# Viral Diagnostics Lab

# Beating the Next Pandemic

Viral Diagnostics Lab:
Beating the Next Pandemic
KT-1503-01

Version: 1.3.
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# Contents

# At a glance

## Lab overview

A new virus is spreading across the globe. You must use molecular techniques to diagnose your patients!

In this lab, students act as clinicians, diagnosing four patients who have arrived at their clinics complaining of flu-like symptoms. Are these patients suffering from seasonal flu, the new and dangerous novel influenza Q virus, or something else entirely? Students will use gel electrophoresis to learn how molecular tools allow healthcare providers to diagnose hard-to-distinguish infections.

**Disclaimer: no pathogenic materials are used.** This experimental protocol engages students in a *simulated* patient diagnosis exercise. None of the materials provided in this lab kit pose a pathogenic risk.

**TECHNIQUES**

Micropipetting
Gel electrophoresis

**TOPICS**

Infectious disease
Molecular and clinical diagnosis
Evolution
Biotechnology

**LEVEL**

General high school
Advanced high school

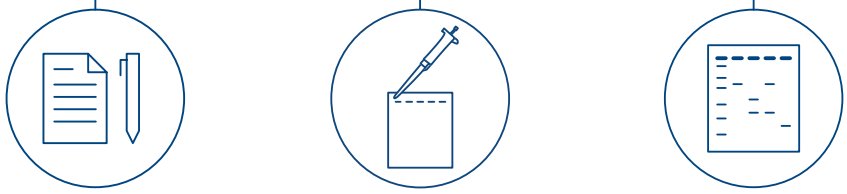
## Planning your time

**SINGLE CLASS: 45 min.**

See the next page for detailed class time requirements.

**Teacher prep (30 min.)** → **Review patient cases** → **Gel electrophoresis** → **Interpret results**

See page 7 for details.
We recommend that the teacher prepare the gels before class.



## Technical support

If you have any questions about implementing this activity, contact support@minipcr.com.

# Class time requirements

This activity can be completed in a single 45-minute class period if the gels have been prepared in advance.

| Steps | | Time required |
|---|---|---|
| **Prep** | Make gels | We recommend the teacher prepare the gels outside of class (see page 7). Allot 30 minutes of class time if you opt to have students prepare the gels. |
| **1** | Load gel | 10 minutes |
| **2** | Run gel | 15-20 minutes<br><br>The gel does not need to be actively monitored. We recommend that you complete the Pre-lab patient assessments during this time. |
| **3** | Interpret results | 5 minutes |

# Materials needed

## Supplied in kit (KT-1503-01)

- Kit contains DNA samples for eight lab groups.
- If kept in the freezer, reagents can be stored for 12 months after receipt. If kept in the refrigerator, reagents can be stored for 6 months after receipt.
- Reagents for preparing gels, plastic tubes for distributing samples to individual groups, and pipette tips are sold separately. Refer to the section below for details.

| Contents | Provided | Required per group | Storage |
|---|---|---|---|
| Simulated DNA Samples<br>• Patient K.T. DNA<br>• Patient O.G. DNA<br>• Patient B.D. DNA<br>• Patient D.Z. DNA<br>• Control DNA | 150 µl each | 15 µl each | Freezer |
| Fast DNA Ladder 1 | 150 µl | 15 µl | Freezer |

## Electrophoresis reagents and plastics sold separately

- This lab requires 2% agarose gels with a fluorescent DNA stain (*e.g.,* SeeGreen™ or GelGreen®) and plastic tubes to distribute reagents to individual groups.
- The Learning Lab Companion Kit (KT-1510-01) provides sufficient reagents to prepare and run eight gels when using the blueGel electrophoresis system, as well as plastic tubes to distribute samples to student groups.
- Alternatively, bulk electrophoresis reagents and plastics (tubes, pipette tips) are available for purchase from miniPCR bio.
- Gel electrophoresis reagents and plastics can also be purchased from other suppliers.

## Required equipment

- This lab is compatible with any horizontal gel electrophoresis system in combination with:
  - A fluorescent DNA stain (*e.g.,* SeeGreen™ or GelGreen®).
  - A transilluminator that is compatible with the DNA stain used. Fluorescent DNA stains typically require blue light (~470 nm) or UV (~260 nm) illumination.
- The table below outlines gel electrophoresis equipment from miniPCR bio that meets these requirements.

AVAILABLE AT MINIPCR.COM

| Item | Recommended quantity |
|---|---|
| **Gel electrophoresis and visualization system**<br>Option 1: BlueGel™ OR GELATO™ electrophoresis systems with integrated blue light transilluminator | 1 blueGel can be shared by two groups<br>1 GELATO can be shared by four groups |
| Option 2: Bandit™ STEM electrophoresis kit paired with the Viewit™ Illumination Kit | 1 Bandit + 1 Viewit per group |
| Option 3: Bandit™ STEM electrophoresis kit paired with a blueBox™ blue light transilluminator | 1 Bandit per group + 1 blueBox for the class to share |
| **Micropipettes and tips**<br>2-20 µl adjustable or 10 µl fixed volume | 1 pipette per group |

## Other materials supplied by user

- Distilled water
- Microwave or hot plate
- Heat-resistant flask or beaker
- Disposable laboratory gloves
- Protective eyewear
- Fine-tipped permanent marker

# Teacher prep

Protective gloves and eyewear should be worn for the entirety of this experiment.

## Overview

The table below provides an overview of the teacher prep, and the subsequent pages provide detailed instructions.

| Prep | Time required | Timeline |
|---|---|---|
| Dispense reagents | 10 minutes | Can be completed up to one week before before use. |
| Prepare electrophoresis buffer and agarose gels | 20 minutes | Varies - If using gel reagents from miniPCR, gels can be prepared up to five days before use. |

## Dispense reagents

- DNA samples can be dispensed up to one week in advance and stored in the refrigerator until use.
- This kit provides sufficient reagents for eight lab groups.

**Materials needed**

From the lab kit (stored in the freezer):

- Patient K.T. DNA
- Patient O.G. DNA
- Patient B.D. DNA
- Patient D.Z. DNA
- Control DNA
- Fast DNA Ladder 1

Supplied by user:

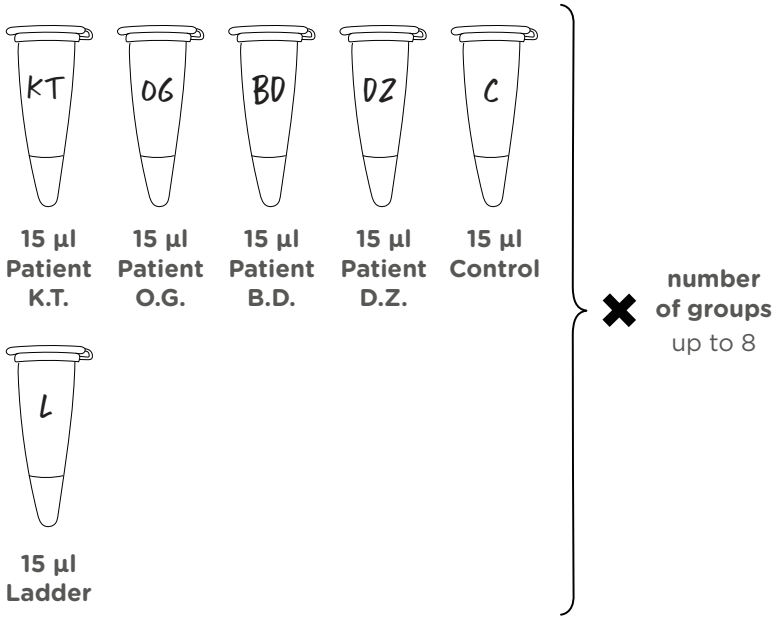
- Plastic tubes for dispensing reagents. 1.5 ml or 0.2 ml tubes can be used
- 2-20 µl micropipette and tips
- Fine-tipped permanent marker

1. Thaw reagents by placing tubes at room temperature.

2. Collect the liquid at the bottom of each tube. Either spin briefly in a microcentrifuge or shake the liquid down with a flick of the wrist.

