



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



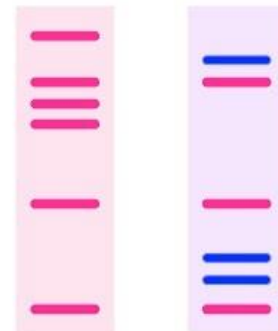
DNA Fingerprinting



- 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

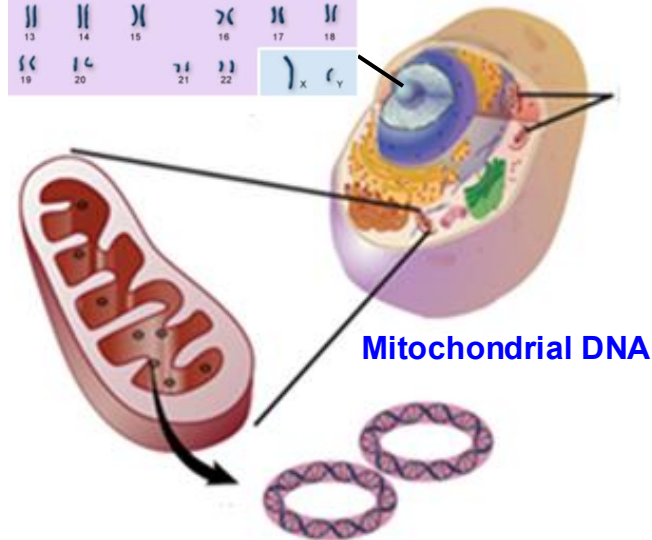
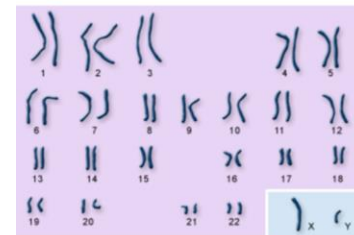



Types of DNA



- 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)

Chromosomal DNA





Gene

Gene integrates information from a wide range of species. A record may include nomenclature, Reference Sequences (RefSeqs), maps, pathways, variations, phenotypes, and links to genome-, phenotype-, and locus-specific resources worldwide.

Using Gene Gene Quick Start FAQ Download/FTP RefSeq Mailing List Gene News Factsheet	Gene Tools Submit GeneRIFs Submit Correction Statistics BLAST Splign	Other Resources OMIM RefSeq RefSeqGene Protein Clusters
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Representative queries

Find genes by... Search text

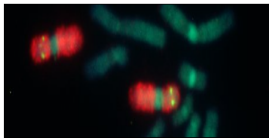
free text [human muscular dyst](#)

chromosome and symbol [\(11|chr1_OR_2|chr1\) ANI](#)

here to search



Gene Search



Using Gene

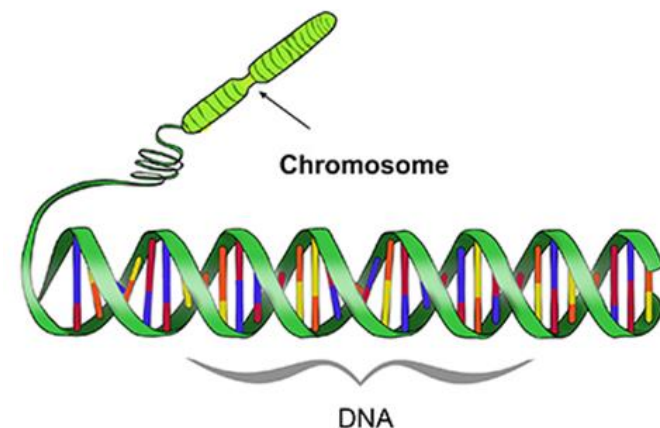
[Gene Quick Start](#)
[FAQ](#)
[Download/FTP](#)
[RefSeq Mailing List](#)
[Gene News](#)
[Factsheet](#)

