



Lab Manual

Introductory Biology (Version 1.4)



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Common Labware found in ESL Kits

A—Beakers
 B—Measuring Cup
 C—Measuring Spoon
 D—Mortar and Pestle
 E—Wash Bottle

F—Canning Jar
 G—Graduated Cylinder
 H—Standing Test Tube
 I—Funnel
 J—Micropipettes

K—Weigh Boats
 L—Pipettes
 M—Glass Test Tube (17 x 100mm)
 N—Plastic Test Tube (17 x 100mm)
 O—Plastic Test Tube (13 x 100mm)

P—Petri Dish
 Q—Syringe
 R—Thermometer
 S—Spray Lid





Lab Safety

Always follow the instructions in your laboratory manual and these general rules:

eScience Labs, Inc. designs every kit with safety as our top priority. Nonetheless, these are science kits and contain items which must be handled with care. **Safety in the laboratory always comes first!**

Lab preparation

- **Please thoroughly read the lab exercise before starting!**
- If you have any doubt as to what you are supposed to be doing and how to do it safely, please STOP and then:
 - ✓ Double-check the manual instructions.
 - ✓ Check www.esciencelabs.com for updates and tips.
 - ✓ Contact us for technical support by phone at 1-888-ESL-Kits (1-888-375-5487) or by email at Help@esciencelabs.com.
- Read and understand all labels on chemicals.
 - ✓ If you have any questions or concerns, refer to the Material Safety Data Sheets (MSDS) available at www.esciencelabs.com. The MSDS lists the dangers, storage requirements, exposure treatment and disposal instructions for each chemical.
- Consult your physician if you are pregnant, allergic to chemicals, or have other medical conditions that may require additional protective measures.

Proper lab attire

- Remove all loose clothing (jackets, sweatshirts, etc.) and always wear closed-toe shoes.
- Long hair should be pulled back and secured and all jewelry (rings, watches, necklaces, earrings, bracelets, etc.), should be removed.
- Safety glasses or goggles should be worn at all times. In addition, wearing soft contact lenses while conducting experiments is discouraged, as they can absorb potentially harmful chemicals.
- When handling chemicals, always wear the protective goggles, gloves, and apron provided.



Performing the experiment

- Do not eat, drink, chew gum, apply cosmetics or smoke while conducting an experiment.
- Work in a well ventilated area and monitor experiments at all times, unless instructed otherwise.
- When working with chemicals:
 - ✓ Never return unused chemicals to their original container or place chemicals in an unmarked container.
 - ✓ Always put lids back onto chemicals immediately after use.
 - ✓ Never ingest chemicals. If this occurs, seek immediate help.

Call 911 or "Poison Control" 1-800-222-1222

- Never pipette anything by mouth.
- Never leave a heat source unattended.
 - ✓ If there is a fire, evacuate the room immediately and dial 911.

Lab Clean-up and Disposal

- If a spill occurs, consult the MSDS to determine how to clean it up.
- Never pick up broken glassware with your hands. Use a broom and a dustpan and discard in a safe area.
- Do not use any part of the lab kit as a container for food.
- Safely dispose of chemicals. If there are any special requirements for disposal, it will be noted in the lab manual.
- When finished, wash hands and lab equipment thoroughly with soap and water.

Above all, USE COMMON SENSE!

The Cell



Lab 13

DNA & RNA



Concepts to explore:

- DNA structure
- Nucleotides
- Amino acids
- Proteins
- Genetic code
- Mutation
- RNA
- Transcription
- Translation

Introduction

Long before we had any understanding of how, we knew that traits were passed on from generation to generation. We knew traits were expressed as heritable proteins, but we had no idea of the mechanism. Whatever the mechanism, it needed to meet three criteria:

- It needed to carry information between generations.
- It needed to express that information.
- It needed to be easily replicable.

