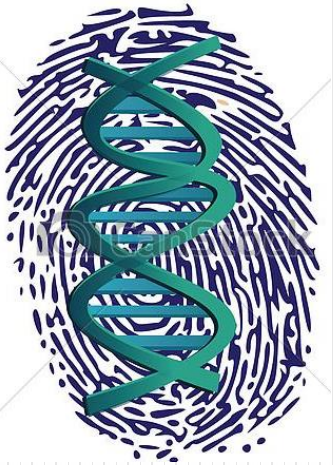
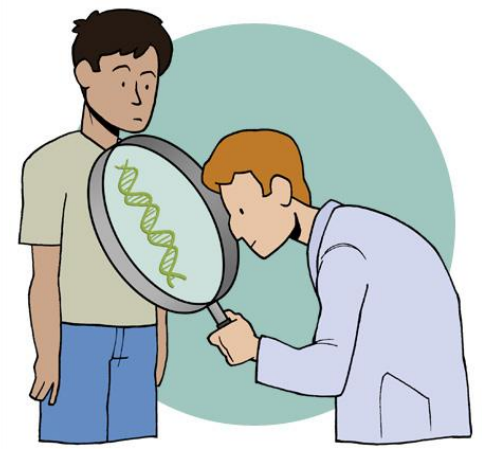
Genetic Screening.
Genetic fingerprinting.

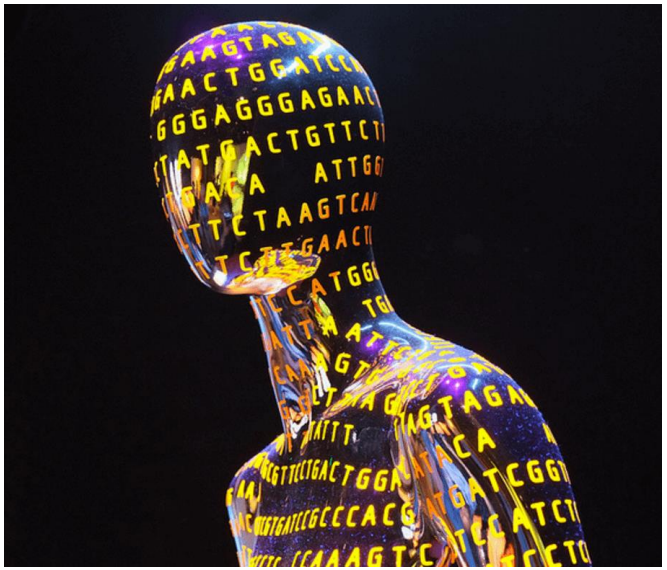
Lecture

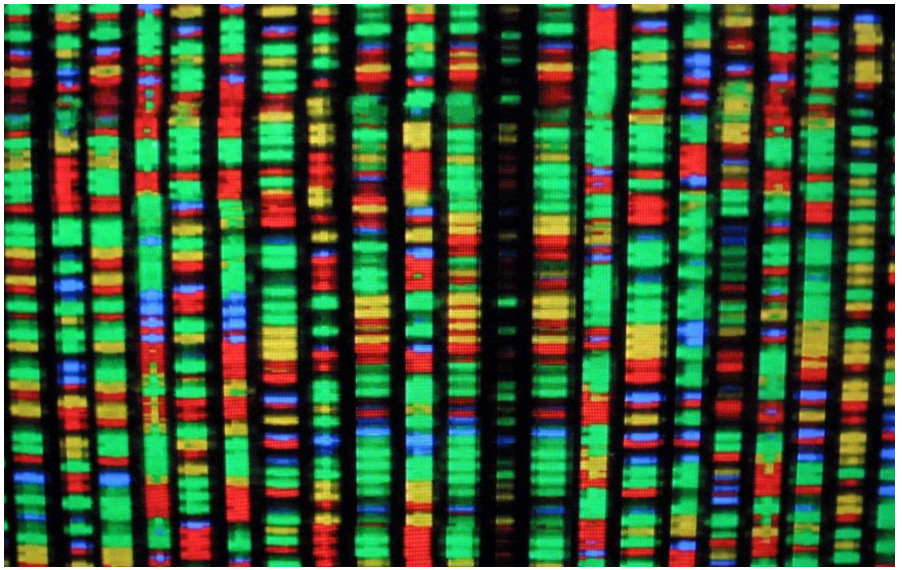




Human Genome Project

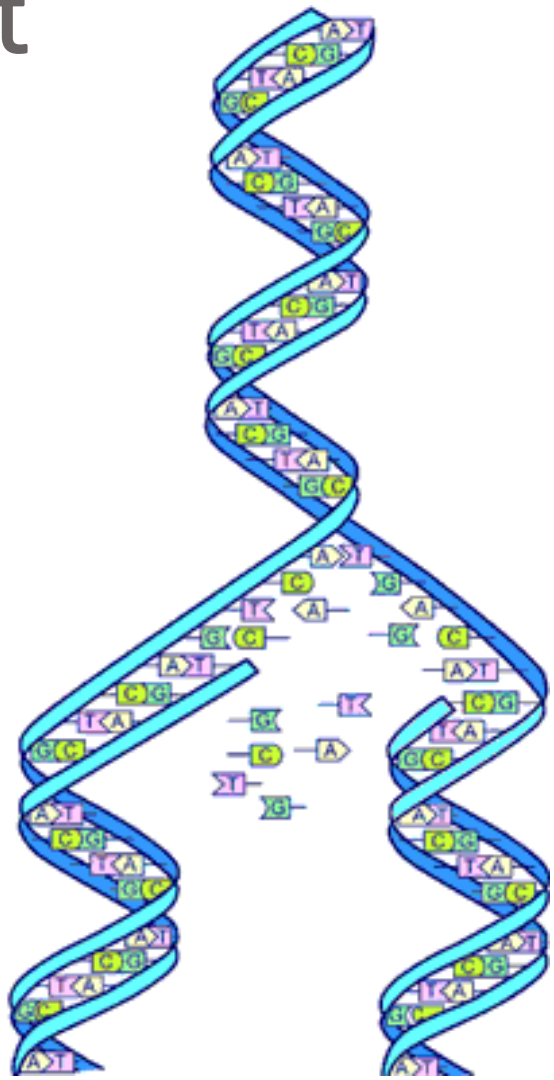
- In 1990 this project was initiated as joint effort of U.S. Department of Energy and the National Institutes of Health. In April 2003 Human Genomic Project sequencing is completed and Project is declared finished two years ahead of schedule.





Human Genome Project

- Improve diagnosis of disease.
- Detect genetic predispositions to disease:
- screening,
- advice,
- risk factor modification.
- Create drugs based on molecular information.
- Design “custom drugs” (pharmacogenomics) based on individual genetic profiles.
- Use gene therapy for treatment.
- Identify potential suspects whose DNA may match evidence left at crime scenes.
- Exonerate persons wrongly accused of crimes.



What is genetic screening?

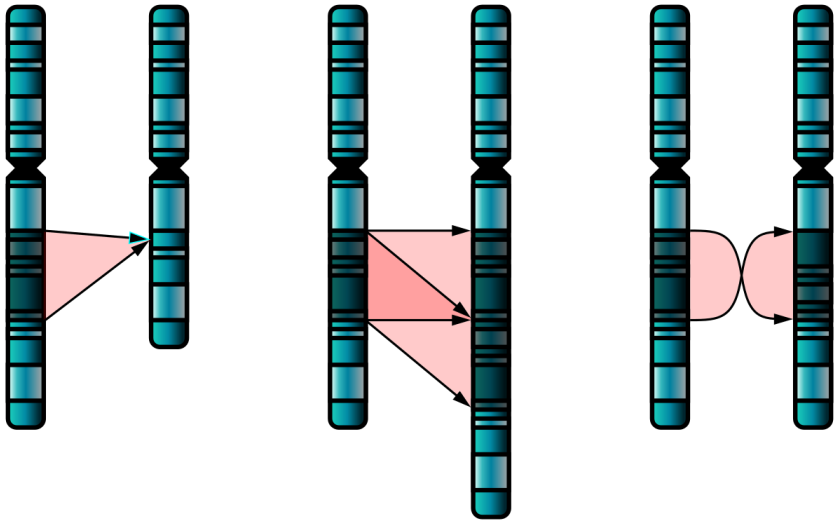
- Genetic screening provides a way to predict whether or not a specific phenotype will occur.

Methods of Genetic screening:

Determining abnormalities	Screening Embryos
Karyotyping	Amniocentesis
Polymerase Chain Reaction (PCR)	Preimplantation genetic diagnosis (PGD)
Restriction Fragment Length Polymorphisms (RFLPS) / Southern Blotting	Chorionic villus sampling

Karyotyping

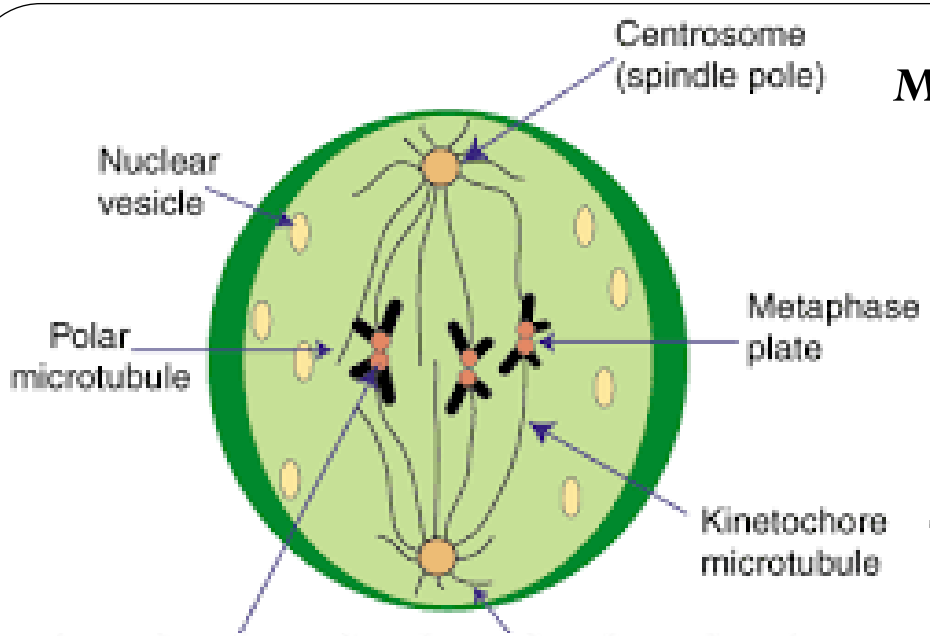
- **Definition:** "Karyotyping is the process of pairing and ordering all the chromosomes of an organism, thus providing a genome-wide image of an individual's chromosomes."
- Karyotypes are prepared from mitotic cells which are frozen in metaphase.
- Characteristic structural features for each chromosome are revealed.
- Can reveal changes in chromosome numbers linked to conditions such as Down's syndrome.



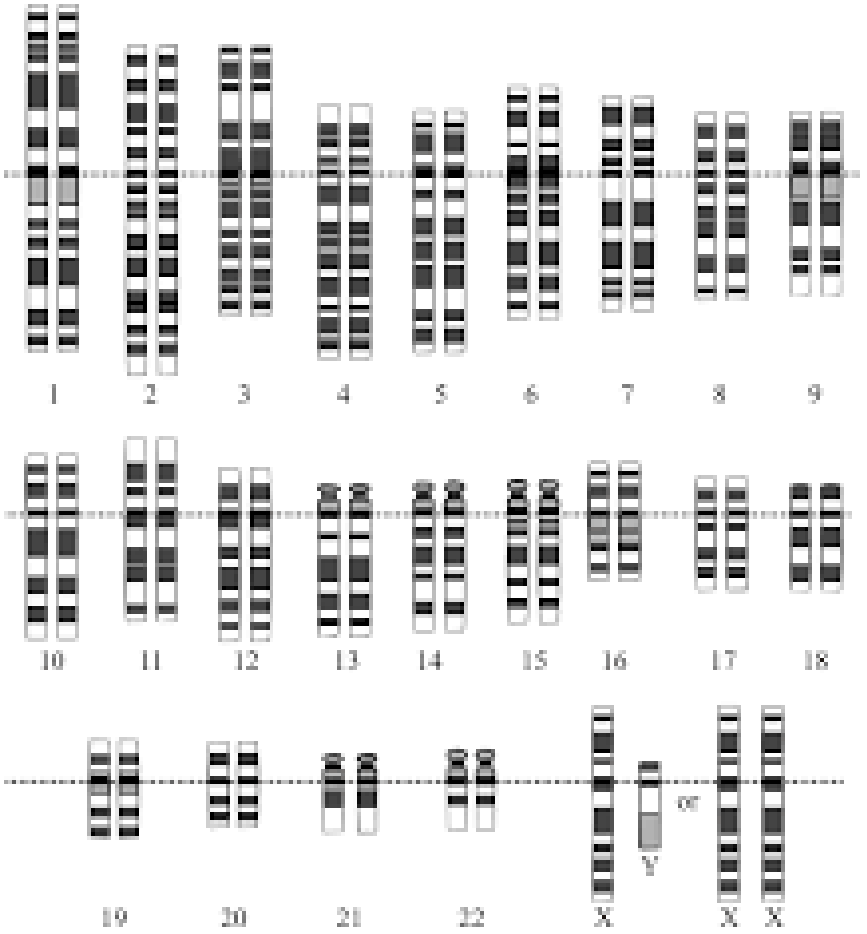
Deletions Duplications Inversions

- Careful analysis can show more subtle changes as chromosomal deletions, duplications, translocations or inversions.
- Increasing use of karyotyping for diagnosis of specific birth defects and genetic disorders.

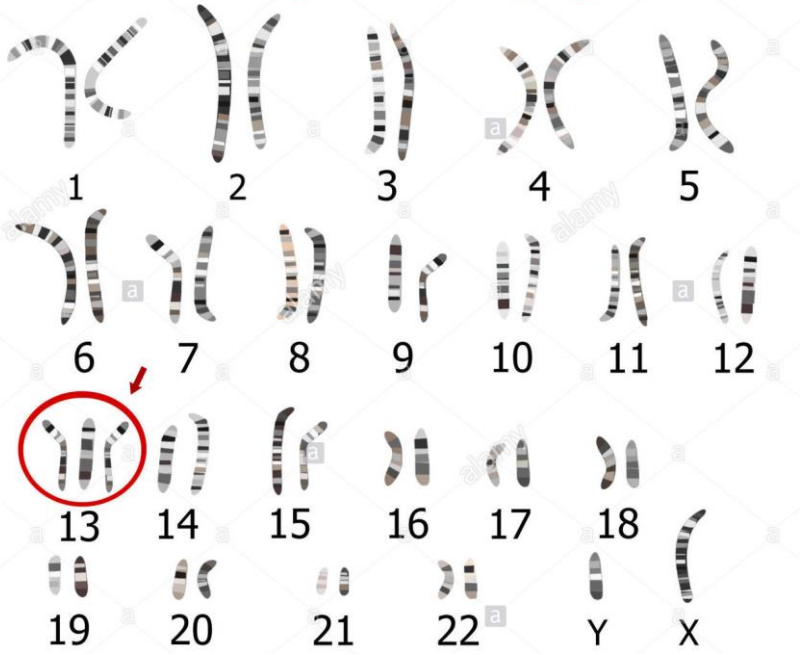
Metaphase



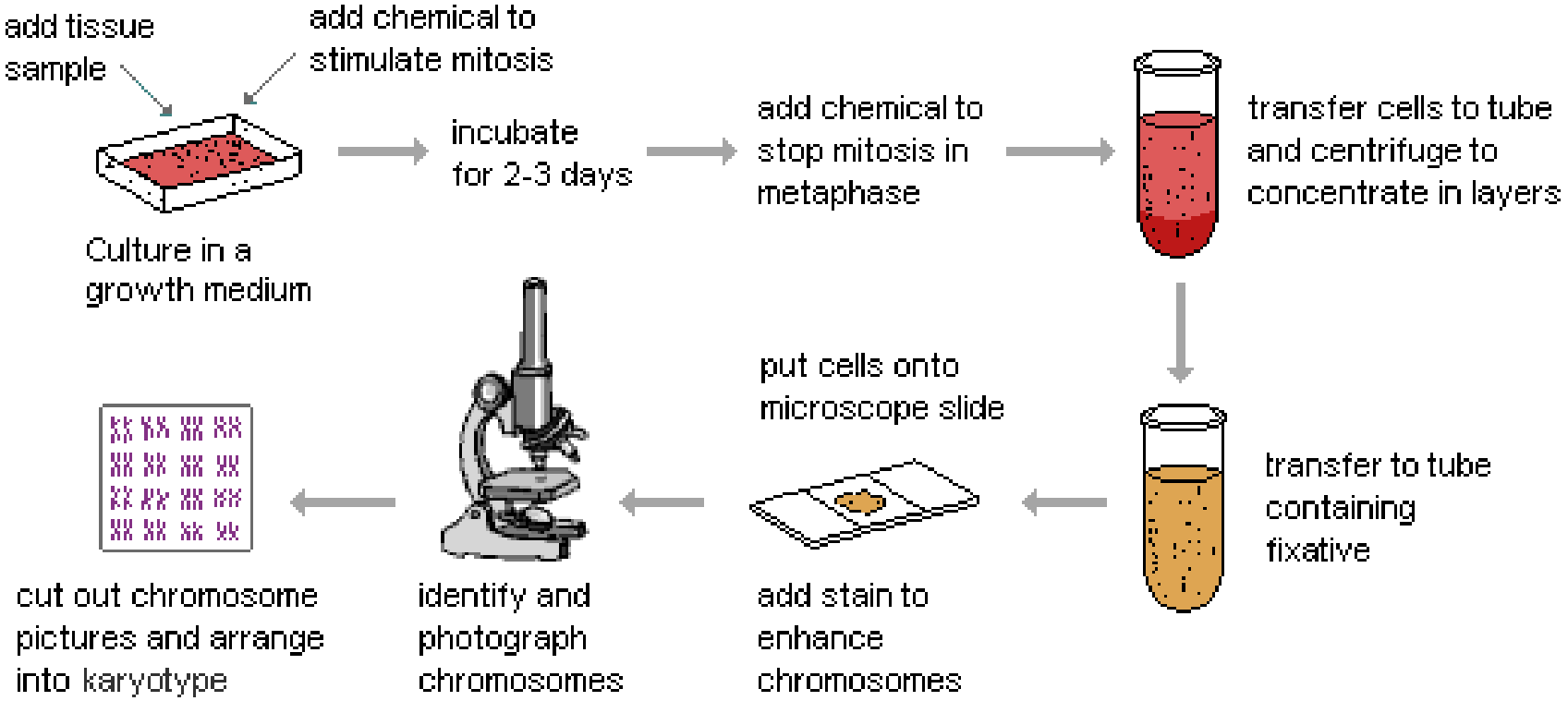
Karyotyping



Patau syndrome karyotype



Process of Karyotyping



Blood, skin, tumors (for identifying cancer) are some of the tissue types used for diagnosis.

