EDVOTEK®

PLTW

STUDENT VERSION

EDVOTEK & PLTW Experiment #430

DNA Analysis

(PBS 1.1.6)

Experiment Objective:

The objective of this experiment is to develop a basic understanding of DNA Fingerprinting. First, students will master micropipetting, an essential biotechnology technique. Next, they will analyze PCR reactions obtained from different suspects and compare them to a crime scene sample.

See page 3 for storage instructions.

Version 430.200430

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Experiment Components

MODULES I & II:

All components for Modules I and II can be stored at room temperature.

Components	Check (✓)
• Red Dye	❑
• Blue Dye	❑
• Purple Dye	❑
• Yellow Dye	❑
• Navy Dye	❑
• Test strips, Pipetting by Numbers Canvas, and Blank Canvases	❑
• Microcentrifuge tubes	❑

Experiment #430 is designed for 10 groups.

STORAGE:

Store QuickStrip™ samples in the refrigerator immediately upon receipt. All other components can be stored at room temperature.

MODULES III & IV:

Store QuickStrip™ samples in the refrigerator immediately upon receipt.
All other components can be stored at room temperature.

Components (in QuickStrip™ format)	Check (✓)
A Standard DNA Marker	❑
B "Blood" from Crime Scene: Table	❑
C "Blood" from Crime Scene: Floor	❑
D Eric Piedmont	❑
E Dominique Hall	❑
F Anna Garcia	❑

REAGENTS & SUPPLIES

• UltraSpec-Agarose™	❑
• Electrophoresis Buffer (50x)	❑
• SYBR® Safe Stain	❑
• Practice Gel Loading Solution	❑
• FlashBlue™ DNA Stain	❑

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

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Requirements

For Modules I and II:

- Automatic micropipettes* with tips
- Small containers for discarding used tips
- Scissors
- Tape

For Modules III and IV:

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipettes with tips
- Balance
- Microwave, hot plate or burner
- Pipet pump
- 250 mL flasks or beakers
- Hot gloves
- Safety goggles and disposable laboratory gloves
- Small plastic trays or large weigh boats (for optional gel staining/destaining)
- Distilled or deionized water
- Blue light or UV transilluminator
- White light visualization system (optional)

* *The provided pipetting templates require students to pipette 10-60 µL. Several pipette combinations can accommodate these different volumes. The Pre-Lab Preparations will also require a 100-1000 µL micropipette.*

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Background Information

Deoxyribonucleic acid (DNA) is present in every living cell. It is the genetic material that acts as the blueprint for protein synthesis by cells. Your DNA is inherited from your parents, and is in turn passed down to future generations. Importantly, everyone's DNA is unique. Polymorphic DNA refers to chromosomal regions that vary among individuals. By examining several of these variable regions within genomic DNA, one can determine a "DNA Fingerprint" for an individual. DNA polymorphisms are now widely used for determining paternity/maternity, kinship, identification of human remains, and to determine the genetic basis of various inherited diseases. The most widely used and far-reaching application has been to the field of criminal forensics. DNA from crime victims and offenders can now be definitively matched, affecting outcomes of criminal and civil trials.

DNA fingerprinting was first used as a forensic tool in the United Kingdom in 1984, following the pioneering work of Dr. Alex Jeffreys. Analysis by Jeffreys led to the apprehension of a murderer in the first DNA fingerprinting case in September 1987. The first U.S. conviction occurred on November 6, 1987 in Orlando, FL. Since then, DNA analysis has been used in thousands of convictions. Additionally, DNA evidence has exonerated numerous convicted inmates, including multiple death row inmates.

In 1990, the Federal Bureau of Investigation (FBI) established the Combined DNA Index System (CODIS), a system that allows comparison of crime scene DNA to DNA profiles of convicted offenders. CODIS has now been used to solve dozens of cases where authorities had no suspect for the crime under investigation.

The first step in forensic DNA fingerprinting is the collection of blood or other tissue samples from the crime scene or victim (Figure 1). A blood sample, often present as a stain, is treated with a buffer solution that contains detergent to rupture the cell membrane and obtain DNA for further analysis. When this technology was in its early stages, a method, called restriction fragment length polymorphism (RFLP) analysis, was used.

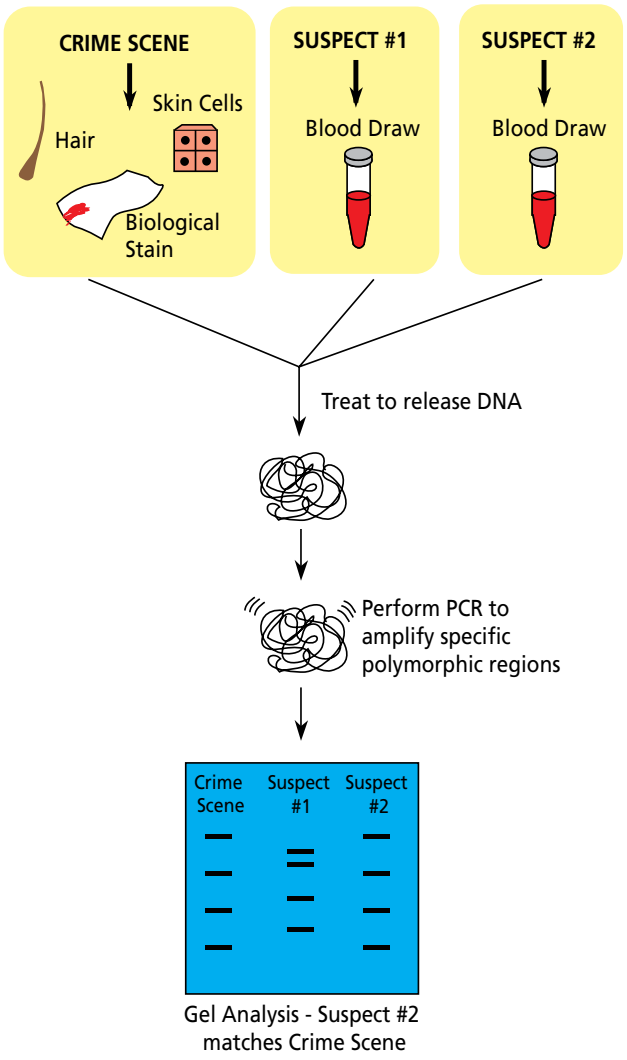

Figure 1:
Comparison of a crime scene DNA fingerprint with those of two suspects.

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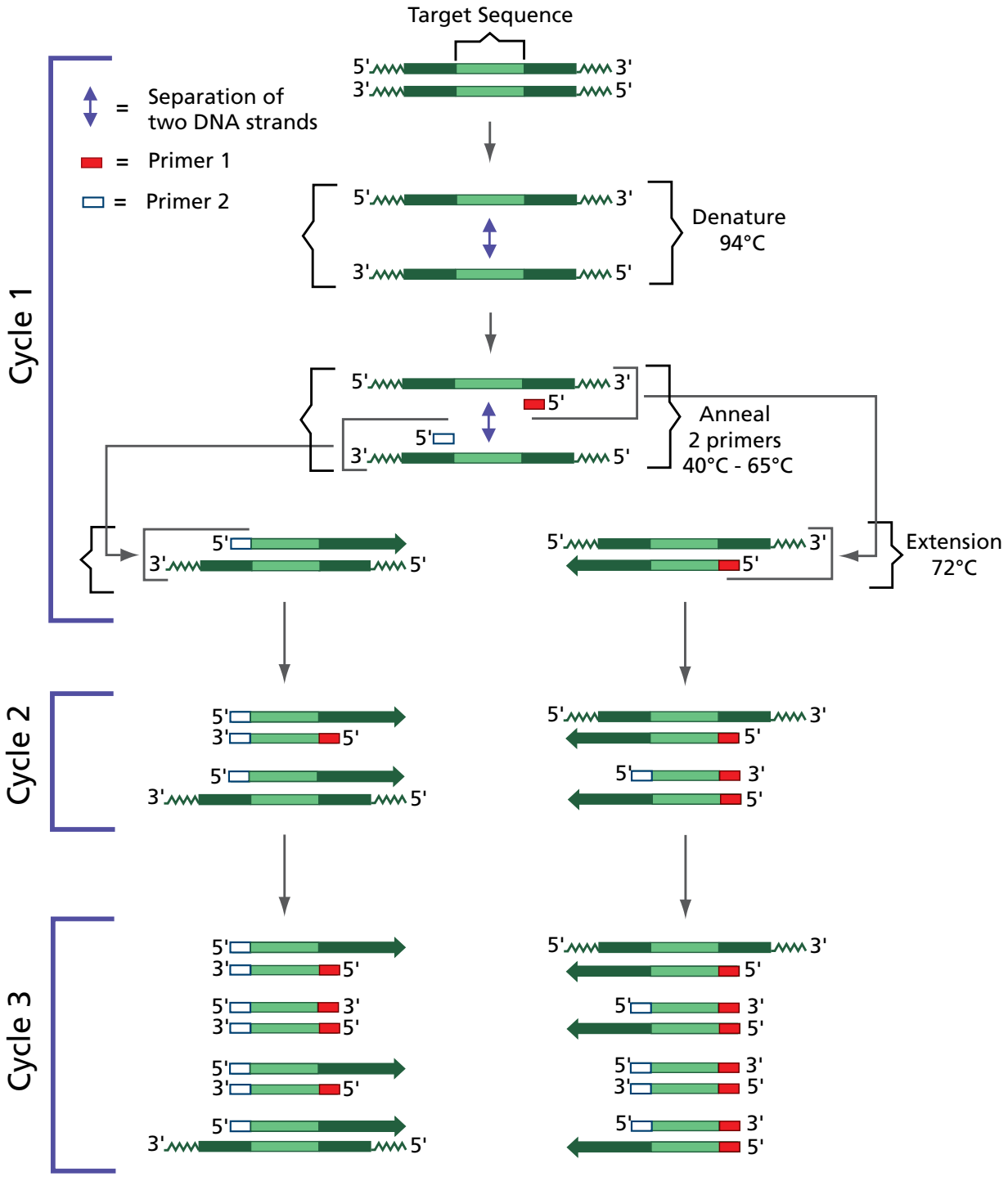

