# APPLYING MOLECULAR BIOLOGY TECHNIQUES TO ASSESS BACTERIAL INFECTION

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## I. Science Background

### Content Knowledge and Laboratory Procedures

A major concept associated with this project allows students to connect how the body's inflammatory response, as uncomfortable as it makes us feel, is actually helping eliminate the pathogen. Fever, induced by a bacterium's lipopolysaccharide or an activated leukocyte's interleukin-1, followed by prostaglandin-E2, enhances motility, phagocytosis or proliferation of various innate and adaptive white blood cells. Swelling further aids in our battle against infection. Here, a macrophage's released vasoactive amine (i.e. histamine) induces the vasodilation and permeability of blood vessels, enabling antigen presenting cells to enter the site of infection from circulation, and phagocytose the pathogen. Students will learn about these concepts when they research and discuss their assigned patient's case study among team members. The teacher will present them with an individual case study of information, along with the report form that asks the students to analyze their patient's remarks and blood cell count profile, as well as questions about the immune system (a grading rubric is provided). In addition to their textbooks, students may access the following online resources: Biol 1406 interactive tutorial, NCBI, HHMI, Biotechniques.com, and Science Direct.

Another important concept emphasizes the molecular specificity of the immune system's leukocytes. Critical mechanisms of defense, such as phagocytosis or clonal expansion both result from a specific interaction between the host cell's surface receptor and an external ligand. There is also specificity between the penicillin-resistant bacterium's beta-lactamase and its substrate; this enzyme will not be effective against any another antibiotic (i.e. kanamycin). Students will learn that the enzyme, beta-lactamase, is specific because of its active site's physical and chemical makeup. It does undergo a temporary alteration to accommodate its substrate better but not nearly enough to accommodate other substrates. Students will notice no bacterial growth in a test tube of media that contains kanamycin.

One of the central dogmas of biology is that a gene that encodes information for the cell to synthesize a polypeptide, via an RNA transcript, can greatly influence the organism's phenotype if it confers special survival or reproductive properties. In this case, the specific gene product, beta-lactamase allows the bacterium to inactivate penicillin (hydrolyzing the beta-lactam ring). A powerful selection agent, penicillin gives the bacteria a great advantage in the population. Students will visualize the release of this gene through restriction endonuclease digestion of the plasmid DNA, which is where the gene resides during gel electrophoresis. Students will notice that the penicillin-vulnerable bacteria, do not possess this gene, and can only survive in an antibiotic-free test tube culture.

**REFERENCES:**

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2. Back to Basics: Validation of the Admission Systemic Inflammatory Response Syndrome Score in Predicting Outcome in Trauma: Malone, Debra L. MD; Kuhls, Deborah MD; Napolitano, Lena M. MD; Journal of Trauma-Injury Infection & Critical Care: September 2001 - Volume 51 - Issue 3 - pp 458-463

3. Inflammation in Wound Repair: Molecular and Cellular Mechanisms: Sabine A. Eming, Thomas Krieg: Journal of Investigative Dermatology: Vol 127, Issue 3, March 2007, pp 514–525

## II. Student Outcomes

### Science Concepts

These project-based lessons are aimed at aiding high school teachers to review with their students the more salient concepts in immunology such as the mechanisms and effects associated with inflammation, through the use of molecular biology techniques. Students will be assessed on their knowledge of the innate immune system, the purpose of the inflammatory response, gene expression, and directional selection in the form of worksheets, quizzes, a physician's final report and a concluding research conference day where they exchange information and new ideas about their patients' illnesses.

Teams of students (2-4) will read and assess a case study describing a fictional patient's symptoms and blood chemistry. There is not enough information on paper to make an accurate diagnosis; the inflammatory-associated symptoms could be the result of a bacterial based infection or something else (virus, toxin, allergen, or possibly cancer). Students will conclude that they must perform polymerase chain reaction (pcr) amplification of the 16S rRNA gene to confirm that the infection is bacterial based before prescribing a treatment. The resulting 630 base pair (bp) amplicon will be visualized via gel electrophoresis. In addition to the pcr-electrophoresis results, students will review the report's blood cell count profile as well as patient's symptoms to make a final diagnosis and prescribe a treatment for their patient.

The first day, students review and discuss a patient's profile, furnished by the referring physician. The profile includes the patient's symptoms and a blood cell count profile. The second day, student teams will perform a micro-pipetting exercise in preparation for the following day's actual pcr-amplification experiment. The third day, students will attempt to pcr-amplify the 16S rRNA gene from their patient's bodily fluid along with positive and negative controls. The fourth day, students will gel electrophorese their pcr-amplification results along with a molecular weight standard.

In a follow up case, students will read about another patient, who is infected with a bacterium. The patient normally responds to penicillin, but for an unknown reason remains ill, and is getting worse. Here students perform a restriction enzyme digestion (EcoRI and HindIII) on the bacteria's plasmid DNA. If a 2635 bp band appears during the gel electrophoresis (see plasmid's restriction enzyme map), it will confirm that this pathogen has evolved penicillin resistance, in the form of beta-lactamase. (If not, it just may be that the penicillin was bad). The band will appear, and students will be asked to suggest an alternate antibiotic or a different treatment altogether. The next day, the teacher will display test tubes of cultures from a fictional laboratory where bacterial growth is only apparent in the penicillin-treated broth but not the other test tubes carrying different antibiotics (kanamycin or chloramphenicol). After viewing this phenomenon, students will work on a worksheet that prompts thinking and discussion about evolution of ampicillin resistance, both at the species and molecular level.
Students will realize, according to the rules of evolution, that penicillin-resistant bacteria react to a change in the environment (i.e. kanamycin-containing media) through random mutation of DNA, the true fuel for speciation. Another lesson learned is that if the penicillin-resistant bacteria live long enough, without the presence of penicillin as a selecting agent, they may lose the ability to express beta-lactamase gene and subsequently die if later exposed to penicillin. Further, students will be trained in performing very fundamental molecular biology techniques. Today's commercial and academic biology-based laboratories, for a variety of purposes, are always performing pcr-amplifications and restriction endonuclease analyses followed by gel electrophoresis. Students will measure small quantities of solutions (micro-liters) as well as handle delicate pieces of equipment such as a micro-pipette, a pcr-thermocycler and an electrophoresis box. Students will perform a polymerase chain reaction of the 16S rRNA gene

(630 bp) amplicon. Students will understand that the restriction endonuclease digestion (EcoRI and HindIII) will result in the release of the beta-lactamase gene (2635 bp).

To conclude this unit of lessons, students will participate in a research poster conference where there will be an active exchange of new ideas about their research findings, including the information about their patients' diagnoses.

This project is in agreement with many of the Next Generation Science Standards for the living environment curriculum. Planning and preparation, for instance, is a critical skill that students will learn in their team setting. They will discuss the role each member will play in these projects, including diagnosis of their patient's case study, and how to perform a molecular biology experiment (polymerase chain reaction, restriction enzyme analysis, and gel electrophoresis). Analyzing and interpreting data will be another major feature of these projects, for instance if their 630 bp pcr-amplicon does not work or the 2635 bp beta-lactamase gene is not released, as is often the case for first attempts, students will have to come up with solutions (i.e. lower the annealing temperature for the amplification or using a plasmid DNA for the double restriction endonuclease digest). Lastly, students will communicate continuously throughout the projects as they plan strategies for their patient's initial diagnosis, interpreting their experimental results, and informing other classmates about the case study final diagnosis and prescription. Students will also participate in a concluding research conference day where they exchange information and new ideas about their patients' illnesses.

