Biotechnology Tools: Emerging Pathogens
October 23, 2009
A Day-Long Workshop sponsored by Volusia County Schools and the University of Florida Center for Precollegiate Education and Training

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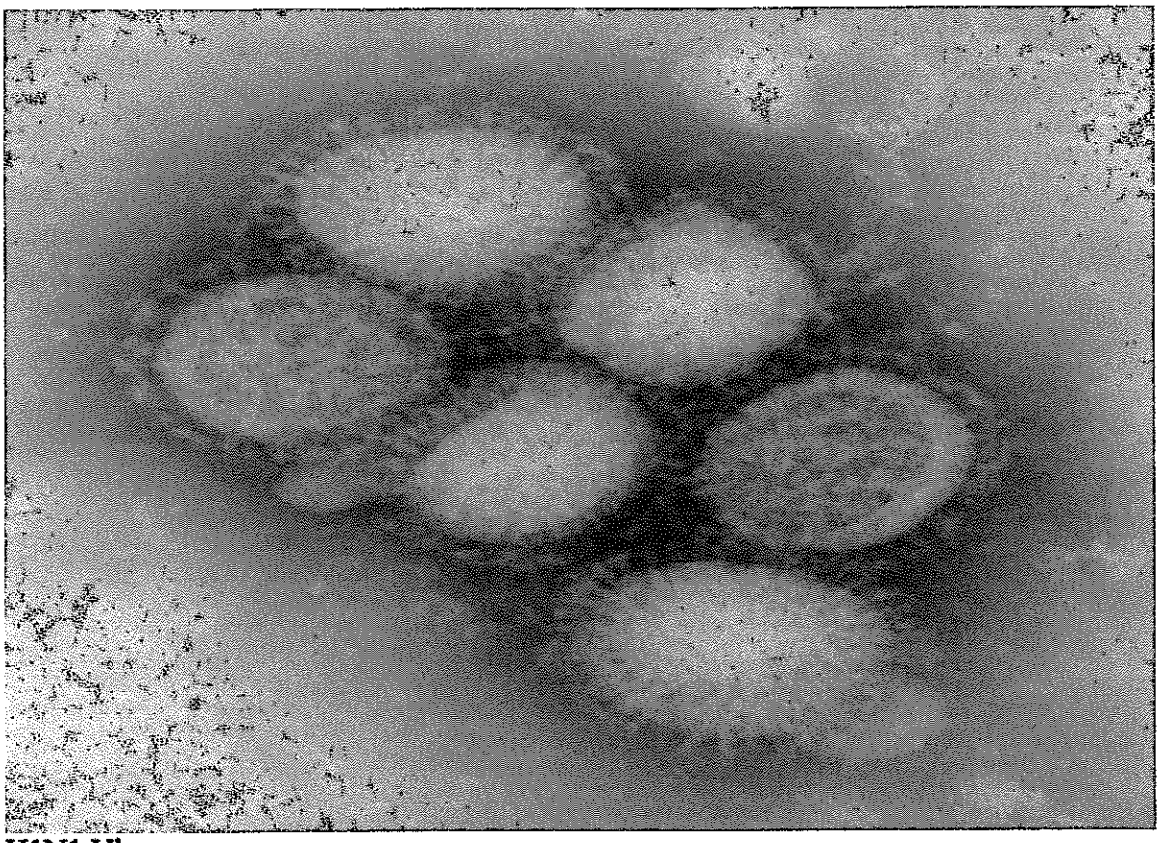

H1N1 Virus

Name _____

Emerging Pathogens and Biotechnology

Pre-Test

1. Emerging Pathogens are

a) a disease-causing microorganism that is newly recognized b) a disease-causing microorganism that is increasing in frequency c) a disease-causing microorganism found in human, animal, or plant populations d) a and b e) a, b and c

2. Pathogens emerge or re-emerge when

a) environmental change occurs b) genetic modifications permit crossing of species barriers c) rural urbanization allows infectious to arise in isolated rural areas and spread d) a and b e) a, b and c

3. The global reservoir for Influenza A (Swine Flu)

a) humans b) pigs c) domesticated poultry d) aquatic birds e) sea mammals

4. Influenza A (Swine Flu)

a) is an RNA virus b) is a DNA virus

5. E.coli can be

a) a foodborne illness b) may contaminate the surface of meat during slaughter c) is a virus d) a and b e) a, b and c

6. Plant disease(s) that are caused by fungi

a) rusts b) blights c) wilts d) a and b e) a, b and c

7. Tomato Spotted Wilt Virus is vectored by

a) mosquitos b) birds c) thrips d) a and b e) a,b, and c

8. Microarrays

a) can be used to diagnose which genes are active in a disease b) can be used to determine if an antigen to a specific antibody is present in a sample c) can be printed with DNA or protein d) a and b e) a, b and c

Name _____

Emerging Pathogens and Biotechnology

Post-Test

1. Emerging Pathogens are

a) a disease-causing microorganism that is newly recognized b) a disease-causing microorganism that is increasing in frequency c) a disease-causing microorganism found in human, animal, or plant populations d) a and b e) a, b and c

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Outline of the Professional Development Day and Activities: Emerging Pathogens and Biotechnology

Andrea M. White, Ph.D. and Erin Kelso, Ph.D. along with SCHS IB 12th grade student mentors

Spruce Creek High School University of Florida, Center for Pre-Collegiate Education and Training

7:30 - 8:00 am Optional: Breakfast and coffee

8:00 – 8:15 am Introductions and Orientation to the Day

8:15 – 9:30 am Introduction to Micropipetting Techniques and Practice

9:30 - 9:45 am Short Break

9:45 – 11:00 am DNA Extraction from peanuts (seeds) and PCR Preparation

11:00 – 12:00 pm Introduction to Emerging Pathogens (PCR ongoing for 2 hrs. 40 minutes)

12:00 – 12:45 pm Lunch provided

1:00 – 1:30 pm Introduction to Tomato Spot Wilted Virus and Test Strip Analysis if possible/ Emerging Pathogens Classroom Simulation Activities

1:45 – 2:45 pm Agarose Gel Electrophoresis of DNA using Invitrogen E-gels and visualization of bands

2:45 – 3:00 pm Workshop Evaluation

Measurements, Micropipetting, and Sterile Techniques

LABORATORY 1 INTRODUCES MICROPIPETTING AND STERILE PIPETTING techniques used throughout this course. Mastery of these techniques is important for good results in all of the experiments that follow. Most of the laboratories are based on *microchemical* protocols that use very small volumes of DNA and reagents. These require use of an adjustable micropipettor (or microcapillary pipette) that measures as little as one microliter (μl)—a millionth of a liter.

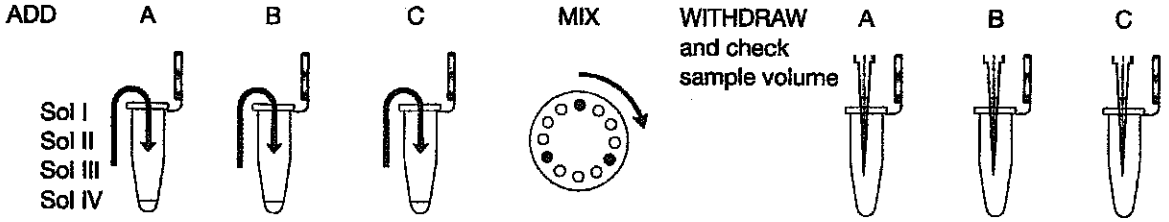
Many experiments require growing *Escherichia coli* in a culture medium that provides an ideal environment for other microorganisms as well. Therefore, it is important to maintain sterile conditions to minimize the chance of contaminating an experiment with foreign bacteria or fungi. *Sterile conditions* must be maintained whenever living bacterial cells are to be used in further cultures. Use sterilized materials for everything that comes in contact with a bacterial culture: nutrient media, solutions, pipettes, micropipettor tips, inoculating and spreading loops, flasks, culture tubes, and plates.

Remember this rule of thumb: Use sterile technique if live bacteria are needed at the end of a manipulation (general culturing and transformations). Sterile technique is not necessary when the bacteria are destroyed by the manipulations in the experiment or when working with solutions for DNA analysis (plasmid isolation, DNA restriction, and DNA ligation).

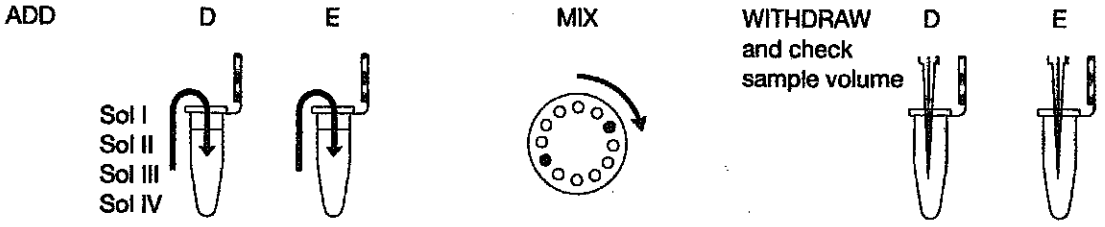
Equipment and materials for this laboratory are available from the Carolina Biological Supply Company (see Appendix 1).

Taken from: DNA Science A First Course, 2nd Edition
D. A. Micklos, G. A. Freyer with David Crotty
Cold Spring Harbor Laboratory Press. 2003.

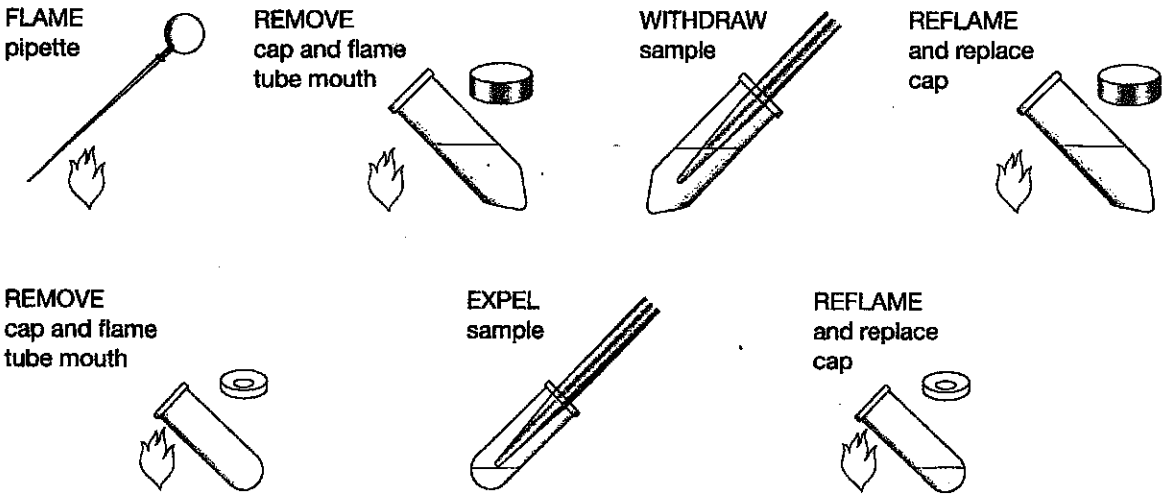
I. Small-volume Micropipettor Exercise



II. Large-volume Micropipettor Exercise



III. Sterile Use of 10-ml Standard Pipette



PRELAB NOTES

Digital Micropipettors

The volume range of digital micropipettors varies from manufacturer to manufacturer. Select both a small-volume micropipettor with a range of 0.5–10 µl or 1–20 µl and a large-volume micropipettor with a range of 100–1000 µl.

Microcapillary Pipettes

Microcapillary pipettes are an inexpensive alternative to adjustable micropipettors. These disposable glass capillary tubes come in sizes that cover the range of volumes used in this course. Several types of inexpensive micropipette aids are available. A thumbscrew micrometer may be easier to use than a pipettor bulb. A no-cost and easily controllable pipette bulb can be made by tying a knot in a length of latex tubing, which is usually provided with the capillary pipettes.

Under conditions of high static electricity, capillary pipetting can be very difficult and, at times, impossible. The reagent droplet adheres stubbornly to the side of the pipette and cannot be transferred to the side of a polypropylene reaction tube. Even under the best of circumstances, microcapillary pipettes are more difficult to master. Allow students sufficient time to become competent with them before attempting any experiments.

Transfer Pipettes

Small polypropylene transfer pipettes are handy because they have an integrated bulb. The smallest size, which holds a *total* volume of approximately 1 ml, has a thin tip that can be used to measure microliter amounts. Before use, calibrate the transfer pipette using a digital micropipettor or microcapillary pipette. Pressing on the pipette barrel, rather than the bulb, creates less air displacement and makes measuring small volumes easier.

10-ml Pipettes

Presterilized, disposable 10-ml plastic pipettes are most convenient and are supplied in bulk pack or individually wrapped. Bulk-packed pipettes should be opened immediately before use. To dispense, cut one corner of the plastic wrapper at the end opposite the pipette tips. Avoid touching and contaminating the wrapper opening; tap bag to push the pipette end through the cut opening. Reclose with tape to keep sterile for future use. To use individually wrapped pipettes properly, peel back only enough of the wrapper to expose the wide end of the pipette and affix the end into the pipette aid or bulb. Completely peel back the wrapper immediately before use.

To Flame or Not to Flame?

There is general disagreement about whether it is necessary to flame pipettes and mouths of tubes as part of the sterile technique. Flaming warms the air at

the mouth of the container, creating an outward convection current that prevents microorganisms from falling in. Even so, the effect of flaming may be primarily psychological when fresh sterile supplies are used and manipulations are done quickly. Especially when using individually wrapped supplies, flaming can be omitted without compromise to sterility. When flaming plasticware, do so briefly to avoid melting the plastic.

Microfuge

Although not essential, a microfuge is very useful for pooling and mixing droplets of pipetted solutions in the bottom of a 1.5-ml reaction tube.

PRELAB PREPARATION

1. To simplify initial practice with a micropipettor, use colored solutions that are easily visible. Prepare five colored solutions using food coloring or other dyes mixed with water.
2. Prepare for each experiment:
   - Four 1.5-ml tubes, each containing 1 ml of a different colored solution, marked I, II, III, and IV.
   - One 50-ml conical tube containing 25 ml of colored solution, marked V.

MATERIALS

REAGENTS

Solution I (1 ml), colored
Solution II (1 ml), colored
Solution III (1 ml), colored
Solution IV (1 ml), colored
Solution V (25 ml), colored

SUPPLIES AND EQUIPMENT

Beaker for waste/used tips
Bunsen burner (optional)
Conical tube (50-ml)
Culture tube (15-ml)
Microfuge (optional)
Micropipettor (0.5–10-µl) + tips
Micropipettor (100–1000-µl) + tips
Permanent marker
Pipette (10-ml)
Pipette aid or bulb
Test tube rack
Tubes (1.5-ml)

METHODS

Metric Conversions

Become familiar with metric units of measurement and their conversions. We concentrate on liquid measurements based on the liter, but the same prefixes also apply to dry measurements based on the gram. The two most useful units of liquid measurement in molecular biology are the milliliter (ml) and microliter (µl).

1 ml	=	0.001 liter	1,000 ml	=	1 liter
1 µl	=	0.000001 liter	1,000,000 µl	=	1 liter

Complete the following conversions:

1 µl	=	_____ ml	_____ µl	=	1 ml
10 µl	=	_____ ml			
100 µl	=	_____ ml			

Use of Digital Micropipettors

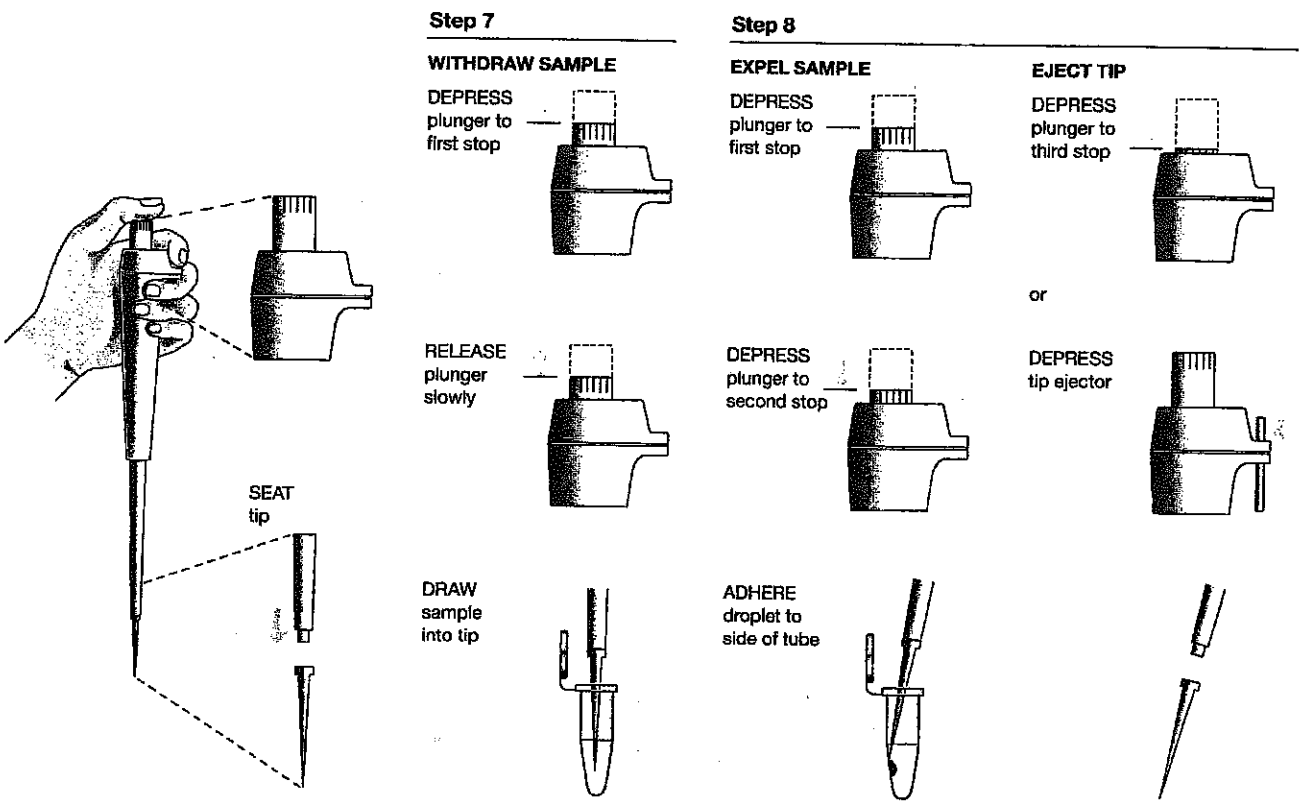
(10 minutes)

"Nevers"

- Never rotate volume adjustor beyond the upper or lower range of the micropipettor, as stated by the manufacturer.
- Never use a micropipettor without the tip in place; this could ruin the precision piston that measures the volume of fluid.
- Never lay down a micropipettor with a filled tip; fluid could run back into the piston.
- Never let the plunger snap back after withdrawing or ejecting fluid; this could damage the piston.
- Never immerse the barrel of the micropipettor in fluid.
- Never flame the micropipettor tip.

Micropipetting Directions

1. Rotate the volume adjustor to the desired setting. Note the change in the plunger length as the volume is changed. Be sure to locate the decimal point properly when reading volume setting.
2. *Firmly* seat proper-sized tip on the end of the micropipettor.
3. When withdrawing or expelling fluid, always hold the tube firmly between thumb and forefinger. Hold the tube at nearly eye level to observe the change in the fluid level in the pipette tip. Do not pipette with the tube in the test tube rack or have another person hold the tube while pipetting.
4. Each tube must be held in the hand during each manipulation. Grasping the tube body, rather than the lid, provides more control and avoids contamination from the hands.
5. Hold micropipettor almost vertical when filling.



Use of Digital Micropipettor (Steps 7 and 8)

6. Most digital micropipettors have a two-position plunger with friction "stops." Depressing to the first stop measures the desired volume. Depressing to the second stop introduces an additional volume of air to blow out any solution remaining in the tip. Pay attention to these friction stops, which can be felt with the thumb.
7. To withdraw sample from reagent tube:
   a. Depress the plunger *to the first stop* and hold in this position. Dip the tip into the solution to be pipetted, and draw the fluid into the tip by *gradually* releasing the plunger.
   b. Slide the tip out along the inside wall of the reagent tube to dislodge excess droplets adhering to the outside of the tip.
   c. Check that there is no air space at the very end of the tip. To avoid future pipetting errors, learn to recognize the approximate level that particular volumes reach in the tip.
8. To expel sample into reaction tube:
   a. Touch the tip to the inside wall of the reaction tube into which the sample will be emptied. This creates a capillary effect that helps draw fluid out of the tip.
   b. *Slowly* depress the plunger to the first stop to expel sample. Depress to the second stop to blow out the last bit of fluid. Hold plunger in depressed position.

c. Slide the pipette out of the reagent tube with the plunger depressed to avoid sucking any liquid back into the tip.

d. Manually remove or eject the tip into a beaker kept on the lab bench for this purpose. The tip is ejected by depressing the measurement plunger beyond the second stop or by depressing a separate tip-ejection button, depending on the particular micropipettor being used.

9. To prevent cross-contamination of reagents:

a. Always add appropriate amounts of a single reagent sequentially to all reaction tubes.

b. Release each reagent drop onto a new location on the inside wall, near the bottom of the reaction tube. In this way, the same tip can be used to pipette the reagent into each reaction tube.

c. Use a *fresh tip* for each new reagent to be pipetted.

d. If the tip touches one of the other reagents in the tube, switch to a new tip.

10. Eject used tips into a beaker kept on the lab bench for this purpose.

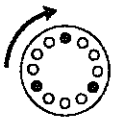
I. Small-volume Micropipettor Exercise (15 minutes)

This exercise simulates setting up a reaction, using a micropipettor with a range of 0.5–10 µl or 1–20 µl.

1. Use a permanent marker to label three 1.5-ml tubes A, B, and C.
2. Use the matrix below as a checklist while adding solutions to each reaction tube.

Tube	Sol. I	Sol. II	Sol. III	Sol. IV
A	4 µl	5 µl	1 µl	–
B	4 µl	5 µl	–	1 µl
C	4 µl	4 µl	1 µl	1 µl

3. Set the micropipettor to 4 µl and add Solution I to each reaction tube.
4. Use a *fresh tip* to add appropriate volume of Solution II to a clean spot on reaction Tubes A, B, and C.
5. Use a *fresh tip* to add 1 µl of Solution III to Tubes A and C.
6. Use a *fresh tip* to add 1 µl of Solution IV to Tubes B and C.
7. Close tops. Pool and mix reagents by using one of the following methods:



An empty 1.5-ml tube can be used to balance a sample with a volume of 20 µl or less.

a. Sharply tap the tube bottom on the bench top. Make sure that the drops have pooled into one drop at the bottom of the tube.

or

b. Place the tubes in a microfuge and apply a short, few-second pulse. Make sure that the reaction tubes are placed in a *balanced* configuration in the microfuge rotor. Spinning tubes in an unbalanced position will damage the microfuge motor.

8. A total of 10 µl of reagents was added to each reaction tube. To check that the measurements were accurate, set the pipette to 10 µl and very carefully withdraw solution from each tube.
   a. Is the tip just filled?

      or

   b. Is a small volume of fluid left in tube?

      or

   c. After extracting all fluid, is an air space left in the tip end? (The air can be displaced and actual volume determined simply by rotating volume adjustment to push fluid to very end of tip. Then, read the volume directly.)
9. If several measurements were inaccurate, repeat the exercise to obtain a near-perfect result.

II. Large-volume Micropipettor Exercise

(10 minutes)

This exercise simulates a bacterial transformation or plasmid preparation, for which a 100–1000-µl micropipettor is used. It is far easier to mismeasure when using a large-volume micropipettor. If the plunger is not released slowly, an air bubble may form or solution may be drawn into piston.

1. Use a permanent marker to label two 1.5-ml reaction tubes D and E.

2. Use the matrix below as a checklist while adding solutions to each reaction tube.

Tube	Sol. I	Sol. II	Sol. III	Sol. IV
D	100 µl	200 µl	150 µl	550 µl
E	150 µl	250 µl	350 µl	250 µl

3. Set the micropipettor to add appropriate volumes of Solutions I–IV to reaction tubes D and E. Follow the same procedure as for the small-volume micropipettor.

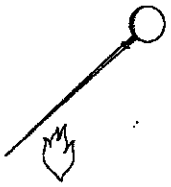
4. A total of 1000 µl of reactants was added to each tube. To check that the measurements were accurate, set the micropipettor to 1000 µl and carefully withdraw solution from each tube.
   a. Is the tip just filled?

      or

   b. Is a small volume of fluid left in tube?

      or

   c. After extracting all fluid, is an air space left in the tip end? (The air can be displaced and actual volume determined simply by rotating volume adjustment to push fluid to very end of tip. Then, read the volume directly.)
5. If the measurements were inaccurate, repeat the exercise to obtain a near-perfect result.



III. Sterile Use of 10-ml Standard Pipette (10 minutes)

The following directions include flaming the pipette and tube mouth. It is probably best to learn to flame, then omit flaming when safety or situation dictates. The directions also assume one-person pipetting, which is rather difficult. The process is much easier when working as a team: One person handles the pipette, while the other removes and replaces the caps of the tubes.

The key to successful sterile technique is to work quickly and efficiently. Before beginning, clear off the lab bench and arrange tubes, pipettes, and culture medium within easy reach. Locate Bunsen burner in a central position on the lab bench to avoid reaching over the flame.

Loosen caps so that they are ready for easy removal. Remember, the longer the top is off the tube, the greater the chance of microbe contamination. Do not place a sterile cap on a nonsterile lab bench.

> **CAUTION**
> Always use a pipette aid or bulb to draw solutions up the pipette. Never pipette solutions using mouth suction: This method is not sterile and can be dangerous.

Nonsterile pipettes may be used for this practice exercise.

1. Light Bunsen burner.

This expels contaminated air and prepares vacuum to withdraw fluid.

2. Set pipette aid to 5 ml or depress pipette bulb if using a pipette.
3. Select a sterile 10-ml pipette and insert into pipette aid or bulb. *Remember to handle only the large end of the pipette; avoid touching the lower two thirds.*

When using an individually wrapped pipette, be careful to open wrapper end opposite the pipette tip. Unwrap only enough of the pipette to attach end into pipette aid or bulb.

4. Quickly pass the lower two thirds of the pipette cylinder through the Bunsen flame several times. *Be sure to flame any portion of the pipette that will enter the sterile container.* Pipette should become warm, but not hot enough to melt the plastic pipette or to cause the glass pipette to crack when immersed in solution to be pipetted.

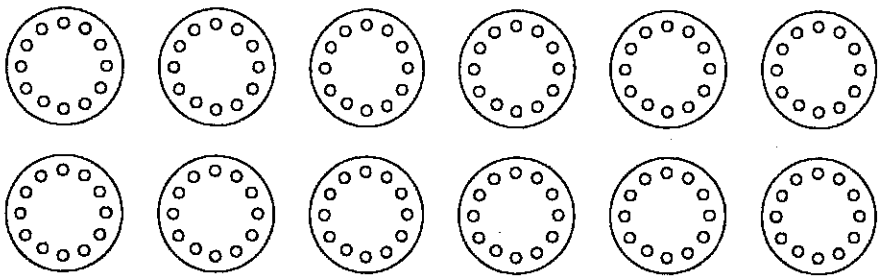
5. Hold a 50-ml conical tube containing Solution V in free hand and remove cap using little finger of hand holding pipette aid or bulb. *Do not place cap on lab bench.*
6. Quickly pass mouth of conical tube through the Bunsen flame several times, being careful not to melt the plastic.
7. Withdraw 5 ml of Solution V from the conical tube.
8. Reflame the mouth of the tube and replace top.

9. Remove the top of a sterile 15-ml culture tube with little finger of hand holding the pipette. Quickly flame the mouth of the tube.
10. Expel fluid into the culture tube. Reflame the mouth of the tube and replace top.

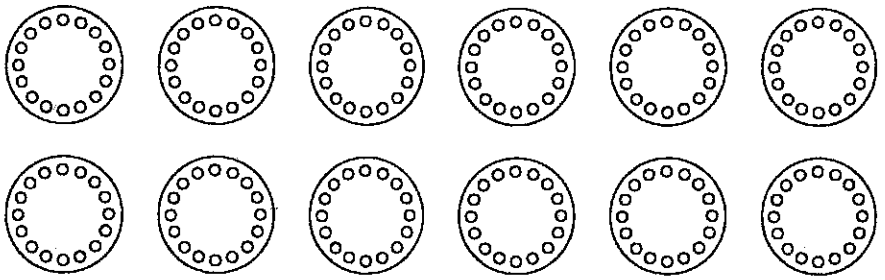
RESULTS AND DISCUSSION

Inaccurate pipetting and improper sterile technique are the chief contributors to poor laboratory results. If the handling of micropipettors and/or the use of sterile technique are still uncomfortable or difficult, take time now for additional practice. These techiques will soon become second nature.

1. Why must tubes be balanced in a microfuge rotor?
2. Use the rotor diagrams below to show how to balance 3–11 tubes (for the 12-place rotor) or 3–15 tubes (for the 16-place rotor). When balancing an odd numbers of tubes, begin with a balanced triangle of three tubes, and then add balanced pairs. Which number of tubes cannot be balanced in the 12- or 16-place rotors?



12-Place Rotor



16-Place Rotor

3. What common error in handling a micropipettor can account for pipetting too much reagent into a tube? What errors account for underpipetting?
4. When is it necessary to use sterile technique?
5. What does flaming accomplish?
6. Convert the following into microliters (μl):

   0.130 ml 0.025 ml
   0.002 ml 1.034 ml

   Convert the following into milliliters (ml):

   0.036 liter 0.803 liter
   345 μl 1345 μl

FOR FURTHER RESEARCH

Devise a method to determine the percentage of error in micropipetting. Then determine the percentage of error when purposely make pipetting mistakes with small- and large-volume micropipettors.

[redacted]

Investigating Tomato Spotted Wilt Virus: Can We Stop It?

This curriculum unit is devoted to the plant pathogen tomato spotted wilt virus. It consists of several lessons that build the students' knowledge of viruses and technologies used to diagnose and combat plant pathogens. The lessons have increasing levels of complexity and equipment requirements allowing instructors to customize the experience for their students. The concept of plant pathogens and emerging pathogens in particular, is an interdisciplinary topic, one that incorporates multiple science subjects as well as social sciences, humanities, and English.

The curriculum is still in development. Only the first lesson has a complete lesson plan associated with it. It is anticipated to be completed for the 2010 ICORE program and include revisions suggested by the 2009 ICORE program participants and researchers.

First printing and teacher review: June 2009

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Teacher Manual: Investigating Tomato Spotted Wilt Virus

Lesson 1: ImmunoStrip Assays Lesson Plan

Key Question: To what extent can TSWV be identified by direct observations? Is there a quick diagnostic tool that can be used to confirm the field observations?

Science Subject: Biology, Biotechnology, Environmental Science

Grade: 9-12

Science Concepts: Viruses, plant pathogens, insect vectors, biotechnology, immunoassays

Overall Time Estimate: 45 minutes plus 30 minute preparation

Learning Styles: Visual and kinesthetic

Vocabulary:
Immunoassay- biochemical test that measures the concentration of a substance in a biological substance, using the reaction of an antibody to its antigen.
Thrips (singular and plural)- small (~millimeter in length) insect vector of tospoviruses
TSWV- tomato spotted wilt virus
Tospoviruses- negative, single stranded RNA viruses in the genus *Tospovirus*; arbovirus, vectored by thrips
Vector- an organism that transfers (but does not cause) disease from one host to another
Virus- infectious agent unable to grow and reproduce without a host cell

Lesson Summary:

Students are given peanut plants suspected of having TSWV, and they are to observe stems, leaves, and roots to determine if they are infected. The students will perform an immunoassay to test the leaf material for the presence of TSWV.

Student Learning Objectives:
The student will be able to:

1. Describe the physical characteristics of a plant suspected of viral infection
2. Use technology to test for the presence of TSWV.

New Generation Florida Science Standards:

Nature of Science Body of Knowledge: Standard 1: The Practice of Science
Benchmark (SC.912.N.1.6): Describe how scientific inferences are drawn from scientific observations and provide examples from the content being studied

Life Science Body of Knowledge: Standard 16: Heredity and Reproduction
Benchmark (SC.912.L.16.7): Describe how viruses and bacteria transfer genetic material between cells and the role of this process in biotechnology

Life Science Body of Knowledge: Standard 17: Interdependence
Benchmark (SC.912.L.17.6): Compare and contrast the relationships among organisms, including predation, parasitism, competition, commensalism, and mutualism.

Materials:

Peanut plants showing signs of TSWV infection
Agdia ImmunoStrip™ Tests, 2-4 per group
Scissors, 1 per group
Student Manual, Lesson 1

Background Information:

Viruses are extremely small organisms that consist simply of nucleic acid and protein. These organisms are not capable of living independent of another organism; therefore, they are considered obligate parasites. They are completely dependent upon their host's living cells to produce new virus nucleic acid and structural proteins. Individual viruses cannot be seen with a standard light microscope, yet they cause tremendous problems in animals and plants.

Most of the more familiar viruses are those that cause disease in humans. From cold sores, warts and the common cold and to influenza, polio, rabies and yellow fever, viruses can cause diseases in humans that range from minor irritation to death. Likewise with plants, viruses cause diseases that have a tremendous range in their effects.

Plant viruses enter their host plants only through wounds, via pollen transmission, or by vectors. **Vectors** are animals, plants and fungi that are able to transmit the virus from an infected plant to a healthy one, such that the second plant also becomes infected. Insects are the most common and most economically important vectors of plant viruses. Aphids, leafhoppers, plant hoppers, treehoppers, whiteflies, mealybugs, plant bugs, beetles, grasshoppers and **thrips** can transmit one or more plant viruses. Aphids are the most common insect vectors, but all of the insects mentioned can be extremely important for transmission of specific viruses or virus groups.

Members of the group of plant viruses called "Tospoviruses" cause severe problems in many of the world's agricultural production systems. The **tospoviruses** in many ways are more similar to some of the viruses that are pathogens of humans and other animals than they are to other plant viruses. In fact, the tospoviruses are closely related to the hantavirus that has caused disease and death in humans in the Four Corners area of the southwestern U.S. Tomato spotted wilt virus can be thought of as the HIV of the plant world: tiny RNA viruses that are spread rapidly, quite infectious, and detrimental to the new host.

Tospoviruses are typically vectored or spread by thrips, tiny insects that live, feed and reproduce on plant leaves and flowers. Several species of thrips have been proven to transmit one or more tospovirus(es). Tomato spotted wilt virus (TSWV) is the virus for which the tospovirus was named. It is spread by thrips. To be able to transmit the virus, a thrips must acquire the virus from an infected plant as an immature larva. Once the larva has acquired the virus, it can transmit the virus to healthy plants as long as it lives. Tomato spotted wilt virus infects approximately 800 different species of plants. Many of

these are important food and oil crops. As the name indicates, this virus does infect tomato, and causes serious damage to tomato crops. It is not, however, just a tomato virus.

Peanut is an extremely important crop to areas of southern Georgia and Alabama, and northern Florida. Spotted wilt of peanut, caused by TSWV, has become one of the most serious and complex disease problems in production of peanut (*Arachis hypogaea L.)* and other crops since 1985 in the southeastern U.S. Spotted wilt is now common across the peanut growing areas of Georgia, Florida, and Alabama. In 1997 and 1998, losses to spotted wilt in peanut were estimated at $40 million/year for Georgia alone.

Spotted wilt and the thrips that vector TSWV present a perplexing challenge in epidemiology and disease control. Peanut appears to be an extremely good host for TSWV in the field. Tobacco thrips, *Frankliniella fusca* and western flower thrips, *Frankliniella occidentalis*, are both good vectors of TSWV and both infest peanut plants in Georgia and the southeastern U.S. Since the virus is spread by thrips, it follows logically that growers should be able to control the disease with insecticides that kill the thrips. However, this has not been the case. Since the emergence of spotted wilt as an important problem in peanut production in the U.S., numerous studies have generally failed to show that even long term intensive insecticide applications for control of thrips gives a reduction in incidence of spotted wilt in peanut. Growers have been educated to the fact that intensive applications of insecticides for thrips control are ineffective for preventing spotted wilt, wasteful of time and money, and serve as an unnecessary risk to the environment.

Unlike the situation with many of the fungal disease and insect problems that are controlled by fungicides or insecticides, there are no viricides for control of this disease in peanut. Although several factors have been shown to provide some suppression of spotted wilt in peanut, no single measure alone has been effective in field situations of heavy disease pressure.

A team approach in research and extension has been employed to improved management of spotted wilt in Georgia and Florida. This team has succeeded in identifying and combining some critical management inputs that collectively minimize the risk of losses to spotted wilt. The most important inputs employ genetic resistance and other cultural practices (e.g., planting time and more resistant strains). Initial experiments to elucidate the effects of these inputs were done with only one or combinations of two factors. Results from intensive cooperative research have shown that the cumulative effects of multiple management practices can have a huge impact on epidemics of spotted wilt, whereas one input alone may result in only marginal suppression of the disease.