Figure 2:
DNA Amplification by the Polymerase Chain Reaction

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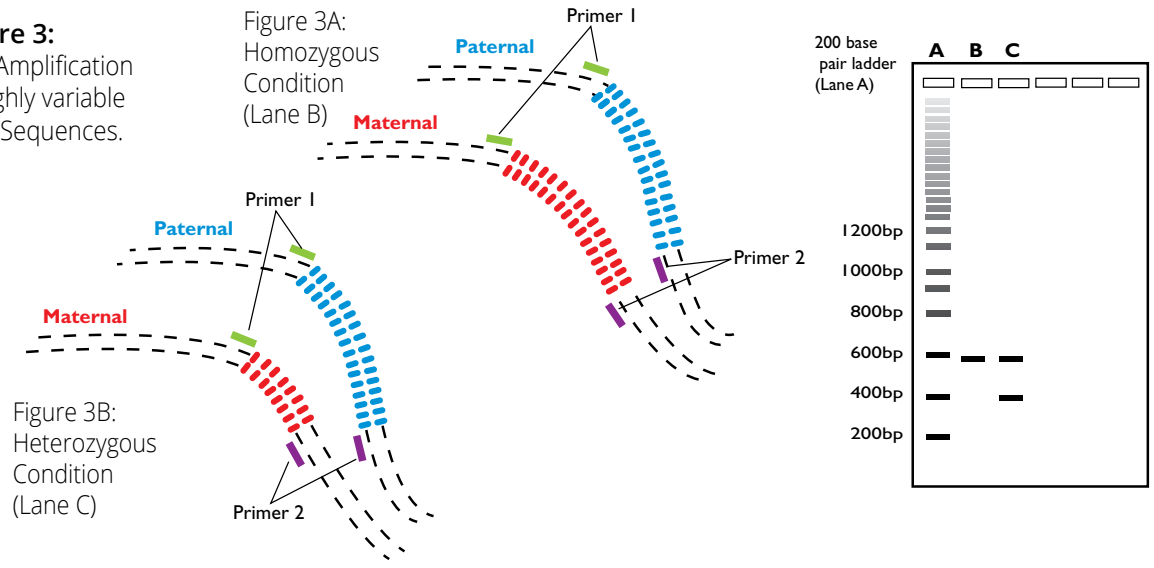
RFLP involves digesting the DNA with restriction enzymes, separation on an agarose gel, transferring the DNA to a membrane, and hybridizing the DNA on the membrane with probes to detect polymorphic regions. This procedure, known as a Southern Blot, requires relatively large amounts of DNA and takes several days to complete.

More recently, the polymerase chain reaction (PCR) has been used in forensics to analyze DNA (See Figure 2). This technique requires about 500-fold less DNA than RFLP analysis and is cheaper and less time-consuming. PCR amplification (Figure 2) uses an enzyme known as *Taq* DNA polymerase. This enzyme was originally purified from a bacterium that inhabits hot springs and is stable at very high (near boiling) temperatures. Also included in the PCR reaction mixture are two short synthetic pieces of DNA known as "primers" and the extracted DNA. The region in DNA to be amplified is known as the "target".

In the first step of the PCR reaction, the template DNA strands are separated (denatured) from each other at 94°C. The *Taq* DNA polymerase remains stable at this high temperature. In the second step, known as annealing, the sample is cooled to an intermediate temperature, usually 40° - 65° C, to allow hybridization of the two primers, one to each of the two strands of the template DNA. In the third step, known as extension, the temperature is raised to 72° C and the *Taq* polymerase adds nucleotides to the primers to complete the synthesis of the new complementary strands. These three steps - denaturation, annealing, and extension - constitute one PCR "cycle". This process is typically repeated for 20-40 cycles, amplifying the target sequence of DNA exponentially (Figure 2). PCR is performed in a thermal cycler, an instrument that is programmed to rapidly heat, cool and maintain samples at designated temperatures for varying amounts of time.

In forensics, PCR is used to amplify and examine highly variable (polymorphic) DNA regions (Figure 3). These are regions that vary in length from individual to individual and fall into two categories: 1) variable number of tandem repeats (VNTR) and 2) STR (short tandem repeats). A VNTR is a region that varies amongst individuals and is typically composed of 15 to 70 base pair sequences, repeated 5 to 100 times. An STR is similar to a VNTR except that the repeated unit is only 2 to 4 nucleotides in length. The shorter length of STRs often makes PCR analysis easier, making them the preferred genetic marker for DNA fingerprinting. By examining several different VNTRs or STRs from the same individual, investigators obtain a unique DNA profile for that individual that is unlike that of any other person (except for identical twins).

Figure 3:
PCR Amplification of highly variable DNA Sequences.



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TOOLS FOR DNA FINGERPRINTING: THE MICROPIPETTE

Modern forensic scientists use advanced equipment to analyze samples, detect trace chemicals, and identify suspects. One of the most essential laboratory tools is the adjustable volume micropipette (Figure 4). These devices allow scientists to accurately and safely work with experimental solutions. Consequently, they are used daily in almost all biotech labs.

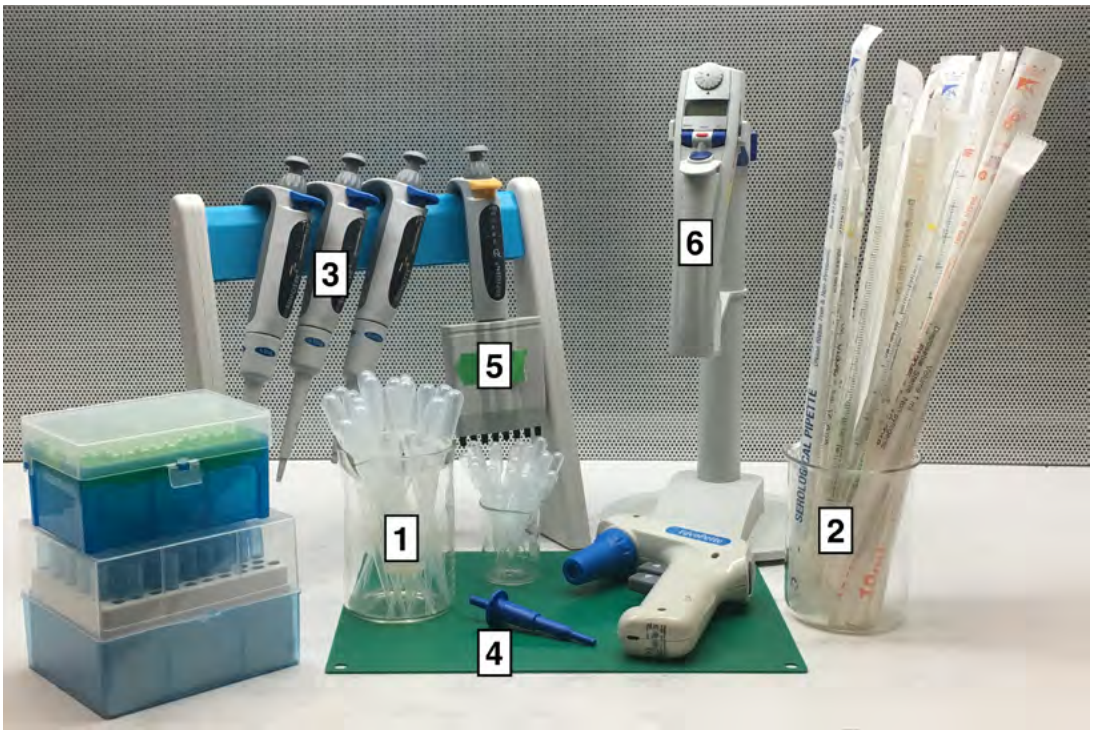

Figure 4: Common pipettes used in the lab. (1) Pasture pipettes, (2) Graduated pipettes, (3) Adjustable Micropipettes, (4) Fixed Micropipettes, (5) Multichannel Pipette, (6) Robotic Pipette.