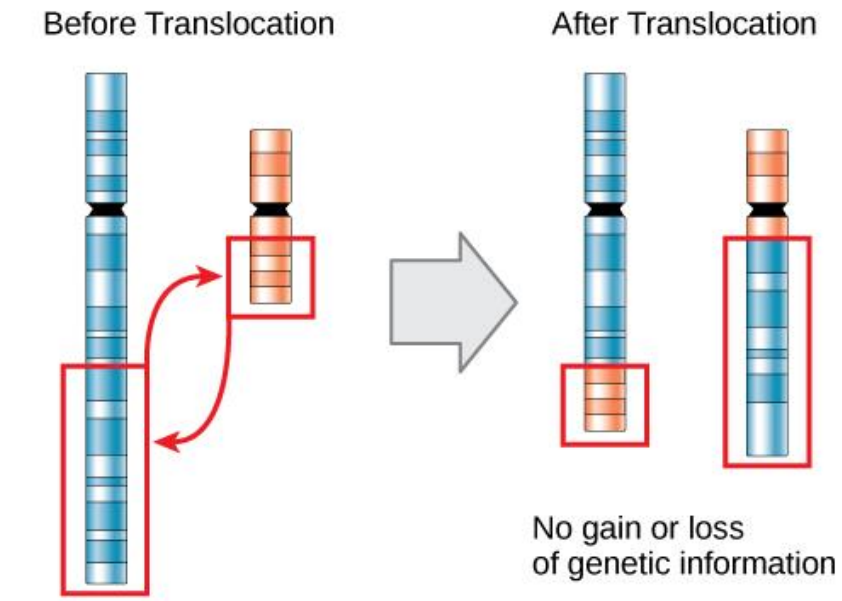
Amniotic fluid or chorionic villus specimens are used for prenatal diagnosis.

Applications of Karyograms

- Now used to diagnose a wide range of chromosomal abnormalities in humans.

Examples

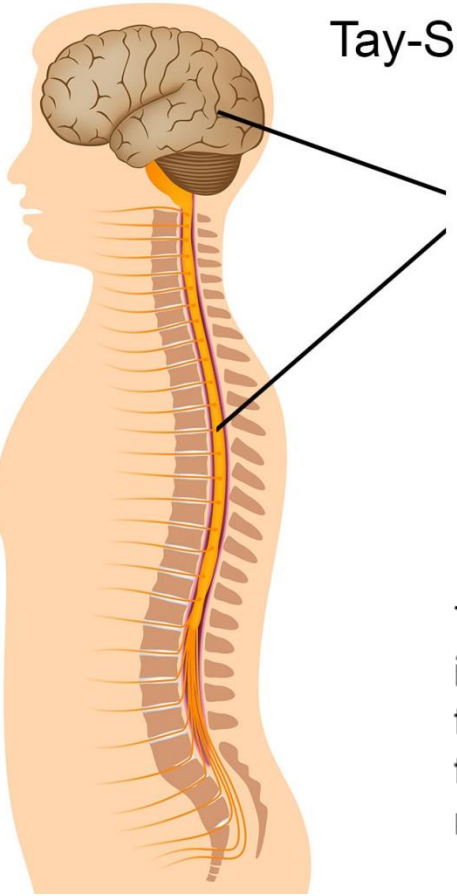
- Aneuploidy: a disease caused by the absence or addition of a chromosome. (e.g. Down syndrome)
- Translocations: an exchange of chromosome parts.
- Researches use karyotypes to identify genes within critical regions of which mis-expression would cause symptoms in patients.



Prospectives & Concerns of genetic screening

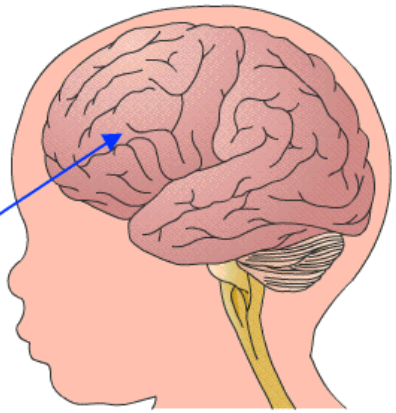
Prospectives	Concerns
Prevention of the birth of babies with lethal diseases (i.e. Tay-Sachs disease)	"Designer babies"
Can determine ancestry	which genes to screen: seriously harmful genes vs. harmless genes. (can we go too far?)
Can predict forthcoming diseases (cancer)	Economic and social consequences of the test results

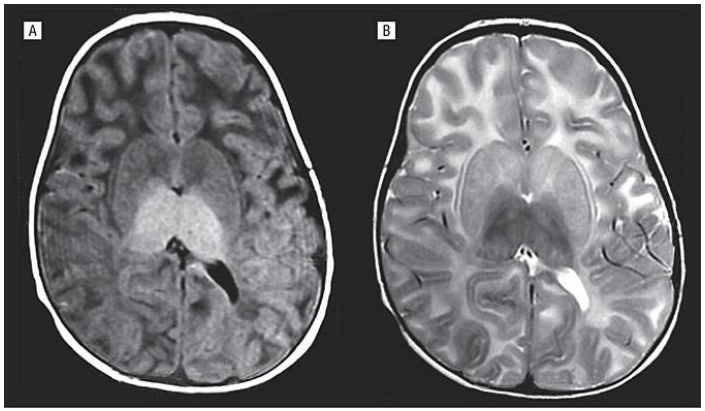
Tay-Sachs Disease



Degeneration of neurons in the brain and spinal cord

Tay-Sachs disease is a rare, inherited disorder. It causes too much of a fatty substance to build up in tissues and nerve cells of the brain.





Thalamic Changes in Tay-Sachs' Disease

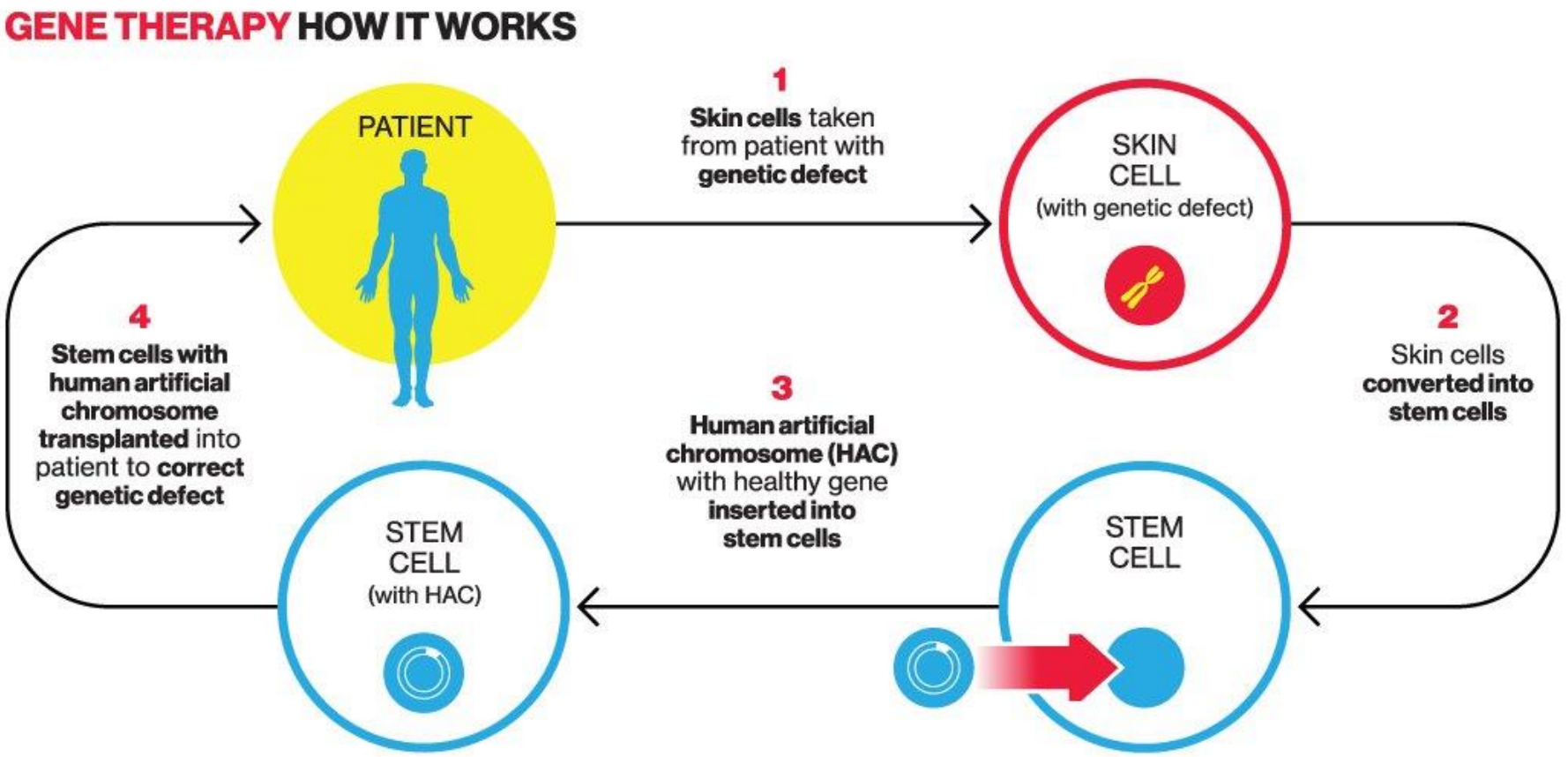
Pharmacogenomics

- The development of drugs tailored to specific subpopulations based on genes. Pharmacogenomics has the potential to:
- Decrease side effects of drugs
- Increase drug effectiveness
- Make drug development faster and less costly
- Use of medications otherwise rejected because of side effects
- New medications for specific genotypic disease subtypes.

Gene Therapy

- **Definition:** Introduction of normal genes into cells that contain defective genes to reconstitute a missing protein product.
- Gene therapy is used to correct a deficient phenotype so that sufficient amounts of a normal gene product are synthesized to improve a genetic disorder.
- Modification of cells by transferring desired gene sequences into the genome. Delivery systems available:
- In vivo: delivery of genes takes place in the body.
- Ex vivo: delivery takes place out of the body, and then cells are placed back into the body.

Gene Therapy



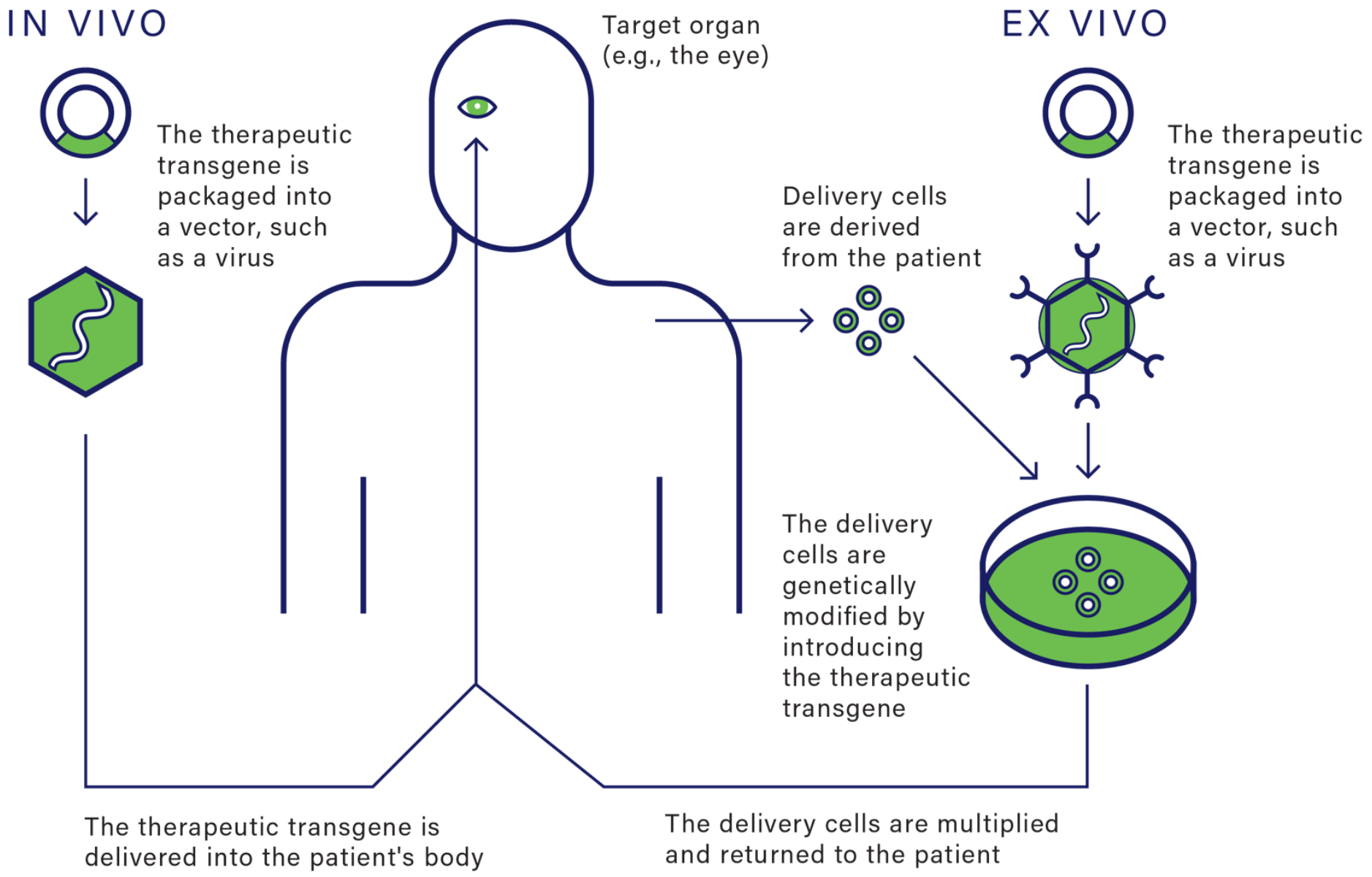
Gene Therapy

In vivo techniques

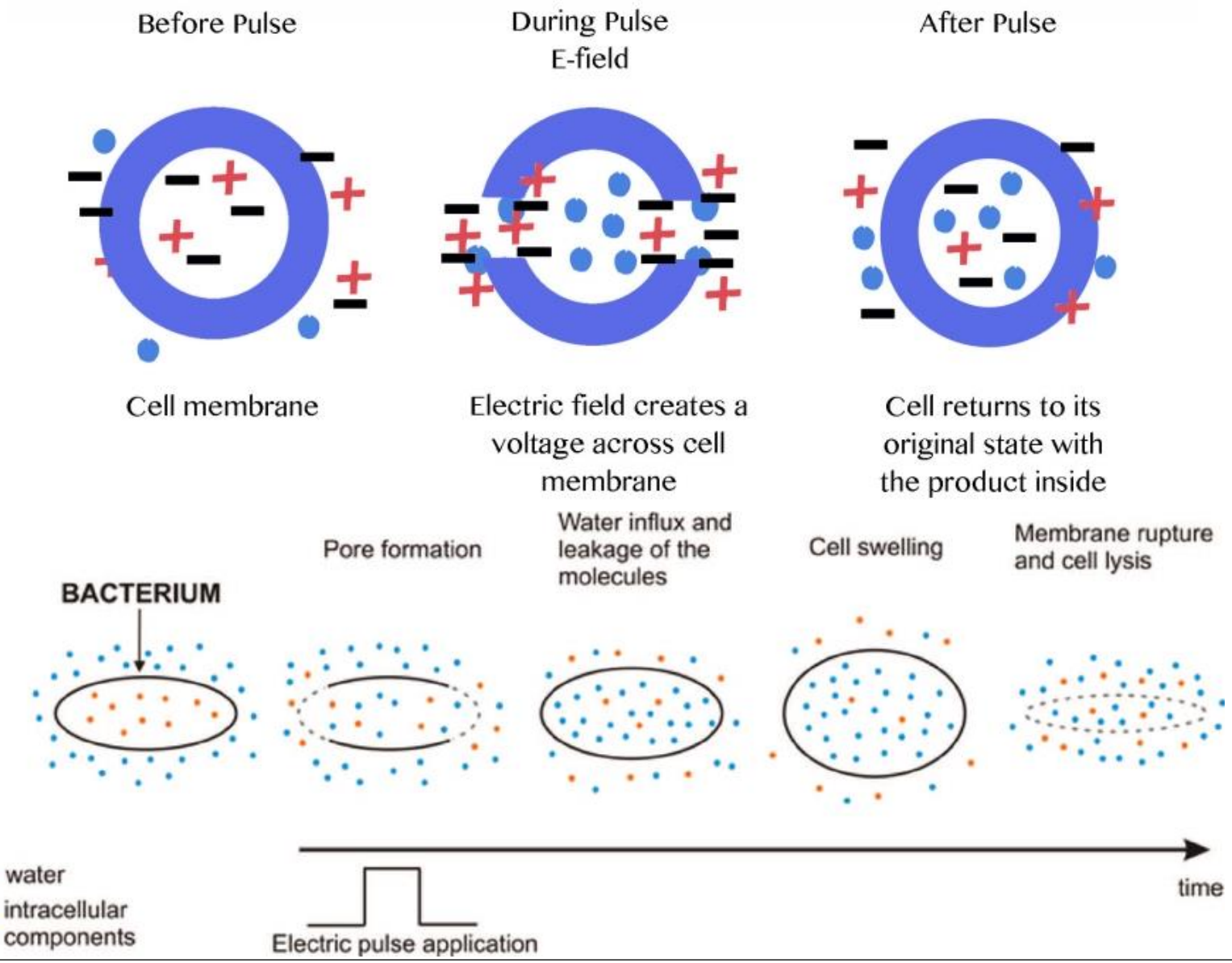
- usually utilize viral vectors,
- Virus carrier of desired gene, e.g. adenovirus, retroviruses, herpes simplex virus.
- Virus is usually "crippled" to disable its ability to cause disease.
- Viral methods have proved to be the most efficient to date.
- Many viral vectors can stably integrate the desired gene into the target cell's genome.

