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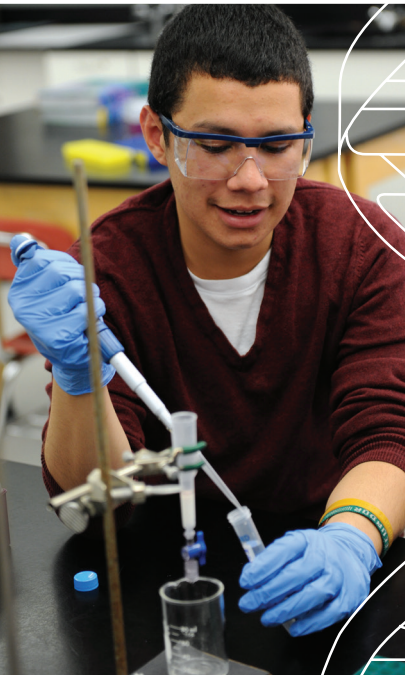
Scientific Discovery for the Classroom

Teacher Guide



Colony PCR

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TABLE OF CONTENTS

OVERVIEW

This chapter introduces students to the applications and practice of polymerase chain reaction (PCR), a method that amplifies a DNA sequence—often a gene—by making billions of copies of a specific DNA sequence through successive rounds of DNA replication. PCR is a standard protocol in molecular biology, and hundreds of scientific papers are published each year about research that incorporates this technique. The purpose of this colony PCR lab is to confirm that the cells producing RFP from Laboratory 5 have been transformed with the plasmid carrying the *rfp* gene, pARA-R.

ASSUMPTIONS OF PRIOR KNOWLEDGE

Students should already know the following:

- Charged objects, including molecules, move through an electric field.
- DNA is a double-stranded molecule, and each strand of DNA is made up of covalently linked subunits called nucleotides, which contain a sugar, a phosphate group, and a nitrogenous base.
- Nucleotides are attached to one another by a sugar-phosphate backbone; the nitrogenous bases jut out from this backbone.
- The two strands of DNA are connected by hydrogen bonds between adjacent nitrogenous bases, which are called base pairs; cytosine is always paired with guanine (C–G), and adenine is always paired with thymine (A–T).

LEARNING GOALS

By the end of this chapter, students will be able to do the following:

- Carry out the PCR method
- Describe applications of PCR
- Explain the role of DNA polymerase and DNA primers in PCR

ASSESSED OUTCOMES

- Assess students' abilities to carry out PCR by reviewing their work in Laboratory 5-EXT, Part A (pages 9–12 of the Student Guide) and by reviewing their responses to questions 1 and 2 in *Chapter 5-EXT Questions, Part A* (page 15 of the Student Guide).
- Assess students' abilities to describe the applications of PCR by reviewing their responses to question 1 in the *Chapter 5-EXT Questions, Part B* (page 15 of the Student Guide).
- Assess students' abilities to explain the role of DNA polymerase and DNA primers in PCR by reviewing their responses to questions 2–6 in the *Chapter 5-EXT Questions, Part B* (page 15 of the Student Guide).

SUGGESTED SEQUENCE OF ACTIVITIES

SESSION 1

- Review the **Introduction** and *Chapter 5-EXT Goals* with students. (5 min.)
- Have students answer the *What Do You Already Know?* questions and share their responses. (10 min.)
- Have students read **What Is PCR?** (20 min.)
- Have students read the introduction to Laboratory 5-EXT and answer the *Before the Lab* questions. (10 min.)

SESSION 2

- Review the steps of the PCR procedure with students. (10 min.)
- Have students complete Laboratory 5-EXT, Part A. (25 min.)
- Have students answer the *Chapter 5-EXT Questions, Part A*. (10 min.)

SESSION 3

- Have students complete Laboratory 5-EXT, Part B. (35 min.)
- Have students read **Some Like It Hot.** (10 min.)

SESSION 4

- Have students discuss *Chapter 5-EXT Questions, Part B* in small groups and record their answers individually. Discuss students' answers as a class. (45 min.)

PREPARATION

Familiarize yourself with the laboratory procedures, the preparation required, and the materials you'll need. The instructions assume 12 groups of 2–4 students. Multiply the amounts as necessary depending on the number of students and the number of classes you are teaching. See pages 23–24 for a Materials list for this laboratory.

NOTE: The following directions may vary based on the gel electrophoresis system you have.

MAKE AGAROSE GELS FOR CHAPTER 5-EXT, PART B

RESOURCES: The video *Making an Agarose Gel* (available on the program website) goes through the process of making and casting an agarose gel as described below.

NOTE: The gels can be made several days in advance.

1. Prepare electrophoresis gel trays:
   a. Gather the following materials:
      - 6 gel electrophoresis trays
      - 12 10-well combs
      - Optional: Tape
   b. Prepare the electrophoresis gel trays for casting by securing the gates on the ends of each tray in the "up" position or taping the ends of each tray. Place two combs in each tray—one at the end and one in the middle—before adding the agarose solution.