WHAT IS A PIPETTE?

Pipettes are a large group of laboratory tools that scientists use to measure and manipulate liquids. The simplest pipettes closely resemble eye droppers with squeezable bulbs that can suction liquid up a narrow cylinder. These are known as ***Pasteur pipettes*** after their inventor Louis Pasteur. While Pasteur's primary goal was to protect his liquid samples from contamination during transfer, Pasteur pipettes can also be used to make approximate measurements.

Graduated pipettes are similar to Pasteur pipettes but designed for higher volumes. Consequently, graduated pipettes tend to be used when preparing larger amounts of general use buffers, mass producing products, performing analytical chemistry experiments, and working with cells. In order to draw in large volumes of liquids, most graduated pipettes are attached to either a very large silicone bulb or an electronic pump.

For smaller volume reactions - such as those required for most molecular and microbial biology experiments - scientists use ***micropipettes***. These were originally miniature versions of a graduated pipette attached to a bulb or even a suction piece. However, today, most labs are equipped with *piston displacement micropipettes*. Inside these pipettes are a solid disk and tightly fitted tube (Figure 5). When the user depresses a plunger the disk moves

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down which pushes air out of the tube and the connected tip. Next, the user immerses the tip into a solution and releases the plunger which creates a vacuum. This vacuum causes the liquid in the sample to flow up into the tip. The volume of this liquid (sometimes called the load volume) is equivalent to the volume of air displaced by the downward moving piston. Therefore, load volumes can be set to a specific value by calibrating the piston's height. Early displacement pipettes had pistons that were ***fixed*** at a single height and so could measure one specific volume. However, later displacement pipettes were redesigned so that the user could repeatedly ***adjust*** the piston height in order to measure out a range of volumes (Figure 6).

Continued innovation has created several additional pipettes. For example, ***multichannel pipettes*** can hold a whole row of tips, which allows scientists to prepare multiple samples at once, and ***positive displacement pipettes*** have disposable pistons for when scientists are working with hot, hazardous, thick, or corrosive solutions. ***Robotic pipettes*** also exist. At their simplest, robotic pipettes repeatedly release a specified volume to multiple tubes or wells (a process known as aliquoting) in response to a light tap or time interval. More advanced robot pipettes take advantage of artificial intelligence to carry out whole experiments - measuring and mixing multiple volumes, examining the results, and then revising the volumes based on their interpretation!

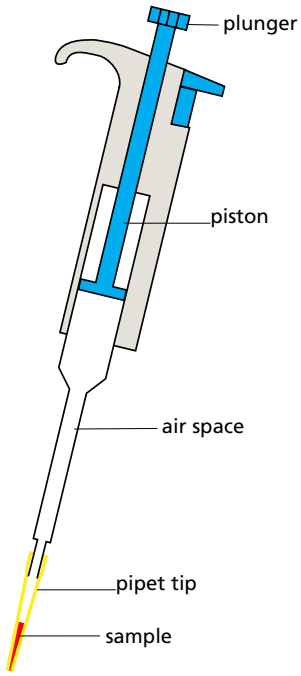

Figure 5: Inside of a piston pipette.

HOW TO USE AN ADJUSTABLE MICROPIPETTE

Because pipettes are so frequently used in the lab, pipetting quickly becomes an almost instinctive action. However, the pipetting process can be far from intuitive and fluid at first. The major steps of pipetting are: prepare, aspirate, dispense, re-aspirate and re-dispense, and finally purge. These are briefly described below. In addition, Module I of the experiment will walk you through this process in detail.

1. **PREPARE:** Set the volume by dialing the pipette to the appropriate value and adding a tip.

2. **ASPIRATE:** Create a vacuum by pushing the plunger down and placing the tip into the sample. Then slowly release the plunger. Finally, pause to make sure the liquid has moved into the tip.

   *This step is slightly complicated by the fact that most micropipettes have two plunger positions (Figure 7). Depressing the button to the first "soft" stop (red brackets in Figure 7) creates a vacuum that matches the set volume. This will result in the correct amount being aspirated. However, depressing the button to the second "hard" stop (red + blue brackets in Figure 7) will expel additional air and create a larger vacuum

Figure 6: Parts of an adjustable micropipette.

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than desired. While a great feature for purging, using the second stop when aspirating will create an inaccurate measurement.

3. **DISPENSE:** Place the tip where you want to add the sample and push the plunger down. This increases the pressure inside the pipette which causes the liquid to flow out. However, it also creates a new vacuum so make sure to lift the tip out of the solution before releasing the plunger.

4. **RE-ASPIRATE and RE-DISPENSE:** Repeat steps 2 and 3 if you are pipetting the same amount from the same start solution. Change tips as needed.

5. **PURGE:** Ensure that all the sample is released by pushing the plunger down to the second hard stop. After all the liquid has been ejected, remove the pipette and tip from the solution, release the plunger, eject the tip, and finally store the pipette upright in an easy to access location.

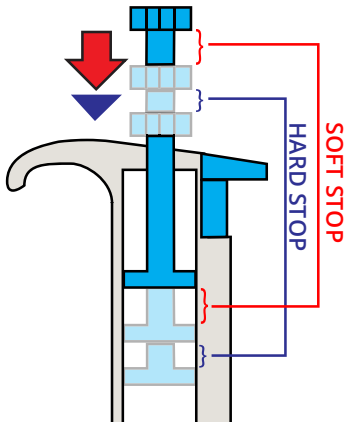

Figure 7: Two stops in a pipette.

Most biotechnology experiments involve preparing reactions by going through these steps multiple times for multiple reagents. Furthermore, most reactions get duplicated or even triplicated in order to confirm the reproducibility of the result. Such practice quickly builds pipetting familiarity!

In addition to the steps outlined above, there are several good laboratory practices to keep in mind when working with pipettes. These practices reduce the chance of cross contamination and increase experimental accuracy and reproducibility. They also help maintain the functionality of the equipment and reduce the risk of injury to you, the scientists. Below are seven "golden rules" of pipetting:

- Use the correct pipette for the volume you are pipetting. Micropipettes come in different sizes which have a specific range. Never adjust the volume beyond the upper or lower limit of this range as it can compromise the accuracy of the experiment and the integrity of the pipette.

- Use the correct tip for the pipette you are using. Micropipette tips come in different sizes and like their pipette counterparts each size has an optimal range. Using the correct tip helps to maintain a buffer between the solution and the internal pipette tubing and ensures a tight seal between the tip and pipette. (A poorly sealed pipette tip can affect accuracy by up to 50%!)

- Switch pipette tips between samples to avoid contamination. Also, switch pipette tips if the tip comes in contact with anything other than the samples or sample vessels.

- Keep pipettes vertical or vertically angled with the tip or tip cone facing down. This is especially important when there is liquid in the tip. Laying a pipette down that has a liquid filled tip can contaminate both the sample and pipette. In some cases, it can also damage the pipette.

- For maximum accuracy and to avoid contamination, depress and release the plunger using smooth and slow movements.

- Apply a consistent technique between samples. Small differences in pipetting (the depth that a tip is immersed, the angle that the pipette is held at, the force used to lower the plunger, the speed used to raise the plunger, etc.) can quickly add up to larger differences in volume.

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- Remember that you are a key component of the pipetting operation! Practice good ergonomics especially when pipetting multiple times - sit or stand so that you are comfortable and so that you can see your samples, hold the pipette safely but loosely, and when possible keep both wrists in a neutral position. Also wear gloves, a lab coat, and goggles when needed to protect yourself and avoid contamination.

ACCURACY AND PRECISION

Pipetting is such an important technology and technique because it enables scientists to accurately and precisely measure volumes, which in turn results in successful and repeatable experiments. What are these two traits? Accuracy describes how close a measurement is to the true value of a given quantity while precision describes the reproducibility of the measurement. One way to think of this is to imagine a basketball player shooting baskets. If the player has a high accuracy then the ball will always be close to or in the basket. If the player is precise then the ball will always go to the same location - which may or may not be close to the basket. In the case of multiple pipette measurements, the set of pipetted volumes would be accurate if their average was close to the amount intended to be measured and would be precise if all the values were close together. Accuracy and precision are the ideal duo but they do not necessarily go hand in hand. Measurements can be either accurate, or precise, or both, or neither (Figure 8).