Ex vivo manipulation techniques

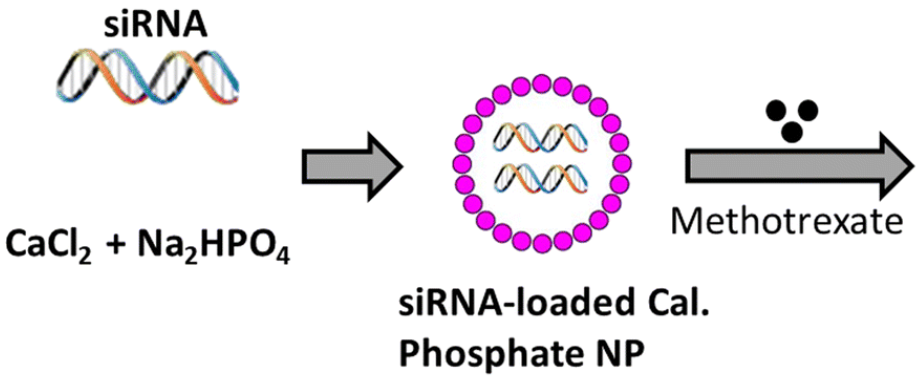
- Electroporation,
- Liposomes Calcium phosphate,
- Gold bullets (fired within helium pressurized gun),
- Retrotransposons (jumping gene),
- Human artificial chromosomes.

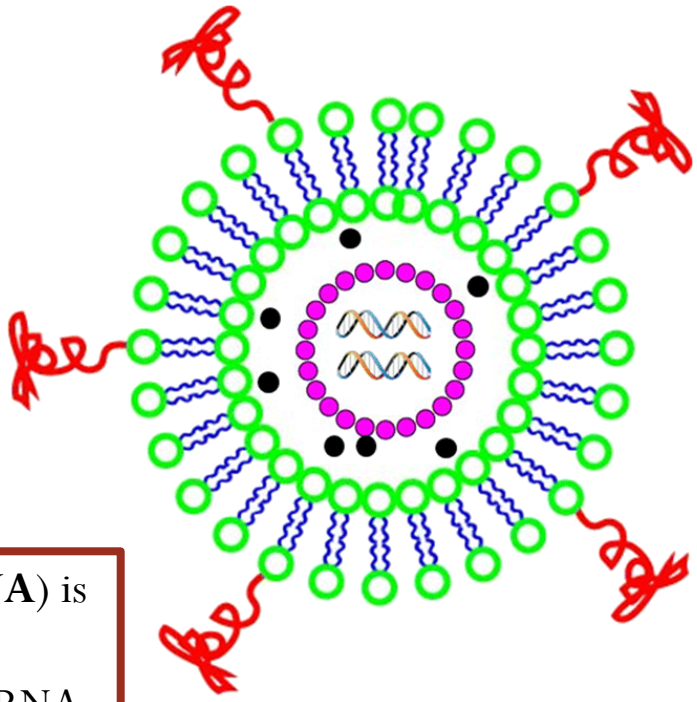

IN VIVO
The therapeutic transgene is packaged into a vector, such as a virus
Target organ (e.g., the eye)
Delivery cells are derived from the patient
EX VIVO
The therapeutic transgene is packaged into a vector, such as a virus
The delivery cells are genetically modified by introducing the therapeutic transgene
The therapeutic transgene is delivered into the patient's body
The delivery cells are multiplied and returned to the patient

Electroporation

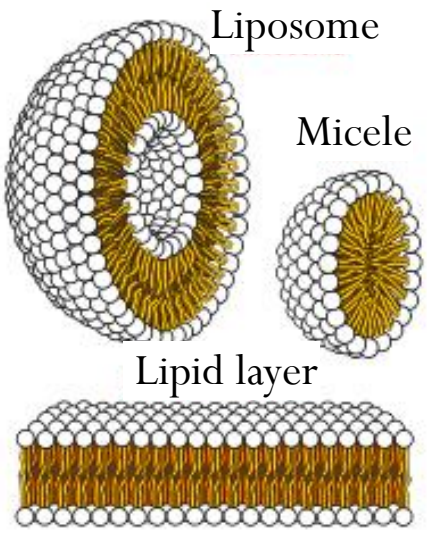


Liposomes Calcium phosphate



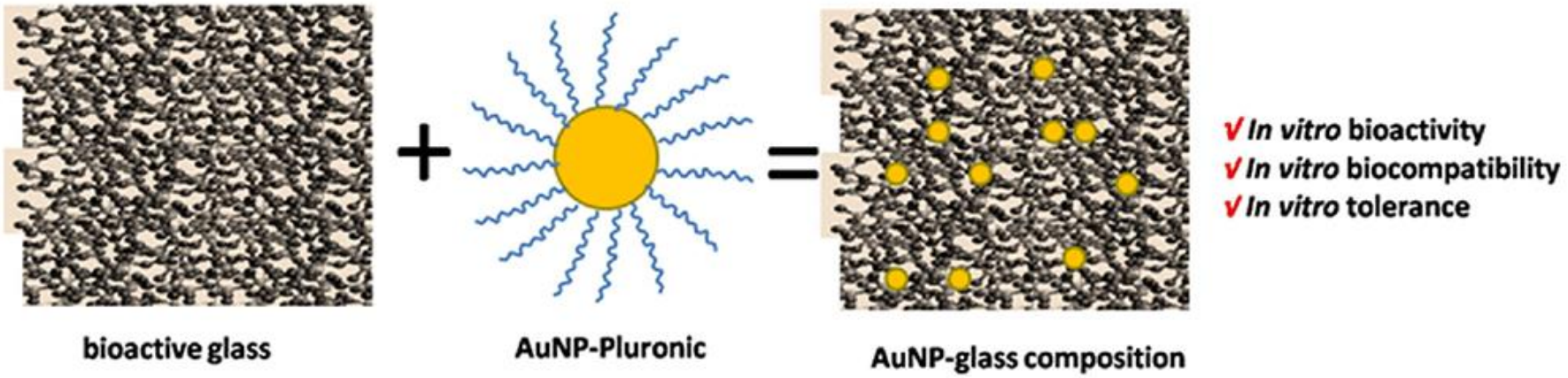


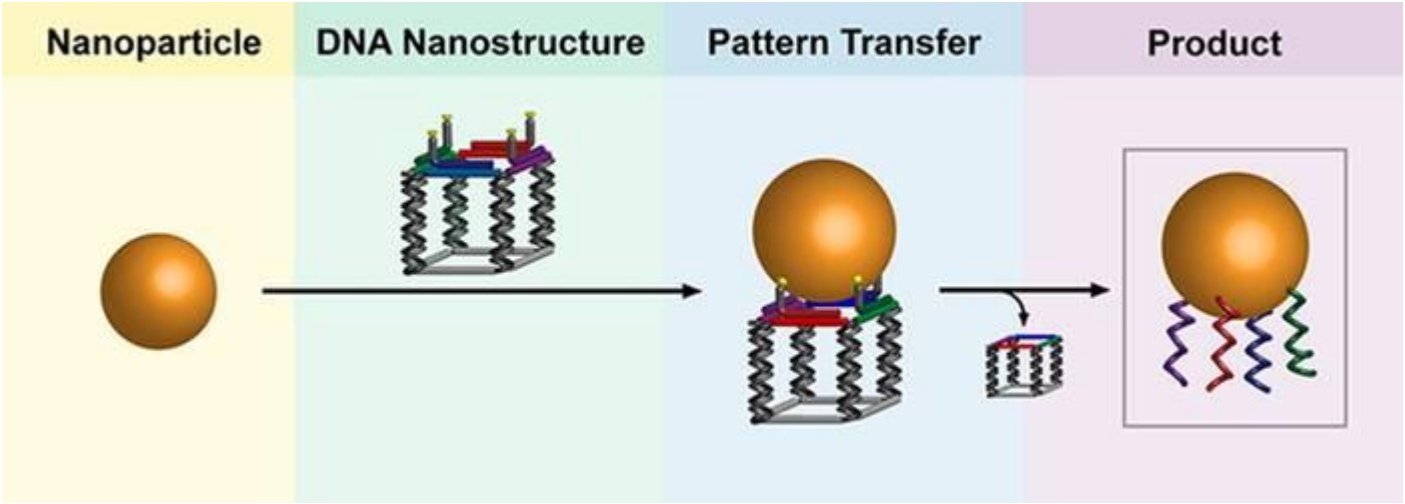
Folate-conjugated MTX/siRNA-loaded liposome

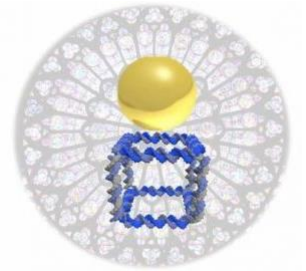


Small interfering RNA (**siRNA**) is a class of double-stranded RNA molecules, operating within the RNA interference (RNAi) pathway. It interferes with the expression of specific genes with complementary nucleotide sequences by degrading mRNA after transcription, preventing translation.

Gold bullets (fired within helium pressurized gun)

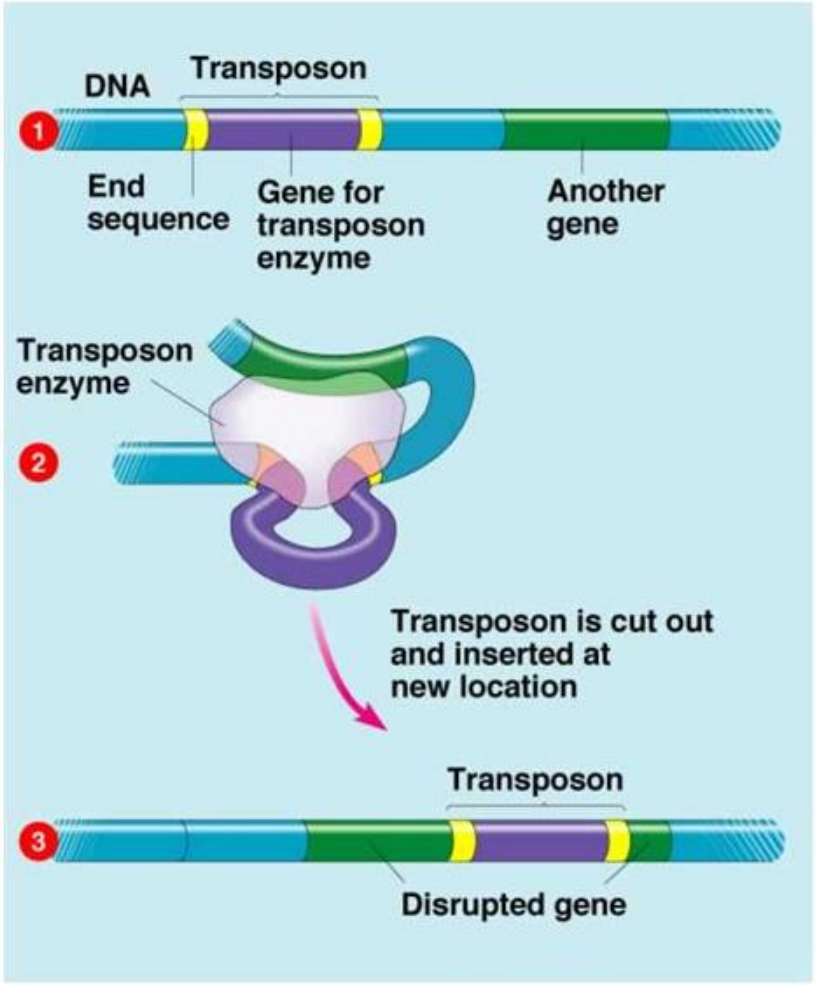






Retrotransposons (jumping gene)

- In the 1940's, while studying corn, Barbara McClintock discovered that sometimes genes could move from one location to another in a chromosome or even to other chromosomes.
- The movement could result in the genes landing in the middle of another gene and disrupting them.
- These "jumping genes" are now called transposons.



Human artificial chromosomes

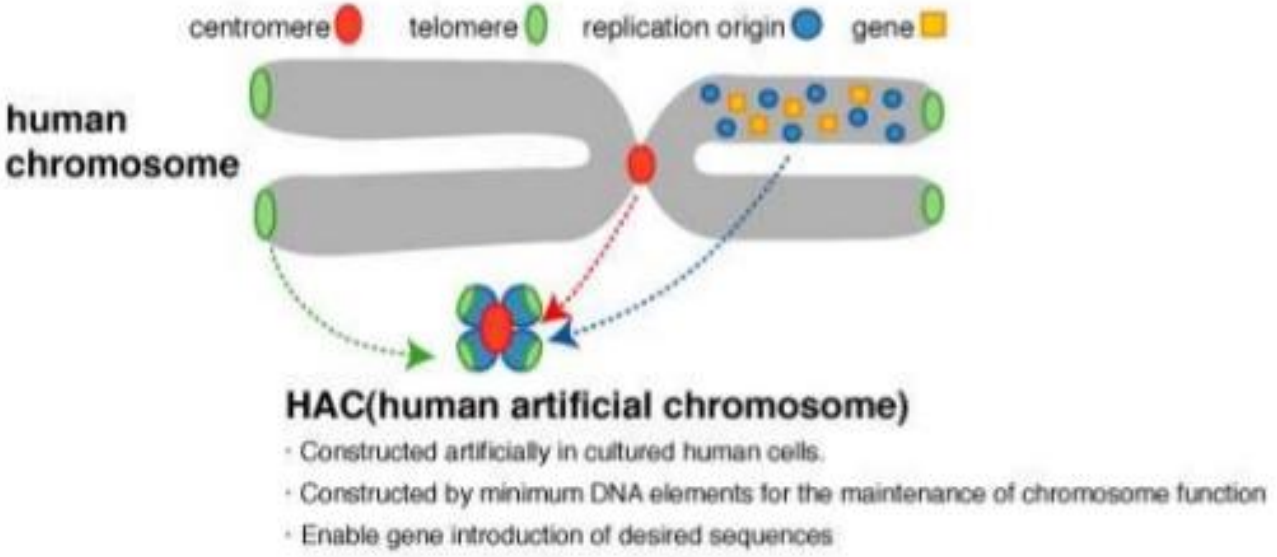
•A human artificial chromosome (HAC) is a mini-chromosome that is constructed artificially in human cells. Using its own self-replicating and segregating systems, a HAC can behave as a stable chromosome that is independent from the chromosomes of host cells.

The essential elements for chromosome maintenance and transmission are the following three regions:

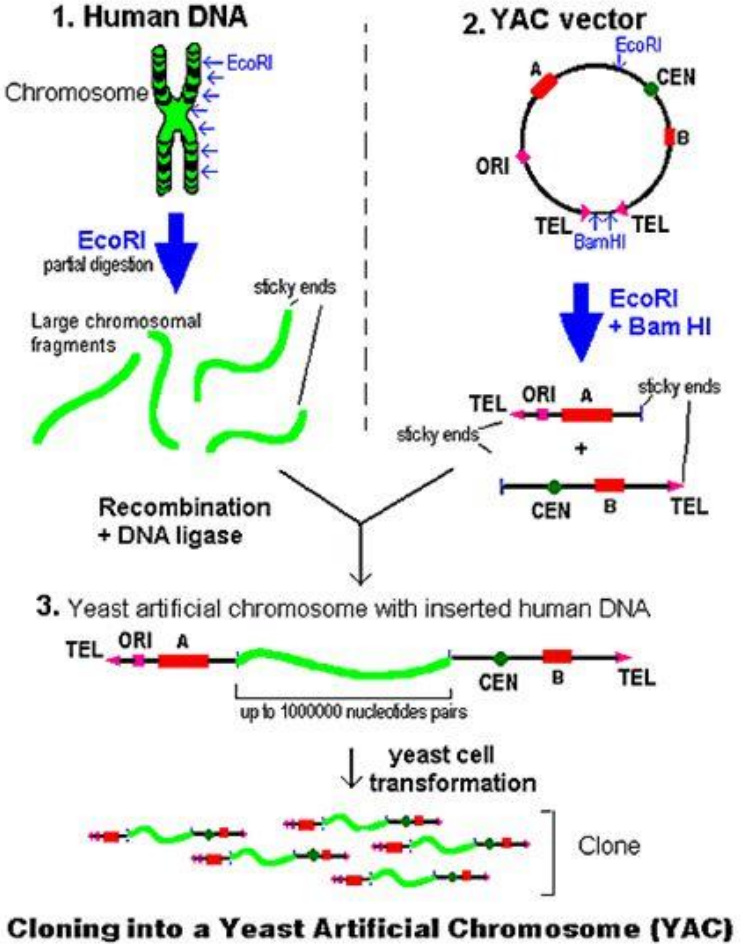
(1) the "replication origin," from which the duplication of DNA begins,

(2) the "centromere," which functions in proper chromosome segregation during cell division, and

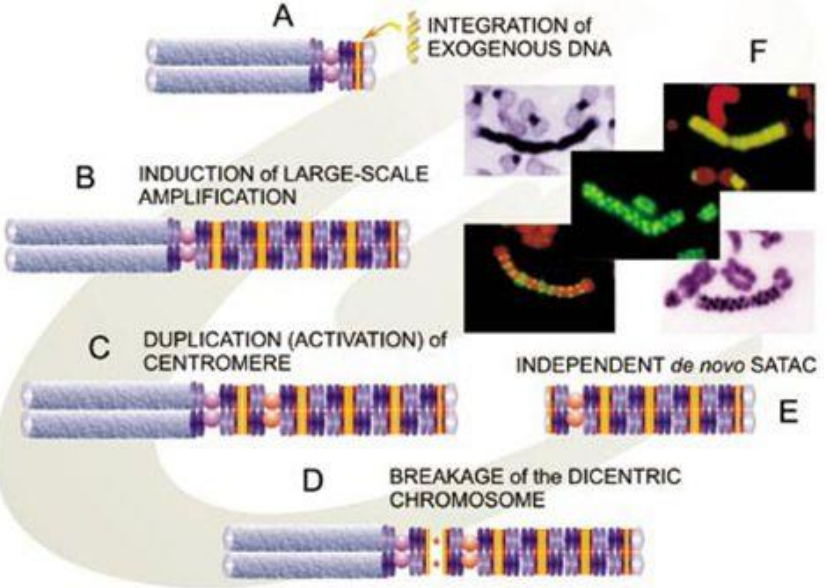
(3) the "telomere," which protects the ends of linear chromosomes.