This project is recommended to be implemented after the conclusion of the molecular biology unit, in the spring. At that time, the students are well versed in the basics of immunology and molecular biology techniques. The concepts and techniques covered in this project are invaluable to the student in real-life circumstances. For instance, students may be able to more effectively communicate symptoms of an illness to a physician as well as understand the logic behind the prescribed antibiotic. Further, in college or the professional workforce, students will be better prepared to understand and perform laboratory exercises associated with the complexity of the immune system.

**III. Learning Objectives**

One measureable learning objective connects an infection with the inflammatory response's fever and swelling and rise in leukocytes. Students will learn about these processes by reading and analyzing the fictional patient's profile that describes symptoms as well as blood cell count profile with any unusual values being underscored. Students will be asked to fill out an initial report where they use the case study's information to argue whether they think the infection is bacterial or something else (virus, toxin, allergen or possibly cancer). In addition to their initial diagnosis, they will answer questions regarding the effects of inflammation and the effects they have on certain leukocytes.

A second learning objective will emphasize the connection between the bacteria's ability to resist penicillin's deadly effects and the expression of the gene, beta-lactamase (hydrolyzes ampicillin's beta-lactam ring) responsible for this newly favored phenotype. Students will visualize this released gene through restriction endonuclease digestion (EcoRI, HindIII) of the bacteria's plasmid DNA, where it resides, followed by gel electrophoresis. Further, they will learn that this enzyme is very specific and confers resistance only against this particular antibiotic. For instance there will be no noticeable bacterial growth in a test tube of media that contains kanamycin or chloramphenicol. Students will also learn that penicillin-resistant bacteria are not capable of reacting to a change in the environment by sheer desire but through random mutation of DNA, the true fuel for speciation.

A third learning objective focuses on the mechanics and applications of the molecular biological techniques being implemented in these lessons. Students will demonstrate their understanding of the polymerase chain reaction via the successful amplification of the 16S rRNA gene (630 bp) amplicon either in the experimental or the positive control test tube. They will also demonstrate their understanding of the speed and specificity of how restriction endonucleases work through the digestion-release (EcoRI and HindIII) of the beta-lactamase gene (2564 bp fragment). If, for whatever reason, these experiments are not successful, students will have the opportunity to demonstrate their understanding of the theory of these techniques and their applications in scientific research in the formative assessment such as an open-ended response quiz or homework assignment.

The project's overall concepts including the inflammatory response, the mechanism of gene expression and the evolution of an antibiotic-resisting bacteria, will be formatively assessed daily through open-ended response quizzes, worksheets and homework assignments. Summative assessments include the student teams' final diagnosis reports and conference poster presentations (Grading rubrics will be provided). On a less quantitative scale, I will be frequently walking around the laboratory room observing the quality and productivity of the discussions among the various team members as they perform the daily activities.

If these experiments are not successful, students will have the opportunity to demonstrate their understanding of the theory of these techniques and their applications in scientific research via formative assessments such as an open-ended response quizzes. These projects together will help students realize how powerful these techniques are in the fight against disease.

**IV. Time Requirements**
Most of the lessons in this project only require a 41 minute period. The exceptions are the fourth and sixth days, a double period (82 minutes) is needed to perform gel electrophoresis visualize and photograph the results.

**V. Advance Preparation and VI. Materials and Equipment**
Project's required items, vendors (catalogue number) and costs

| item | price | | cat# | vendor |
|---|---|---|---|---|
| **BACTERIA** | | | | |
| MM294/pAMP E. coli Slant Culture | $12 | | 211540 | Carolina |
| Ampicillin solution | $11 | $5.50x2 | 216858 | Carolina |
| kanamycin solution | $11 | $5.50x2 | 216862 | Carolina |
| LB-broth | $94 | | 12780052 (500 g) | thermofisher scientific |
| distilled water | $35 | | 10977015 (500 ml) | thermofisher scientific |
| 14 ml snap-cap test tubes | $172 | | 14-959-1B (500) | fisher scientific |
| Inoculating Loop | $211 | | 22-363-595 | fisher scientific |
| culture shaker | $1,995 | | SH1000 IncuShaker | southwest science |
| magnetic platform-mat | $209 | | SH1000-MR | southwest science |
| magnetic 250 ml flask Clamp | $112 | $27x4 | FM-CLAMP-250 | southwest science |
| micro-centrifuge | $2,000 | | S98645 | fisherscientific |
| 1.5 ml micro-centrifuge tubes | $126 | $63x2 | 05-408-129 (500/cs) | thermofisher scientific |
| **PCR-AMPLIFICATION** | | | | |
| genomic DNA purification kit | $235 | | 158567 | QIAGEN |
| App Bio 2720 Thermal Cycler | $2,995 | | 4359659 | thermofisher scientific |
| 2x pcr mix | $120 | | K0171 200x rxns | thermofisher scientific |
| Laboratory Pipettor (20-200 µl) | $750 | $150 x5 | 214627 | Carolina |
| Laboratory Pipettor (2-20 µl) | $750 | $150 x5 | 214623 | Carolina |
| Pipette tips (1-200 µl) | $67 | | 02-707-419 (960) | thermofisher scientific |
| vortex | $394 | | 88880017TS | thermofisher scientific |
| 16S rRNA pcr primers | $50 | $25x2 | cat# 10336022-254528-C10, 10336022-254528-D07 | invitrogen-thermofisher scientific |

**RESTRICTION ENZYME DIGESTS**

| | | | |
|---|---|---|---|
| plasmid DNA miniprep kit | $91 | 27104 | QIAGEN |
| EcoRI | $56 | ER0275 10 U/µl | thermofisher scientific |
| HindIII | $56 | ER0505, 10 U/µl | thermofisher scientific |
| 10x Buffer R | $15 | BR5 | thermofisher scientific |

**GEL ELECTROPHORESIS**

| | | | |
|---|---|---|---|
| Gel loading dye | $76 | 38x2 R0611 (5 x 1 ml) | thermofisher scientific |
| SYBR® Safe DNA Gel Stain | $136 | 68x2 S33102 (400 µl @10,000X) | thermofisher scientific |
| electrophoresis agarose | $602 | 16500500 (500 g) | thermofisher scientific |
| 10X TAE buffer | $125 | 15558026 (4 l) | thermofisher scientific |
| TE buffer | $41 | AM9849 (500 ml) | thermofisher scientific |
| electrophoresis box (hexa-gel) | $325 | cat# 515 | edvotek |
| power supply | $179 | cat #509 (2 outlets) | edvotek |
| Midrange UV Transilluminator | $549 | cat# 558 | edvotek |
| Lambda DNA x EcorI/HindIII markers) | $79 | SM0191 (5 x 50 µg) | thermofisher scientific |

**V. Advance Preparation and VI. Materials and Equipment**
**Directions for preparing solutions and other reagents:**

**DAY 3: "PERFORMING A POLYMERASE CHAIN REACTION" LESSON (41 min)**
**Bacterial culture growth (teacher prepares 1 week prior)**
Reference: CAROLINA - MM294/pAMP E. coli Slant Culture (Item# 211540)
http://www.carolina.com/biotechnology-bacterial-strains/mm294/FAM_211530.pr

Materials: sterilized Luria Broth (thermofisher scientific), Ampicillin solution (Carolina), kanamycin solution (Carolina), 14 ml snap-cap test tubes (fisher scientific), Inoculating Loop (fisher scientific), culture air shaker (Southwest)

**Procedure:** (sterile technique)

1. Uncap bottle of sterile Luria Broth.

2. Flame mouth and pour 2-4 ml into uncapped 14 ml snap-cap test tube.

3. Add 20-40 µl 10,000 µg/ml ampicillin (100 µg/ml final).

4. Use disposable inoculating loop to transfer sample of MM294/pAMP slant culture into Luria Broth-ampicillin solution, replace snap cap onto test tube.