Accuracy in pipetting is best ensured by using the pipette correctly, keeping the pipette (and in particular the internal piston and tube) in good working order, and by periodically recalibrating the volume. During calibration, an outside measurement is made - in pipetting it is the volume of water based on weight - and then used to determine how far off the pipette measured is from this alternative or "true" value. Precision is also related to having a well functioning and well-maintained pipette. Precise pipetting also depends on using a correct and consistent technique. At the end of this experiment, you should feel confident about using a pipette to maximize the accuracy and precision of all your experiments.

In this experiment, you will start by mastering the use of a micropipette in a pipetting-by-numbers practice scenario. Next, you will use your newly developed skills to perform an agarose gel electrophoresis experiment which will analyze DNA fingerprinting samples from a crime scene.

A useful mnemonic:

A**C**curate is **C**orrect. (or **C**lose to real value)
P**R**ecise is **R**epeating. (or **R**epeatable)

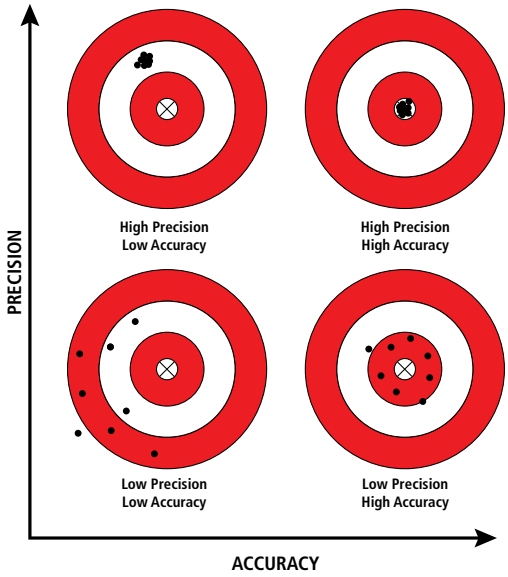

Figure 8: Different accuracy and precision combinations.

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Experiment Overview

EXPERIMENT OBJECTIVE:

The objective of this experiment is to develop a basic understanding of DNA Fingerprinting. First, students will master micropipetting, an essential biotechnology technique. Next, they will analyze PCR reactions obtained from different suspects and compare them to a crime scene sample.

LABORATORY SAFETY

Wear gloves and safety goggles

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. Exercise caution when using any electrical equipment in the laboratory.
4. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations and experiences.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Module I
Introduction to Micropipetting
15 min.

↓

Module II
Pipetting by Numbers
30 min.

↓

Module III
DNA Fingerprinting
45 min.

↓

Module IV
OPTIONAL: Staining with FlashBlue™
15 min. + Overnight

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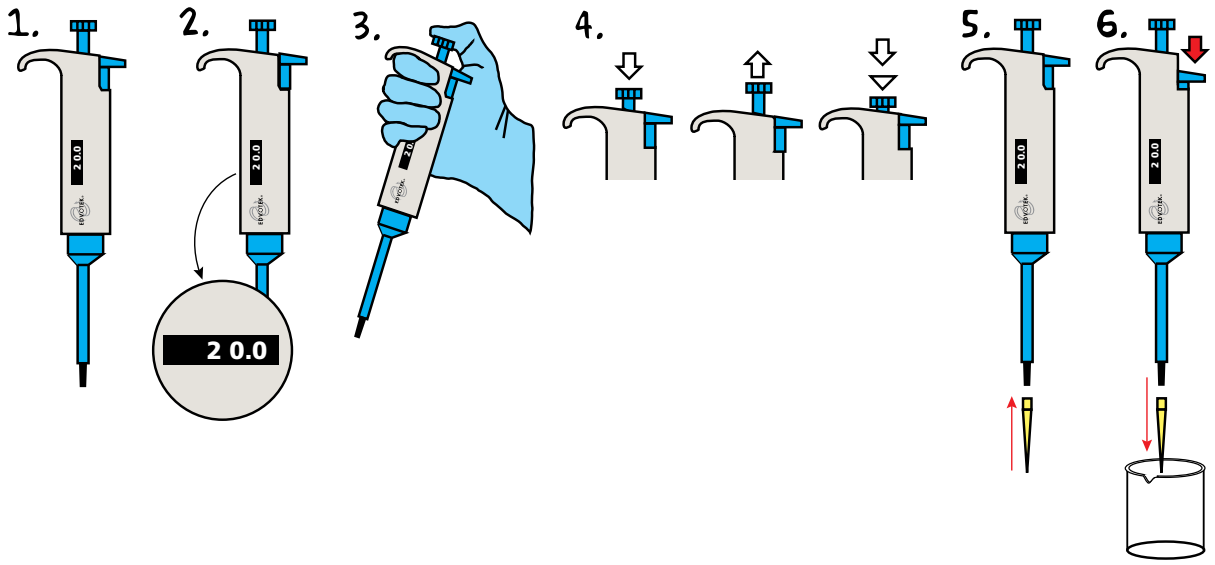
Module I: Introduction to Micropipetting



1. Examine the pipette(s) on your bench. Use Figure 6 on page 9 to **IDENTIFY** the following: pipetting button, volume setting wheel, tip ejection key, and tip cone.

2. Next, **IDENTIFY** the volume range and the volume display. In your lab notebook or below answer the following:
   - What is the maximum amount that you would pipette with this instrument?
   - What is the minimum amount that you would pipette with this instrument?
   - What volume is the pipette currently collecting?
   - What tip would you use with this pipette?

3. **PICK UP** your pipette. For best control, grasp the pipette in your palm and **WRAP** your fingers around the barrel. Rest your thumb on the pipetting button. Remember to always hold the micropipette so that the tip cone is facing down.

4. **PRACTICE** the motions for **ASPIRATING**, **DISPENSING**, and **PURGING** liquids. Push the pipetting button down to the first (friction) stop and then the second (hard) stop. Also practice *slowly* raising the button back to the original height.

5. **PRACTICE ATTACHING** a disposable tip. First, **BRING** the pipette above the tip. Next, gently **LOWER** the pipette so that the tip fits around the tip cone. Slightly **TWIST** the pipette to ensure that the tip is sealed. Finally, **LIFT** the pipette. The tip should come out of the rack and stay on the pipette. Remember not to let the tip touch objects other than the sample and its container.

6. **PRACTICE EJECTING** the tip. First, **HOVER** the pipette over a beaker or similar waste container. Next, use your thumb to **PRESS DOWN** on the tip ejector button. This will propel the tip off of the pipette and into the beaker - likely with some force!

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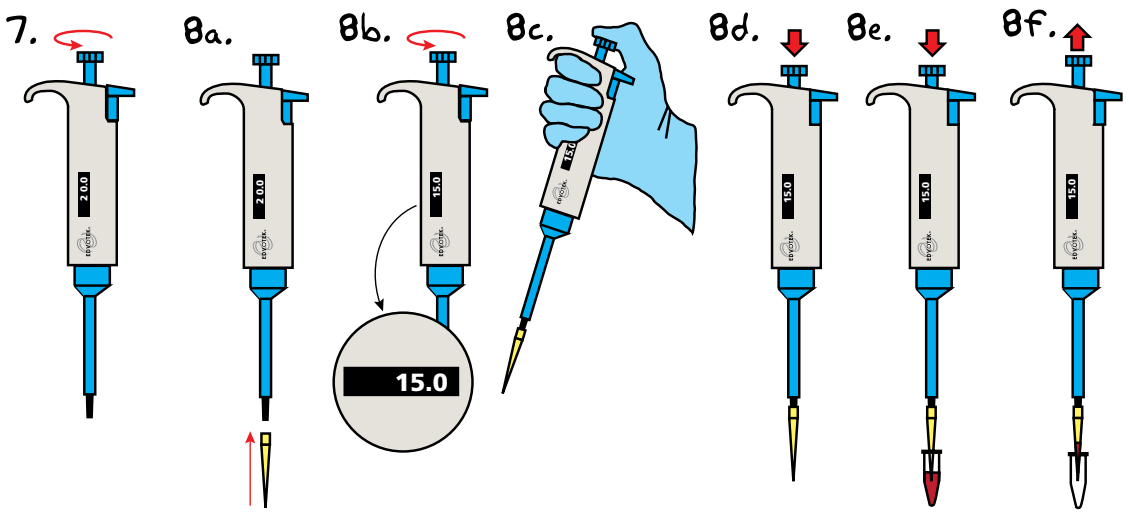
Module I: Introduction to Micropipetting, continued



7. **PRACTICE ADJUSTING** the volume. Use your free hand to **TURN** the volume-setting wheel clockwise to increase the volume and counter clockwise to decrease the volume (see Box 1). **OBSERVE** the volume display (see Box 2). Remember to always stay within the volume range of the pipette.

