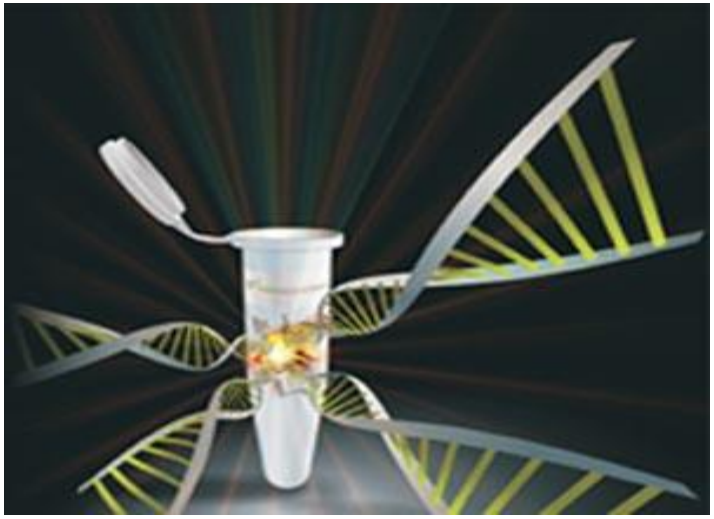
Genomic DNA Extraction



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Part I

Introduction

Extraction of DNA

- **DNA Extraction:** is a process of isolation and purification of DNA from a sample using a combination of physical and chemical methods
- Routine procedure widely used in:

1. Molecular biology labs (scientific research labs) for example to study a gene involved in a cancer

Extraction of DNA

2. Genetic testing is used to diagnose or rule out suspected genetic or inherited diseases. Also to identify disease carriers or to predict those at high risk for specific conditions