3. When you open each tube, check for liquid stuck inside the cap. If necessary, put the cap back on and repeat step 2.

4. For each lab group, dispense the following reagents into labeled plastic tubes. 1.5 ml or 0.2 ml plastic tubes can be used.
   - Patient K.T. DNA (label tube as “KT”) 15 µl
   - Patient O.G. DNA (tube OG) 15 µl
   - Patient B.D. DNA (tube BD) 15 µl
   - Patient D.Z. DNA (tube DZ) 15 µl
   - Control DNA (tube C) 15 µl
   - Fast DNA Ladder 1 (tube L) 15 µl




5. If you are preparing the DNA samples more than 24 hours before class, store the tubes in the refrigerator until use. Dispensed DNA samples can be stored in the refrigerator for up to one week before use.

## Prepare gel electrophoresis buffer and agarose gels

1. Prepare electrophoresis buffer.
   - Follow the manufacturer's instructions to prepare buffer solution.
   - The volume of buffer needed varies based on the gel electrophoresis system.
     - For the blueGel and Bandit electrophoresis systems, 600 ml of TBE buffer is sufficient for at least eight gel runs.
     - For other systems, refer to the manufacturer's instructions for:
       (1) The buffer volume needed to prepare agarose gels.
       (2) The buffer volume needed for use as a running buffer.

2. Prepare 2% agarose gels with fluorescent DNA stain.
   - Each group will need five lanes, plus one lane for ladder. If groups are sharing gels, a single lane for ladder per gel is sufficient.
   - This lab kit is compatible with any molecular grade agarose and fluorescent DNA stain (*e.g.,* SeeGreen™ or GelGreen®).
   - The volume of gel needed varies based on the gel electrophoresis system you are using. Refer to the manufacturer's instructions.
   - If using gel electrophoresis reagents from miniPCR bio, gels can be prepared up to five days in advance. Store prepared gels at room temperature in an airtight container protected from light. Do NOT soak the gels in buffer or wrap them in paper towels.

**Detailed instructions for preparing buffer and gels for miniPCR electrophoresis systems**

**blueGel**
https://links.minipcr.com/gelpouring

**Bandit**
https://links.minipcr.com/BanditDNAgel

# Student workstation setup

| At the start of this experiment, every lab group should have: | |
|---|---|
| DNA samples:<br>• Patient K.T. DNA (tube KT)<br>• Patient O.G. DNA (tube OG)<br>• Patient B.D. DNA (tube BD)<br>• Patient D.Z. DNA (tube DZ)<br>• Control DNA (tube C) | 15 µl each |
| Fast DNA Ladder 1 (tube L) | 15 µl |
| 2-20 µl micropipette or 10 µl fixed volume micropipette | 1 |
| Micropipette tips | At least 6 |
| Electrophoresis buffer<br>*Volume depends on your electrophoresis system | 30 ml TBE if using a blueGel or Bandit |
| 6 wells in a 2% agarose gel with fluorescent DNA stain | |

# Student guide

# Background information

## Scenario overview

As a healthcare provider, your job is to diagnose your patients and provide the best treatment possible based on that diagnosis. Sometimes, however, illnesses can be difficult to discern. Symptoms for many diseases are similar and untangling whether a patient has a run-of-the-mill cold, seasonal allergies, or a dangerous virus is not always easy. To complicate things even more, new viruses entering the human population are always a potential threat. Now, four patients have just arrived at your clinic complaining of flu-like symptoms. Are they suffering from a typical case of seasonal flu, the new and dangerous novel influenza Q virus (or nIQV – pronounced "nick vee")[1] , or something else entirely?

Previously limited to infecting cattle and other livestock, nIQV caused mild respiratory symptoms in animals. In humans, however, nIQV has wreaked havoc; though the disease has only just begun to spread, scientists have estimated that up to 40% of patients will require hospitalization and for 4% to 8% of patients, the disease will be fatal.

Today, four patients have come to your clinic complaining of flu-like symptoms. As their healthcare provider, you'll use molecular techniques to determine which viruses have infected your patients and arrive at conclusive diagnoses.

## What are viruses?

Many common illnesses, including the flu and the common cold, are caused by viruses. Viruses are infectious agents with simple structures; at their most basic, they are little more than a protein coat wrapped around a small genome made of RNA or DNA (Figure 1). Because they lack organelles and other cellular machinery, they are unable to do most of the things a typical cell does—including reproduce independently of a host. Because of this, viruses are generally considered nonliving.

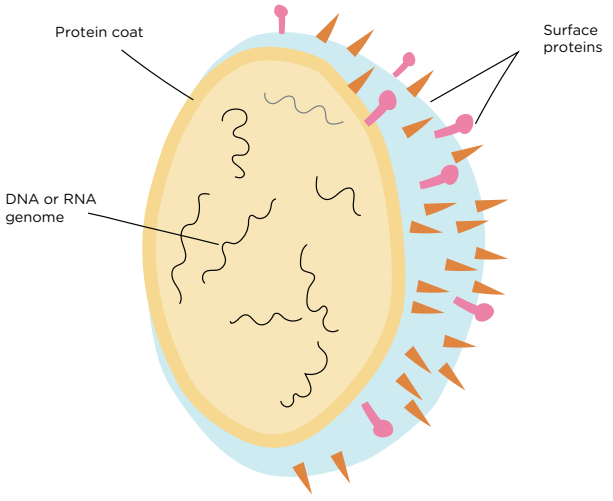



**Figure 1** Structure of a virus

[1] nIQV is a fictional virus, but its story, as outlined here, represents how real diseases may emerge and spread through the human population.

To reproduce, a virus must invade a living cell and use its machinery to make copies of itself. It does this by physically attaching to and emptying its genetic material into a host cell. How exactly it does this varies from virus to virus, but the end result is the same: with the viral genome unleashed, the host cell begins to manufacture viral proteins and replicate the viral DNA or RNA. Newly made proteins and copies of the viral genome then come together to form an army of new virus particles (Figure 2). These viruses emerge from the host cell, moving on to infect new cells—and new hosts, if they're able to find their way out of the body, say though a cough or sneeze —and the cycle repeats itself.

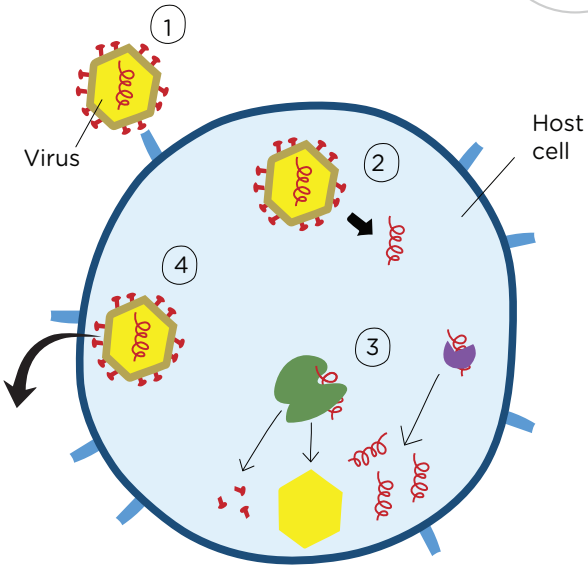



**Figure 2** Viral replication cycle. 1 – Virus particle binds to and enters host cell. 2 – Virus particle sheds its coat, releasing its genome into the host. 3 – Host cell copies viral genome and expresses viral proteins, which come together to make new virus particles. 4 – Newly assembled virus particles emerge from cell, ready to infect new host cells.

