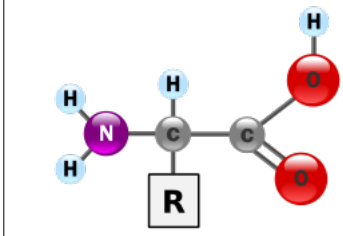
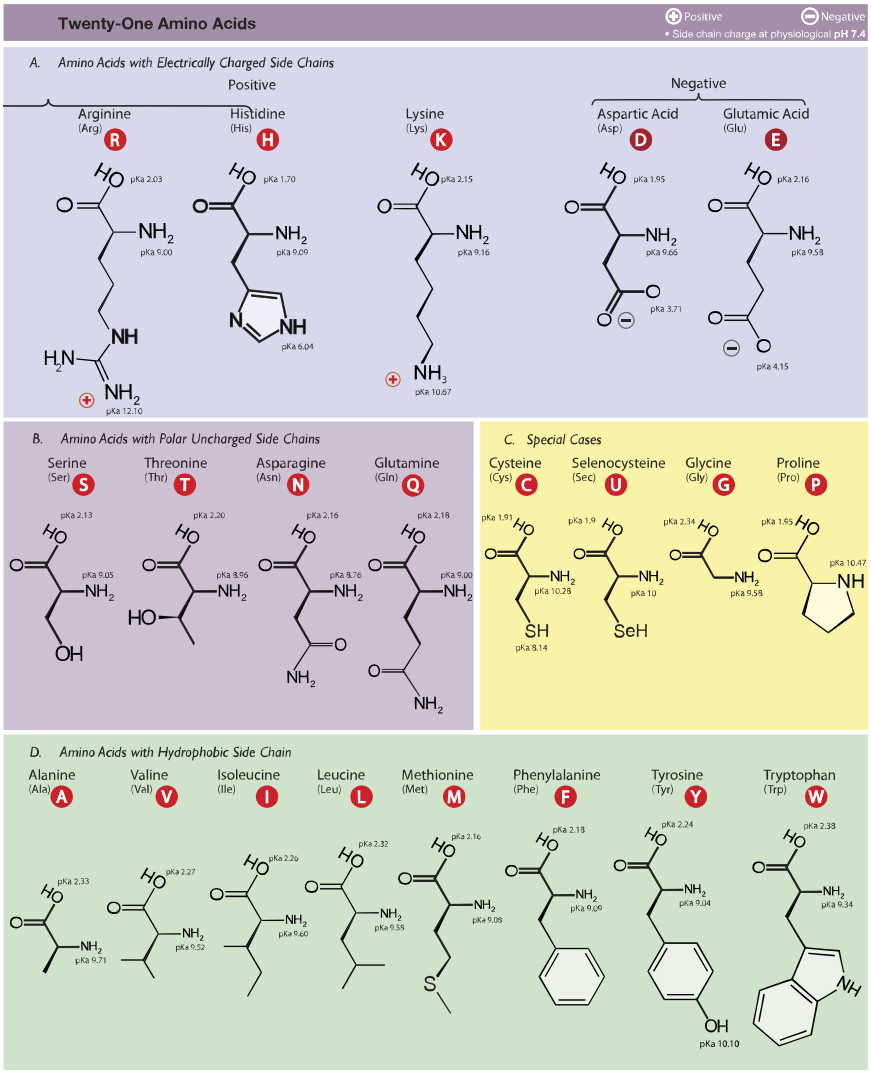
Proteins are polymers of Amino Acids



Credit: By GYassineMrabetTalk [Public domain]