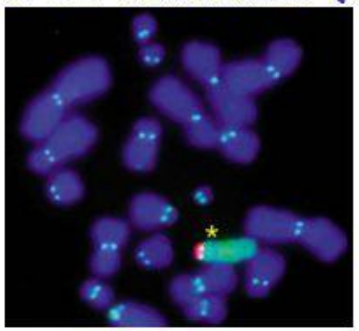
Artificial Chromosomes

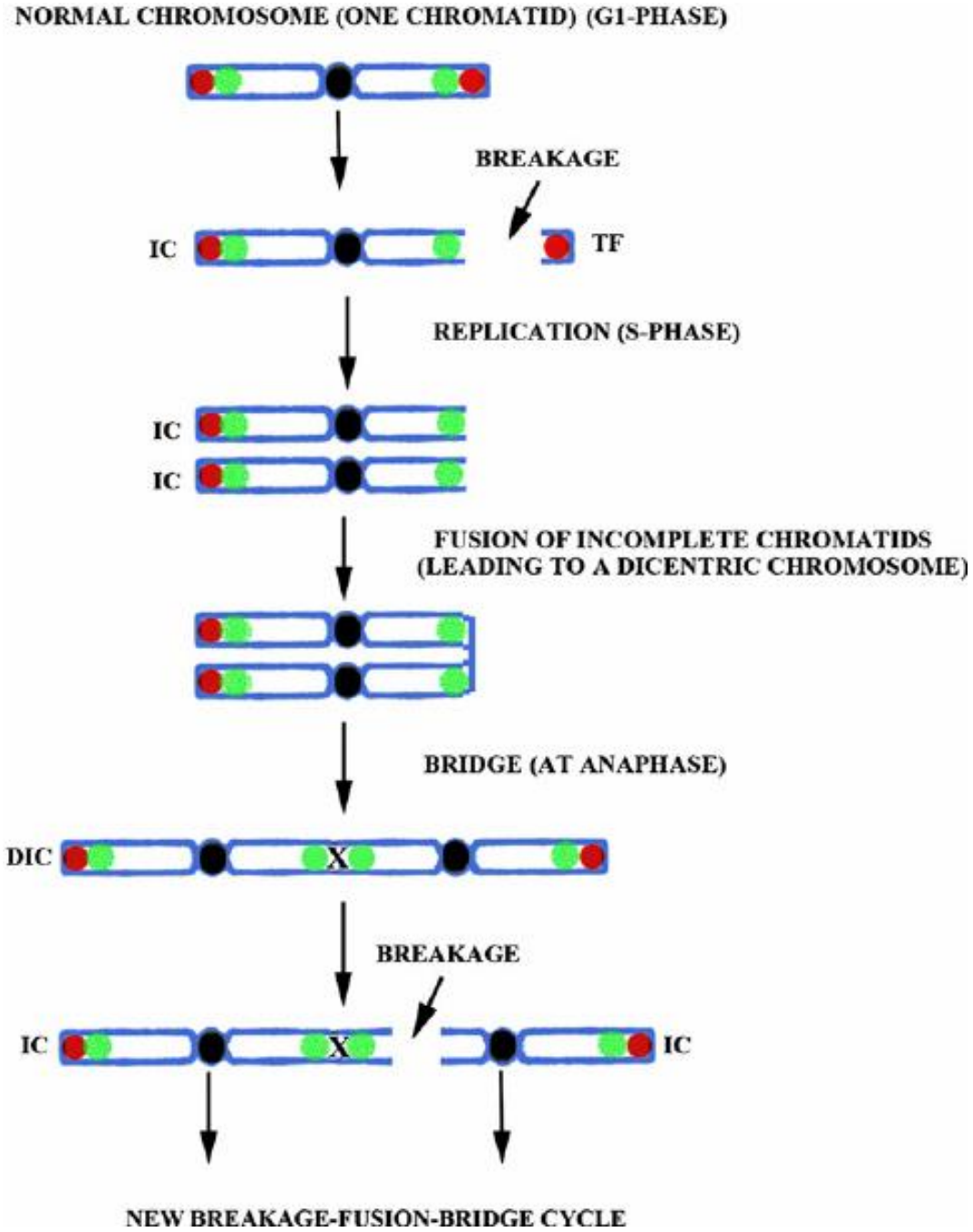


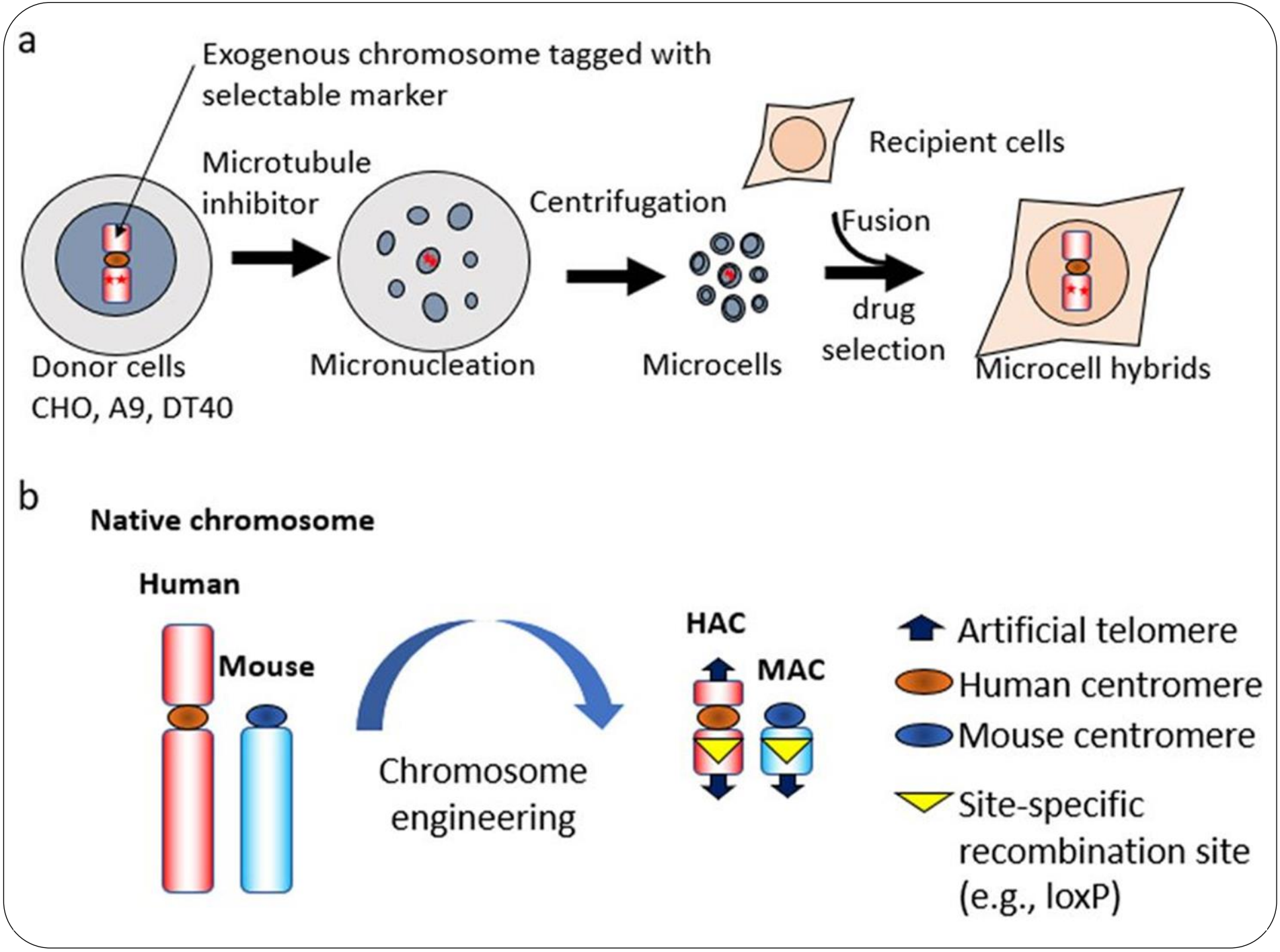
Cloning into a Yeast Artificial Chromosome (YAC)



Mammalian satellite DNA-based artificial chromosomes (SATACs)

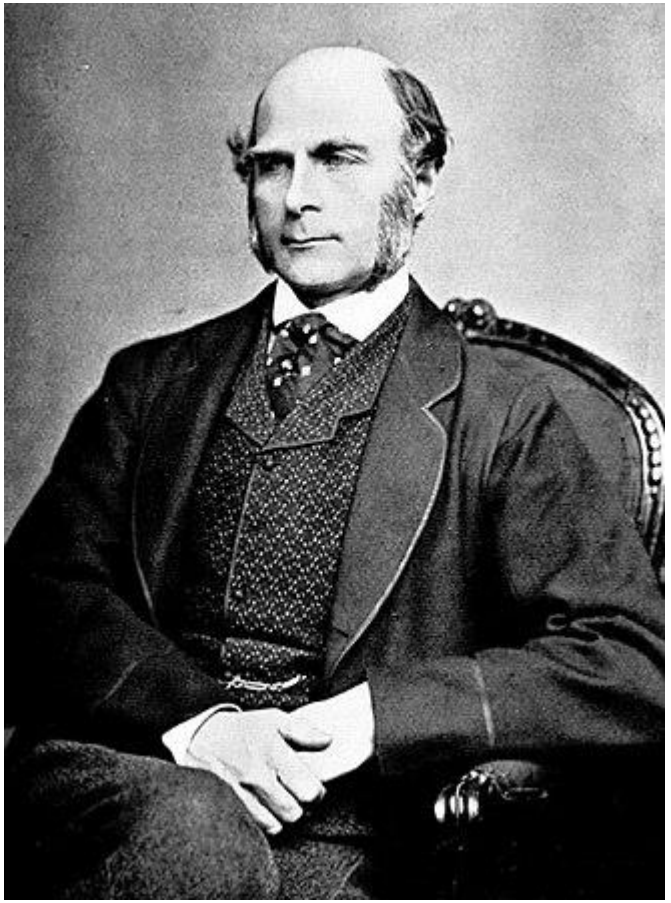



NORMAL CHROMOSOME (ONE CHROMATID) (G1-PHASE)
BREAKAGE
IC
TF
REPLICATION (S-PHASE)
IC
IC
FUSION OF INCOMPLETE CHROMATIDS
(LEADING TO A DICENTRIC CHROMOSOME)
BRIDGE (AT ANAPHASE)
DIC
X
BREAKAGE
IC
X
IC
NEW BREAKAGE-FUSION-BRIDGE CYCLE


a
Exogenous chromosome tagged with selectable marker
Microtubule inhibitor
Recipient cells
Centrifugation
Fusion
drug selection
Donor cells
CHO, A9, DT40
Micronucleation
Microcells
Microcell hybrids
b
Native chromosome
Human
Mouse
Chromosome engineering
HAC
MAC
Artificial telomere
Human centromere
Mouse centromere
Site-specific recombination site (e.g., loxP)

Eugenics

- In 1883 Fracis Galton coins the word 'Eugenics' from the Greek for good ('eu') and born ('genics').
- It is defined as "the science of improvement of the human race through better breeding."
- It is of two types, Positive eugenics and negative eugenecis.



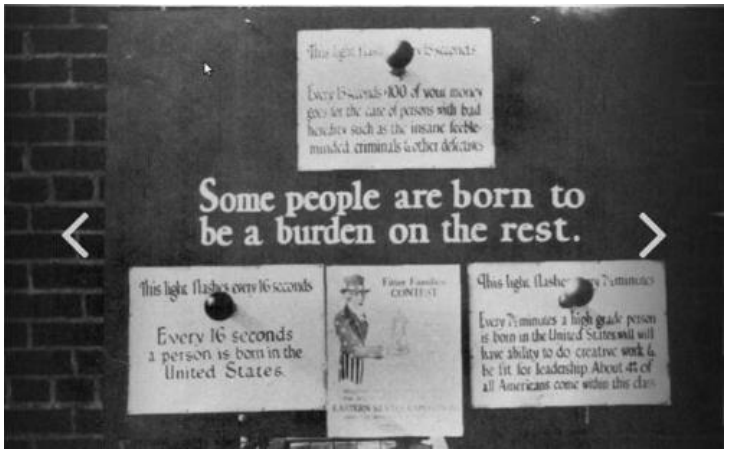
Sir Francis Galton

He was a pioneer in Eugenics. His book *Hereditary Genius* (1869) was the first social scientific attempt to study genius and greatness.

Positive Eugenics

- promotes marriage and breeding between people considered "desirable", and though a positive Eugenist may view certain persons as "undesirable", they will not initiate in such practices as nonvoluntary sterilization, genocide, active euthanasia, or any other forms of violence.

Negative Eugenics



- Negative eugenics: improving the quality of the human race by eliminating or excluding biologically inferior people from the population.
- This goal required severe restrictions on reproductive rights, for those with "defects" had to be kept from reproducing, if necessary through the forceful sterilization.
- Elderly and sick people killed under Hitler's policy of eugenics.

Genetic Counselling

- The genetic counselor communicates
- Genetic, Medical and Technical information
- in a comprehensive, understandable manner with knowledge of psychosocial and cultural background of each client and their family.

Indications for Prenatal Diagnosis

- Advanced maternal age
- Previous child with a chromosome abnormality
- Family history of a chromosome abnormality
- Family history of single gene disorder
- Family history of neural tube defect (NTD)
- Family history of other congenital structural abnormalities
- Abnormalities identified in pregnancy
- Other high risk factors (consanguinity, poor obstetric history, maternal illnesses)

Prenatal Screening For Genetic Disorders

Invasive testing:

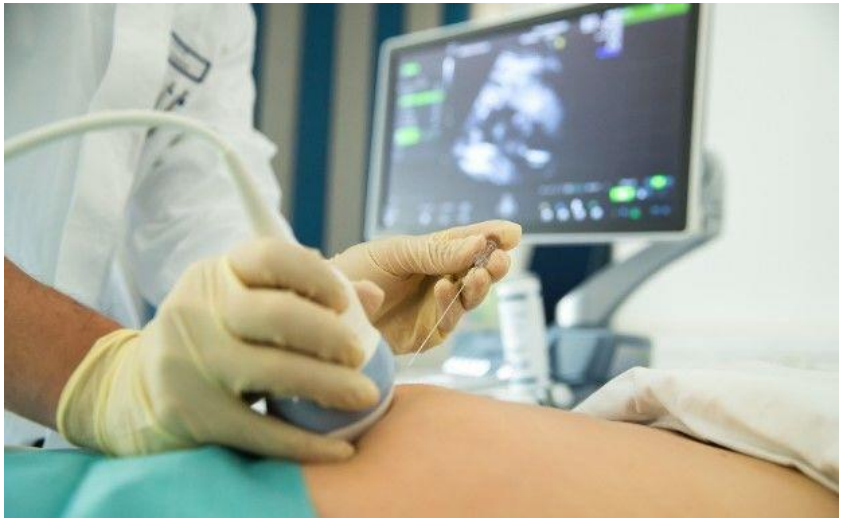
- Amniocentesis
- Chorionic villus sampling (CVS)
- Cordocentesis
- Preimplatation genetic diagnosis
- Fetoscopy

Non-Invasive testing:

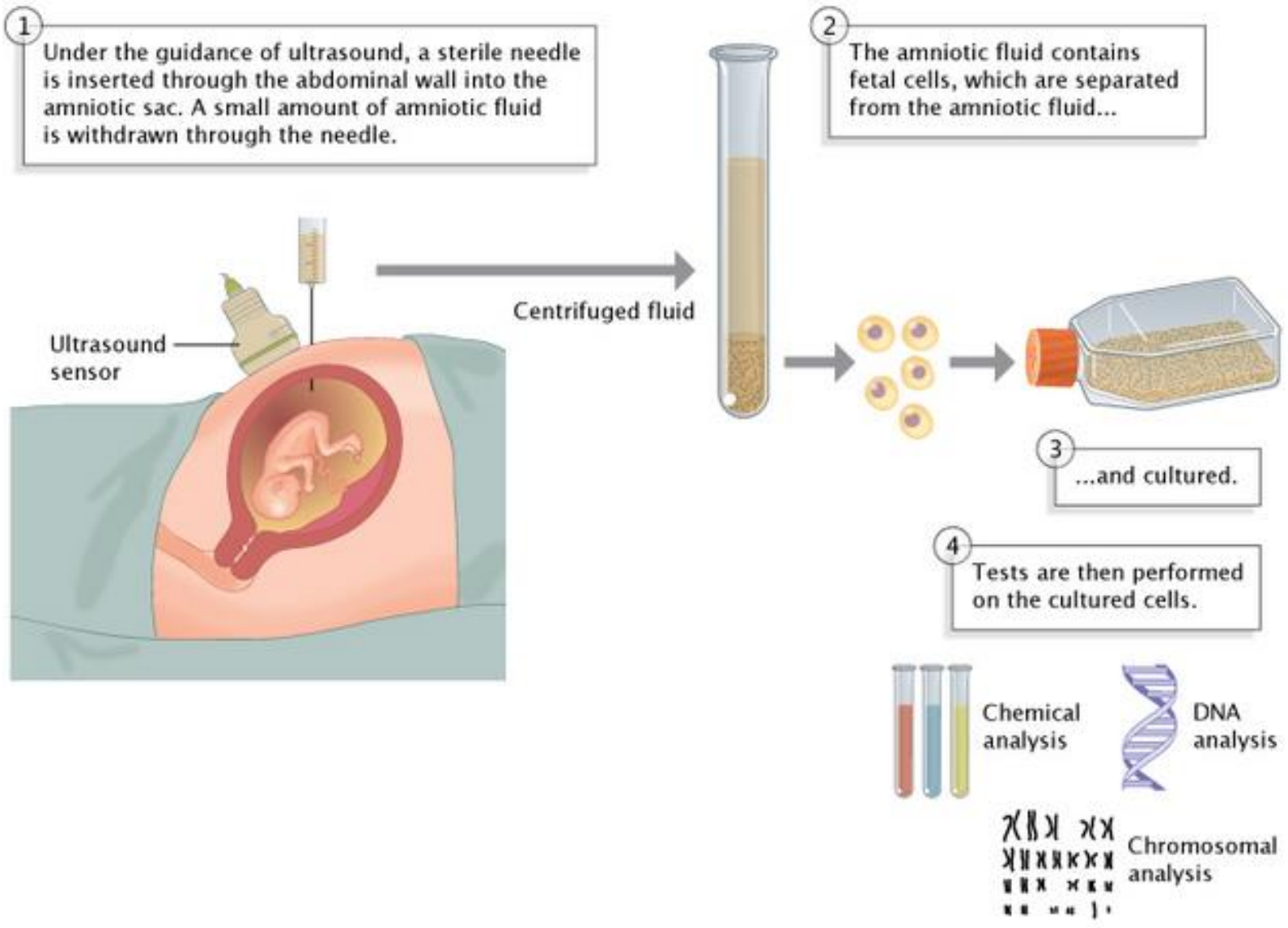
- Ultrasonography
- Maternal serum AFP
- Isolation of fetal cells from maternal circulatio

Amniocentesis

- **Definition:** "Procedure used for prenatal genetic testing to obtain a sample of amniotic fluid from a pregnant woman. A long sterile needle is inserted through the abdominal wall into the amniotic sac to obtain the fluid."
- Oldest procedure for obtaining DNA from an unborn child
- Usually performed between 15 - 18 weeks of pregnancy
- Despite improvements, still carries a risk of miscarriage or infection at a ratio about 1:400.

