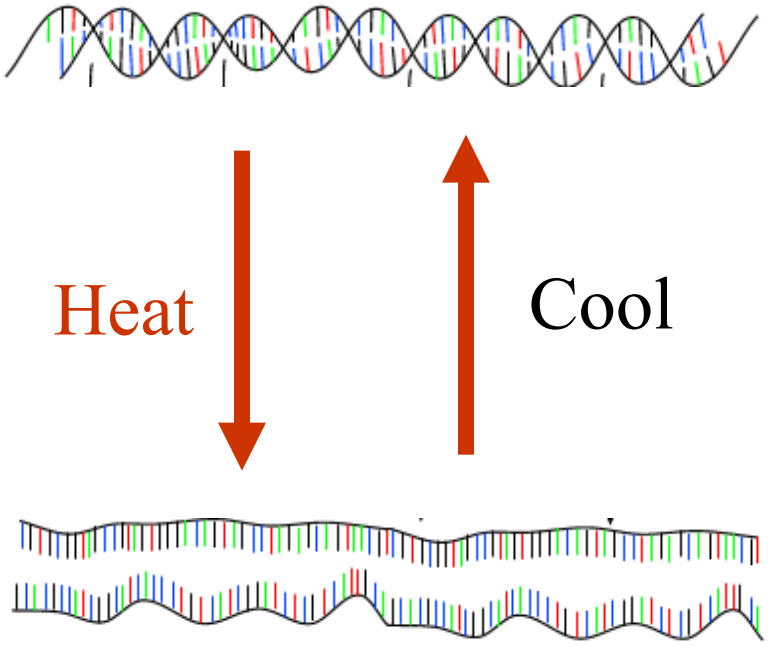


DNA polymerase incorporates A, T, G, C in the new strand according to hydrogen bonding

- The new DNA strands act as template DNA in the next PCR cycle

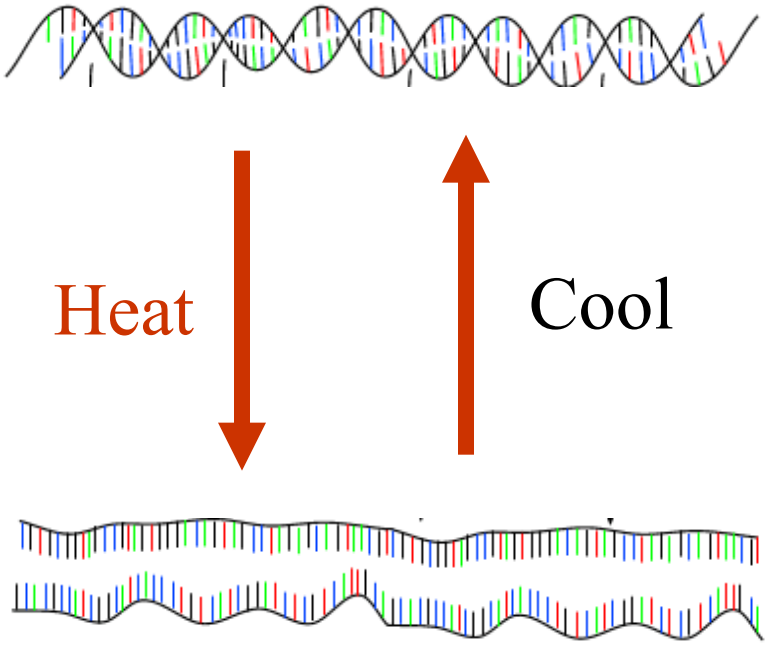
- Η συσκευή PCR ρυθμίζει τις θερμοκρασίες
- Η τυπική PCR πραγματοποιείται σε τρία βήματα
  - Αποδιάταξη
  - Επαναδιάταξη
  - Επέκταση

- Η θέρμανση χωρίζει το δίκλωνο DNA
  - Αποδιάταξη
- Αργή ψύξη επαναδιατάσσει τους δύο κλώνους
  - Επαναδιάταξη

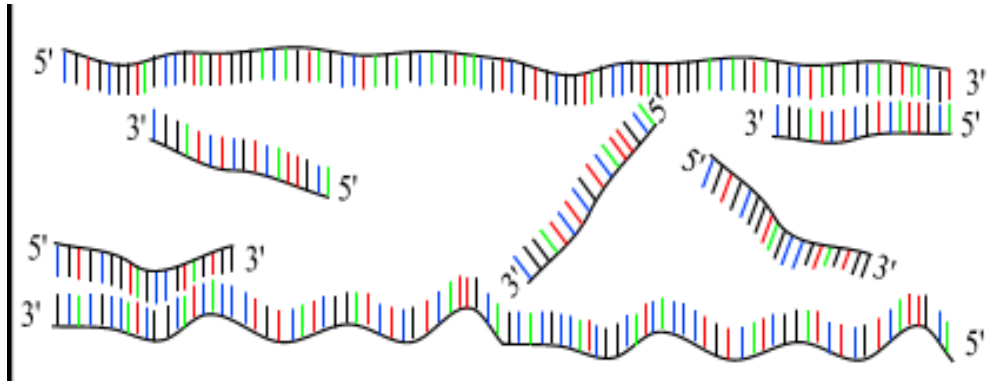


- PCR thermal cycler regulates the temperature of each step of the cycle
- A typical PCR is divided to three steps
  - Denaturation
  - Annealing
  - Elongation

- Increase in temperature denatures double-stranded DNA
  - Denaturation
- Mild cooling anneals the two strands
  - Annealing



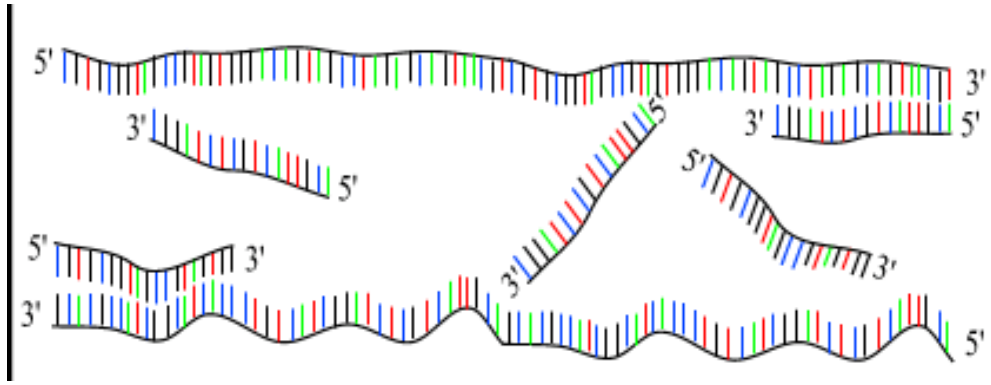
Επαναδιάταξη



- Οι δύο εκκινητές εφαρμόζονται σε μοριακή περίσσεια
- Σύνδεση των συμπληρωματικών περιοχών
- Καθώς το DNA ψύχεται, παρεμβάλλονται μεταξύ των δύο προτύπων κλώνων
- Η βέλτιστη θερμοκρασία ποικίλει ανάλογα με το μήκος του εκκινητή κ.λ.π.

Τυπική θερμοκρασία από 40 έως 60^{o}C

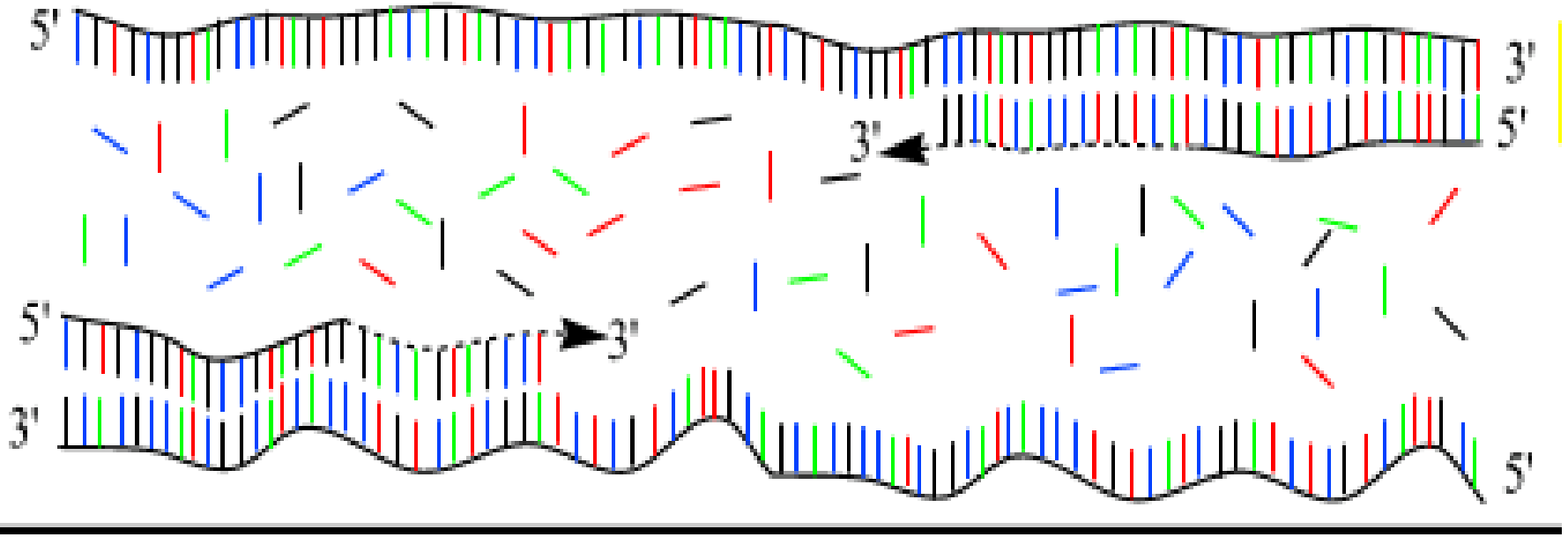
Annealing



- Both primers are applied in molar excess
- Connection of complementary areas
- As the DNA cools, primers are easily interacted with the two template strands
- The optimum temperature varies depending on the length of the starter etc.
- Typical temperatures are between 40 and 60°C

