Biotechnology in Molecular Biology

Dr Piyush B. Tailor

Associate Professor

Department of Biochemistry

Govt. Medical College, Surat

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What is Biotechnology?

With (10^9) DNA Base Pair & (20,000 to 30,000) Gene

- Restricted Endonuclease Enzyme
- DNA Fragement
- Identification of Gene
- Human Genomic Project
- Gene Library
- **Use of All of Above for Clinical – Diagnosis and Management**

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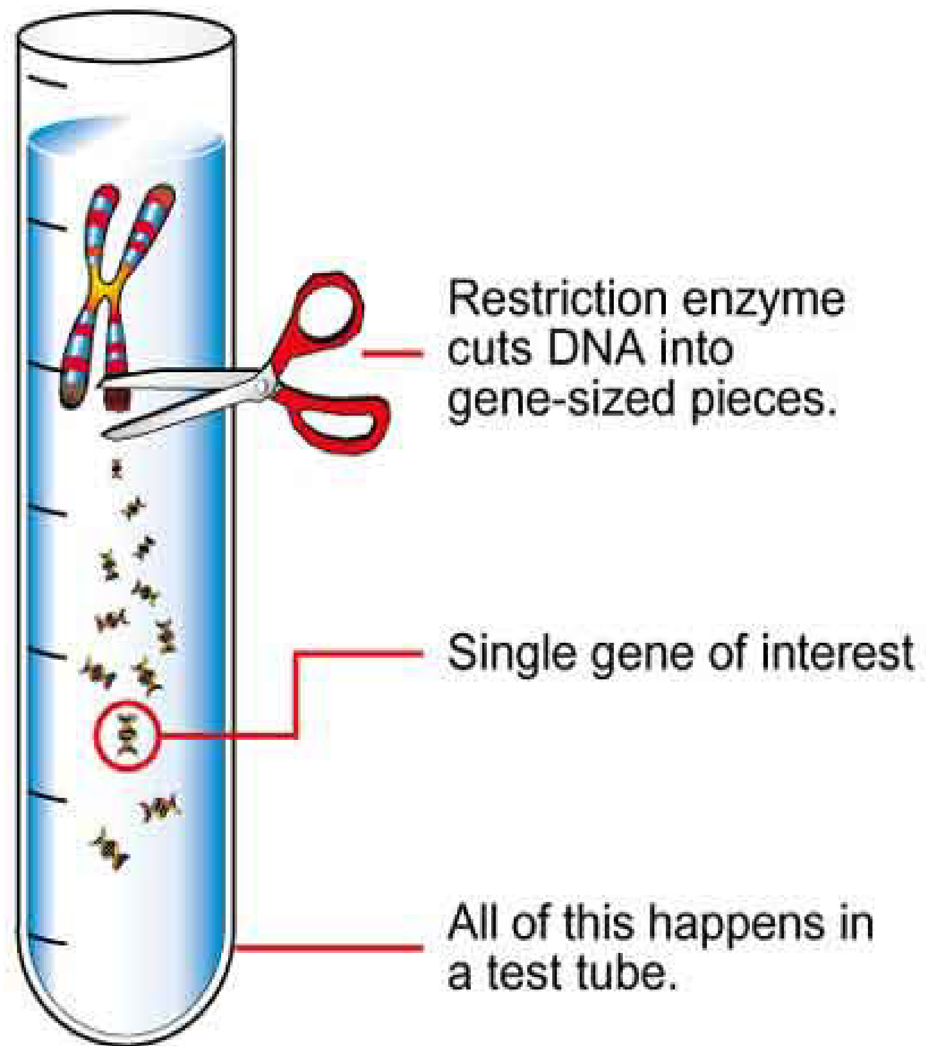
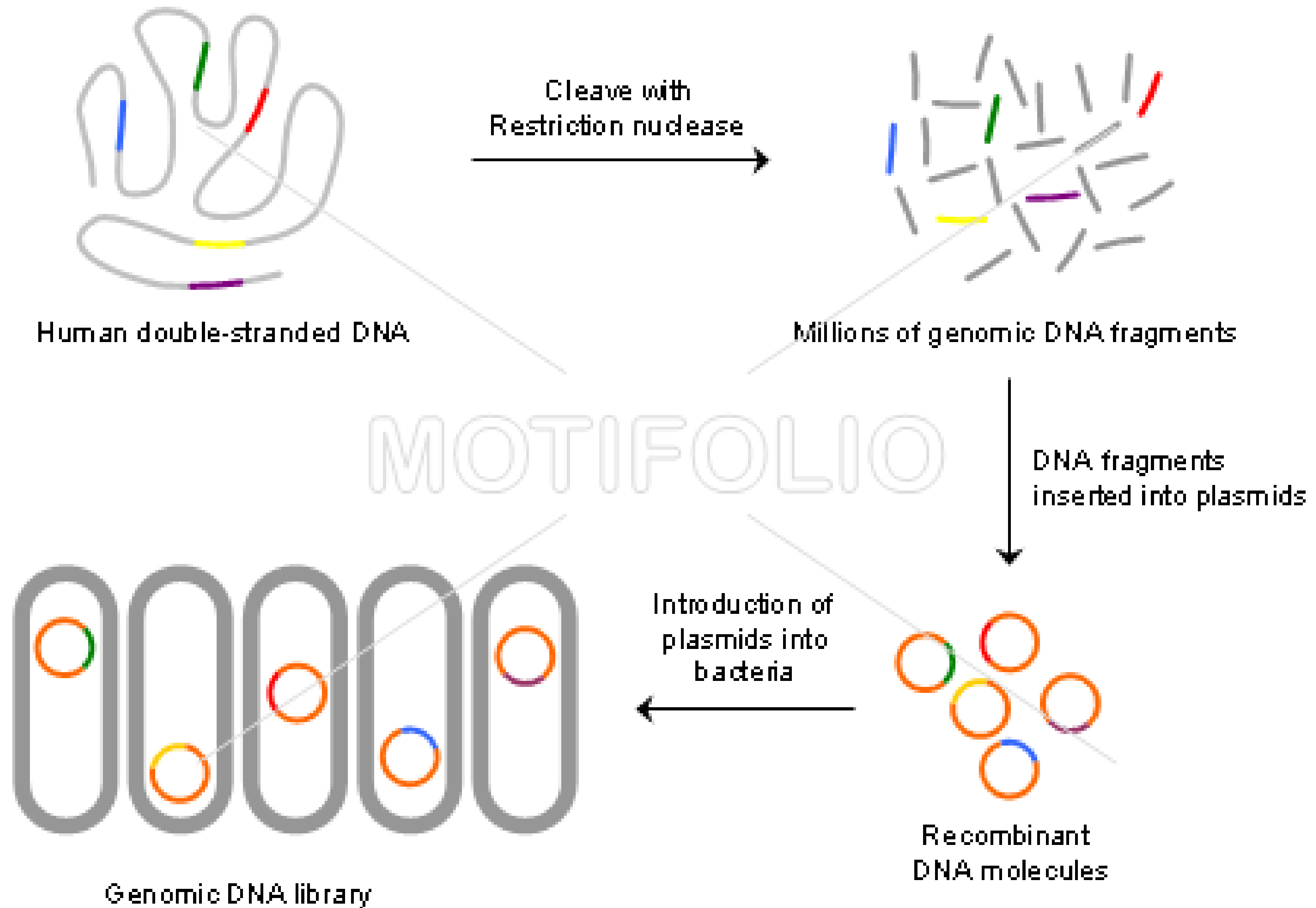
Molecular Biotechnology




Construction of a human genomic DNA library
Cleave with
Restriction nuclease
Human double-stranded DNA
Millions of genomic DNA fragments
DNA fragments
inserted into plasmids
Introduction of
plasmids into
bacteria
Recombinant
DNA molecules
Genomic DNA library
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Goal of Human Genomic Project

- Identify all of the genes in human DNA.
- Determine the sequence of the 3 billion nucleotide bases of DNA
- Store this information in Data bases.
- To Know function of DNA gene
- Develop efficient sequencing technologies.
- Develop tools for data analysis.

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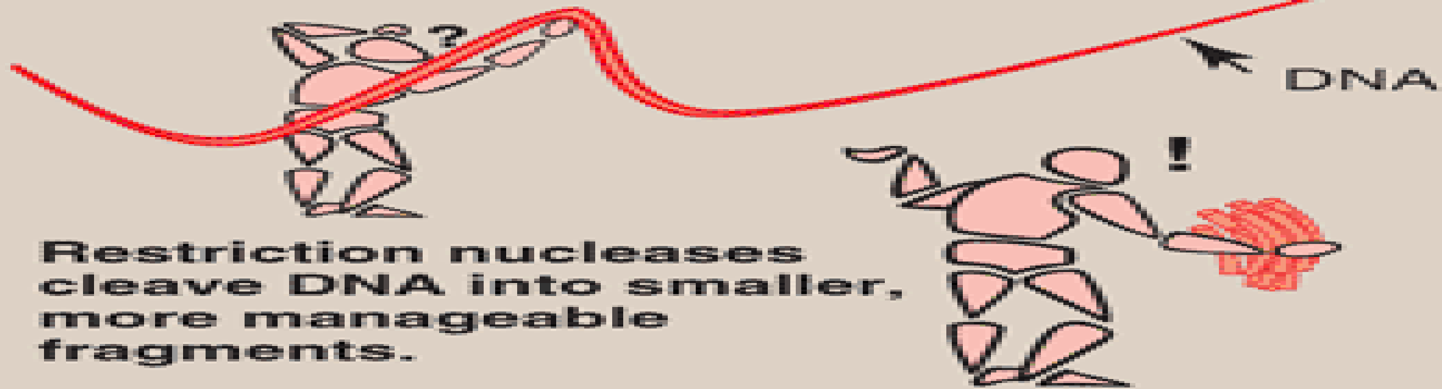
Restriction endonucleases
?
DNA
!
Restriction nucleases cleave DNA into smaller, more manageable fragments.

Cloning of DNA
?
!
Amplified DNA fragments
DNA fragments must be amplified to be more useful.

Probes
DNA fragment
CTCCCCCTCCTTCCC
GAGGGGGAGGAAGGG
?
Probe
A specific fragment can be identified using a complementary probe.

Restricted Endonuclease

- Why called "Restriction" Enzymes
  - Restrict Growth of Bacteriophages
- Each cleaves at a specific nucleotide sequence
- Used to obtain defined DNA segments
  - Restriction Fragment.
- Cleave double-stranded (ds) DNA
- Easy for DNA analysis.

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Action of Restricted Endonuclease

A

ATCGATCGATCGATCGAATTCATCGATCGCTAG
TAGCTAGCTAGCTAGCTTAAGTAGCTAGCGATC

B

ATCGATCGATCGATCGAATTCATCGATCGCTAG
TAGCTAGCTAGCTAGCTTAAGTAGCTAGCGATC

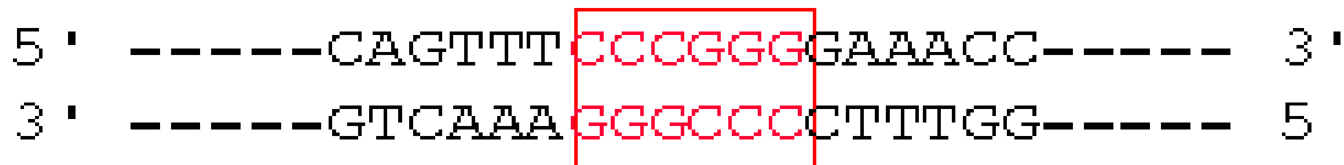
C

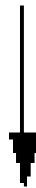
ATCGATCGATCGATCG AATTCATCGATCGCTAG
TAGCTAGCTAGCTAGCTTAA GTAGCTAGCGATC

- **Which bond formation is require to rejoin the sequence?**
- **Which enzyme can make that bonding?**

Action of Restricted Endonuclease

Sma I





- **Which bond formation is require to rejoin the sequence?**
- **Which enzyme can make that bonding?**
- **What is advantage or disadvantage of both type of R.E.?**

Specificity of Restriction Endonuclease

- Recognize short specific nucleotide sequences
- Generally Four or Six base pairs
- 4 nucleotide pair = cut at every 256 bp
- 6 nucleotide pair = cut at every 4096 bp
- Mostly " **Palindrome Sequence** "

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A Palindrome

When read in the 5' ⟶ 3' direction, the sequence on the "top" strand is identical to that of the "bottom" strand.

3' -CTTAAG- 5'

"Sticky" and "Blunt" ends

STICKY END

- TaqI = produce "sticky" end
- Resulting DNA fragments = single-stranded (ss) sequences
- Sequence is complementary to each other

BLUNT END

- HaeIII = produce "blunt" ends
- Resulting fragment is double-stranded
- Do not form hydrogen bond
- DNA ligase help to rejoin sticky ends of a DNA fragment

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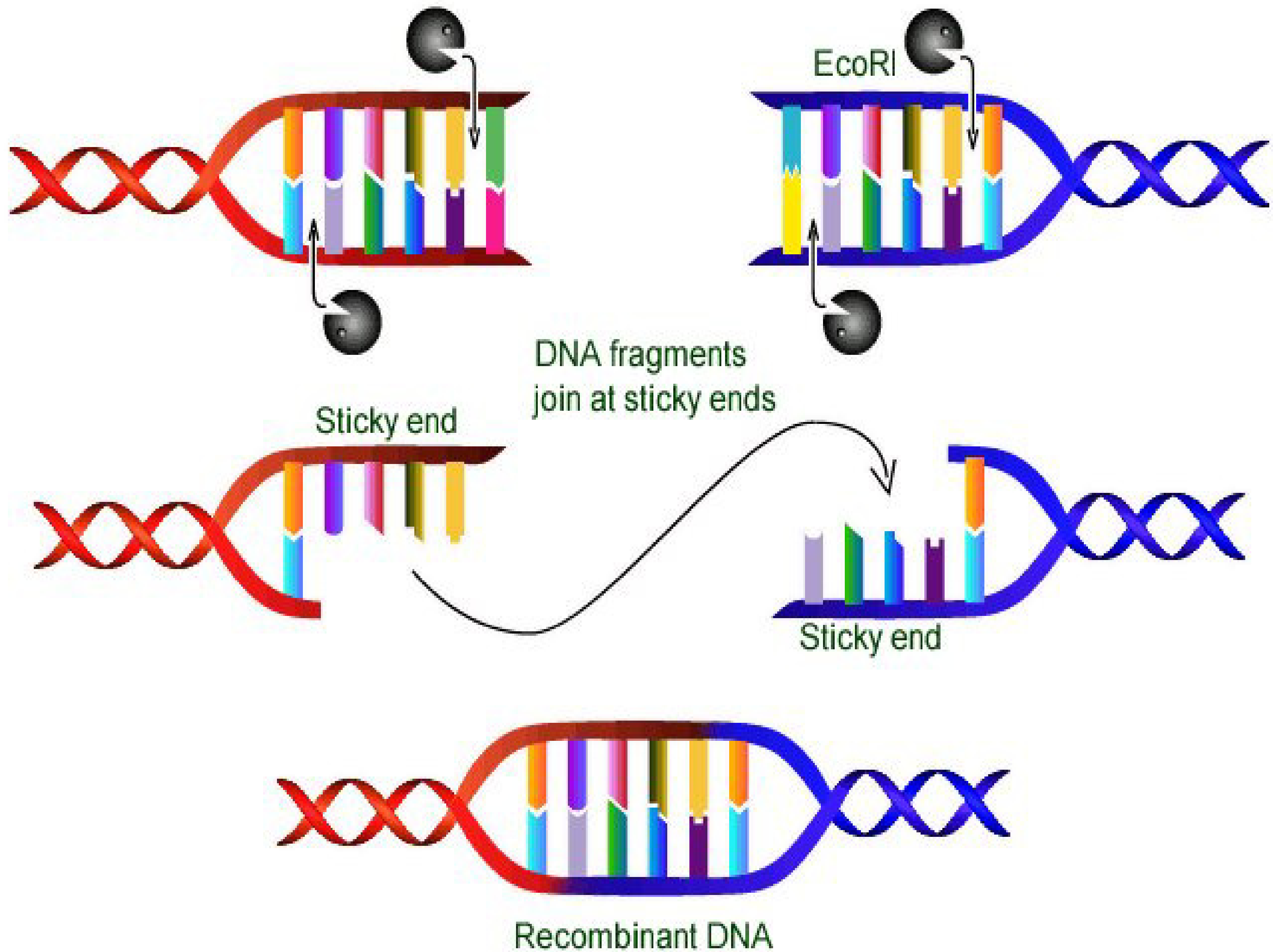
Restriction Enzyme