A single virus particle can turn into thousands very quickly. Sometimes, the newly manufactured virus particles are packaged into capsules and released from the cell in a slow trickle. In other cases, though, replication is so fast and relentless, it continues until the host cell literally explodes, releasing a swarm of newborn virus particles. Regardless of how they exit the host cell, each of these newly released virus particles has a chance to infect new cells and, in turn, make thousands of copies of itself. In this way, the growth of the viral population is exponential. While an infection can be disastrous for the host, the speed and efficiency of viral replication represents a success from the point of view of the virus. Despite their small size, simple construction, and total dependence on living cells, viruses are remarkably efficient in achieving the primary functions of all biological entities: to persist and replicate.

## The shape-shifting virus

Viruses are typically limited in the range of hosts they are able to infect. Viruses use dedicated proteins on the surface of their coats to recognize host cells. These surface proteins attach to a specific host cell or cells by binding to receptor molecules that stick out from the host cell's membrane. Receptors are normally used by host cells for cell-to-cell communication, sensing the environment, and adhering to other cells, but viruses hijack these structures for their own purposes. For this reason, a virus can only infect a given cell if that cell displays specific receptors on its surface (Figure 3). This is why you can't typically catch a cold from your dog—the receptors on your cells and a dog's cells are different enough that viruses can only recognize one or the other.

If the surface proteins on a virus change, however, they may gain the ability to bind to slightly different types of receptors, and therefore may be able to infect new hosts. This can happen as the result of a *mutation*. Viral genomes, like the genomes of living things, are subject to occasional errors in DNA or RNA copying that may be passed down to subsequent generations. While many of these mutations make it harder for a virus to replicate, and others have no effect on how a virus behaves, some may confer traits that help a virus infect a new species. Mutations in the genes encoding viral surface proteins may enable viruses to bind to host cell receptors they could not recognize before. Thus, through random mutations, the virus may gain the ability to infect a new species that it was previously unable to infect. A mutation event like this is thought to have triggered the COVID-19 pandemic; scientists believe a mutation in a strain of coronavirus carried by bats allowed the virus to jump species and eventually infect human cells. The subsequent spread of this virus from human to human led to a wave of disease that infected millions across the world.

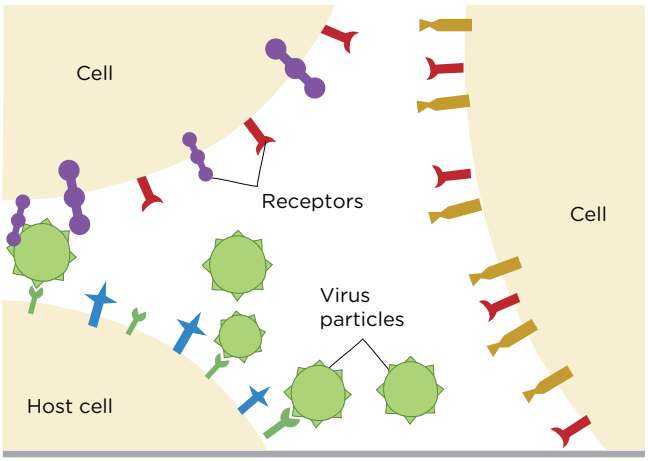



**Figure 3** Virus particles can only enter cells expressing specific receptors

You may wonder why we rarely, or never, witness the emergence of a new living animal or plant species in our lifetime, yet the past few decades alone have seen the emergence of Zika, MERS, swine flu, and the COVID-19 pandemic—all of which are viral illnesses. While animal evolution tends to happen on long timescales well beyond the scope of a single human lifetime, viral evolution can proceed so quickly that it can transform the way entire human societies live.

There are three main reasons why viruses evolve so rapidly. The first is their abundance. Scientists have estimated that $10^{31}$ virus particles exist on Earth. That means viruses outnumber even a very abundant population of organisms—say, insects—by a factor of 1 trillion. With so many individual virus particles, there are that many more opportunities mutations to occur. The second reason is that, as we've seen, viruses reproduce prolifically. A single virus particle typically makes hundreds to thousands of copies of itself upon infecting a cell; each of its "offspring" can in turn multiply itself hundreds to thousands of times. This scale of replication offers the chance for mutations to spread widely in just a single generation. Compare this to human reproduction, where a mutation in one individual is spread to just a handful of individuals over one generation. The third reason is that in making copies of themselves, viruses make frequent errors. This is especially true for viruses with an RNA-based genome. RNA replication is dependent on a different enzyme than the one that guides DNA replication—one that is more likely to make errors while copying. So even if viral populations were similar in size to animal and plant populations, the likelihood of mutation would still be greater among viruses.

# Identifying viral infections

Viruses cause different symptoms depending on what body systems they infect. Even within the body of an infected individual, the fact that different types of receptors are expressed by different cell types means a virus will invade only certain tissues. Some viruses, like the influenza viruses that cause seasonal flu, infect cells in the respiratory tract. As they take over the cells that line our airways, they cause respiratory symptoms like sneezing and coughing. The fact that these symptoms promote the spread of the virus from person to person may be no coincidence, since sneezing and coughing help the virus reach new hosts they can infect.

Viruses that infect the same body systems can produce very similar symptoms, making it difficult to tell what virus underlies a disease. This is particularly true for respiratory viruses. A cold and a case of the flu may appear quite similar, both causing a cough, nasal congestion, and body aches. In fact, the term "a cold" is a catch-all term that describes several different types of infections—both viral and bacterial— all with similar symptoms and severities.

Doctors often use laboratory tests to determine the identity of the pathogen underlying a disease. Among the most common viral tests are nucleic acid detection tests. You will be using this method to test patient samples today. Nucleic acid tests look for the presence of viral DNA or RNA in a sample, taking advantage of unique genetic sequences present in the virus. If we find a viral sequence in a sample from a human patient, we can presume the patient is infected with that virus.

A nucleic acid detection test typically involves the following four steps:

- **Step 1: Collect patient sample.** Technicians must sample patient tissue that the virus of interest will have infected. For a virus like seasonal influenza, a technician might swab the back of the throat to collect some of the respiratory cells the virus is equipped to invade.

- **Step 2: Extract genetic material.** To isolate nucleic acids from the surrounding biological material, cells are ruptured to release their DNA and RNA, and lipids and proteins are filtered out of the sample. The DNA and RNA is also stabilized during this step so it isn't broken down by subsequent processing.

- **Step 3: Amplify viral sequence.** In this step, a gene segment specific to the virus of interest is *amplified,* or repeatedly copied, so it is much more abundant than the genetic material we aren't interested in (*e.g.,* the patient's own DNA and RNA, which is also present in the sample). Most commonly, the technique polymerase chain reaction (PCR) is used for amplification; because of this, nucleic acid detection tests are sometimes referred to as PCR tests. Because PCR can only be used to amplify DNA and not RNA, detection of RNA viruses requires an additional *reverse transcription (RT)* step where RNA is converted to DNA before PCR. The process of reverse transcription followed by PCR is called RT-PCR.

