TOPIC 34:

BIOTECHNOLOGY AND RECOMBINANT DNA

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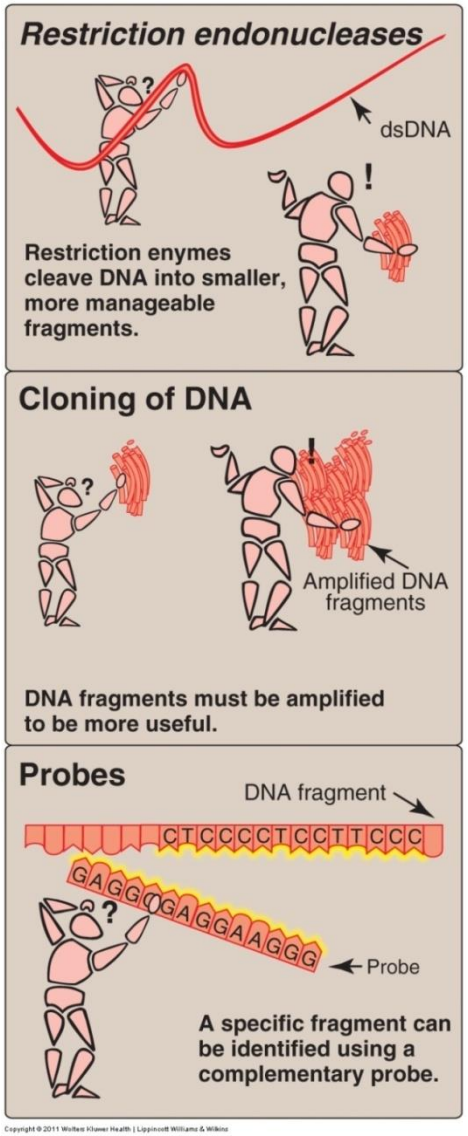
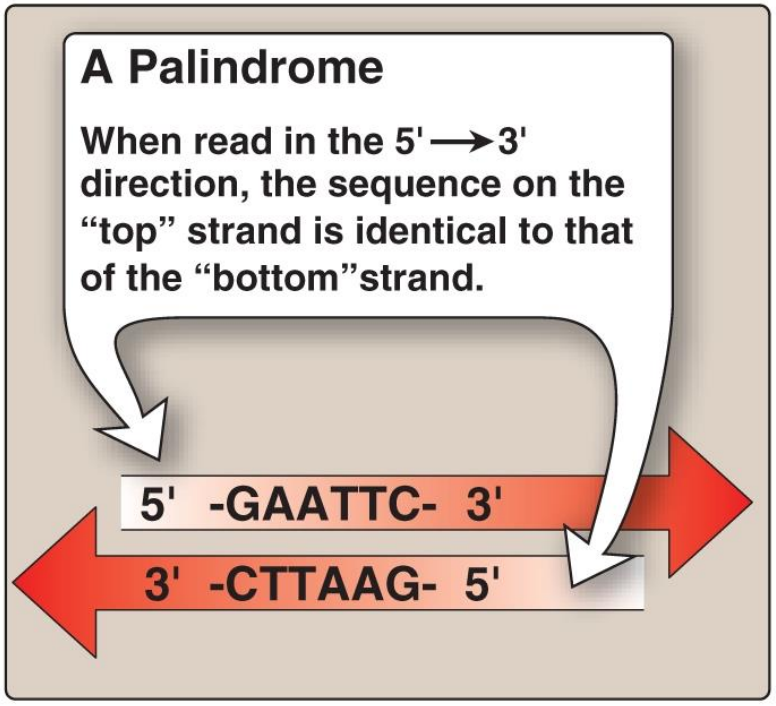
Techniques to Analyze the DNA



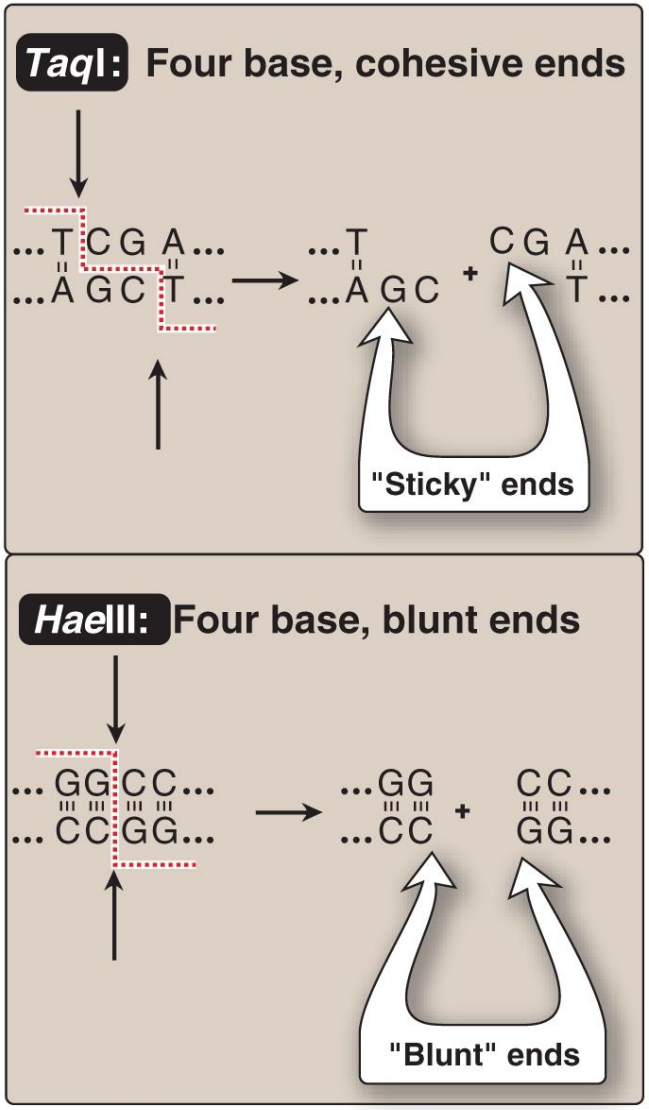
Restriction enzymes recognize palindrome DNA sequences



Nomenclature:

genus, species, strain, the final number indicate the order in which the enzyme was discovered:
HaeIII: The third restriction endonuclease isolated from the bacterium *Haemophilus aegyptius.*

Restriction enzymes produce sticky or blunt ends



Restriction sites:

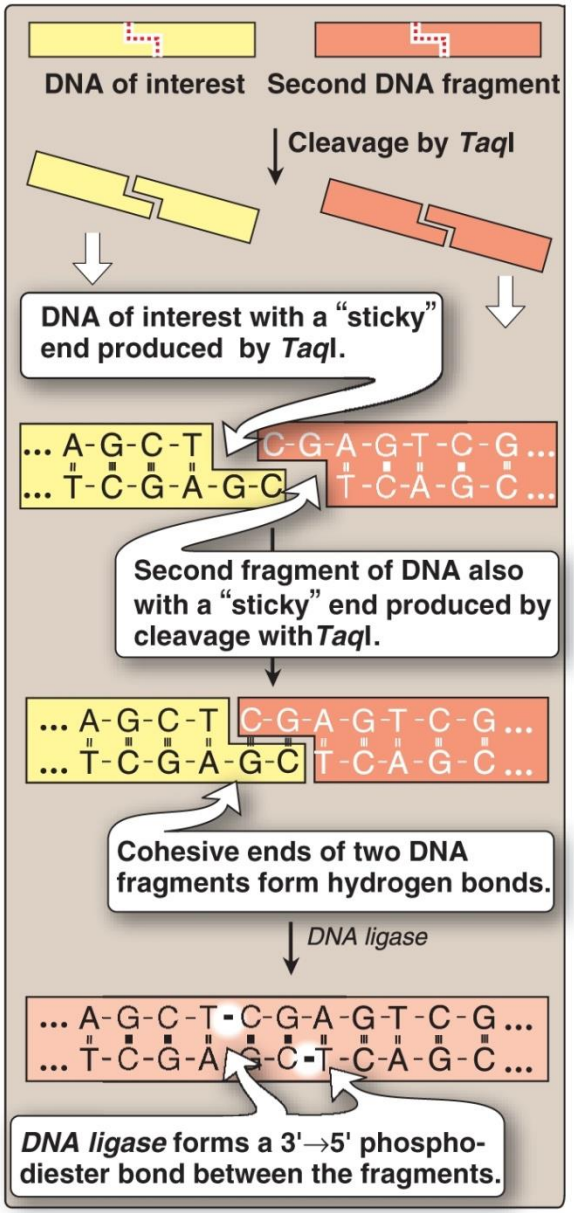
Four-base pairs: 4^4

Six-base pairs: 4^6

Example: Human genome: 3 x10^9 bp

How many pieces with each enzyme?