Spotted wilt in peanut displays a wide array of symptoms that range from minor spotting on leaves to severe plant stunting and death as the root crown can become severely infected. Standard detection of spotted wilt in the field is through a visual disease intensity rating that corresponds to both incidence and severity. However, peanuts can be asymptomatic, yet still contain TSWV. **Immunoassays** of peanut leaf and root tissues from plants not displaying visible symptoms have detected the presence of TSWV. A common type of immunoassay is an enzyme-linked immunosorbant assay (ELISA) which has an enzyme that causes a color change when a specific antigen and antibody bind. The ELISA test system for tomato spotted wilt virus utilized in this activity is an immunostrip: the components (antibodies and

color-linked enzyme) are in a strip that if positive (antigens in leaf tissue) causes a colored line to appear, not unlike a pregnancy test kit. Other widely used applications of immunoassays include diagnostics of other infectious diseases such as HIV and the rapid strep test.

Advanced Preparation:

Order Agdia ImmunoStrip™ Tests (see resources below)

Obtain peanut plants suspected of infection (see resources below)

Procedure:

1. (3 minutes) Have students form groups around the student workstations (see student workstations below).
2. (5 minutes) Ask students to read the vignette to themselves, or read aloud to the class.
3. (15 minutes) Allow the students to work through *Lesson 1: ImmunoStrip Assays* in groups.
4. (15 minutes) When groups have finished and cleaned up, ask each group to report their findings on the board, making a chart with the class results. Have the students discuss their conclusions and establish that visual observation correlates with TSWV infection.
5. Assign the assessment (summary report to Farmer John) for homework, or if time allows, students may work on it while all groups are finishing.

Assessment:

Students write a report for Farmer John summarizing the steps taken to diagnose the pathology in the samples he provided.

Extension activities:

Design a webquest for students to investigate tomato spotted wilt virus.

Using the internet, have groups of students identify 5 consumer products that are made from peanuts or peanut by-products. Have groups share findings to produce one master list.

Discuss economic and socio-political impacts if the peanut industry was decimated.

Trace the epidemiology of TSWV and develop a timeline. Discuss the global impacts of emerging pathogens and infectious diseases.

Research the history of the peanut plant and the role George Washington Carver played in promoting peanut agriculture.

Extension literature:

Students read actual research journals and/or fact sheets to gather background information about tomato spotted wilt virus. Goggle scholar or a websearch for TSWV produces quite a few. UF and UGA extension offices have fact sheets accessible on the web.

Student groups read portions of research reports, summarize findings, and share with class.

Resources:

Agdia ImmunoStrip™ Tests: Item ISK 39300/0025 www.agdia.com

Peanut plants provided by Dr. Maria Gallo, University of Florida

Tomato Spotted Wilt Virus PowerPoint: Presented by Dr. Maria Gallo

Background information: http://www.caes.uga.edu/topics/diseases/tswv/peanut/intro.html
TSWV images: http://www.caes.uga.edu/topics/diseases/tswv/peanut/images.html

References:

Culbreath, A.K., Todd, J.W., and Brown, S.L. 2003. Epidemiology and management of tomato spotted wilt in peanut. Annu. Rev. Phytopathol. 43: 53-78

Murakami, M., Gallo-Meagher, M., Gorbet, D.W., and Meagher, R.L. 2006. Utilizing immunoassays to determine systemic tomato spotted wilt virus infection for elucidating field resistance in peanut. Crop Protection. 25: (235-243).

Technical sheet for Agdia ImmunoStrip™ Tests http://www.agdia.com/cgi_bin/catalog.cgi/39300

Tomato Spotted Wilt Virus PowerPoint: Presented by Dr. Maria Gallo

UF IFAS Extension Fact Sheets: http://edis.ifas.ufl.edu/

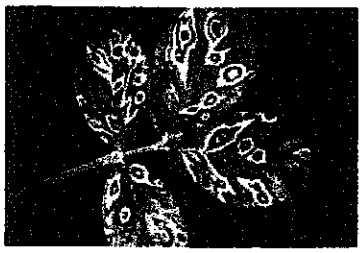
Lesson 1 Vignette:

Farmer John comes to see you at the Plant Pathogen Diagnostic Laboratory (PPDL). He has observed that some of his peanut plants are not thriving and brings samples of them to the PPDL. Based on years of field experience, Farmer John has a good idea what the culprit is, but it is suggested that laboratory confirmation be made so the best management practices can be utilized. He has asked you to determine what is afflicting his peanut plants.

Focus Questions

1. List and illustrate all observable traits or characteristics for each sample. Consider the whole plant as well as individual portions of the plant.

   Answers and illustrations will vary. Likely answers: yellow spots, stunted growth, yellow rings



Peanut leaf with concentric ring spots caused by *Tomato spotted wilt virus* (TSWV). (Courtesy A. Culbreath, J. Todd, and H. Pilcher)

2. Using your observations and knowledge, write an inference indicating the cause of Farmer Jones' problem.

   Tomato Spotted Wilt Virus
   **students will not know this answer unless they have been previously introduced to TSWV*

ImmunoStrip Assay Laboratory

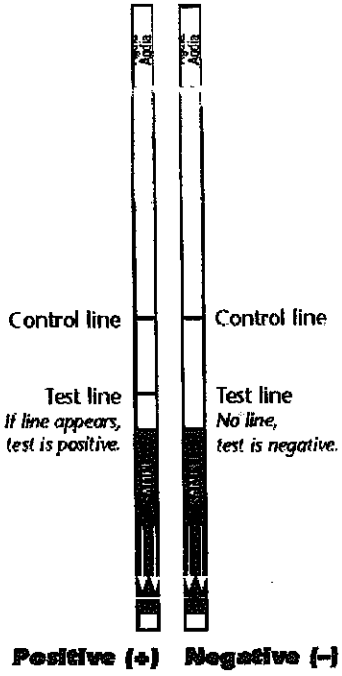
Workstation Checklist

Student workstation	Number required
Farmer John's plant samples	Variable
Sample extract bags	4
Test strips	4
Sharpie	1
Scissors	1

Procedure for Agdia ImmunoStrip™ Test

1. Each sample bag contains 3 ml of sample extract buffer. For optimal performance, the sample to be tested should be about 0.15 g (leaf area about 3 to 5 cm^2 or 1 $inch^2$).
2. Cut off the top of the sample extract bag, being careful not to spill the buffer.
3. Place sample between the mesh linings of the bag.
4. Rub the bag with a pen or blunt object to completely crush sample. Use only one sample per bag and be sure to label each bag.
5. Remove one strip from the packaging. When handling the strip, always grasp the top of the strip marked with the test name. Do not remove protective covering.
6. Keep the strip in a vertical position; insert the end of the strip marked "sample" into the extract.
7. Do not allow much more than 0.5 cm or 1/4 inch of the end of the strip to be submerged in the extract. Be sure the strip remains in the extract during the test.

RESULTS:



Agdia ImmunoStrip™ Test Results

Control line: will usually appear in 3 to 5 minutes. The control line assures that the test is working properly. If the control line does not appear, the test is invalid.

Positive result: the test line will also appear. The test line will be red to purple in color just as the control line. The color intensity of the test line will vary.

Negative result: the test line will not appear.

If you wish to keep the strips as permanent records, cut off the sample pads (green ends marked "sample") and discard. Then blot the ImmunoStrips between paper towels to remove excess liquid.

Data Collection and Analysis

1. a. What do you observe?
   b. Draw or affix your strips and describe them below.

   Answers will vary. Hopefully students will have at least one positive result and be able to describe how they know it is positive.

2. What are the results of the tests?

Extension Activity

Write a report to send to Farmer John detailing your findings. Be sure to include the methods used to reach your conclusion.

Rubric should be designed to detail the information to be included and the style the summary report should be written.

Students should be able to explain their visual observations that suggested the use of the immunoassay and how the immunoassay was performed.

Lesson 2: Genetic Engineering -- DNA Extraction

Farmer John is a very successful peanut farmer, who is farming the same land his grandfather planted many years ago. He has many friends and fellow growers across the country that call on him for advice and assistance. One such collaboration is with a researcher at a local university. Farmer John helps in the research effort of testing new seeds by donating some of his land and labor. Farmer John received peanut seeds from a researcher to plant a test field according to the research design. Both genetically engineered and wild-type seed were sent. Unfortunately, the labels that indicated which bag contained which seeds came off during the shipping process. The researcher is in another state, and it will take considerable time to have her lab analyze them. Since you did such a good job detecting TSWV in his previous peanut plants, Farmer John is again asking for your help. You are familiar with the research project, and your task is to set up an experiment to extract DNA and amplify a portion of the peanut genome to determine which seeds are genetically engineered.

Focus Questions

1. Why does the research design include genetically engineered and wild-type peanut seeds?

   Wild-type seeds function as the control group to compare what the normal infectivity rate for the field would be. The genetically engineered seeds are the test group.

2. Where in a peanut seed is the DNA located? Draw a picture of a plant cell, labeling key structures related to DNA instruction.

   Illustrations will vary. Instructor may wish to indicate key structures important in DNA extraction such as cell wall, cellular membrane, nuclear membrane, nucleus, and DNA.

3. How might you extract the DNA from the plant cell?

   Answers will vary based on the prior knowledge and experience of the students. If they have performed a DNA extraction from fruit, wheat germ, or cheek cells, they should be able to explain the steps well.

   a) *Break open the cell wall using mechanical force.*
   b) *Break open the cell membrane and nuclear membrane using detergent which disrupts the phospholipid bi-layer.*
   c) *DNA is now free in solution. Filter plant debris (cell wall, seeds, etc) to separate the DNA.*
   d) *Precipitate the DNA with ethyl alcohol.*
   e) *Collect the DNA.*

Workstation Checklist

Materials and supplies that should be present at your workstation prior to beginning this lab are listed below:

Student workstation	Number required
Seed extraction buffer	1 tube
Seed preparation buffer	1 tube
Neutralization buffer	1 tube
1.5ml eppendorf tubes	2
Sterile blue micropestle	2
0.2µl PCR tubes	2
Sterile distilled H_2O	1 tube
P20 pipette	1
P200 pipette	1
P20 pipette tips	1 box
P200 pipette tips	1 box

Instructor's (common) workstation	Number required
Thermal cycler	1
70% ethanol	1 squeeze bottle
Analytical scale	1
Scalpel	1
Kimwipes	1 box

DNA Extraction Procedure

Since even the slightest trace of unwanted DNA will give a false positive result in the subsequent PCR, always wear gloves, clean scalpel between samples, and use new pipette tips for different samples or if tip accidentally touches anything.

1. Obtain peanut seeds and identify one as Seed A and the other as Seed B.

Note: The DNA extraction steps below should be performed for each sample.

2. Remove 0.02g peanut seed using a clean scalpel. Clean blade between samples using 70% ethanol.
3. Place sample in a 1.5ml eppendorf tube. Be sure to label the tube.
4. Add 80µl sterile distilled H_20 to eppendorf tube.
5. Using a sterile blue micropestle, grind seed to a white, milky solution.
6. Label a sterile 0.2ml PCR tube with the seed sample id and group number.

7. Place 45µl Seed Extraction Solution + 5µl Seed Preparation Solution into the labeled PCR tube.
8. Add 5µl white peanut seed extract to the labeled PCR tube and mix by pipetting up and down.
9. Place sample in thermal cycler. Once all samples are collected, the instructor will start the thermal cycler "Extraction" program. (55°C for 10 minutes; 95°C for 3 minutes)
10. Remove samples from thermal cycler and add 50µl Neutralization Buffer.
11. Mix by pipetting up and down.
12. Samples can be stored at 4°C or used immediately.

Review Questions

1. What was the purpose of using the micropestle?

   Grind the peanut seed, mechanically breaking open the cell wall.

2. What might heating at 55°C accomplish? 95°C?

   55°C softens the membranes and breaks-up connective materials in the cells.
   95°C destroys DNase -- an enzyme that chews up DNA.

Lesson 3: Genetic Engineering -- DNA amplification

More resistant strains of peanuts have been produced through traditional breeding programs. However, even these are still susceptible to TSWV. Research is ongoing to create a viable and high-yielding genetically engineered peanut plant that is resistant to TSWV. Inserting part of the viral construct into the plant genome conveys increased resistance to tomato spotted wilt virus. One method that can be used to analyze seeds and determine if the viral construct has been incorporated into the peanut genome is PCR.

Focus Questions

1. What is PCR?

   Polymerase chain reaction: a method for amplifying a portion of a genome. The great power of PCR is the small amount of template needed which yields millions of copies of the targeted section of the genome.

2. What are the key steps in the PCR process?

   Denature: the reaction is heated to ~94°C, causing the double stranded DNA template to separate into single strands

   Anneal: temperature is brought down to ~60°C and the forward and reverse primers bind to the single stranded DNA

   Extend: temperature is raised to 72°C and Taq polymerase extends the single strands of DNA to form the complement strand

   These steps cycle 30-35 times, producing millions of copies of DNA, much like a photocopier copying a page from a book.

DNA Amplification Laboratory

Workstation Checklist

Materials and supplies that should be present at your workstation prior to beginning this lab are listed below:

Student workstation	Number required
DNA samples (Seed A, Seed B, Positive Control)	3
PCR reaction mix (Contains reaction buffer,Mg2+, dNTPs, Taq polymerase)	1 tube
Primer 1	1 tube
Primer 2	1 tube
1.5ml eppendorf tubes	1
0.2ml PCR tubes	5
Sterile distilled H_2O	1 tube
P20 pipette	1
P200 pipette	1
P20 pipette tips	1 box
P200 pipette tips	1 box
Vortex	1

Instructor's (common) workstation	Number required
Thermal cycler	1
Mini centrifuge (0.2ml tubes)	1

DNA Amplification Procedure

Since PCR amplifies a very small amount of DNA to a quantity that can easily be detected, even trace amounts of unwanted DNA can produce false positive results. Take care not to cross-contaminate samples and reagents by changing pipette tips between reagents and DNA extracts. Barrier or filter tips are used to prevent DNA contamination of pipettors. Always wear gloves.

1. Obtain DNA samples to be amplified from 4°C.
2. Label 0.2ml PCR tubes with DNA sample identifier and your group number.
   - Seed A DNA
   - Seed B DNA
   - Positive control
   - Negative control
3. Vortex and centrifuge all master mix reagents (PCR reaction mix, Primers, H_2O) and return to ice.
4. Prepare one master mix containing all of the master mix reagents (PCR reaction mix, Primers, H_2O). Calculate the amount of master mix reagents needed using the volumes for the basic reaction for one sample below.

   # PCR samples + one (to allow for pipetting error) = 5 total sample prep

1 PCR reaction	
PCR reaction mix	10µl
Primer 1	0.5µl
Primer 2	0.5µl
Sterile distilled H_2O	5µl
Total master mix volume per sample	16µl

5 PCR reactions	
PCR reaction mix	µl
Primer 1	µl
Primer 2	µl
Sterile distilled H_2O	µl
Total master mix volume	µl

5. Add master mix reagents to a 1.5ml eppendorf tube.
6. Mix by vortexing briefly.
7. Aliquot 16µl master mix into each labeled PCR tube.
8. Add 4µl DNA to correspondingly labeled PCR tube.
9. Add 4µl Sterile distilled H_2O to negative control PCR tube.
10. Mix samples by pipetting up and down or flicking the tube.
11. Centrifuge samples in the mini centrifuge and place in thermal cycler.
12. Once all samples are collected, the instructor will start the thermal cycler "TSWV" program.

PCR tube	Master Mix	DNA	H_2O
Seed A	16µl	4µl	--
Seed B	16µl	4µl	--
Positive control	16µl	4µl	--
Negative control	16µl	--	4µl

PCR cycling conditions

Step 1:	94°C	5 mins
Step 2:	94°C	1 min
Step 3:	60°C	30 sec
Step 4:	72°C	2 mins
Step 5:	Go to step 2; repeat 34 times	
Step 6:	72°C	3 mins
Step 7:	4°C	forever

The complete cycle takes around 2h 40 mins.

Review Questions

1. Why is it important to keep PCR reagents cold?

   Keeping the reagents cold prevents them from 'working' before all components of the PCR reaction are mixed.

2. How might incorporating viral constructs into the peanut genome confer resistance?

   The single stranded virus will bind to the complimentary sequence. Once it is bound, it cannot be transcribed and translated → no infection can occur.

Extension Activity

Review primary research literature to see the progress toward genetically engineered peanut plants.

Students can be provided research articles and/or web addresses. Genetically engineered peanut plants are making progress, although there is not a GMO peanut used in consumer goods as yet.

Lesson 4: Genetic Engineering – Gel Electrophoresis

Polymerase chain reaction amplifies a targeted region of a genome, creating millions of copies of the target. To determine if amplification was successful, gel electrophoresis is performed. The fragment length and band intensity reveals an approximate qualitative estimate of PCR.

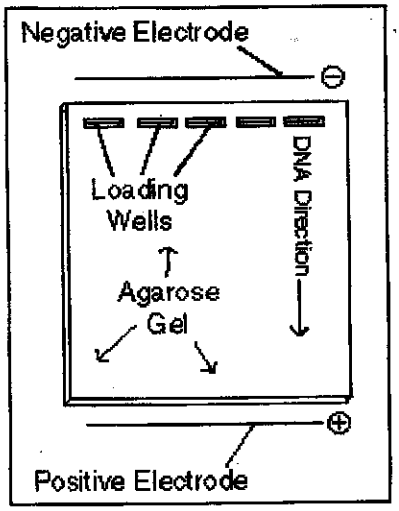
Focus Questions

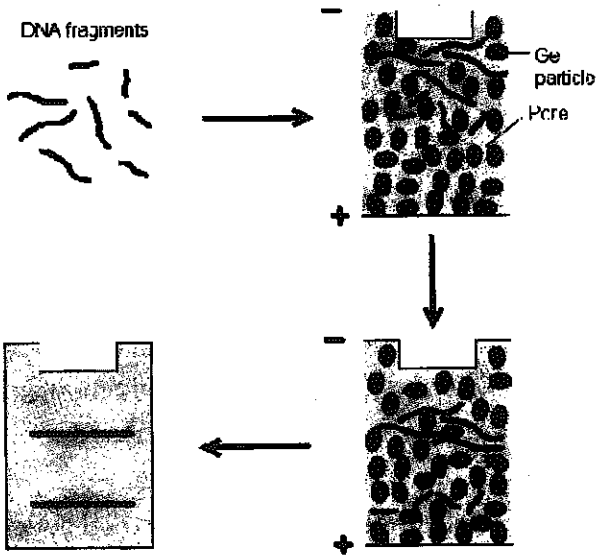
1. a. What is gel electrophoresis?

   b. Describe how gel electrophoresis works.

   Gel electrophoresis is a method used to separate nucleic acids and/or proteins. Nucleic acids are generally separated using an agarose gel. Protein separation makes use of acrylamide gels.

   The agarose gel is a colloidal mixture, with small agarose beads essentially suspended as a matrix. DNA samples are added at the top of the gel in wells. The wells are small divots in the gel that allow the DNA to sit and then move through the gel once an electrical current is applied. DNA is negatively charged, so it will move toward the positive electrode. As DNA fragments move through the matrix, smaller fragments move through faster. This is what allows DNA to separate based on size.





Workstation Checklist

Materials and supplies that should be present at your workstation prior to beginning this lab are listed below:

Student workstation	Number required
PCR samples (Seed A, Seed B, Positive Control, Negative Control)	4
Loading dye	1 tube
Molecular weight marker	1 tube
Sterile distilled H_2O	1 tube
P20 pipette	1
P20 pipette tips	1 box
E-Gel® PowerBase™ and adaptor plug	1
E-Gel® 1.2% with SYBR Safe™	1
Vortex	1

Instructor's (common) workstation	Number required
Mini centrifuge (0.2ml tubes)	1
UV Transilluminator	1
E-Gel Safe Imager Transilluminator	4

Prepare samples for loading

1. Obtain PCR samples from thermal cycler or 4°C.
2. Add 2µl loading dye to each PCR sample.
3. Mix samples by pipetting up and down or flicking tube.
4. Centrifuge samples in the mini centrifuge.

Prepare gel

1. Plug PowerBase™ into an electrical outlet.
2. Remove gel cassette from package and insert the gel (with comb in place) into the base right edge first. The Invitrogen logo should be located at the bottom of the base. Press firmly at the top and bottom to seat the gel cassette in the PowerBase™. A steady, red light will illuminate if the gel cassette is correctly inserted.
3. Pre-run the gel (with comb in place) by pressing and holding either the 15 minute or 30 minute button until the red light turns to a flashing green light indicating the start of the 2 minute pre-run. Release the button. At the end of the pre-run, the current will automatically shut off and the power base will display a flashing red light and beep rapidly. Press either button to stop the beeping.

Load prepared samples

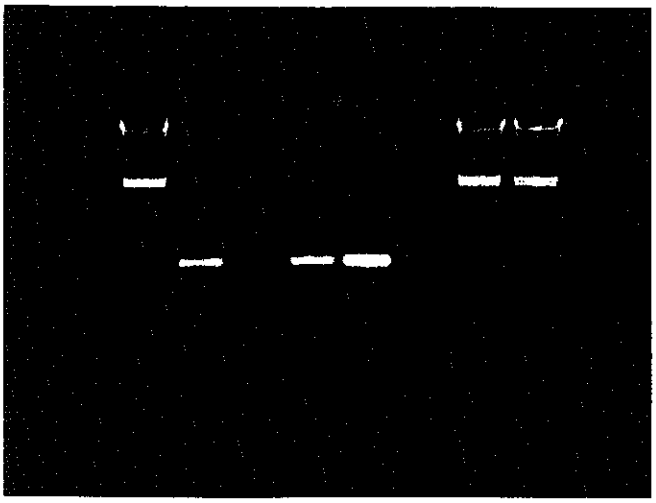
1. Remove and discard comb from the E-Gel® cassette.
2. Add 10µl prepared samples to wells 5-8 (Seed A, Seed B, Positive Control, Negative Control).
3. Add 10µl molecular weight marker to wells 4 and 9.
4. Add 10µl sterile distilled H_2O to wells 4-9.
5. Add 20µl sterile distilled H_2O to all remaining empty wells.

Run gel

1. Press and release the 30 minute button on the E-Gel® PowerBase™ to begin electrophoresis.
2. At the end of the run, the current will automatically shut off and the power base will display a flashing red light and beep rapidly. Press either button to stop the beeping.
3. Remove the gel cassette and analyze your results by viewing on one of the transilluminators. The instructor will take a picture of your gel as well.

Data Collection and Analysis

1. Draw or affix a picture of your gel. Label each of the lanes.



Lane 1: Molecular weight marker
Lane 2, 7, 8: Seed A
Lane 3: Seed B
Lane 4, 5: Positive control
Lane 6: Negative control

2. Discuss your results and explain any results not expected.

   Answers will vary. Possible errors: incorrect DNA used during PCR, not changing tips during PCR set-up causing cross-contamination, general procedural errors.

3. Which if either of the seeds is genetically engineered?

   Seed A is genetically engineered.

Review Questions

1. What is the chemical is used to visualize the DNA bands in the agarose gel? What type of light must be used?

   SYBR Safe is used to stain the DNA in this activity. This is a safe alternative to ethidium bromide, with integrates with the DNA and fluoresces. UV or blue light needs to be used to visualize SYBR Safe.

2. What is the function of the molecular weight marker?

   The molecular weight marker acts as an internal ruler for the gel with bands of known size. Since all gels will run differently, the standard allows us to determine the approximate size of our unknown DNA samples.

3. How does the band intensity relate to the amount of DNA present?

   The SYBR Safe binds to the DNA. The more DNA present, the more SYBR Safe is bound, causing the band to fluoresce more brightly.

Extension Activity

Prepare report to send to Farmer John and Professor M detailing your findings. Be sure to include the methods used to reach your conclusion.

Rubric should be designed to detail the information to be included and the style the summary report should be written.

Students should be able to describe the steps used in extraction, amplification, and electrophoresis. Students will need to be informed of what they amplified or the bioinformatics extension can be used to generate this information and the report written afterwards.

Extension Activity: Using Bioinformatics

To verify results, you send the amplified sample to the sequencing core laboratory. They send back the following 228 base sequence.

TGACCTTCAGAAGGCTTGATAGCTTGATCAGGGTCAGGCTTGTTGAAGAAACTGGGAATTCTGAGAATCTCAATACTATCAAA
TCTAAGATTGCTTCCCATCCTTTGATTCAAGCCTATGGATTACCTCTCGATGATGCAAAGTCTGTGAGACTTGCCATAATGCTGG
GAGGTAGCTTACCTCTTATTGCTTCAGTTGATAGCTTTGAGATGATCAGTGTTGTCTTGGC

1. Is the size of the sequence consistent with your gel electrophoresis results?

   Yes it is. The gel indicated a fragment less than 300bp. It was difficult to tell how large it was, but the students should have been able to estimate based on the molecular weight marker.

2. Perform a *nucleotide* BLAST of the sequence against the *nucleotide collection* database. What gene does this sequence most closely align with?
   http://blast.ncbi.nlm.nih.gov/Blast.cgi

   - Tomato spotted wilt virus N mRNA for Nucleocapsid (N) protein

   The entire nucleocapsid sequence is below. The primers are highlighted in yellow and the sequenced region includes the yellow and blue.

ATGTCTAAGGTTAAGCTCACTAAGGAAAGCATTGTTGCTTTGTTGACACAAGGCAAAGACCTTGAGTTTGAGGAAGATCAGAA
TCTGGTAGCATTCAACTTCAAGACTTTTTGTCTGGAAAACATCGACCAGATCAAGAAGATGAGCGTTATTTCATGTCTGACATTC
CTAAAGAATCGTCAGAGCATAATGAAGGTTATTAAGCAAAGCGATTTTACTTTTGGTAAAATTACCATAAAGAAAACTTCAGAC
AGGATTGGAGGCACTGACATGACCTTCAGAAGGCTTGATAGCTTGATCAGGGTCAGGCTTGTTGAAGAAACTGGGAATTCTG
AGAATCTCAATACTATCAAATCTAAGATTGCTTCCCATCCTTTGATTCAAGCCTATGGATTACCTCTCGATGATGCAAAGTCTGT
GAGACTTGCCATAATGCTGGGAGGTAGCTTACCTCTTATTGCTTCAGTTGATAGCTTTGAGATGATCAGTGTTGTCTTGGCTAT
ATATCAGGATGCAAAATACAAGGACCTCGGGATCGACCCAAAGAAGTATGACACCAAGGAAGCCTTAGGAAAAGTTTGCACT
GTGCTGAAAAGCAAAGCATTTGAAATGAATGAAGATCAGGTGAAGAAGGGGAAAGAGTATGCTGCTATACTTAGCTCCAGCA
ATCCTAATGCTAAAGGGAGTGTTGCTATGGAACATTACAGTGAAACTCTTAACAAGTTCTATGAAATGTTCGGGGTTAAAAAG
CAGGCAAAACTCGCAGAACTTGCTTGA

Student Manual: Investigating Tomato Spotted Wilt Virus

Lesson 1: ImmunoStrip Assays

Farmer John comes to see you at the Plant Pathogen Diagnostic Laboratory (PPDL). He has observed that some of his peanut plants are not thriving and brings samples of them to the PPDL. Based on years of field experience, Farmer John has a good idea what the culprit is, but it is suggested that laboratory confirmation be made so the best management practices can be utilized. He has asked you to determine what is afflicting his peanut plants.

Focus Questions

1. List and illustrate all observable traits or characteristics for each sample. Consider the whole plant as well as individual portions of the plant.

2. Using your observations and knowledge, write an inference indicating the cause of Farmer Jones' problem.

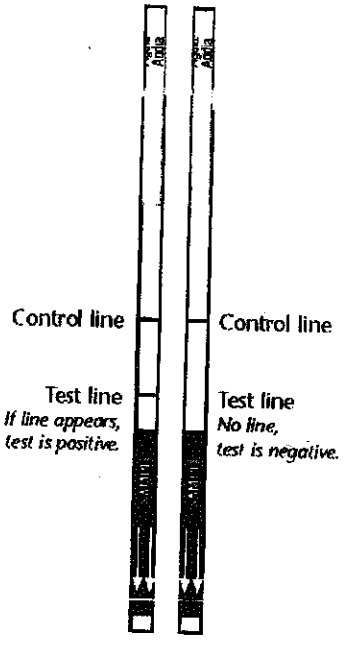
ImmunoStrip Assay Laboratory
Workstation Checklist

Student workstation	Number required
Farmer John's plant samples	Variable
Sample extract bags	4
Test strips	4
Sharpie	1
Scissors	1

Procedure for Agdia ImmunoStrip™ Test

8. Each sample bag contains 3 ml of sample extract buffer. For optimal performance, the sample to be tested should be about 0.15 g (leaf area about 3 to 5 cm^2 or 1 $inch^2$).
9. Cut off the top of the sample extract bag, being careful not to spill the buffer.
10. Place sample between the mesh linings of the bag.
11. Rub the bag with a pen or blunt object to completely crush sample. Use only one sample per bag and be sure to label each bag.
12. Remove one strip from the packaging. When handling the strip, always grasp the top of the strip marked with the test name. Do not remove protective covering.
13. Keep the strip in a vertical position; insert the end of the strip marked "sample" into the extract.
14. Do not allow much more than 0.5 cm or 1/4 inch of the end of the strip to be submerged in the extract. Be sure the strip remains in the extract during the test.

RESULTS:



Agdia ImmunoStrip™ Test Results

Control line: will usually appear in 3 to 5 minutes. The control line assures that the test is working properly. If the control line does not appear, the test is invalid.

Positive result: the test line will also appear. The test line will be red to purple in color just as the control line. The color intensity of the test line will vary.

Negative result: the test line will not appear.

If you wish to keep the strips as permanent records, cut off the sample pads (green ends marked "sample") and discard. Then blot the ImmunoStrips between paper towels to remove excess liquid.

Data Collection and Analysis

1. a. What do you observe?
   b. Draw or affix your strips and describe them below.

2. What are the results of the tests?

Extension Activity

Write a report to send to Farmer John detailing your findings. Be sure to include the methods used to reach your conclusion.

Lesson 2: Genetic Engineering -- DNA Extraction

Farmer John is a very successful peanut farmer, who is farming the same land his grandfather planted many years ago. He has many friends and fellow growers across the country that call on him for advice and assistance. One such collaboration is with a researcher at a local university. Farmer John helps in the research effort of testing new seeds by donating some of his land and labor. Farmer John received peanut seeds from a researcher to plant a test field according to the research design. Both genetically engineered and wild-type seed were sent. Unfortunately, the labels that indicated which bag contained which seeds came off during the shipping process. The researcher is in another state, and it will take considerable time to have her lab analyze them. Since you did such a good job detecting TSWV in his previous peanut plants, Farmer John is again asking for your help. You are familiar with the research project, and your task is to set up an experiment to extract DNA and amplify a portion of the peanut genome to determine which seeds are genetically engineered.

Focus Questions

1. Why does the research design include genetically engineered and wild-type peanut seeds?

2. Where in a peanut seed is the DNA located? Draw a picture of a plant cell, labeling key structures related to DNA instruction.

3. How might you extract the DNA from the cell?

Workstation Checklist

Materials and supplies that should be present at your workstation prior to beginning this lab are listed below:

Student workstation	Number required
Seed extraction buffer	1 tube
Seed preparation buffer	1 tube
Neutralization buffer	1 tube
1.5ml eppendorf tubes	2
Sterile blue micropestle	2
0.2µl PCR tubes	2
Sterile distilled H_2O	1 tube
P20 pipette	1
P200 pipette	1
P20 pipette tips	1 box
P200 pipette tips	1 box

Instructor's (common) workstation	Number required
Thermal cycler	1
70% ethanol	1 squeeze bottle
Analytical scale	1
Scalpel	1
Kimwipes	1 box

DNA Extraction Procedure

Since even the slightest trace of unwanted DNA will give a false positive result in the subsequent PCR, always wear gloves, clean scalpel between samples, and use new pipette tips for different samples or if tip accidentally touches anything.

1. Obtain peanut seeds and identify one as Seed A and the other as Seed B.

Note: The DNA extraction steps below should be performed for each sample.

2. Remove 0.02g peanut seed using a clean scalpel. Clean blade between samples using 70% ethanol.
3. Place sample in a 1.5ml eppendorf tube. Be sure to label the tube.
4. Add 80µl sterile distilled H_20 to eppendorf tube.
5. Using a sterile blue micropestle, grind seed to a white, milky solution.
6. Label a sterile 0.2ml PCR tube with the seed sample id and group number.

7. Place 45µl Seed Extraction Solution + 5µl Seed Preparation Solution into the labeled PCR tube.
8. Add 5µl white peanut seed extract to the labeled PCR tube and mix by pipetting up and down.
9. Place sample in thermal cycler. Once all samples are collected, the instructor will start the thermal cycler "Extraction" program. (55°C for 10 minutes; 95°C for 3 minutes)
10. Remove samples from thermal cycler and add 50µl Neutralization Buffer.
11. Mix by pipetting up and down.
12. Samples can be stored at 4°C or used immediately.

Review Questions

1. What was the purpose of using the micropestle?

2. Different solutions were used during the extraction process. Why?

3. What might heating at 55°C accomplish? 95°C?

Lesson 3: Genetic Engineering – DNA Amplification

More resistant strains of peanuts have been produced through traditional breeding programs. However, even these are still susceptible to TSWV. Research is ongoing to create a viable and high-yielding genetically engineered peanut plant that is resistant to TSWV. Inserting part of the viral construct into the plant genome conveys increased resistance to tomato spotted wilt virus. One method that can be used to analyze seeds and determine if the viral construct has been incorporated into the peanut genome is PCR.

Focus Questions

1. What is PCR?

2. What are the key steps in the PCR process?

DNA Amplification Laboratory
Workstation Checklist

Materials and supplies that should be present at your workstation prior to beginning this lab are listed below:

Student workstation	Number required
DNA samples (Seed A, Seed B, Positive Control)	3
PCR reaction mix (Contains reaction buffer,Mg2+, dNTPs, Taq polymerase)	1 tube
Primer 1	1 tube
Primer 2	1 tube
1.5ml eppendorf tubes	1
0.2ml PCR tubes	5
Sterile distilled H_2O	1 tube
P20 pipette	1
P200 pipette	1
P20 pipette tips	1 box
P200 pipette tips	1 box
Vortex	1

Instructor's (common) workstation	Number required
Thermal cycler	1
Mini centrifuge (0.2ml tubes)	1

DNA Amplification Procedure

Since PCR amplifies a very small amount of DNA to a quantity that can easily be detected, even trace amounts of unwanted DNA can produce false positive results. Take care not to cross-contaminate samples and reagents by changing pipette tips between reagents and DNA extracts. Barrier or filter tips are used to prevent DNA contamination of pipettors. Always wear gloves.

1. Obtain DNA samples to be amplified from 4°C.
2. Label 0.2ml PCR tubes with DNA sample identifier and your group number.
   - Seed A DNA
   - Seed B DNA
   - Positive control
   - Negative control
3. Vortex and centrifuge all master mix reagents (PCR reaction mix, Primers, H_2O) and return to ice.
4. Prepare one master mix containing all of the master mix reagents (PCR reaction mix, Primers, H_2O). Calculate the amount of master mix reagents needed using the volumes for the basic reaction for one sample below.

   # PCR samples + one (to allow for pipetting error) = 5 total sample prep

1 PCR reaction	
PCR reaction mix	10µl
Primer 1	0.5µl
Primer 2	0.5µl
Sterile distilled H_2O	5µl
Total master mix volume per sample	16µl

5 PCR reactions	
PCR reaction mix	µl
Primer 1	µl
Primer 2	µl
Sterile distilled H_2O	µl
Total master mix volume	µl

5. Add master mix reagents to a 1.5ml eppendorf tube.
6. Mix by vortexing briefly.
7. Aliquot 16µl master mix into each labeled PCR tube.
8. Add 4µl DNA to correspondingly labeled PCR tube.
9. Add 4µl Sterile distilled H_2O to negative control PCR tube.
10. Mix samples by pipetting up and down or flicking the tube.
11. Centrifuge samples in the mini centrifuge and place in thermal cycler.
12. Once all samples are collected, the instructor will start the thermal cycler "TSWV" program.

PCR tube	Master Mix	DNA	H_2O
Seed A	16µl	4µl	–
Seed B	16µl	4µl	–
Positive control	16µl	4µl	–
Negative control	16µl	--	4µl

PCR cycling conditions (The complete cycle takes around 2h 40 mins.)

Step 1: 94°C	5 mins
Step 2: 94°C	1 min
Step 3: 60°C	30 sec
Step 4: 72°C	2 mins
Step 5: Go to step 2; repeat 34 times	
Step 6: 72°C	3 mins
Step 7: 4°C	forever

Review Questions

1. Why is it important to keep PCR reagents cold?

2. How might incorporating viral constructs into the peanut genome confer resistance?

Extension Activity

Review primary research literature to see the progress toward genetically engineered peanut plants.

Lesson 4: Genetic Engineering – Gel Electrophoresis

Polymerase chain reaction amplifies a targeted region of a genome, creating millions of copies of the target. To determine if amplification was successful, gel electrophoresis is performed. The fragment length and band intensity reveals an approximate qualitative estimate of PCR.

Focus Questions

1. a. What is gel electrophoresis?
   b. Describe how gel electrophoresis works?

DNA Gel Electrophoresis Laboratory
Workstation Checklist

Materials and supplies that should be present at your workstation prior to beginning this lab are listed below:

Student workstation	Number required
PCR samples (Seed A, Seed B, Positive Control, Negative Control)	4
Loading dye	1 tube
Molecular weight marker	1 tube
Sterile distilled H_2O	1 tube
P20 pipette	1
P20 pipette tips	1 box
E-Gel® PowerBase™ and adaptor plug	1
E-Gel® 1.2% with SYBR Safe™	1
Vortex	1

Instructor's (common) workstation	Number required
Mini centrifuge (0.2ml tubes)	1
UV Transilluminator	1
E-Gel Safe Imager Transilluminator	4

Prepare samples for loading

1. Obtain PCR samples from thermal cycler or 4°C.
2. Add 2µl loading dye to each PCR sample.
3. Mix samples by pipetting up and down or flicking tube.
4. Centrifuge samples in the mini centrifuge.