8. **PRACTICE** using the pipette! (For this step, you will need a test strip and red dye which you will collect from your teacher before starting.)

   a. **PLACE** a new tip on your pipette.

   b. **SET** the volume to 15 µL.

   c. With your free hand **PICK UP** the test tube. **HOLD** the tube between your thumb and forefinger and at or near eye level to best observe the liquid moving into the pipette during the next few steps.

   d. **PRESS** the plunger down to the first soft stop and **HOLD** it in this position.

   e. **DIP** the tip into the solution. Immerse the tip enough to cover the end but not so deep that it obscure your view of the liquid ~ 5 mm.

   f. Keeping the tip in the solution, slowly **RELEASE** the plunger until it is in its original position. You should see the liquid flowing into the pipette tip (see Box 3).

continued

Box 1:
If the volume-setting wheel is not moving, it may be that your pipette is locked at a certain volume. In many pipettes the volume lock is a small button that is located just below the tip ejector. This button can be disengaged by pushing upwards and relocked by pushing downwards.

Box 2:
Most pipettes have volume displays that are read top down. Some volume displays will contain a white dash line representing the decimal point. Account for this decimal place when setting your volume!

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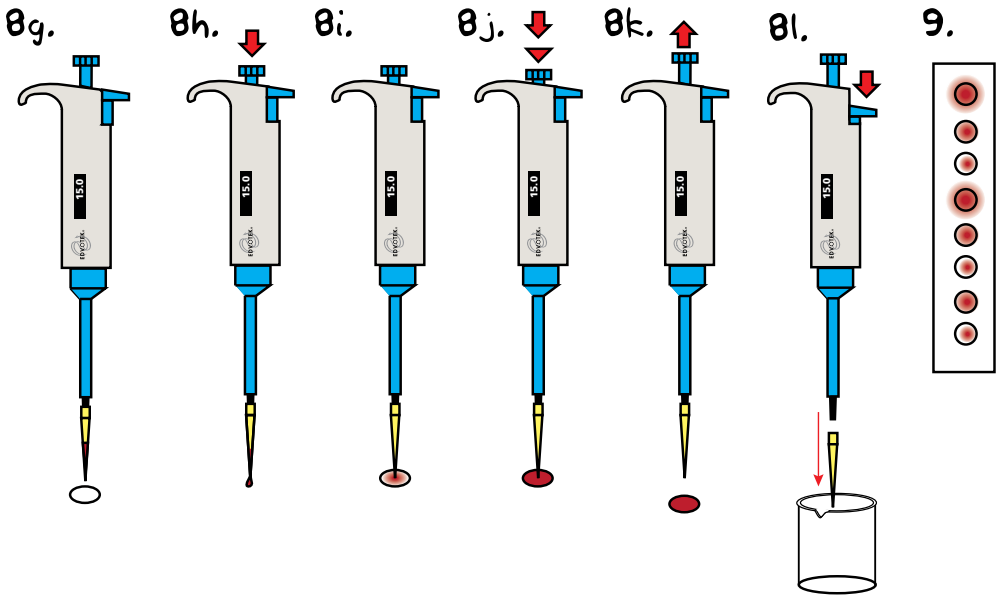
Module I: Introduction to Micropipetting, continued



g. **SLIDE** the pipette and tip out of the tube using the inside wall to dislodge any excess droplets that may be adhering to the outside of the tip. Then **MOVE** your pipette to just above the first practice circle.

h. Slowly **DEPRESS** the plunger to the first stop. You should see the liquid flowing out of the pipette tip.

i. Gently **TOUCH** the tip to the paper to create a capillary effect that will help draw any remaining fluid out of the tip.

j. Keeping the tip on the paper, **DEPRESS** the pipette plunger to the second hard stop to ensure all liquid has been ejected.

Box 3:
Watch out for bubbles in the tip or air space at the end of the tip. These can significantly offset the measurement! If you observe either, expel the liquid back into the tube (Steps 8h through 8k) and then repeat the process starting at Step 8c. Often air is introduced when pipetting quickly so perform these steps slowly.

k. **RAISE** the pipette so that the tip is no longer on the paper and then **RELEASE** the plunger to its original position. (This is more important when pipetting from one liquid solution into another but also good practice here.)

l. **EJECT** the tip into a beaker for used tips.

9. **REPEAT** Step 8 for the remaining circles. As this is practice, you may want to experiment and observe what happens when common micropipetting errors are made. For instance, you could intentionally press down to the second stop while sucking up the liquid or intentionally skip pressing down to second stop when ejecting the liquid to see how this affects circle size.

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Module II: Pipetting By Numbers

1. Your teacher will provide you with a segment of the Pipetting By Numbers canvas. Each segment will have a slightly different pattern.

2. Colors and pipetting volumes are indicated inside each circle. Color abbreviations are Red (R), Blue (B), Purple (P), Yellow (Y), and Navy (N). Volumes are given in microliters. So a circle marked "R20" would get 20 µL of the red dye.

3. Use the techniques described in Module I Step 8 to pipette the specified color and volume.

   For Best Results:
   - Use a small amount of tape to secure the top and bottom corners of the canvas to your lab bench. Fasten the tape to the back side (bench facing side) by wrapping the tape into a cylinder.
   - Pipette into the center of each circle and hold the pipette perpendicular to the paper.
   - When possible, start with the largest circles / highest volumes.
   - To save time and tips, pipette all the same colored dots at once using the same tip. Then switch tips and move to the next color.
   - Dyes vary in color and in viscosity (thickness). High viscosity dyes like the Navy and Red will require slower pipetting.

4. Combine your finished art with other groups by matching the edge patterns to create a single poster. Hang this in a prominent place!

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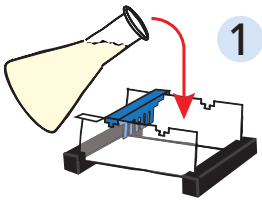
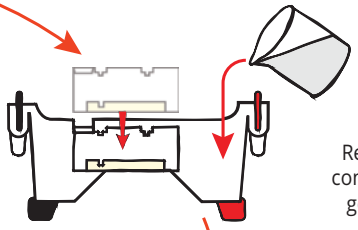
Overview

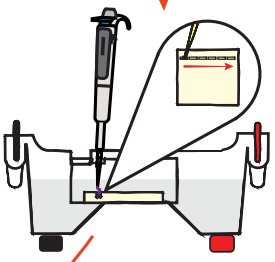
Module III: DNA Fingerprinting



1 Prepare agarose gel in casting tray.

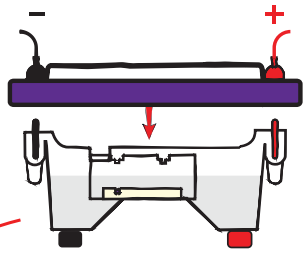


2 Remove end caps & comb, then submerge gel under buffer in electrophoresis chamber.



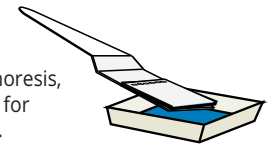
3 Load each sample in consecutive wells

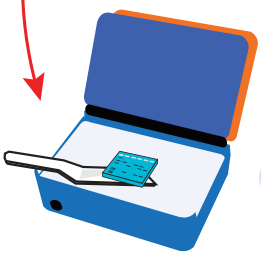
Module IV: Optional Staining Using FlashBlue™



4 Attach safety cover & connect leads to power source to conduct electrophoresis.

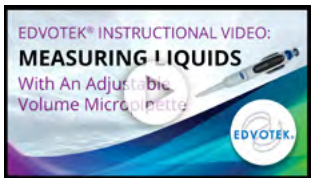
5 After electrophoresis, transfer gel for staining.