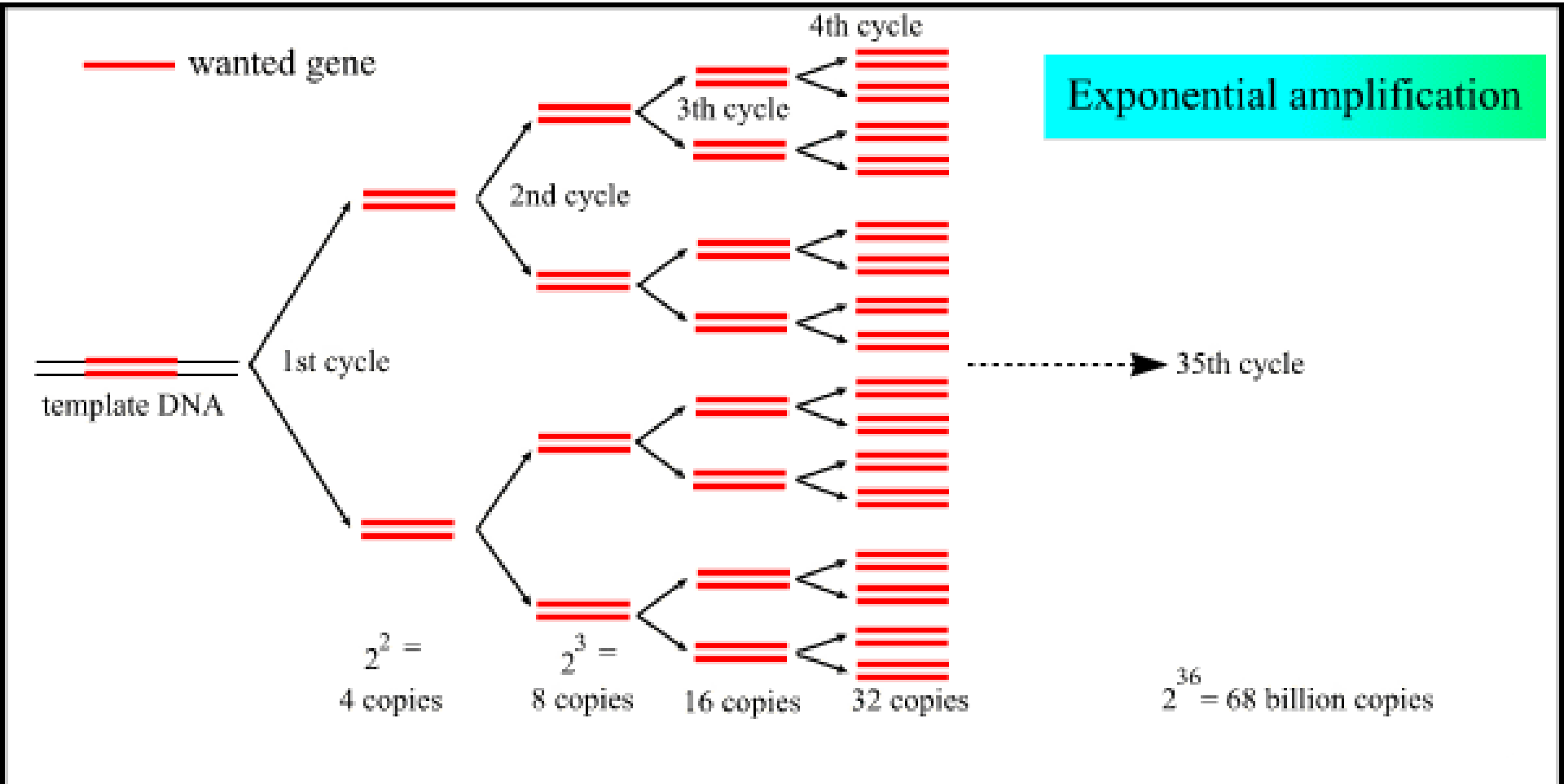
Elongation

- DNA polymerase replicates DNA
- Optimum temperature 72°C

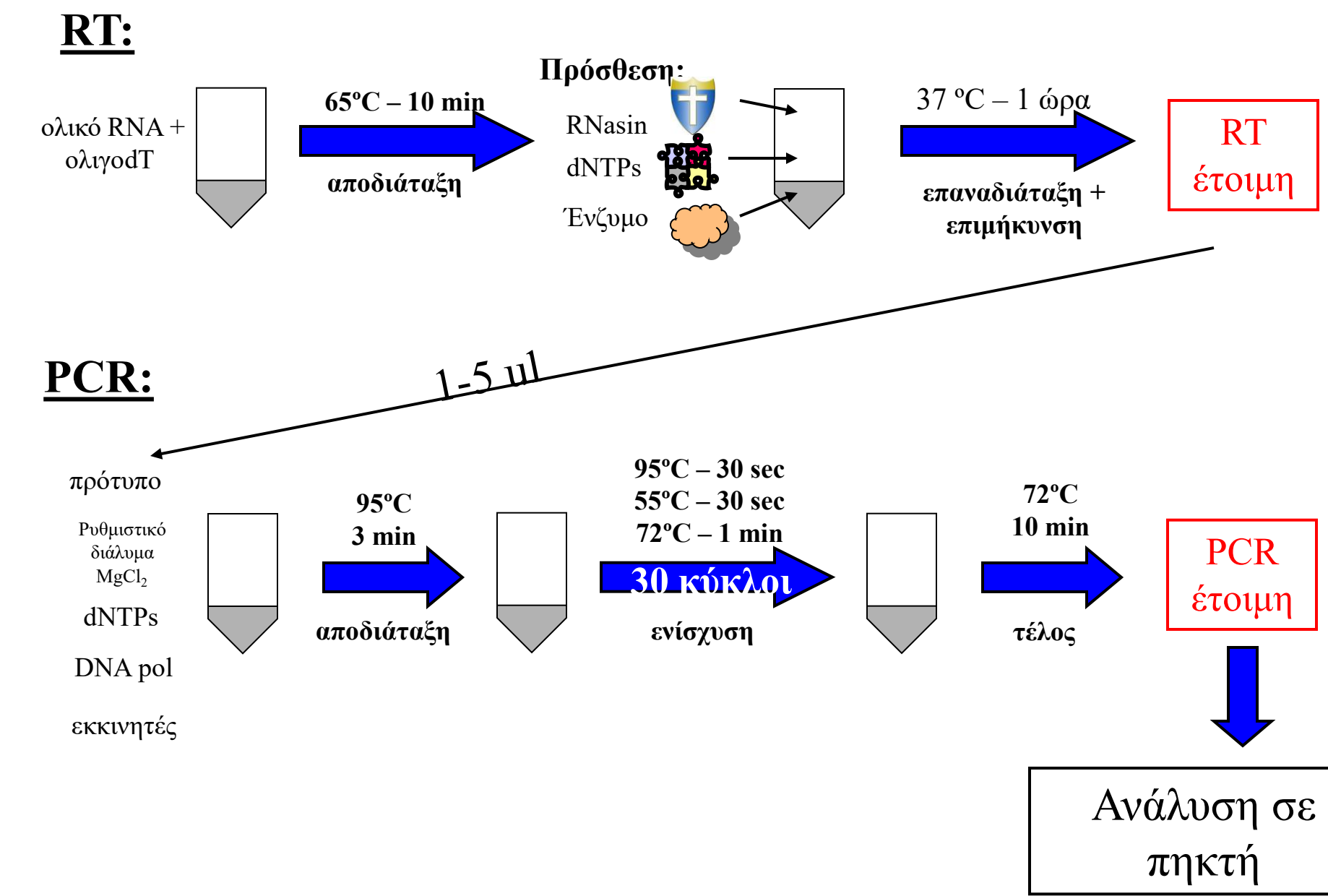


PCR Amplification

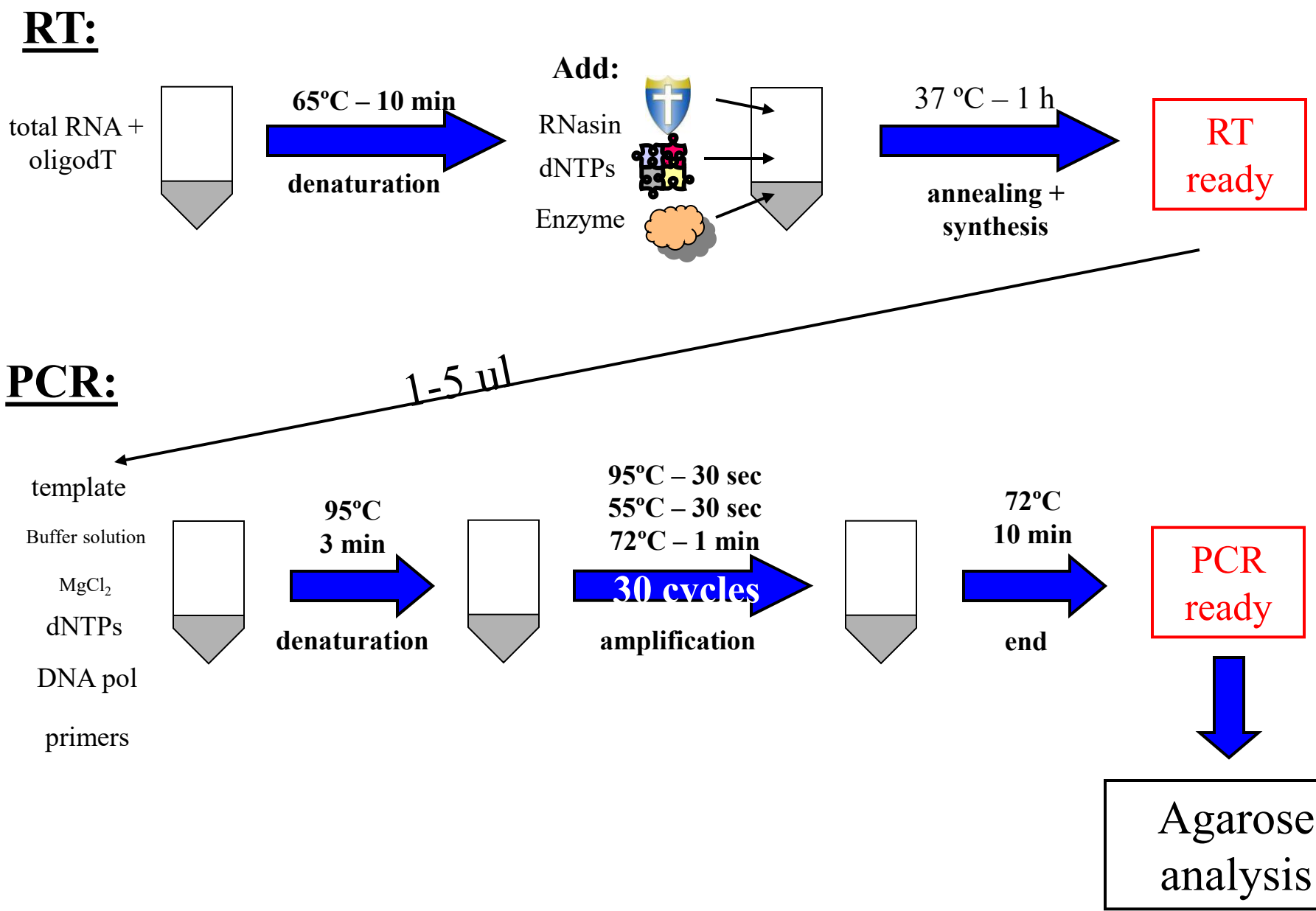
Exponential amplification of DNA template



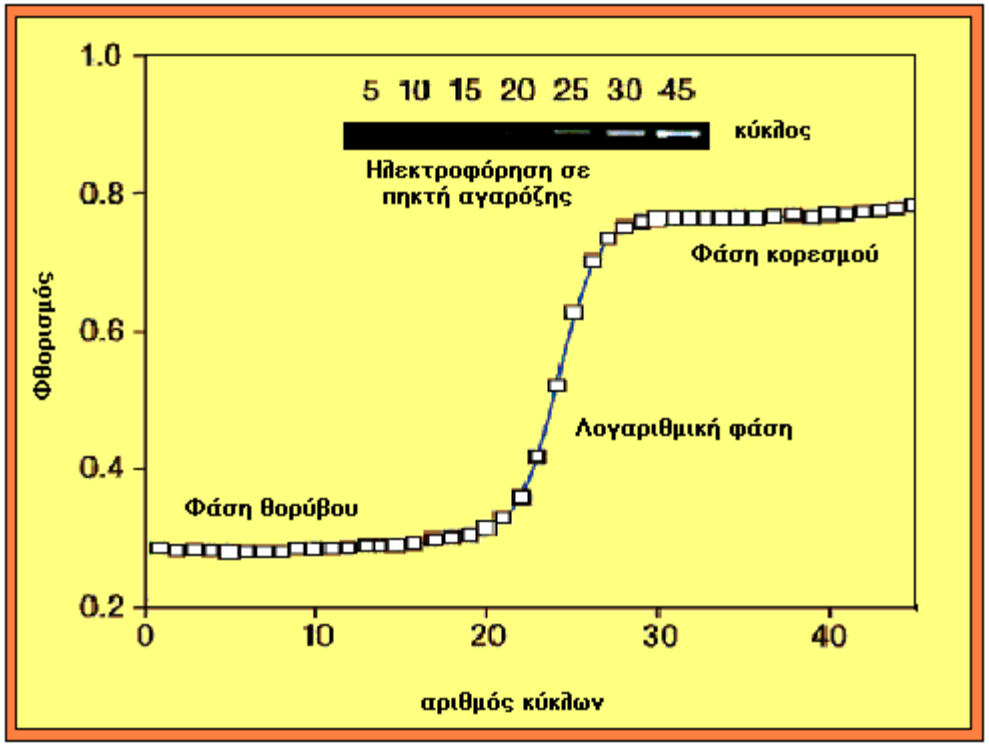
Τα βήματα της RT–PCR



RT–PCR steps


RT:
total RNA +
oligodT
65ºC – 10 min
denaturation
Add:
RNasin
dNTPs
Enzyme
37 ºC – 1 h
annealing +
synthesis
RT
ready
1-5 ul
PCR:
template
Buffer solution
MgCl2
dNTPs
DNA pol
primers
95ºC
3 min
denaturation
95ºC – 30 sec
55ºC – 30 sec
72ºC – 1 min
30 cycles
amplification
72ºC
10 min
end
PCR
ready
Agarose
analysis

Φάση κορεσμού (plateau phase)



Major variants of PCR

- PCR αντίστροφης μεταγραφής (Reverse transcription-PCR, RT-PCR)
- Ασύμμετρη PCR (Asymmetric PCR)
- Διπλή PCR (Nested PCR)
- Πολλαπλή PCR (Multiplex PCR)
- PCR-ELISA
- PCR σε πραγματικό χρόνο (Real time PCR)

Asymmetric PCR

- Single-stranded DNA is produced
- It is used for DNA-sequencing
- Non-equal (asymmetric) amounts of primers are used (ratios may be 1:10, 1:2, 1:50)