Prepare gel

1. Plug PowerBase™ into an electrical outlet.
2. Remove gel cassette from package and insert the gel (with comb in place) into the base right edge first. The Invitrogen logo should be located at the bottom of the base. Press firmly at the top and bottom to seat the gel cassette in the PowerBase™. A steady, red light will illuminate if the gel cassette is correctly inserted.
3. Pre-run the gel (with comb in place) by pressing and holding either the 15 minute or 30 minute button until the red light turns to a flashing green light indicating the start of the 2 minute pre-run. Release the button. At the end of the pre-run, the current will automatically shut off and the power base will display a flashing red light and beep rapidly. Press either button to stop the beeping.

Load prepared samples

1. Remove and discard comb from the E-Gel® cassette.
2. Add 10µl prepared samples to wells 5-8 (Seed A, Seed B, Positive Control, Negative Control).
3. Add 10µl molecular weight marker to wells 4 and 9.
4. Add 10µl sterile distilled H_2O to wells 4-9.
5. Add 20µl sterile distilled H_2O to all remaining empty wells.

Run gel

1. Press and release the 30 minute button on the E-Gel® PowerBase™ to begin electrophoresis.
2. At the end of the run, the current will automatically shut off and the power base will display a flashing red light and beep rapidly. Press either button to stop the beeping.
3. Remove the gel cassette and analyze your results by viewing on one of the transilluminators. The instructor will take a picture of your gel as well.

Data Collection and Analysis

1. Draw or affix a picture of your gel. Label each of the lanes.

2. Discuss your results and explain any results not expected.

3. Which if either of the seeds is genetically engineered?

Review Questions

1. What is chemical is used to visualize the DNA bands in the agarose gel? What type of light must be used?

2. What is the function of the molecular weight marker?

3. How does the band intensity relate to the amount of DNA present?

Extension Activity

Prepare report to send to Farmer John and Professor M detailing your findings. Be sure to include the methods used to reach your conclusion.

Extension Activity: Using Bioinformatics

To verify results, you send the amplified sample to the sequencing core laboratory. They send back the following 228 base sequence.

TGACCTTCAGAAGGCTTGATAGCTTGATCAGGGTCAGGCTTGTTGAAGAAACTGGGAATTCTGAGAATCTCAATACTATCAAA
TCTAAGATTGCTTCCCATCCTTTGATTCAAGCCTATGGATTACCTCTCGATGATGCAAAGTCTGTGAGACTTGCCATAATGCTGG
GAGGTAGCTTACCTCTTATTGCTTCAGTTGATAGCTTTGAGATGATCAGTGTTGTCTTGGC

3. Is the size of the sequence consistent with your gel electrophoresis results?

4. Perform a *nucleotide* BLAST of the sequence against the *nucleotide collection* database. What gene does this sequence most closely align with?
   http://blast.ncbi.nlm.nih.gov/Blast.cgi

Introduction to Emerging Pathogens

Compiled from the UF-HHMI
ICORE Institute 6/2009

Information provided by

- J. Glenn Morris, Jr., MD, MPH&TM
- *UF Emerging Pathogens Institute*
- Maria Gallo, Ph.D.

What is an "Emerging Pathogen"?

A disease-causing microorganism that is newly recognized and/or increasing in frequency in human, animal, or plant populations

- Examples:
  - West Nile, Dengue, Chikungunya
  - Avian Flu/Pandemic Flu
  - Multi-drug resistant TB
  - MRSA (methicillin-resistant *Staphylococcus aureus*)
  - AIDS
  - Sars: Severe Acute Respiratory Syndrome
  - Plant diseases such as: Citrus greening
  - Soybean rust
  - Tomato Spotted Wilt Virus (TSWV)

Common Disease Organisms

- Bacteria, Fungi, Viruses
- Can be host specific or have a broad host range (more difficult to control)
- Over 250 known water, soil, and foodborne human diseases

Why Do Pathogens Emerge?

- Appearance of new/genetically different strains
  - New virulence factor(s)
  - Modifications permitting crossing of species barriers
  - Antigenic change
- Changes in opportunities for pathogen growth and spread (often anthropogenic)
  - Environmental change, changes in ecologic niches, changes in host behavior
  - Introduction of a pathogen into a new geographic area (intentional or otherwise)
- Change in host susceptibility
  - loss of herd immunity
  - immunosuppression

Contributing Factors: Changes in Human Demographics and Behavior

- Increasing numbers of people susceptible to infections with specific potential pathogens
- Rural urbanization allows infections to arise in isolated rural areas
- Decay of basic sanitation practices
- Breakdown of Public Health Measures (Pathogens reemerge when classic measures breakdown)
- Microbial Adaptation
- Changes in Agricultural Practices (increased river and stream pollution by agricultural waste and runoff)

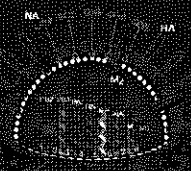
Pandemic: explosive, frequently recurring, disease that spreads rapidly through populations and across continents

- Why do pandemics occur?
- Often environmental or animal reservoir
- Genetic structure that encourages reassortment/ recombination resulting in appearance of new sub-types
- Easy person-to-person transmission in a sustained manner
- Ability to cause serious illness

Swine Flu!!

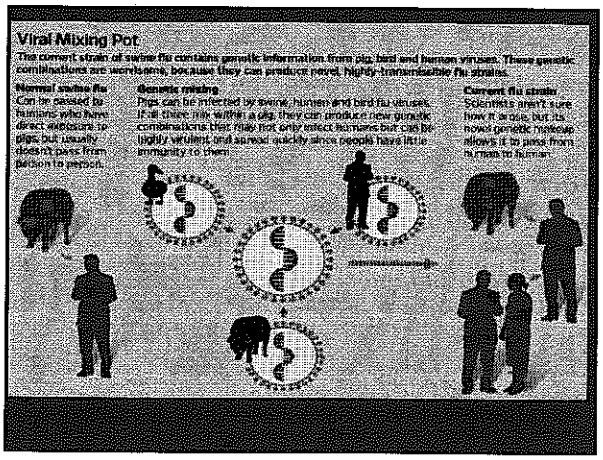
Influenza A

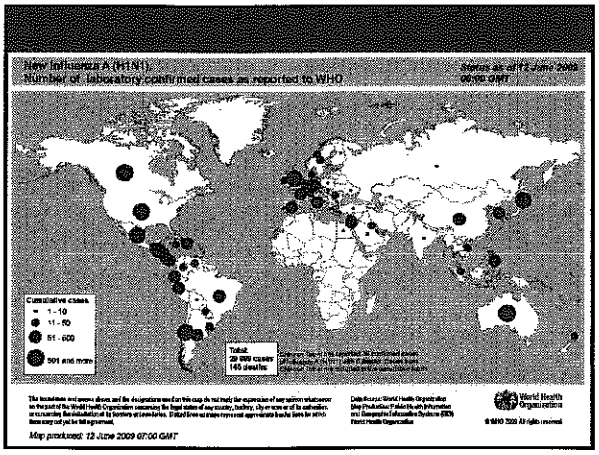
- Orthomyxovirus
  - Enveloped
  - Single strand RNA (negative sense)
  - 8 segmented genes
  - Surface proteins used for subtyping
    - Hemagglutinin (HA)
    - Neuraminidase (NA)



Influenza A Ecology

- Aquatic birds are the global reservoir
  - Source of all HA and NA subtype combinations
  - New hosts established by interspecies transmission
    - Virus adaptation to new host species
    - Sustained in new host by virus spread between individuals
- Natural host range
  - Avian
    - Wild birds
    - Domesticated poultry
  - Mammalian
    - Human, pig, horse, sea mammals
    - In 2004 work at UF College Vet Med added dogs to this list





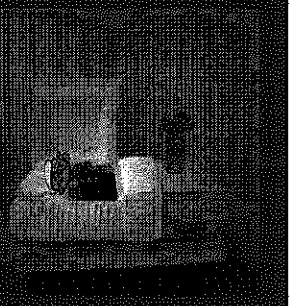
"Swine Flu"

- Appears to be very similar to "seasonal" flu
  - Similar/lower transmission rate
  - Similar virulence
    - Normally mortality rate of 1/400-500 cases
    - Lacks critical genes found in high virulence strains
    - ? Increased virulence in Mexico
  - Appear to be more cases in young adults
- Susceptible to antiviral agents oseltamivir (TamiFlu) and zanamivir (Relenza)

Management of Influenza in the Community

- Schools are virus exchange systems
- Children are highly efficient spreaders

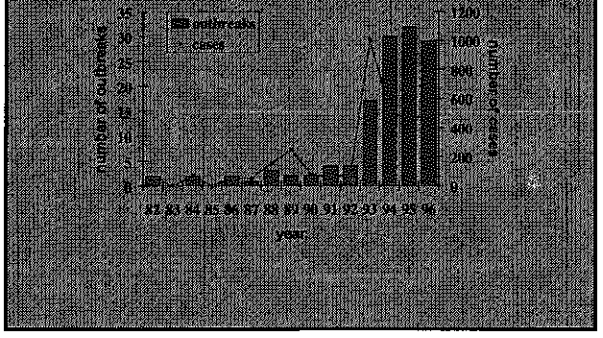
Foodborne Illness



E. coli O157:H7: Microbiology

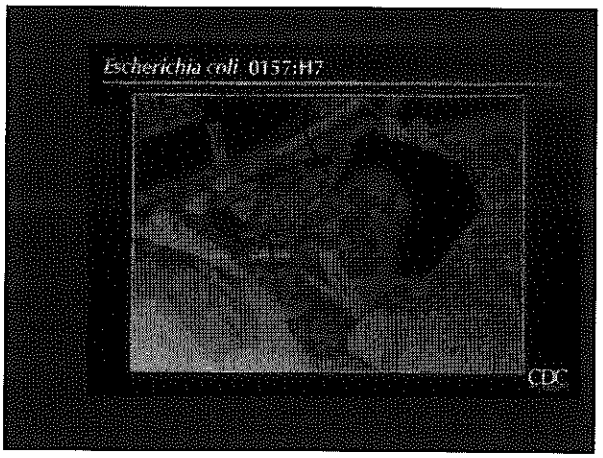
- Serologic classification
  - O group 157 (somatic antigen), H group 7 (flagellar antigen)
  - In other countries (particular south of equator) other serotypes more common
- Carriage of critical virulence genes: Enterohemorrhagic *E. coli* (EHEC)
  - Attaching and effacing genes
  - Shiga toxin
  - Other plasmid associated virulence genes

Reported Outbreaks/Cases of *E. coli* O157:H7, 1982-96



E. coli O157:H7: Clinical Features

- 30-95% of patients have bloody diarrhea
- Patients generally afebrile, but with abdominal tenderness, leukocytosis
- 2-7% develop hemolytic-uremic syndrome (up to 22% in some high risk populations)
- Mortality rates of <1%-35%, depending on affected population



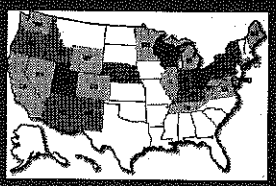
E. coli O157:H7 Outbreaks, 1982-1996 Vehicles/Routes of Transmission

Source	# Outbreaks (%) n = 126
ground beef	40 (32%)
water (swimming/drinking)	13 (10%)
vegetables/salads/salad bars	11 (9%)
apple cider	4 (3%)
roast beef	3 (2%)
venison	3 (2%)
person-to-person	21 (17%)
misc/unknown	30 (24%)

E. coli O157:H7: Epidemiology

- Present as asymptomatic colonizer in intestinal tract of cattle
  - isolated from 28% of cattle at time of slaughter
  - Some farms appear to be "hot spots"
- May contaminate surface of meat during slaughter/processing
- Present in a food (ground beef) which is not always fully cooked by the consumer
- Low infectious dose
  - <100 bacteria can cause illness
  - can be transmitted person-to-person

Spinach and *E. coli* O157:H7

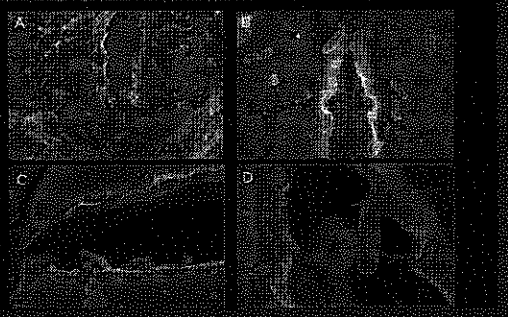


Number of cases occurring between Aug. 19 and October 6, 2006, by state

Spinach and *E. coli* Outbreak

- 199 persons infected with outbreak strain of *E. coli* O157:H7
  - 11% children under 5 years old
  - 71% female
- 102 (51%) hospitalized
- 31 (16%) developed HUS
  - 29% in children (<18 years old)
  - 8% in persons aged 18-59
  - 14% in persons aged 60 and older
- 3 deaths (2 elderly, one 2-year-old child)
- 11 of 13 spinach packages positive for the outbreak strain were from a single manufacturing facility on a single day

Spinach and EHEC



Plant Diseases: Bacteria

- 10-15% crop loss
- Spots: fruits, leaves or stems
  - decrease photosynthesis
  - disfigure fruit

Softrots: enzymes produced by the bacteria cause tissue to become soft and liquid
- leads to post-harvest loss

Wilts: clog conductive tissue so that water and minerals cannot get from roots to leaves.

Plant Disease: Fungi

- Can have devastating losses
  - produce toxin, secrete a slime, attack seedlings at germination, dead spots

100,000 known species with more than 8000 causing plant disease

Plant Disease Fungi

- Toxic Fungus: Aspergillus flavus
- Corn, peanut and others – aflatoxin
- Rusts: most destructive – famines, economic depression
- Blights: e.g. Southern corn leaf flight
  1970: Destroyed 15% of US corn crop
  1846: great potato famine in Ireland with entire crop wiped out in 1 week > 1 million deaths Initiated emigration to US 4-8 million people in 10 years

More Plant Disease Fungi

- Blights: Ergot of grains
  - Salem witch trials (rye ergot)
  - Forms hallucinogenic drugs in bread
  - Crazy behavior, "bewitched" (stoned)

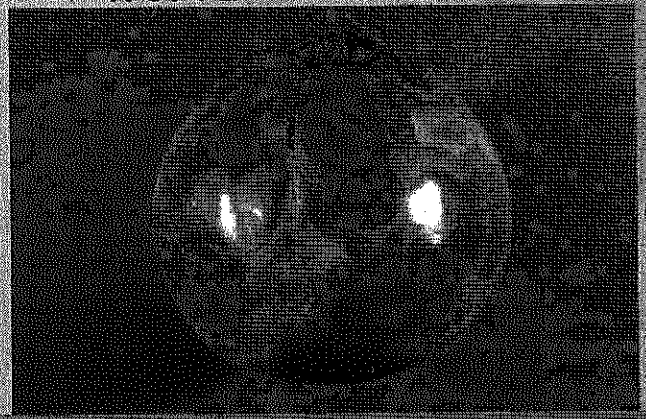
Plant Disease Viruses

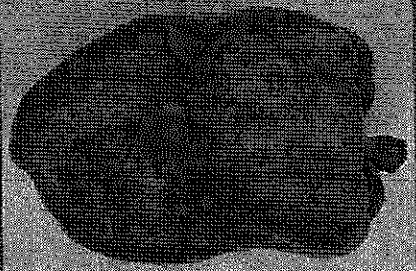
- Smallest infectious agents
- Need electron microscope to see them
- Few genes, few proteins produced
- Many are parasitic
- Many are vectored by insects:
  - aphids, thrips, leafhoppers, whiteflies
  - probing mouth parts

Tomato Spotted Wilt Virus
(TSWV)

Tomato Spotted Wilt Virus

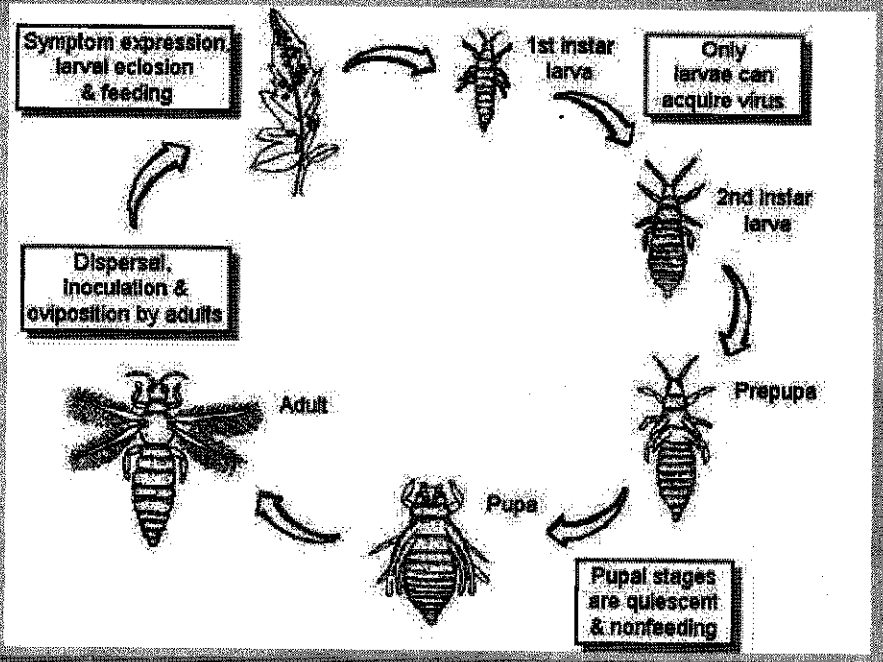
- Spotted wilt of tomato was first described in 1915 in Australia
- Today it is one of the 10 most economically destructive plant viruses
  - Worldwide losses exceed one billion dollars annually
- Host Plants
- 800+ known host species (80+ plant families)
  - Both monocots and dicots, includes horticultural and agronomic crops
  - Found in temperate, subtropical and tropical regions of the world





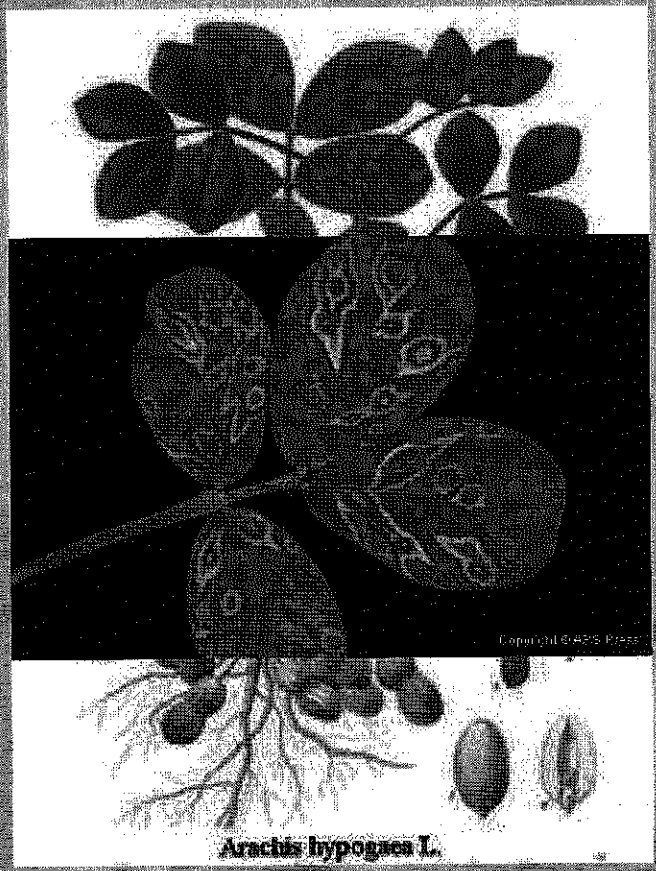
Tomato Spotted Wilt Virus (cont.)

- Vectored and spread by thrips
  - Tobacco thrips (*Frankliniella fusca*)
  - Western flower thrips (*Frankliniella occidentalis*)
- Lifecycle (20-30 days from egg to adult)



TSWV in Peanut (*Arachis hypogaea L.*)

- Spotted wilt caused by TSWV in peanut
  - First observed in 1971
  - Became one of the most serious diseases in 1980s
- In Georgia, spot wilt lead to a 7.5% reduction in crop value that equals $31.7 million in losses (2005)

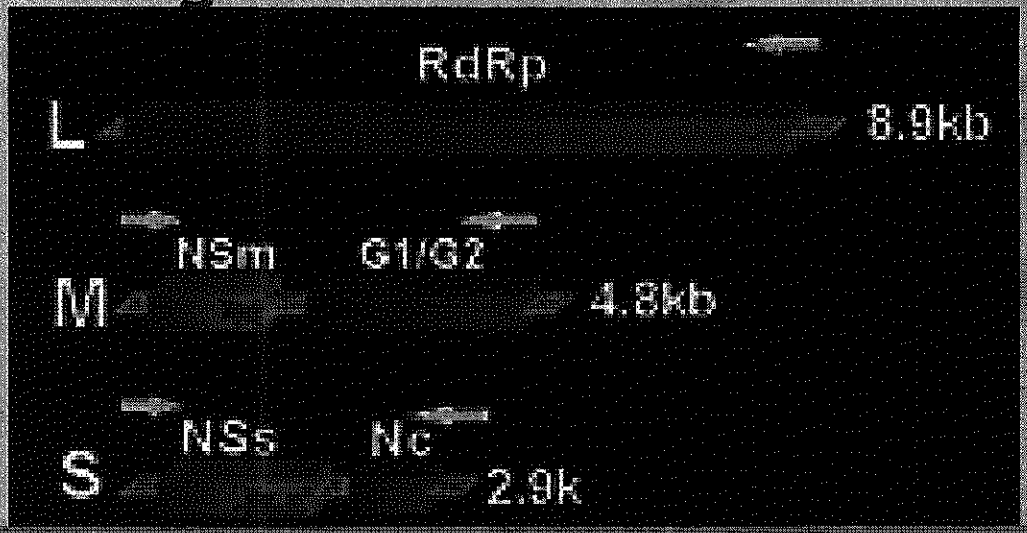


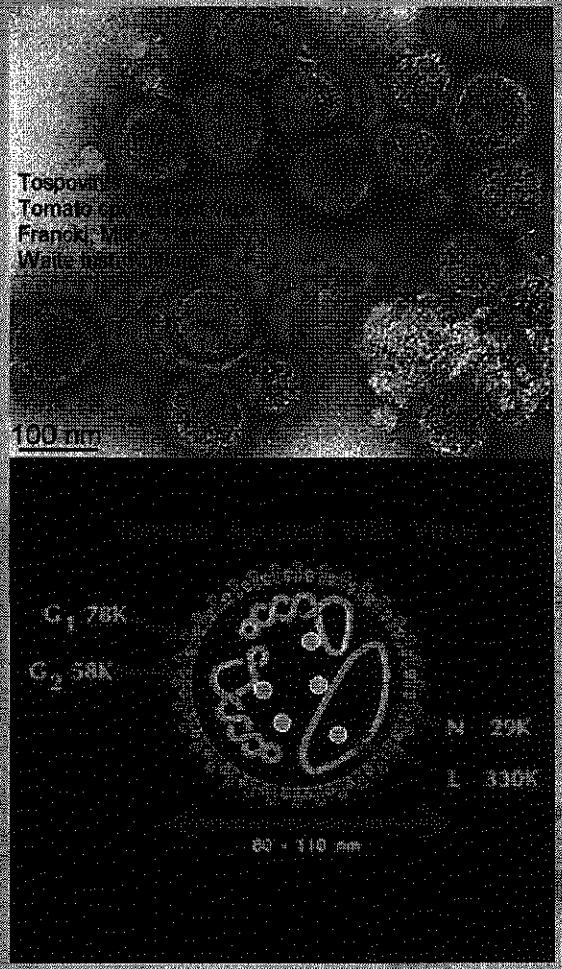
Management of TSWV in Peanut

- Single measure: control of the thrips vectors
- Integrated management
  - Make use of highly resistant cultivars
  - Chemical practices
  - Cultural practices
- One of the most important factors
  - Deployment of resistant cultivars

Tomato Spotted Wilt Virus (cont.)

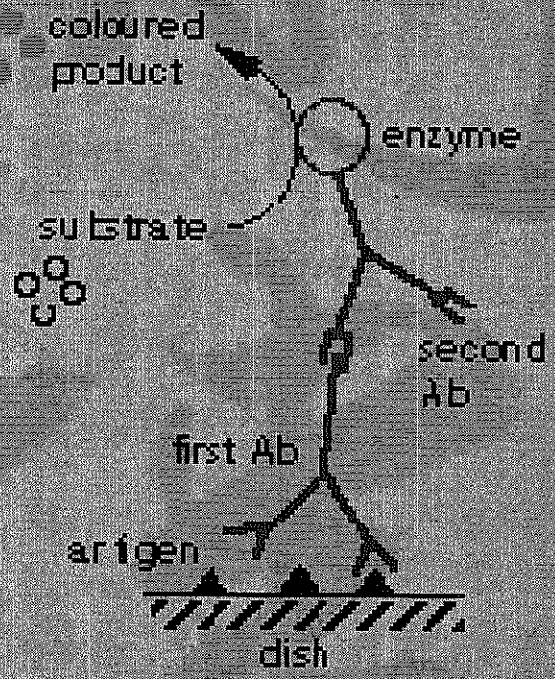
- Family: *Bunyaviridae*
  Genus: *Tospovirus*
- Virions
  - 80-120 nm
  - Pleomorphic particles
- Single-stranded RNA





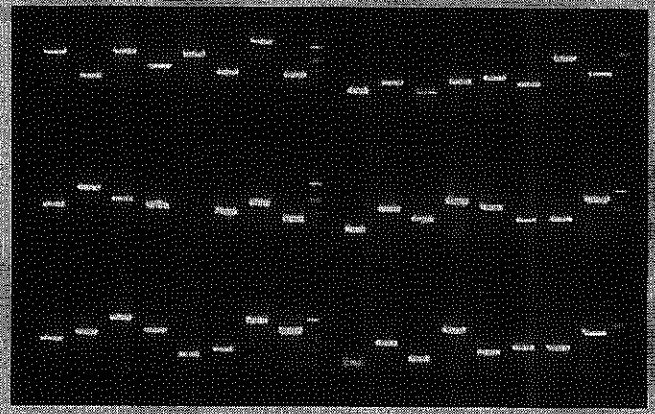
Detection Tools

- 1. Traditional Detection Techniques



- 2. Nucleic Acid-Based Detection Methods

Gene-specific PCR, restriction fragment length polymorphism (RFLP), RT-PCR, etc.



ICORE TSWV Experiment

University of Florida
Center for Precollegiate Education and Training

Polymerase Chain Reaction

Overview

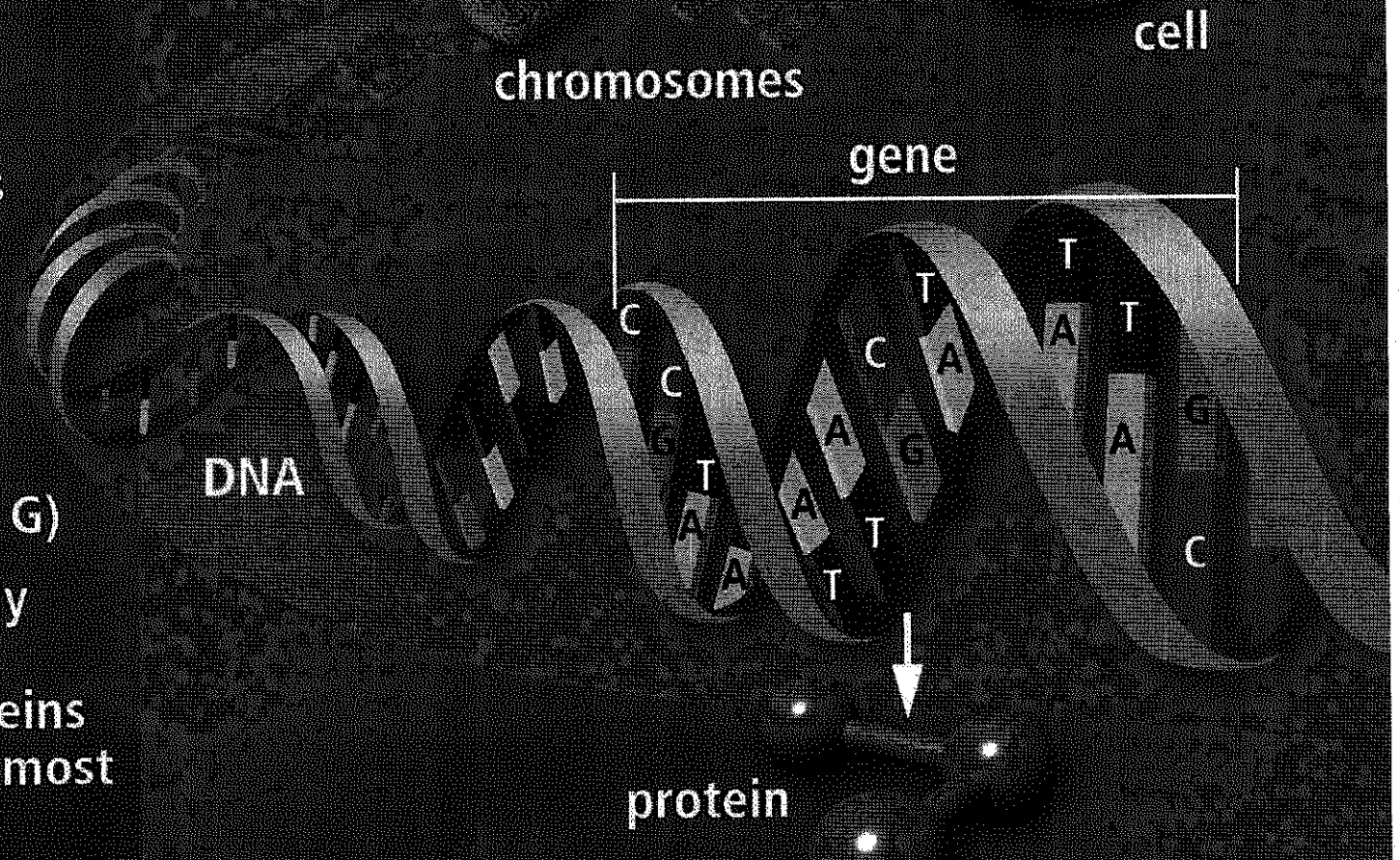
- Amplify a specific sequence of your own DNA in a tube
- Looking for a particular piece of DNA that is present in the genes of many, but not all, people
- Analysis of the data generated in this experiment will enable you to determine if you carry this specific DNA sequence

DNA the molecule of life

Trillions of cells

Each cell:

- 46 human chromosomes
- 2 meters of DNA
- 3 billion DNA subunits (the bases: A, T, C, G)
- Approximately 30,000 genes code for proteins that perform most life functions



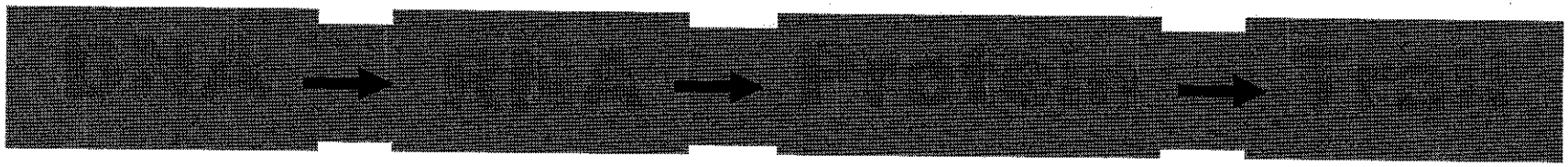
Y-GG (1-1085)

U.S. Department of Energy Genomics:GTL Program http://www.ornl.gov/hgmis

Introduction to PCR

- Genome: composed of DNA:
  - Number of genes, size

- Molecular biology: the study of genes and the molecular details that regulate the flow of genetic information from generation to generation:



Introduction to PCR

- 1983 Kary Mullis (Cetus Corp) developed the molecular biology technique that has revolutionized genetic research: the Polymerase Chain Reaction

- PCR quickly transformed molecular biology into a multidisciplinary research field

Introduction to PCR

- Object of PCR: produce a large amount of DNA in a test tube (*in vitro*) starting with only a trace amount
- Controlled enzymatic amplification of a DNA sequence, or gene, of interest
- Tiny amounts of genomic DNA from a drop of blood, single hair, or cheek cell can generate enough DNA to study

Introduction to PCR

- PCR impacted several areas of genetic research:
  - used as a medical diagnostic tool to detect specific mutations that may cause genetic disease
  - used in criminal investigations and courts of law to identify suspects
  - used in the sequencing of the human genome

Introduction to PCR

- Prior to PCR, the use of molecular biology techniques for therapeutic, forensic, pharmaceutical, agricultural, or medical diagnostic purposes was neither practical nor cost-effective

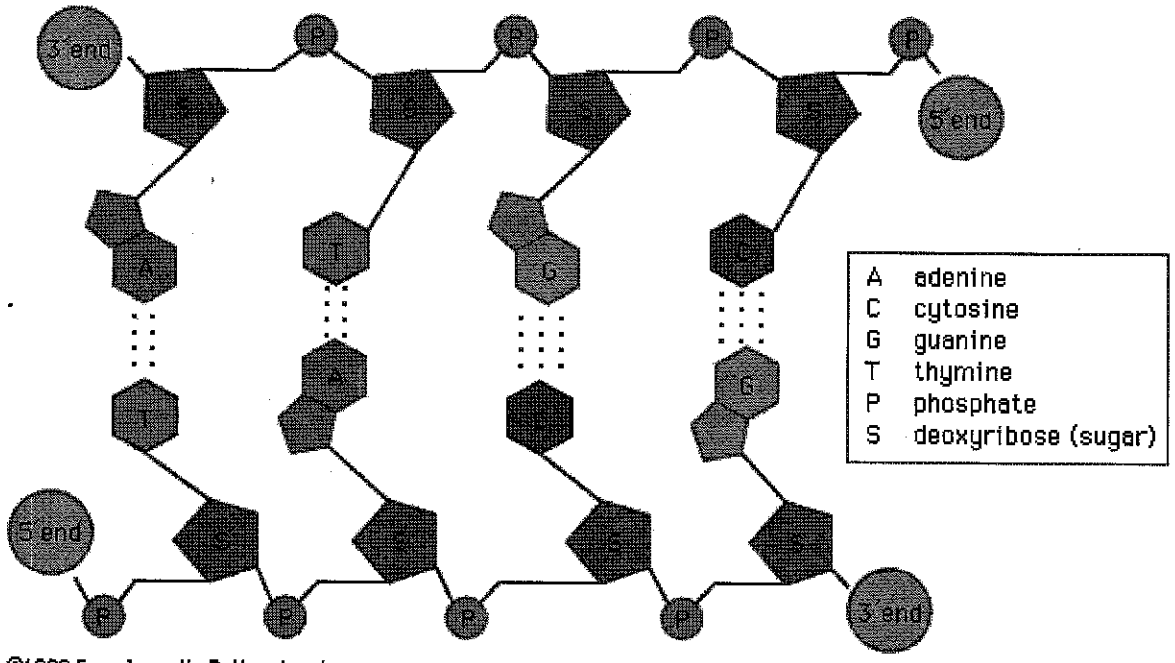
- The development of PCR transformed molecular biology from a difficult science to one of the most accessible and widely used disciplines of biotechnology

PCR Amplification

- DNA basics:
  - Deoxyribonucleic acid
  - Phosphate, sugar, ATCG
  - 3 billion letters → ~30,000 genes

DNA structure

- Double Helix
- Ladder
  - Sides = Phosphate/ Sugar backbone
  - Rungs (steps) = Nucleotides
    - A, T, C, G
    - Also called bases



PCR amplification

- With PCR, you can target and make millions of copies (amplify) a specific piece of DNA (or gene) out of a complete genome

- In our experiment, you will amplify a region of the peanut genome

PCR amplification

- PCR makes use of the same basic processes that cells use to duplicate their DNA (replication)
  - Complementary DNA strand hybridization
  - DNA strand synthesis via DNA polymerase