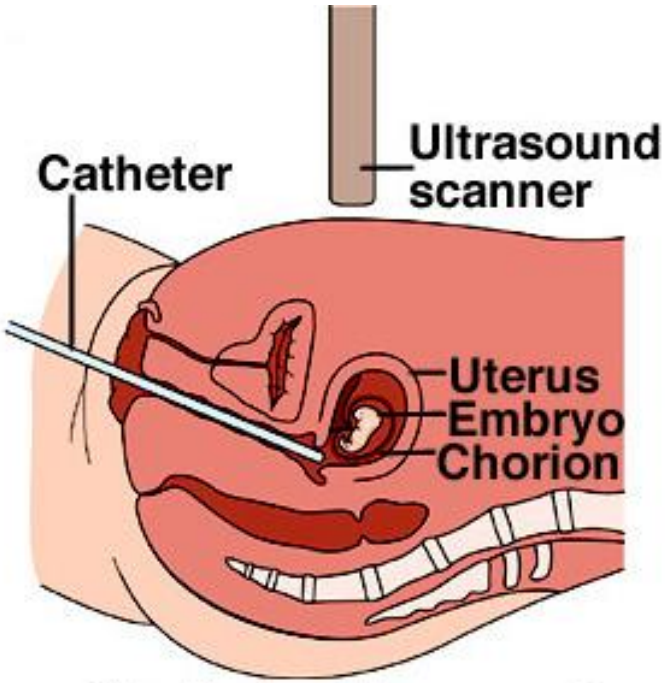


Process of Amniocentesis



Chorionic villus sampling (CVS)

- **Definition**: Procedure used for prenatal genetic testing in which a small piece of the chorion is removed from a pregnant woman. A catheter is inserted through the vagina and cervix into the uterus. Suction is then applied to remove the sample.
- Performed between 10 - 12 weeks of conception.
- Testing for abnormalities in Fetal DNA:
- Can test for: the child's sex, down syndrome…etc.
- Fetal DNA can also be used for karyotyping.

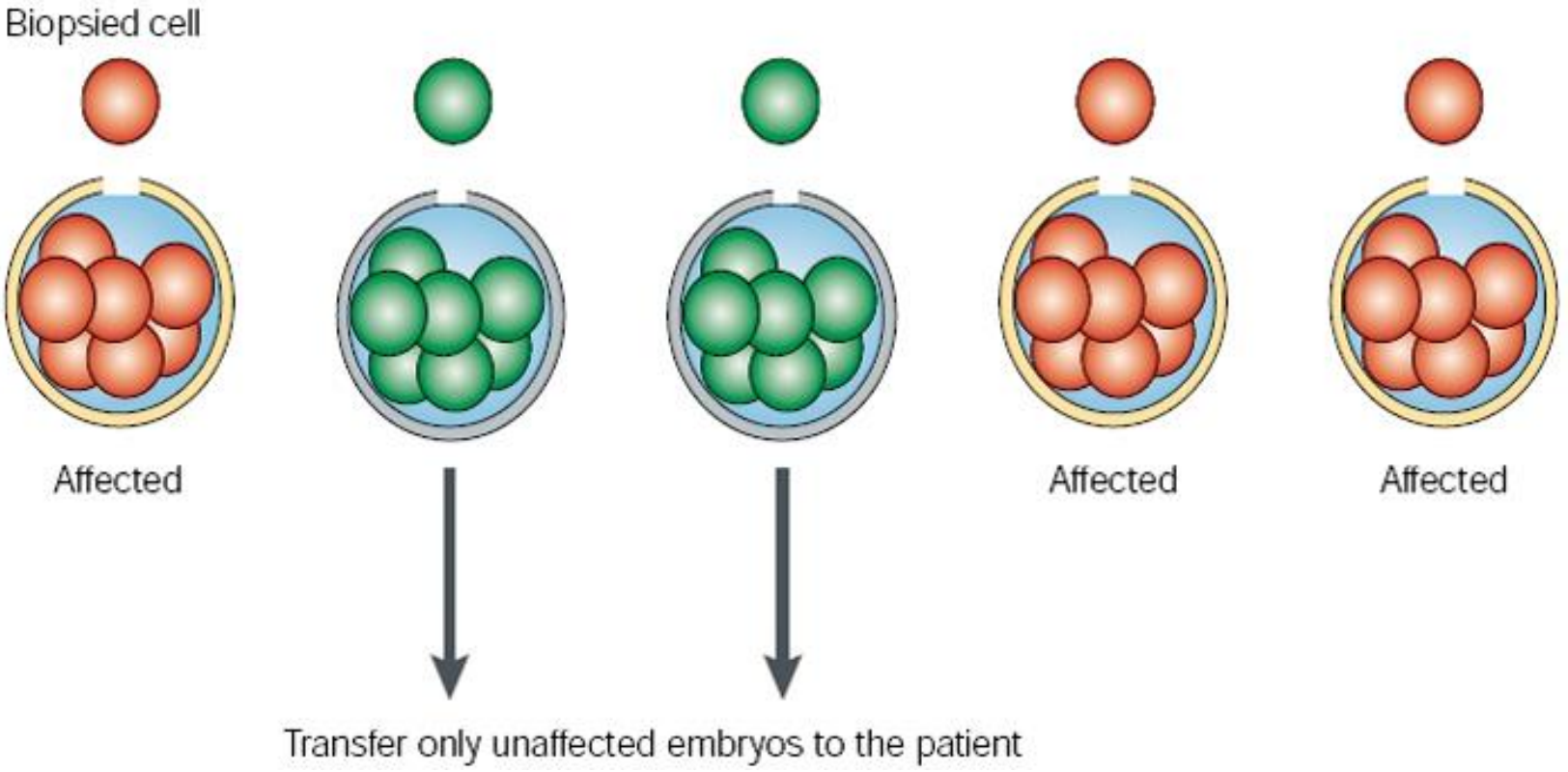


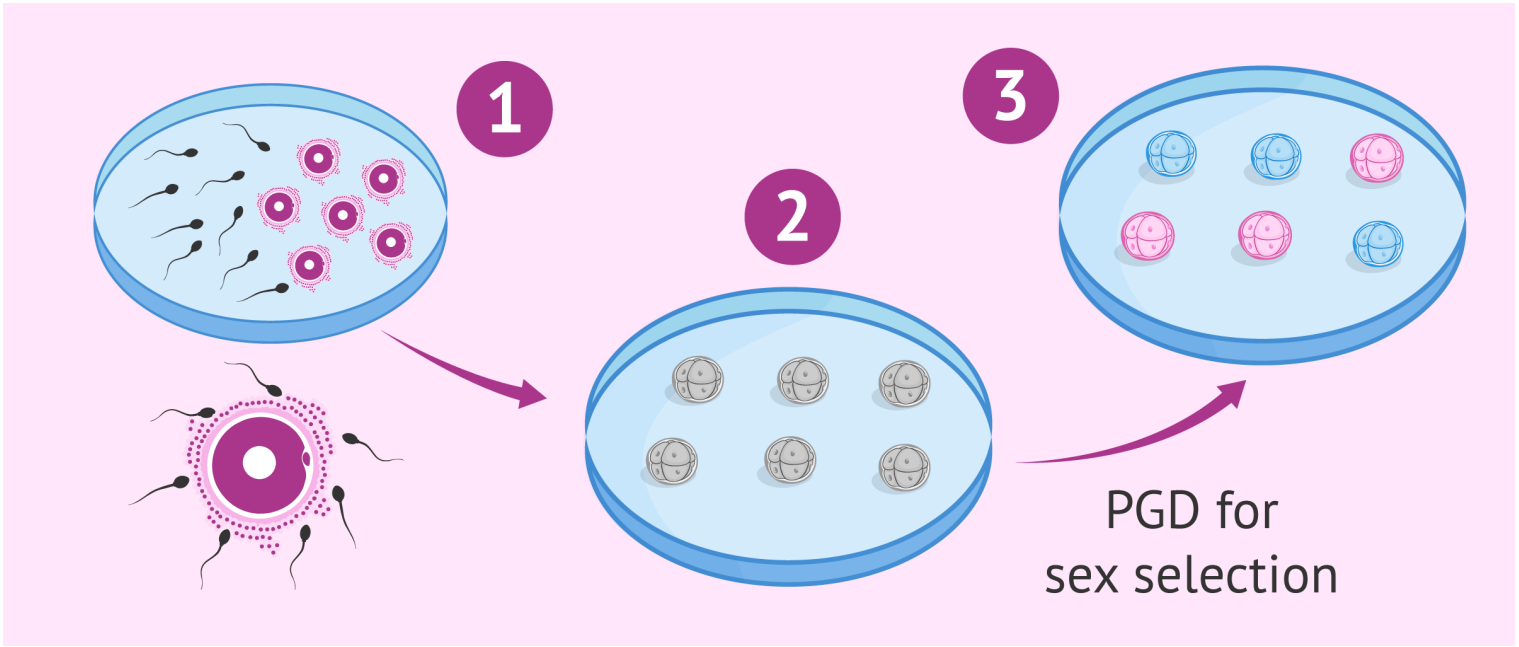
Chorionic villus sampling

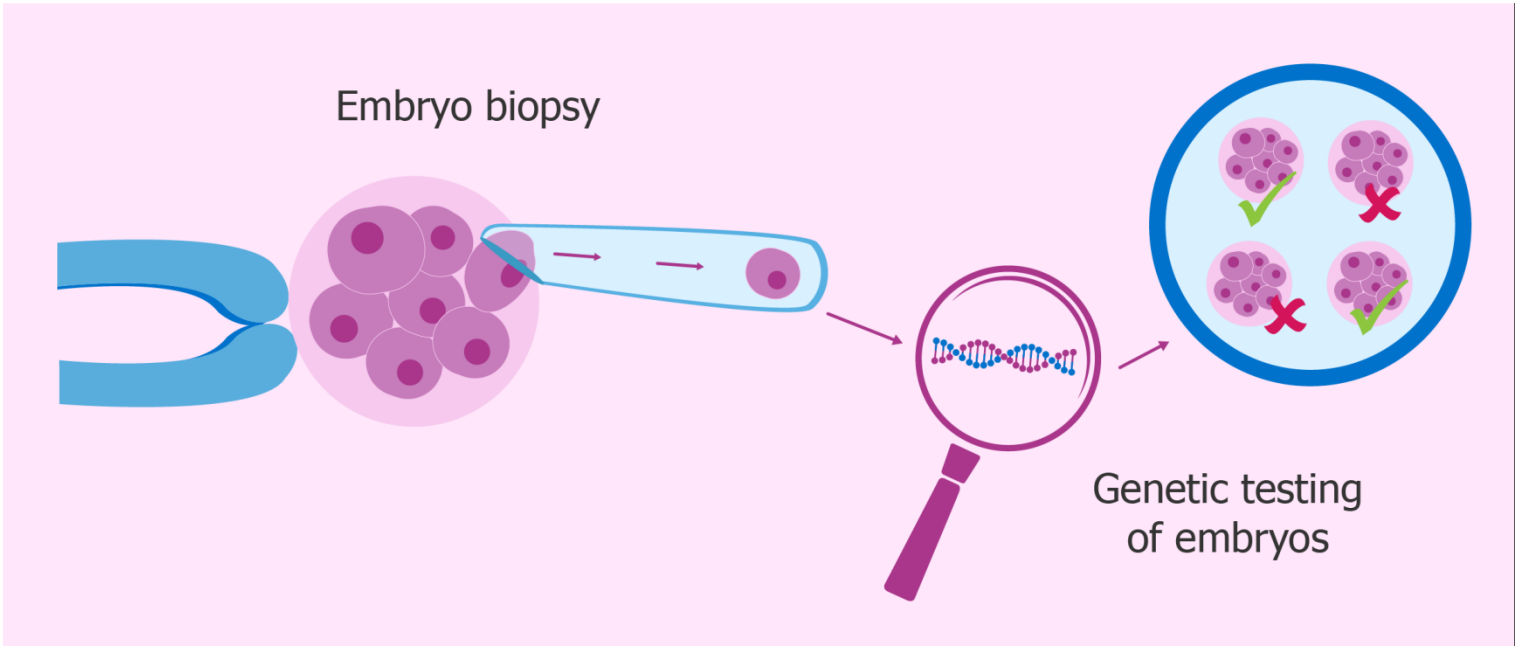
An alternative method to amniocentesis which is more modern but more risky.

Preimplantation Genetic Diagnosis

- **Definition:** "A process which allows parents to have the option of detecting potential defects in an embryo within days after conception."



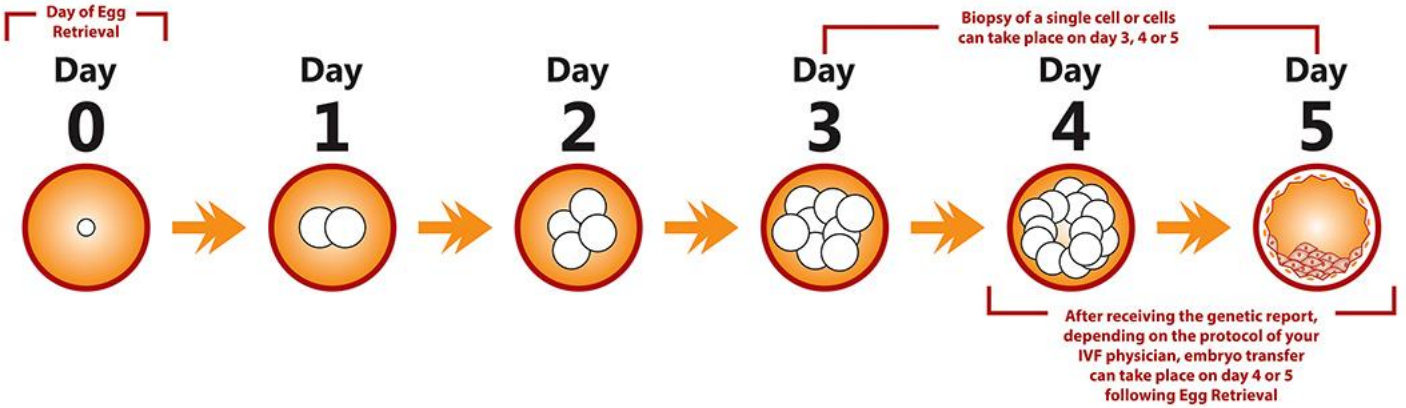




PGD depends on methods that are routinely used for in vitro fertilizatio n (IVF).

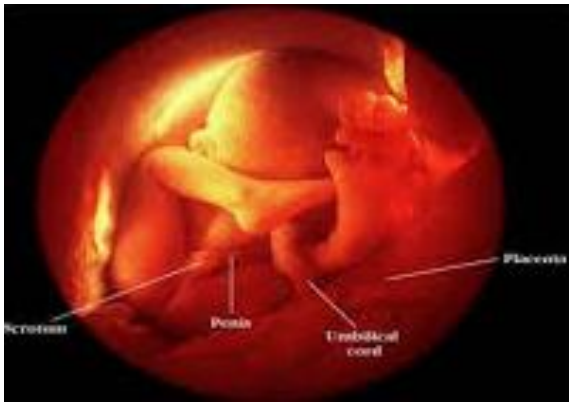
Preimplantation Genetic Diagnosis: The most common method:

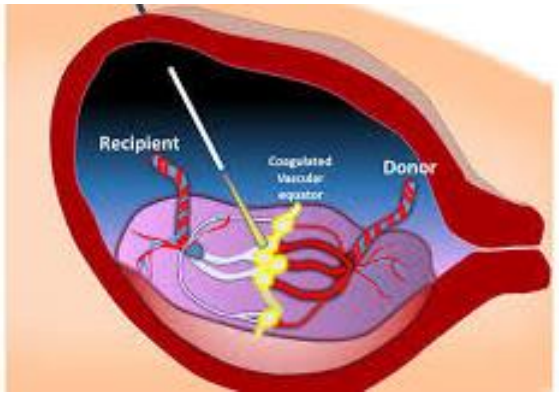
- A blastomere (type of cell produced by division of the egg postfertilization), is removed from the embryo about 3 days after fertilization. Once the blastomere is removed for analysis, it must be transferred to the mother's uterus within the next one or two days.
- PGD has a significantly higher error rate than amniocentesis or CVS; those two methods are often recommended to be used as follow up procedures to PGD.



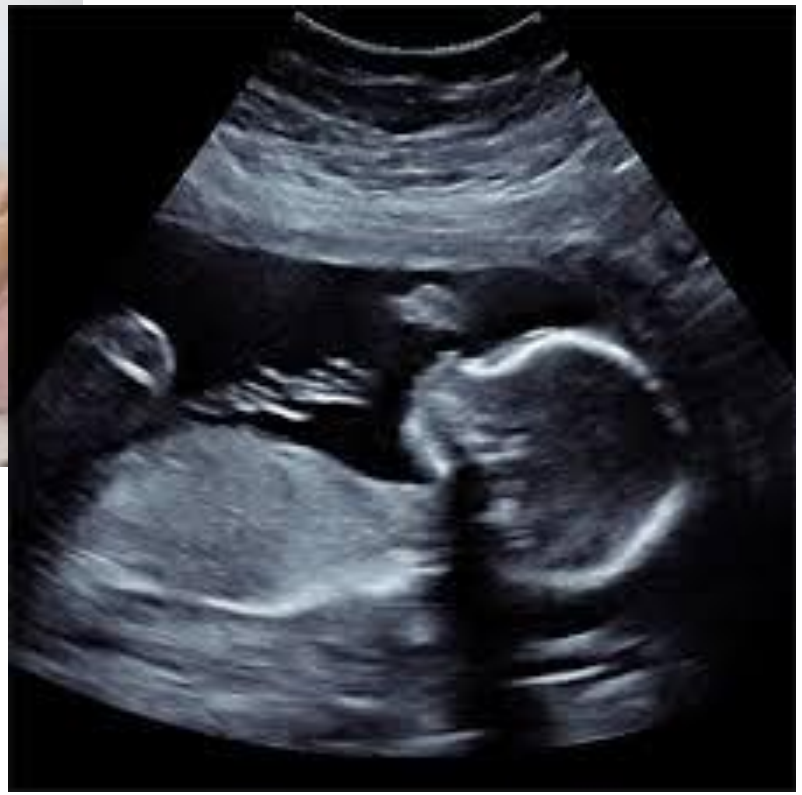
Fetoscopy

- **Fetoscopy** is an endoscopic procedure during pregnancy to allow surgical access to the fetus, the amniotic cavity, the umbilical cord, and the fetal side of the placenta.
- Fetoscopy allows for medical interventions such as a biopsy (tissue sample) or a laser occlusion of abnormal blood vessels.
- Fetoscopy is usually performed in the second or third trimester of pregnancy. The procedure can place the fetus at increased risk of adverse outcomes, including fetal loss or preterm delivery, so the risks and benefits must be carefully weighed in order to protect the health of the mother and fetus(es).