- In the first15-25 cycles, most of the product is double-stranded
- Then, as the primer of lower amount runs out, the main product of the next cycles is single-stranded
- single-stranded DNA accumulates linearly

Διπλή PCR (nested PCR)

- It is used to increase the specificity of the PCR reaction
- two PCRs where different primer pairs are used (outer and inner)
- the inner pair hybridizes to a region amplified by the other
- in the first round of PCR, the outer pair of primers is used
- in the second round, fragments containing the target sequence are selectively amplified

Πολλαπλή PCR (Multiplex PCR)

- different primer pairs are used to simultaneously amplify multiple regions of the target sequence

- is used to diagnose genetic diseases caused by genetic deletions of genes, such as Duchenne muscular dystrophy

- much simpler than Southern blot

Αλυσιδωτή αντίδραση της πολυμεράσης σε πραγματικό χρόνο (Real time PCR)

Ποσοτική PCR (qPCR-quantitative PCR)

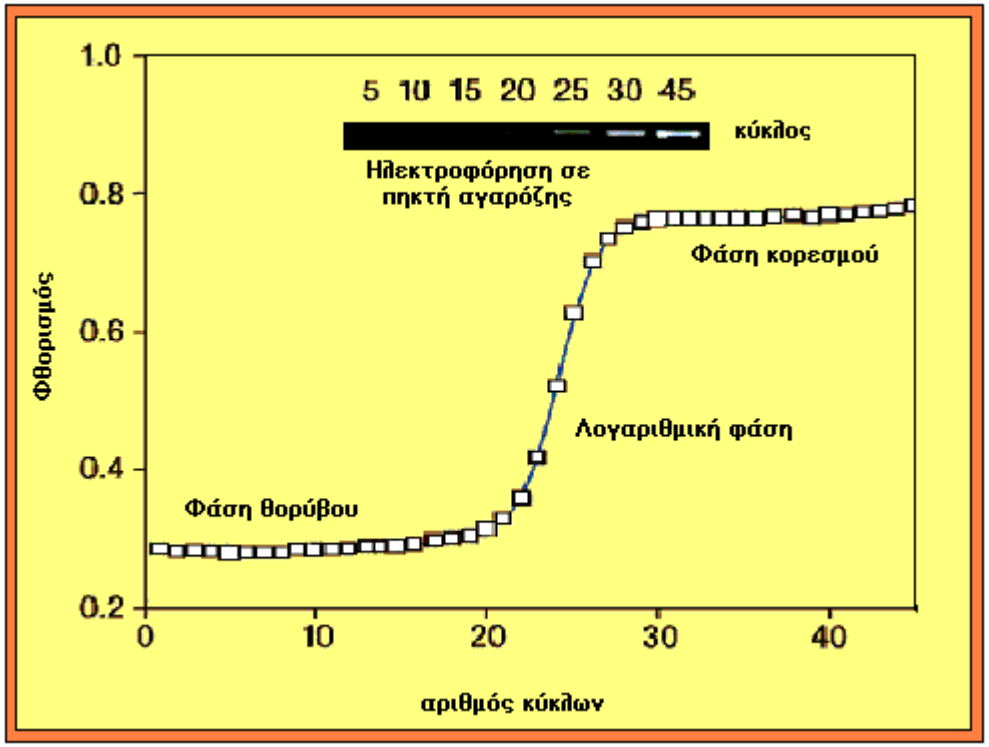
Αρχή ποσοτικού προσδιορισμού – Quantitative determinations