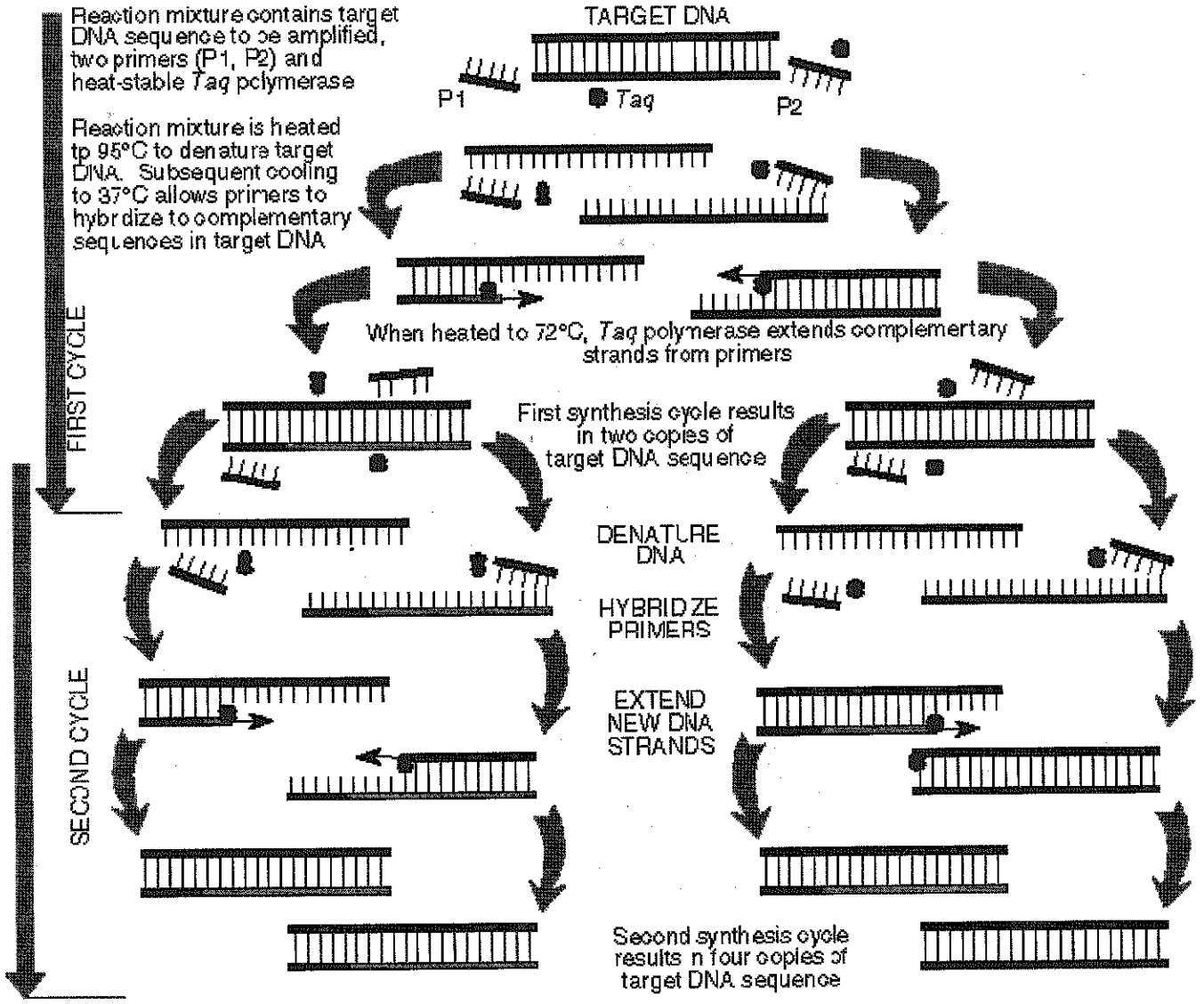
PCR amplification

- Recipe for a PCR amplification of DNA:
  - DNA template: Your DNA, containing the intact sequence of DNA to be amplified
  - Deoxynucleotides: raw material of DNA
  - DNA polymerase: enzyme that assembles the nucleotides into a new DNA chain
  - Magnesium ion (Mg^{2+}): cofactor (catalyst) required by DNA polymerase to create the DNA chain
  - Primers: pieces of DNA complementary to the template that tell DNA polymerase exactly where to start
  - Salt buffer: provides the optimum ionic environment and pH for the PCR reaction

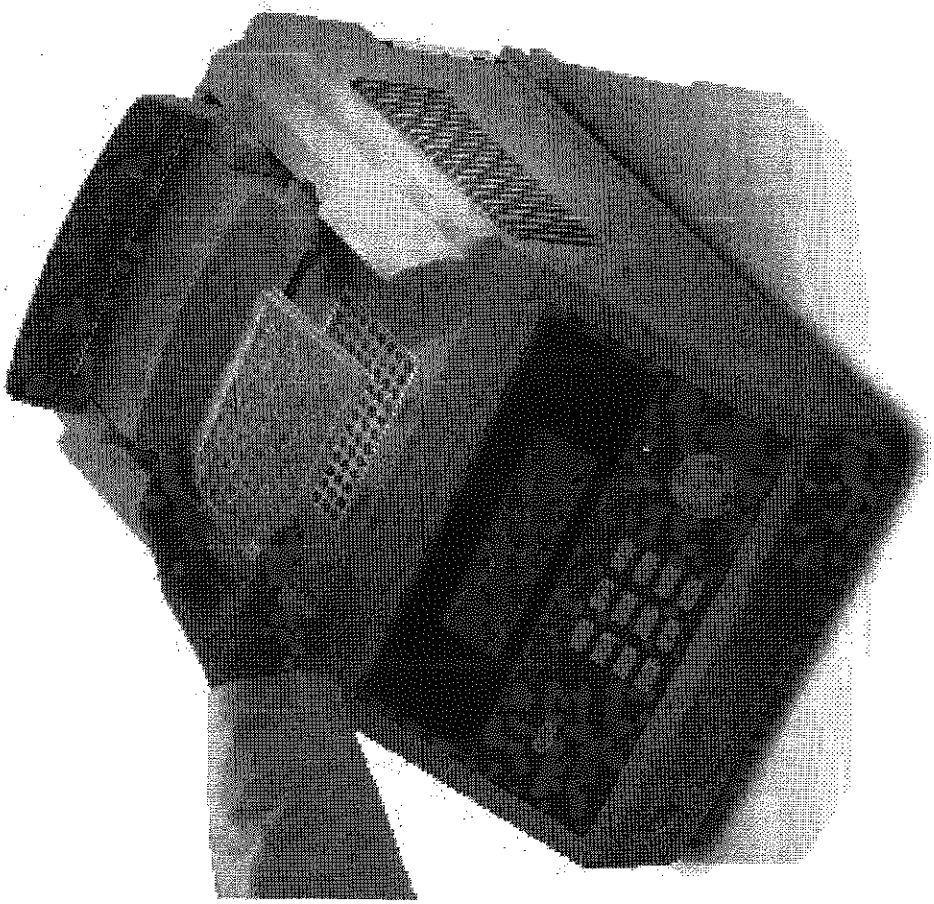
PCR amplification

- PCR: three main steps
  - Denaturation (94 degrees, 1min)
  - Annealing (60 degrees, 1min)
  - Extension (72 degrees, 2min)
    - 40 cycles
    - Amplified exponentially
    - Results in 1.1×10^{12} sets of precise-length DNA

DNA Amplification Using Polymerase Chain Reaction



Source: *DNA Science*, see Fig. 13.

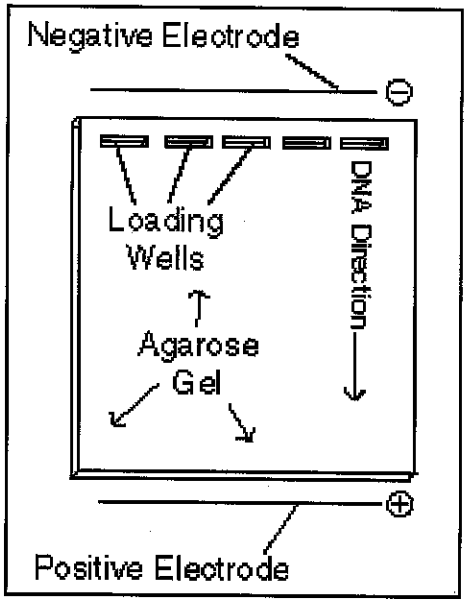


PCR amplification

- Now…time to amplify your DNA
- Please follow the protocol

Gel electrophoresis

- Migration of charged particles under the influence of an electric field
  - DNA is negatively charged
  - Moves toward positive electrode



Gel electrophoresis

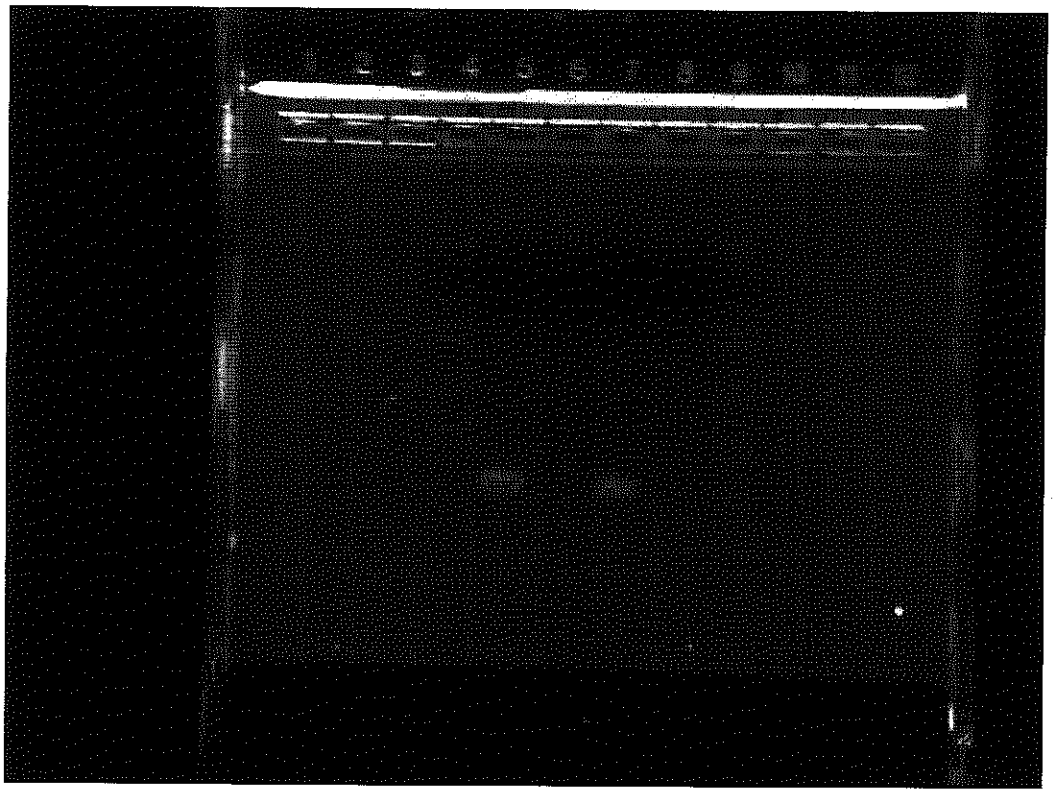
- Horizontal gel made of agarose
  - Agarose is a substance extracted from seaweed
  - The agarose powder is mixed with a liquid, heated to boiling, poured into a tray with a comb to form a rigid gel, much like making a Jell-O mold
  - DNA moves through the agarose gel because of the wells the comb makes
  - Different size fragments of DNA move at different speeds

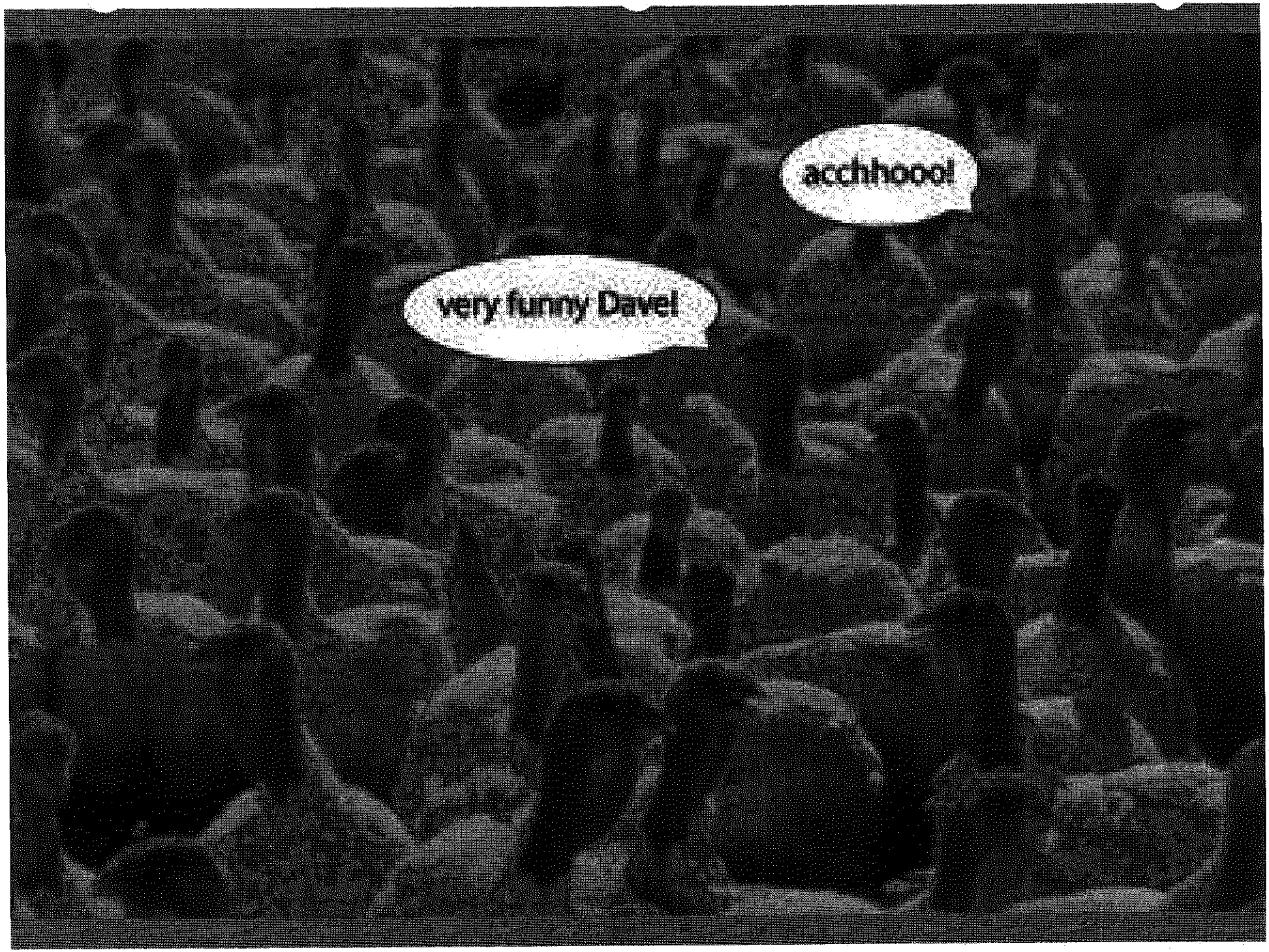
Adding DNA to agarose gel

- DNA is suspended in a liquid
- Blue dye is added to the DNA
  - This makes the DNA sink to the bottom of the well
  - Allows visualization of DNA sample
  - Track the progress of the DNA

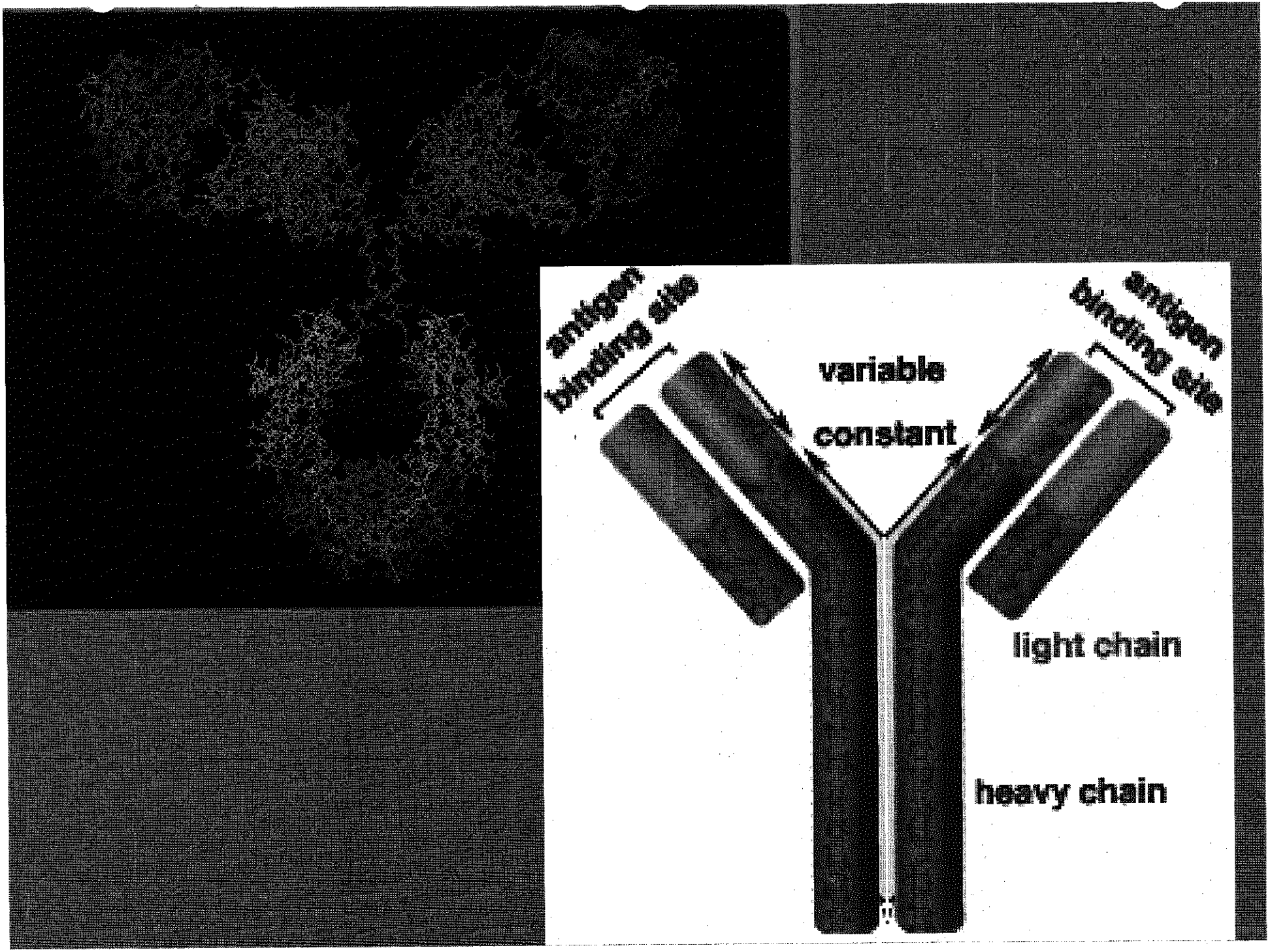
Gel electrophoresis of amplified PCR samples

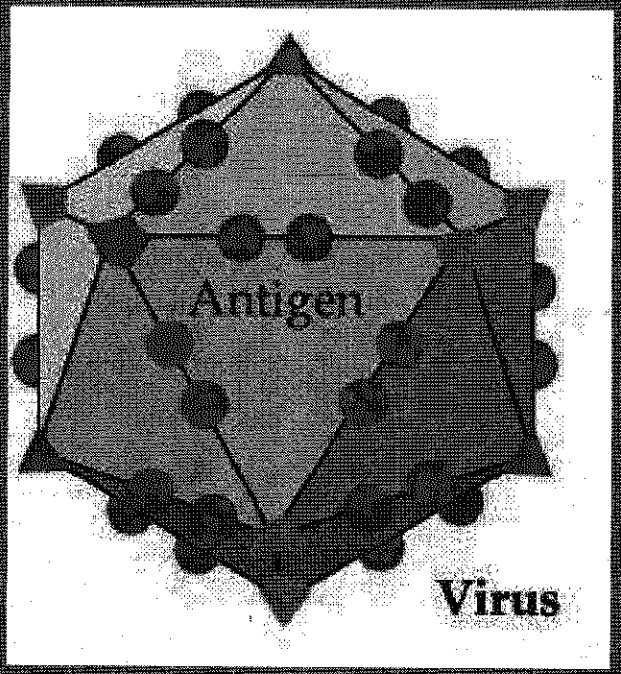
- Results with E-Gel®

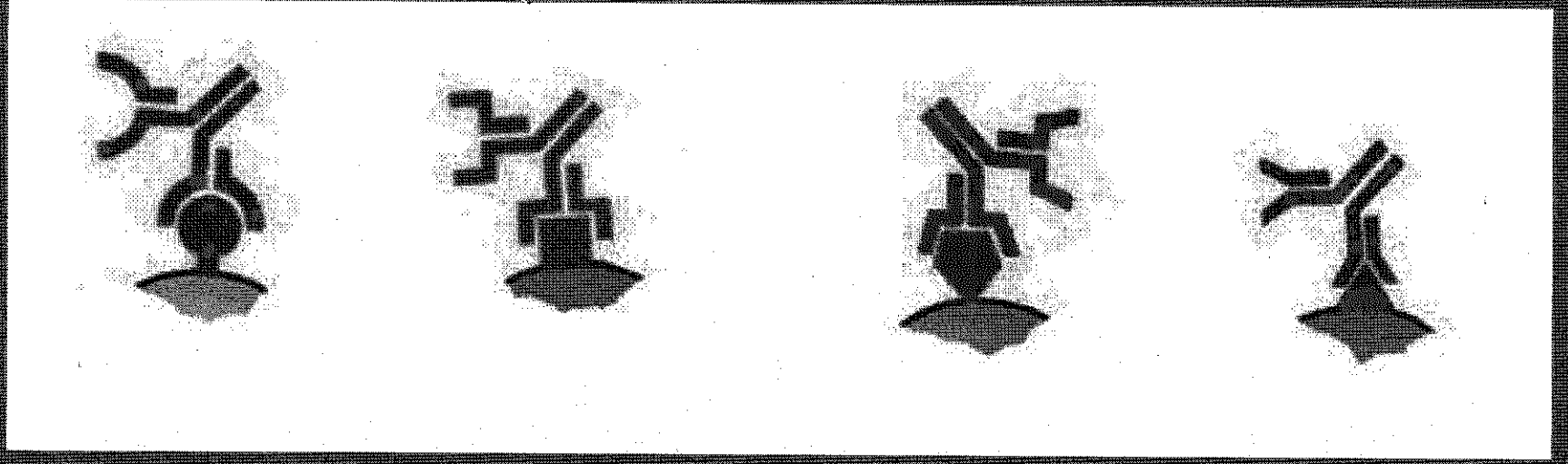



acchhooo!
very funny Dave!

Florida Bird Flu


antigen binding site
antigen binding site
variable
constant
light chain
heavy chain

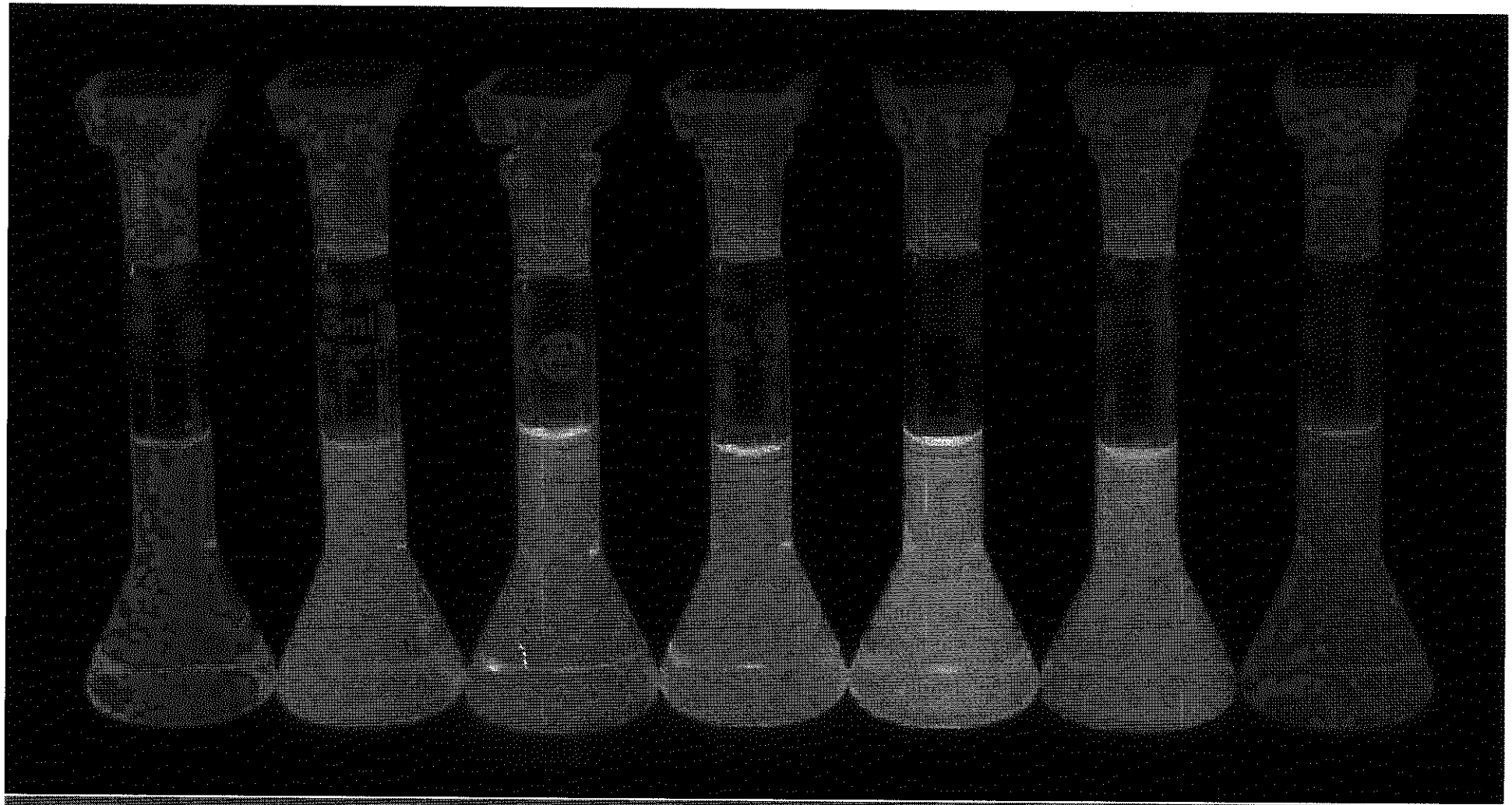

Antigen
Virus

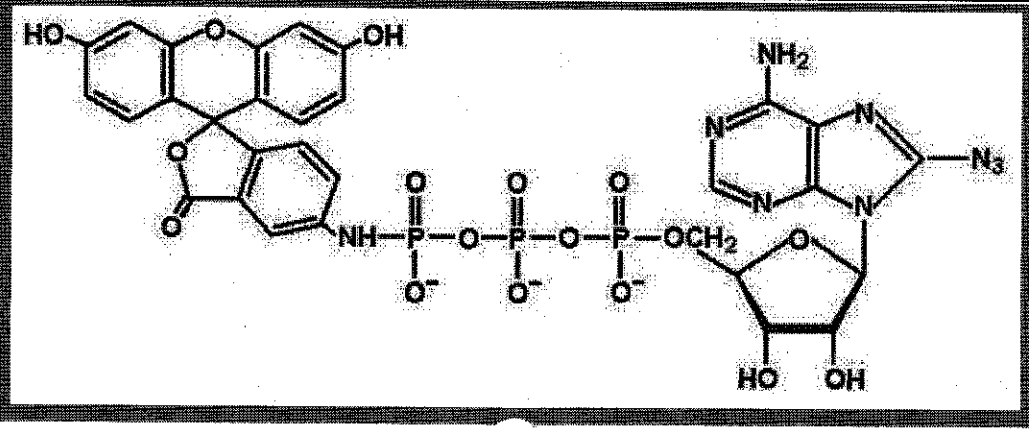


136832
arrayit.com
Antibody Array

Attach specific Antigen
artnyt.com
136832

Sandwiched Array




HO
O
OH
O
O
NH
P
O
O⁻
O
P
O
O⁻
O
P
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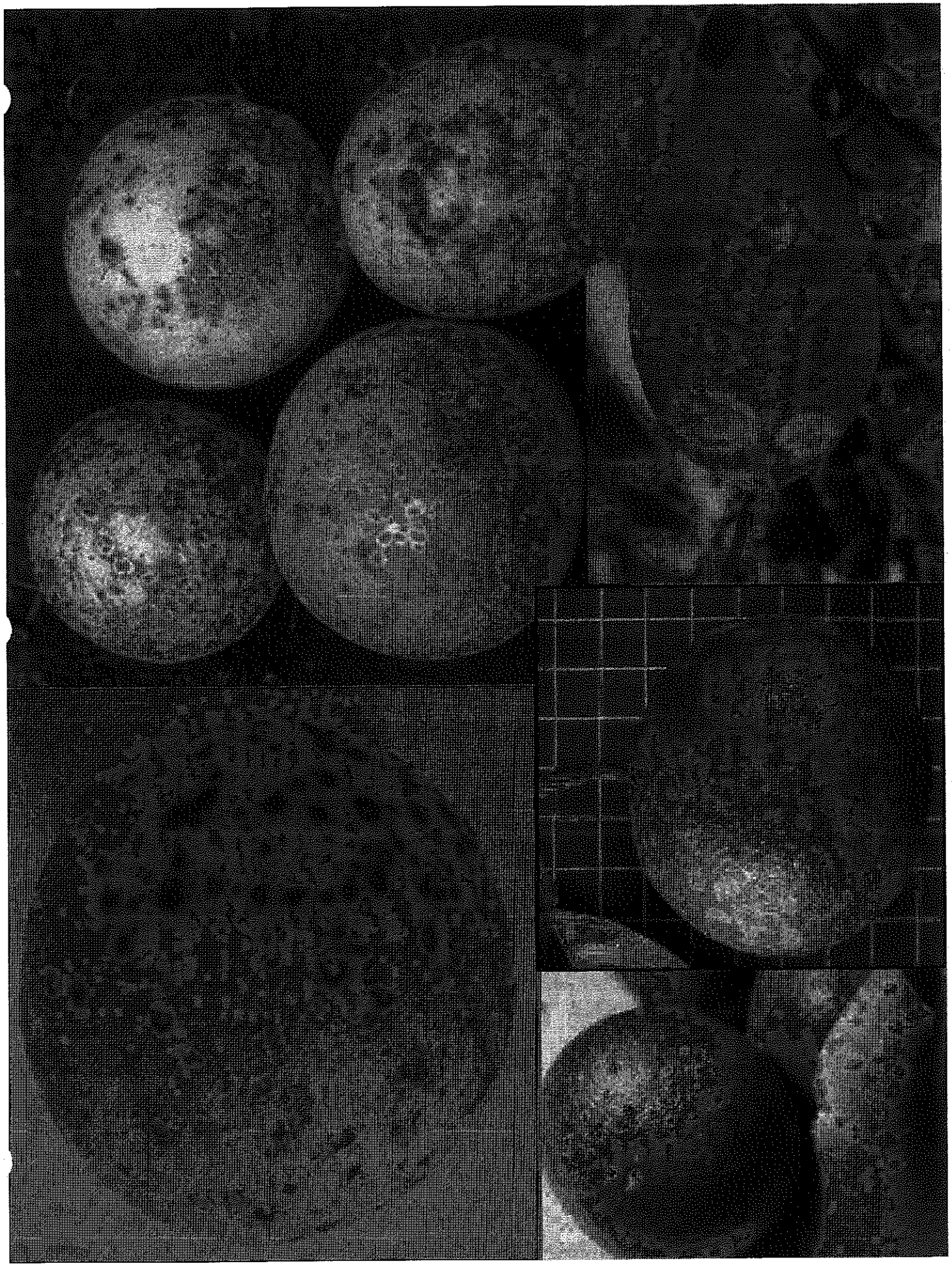
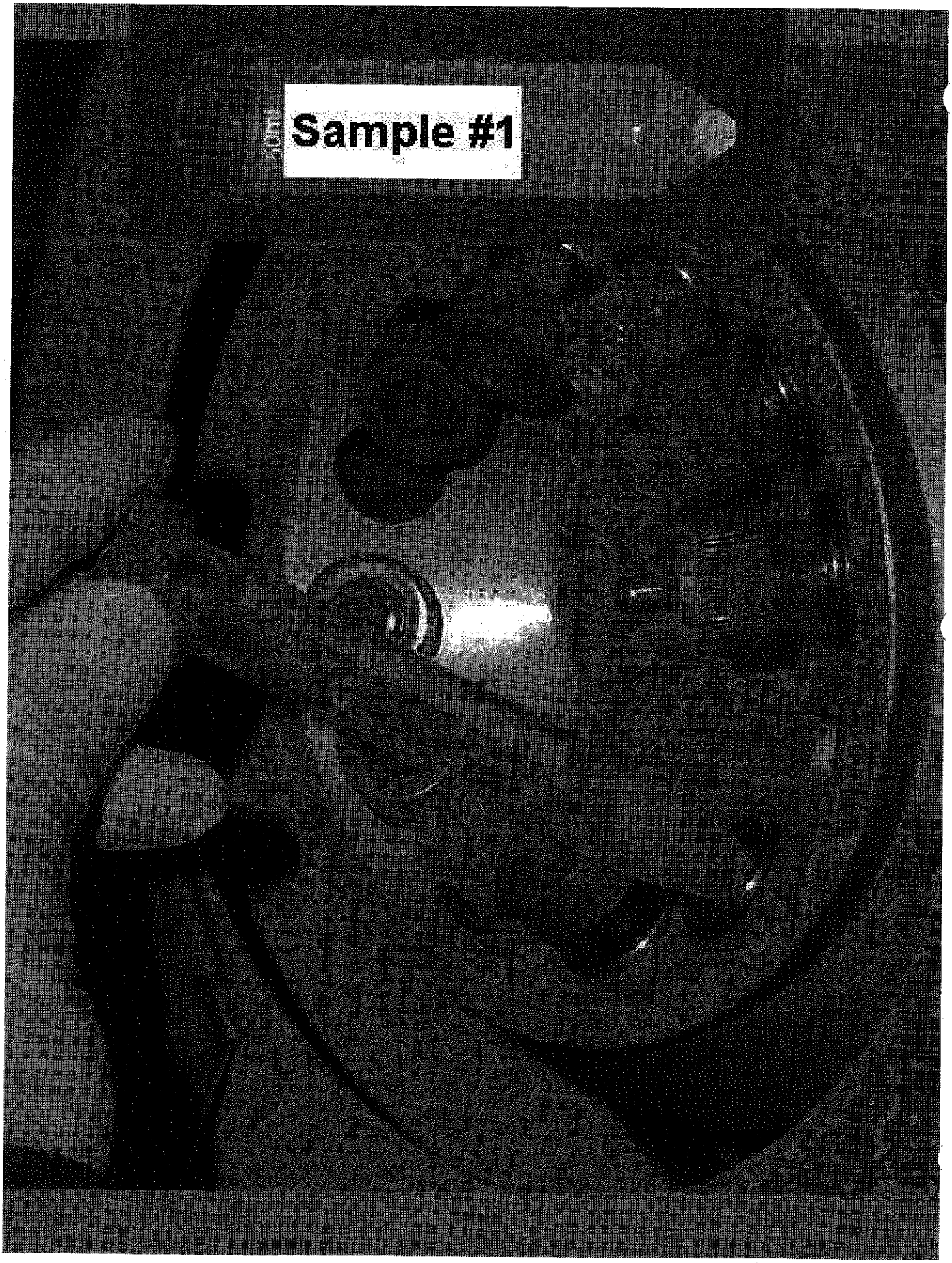
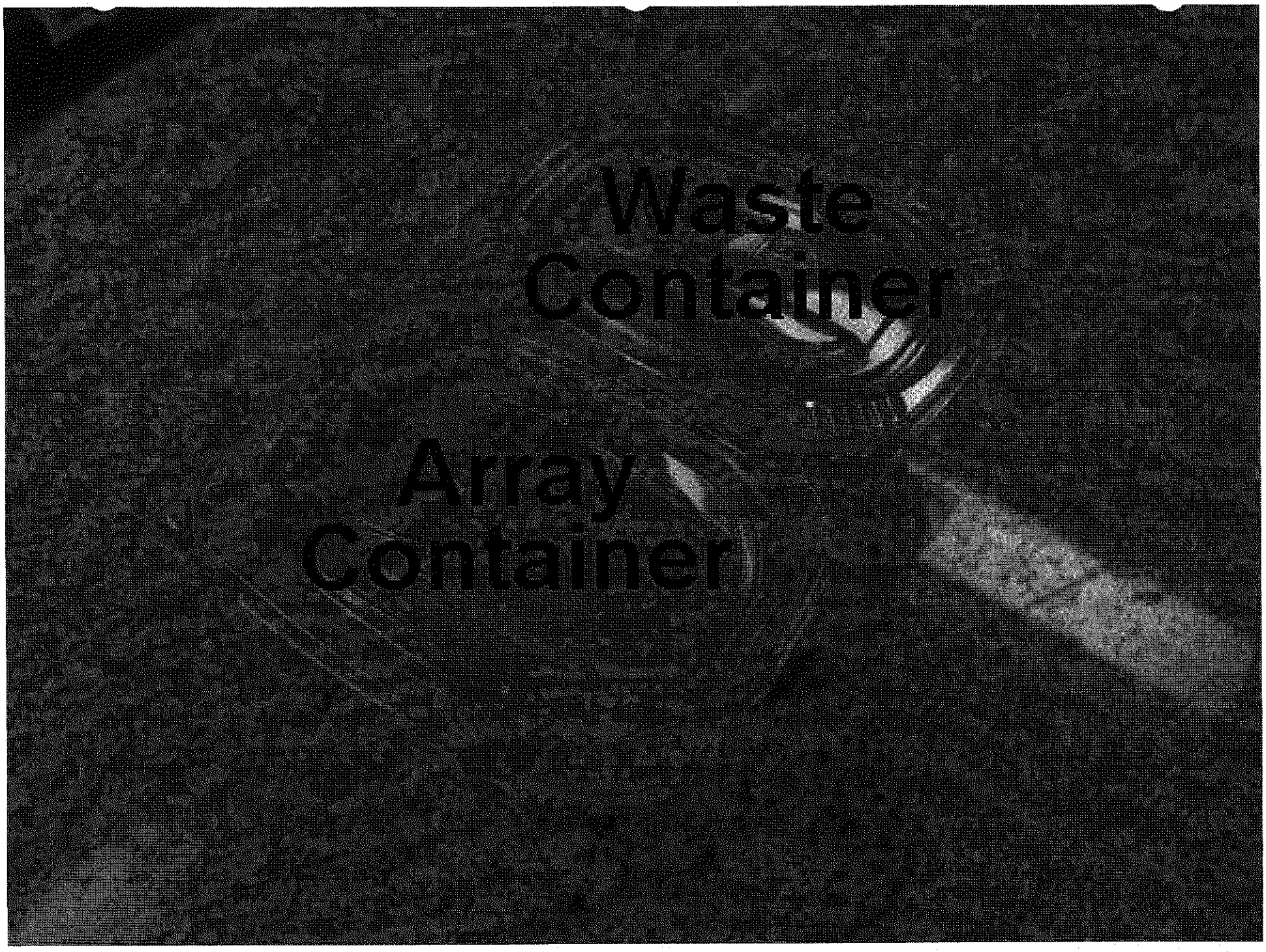
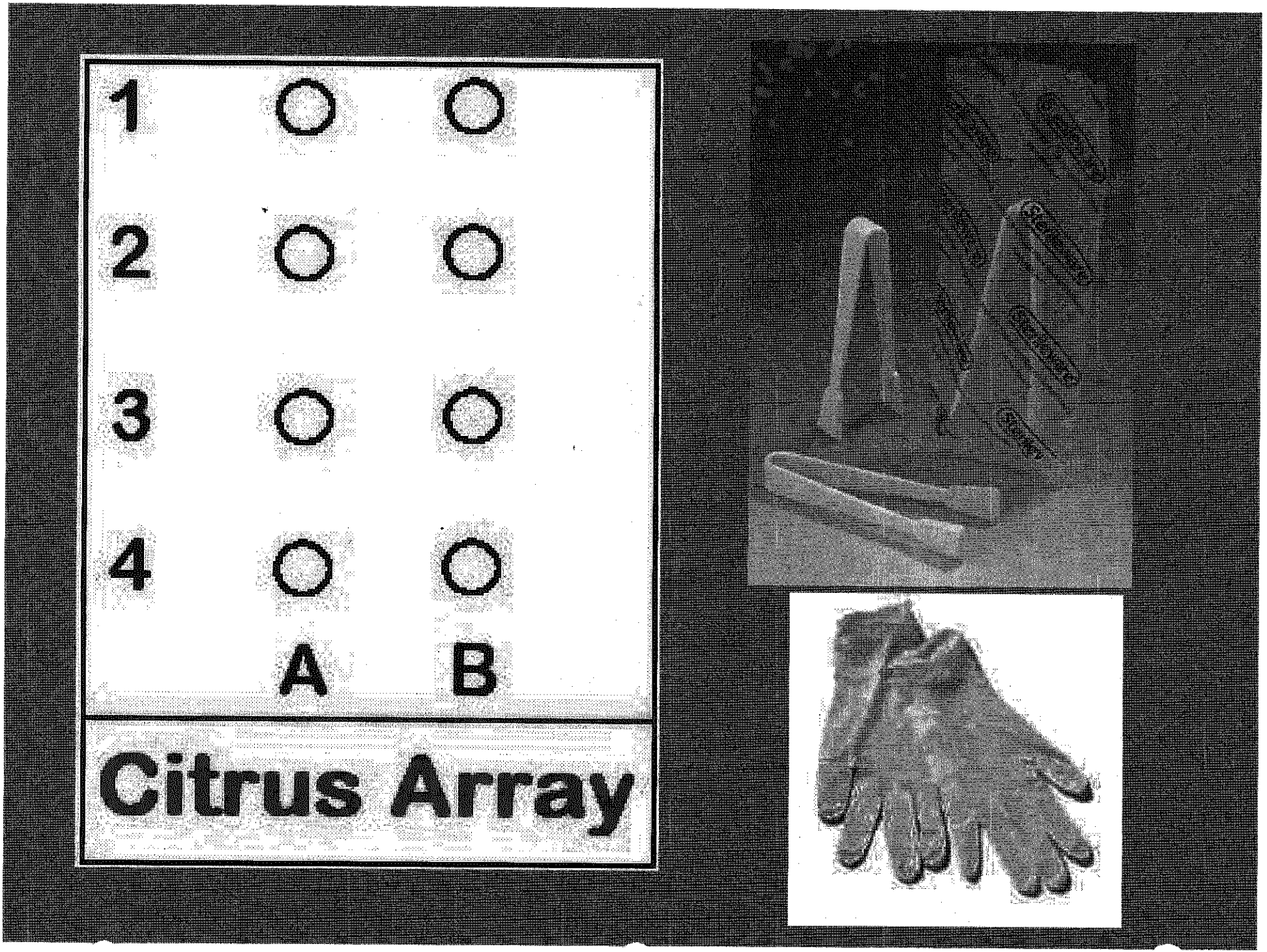
O⁻
OCH₂
O
NH₂
N
N
N
N
N₃
HO
OH

