

Edvo-Kit #

344

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Tick Talk: Tick-Borne Disease Diagnostics

Experiment Objective:

In this experiment, students will work to identify several tick species found in a geographic region using physical attributes and characteristics. Once the species is identified, students will work to extract DNA from the simulated tick species. Finally, students will then use PCR analysis to determine what tick-borne diseases are present in this particular tick population.

See page 3 for storage instructions.

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EDVOTEK®

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Experiment Components

Perishable Components

	Storage	Check (✓)
• PCR EdvoBeads™ PLUS*	NEW Update: -20°C	☐
A Universal DNA Buffer	-20°C Freezer	☐
B TE Buffer	-20°C Freezer	☐
C LyphoPrimer™ Mix	-20°C Freezer	☐
D LyphoControl™	-20°C Freezer	☐
E Lone Star Tick DNA template	-20°C Freezer	☐
F Blacklegged Tick DNA template	-20°C Freezer	☐
G American Dog Tick DNA template	-20°C Freezer	☐
• EdvoQuick™ DNA ladder	-20°C Freezer	☐
• SYBR® Safe Stain	-20°C Freezer	☐

NOTE: Components C and D are supplied in our LyphoPrimer™ and LyphoControl™ format. They will require reconstitution prior to use. Be sure to review the Instructor's Guide for more details.

*Each PCR EdvoBead™ PLUS contains: dNTP Mixture, Taq DNA Polymerase Buffer, Taq DNA Polymerase, MgCl₂ and Reaction Buffer).

This kit contains enough reagents for 24 PCR samples. This is enough for 6 groups of three samples and one control.

Sample volumes are very small. It is important to quickly spin the tube contents in a microcentrifuge to obtain sufficient volume for pipetting. Spin samples for 10-20 seconds at maximum speed.

Reagents & Supplies

Store all components below at room temperature.

• UltraSpec-Agarose™	☐
• TAE Electrophoresis Buffer Powder	☐
• 0.2 mL PCR Tubes	☐
• Plastic Micropestles	☐
• Screw-top Tubes	☐
• Snap-top Microcentrifuge Tubes	☐
• Simulated "ticks"	☐



NEW Update:
PCR EdvoBeads™ are now conveniently stored at -20°C with the other perishable components.



NOTE: Check that your PCR program matches the protocol found in the student and instructor's guides before starting the experiment!

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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Experiment Requirements *(NOT included in this experiment)*

- Thermal cycler (EDVOTEK® Cat. [#540](#) or [#541-542](#) highly recommended) or three water baths
- Water bath 56°C and 99°C (EDVOTEK Cat. [#539](#) highly recommended)
- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Balance
- Microcentrifuge
- UV Transilluminator or Blue light visualization (EDVOTEK® [Cat #557](#) recommended)
- UV safety goggles
- Automatic micropipettes (5-50 µL) with tips
- Microwave or hot plate
- Pipet pump
- 250 mL flasks or beakers
- Hot gloves
- Disposable laboratory gloves
- Ice buckets and ice
- Distilled or deionized water - 3.7 L (*NOTE: for ease of preparation, we recommend purchasing one gallon [3.78 L] of distilled water.*)
- Bleach solution



Background Information

Ticks are among the most patient hunters on Earth. These minuscule arachnids, often no bigger than a sesame seed, can go unnoticed for days while feeding on blood and, in doing so, transmit some of the most serious infections in the world. Despite their small stature, ticks play a surprisingly powerful role in ecosystems and represent one of the most significant vector threats to human and animal health. Understanding their anatomy, behavior, and life cycle is not just an academic exercise — it is a critical step toward protecting communities from disease.

There are over 900 known species of ticks worldwide, divided into three main categories (Figure 1). **Hard ticks (*Ixodidae*)** are the most numerous, with roughly 700 species, and are distinguished by a tough, armor-like dorsal shield called a scutum. **Soft ticks (*Argasidae*)** are another family of ticks characterized by their soft bodies resulting in the lack of that hard back plate which gives them a wrinkled, leathery appearance. There are about 200 species of soft ticks worldwide. A third family, ***Nuttalliellidae***, contains only a single known species (*Nuttalliella nam-aqua*) only in southern Africa. Ticks are found on every continent except for Antarctica and have adapted to a wide variety of hosts and environments. Ticks are becoming even more of a problem as climate change plagues the globe. Warmer climates and changes in humidity can allow ticks to expand into new areas that were previously too cold or dry for them. For example, species like the black-legged tick (*Ixodes scapularis*), responsible for spreading Lyme disease, are moving farther north in North America. A longer, warmer tick season means more feeding, more reproduction, and greater disease risk. The threat is growing, and so is the urgency to understand it.



Figure 1 (From left to right):
Ixodidae, *Argasidae*, and *Nuttalliellidae* ticks.

Tick Anatomy

Adult hard ticks have a unique body structure that is specially adapted for feeding on hosts and surviving in their environment. Understanding the anatomy of ticks reveals how their specialized features support feeding, movement, and disease transmission. The body of the tick is divided into two main parts: the head/feeding part and the main body. The head/feeding part is known as the **capitulum**. The capitulum consists of the:

- **Basis capituli:** Base of the feeding apparatus; attaches the mouthparts to the body.
- **Chelicerae:** Paired cutting organs, like tiny blades, used to pierce the host's skin.
- **Hypostome:** Barbed, harpoon-like structure that anchors the tick while feeding.
- **Palps (pedipalps):** sensory appendages on either side of the hypostome that detect heat, odors, and chemicals to guide the tick to the host.

The main body of the tick is called the **idiosoma**. This section makes up the majority

Tick Talk: Overview

- Ticks are arachnids, not insects. Like spiders, adult ticks have 8 legs.
- A single female tick can lay up to 3,000 eggs at one time.
- Ticks have been around for at least 100 million years — they fed on dinosaurs.

Tick Talk: Anatomy

- A tick's hypostome is covered in backward-pointing barbs — like a fish-hook — making removal difficult.
- Tick saliva contains anesthetics, so most hosts feel nothing during attachment.
- Haller's organ can detect CO₂ exhaled by a potential host from several meters away.
- Female ticks can expand to 200–600 times their unfed body weight after a full blood meal.
- Unlike insects, ticks do not jump or fly — they "quest," extending their front legs to latch onto passing hosts.

of the tick and houses most of its internal organs. The ticks idiosoma is comprised of:

- **Scutum (dorsal shield):** Hard plate on the back. Covers entire dorsal surface in males, partial in females.
- **Festoons:** Small notches along the rear edge of the back useful for identification.
- **Legs:** Nymph and adults have 8 legs; each leg is broken down into seven segments called the v_l coxa or the base of the leg, trochanter, femur, patella, tibia, metatarsus, and tarsus.
- **Haller's organ** is found on the end of the first leg or the tarsus, and is a sensory organ detecting the host's body heat, humidity, and CO₂.
- **Eyes:** Some hard ticks have simple lateral eyes.
- **Spiracles:** Openings that allow for respiration located near the base of the fourth pair of legs

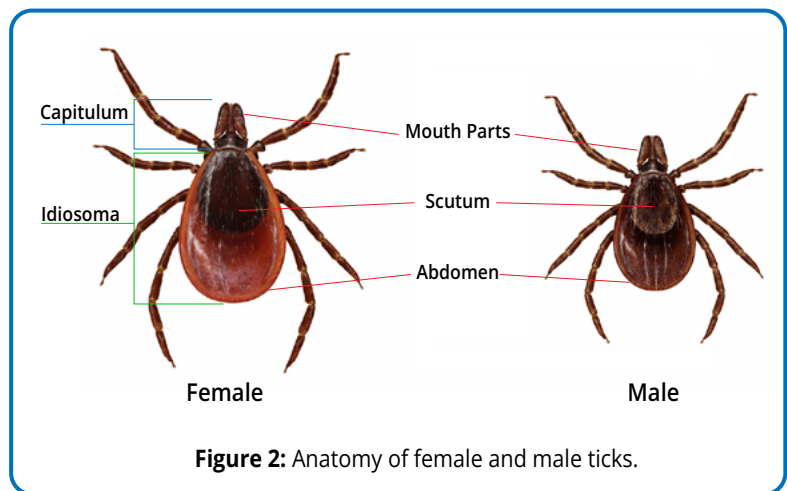


Figure 2: Anatomy of female and male ticks.