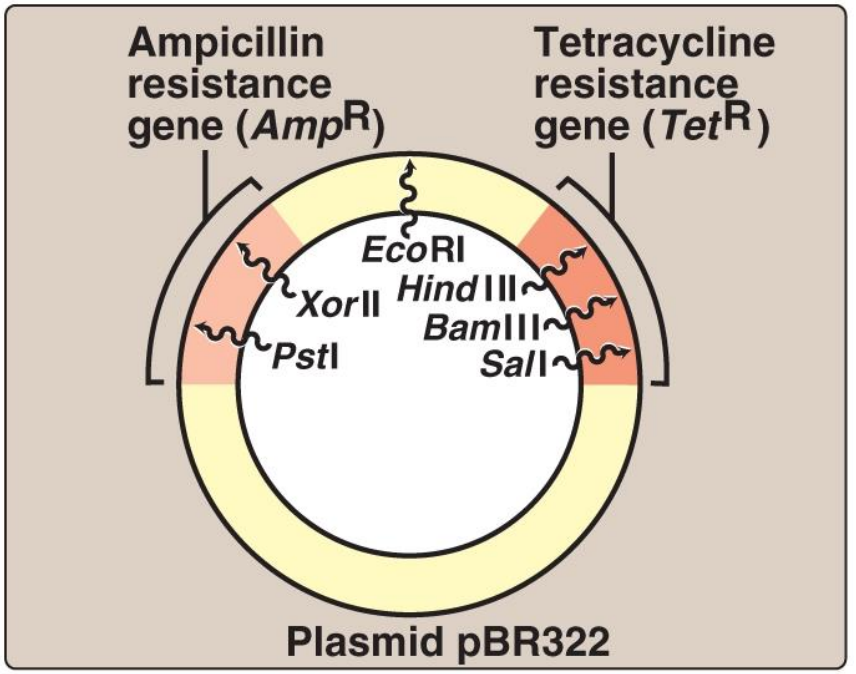
Formation of an recombinant DNA



Plasmids are used to clone DNA fragments

Properties of a vector:

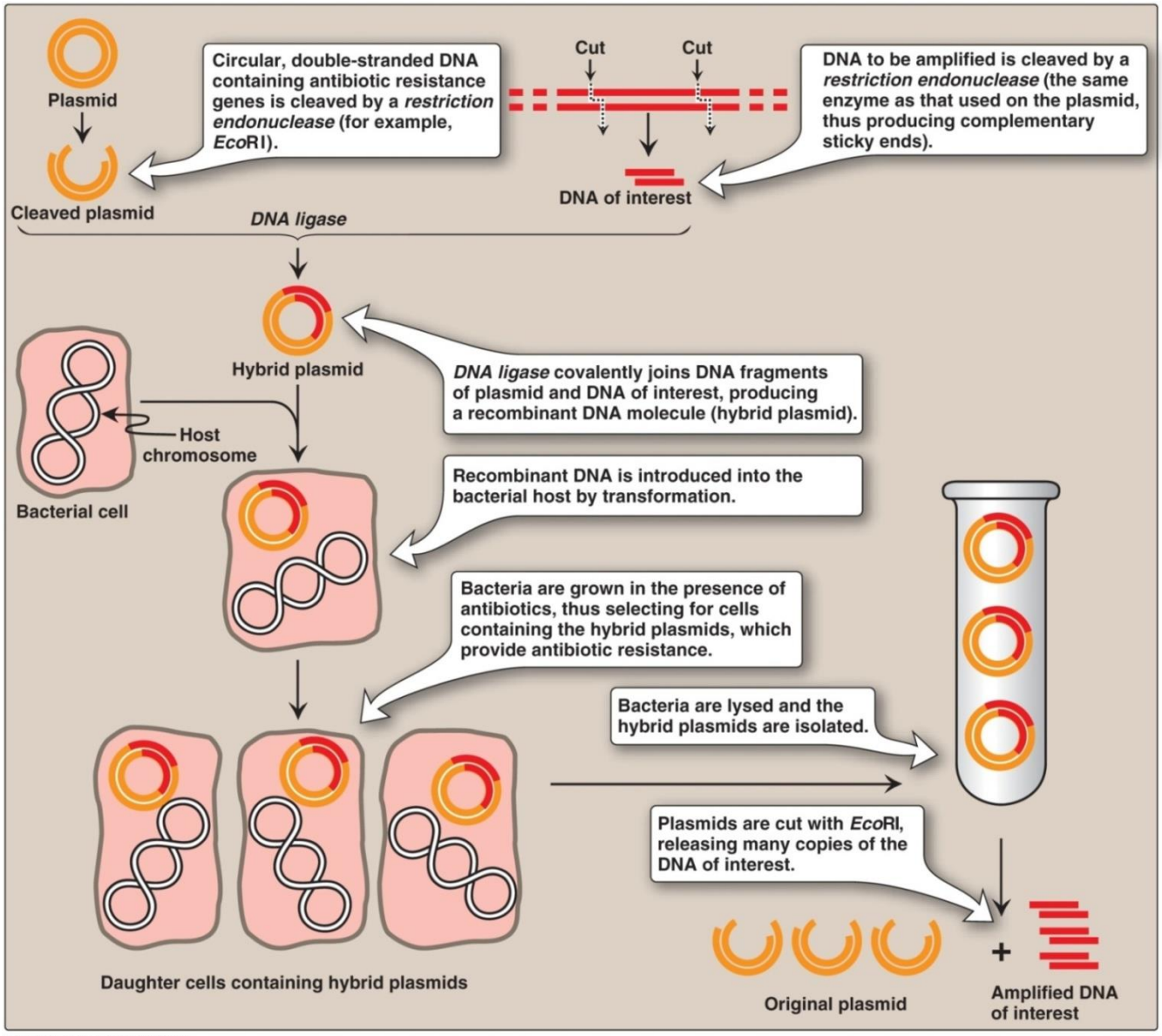
- Autonomous replication.
- Nucleotide sequence recognized for a restriction enzyme.
- Gene for vector selection.



Common vectors:

- Plasmids (10 Kb)
- Viruses
- Cosmids (37-53 Kb)
- Bacterial and yeast artificial chromosomes (BACs and YACs) 100-200 Kb and 200-500 Kb

Gene cloning

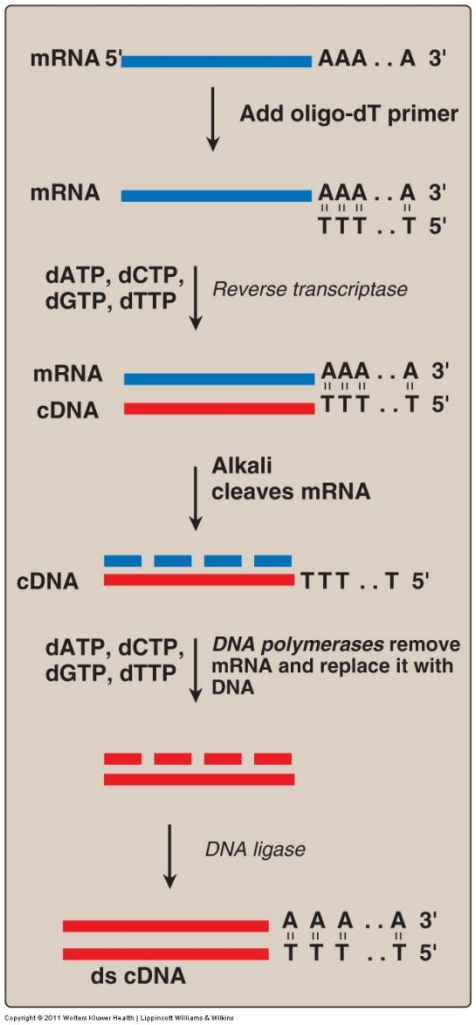


DNA libraries

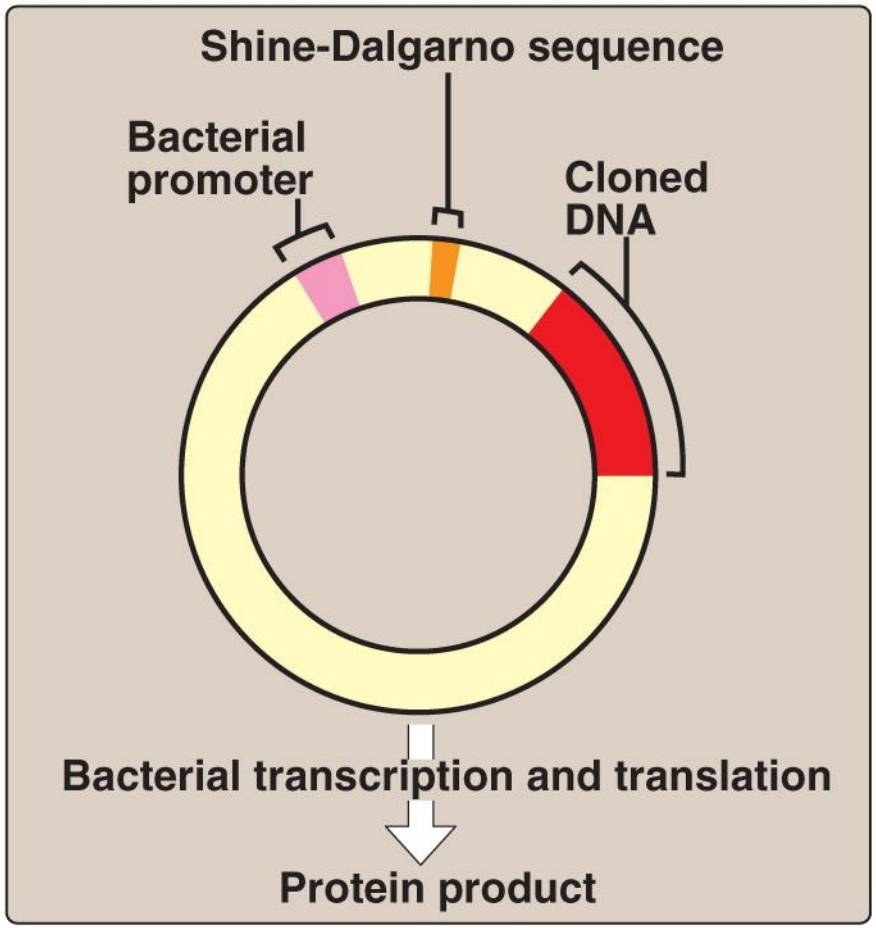
Genomic DNA libraries: Total DNA of an organism.

cDNA libraries: cDNA generated of mRNA sequences.