Quantitative PCR makes it possible to monitor the PCR reaction during its progress

Advantages:

- Automation
- Ability to quantify
- Avoiding electrophoresis
- Avoiding contaminations
- High sensitivity
- Suitable for large number of samples

Αλυσιδωτή αντίδραση της πολυμεράσης σε πραγματικό χρόνο (Real time PCR)

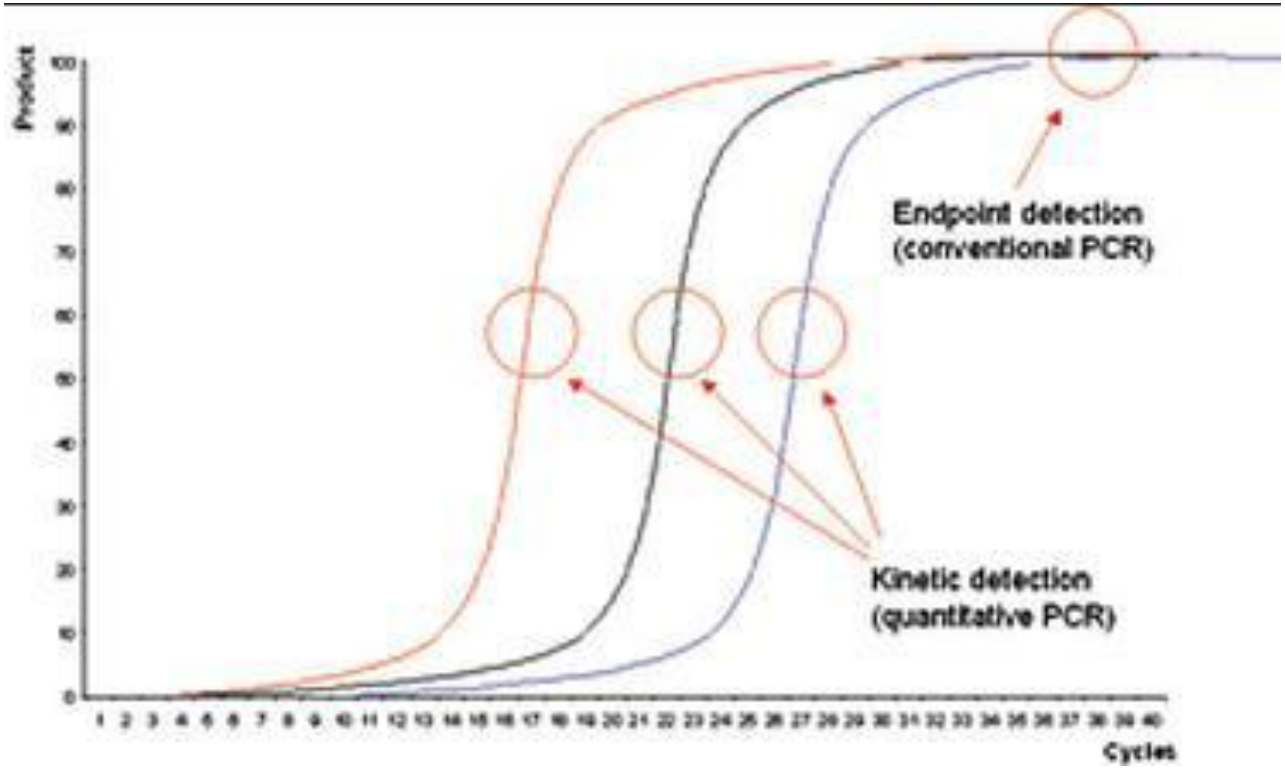


Tracking the PCR products with fluorescent molecules and measuring the fluorescence intensity emitted during the reaction

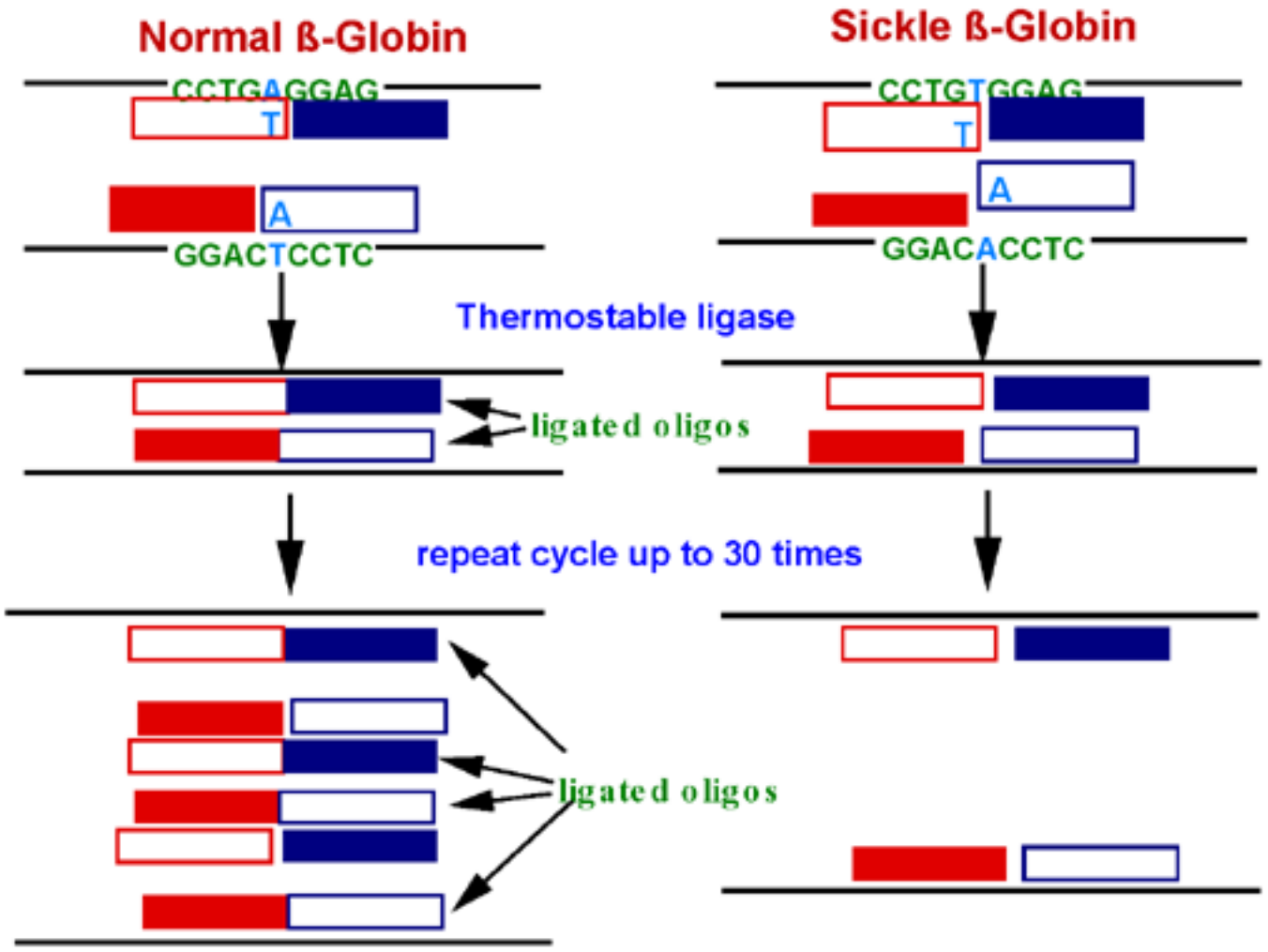
Αλυσιδωτή αντίδραση της πολυμεράσης σε πραγματικό χρόνο (Real time PCR)

Ποσοτική PCR (qPCR)

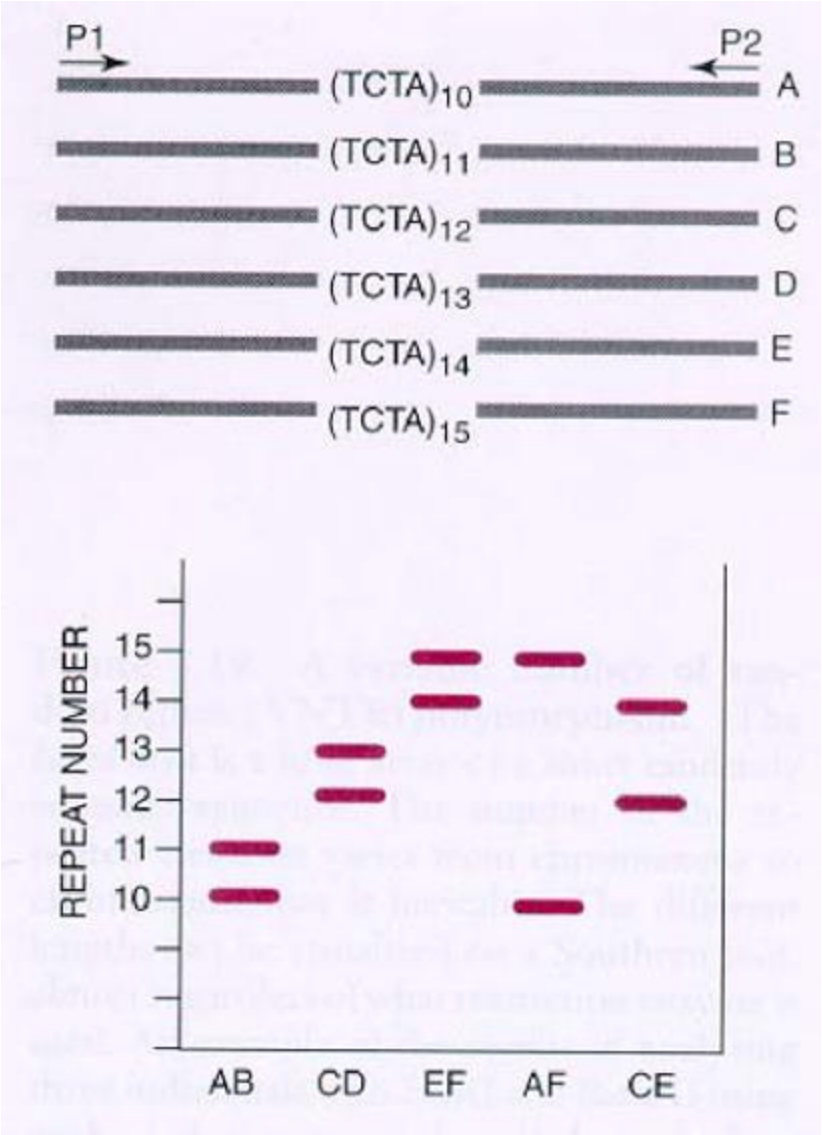
Αρχή ποσοτικού προσδιορισμού – Quantitative determinations