- Adult
- Newborn
- Fetus

Extraction of DNA

3. Forensic analyses to gather evidences from the crime scene that can be used in the court

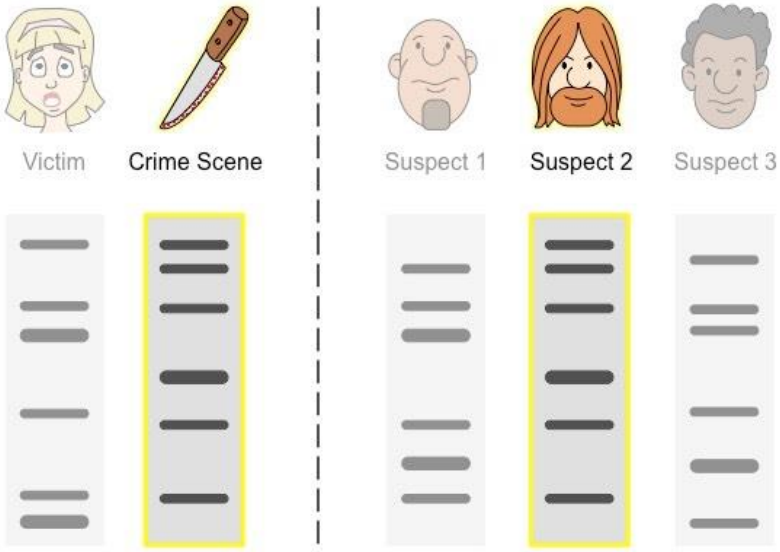
DNA Fingerprinting

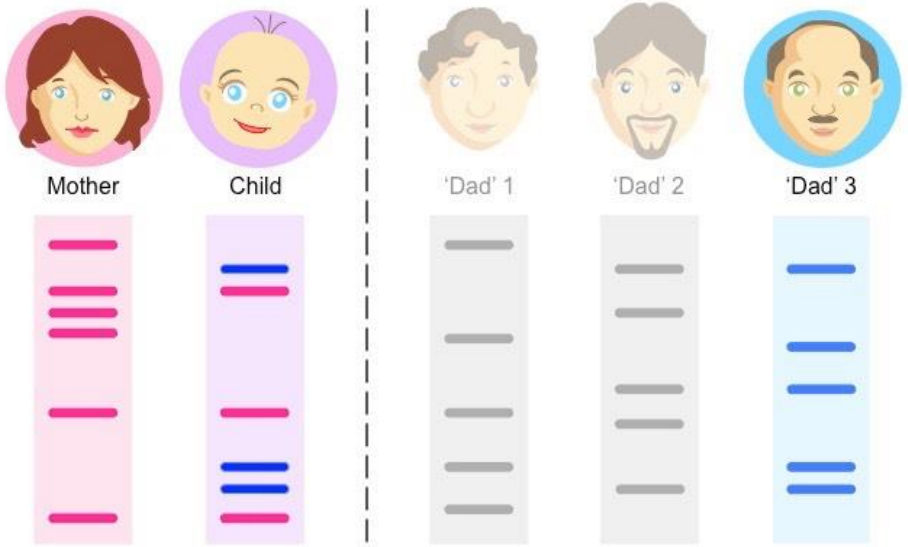
- DNA fingerprinting (DNA profiling or typing): is a technique to identify individuals by features of their DNA

- 99.9% of Human DNA sequences are identical in every person. Only 0.1% of our DNA sequences that make us unique
- DNA profiles: are small set of DNA variations (fragments) that are very likely to be different in all unrelated individuals **(except identical twins)**

- DNA fingerprinting is used in criminal investigations and paternity or parentage testing