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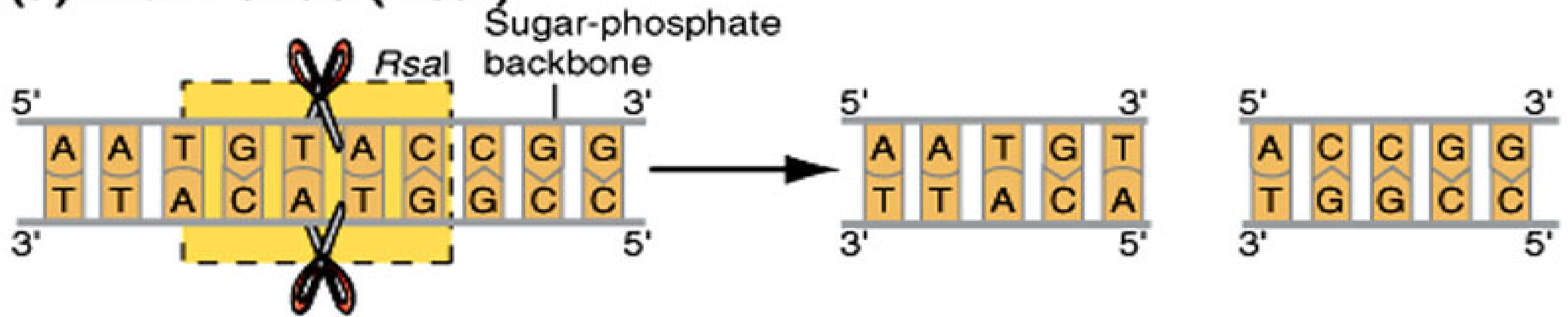
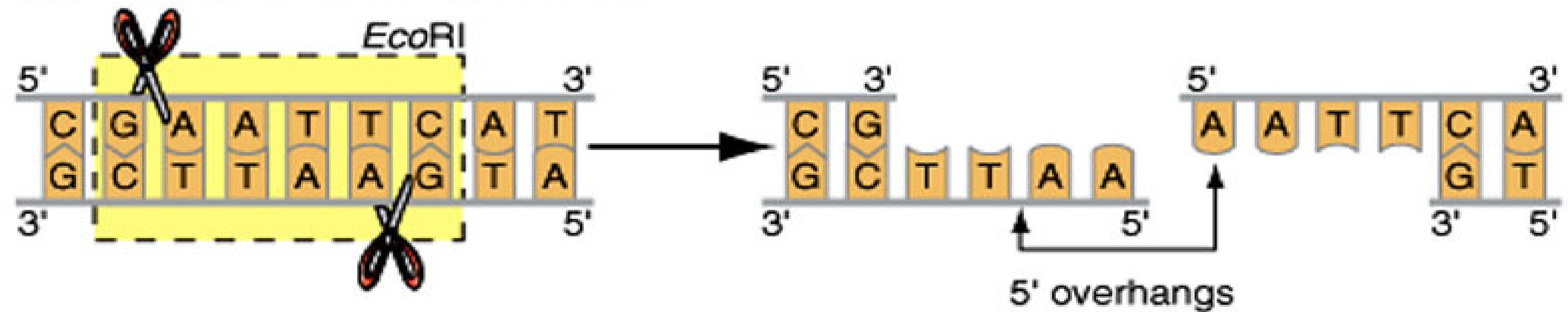
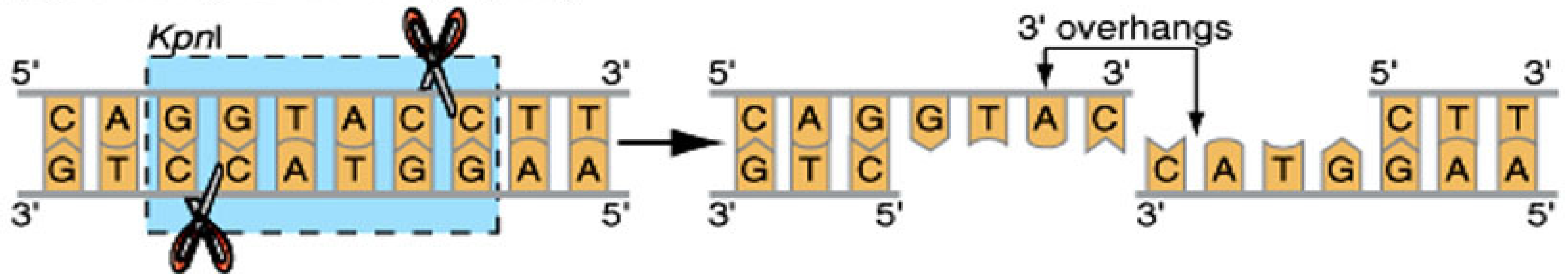
(a) Blunt ends (R*sa*I)



(b) Sticky 5' ends (E*co*RI)

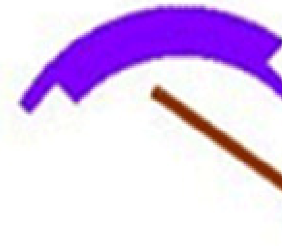


(c) Sticky 3' ends (K*pn*I)

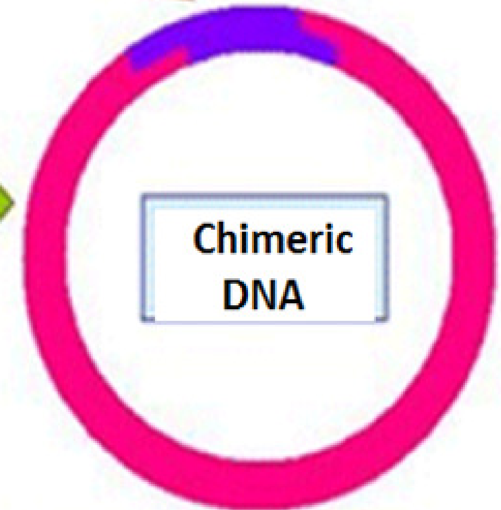


Foreign DNA
(Target Gene containing DNA)

Cutting of Foreign DNA through R.E. to extract Target Gene

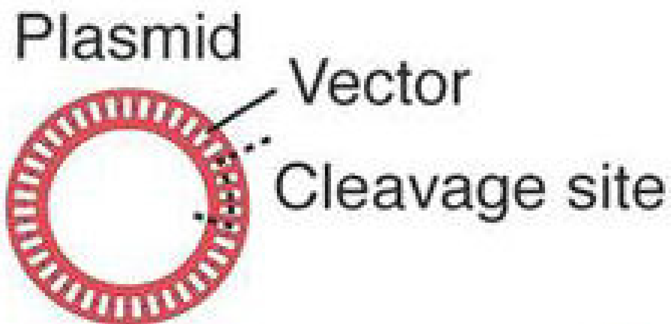


Same
Restricted Endonuclease enzyme for
Vector & Foreign DNA



Plasmid vector has a single restricted site, hence there is only one nick

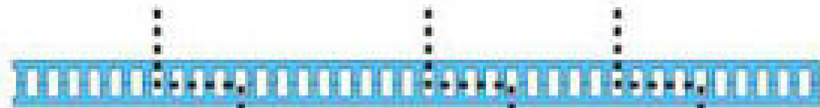
Cleaved foreign DNA anneales with Plamid vestor & Ligated


Plasmid
Vector
Cleavage site

Cleavage
by *Eco*RI
endonuclease

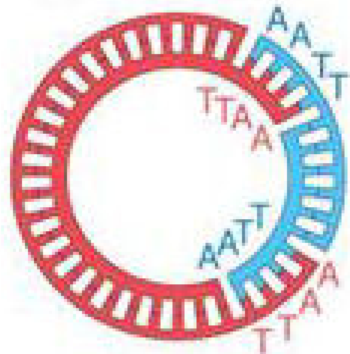
TTAA
AATT

Donor DNA

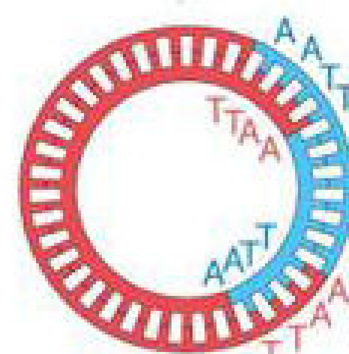

Cleavage
sites
Cleavage by *Eco*RI
endonuclease

AATT
TTAA
AATT
TTAA
AATT
TTAA

Hybridization


AATT
TTAA
AATT
TTAA

DNA ligase


AATT
TTAA
AATT
TTAA
Recombinant plasmid

Restriction Site of DNA

- Sites are recognized by restriction endonuclease
- Cleave DNA into fragments of different sizes

➢ **Example 1**

- Enzyme that recognizes a specific four-base-pair sequence
- Produces Many cuts and Shorter pieces in DNA.

➢ **Example 2**

- Enzyme requiring sequence of six base pairs
- Produces Fewer cuts & Longer pieces.
- Different enzyme = Different cleavage

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DNA of interest

Second DNA fragment

Cleavage by *TaqI*

DNA of interest with a "sticky" end produced by *TaqI*.

Second fragment of DNA also with a "sticky" end produced by cleavage with *TaqI*.

Cohesive ends of two DNA fragments form hydrogen bonds.

DNA ligase

DNA ligase forms a 3'⟶5' phosphodiester bond between the fragments.

Nomenclature of R.E.

- 1st Letter = Genus of bacterium.
- 2nd Letters = Name of species.
- Additional letter = Type or Strain
- Final number = order discovered

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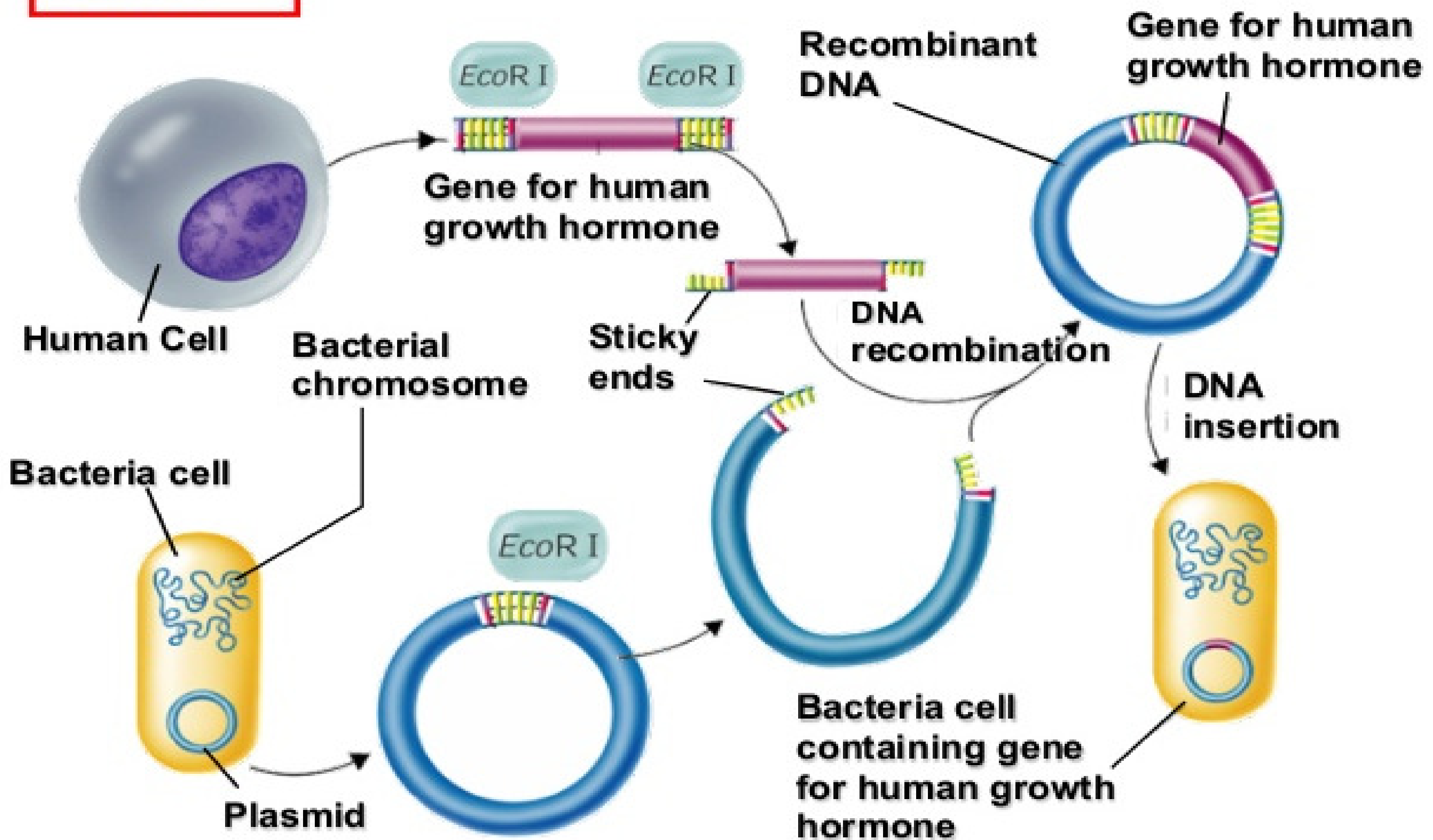
EcoRI

Gender | Species | Strain | Enzyme number

Figure 2.4. Standardized nomenclature system for restriction enzymes.

Basic Principle of Recombinant DNA Technology



DNA Cloning

- Insert target DNA into a replicating cell
- Permits Amplification.
- "**Cloning Vector**" = Target Gene + Vector
- For example,
- The process of introducing foreign DNA into a cell
  - **Transformation** for bacteria
  - **Transfection** for eukaryotes.
- As Cell / Bacteria multiplies = Copies of clone DNA.
- Cloned DNA released from its vector by cleavage (using the appropriate restriction endonuclease) and is isolated.

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Vector

- It is DNA to which target gene is joined, for cloning.
- **Essential properties**
  1. **Autonomous replication**
  2. **Atleast one specific nucleotide sequence recognized by a restriction endonuclease in it**
  3. **Atleast one gene that confers the ability to select for the vector**
     - **such as an antibiotic resistance gene.**

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Vector

1. **Plasmid**
   - Use to transfer 6-10 kbp gene
2. **Bacteriophage**
   - Use to transfer 10-20 kbp gene
3. **Cosmid**
   - Use to transfer > 20 kbp gene
4. **Bacterial Arteficial Chromosome (BAC)**

   Yeast Arteficial Chromosome (YAC)

   Bacteriophage P1 Based Vector
   - Several hundred kbp gene

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PLASMID

- Double stranded circular DNA.
- Easily trans-infect = easily enter from one bacteria to another bacteria
- Self Replicating.
- Antibiotic Resistant Property

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Bacteriophage & Cosmid

- Bacteriophage = Virus that infect bacteria
- Cosmids = Artificial constructs
- More efficiently
- Accommodate large DNA segments

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Is it only target gene require to clone,
when we like to produce target protein
(e.g. deficient enzyme)
from recombinant DNA technic
or
gene therapy?

