COVID-19 qPCR Lab

Detecting SARS-CoV-2 Infection

For educational use only. Not for diagnostic use.

COVID-19 qPCR Lab
Detecting SARS-CoV-2 Infection
Instructor's and Student's Guide

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Instructor's Guide Contents

At a glance

Practice the gold standard technique used to test patients for COVID-19!

Students will use low-cost tools to get hands-on experience with qPCR principles and determine if fictional patients are infected with the SARS-CoV-2 virus.

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. **For educational use only. Not for diagnostic use.**

TECHNIQUES

Micropipetting
Quantitative PCR
Fluorescence visualization
Gel electrophoresis (optional)

TOPICS

Infectious disease
Molecular diagnostics
Biotechnology

LEVEL

General high school
Advanced high school
College

WHAT YOU NEED

Micropipettes
PCR thermocycler
P51™ molecular fluorescence viewer or other blue light illuminator
Optional: Gel electrophoresis and visualization system

AP CONNECTION

AP Biology units 6.8

Skills and Practices 1.A-1.C, 2.A, 2.D, 3.A-3.D, 4.A-B, 6.A-6.E

Planning your time

The data for this lab can be collected two ways: endpoint observation or qPCR time point observations. See pages 4-5 for details.

Option 1: Endpoint observation

SINGLE CLASS PERIOD: 50 MINUTES

OR

PERIOD 1: 35 MINUTES → **PERIOD 2: 5 MINUTES**

Option 2: qPCR time point observations

SINGLE CLASS PERIOD: 80 MINUTES

Additional supports

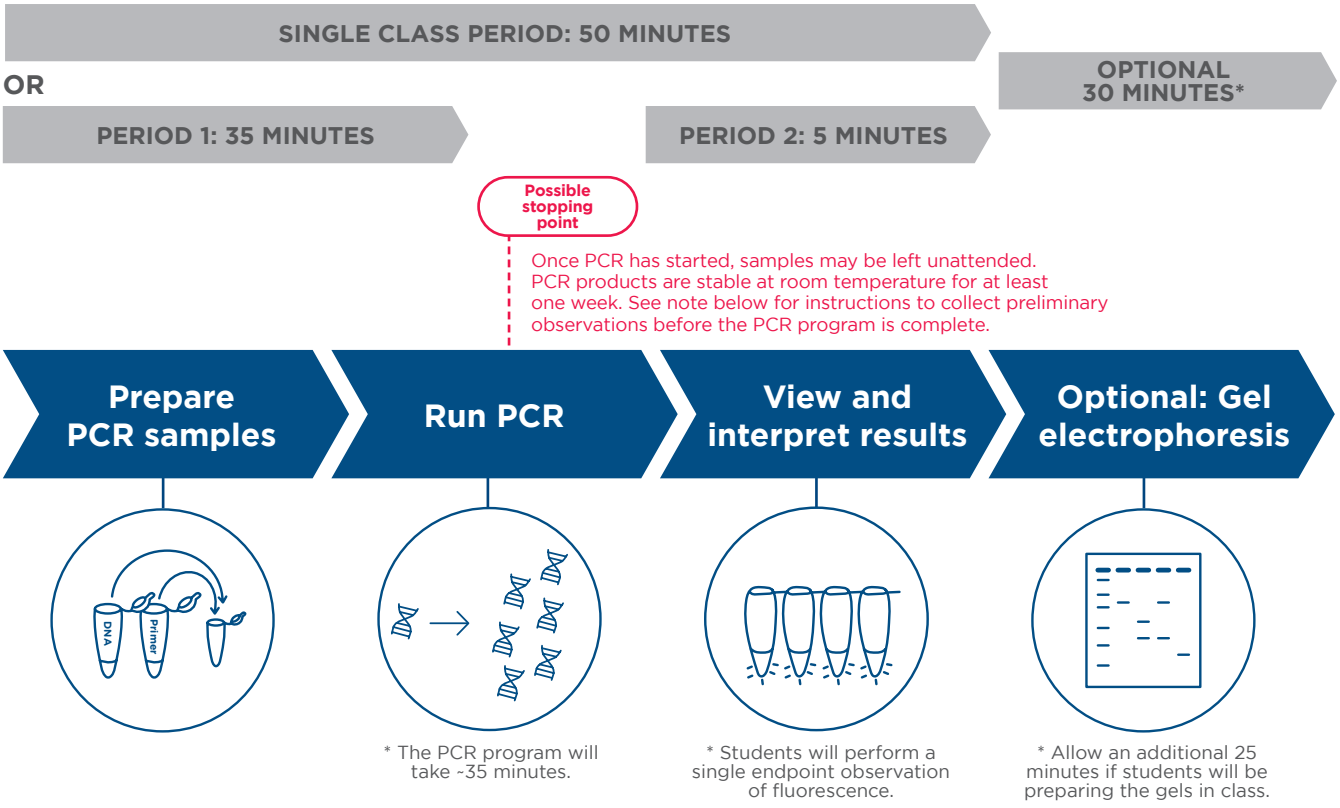
Help your students build proficiency in pipetting, PCR, and gel electrophoresis with additional instructional videos, worksheets, and activities available at: https://www.minipcr.com/tutorials/

