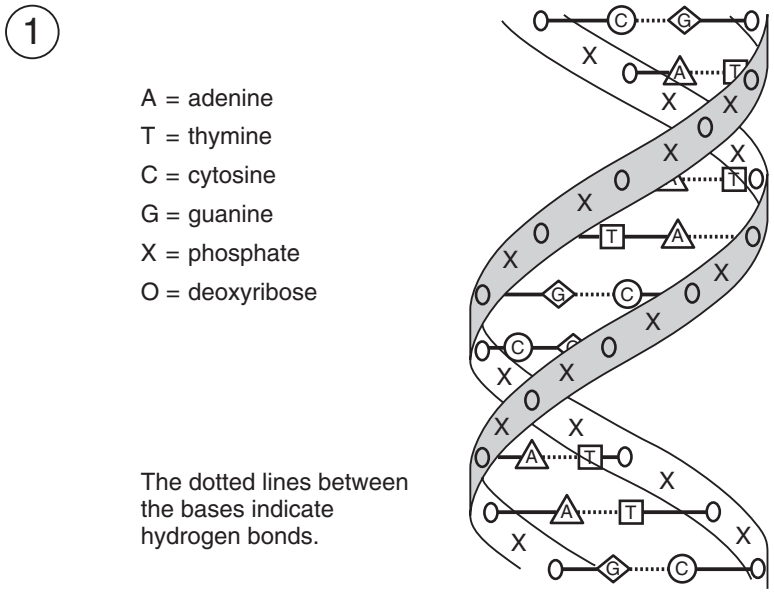
The Value Of DNA

Topic

DNA recovery

Introduction

Deoxyribonucleic acid (DNA) is present in the nuclei of cells of all living things. It consists of very long strands of nucleic acid, which are formed of alternating groups of a carbohydrate (called deoxyribose) and a phosphate group. One of four different bases – adenine, guanine, thymine, or cytosine – attaches itself to each carbohydrate unit. The bases on adjoining strands of nucleic acid link to each other via hydrogen bonds to form the two strands into the structure known as a double helix; the structure of DNA was discovered in 1953 by James Watson (1928–) and Francis Crick (1916–). The bases will only link in certain combinations – adenine with thymine, and guanine with cytosine. Each molecule of DNA contains millions of these base pairs. There is an almost infinite potential for different combinations of these base pairs, making it very unlikely that DNA from two people will share the same pattern (except for identical twins). This is very useful for forensic scientists because they can compare DNA found at a crime scene with that taken from a suspect. In this experiment, you will extract DNA from a selection of different materials. You will break down the cell structure of each material using salt, detergent, and mechanical force; this will release DNA from the cells. You will remove proteins adhering to the DNA using the enzyme papain, which is present in pineapple juice. You may be able to produce long strands of DNA, but you are more likely to find that the DNA has fractured into shorter lengths that clump together in a mass.



The structure of DNA

Time required

at least 1 hour for each extraction

Materials

For each extraction:
20 g of a source of DNA (e.g., chopped chicken liver, peas, chopped onion, or yeast)
pinch of table salt
50 ml tap water
2 ml detergent
2 ml pineapple juice
10 ml isopropyl alcohol (propan-2-ol)
2 test tubes and stoppers
test tube rack
two 400 ml beakers
two 10 ml graduated cylinders
funnel (to fit into a 400 ml beaker)
filter paper
hand blender
2 eyedroppers
20 cm glass rod
watch or clock (measuring minutes)
safety glasses

Safety note

Isopropyl alcohol is poisonous and flammable. If using meat products such as chicken liver, wash hands carefully after use.

Procedure

1. Put on your safety glasses. Place the DNA source, the water, and the salt in a beaker. Blend for 30 seconds (see diagram 2 below).
2. Place the filter paper in the funnel and then put the funnel in the second beaker. Pour the contents of the first beaker into the filter paper and allow the filtrate to drip through into the second beaker (see diagram 3 below). This stage takes longer for some DNA sources than others, e.g., the onion mixture takes about 10 minutes, but the chicken liver mixture can take about 1 hour.
3. Pour 10 ml of the filtrate into a test tube.
4. Using an eyedropper, add about 2 ml of detergent to the test tube. Put a stopper in the neck of the test tube and shake well for 5 minutes. Leave to settle for 5 minutes.
5. Using an eyedropper, add about 2 ml of pineapple juice to the test tube. Stir very carefully with the glass rod.