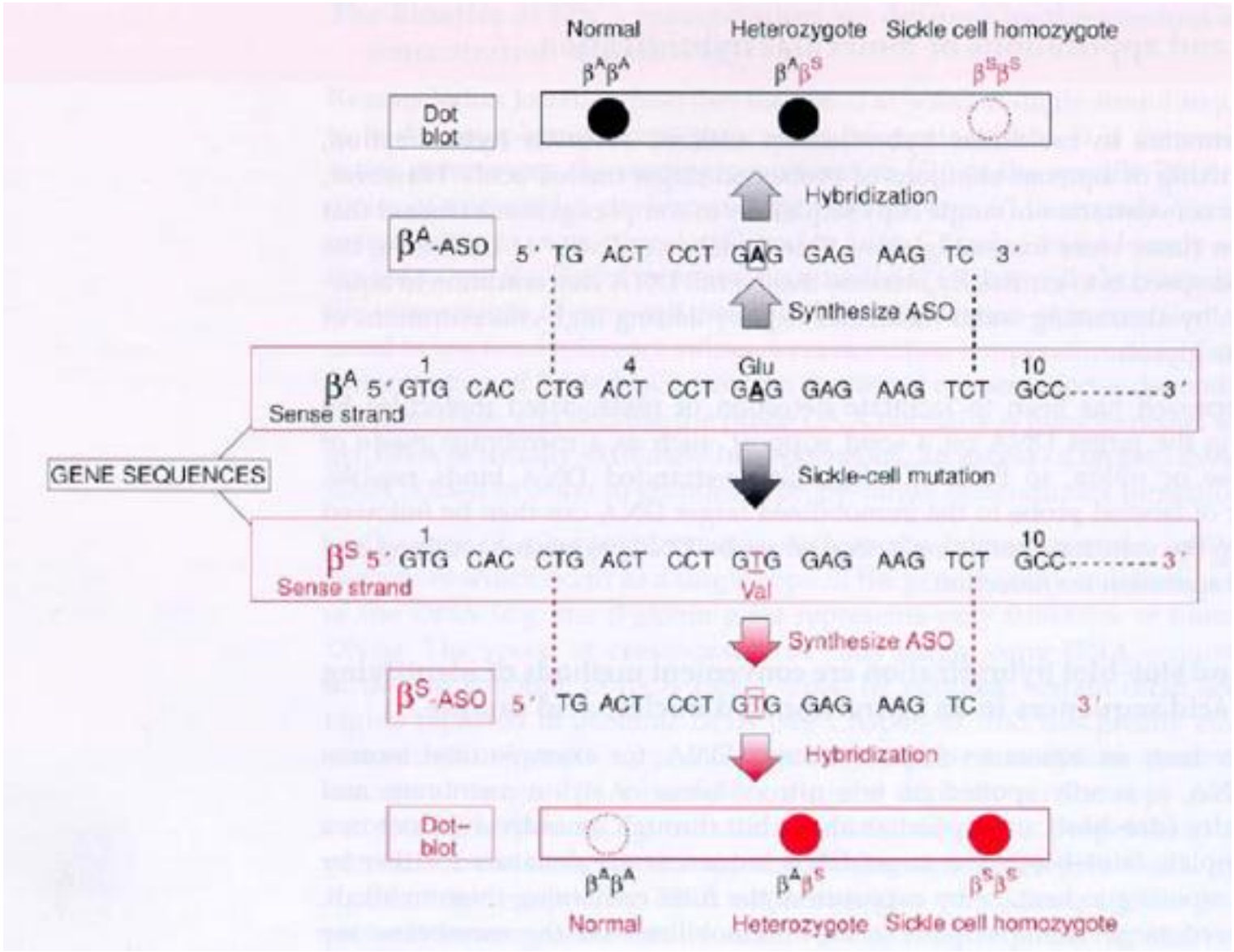
Ligase Chain Reaction, LCR


Normal ß-Globin
CCTGAGGAG
T
A
GGACTCCTC
Sickle ß-Globin
CCTGTGGAG
T
A
GGACACCTC
Thermostable ligase
ligated oligos
repeat cycle up to 30 times
ligated oligos

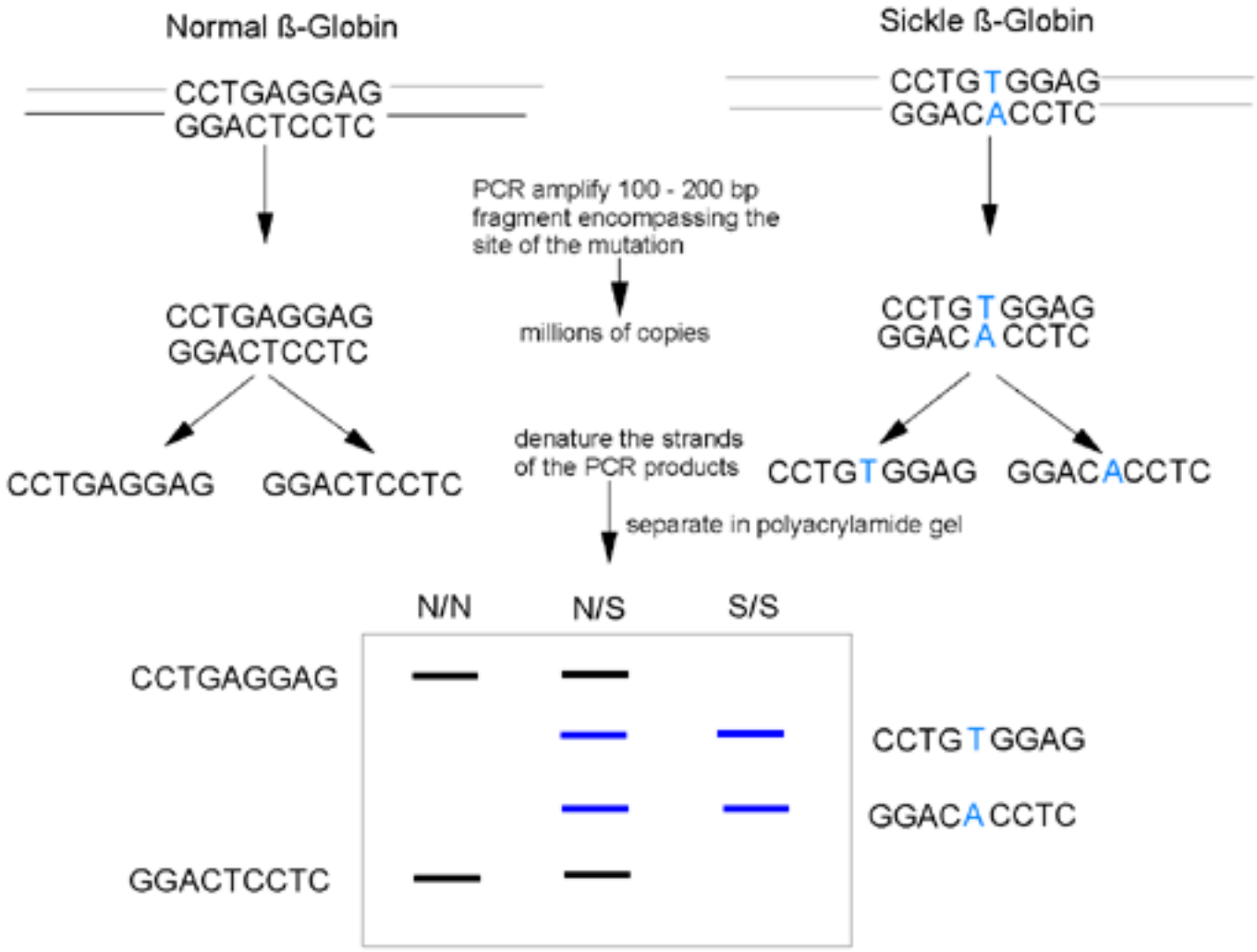
Simple Sequence Repeat {SSR} Polymorphisms