Visit https://www.minipcr.com/covid-resources/ to access the complete miniPCR bio™ COVID-19 resource library.

For answers to the lab study questions and extensions, email answers@minipcr.com. Please include the name of the lab, as well as your name, school, and title in the body of the email.

Option 1: Endpoint observation

For classes with shorter class periods or where an emphasis on the quantitative aspects of qPCR is unnecessary, we recommend students collect a single endpoint observation (instructions on pages 22-27). This approach takes less class time and still allows students to diagnose their patients as being infected or uninfected. However, it does not allow students to compare the relative viral loads between samples. This approach can be completed in a single 50 minute class period, or split across two class periods. Further, this method of data collection can completed using a single thermal cycler that is shared across lab groups if the machine is large enough. For eight lab groups, you will have 48 samples..



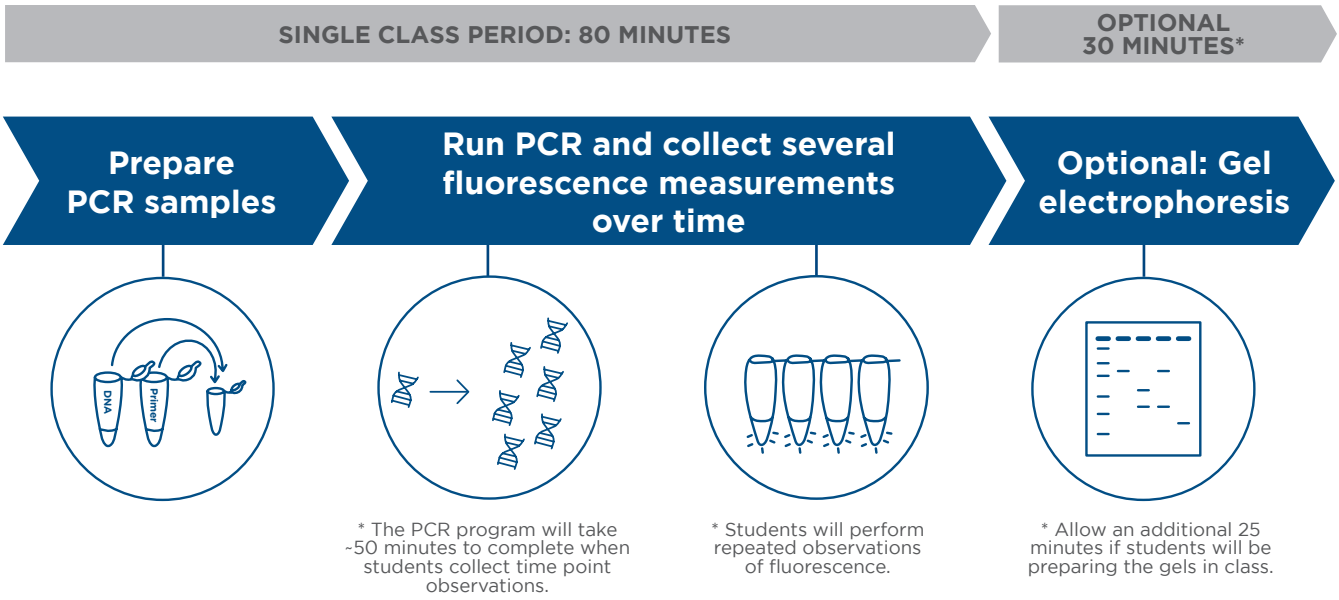
Note: If students run out of class time before their PCR is done, they can pause the PCR program during the extension step (72°C) and collect initial observations. Students can then return their samples to the thermal cycler for the PCR program to finish and observe their final results the next class. See page 65 for details.