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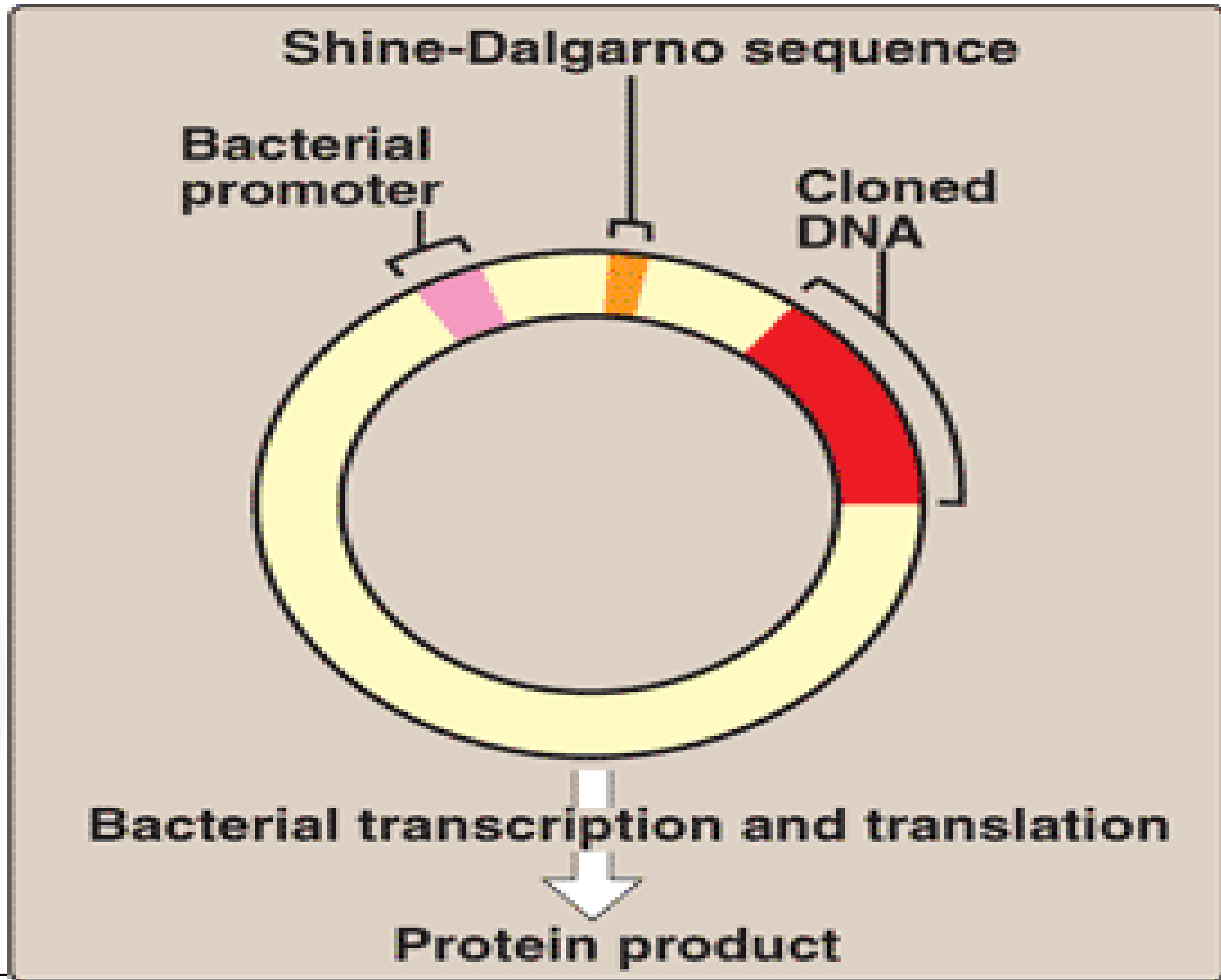
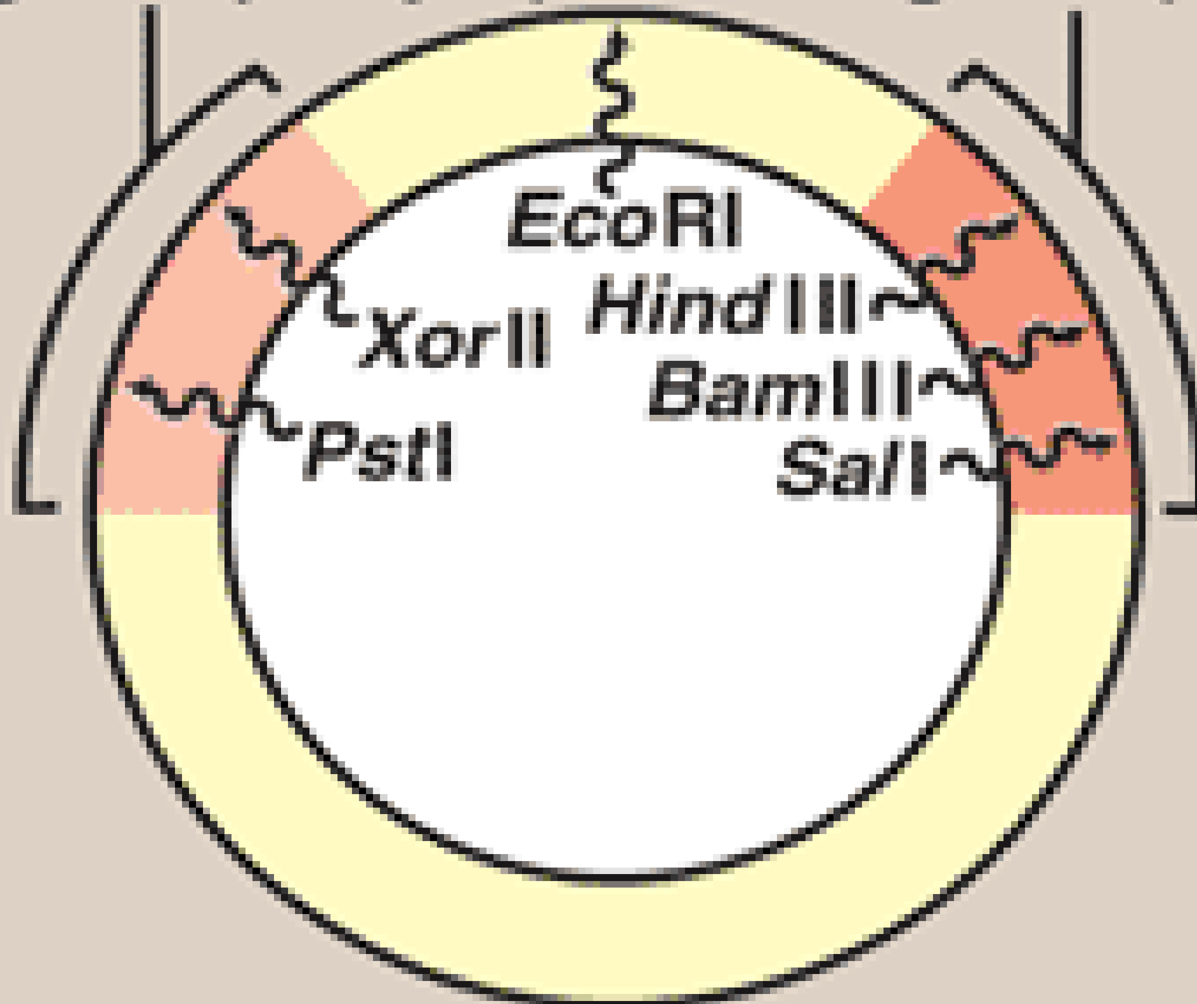
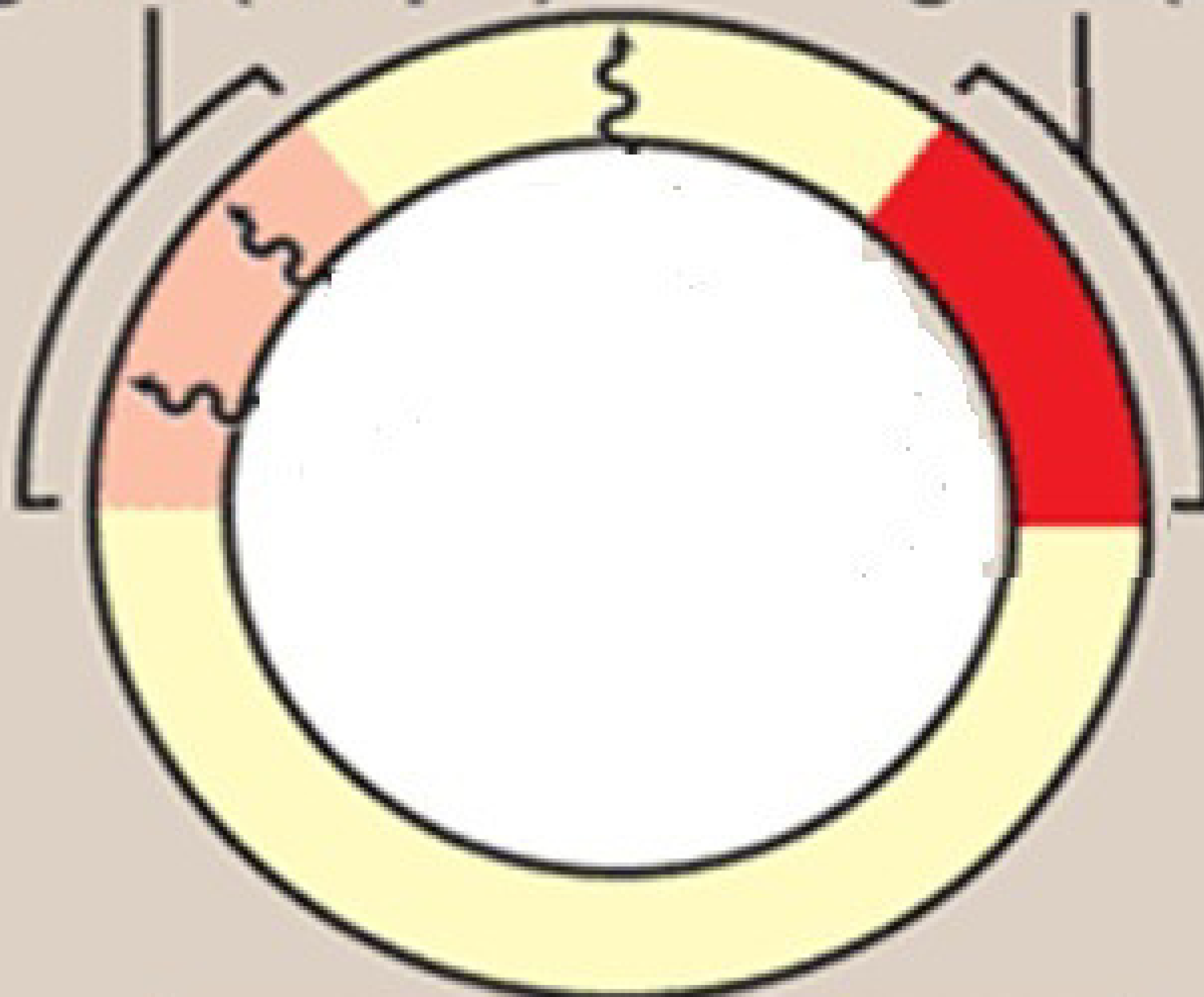
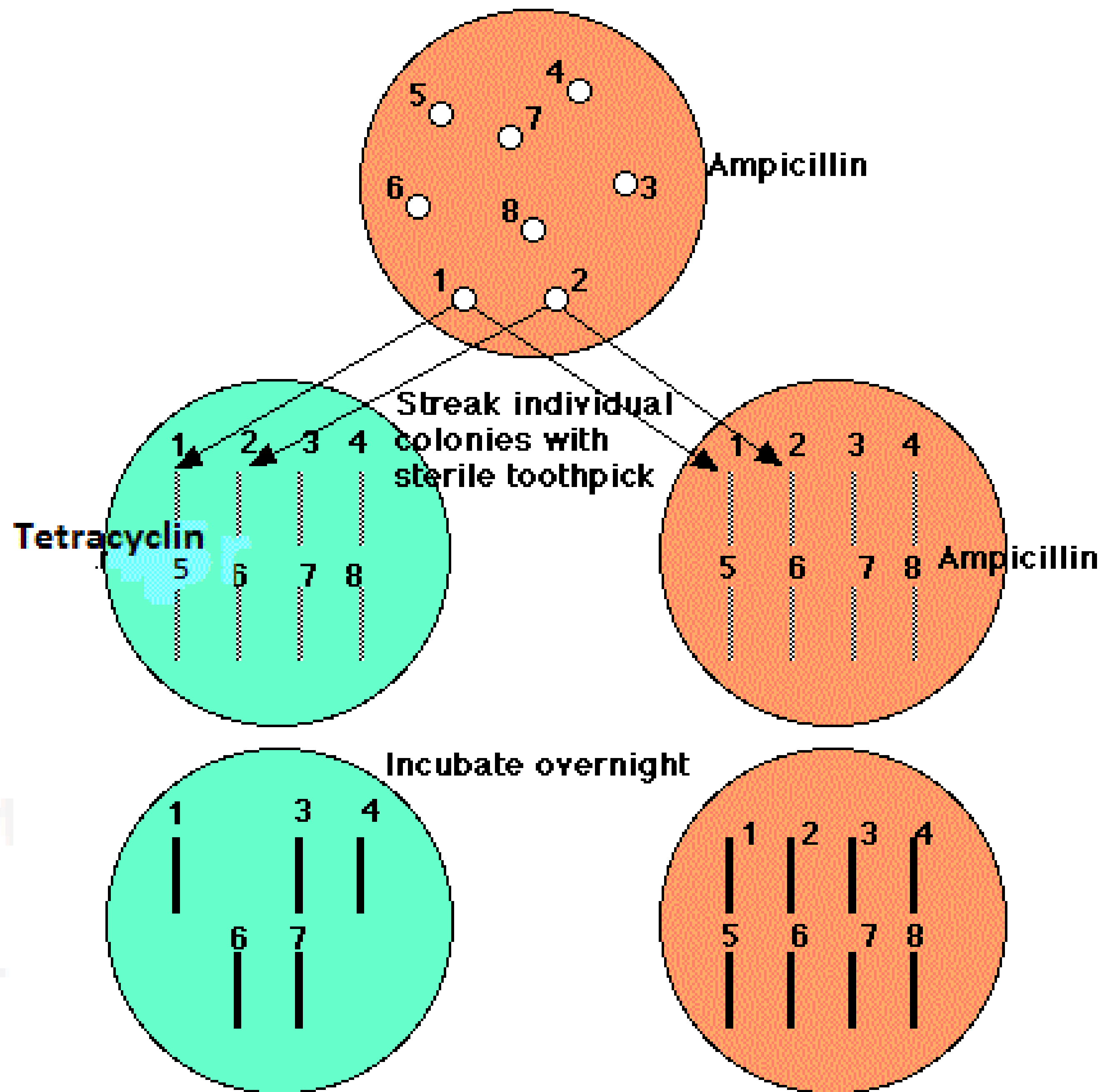
Expression Vector

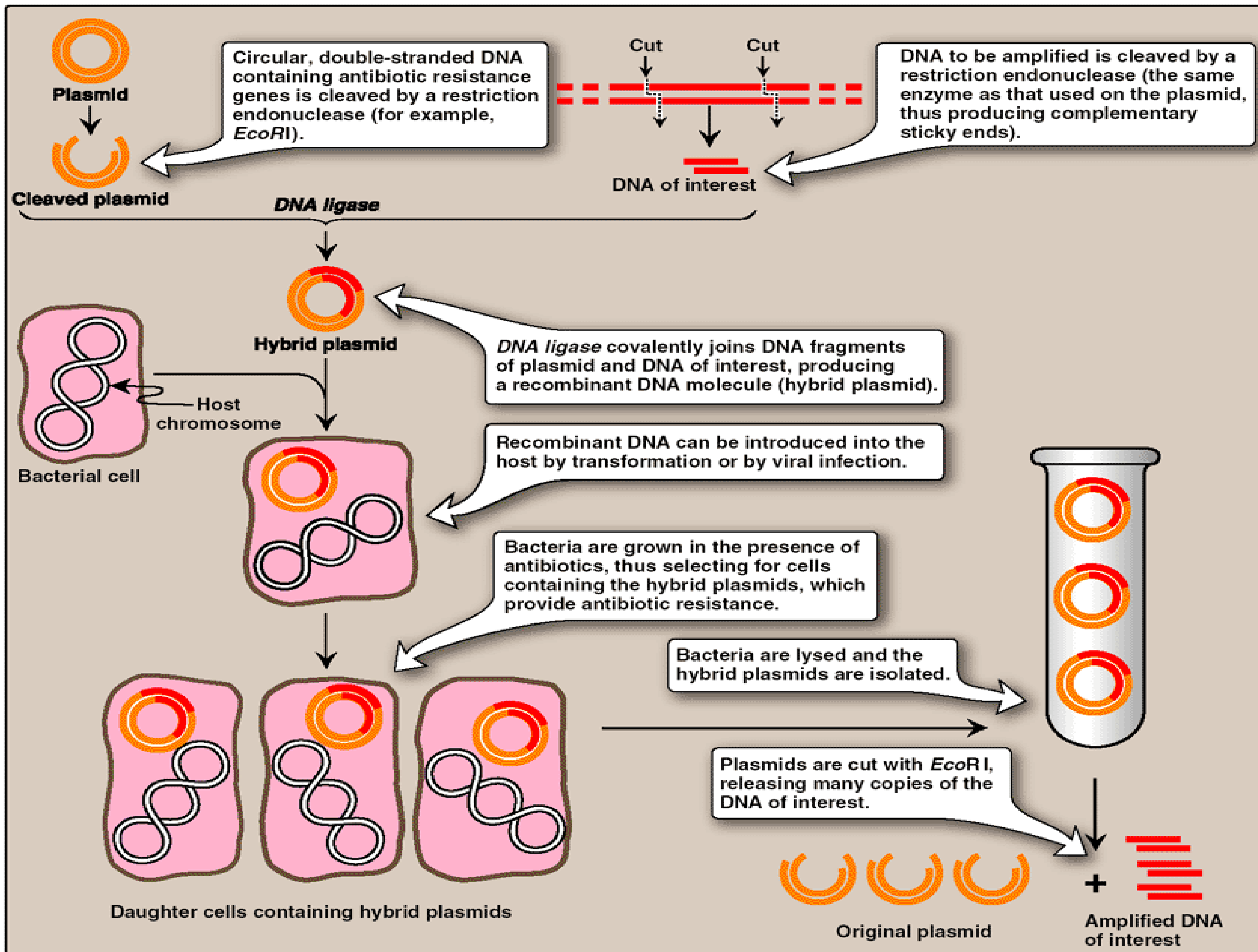



Ampicillin resistance gene (Amp^R)
Tetracycline resistance gene (Tet^R)
EcoRI
HindIII
XorII
BamIII
PstI
SalI
Plasmid pBR322



Chimeric DNA Plasmid


Ampicillin
5
4
7
6
3
8
1
2
Streak individual colonies with sterile toothpick
Tetracyclin
1 2 3 4
5 6 7 8
Ampicillin
1 2 3 4
5 6 7 8
Incubate overnight
1 3 4
6 7
1 2 3 4
5 6 7 8


Plasmid
Circular, double-stranded DNA containing antibiotic resistance genes is cleaved by a restriction endonuclease (for example, *Eco*RI).
Cut
Cut
DNA to be amplified is cleaved by a restriction endonuclease (the same enzyme as that used on the plasmid, thus producing complementary sticky ends).
Cleaved plasmid
DNA of interest
DNA ligase
Hybrid plasmid
DNA ligase covalently joins DNA fragments of plasmid and DNA of interest, producing a recombinant DNA molecule (hybrid plasmid).
Host chromosome
Bacterial cell
Recombinant DNA can be introduced into the host by transformation or by viral infection.
Bacteria are grown in the presence of antibiotics, thus selecting for cells containing the hybrid plasmids, which provide antibiotic resistance.
Bacteria are lysed and the hybrid plasmids are isolated.
Plasmids are cut with *Eco*R I, releasing many copies of the DNA of interest.
Daughter cells containing hybrid plasmids
Original plasmid
Amplified DNA of interest

Genomic Library

Human DNA

CLEAVE WITH
RESTRICTION
NUCLEASE

Millions of genomic
DNA fragments

DNA FRAGMENTS
INSERTED INTO
PLASMIDS USING LIGASE

Recombinant
DNA molecules

INTRODUCTION OF
PLASMIDS INTO BACTERIA

Genomic library

DNA Libraries

- Collection of cloned restriction fragments of the DNA
- Two type of libraries

1. **Genomic libraries**
   - ✓ Copy of every DNA nucleotide sequence.
   - ✓ Intron & Regulatory gene present.
2. **Complementary DNA (cDNA) libraries.**
   - ✓ cDNA made from mRNA.
   - ✓ Appear like mRNA molecules
   - ✓ Intron & Regulatory genes absent.

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Genomic DNA libraries

Collection of fragments of total dsDNA

- Digestion of total DNA with restriction endonuclease
- Accordingly RE enzymes = more than one restriction site recognized
- If completion fragmentation
- Gene of interest is also fragmented
- No Gene for library.
- So, Partial digestion of DNA is performed
  - Limit - Amount of enzyme
  - Limit - Time of action of the enzyme.