Tick Life Cycle

Before they can reach adulthood, ticks go through three prior stages in which they have different anatomy. The journey can take anywhere from several months to several years depending on species and environment, but each stage is defined by a single, critical requirement: a blood meal. Ticks have a complex life cycle that includes four stages: egg, larva, nymph, and adult. The life cycle begins when a female tick lays hundreds to thousands of eggs in a protected environment, such as leaf litter or soil. Once the eggs hatch, tiny six-legged larvae emerge, often called “seed ticks,” and take their first blood meal from a small host like a rodent or bird. After feeding, the larva molts into an eight-legged nymph, which is larger, more active, and potential pathogens acquired during the larval stage. Nymphs are particularly dangerous to humans as they are small enough to go unnoticed yet capable of transmitting disease after only a few hours of feeding.

Following another blood meal of humans or other mammals, the nymph molts into an adult tick, and develops dimorphic reproductive organs and distinct adult markings. Adult females require a significant blood meal to develop eggs, while males often feed less and focus on mating. Each stage depends on host interactions for growth and survival, and the feeding behavior at each stage is a key factor in the spread of tick-borne diseases such as Lyme disease, Rocky Mountain spotted fever, and babesiosis.

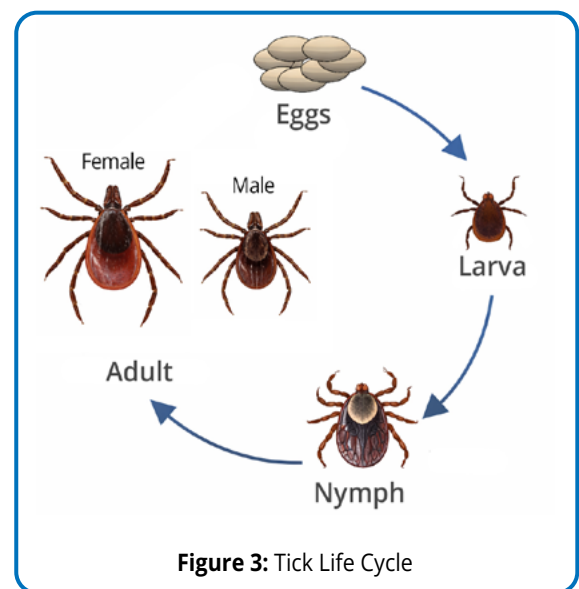


Figure 3: Tick Life Cycle

Tick Talk: Life Cycle

- Ticks can survive over a year without a blood meal between life stages.
- Nymph ticks cause the majority of Lyme disease cases in humans — they are poppy-seed sized and often go undetected.
- The blacklegged tick's full life cycle takes about 2 years to complete.
- Pathogens acquired by larvae can be passed on during the nymph stage — a process called transstadial transmission.

Common Tick Species

While hard tick species share a common bodily anatomy, they vary widely in appearance, behavior, and the diseases they can transmit. In North America, three species are of particular note being the most commonly found as well as being the most prevalent in disease spreading: the blacklegged or deer tick, lone star tick, and the American dog tick. Each species occupies different geographic regions, prefers different hosts, and carries specific pathogens, making it important to understand their distinguishing characteristics.

- **Blacklegged Tick (*Ixodes scapularis*)**

Also known as the deer tick, the blacklegged tick is smaller and less conspicuous than many other hard ticks (Figure 4). Adult females have a dark reddish-brown body with a black scutum, while males are darker and smaller. This species is found mainly in the northeastern, mid-Atlantic, and upper Midwestern United States. Blacklegged ticks are notorious for transmitting Lyme disease, as well as anaplasmosis and babesiosis. They feed on a wide range of hosts, including deer, rodents, and humans, and their nymphs are particularly important in spreading disease because they are tiny and often go unnoticed.



Figure 4:
Blacklegged Tick
(*Ixodes scapularis*)

- **Lone Star Tick (*Amblyomma americanum*)**

The lone star tick is easily recognized by the distinctive white “star” marking on the back of adult females (Figure 5). It is an aggressive feeder and will actively seek hosts, including humans. This species is primarily found in the southeastern and eastern United States, but its range is expanding northward. Lone star ticks are vectors for diseases such as ehrlichiosis and Southern tick-associated rash illness (STARI). Both nymphs and adults can feed on a variety of hosts, including deer, birds, and humans.



Figure 5:
Lone Star Tick
(*Amblyomma americanum*)

- **American Dog Tick (*Dermacentor variabilis*)**

The American dog tick is a hard tick with a broad, ornate scutum and a reddish-brown body (Figure 6). Adult females are larger than males and can engorge significantly after feeding. This species is commonly found in the eastern and central United States, often in grassy fields, along trails, and in areas where dogs and other mammals roam. American dog ticks are known vectors of Rocky Mountain spotted fever and tularemia, and they primarily feed on dogs, humans, and other medium to large mammals.



Figure 6:
American Dog Tick
(*Dermacentor variabilis*)

Tick-Borne Diseases

The tick does not bite and run. It settles in, secretes a chemical cocktail of anticoagulants and immune-suppressing compounds into its host's skin, and feeds, sometimes for days, without the host ever feeling a thing. This prolonged, stealthy feeding is precisely what makes tick-borne disease transmission so effective. If the tick's salivary glands harbor pathogens such as bacteria, viruses, or protozoa, those agents flow into the host's bloodstream alongside the saliva. In some cases, pathogens can also be regurgitated from the tick's midgut into the wound. The result is a remarkably efficient delivery system for over twenty different diseases in the United States alone.

Not every tick bite leads to infection; the tick must be infected, and most pathogens require hours to days of attachment before transmission occurs. But when conditions align, the consequences can be severe.

- **Lyme Disease**

The most common vector-borne disease in the United States is Lyme disease which is caused by *Borrelia burgdorferi* (and *B. mayonii* in some regions). Lyme disease is transmitted primarily by the blacklegged tick in the northeastern, mid-Atlantic, and upper Midwestern United States, and by the Western blacklegged tick along the Pacific coast. Infection occurs when an infected tick attaches and feeds on a host, typically for at least 24 hours, allowing the bacteria to enter the bloodstream. Its identifier is the erythema migrans, a spreading, bulls eye-shaped rash, accompanied by

fever, fatigue, and joint pain. Left untreated, the infection can progress to more serious complications such as arthritis, neurological symptoms (e.g., facial palsy, meningitis), and heart problems. Caught early, it responds well to oral antibiotics while severe cases may require intravenous antibiotics. Some patients experience lingering symptoms even after treatment, a condition known as Post-Treatment Lyme Disease Syndrome (PTLDS).

- **Rocky Mountain Spotted Fever**

Rocky Mountain Spotted Fever (RMSF) is found throughout the United States but is most common in the southeastern United States. Caused by *Rickettsia rickettsii* and transmitted by American dog ticks, Rocky Mountain wood ticks, and occasionally brown dog ticks. RMSF begins with fever, headache, and muscle pain, followed days later by a distinctive spotted rash. Without prompt doxycycline treatment, it can escalate rapidly to organ failure, internal bleeding, and death. It is a genuine medical emergency, and early recognition is everything.

- **Anaplasmosis**

Anaplasmosis is a bacterial infection caused by *Anaplasma phagocytophilum* and is primarily transmitted by the black-legged tick (*Ixodes scapularis*) in the eastern and Midwestern United States and the Western blacklegged tick on the Pacific coast. Its early symptoms, fever, chills, headache, muscle aches, resemble countless other illnesses. In most healthy patients, doxycycline leads to a full recovery, but in elderly or immunocompromised individuals, it can spiral into respiratory failure or organ damage.

- **Babesiosis**

Unlike most tick-borne diseases caused by bacteria, babesiosis is caused by *Babesia microti*, a protozoan parasite that invades and destroys red blood cells. Transmitted by the blacklegged tick in the Northeast and upper Midwest, it causes fever, fatigue, chills, and hemolytic anemia. Treatment depends on the severity of the infection with the use of different antibiotic combinations. Most healthy individuals make a full recovery but in those without a spleen, the elderly, or the immunocompromised, babesiosis can become life-threatening.