2. Prepare the agarose solution:
   a. Gather the following materials:
      - 2 250-mL graduated flasks, one labeled "1x SB"
      - 12.5 mL of 20x sodium borate buffer (20x SB)
      - 237.5 mL of distilled or deionized water
      - ~~1.44 g of agarose~~ 1.2 g of Agarose
      - Mass scale
      - 500-mL flask labeled "Gel"
      - Plastic wrap
      - Disposable pipette tip
      - Microwave
      - Heat-resistant gloves or tongs
      - 25 µL 10,000X GelGreen™ (or other stain provided)
      - 6 sandwich- or quart-sized resealable bags
      - Waste container for used tips and microfuge tubes

Recommended to make 2L-3L of 1X SB for class of 24 students.

Highlighted reagents provided

b. Prepare 250 mL of 1x SB by adding 12.5 mL of 20x SB to the 250-mL graduated flask labeled "1x SB," adding distilled or deionized water to the 250-mL mark, and mixing.

c. Pour 180 mL of 1x SB into the second 250-mL graduated flask.

we prove 1.2gr of Agarose in a conical. Add 150mL of 1XSB

d. Add ~~1.44~~ g of agarose (already measured in conical tubes unless otherwise noted) to the 500-mL flask labeled "Gel." Add the ~~180~~ mL of 1x SB previously measured to make 0.8% agarose solution.

e. Cover the opening of the 500-mL flask with plastic wrap. Use the pipette tip to poke a small hole in the plastic wrap.

f. Place the covered flask in a microwave and heat for one minute on high. With a gloved hand, gently swirl the flask. (Alternatively, a hot plate can be used to melt the agarose, but you will need to use a double boiler.)

SAFETY: Wear heat-resistant gloves or use tongs to hold the flask.

g. Continue microwaving the flask for 5–15-second intervals until all the agarose has dissolved. To check this, hold the flask to the light and swirl the solution. Look carefully for "lenses" of agarose crystals suspended in the liquid. If no lenses are visible, the agarose is dissolved.

Either Gell Green or SYber Safe will be given. Same dilition.

h. Wait until the agarose solution has cooled to 60–65°C. Once the gel has cooled to 60–65°C, add 25 µL 10,000X GelGreen™ and swirl the solution.

LAB TECHNIQUE: If the solution cools to the point that the agarose begins to re-solidify, simply reheat the solution as described above. It will be necessary to add more stain to the re-melted gel, as GelGreen™ is heat sensitive.

3. Cast the gels in the trays:

a. When the agarose solution has cooled to the point that you can safely touch the bottom of the flask (approximately 60°C; this will take around five minutes), pour 25–30 mL of the agarose solution into each electrophoresis tray. The solution should cover about 2 mm of the comb.

b. Once the gels solidify (which will take around 30 minutes), pull the combs out of each gel. Pull each comb straight out without wiggling it back and forth; this will minimize damage to the front wall of the well.

c. Remove the gels from the gel electrophoresis trays, and store them in individual resealable bags with a small amount of 1x SB. If using GelGreen™, store them in a dark, room-temperature location until ready to use. Otherwise, store in the refrigerator until ready to use. Be sure to keep them flat and not on a textured surface, as textured surfaces will imprint onto the gels and impact how molecules move through them. Discard the used tips and microfuge tubes.

REVIEW THE SEQUENCES AMPLIFIED BY PCR IN LABORATORY 5-EXT

This laboratory uses PCR to amplify sections of the pARA and pARA-R plasmids. Only the sequence that is amplified from pARA-R plasmids contains the *rfp* gene. The sizes of the amplified sequences in each plasmid vary and therefore can be used to identify the two plasmids. The following figures show the important sequences, the position of the primer binding sites, and the size of the amplified fragments. Features of the pARA plasmid include the *Bam*HI and *Hin*dIII restriction enzyme sites, the forward and reverse primer binding sites, the beta-lactamase gene encoding the protein for ampicillin resistance, and the *araC* gene encoding the AraC protein that inhibits expression of the *rfp* gene in the absence of arabinose. The amplified sequence is 662 bp in size.

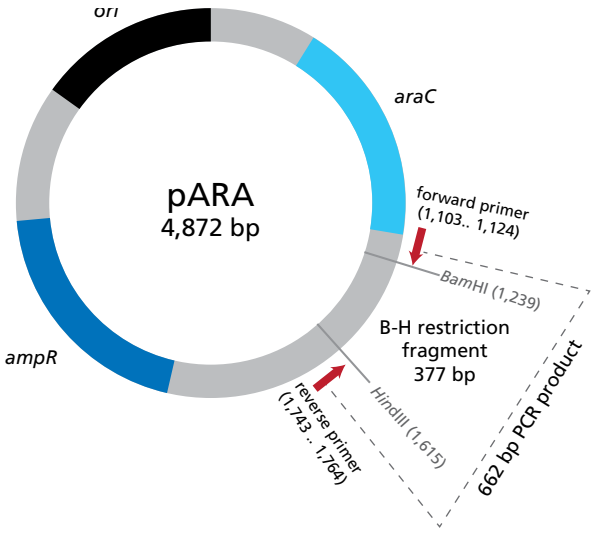


Figure 5-EXT.1: The pARA plasmid

Features of the pARA-R plasmid include the *Bam*HI and *Hin*dIII restriction enzyme sites, the forward and reverse primer binding sites, the beta-lactamase gene encoding the protein for ampicillin resistance, the *araC* gene encoding the AraC protein that inhibits expression of the *rfp* gene in the absence of arabinose, the pBAD promoter that the AraC protein binds to, and the *rfp* gene encoding the red fluorescent protein. The amplified sequence is 1,092 bp in size.