5. Place test tube into shaking air or water bath at $37^{o}$C overnight.

**Bacterial genomic DNA isolation (teacher prepares 1 week prior)**
**Reference:** QIAGEN - Gentra Pure Gene Yeast/Bacteria (Kit #158567) – see attachment
https://www.qiagen.com/us/shop/sample-technologies/dna/gentra-puregene-yeastbact-kit/

**Materials:** 1-20 µl and 20-200 µl micro-pipettes (carolina), 1-200 µl pipette tips (thermofisher scientific), microcentrifuge (thermofisher scientific), microcentrifuge tubes (thermofisher scientific), vortex (thermofisher scientific), genomic DNA isolation kit (Qiagen)

**Procedure:**
"Gentra Pure Gene Yeast/Bacteria (Qiagen)" handbook for protocol
Expected yield from 2 ml culture (MM294/pAMP E. coli is gram negative)
**= 100 µg suspended in 100 µl (1 µg/µl)**

This protocol is for purification of genomic DNA from fresh or frozen samples of 0.5 ml Gram-negative bacterial cultures. An overnight culture contains 1–3 x $10^9$ cells/ml. Due to the small genome size of Gram-negative bacteria, up to 3 x $10^9$ cells may be used for the protocol. Thus, culture can either be used directly, or, if necessary, concentrated by centrifuging. To concentrate, pellet 1 ml of overnight culture at 13,000–16,000 x g for 1 min. Remove the supernatant, leaving 200 µl residual fluid. Thoroughly suspend the pellet in the residual fluid by pipetting up and down 10 times. Place the sample on ice for immediate use or store frozen at –80°C.

1. Prepare an overnight culture.

2. Transfer 500 µl of culture (0.5–1.5 x $10^9$ cells) to a 1.5 ml microcentrifuge tube on ice.

3. Centrifuge for 5 s at 13,000–16,000 x g to pellet cells.

4. Carefully discard the supernatant by pipetting or pouring.

5. Add 300 µl Cell Lysis Solution, and mix by pipetting up and down. Incubate sample at 80°C for 5 min to lyse the cells. Samples are stable in Cell Lysis Solution for at least 2 years at room temperature.

6. Add 1.5 µl RNase A Solution, and mix by inverting 25 times. Incubate for 15–60 min at 37°C.

7. Incubate for 1 min on ice to quickly cool the sample.

8. Add 100 µl Protein Precipitation Solution, and vortex vigorously for 20 s.

9. Centrifuge for 3 min at 13,000–16,000 x g. The precipitated proteins should form a tight pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation.

10. Pipet 300 µl isopropanol into a clean 1.5 ml microcentrifuge tube and add the supernatant from the previous step by pouring carefully.
Be sure the protein pellet is not dislodged during pouring.

11. Mix by inverting gently 5 times.

12. Centrifuge for 1 min at 13,000–16,000 x g. The DNA is a small white pellet.

13. Carefully discard the supernatant, and drain the tube by inverting on a clean piece of absorbent paper, taking care that the pellet remains in the tube.

14. Add 300 µl of 70% ethanol and invert several times to wash the DNA pellet.

15. Centrifuge for 1 min at 13,000–16,000 x g.

16. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube. Allow to air dry for 5 min.
The pellet might be loose and easily dislodged. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.

17. Add 100 µl DNA Hydration Solution and vortex for 5 s at medium speed to mix.

18. Incubate at 65°C for 1 h to dissolve the DNA.

19. Incubate at room temperature overnight with gentle shaking. Ensure tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.

**DAY 4: "GEL ELECTROPHORESIS" LESSON (82 min – two periods)**
**1.5 % agarose gel and molecular markers (teacher prepares 1 day prior)**

Reference: http://www.thermofisher.com/us/en/home.html

Materials: Lambda DNA x EcorI/HindIII molecular weight markers (thermofisher scientific), 1.5 ml micro-centrifuge tubes (fisher scientific), 6x gel loading dye and 10,000x SYBR gel stain (thermofisher scientific), electrophoresis agarose (thermofisher scientific), 10X TAE buffer (thermofisher scientific), electrophoresis box M36 HexaGel apparatus (edvotek), power supply (edvotek), transilluminator 7 x 14 cm (edvotek)

**Procedure:**

**1.5% agarose gel (teacher prepares 1 day prior)**

1. Add 3.75 g agarose to 250 ml 1x TAE to 500 ml glass flask (25 ml 10x TAE + 225 ml deionized $H_2O$).

2. Add 25 µl 10,000x SYBR gel stain.

3. Swirl and microwave for 2 min… let cool for 5 min.

4. Pour into tray with comb and bumpers affixed at ends, ready to use when gel is cloudy.

5. Remove comb and bumpers and submerge into gel box with 1x TAE.

**molecular weight markers (teacher prepares 1 day prior)**

1. Add 1 µl (0.5 µg) Lambda DNA x EcoRI/HindIII molecular weight markers to 1.5 ml micro-centrifuge tube.

2. Add 1 µl of 6X DNA loading dye.

3. Add 28 µl $H_2O$ to 1.5 ml micro-centrifuge tube.

4. 65°C for 5 min and then ice for 3 min.

**DAY 5: "FIGHTING ANTIBIOTIC-RESISTANT BACTERIA" LESSON (41 min)**

**Bacterial plasmid DNA isolation (teacher prepares 1 week prior)**

Reference: QIAprep Spin for 50 plasmid minipreps cat# 27104
https://www.qiagen.com/us/shop/sample-technologies/dna/gentra-puregene-yeastbact-kit/

Materials: 1-20 µl and 20-200 µl micro-pipettes (carolina), 1-200 µl pipette tips (thermofisher scientific), microcentrifuge (thermofisher scientific), microcentrifuge tubes (thermofisher scientific), vortex (thermofisher scientific), plasmid DNA isolation kit (Qiagen)

**Procedure:**

Inoculate a bacterial culture from 2 ml LB medium containing ampicillin (100 µg/ml final).

Incubate for 12–16 h at 37°C with vigorous shaking.

Pellet bacterial cells in 14 ml centrifuge tubes at 5400 x *g* for 10 min at 4°C.

Remove all traces of supernatant by inverting tube.
(This protocol purifies up to 20 µg plasmid DNA.)

1. Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube. Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible.

