DEOXYRIBONUCLEIC ACID (DNA) SPOOLING
EDUCATIONAL KIT

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Technical Bulletin ED-100

TECHNICAL BULLETIN

Product Description
Deoxyribonucleic acid, usually abbreviated as DNA, is a
long double-helical molecule containing genetic
information. DNA is mainly found as tightly-coiled
chromosomes in the nucleus of the cell, or as more
loosely coiled strands in a bacterial cell which has no
nucleus. If you uncoiled all the DNA in one human cell,
the filament which is invisible to the human eye would
measure about 1.5 meters. If it were multiplied a
thousand times, it would be 1500 meters long (almost a
mile), but still thinner than a fine hair.

A DNA molecule does not exist in humans as a long
loose rope, but is coiled and twisted much like an
extra-long telephone cord that has not been untangled
for years. The thick twisted bundles of DNA are the
chromosomes that can be seen with a powerful
microscope. DNA is a double-stranded molecule.
Each strand is composed of an ordered combination of
four nucleotides, each nucleotide consisting of a purine
or pyrimidine base (adenine, guanine, thymine or
cytosine) associated with a deoxyribose sugar molecule
and a phosphate group. A base in one strand can form
weak, but significant, hydrogen bonds to a
complementary base in the other strand (or even to a
complementary base somewhere in the same strand).
These base-pairs are like rungs in a spiral rope
ladder. The bends and twists in this ladder all are
important to the functioning of the DNA molecule. The
folded shape is so complex and sensitive that other
molecules involved in the transfer of genetic information
migrate to the DNA, rather than the other way around.
If the structure of DNA is altered, so is its function. If it
were to completely uncoiled, it would no longer function
properly in human cells.

The sequence of bases in a DNA molecule from any
organism is a code for the synthesis of each and every
molecule of that organism. In general, the length of the
DNA is related to the complexity of the creature with
which it is associated. A virus DNA may have only
3000 base pairs, a bacterium has about 3 million base
pairs. Mammals, including humans, have 3-5 billion
base pairs.

Extracting DNA from cells is not an easy task. The cell
walls and nuclear membranes must be lysed (broken)
so that the DNA molecules can be dissolved into a
solvent. The DNA must then be separated from cell
debris, substructures, and other molecules. Cells also
contain a variety of enzymes, some of which attack and
destroy nucleic acids. The DNA must be protected
from these nucleases as it is being isolated.

From a practical viewpoint, a commercial source of
DNA must be inexpensive and rich in DNA. Although
sirloin steak contains DNA, it is extremely expensive
and contains lipids, saccharides, and protein material
which must be discarded. Not practical! Two excellent
sources of DNA are sperm cells and thymus glands of
very young animals (their function is the development of
immune systems; they become dysfunctional in older
animals). Since these cells contain almost nothing but
DNA, this makes the isolation procedure much simpler.
Calf thymus DNA is available from Sigma, but thymus
glands are very small; often called sweetbreads, they
are a restaurant delicacy priced like steak. By
comparison, salmon sperm is more readily available.

The isolation process for DNA is straightforward, but
too long for most high school class periods, and highly
dependent on good laboratory skills. Most college
biology laboratories have procedures for isolation of
DNA from yeast or bacterial strains. These procedures involve lysing cell walls, centrifuging fragments, and carefully separating the DNA from the proteins, carbohydrates, lipids, and other molecules present. The DNA solution must be buffered (that is, protected from extreme changes in acidity). The long chain must be untangled using substances which disrupt the cross-links between coils, so the twisted rope can relax. DNA is soluble in water, but not in alcohol. Alcohol can denature macromolecules, i.e., push them out of solution and disrupt the cross-linking that maintains the coiled structure.

This kit allows you to take the final step in the isolation of DNA and to have the success of seeing a macromolecule without the often frustrating problems associated with cell lysis, etc. The addition of alcohol to a solution of DNA will precipitate it, allowing you to retrieve strands of DNA with a glass rod. One molecule of DNA is not visible by itself, but if enough molecules are entangled with each other, you will be able to see the strands of DNA.

Procedure
2. Pipette 1 ml of DNA solution into each test tube.
3. Slowly add 1 ml of sodium acetate 3 M solution, to the DNA solution while tilting tube and letting the sodium acetate run down side of tube. Mix by gently swirling.
4. Slowly add 2 ml of isopropanol and observe the layering. NOTE: If isopropanol is not available, use 4 ml of ethanol. Results will be the same.
5. With a glass stirring rod or glass pipet, gently mix the aqueous and organic phases. DNA will start to precipitate. Gently spool the DNA around the glass rod.
6. Carefully remove the DNA and place on a petri dish or other suitable surface and let dry overnight. The DNA you have isolated is ready for storage or examination under a microscope. It may also be used for other laboratory projects.

References
You may enjoy reading more about what DNA does and how genetic codes can help anticipate development of disease, answer legal questions of identity, solve crimes and more. Most current biology and biochemistry textbooks contain basic background information about DNA technology. Some of the information above was derived from the following books. Their excellent illustrations would add a great deal to your understanding of DNA. These are available from Sigma Chemical Company; see our catalog for more information and additional book listings.

A very entertaining introduction to the mechanism of DNA/RNA and protein synthesis. Even has a Biodisc which allows you to assemble your own proteins! Appropriate for high school or college; clever cartoon illustrations add considerable appeal. (Product No. BIO-1)

Intended for a college biology class: a special unit about DNA technology. Good background information with lab experiments included in the book. Could be used in advanced high school special topics science course. (Product No. D4667)
The fourth edition is a challenging reappraisal of the discipline by an active research scientist and teacher. Readers will see how dramatically the field has been transformed by recombinant DNA technology.

The next page of the bulletin has a student report form which may be photocopied for classroom usage.

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MAM 02/02
STUDENT REPORT FORM: SPOOLING DNA

NAME: _____________________________________

CLASS HOURS: ______________________________

INSTRUCTOR: ________________________________

The nucleic acid you will see today is deoxyribonucleic acid (DNA). As you perform the investigation note your findings.

1. When alcohol is added to the DNA/Sodium Acetate Solution which liquid formed the top layer?

________________________________________________________________________________________________________

2. What was the purpose of the alcohol?

________________________________________________________________________________________________________

3. What did the DNA look like?
   a. Describe the solution of DNA (color/viscosity)
   b. Describe it as it came out of the solution (color, etc.)
   c. Describe the DNA after it has been exposed to air to dry.

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4. What did you think DNA would look like before you did this experiment? Were you surprised?

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