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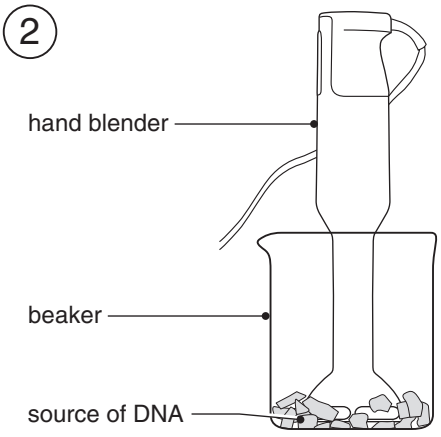
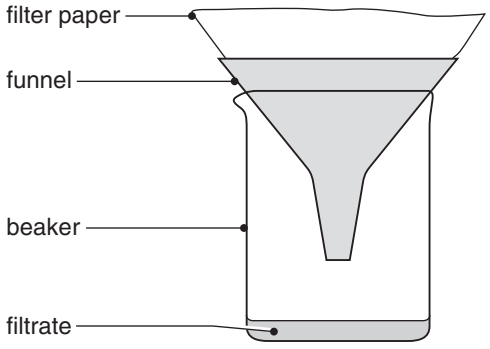


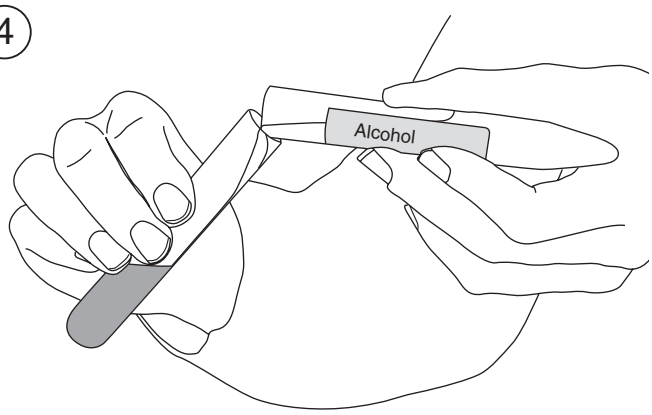
Blending the mixture

3



Filtering the mixture

6. Pour about 10 ml isopropyl alcohol into a second test tube (i.e., approximately the same volume as the contents of the first test tube). Then pour the isopropyl alcohol slowly and carefully from the second test tube into the first test tube (see diagram 4 opposite). The aim is to form a layer of isopropyl alcohol on top of the watery liquid.



Adding the isopropyl alcohol to the filtrate

7. Place both test tubes carefully in the rack and observe the first test tube. Draw the contents of the test tube in the data table below after 5, 10, and 30 minutes.

DATA TABLE		
After 5 minutes	**After 10 minutes**	**After 30 minutes**

Analysis

1. Did whitish strands appear in the top (isopropyl alcohol) layer? What do you think these were?
2. If whitish strands appeared, can you wrap them around the glass rod? Or if this is not possible, can you move them around with the glass rod?
3. What happened to the contents of the first test tube?
4. If you performed this experiment using more than one DNA source, which produced the most DNA?

Want to know more?

1. The whitish strands are DNA.
2. If the whitish strands are clumped together and will not wrap around the rod, the DNA strands have sheared (broken up). Unbroken DNA strands should wrap around the rod.
3. Of the sources suggested, chicken liver produces the most DNA; onion and peas produce a moderate amount of DNA, but yeast does not produce very much DNA.

Special Safety Note To Experimenters

Each experiment includes any special safety precautions that are relevant to that particular project. These do not include all of the basic safety precautions that are necessary whenever you are working on a scientific experiment. For this reason, it is absolutely essential that you read, copy, and remain mindful of the General Safety Precautions that follow this note. Experimental science can be dangerous, and good laboratory procedure always includes carefully following basic safety rules. Things can happen very quickly while you are performing an experiment. Things can spill, break, even catch fire. There will be no time after the fact to protect yourself. Be prepared for unexpected dangers by following basic safety guidelines the entire time you are performing the experiment, whether or not something seems dangerous to you at a given moment.

We have been quite sparing in prescribing safety precautions for the individual experiments. We made this choice for one reason: We want you to take very seriously every safety precaution that is printed in this book. If you see it written here, you can be sure that it is here because it is absolutely critical to your safety.