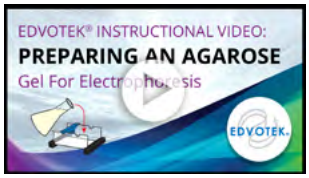




6 Analysis on white light source.

Related EDVOTEK® Instructional Videos





www.youtube.com/edvotekinc

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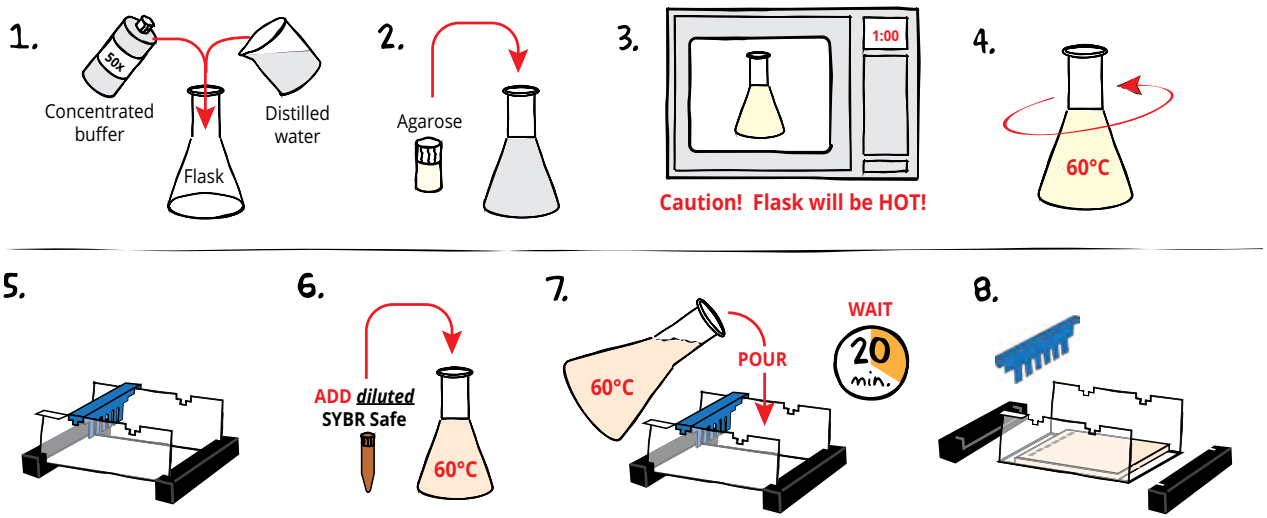
Module III: DNA Fingerprinting



NOTE: If you are casting your own gels, review the following instructions. If you are using pre-cast gels, proceed to Step 8.

1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** agarose powder with 1X buffer in a 250 mL flask (see Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60 °C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. Before casting the gel, **ADD** diluted SYBR® Safe to the molten agarose and swirl to mix (see Table A).
7. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
8. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Wear gloves and safety goggles

Reminder:
This experiment requires 0.8% agarose gels cast with 6 or 8 wells.

Table A — **Individual 0.8% UltraSpec-Agarose™ with SYBR® Stain**

Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume	Diluted SYBR® (Step 6)
7 x 7 cm	0.6 mL	29.4 mL	0.24 g	30 mL	30 µL
10 x 7 cm*	0.9 mL	44.1 mL	0.36 g	45 mL	45 µL
14 x 7 cm	1.2 mL	58.8 mL	0.48 g	60 mL	60 µL

** Recommended gel volume for the EDGE™ Integrated Electrophoresis System.*

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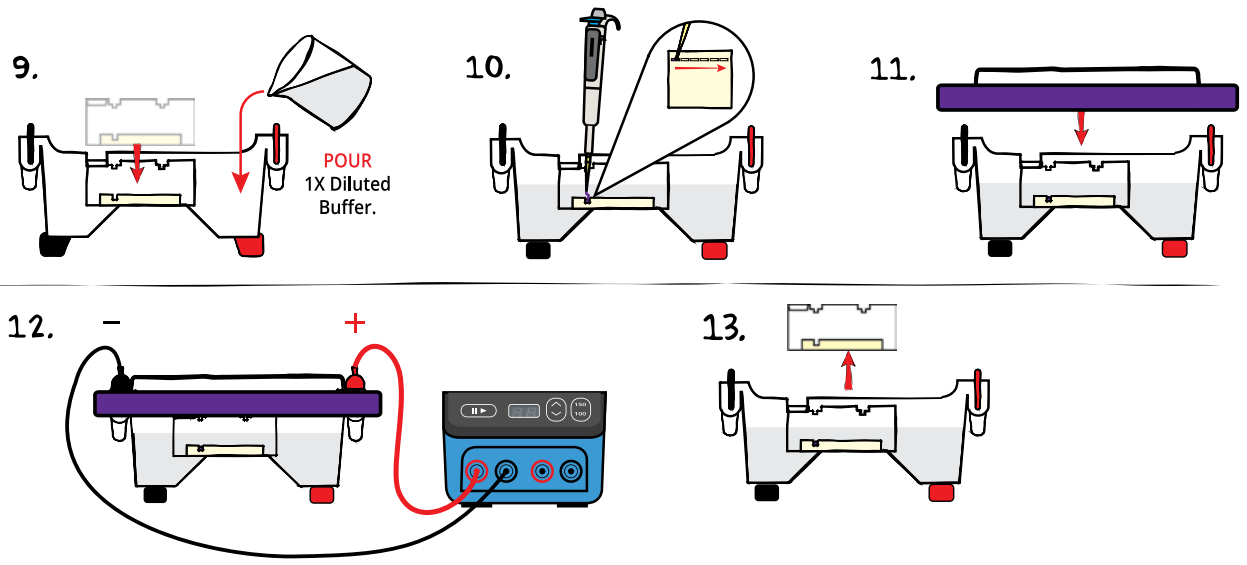
Module III: DNA Fingerprinting, continued



9. **PLACE** gel (on the tray) into electrophoresis chamber. **COVER** the gel with 1X electrophoresis buffer (See Table B for recommended volumes). The gel should be completely submerged.
10. **LOAD** the entire sample (35 µL) into the well in the order indicated by Table 1.
11. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
12. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines).
13. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber.

REMINDER:
Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.

TABLE 1: Gel Loading

Lane 1	Tube A	Standard DNA Marker
Lane 2	Tube B	"Blood" from Crime Scene: Table
Lane 3	Tube C	"Blood" from Crime Scene: Floor
Lane 4	Tube D	Eric Piedmont
Lane 5	Tube E	Dominique Hall
Lane 6	Tube F	Anna Garcia

Table B — **1x Electrophoresis Buffer (Chamber Buffer)**

EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
EDGE™	150 mL	3 mL	147 mL
M12	400 mL	8 mL	392 mL
M36	1000 mL	20 mL	980 mL

Table C — **Time and Voltage Guidelines**
(0.8% Agarose Gel)

	Electrophoresis Model: EDGE™	M12 & M36
Volts	Min/Max (minutes)	Min/Max (minutes)
150	10/20	20/35
125	N/A	30/45
100	15/25	40/60

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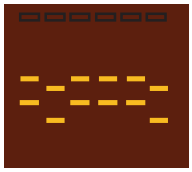
Module III: DNA Fingerprinting, continued

14.

15.



16.

VISUALIZING THE SYBR® GEL

14. **SLIDE** gel off the casting tray onto the viewing surface of the transilluminator and turn the unit on. **ADJUST** the brightness to the desired level to maximize band visualization. DNA should appear as bright green bands on a dark background.
15. **PHOTOGRAPH** results.
16. **REMOVE** and **DISPOSE** of the gel and **CLEAN** the transilluminator surfaces with distilled water.

Be sure to wear UV goggles if using a UV transilluminator.

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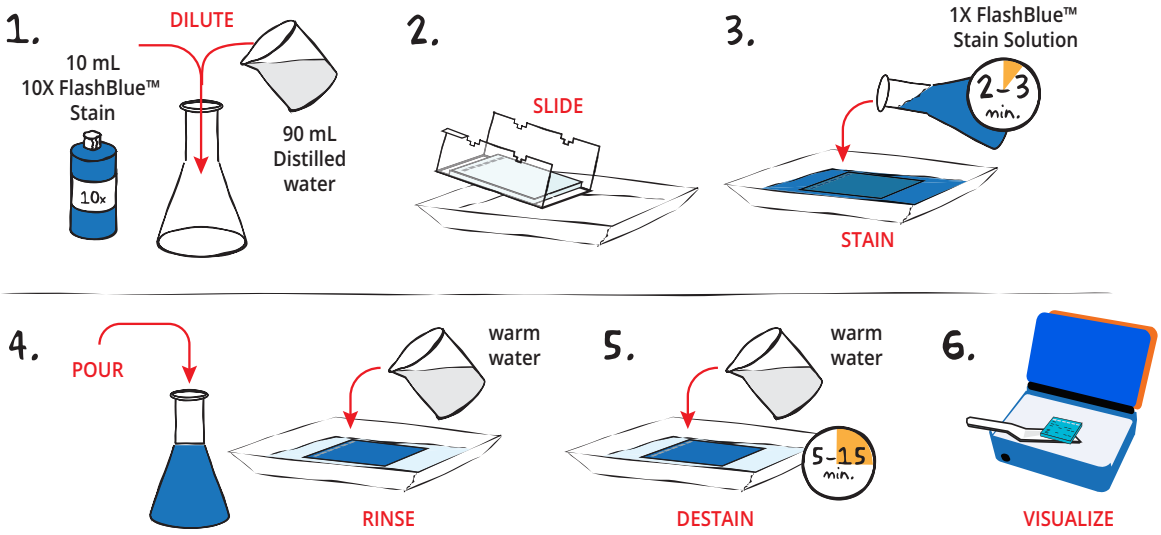
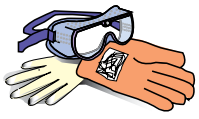
Module IV: Optional Staining Using FlashBlue™