- **Ehrlichiosis**

Ehrlichiosis is a bacterial infection caused by species of Ehrlichia, most commonly *Ehrlichia chaffeensis* and *Ehrlichia ewingii* in the United States. It is primarily transmitted by the lone star tick, which is found in the southeastern and eastern United States, with its range expanding northward. In some places, the blacklegged tick has also been known to spread the disease. Early symptoms, including fever, headache, fatigue, muscle aches, and sometimes a rash, are frustratingly nonspecific. Oral doxycycline is highly effective when started early. With its geographic range expanding northward alongside its primary vector, ehrlichiosis is a disease whose footprint is still growing.

- **Alpha-Gal Syndrome**

Not a tick borne pathogen, but another example of some of the danger ticks can cause. Alpha-gal syndrome (AGS) is a striking example of a tick bite triggering an immune response that can last a lifetime. When a lone star tick bites a human, it can introduce a sugar molecule called alpha-galactose (alpha-gal) into the bloodstream, a molecule found in the cells of most mammals, but not in humans. Some people's immune systems mount an allergic response to this molecule and to anything else that contains it — such as red meat such as beef, pork, and lamb. The result is a delayed allergic reaction, typically occurring 2–6 hours after eating red meat, ranging from hives and stomach pain to life-threatening anaphylaxis. What makes AGS especially disorienting for patients is that they may have eaten red meat their entire lives without issue, then suddenly find themselves unable to tolerate it after a tick bite. There is currently no cure; management centers on strict dietary avoidance and carrying emergency epinephrine. Cases of alpha-gal syndrome have been rising steadily alongside expanding lone star tick populations, making it an emerging public health concern.

Tick Talk: Diseases

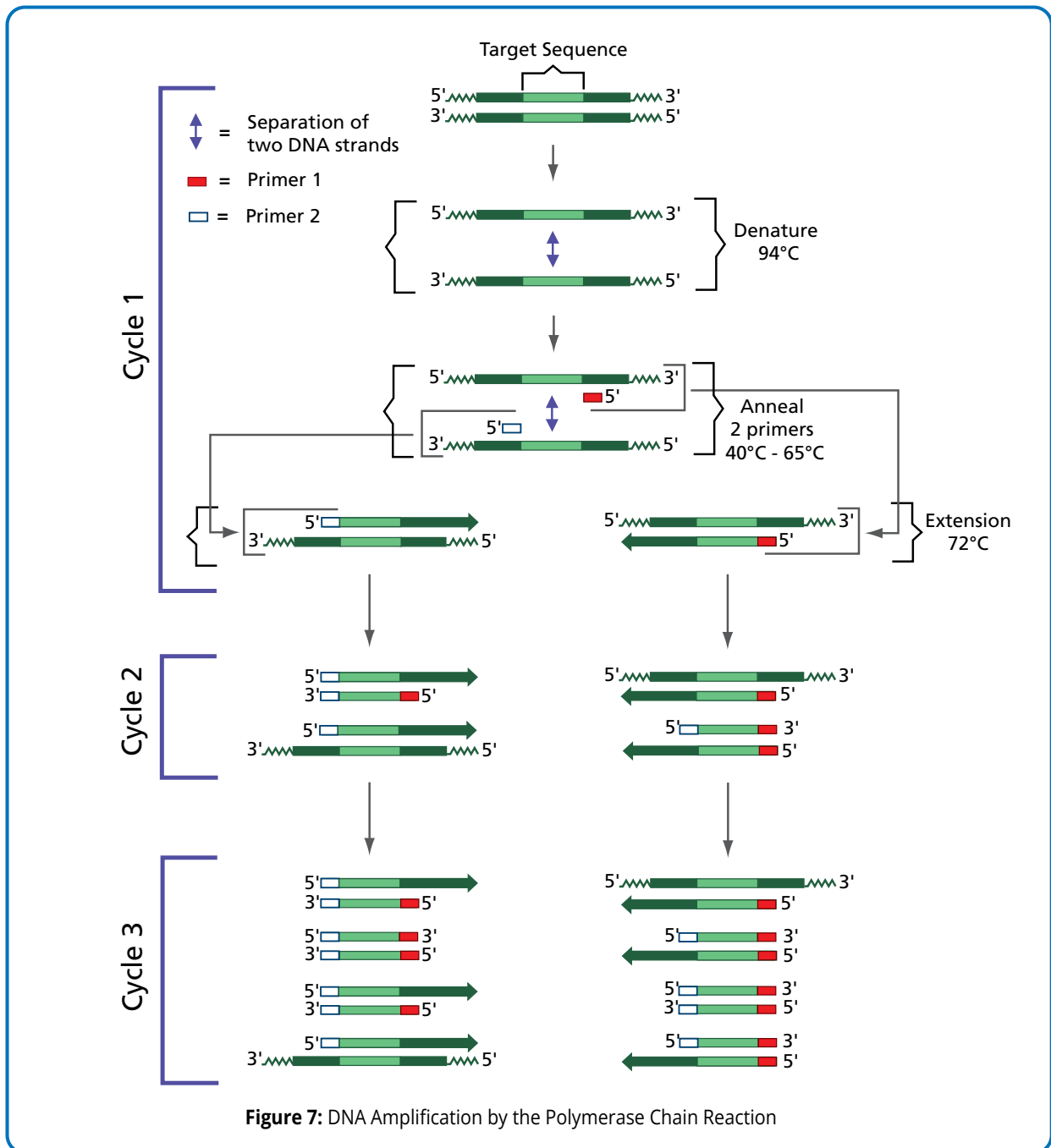
- Ticks transmit more diseases to humans in the U.S. than any other arthropod.
- Over 476,000 Americans are diagnosed with Lyme disease each year (CDC estimate).
- Rocky Mountain Spotted Fever can be fatal within 8 days if untreated.
- A single tick can be co-infected with multiple pathogens simultaneously.
- Most pathogens require 24–48 hours of tick attachment to be transmitted — early removal saves lives.

Not all ticks carry disease; infection rates vary widely by species and region.

Preventive measures include avoiding tick-infested areas, using tick repellents, performing regular tick checks, and promptly removing attached ticks. Awareness of tick habitats and early recognition of symptoms are key to preventing serious complications and ensuring a full recovery.

Tick Testing

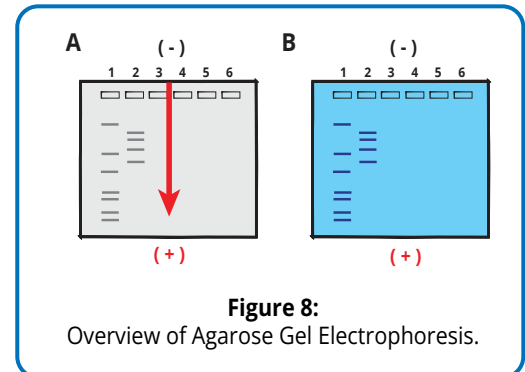
A tick collected from the field tells us very little on its own. It might look completely unremarkable under a magnifying glass, yet harbor a dangerous pathogen in its salivary glands. This is where polymerase chain reaction (PCR) becomes a transformative tool (Figure 7). PCR can detect specific pathogen DNA within a tick sample with extraordinary sensitivity.



The process works in three repeating steps. First, the DNA sample is heated to 94°C, forcing the double helix to unzip into two single strands in a process called denaturation. Next in the annealing step, the temperature drops to 45°C–60°C, allowing short DNA sequences called primers to bind specifically to the target pathogen sequence. Finally, the temperature rises to 72°C, where *Taq* polymerase builds a new complementary strand, doubling the target DNA in the extension step. After 35–40 cycles, a single molecule of pathogen DNA can be amplified into billions of copies.

Once amplified, PCR products are separated by size using agarose gel electrophoresis (Figure 8). DNA fragments are loaded into wells in a gel matrix and driven by an electrical current toward a positive electrode. Smaller fragments move faster; larger ones lag behind. The result is a series of distinct “bands”, a visual barcode that reveals which pathogens are present, allowing researchers to identify diseases in the tick population with speed and precision.

By systematically PCR-testing tick populations across different habitats and seasons, public health scientists can track disease prevalence, map geographic spread, detect co-infections, and issue timely warnings before human cases spike. In a world where tick ranges are expanding and tick seasons are lengthening, this kind of molecular surveillance is not just useful but also essential.