2. Add 250 µl Buffer P2 and mix by inverting the tube 4–6 times. Do not vortex, as this will shear genomic DNA; continue until solution is viscous and slightly clear.

3. Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times. The solution should become cloudy.

4. Centrifuge for 10 min at 13,000 rpm (~17,900 x *g*) in a table-top microcentrifuge. A compact white pellet will form.

5. Apply 800 µl of supernatant from step 4 to QIAprep 2.0 spin column by pipetting.

6. Centrifuge for 30–60 s. Discard the flow-through.

7. Recommended: Wash the QIAprep 2.0 spin column by adding 0.5 ml Buffer PB and centrifuging for 30–60 s. Discard the flow-through (remove trace nuclease activity).

8. Wash QIAprep 2.0 spin column with 0.75 ml Buffer PE and centrifuging for 30–60 s.

9. Discard flow-through and centrifuge at full speed for 1 min to remove residual wash buffer. (Residual ethanol from Buffer PE inhibits restriction enzyme reactions)

10. Place the QIAprep 2.0 column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of each QIAprep 2.0 spin column, let stand for 1 min, and centrifuge for 1 min.

**~0.25 µg/µl in 50 µl (use 5 µl per digest)**

**DAY 6: "GEL ELECTROPHORESIS" LESSON (82 min – two periods)**
**1% agarose gel and molecular markers (teacher prepares 1 day prior)**

Reference: http://www.thermofisher.com/us/en/home.html

Materials: Lambda DNA x EcorI/HindIII molecular weight markers (thermofisher scientific), 1.5 ml micro-centrifuge tubes (fisher scientific), 6x gel loading dye and 10,000x SYBR gel stain (thermofisher scientific), electrophoresis agarose (thermofisher scientific), 10X TAE buffer (thermofisher scientific), electrophoresis box M36 HexaGel apparatus (edvotek), power supply (edvotek), transilluminator 7 x 14 cm (edvotek)

**Procedure:**

**1% agarose gel (teacher prepares 1 day prior)**

1. Add 2.5 g agarose to 250 ml 1x TAE to 500 ml glass flask.
(25 ml 10x TAE + 225 ml deionized $H_2O$)

2. Add 25 µl 10,000x SYBR gel stain.

3. Swirl and microwave for 2 min… let cool for 5 min.

4. Pour into tray with comb and bumpers affixed at ends, ready to use when gel is cloudy.

5. Remove comb and bumpers and submerge into gel box with 1x TAE.

**molecular weight markers (teacher prepares 1 day prior)**

1. Add 1 µl (0.5 µg) Lambda DNA x EcoRI/HindIII molecular weight markers to 1.5 ml micro-centrifuge tube.

2. Add 1 µl of 6X DNA Loading Dye.

3. Add 22 µl $H_2O$ to 1.5 ml micro-centrifuge tube.

4. 65°C for 5 min and then ice for 3 min.

**undigested plasmid (teacher prepares 1 day prior)**

1. Add 1 µl (0.25 µg) undigested plasmid.

2. Add 1 µl of 6X DNA loading dye.

3. Add 22 µl $H_2O$ to 1.5 ml micro-centrifuge tube.

4. Incubate 65°C for 5 min and then ice for 3 min.

**DAY 7: "FIGHTING ANTIBIOTIC-RESISTANT BACTERIA" LESSON (41 min)**
**Bacterial culture growth (teacher prepares 1-2 days prior)**
Reference: CAROLINA - MM294/pAMP E. coli Slant Culture (Item# 211540)
http://www.carolina.com/biotechnology-bacterial-strains/mm294/FAM_211530.pr

Materials: sterilized Luria Broth (thermofisher scientific), ampicillin solution (Carolina), kanamycin solution (Carolina), 14 ml snap-cap test tubes (fisher scientific), inoculating loop (fisher scientific), culture air shaker (southwest)

**Procedure:** (use sterile technique)

1. Uncap bottle of sterile Luria Broth and flame mouth.

2. Pour 2-4 ml into 3 uncapped 14 ml snap-cap test tube.

3. Test tube #1 (neg ctrl) Luria Broth alone – bacterial growth.

4. Test tube #2 add 20-40 µl 100x ampicillin (100 µg/ml final) - bacterial growth.

5. Test tube #3 add 10-20 µl 200x kanamycin (100 µg/ml final) – no bacterial growth.

6. Use disposable inoculating loops to transfer sample of MM294/pAMP E. coli slant culture into each of the three test tubes.

7. Replace snap caps onto test tubes.

8. Place test tubes into shaking air or water bath at 37$^{o}$C overnight.

**VII. Student Prior Knowledge and Skills**

Because this project is recommended to be implemented at the conclusion of the molecular biology unit (and well after the immune system unit), the students are expected to be well versed in the basics of immunology, including the inflammatory effects following infection as well as the general mechanics of the molecular biology techniques (pcr-amplification, restriction endonuclease digestion and gel electrophoresis).

Further, in regards to the immune system, students are expected to familiarize themselves, via their assigned text book or an online tutorial, the three lines of the immune system, in particular the inflammatory response to infection. They should also be aware that sometimes the immune system is not effective against all pathogens and needs assistance in the form of antibiotics if it's bacteria (or vaccine if a virus).

With regards to the molecular biology techniques (polymerase chain reaction amplification, restriction endonuclease digestion and electrophoresis resolution) involved in this project, the teacher needs to first demonstrate proper technique before allowing the students to participate in order to reduce erroneous reaction results or possible damage to the equipment.

One predictable student misconception to address and correct is "directional selection of a bacterial population towards antibiotic resistance is not through random mutation but by sheer determination."

**VIII. Daily Unit Plans and IX. Summative Assessments**

Student performance in the project's overall concepts including the inflammatory response, the mechanism of gene expression and the evolution of an antibiotic-resisting bacteria, will be formatively assessed daily through open-ended response quizzes, worksheets and homework assignments. Summative assessments will be in the form of teams' final diagnosis reports and conference poster presentations where they exchange information and new ideas about their patients' illnesses (grading rubrics will be provided.)

On a less quantitative scale, I will be frequently walking around the laboratory room observing the quality and productivity of the discussions among the various team members as they perform molecular biology technique.

**VIII. Daily Unit Plans and IX. Summative Assessments**
**DAY 1: "REVIEWING THE IMMUNE SYSTEM AND DIAGNOSING PATIENT'S SYMPTOMS" LESSON (41 min – see attachment)**

***Note:** All students are to be given the initial and final diagnosis report, references and grading rubrics. Handout only one, of the eight, patient profiles to each student team (2-4 students).*

**Objectives: students will be able to …**
give examples of the immune system's first line of defense
describe features of inflammation during an infection
describe types of leukocytes associated with the immune system's second and third lines of defense
assess a patient's symptoms and make an initial diagnosis

**Aim:** What are the symptoms of a bacterial infection and how do they differ from other illnesses (i.e. viral infection, cancer or allergies)?

**1st 15'** Administer a formative diagnostic in the form of a short-essay response quiz on the body's immune system and signs of inflammation (ee attachment).

**2nd 5'** Review answers to the quiz with the class.

**3rd 10'** Handout a different patient profile to each student team. The profile contains information on patient's background and symptoms they are experiencing and an erroneous diagnosis by the referring physician Dr. Areal Kwak.