FlashBlue™ Stain is a simple and effective visible DNA stain that can be used as an alternative, or in addition to, UV-reactive DNA stains like SYBR® Safe. ***IF staining with both SYBR® Safe and FlashBlue™, you must examine and record the SYBR® Safe bands before beginning the FlashBlue™ Staining.***



1. **DILUTE** 10 mL of 10X concentrated FlashBlue™ with 90 mL of distilled water in a flask. **MIX** well.
2. **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off the casting tray into a small, clean gel-staining tray.
3. **COVER** the gel with the 1X FlashBlue™ stain solution. **STAIN** the gel for 2-3 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. **STAINING THE GEL FOR LONGER THAN 3 MINUTES WILL REQUIRE EXTRA DESTAINING TIME.**
4. **POUR** the 1X FlashBlue™ back into the flask (the stain can be reused). **COVER** the gel with warm water (40-45 °C). Gently **RINSE** the gel for 20-30 seconds. **POUR** off the water.
5. **COVER** the gel with clean, warm water (40-45 °C). **DESTAIN** for 5-15 minutes with gentle shaking (longer periods will yield better results). DNA bands will start to appear after 5 minutes of destaining. Changing the water frequently will accelerate destaining.
6. Carefully **REMOVE** the gel from the destaining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.



Wear gloves and safety goggles

ALTERNATIVE FLASHBLUE™ STAINING PROTOCOL:

1. **DILUTE** 1 mL of 10X FlashBlue™ stain with 149 mL distilled water.
2. **COVER** the gel with diluted FlashBlue™ stain.
3. **SOAK** the gel in the staining liquid for at least three hours. For best results, stain gels overnight.
4. Carefully **REMOVE** the gel from the staining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

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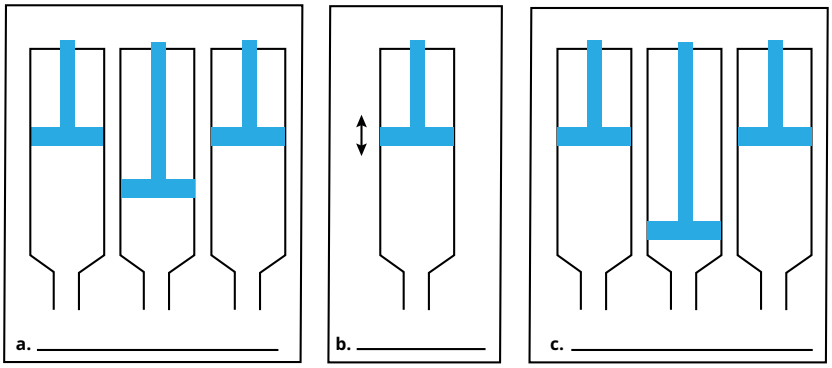
Study Questions

PRELAB QUESTIONS

1. Define, draw or describe the difference between the terms below.
   - Pasteur Pipette, Graduated Pipette, Displacement Micropipette
   - Soft Stop, Hard Stop
   - Accuracy, Precision

2. Below each image of a piston and tube, write the appropriate pipetting step (prepare, aspirate/dispense, purge).



3. Why do you think that so many biotechnology experiments involve small volumes of reactions?

4. Even with the help of pipettes, scientists can still introduce measurement errors into their experiments with improper technique. What are some user related sources of pipetting variability?

5. A standard practice in many biotechnology experiments is to have replicates - multiple copies of the sample or mixture being analyzed. What can replicates tell you about the precision of the experiment? The accuracy of the experiment?

POSTLAB QUESTIONS

6. Examine the replicate dots you created in Module I or any same sized dots in the Pipetting By Numbers canvas. Do they match exactly? If not what could be the source of variability? Next, compare same sized dots from your canvas with those of another group.

7. Go back to the list of golden rules presented in the introduction. Which of these came intuitively or were easy to remember? Which were harder to remember or implement?

8. What is polymorphic DNA? How is it used for identification purposes?

9. What is CODIS? How is it used to solve crimes?

10. What is an STR? A VNTR? Which (STR or VNTR) is predominantly now used in law enforcement? Why?

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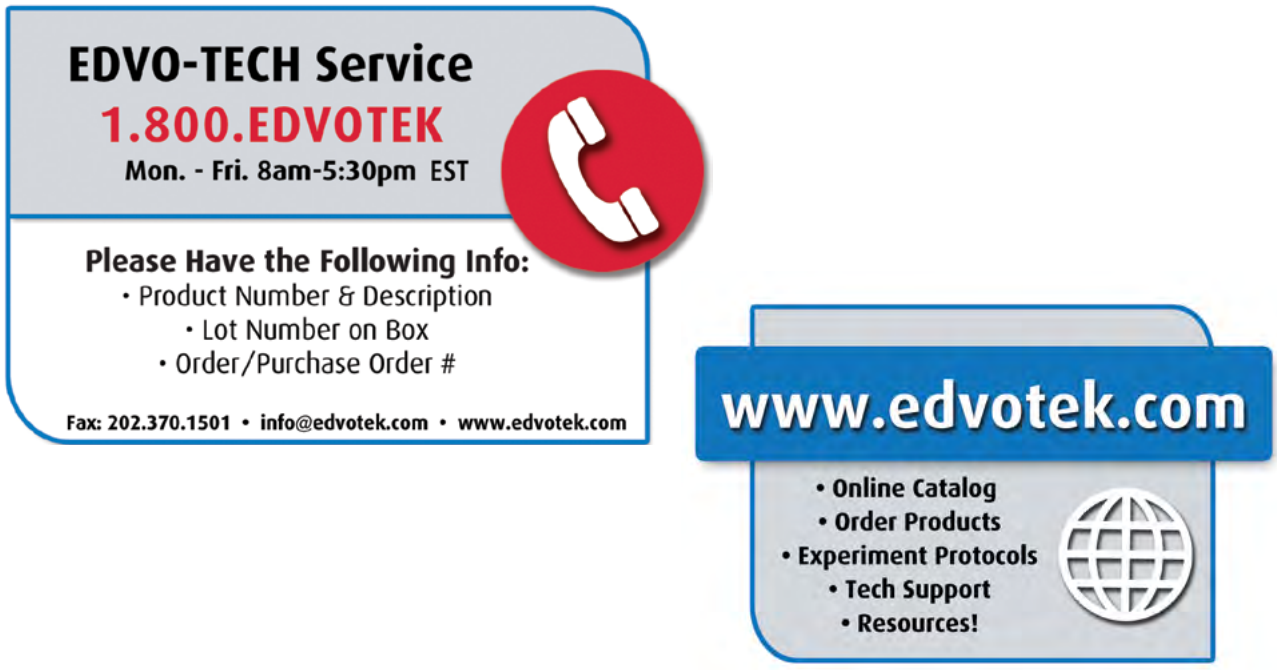
Appendices

C Data Analysis Using a Standard Curve

Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets



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Appendix C

Data Analysis Using a Standard Curve

Agarose gel electrophoresis separates biomolecules into discrete bands, each comprising molecules of the same size. How can these results be used to determine the lengths of different fragments? Remember, as the length of a biomolecule increases, the distance to which the molecule can migrate decreases because large molecules cannot pass through the channels in the gel with ease. Therefore, the migration rate is inversely proportional to the length of the molecules—more specifically, to the $\log_{10}$ of molecule's length. To illustrate this, we ran a sample that contains bands of known lengths called a "standard". We will measure the distance that each of these bands traveled to create a graph, known as a "standard curve", which can then be used to extrapolate the size of unknown molecule(s).

Figure 9: Measure distance migrated from the lower edge of the well to the lower edge of each band.

1. Measure and Record Migration Distances

Measure the distance traveled by each Standard DNA Fragment from the lower edge of the sample well to the lower end of each band. Record the distance in centimeters (to the nearest millimeter) in your notebook. Repeat this for each DNA fragment in the standard.

Measure and record the migration distances of each of the fragments in the unknown samples in the same way you measured the standard bands.