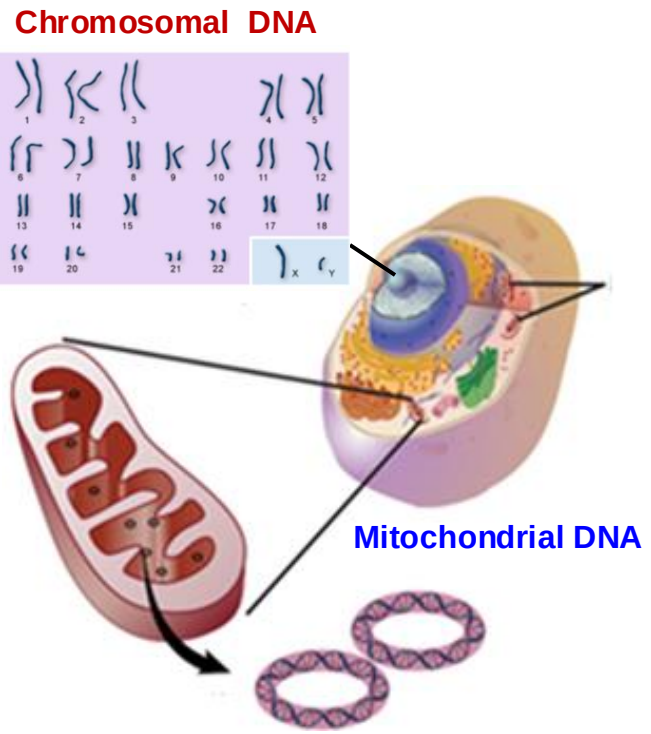
DNA Fingerprinting

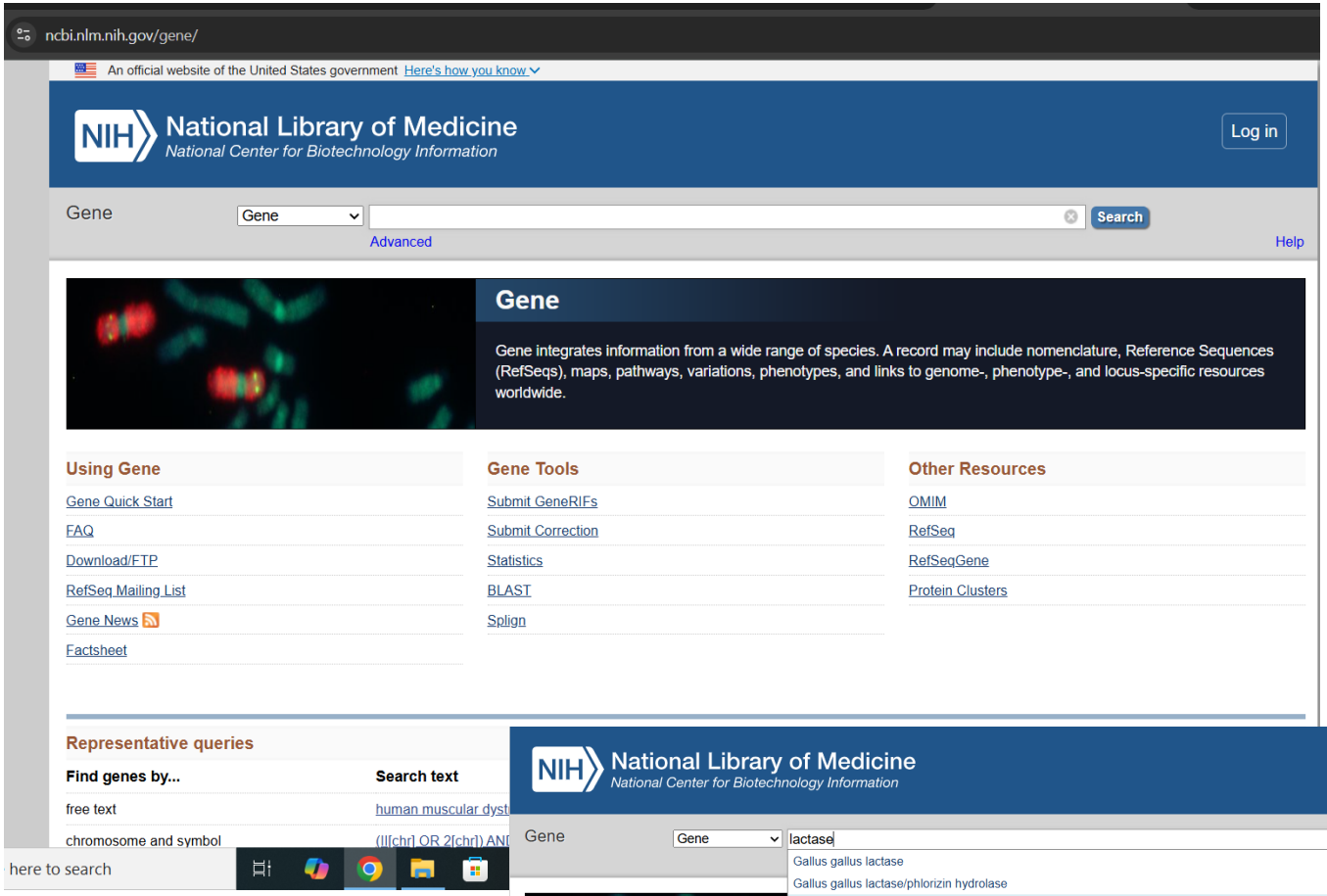




Types of DNA

- We need cells to isolate DNA. In eukaryotes (multicellular) like human (*Homo sapiens*), **genomic DNA or chromosomal DNA** is found inside the nucleus of every cell (exceptions like the anucleated **RBCs**). Also, DNA is found within mitochondria **(mitochondrial DNA),** the energy producing organelles
- Mitochondrial DNA is 16Kbp small circular dsDNA (2-10 copies) codes for 37 genes (inherited from mother)
- Genomic DNA contains genetic data of organism with size of 300 million bp and codes for 25,000 genes arranged on 23 chromosomes (haploid cell, gametes/sex cells)