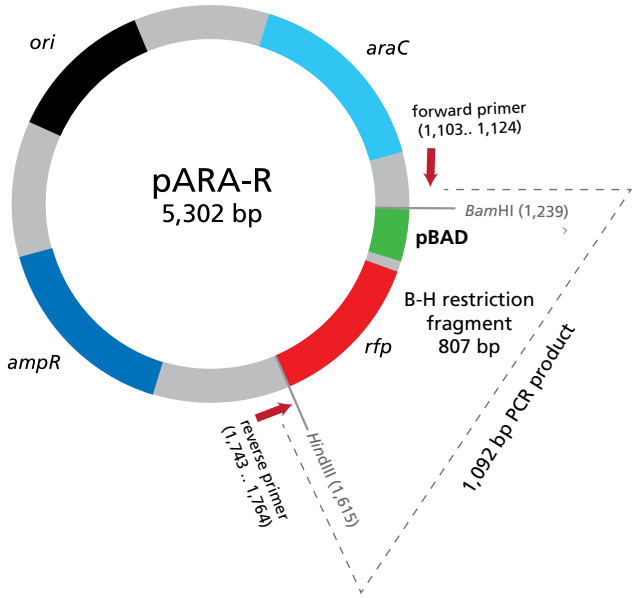


Figure 5-EXT.2: The pARA-R plasmid

In this lab, students use a pipette tip to transfer cells from a red colony and a white colony to separate PCR microfuge tubes, each of which contains a "cocktail" of reagents, called a master mix. The master mix contains all the reagents necessary for DNA replication, including:

- DNA nucleotides (dNTPs)
- *Taq* polymerase, a DNA polymerase (enzyme) capable of working at high temperatures
- DNA primers that selectively target and flank the plasmid locus targeted for amplification
- A buffer that creates optimal conditions for the reaction

The DNA primers used in this lab flank the *Bam*HI and *Hin*dIII restriction enzyme sites in the pARA and pARA-R plasmids. Information about the primer sequences and the positions in each plasmid where the primers bind is shown in **Table 5-EXT.1**. (Both primers are 22 base pairs long, have a GC [Guanine-Cytosine] content of about 50%, and have a T_m [primer melting point] of about 58°C.)

Table 5-EXT.1: Primer sequences and binding positions in pARA and pARA-R

Direction	Sequence	pARA positions	pARA-R positions
Forward	5'-TGTAACAAAGCGGGACCAAAGC-3'	1103–1124	1103–1124
Reverse	5'-GCGTTTCACTTCTGAGTTCGGC-3'	1764–1743	2194–2173

REVIEW USE OF THE PCR EQUIPMENT

Review how to use the thermocycler using the Colony PCR program, and practice the PCR procedure if you are unfamiliar with it.

COPY HANDOUTS FOR LABORATORY 5-EXT

One copy of **DNA Ladder Diagram (RM 5-EXT.1)** is needed for each student. The reproducible master (RM) for the handout is found at the end of this guide.

ALIQUOT REAGENTS FOR LABORATORY 5-EXT, PART A

NOTE: The reagents can be aliquoted one day before Laboratory 5-EXT, Part A.

1. Gather the following materials:
    - 0.025 ng/µL pARA-R and 0.025 ng/µL pARA controls (store at –20°C)
    - PCR master mix with primers and *Taq* polymerase (store at –20°C)
    - Plastic container full of water and crushed ice
    - Fine-tip marking pen
    - 36 1.5-mL microfuge tubes
    - P-20 pipette
    - P-200 pipette
    - P-20 pipette tips
    - P-200 pipette tips

P10 pipettes and tips will be provided

NOTE: Your site may require you to mix the primers with the master mix. Follow the instructions given by your site if required.

2. Thaw the plasmid controls at room temperature (15–20 minutes). Thaw the master mix in wet ice, and keep it there while you aliquot it.
3. While the PCR master mix and plasmid controls thaw, label the caps of the microfuge tubes: 12 as "PCR," 12 as "+" (for pARA-R), and 12 as "–" (for pARA).

LAB TECHNIQUE: Once the master mix is thawed, it's very important to keep the mixture in wet ice. If allowed to sit at room temperature, it's possible to produce unintended amplification products.

4. Pipette the PCR master mix up and down several times to mix it thoroughly, then aliquot 100 µL into each microfuge tube marked "PCR." Store at 4°C.
5. Aliquot 3 µL of pARA-R into each microfuge tube marked "+" and 3 µL of pARA into each microfuge tube marked "–." Store at 4°C.

GATHER MATERIALS FOR LABORATORY 5-EXT, PART A

NOTE: Gather materials on the day of the lab. Note that the PCR materials need to stay cold. Leave them at 4°C until needed. Prepare ice cups with tubes just before class to prevent the ice from melting.

1. Gather the following materials:
   - 12 cups
   - Ice
   - Water
   - 12 tubes of PCR master mix
   - 24 plasmid control tubes (12 + tubes and 12 – tubes) (stored at 4°C)
   - 48 0.25-ml PCR tubes with caps (tubes may be provided in strips of 8, which will need to be cut apart; you need 4 tubes and caps per group)
   - 3–4 LB/amp/ara plates with transformed colonies (stored at 4°C)—1 plate can be shared by 3–4 groups
   - Disposable gloves
   - Thermocycler (use the Colony PCR program)

Ice and water not provided

2. Set up 12 cups with wet ice.
3. Remove tubes of PCR master mix and plasmid control tubes from the refrigerator, and place one tube of PCR master mix and one of each of the two tubes of plasmid controls into a cup of ice for each group.

