

DNA Extraction... at the kitchen table??

Lee Ellen Brunson-Sicilia, MHS, MLS(ASCP)^{CM}

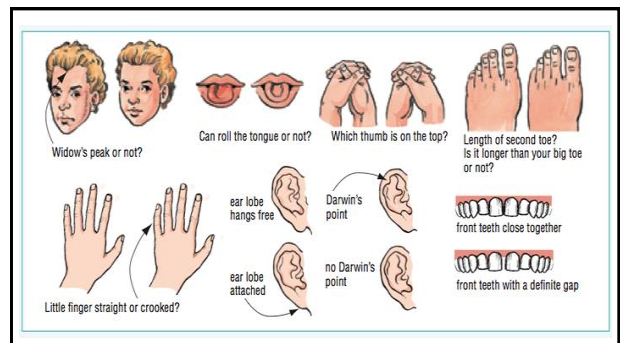


Objectives

1. Review DNA and its function.
2. Discuss various types of genetic testing.
3. Discuss the purpose of DNA extraction.
4. Discuss the basic steps in DNA extraction.

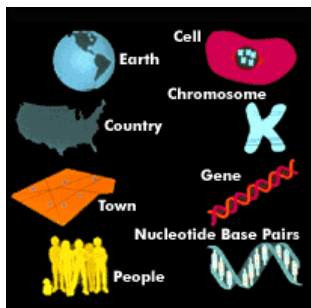
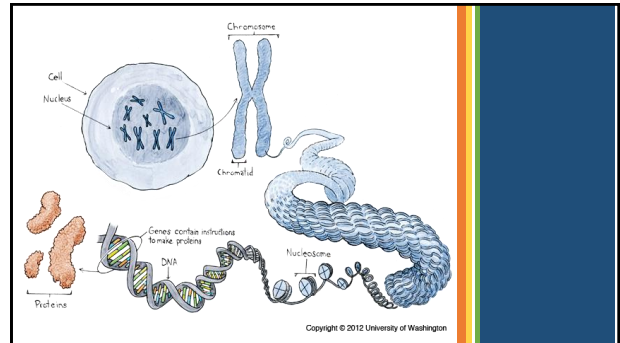
DNA

- Deoxyribonucleic acid
- "Blueprint of life"
- Contains instructions that guide the development and function of all living organisms and many viruses
- Instructions divided into segments called genes, get expressed as various proteins
- Genes determine various traits
 - Visible: hair color, height
 - Not visible: blood type, disease status



DNA, cont.

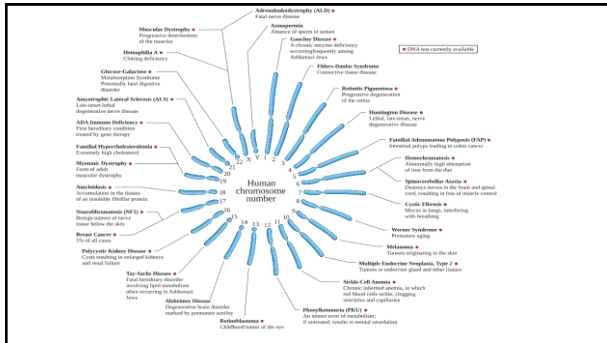
- Two-stranded molecule that is wrapped tightly around proteins called histones
- Packaged into chromosomes
- Located in the nucleus of all human cells, except RBCs
- Must be isolated from cell and purified to be tested



DNA testing

- As we learn more and more about genetic defects that cause disease, more and more types of genetic tests have been developed.
- Sensitive and specific
- Diseases have been now been associated with virtually every human chromosome.





Chromosome-associated diseases

- CHR 4: Huntington Disease
- CHR 6: Hemochromatosis
- CHR 7: Cystic fibrosis
- CHR 9: Melanoma
- CHR 11: Sickle cell anemia
- CHR 15: Tay-Sachs Disease
- CHR 16: Polycystic Kidney Disease
- CHR 17: Breast cancer
- CHR 19: Familial hypercholesterolemia
- CHR X: Muscular dystrophy
- CHR X: Hemophilia A

How is DNA testing used?

- Diagnostic testing
- Predictive genetic tests
- Carrier testing
- Prenatal testing
- Newborn screening
- Pharmacogenomic testing
- Pathogen identification
- Forensic DNA testing
- Direct-to-consumer testing (DTC)
- Research genetic testing

Diagnostic testing

- Used to precisely identify the disease that is making a person ill.
- Results of a diagnostic test may help patients make choices about how to treat or manage their health.

Predictive genetic tests

- Used to find gene changes that increase a person's likelihood of developing diseases.
- Results of these tests provide you with information about your risk of developing a specific disease.
- Such information may be useful in decisions about your lifestyle and healthcare.