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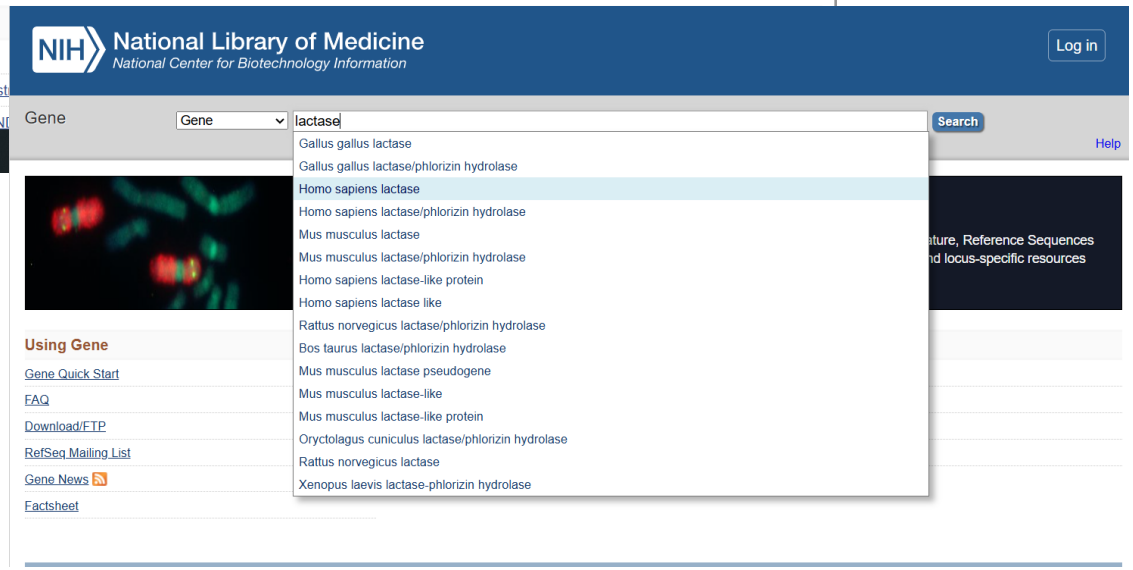
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Mus musculus lactase/phlorizin hydrolase
Homo sapiens lactase-like protein
Homo sapiens lactase like
Rattus norvegicus lactase/phlorizin hydrolase
Bos taurus lactase/phlorizin hydrolase
Mus musculus lactase pseudogene
Mus musculus lactase-like
Mus musculus lactase-like protein
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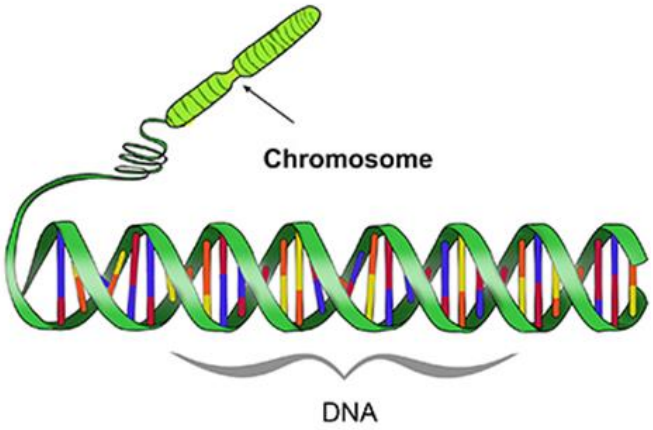
Types of DNA