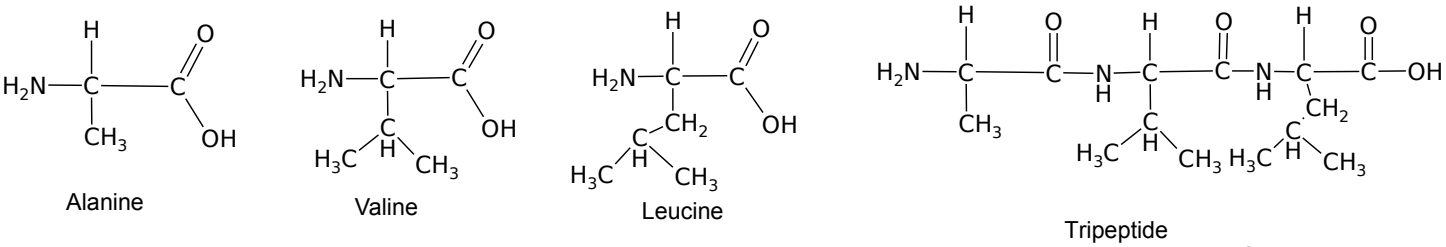
Proteins provide much of the structural and functional capacity of cells. Proteins are composed of monomers called amino acids. **Amino Acids** are hydrocarbons that have an **amino group** (-NH_2) and an acidic **carboxyl group** (-COOH).The R group represents a hydrocarbon chain with a modification that alters the properties of the amino acid. 20 universal amino acids are used to construct proteins. The variation in functional groups along the amino acid chain gives rise to the functional diversity of proteins.



20 amino acids and their properties. A 21st amino acid on this table represents the non-universally found selenocysteine. *Credit:By Dancojocari (CC BY-SA 3.0)*

Monomers bond together through a dehydration synthesis reaction between adjacent amino and carboxyl groups to yield a **peptide bond**.

Jeremy Seto jseto@citytech.cuny.edu



Three amino acids bound into a tripeptide.

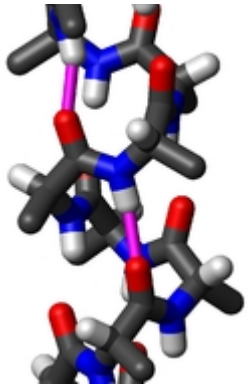
How amino acids interact with each other and the environment

Use the following simulation to test how a polypeptide chain with fold based on the type of solution it is in and the composition of the amino acids.

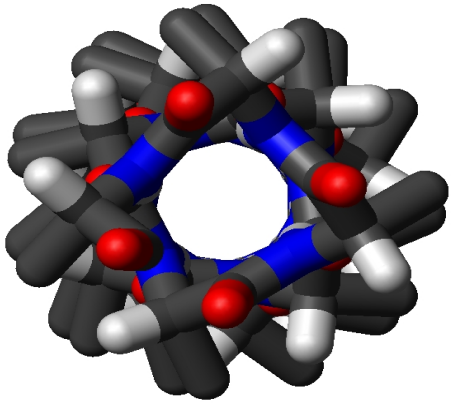
- **Protein Folding Simulation** (CC BY 4.0 Concord Consortium)

Levels of structure

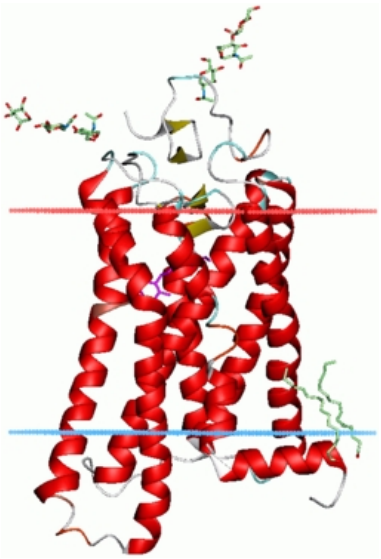
- **Primary Structure (1°)**: The sequence of amino acids read from the Amino or N-terminal end of the molecule to the Carboxyl or C-terminal end
    - Tyr-Cys-Arg-Phe-Leu-Val-....
- **Secondary Structure (2°)**: local three-dimensional structures that form from interactions of amino acids, like hydrogen bonding
    - **Alpha Helix** - coils occurring from the H-bonds between N-H and C=O groups along the backbone of the protein