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cDNA libraries

- Some gene of interest is expressed as mRNA
- Present at high concentrations in the cell.
- For example,
- Reticulocyte mRNA = α-globin and β-globin.
- mRNA used as a template to dsDNA (cDNA).
- Using enzyme reverse transcriptase
- mRNA is isolated from tRNA & rRNA by the presence of its polyA tail.
- cDNA = amplified by cloning or PCR.
- Used as a probe to locate the gene that coded for the original mRNA
- cDNA has no intervening sequences

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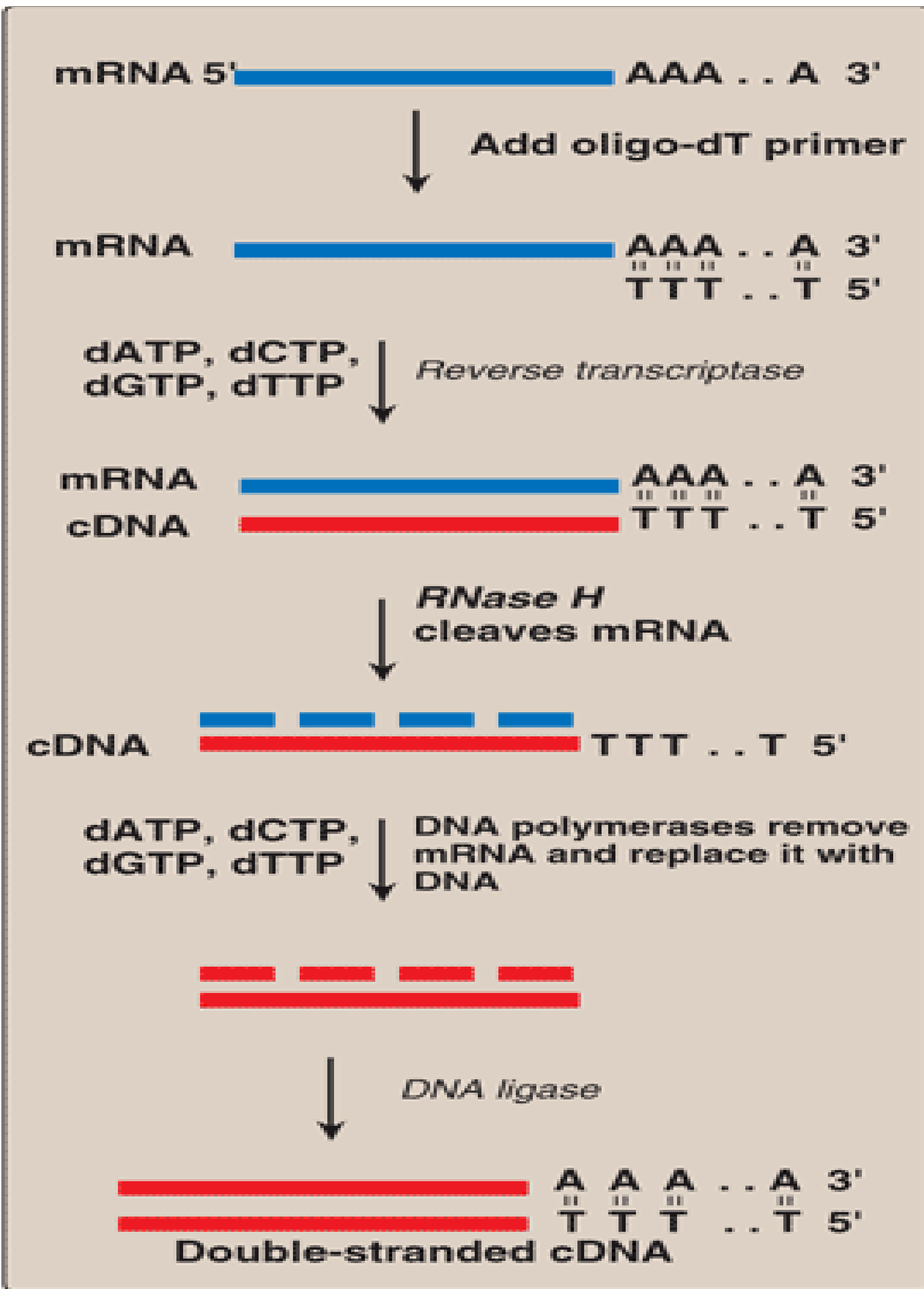

Synthesis of cDNA from mRNA using Reverse Transcriptase

Sequencing of cloned DNA fragments

- Sanger Dideoxy Method
- ssDNA
- DNA polymerase.
- Radioactive primer complementary to the 3′-end of the target DNA
- Four deoxyribonucleoside triphosphates (dNTP).
- Dideoxyribonucleoside triphosphates (ddNTP) .
- Separation of DNA = Polyacrylamide gel electrophoresis
- Followed by autoradiography

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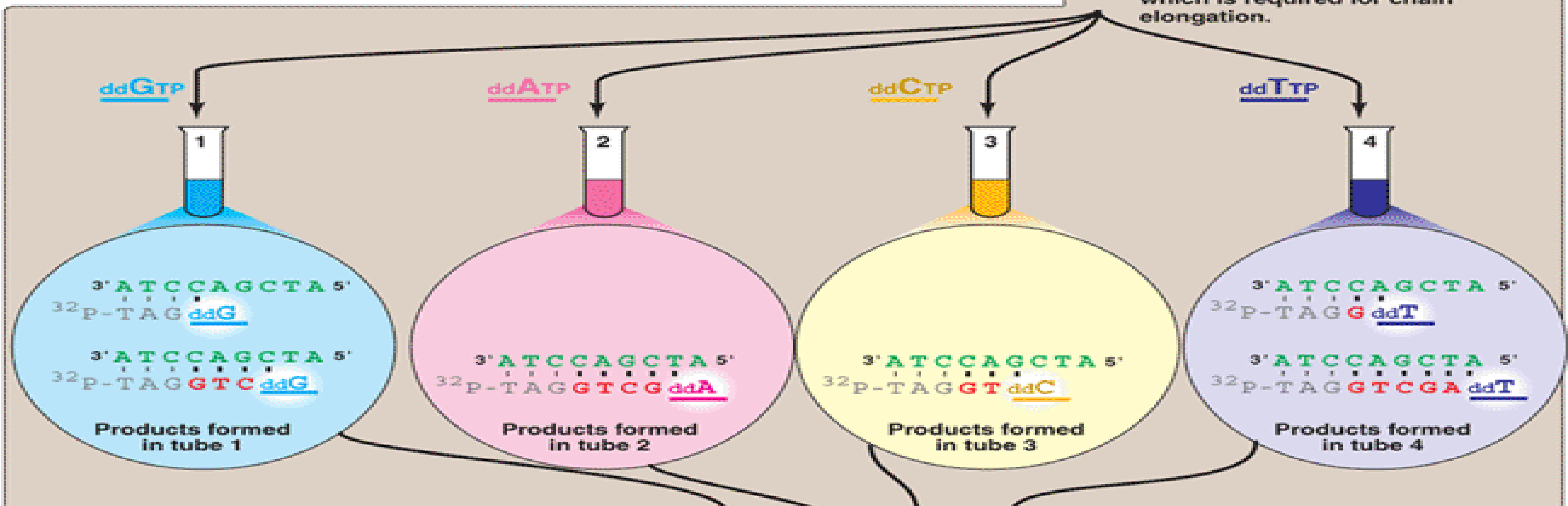
1 Single-stranded DNA of unknown sequence is used as a template.

2 Add primer, *DNA polymerase* + dATP, dGTP, dCTP, dTTP.

3 Split the sample into four tubes, each with one of the four dideoxynucleotides.

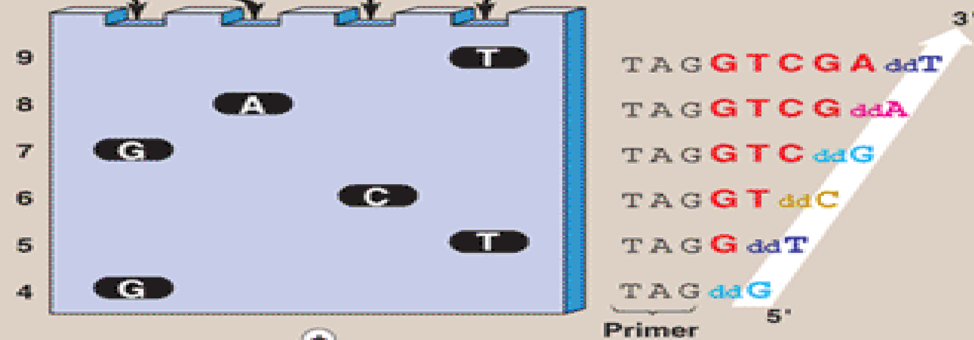
4 Synthesis proceeds until the dideoxynucleotide is incorporated into a DNA strand. DNA terminating in a dideoxynucleotide cannot be elongated because it lacks a 3'-OH, which is required for chain elongation.



5 Perform gel electrophoresis.

6 Read the sequence of the newly synthesized strand (complementary to the original DNA sample).

Length of fragments (base pairs)



Probes

Use

- To find target DNA sequence from DNA fragments
- Used to identify which clone of library or which band on a gel contains target DNA.

Characteristic

- Short sequence
- Single-stranded piece of DNA
- Labeled with a radioisotope or biotin.
- Complementary to the DNA of interest

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Hybridization of a probe to DNA fragments

- denatured of dsDNA = ssDNA
- Bound to solid support = nitrocellulose membrane.
- Hybridization by exogenous, single-stranded, radiolabeled DNA probe (complementary nucleotide sequence)
- Probes hinges to target DNA
- Extent of hybridization is measured by radioactivity.
- Excess Probe = Do not hybridize = Removed by washing.

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Synthetic oligonucleotide probes

- 20–30 nucleotides.
- Used to detect single-base changes

cDNA probes

- Thousands of bases
- It can binding to a target DNA even with single-base change.

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- Amino acid sequence of the protein may be used to construct a probe.
- Because of the degeneracy of the genetic code, it is necessary to synthesize several oligonucleotides.

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Allele-specific oligonucleotide (ASO) probe

A DNA from a patient with sickle cell disease

Portion of the gene for the β^S-chain of hemoglobin S

DNA codes for valine instead of glutamate in the sixth position of β-globin.

5 6 7
····Pro — Val — Glu····

DNA

Oligonucleotide probe hybridizes with a DNA fragment from the gene for the β chain of Hb S.

Probe

*CTCCTGTGGAGAAGT

GAGGACACCTCTTCAGACG

DNA fragment coding for Hb S

B DNA from a normal individual

Oligonucleotide probe fails to hybridize with the DNA fragment from the gene for the β chain of Hb A.

Probe

*CTCCTGTGGAGAAGT

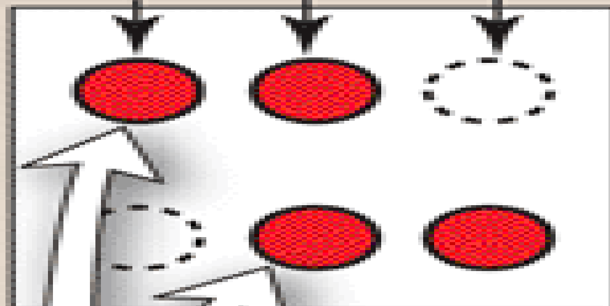
. . . GAGGACTCCTCTTCAGACG . . .

DNA fragment coding for Hb A

Homozygous normal, (AA)

Heterozygous (carrier, AS)

Homozygous mutant (affected, SS)



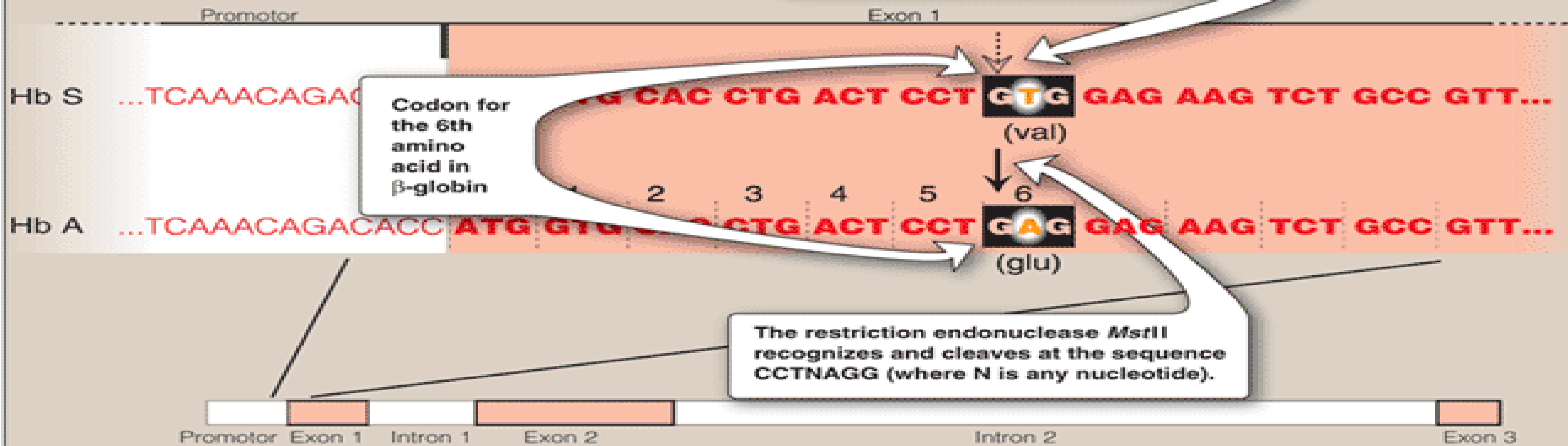
This row was probed with an ASO specific for a normal β^A gene

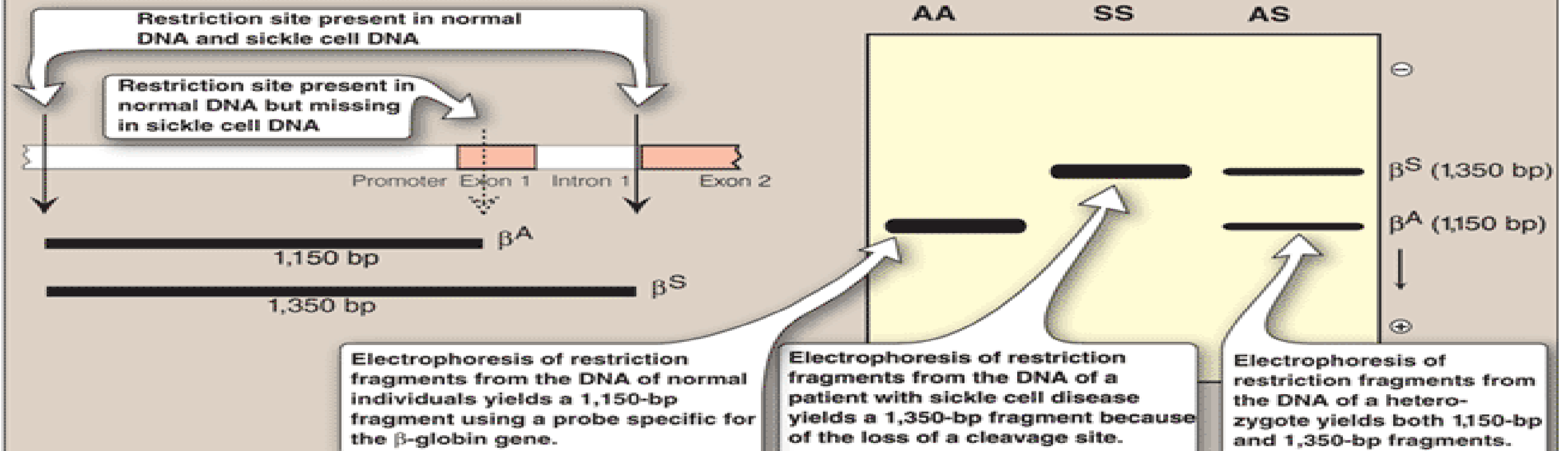
This row was probed with an ASO specific for a mutant β^S gene

= Probe hybridizes with patient's DNA

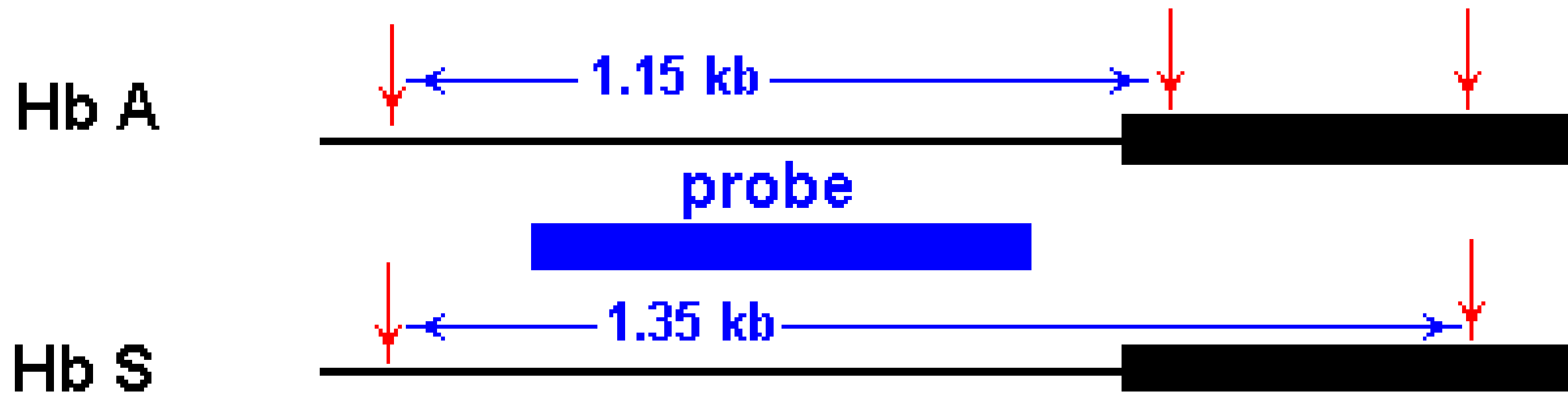
= Probe does not hybridize to patient's DNA