Fluorescent Reporter Molecule

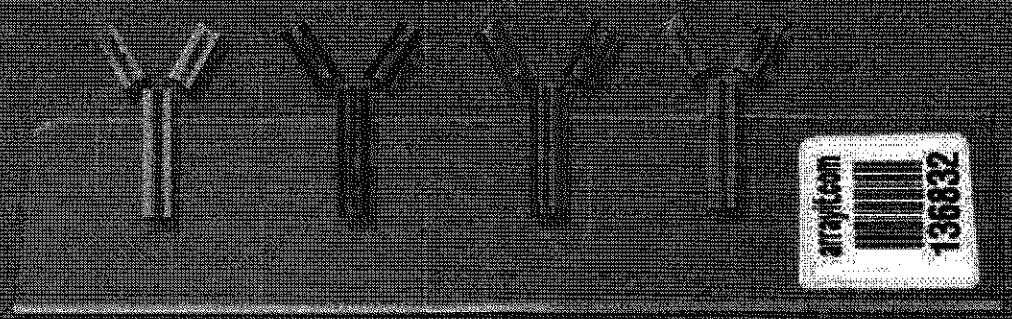


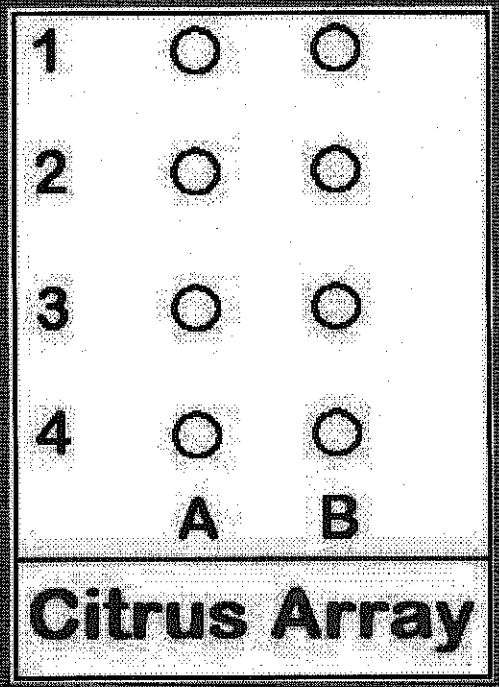

Sample #1

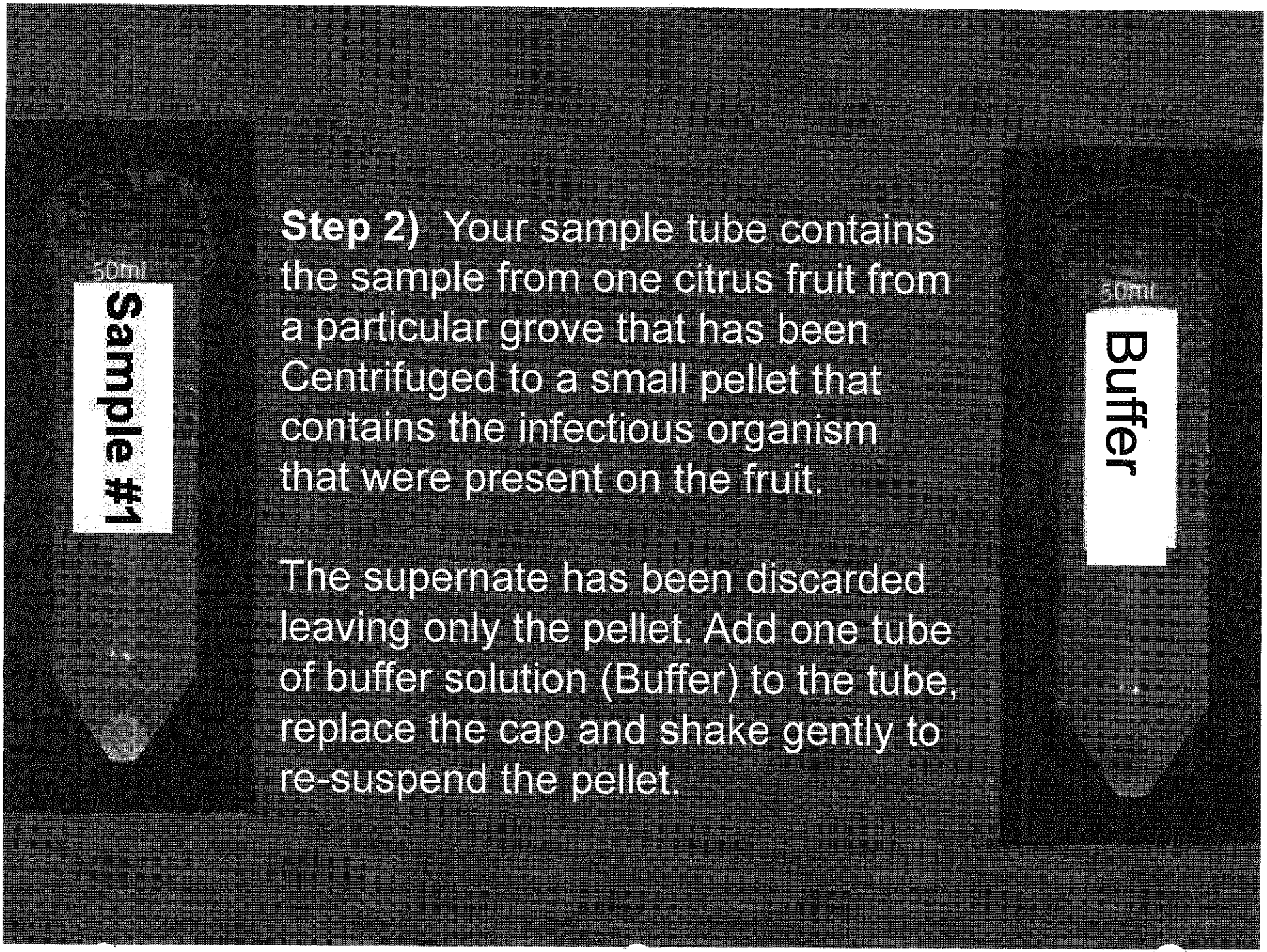

Waste
Container
Array
Container


1
2
3
4
A
B
Citrus Array

The microarray already has 8 different antibodies attached at the 8 circled areas (1 circle = 1 antibody type)







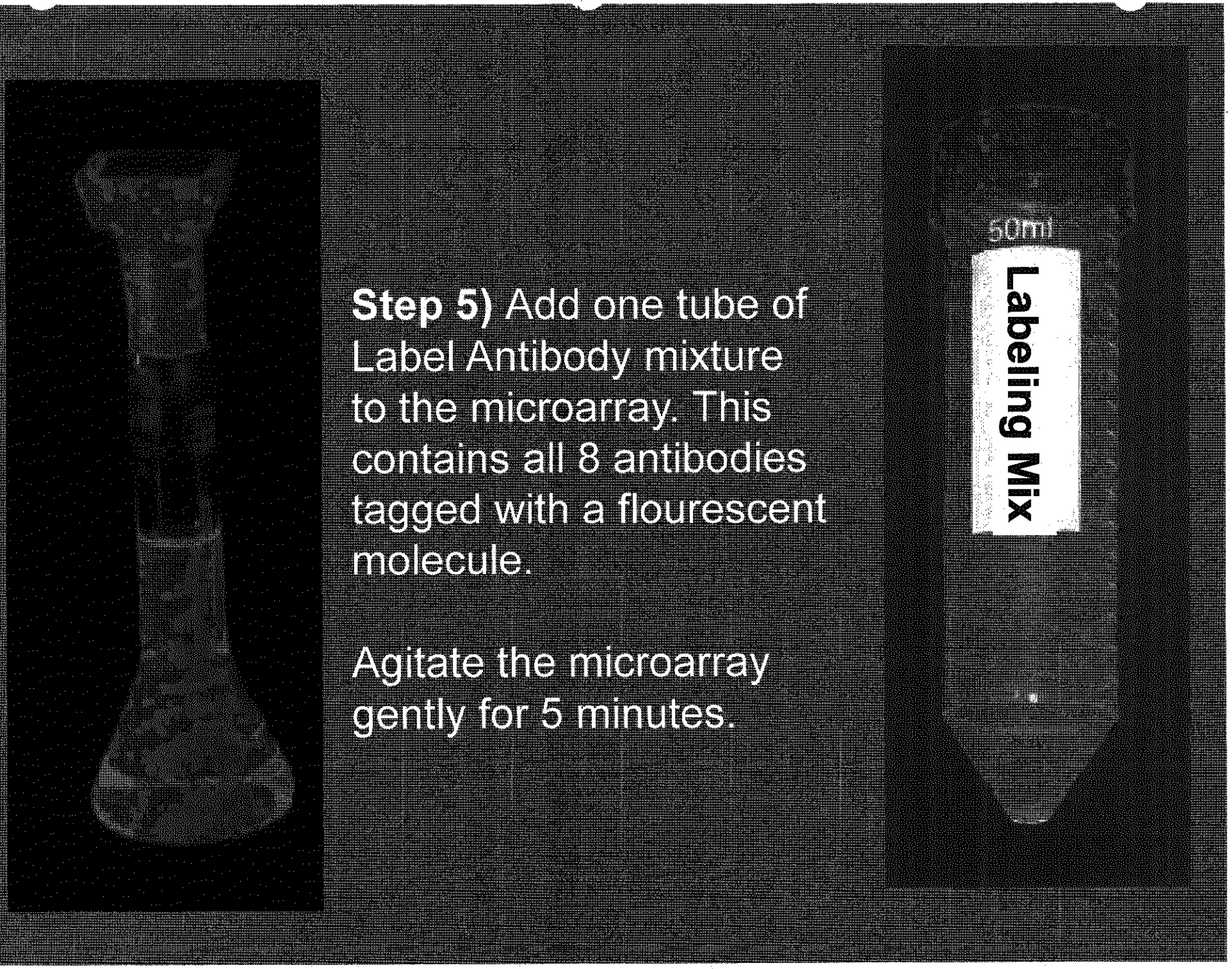
Step 2) Your sample tube contains the sample from one citrus fruit from a particular grove that has been Centrifuged to a small pellet that contains the infectious organism that were present on the fruit.

The supernate has been discarded leaving only the pellet. Add one tube of buffer solution (Buffer) to the tube, replace the cap and shake gently to re-suspend the pellet.

Step 3) Pour the contents of the tube into the microarray container. Agitate the microarray container gently for 5 minutes.

Step 4) Pour the solution from the microarray container into the waste container. Pour the liquid from the microarray container carefully so you not dump out the microarray in the process.

The specific antigens have
Now attached to the antibodies



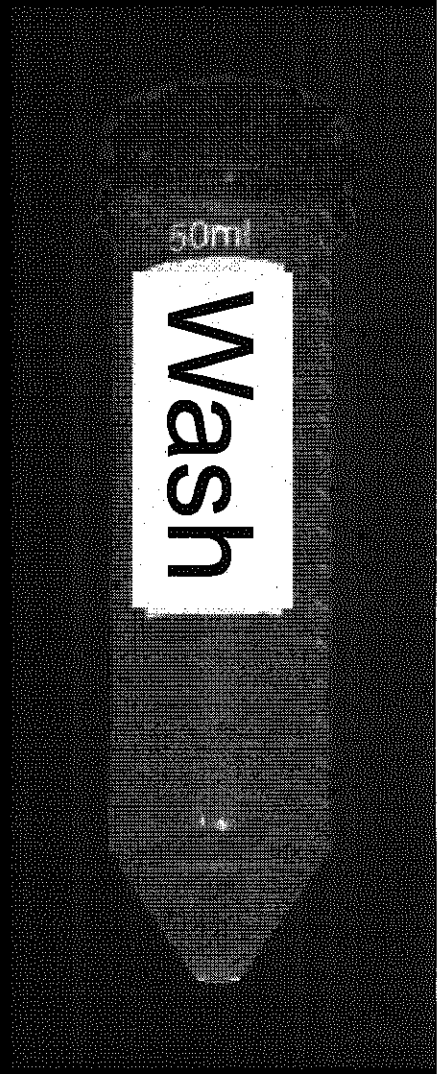
Step 5) Add one tube of Label Antibody mixture to the microarray. This contains all 8 antibodies tagged with a flourescent molecule.

Agitate the microarray gently for 5 minutes.

Fluorescent Tagged Antibody

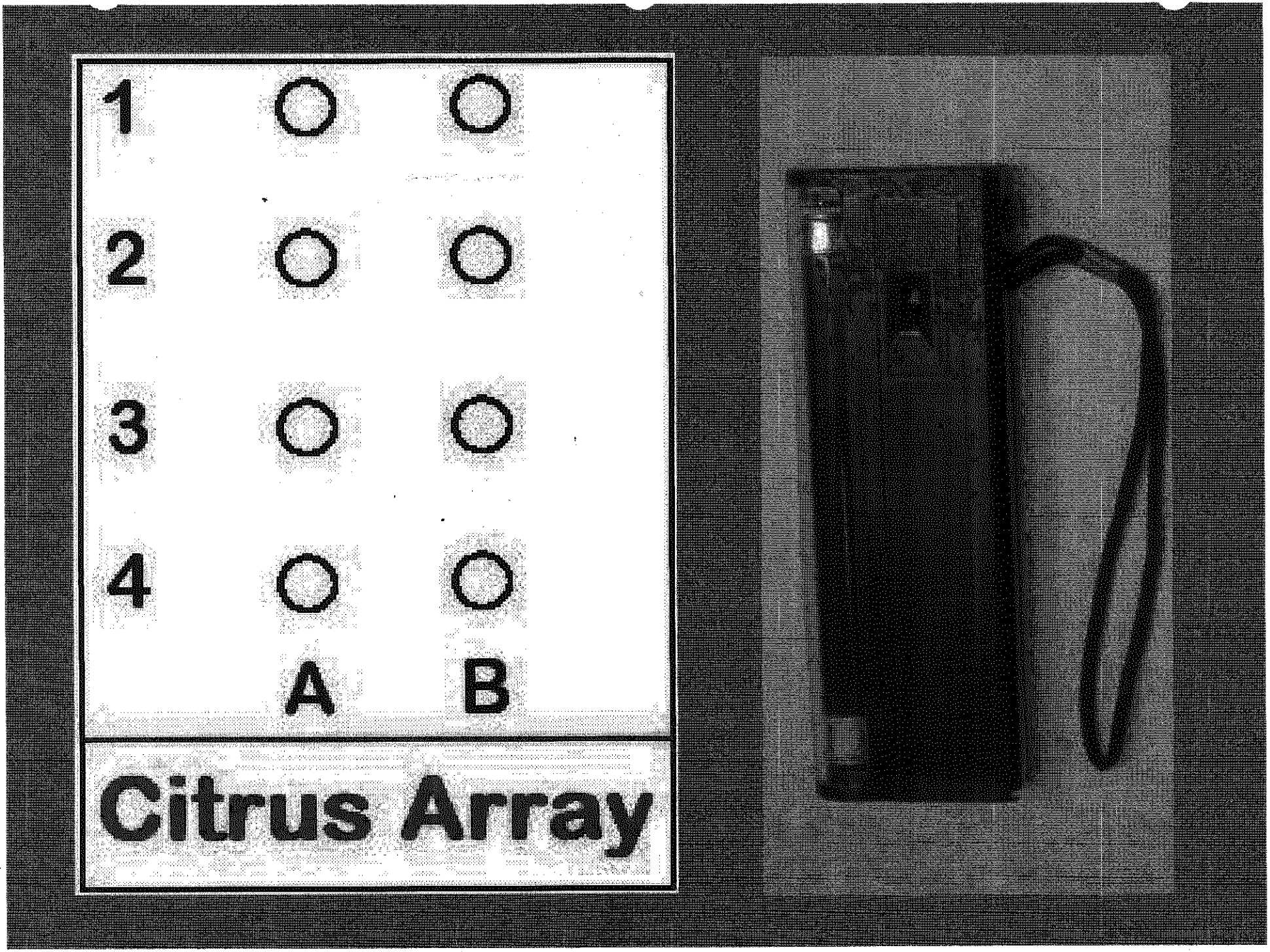
Step 6) Pour the solution from the microarray container into the waste container. Pour the liquid from the microarray container carefully so you not dump out the microarray in the process.

Step 7) Add one tube of wash solution (#56455) to microarray. Agitate the microarray gently for 5 minutes. Pour the wash solution into waste container. Pour the liquid from the microarray container carefully so you not dump out the microarray in the process.



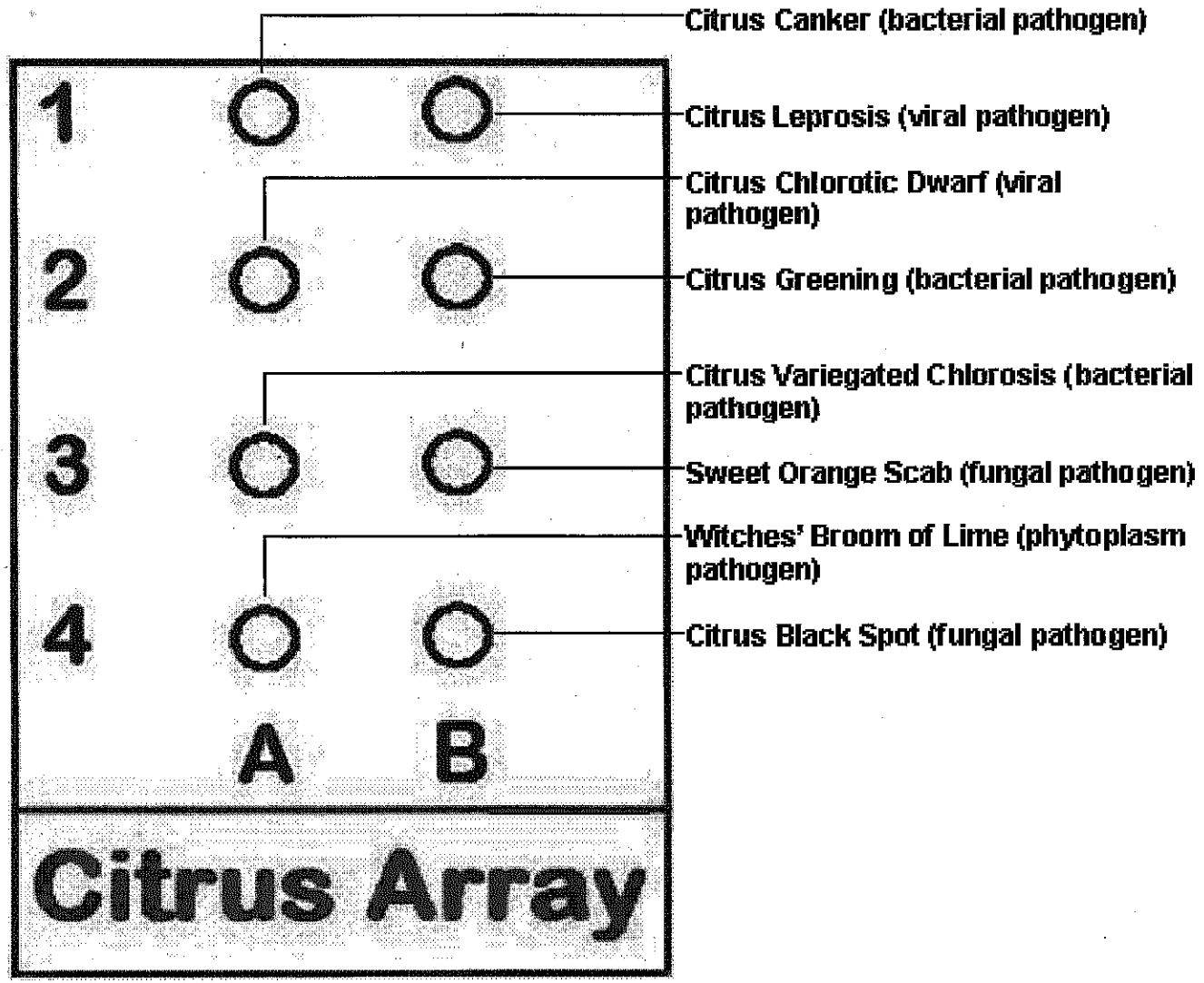
Step 8) Remove the microarray from the container being careful to touch only the end with the CDL-7 label. Shake off the excess wash solution and place the microarray "**face up**" on a sheet of paper towel.

Dim the room lights and expose the microarray to the ultraviolet light by holding the microarray over the UV light.


1
2
3
4
A
B
Citrus Array

On the microarray data sheet, circle the spot(s) which fluoresce a blue color. Then refer to the Virus Microarray Key to determine which antibodies were present. Write the virus name(s) in the blanks on the right side of the page and draw a line from the fluorescent spot to the name of the virus.

Citrus Microarray



Citrus Diseases

Citrus canker

Citrus canker is a disease affecting citrus species that is caused by the bacterium Xanthomonas axonopodis. Infection causes lesions on the leaves, stems, and fruit of citrus trees, including lime, oranges, and grapefruit. While not harmful to humans, canker significantly affects the vitality of citrus trees, causing leaves and fruit to drop prematurely; a fruit infected with canker is safe to eat but too unsightly to be sold.

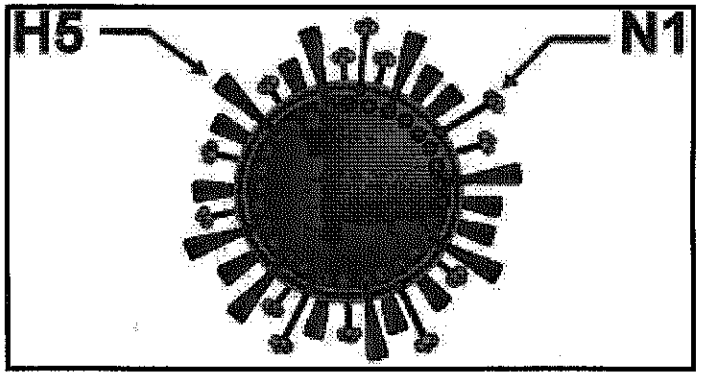
The disease, which is believed to have originated in South East Asia, is extremely persistent when it becomes established in an area. Citrus orchards have been destroyed in attempts to eradicate the disease. Australia, Brazil and the United States are currently suffering from canker outbreaks.

Pathology

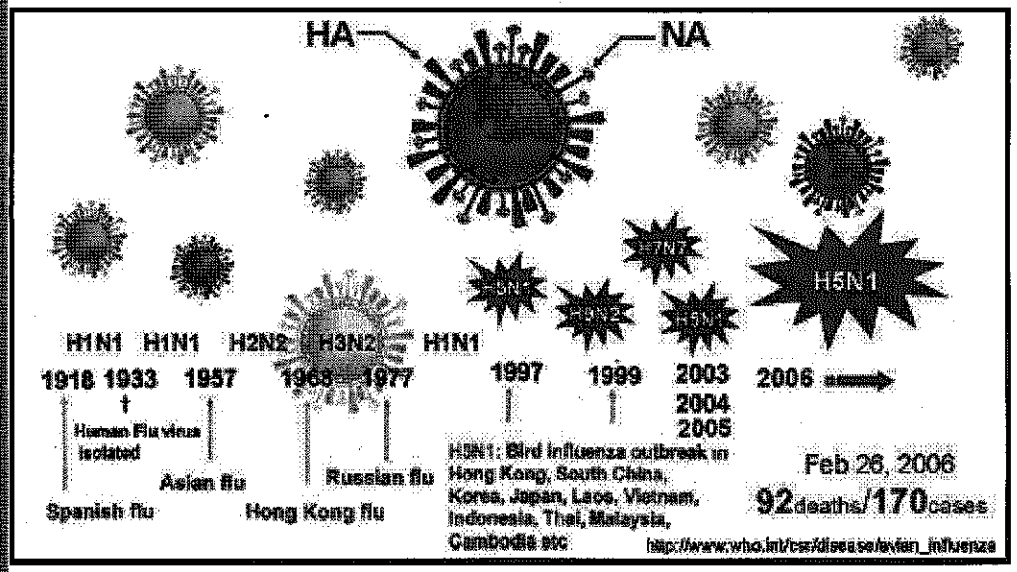
Plants infected with citrus canker have characteristic lesions on leaves, stems, and fruit with raised, brown, water-soaked margins, usually with a yellow halo or ring effect around the lesion. Older lesions have a corky appearance, still in many cases retaining the halo effect. The bacterium propagates in lesions in leaves, stems, and fruit. The lesions ooze bacterial cells that, when dispersed by windblown rain, can spread to other plants in the area. Infection may spread further by hurricanes. The disease can also be spread by from contaminated equipment, and by transport of infected or apparently healthy plants. Due to latency of the disease, a plant may appear to be healthy, but actually be infected.

Citrus canker bacteria can enter through a plant's stomata or through wounds on leaves or other green parts. In most cases, younger leaves are considered to be the most susceptible. Also, damage caused by Citrus Leaf Miner larvae (Phyllocnistis citrella) can be sites for infection to occur. Within a controlled laboratory setting, symptoms can appear in 14 days following inoculation into a susceptible host. In the field environment, the time for symptoms to appear and be clearly discernible from other foliar diseases varies; it may be on the order of several months after infection. Lower temperature increases the latency of the disease. Citrus canker bacteria can stay viable in old lesions and other plant surfaces for several months.

Influenza A virus subtype H5N1



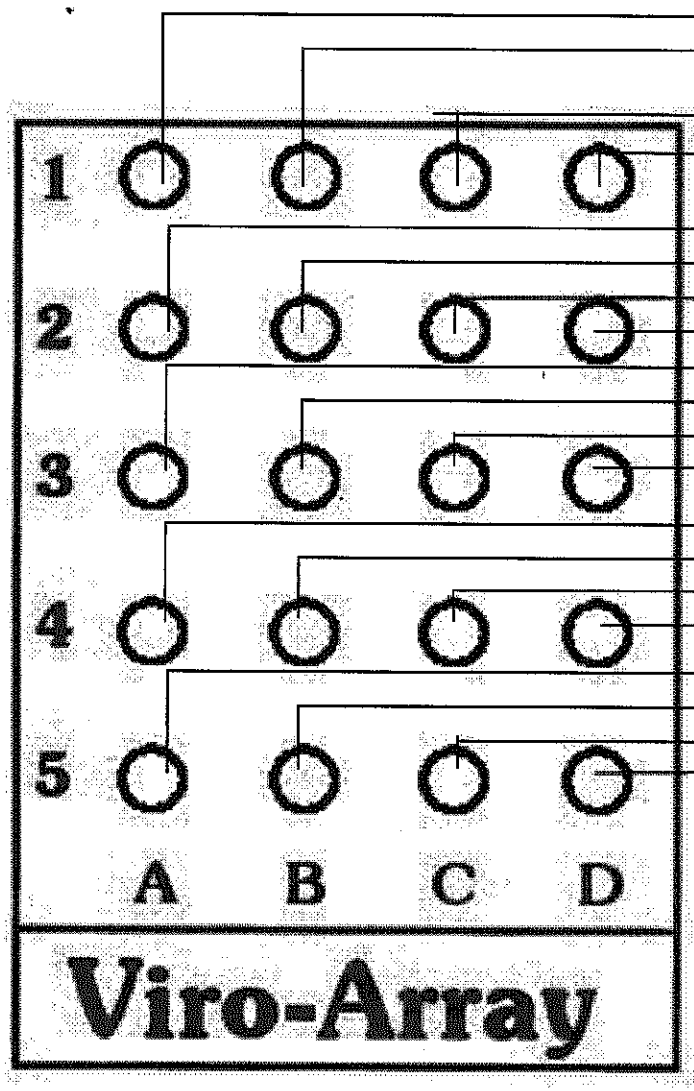
Influenza A virus subtype H5N1, also known as A(H5N1) or simply H5N1, is a subtype of the Influenza A virus which can cause illness in humans and many other animal species. A bird-adapted strain of H5N1, called HPAI A(H5N1) for "highly pathogenic avian influenza virus of type A of subtype H5N1", is the causative agent of H5N1 flu, commonly known as "avian influenza" or "bird flu". It is enzootic in many bird populations, especially in Southeast Asia. One strain of HPAI A(H5N1) is spreading globally after first appearing in Asia. It is epizootic (an epidemic in nonhumans) and panzootic (affecting animals of many species, especially over a wide area), killing tens of millions of birds and spurring the culling of hundreds of millions of others to stem its spread. Most references to "bird flu" and H5N1 in the popular media refer to this strain.



Overview

PAI A(H5N1) is an avian disease. There is no evidence of efficient human-to-human transmission or of airborne transmission of HPAI A(H5N1) to humans. In almost all cases, those infected with H5N1 had extensive physical contact with infected birds.

Virus Microarray Key



1 Herpes simplex type 1 - cold sores
2 SARS - severe acute respiratory syndrome
3 Measles Virus
4 Avian Influenza A (H5N1)
5 Rabies virus
6 Adenovirus - the common cold
7 Herpes simplex type 2 - Genital herpes
8 Rubella virus - Rubella, birth defects
9 Epstein-Barr virus - mononucleosis
10 Papovavirus - Warts
11 Coronavirus - the common cold
12 Hepatitis
13 Orthopoxvirus - smallpox
14 Varicella-zoster - Chicken pox
15 Human herpesvirus 8 (HHV8)
16 Human Papillomavirus - Genital Warts
17 Eastern equine Encephalitis
18 West Nile virus
19 Mumps virus
20 Human immunodeficiency virus - AIDS

Drinking Water Pathogens

Drinking water pathogens may be divided into three general categories: bacteria, viruses and parasitic protozoa. Bacteria and viruses contaminate both surface and groundwater, whereas parasitic protozoa appear predominantly in surface water. The purpose of disinfection is to kill or inactivate microorganisms so that they cannot reproduce and infect human hosts. Bacteria and viruses are well-controlled by normal chlorination, in contrast to parasitic protozoa, which demand more sophisticated control measures. For that reason, parasitic protozoan infections may be more common than bacterial or viral infections in areas where some degree of disinfection is achieved.

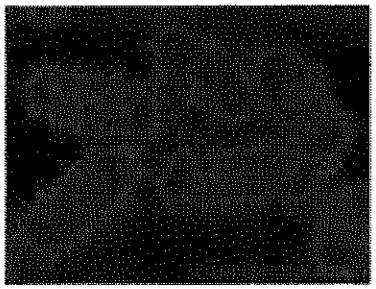
1	Escherichia coli	9	Norwalk Virus
2	Enterococci	10	Rotavirus
3	Fecal coliforms	11	Microsporidia
4	Bacteroides	12	Giardia Lamblia
5	Salmonella	13	Cryptosporidium
6	Clostridium	14	Helicobacter pylori
7	enteroviruses	14	Campylobacter jejuni
8	Hepatitis	16	Shigella

Bacteria

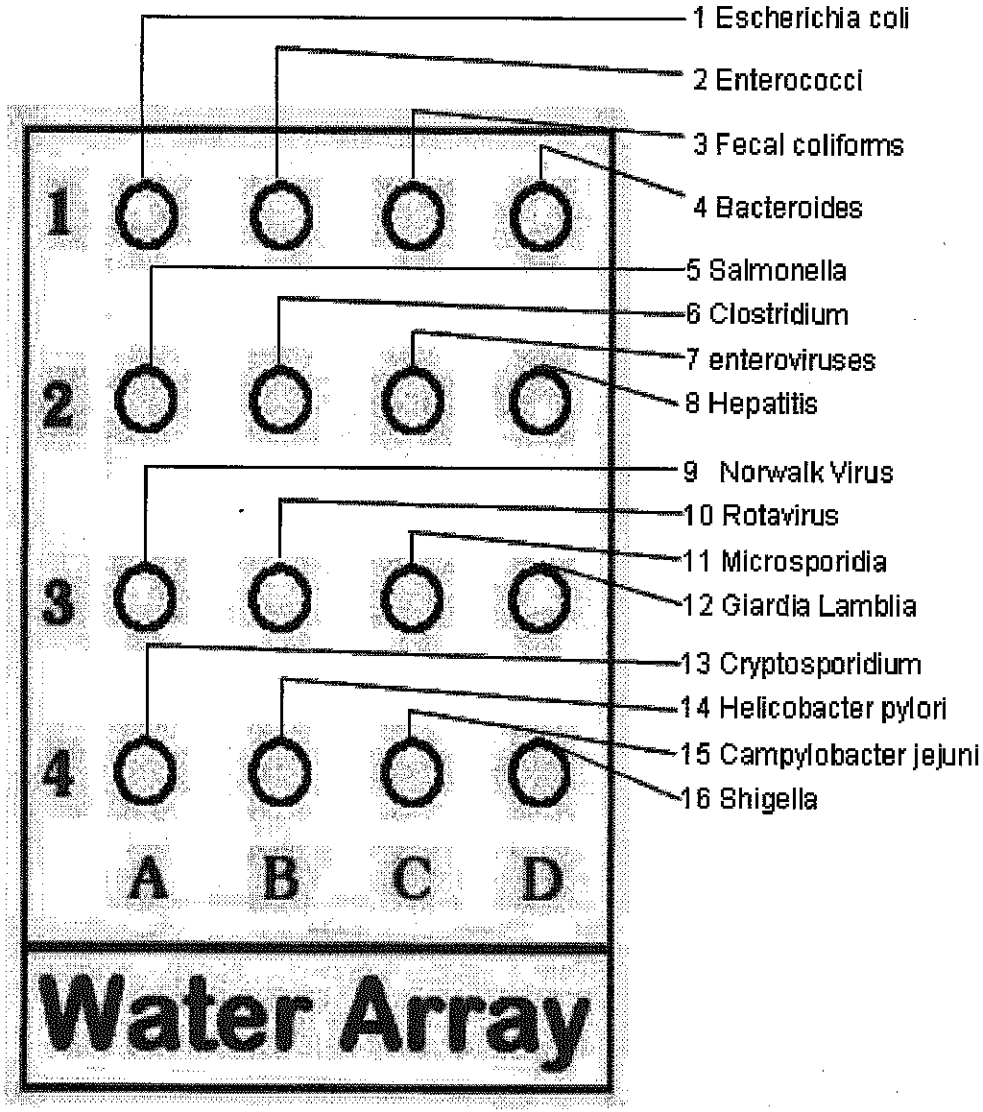
Bacteria are microorganisms often composed of single cells shaped like rods, spheres or spiral structures. Prior to widespread chlorination of drinking water, bacteria like Vibrio cholerae, Salmonella typhii and several species of Shigella routinely inflicted serious diseases such as cholera, typhoid fever and bacillary dysentery, respectively. As recently as 2000, a drinking water outbreak of E. coli in Walkerton, Ontario sickened 2,300 residents and killed seven when operators failed to properly disinfect the municipal water supply. While developed nations have largely conquered waterborne bacterial pathogens through the use of chlorine and other disinfectants, the developing world still grapples with these public health enemies.