cDNA synthesis from mRNA

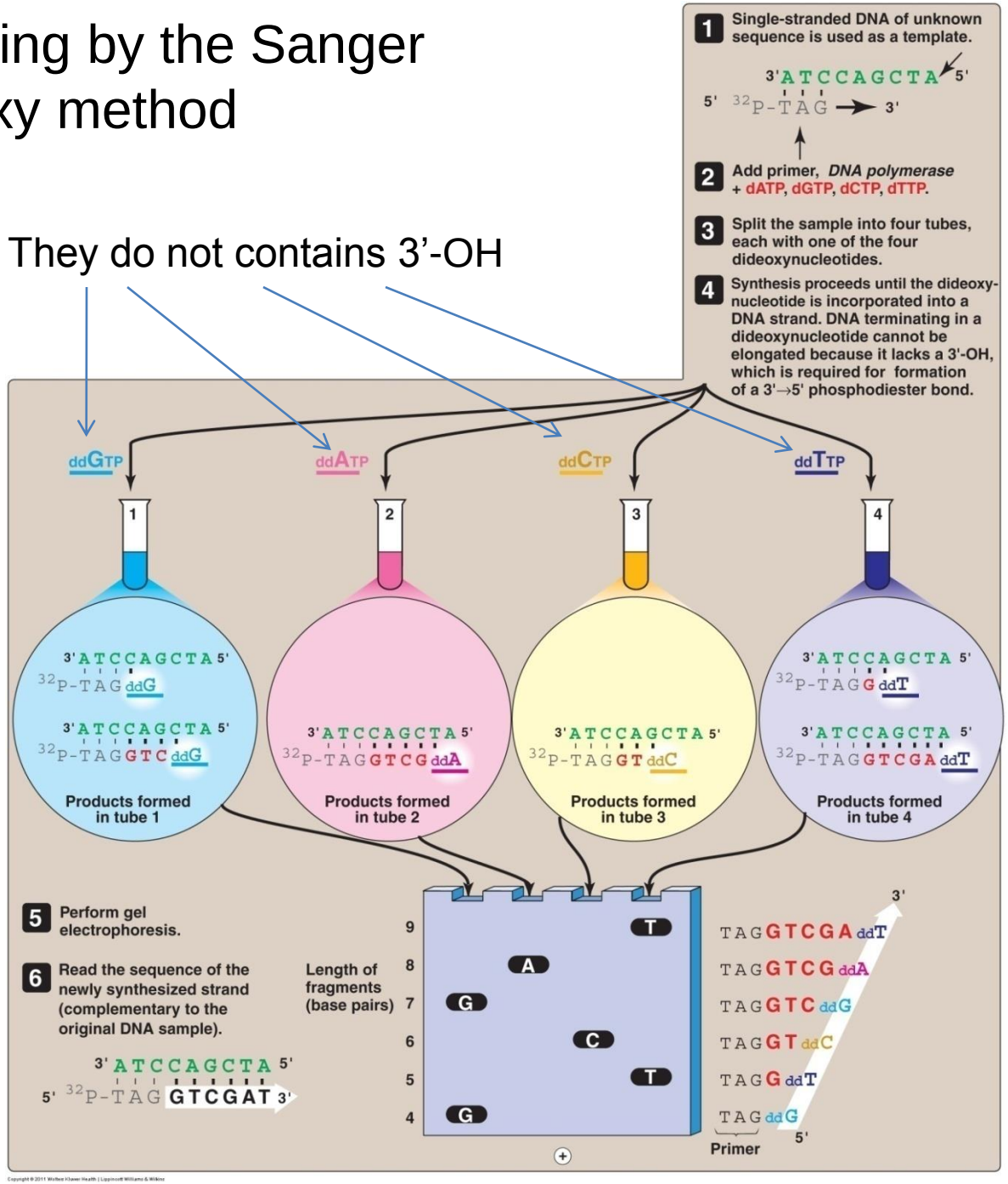


Expression vectors are used to synthesize eukaryotic proteins

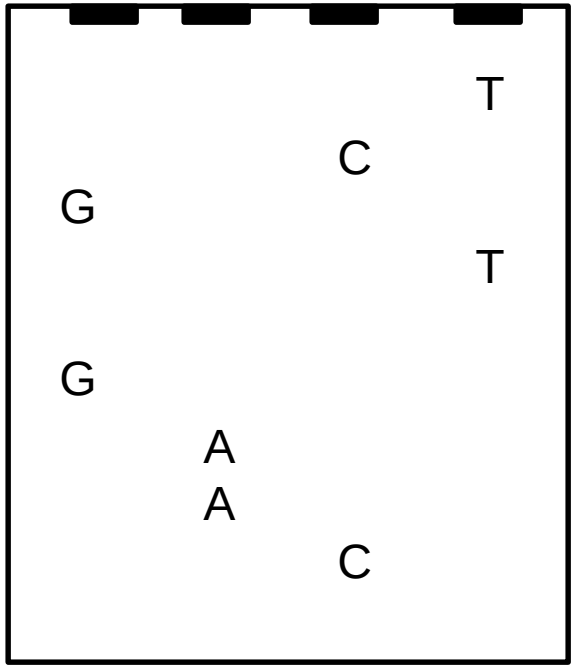


DNA sequencing by the Sanger dideoxy method

They do not contains 3’-OH



Finding the correct sequence



^{32}P-TAG-

Applications of recombinant DNA techniques

- **Recombinant human insulin**. Recombinant insulin is synthesized by inserting the human insulin gene into *E. coli* or yeast which then produces insulin for human use.

- **Recombinant human growth hormone** (HGH, somatotropin). Before recombinant HGH became available, HGH for therapeutic use was obtained from pituitary glands of cadavers. This unsafe practice led to some patients developing Creutzfeldt-Jacob disease. Produced in *E. coli*.

- **Recombinant blood clotting factor VIII**. Recombinant factor VIII is a blood-clotting protein that is administered to patients with forms of the bleeding disorder hemophilia. The protein was obtained from multiple donors.

- **Recombinant hepatitis B vaccine**. Prevention of hepatitis B infection is controlled through the use of a recombinant hepatitis B vaccine, which contains a form of the hepatitis B virus surface antigen that is produced in yeast cells.

- **Golden rice** is a recombinant variety of rice that has been engineered to express the enzymes responsible for β-carotene biosynthesis. This variety of rice holds substantial promise for reducing the incidence of vitamin A deficiency in the world's population.