Ultrasonography



Alpha-fetoprotein (AFP) test

Blood is withdrawn from vein between the 16-18 weeks of pregnancy

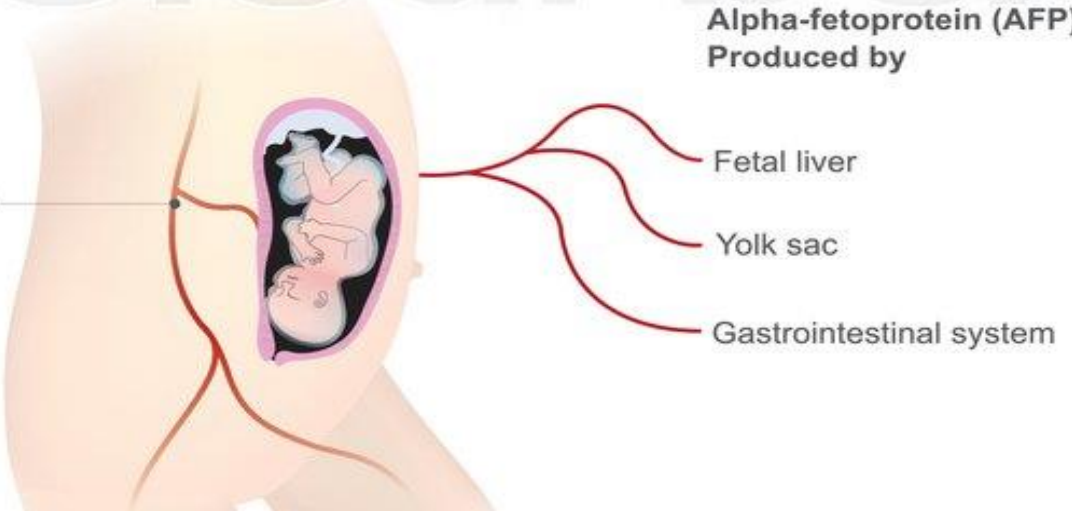
AFP : ☐ –

☐ +

- To detect liver cancer
- Other chromosomal abnormalities
- Defects in the abdominal wall of the fetus
- To screen for neural tube defect (high level AFP)
- To screen for Down's syndrome (low level AFP)

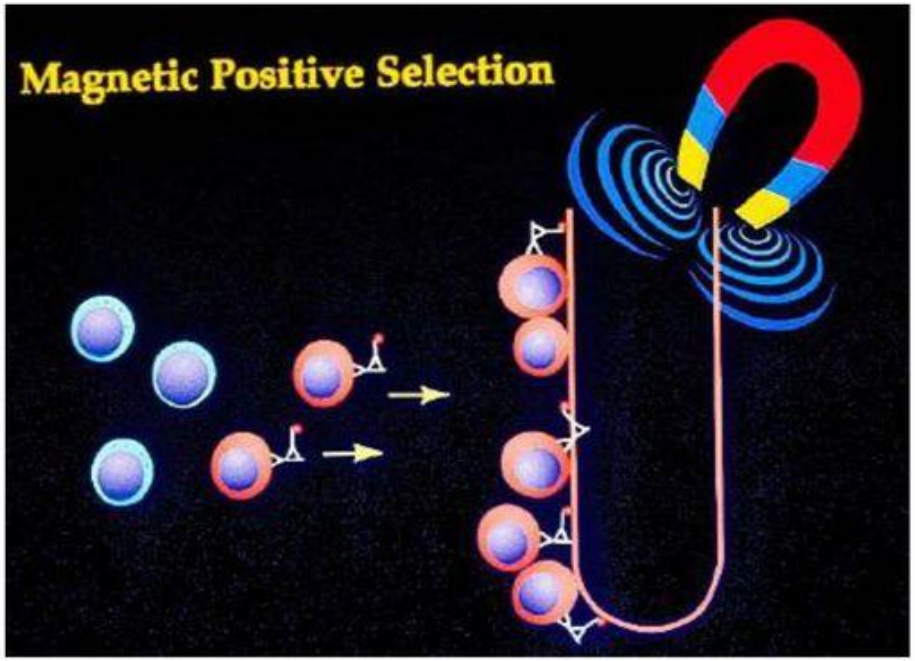
Right radial artery

Alpha-fetoprotein (AFP) Produced by



Other Sources of fetal tissues for Non-Invasive Prenatal Diagnosis

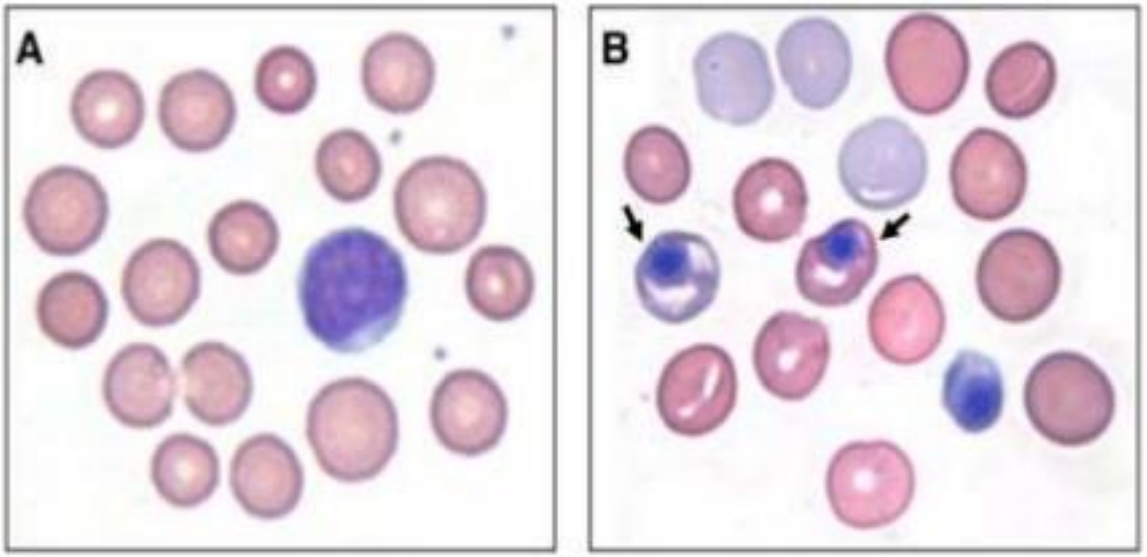
- **Fetal Cells in maternal circulation**
  - Erythrocytes
  - Trophoblastic Cells
  - Leukocytes
    - ✓ Difficult to Isolate
    - ✓ Very low abundance
    - ✓ Persist for years after delivery



Very small number of fetal cells migrate into the mother's circulation
1 out of 10^7 nucleated cells

Sorting using CD-71 (transferrin receptor to separate nucleated red blood cells.
FISH –for X and Y Signals

- ✓ Fetal blood cells can then be analyzed for the diagnosis of genetic disorders using FISH, PCR etc.
- ✓ Fetal cells separated from a mother's blood have been successfully used in the diagnosis of cystic fibrosis, sickle cell anemia, and thalassemia in a fetus.

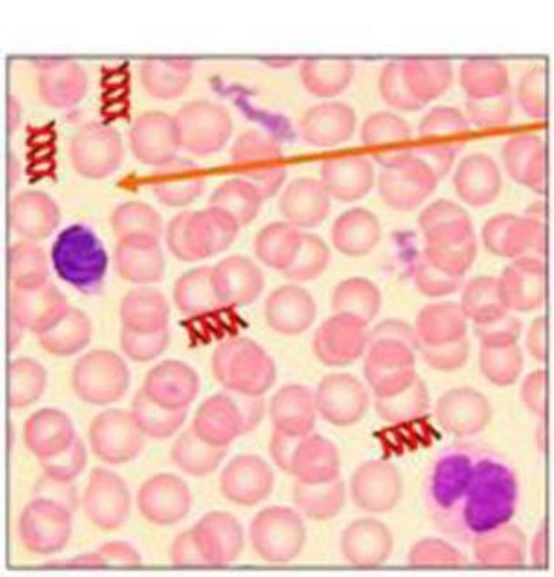


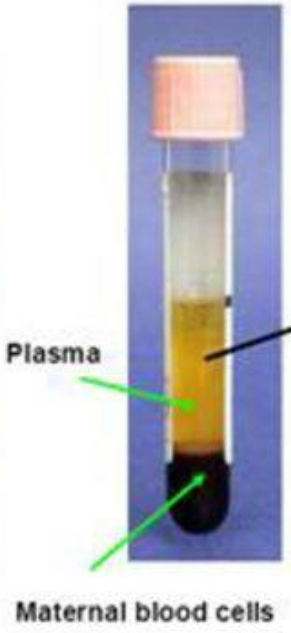
A. Maternal RBCs

B. Fetal RBCs (nucleated)

Cell free fetal nucleic acids from maternal plasma

- 1977: Small quantities of free DNA observed in cancer patients
- 1997: Cell free DNA isolated from the plasma of pregnant women





Cell free maternal DNA (96.6%)

Cell free fetal DNA (3.4%)

Amount of cell free fetal DNA extracted is equivalent to 25 cells / ml plasma

Cell free maternal RNA

Cell free fetal RNA

Process of Genetic Counselling

Benificeries: Individual or couple

- Have affected child
- Are carriers
- Have genetic disease in family
- Have recurrent abortions
- High maternal/paternal age
- Exposed to a mutagen/teratogenic
- Are consanguineous.

Genetic Counseling

- Available options
- Risk calculations
- New developments
- Disease course
- Treatment availability

Process of Genetic Counselling

Reaching accurate Diagnosis

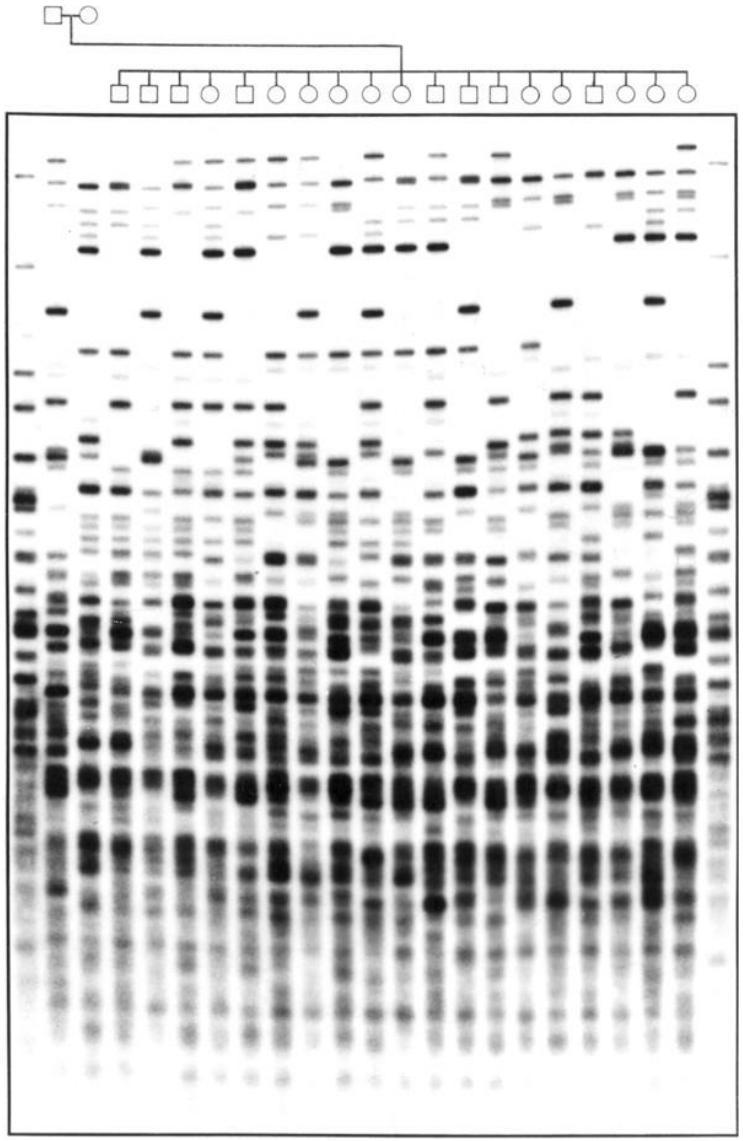
- Family history
- Physical/clinical examination
- Cytogenetic studies/radiology
- Laboratory/DNA analysis

Estimation of Recurrence Risk

- Family pedigree
- Applying various methods
- Risk calculation
- Mendel's

DNA Fingerprinting and Forensic Analysis

- What Is a DNA Fingerprint?
- Preparing a DNA Fingerprint
- Putting DNA to Use
- DNA and the Rules of Evidence
- Familial Relationships and DNA Profiles
- Nonhuman DNA Analysis

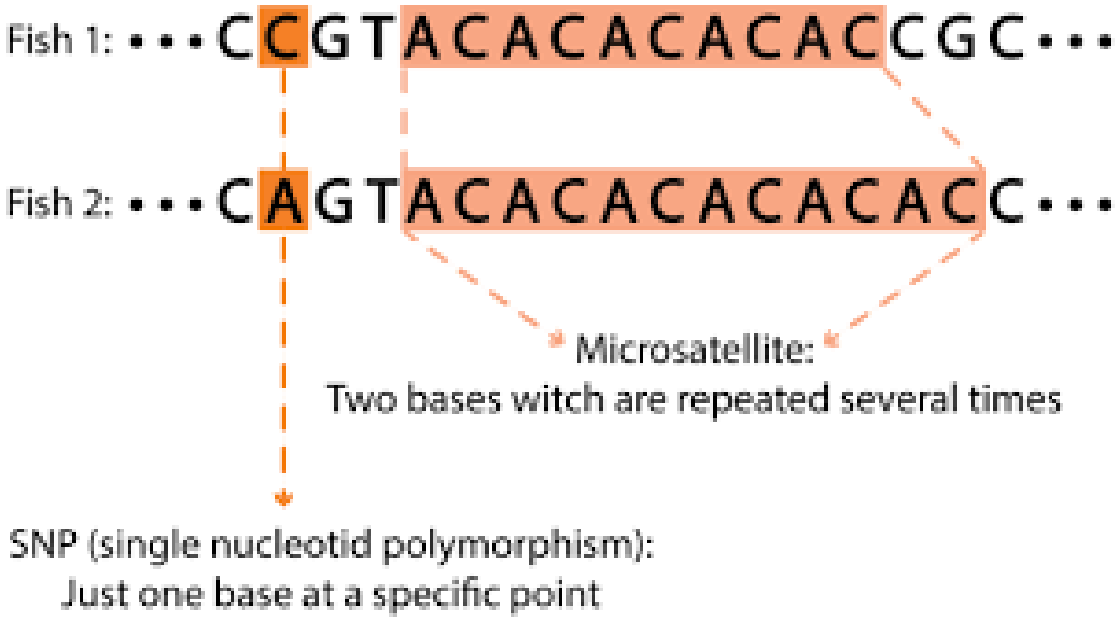


What Is a DNA Fingerprint?

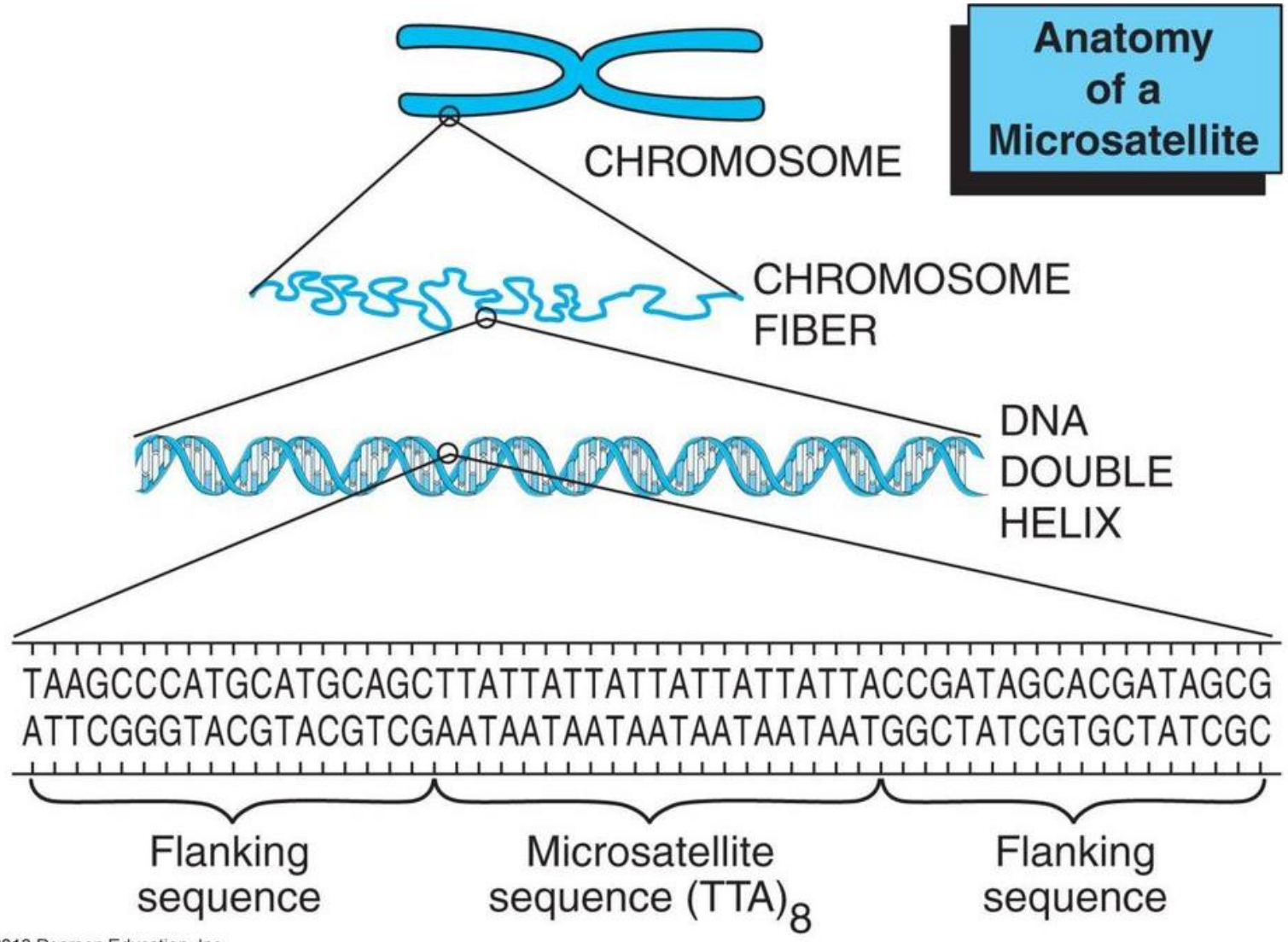
- Every individual carries a unique set of genes.
- Chemical structure of DNA is same, but the order of the base pairs differs.
- Every cell contains a complete set of DNA that identifies the organism as a whole.
- Only one tenth of 1% of DNA differs from person to person.
- Two Main Types of Forensic DNA Testing:
- Restriction fragment length polymorphism (RFLP) requires larger amounts of DNA and DNA cannot be degraded.
- Polymerase chain reaction (PCR) requires less DNA and DNA can be partially degraded. Extremely sensitive to contaminating DNA.