Option 2: qPCR time point observations

For classes where it is important to teach about quantitative PCR in detail, we also include instructions for students to collect data throughout the PCR (pages 37-44). This approach allows students to generate qPCR curves and compare viral loads across samples.

This option requires:

- A single 80 minute class period as students need to both set up the PCR and then monitor the fluorescence throughout the PCR program.
- Thermal cyclers must have the capability to pause a PCR program while it is running (e.g. miniPCR®)
- Each student group will also require their own blue light illuminator to make frequent observations (e.g., P51™ molecular fluorescence viewer or blueGel™).



Materials needed

Supplied in kit (KT-1900-06)

Reagents and supplies	Amount provided in kit	Amount needed per lab group	Storage	Teacher's checklist
2X qGRN Master Mix	**1000 µl**	**110 µl**	Freezer	
COVID Lab Primers	**550 µl**	**55 µl**	Freezer	
Simulated Patient Samples • Patient AH • Patient BH • Patient CH • Patient DH	**100 µl each**	**10 µl each**	Freezer	
Simulated Control Samples • Positive Control • Negative Control	**100 µl each**	**10 µl each**	Freezer	
PCR strip tubes	**Ten 8-tube strips**	**One 8-tube strip**	Room temp.	
For optional gel electrophoresis:				
6X Loading Dye	**300 µl**	**25 µl**	Freezer	
Fast DNA Ladder 1	**150 µl**	**15 µl**	Freezer	

Materials needed (cont.)

Supplied by teacher

Available at miniPCR.com

Reagents and supplies	Amount needed per lab group	Teacher's checklist
PCR thermal cycler: e.g. miniPCR® machine Note: If students are collecting time point qPCR observations, the machine must have the capability to pause while running a PCR program	**1** **Six reactions per group** **Can be shared between groups if performing endpoint observation**	
P51™ molecular fluorescence viewer or other blue light illuminator: e.g. blueGel™ or blueBox™	**1** **Can be shared between groups if performing endpoint observation**	
Micropipettes • 2-20 µl: one per lab group • 20-200 µl: one for the teacher to dispense reagents	**1**	
Disposable micropipette tips	**At least 18 per group**	
PCR tubes (0.2 ml): Included in the Lab Companion Kit (see next page for details)	**6**	
Plastic tubes (1.7 ml): Included in the Lab Companion Kit (see next page for details)	**8**	

Other supplies:
- Disposable laboratory gloves
- Protective eyewear
- Permanent marker
- Cup to dispose of tips

Additional materials needed for optional gel electrophoresis

Supplied by teacher

Reagents and supplies	Amount needed	Teacher's checklist
Horizontal gel electrophoresis apparatus e.g. blueGel™ electrophoresis system	**1 per group** **Can be shared between groups if you use two combs per gel**	
Distilled water for making agarose gels and diluting TBE buffer	**50 ml per gel**	
Heat-proof flask or beaker to dissolve agarose	**1 per class**	
Microwave or hot plate to dissolve agarose	**1 per class**	

Sold separately in Learning Lab Companion Kit (KT-1510-01)

This lab requires reagents for running and visualizing DNA samples on a 2% agarose gel with a fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®). The Learning Lab Companion Kit provides enough electrophoresis reagents for 8 groups when using the blueGel™ electrophoresis system. Gels can also be prepared with agarose tabs or agarose powder. Refer to www.minipcr.com/agarose-gel/ for detailed instructions..