LAB TECHNIQUE: Place the master mix tubes in wet ice immediately; do not allow them to warm to room temperature. Once the cups are prepared, they can be kept in the refrigerator. Make sure that there is still ice in the cups when you distribute them to groups.

4. Prepare 12 sets of materials that each include the following:
   - 4 empty 0.25-ml PCR tubes and caps
   - Empty tip box to use as a PCR tube rack
   - P-20 micropipette
   - Tip box of disposable pipette tips
   - Fine-tip permanent marker
   - Waste container for used tips and microfuge tubes (1 container per 2 groups)

ALIQUOT REAGENTS FOR LABORATORY 5-EXT, PART B

NOTE: The reagents can be aliquoted one day before Laboratory 5-EXT, Part B.

1. Gather the following materials:
   - DNA ladder (marker, in the freezer, labeled "100")
   - Fine-tip marking pen
   - 12 1.5-mL microfuge tubes
   - P-20 pipette
   - P-20 pipette tips

You can also label M, but don't confuse with 1Kb ladder from lab 4A

P10 pipettes and tips will also be provided

2. Label 12 microfuge tubes "M."
3. Pipette 10 µL of DNA ladder into each tube marked "M." Store the aliquoted DNA ladder in the refrigerator at 4°C.

NOTE: The DNA ladder used in this lab is a 100bp ladder, which is different from the ladder used in previous labs.

GATHER MATERIALS FOR LABORATORY 5-EXT, PART B

NOTE: Gather materials on the day of the lab. Note that the PCR materials need to stay cold. Leave them at 4°C until needed.

1. Gather the following materials:
   - 500-mL graduated flask
   - Labels
   - Fine-tip marking pen
   - 15 mL of 20x SB
   - 285 mL of distilled or deionized water
   - ~~Optional: 6 50-mL flasks~~
   - PCR tubes of amplification products (from Part A)
   - Microcentrifuge with PCR tune adapter

Our electrophoresis units hold 300 mL of Buffer. You should prepare enough buffer to accommodate all the units. 6 units X 300mL=1.5L

2. Prepare 300 mL of 1x SB buffer per class (adjust amounts as needed for the gel electrophoresis system you are using):
   a. Label the 500-mL graduated flask "1x SB." Add 15 mL of 20x SB to the flask, add distilled or deionized water to the 300-mL mark, and mix.
   b. ~~Optional: Label each 50-mL flask "1x SB." Pour 50 mL of 1x SB into each flask.~~
3. Using the PCR tube adapter, centrifuge the PCR tubes from Part A to pool condensation.
4. Prepare 12 sets of materials that each include the following:
   - Plastic microfuge tube rack
   - 4 PCR tubes with amplification products from Part A (in rack)
   - Microfuge tube of DNA ladder, marked "M" (prepared on page 10 of this Teacher Guide)
   - P-20 micropipette
   - Tip box of disposable pipette tips
   - Waste container for used tips and microfuge tubes (1 container per 2 groups)
   - Copies of **DNA Ladder Diagram (RM 5-EXT.1)** (1 per student)

Marker is labeled M or M100

P-10 pipettes and tips will also be provided

5. Set up six electrophoresis boxes, each near a power supply; two groups will share one box. Load each box with 0.8% agarose gel (prepared earlier), and set the flask of buffer at a central location ~~or one 50-mL flask containing 1x SB buffer (prepared above) near each box~~. Keep the resealable bags that held the gels, and label them with each group's number and class period in case you need to store the gels before photo-documenting—see *Completing the Gels for Laboratory 5-EXT* on the following page of this Teacher Guide.
6. ~~Put~~ the microcentrifuge in a central location so that all groups can share it.

COMPLETING THE GELS FOR LABORATORY 5-EXT

1. If you need to continue running the gels after class, here are two options:
    - If you can complete the gels after the class has ended, run the gels until the yellow dye is at the middle of the gel. Once the electrophoresis is complete, you can transfer the gels to the labeled resealable bags.
    - If you need to interrupt the gels, be sure that students have been running them for at least 10 minutes. Ask students to shut off the power to the electrophoresis unit, remove the casting tray, and slide the gel into the labeled resealable bag. Place a new gel in the tray for the next class. When you have time, you can return the partially run gels to the tray and continue the electrophoresis, following the instructions above.
2. After the gels have been run, view them on an appropriate light source (e.g., a blue light or UV light box) to visualize the DNA bands. Take photographs of the gels for documentation and analysis. Gels can be discarded in the regular trash following documentation.

TEACHING

SESSION 1

KEY IDEAS: PCR, which amplifies a specific sequence of DNA by successive rounds of DNA replication *in vitro*, is an important technique that has multiple applications in medicine, forensics, and basic research. Replication of DNA *in vitro* requires denaturing of the DNA to separate its two strands. Because PCR takes place at high temperatures in order to denature the DNA, it requires the use of a temperature-controlled device, the thermocycler, and a specific DNA polymerase found in microorganisms that live in high-heat environments.

Review the Introduction and Chapter 5-EXT Goals with students. (5 min.)