Carrier testing

- Used to find people who "carry" a change in a gene that is linked to disease.
- Carriers often show no signs of the disease but have the ability to pass on the gene change to their children, who may develop the disease or become carriers themselves.
- Usually offered to people who have a family history of a specific inherited disease or who belong to certain ethnic groups that have a higher risk of specific inherited diseases

Prenatal testing

- Offered during pregnancy to help identify fetuses that have certain diseases.
- May provide information about pregnancy risk or viability.

Newborn screening

- Used to test infants one or two days after birth to find out if they have certain diseases known to cause problems with health and development.

Pharmacogenomic testing

- Gives information about how certain medicines are processed by an individual's body.
- This type of testing can help healthcare providers choose the medicines that work best with a patient's genetic makeup genetic makeup.

Pathogen identification

- Genetic material from many microbes may be tested to confirm identity.
- Bacteria, viruses, parasites, fungi
- Provides valuable information about best treatment to be used.

Forensic DNA testing

- Powerful tool used in both convicting and exonerating criminal suspects in both current and cold cases.
- Highly reliable results because each person's genetic makeup is unique.

Direct-to-consumer (DTC) testing

- Genetic testing marketed directly to customers via advertisements, can be purchased online or in stores
- Genetic information without involving healthcare provider or health insurance company
- Use genetic variations to make predictions about health, provide information about common traits, and offer clues about a person's ancestry

Research genetic testing

- Used to learn more about the contributions of genes to health and to disease.
- Sometimes the results may not be directly helpful to participants, but they may benefit others by helping researchers expand their understanding of the human body, health, and disease.
- Often a component of clinical trials for new treatments

How DNA testing works...



The most fun part of DNA testing...extracting the DNA!

Purposes of DNA extraction for lab testing

- To separate DNA-containing cells of interest from their environment and then separate DNA from those cells and their other components
- To obtain useful samples of DNA that are free from contaminants that could impede testing
- To isolate DNA of sufficient quantity and quality to perform successful analysis

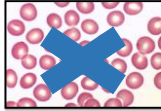


Specimens for testing



• Can use blood or virtually any tissue specimen containing nucleated cells

- Whole blood
- Bone Marrow
- Solid tissue
- Serum/plasma
- Buccal swabs
- Amniocytes
- Urine, Feces, Endocervical swabs
- Sputum, saliva
- Pus



Basic Steps in DNA Extraction

1. Pretreatment of cells, if necessary
2. Lyse nucleated cells
3. Digest proteins
4. Purify DNA
5. Precipitate DNA
6. Resuspend in buffer



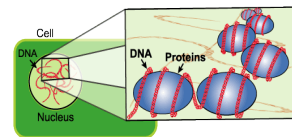
1. Pretreatment

- Blood and bone marrow – remove RBCs
- Tissue – digest wax if paraffin-embedded, grind or mince
- Microorganisms – digest cell walls



2. Lysis

- Detergent - SDS
- Liberates contents of the cells: proteins and nucleic acid
- Separates histone proteins from DNA



3. Digest proteins

- Enzymatic degradation by incubation with an enzyme – protease/proteinase
- Gets rid of all protein and leaves DNA intact



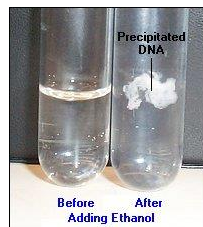
4. Purification

- Protein breakdown products are separated from free DNA
- **AKA protein precipitation**
- Can be done using a solvent (liquid phase extraction) or a filter column (solid phase extraction)



5. Precipitation

- Alcohol used to concentrate DNA
- Usually cold ethanol or isopropanol
- DNA insoluble in alcohol
- Suspension centrifuged, alcohol discarded

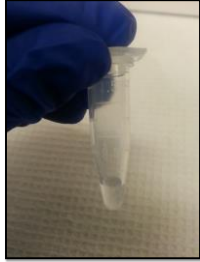


Precipitated DNA

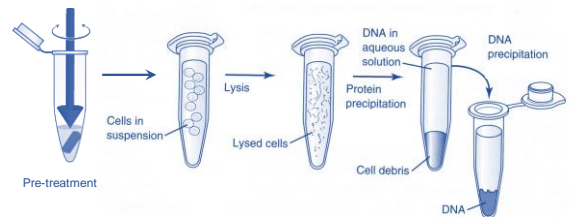


6. Resuspension

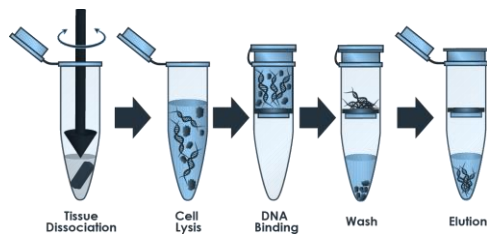
- Concentrated DNA pellet resuspended in buffer
- Concentration measured and adjusted prior to testing
- Purity also assessed