The Scenario

Over several years, a region experiences warmer winters and longer spring seasons, allowing more ticks to survive and remain active for longer periods. At the same time, increased deer and rodent populations provide abundant hosts for ticks to feed and reproduce. As tick populations expand into new areas, residents begin to report more tick encounters during outdoor activities. Public health surveillance shows a rise in ticks carrying disease-causing pathogens, leading to an increase in reported cases of tick-borne illnesses. This combination of climate change, host availability, and human exposure results in a noticeable increase in disease risk within the area.

In this experiment, students will act as acarologists, scientists who specialize in the study of ticks and mites, and work to identify several tick species found in a geographic region using physical attributes and characteristics. Once the species is identified, students will work to extract DNA from the simulated collected tick species. Once the DNA is collected, students will run PCR analysis to determine what tick borne pathogens are present in this particular tick population.

Experiment Overview

EXPERIMENT OBJECTIVE:

In this experiment, students will work to identify several tick species found in a geographic region using physical attributes and characteristics. Once the species is identified, students will work to extract DNA from the simulated tick species. Finally, students will then use PCR analysis to determine what tick-borne diseases are present in this particular tick population.

LABORATORY SAFETY:

Be sure to READ and UNDERSTAND the instructions completely BEFORE starting the experiment.

If you are unsure of something, ASK YOUR INSTRUCTOR!

- Wear gloves and goggles while working in the laboratory.
- Exercise caution when working in the laboratory – you will be using equipment that can be dangerous if used incorrectly.
- Wear protective gloves when working with hot reagents like melted agarose.
- DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
- Always wash hands thoroughly with soap and water after working in the laboratory.
- Be sure to properly dispose of any biological samples according to your institutional guidelines.

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.

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.



NOTE: Check that your PCR program matches the protocol found in the student and instructor's guides before starting the experiment!

Module I: Macroscopic Tick Identification

Upon noting the increase of tick presence and tick based diseases in a community in Tennessee, local health officials set out to collect a sample of ticks present throughout several habitats in the community. Before testing these ticks for pathogens, it is your job to identify the species of the ticks. Using macroscopic (visual) features and information provided, examine the ticks, compare characteristics, record your observations and determine their likely species.

Tick Identification Procedure

For each unidentified tick, follow these steps in order:

1. Examine General Morphology

Observe the tick images carefully and record in Table 1:

- Body color(s)
- Distinctive markings
- Appearance of the scutum (plain, patterned, dark)
- Mouthpart length (long or short)

2. Confirm Developmental and Structural Features

Confirm that the specimen is:

- A hard tick (presence of a scutum)
- An adult/nymph based on leg count (8)

3. Review Habitat Information

Look at the habitat description on the profile card:

- Where was the tick collected?
- What animals were present?
- Was the environment wooded, grassy, brushy, etc.?

4. Compare Data with the Tick Characteristic Table (page 14)

Use features such as mouthpart length, markings, and habitat information to confirm your identification.

5. Record Your Most Likely Species

Enter your conclusion in Table 1.

Tools for Tick Identification

Tick Profile Cards:

Tick 1



Habitat: This tick species was collected from brushy understory with lots of shrubs and young trees. The area was very mossy with lots of fallen, rotting fallen trees and logs. White tailed deer were spotted in the area when collecting ticks.

Tick 2



Habitat: This tick species was found dwelling in leaf piles on the forest floor. There were dense areas of leaf litter on the ground on a trail of bare trees. There were signs of rodents and deer residing in the forest as well.

Tick 3



Habitat: These ticks were collected from an open grassy field. Scientists found the ticks within tall blades of grass. It was noted upon collection that the field was at the base of a nearby forest.

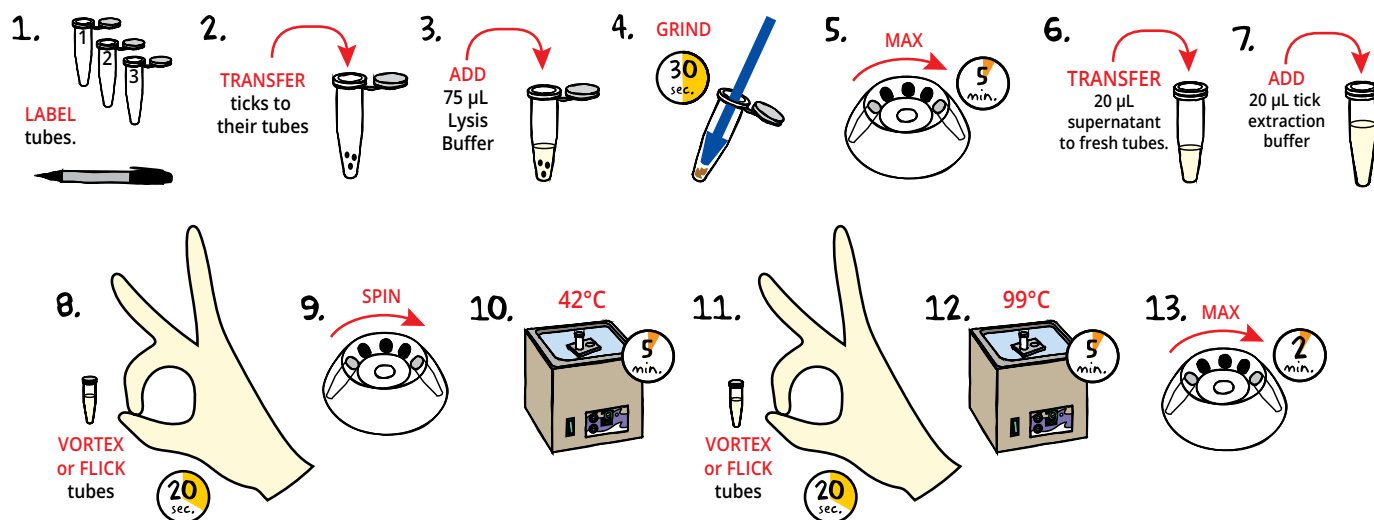
Tick Characteristics Table:

Common Name	Distinctive Markings	Scutum (Back Shield)	Mouthparts	Typical Habitat	Common Hosts	Diseases / Notes
Blacklegged Tick (Deer Tick)	Smooth, dark scutum, no pattern; black legs	Plain, dark	Long	Forests, leaf litter, wooded areas	Deer, mice, humans	Lyme disease, anaplasmosis, babesiosis
American Dog Tick	Mottled/patterned back (lighter & darker areas)	Ornate	Short	Fields, grasslands, trails	Dogs, wildlife, humans	Rocky Mountain spotted fever, tick paralysis
Rocky Mountain Wood Tick	Light gray or white pattern on scutum	Ornate	Short	Rocky, grassy, shrubby areas	Wildlife, livestock, humans	Rocky Mountain spotted fever, tularemia
Lone Star Tick	Reddish-brown; female has single white dot	Plain	Long	Wooded areas, brush	Deer, livestock, humans	Ehrlichiosis, red meat allergy (alpha-gal)
Brown Dog Tick	Uniform reddish-brown; no pattern	Plain	Short	Homes, kennels, indoors	Dogs (all life stages)	Can transmit RMSF; kennel infestations
Asian Longhorned Tick	Plain brown; thick body	Plain	Short	Pastures, wooded edges	Livestock, wildlife, humans	Emerging species; livestock blood loss risk

Table 1: Tick Identification

	General Morphology: colors, distinctive markings, scutum, mouthpart (long or short)	Developmental and Structural Features: Hard/soft tick, adult/larva	Habitat Information: environment, animals present?	Suspected Species
Tick 1		☐ Hard tick ☐ Soft tick ☐ Adult/nymph ☐ Larva		
Tick 2		☐ Hard tick ☐ Soft tick ☐ Adult/nymph ☐ Larva		
Tick 3		☐ Hard tick ☐ Soft tick ☐ Adult/nymph ☐ Larva		

Module II: Tick DNA Extraction



1. **COLLECT** three 1.5 mL microcentrifuge tubes labeled #1, #2, and #3 from your instructor. **LABEL** with your initials or group number.
2. **SELECT** 3-5 Ticks provided by your instructor and **PLACE** them in their respective tubes.
 - a. Tick 1 species: Lone Star Tick
 - b. Tick 2 species: Blacklegged Tick
 - c. Tick 3 species: American Dog Tick
3. **ADD** 75 µL Lysis buffer to each tube.
4. Using a micropestle, **GRIND** the ticks to break up the shell until no large pieces remain (approx. 30 seconds).
5. **CENTRIFUGE** the samples at full speed for 5 minutes.
6. **TRANSFER** 20 µL of the supernatant to fresh, labeled, screw top tubes. **NOTE: Try not to collect any debris.**
7. **ADD** 20 µL of Tick Extraction Buffer to the corresponding tube.
8. **MIX** the tube by flicking or vortexing for 20 seconds.
9. **SPIN or PULSE** the tube to collect material to the bottom.
10. **INCUBATE** at 42°C for five minutes.
11. **MIX** the tube by flicking or vortexing for 20 seconds.
12. **INCUBATE** at 99°C for five minutes.
13. **CENTRIFUGE** for two minutes at full speed.