Student teams read and discuss a patient's profile. Then, they will write an initial report as well as some basic questions about the body's immune system. (See attachment for report and rubric.)

**Teacher's answer key**

**Profile 1** Chronic Myelogenous Leukemia cancer via chromosomal 9:22 translocation

**Profile 2** Human Immunodeficiency Virus infection via dirty needle

**Profile 3** Allergy – via bee sting venom induced IgE-eosinophil

**Profile 4** Systemic lupus erythematosus – autoimmune disorder

**Profile 5** Vibrio cholerae – cholera via contaminated water

**Profile 6** Botulism - Botulinum injections

**Profile 7** Influenza – "flu" via sneezing from infected person

**Profile 8** Clostridium tetani - tetanus via rusty nail puncture

***Note:** Patient profiles #5, 8 will give a positive result (630 bp amplicon) for the pcr-amplification. Students given patient profiles #1-4, 6, 7, 8 will get a negative result but they will see the 630 bp amplicon in the positive control test tube.*

**DAY 1: REVIEWING THE IMMUNE SYSTEM AND DIAGNOSING PATIENT'S SYMPTOMS**
**Student Name:**_____
**QUIZ**

**1.** Give two examples of our body's first line of defense.

**2.** Discuss one way a person can become infected.

**3.** Explain the mechanism underlying one of our body's inflammatory responses to an infection.

**4.** Briefly describe a type of leukocyte (white blood cell) associated with the immune system's second and third line of defense.

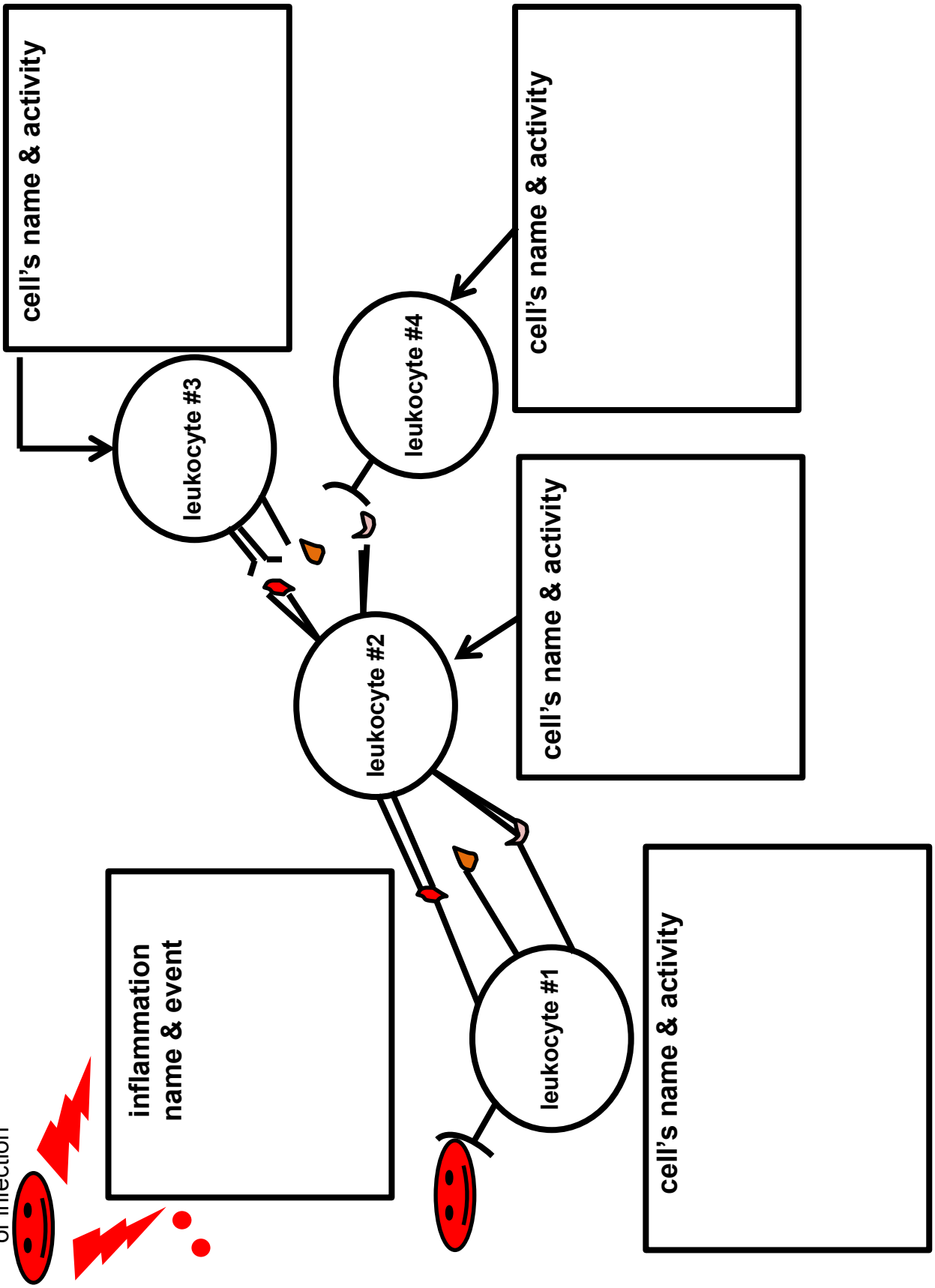
**5.** If you examined the blood cell profile (wbc, rbc, platelets) of a patient infected with a pathogenic bacterium what would you expect to find?

**REVIEWING THE IMMUNE SYSTEM AND DIAGNOSING PATIENT'S SYMPTOMS**

**Student Name:**_____

**Homework**

Lesson #1 – Homework – Discuss within the "boxes" names and events associated with our immune response to this pathogenic infection (Use textbook or other sources to complete the diagram below)



**Profile 1 Team's Name:** _____

**Background:** Your team runs a very promising diagnostic research center, "Disease Busters," that has rapidly gained a very favorable reputation among the physicians for quickly and accurately diagnosing difficult cases. Use the following information compiled by the physician and the complete blood count your team prepared to figure out this puzzling case.

**Patient's name:** Luke Mya

**What is your major complaint?** fever, very weak and ache all over

**How long have you had this condition?** on and off for months

**Have you experienced this condition in the past?** no, not for this long

**What do you think caused this condition?** can't shake off this cold since last winter

**Dr. Areal Kwak (primary physician) notes:** Mr. Mya is 78 years old and has been coming in regularly this winter for minor aches with fever. I prescribed an antibiotic, penicillin but it was not effective.

**LABORATORY REPORT**

***Compare patient's blood count values to the normal, acceptable ranges.**

1. http://emedicine.medscape.com/article/199425-overview#showall
2. https://www.lls.org/managing-your-cancer/lab-and-imaging-tests/understanding-blood-counts
3. Provan, D; Gribben, JG (2010). "Chapter 7 Chronic myelogenous leukemia". *Molecular Hematology* (3rd ed.).

| RBC per µl blood | WBC per µl blood | Platelets per µl blood | Hematocrit % of blood composed of red cells | Hemoglobin g/dl |
|---|---|---|---|---|
| 3,000,000 | 25,000 | 100,000 | 23.0 | 78.0 |

**Profile 2 Team's Name:** _____

**Background:** Your team runs a very promising diagnostic research center, "Disease Busters" that has rapidly gained a very favorable reputation among the physicians for quickly and accurately diagnosing difficult cases. Use the following information compiled by the physician and the complete blood count your team prepared to figure out this puzzling case.