Prior to the 1950's, there was much debate over what the structure of a molecule that met all three criteria would look like. Though a number of people made significant contributions, in 1953 James Watson and Francis Crick won the Nobel Prize for their model of what we now know as DNA (deoxyribonucleic acid). The features of this model satisfied all of the necessary criteria.

DNA takes the form of what is commonly referred to as a “double helix”, or perhaps more simply, a long twisted ladder with rungs (Figure 1). The sides of the ladder consist of a sugar-phosphate “backbone” and the strand itself has directionality. In other words, like the words on this page, there is a set order in which they are read. In the case of DNA, it is from the 5' (five prime) to 3' (three prime) end.

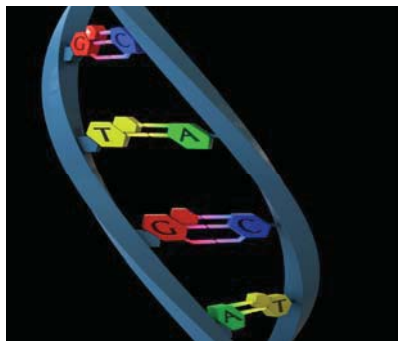


Figure 2: Nucleotides

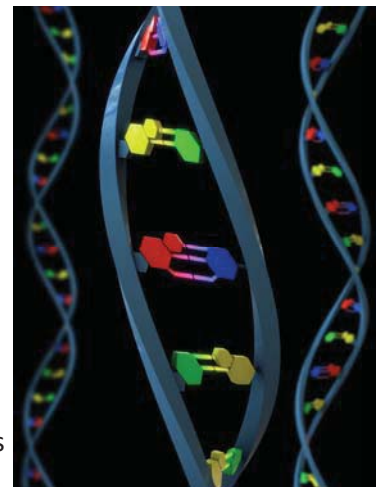


Figure 1: DNA Double Helix

The rungs of the ladder carry information in a sequential series of four different nucleotides (small molecules): Guanine (G), Adenine (A), Thymine (T) and Cytosine (C). These nucleotides pair up in a very precise manner (specificity); A with T, and G with C (Figure 2). No other combinations are ever made because of the chemical and electrical forces within the nucleotide.



As a cell divides, the DNA double helix splits into a single helix (Figure 3). Each single helix then serves as a template for a new strand. Neighboring nucleotides then bind to the single strand helix after which a new sugar-phosphate backbone is formed.

The specificity in which the nucleotides pair means the two new double helices (DNA) are identical to the original. It is the sequence of these nucleotides that are passed on from one generation to another, as heritable information.

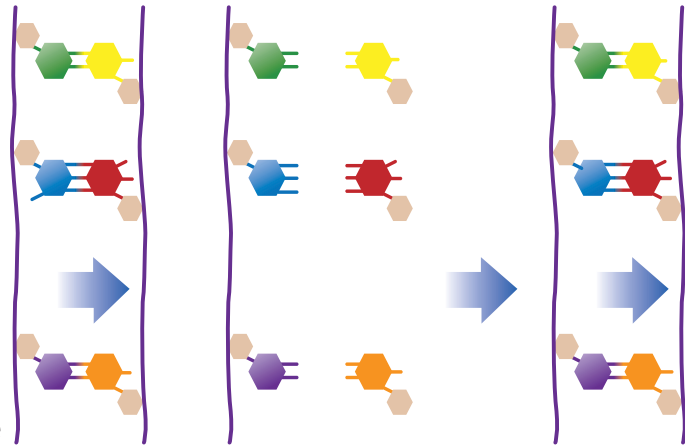


Figure 3: DNA replication

So the question remains, why is the sequence of these different four nucleotides so important? Simply put, they instruct your cells what proteins to make and how to make them (your body is made of proteins). If the protein is wrong you are likely either very sick or dead.

Proteins are simply chains of amino acids (small molecular building blocks) that are linked together. Twenty different amino acids are available to produce all the proteins in the body. Each amino acid is coded for by a three nucleotide sequence (codon). The sequence of the amino acids determines the size of the protein and how it will fold, both factors that determine its function. Other factors such as charge and hydrophobicity (an aversion to water molecules) play a role in determining how a protein folds. Consider the following analogy:

“The earth revolves around the sun.”