NOTE: Wear gloves and safety goggles.

The extracted DNA is now ready for Module III: PCR Amplification. If you are ready to proceed, turn to page 16. Alternatively, the extracted DNA may be stored in the **FREEZER** (-20°C) until needed.

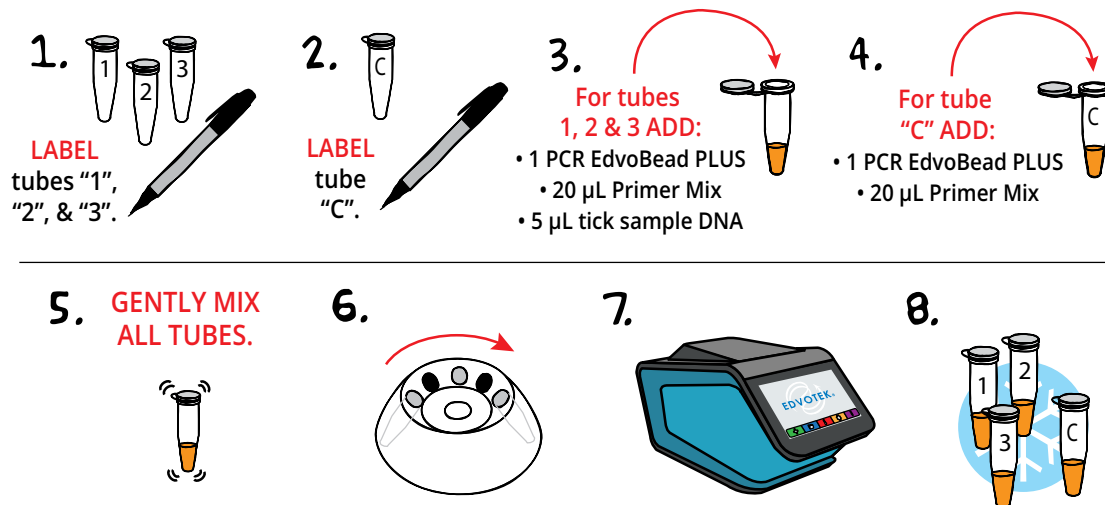


OPTIONAL STOPPING POINT:

The extracted DNA may be stored in the freezer (-20°C) until needed.



Module III: Amplification of the Pathogen DNA



- 1. OBTAIN** three PCR tubes from your instructor. **LABEL** these tubes as follows: 1, 2, 3. Put your initials or group number on the tubes.
- 2. OBTAIN** a PCR tube of control DNA from your instructor. Label this tube "C" and with your initials or group number.
- To tubes 1, 2 and 3, **ADD** one PCR EdvoBead™ PLUS, 20 μ L primer mix and 5 μ L of its corresponding tick sample DNA prepared in Module II.
- To tube "C", **ADD** one PCR EdvoBead™ PLUS and 20 μ L primer mix. **NOTE: The control tube already contains DNA and should only receive primers and an EdvoBead™.**
- MIX** the PCR samples gently. Make sure the EdvoBeads™ are completely dissolved.
- CENTRIFUGE** to collect the samples at the bottom of the tubes.
- AMPLIFY** DNA using PCR:



- Initial denaturation 94°C for 3 minutes.
 - 94°C for 30 seconds
 - 55°C for 65 seconds
 - 72°C for 30 seconds
- 35 cycles
- Final extension at 72°C for 4 minutes.

- After PCR, **PLACE** the tubes on ice. **PROCEED** to Module IV. Alternatively, the amplified DNA may be stored in the **FREEZER** (-20°C) until needed.



OPTIONAL STOPPING POINT:

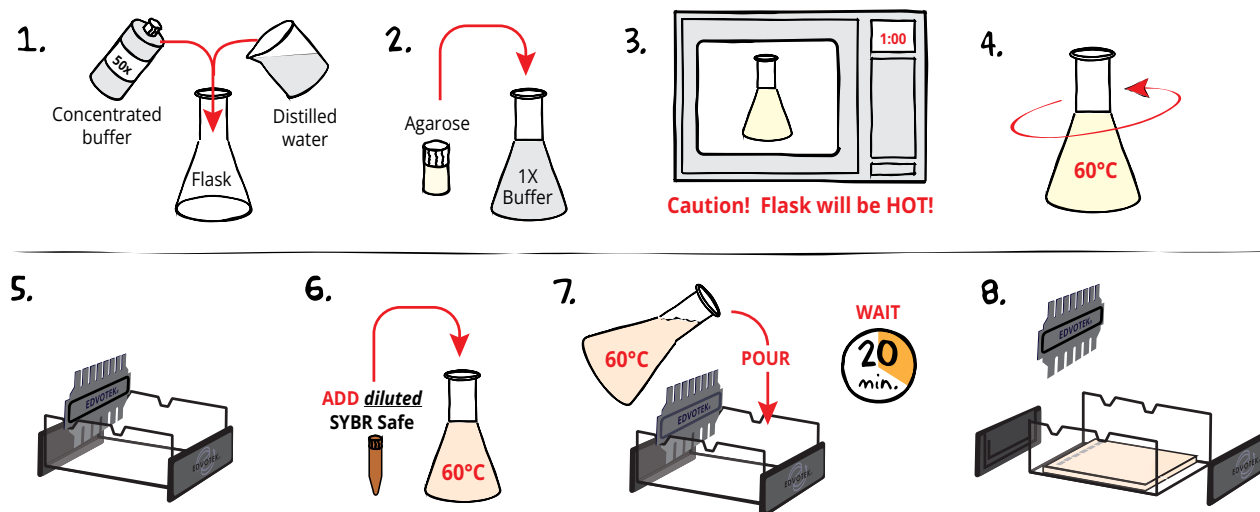
The PCR samples may be stored at -20°C for restriction digest at a later time.



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Module IV: Separation of DNA Fragments by Electrophoresis



PREPARING THE AGAROSE GEL with SYBR® safe stain

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** the agarose to 60° C by carefully swirling the flask to promote even dissipation of heat.
5. While the agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the 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** the end caps and comb. Take particular care when removing the comb to prevent damage to the wells.



Reminder:
Each group will require one 1.0% agarose gel with at least five sample wells for electrophoresis. For this experiment, we recommend casting 7 x 7 cm gels.