**Patient's name:** Horatio Ignacio Victorio

**What is your major complaint?** fever and sore throat

**How long have you had this condition?** on and off for months

**Have you experienced this condition in the past?** no, not for this long

**What do you think caused this condition?** caught it from my friend

**Dr. Areal Kwak (primary physician) notes:** Mr. Victorio is 23 years old and experiences fever, large tender lymph nodes, throat inflammation, headache, sores on mouth. He looks really high and is arm is covered with needle marks! I prescribed an antibiotic, penicillin but it was not effective. He has other issues, none of which were cleared up; I'm baffled.

**LABORATORY REPORT**

***Compare patient's blood count values to the normal, acceptable ranges.**

1. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4383537/
2. "How does HIV cause AIDS?". *Science*. **260** (5112): 1273–9.
3. https://www.lls.org/managing-your-cancer/lab-and-imaging-tests/understanding-blood-counts

| blood cells | patient | normal range |
|---|---|---|
| T helper cells (CD3) | 92%, 2,600 | 54-78%, 785-1950 |
| T helper cells (CD4) | 14%, 400 | 30-50%, 425-1050 |
| T regulatory cells (CD8) | 82%, 2,320 | 18-35%, 280-650 |
| CD4 / CD8 ($T_h/T_s$) ratio | 0.17 | 0.8-2.5 |

**Profile 3 Team's Name:** _____

**Background:** Your team runs a very promising diagnostic research center, "Disease Busters" that has rapidly gained a very favorable reputation among the physicians for quickly and accurately diagnosing difficult cases. Use the following information compiled by the physician and the complete blood count your team prepared to figure out this puzzling case.

**Patient's name:** Ally Genn

**What is your major complaint?** Sneezing, dizziness and shortness of breath

**How long have you had this condition?** No, not long - they're sporadic but intense, mostly in the mornings

**Have you experienced this condition in the past?** no, not for this long

**What do you think caused this condition?** mommy says it's probably from eating too much junk food

**Dr. Areal Kwak (primary physician) notes:** Ally is 8 years old and came in with her mom. Both claim Ally experiences sporadic fever and achiness. I prescribed an antibiotic, penicillin but it was not effective. She could not stop talking about helping mommy collect honey from the family bee hives.

**LABORATORY REPORT**

***Compare patient's blood count values to the normal, acceptable ranges.**

1. Bope, Edward T. (2005). Conn's Current Therapy.
2. "Immunoglobulin E: importance in parasitic infections and hypersensitivity responses". Arch. Pathol. Lab. Med. 124 (9): 1382–5. 124
3. https://www.lls.org/managing-your-cancer/lab-and-imaging-tests/understanding-blood-counts

| blood cells | patient | normal range |
|---|---|---|
| Lymphocytes | 50% | (20% to 40%) |
| Monocytes | 2% | (2% to 8%) |
| Eosinophils | 9% | (1% to 4%) |
| Basophils | 5% | (0.5% to 1%) |
| T regulatory cells (CD8) | 55% | (18 to 35%) |

| blood pressure | patient | normal systolic/diastolic |
|---|---|---|
| 6-13 years | 70/30 | 85-120/55-80 |

**Profile 4 Team's Name:** _____

**Background:** Your team runs a very promising diagnostic research center, "Disease Busters" that has rapidly gained a very favorable reputation among the physicians for quickly and accurately diagnosing difficult cases. Use the following information compiled by the physician and the complete blood count your team prepared to figure out this puzzling case.

**Patient's name:** Lupita Loopes

**What is your major complaint?** Fever and malaise

**How long have you had this condition?** A few months

**Have you experienced this condition in the past?** No

**What do you think caused this condition?** No idea

**Dr. Areal Kwak (primary physician) notes:** Ms. Loopes is an 18 year old African American. She seems anemic and complains of pains in certain joints (ie. fingers). Noticeable bruising on arms with rashes on face. I prescribed an antibiotic, penicillin but it was not effective.

**LABORATORY REPORT**

***Compare patient's blood count values to the normal, acceptable ranges.**

1. "Handout on Health: Systemic Lupus Erythematosus". www.niams.nih.gov. June 2016.
2. "Article on the classification of rheumatic diseases". Rheumatology.org. 2011-06-08.
3. https://www.lls.org/managing-your-cancer/lab-and-imaging-tests/understanding-blood-counts

| blood cells | patient | normal range |
|---|---|---|
| White Blood Cells (per µl blood) | 1,000 | 5,000 to 10,000 |
| Platelets (per µl blood) | 90,000 | 150,000 to 400,000 |

**Profile 5 Team's Name:** _____

**Background:** Your team runs a very promising diagnostic research center, "Disease Busters" that has rapidly gained a very favorable reputation among the physicians for quickly and accurately diagnosing difficult cases. Use the following information compiled by the physician and the complete blood count your team prepared to figure out this puzzling case.

**Patient's name:** Cole Vibriole

**What is your major complaint?** diarrhea and vomiting

**How long have you had this condition?** One week

**Have you experienced this condition in the past?** not that I can remember

**What do you think caused this condition?** a new diner my husband took me to for my birthday! Great low prices but the water tasted funny.

**Dr. Areal Kwak (primary physician) notes:** Ms. Vibriole is 35 years old and looks awful; bluish skin and sunken eyes. I prescribed an antibiotic, penicillin but it was not effective.

**LABORATORY REPORT**

***Compare patient's blood count values to the normal, acceptable ranges.**

1. "Laboratory Methods for the Diagnosis of Vibrio cholerae". Centre for Disease Control. 29 October 2013
2. Howard-Jones, N (1984). "Robert Koch and the cholera vibrio: a centenary". BMJ. 288 (6414): 379–81.
3. http://emedicine.medscape.com/article/213311-workup
4. https://www.lls.org/managing-your-cancer/lab-and-imaging-tests/understanding-blood-counts

| blood | patient | normal range |
|---|---|---|
| White Blood Cells (per µl blood) | 20,000 | 5,000 to 10,000 |
| Blood pressure (systolic / diastolic) | 80/ 55 | 120/80 mm Hg |

**Profile 6 Team's Name:** _____

**Background:** Your team runs a very promising diagnostic research center, "Disease Busters" that has rapidly gained a very favorable reputation among the physicians for quickly and accurately diagnosing difficult cases. Use the following information compiled by the physician and the complete blood count your team prepared to figure out this puzzling case.

**Patient's name:** Claus Botulyn, the one and only!!!

**What is your major complaint?** blurred vision and headaches

**How long have you had this condition?** just the last couple of days

**Have you experienced this condition in the past?** Not that I can remember

**What do you think caused this condition?** just celebrated a promotion by going to my plastic surgeon for a little touch up work (I'm a television news reporter); he was coughing and sneezing a lot, maybe I caught something from him.