Side view of α-helix illustrating H-bonds in magenta between carboxyl oxygen (red) and amine nitrogen (blue) Credit: By WillowW (CC-BY-SA-3.0)



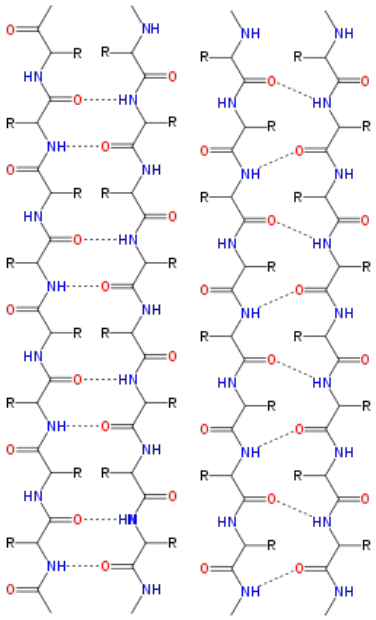
Top-down view of an α-helix
Credit: By WillowW (CC-BY-SA-3.0)



Side view of ribbon diagram of α-helices traversing a membrane.
Credit: By Andrei Lomize (CC-BY-SA-3.0)

- **Beta Sheets** - laterally connected strands or sheets of amino acids occurring from the H-bonds between N-H and C=O groups along the backbone of the protein

Jeremy Seto jseto@citytech.cuny.edu



Credit: Fvasconcellos [Public domain]

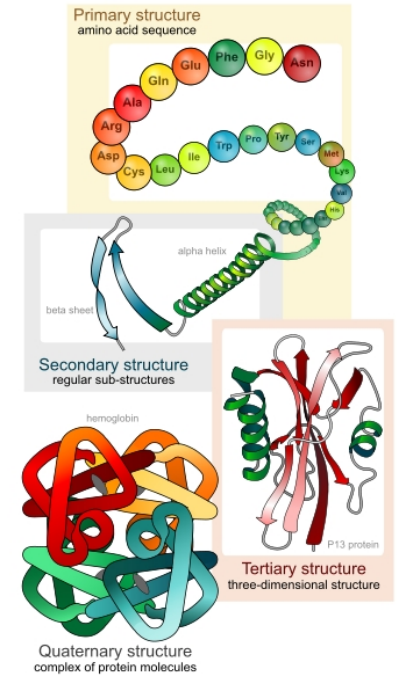
Ribbon diagram of β-sheets

Credit: Xenonblast [Public domain]

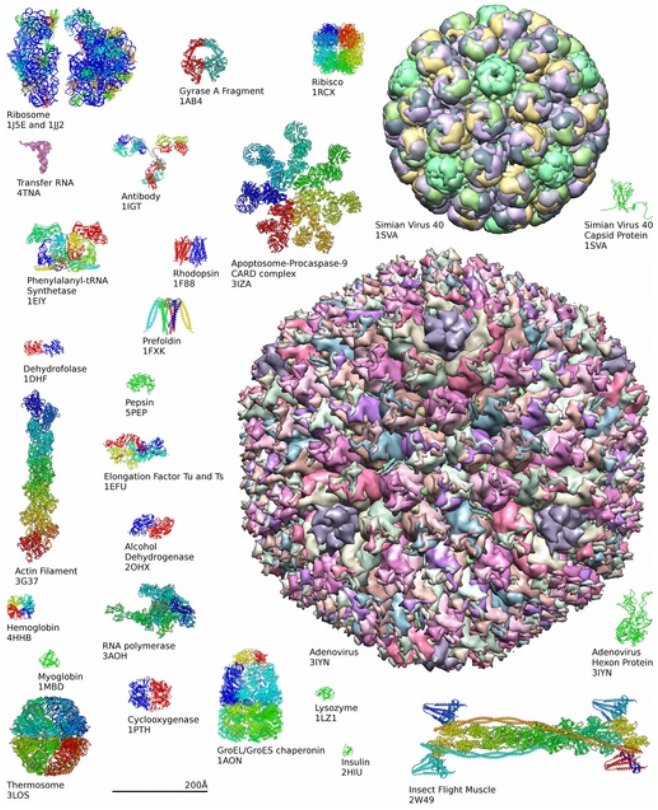
- **Tertiary structure (3°)**: overall 3-D structure of the peptide chain
- **Quaternary structure(4°)**: multimeric protein structure from assembling multiple peptide subunits