P1
P2
(TCTA)10
A
(TCTA)11
B
(TCTA)12
C
(TCTA)13
D
(TCTA)14
E
(TCTA)15
F
REPEAT NUMBER
15
14
13
12
11
10
AB
CD
EF
AF
CE

Allele-Specific Oligonucleotide {ASO} Mapping


Normal
Heterozygote
Sickle cell homozygote
$\beta^A\beta^A$
$\beta^A\beta^S$
$\beta^S\beta^S$
Dot blot
Hybridization
β^A-ASO
5' TG ACT CCT GAG GAG AAG TC 3'
Synthesize ASO
1 4 Glu 10
β^A 5' GTG CAC CTG ACT CCT GAG GAG AAG TCT GCC ..... 3'
Sense strand
GENE SEQUENCES
Sickle-cell mutation
1 10
β^S 5' GTG CAC CTG ACT CCT GTG GAG AAG TCT GCC ..... 3'
Sense strand
Val
Synthesize ASO
β^S-ASO
5' TG ACT CCT GTG GAG AAG TC 3'
Hybridization
Dot-blot
$\beta^A\beta^A$
$\beta^A\beta^S$
$\beta^S\beta^S$
Normal
Heterozygote
Sickle cell homozygote

PCR-Single Strand Conformational Polymorphism, PCR-SSCP


Normal ß-Globin
CCTGAGGAG
GGACTCCTC
Sickle ß-Globin
CCTGTGGAG
GGACACCTC
PCR amplify 100 - 200 bp fragment encompassing the site of the mutation
millions of copies
CCTGAGGAG
GGACTCCTC
CCTGTGGAG
GGACACCTC
CCTGAGGAG
GGACTCCTC
denature the strands of the PCR products
CCTGTGGAG
GGACACCTC
separate in polyacrylamide gel
N/N
N/S
S/S
CCTGAGGAG
GGACTCCTC
CCTGTGGAG
GGACACCTC

Gene arrays

- Gene arrays are solid supports upon which a collection of gene-specific nucleic acids have been placed at defined locations, either by spotting or direct synthesis
- In array analysis, a nucleic acid-containing sample is labeled and then allowed to hybridize with the gene-specific targets on the array
- Based on the amount of probe hybridized to each target spot, information is gained about the specific nucleic acid composition of the sample
- The major advantage of gene arrays is that they can provide information on thousands of targets in a single experiment.

Experimental characteristics

- The solid supports upon which nucleic acids are arrayed are either glass slides or nylon membranes
- Fluorescently labeled probes are used with glass arrays, while radiolabeled probes are used with membranes
- The arrayed nucleic acids may be composed of oligonucleotides, PCR products or cDNA vectors or purified inserts
- The sequences may represent entire genomes and may include both known and unknown sequences or may be collections of sequences such as apoptosis-related genes or cytokines

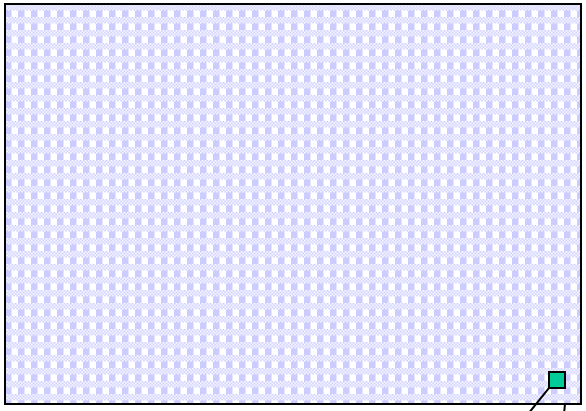
Experimental characteristics

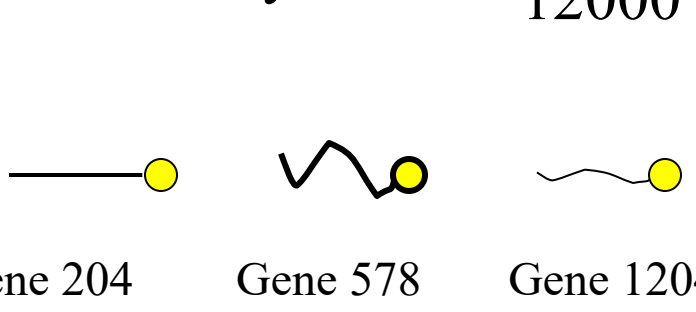
- Many terms exist for naming gene arrays, including biochip, DNA chip, GeneChip®, DNA array, microarray and macroarray
- Generally when biochip, DNA chip or GeneChip is used, it refers to arrays on glass supports
- Microarray and macroarray may be used to differentiate between spot size or the number of spots on the support
- Gene arrays may be used for sequence identification (e.g. mutation analysis) or differential expression analysis of two or more RNA samples

DNA arrays

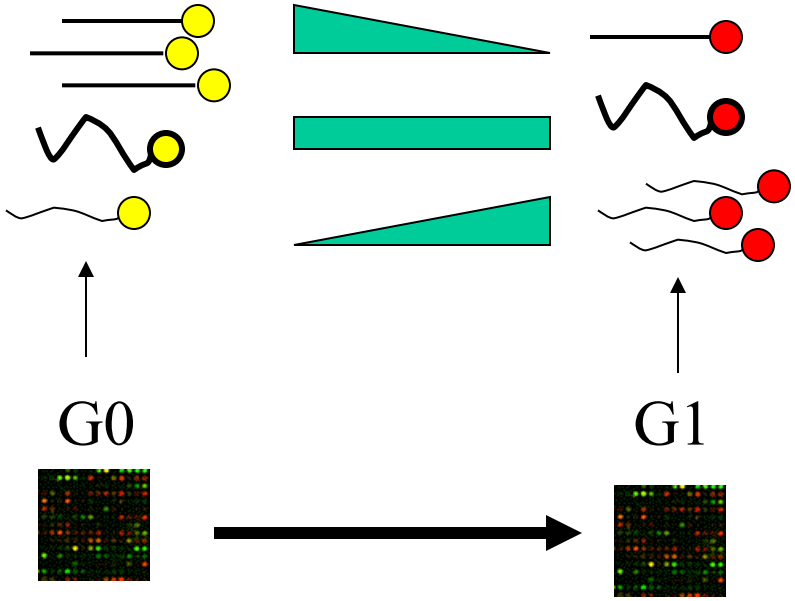
Gene arrays

12000 cDNA/oligo sets



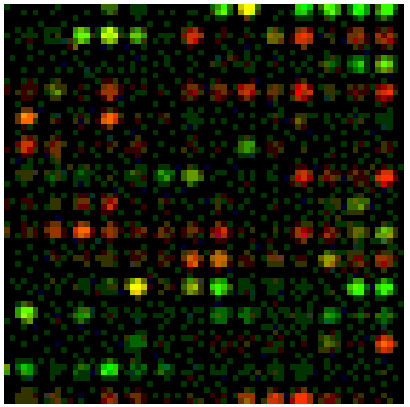


Gene 204 Gene 578 Gene 1204



G0 G1

- Induced
- Unchanged
- Repressed



Gene Arrays for Expression Analysis

- Gene arrays have become a powerful approach for comparing complex sample RNA populations
- Using array analysis, the expression profiles of normal and tumor tissues, treated and untreated cell cultures, developmental stages of an organism or tissue, and different tissues can be compared
- A typical gene array experiment involves:

- Isolating RNA from the samples to be compared
- Converting the RNA samples to labeled cDNA via reverse transcription; this step may be combined with aRNA amplification
- Hybridizing the labeled cDNA to identical membrane or glass slide arrays
- Removing the unhybridized cDNA
- Detecting and quantitating the hybridized cDNA
- Comparing the quantitative data from the various samples

- Some array manufacturers offer custom analysis services and may perform the probe labeling and hybridization reactions as a service

Nylon Membrane Arrays

- Nylon membrane arrays are typically hybridized with ^{33}P-dNTP labeled probes and analyzed by a phosphorimager along with the appropriate software
- A different array must be used for each sample analyzed
- A typical experiment involves isolating RNA from two tissue or cell samples
- The RNAs are reverse transcribed using labeled nucleotides and target specific, oligo dT, or random-sequence primers to create two labeled cDNA populations
- The two cDNAs are hybridized to two identical arrays
- After washing, the hybridized signal on each array is detected and analyzed
- The signal emitting from each gene-specific spot is compared between the populations
- Genes expressed at different levels in two samples generate different amounts of labeled cDNA and this results in spots on the array with different amounts of signal