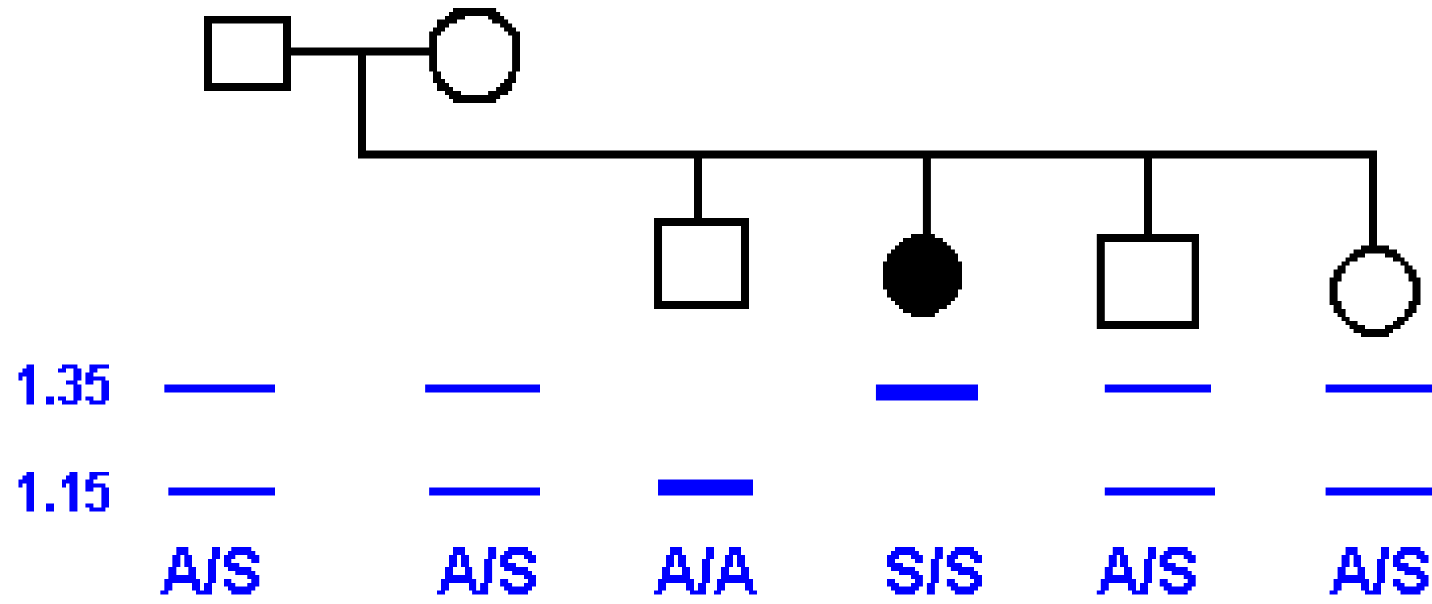
Two samples of DNA from each individual are applied to the membrane.


A
Details of a portion of the β-globin gene
The A to T mutation within codon six of the βS-globin gene eliminates a cleavage site for the enzyme, MstII.
Promotor
Exon 1
Hb S
...TCAAACAGAC
CAC CTG ACT CCT GTG GAG AAG TCT GCC GTT...
(val)
Codon for the 6th amino acid in β-globin
1 2 3 4 5 6
Hb A
...TCAAACAGACACC ATG GTG CTG ACT CCT GAG GAG AAG TCT GCC GTT...
(glu)
The restriction endonuclease MstII recognizes and cleaves at the sequence CCTNAGG (where N is any nucleotide).
Promotor
Exon 1
Intron 1
Exon 2
Intron 2
Exon 3


B
Cleavage of the β-globin gene with a restriction endonuclease
Restriction site present in normal DNA and sickle cell DNA
Restriction site present in normal DNA but missing in sickle cell DNA
Promoter
Exon 1
Intron 1
Exon 2
βA
1,150 bp
βS
1,350 bp
AA
SS
AS
⊖
βS (1,350 bp)
βA (1,150 bp)
⊕
Electrophoresis of restriction fragments from the DNA of normal individuals yields a 1,150-bp fragment using a probe specific for the β-globin gene.
Electrophoresis of restriction fragments from the DNA of a patient with sickle cell disease yields a 1,350-bp fragment because of the loss of a cleavage site.
Electrophoresis of restriction fragments from the DNA of a hetero-zygote yields both 1,150-bp and 1,350-bp fragments.

restriction site locations





Biotinylated probes

- **Disposal of radioactive = expensive**.
- Biotin + 4 Avidin (Egg-Protein)
- Avidin can be attached to a fluorescent dye
- Great sensitivity.

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Antibodies

- When amino acid sequence is unknown for synthesis of a probe.
- A labeled antibody is used to identify which bacterial colony produces the protein
- Gene can be identified indirectly by cloning cDNA in an expression vector that allows the cloned cDNA to be transcribed and translated.
- [Note: A library created using expression vectors is called an expression library.]

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(Edward)Southern Blotting

- “Southern” = Detect in DNA.
- “Northern” = mRNA
- “Western” = Protein
- It combines the use
  - Restriction enzymes
  - Electrophoresis
  - DNA probes
  - Blotting – Nitrocellulose paper

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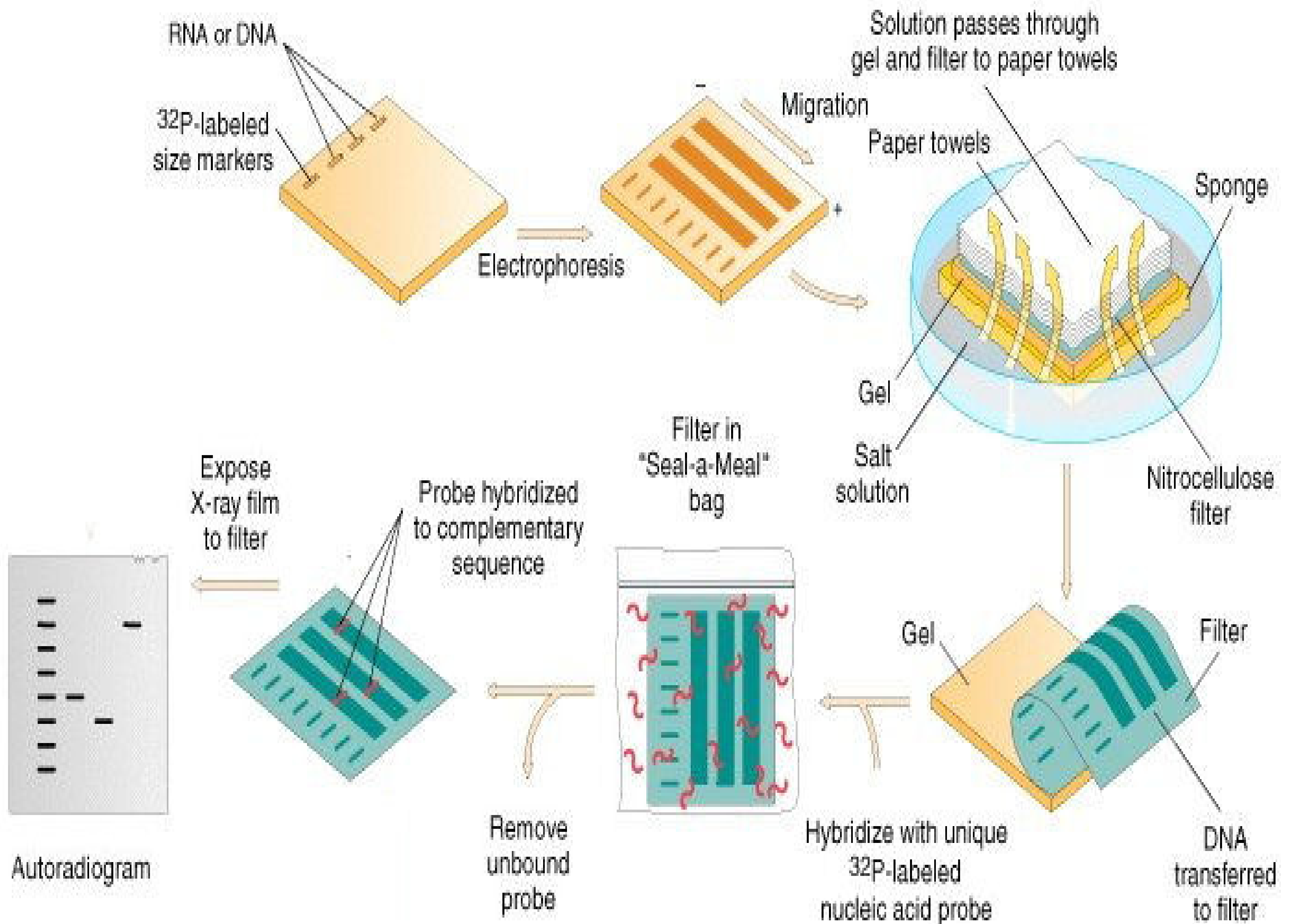

RNA or DNA
^{32}P-labeled size markers
Electrophoresis
–
Migration
+
Solution passes through gel and filter to paper towels
Paper towels
Sponge
Gel
Salt solution
Nitrocellulose filter
Gel
Filter
DNA transferred to filter
Hybridize with unique ^{32}P-labeled nucleic acid probe
Filter in "Seal-a-Meal" bag
Remove unbound probe
Probe hybridized to complementary sequence
Expose X-ray film to filter
Autoradiogram

Southern Blot Procedure

- DNA is extracted from cells
- Cleaved into many fragments = Restriction Enzyme.
- Separated on basis of size by Electrophoresis.
- Denatured and transferred (blotted) to a nitrocellulose membrane
- If Whole DNA = Millions of Fragment copy
- Blure & Overlapping bands.
- To avoid this = Uses a probe to identify Target DNA.
- Expose to X – ray film
- Comparison of the position of the band to standard fragments.
- Band pattern depend = on Restriction Endonuclease

  = on Probe

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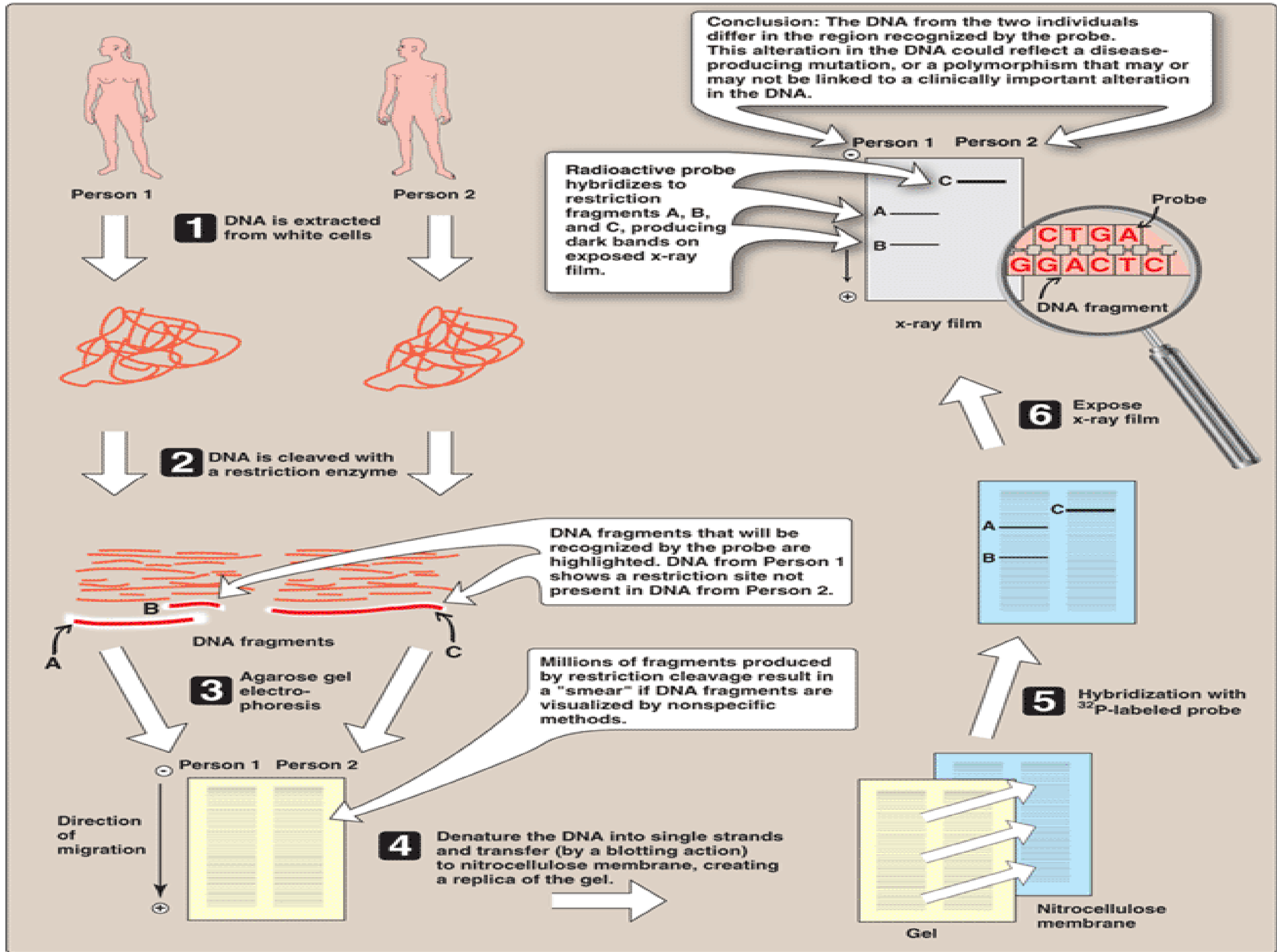
Person 1
Person 2
1 DNA is extracted from white cells
2 DNA is cleaved with a restriction enzyme
B
A
C
DNA fragments
DNA fragments that will be recognized by the probe are highlighted. DNA from Person 1 shows a restriction site not present in DNA from Person 2.
3 Agarose gel electro-phoresis
Millions of fragments produced by restriction cleavage result in a "smear" if DNA fragments are visualized by nonspecific methods.
Person 1 Person 2
Direction of migration
4 Denature the DNA into single strands and transfer (by a blotting action) to nitrocellulose membrane, creating a replica of the gel.
Gel
Nitrocellulose membrane
5 Hybridization with ^{32}P-labeled probe
6 Expose x-ray film
Conclusion: The DNA from the two individuals differ in the region recognized by the probe. This alteration in the DNA could reflect a disease-producing mutation, or a polymorphism that may or may not be linked to a clinically important alteration in the DNA.
Radioactive probe hybridizes to restriction fragments A, B, and C, producing dark bands on exposed x-ray film.
x-ray film
Probe
C T G A
G G A C T C
DNA fragment

Detection of mutations

- Mutation = Pattern of bands is different.
- *Sometime Mutation may not affect a restriction site, with one specific restriction enzyme.*
- *It may be done by using a different restriction enzyme, those can recognize sequence affected by mutation.*

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Restriction Fragment Length Polymorphism

- Genomic variation in DNA among more than 1% of population.
- Differ in 0.1% of genome.
- Genome variations include both polymorphisms and mutations.
- Polymorphism = not always harmful
- Mutation = harmful
- Polymorphisms = in the Introns = that do not code for proteins.
- RFLP can be examined by cleaving DNA into fragments with R.E.
- **Length of restriction fragments is altered if the genetic variant alters the DNA**

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Using EcoRI to cut example DNA at GAATTC produces the following four strand fragments.

DNA variations resulting in RFLP

- Two types of DNA RFLP
  1. Single-base changes
  2. Tandem Repeats

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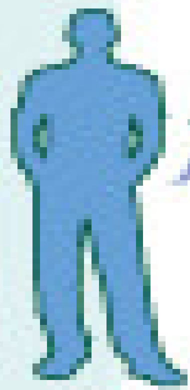
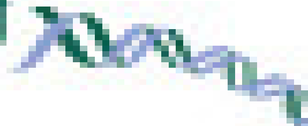
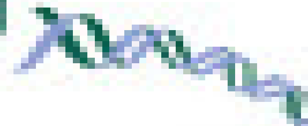
SNP

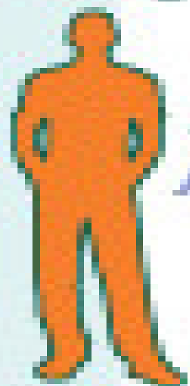


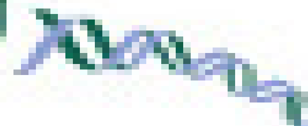


G C A **A** C G T T A G A



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





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

Single Nucleotide Polymorphism (SNP)

Individual 1

Chr 2 ...CGATATTCCTATCGAATGTC...
copy1 ...GCTATAAGGATAGCTTACAG...

Chr 2 ...CGATATTCCCATCGAATGTC...
copy2 ...GCTATAAGGGTAGCTTACAG...

Individual 2

Chr 2 ...CGATATTCCCATCGAATGTC...
copy1 ...GCTATAAGGGTAGCTTACAG...

Chr 2 ...CGATATTCCCATCGAATGTC...
copy2 ...GCTATAAGGGTAGCTTACAG...

Individual 3

Chr 2 ...CGATATTCCTATCGAATGTC...
copy1 ...GCTATAAGGATAGCTTACAG...

Chr 2 ...CGATATTCCTATCGAATGTC...
copy2 ...GCTATAAGGATAGCTTACAG...

Individual 4

Chr 2 ...CGATATTCCTATCGAATGTC...
copy1 ...GCTATAAGGATAGCTTACAG...

Chr 2 ...CGATATTCCCATCGAATGTC...
copy2 ...GCTATAAGGGTAGCTTACAG...