Diversity of Proteins

Learn more about complexity of protein structures at the Protein Data Bank



Credit: LadyofHats [Public domain]

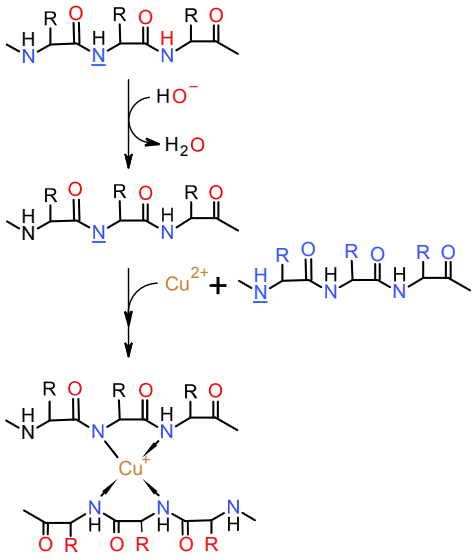


Credit: Axel Griewel (CC-BY-SA 3.)

Jeremy Seto jseto@citytech.cuny.edu

Protein Detection Theory

Proteins can be detected through the use of the **Biuret test**. Specifically, peptide bonds (C-N bonds) in proteins complex with Cu^{2+} in Biuret reagent and produce a violet color. A Cu^{2+} must complex with four to six peptide bonds to produce a color; therefore, free amino acids do not positively react. Long polypeptides (proteins) have many peptide bonds and produce a positive reaction to the reagent. **Biuret reagent** is an alkaline solution of 1% $CuSO_4$, copper sulfate. A violet color is a positive test for the presence of protein, and the intensity of color is proportional to the number of peptide bonds in the solution.



purple complex, λ_{max} = 540 nm

By Ebuxbaum (Own work) [CC BY-SA 3.0]

By Ozone aurora/Philip Evans [CC BY-SA 3.0]

Biuret Test

1. Examine the table below. Indicate if the sample is a negative control, positive control or an experimental.
2. Predict the color change of the solution.
    - Formulate a hypothesis about the components of the experimentals
3. Obtain 6 test tubes and number them 1-6.
4. Add the materials listed in the table.
5. Add 3 drops of Biuret reagent (1.0% $CuSO_4$ with NaOH) to each tube and mix
6. Record the color of the tubes' contents in Table

Jeremy Seto jseto@citytech.cuny.edu

Tube	Solution	Control (+/-) or Experimental	Predicted Color Change	Actual Color Change
1	2 ml urine sample 1			
2	2 ml urine sample 2			
3	2 ml egg albumin			
4	2 ml amino acid solution			
5	2 ml distilled water			
6	2 ml protein solution			

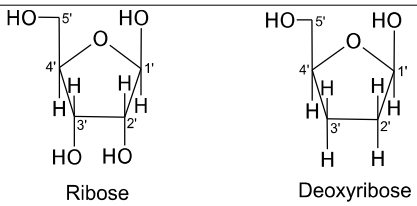
Conclusions about the Urine Samples

Based on the results of the Benedict's test and the Biuret test, can we make any conclusions?