- Gallus gallus lactase
- Gallus gallus lactase/phlorizin hydrolase
- Homo sapiens lactase**
- Homo sapiens lactase/phlorizin hydrolase
- Mus musculus lactase
- 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
- Oryctolagus cuniculus lactase/phlorizin hydrolase
- Rattus norvegicus lactase
- Xenopus laevis lactase-phlorizin hydrolase

Types of DNA



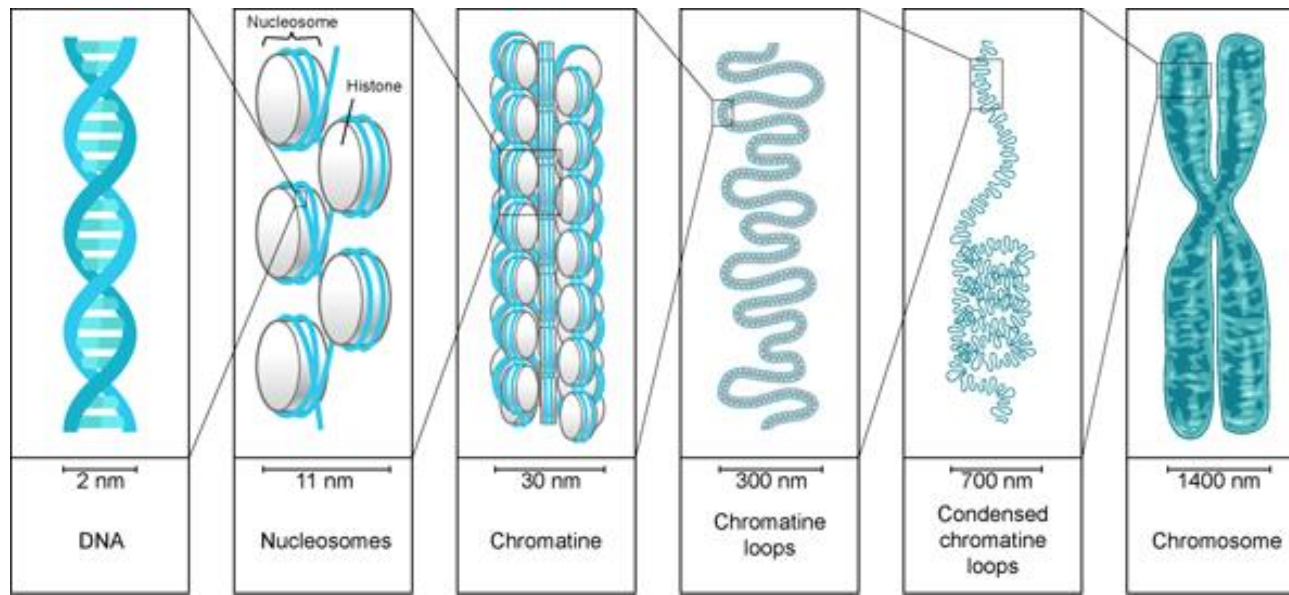
- Somatic cell (diploid) consists of 23 pairs of chromosomes (46 chromosomes) code for different 25,000 genes (duplicate, one copy from each parent) with total size of 600 million bp
- Genomic DNA is inherited from both parents (half from the mother and half from the father)
- In diploid cell, if DNA 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