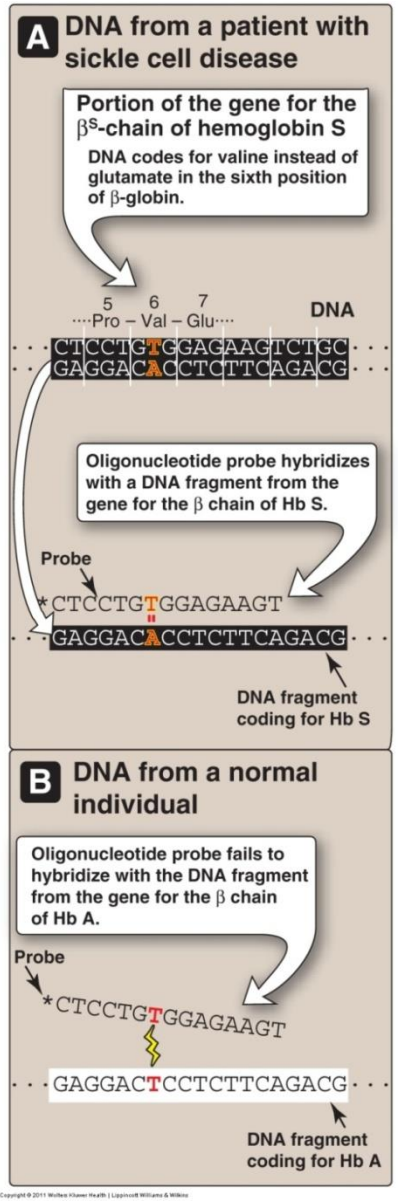
Probes

- DNA fragments
- Synthetic oligonucleotide
- Biotinylated probes
- Antibodies

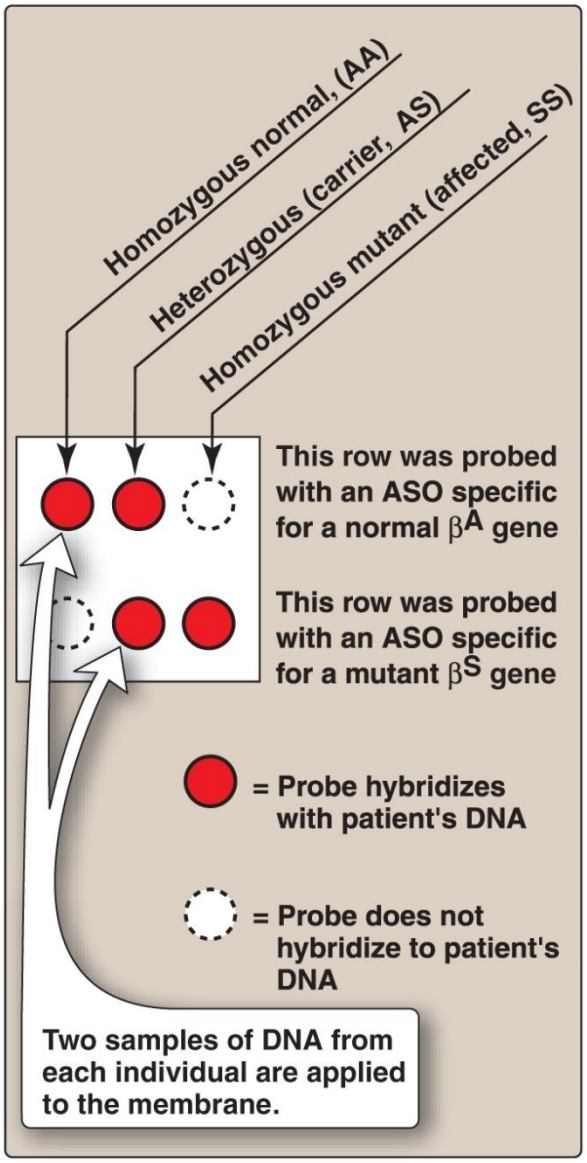
GAG ⟶ GTG

Glutamate ⟶ valine

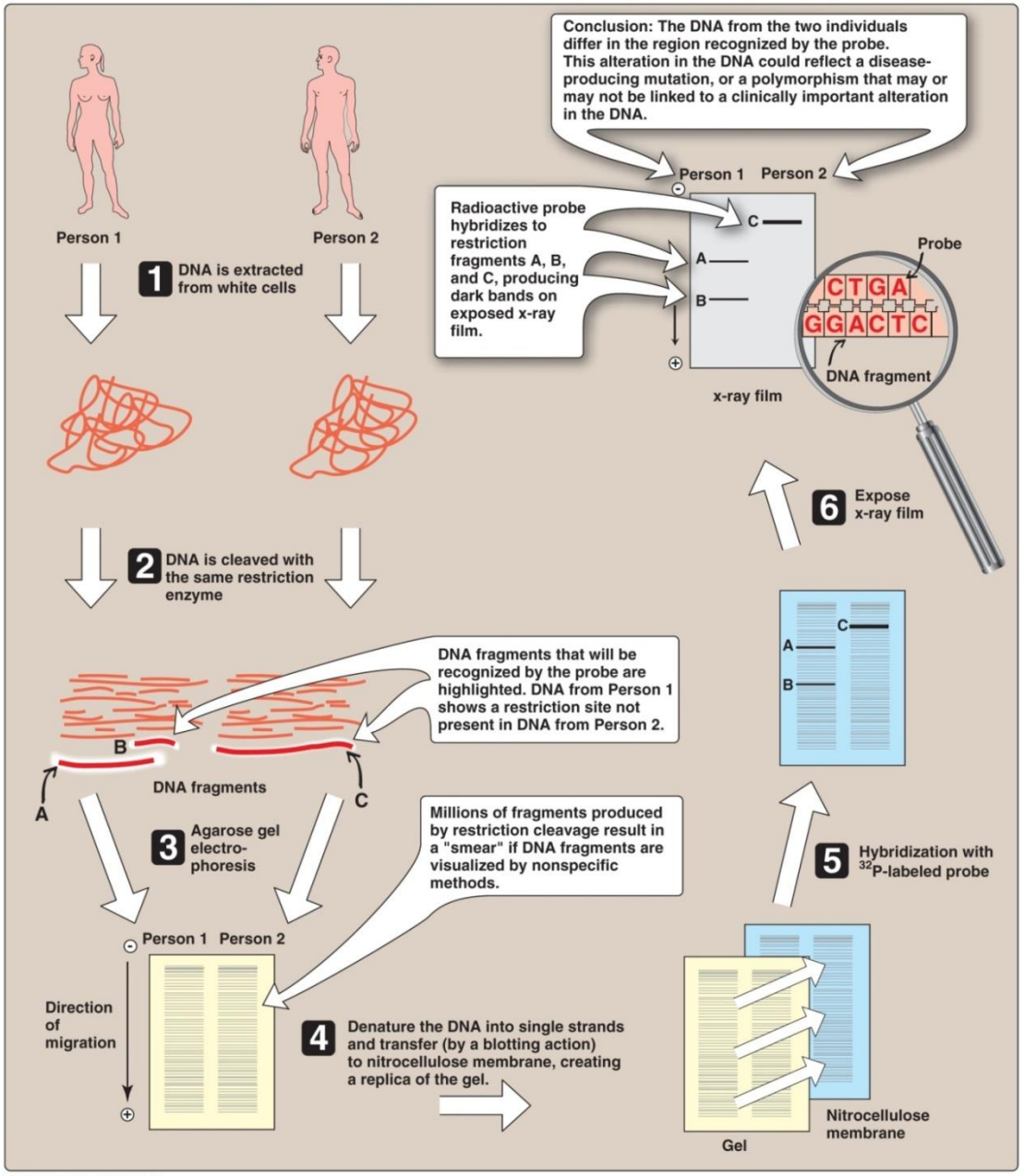
Allele-specific probe to detect hemoglobin mutation



Allele specific oligonulceotide probe



Southern Blotting

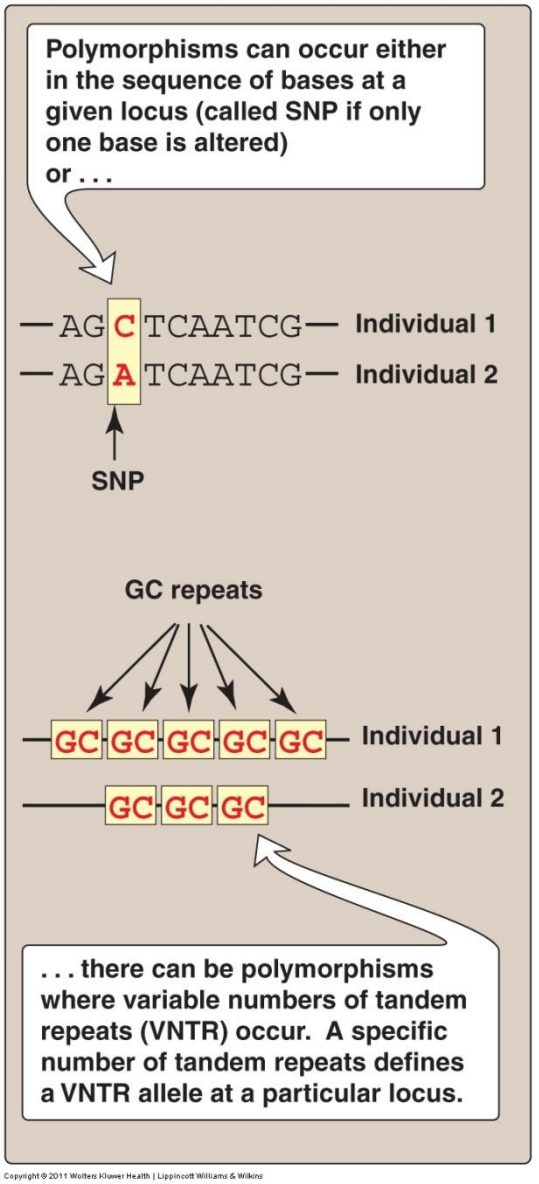


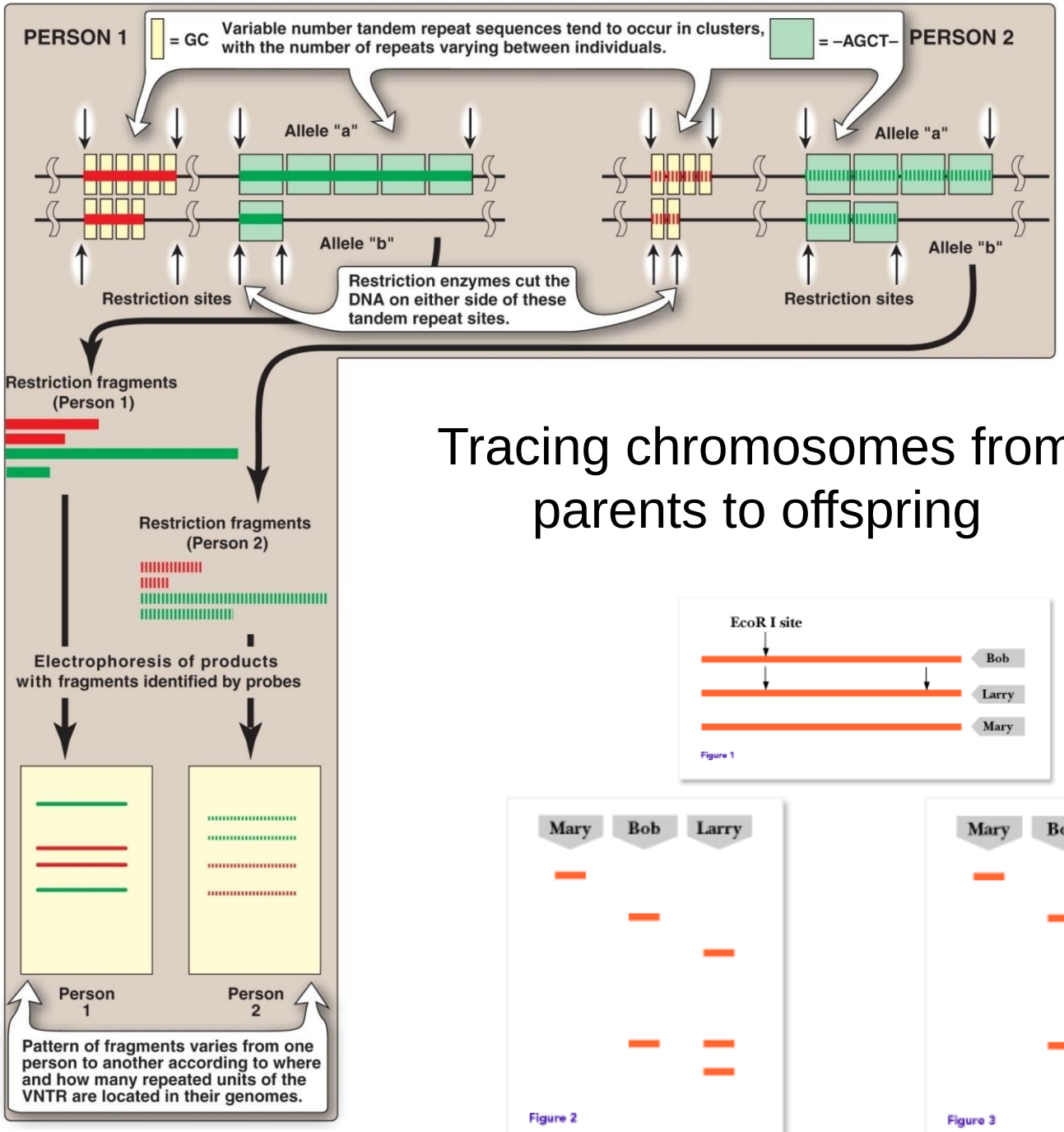
Restriction Fragments Length Polymorphism (RFLP)

Genome of non-related people: 1,200 bp or 0.1% genome: mutations and polymorphisms.