- Each of the 12 different letters (codons) in the preceding sentence is largely uninformative.
- When letters are assembled they create words, which have meaning.
- Linked words create a sentence (protein), which is then informative.

Consider a protein that is five amino acids long. Picking from the 20 available amino acids there are 5^{20} different possible combinations (3,200,000). Even small proteins are typically several hundred amino acids long. The number of different proteins that can ultimately be coded for by 20 amino acids is virtually limitless.

The next obvious question is how do the 4 nucleotides “code” for 20 different amino acids. Each “letter” (codon) in the genetic code is made up of three nucleotides which codes for a specific amino acid. If we start with four possible nucleotides (A, T, G, C), how does your body make twenty different amino acids?

- If the “letter” is two nucleotides long, there are 16 possible “letters” (2^4) - not enough.
- If the “letter” is three nucleotides long, there are 64 possible combinations (3^4) - more than what’s needed for twenty amino acids.



Like a sentence, the reader (a cell) needs to know where to start and where to stop (two more codons, for a total of 22). The remaining 42 possible combinations make up what is referred to as “the redundancy of the code”. In other words; Tim, Tom, Tam would all be the same person, it is simply three different spellings for his name. Each combination of three nucleotide is known as a “codon”.

Experiment 1: Coding

Materials

Red beads
Blue beads
Yellow beads
Green beads

For the following exercises:

- Regular beads are used as nucleotides
- Pop-it beads are used as amino acids

Procedure

- A) Using red, blue, yellow and green beads, devise and lay out a three color code for each of the following letters (codon). For example Z = green:red:green.

In the spaces below the letter, record your “code”.

C:	E:	H:	I:	K:	L:
---	---	---	---	---	---
M:	O:	S:	T:	U:	
---	---	---	---	---	
Create codons for:	Start:	Stop:	Space:		
	---	---	---		

- B) Using this code, align the beads corresponding to the appropriate letter to write the following sentence (don't forget start, space and stop):

The mouse likes most cheese

1. How many beads did you use?



There are multiple ways your cells can read a sequence of DNA and build slightly different proteins from the same strand. We will not go through the process here, but as an illustration of this “alternate splicing”, remove codons (beads) 52-66 from your sentence above.

2. What does the sentence say now? (re-read the entire sentence)

Mutations are simply changes in the sequence of nucleotides. There are three ways this occurs:

Change one, remove one, or add one

Using the sentence from exercise 1B:

- C) Change the 24th bead to a different color.

3. What does the sentence say now? (re-read the entire sentence)

4. Does it make sense?

- D) Replace the 24th bead and remove the 20th bead (remember what was there).

5. What does the sentence say (re-read the entire sentence)?

6. Does it make sense?

7. Where does it make sense?

- E) Replace the 20th bead and add one between bead numbers 50 and 51.

8. What does the sentence say?

9. Does it make sense?

10. In “C” we mutated one letter. What role do you think the redundancy of the genetic code plays, in light of this change?



11. Based on your observations, why do you suppose the mutations we made in “D” and “E” are called frame shift mutations.

12. Which mutations do you suspect have the greatest consequence? Why?

DNA codes for all of the proteins manufactured by any organism (including you!). It is valuable, highly informative and securely protected in the nucleus of every cell.

Consider the following analogy:

An architect spends months or years designing a building. His original drawings are valuable and informative. He will not provide the original copy to everyone involved in constructing the building.

Instead, he gives the electrician a copy with the information he needs to build the electrical system. He will do the same for the plumbers, the framers, the roofers and everyone else who needs to play a role to build the structure. These are subsets of the information contained in the original copy. Your cell does the same thing. The “original drawings” are contained in your DNA which is securely stored in the nucleus.