Liquid phase extraction

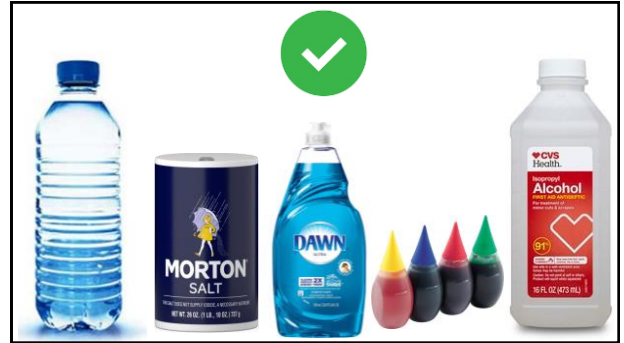


Solid Phase Extraction



New challenges: POC extraction

- DNA extraction at point-of-care
- One device performs DNA extraction, amplification, and detection
- Two most widely used POC extraction methods
 - Membrane separation
 - Magnetic bead separation
- Currently most widely used for pathogen detection
 - Viruses
 - Bacteria
 - Greater amounts of nucleic acid present for isolation



Extract your own DNA at home!

- From cheek cells



1. Make salt solution

- Add about 2 tablespoons of water to a small, clean glass or cup.
 - Clear works best.
- Add about 1/8 teaspoon of table salt.
- Stir gently until salt is dissolved.



2. Swish!

- Swish the liquid around in your mouth as you would mouthwash, for about a minute.
- **DO NOT SWALLOW!**
- GENTLY graze your tongue and insides of cheeks with your teeth to help cells slough off.
- After one minute of swishing, spit the salt solution back into the cup.



3. Add detergent and color

- Add about ½ teaspoon of dish soap and two drops of food coloring to the cup containing the salt water-cheek cell solution.
- Stir gently and avoid creating bubbles.



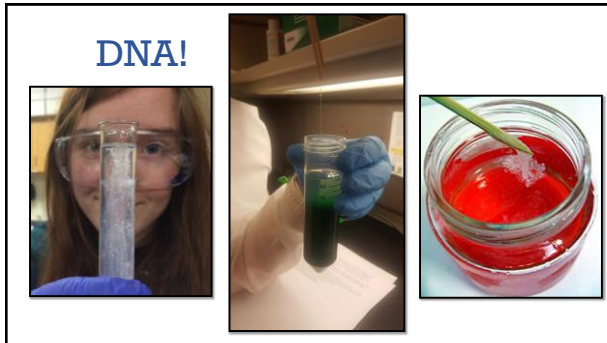
4. Add ice-cold alcohol

- Tilt the cup slightly and add about a tablespoon of very cold rubbing alcohol slowly by letting it drip down the inside of the cup, so the two liquids do not mix.
 - Put alcohol in the freezer ahead of time.
 - The higher the % of alcohol, the better (90%+).
- Upper alcohol layer should remain colorless and bottom layer should be the color of the food coloring.



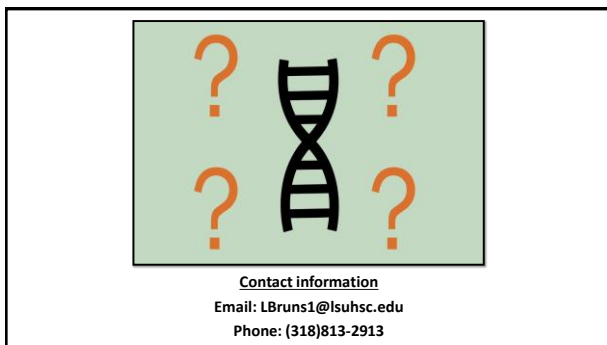
5. Wait, stir, and watch!

- DNA will begin to condense in the alcohol layer.
- It will appear cloudy and feel slimy to the touch.
- This may take a few minutes to occur.
- With gentle stirring motions, draw the DNA up toward the top of the cup with a wooden stir stick, toothpick or a straw.
- The clear "strands" that may be drawn upward are DNA!



What just happened?

1. Salt and water AND FRICTION allow collection of DNA-containing cells.
2. Detergent breaks cells open; DNA is released.
3. Alcohol causes DNA to form a semi-solid clump/strand.



Don't forget to do your evaluation!