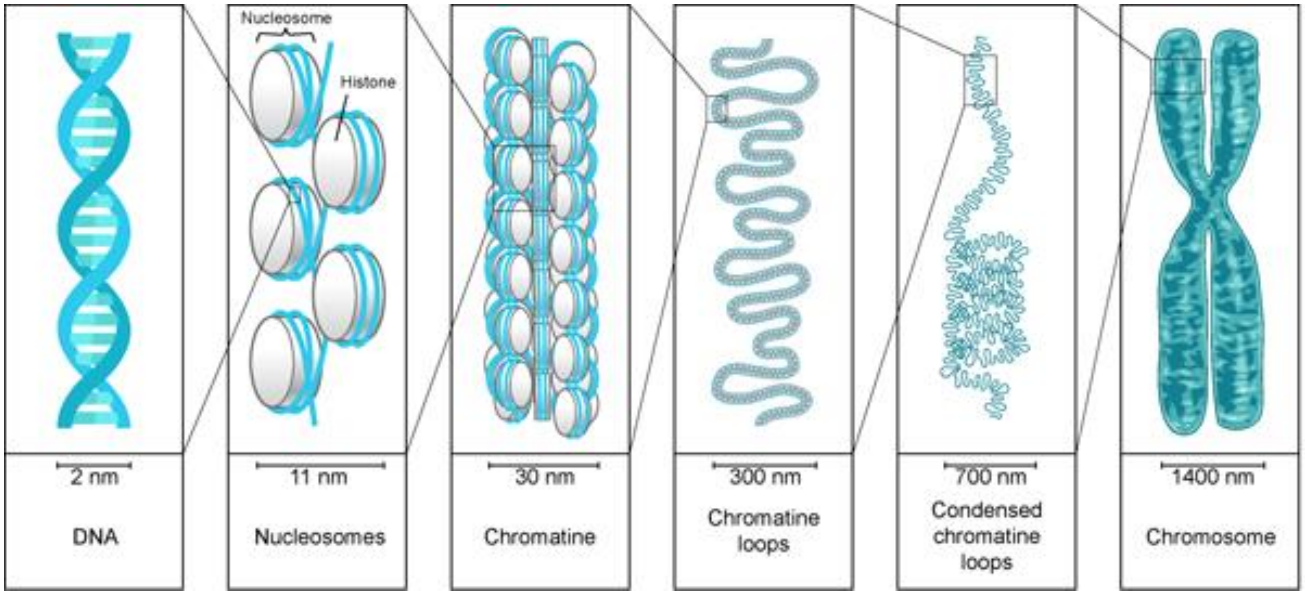
- Somatic cell (diploid) consists of 23 pairs of chromosomes (46 chromosomes) code for different 25,000 genes (duplicate, one copy from each parent) with total size of 600 million bp
- Genomic DNA is inherited from both parents (half from the mother and half from the father)
- In diploid cell, if DNA is laid out end to end, the total length will be approximately 2 meters
- We have enough DNA to make 70 round trips from earth to sun
- Human genome project (1990-2003)
- 98.5% of human genome is junk DNA or non-coding DNA (1.5% only is gene-coding DNA)



DNA Packaging

- DNA is arranged into three different levels of organization:

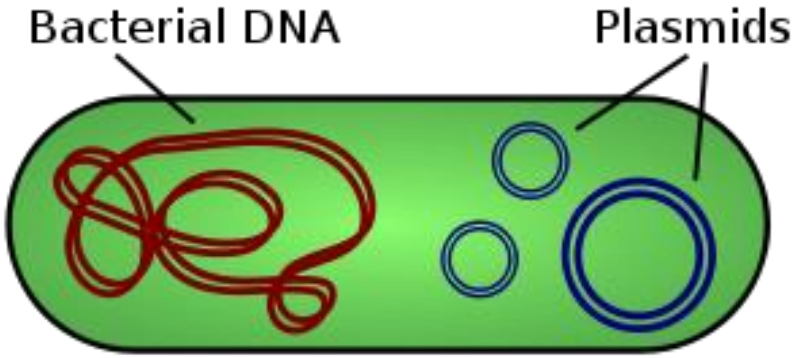
1. Nucleosome: DNA is wrapped around histone proteins (octamer)
2. Chromatin fibers: nucleosomes coil and form loops
3. Chromosomes: chromatin fiber is further condensed



Bacterial DNA

- In prokaryotes (unicellular) like bacterial cells, there are a single circular chromosome (bacterial DNA) found in the cytoplasm (with size of 5 million bp)
- Extrachromosomal DNA called plasmid also found in the cytoplasm:

1. Small circular dsDNA (e.g. A.B resistance genes)
2. Replicate independently of chromosomal DNA
3. Hundred of copies of single plasmid
4. Synthetically modified plasmids are used as vectors in genetic engineering



DNA Extraction Kits

- Obtaining high quality ***(purity)*** and quantity ***(concentration/amount)*** of ***intact DNA*** **(not degraded)** is often the first and most critical step in many fundamental molecular biology applications, such as: DNA cloning, gene sequencing, PCR, DNA fingerprinting …… etc

- Different kinds of kits are available from different companies: Qiagen, Invitrogen, Promega and Bio Basic Inc.

DNA Extraction Kits

QIAGEN®

BIO BASIC INC.

invitrogen™
by life technologies™

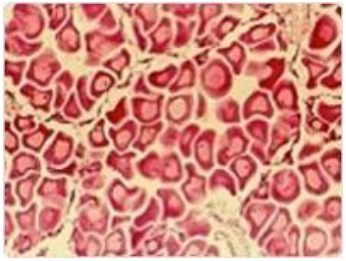
Promega

DNA Isolation from Various Samples

- DNA can be extracted from variety of samples:

1. Human Samples

Forensic Sample



Tissues

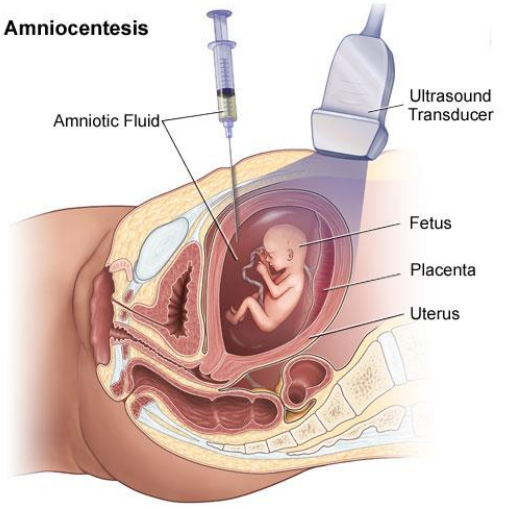
Cells

Buccal swab

Embryonic or Fetal biopsy for Prenatal genetic testing

Human Samples

- Genomic material can be extracted from different human samples like cells or tissues (extraction from the liver tissue is easier than spleen or brain tissues depending on the biochemical nature of that tissue)
- Buccal swab (cheek cells) is the most convenient and easiest way (non-invasive) because the squamous epithelial cells of buccal mucosa divide every 24 hours
- Fetal sample (screening/diagnostic): cells of the placenta (in late first trimester) or from amniotic fluid (in second trimester) to do chromosomal analysis