Individual 5

Chr 2 ...CGATATTCCCATCGAATGTC...
copy1 ...GCTATAAGGGTAGCTTACAG...

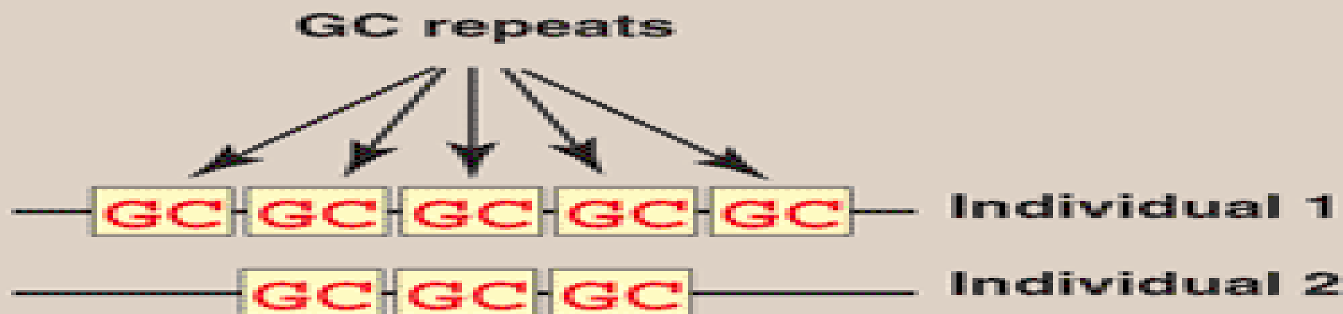
Chr 2 ...CGATATTCCTATCGAATGTC...
copy2 ...GCTATAAGGATAGCTTACAG...

Individual 6

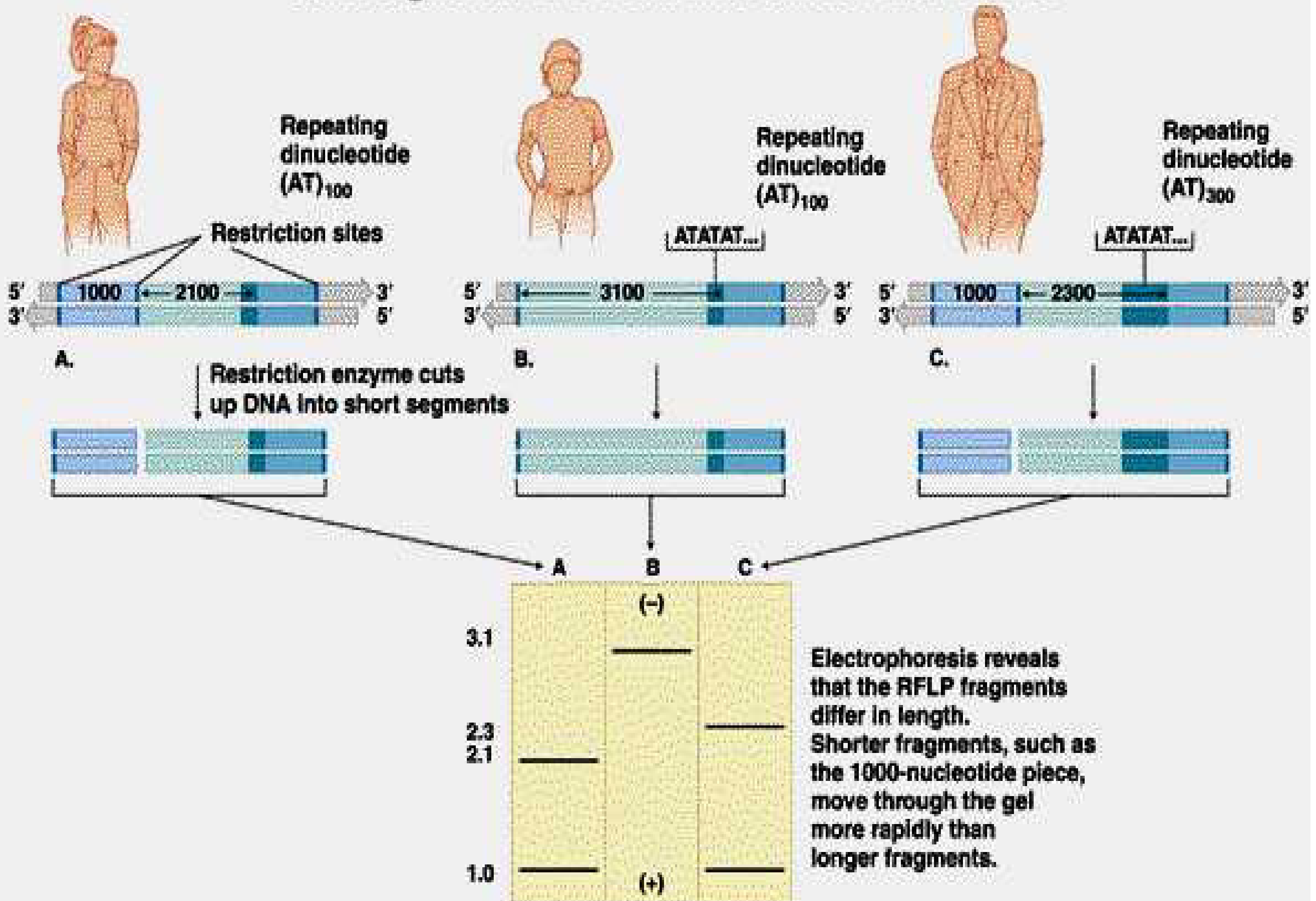
Chr 2 ...CGATATTCCCATCGAATGTC...
copy1 ...GCTATAAGGGTAGCTTACAG...

Chr 2 ...CGATATTCCTATCGAATGTC...
copy2 ...GCTATAAGGATAGCTTACAG...

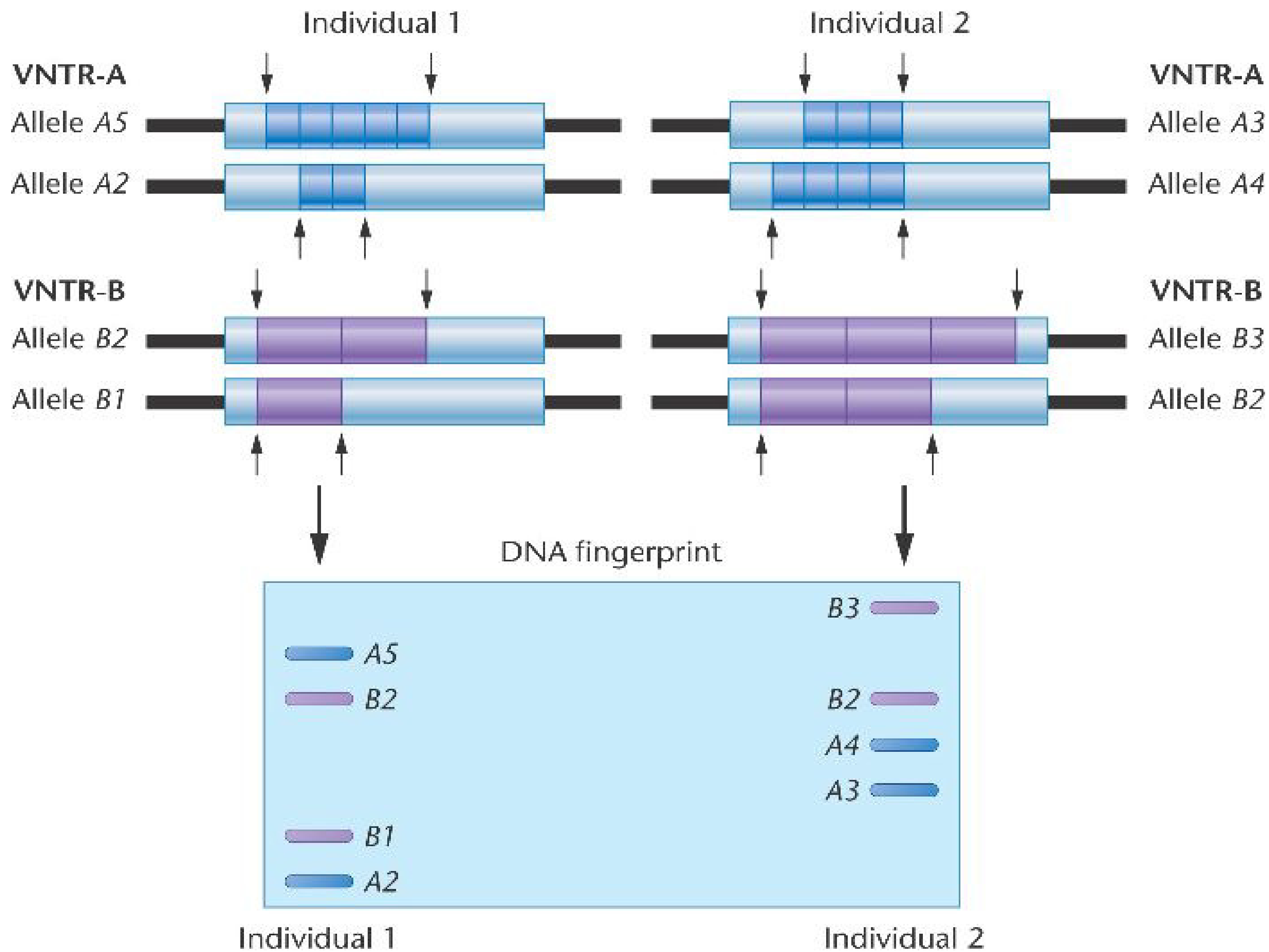
Polymorphisms can occur either in the sequence of bases at a single nucleotide locus (called SNP if only one base is altered) or . . .

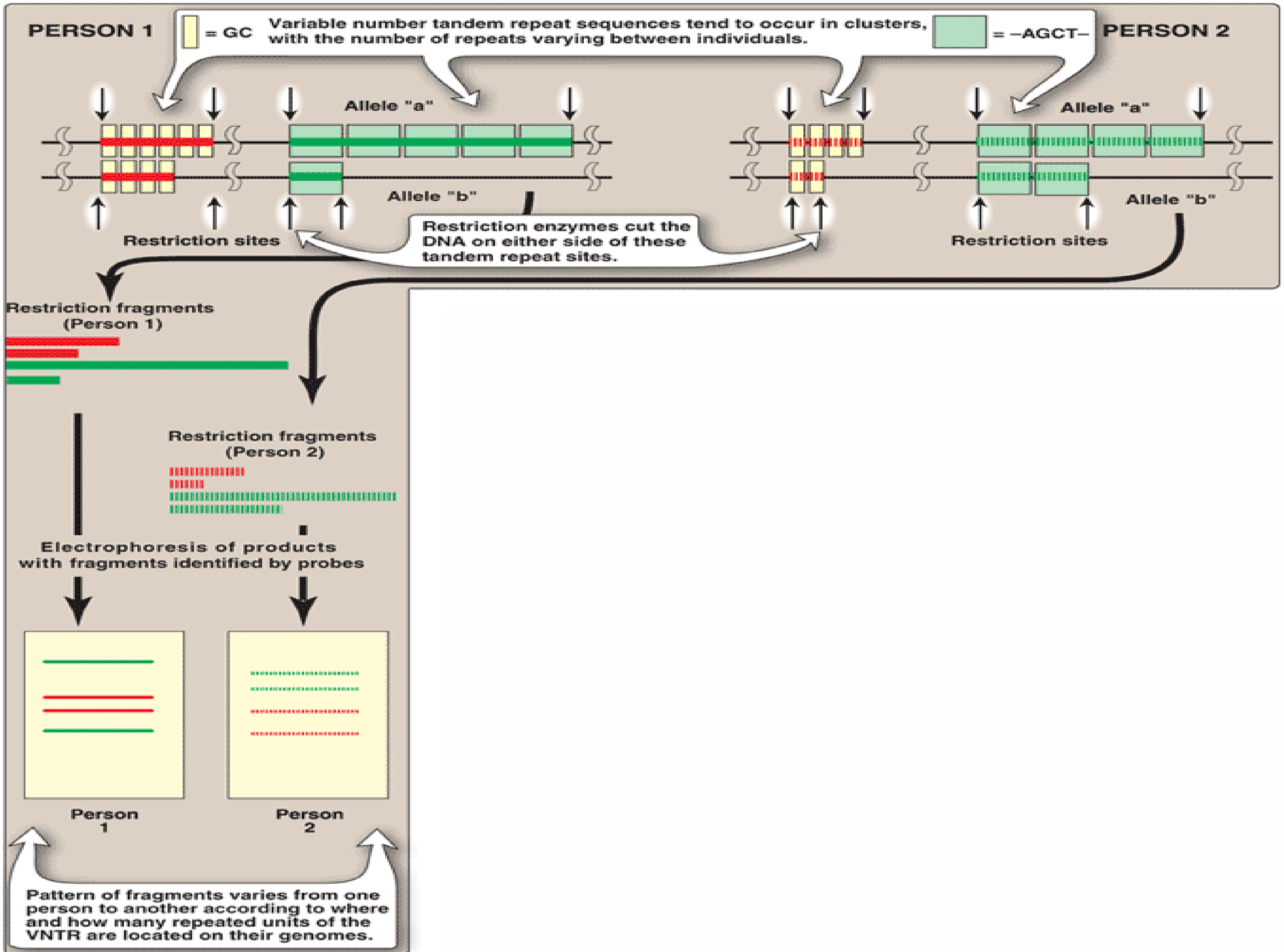


. . . there can be polymorphisms where variable numbers of tandem repeats (VNTR) occur. A specific number of tandem repeats defines a VNTR allele at a particular locus.



Same segment of DNA from three different individuals:
Repeating dinucleotide (AT)100
Restriction sites
5' 1000 2100 3'
3' 5'
A.
Restriction enzyme cuts up DNA into short segments
Repeating dinucleotide (AT)100
ATATAT...
5' 3100 3'
3' 5'
B.
Repeating dinucleotide (AT)300
ATATAT...
5' 1000 2300 3'
3' 5'
C.
A
B
C
(−)
(+)
3.1
2.3
2.1
1.0
Electrophoresis reveals that the RFLP fragments differ in length.
Shorter fragments, such as the 1000-nucleotide piece, move through the gel more rapidly than longer fragments.


Individual 1
Individual 2
VNTR-A
Allele A5
Allele A2
Allele A3
Allele A4
VNTR-B
Allele B2
Allele B1
Allele B3
Allele B2
DNA fingerprint
B3
A5
B2
B2
A4
A3
B1
A2
Individual 1
Individual 2



PERSON 1
= GC
Variable number tandem repeat sequences tend to occur in clusters, with the number of repeats varying between individuals.
= –AGCT–
PERSON 2
Allele "a"
Allele "b"
Allele "a"
Allele "b"
Restriction sites
Restriction enzymes cut the DNA on either side of these tandem repeat sites.
Restriction sites
Restriction fragments (Person 1)
Restriction fragments (Person 2)
Electrophoresis of products with fragments identified by probes
Person 1
Person 2
Pattern of fragments varies from one person to another according to where and how many repeated units of the VNTR are located on their genomes.

Single base changes in DNA

- About 90%
- SNPs = Single Base Polymorphism ("snips")
- New restriction site created
- Results in fragments of lengths differing from the normal
- Detected by DNA hybridization

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Tandem Repeats

- Variable number of tandem repeats (VNTR)
  - Short sequences
  - Scattered location
  - Repeated in tandem (one after another).
  - Number of VNTR units varies from person to person
  - unique for any given individual.
- Useful for DNA fingerprint
- In forensic and paternity identity cases.
- No known effect on the structure or rate of production of any particular protein.