The Introduction explains the main purpose of this chapter. The Goals tell students what they should focus on as they work through the chapter. Explain to students what you will assess in this chapter and what your expectations are for students' performance.

Have students answer the *What Do You Already Know?* questions and share their responses. (10 min.)

The *What Do You Already Know?* section activates students' prior knowledge of DNA cloning and PCR, and reveals gaps or misconceptions in that knowledge. Have students answer the questions in pairs and record and share their ideas so you can evaluate what they know and don't know about DNA cloning and PCR.

Possible answers:

1. What might be some reasons to make many copies of a gene? *Answers will vary. Students might suggest that many copies would be easier to analyze than just one, or that copies could be used for different procedures and experiments.*
2. Under what circumstances might it be important to copy DNA quickly using PCR? *Answers will vary. Students may propose that PCR can be used for genetic testing, for matching DNA at a crime scene, or for cross-species gene analysis.*

STRATEGY: If students struggle with this question, ask them to consider situations—such as in medicine, forensics, or basic research—where it might be important to be able to analyze or compare a known DNA sequence, but only a small amount of DNA is available.

3. In what situations might you want to know if an organism carries a particular gene or DNA sequence? *Answers will vary. Students may suggest that they might need to know if a bacterial transformation had been successful or if a person carried a particular disease gene.*

Have students read What Is PCR? (20 min.)

In this reading, students learn about PCR and some of its applications. Remind students to use the *Glossary* to look up scientific terms if they need help understanding the reading.

Have students read the introduction to Laboratory 5-EXT and answer the *Before the Lab* questions. (10 min.)

Have students share their answers to the *Before the Lab* questions with their groups and resolve any differences. Then have students share their answers and their thinking for each question with the class.

Possible answers:

1. In this lab, we use PCR and gel electrophoresis to confirm whether the bacteria have been transformed with the correct plasmid. Why is it necessary to perform PCR before gel electrophoresis? What might happen if you attempted to perform gel electrophoresis on the sample from the agar plate? *Most likely there would not have been enough DNA material to show distinct bands on the gel.*
2. You read about the importance of using specific primers to frame the target sequence for PCR. How might it impact the products of PCR if a scientist added very short primers to the DNA? *The primers might not be specific enough to only cut out the desired fragment and might cut the plasmid in*

many places, making it impossible to determine whether the transformation had or had not happened using gel electrophoresis. You would get a variety of bands of different sizes when you perform gel electrophoresis.

3. Read through the *Methods* sections for Part A (on pages 10 through 12 of the Student Guide) and for Part B (on page 13 of the Student Guide), and briefly outline the steps for Part A and Part B, using words and a flowchart. *Students' flowcharts should show all applicable steps.*