2. Generate a Standard Curve.

Because migration rate is inversely proportional to the $\log_{10}$ of band length, plotting the data as a semi-log plot will produce a straight line and allow us to analyze an exponential range of fragment sizes. You will notice that the vertical axis of the semi-log plot appears atypical at first; the distance between numbers shrinks as the axis progresses from 1 to 9. This is because the axis represents a logarithmic scale. The first cycle on the y-axis corresponds to lengths from 100-1,000 base pairs, the second cycle measures 1,000-10,000 base pairs, and so on. To create a standard curve on the semi-log paper, plot the distance each Standard DNA fragment migrated on the x-axis (in mm) versus its size on the y-axis (in base pairs). Be sure to label the axes!

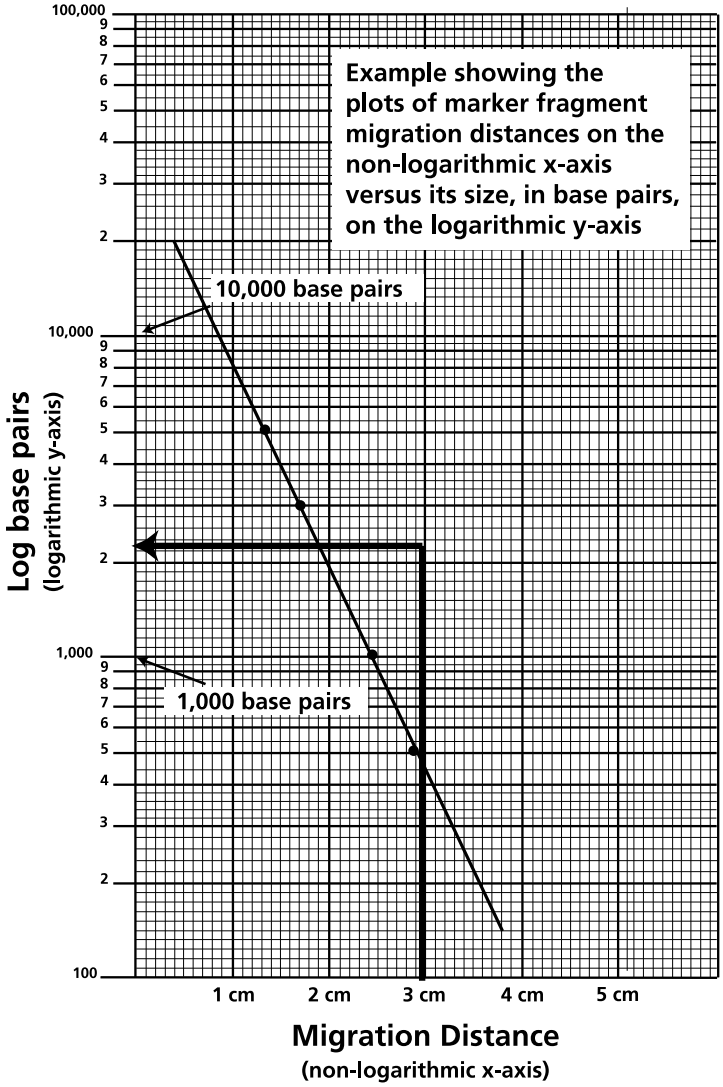

Figure 10: Semilog graph example

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Appendix C
Data Analysis Using a Standard Curve

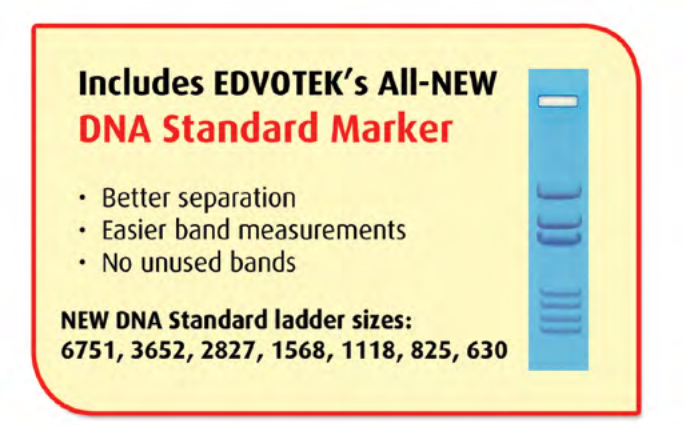
After all the points have been plotted, use a ruler or a straight edge to draw the best straight line possible through the points. The line should have approximately equal numbers of points scattered on each side of the line. It is okay if the line runs through some points (see Figure 10 for an example).

3. Determine the length of each unknown fragment.

a. Locate the migration distance of the unknown fragment on the x-axis of your semi-log graph. Draw a vertical line extending from that point until it intersects the line of your standard curve.

b. From the point of intersection, draw a second line, this time horizontally, toward the y-axis. The value at which this line intersects the y-axis represents the approximate size of the fragment in base pairs (refer to Figure 10 for an example). Make note of this in your lab notebook.

c. Repeat for each fragment in your unknown sample.



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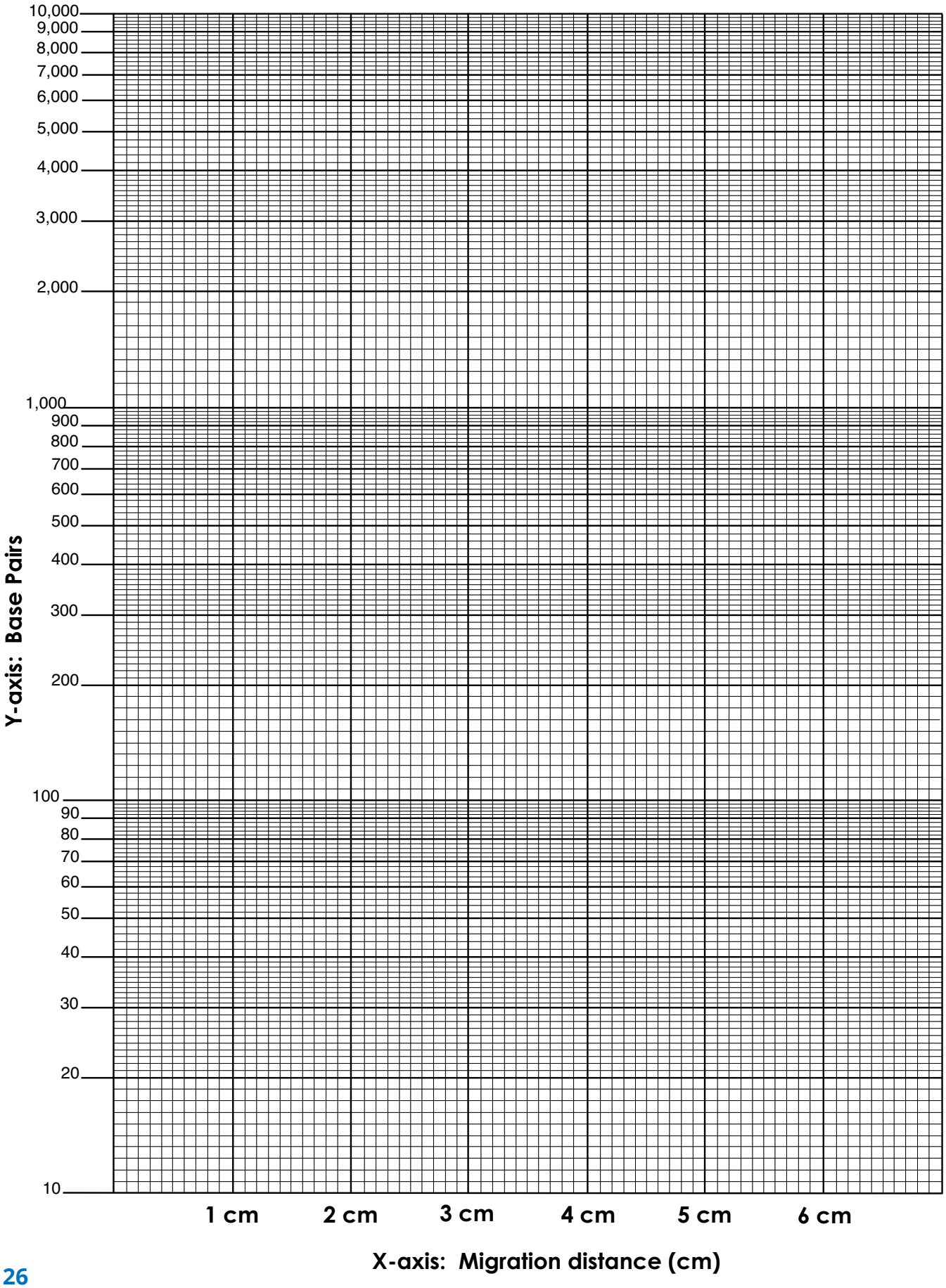
Appendix C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Y-axis: Base Pairs
1 cm
2 cm
3 cm
4 cm
5 cm
6 cm
X-axis: Migration distance (cm)