DNA Extraction… at the kitchen table??

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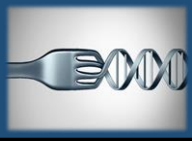
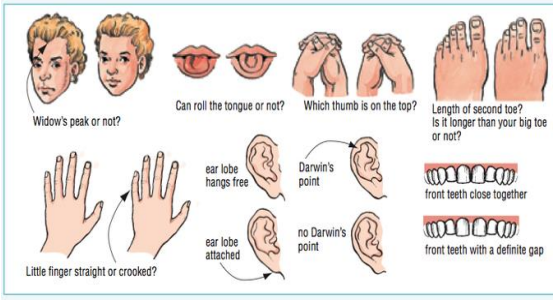


Objectives

1. Review DNA and its function.
2. Discuss the purpose of DNA extraction.
3. Discuss the basic steps in DNA extraction.

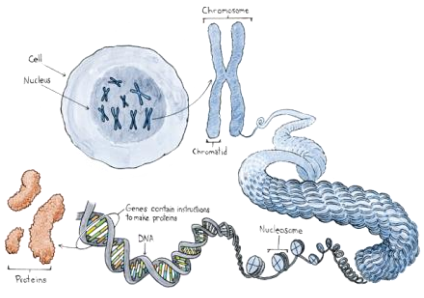
DNA

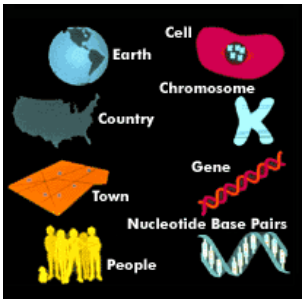
- Deoxyribonucleic acid
- "Blueprint of life"
- Contains instructions that guide the development and function of all living organisms and many viruses
- Instructions divided into segments called genes, get expressed as various proteins
- Genes determine various traits
  - Visible: hair color, height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

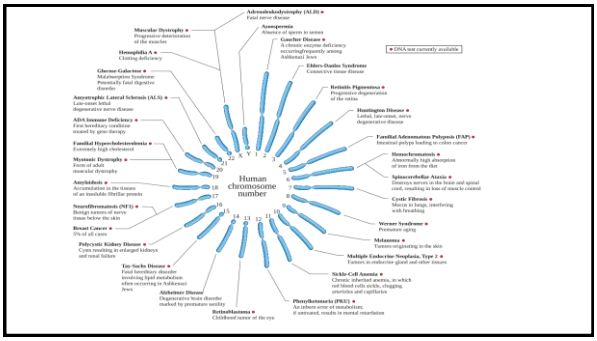


DNA, cont.

- 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







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: Hemophila A

How is DNA testing used?

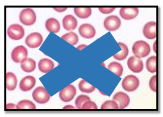
- Diagnostic testing
- Predictive genetic tests
- Carrier testing
- Prenatal testing
- Newborn screening
- Pharmacogenomic testing
- Pathogen identification
- Forensics
- Direct-to-consumer testing (DTC)
- Research genetic testing

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

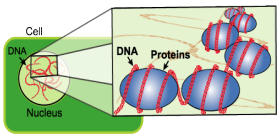
1. Pretreatment of cells, if necessary
2. Lyse nucleated cells
3. Digest proteins
4. Purify DNA
5. Precipitate DNA
6. Resuspend in buffer

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

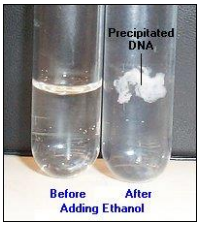
- Enzymatic degradation by incubation with an enzyme – protease/proteinase
- Gets rid of all protein and leaves DNA intact

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

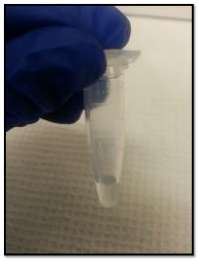
- Alcohol used to concentrate DNA
- Usually cold ethanol or isopropanol
- DNA insoluble in alcohol
- Suspension centrifuged, alcohol discarded



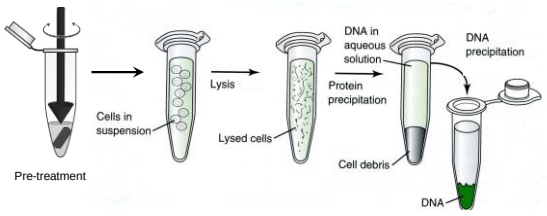
Precipitated DNA

6. Resuspension

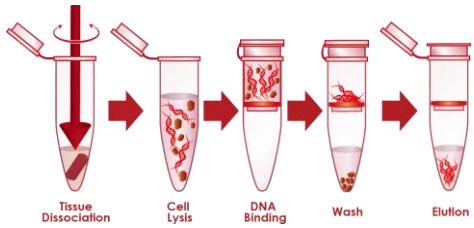
- Concentrated DNA pellet resuspended in buffer
- Concentration measured and adjusted prior to testing
- Purity also assessed

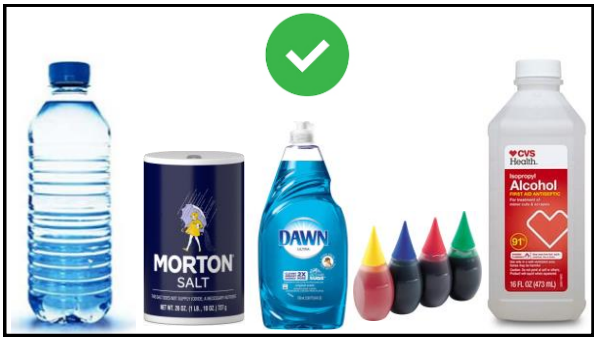


Liquid phase extraction



Solid Phase Extraction





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.

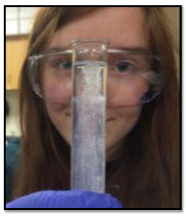
4. Add ice-cold alcohol

- 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!

DNA!



What just happened?

1. Salt and water 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.

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