- **Step 4: Visualize results.** Finally, the test result is ready to be read. The amplified DNA is visualized to determine whether the virus of interest was present in the patient sample. A patient is said to "test positive" for a virus when we see amplified viral DNA in their sample. If we don't see amplified viral DNA, we would say they have "tested negative" and conclude that they are not infected. Often, a machine is used to detect amplified DNA in a process called quantitative PCR (qPCR). Today, however, we will directly visualize amplified PCR product ourselves using gel electrophoresis.

Today you'll experience how fast, precise, and powerful nucleic acid tests can be. In recent decades, development of nucleic acid detection methods has expanded our ability to rapidly and specifically detect infectious agents, allowing us to combat infectious disease, inform patient treatment, and better understand the processes by which viral pathogens emerge and spread.

# Today's lab

In today's lab, you will use a nucleic acid detection test on samples taken from four patients displaying flu-like symptoms. Your patients may be experiencing ordinary seasonal flu or they may have been infected with the novel influenza Q virus (nIQV), an emergent RNA virus that, through a mutation, recently jumped from cattle into the human population.

With a fatality rate of 4% to 8%, nIQV is much deadlier than the seasonal flu, which kills roughly 0.1% of infected individuals. But despite this difference in severity, early nIQV symptoms look remarkably like the seasonal flu. Both viruses cause fever, aches and pains, fatigue, and a cough. Occasionally, both viruses cause nasal congestion, leading some sufferers to believe they are experiencing seasonal allergies or a particularly nasty case of the common cold. Your task will be to use molecular techniques to get your patients clear answers on the nature of their infections.

The patient samples you will be given were prepared as follows. A technician first took a nasal swab to collect tissue from the patient's airways. RNA was extracted from the sample and RT-PCR was used to convert this RNA to DNA, then amplify viral sequences from the samples (Figure 4). For every sample, two viral sequences were amplified:

1. A 250-base pair portion of the **seasonal influenza virus gene** for matrix protein M.
2. A 400-base pair portion of the **nIQV gene** for the surface protein HEF.

If either of these sequences is present in a patient sample, we can conclude the patient was infected with the corresponding virus. That is, if we find gene M in a sample, we will diagnose that patient with seasonal influenza. The presence of gene HEF would indicate a nIQV infection.

What if a patient tests negative for both nIQV and seasonal influenza? That could be great news: a clean bill of health! But it could also mean our experiment did not work properly: perhaps our patient has nIQV, but our chemicals went bad and we were unable to extract nIQV RNA from our nasal swabs. To rule out the possibility of such a *false negative* (a result that incorrectly indicates that an infection is not present), a third sequence was also amplified from each patient sample:

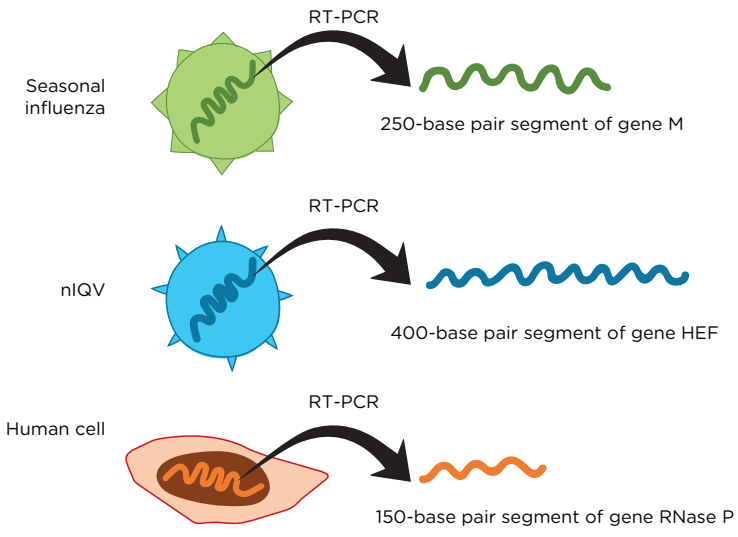



**Figure 4** A schematic showing the gene sequences you will test for in this lab.

3. A 150-base pair segment of the **human gene for ribonuclease P** (RNase P), which is con tinually expressed by our cells, as it is involved in the process of translating RNA into protein. This sequence serves as an experimental control. It should be present in all of the patient samples—even healthy ones—since we inevitably collected some of the patients' own cells on every nasal swab. We will know our experiment has worked correctly, from sample collec tion through detection, if this sequence is successfully amplified.

What if all four of our patients test positive for nIQV? It could be that our clinic has been hit by a genuine public health disaster. Or again, it could mean our experiment did not work properly: perhaps none of our patients have nIQV, but the materials we used to collect our patients' samples were contaminated. To rule out the possibility of such a *false positive* (a result that incorrectly indicates that an infection is present), we will test a sample of human tissue known to be free from any viral agents, termed a *control* sample. If we were to find viral RNA in this sample, we would know our experiment must not be working properly, and we should re-test our patients to be sure we're getting trustworthy results.

Today, you will use gel electrophoresis to determine whether viral RNA was present in your patients' samples. Your task will be to use the data you collect to confirm your patients' diagnoses. Have they fallen ill with nIQV? The flu? Or something else?

# Pre-lab patient assessments

## Patient descriptions

**Patient K.T.**

Patient K.T. is a 66-year-old male retiree. He presents with muscle aches throughout his body and a persistent cough, with a fever of 102°F. His symptoms set in nearly a week ago, but were mild enough to ignore until today. Patient K.T. has asthma and seasonal allergies, and was hospitalized two years ago following an asthma attack.

**Patient O.G.**

Patient O.G. is a 37-year-old father of three. He works part-time as a nurse. He presents with wheezing and a runny nose. He does not have a fever. His symptoms began two days ago and have been steady since their onset. Patient O.G. received a flu vaccination this season and is generally in good health, aside from seasonal allergies.

**Patient B.D.**

Patient B.D. is a 15-year-old female who is currently a sophomore in high school. Her mother brought her to the emergency room after patient B.D. complained of being unusually tired for two consecutive days, despite sleeping 9 to 10 hours each night. Patient B.D. presents with a persistent cough and a fever of 104 °F. Aside from her present illness, she is generally in good health.

**Patient D.Z.**

Patient D.Z. is a 42-year-old female who is visiting the United States on a family vacation from Australia. Four days into her trip, she began experiencing fatigue and shortness of breath with mild wheezing and a cough. Her symptoms have progressively worsened over the past two days, leading her to seek medical treatment today. Patient D.Z. has a fever of 102 °F. She suffers from lupus, a chronic autoimmune disease, and did receive a flu shot this season, although note that the Australian flu vaccine may differ slightly from the vaccine administered in the U.S.

# Preliminary diagnosis

In this lab, you won't make your final diagnosis until you've run your gel electrophoresis experiment, but like any doctor, you may have hypotheses about the viruses your patients are suffering from based only on their symptoms. Consider your patients' possible diagnoses and answer the questions below. Use this chart, which outlines some of the symptoms associated with each potential diagnosis, to help you.

| | Common cold | Seasonal influenza | Allergies | nIQV |
|---|---|---|---|---|
| **Fever** | Rare | Common | Never | Common |
| **Aches & pains** | Slight | Common and severe | Never | Occasional |
| **Fatigue** | Occasional | Common | Occasional | Common |
| **Stuffy or runny nose** | Common | Occasional | Common | Rare |
| **Cough** | Common | Common | Sometimes | Common |

Q1. Patient O.G. is certain he has nIQV. As a healthcare worker at a neighboring hospital, he has cared for nIQV patients and fears he has been exposed to the virus. Based on his symptoms, do you agree or disagree with his self-assessment? Why or why not?