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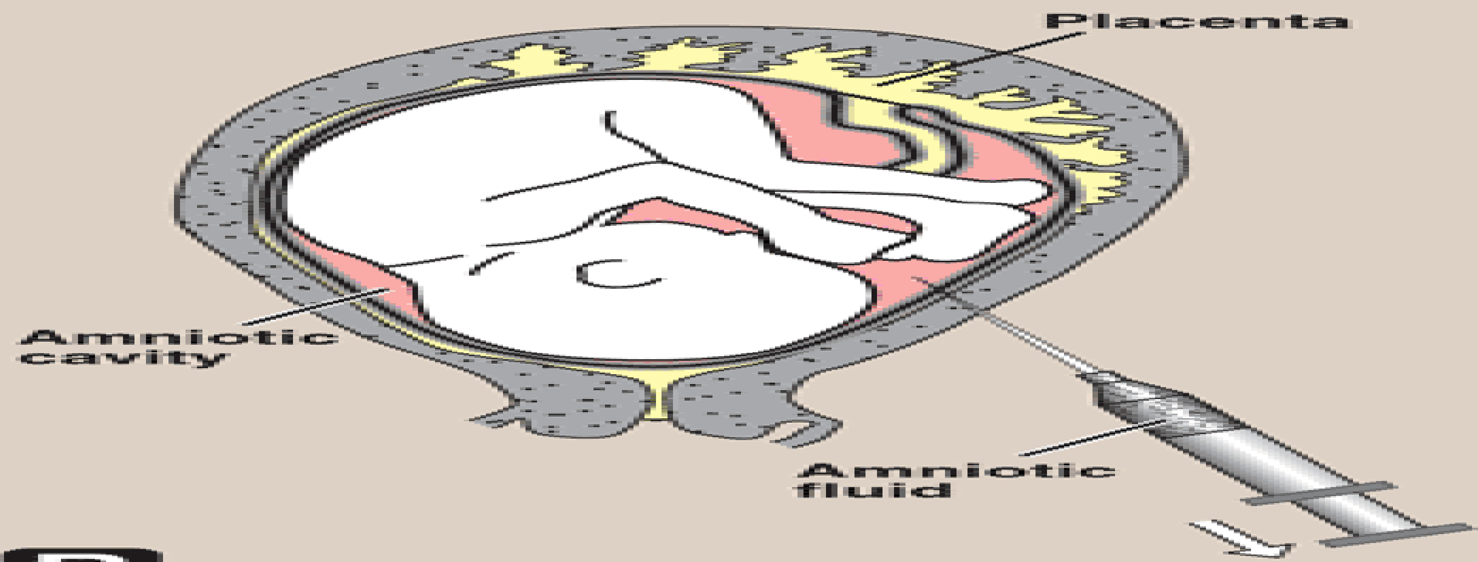
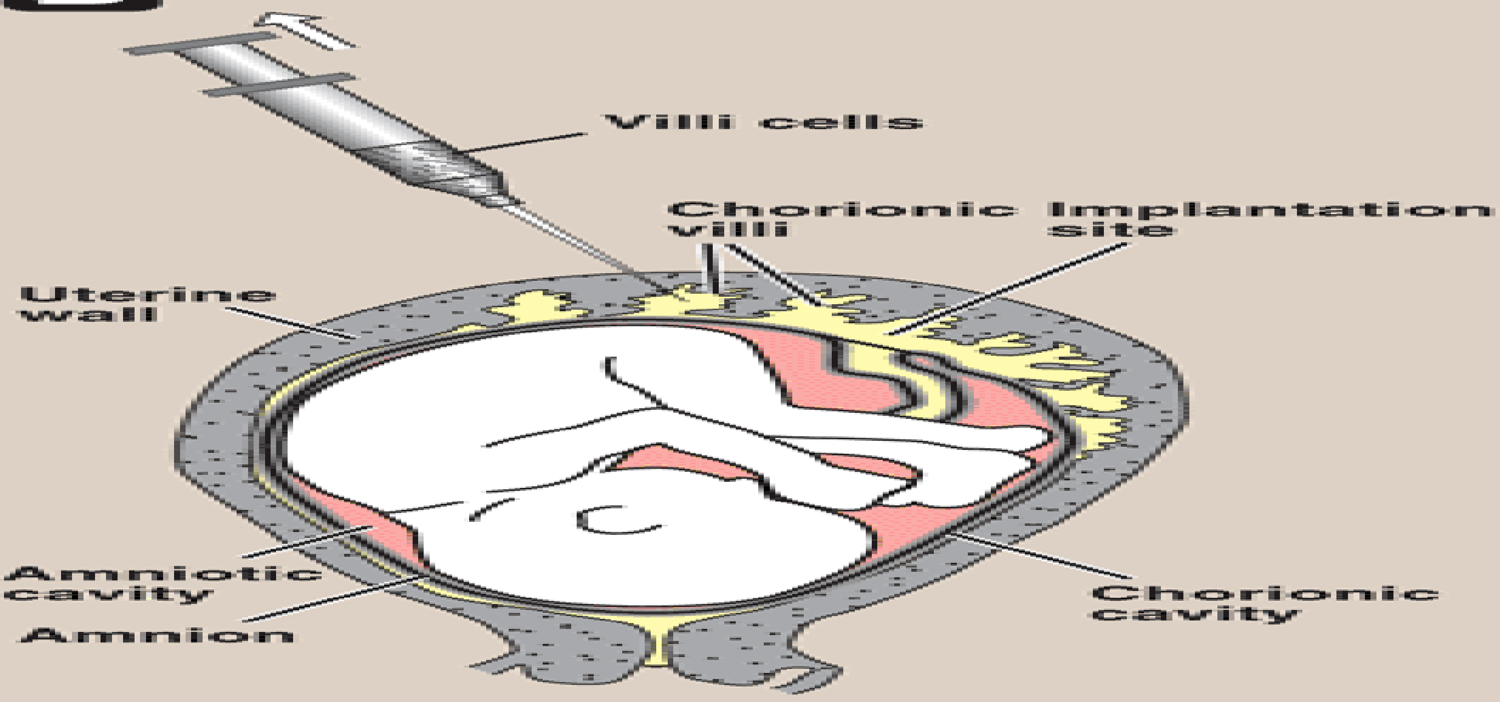

A
Placenta
Amniotic
cavity
Amniotic
fluid


B
Villi cells
Chorionic
villi
Implantation
site
Uterine
wall
Amniotic
cavity
Amnion
Chorionic
cavity

Pre-natal diagnosis by RFLP

- Direct diagnosis : Sickle cell disease
- Indirect diagnosis : Phenylketonuria
  - Phenylalanine hydroxylase (PAH) gene deficient
  - On chromosome 12
  - 90kb of genomic DNA & 13 exons
  - Mutations in this gene usually do not directly affect any restriction endonuclease recognition site.
  - To establish a diagnostic protocol for this genetic disease, one has to analyze DNA of family members of the afflicted individual. The key is to identify markers (RFLP) that are tightly linked to the disease trait.
  - Once these markers are identified, RFLP analysis can be used to carry out prenatal diagnosis.

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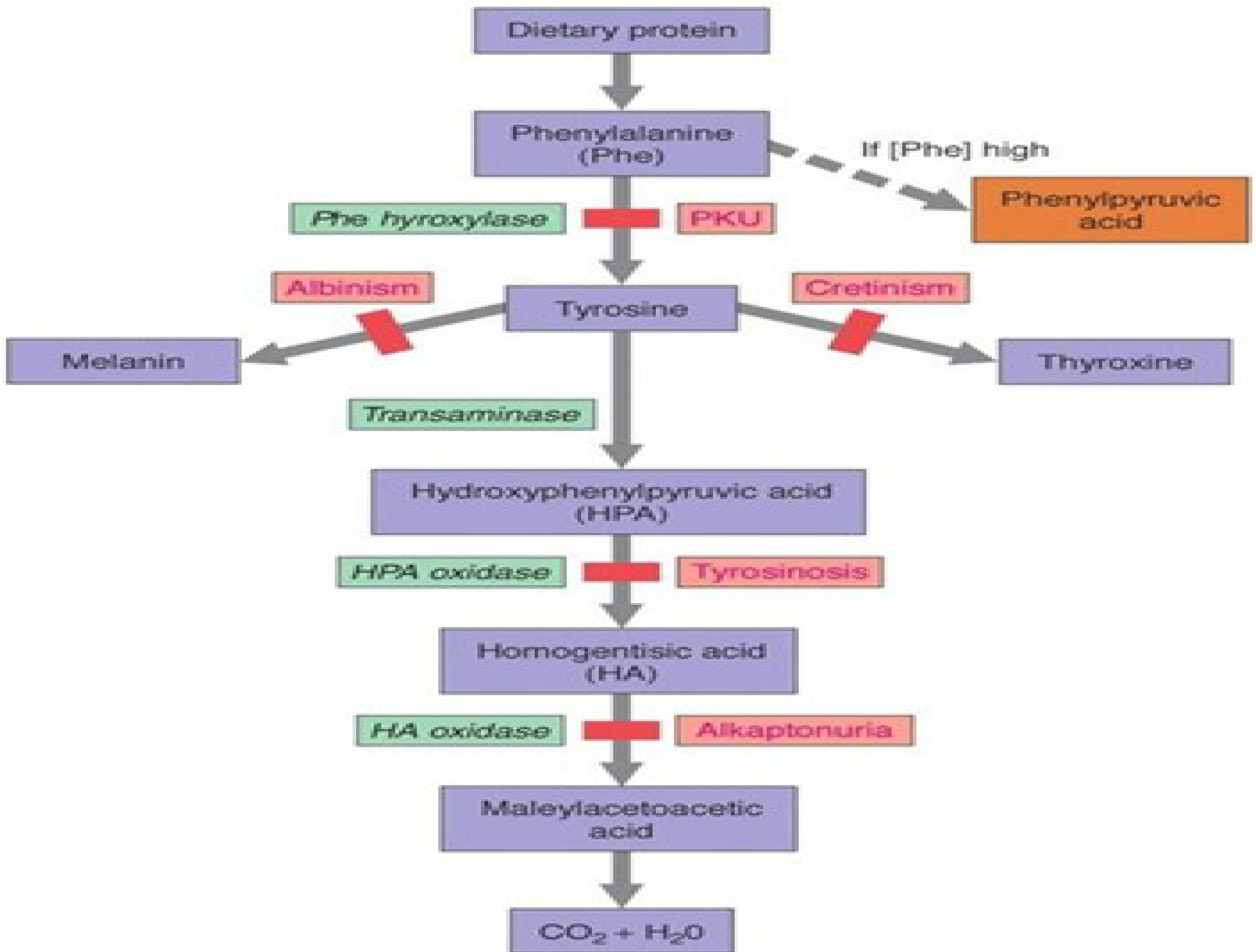
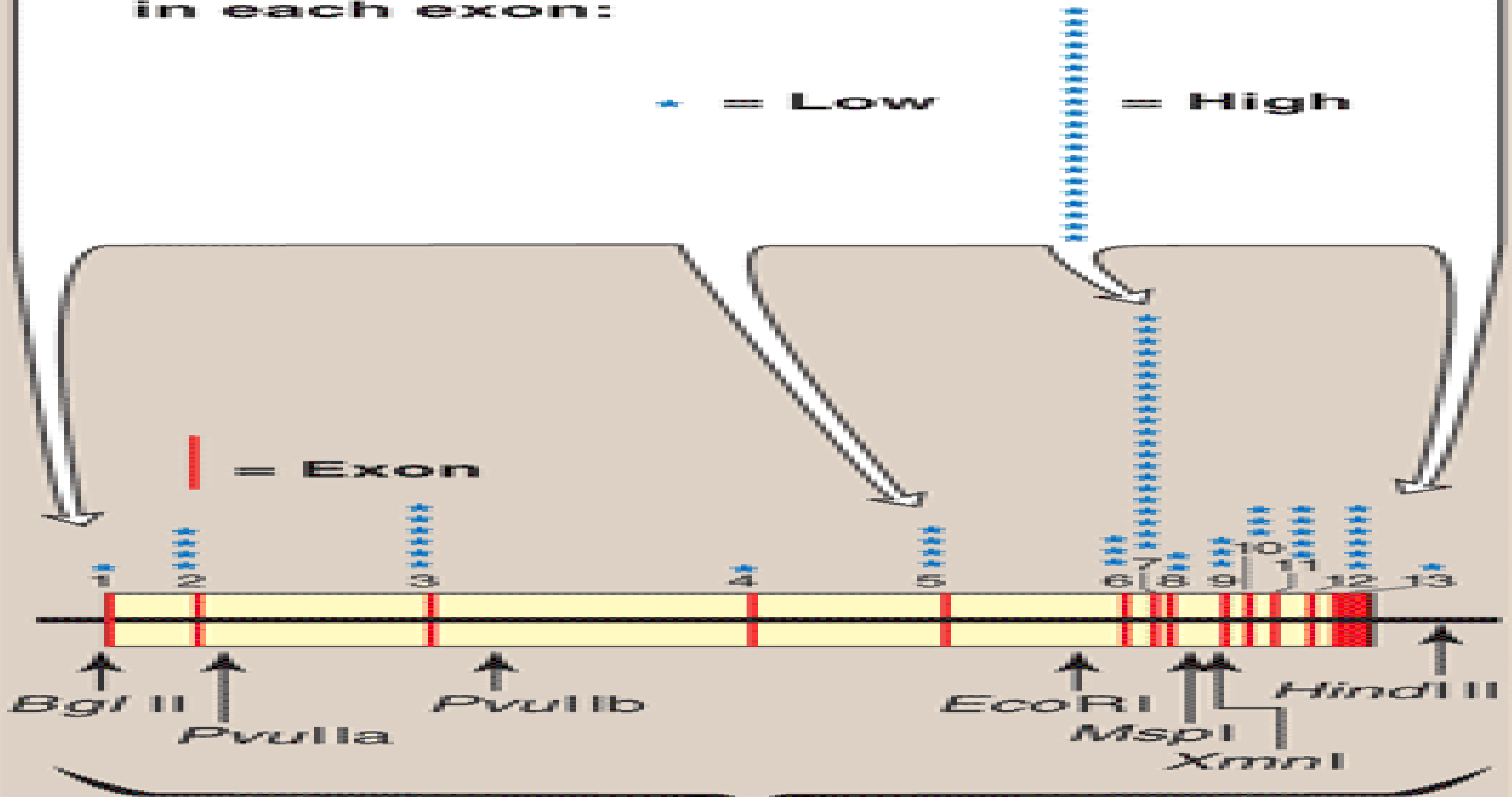
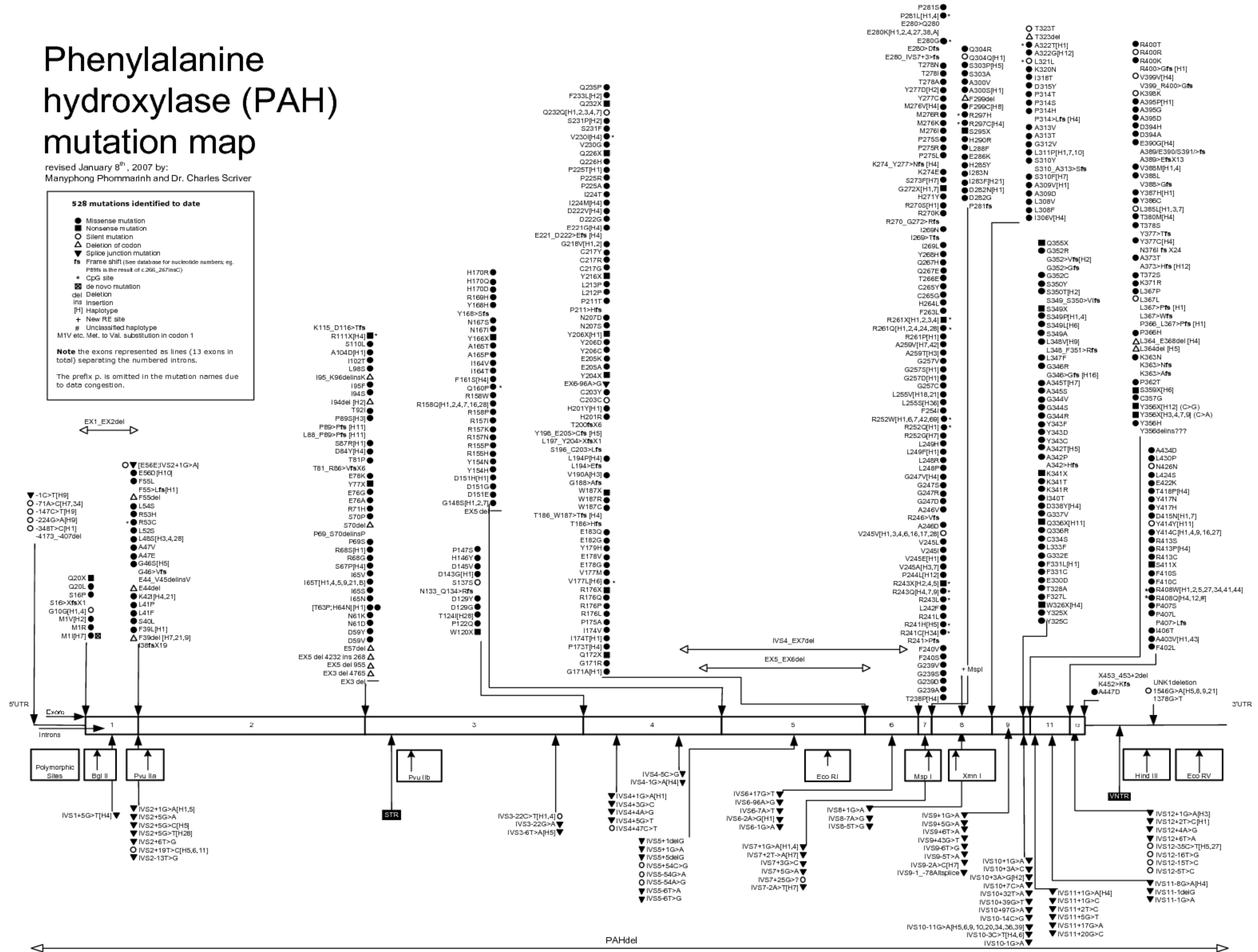


Dietary protein
Phenylalanine (Phe)
If [Phe] high
Phenylpyruvic acid
Phe hyroxylase
PKU
Albinism
Tyrosine
Cretinism
Melanin
Thyroxine
Transaminase
Hydroxyphenylpyruvic acid (HPA)
HPA oxidase
Tyrosinosis
Homogentisic acid (HA)
HA oxidase
Alkaptonuria
Maleylacetoacetic acid
$CO_2 + H_20$



Mutations in the *phenylalanine hydroxylase* gene occur in all thirteen exons of the gene. The majority are missense mutations, although splice, nonsense, and silent mutations, as well as deletions and insertions, have been found.
Relative frequency of mutation in each exon:
= Low
= High
= Exon
1
2
3
4
5
6
7
8
9
10
11
12
13
Bgl II
*Pvu*IIa
*Pvu*IIb
*Eco*RI
*Msp*I
*Xmn*I
*Hind*III
Some sites cleaved by restriction enzymes