What Is a DNA Fingerprint?

- DNA fingerprinting is restricted to the detection of microsatellites 1 to 6 nucleotide repeats dispersed throughout the chromosomes.
- Probes used to identify the microsatellite surround the specific microsatellite being analyzed.
- Also called short tandem repeats (STR).

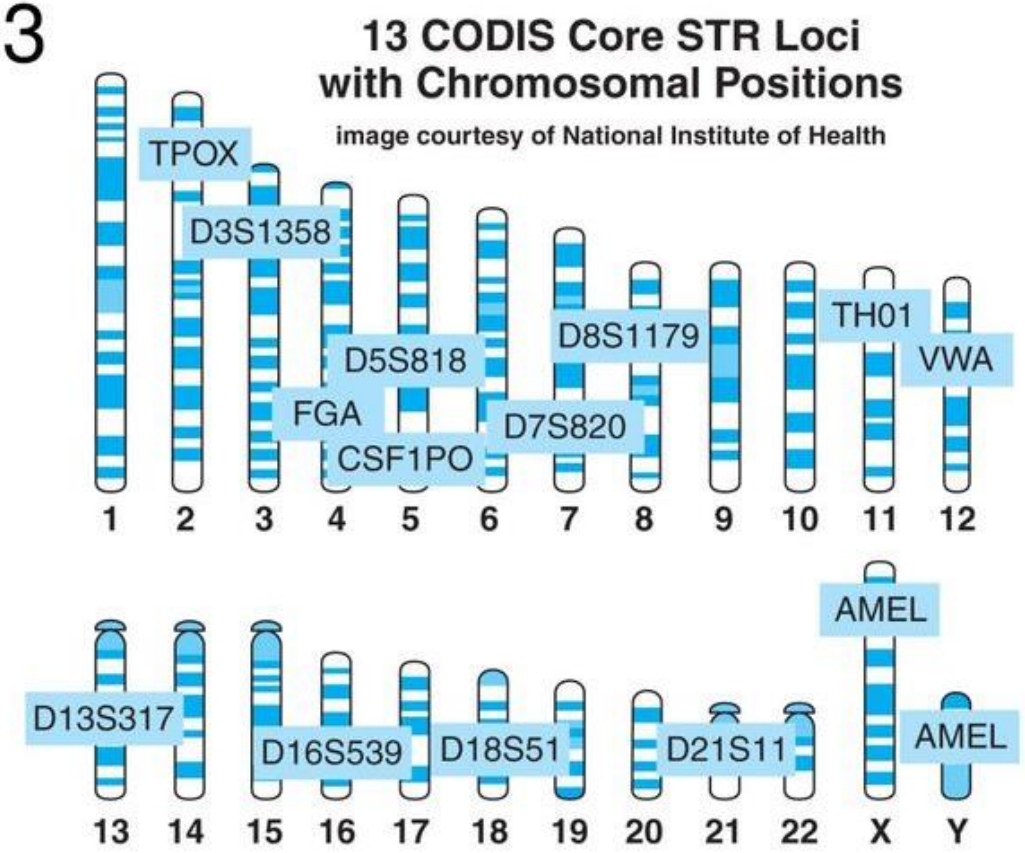


What Is a DNA Fingerprint?



What Is a DNA Fingerprint?

- FBI has chosen 13 unique STRs for analysis and comparison in its library of DNA fingerprints
  - Combined DNA Index System (CODIS)



Preparing a DNA Fingerprint. Specimen Collection

- Search for sources of DNA Collection requires scrupulous attention to detail.
- Wear disposable gloves; change them frequently.
- Use disposable instruments.
- Avoid talking, sneezing, and coughing.
- Avoid touching any item that might contain DNA (face, nose, or mouth).
- Air-dry evidence before packaging; mold can contaminate a sample

Preparing a DNA Fingerprint

Enemies of Evidence

- Sunlight and high temperature
- Bacteria
- Moisture

DNA fingerprinting is a comparative process

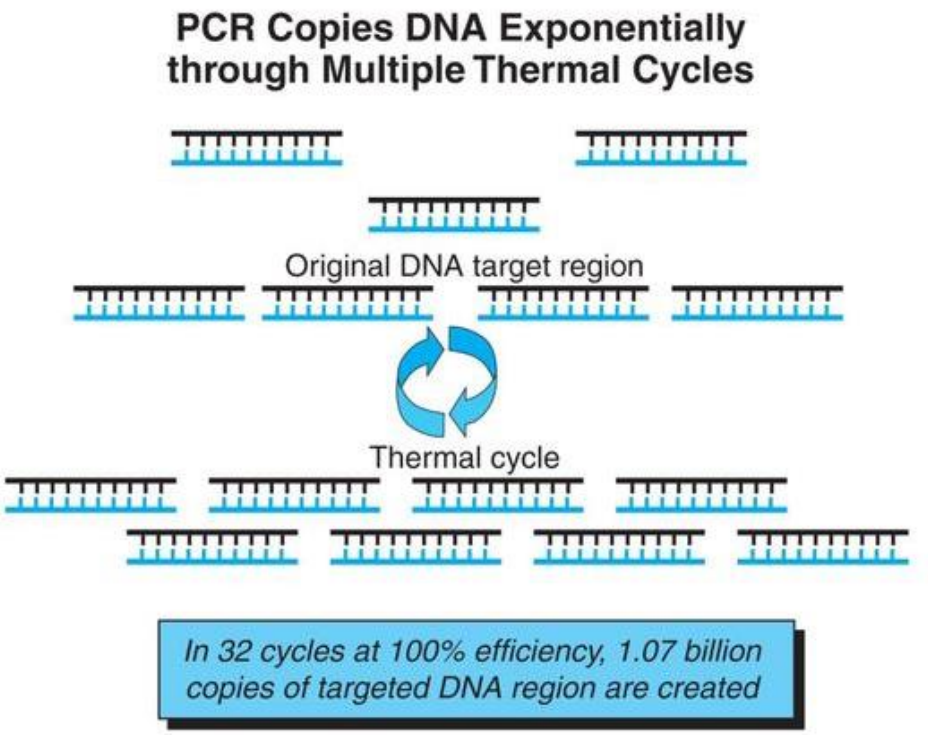
- Samples from crime scene must be compared to suspect DNA
- Best sample from suspect DNA is fresh, whole blood

Preparing a DNA Fingerprint. Extraction of DNA

- DNA can be purified.
- Chemically (using detergents)
- Mechanically (pressure to force DNA out of cell).
- RFLP Analysis (Restriction Fragments Length Polymorphism)
- Treat DNA with restriction enzyme.
- Restriction enzyme cuts DNA at restriction sites.
- Use several restriction enzymes in sequence or combined.
- Use agarose gel electrophoresis to separate the piece.
- Gel is chemically treated or heated to denature the DNA
- Allows the binding of a single-stranded probe

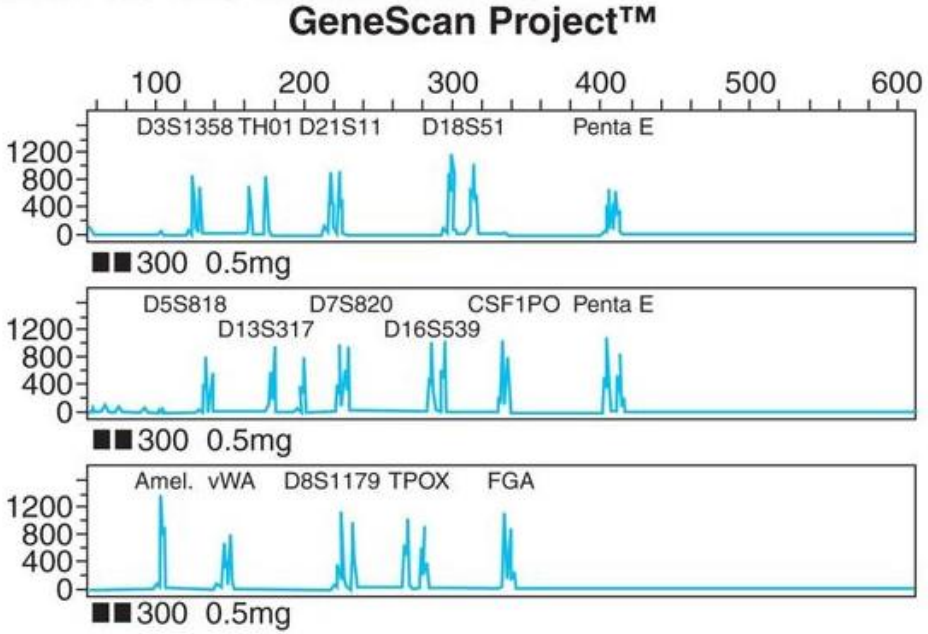
Preparing a DNA Fingerprint

- **PCR** – used to amplify DNA found at crime scene into an amount that can be analyzed
  - DNA primers for the flanking regions of CODIS sites results in DNA amplification at specific STR sites



Preparing a DNA Fingerprint

- **STR Analysis**
  - After STRs are amplified by PCR, alleles are separated and detected using capillary electrophoresis, allowing for the number of repeats in each of the two alleles on a homologous chromosome to be determined



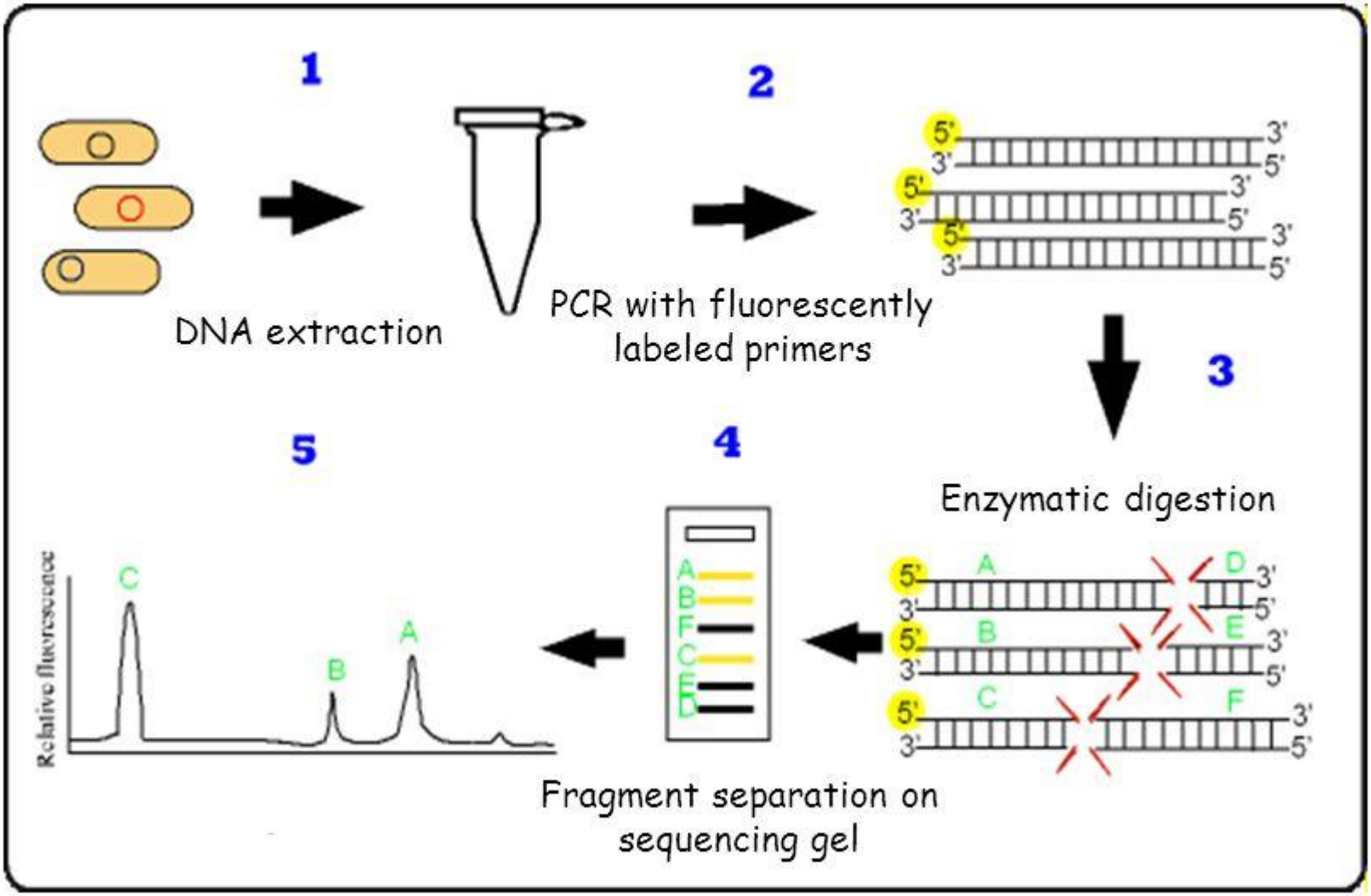
Preparing a DNA Fingerprint

- **STR Analysis, continued**
  - The number of repeats within an STR is referred to as an allele
  - Ex. The D7S820 STR on chromosome 7 contains between 5 and 16 repeats of GATA
  - An individual with D7S820 alleles 10 and 15 would have inherited a copy with 10 GATA repeats from one parent and a copy with15 GATA repeats from the other parent
  - There are 12 different alleles for this STR, and 78 different possible genotypes

Preparing a DNA Fingerprint

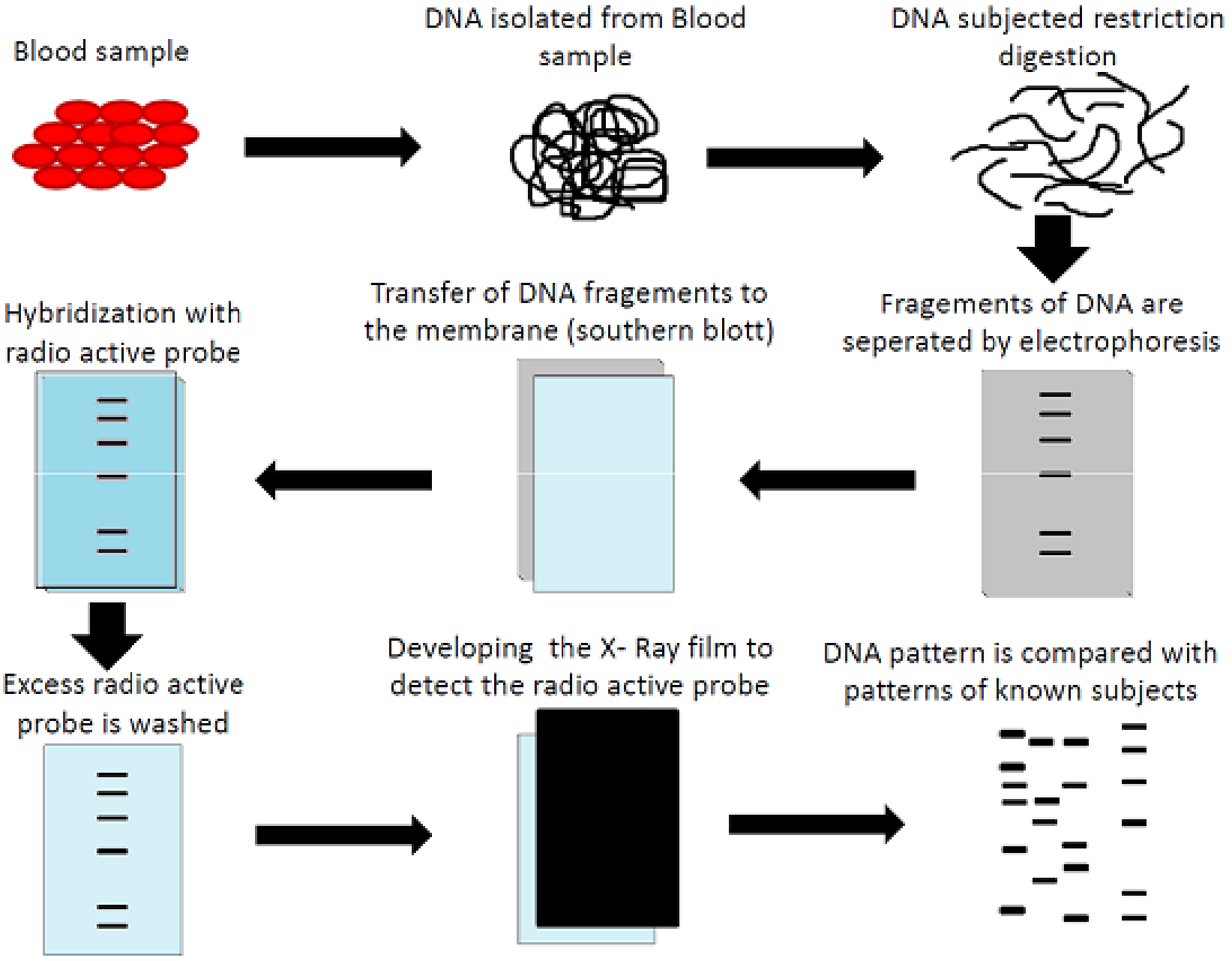
- FBI uses 13 STR regions
  - Odds that two individuals will have the same 13-loci DNA profile are more than one in a billion

Terminal Restriction Fragment Length Polymorphism (T-RFLP)



Southern Blot Technique

- Transfer DNA fragments from gel to nitrocellulose or nylon membrane
- Membrane incubated with a probe
- Short strand of complementary DNA with a radioactive or fluorescent tag
- Targeted area on the DNA fragment is called a locus
- Expose X-ray (photo) film to membrane to obtain permanent record of results