Q2. Your colleague, Nurse Li, put on a double layer of gloves and a face mask before treating patient D.Z. She is certain this patient has nIQV and is doing all she can to keep from being exposed to the virus. What evidence may have led Nurse Li to believe patient D.Z. has nIQV? What other potential diagnoses can you not rule out at this point?

Q3. At this point, what is your hypothesis about each of your patients' diagnoses? Of course, your diagnoses won't be final until you've received the results of your gel electrophoresis experiment.

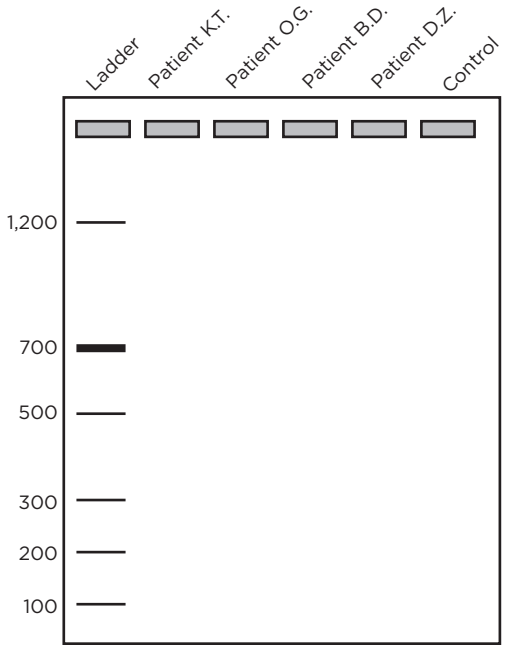
Patient K.T.:
Reasoning:

Patient O.G.:
Reasoning:

Patient B.D.:
Reasoning:

Patient D.Z.:
Reasoning:

Q4. The illustration to the right depicts an electrophoresis gel. Lane 1 contains a DNA ladder, showing how far bands of different size will migrate on the gel. Using the DNA segment sizes we expect from our PCR test (described on pages 15-16), predict what your gel will look like. Draw in the bands you expect to see for each patient based on your predictions from the previous question.



# Student lab protocol

Protective gloves and eyewear should be worn for the entirety of this experiment.

1. Place the prepared gel into the electrophoresis chamber.

2. Add enough electrophoresis buffer to fill the chamber and just cover the gel.
   - You will need 30 ml of TBE buffer for a blueGel™ or Bandit™ electrophoresis system. Do not overfill the chamber.
   - If using another electrophoresis system, refer to the manufacturer's instructions for the recommended buffer type and volume.

3. Use a micropipette to load samples in the following order. To prevent contamination, use a new tip for each sample.
   - Well 1: 10 µl Fast DNA Ladder 1 (tube L)
   - Well 2: 10 µl Patient K.T. (tube KT)
   - Well 3: 10 µl Patient O.G. (tube OG)
   - Well 4: 10 µl Patient B.D. (tube BD)
   - Well 5: 10 µl Patient D.Z. (tube DZ)
   - Well 6: 10 µl Control DNA (tube C)

**Detailed operating instructions for miniPCR electrophoresis systems**

**blueGel**
https://links.minipcr.com/blueGelRun

**Bandit**
https://links.minipcr.com/BanditViewit

4. Run the gel for 15-20 minutes.
   - The blueGel™ and Bandit™ electrophoresis systems run at a fixed voltage.
   - If using another gel electrophoresis system, set the voltage in the 70-90 V range.

5. To visualize the DNA samples, turn on the blue light in your electrophoresis system, or move the gel to a transilluminator.

6. If needed, continue to run the gel until there is sufficient separation between the 100-500 bp bands in the ladder to interpret the results.

7. If desired, take a photo to document the results.

8. Compare the bands from the DNA samples to the DNA ladder to obtain size estimates.

# Pre-lab questions

## Review

1. Why must a virus enter a living cell in order to replicate?

2. Your friend has a cold, but insists she doesn't need to cover her mouth when she coughs. Using what you know about virus biology, explain how covering your cough helps prevent the spread of viral infections.

3. Why don't humans typically pass viruses to their pets and vice versa?

4. How was nIQV able to jump from cattle to humans? What would have had to happen to allow it to do so?

5. What are three reasons viruses mutate and change more rapidly than, say, trees do?

6. We will carry out a nucleic acid detection test on samples from each of our four patients. The samples will contain DNA and RNA from different sources: from patients' own cells, from bacteria present in the nasal passage, and potentially from viruses, too. How can we be sure that we are testing specifically for our virus(es) of interest?

7. In this lab, we use experimental controls to ensure that we don't see false positive or false negative results.
   a. Explain what is meant by the term "false negative."

   b. Explain what is meant by the term "false positive."

## Critical thinking

8. Viruses are generally considered nonliving. What about viruses might lead someone to argue that they are actually alive?

9. Malaria is a disease caused by a parasite that invades the cells of the blood. In malaria, a single celled organism, *Plasmodium falciparum*, enters red blood cells and begins to reproduce, using the resources of the cell. Eventually the red blood cells rupture, releasing scores of new malaria parasites into the blood where they infect new blood cells. *Plasmodium falciparum* is not a virus, but in some ways it behaves like one. What are two ways malaria is like a viral infection?

10. Why do we say a video "goes viral" if it is circulated to a wide audience in a short time? Connect this to your knowledge of virus biology.

11. For each scenario below, identify whether the test result described is a false negative or a false positive. Explain your answer.
    a. A nucleic acid detection test indicates a patient is not infected with nIQV when they actually are.

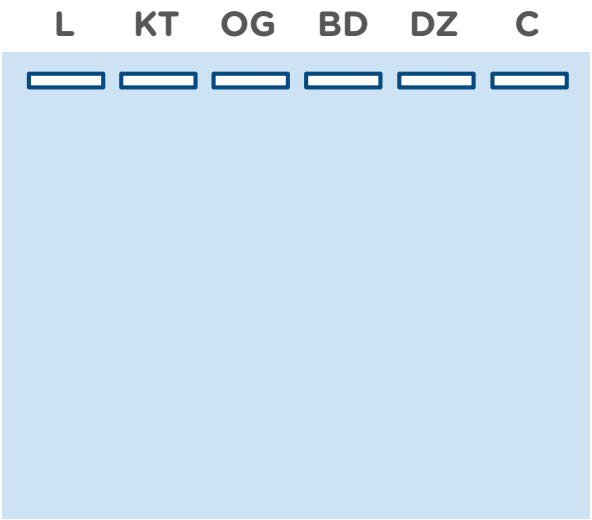
    b. A nucleic acid detection test indicates that a patient is infected with seasonal influenza, but in fact they are healthy.

12. In your opinion, is it more dangerous to have a false negative or a false positive result? In which case are the potential consequences worse? Justify your answer.

13. In diagnosing a patient with a viral infection, why is it better to use a nucleic acid detection test than make a diagnosis based only on the patient's symptoms?

# Post-lab questions

## Interpreting results




1. Use the image on the right to draw what your gel looks like. For each sample, draw the bands that you see on your actual gel.

2. Next to each band, write approximately how long (in base pairs) the DNA in that band is. Use the image of the ladder from page 22 to help you.