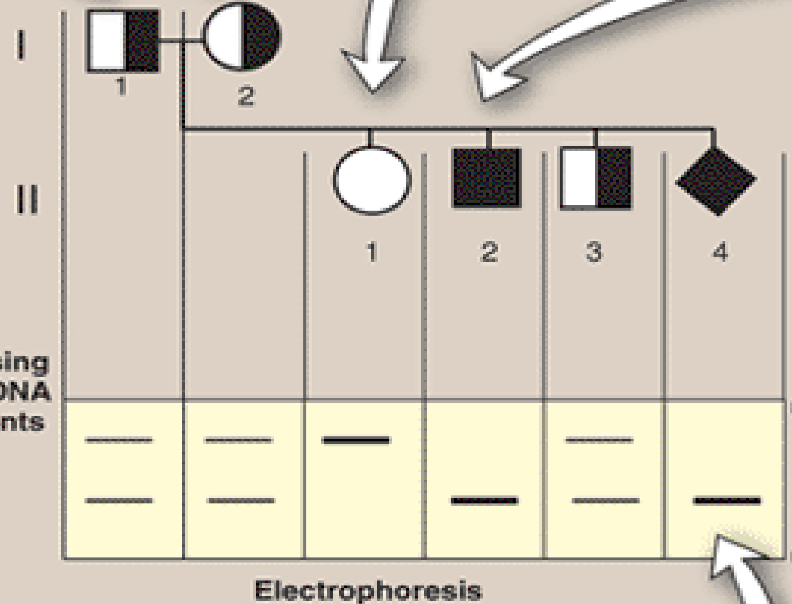
Phenylalanine hydroxylase (PAH) mutation map
revised January 8th, 2007 by:
Manyphong Phommarinh and Dr. Charles Scriver
528 mutations identified to date
Missense mutation
Nonsense mutation
Silent mutation
Deletion of codon
Splice junction mutation
fs Frame shift (See database for nucleotide numbers; eg. P89fs is the result of c.266_267insC)
* CpG site
de novo mutation
del Deletion
ins Insertion
[H] Haplotype
+ New RE site
# Unclassified haplotype
M1V etc. Met. to Val. substitution in codon 1
Note the exons represented as lines (13 exons in total) separating the numbered introns.
The prefix p. is omitted in the mutation names due to data congestion.
EX1_EX2del
IVS4_EX7del
EX5_EX6del
5'UTR
3'UTR
Exons
Introns
Polymorphic Sites
Bgl II
Pvu IIa
Pvu IIb
STR
Eco RI
Msp I
Xmn I
Hind III
Eco RV
VNTR
PAHdel

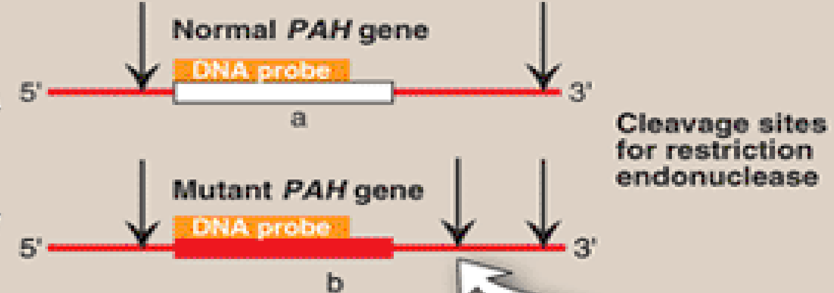
The parents are both heterozygous for the phenylalanine hydroxylase gene. They, thus, have both fragment "a" (normal) and fragment "b" (defective) cleaved by a restriction nuclease.

This child is unaffected and shows only fragment "a" when DNA is digested with the same restriction endonuclease as her parents' DNA. Thus, the normal gene is associated with the polymorphism giving fragment "a."

This child is affected (lacks *phenylalanine hydroxylase* activity) and shows only fragment "b" when DNA is digested with the same restriction endonuclease. Thus, the defective gene is associated with the polymorphism giving fragment "b."

A radioactive probe hybridizes with DNA fragments "a" and "b".





Fetal DNA shows only fragment "b" when DNA is digested with the same restriction endonuclease. This means that the fetus is affected because it has inherited two abnormal genes from its parents and shows the genotype "bb."

The presence of a polymorphic site permits cleavage by the enzyme and, therefore, yields fragment "b." [Note: The polymorphic site is, in general, not the structural alteration that causes the disease and in this case is not even in the coding part of the gene.]

Legend:

Male Female Fetus Heterozygote Homozygote

Microarray

- Contain thousands of immobilized DNA sequences
- Used to analyze a sample for the gene variations & mutation
- Analyzing thousands of genes at the same time.
- mRNA = converted to cDNA
- labeled with a fluorescent tag
- This mixture is then exposed to a gene chip, which is a glass slide or membrane containing thousands of tiny spots of DNA, each corresponding to a different gene.
- Amount of fluorescence is measure

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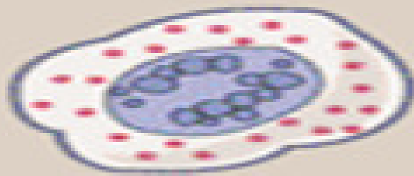
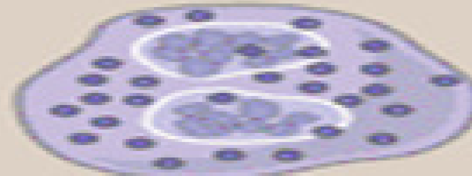
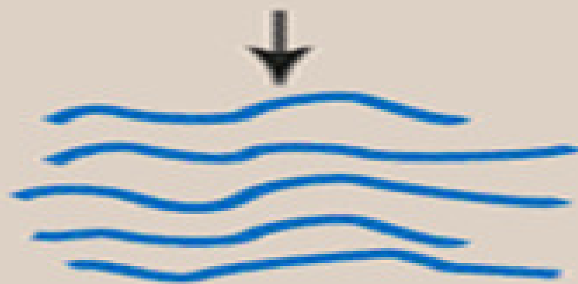
Normal cell

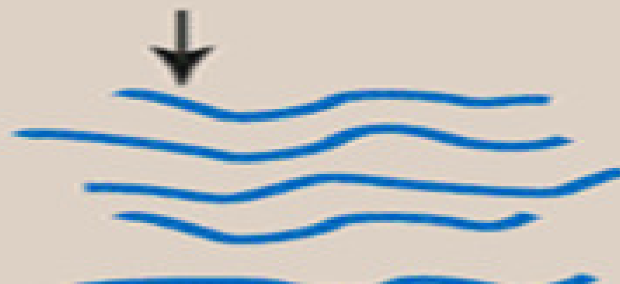
Cancer cell



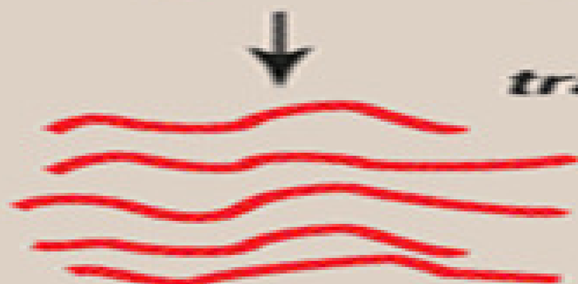




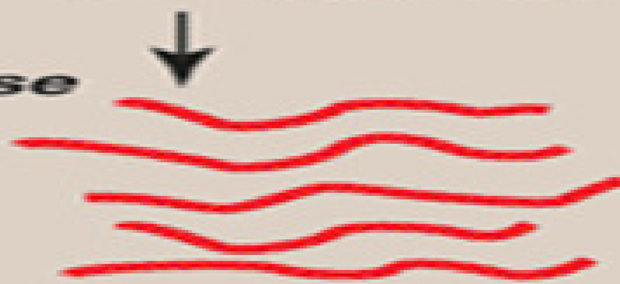
mRNA



Reverse transcriptase



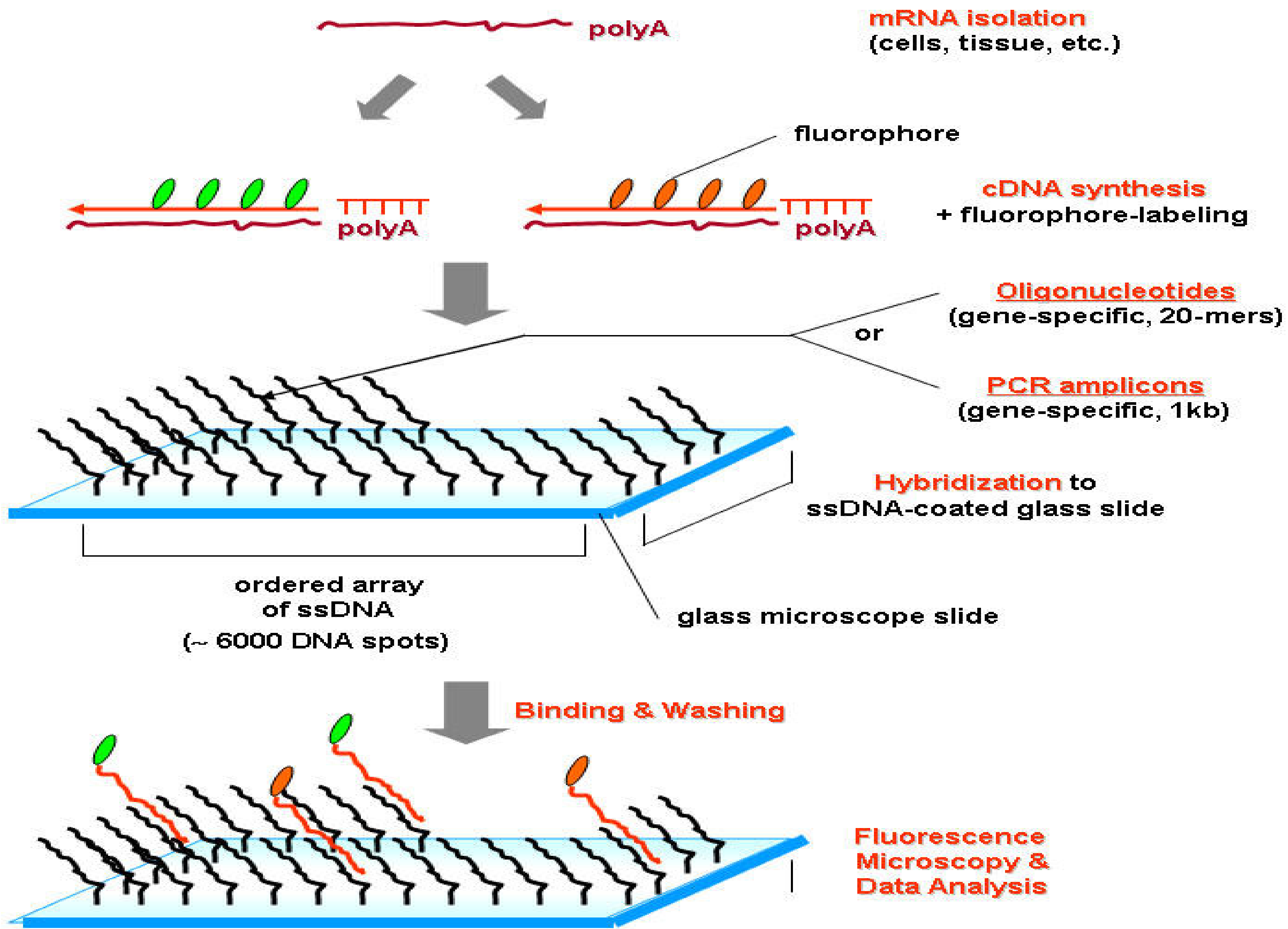
cDNA



Label with green fluorescent molecule

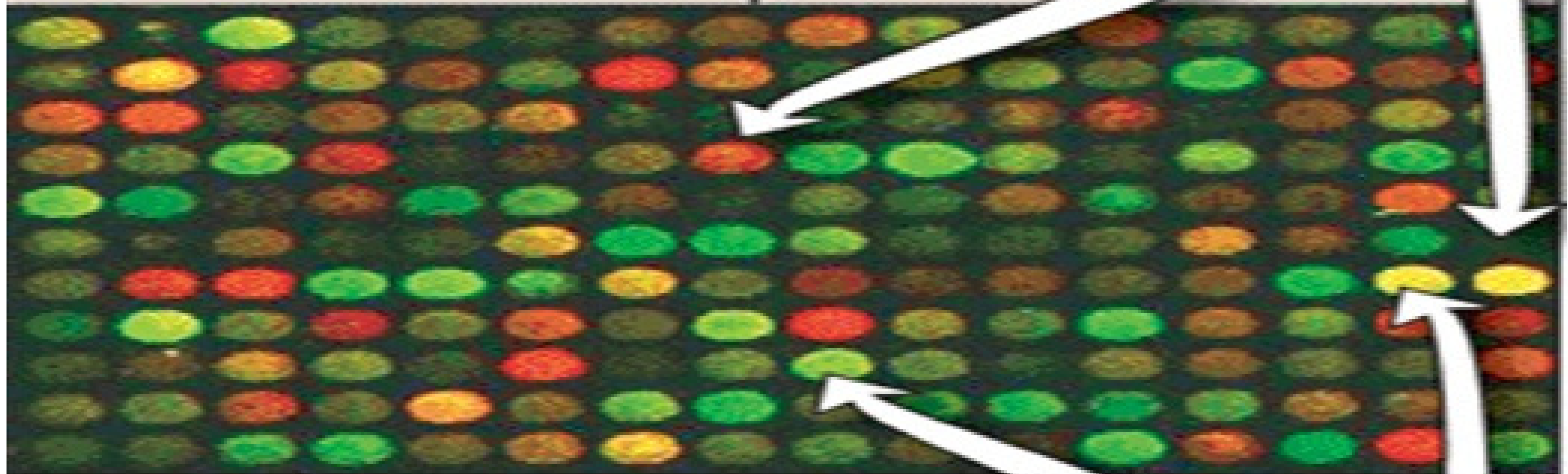
Label with red fluorescent molecule

Mix cDNAs and hybridize to microarray


polyA
mRNA isolation
(cells, tissue, etc.)
fluorophore
polyA
polyA
cDNA synthesis
+ fluorophore-labeling
Oligonucleotides
(gene-specific, 20-mers)
or
PCR amplicons
(gene-specific, 1kb)
Hybridization to
ssDNA-coated glass slide
ordered array
of ssDNA
(~ 6000 DNA spots)
glass microscope slide
Binding & Washing
Fluorescence
Microscopy &
Data Analysis
Graphic©E.Schmid-2005

Dark (black) spot: Neither cell produces this message.

Red spot: Cancer cell produces more of its message.

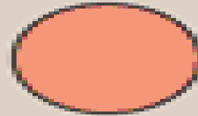
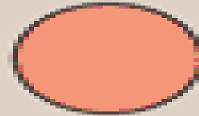
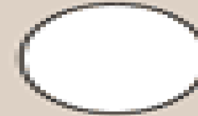


Green spot: Normal cell produces more of its message.

Yellow spot: Both cells produce the same amount of message.

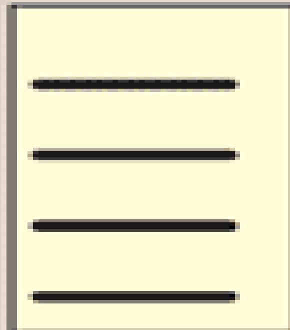
Patient 1 | Patient 2 | Patient 3

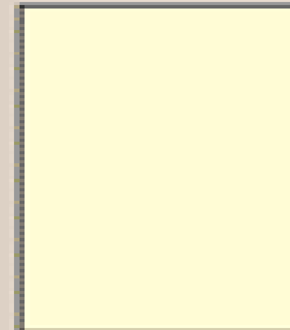
Test blood samples by ELISA assay

Positive | Positive | Negative

Retest using Western blots





Positive

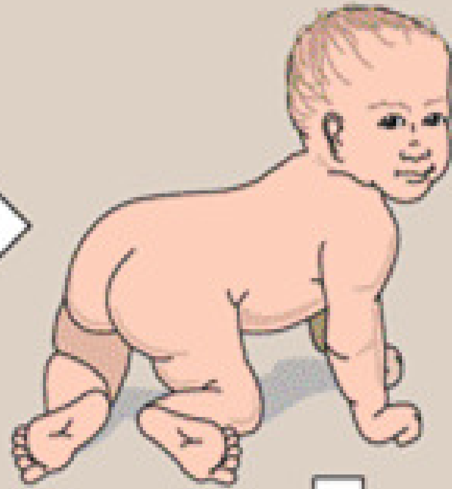
The patient's serum contains antibodies to HIV

Negative

The patient's serum gave a false-positive response in the ELISA assay

Negative

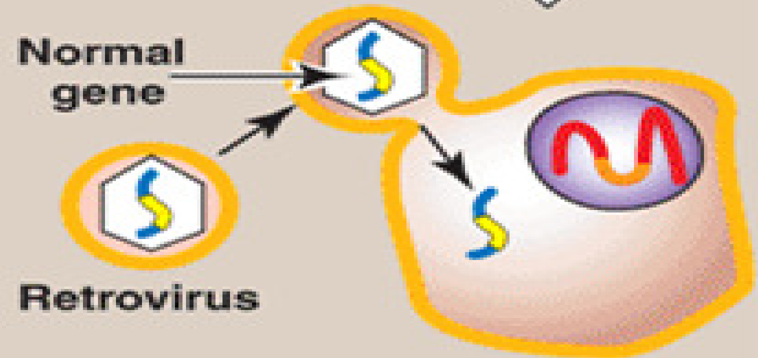
TECHNIQUE	SAMPLE ANALYZED	GEL USED	PURPOSE
Southern blot	DNA	Yes	Detects DNA changes
Northern blot	RNA	Yes	Measures mRNA amounts and sizes
Western blot	Protein	Yes	Measures protein amounts
ASO	DNA	No	Detects DNA mutations
Microarray	RNA or cDNA	No	Measures many mRNA levels at once
ELISA	Proteins or antibodies	No	Detects proteins (antigens) or antibodies
Proteomics	Proteins	Yes	Measures abundance, distribution, posttranslational modifications, functions, and interactions of cellular proteins



1 Remove some of the patient's blood. Lymphocytes are separated from other cells in the blood)

4 Return the cells to the patient, and the patient regains immune function

2 Infect the lymphocytes with a retrovirus modified to carry the normal adenosine deaminase gene.



3 The gene becomes integrated into the cell's chromosomes and is expressed. Some of the patient's lymphocytes now synthesize *adenosine deaminase*.