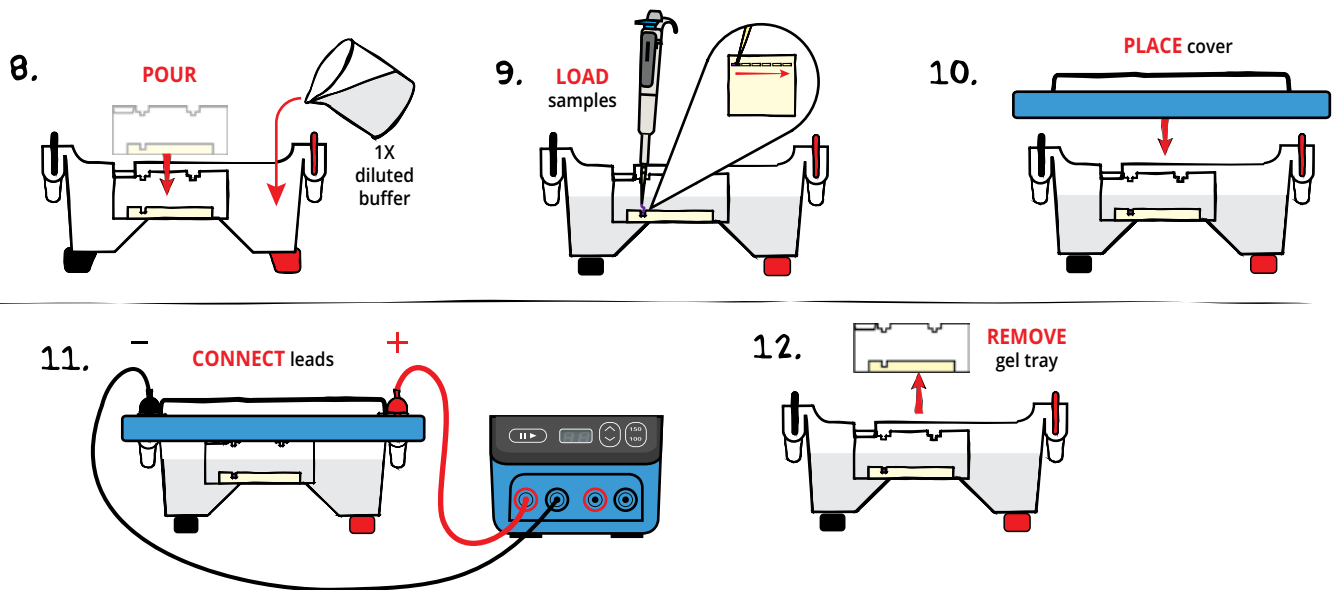
Table
A

Individual 1.0% 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	0.5 mL	29.5 mL	0.3 g		30 mL	30 µL
10 x 7 cm*	1.0 mL	49.0 mL	0.5 g		50 mL	50 µL
14 x 7 cm	1.2 mL	58.8 mL	0.6 g		60 mL	60 µL

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

Module IV: Separation of DNA Fragments by Electrophoresis, continued



RUNNING THE GEL

- PLACE** gel (on the tray) into the electrophoresis chamber. **COVER** the gel with 1X electrophoresis buffer (See Table B for recommended volumes). The gel should be completely submerged.
- Using Table 3 as a guide, **LOAD** the entire sample (~25 μ L) into the wells in consecutive order.
- PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
- CONNECT** the leads to the power source and **PERFORM** electrophoresis. (See Table C for time and voltage guidelines.)
- 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 3: Gel Loading

Lane	Sample
1	EdvoQuick™ DNA Ladder
2	Control
3	Tick 1 DNA
4	Tick 2 DNA
5	Tick 3 DNA

OPTIONAL STOPPING POINT:

Gels can be stored for several days. Place gel in a watertight plastic bag with 2 mL of electrophoresis buffer and store in the refrigerator. Limit light exposure.

Table B

1x Electrophoresis Buffer (Chamber Buffer)

EDVOTEK Model #	Total Volume Required	50x Conc. Buffer	Dilution + 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
(1.0% - 7 x 7 cm Agarose Gel)

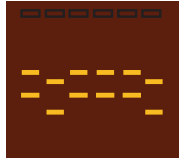
Volts	Recommended Time	
	Minimum	Maximum
150	15 min.	20 min.
125	20 min.	35 min.
70	35 min.	1 hour

Module IV: Separation of DNA Fragments by Electrophoresis, continued

14.



15.



16.



VISUALIZING THE SYBR® GEL

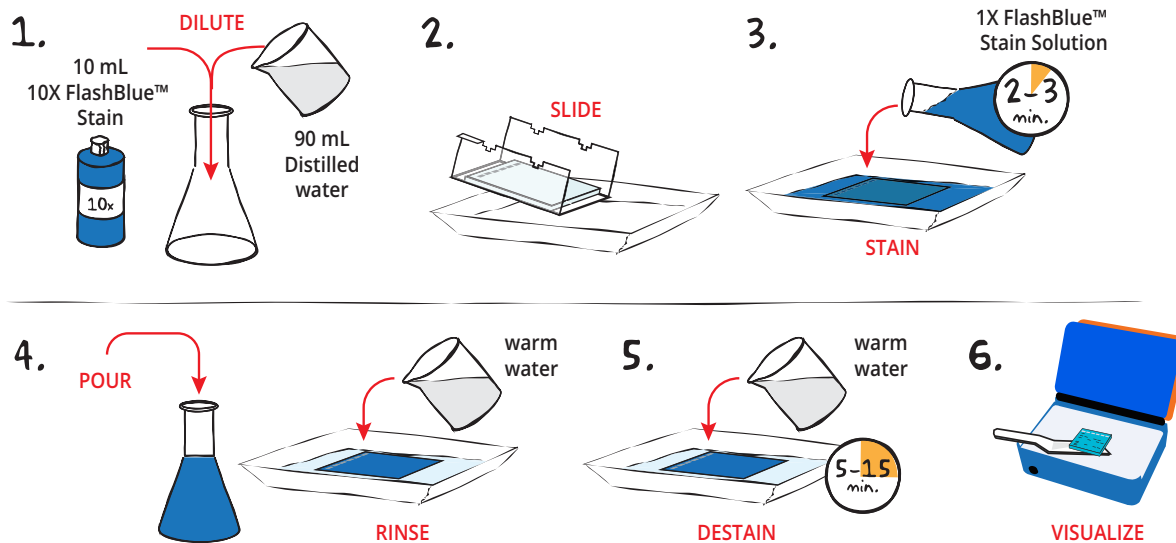
14. **SLIDE** gel off the casting tray onto the viewing surface of the transilluminator.
15. **TURN** the unit on. DNA should appear as bright green bands on a dark background. **DOCUMENT** the results of the experiment by using a camera or smartphone to take an image through the orange safety lid. Dimming the lights in the room can increase the visibility if needed.
16. **TURN** the unit off. **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.

Module V: OPTIONAL Staining with 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.



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.

Study Questions

1. Why are ticks important to study in human and animal health?
2. Why are nymph ticks often more dangerous to humans than adult ticks?
3. Why are ticks considered a major concern even though they are small and often unnoticed?
4. Why is climate change expected to increase the risk of tick-borne diseases?
5. What pathogens are present in each tick species and what diseases do they cause?

Instructor's Guide

OVERVIEW OF INSTRUCTOR'S PRELAB PREPARATION:

This experiment will require 3-4 class periods to complete the student experiment. Many reagents can be prepared in advance, but others require special considerations. Please consult the table below and the detailed instructions in the Instructor's Guide for more information.

Preparation For	What to do	When	Time Required
Module I: Macroscopic Tick Identification	Print out handouts and tables	Anytime before Module I.	5 min.
Module II: Tick DNA Extraction	Prepare and aliquot samples and plastic tubes	Anytime before performing the experiment.	30 min.
	Prepare and aliquot Lysis Buffer.	Prepare on the day the students will be performing the experiment OR freeze for up to one week.	15 min.
	Equilibrate water baths at 55° C and boiling.	Anytime before performing the experiment.	5 min.
Module III: Amplification of the Pathogen DNA	Prepare and aliquot various reagents (Primer, PCR Edvo-Beads™, etc.).	Up to one week before performing the experiment. Diluted primers should be frozen until needed.	30 min.
	Program Thermal Cycler.	Anytime before performing the experiment.	15 min.
Module IV: Separation of DNA Fragments by Electrophoresis	Prepare TAE buffer.	Up to one week before performing the experiment.	45 min.
	Prepare molten agarose and pour gels (OPTIONAL) or aliquot agarose, TAE, and SYBR® Safe.	Up to one week before performing the experiment.	45 min.
	Aliquot EdvoQuick™ DNA ladder.	Up to one week before performing the experiment.	45 min.



Red = Prepare immediately before module.



Yellow = Prepare shortly before module.



Green = Flexible / prepare up to a week before the module.



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Pre-Lab Preparations: Module II

There are 3 different simulated tick samples and one provided control for a total of 18 experimental reactions and 6 controls.

Preparation of the Tick Samples

1. Retrieve the three bags of simulated tick samples labeled "Lone Star Tick", "Blacklegged Tick", and "American Dog Tick".
2. Obtain 24 1.5 mL microcentrifuge tubes. LABEL 6 "Tick 1", 6 "Tick 2", and 6 "Tick 3".
3. ADD tick samples into their corresponding tubes as follows:
 - a. ADD ~10 Lone Star Tick Ticks to each of the "Tick 1" tubes.
 - b. ADD ~10 Blacklegged Ticks to each of the "Tick 2" tubes.
 - c. ADD ~10 American Dog Ticks to each of the "Tick 3" tubes.