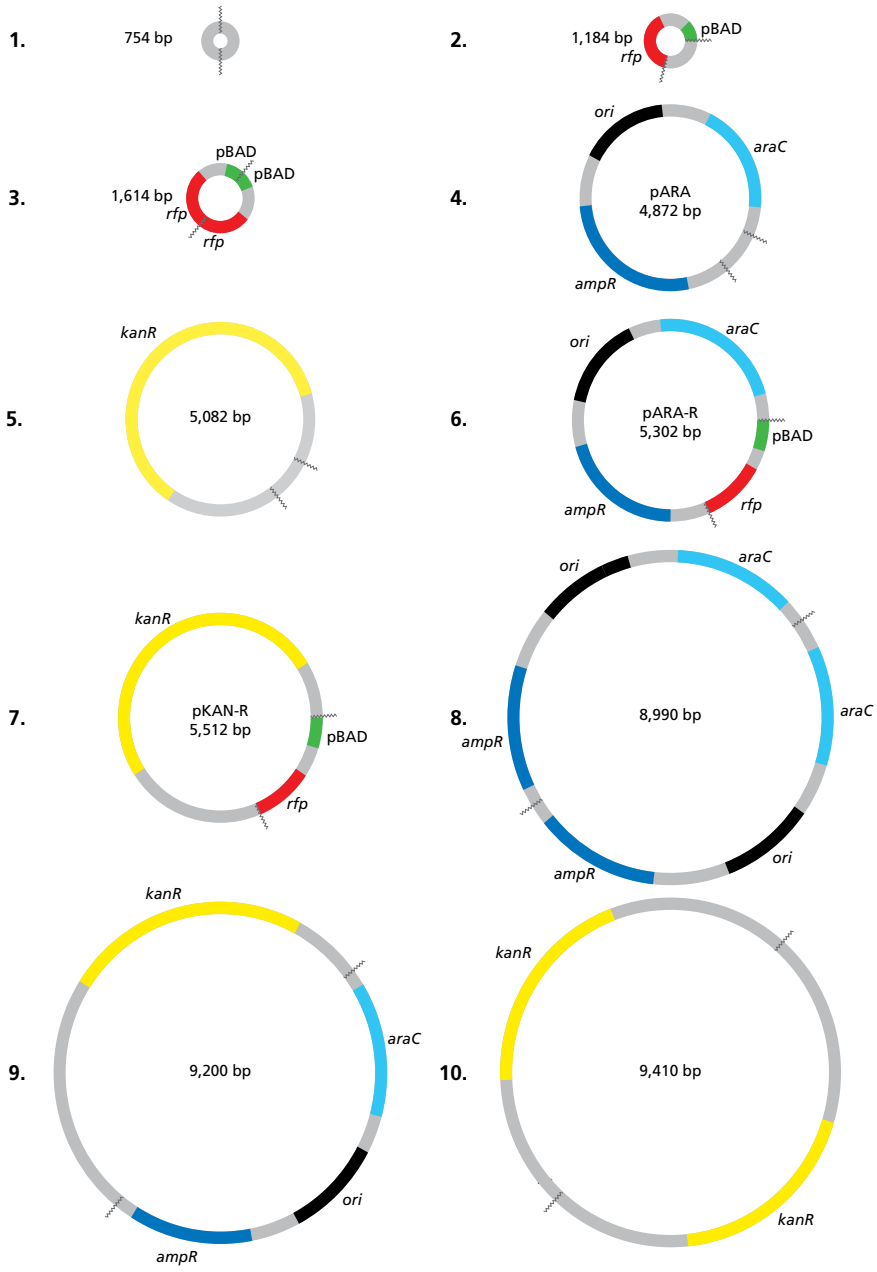
SESSION 2

KEY IDEAS: PCR requires the use of a DNA sample, short pieces of single-stranded DNA called primers that anneal (attach) to a specific DNA sequence in the sample, individual nucleotides that are used to build the DNA copies, and DNA polymerase. Because the primers bind to specific sites in the DNA that is being amplified, you need information about the DNA sequence you are copying.

Review the steps of the PCR procedure with students. (10 min.)

Review the PCR procedure and equipment with students, and review what will be added to each PCR tube. Go over the two controls—the positive plasmid control (pARA-R) and the negative plasmid control (pARA). Review the plasmid diagrams (shown in **Figures 5-EXT.1** and **5-EXT.2** on pages 7 and 8 of this Teacher Guide, and in **Figure 5-EXT.4** on page 9 of the Student Guide), and have students predict the size of the PCR product for each sample. Review the possible plasmids that would create a white colony.

Figure 5-EXT.3: All two-fragment plasmids that can form during ligation



RESOURCES: You will find videos related to PCR, colony transformation, and gel electrophoresis on the program website.

Have students complete Laboratory 5-EXT, Part A. (25 min.)

During the lab, monitor students' work as needed. Emphasize keeping materials on ice to prevent warming them.

NOTE: When students have set up PCR reactions and are ready to place their tubes in the thermocycler:

1. **Remind students to make sure that they have tightly closed their PCR tubes. This is important, as a loose cap will allow the reagents to evaporate during the PCR run.**
2. **If you have several classes doing this lab, groups can place their tubes in the thermocycler once they are ready, as it has been programmed to keep the tubes at 4°C. Remind students to mark the location of their tubes on the heat block template so they can locate their tubes following the PCR run.**

After the amplification, transfer samples to the refrigerator (4°C). Do not leave the thermocycler running longer than necessary.

Have students answer the *Laboratory 5-EXT Questions, Part A.* (10 min.)

You may wish to have students discuss the questions first, and then write individual answers in class or as a homework assignment.

Possible answers:

1. Why are multiple cycles of denaturation, annealing, and extension required in PCR? *In each cycle, the DNA sequence of interest is doubled. Multiple cycles therefore lead to significant amplification of that DNA sequence.*
2. The recombinant plasmids used to transform the colonies in this lab were made in the gene cloning lab (Lab 2/2A) and the building a recombinant plasmid lab (Lab 3). The initial plasmid restriction digest resulted in the formation of four different fragments, each with a *Bam*HI and a *Hin*dIII sticky end.
    a. How many different two-fragment recombinant plasmids would you expect to have formed in Lab 3 (the ligation lab)? *We expect nine different two-fragment recombinant plasmids.*
    b. Of the two-fragment recombinant plasmids that could form while building the recombinant plasmid, which would you expect could be carried by the cells growing on the LB/amp/ara plates used in this lab?

NOTE: The large pARA and large pKAN-R fragment combination is unlikely because of its large size and multiple origins of replication.

The large fragment of pARA + rfp *or the small pARA fragment are the two most likely to be carried by the cells. A plasmid with two large pARA fragments would be too large and would have two different origins of replication.*

    c. What single restriction fragment would each plasmid need to be present in all the cells that grew on the LB/amp/ara plates? *All cells must carry the large pARA fragment, which allows them to grow on a plate with ampicillin.*

d. Of the most likely plasmids, what would be the size of the amplification product from a red colony? *1,092 bp* From a white colony? *662 bp*

NOTE: Figure 5-EXT.3 on page 15 of this Teacher Guide should help you determine the sizes.

SESSION 3

KEY IDEAS: DNA copies from PCR can be separated by gel electrophoresis. DNA cannot be seen on the gel, so loading dye is mixed with the DNA samples to monitor the progress of the gel electrophoresis procedure. To help determine the sizes of the pieces of DNA, a DNA ladder is run on the gel. After the gel electrophoresis is complete, a gel stain and a visualization system are used to show the location of the DNA fragments (this laboratory uses a stain that is added before the gel is cast, and visualized with blue light or UV light after the gel is run).

Have students complete Laboratory 5-EXT, Part B. (35 min.)