Several bacterial species (E. coli, Enterococci, fecal coliforms, the Bacteroides-Prevotella group, Salmonella, Clostridium) and viral species (coliphage, Enterovirus, Hepatitis, Norwalk, Rotavirus) have been studied to determine their suitability as indicator organisms of ambient water quality.

Escherichia coli is a bacterium that is commonly found in the lower intestine of warm-blooded animals. Most E. coli strains are harmless, but some, such as serotype O157:H7, can cause serious food poisoning in humans, and are occasionally responsible for costly product recalls. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K2, or by preventing the establishment of pathogenic bacteria within the intestine.



Water Microarray Key

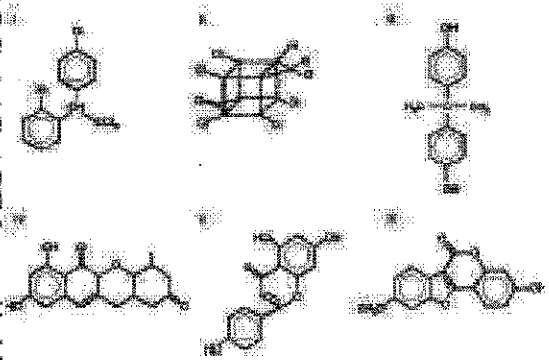


Environmental Microarray And Biomarkers

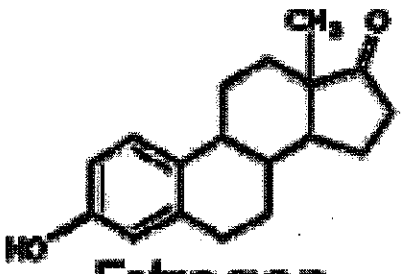
In recent years, some scientists have proposed that chemicals might inadvertently be disrupting the endocrine system of humans and wildlife. There is mounting concern in the scientific, environmental, private and governmental sectors concerning a wide range of substances, known as endocrine disruptors, that have the potential to interfere with the normal functioning of a living organism's hormone system. Endocrine disruption has the potential to compromise proper development in organisms, leading to reproductive, behavioral, immune system and neurological problems, as well as the development of cancer.

A variety of chemicals have been found to disrupt the endocrine systems of animals in laboratory studies, and compelling evidence shows that endocrine systems of certain fish and wildlife have been effected by chemical contaminants, resulting in developmental and reproductive problems. However, the relationship of human diseases of the endocrine system and exposure to environmental contaminants is poorly understood and scientifically controversial. Effects often do not show up until later in life.

Scientists have identified dozens of human made chemicals that can disrupt an animal's endocrine system -- a group of organs that release chemicals into the body that are important for a wide range of activities -- including growing, managing stress and reproducing. One of the chemicals released by the endocrine system is estrogen. Estrogen helps females develop into adults with the ability to reproduce -- it helps prepare the body for pregnancy -- and is active during pregnancy.

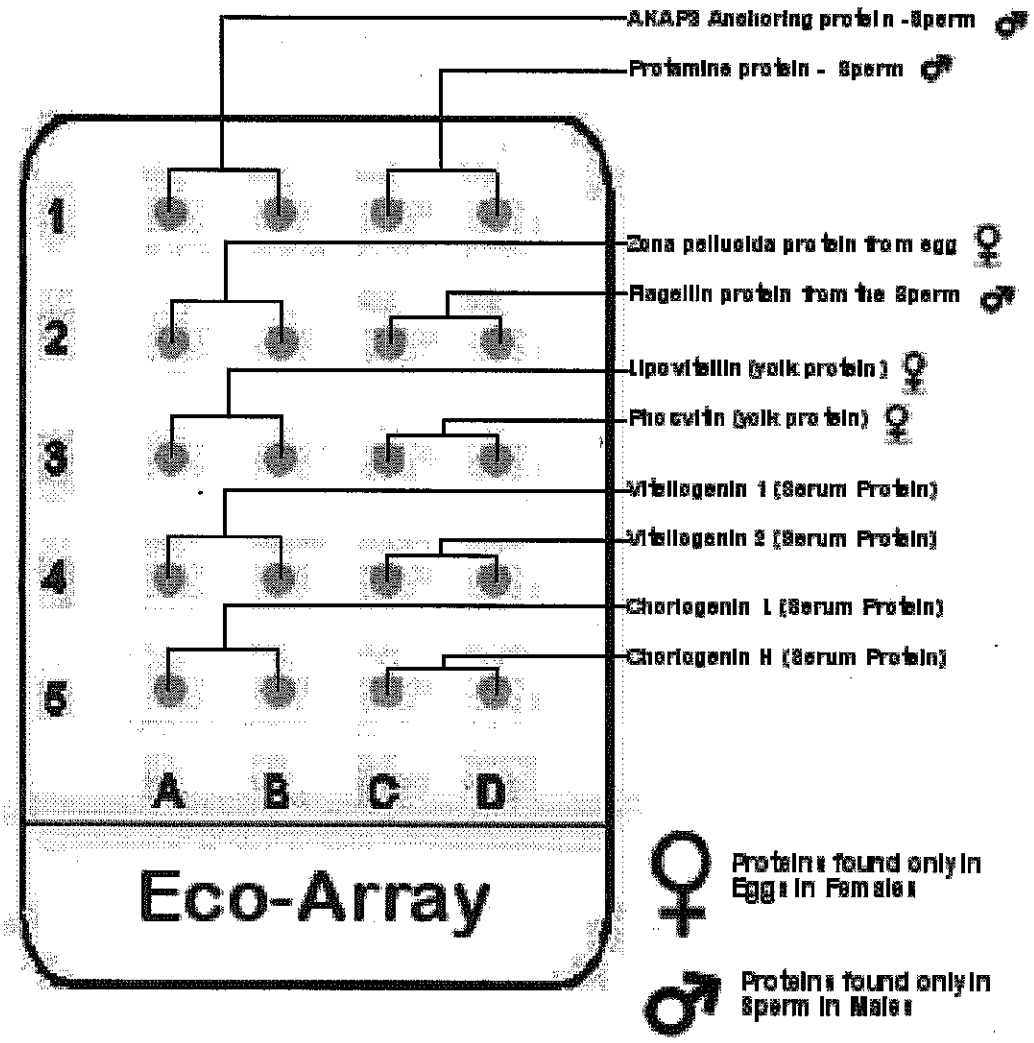


Some human made chemicals can act like estrogen in an animal's body. These so called "estrogen mimics" are being increasingly detected in agricultural runoff, effluent from water treatment plants often from drugs flushed down the toilet in homes and pollution from manufacturers including plastics factories and paper pulp mills. These chemicals are also turning up in aquatic animals and birds that live in or near streams, rivers and the ocean. They are known -- in certain concentrations -- to disrupt the ability of alligators, frogs, birds and fish to mature and reproduce.

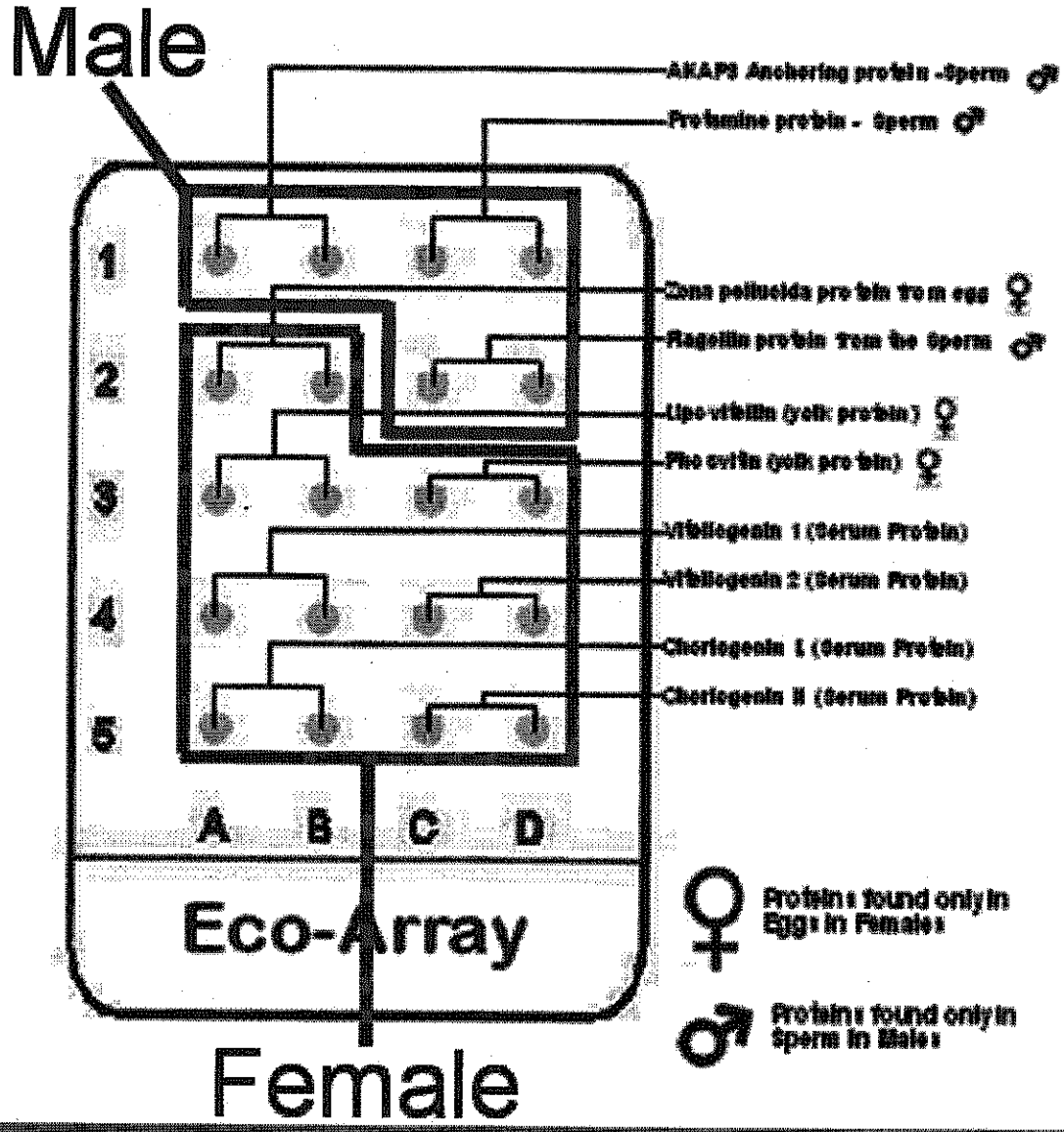


Estrogen

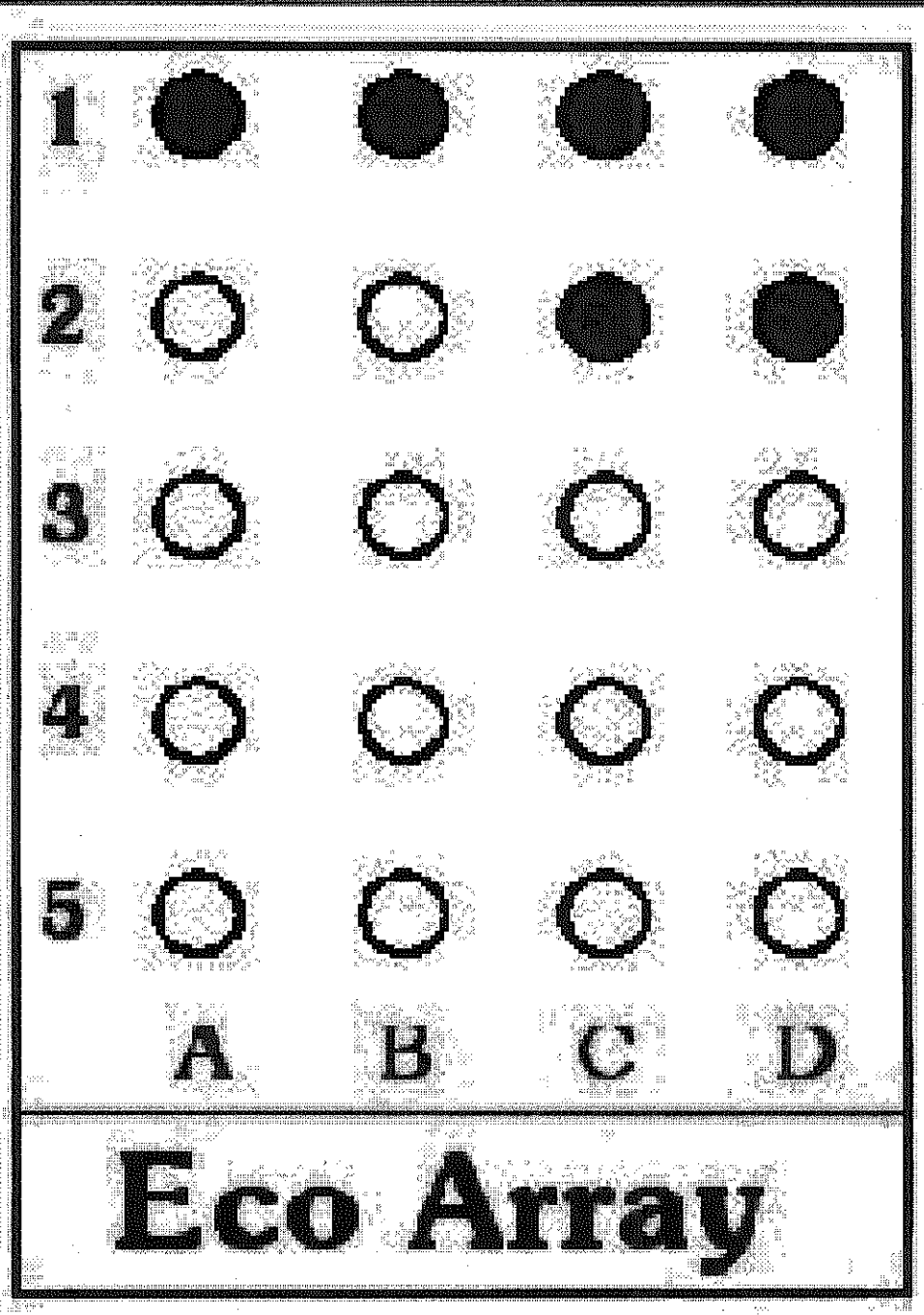
Environmental Microarray Key



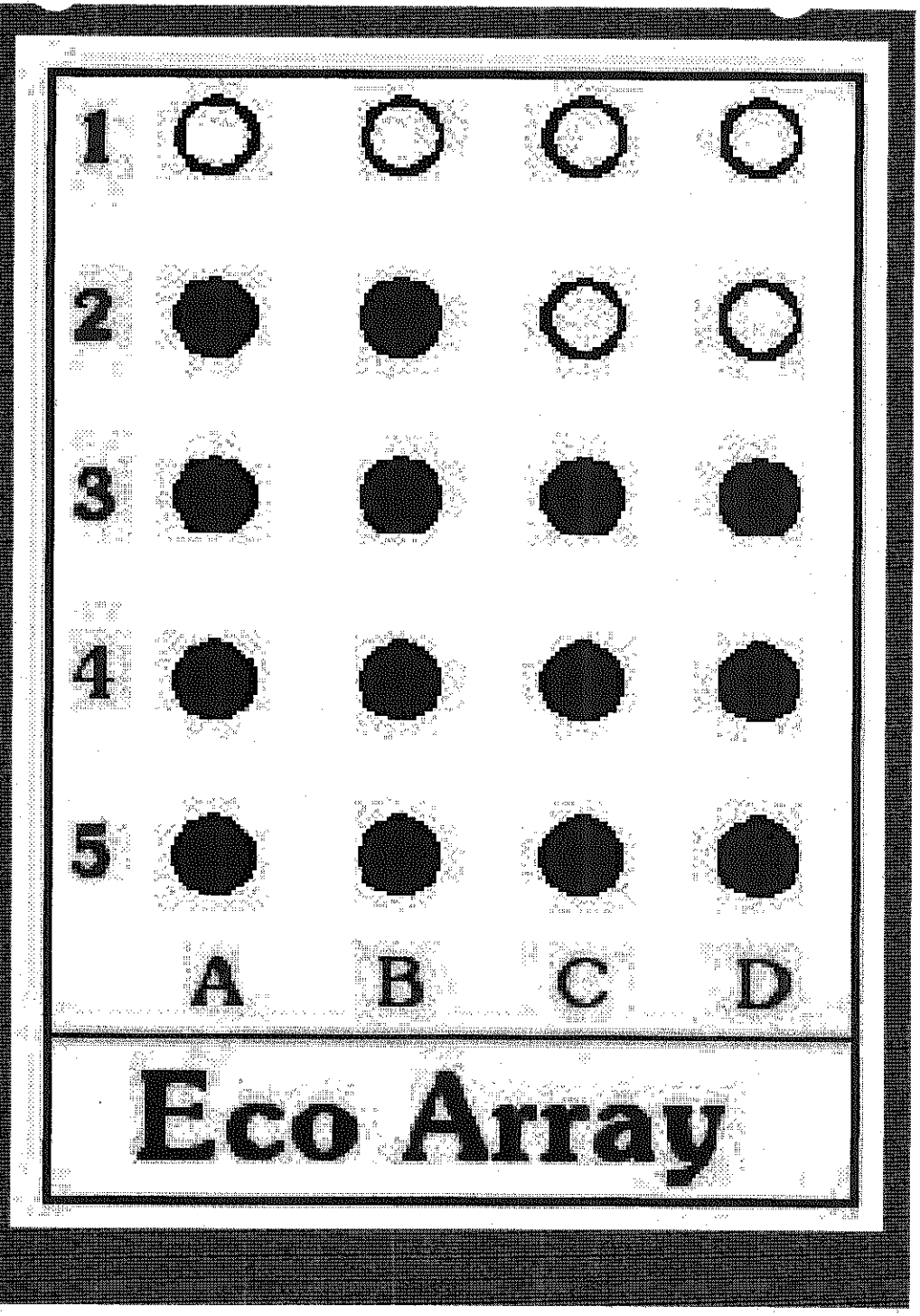
Environmental Microarray Key



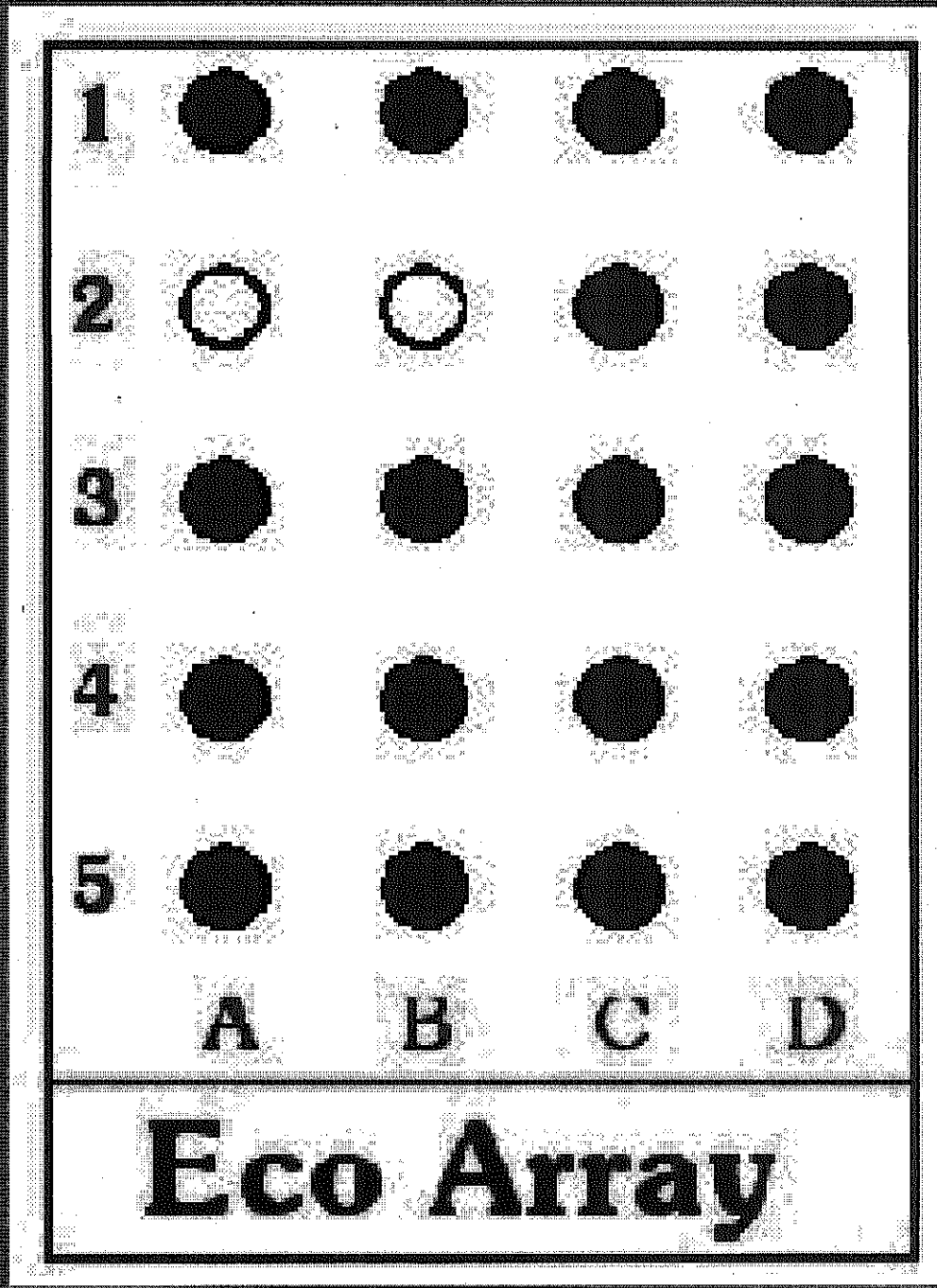
Male Fish



Female Fish



Male Fish Exposed To Estrogen Mimic

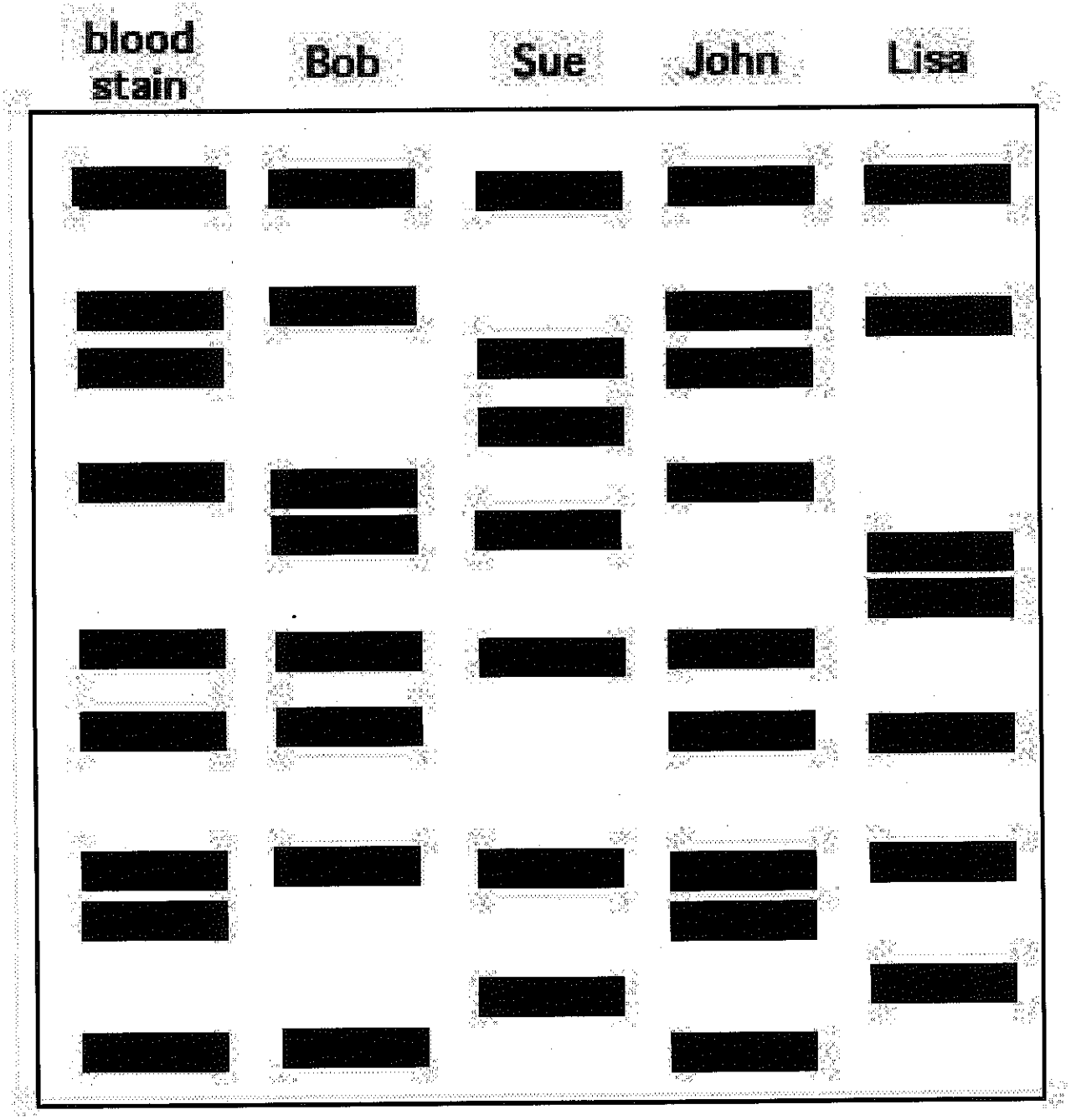


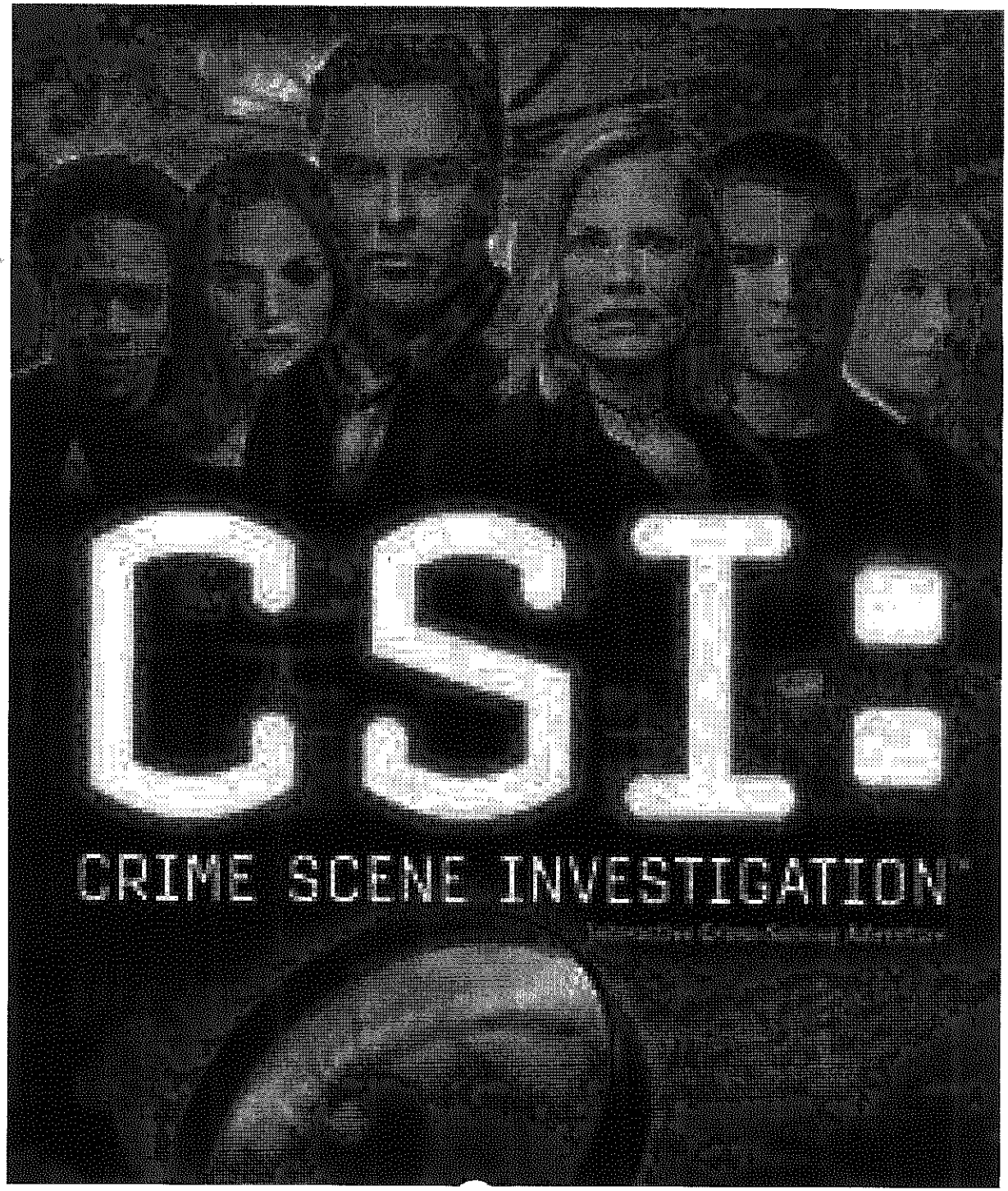
Southern Blot

DNA Fingerprint

RFLP Analysis

Restriction Fragment Length Polymorphism


blood stain
Bob
Sue
John
Lisa


C.S.I.:
CRIME SCENE INVESTIGATION

GRADE 9 - 12

LIFE SCIENCE BODY OF KNOWLEDGE

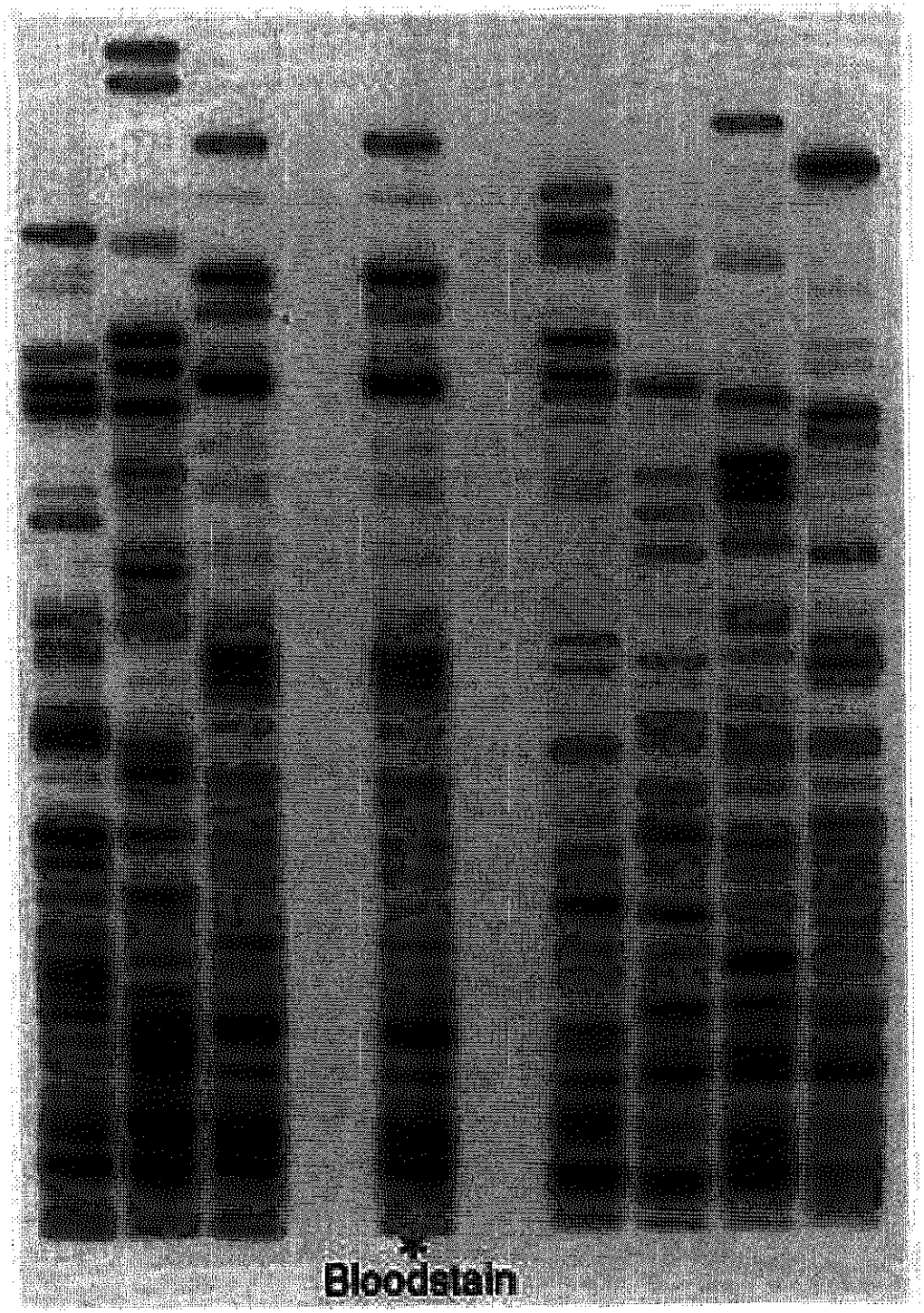
Standard 16: Heredity and Reproduction
A. DNA stores and transmits genetic information. Genes are sets of instructions encoded in the structure of DNA.
B. Genetic information is passed from generation to generation by DNA in all organisms and accounts for similarities in related individuals.
C. Manipulation of DNA in organisms has led to commercial production of biological molecules on a large scale and genetically modified organisms.
D. Reproduction is characteristic of living things and is essential for the survival of species.