3. Use your gel electrophoresis results to complete the table below:
   a. Use checkmarks to record the gel electrophoresis results in the first three rows of the table.
   b. Use the results to determine each patient's diagnosis.

| | Patient K.T. | Patient O.G. | Patient B.D. | Patient D.Z. | Control human DNA |
|---|---|---|---|---|---|
| niQV (400 bp) | | | | | |
| Seasonal influenza (250 bp) | | | | | |
| Human RNase P control (150 bp) | | | | | |
| Diagnosis | | | | | |

4. For any of your patients, is your diagnosis different from your original prediction? If so, explain how.

5. Are there any patients for whom you cannot give a definitive diagnosis? If so, identify them and list as many possible diagnoses as you can think of.

## Critical thinking

6. Imagine you observed two bands in your control lane: one at 250 bp and one at 150 bp. What concern would this raise? If you were to repeat this test, what might you do differently to ensure this doesn't happen again?

7. Imagine you had four patients test positive for seasonal influenza. Your colleague insists at least some of these must be false positives; after all, what are the chances all four patients would be infected with the flu? In your control lane, you see just one band measuring 150 bp. Do you agree or disagree with your colleague's assessment?

8. What would it mean if, for one patient, you observed 3 bands: one at 150 bp, one at 250 bp, and one at 400 bp? What would you want to see in your control lane to ensure this is a reliable result?

# CER table

Fill in the table based on your results from the lab. Refer to the rubric on the next page.

## Question:

*Are any of your patients infected with nIQV?*

### Claim

Make a clear statement that answers the above question.

### Evidence

Provide data from the lab that supports your claim.

### Reasoning

Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.

| Score | 4 | 3 | 2 | 1 |
|---|---|---|---|---|
| **CLAIM**<br>A statement that answers the original question/ problem. | Makes a clear, accurate, and complete claim. | Makes an accurate and complete claim. | Makes an accurate but incomplete or vague claim. | Makes a claim that is inaccurate. |
| **EVIDENCE**<br>Data from the experiment that supports the claim.<br>Data must be relevant and sufficient to support the claim. | All of the evidence presented is relevant and sufficient to support the claim. | Provides evidence that is relevant and sufficient to support the claim. May include some non-relevant evidence. | Provides relevant but insufficient evidence to support the claim. May include some non-relevant evidence. | Only provides evidence that does not support claim. |
| **REASONING**<br>Explain why your evidence supports your claim. This must include scientific principles/ knowledge that you have about the topic to show why the data counts as evidence. | Provides reasoning that clearly links the evidence to the claim. Relevant scientific principles are well integrated in the reasoning. | Provides reasoning that links the evidence to the claim. Relevant scientific principles are discussed. | Provides reasoning that links the evidence to the claim, but does not include relevant scientific principles or uses them incorrectly. | Provides reasoning that does not link the evidence to the claim. Does not include relevant scientific principles or uses them incorrectly. |

We recommend that teachers use the following scale when assessing this assignment using the rubric. Teachers should feel free to adjust this scale to their expectations.

| Rubric score | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|
| Equivalent Grade | 55 | 60 | 65 | 70 | 75 | 80 | 85 | 90 | 95 | 100 |

# Extension: Using genetic data to track outbreaks

To trace the spread of a pathogen like novel influenza Q virus (nIQV) across the globe, scientists take advantage of the fact that as viruses spread, their genetic sequences mutate. These mutations give rise to unique *variants*, or versions of the same virus with variations in their genetic sequences. By comparing the sequences of different viral samples and tracking where and when closely related variants were found, researchers can trace the geographical path of a virus through a population.

Tracking viruses requires researchers to collect patient samples just as a clinician would do to carry out nucleic acid detection tests. Researchers tracking outbreaks collect samples of infected patient tissue and extract the viral genetic material, but instead of testing for the mere presence or absence of a particular gene, they read the nucleotide sequence of the entire viral genome or vast portions of it. The more of the genome a researcher can read—or sequence—the more information they will have about the relationships between variants.

Determining how closely related two viral variants are by comparing their genetic sequences is rather simple. In short, if two variants share the same sequence at a given position in their genomes while other variants share a different one—say two guanines (Gs) where every other virus has an adenine (A) followed by a cytosine (C)—scientists make the assumption that the two viral sequences share an ancestor in which that mutation first occurred, and are more closely related to one another than other variants. While this principle is fundamentally simple, it can become complex when applied to a dataset consisting of dozens or hundreds of different sequences, each thousands of nucleotides long. Because of the sheer amount of data involved, this type of analysis is handled by computers.

```
Variant 1: AUUGGUACAC
Variant 2: AUUGGUACAC
Variant 3: AUUGGUACAC
Variant 4: AUUGGUGGAC
Variant 5: AUUGGUGGAC
Variant 6: AUUGGUACAC
```

Genome sequences from 6 related viral variants. Because variants 4 and 5 share a common mutation (GG where other variants have AC), we can presume they are more closely related.

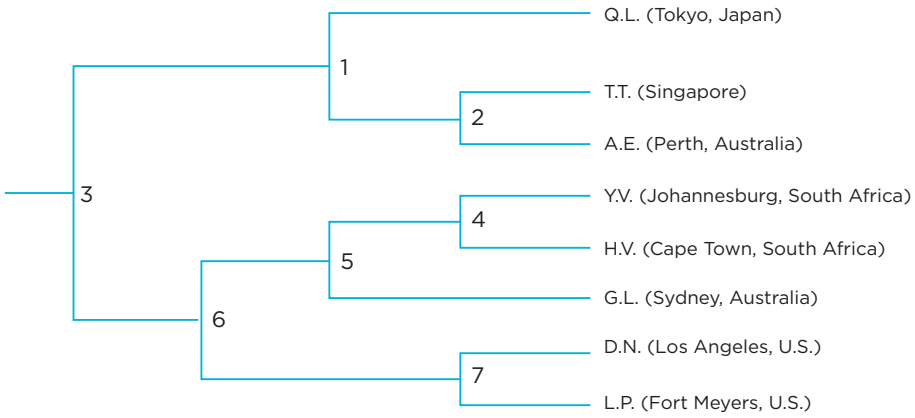
## Tree thinking

Understanding how viruses are related and how they spread uses the tools of evolutionary biology and more specifically, *phylogenetics*: the study of how different species are related to each other evolutionarily. You may have experience with some of these tools; phylogenetic trees or cladograms are diagrams that depict evolutionary relationships between living things—or viruses.

Below, you can see a tree showing different nIQV virus variants. As you trace the paths of the tree from left to right, you are advancing in evolutionary time. Nodes—the points where one branch splits into two—represent the most recent common ancestor shared by the organisms on the branches.

Tracing from right to left, you'll see that the sooner two organisms meet at a node, the more closely related they are.

You may see trees drawn in a variety of ways. Sometimes lines are drawn at an angle, making a series of connected "V"s. Sometimes they advance from left to right as in the tree below, but they can be oriented in any direction. Sometimes they are even drawn as a circle. There can be different reasons for drawing trees all these different ways, but universally, the most important things to look for in a tree are the nodes. Understanding at what nodes two branches connect will tell you how organisms are related to each other.




The tree above represents eight nIQV viral sequences collected from patients around the world. Each sequence bears the initials of the patient from whom the virus was isolated. In this tree the nodes have been numbered so we can easily reference them.

When reading a tree, remember that the order in which the names are written in is not important for determining relationships. What is important is determining the node at which branches meet. For example, A.E and Y.V. are written next to each other, but their branches don't meet until the deepest node in the tree (3), showing that they are only distantly related. On the other hand, Y.V. and L.P. are not close to each other the way this tree is written, but their branches meet at node 6. This tells us their viral samples are more closely related to each other than those of Y.V. and A.E.