After the gels are run, you will need to photo-document them for students to use to answer question 2 in *Chapter 5-EXT Questions, Part B*. You may wish to review the controls you have prepared and run, and ask students to describe the expected results. The expected control 1 product is 662 bp, the expected control 2 product is 1,092 bp, and there should be no control 3 product. Students make predictions on the **DNA Ladder Diagram (RM 5-EXT.1)** while performing the lab. Students' predicted answers will vary.

Have students read *Some Like It Hot*. (10 min.)

Students can read and discuss the reading in pairs or groups, or complete the reading as a homework assignment.

SESSION 4

KEY IDEAS: The size of PCR DNA products that have been separated by gel electrophoresis can be identified by comparison to a DNA ladder. This method can be used to verify if the recombinant plasmid needed is present and can also give students information about how well their procedures worked. Steps in the PCR method are denaturing to separate the strands of double-stranded DNA, annealing of primers at specific sequences, and replication of DNA by DNA polymerase.

Have students discuss the *Laboratory 5-EXT Questions, Part B* in small groups, and record their answers individually. Discuss students' answers as a class. (45 min.)

To answer many of the questions, students will need to analyze their gels. Be prepared to help students make sense of what they see as they answer the questions, and to talk through possible reasons for inconclusive results. Possible sources of inconclusive results include a bad batch of plasmids, pipetting or labeling errors in the PCR procedure, inactive enzymes, and adding the wrong samples to the wells in the gel. Explain that scientists check their results after each step, but students did not have enough time to carry out multiple checks.

STRATEGY: As you lead the discussion, the following practices may be helpful:

- **Give students time to consider one another's responses.**
- **Ask for clarification.**
- **Ask for an explanation.**
- **Restate or rephrase.**
- **Ask for an example.**
- **Ask for evidence.**
- **Provide examples and counterexamples.**
- **Ask students to add to an explanation.**
- **Ask students to evaluate a response.**

Possible answers:

1. Why is it important to examine the PCR products? *In this application of PCR, you must examine the product in order to verify the identity of the plasmid.*

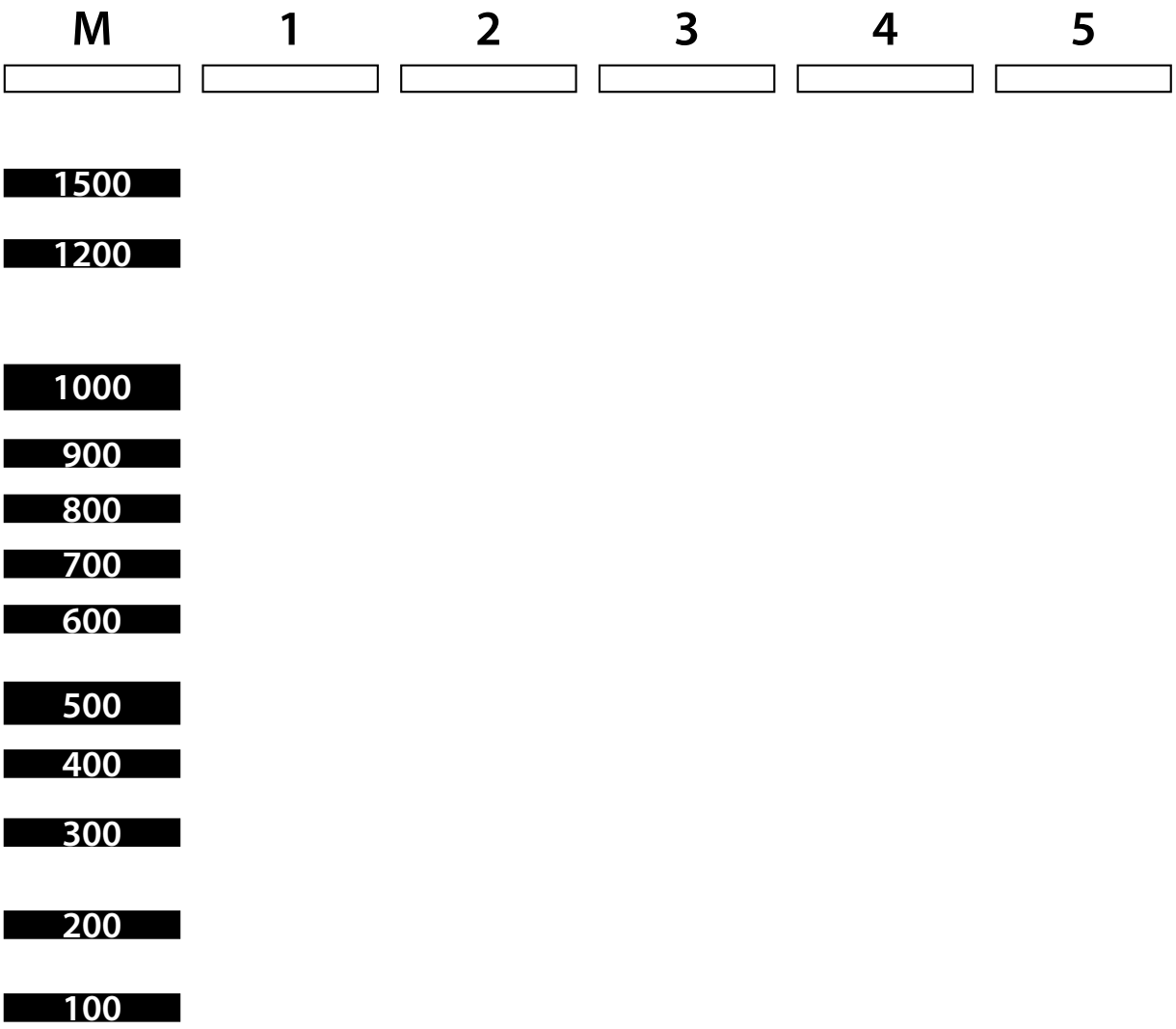
NOTE: Students' answers to questions 2–4 will vary, depending on their success in carrying out the procedures, including the gel electrophoresis procedure. These possible answers may not be applicable if their procedures were not successful.