**Dr. Areal Kwak (primary physician) notes:** Mr. Botulyn is 67 years old and a very demanding celebrity. He probably did catch whatever his surgeon had but then I noticed certain, specific areas of his face were bruised and swollen. I prescribed an antibiotic, penicillin, but it was not effective.

**LABORATORY REPORT**

***Compare patient's blood count values to the normal, acceptable ranges.**

1. http://www.emedicinehealth.com/clostridium
2. Sobel, J. (2005). "Botulism". Clinical Infectious Diseases. 41 (8): 1167–73.
3. https://www.lls.org/managing-your-cancer/lab-and-imaging-tests/understanding-blood-counts

| blood | patient | normal range |
|---|---|---|
| White Blood Cells (per µl blood) | 20,000 | 5,000 to 10,000 |

**Profile 7 Team's Name:** _____

**Background:** Your team runs a very promising diagnostic research center, "Disease Busters" that has rapidly gained a very favorable reputation among the physicians for quickly and accurately diagnosing difficult cases. Use the following information compiled by the physician and the complete blood count your team prepared to figure out this puzzling case.

**Patient's name:** Influr Enza

**What is your major complaint?** runny nose and shivering

**How long have you had this condition?** Last two days

**Have you experienced this condition in the past?** My business trip?

**What do you think caused this condition?** On the way home I got bumped up to first class! Felt sorry for the stewardess, she was sneezing non-stop. She brought me a great turkey club sandwich.

**Dr. Areal Kwak (primary physician) notes:** Ms. Enza is a 29 year old clothing designer; looks absolutely awful. Shows classic signs of a common bacterial infection; probably from the airplane food not being well cooked. I prescribed an antibiotic, penicillin but it was not effective.

**LABORATORY REPORT**

***Compare patient's blood count values to the normal, acceptable ranges.**

1. http://www.aafp.org/afp/2005/1101/p1789.html
2. "Key Facts about Influenza (Flu) & Flu Vaccine". cdc.gov. 9 September 2014. 26 November 2014.
3. https://www.lls.org/managing-your-cancer/lab-and-imaging-tests/understanding-blood-counts

| blood | patient | normal range |
|---|---|---|
| White Blood Cells (per µl blood) | 4,000 | 5,000 to 10,000 |

**Profile 8 Team's Name:** _____

**Background:** Your team runs a very promising diagnostic research center, "Disease Busters" that has rapidly gained a very favorable reputation among the physicians for quickly and accurately diagnosing difficult cases. Use the following information compiled by the physician and the complete blood count your team prepared to figure out this puzzling case.

**Patient's name:** Tano Spasmoni

**What is your major complaint?** fever, sweating, and headaches

**How long have you had this condition?** just the last two days

**Have you experienced this condition in the past?** Not that I can remember

**What do you think caused this condition?** Not sure – probably at the construction site; a couple of the guys have been sneezing a lot.

**Dr. Areal Kwak (primary physician) notes:** Mr. Spasmoni is 49 years old and looks absolutely awful. Shows classic signs of a common bacterial infection; probably from a colleague. Noticed his right hand had a fresh looking wound, like a puncture. I prescribed an antibiotic, penicillin, but it was not effective. He's now exhibiting mild shaking; I'm concerned!

**LABORATORY REPORT**

***Compare patient's blood count values to the normal, acceptable ranges.**

1. Todar, Ken (2005) Pathogenic Clostridia, Ken Todar's Microbial World, University of Wisconsin - Madison.
2. Centers for Disease Control and Prevention (2006). "Tetanus" (PDF). (10th ed.). Public Health Foundation.
3. https://www.lls.org/managing-your-cancer/lab-and-imaging-tests/understanding-blood-counts

**white blood cell count 6,800 / µl**

**72 % neutrophils**
**11 % lymphocytes**
**15 % monocytes**

**hemoglobin 10.5 g / dl**

**platelet count of 214,000 / ml**

**Student Name:** _____

# I. Initial Diagnosis Report

**Cite evidence** from referring doctor, patient's comments or journal articles for all responses
**Suggested References:** Biol 1406 tutorial, NCBI, HHMI, Biotechniques, Science Direct

**1.** Regarding the patient's complaints and Dr. Kwak's notes, what is your diagnosis?

**2.** Why did Dr. Kwak's prescribe penicillin? (Describe the mechanism of action.)

**3.** Do you agree with Dr. Kwak's decision? If not, what would you have prescribed?

**4.** What do expect to "see" in a blood sample of someone experiencing an infection? Discuss the events along with the cells and molecules associated with an inflammatory response?

**(Q# 5-7) Define these terms and what they indicate to a physician when out of the normal range.**

**5.** Platelets (definition and diagnosis if low)

**6.** Hematocrit & Red blood cells & Hemoglobin (definition and diagnosis if low)

**7.** Granulocytes – Neutrophils, Monocytes, Eosinophils, Basophils
(definition and diagnosis if high)

**8.** What technique(s) do you think the Disease Busters technicians used to isolate and identify these individual blood cell types?

**9.** In addition to attempting to PCR amplify the bacterial 16S rRNA gene (variable genomic regions 3 and 4), what other ways could you identify this microbe?

**10.** In reviewing all of the report, including patient remarks, Dr. Kwak's notes and any other data provided, what do you think is wrong with this patient?

**Student Name:** _____

# II. Final Diagnosis and Prescription Report (post PCR analysis of 16S rRNA gene)

**Cite evidence** from referring doctor, patient's comments or journal articles for all responses
**Suggested References:** Biol 1406 tutorial, NCBI, HHMI, Biotechniques, Science Direct

**11.** What were your PCR 16S rRNA gene test results?

positive control:

experimental (patient's fluid):

negative control:

**12.** What would you conclude if the positive control did not work?

**13.** In addition to PCR amplifying the 16S rRNA gene's variable regions 3 and 4 (genomic) what other ways could you detect this microbe?

**14.** If the PCR results are positive for a bacterium, how would you identify the species? (Describe a technique.)

**15.** Based on the information available, including the PCR test results, what is your final diagnosis and what treatment(s) would you prescribe?

## REFERENCE DATA FOR PHYSICIANS ONLY!

### Normal Ranges of Blood Cell Counts for Healthy Adults and Children

(https://www.lls.org/managing-your-cancer/lab-and-imaging-tests/understanding-blood-counts)

| | Red Cells per µl blood | White Cells per µl blood | Platelets per µl blood | Hematocrit[1] % of blood composed of Red cells | Hemoglobin[1] g/dl |
|---|---|---|---|---|---|
| **Men** | 4.7 to 6.1 million | 5,000 to 10,000 | 150,000 to 400,000 | 42 to 52 | 14 to 18 |
| **Women[2]** | 4.2 to 5.4 million | 4,500 to 11,000 | 150,000 to 400,000 | 37 to 47 | 12 to 16 |
| **Children[3]** | 4.0 to 5.5 million | 5,000 to 10,000 | 150,000 to 400,000 | 32 to 44 | 9.5 to 15.5 |

[1]The ratio of hematocrit to hemoglobin is about 3 to 1.
[2]Normal ranges for women who are pregnant differ from these ranges.
[3]These ranges are for children from infancy to adolescence.