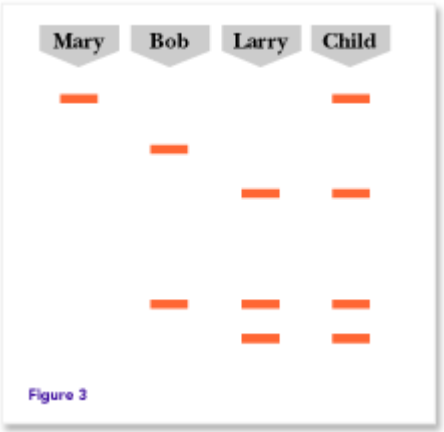
- Single nucleotide polymorphisms: single base changes in DNA (90% of variations)

- Tandem repeats (VNTR)



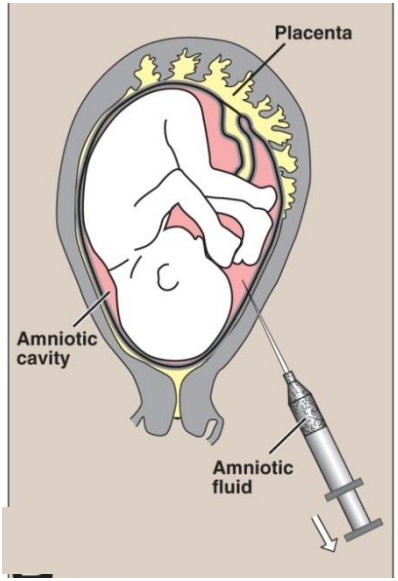


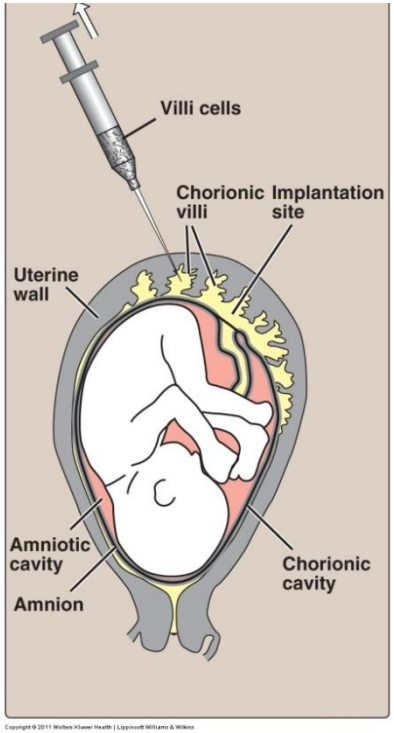
Tracing chromosomes from parents to offspring



Prenatal diagnosis

Ultrasound: gross anatomic defects
Amniotic fluids : chemical composition (α-fetoprotein), karyotyping
Chorionic villi: Karyotyping (chromosome abnormalities)
DNA: blood cells, amniotic fluid or chorionic villi

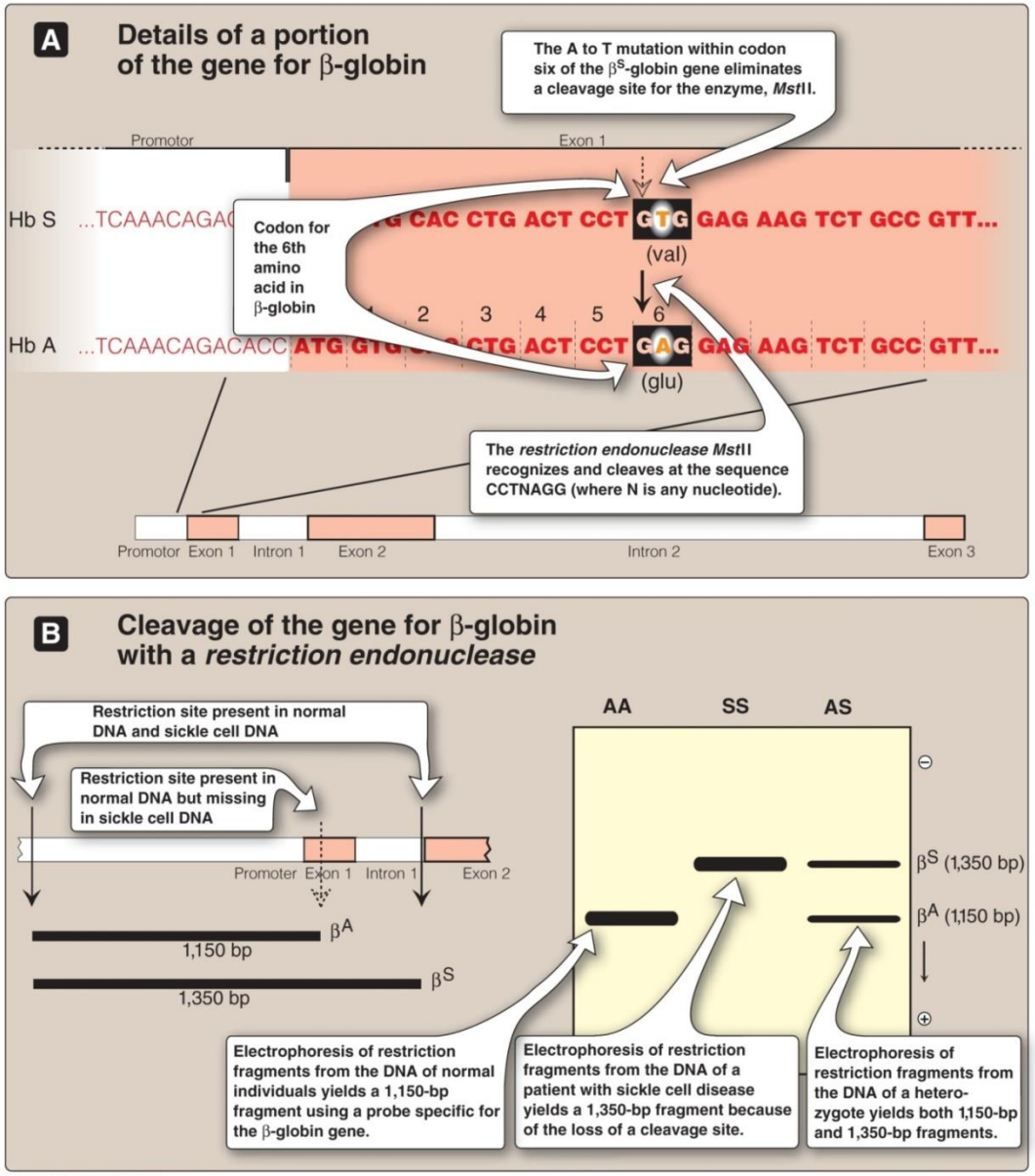




Detection of β^s-globulin mutation

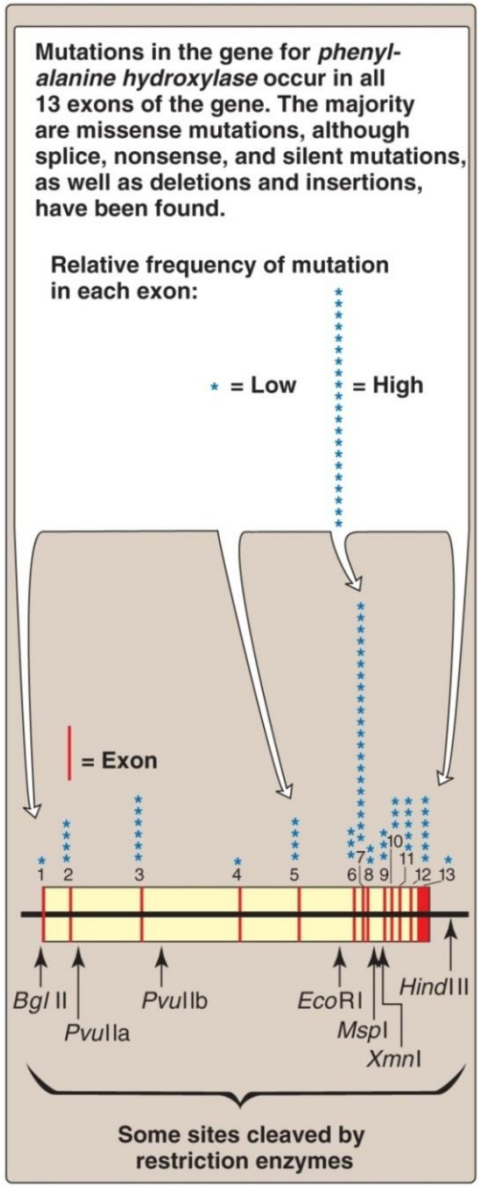
Sickle cell anemia detection by RFLP.