One further note: The book assumes that you will read the safety precautions that follow, as well as those in the box within each experiment you are preparing to perform, and that you will remember them. Except in rare instances, the general precautions listed below will not be repeated in the procedure itself. It is up to you to use your good judgment and pay attention when performing potentially dangerous parts of the procedure. Just because the book does not say **BE CAREFUL WITH HOT LIQUIDS or DON'T CUT YOURSELF WITH THE KNIFE** does not mean that you should be careless when boiling water or cutting a section of a stem for microscope work. It does mean that when you see a special note to be careful, it is extremely important that you pay attention to it. If you ever have a question about whether a procedure or material is dangerous, wait to perform it until you find out from a qualified adult that it is safe.

GENERAL SAFETY PRECAUTIONS

Accidents caused by carelessness, haste, insufficient knowledge, or taking unnecessary risks can be avoided by practicing safety procedures and being alert while conducting experiments. Be sure to check the individual experiments in this book for additional safety regulations and adult supervision requirements. If you will be working in a lab, do not work alone.

PREPARING:

—Clear all surfaces before beginning experiments
—Read the instructions before you start
—Know the hazards of the experiments and anticipate dangers

PROTECTING YOURSELF:

—Follow the directions step-by-step; only do one experiment at a time
—Locate exits, fire blanket and extinguisher, gas and electricity shut-offs, eyewash, and first-aid kit
—Make sure there is adequate ventilation
—Act sensibly at all times
—Wear an apron and safety glasses
—Do not wear open shoes, loose clothing, or loose hair
—Keep floor and workspace neat, clean, and dry
—Clean up spills immediately, being careful to follow the recommended procedure for dealing with the spilt substance
—Never eat, drink, or smoke in the laboratory or workspace
—Do not eat or drink any substances tested unless expressly permitted to do so by a knowledgeable adult

USING EQUIPMENT WITH CARE:

—Set up apparatus far from the edge of the desk
—Use knives and other sharp or pointed instruments with caution
—Pull plugs, not cords, when removing electrical plugs

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—Don't use your mouth to pipette liquids; use a suction bulb
—Check glassware is clean and dry before use
—Check glassware for scratches, cracks, and sharp edges
—Report broken glassware immediately so that it can be cleaned up by a responsible person
—Do not use reflected sunlight to illuminate your microscope
—Use only low voltage and current materials such as lantern batteries
—Be careful when using stepstools, chairs, and ladders

USING CHEMICALS AND BIOLOGICAL MATERIALS:
—Never taste or inhale chemicals
—Label all bottles and apparatus containing chemicals
—Read labels carefully
—Avoid chemical contact with skin and eyes (wear safety glasses, lab apron, and gloves)
—Do not touch chemical solutions
—Wash hands before and after using solutions
—Wipe up spills thoroughly
—Use sterile procedures when handling even common and harmless microorganisms
—Avoid contact with human blood
—Treat all living organisms with appropriate respect

HEATING SUBSTANCES:
—Wear safety glasses, apron, and gloves when boiling water
—Keep your face away from test tubes and beakers
—Use test tubes, beakers, and other glassware made of Pyrex™ or borosilicate glass
—Use alcohol-filled thermometers (do not used mercury-filled thermometers)
—Never leave apparatus unattended
—Use safety tongs and heat-resistant mittens
—If your laboratory does not have heat-proof workbenches, put your Bunsen burner on a heat-proof mat before lighting it
—Take care when lighting your Bunsen burner; use a Bunsen burner lighter in preference to wooden matches
—Turn off hot plates, Bunsen burners, and gas when you are done
—Keep flammable substances away from heat
—Keep sheets of paper and other flammable objects away from your Bunsen burner
—Have a fire extinguisher on hand

FIELDWORK:
—Be aware of environmental dangers (e.g., do not carry out fieldwork near dangerous roads, cliffs, or water)
—Remember that strong sunlight can be dangerous – pack sunscreen and a good supply of drinking water if you will be outside all day
—Never carry out fieldwork in areas where you cannot find your way to safety easily and quickly and never wander off on your own in search of new areas to study

FINISHING UP:
—Clean your work area and glassware (follow any instructions given by a supervising adult)
—Be careful not to return chemicals or contaminated reagents to the wrong containers
—Don't dispose of materials in the sink unless instructed to do so
—Wash your hands
—Clean up all residues and put in proper containers for disposal
—Dispose of all chemicals according to all local, state, and federal laws
—Dispose of all microbiological cultures by treatment with an appropriate disinfectant

BE SAFETY CONSCIOUS AT ALL TIMES