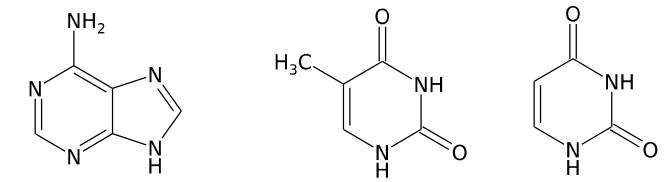
Jeremy Seto jseto@citytech.cuny.edu

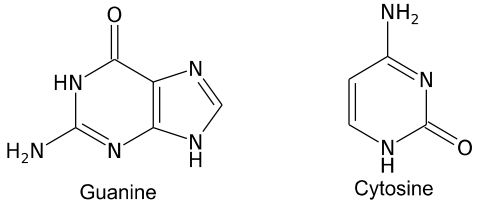
Introduction: NUCLEIC ACIDS

DNA and RNA are nucleic acids and make up the genetic instructions of an organism. Their monomers are called **nucleotides**, which are made up of individual subunits. Nucleotides consist of a 5-Carbon sugar (a **pentose**), a charged phosphate and a **nitrogenous base** (Adenine, Guanine, Thymine, Cytosine or Uracil). Each carbon of the pentose has a position designation from 1 through 5. One major difference between DNA and RNA is that DNA contains deoxyribose, and RNA contains ribose. The discriminating feature between these pentoses is at the 2' position where a hydroxyl group in ribose is substituted with a hydrogen.



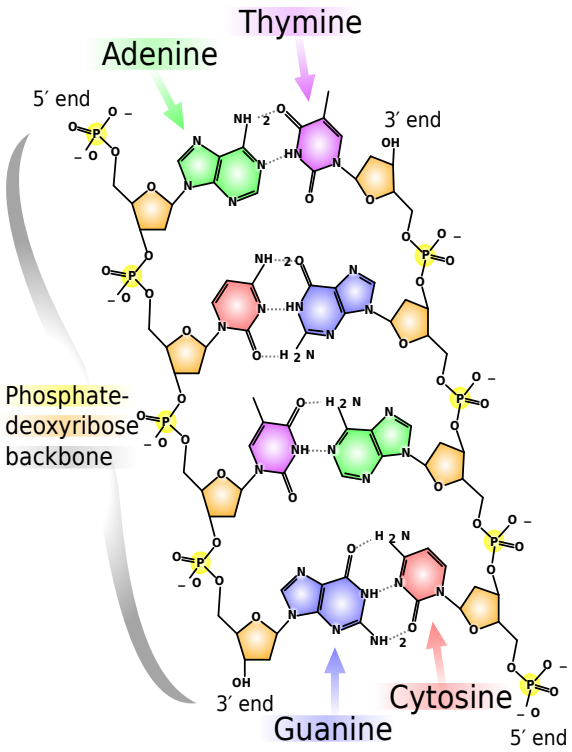
The Two Sugar Subunits Of Nucleic Acids





The Five Nitrogenous Bases of Nucleic Acids

Nucleic acids are composed of linked nucleotides. DNA includes the sugar, deoxyribose, combined with phosphate groups and combinations of thymine, cytosine, guanine, and adenine. RNA includes the sugar, ribose with phosphate groups and combinations of uracil, cytosine, guanine, and adenine.



DNA has a double helical structure. Two anti-parallel strands are bound by hydrogen bonds.

DNA is a double helical molecule. Two anti-parallel strands are bound together by hydrogen bonds. Adenine forms 2 H-bonds with Thymine. Guanine forms 3 H-bonds with Cytosine. This AT & GC matching is referred to as **complementarity**. While the nitrogenous bases are found on the interior of the double helix (like rungs on a ladder), the repeating backbone of pentose sugar and phosphate form the backbone of the molecule. Notice that phosphate has a negative charge. This makes DNA and RNA, overall negatively charged.