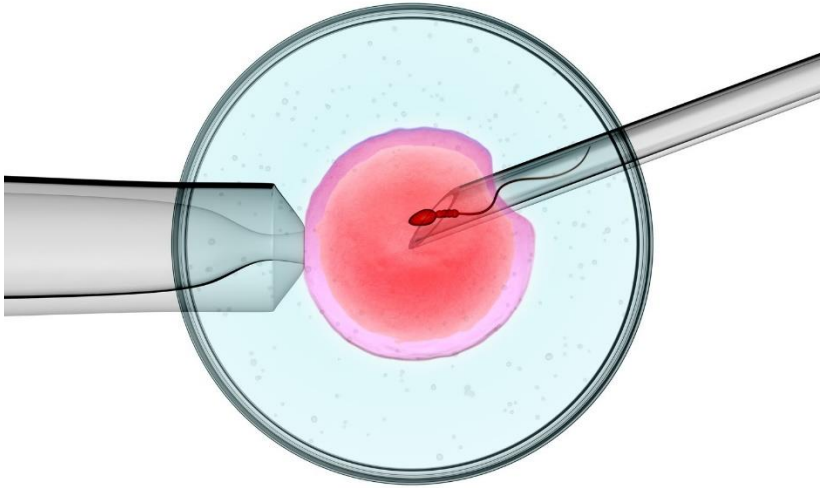


DNA Isolation from Various Samples

- In Vitro Fertilization (IVF) technique

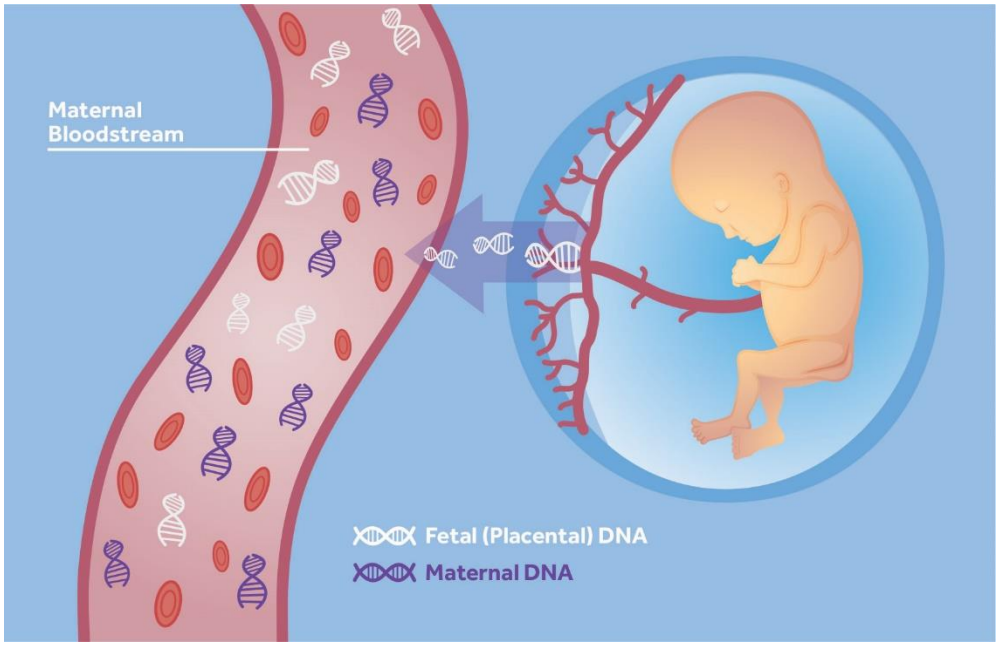
Preimplantation Genetic Diagnosis (PGD) test

Three days embryo biopsy
for PGD test



DNA Isolation from Various Samples

- Non-invasive prenatal testing (NIPT) is a test to determine the risk that the fetus will be born with certain abnormalities like Down syndrome (trisomy 21)
- A blood sample from pregnant woman is taken at the beginning of week 10
- Free DNA molecules from placenta and mother are circulating in mother's blood
- Placental DNA is identical to fetal DNA

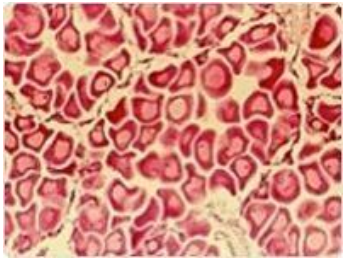


DNA Isolation from Various Samples

- DNA can be extracted from variety of samples:

1. Human Samples

Forensic Sample



Tissues

Blood

Cells

Buccal swab

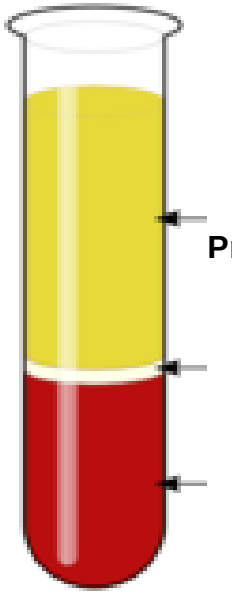
Embryonic or Fetal biopsy for Prenatal genetic testing