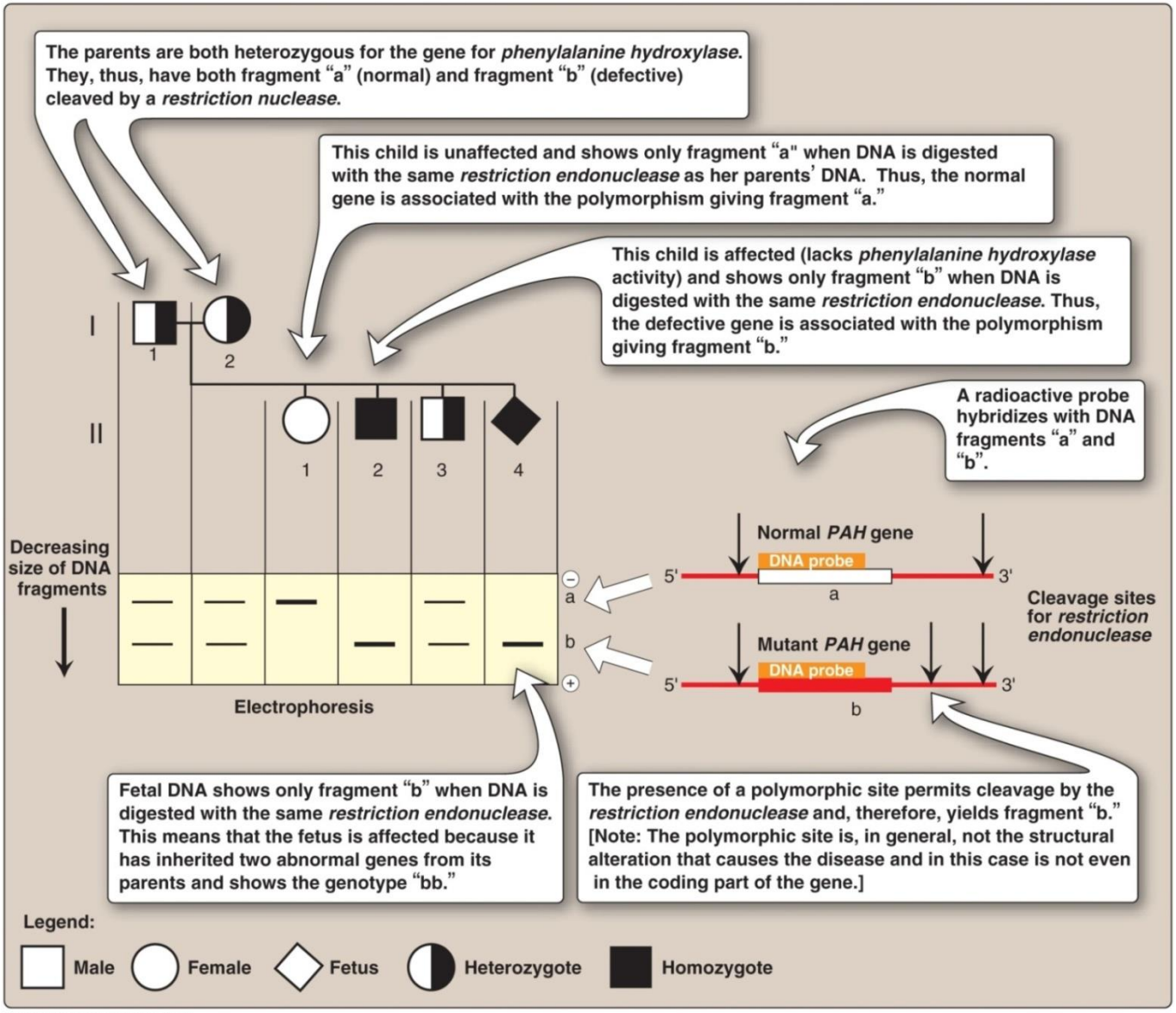
Using the enzyme MstII:
CCTNAG: A to T mutation



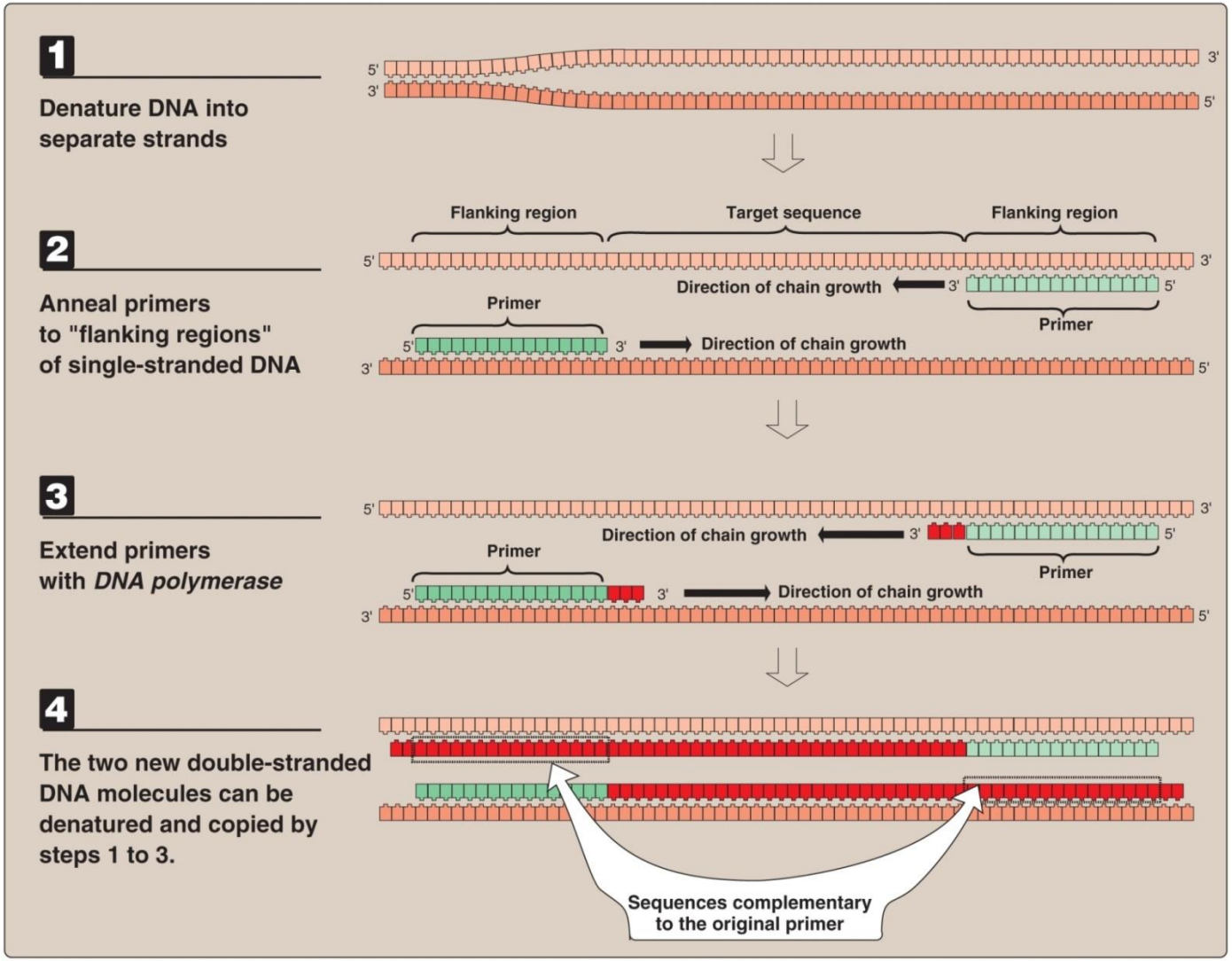
Indirect, prenatal diagnosis of phenylketonuria using RFLP

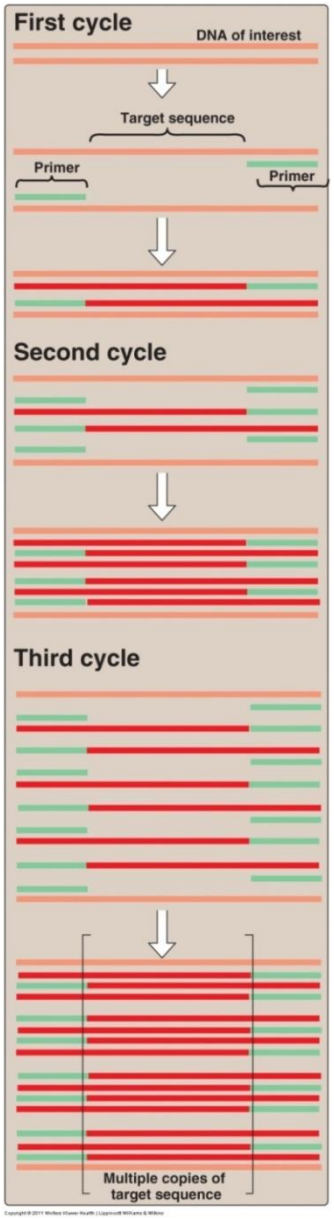
PAH: phenylalanine (Phe) to the amino acid tyrosine





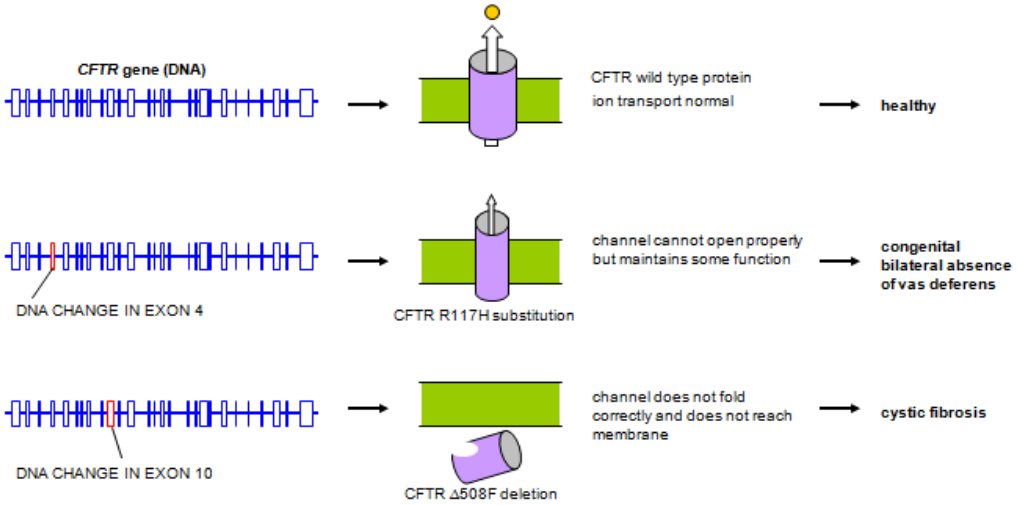
The Polymerase Chain Reaction (PCR)