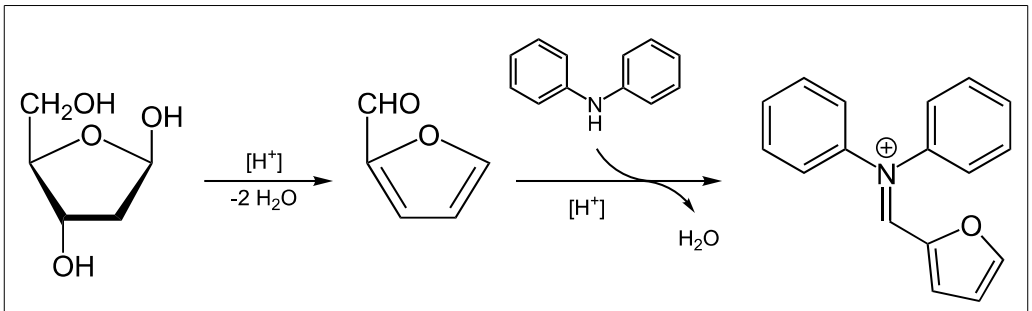
Jeremy Seto jseto@citytech.cuny.edu

Nucleic Acids: DNA extraction and Dische's Diphenylamine test (Activity)

Prelab Questions

1. What are fruits?
    a. Where do they come from?
    b. What are they made of?
2. Use phylogeny to classify plants (DKPCOFGS)
3. Where is DNA located within the fruits? Where is it located in you?
4. Why would you want to extract DNA from an organism?
5. What class of molecule is DNA?

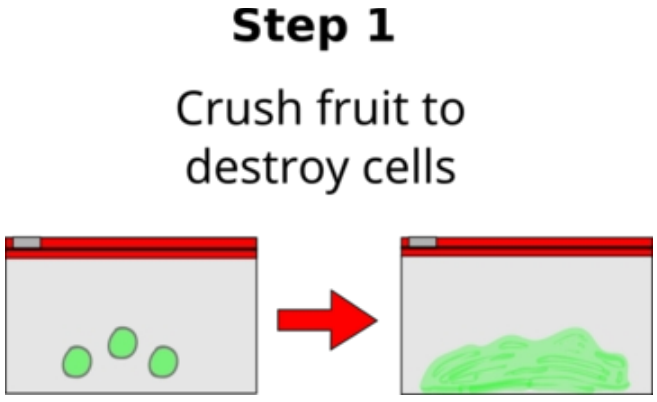
DNA can be identified chemically with the **Dische diphenylamine test**. Acidic conditions convert deoxyribose to a molecule that binds with diphenylamine to form a blue complex. The intensity of the blue color is proportional to the concentration of DNA. The Dische's Test will detect the deoxyribose of DNA and will not interact with the ribose in RNA. The amount of blue corresponds to the amount of DNA in solution.



The diphenylamine compound of the Dische's test interacts with the deoxyribose of DNA to yield a blue coloration.