Nuclear DNA is “opened up” by an enzyme (Helicase) and a subset of information is transcribed (copied) into RNA. RNA is a single strand version of DNA, where the nucleotide uracil, replaces thymine. The copies are sent from the nucleus to the cytoplasm in the form of mRNA (messenger RNA). Once in the cytoplasm, tRNA (transfer RNA) links to the codons and aligns the proper amino acids, based on the mRNA sequence.

The ribosomes (protein builders) that float around in the cytoplasm, latch onto the strand of mRNA and sequentially link the amino acids together that the tRNA has lined up for them. This construction of proteins from the mRNA is known as translation.



Experiment 2: Transcription and translation

Materials

Red beads
Blue beads
Green beads
Yellow beads
Pop-it beads (8 different colors)

Procedure

- A) Write a five word sentence using no more than 8 different letters.
- B) Using the four colored beads, create “codons” (3 beads) for each letter in your sentence, plus ones for “start, “space” and stop”.
- C) “Write” the sentence using the beads.
 1. How many beads did you use?

Using your pop-it beads, assign one bead for each codon (you do not need beads for start, stop and space). These will be your amino acids.

Connect the “Pop it” beads to build the chain of amino acids that codes for your sentence (leave out the “start”, “stop” and “space”).

2. How many different amino acids did you use?
3. How many total amino acids did you use?



Experiment 3: DNA Extraction

Materials

Fresh soft fruit (i.e. grapes, berries or banana)*

Cheesecloth

Rubber band

Plastic zipper bag

2 100mL Beaker

DNA extraction solution**

Standing test tube

Scissors*

Ethanol (ice cold)***

Stir stick

Rubber band

***You must provide**

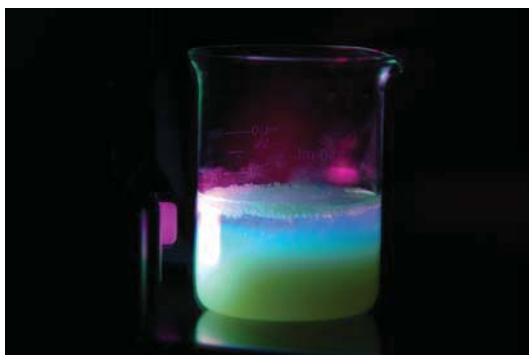


Figure 4: DNA extracted from fruit was dyed with a substance that glows under black light.

** Sodium chloride, detergent and water

***For ice cold ethanol, store in the freezer 60 minutes before use.

Procedure

1. Put pieces of soft fruit (approximate size of 5 grapes) into a plastic zipper bag and mash with your fist.
2. Using a 100ml beaker, measure out 10ml of the DNA extraction solution and pour it into the bag with the fruit it in. Seal the bag completely.
3. Mix well by kneading the bag for 2 minutes.
4. Create a filter by placing the center of the cheesecloth over the mouth of the standing test tube, pushing it into the tube about 2 inches, and securing the cheesecloth with a rubber band around the top of the test tube.
5. Cut a hole in the corner of the bag and filter your extraction by pouring it into the cheese-



cloth (the filtered solution in the beaker is what you keep).

6. While holding the test tube at a 45° angle, slowly pour 5ml ice-cold ethanol into the test tube.
7. As the DNA enters the ethanol it will precipitate (come out of solution). Let the test tube sit for 2-3 minutes. You should see air bubbles on DNA, which will eventually float to the top of the ethanol.
8. Gently insert the stir stick into the test tube and slowly raise and lower the tip several times, to spool and collect the DNA.

Questions

1. Which DNA bases pair with each other?
2. How is information to make proteins passed on through generations?
3. Why did we use a salt in the extraction solution?
4. What else might be in the ethanol/aqueous interface? How could you eliminate this?
5. What is the texture and consistency of DNA?
6. Is the DNA soluble in the aqueous solution or alcohol?
7. What surprised you about DNA replication and protein synthesis?



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