2. How did your gel results from this laboratory compare to your gel predictions? Did you see any bands that were not expected? What could explain the origin of these unexpected bands? *Answers will vary depending on what sizes students predicted. During trials developing this lab, a white colony showing a 1,092 bp fragment was observed. This may be the result of an early mutation that prevents cells from expressing the* rfp *gene. Because these cells can divert their energy into growth and cell division, they will outcompete cells that do express the gene. Unexpected bands could appear if either incorrect or expired reagents were used in the procedures, if students loaded the gels in a different order than suggested in the protocol, or if students double-loaded a lane.*
3. Does the gel photograph show that your PCR procedure was successful? Describe the evidence you used to make this conclusion. *Answers will vary depending on what DNA sample was used. Students should use the DNA ladder to confirm that an amplified DNA sequence of the right size was made during the procedure.*
4. In this laboratory, you used two controls. Can you think of any additional controls this laboratory might have included? Explain. *Answers will vary,*

but one control that is sometimes included with PCR is a DNA control, which would include all the reagents minus the DNA templates or plasmids. This control was not included because we wanted two groups to fit all their PCR samples into a gel with 10 wells.

5. Why does DNA have to be denatured to carry out PCR? *The primer must attach to a single strand of DNA, and denaturing at high heat causes the DNA that is being amplified to separate into two single strands of DNA.*
6. What are the roles of DNA polymerase and DNA primers in the PCR method? *The DNA polymerase replicates (copies) the DNA. The DNA primers must be attached to two specific sequences in the DNA and provide the starting point for the replication.*

DNA LADDER DIAGRAM



MATERIALS LIST

Session/ Laboratory	Preparation Step	Action	Material	Amount Needed per Group
Set up for each group of students. 2-4 students per group.	~~Aliquot reagents for Laboratory 5-EXT, Part A *(can be done one day in advance)*~~	Label three 1.5-m microfuge tubes as follows:	a. Microfuge tube marked "PCR" b. Microfuge tube marked "+" c. Microfuge tube marked "–"	1 of each
		Pipette reagents into the microfuge tubes as follows:	a. PCR master mix into tube marked "PCR"	100.0 µL
			b. pARA-R into tube marked "+"	3.0 µL
			c. pARA into tube marked "–"	3.0 µL
Chapter 5-EXT, Session 2, Laboratory 5-EXT, Part A	Please refer to the Gel preparation instructions in the Resource Binder	Prepare the agarose solution	a. 250-ml graduated flasks	2
			b. 20x SB	1.5 mL
			c. Distilled water (dH2O)	28.5 mL
			d. Agarose	0.24 g
			e. 10,000X GelGreen™ (or other stain provided)	3.0 µL
	Make agarose gels for Laboratory 5-EXT (can be done several days in advance)		f. Sandwich- or quart-sized resealable bags	6
	P10 micropipettes and tips will be provided	Prepare sets of materials that each include the following:	a. Plastic microfuge tube rack with: i. Microfuge tube of PCR master mix ii. Microfuge tube of pARA-R iii. Microfuge tube of pARA	1
			b. Microfuge tubes	4
			c. Permanent marker	1
			d. P-20 micropipette	1
			e. Tip box of disposable pipette tips	1
			f. Waste container	1 for every two groups

We provide 1.2gr in a 50mL conical. Add contents to 150mL of 1X SB. ~ 30mL @ 5 gels.

Session/ Laboratory	Preparation Step	Action	Material	Amount Needed per Group
Chapter 5-EXT, Session 3, Laboratory 5-EXT, Part B	Aliquot reagents for Laboratory 5-EXT, Part B *(can be done one day in advance)*	Label one 1.5-m microfuge tube "M"	a. Microfuge tube marked "M"	1
		Pipette reagent into the microfuge tube marked "M"	a. Microfuge tube of DNA ladder	10.0 µL
	Additional preparation and materials for Laboratory 5-EXT, Part B	Prepare 1x SB *(can be done several days in advance)*	a. 20x SB	~~2.5 mL~~
			b. dH2O	~~47.5 mL~~
			c. ~~50 mL~~ flask labeled "1x SB"	1
		Copy the Reproducible Master and prepare sets of materials that each include the following:	a. Plastic microfuge tube rack with: i. Microfuge tube of M ii. 4 PCR tubes from Part A	1
			b. Permanent marker	1
			c. P-20 micropipette	1
			d. Tip box of disposable pipette tips	1
			e. Waste container	1 for every two groups
			f. **DNA Ladder Diagram (RM 5-EXT-1)**	1 copy per student

Set up per group. Each group ~2-4 students

Do not confuse with 4A 1kb DNA marker.

Our electrophoresis units require 300mL of SB buffer

15mL

285 mL