Reagents and supplies	Amount provided in kit	Amount needed per lab group	Storage	Teacher's checklist
All-in-one agarose tabs with DNA stain and TBE	**8**	**One tab per agarose gel** (2% agarose gel)	Room temp., protected from light	
TBE electrophoresis buffer	**Supplied as liquid concentrate or powder** Sufficient for 600 ml of 1X working solution	**30 ml 1X solution** per blueGel™ system	Room temp.	
PCR tubes (0.2 ml)	**100**			
Plastic tubes (1.7 ml)	**50**			

Lab setup: PCR and fluorescence visualization

Note: The reagents setup for both endpoint observation and qPCR time point observations is the same.

The following activities can be carried out by the instructor ahead of class. Reagents are sufficient to be used with 8 student groups. Reagents are stable at room temperature for 24 hours but should remain cold for short-term storage and frozen for long-term storage.

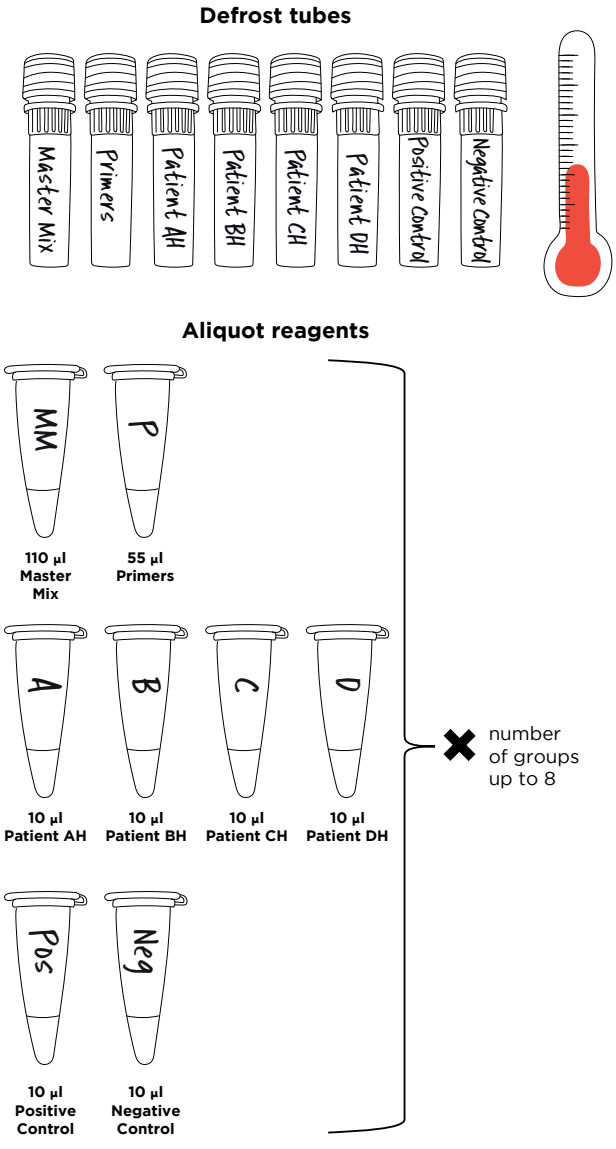
Gloves and protective eyewear should be worn for the entirety of this lab.

Preparation for PCR

- Thaw tubes containing 2X qGRN Master Mix, COVID Lab Primers, Simulated Patient Samples, and Simulated Control Samples by placing them on a rack or benchtop at room temperature.