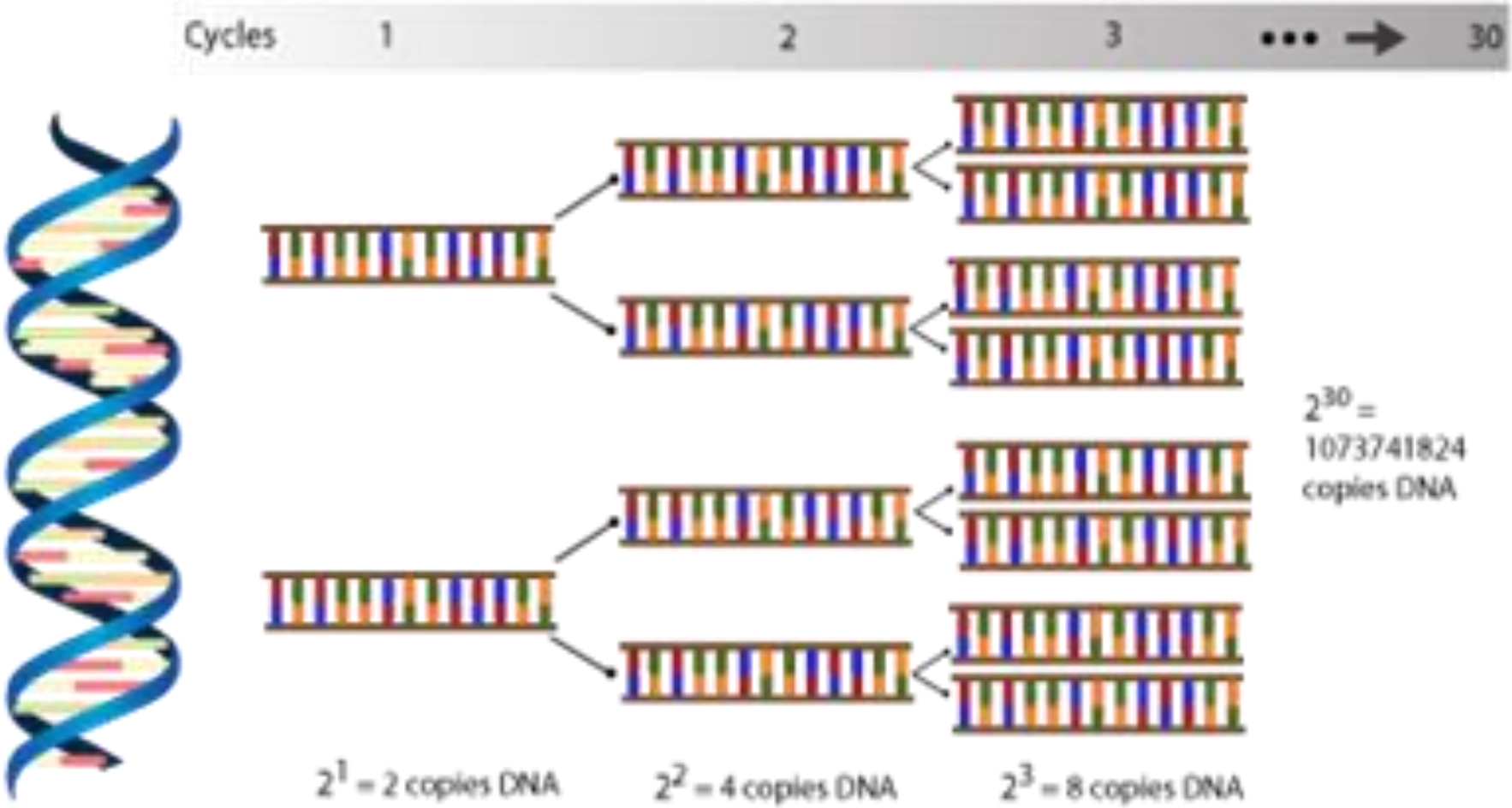

Blood sample
DNA isolated from Blood sample
DNA subjected restriction digestion
Fragements of DNA are seperated by electrophoresis
Transfer of DNA fragements to the membrane (southern blott)
Hybridization with radio active probe
Excess radio active probe is washed
Developing the X- Ray film to detect the radio active probe
DNA pattern is compared with patterns of known subjects

PCR analysis

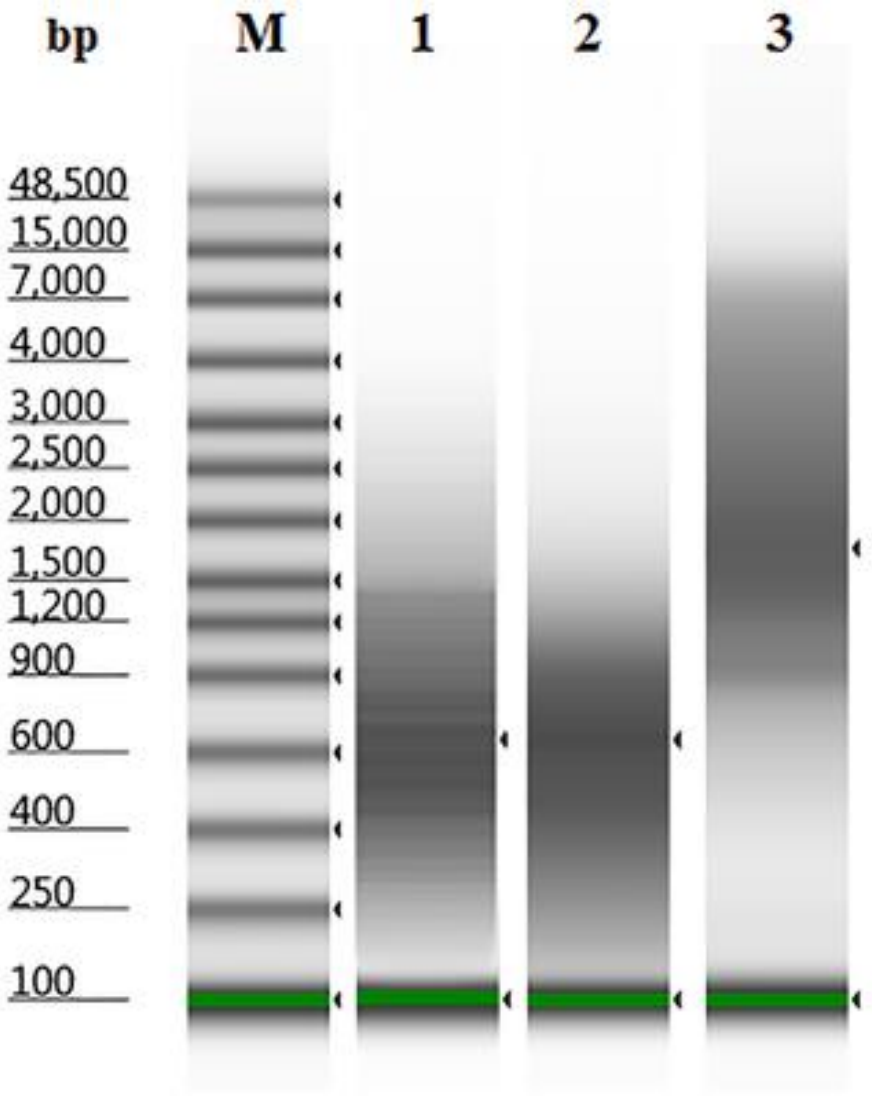
PCR analysis stands for '**polymerase chain reaction**.' It is a technique that allows technicians to create millions of precise DNA replications from a single sample of DNA. In fact, DNA amplification alongside **PCR** can let forensic scientists perform DNA **analysis** on samples that are as tiny as only a couple of skin cells.

- PCR – used to amplify DNA found at crime scene into an amount that can be analyzed.
- DNA produced is identical to the original sample.
- Use amplified DNA in a Dot Blot Analysis.
- DNA amplified by PCR is blotted onto specially prepared blot strips.
- Each dot on the strip is a different DNA probe from human DNA.

PCR amplification



Chain Reaction, copies from copies produced



Electrophoretic analysis of WGA libraries by Agilent 2200 Tape Station Instrument.

The libraries were obtained by DOP-PCR (lane 1), PicoPlex (lane 2), and iDOP-PCR (lane 3) from 15 pg of human gDNA. **M**–DNA marker "Genomic DNA ScreenTape".

Looking for alignment of bands or dots in the fingerprint. All tests are based on exclusion.

Testing continues only until a difference is found.

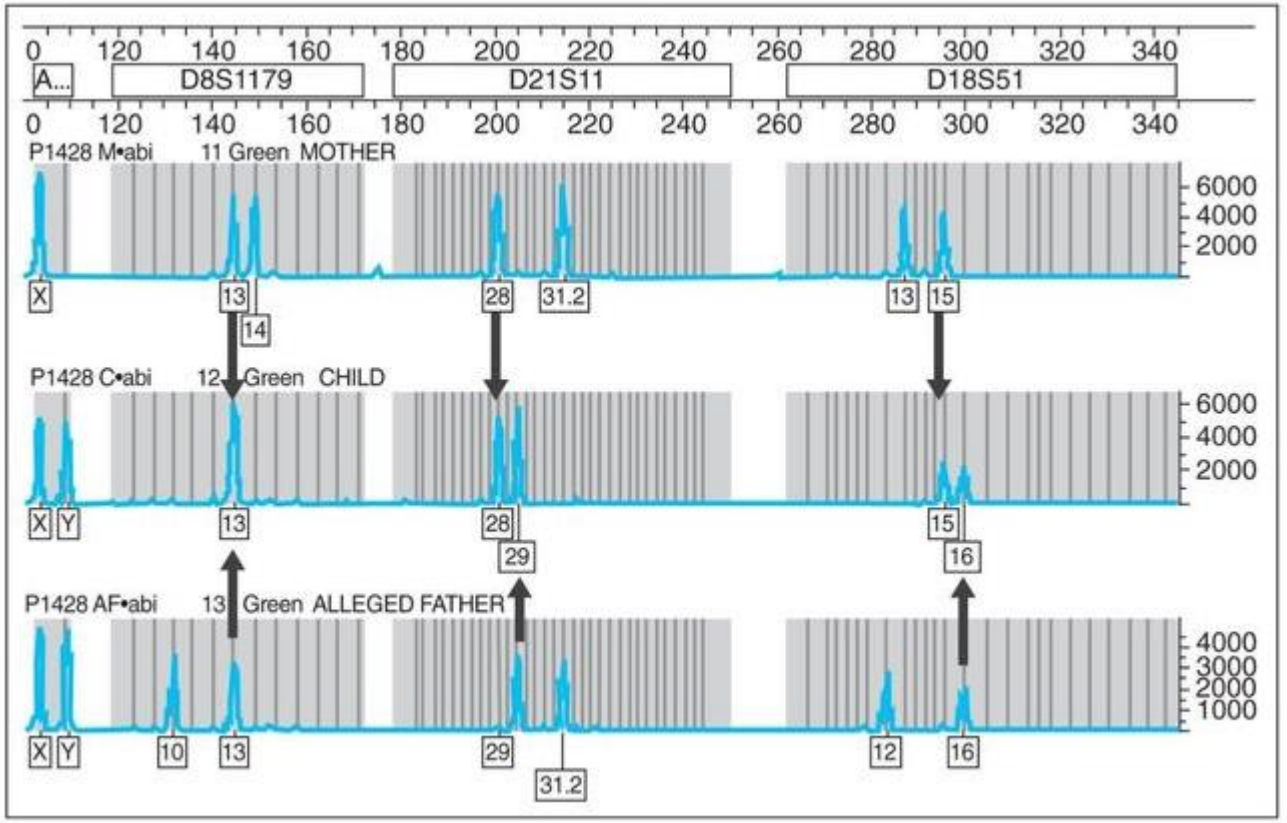
If no difference is found after a statistically acceptable amount of testing, the probability of a match is high.

Putting DNA to Use

- Putting DNA to search criminals, murderers
- Putting DNA to search victims Terrorism and Natural Disasters (World Trade Center (2001), South Asian Tsunami December (2004)
- **Human Error and Sources of Contamination**
- Chain of custody of samples is compromised
- Collection of evidence must be systematically recorded and access to evidence must be controlled
- Follow defined standards of laboratory practice and procedures to prevent DNA damage during the analysis
- **DNA and Juries**
- Must make sense to the jury
- Statistics can be confusing

Familial Relationships and DNA Profiles

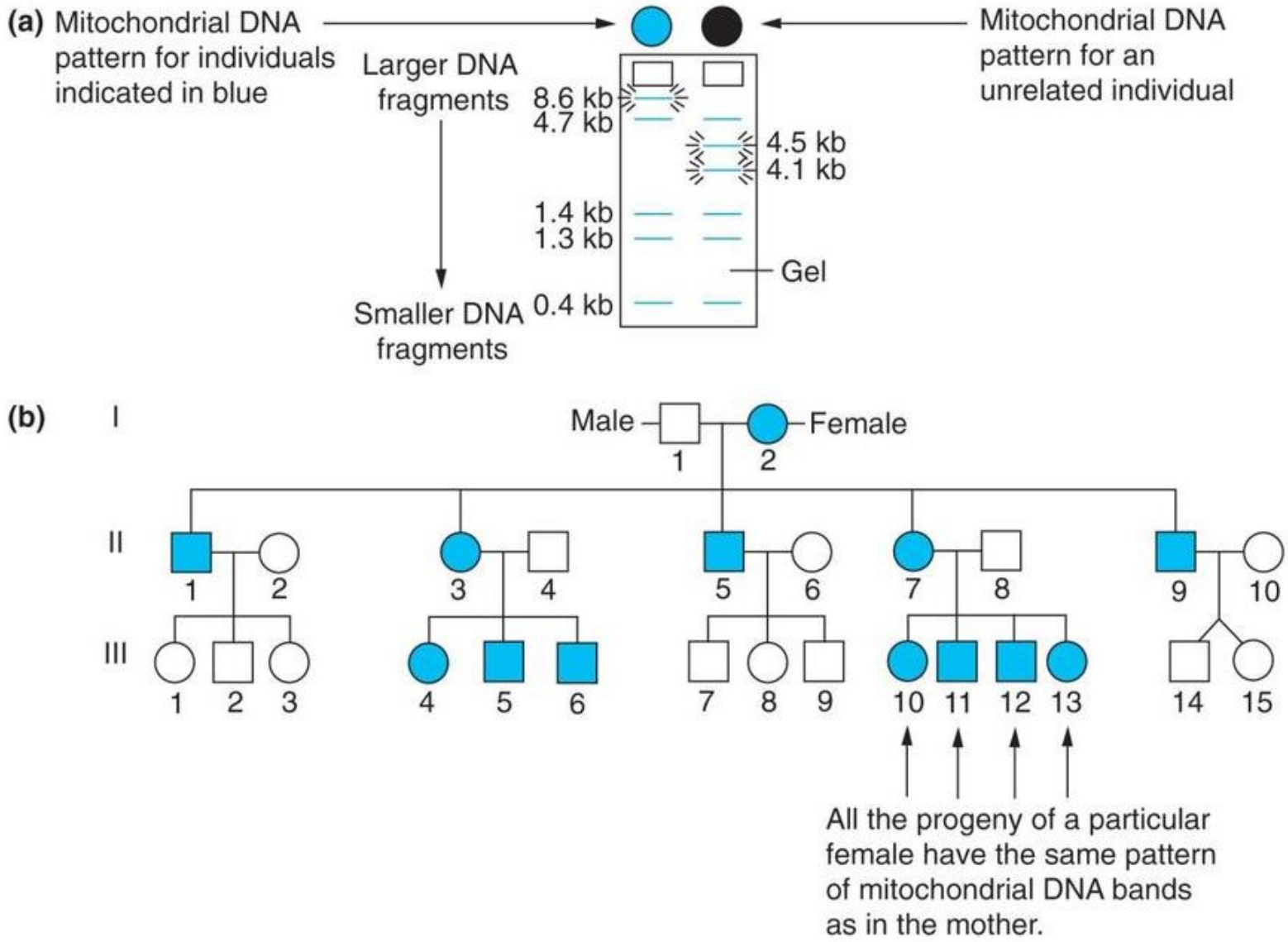
- Paternity Testing
- Analyze samples from child and adults involved



Familial Relationships and DNA Profiles

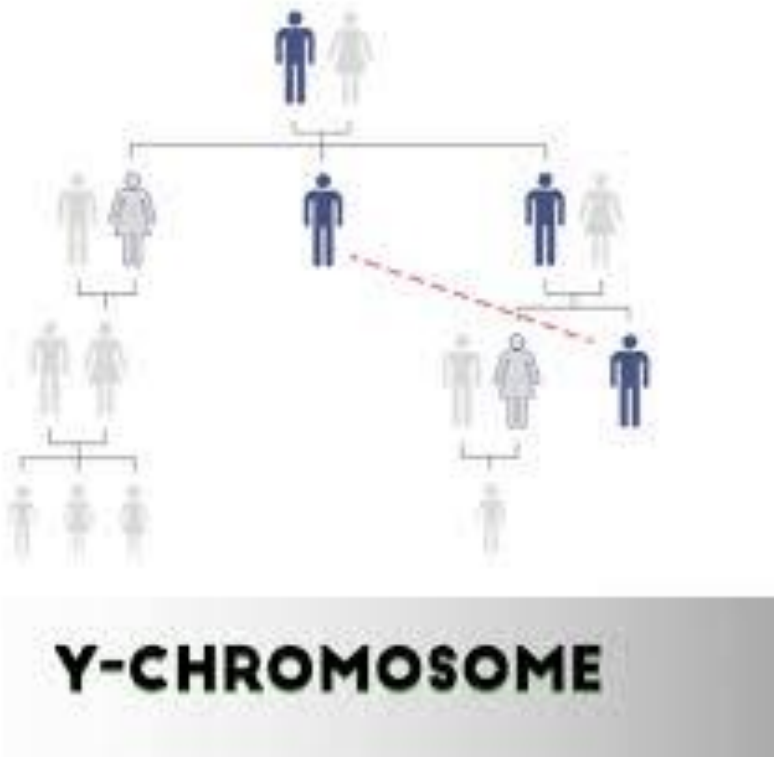
- **Mitochondrial DNA**
- Used to examine samples that cannot be analyzed by PCR or RFLP.
- Older samples that lack nucleated cellular material (hair, bones, and teeth)
- Inherited from the mother only .
- Changes only about 1% every million years due to random mutation.

Familial Relationships and DNA Profiles



Familial Relationships and DNA Profil

- Y-Chromosome Analysis
- Passed directly from father to son
- Useful for tracing relationships among males or analyzing biological evidence involving multiple male contributors.



Nonhuman DNA Analysis

- **Ginseng**
- $3 million market in U.S. alone
- Two major herbal products are referred to as ginseng
- One native to North America, the other native to Asia
- Asian ginseng boosts energy;
- American ginseng calms nervesAmerican variety is rarer and more valuable
- **Ancestry of Cabernet Sauvignon Grapes**
- Hybrid grapes are considered inferior and are legally excluded from bearing the prestigious dinstinction appellation *d'origine contrôlée* in France
- DNA evidence determined that the ancestors of cabernet sauvignon grapes are cabernet franc and sauvignon blanc/

Nonhuman DNA Analysis

- **Prove a hunter killed a bear illegally in PA**
- A law makes it illegal to kill a bear in a den
- Witness reported seeing a hunter discharge gun into den
- Bear's premolars were removed at registration station to confirm sex and age of bear
- Collected blood samples from the den and compared with DNA from bear; were a match
- DNA Tagging to Fight Fraud
- Use DNA as an authentication label hidden in a wide variety of products
- Footballs in 2003 Super Bowl
- 2000 Sydney Summer Olympics