Human Samples

- In vitro fertilization (IVF): is a technique of doing fertilization of eggs in the lab.
- One cell sample can be taken from the zygote to perform genetic testing called preimplantation genetic diagnosis (PGD).
- Blood sample is more common although RBCs are anucleated
- After centrifugation of the sample, we take the buffy coat layer containing the WBCs

Blood Sample

centrifugation



Plasma (55% of total blood)
Proteins, electrolytes, clotting factors, hormones , etc…

Buffy coat (<1% of total blood)
contains WBC's

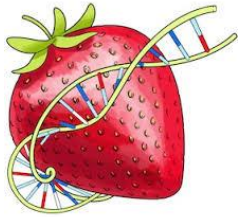
RBC's (45% of total blood)

Human Samples

- In vitro fertilization (IVF): is a technique of doing fertilization of eggs in the lab.
- A one cell sample can be taken from the zygote for genetic testing
- Blood sample is more common although RBCs are anucleated
- After centrifugation of the sample, we take the buffy coat layer containing the WBCs
- Nowadays, there are kits available for extraction of DNA from whole blood sample

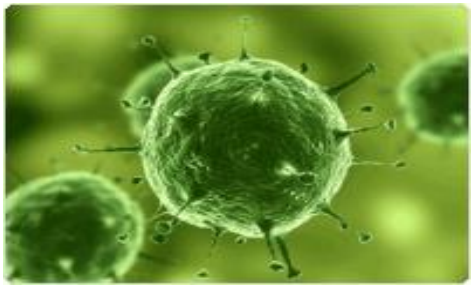
DNA Isolation from Various Samples

2. **Animal cells/tissues**
3. **Plant material (e.g. banana and strawberry)**

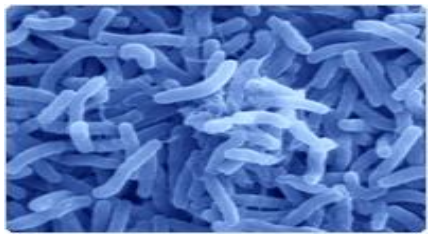


4. **Viral and Bacterial cells**

- Nasal swab like COVID-19
- Oral swab (throat)
- Blood sample (serum)
- Stool sample



Viral cells

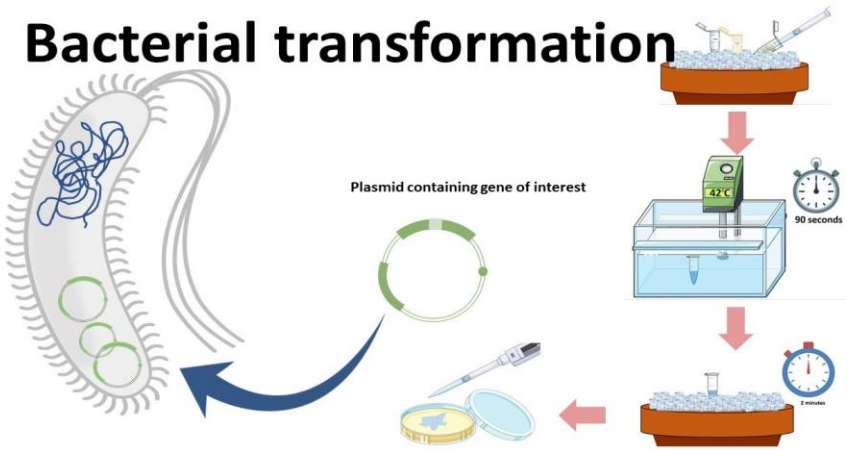


Bacterial cells

DNA Isolation from Various Samples

5. Plasmid DNA (containing the gene of interest)

- After transformation of bacterial cells (competent cells), the amplified plasmid is extracted using different types of kits



  - Miniprep (50-100 µg) kits
  - Midiprep (100-350 µg) kits
  - Maxiprep (500-850 µg) kits

- The purified plasmid is stored as stock at -20 ℃