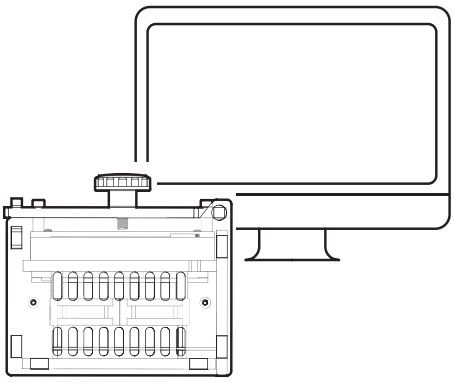
- For each lab group, label and dispense the following reagents into eight labeled 1.7 ml tubes:
  - 2X qGRN Master Mix 110 µl
  - COVID Lab Primers 55 µl
  - Simulated Patient Samples 10 µl each
    - Patient AH
    - Patient BH
    - Patient CH
    - Patient DH
  - Simulated Control Samples 10 µl each
    - Positive Control
    - Negative Control

- Distribute supplies and reagents to lab groups (continued on next page).



Check	At the start of this experiment, every lab group should have:	Amount
	2X qGRN Master Mix	**110 µl**
	COVID Lab Primers	**55 µl**
	Simulated Patient Samples - Patient AH - Patient BH - Patient CH - Patient DH	**10 µl each**
	Simulated Control Samples - Positive Control - Negative Control	**10 µl each**
	PCR tubes (200 µl)	**1 strip of 8 tubes**
	2-20 µl micropipette	**1**
	Micropipette tips	**At least 18**
	Fine tipped permanent marker	**1**
	6 wells in a miniPCR® or other thermocycler Note: If students are collecting time point qPCR observations, the machine must have the capability to pause while running a PCR program	
	Access to a P51™ or other blue light illuminator	

Hardware setup



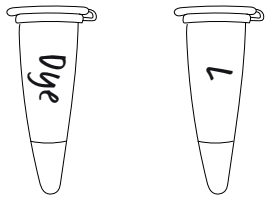
- Thermal cyclers can be programmed ahead of time by the teacher or during class by the students.
- If doing option 1 (endpoint observation):
  - If your thermal cycler is large enough, a single machine can be shared across groups. For eight lab groups, you will have 48 samples.
  - A single, shared blue light illuminator is sufficient, although one per lab group will provide a better student experience.
- If doing option 2 (qPCR time point observations):
  - One thermal cycler per lab group is required.
  - Thermal cyclers must have the capacity to pause during run (e.g., miniPCR® app).
  - One blue light illuminator per lab group is required (e.g., P51™ fluorescence viewer).

Lab setup: Optional gel electrophoresis

We recommend examining the PCR products with gel electrophoresis, but this can be skipped in the interest of time.

Preparation for gel electrophoresis

- For each lab group, label and dispense the following reagents into two labeled 1.7 ml tubes:
  - 6X Loading Dye 25 µl
  - Fast DNA Ladder 1 15 µl
- Have the banding pattern of the Fast DNA Ladder 1 handy (page 57) to help interpret the electrophoresis results.
- Distribute supplies and reagents to lab groups:



25 µl Loading Dye **15 µl Ladder**

Check	At the start of this experiment, every lab group should have:	Amount
	PCR products from previous section of the lab	6 reactions
	6X Loading Dye	25 µl
	Fast DNA Ladder 1	15 µl
	2-20 µl micropipette	1
	Micropipette tips	At least 13
	7 wells in an electrophoresis gel	

Preparing gels

- Prepare 1X TBE buffer.
  - TBE buffer is often provided as liquid concentrate or powder.
  - Follow manufacturer's instructions to prepare 1X TBE buffer solution.
  - Volume to prepare depends on the method used to prepare gels; see "Important Note" below.
- Gels can be poured in advance of the class.
  - This lab requires running and visualizing DNA samples on a 2% agarose gel with a fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®).
  - Pre-poured gels can be stored at ambient temperature, in a sealed container or wrapped in plastic wrap, and protected from light for up to three days.

IMPORTANT NOTE: There are several ways to prepare agarose gels.

- Scan the QR code for detailed instructions on how to prepare agarose gels.
- Both written and video instructions are available.

www.minipcr.com/agarose-gel/