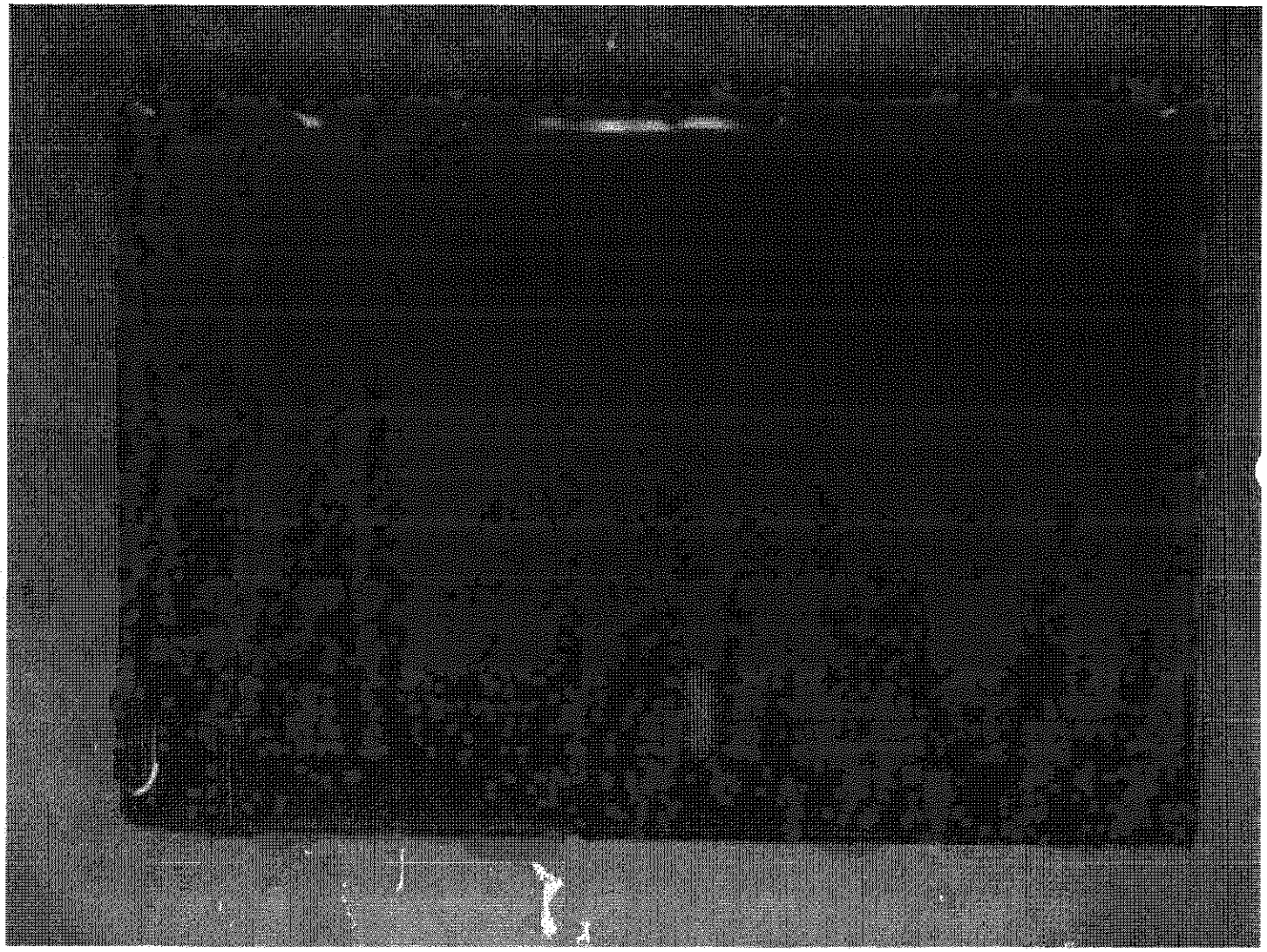
	BENCHMARK CODE	BENCHMARK
*	SC.912.L.16.1	Use Mendel's laws of segregation and independent assortment to analyze patterns of inheritance.
*	SC.912.L.16.2	Discuss observed inheritance patterns caused by various modes of inheritance, including dominant, recessive, codominant, sex-linked, polygenic, and multiple alleles.
*	SC.912.L.16.3	Describe the basic process of DNA replication and how it relates to the transmission and conservation of the genetic information.
*	SC.912.L.16.4	Explain how mutations in the DNA sequence may or may not result in phenotypic change. Explain how mutations in gametes

SC.912.L.16.11 Discuss the technologies associated with forensic medicine and DNA identification, including restriction fragment length polymorphism (RFLP) analysis.

	SC.912.L.16.6	Discuss the mechanisms for regulation of gene expression in prokaryotes and eukaryotes at transcription and translation level.
	SC.912.L.16.7	Describe how viruses and bacteria transfer genetic material between cells and the role of this process in biotechnology.
*	SC.912.L.16.8	Explain the relationship between mutation, cell cycle, and uncontrolled cell growth potentially resulting in cancer.
*	SC.912.L.16.9	Explain how and why the genetic code is universal and is common to almost all organisms.
*	SC.912.L.16.10	Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.
	SC.912.L.16.11	Discuss the technologies associated with forensic medicine and DNA identification, including restriction fragment length polymorphism (RFLP) analysis.
	SC.912.L.16.12	Describe how basic DNA technology (restriction digestion by endonucleases, gel electrophoresis, polymerase chain reaction, ligation, and transformation) is used to construct recombinant DNA molecules (DNA cloning).
*	SC.912.L.16.13	Describe the basic anatomy and physiology of the human reproductive system. Describe the process of human development from fertilization to birth and major changes that occur in each trimester of pregnancy.
*	SC.912.L.16.14	Describe the cell cycle, including the process of mitosis. Explain the role of mitosis in the formation of new cells and its importance in maintaining chromosome number during asexual reproduction.
	SC.912.L.16.15	Compare and contrast binary fission and mitotic cell division.
*	SC.912.L.16.16	Describe the process of meiosis, including independent assortment and crossing over. Explain how reduction division results in the formation of haploid gametes or spores.
*	SC.912.L.16.17	Compare and contrast mitosis and meiosis and relate to the processes of sexual and asexual reproduction and their consequences for genetic variation.

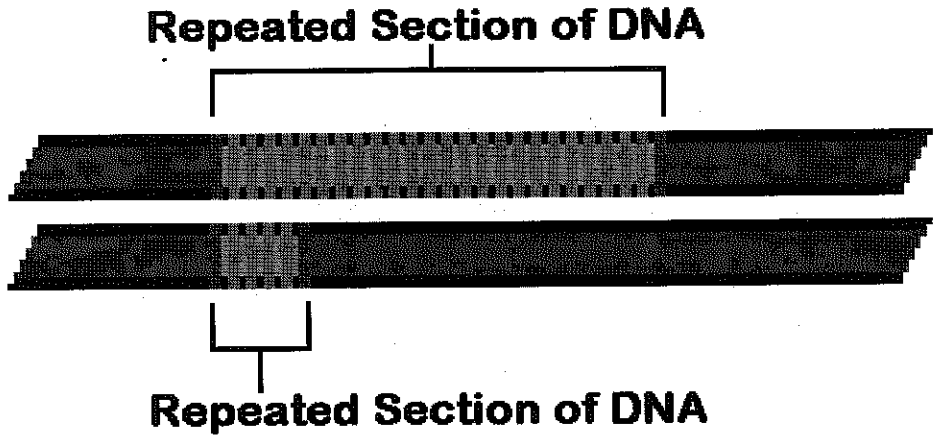
THE HUMAN CHROMOSOMES
A-M Indicate The Thirteen CODIS Sites


Bloodstain



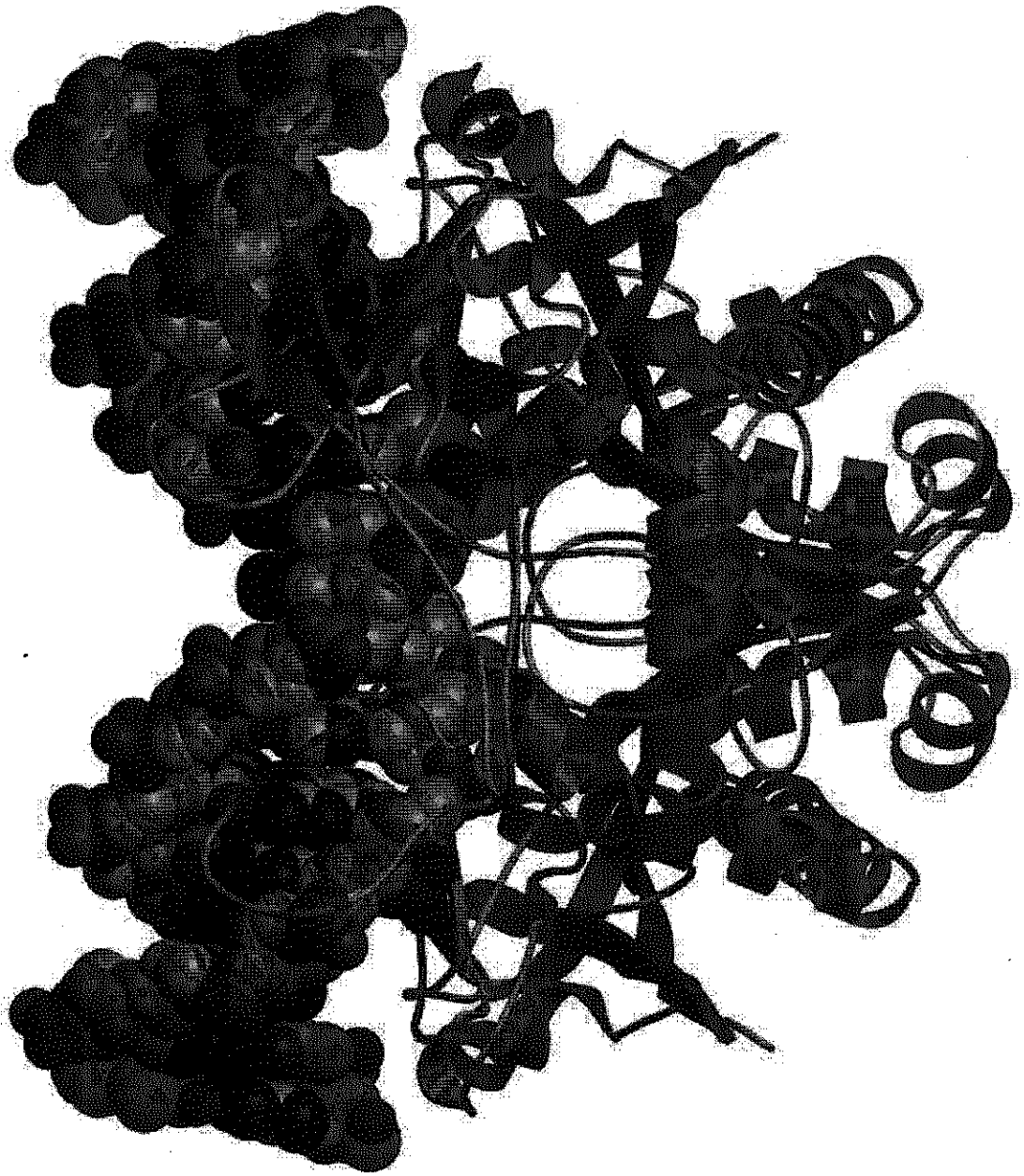
Restriction Fragment Length Polymorphism (RFLP)

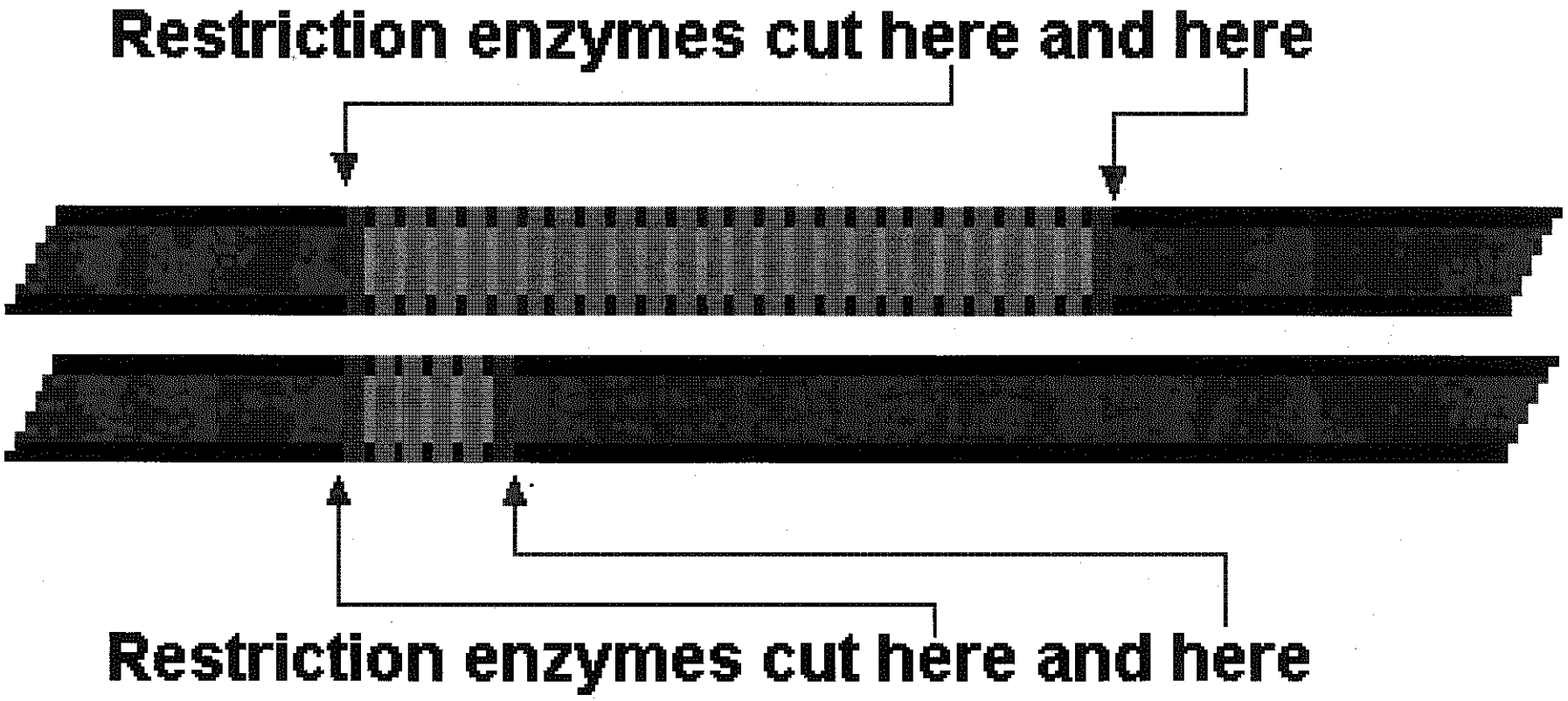
Restriction Fragment Length Polymorphism (RFLP) is a technique in which organisms may be differentiated by analysis of patterns derived from cleavage of their DNA. If two organisms differ in the distance between sites of cleavage of a particular restriction endonuclease, the length of the fragments produced will differ when the DNA is digested with a restriction enzyme. The similarity of the patterns generated can be used to differentiate species (and even strains) from one another.

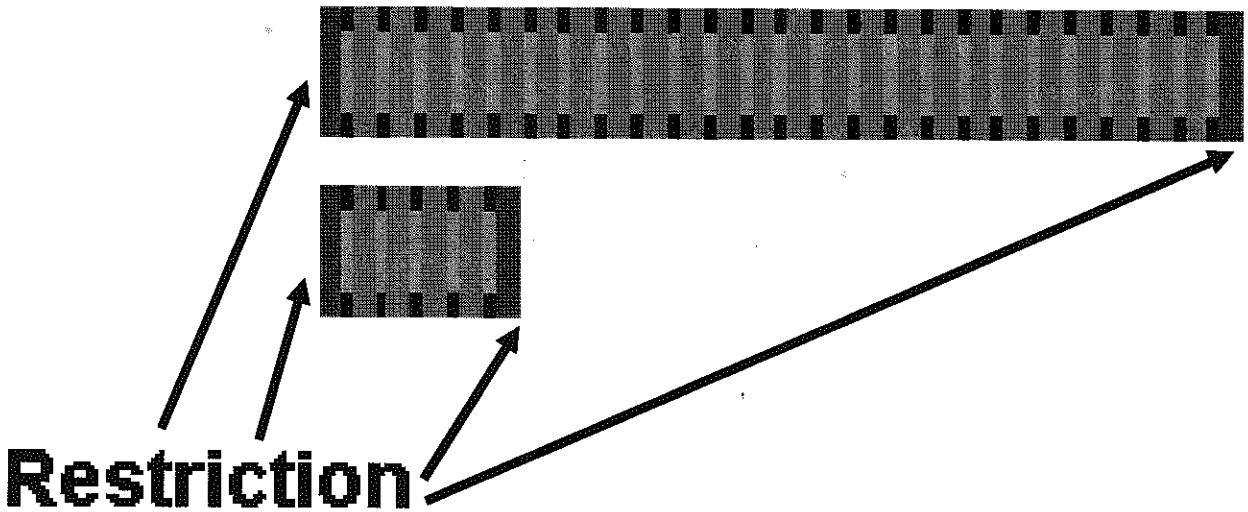


Homologus Chrmosomes

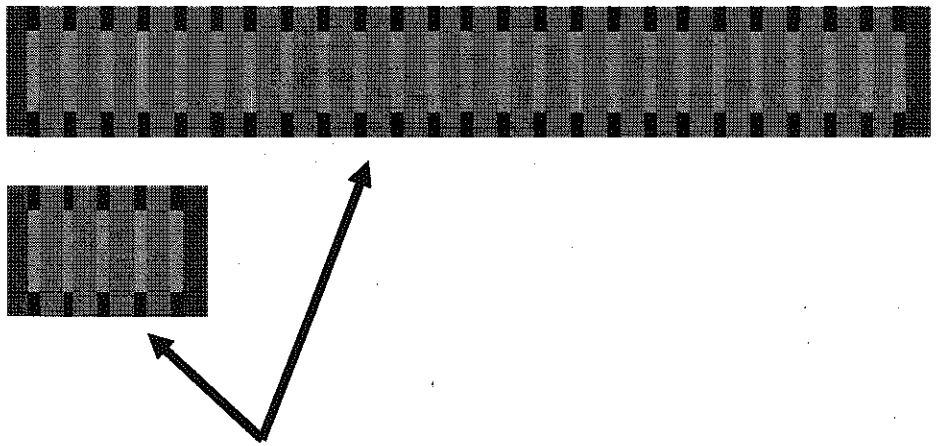
Restriction Enzyme
Attached to DNA




Restriction enzymes cut here and here
Restriction enzymes cut here and here

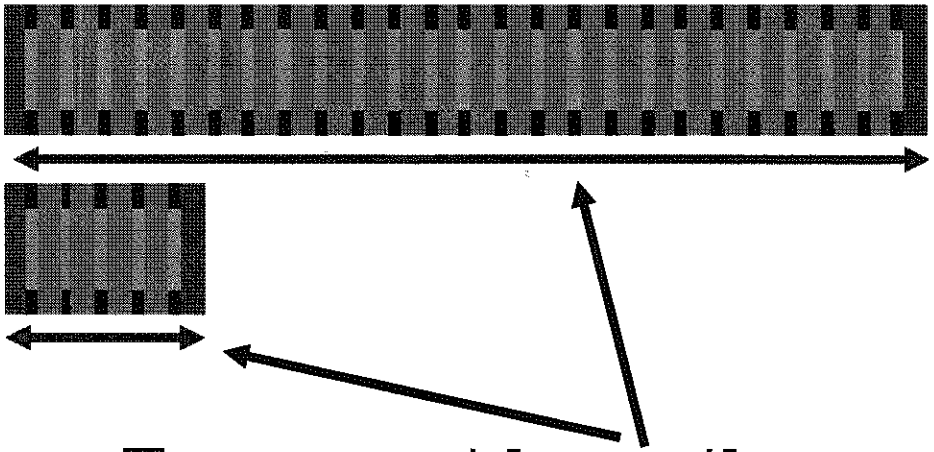


R



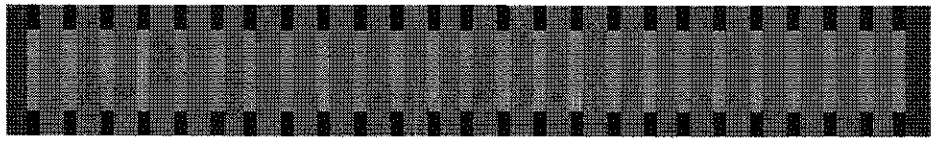
Restriction Fragment

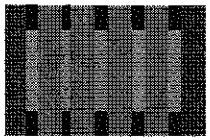
RF



Restriction Fragment Length

RFL

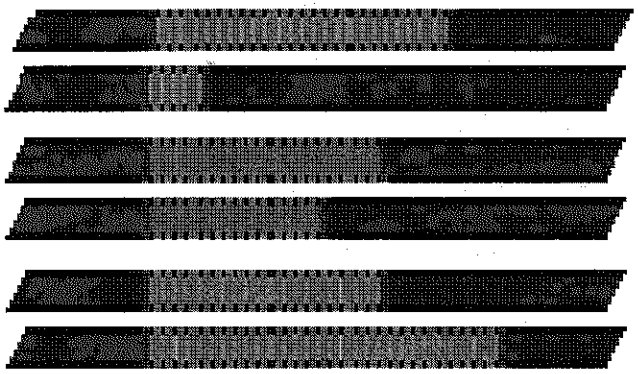




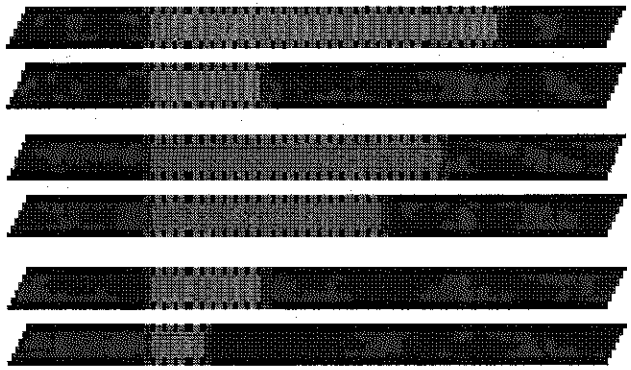
Restriction Fragment Length Polymorphism

RFLP

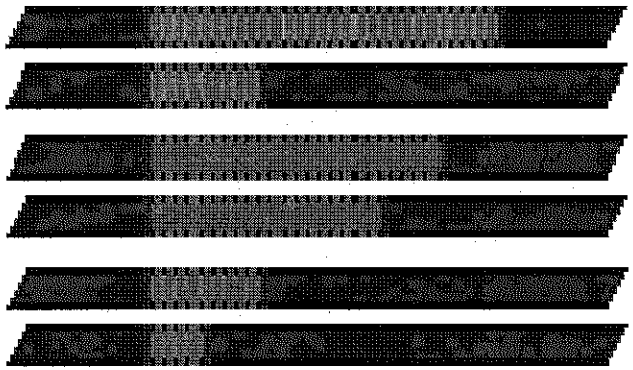
Individual #1



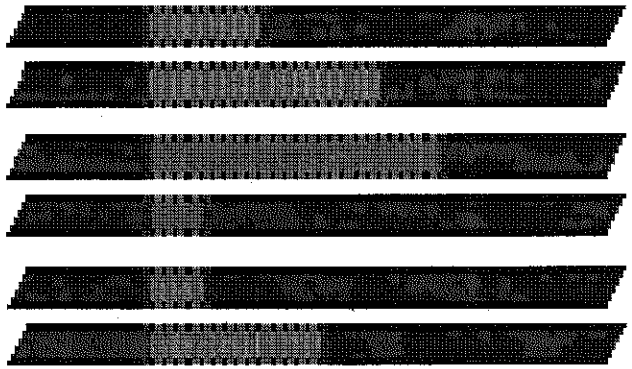
Individual #2

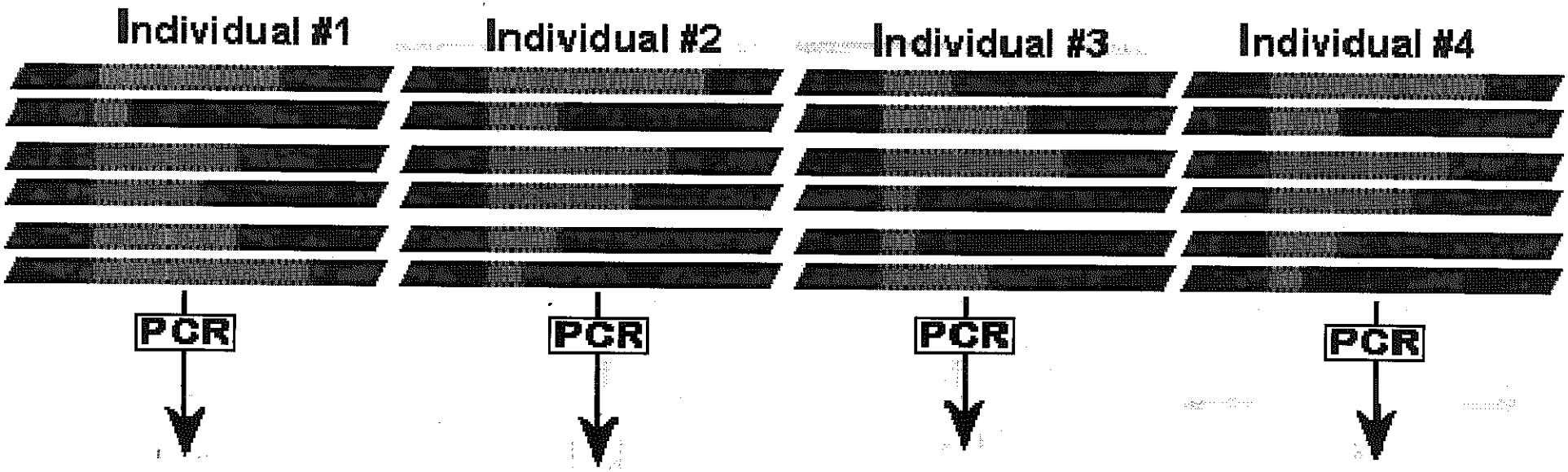


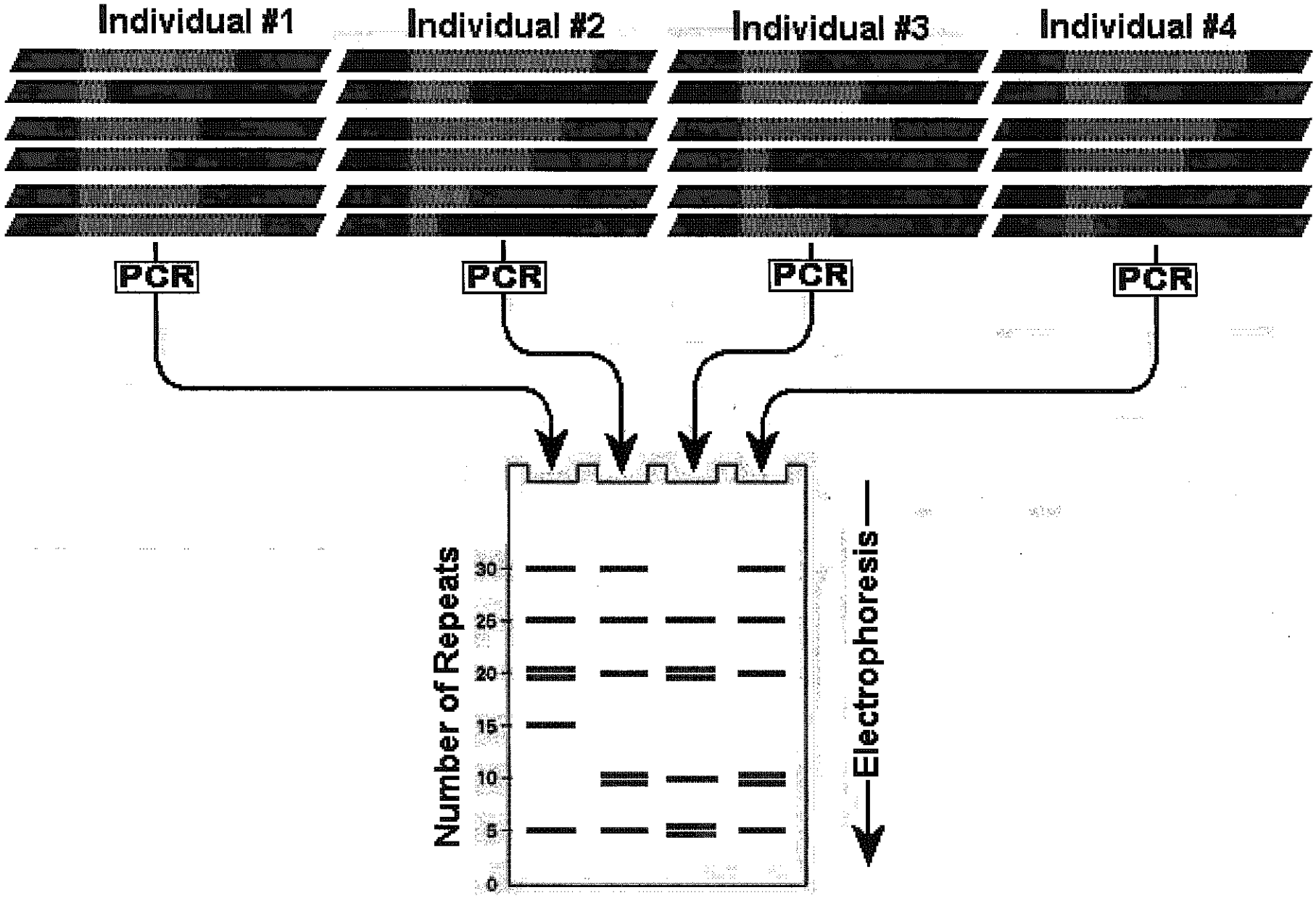
Individual #4



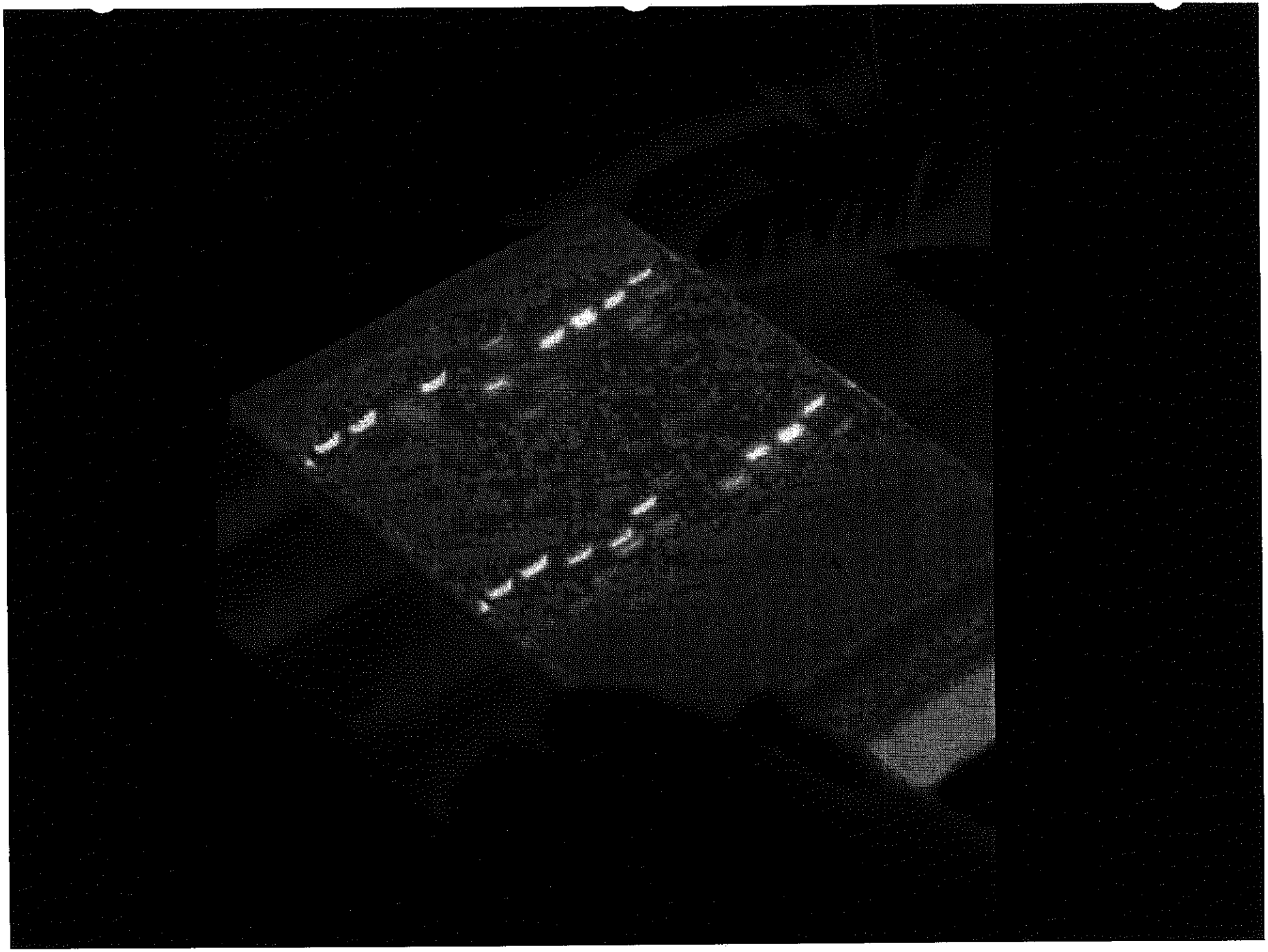
Individual #3

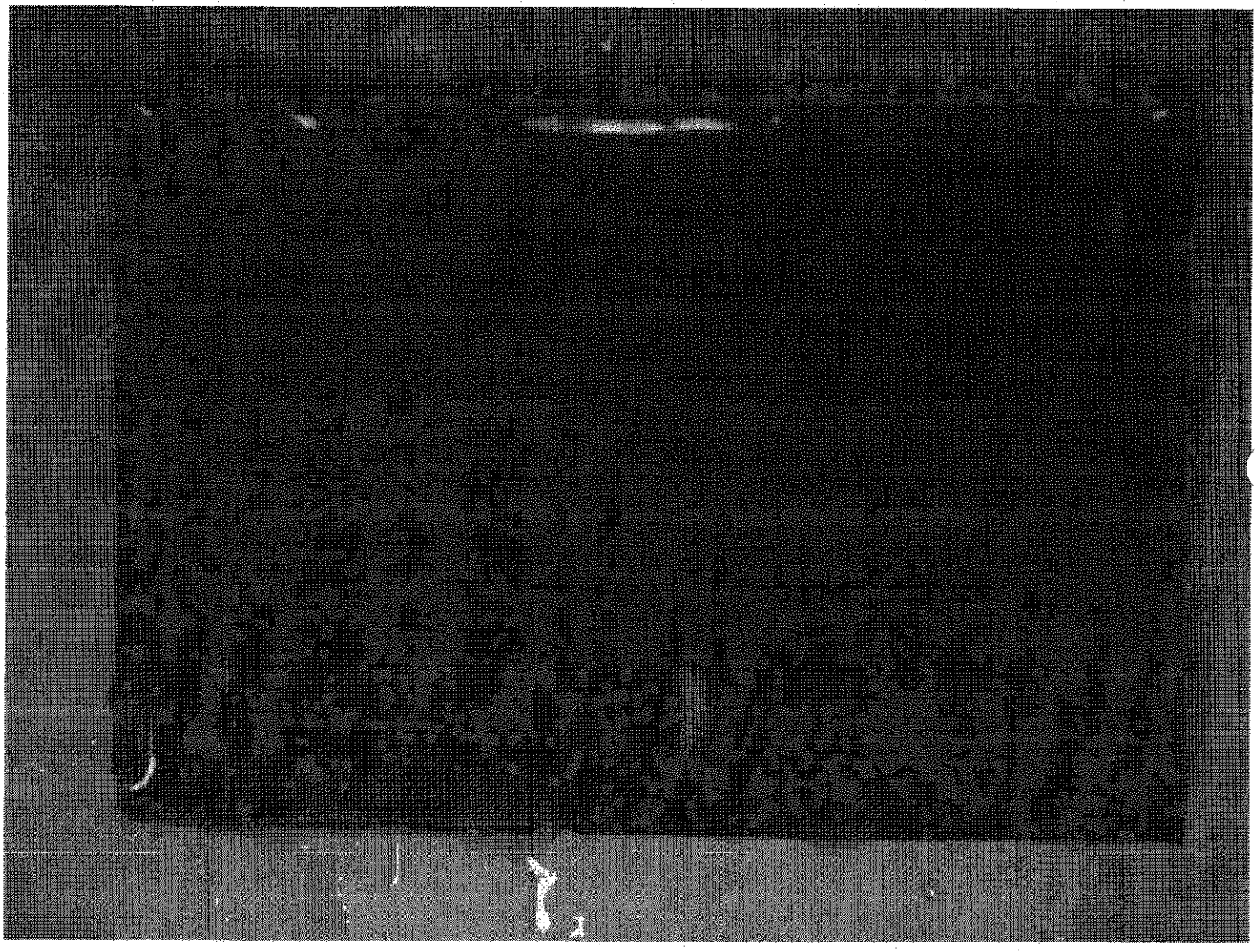



Individual #1
Individual #2
Individual #3
Individual #4
PCR
PCR
PCR
PCR

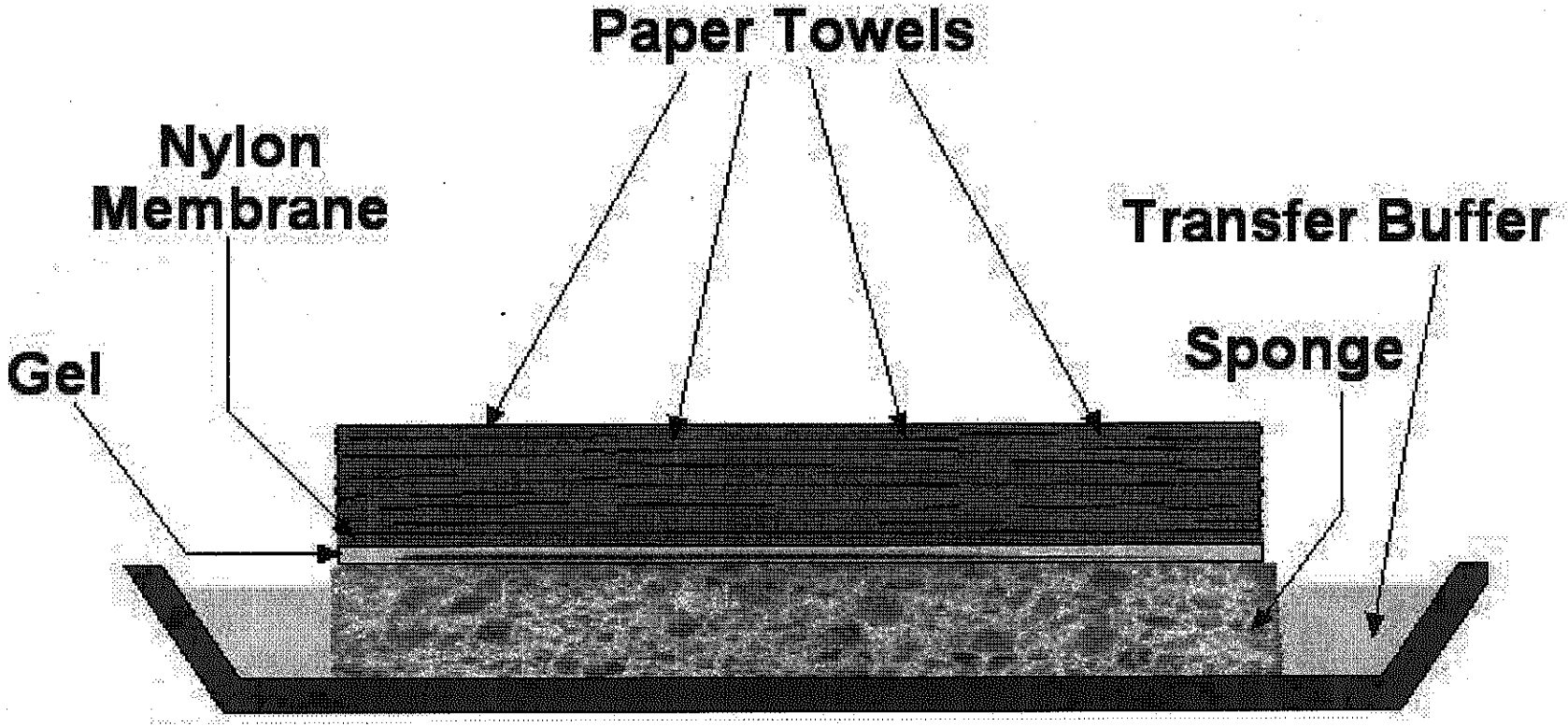


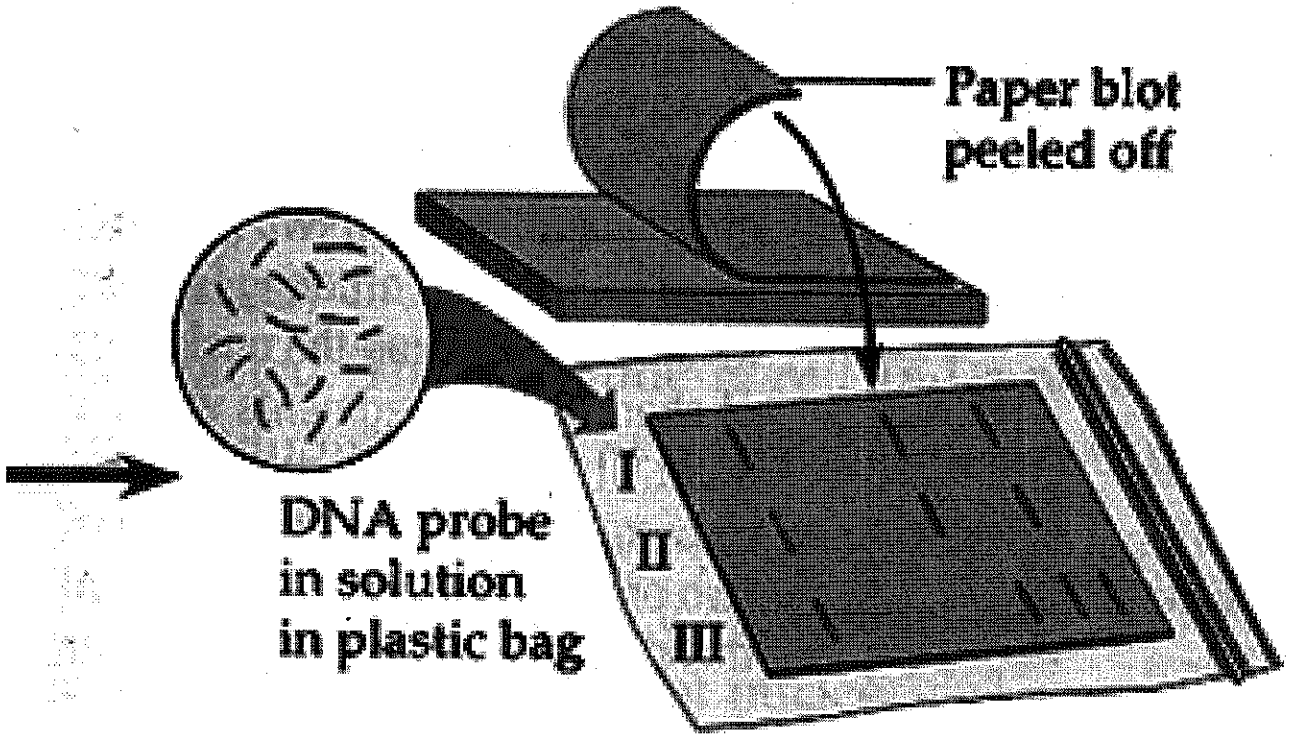
If this were a crime scene DNA fingerprint then lanes 2 & 4 would be the same individual.