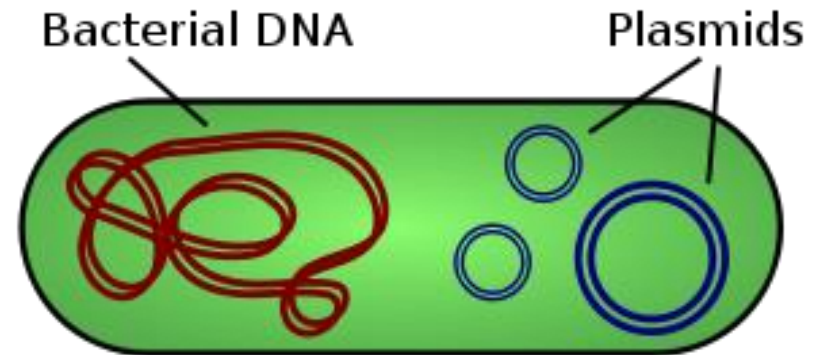
- DNA is arranged into three different levels of organization:
 1. Nucleosome: DNA is wrapped around histone proteins (octamer) like beads on string.
 2. Chromatin fibers: nucleosomes coil and form loops
 3. Chromosomes: chromatin fiber is further condensed



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

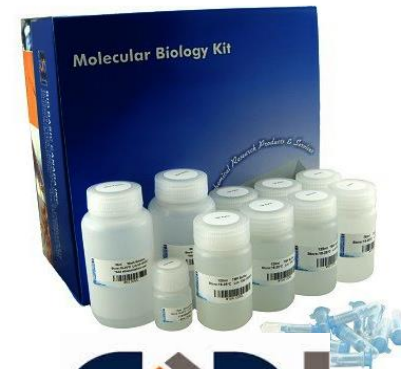


DNA Extraction Kits



- 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



DNA Isolation from Various Samples

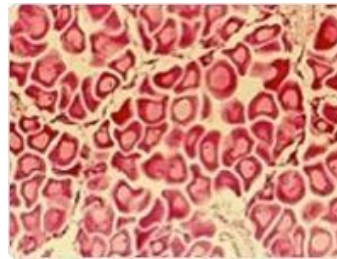


- DNA can be extracted from variety of samples:

1. Human Samples



Forensic Sample



Tissues



Cells



Buccal swab

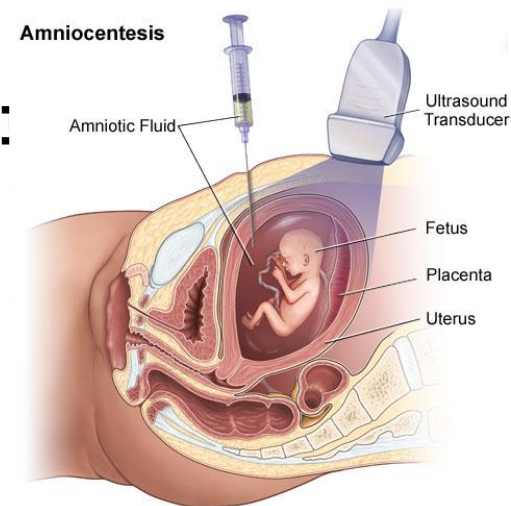


**Embryonic or Fetal biopsy for
Prenatal genetic testing**

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

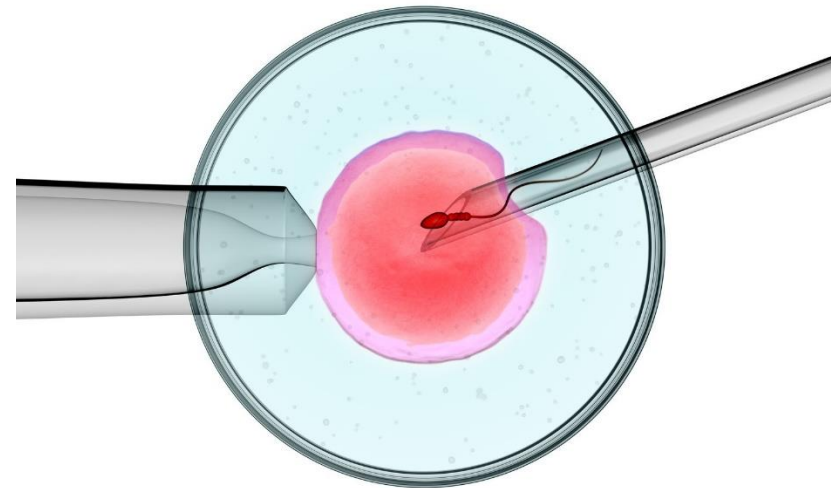


- In Vitro Fertilization (IVF) technique

Preimplantation Genetic Diagnosis (PGD) test



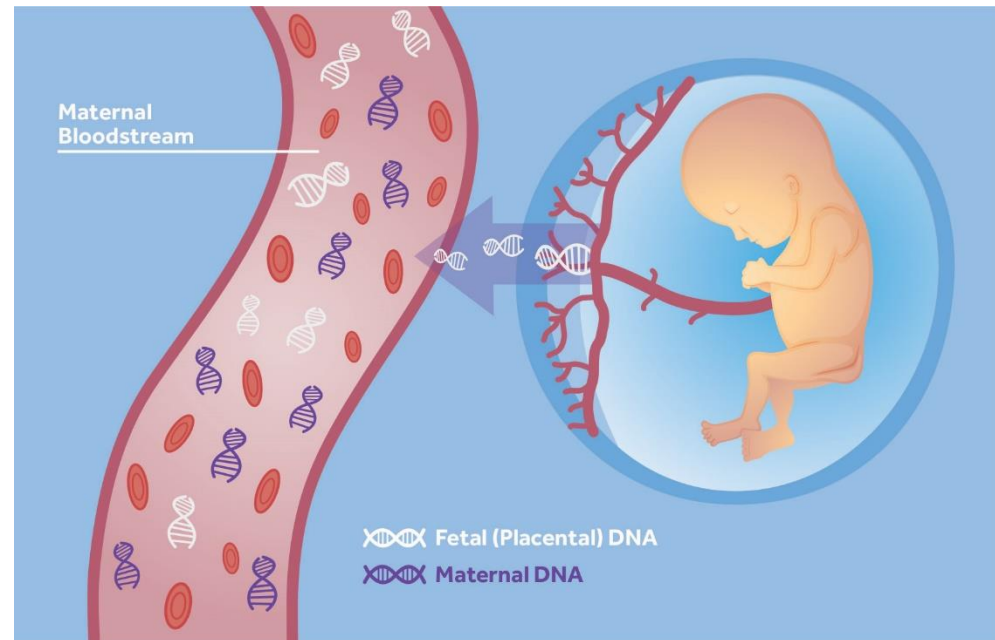
Three days embryo biopsy
for PGD test



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-floating DNA molecules from placenta and mother are circulating in mother's blood
- Placental DNA is identical to fetal DNA



DNA Isolation from Various Samples

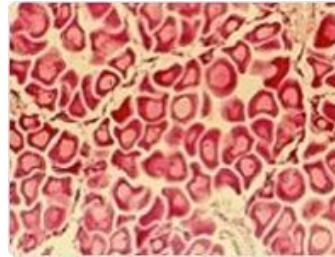


- DNA can be extracted from variety of samples:

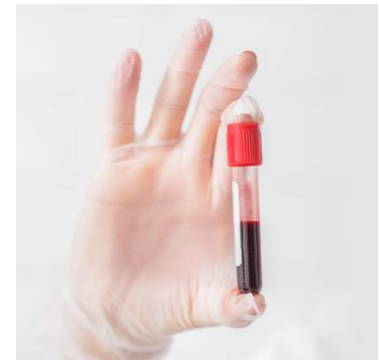
1. Human Samples



Forensic Sample



Tissues



Blood



Cells



Buccal swab



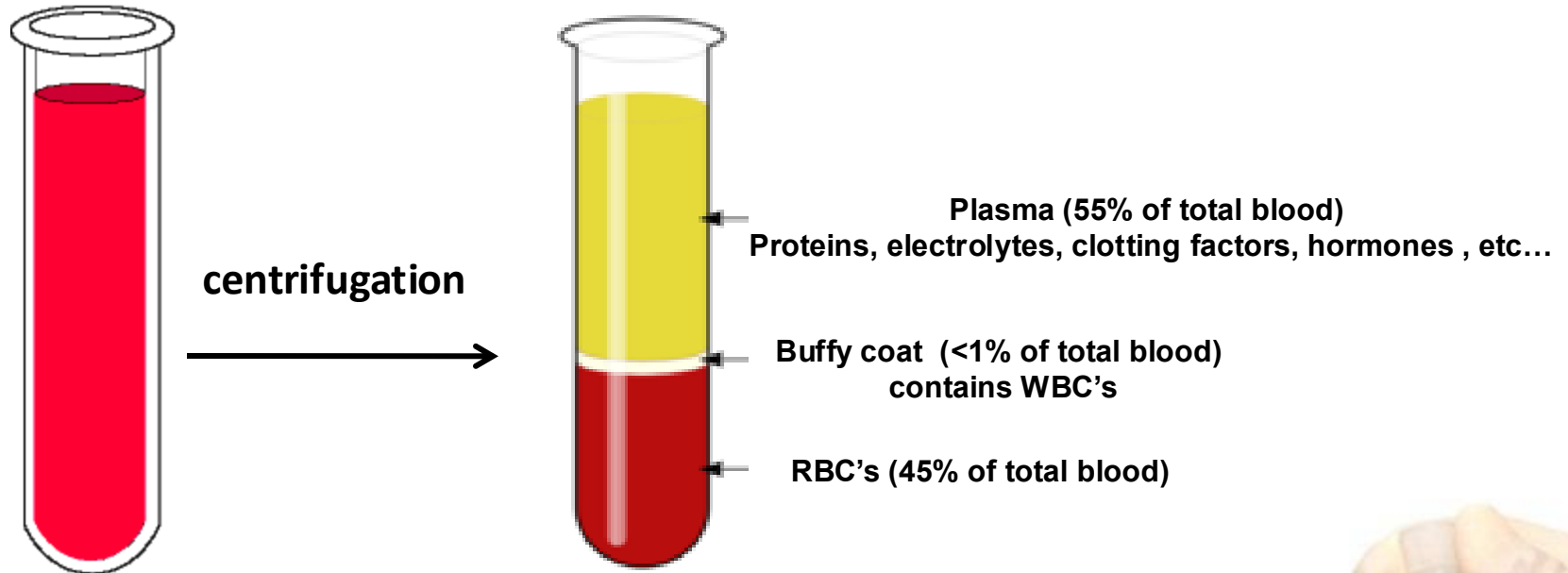
**Embryonic or Fetal biopsy for
Prenatal genetic testing**

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



Human Samples



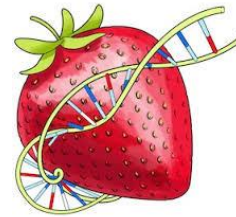
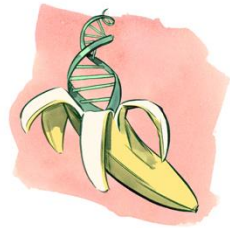
- In vitro fertilization (IVF): is a technique of doing fertilization of eggs in the lab.
- A one cell sample can be taken from the zygote for genetic testing
- Blood sample is more common although RBCs are anucleated
- After centrifugation of the sample, we take the buffy coat layer containing the WBCs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

DNA Isolation from Various Samples



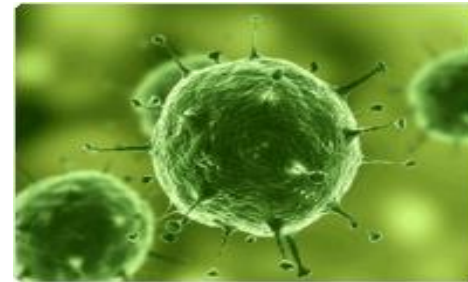
2. Animal cells/tissues

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°C