Note: There are enough materials to perform 24 PCR reactions (18 experimental and 6 controls) and 6 agarose gels. Students can be divided into 6 groups.

For Module II, each group should receive:

- 1 tube of lysis buffer
- 3 snap top tubes
- 3 screw top tubes
- 1 tube of each Tick extraction buffer
- 3 micropestles

Prepare the Regents

1. Label 6 snap top microcentrifuge tubes "Lysis buffer".
2. Aliquot 300 µL Universal DNA Buffer into each tube.
3. Obtain 18 1.5 mL microcentrifuge tubes. Label 6 "Tick 1 EB", 6 "Tick 2 EB" and 6 "Tick 3 EB". (Note: EB=Extraction Buffer)
4. Resuspend each tick DNA template in 130 µL TE buffer. Allow to rehydrate for one minute.
5. Aliquot 20 µL of Lone Star Tick DNA template into each of the "Tick 1 EB" tubes, 20 µL of the Blacklegged DNA template into "Tick 2 EB" tubes, and 20 µL of the American dog tick into each of the "Tick 3 EB" tubes.

Pre-Lab Preparations: Module III

In Module III, the students will perform PCR on the DNA that they extracted in Module II.

The PCR primers are provided as a lyophilized mixture that must be rehydrated by the instructor before performing the experiment. The PCR EdvoBeads™ PLUS can be distributed prior to setting up the PCR – students or instructors can gently transfer the PCR EdvoBeads™ PLUS using gloved hands. Alternatively, beads can be gently "poured" from the vial into individual PCR tubes. After distributing the beads, it is important to quickly close the PCR tubes securely to prevent the beads from absorbing moisture and becoming hard to resuspend prior to the experiment.

For Module III, each group should receive:

- 3 EdvoBeads™
- 1 tube of 100 µL primer
- 1 tube of control DNA
- 3 PCR tubes

This experiment contains LyphoControl™ Complete PCR control samples that should be prepared and run alongside the student PCR samples. LyphoControl™ samples are premixed with all PCR components and are ready to run immediately after rehydration. We highly recommend running the controls alongside student samples if possible, although they can also be run before or after the student PCR.

Preparation of the Primer Mix

1. Thaw the TE Buffer (B) and mix well.
2. Ensure that the lyophilized solid is at the bottom of the LyphoPrimer™ tube (C). If not, centrifuge the tube at full speed for 10 seconds.
3. Add 1 mL of TE Buffer (B) to the tube. Cap and mix well, then place on ice. The solution should be light orange and clear of any solid pieces.
4. Label 6 microcentrifuge tubes "Pathogen Primers". Aliquot 100 µL of the diluted Primer Mix into the 6 microcentrifuge tubes. Place the tubes on ice until they are needed. If you would like to prepare the primers ahead of time, they can be aliquoted and then frozen until needed.
5. Distribute one tube of diluted Tick Primer to each student group.

Preparation of the LyphoControl™

NOTE: This kit includes enough reagents to set up 6 control reactions. At least one control reaction should be performed per class to confirm that PCR was successful.

1. Ensure that the lyophilized solid is at the bottom of the LyphoControl™ tube (D). If not, centrifuge the tube at full speed for 10 seconds.
2. Add 35 µL of TE Buffer (B) to the tube. Cap and mix well then place on ice. The solution should be light red and clear of any solid pieces.
3. Dispense 5 µL of the diluted control mixture into six 0.2 mL PCR tubes for each control reaction.

Pre-Lab Preparations: Module III, continued

IMPORTANT:

The PCR EdvoBeads™ PLUS absorb moisture if left open, even for a short period of time. Only open the vial when you are ready to use the beads. If you are not using all of the beads in one session, quickly remove the required number and promptly secure the gray stopper back into the vial. Store the vial of unused beads at -20°C (desiccated) until needed.

PCR EdvoBeads™ PLUS

NOTE: The beads are vacuum-sealed and may move around once the stopper is removed. Only open the vial when you are ready to use the beads and quickly recap the vial.

Dispense the PCR EdvoBeads™ PLUS just prior to the students performing their PCR reactions. Alternatively, student pairs can dispense PCR EdvoBeads™ PLUS during Module III. Each group will require 4 beads.

1. Open the vial of PCR EdvoBeads™ PLUS (carefully remove metal crimp) and gently/slowly remove the gray rubber stopper.
2. With gloved hand, forceps or sterile loop, remove one single bead and transfer to a PCR tube. Do this for the number of reactions you wish to perform. Quickly cap each of the tubes and distribute to the student groups.

PCR Amplification:

The Thermal cycler should be programmed as outlined in Module III in the Student's Experimental Procedure.

- Accurate temperatures and cycle times are critical. A pre-run for one cycle (takes approximately 3 to 5 minutes) is recommended to check that the thermal cycler is properly programmed.
- For thermal cyclers that do not have a heated lid, it is necessary to place a layer of oil or wax above the PCR reactions in the microcentrifuge tubes to prevent evaporation.

IMPORTANT NOTE: Before running the experiment, confirm that your PCR program matches the settings below.



- Initial denaturation 94°C for 3 minutes.
 - 94°C for 30 seconds
 - 55°C for 65 seconds
 - 72°C for 30 seconds
- } 35 cycles
- Final extension at 72°C for 4 minutes.

Pre-Lab Preparations: Module IV

Separation of PCR Products by Electrophoresis

This experiment requires one 1.0% agarose gel per lab group. For best results, we recommend using 7 x 7 cm gels. You can choose whether to prepare the gels in advance or have the students prepare their own. Allow approximately 30-40 minutes for this procedure.

Prepare SYBR® Safe Stain:

Prepare diluted SYBR® Safe by adding 250 µL of 1X TAE electrophoresis buffer to the tube of concentrated SYBR® Safe and tapping the tube several times to mix. For individual gel preparation, each group will need 30 µL of the diluted SYBR® Safe for a 7 x 7 cm gel. For Batch Gel Preparation, you will use the entire tube of SYBR® Safe (see Appendix B).

For Module IV, each group should receive:

- 50x concentrated buffer
- Distilled Water
- UltraSpec-Agarose™ Powder
- 30 µL **diluted** SYBR® Safe Stain
- 30 µL EdvoQuick DNA ladder
- PCR Samples from Module III

Individual Gel Preparation:

Each student group can be responsible for casting their own individual gel prior to conducting the experiment. See Module III in the Student's Experimental Procedure. Students will need 50X electrophoresis buffer, distilled water, agarose powder, and **diluted** SYBR® Safe Stain.

Batch Gel Preparation:

To save time, a larger quantity of agarose solution can be prepared for sharing by the class. Electrophoresis buffer can also be prepared in bulk (see Appendix B).

Preparing Gels in Advance:

Gels may be prepared ahead and stored for later use. Solidified gels can be stored in the refrigerator for up to 2 weeks. Place 1-2 mL of electrophoresis buffer in a sealable bag with the gels to prevent them from drying out. Excessive buffer will cause SYBR® Safe to diffuse out of the gels.

Do not freeze gels at -20° C as freezing will destroy the gels.

Gels that have been removed from their trays for storage should be "anchored" back to the tray with a few drops of molten agarose before being placed into the tray. This will prevent the gels from sliding around in the trays and the chambers.

Additional Materials:

Aliquot 30 µL of the EdvoQuick™ DNA ladder (B) into labeled microcentrifuge tubes and distribute one tube of EdvoQuick™ DNA ladder per gel.

Visualizing SYBR® Safe-Stained Gels:

SYBR® Safe is a DNA stain that fluoresces when bound to double-stranded DNA, allowing us to visualize our samples. This DNA stain is compatible with both UV and blue-light transilluminators. For best results, we recommend the TruBlu™ 2 Blue Light Transilluminator ([Cat #557](#)).



Pre-Lab Preparations: Module V (Optional)

Staining Using FlashBlue™

FlashBlue™ stain is optimized to shorten the time required for both staining and destaining steps. Agarose gels can be stained with diluted FlashBlue™ for 5 minutes and destained for only 20 minutes. For the best results, leave the gel in liquid overnight. This will allow the stained gel to “equilibrate” in the destaining solution, resulting in dark blue DNA bands contrasting against a uniformly light blue background. A white light box ([Cat. #552](#)) or the white light feature of the TruBlu™ 2 ([Cat #557](#)) is recommended for visualizing gels stained with FlashBlue™.