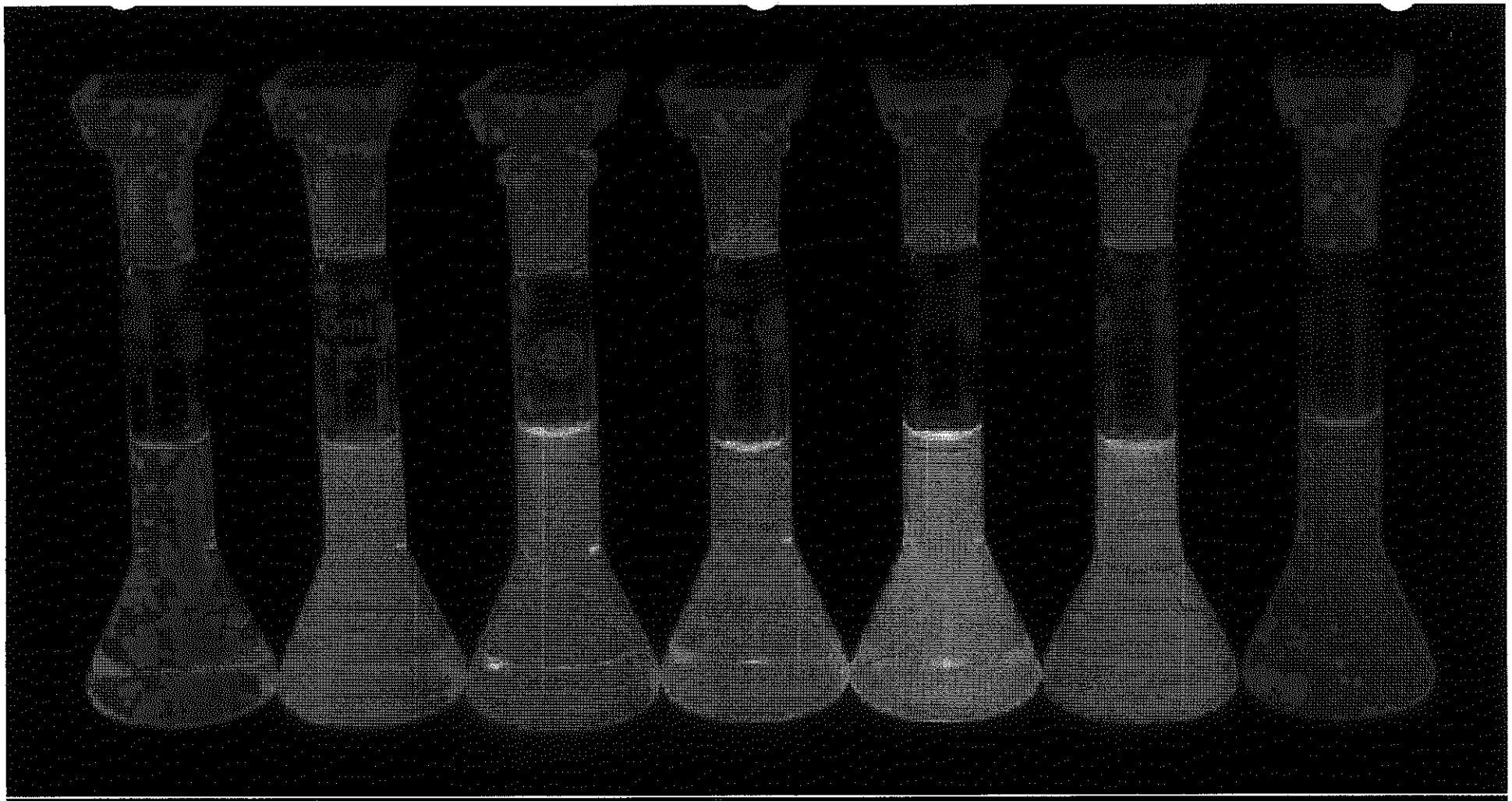


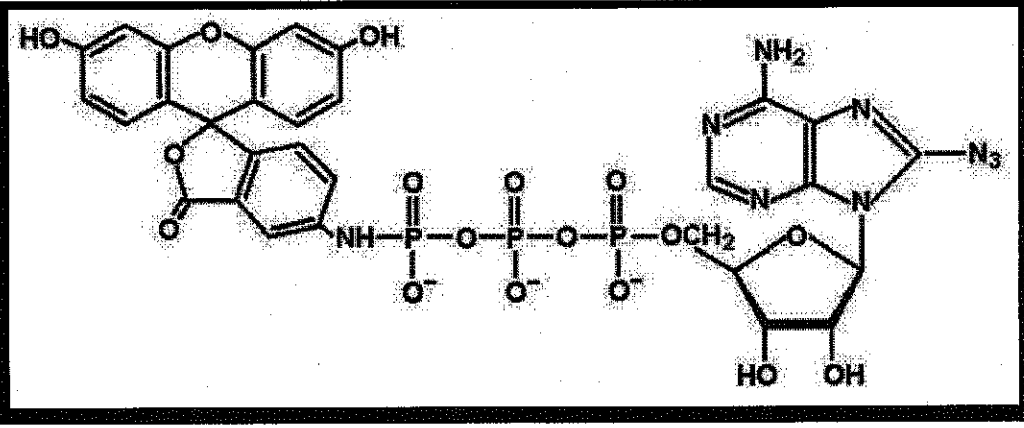
Southern Blot Setup

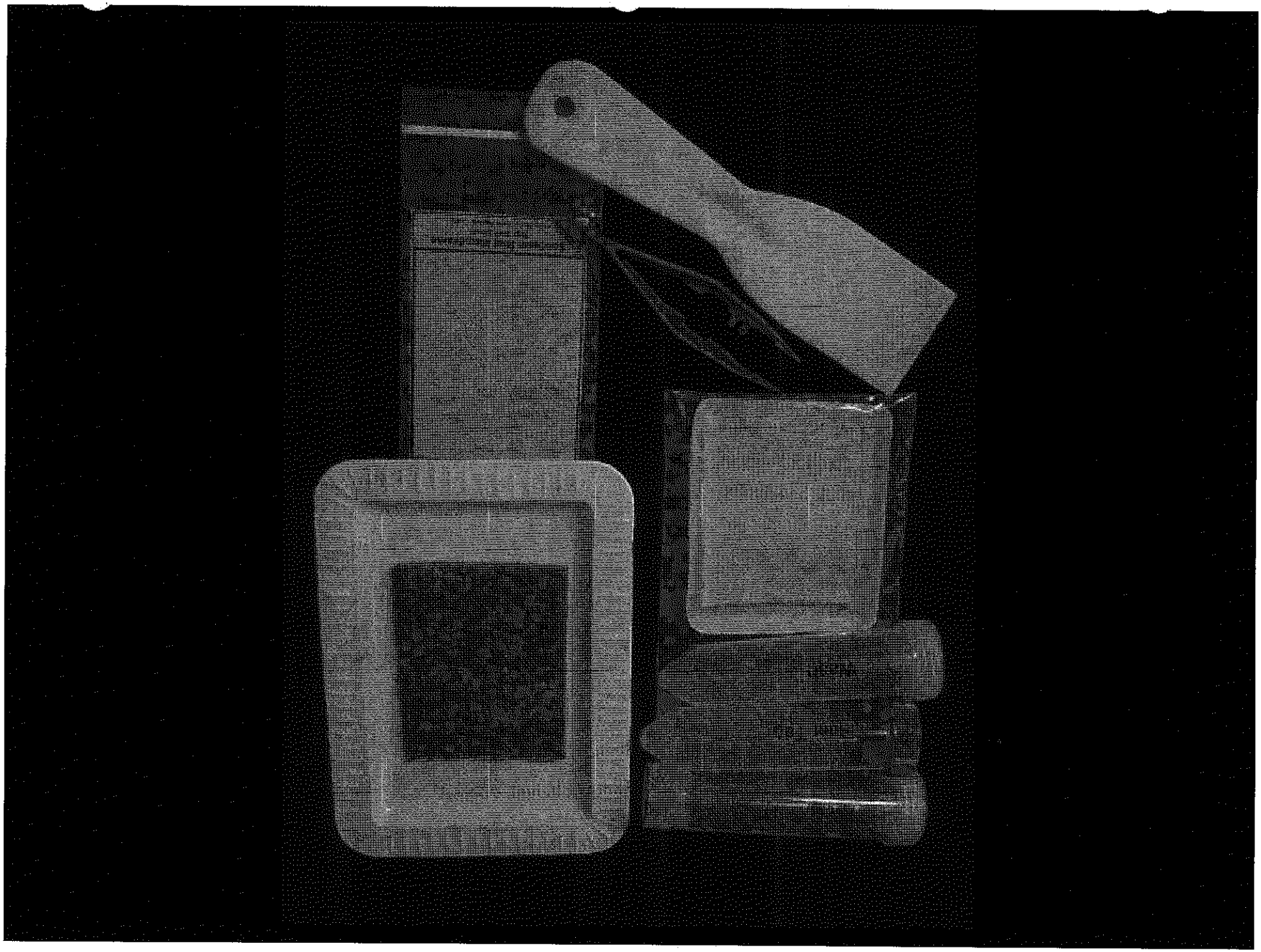




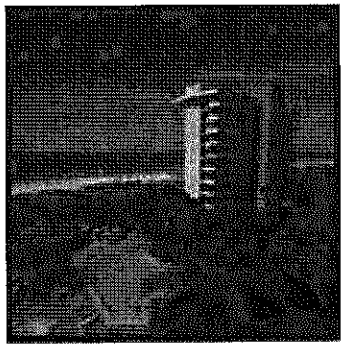
6 **Hybridization with radioactive probe.** The paper blot is exposed to a solution containing radioactively labeled probe. The probe is single-stranded DNA complementary to the DNA sequence of interest, and it attaches by base pairing to restriction fragments of complementary sequence.




HO
OH
NH
P
O
O⁻
OCH₂
NH₂
N
N₃
HO
OH

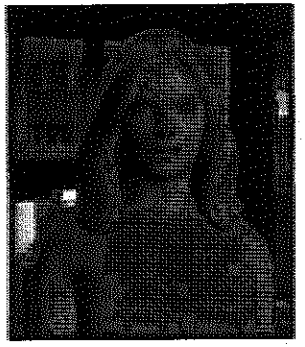


The High Rise Killer

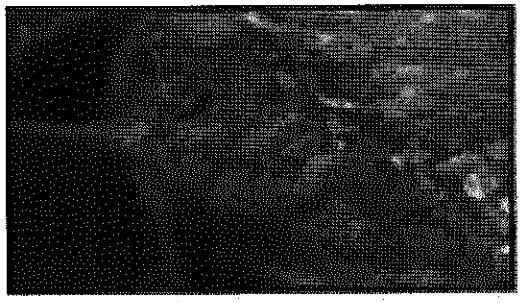


Tampa, Fl - May 3, 2005

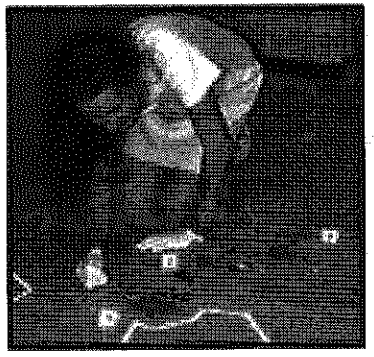
A maid, doing her daily cleaning at a posh condo, found Sarah Elizabeth Jones, 35, dead in her bathtub. The maid said that the doors were locked when she arrived.



Ms. Jones, who worked for an import company in St. Petersburg, had been on vacation at the time her body was found. She was not known to have a boyfriend but one man had been by seen by neighbors entering her condo on at least two occasions.

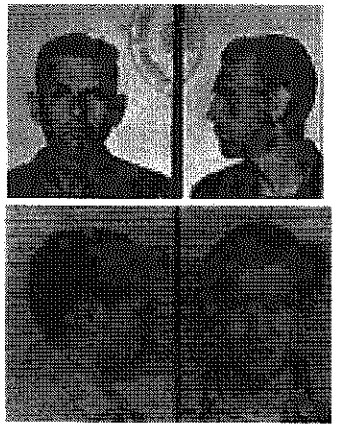


The victim appeared to have been killed after a struggle at her condo near the beach. Police found items strewn about the condo as well as blood in three different places including the bathroom where the body was found.



The numerous areas of blood were analyzed by crime scene technicians and were found to have come from two different people. Police speculate that the killer was injured in the struggle with the victim leaving his or her blood at the condo. Some of the blood was matched to the victim, but other samples came from an individual other than the victim.

Police also found fingerprints belonging to at least three persons, including the victim. The fingerprints were sent to the Integrated Automated Fingerprint Identification System (IAFIS) run by the FBI. IAFIS returned a match on two different men.



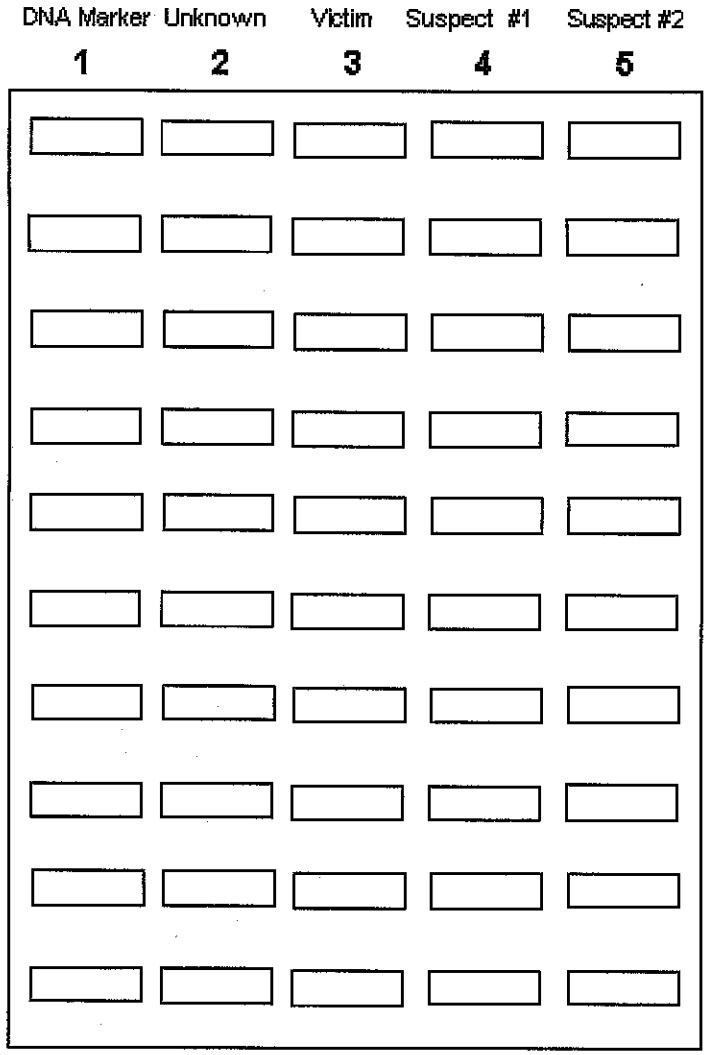
The first set of fingerprints belonged to Gerald L Goss, 45, of Nashville, Tennessee, who listed his occupation as librarian.

The second set belonged to John Kennedy, 35, of Denver, who listed his occupation as writer.

Search warrants were obtained for blood samples from each man. The samples were then compared using Restriction Fragment Length Polymorphism (RFLP) technology. The samples were run on an electrophoretic gel for comparison.

From these samples police hoped to determine which man's blood matched the blood found at the scene that would identify the killer.

A DNA length reference marker mix of all the DNA fragment lengths was placed in the well for lane 1 of the gel. DNA from the four blood samples were treated with restriction enzyme and placed in the four remaining lanes of the gel: lane 2) the unknown blood found at the site, lane 3) the blood of the victim, lane 4) the blood of John Kennedy and lane 5) the blood of Gerald L Goss.



Southern Blot Instructions

1. Pour 15 milliliters of buffer into the weigh boat.

2. Remove the nylon membrane from its plastic bag. Handle the nylon with forceps using the end with the label.

3. Place the nylon membrane into the weigh boat face down.

4. Soak the nylon in the buffer for 5 minutes. While you are waiting proceed with steps 5-7.

5. Place the sponge into the plastic tray.

6. Pour 50 milliliters of buffer into the tray.

7. With your fingers, carefully remove the gel from its plastic bag and place it on the sponge.

8. Place the wetted nylon membrane **face-down** onto the gel **with the printed part at the bottom of the gel** (the wells are at the top of the gel). Smooth out bubbles with a gloved finger.

9. Place a two inch stack of paper towels on top of the membrane.

10. Let the DNA transfer proceed for 20 minutes. During this time, empty the weigh boat in preparation of step 13.

11. Remove the paper towels.

12. Remove the nylon with forceps using the end with the label.

13. Place the nylon membrane face up in the weigh boat.

14. Pour the tube of fluorescent probes into the weigh boat.

15. Wait 5 minutes.

16. Remove the nylon with forceps using the end with the label.

17. Place the nylon membrane face up onto a paper towel.

18. Examine the nylon membrane with a fluorescent light and fill in the Southern Blot data sheet.

Southern Blot Instructions

Wear Gloves

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5. Place the sponge into the plastic tray.

6. Pour up to 50 milliliters of buffer into the tray but do not cover the sponge.

7. With your fingers, carefully remove the gel from its plastic bag and place it on the sponge.

13. Place the nylon membrane face up in the weigh boat.

14. Pour the tube of fluorescent probes into the weigh boat.

15. Wait 5 minutes.

16. Remove the nylon with forceps using the end with the label.

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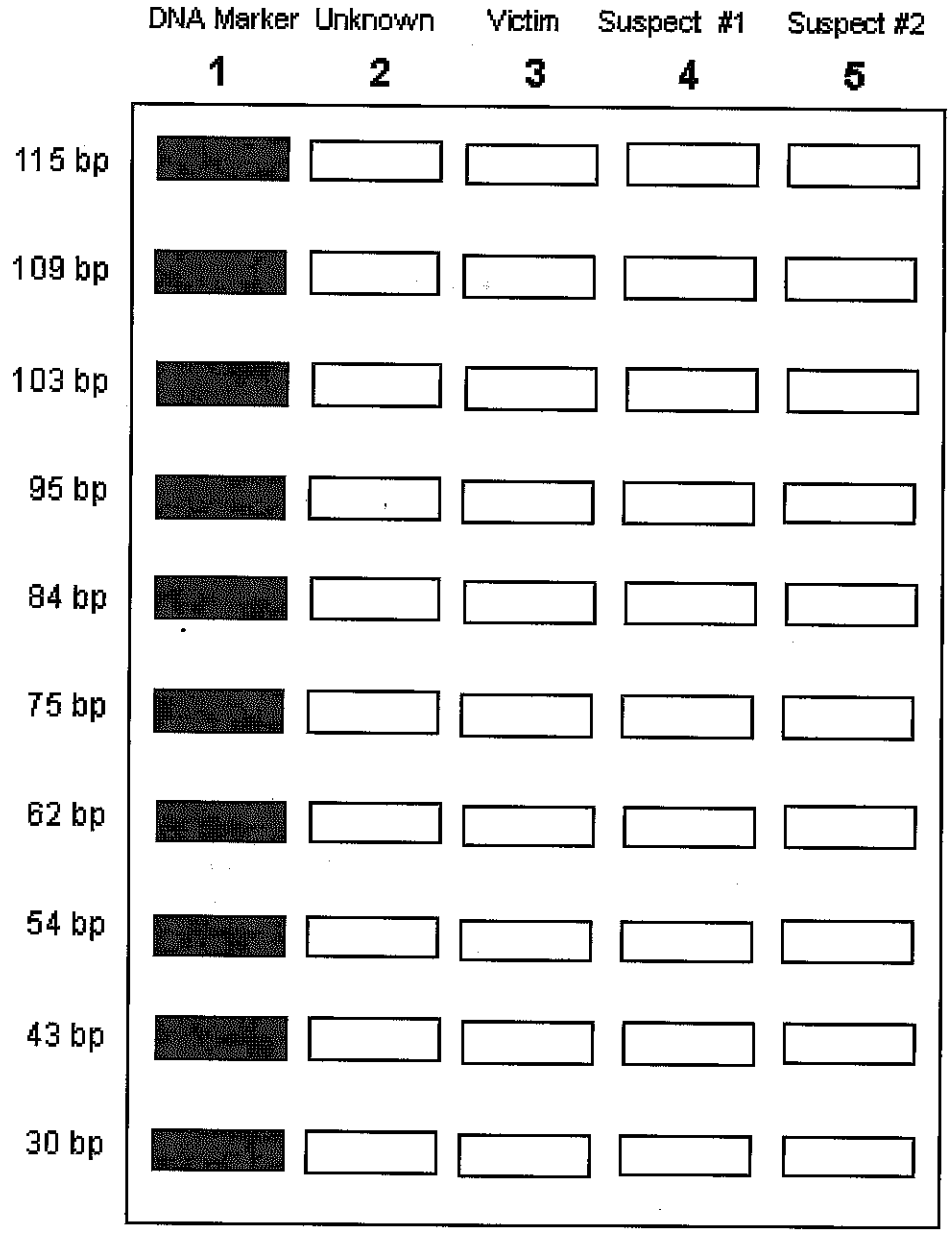
15. Wait 5 minutes.

16. Remove the nylon with forceps using the end with the label.

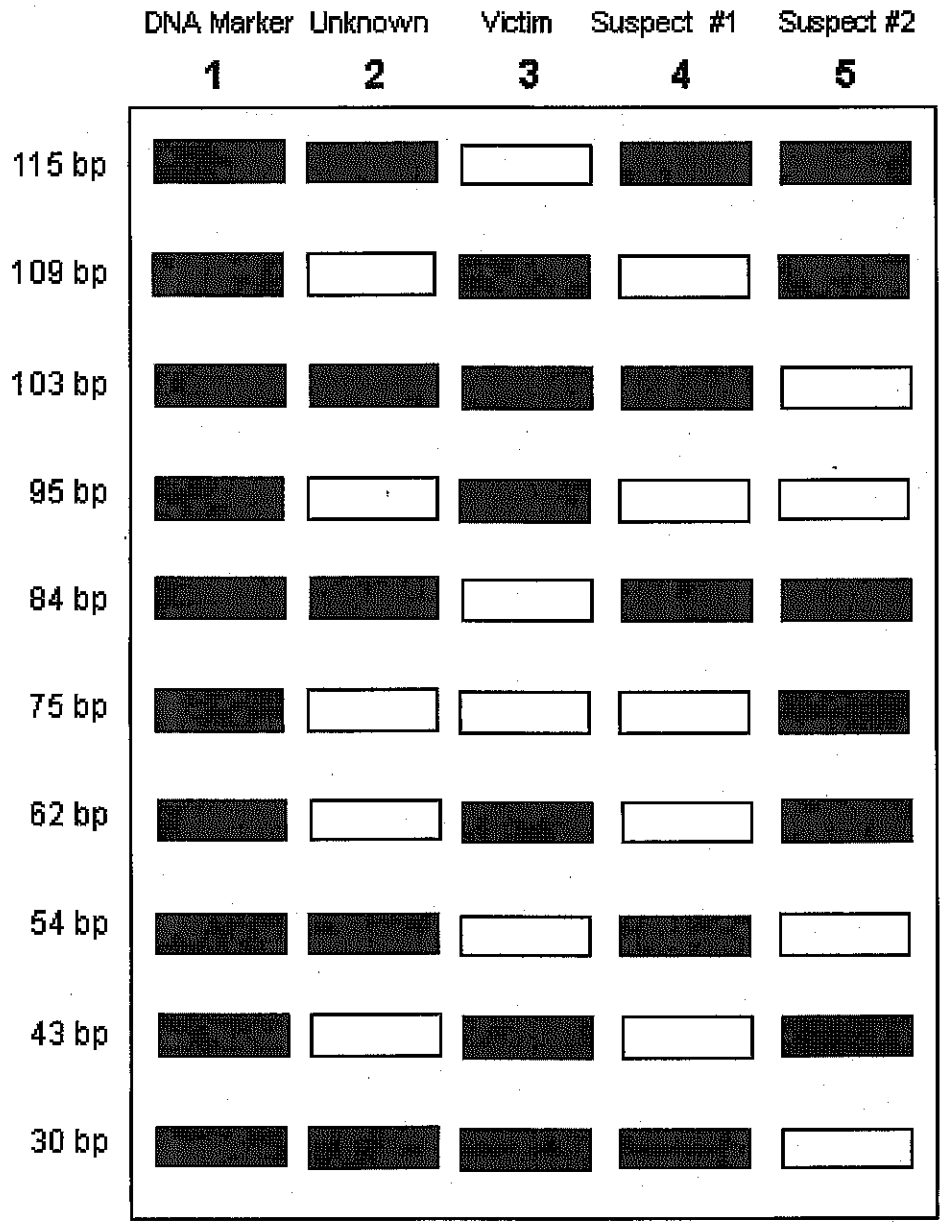
17. Place the nylon membrane face up onto a paper towel.

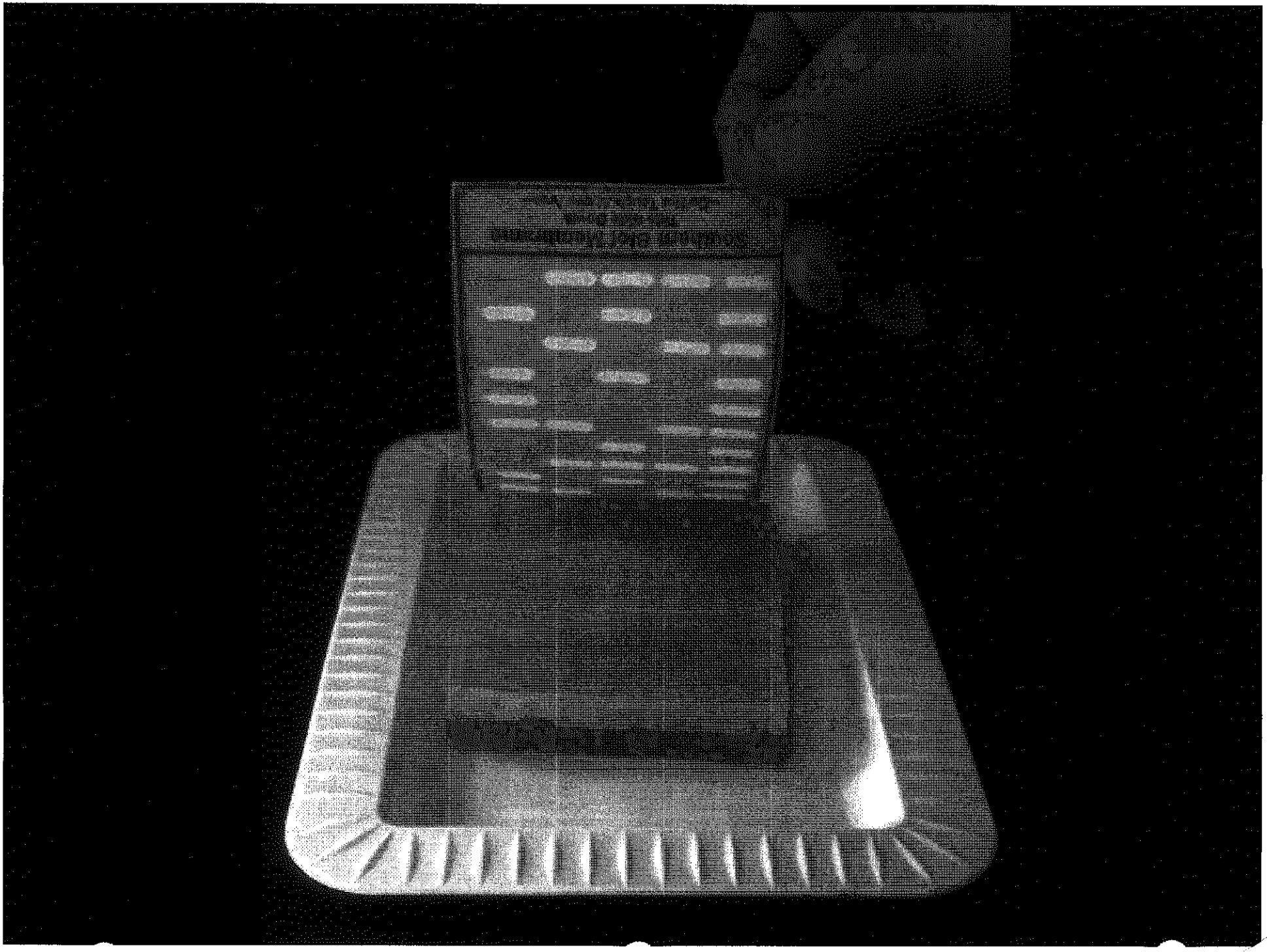
18. Examine the nylon membrane with a fluorescent light and fill in the Southern Blot data sheet.

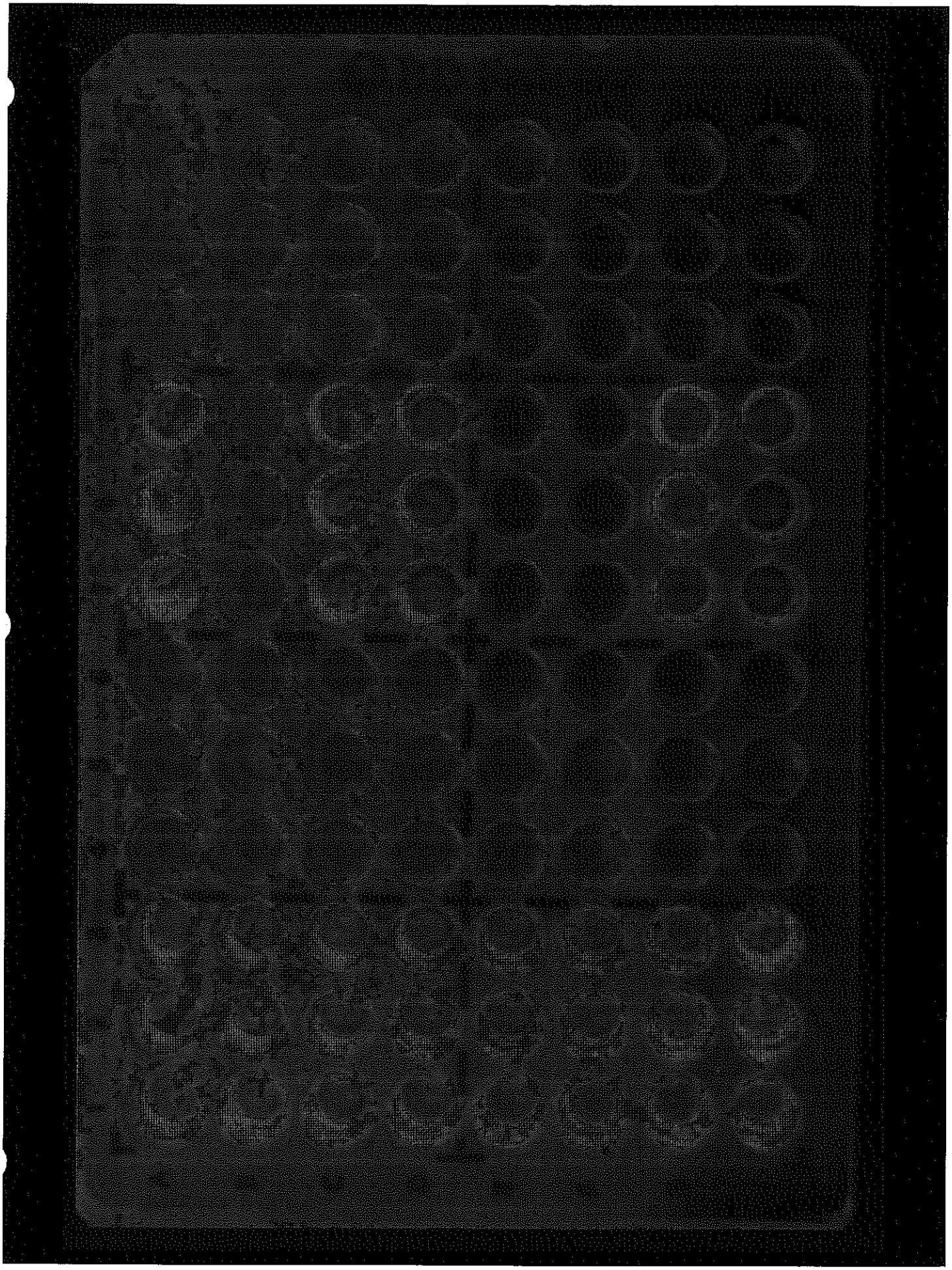
Student Worksheet
"The High Rise Killer"



Teacher Key
"The High Rise Killer"







ELISA Test Key