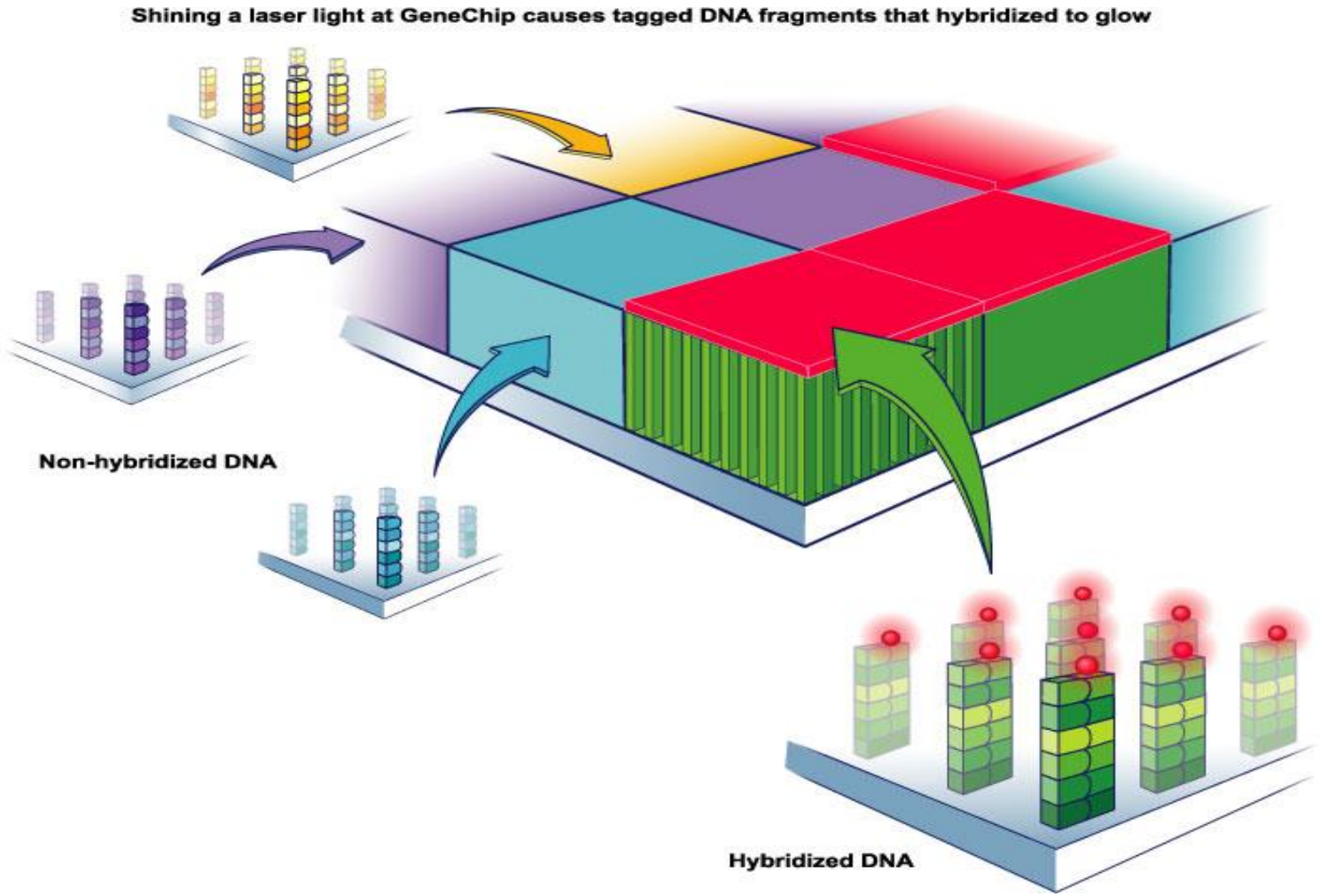
Glass Slide Arrays

- Glass slide arrays analysis involves the same steps, but rather than labeling with isotopes during reverse transcription, probes for glass arrays are labeled with two distinct fluorescently labeled nucleotides and both probes are hybridized to the same array
- Typically, one sample RNA is labeled with Cyanine 3-dNTP (Cy3) and the other with Cyanine 5-dNTP (Cy5)
- Each dye produces different color fluorescence
- The two labeled RNA populations are hybridized to one glass slide and scanned using a fluorescent imager

GeneChip

- Affymetrix's GeneChips are glass slide arrays manufactured using special photolithographic methods and combinatorial chemistry, which allow the oligonucleotide spots to be synthesized directly onto the array substrate
- The analysis procedure specifies that the RNA samples are converted to biotin-labeled cDNA, and each sample is hybridized to a separate GeneChip
- The hybridized cDNA is then stained with a streptavidin-phycoerythrin conjugate and visualized with an array scanner

Affymetrix model for DNA chip



Now, we can infer which of the genes were expressed and in what intensity.

Due to some biological processes, not always the correct sequence will hybridized to the oligo.

Data Analysis

- Where once the bottleneck in gene expression analysis was the benchwork, with array analysis, it is the computer work
- Because a single array experiment can generate thousands of data points, the primary challenge of the technique is making sense of the data
- Many commercial companies provide image analysis software, including BioDiscovery (ImaGene) and Imaging Research (ArrayVision)
- Furthermore, many array manufacturers offer software specifically for the analysis of their arrays and offer the analysis as a service
- For membrane array analysis, a file of the data is generated by phosphoimaging and that file is then analyzed using software
- The software will correlate spots to genes and can compare spot intensities for differential expression studies
- Glass array data is treated in much the same way, but the image's fluorescence is scanned and the software allows detection of each samples' fluorescence individually or simultaneously for analysis
- Most software packages can analyze several arrays simultaneously

Validation

- Differences in expression of specific sequences are often validated by another method of analysis such as RT-PCR, Northern analysis or nuclease protection assays
- These same methods can be used for relative or absolute quantitation of specific messages of interest identified by array analysis

Design of a DNA Microarray System

- There are several steps in the design and implementation of a DNA microarray experiment
- Many strategies have been investigated at each of these steps
  - DNA types
  - Chip fabrication
  - Sample preparation
  - Assay
  - Readout
  - Software (informatics)

Probe (cDNA/oligo with known identity)	**Chip** fabrication (Putting probes on the chip)	**Target** (fluorecently labeled sample)	**Assay**	**Readout**	**Informatics**
Small oligos, cDNAs, chromosome,…. (whole organism on a chip?)	Photolithography, pipette, drop-touch, piezoelectric (ink-jet), electric, ...	RNA, (mRNA==>) cDNA	Hybridization, long, short, ligase, base addition, electric, MS, electrophoresis, fluocytometry, PCR-DIRECT, TaqMan, ...	Fluorescence, probeless (conductance, MS, electrophoresis), electronic, ...	Robotics control, Image processing, DBMS, WWW, bioinformatics, data mining and visualization

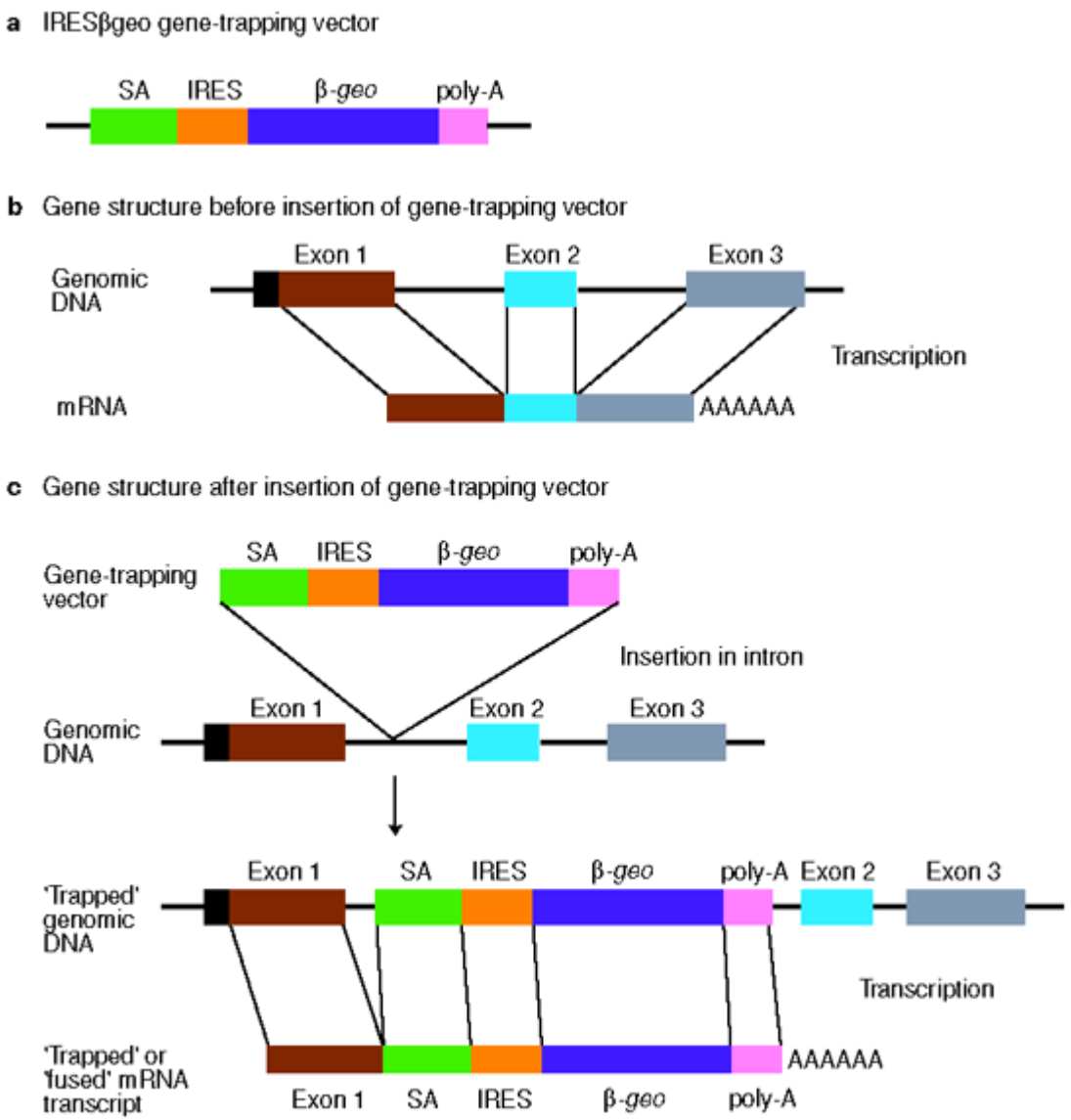
Applications of DNA Microarray Technology

- **Gene discovery** (Many, many applications)
- **Disease diagnosis** (Many, many applications)
- **Drug discovery: *Pharmacogenomics***
    - Why some drugs work better in some patients than in others? And why some drugs may even be highly toxic to certain patients?
    - *Pharmacogenomics* is the hybridization of functional genomics and molecular pharmacology. The goal of pharmacogenomics is to find correlations between therapeutic responses to drugs and the genetic profiles of patients.
- **Toxicological research: *Toxicogenomics***
    - *Toxicogenomics* is the hybridization of functional genomics and molecular toxicology. The goal of toxicogenomics is to find correlations between toxic responses to toxicants and changes in the genetic profiles of the objects exposed to such toxicants.