1. From looking at the tree, which viral sequence is most closely related to the variant isolated from patient A.E.? Justify your answer by referencing a node on the tree.

2. Is the sequence isolated from G.L. more closely related to the one isolated from D.N. or H.V.? How do you know?

3. Is the sequence isolated from G.L. more closely related to either H.V. or Y.V.? Or is it equally related to both? How do you know?

4. Which node represents the most recent common ancestor of Y.V. and G.L.?

When looking at infections in a particular country, scientists will assume they all stemmed from the same outbreak if the viral sequences appear to be closely related—that is, if the number of matching nucleotides is high and the pattern of mutations is comparable. If, on the other hand, the sequences do not appear to be closely related, it is more likely that the virus has entered the country on more than one occasion.

5. South Africa, the United States, and Australia all have more than one case of nIQV. For which countries would you hypothesize that the virus entered only once and spread inside its borders? Explain your reasoning.

6. For which countries would you hypothesize that the virus entered the country more than once? Explain your reasoning.

Normally, trees like the one above would be made using hundreds or thousands of nucleotides. For simplicity, the tree above was made using 50-nucleotide segments of the nIQV genome. Sequences for each patient are listed below in the order in which they were uploaded to the database.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Q.L. | U | A | U | G | C | A | C | U | A | C | C | A | C | C | U | C | C | G | C | A | A | A | G | C | G | A | G | A | G | A | U | G | G | G | A | A | C | G | A | U | U | U | G | G | A | A | A | U | U | G |
| T.T. | U | A | U | G | C | A | C | U | A | G | C | C | G | C | A | A | C | G | C | C | U | A | G | C | G | A | G | A | G | A | U | G | G | G | A | A | C | G | A | U | U | U | C | G | A | A | A | U | U | G |
| D.N. | A | U | U | C | G | A | C | U | U | U | A | C | G | C | A | A | C | C | C | A | A | A | C | G | C | A | C | U | A | G | U | G | G | C | U | G | A | G | U | C | U | G | C | G | A | A | A | A | A | G |
| H.V. | A | U | U | C | G | U | C | C | U | U | A | C | G | C | A | A | C | C | C | A | A | A | C | G | C | A | G | U | A | G | U | G | G | C | U | G | C | G | U | U | G | G | C | G | G | G | A | A | C | G |
| A.E. | U | A | U | G | C | A | C | U | A | C | C | C | G | C | A | A | C | G | C | C | U | A | G | A | G | A | G | A | G | A | U | G | G | G | A | A | C | G | A | U | U | U | C | G | A | A | A | U | U | G |
| L.P. | A | U | U | C | G | A | C | U | C | C | A | C | G | C | A | A | C | C | C | A | A | A | C | G | C | A | C | U | A | G | U | G | G | C | U | G | A | G | U | C | U | G | C | G | A | A | A | A | A | G |
| Y.V. | A | U | U | C | G | U | C | U | C | C | A | C | G | C | A | A | C | C | C | A | A | A | C | G | C | A | G | U | A | G | U | G | G | C | U | G | C | G | U | U | G | G | C | G | G | G | A | A | G | G |
| G.L. | A | U | U | C | G | U | C | U | C | C | A | C | G | C | A | A | C | C | C | A | A | A | C | G | C | A | G | U | A | G | U | G | G | C | U | G | C | U | U | U | G | G | C | G | A | A | A | A | A | G |
| D.Z. | U | A | U | G | C | A | C | U | A | C | C | A | C | C | A | A | C | G | C | A | A | A | G | C | G | A | G | A | G | A | C | C | G | G | A | A | C | G | A | U | U | U | G | G | A | A | A | U | U | C |

7. The tree shows that T.T. and A.E. are very closely related. Can you find evidence for this from their sequences? Are there any segments that they share that are not seen in the other sequences?

8. There are two major groups on the tree that arise from a split at node 3. Looking at the sequences, can you see this split? Can you identify any nucleotides or groups of nucleotides that are shared by all members of one group but no members of the other group?

In this lab, you diagnosed Patient D.Z. with nIQV. D.Z. is from Australia, but was tested in the United States. To get to the United States, she had a flight connection in Tokyo. All three of those locations have seen outbreaks of nIQV. Compare her viral sequence, which appears in the bottom row of the table above, to the sequences above it.

9. Can you say where you think D.Z. most likely caught the virus? Identify specific nucleotides that help you make your decision.

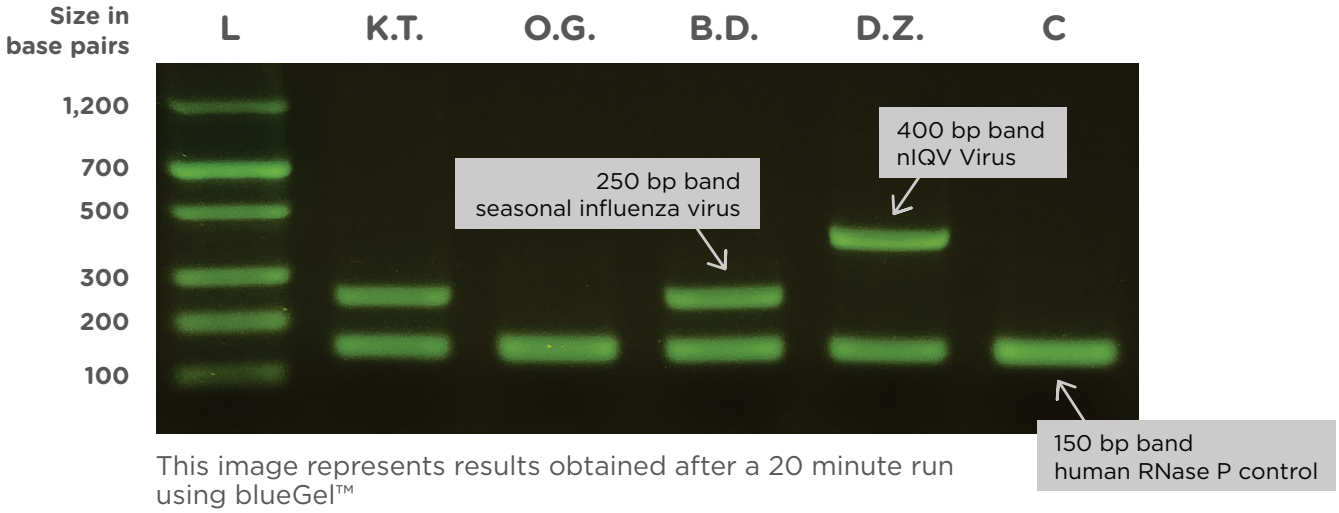
10. Add patient D.Z. to the tree. Create a new node and add a branch where you think D.Z. best fits.