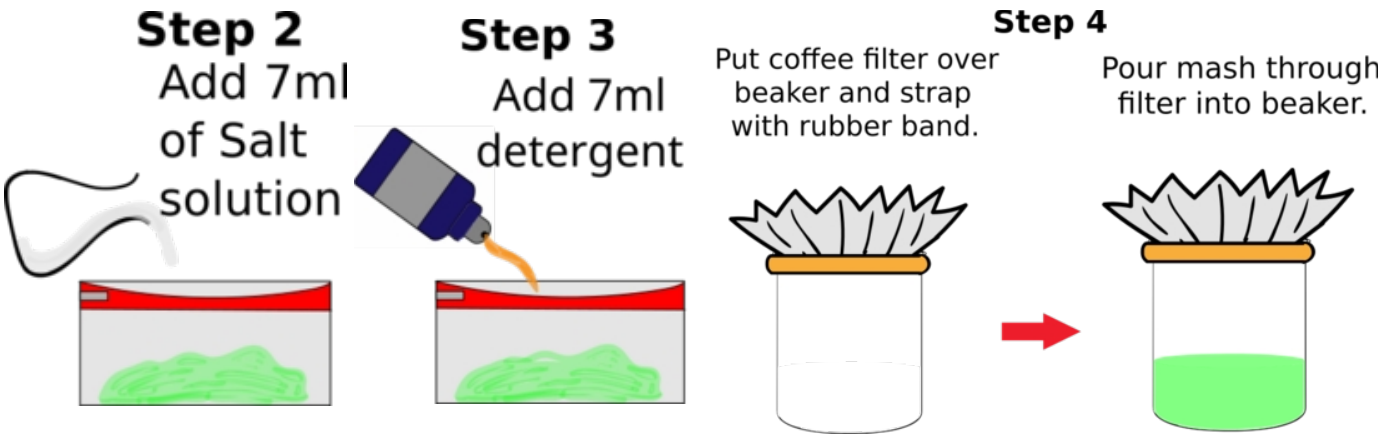
Methods: Extraction of DNA from fruit

1. Mash about 10g or 3cm of over-ripe banana OR 3 grapes OR 1 strawberry in zip-top bag

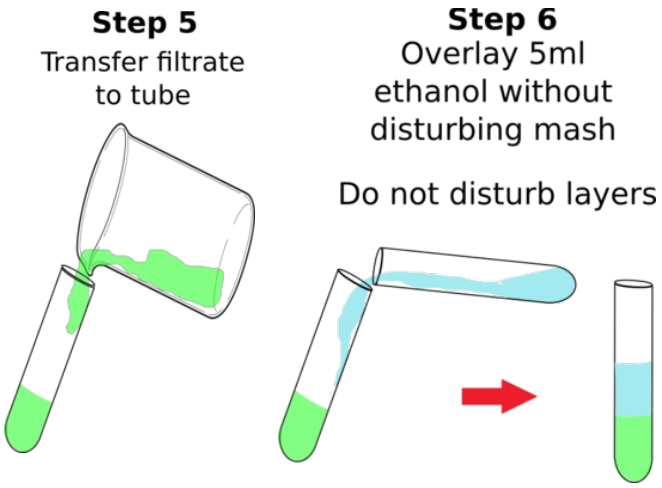


Jeremy Seto jseto@citytech.cuny.edu

2. Add 7ml of salt solution
    - The salt solution helps the DNA to aggregate (clump together).
3. Add 7 ml of liquid detergent and mix
    - dissolves the lipids in the cell and nuclear membranes
    - releases DNA into the salt solution
4. Place a coffee filter over a cup or beaker and fasten with an elastic band



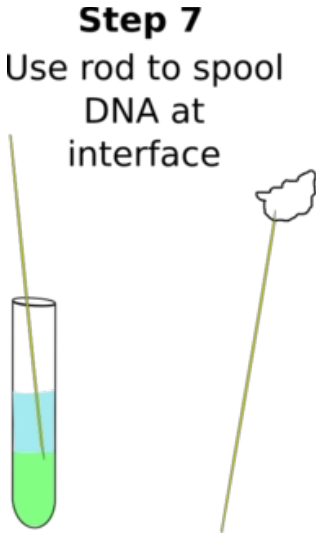
5. Pour about 5 ml of filtrate into a test tube



6. Slowly pour an **EQUAL** volume of cold ethanol down the side of tube to form a layer on top of the fruit fluid.
    - carefully run the alcohol down the side to form a separate layer on top of the fruit solution
    - Do not mix the alcohol and banana solution.
    - Ice-cold 100% ethanol works best

Jeremy Seto jseto@citytech.cuny.edu

7. Spool the DNA: use a plastic loop or glass rod to gently swirl at the interface of the two solutions



   - the interface is where the two solutions meet
   - DNA is not soluble in alcohol
   - bubbles may form around a wooly substance (this is the DNA)

8. Transfer the DNA

Dische Diphenylamine Test For DNA

1. Obtain 3 test tubes and number them 1-3.
2. Suspend the spooled DNA in 3 ml of distilled water. MIX.
3. Add to tubes:
    1. 2 ml of DNA solution
    2. 1 ml of DNA solution with 1 ml H_2O
    3. 2 ml of H_2O
4. Add 2 ml of the Dische's diphenylamine reagent to each tube and mix thoroughly.
5. Place in a boiling water bath for 10 minutes.
6. Evaluate your results. A clear tube indicates no nucleic acids. A blue color indicates the presence of DNA. A greenish color indicates the presence of RNA.

Jeremy Seto jseto@citytech.cuny.edu