**For Module V (OPTIONAL),
each group should receive:**

- 10 mL 10X concentrated FlashBlue™
- Small plastic tray or weight boat
- Distilled or deionized water

- Stained gels may be stored in destaining liquid for several days with refrigeration, although the bands may fade with time. If this happens, re-stain the gel.
- Destained gels can be discarded in solid waste disposal. Destaining solutions can be disposed of down the drain.

Photodocumentation of DNA (Optional)

Once gels are stained, you may wish to photograph your results. There are many different photodocumentation systems available, including digital systems that are interfaced directly with computers. Specific instructions will vary depending upon the type of photodocumentation system you are using.

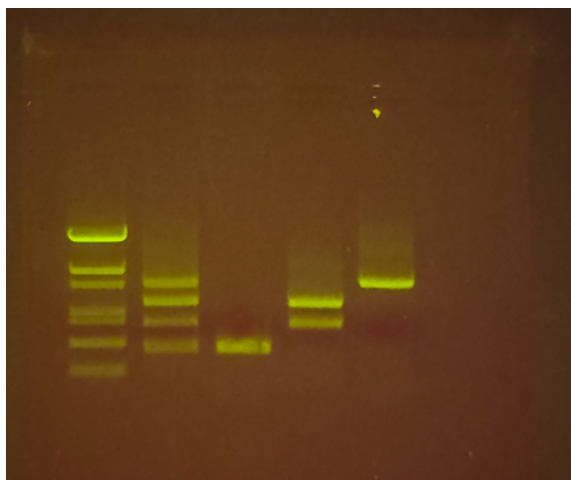
Experiment Results and Analysis

Module I

FINDINGS:

- All three ticks are adult female hard ticks.
- Tick species #1 is the Lone Star tick.
- Tick species #2 is the Blacklegged tick.
- Tick species #3 is the American Dog tick.

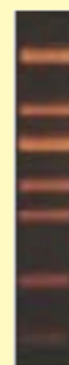
Module II



EdvoQuick™ DNA Ladder

- Better separation
- Easier band measurements
- No unused bands

EdvoQuick™ DNA ladder sizes:
2640, 1400, 1100, 700, 600, 400 and 200



Lane	Sample	Sizes	Pathogen(s) Present	Disease Indicated
1	EdvoQuick™ DNA Ladder	2640, 1400, 1100, 700, 600, 400, 200	---	---
2	Control	1100, 900, 600, 380	---	---
3	Tick 1: Lone Star Tick	380	<i>Ehrlichia chaffeensis</i>	Ehrlichiosis
4	Tick 2: Blacklegged Tick	900, 600	<i>Borrelia burgdorferi</i> and <i>Anaplasma phagocytophilum</i>	Lyme disease and anaplasmosis
5	Tick 3: American Dog Tick	1100	<i>Rickettsia rickettsii</i>	Rocky Mountain spotted fever

Banding patterns in the sample lanes (lanes 3-5) match those in the control (lane 2), allowing identification of pathogen-specific DNA in each tick. Tick 2 shows multiple bands, indicating co-infection. Fragment sizes are approximate and should be interpreted relative to the EdvoQuick™ DNA ladder and control lane.

**Please refer to the kit
insert for the Answers to
Study Questions**

Appendices

- A EDVOTEK® Troubleshooting Guide
- B Bulk Preparation of Electrophoresis Buffer and Agarose Gels

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

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- Online catalog
- Order products
- Experiment protocols
- Tech support
- Additional resources



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Appendix A

EDVOTEK® Troubleshooting Guides

DNA Extraction

Problem	Cause	Answer
Poor DNA extraction	Samples not mixed well enough during extraction	In addition to flicking the tube, vortex or pipet up and down to mix the sample.
	Water baths not at proper temperature	Use a thermometer to confirm water bath set point.
	Not enough DNA	Ensure students are centrifuging saline rinse TWICE during Module I.
The extracted DNA is very cloudy.	Cellular debris from pellet transferred to tube	Centrifuge sample again and move supernatant to a fresh tube. Take care to avoid pellet.
	Cellular debris not separated from supernatant	Centrifuge sample again. If possible, centrifuge at a higher speed. Move cleared supernatant to a fresh tube.

Appendix A

EDVOTEK® Troubleshooting Guides

PCR and Electrophoresis

Problem	Cause	Answer
There is very little liquid left in tube after PCR.	Sample has evaporated.	Make sure the heated lid reaches the appropriate temperature.
	Sample has evaporated.	If your thermal cycler does not have a heated lid, overlay the PCR reaction with oil or wax.
	Sample has evaporated.	Make sure students close the lid of the PCR tube properly.
	Pipetting error.	Make sure students pipet 40 µL primer mix and 10 µL extracted DNA into the 0.2 mL tube.
The ladder, control DNA, and student PCR products are not visible on the gel.	The gel was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.
	The gel was not prepared properly.	Gels of higher concentration (> 0.8%) require special attention when melting the agarose. Make sure that the solution is completely clear of “clumps” and glassy granules before pouring gels.
	The gel was not prepared properly.	The proper buffer was not used for gel preparation. Make sure to use 1x Electrophoresis Buffer.
	The gel was not stained properly.	Ensure that SYBR® Safe was added to the gel before casting. If staining with FlashBlue™, either re-stain with FlashBlue™ solution or destain until bands are seen.
	Malfunctioning electrophoresis unit or power source.	Contact the manufacturer of the electrophoresis unit or power source.
After staining, the ladder and control PCR products are visible on the gel but some student samples are not present.	Wrong volumes of DNA and primer added to PCR reaction.	Practice using micropipets.
Some student samples have more/less amplification than others.	Concentration of DNA varies by sample.	There is an inherent variability in the extraction process.
Low molecular weight band in PCR samples.	Primer dimer	Low concentration of extracted DNA in PCR reaction.
DNA bands were not resolved.	To ensure adequate separation, make sure the tracking dye migrates at least 4 cm on 7 x 7 cm gels and 6 cm on 7 x 14 cm gels.	Be sure to run the gel the appropriate distance before staining and visualizing the DNA.



Appendix B

Bulk Preparation of Electrophoresis Buffer and Agarose Gels

To save time, the electrophoresis buffer and agarose gel solution can be prepared in larger quantities that the whole class can share. Leftover diluted buffer can be used at a later time and solidified agarose gel solution can be remelted.

Bulk Electrophoresis Buffer

Quantity (bulk) preparation for 3 liters of 1x electrophoresis buffer is outlined in Table D.

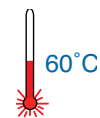
Table D Bulk Preparation of Electrophoresis Buffer			
50x Conc. Buffer	+	Distilled Water	Total Volume Required
60 mL		2940 mL	3000 mL (3 L)

Batch Agarose Gels (1.0%)

For quantity (batch) preparation of 1.0% agarose gels, reference Table E.

1. Use a 500 mL flask to prepare the 1X Diluted gel buffer.
2. Pour the measured UltraSpec-Agarose™ into the prepared buffer. Refer to Table E for the mass. Swirl to disperse clumps.
3. With a marking pen, indicate the level of solution volume on the outside of the flask.
4. Heat the agarose solution as outlined previously for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.
5. Cool the agarose solution to 60° C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.

Table E Batch Preparation of 1.0% UltraSpec-Agarose™						
50x Conc. Buffer	+	Distilled Water	+	Amt of Agarose	=	Total Volume
6.0 ml		294 ml		3.0 g		300 ml
8.0 ml		392 ml		4.0 g		400 ml



6. Add the entire volume of *diluted* SYBR® Safe, prepared on page 25, to the cooled agarose and mix well. This concentration of SYBR® Safe will work for either 300 mL or 400 mL preparations of agarose gels.
7. Dispense the required volume of cooled agarose solution for casting each gel. Measure 25 mL for each 7 x 7 cm gel, 50 mL for each 7 x 14 cm gel. **For this experiment, 7 x 7 cm gels are recommended.**
8. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Then proceed with preparing the gel for electrophoresis. Alternatively, gels can be stored in water-tight plastic bags with 2 mL of 1X electrophoresis buffer for up to 1 week in the refrigerator.

NOTE:

The UltraSpec-Agarose™ kit component is usually labeled with the amount it contains. Please read the label carefully. If the amount of agarose is not specified or if the bottle's plastic seal has been broken, weigh the agarose to ensure you are using the correct amount.