# Instructor guide

# Expected results

Gel electrophoresis results are expected to resemble the gel image below:




This image represents results obtained after a 20 minute run using blueGel™

| | Patient K.T. | Patient O.G. | Patient B.D. | Patient D.Z. | Control human DNA |
|---|---|---|---|---|---|
| niQV (400 bp) | | | | ✓ | |
| Seasonal influenza (250 bp) | ✓ | | ✓ | | |
| Human RNase P control (150 bp) | ✓ | ✓ | ✓ | ✓ | ✓ |
| Diagnosis | Seasonal influenza | No definitive diagnosis can be made other than elimination of nIQV and seasonal influenza | Seasonal influenza | nIQV | |

For technical support, contact **support@minipcr.com**

For answers to the student questions, email **answers@minipcr.com**
Please include in the body of the email:

- The name of the lab
- Your name, school, and job title

# Unexpected results and troubleshooting

**If fluorescent DNA bands are not visible on the gel,** the following may have occurred:

- Failure to use a fluorescent DNA stain: This lab requires agarose gels made with a fluorescent DNA stain (*e.g.,* SeeGreen™ or GelGreen®). DNA stains that reveal DNA with a visible blue compound are less sensitive and are not compatible with this lab kit.
- Incorrect visualization conditions: Fluorescent DNA stains (*e.g.,* SeeGreen™ or GelGreen®) must be viewed using a blue light or UV transilluminator. The blueGel system has an integrated blue light transilluminator. For DNA visualization, ensure that you have turned on the blueGel's blue light by pressing the light bulb button and that the orange lid is in place.
- Samples were run off the gel: If you run the gel too long, the DNA samples will migrate the entire length of the gel and off the far end. You should always monitor the progress of your gel run by occasionally visualizing the DNA samples using a transilluminator or tracking the migration of the loading dye. The DNA samples contain colored dyes that migrate through the gel and are visible to the naked eye. Stop the gel run before the loading dye reaches the end of the gel.
- Reagents were stored improperly and/or are expired: DNA samples can be stored in the freezer for up to twelve months after receipt or in a refrigerator for six months. Storage under different conditions or in excess of this guidance may impair performance.

**If some or all of the bands appear faint,** the following may have occurred:

- Failure to load the DNA samples: Loading DNA samples for gel electrophoresis takes a little practice. The bands will appear faint if students do not successfully deposit the full sample volume into the well. Refer to https://www.minipcr.com/how-to-load-a-gel-electrophoresis/ for gel loading tips.
- Non-optimal visualization conditions: Dimming the lights in the room can make the fluorescent DNA stain easier to see in the transilluminator. If using the blueGel, viewing the gel using the Fold-a-View documentation hood and a smartphone camera will provide the best results.
- Old or improperly stored gels: Agarose gels can generally be prepared in advance, but the storage time and conditions depend on the fluorescent DNA stain being used. If using SeeGreen™ or GelGreen® DNA stain, gels can be prepared up to five days in advance. Store gels at room temperature in an airtight container protected from light. Do not soak the gels in buffer or wrap them in paper towels.
- Old or incorrectly prepared buffer: Gel visualization defects not directly ascribable to other causes may be remedied by using freshly prepared buffer compatible with your gel electrophoresis system. If using the blueGel, TBE buffer is recommended.

**For tips on picture-perfect gels, see** https://www.minipcr.com/gel-electrophoresis-troubleshooting/.

**For additional technical support, contact** support@minipcr.com.

# Notes on lab design

**No pathogenic materials are used.** None of the materials provided in the Viral Diagnostics Lab kit present a pathogenic risk.

This lab serves as an introduction to viral outbreaks. We believe this approach provides the right balance between intellectual engagement, inquiry, and accessibility. The design of this lab has simplified certain elements to achieve these goals. Some of these elements include:

- This lab was written during the COVID-19 pandemic in response to high demand for tools to teach about viral outbreaks in a safe, hands-on way. While the context is fictional, the challenges presented are similar to those that may occur during an outbreak. In the interest of looking forward and empowering students to solve the next outbreak, the underlying principles students will take away from this lab apply broadly to pandemics, including COVID-19 and those that may yet take place.
- This lab uses prepared DNA to simulate the results of PCR amplification of a section of the seasonal influenza viral genome, a section of the nIQV genome, and an RNase P control sequence.
- The DNA samples provided consist of synthetic DNA, not viral DNA or animal tissue. References to viral strains or patient samples are used only to recreate a plausible clinical scenario.
- Novel influenza Q virus (nIQV) is a fictional pathogen. Details provided in the lab about this pathogen's emergence are fabricated. While nIQV is loosely based on the influenza D virus, which was first identified in 2011, this case shares many commonalities with other recent viral outbreaks including COVID-19 and the 2009 swine flu pandemic.
- In this lab, electrophoresis is used to read the results of a nucleic acid detection test. In clinical laboratories, detection of amplified DNA is carried out in an automated fashion using quantitative PCR (qPCR). See Additional Student Supports (page 39) for teaching resources on this method.
- In this lab, students test for one strain of seasonal influenza. In reality, multiple viral strains can cause seasonal flu, so medical professionals would need to test for at least two different viruses to confirm a seasonal flu diagnosis.

# Additional student supports

**E-worksheets:** The student questions accompanying this lab are available for download here as editable text documents you can customize and upload to your LMS. E-worksheets can also be accessed from the Curriculum Downloads tab a https://www.minipcr.com/product/viral-diagnostics-lab-next-pandemic/.

**miniPCR tutorials:** Access an extensive set of free resources to help your students succeed in molecular biology techniques. Visit https://www.minipcr.com/tutorials/. The resources most relevant to this lab are listed below.

- **Micropipetting:** Video, worksheet, and hands-on activity resources to train students in the basic use of a micropipette.
- **Gel electrophoresis:** Video and worksheet activity instructing students on the fundamentals and practice of agarose gel electrophoresis.
- **PCR:** While students do not perform PCR in this lab, the samples they analyze represent PCR products. If you want to discuss PCR in more detail with your students, we have a video and worksheet activity instructing students on the fundamentals and practice of PCR.

**miniPCR Digital:** Interactive tools for experiment-based learning with or without hands-on lab kits. Visit https://digital.minipcr.com/.

# Extension activities

The following optional extension activities are provided for students to explore topics more deeply.

**Using genetic data to track outbreaks:** (page 32): In addition to aiding in diagnostics, genetic sequence information can be used to track the spread of a viral outbreak. In this activity, students will use nIQV sequence data to determine how the virus mutated as it spread geographically, and will use that information to determine where their affected patient acquired the virus. This extension introduces molecular phylogeny and illustrates its use as an epidemiological tool.

**Quantitative Polymerase Chain Reaction DNAdots:** Once students have completed the present lab and mastered the basics of using PCR for viral diagnostics, introduce them to the gold standard method for viral diagnostics in clinical laboratories: quantitative PCR or qPCR. Link includes article as well as classroom questions.

https://dnadots.minipcr.com/dnadots/real-time-polymerase-chain-reaction.

# Learning goals and skills developed

**Student learning goals:**

- Understand viruses as nonliving infectious agents
- Explain how viruses take advantage of cellular mechanisms to replicate
- Understand that different biological entities can be identified by their unique genetic sequences
- Explain the advantages of using molecular testing for patient diagnosis
- Learn how mutations contribute to the spread of a virus through a host population

**Scientific inquiry skills:**

- Identify or pose a testable question
- Formulate hypotheses
- Identify dependent and independent variables and appropriate experimental controls
- Follow detailed experimental protocols
- Create tables or graphs to present their results
- Interpret data presented in a chart or table
- Use data to evaluate a hypothesis
- Make a claim based in scientific evidence
- Use reasoning to justify a scientific claim

**Molecular biology skills:**

- Micropipetting
- Principles of PCR
- Agarose gel electrophoresis

# Standards alignment

The standards alignment document for this activity is available for download here. This document can also be accessed from the Curriculum Downloads tab at https://www.minipcr.com/product/viral-diagnostics-lab-next-pandemic/.

This activity is aligned to the following standards:

- Next Generation Science Standards: High School Life Science
- Advanced Placement Biology
- Texas Essential Knowledge and Skills: Biology
- Texas Essential Knowledge and Skills: Biotechnology
- Biotechnician Assistant Credentialing Exam
- Common Core ELA/Literacy Standards (9-10)

For additional information on alignment to state standards, please contact support@minipcr.com.