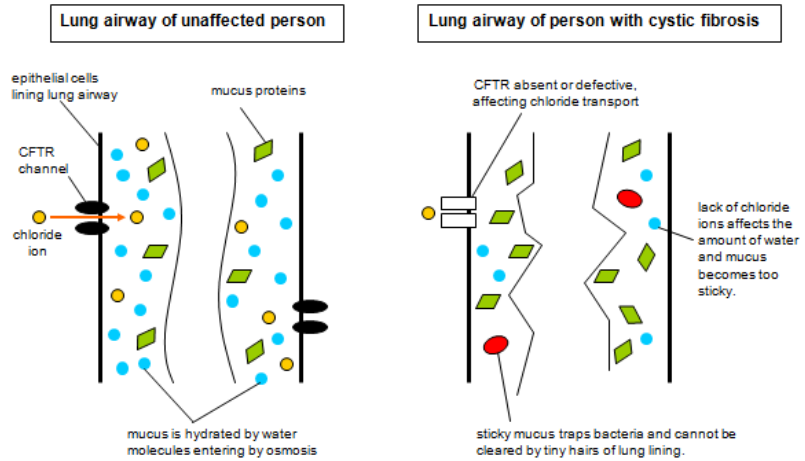


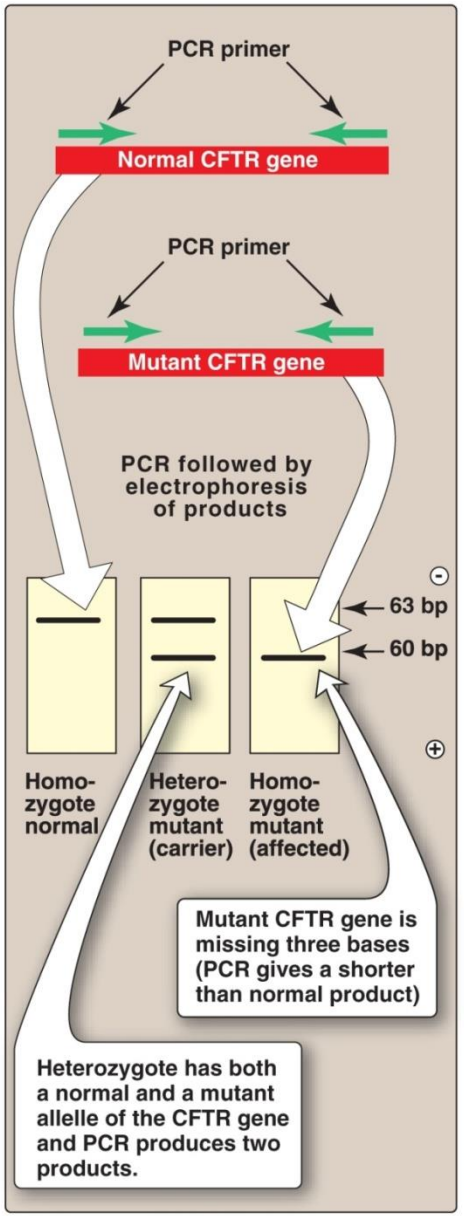


Genetic testing for cystic fibrosis using PCR

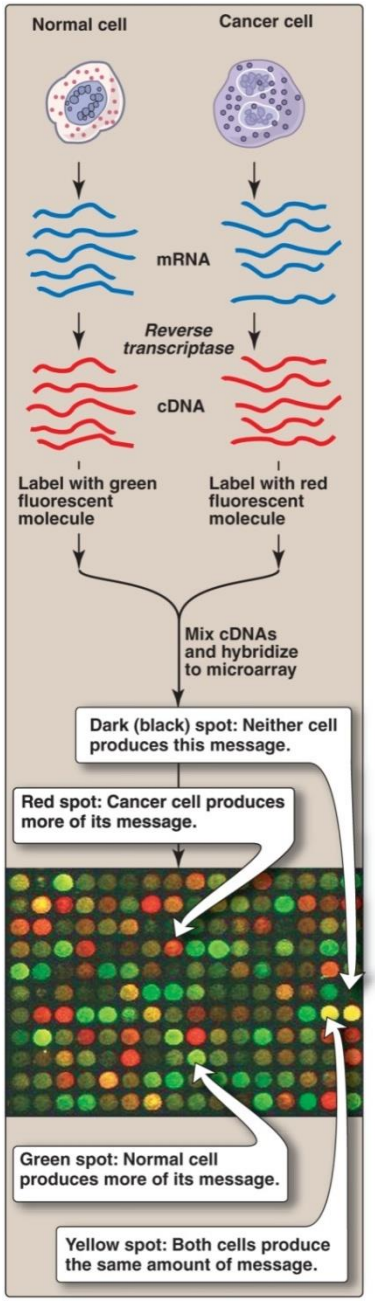
Cystic fibrosis transmembrane conductance regulator (CFTR)



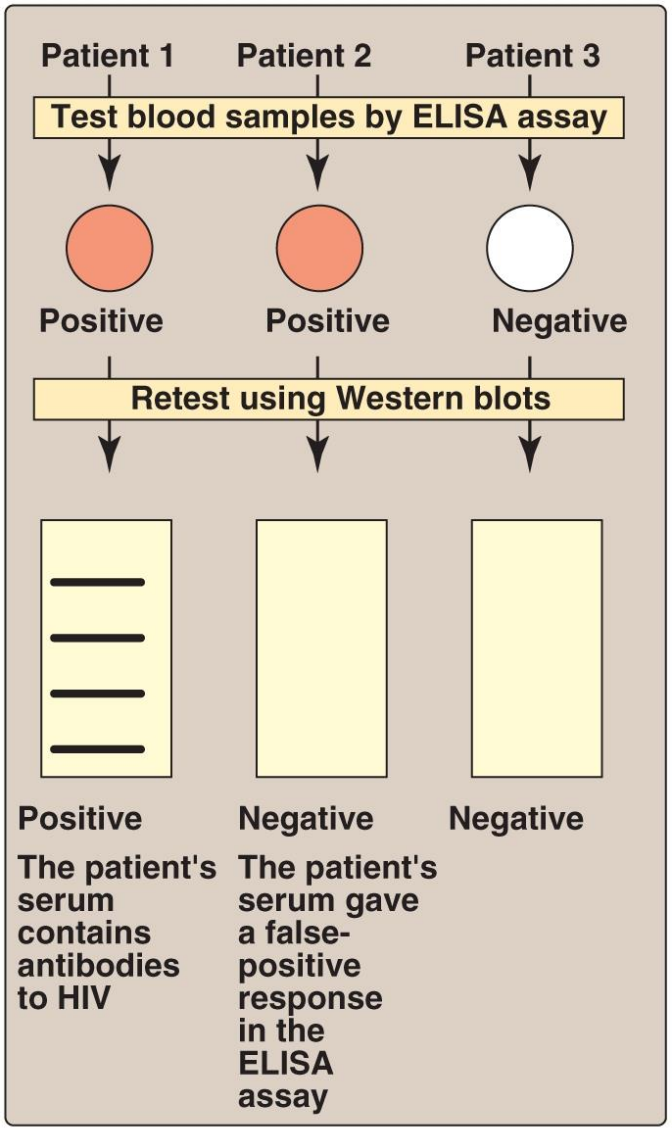




Microarray analysis of gene expression



Testing of HIV exposure by enzyme-linked immunosorbent assays (ELISA)



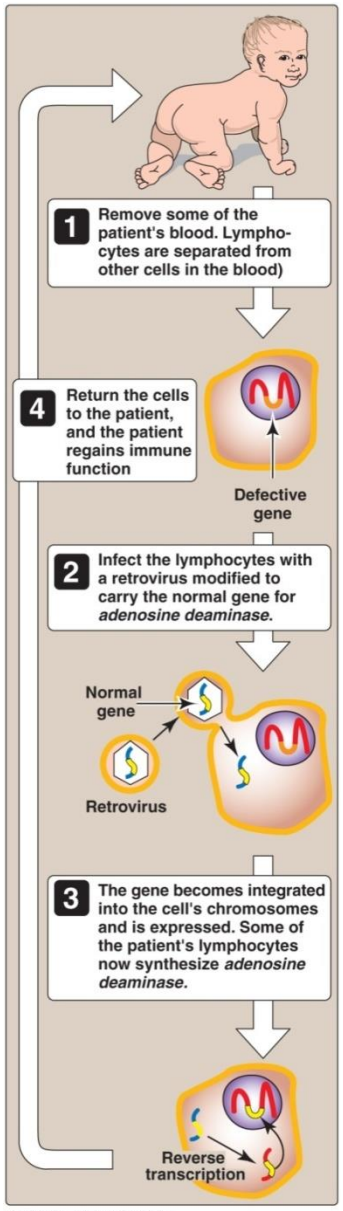
ELISA: specificity: 1,000 HIV test results of healthy individuals, about 15 of these results will be a false positive. repeating the test, could reduce the ultimate likelihood of a false positive to about 1 result in 250,000 tests given.