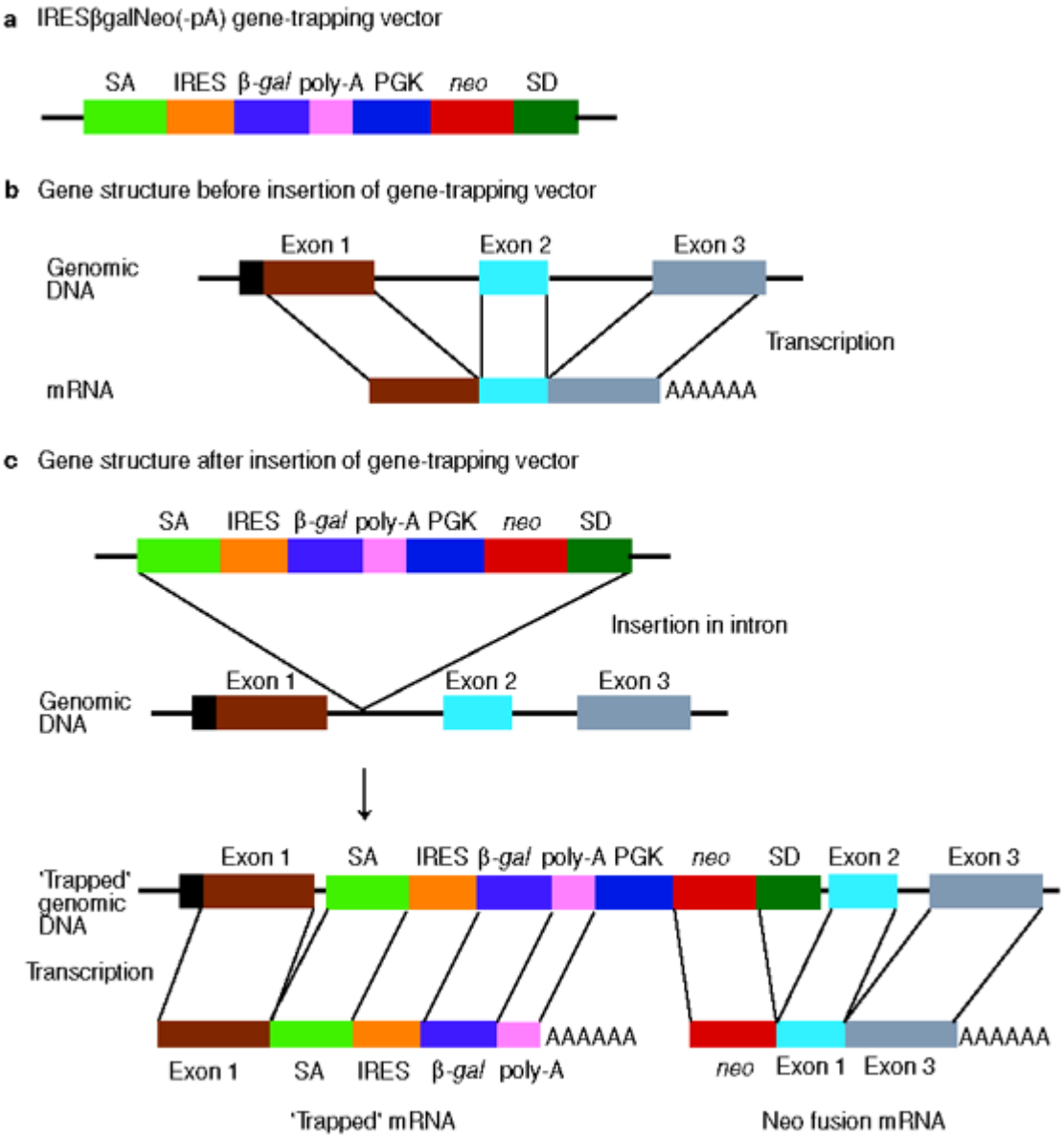
Searching the unknown with gene trapping

- A dramatic increase in sequence information, both in the form of complementary DNA (cDNA) and genomic DNA, has created a huge gap between the discovery of genes and the process of identifying gene function
- To fill this gap, the ‘gene-trapping’ approach has been developed; this combines into a single process the three stages of gene cloning, the study of the pattern of gene expression and the analysis of the respective mutant phenotype
- Recent results indicate that gene trapping can be used successfully to clone specific genes that are involved in the development of the central nervous system, limbs and haematopoietic system
- Continuous improvements in the design of trapping vectors, faster sequencing of cDNA clones and more-efficient in vitro prescreening will certainly aid the large-scale trapping of mammalian genomes

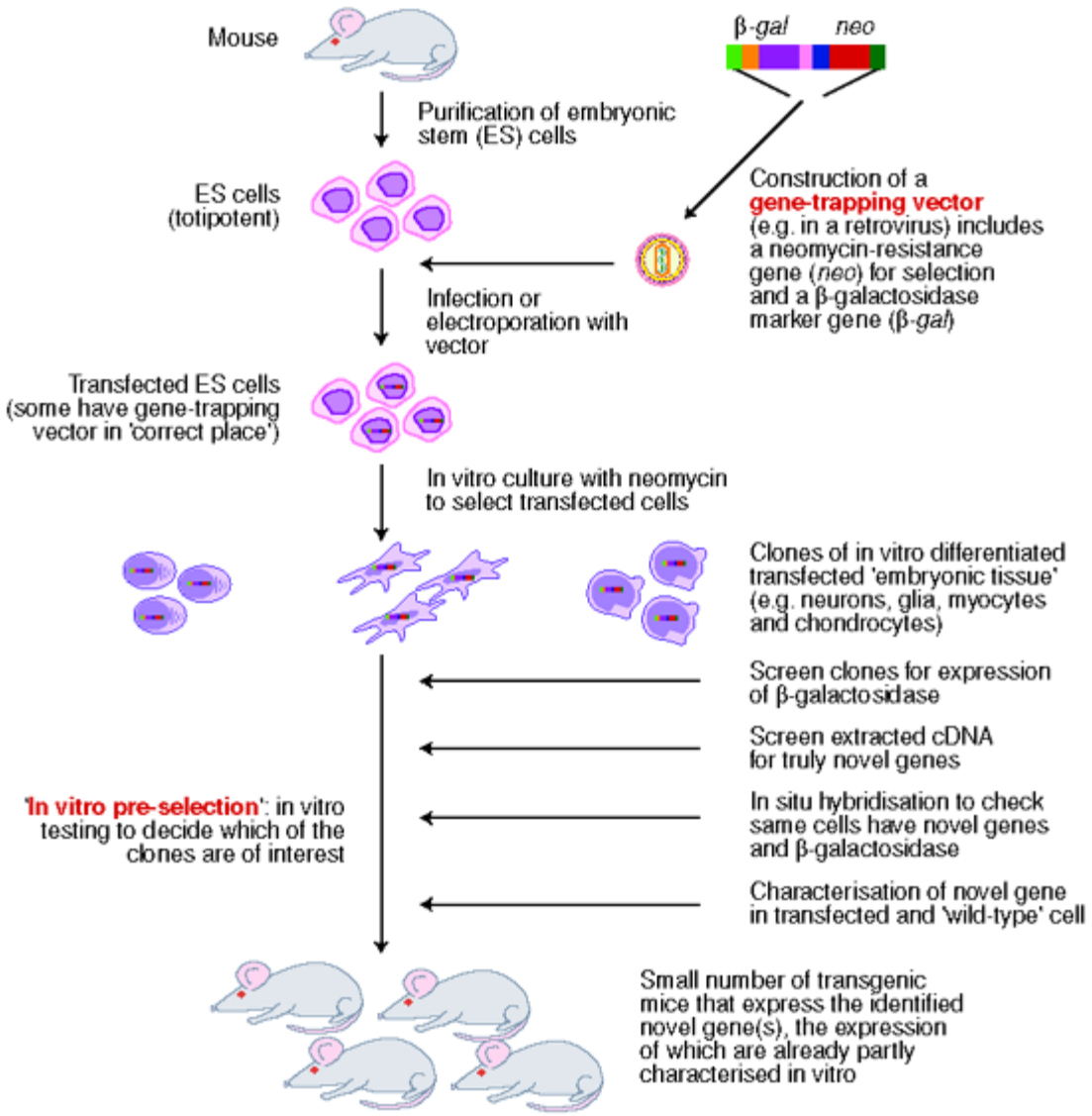
The IRESβgeo gene-trapping vector and gene-trapping strategy



The IRESβgalNeo(-pA) gene-trapping vector and gene-trapping strategy



Principles and advantages of using in vitro pre-selection of gene-trapped embryonic stem cells for characterising novel genes in the mouse



Gene Therapy
“Transient Transgenesis”

Targets for Gene Therapy

- Inherited disorders
  - replacement or prophylactic introduction of non-defective gene
  - utilizes cells of the affected organ
- Cancers
  - expression of immune system modulating molecules eg. TNF, IL-2
  - utilizes cells of the immune system

Delivery Systems

- Retroviral vectors
- Adenoviral vectors
- herpes virus (type I) vectors
- non-viral DNA-based vectors
- nanocarriers

Two Modes of Delivery

- In vivo
  - introduction of the vector directly into the affected tissue to be taken up by and expressed in those cells
- Ex vivo
  - remove cells from affected tissue, introduce vector to cells, reintroduce cells into affected tissue