The sensitivity rating, likewise, 1,000 test results of HIV infected people, 3 will actually be a false negative result.

Summary of the different molecular techniques

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	cDNA or genomic DNA	No	Measures many mRNA levels at once; detects genomic changes
ELISA	Proteins or antibodies	No	Detects proteins (antigens) or antibodies; detects genomic changes
Proteomics	Proteins	Yes	Measures abundance, distribution, posttranslational modifications, functions, and interactions of cellular proteins

Gene therapy

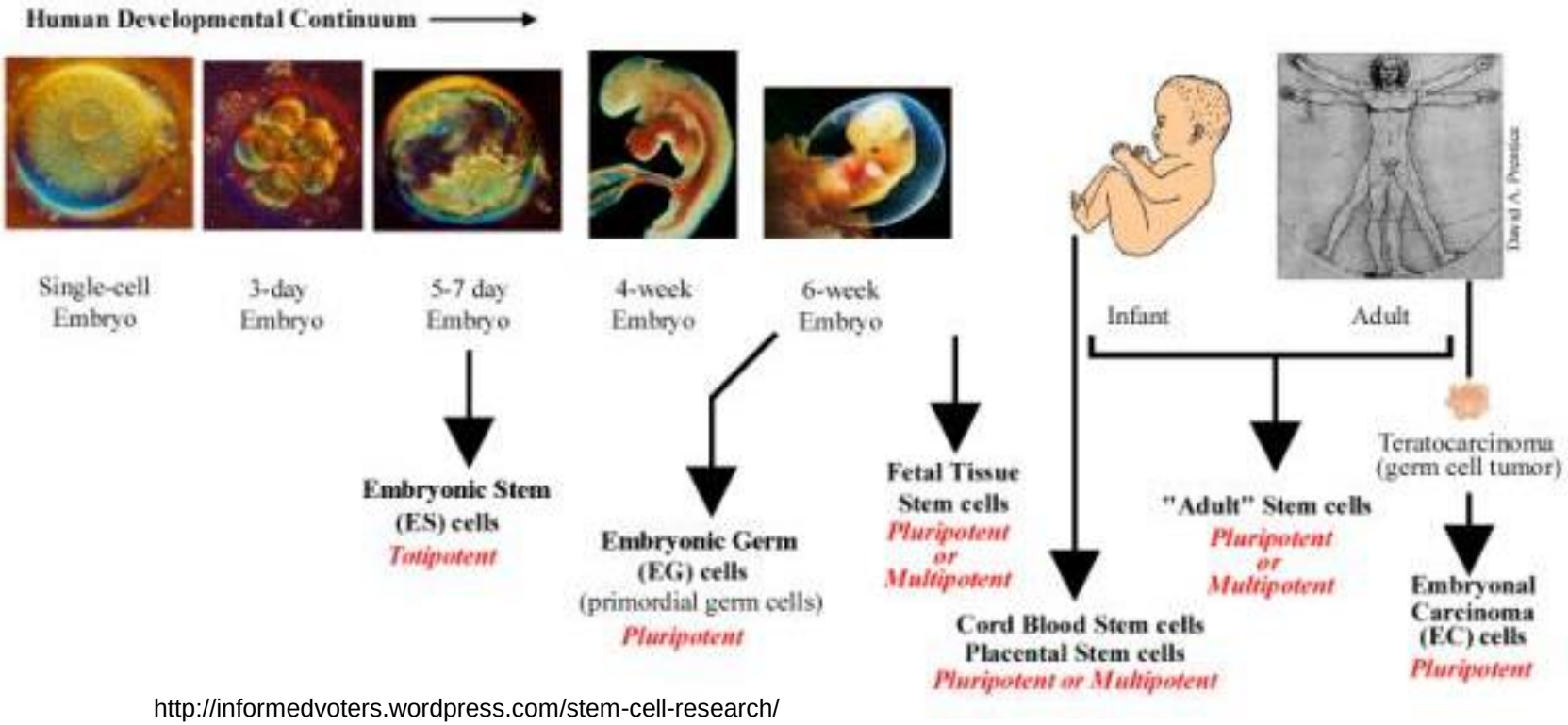


Regenerative Medicine

Regenerative medicine or **stem cell therapy** is the "process of replacing or regenerating human cells, tissues or organs to restore or establish normal function".

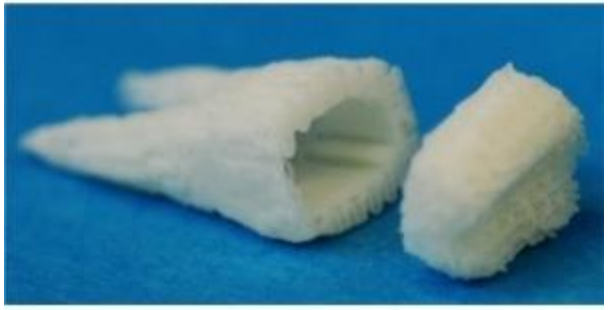
Stem cells are biological cells found in all multicellular organisms, that can divide (mitosis) and differentiate into diverse specialized cell types and can self-renew to produce more stem cells.

Where do stem cells come from?

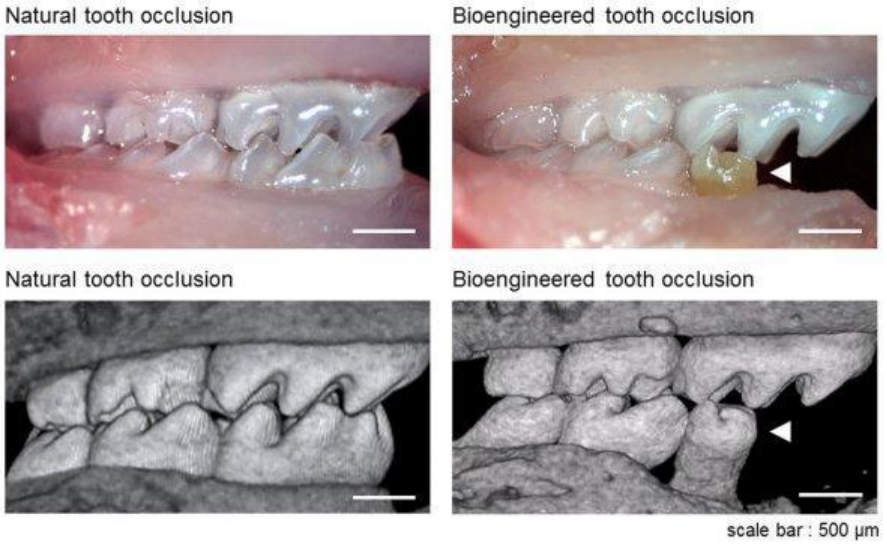


http://informedvoters.wordpress.com/stem-cell-research/

Stem cells used to grow new teeth



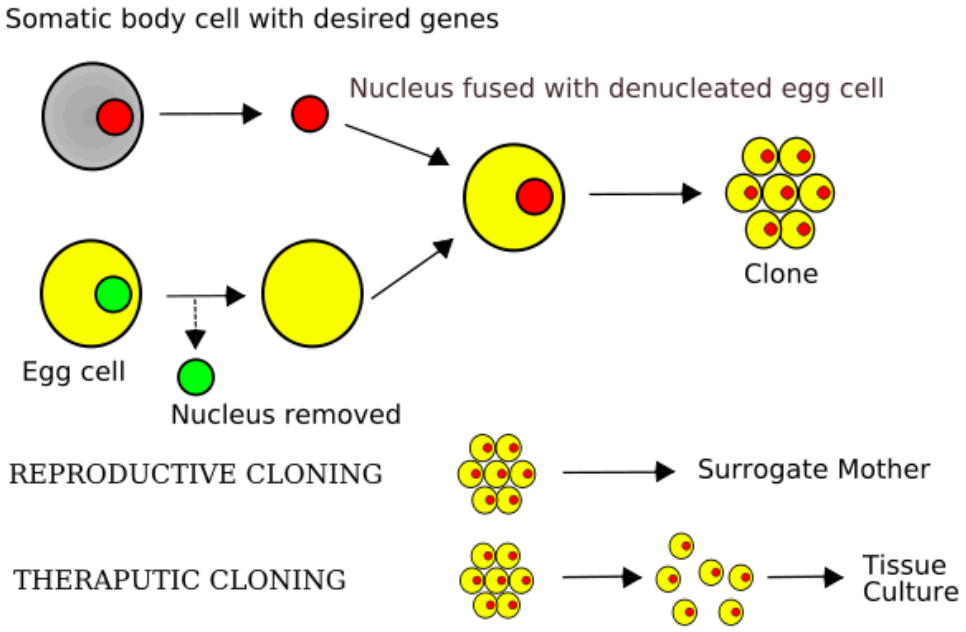
Takashi Tsuji from Tokyo University of Science and his colleagues removed two different stem cells from the molar teeth of mice to build these teeth. They transported these cells to a laboratory where they could grow. To influence how the teeth grew in relation to size and shape, the cells were put inside of a mold to grow.



When the cells developed into full tooth units, the researchers then placed them into the jaws of one-month-old mice. The transplanted choppers attached themselves to the jaw bones and tissues on an average of about 40 days.

Therapeutic cloning

Somatic-cell nuclear transfer (SCNT)



http://en.wikipedia.org/wiki/Somatic-cell_nuclear_transfer

Limitations

Stresses placed on both the egg cell and the introduced nucleus are enormous, leading to a high loss in resulting cells. For example, Dolly the sheep was born after 277 eggs were used for SCNT, which created 29 viable embryos. Only three of these embryos survived until birth, and only one survived to adulthood.

Discuss: The micromanipulation of a nucleus from one cell into another necessarily entails the concomitant transfer of a small quantity of cytoplasm (2%) from the donor cell, and that cytoplasm contains intact mitochondria. What are the consequences of this observation ?