### White Cell Differential

Differential count, sometimes referred to as a "diff," is a breakdown of the different types of white cells. A white cell (WBC) differential also checks whether white cells appear normal. The five types of white cells and the approximate percentage they make up in the blood are:

- Neutrophils (55% to 70%)
- Band neutrophils (0% to 3%)
- Lymphocytes (20% to 40%)
- Monocytes (2% to 8%)
- Eosinophils (1% to 4%)
- Basophils (0.5% to 1%)

Until children are more than 4 years old, they have a higher percentage of lymphocytes in their blood than adults do.

### How Blood Cancers Affect Blood Counts

Blood cancers can affect blood cell counts in a number of ways, either lowering or increasing measurements. If you're currently receiving cancer treatment such as chemotherapy, drug therapy or radiation, your blood counts will be affected. Blood counts usually return to normal after treatment is complete.

## REFERENCE DATA FOR PHYSICIANS ONLY!

### Should You Keep Track of Your Blood Counts?

Some people want to know the results of their blood count tests so they can take preventive measures to protect their health or to what's causing their symptoms. For example:

- If you have anemia as a result of low red cell counts, you'll understand why you have low energy levels or are unable to carry out everyday tasks.
- If you have low white cell counts and develop a fever, you'll know to contact your doctor promptly.
- If your platelet counts are too low, you can bleed or bruise easily, so you may choose to avoid activities that have a risk of injury.

### Noncancerous Conditions

About 5 percent of healthy people will have test results outside of the "normal" range. If one or more of your blood cell counts is higher or lower than normal, your doctor will try to find out why. Many noncancerous conditions can contribute to low or high blood cell counts, such as those in the table below.

| | Red Cells | White Cells | Platelets |
|---|---|---|---|
| **High counts** | • Smoking<br>• Carbon monoxide exposure<br>• Chronic lung disease<br>• Kidney disease<br>• Certain forms of heart disease<br>• Alcoholism<br>• Liver disease<br>• Conditions that affect the body's fluid level | • Infection<br>• Inflammation<br>• Severe physical or emotional stress (such as fever, injury or surgery)<br>• Burns<br>• Kidney failure<br>• Lupus<br>• Rheumatoid arthritis<br>• Malnutrition, thyroid problems<br>• Certain medicines | • Bleeding<br>• Mild to moderate iron deficiency<br>• Problems with bone marrow function |
| **Low counts** | • Anemia from too little iron, folic acid or vitamin B12<br>• Bleeding<br>• Inflammatory bowel disease<br>• Other diseases that might cause malnutrition<br>• Certain drugs | • Infection<br>• Chemotherapy and other medicines<br>• Malaria<br>• Alcoholism<br>• AIDS<br>• Lupus<br>• Enlarged spleen | • Pregnancy<br>• Idiopathic thrombocytopenic purpura<br>• Thrombotic thrombocytopenic purpura<br>• Hemolytic uremic syndrome<br>• Autoimmune diseases |

**REFERENCE DATA FOR PHYSICIANS ONLY!**

**Normal Ranges of White Blood Cell Counts for Healthy Adults**

(http://clinicalgate.com/pediatric-and-geriatric-hematology)

| | TOTAL LEUKOCYTES | | NEUTROPHILS† | | | LYMPHOCYTES | | | MONOCYTES | | EOSINOPHILS | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | Mean | Range | Mean | Range | % | Mean | Range | % | Mean | % | Mean | % |
| Birth | –† | – | 4.0 | 2.0-6.0 | – | 4.2 | 2.0-7.3 | – | 0.6 | – | 0.1 | – |
| 12 hr | – | – | 11.0 | 7.8-14.5 | – | 4.2 | 2.0-7.3 | – | 0.6 | – | 0.1 | – |
| 24 hr | – | – | 9.0 | 7.0-12.0 | – | 4.2 | 2.0-7.3 | – | 0.6 | – | 0.1 | – |
| 1-4 wk | – | – | 3.6 | 1.8-5.4 | – | 5.6 | 2.9-9.1 | – | 0.7 | – | 0.2 | – |
| 6 mo | 11.9 | 6.0-17.5 | 3.8 | 1.0-8.5 | 32 | 7.3 | 4.0-13.5 | 61 | 0.6 | 5 | 0.3 | 3 |
| 1 yr | 11.4 | 6.0-17.5 | 3.5 | 1.5-8.5 | 31 | 7.0 | 4.0-10.5 | 61 | 0.6 | 5 | 0.3 | 3 |
| 2 yr | 10.6 | 6.0-17.0 | 3.5 | 1.5-8.5 | 33 | 6.3 | 3.0-9.5 | 59 | 0.5 | 5 | 0.3 | 3 |
| 4 yr | 9.1 | 5.5-15.5 | 3.8 | 1.5-8.5 | 42 | 4.5 | 2.0-8.0 | 50 | 0.5 | 5 | 0.3 | 3 |
| 6 yr | 8.5 | 5.0-14.5 | 4.3 | 1.5-8.0 | 51 | 3.5 | 1.5-7.0 | 42 | 0.4 | 5 | 0.2 | 3 |
| 8 yr | 8.3 | 4.5-13.5 | 4.4 | 1.5-8.0 | 53 | 3.3 | 1.5-6.8 | 39 | 0.4 | 4 | 0.2 | 2 |
| 10 yr | 8.1 | 4.5-13.5 | 4.4 | 1.8-8.0 | 54 | 3.1 | 1.5-6.5 | 38 | 0.4 | 4 | 0.2 | 2 |
| 16 yr | 7.8 | 4.5-13.0 | 4.4 | 1.8-8.0 | 57 | 2.8 | 1.2-5.2 | 35 | 0.4 | 5 | 0.2 | 3 |
| 21 yr | 7.4 | 4.5-11.0 | 4.4 | 1.8-7.7 | 59 | 2.5 | 1.0-4.8 | 34 | 0.3 | 4 | 0.2 | 3 |

**Student Name:** _____

**DIAGNOSIS REPORT RUBRIC**
**on your 1st patient's illness**

| GRADE/ CATEGORY | 100-90 | 90-80 | 80-70 | 70-60 |
|---|---|---|---|---|
| **1st PATIENT DIAGNOSIS REPORT (100 pts)** | | | | |
| answers formatted in arial font, 12 size, 1.5 spacing | | | | |
| factual answers, with references, for Q# 1-7 and 11, 12 | detailed, accurate & comprehensive answers (5 pts) | content is not comprehensive (missing 1-2 responses) | content is not comprehensive (missing 3-4 responses) | content is not comprehensive (missing ≥ 5 responses) |
| creative (somewhat feasible) answers for Q# 8-10 and 13-15 | | | | |
| followed protocol when performing the pcr-amplification | diagnosis was reasonable and well supported | not all supportive points for diagnosis were reasonable | diagnosis was inaccurate and not well founded | both diagnosis and arguments were very poor |
| got the expected results (at least for the negative & positive controls) | | | | plagiarism; poor grammar or spelling |
| accuracy of your final diagnosis | | | | |

**DAY 2: "MICRO-PIPETTING A SOLUTION" LESSON (41 min)**
**Objectives: students will be able to …**

Answer a set of problems converting volume in metric system
(ie. $10^6$ micro-liter (µl) equal $10^0$ liter (l)

Discuss the advantages of working with in micro-liter volumes versus liters

Perform transfer of various micro-liter volumes with the micro-pipette

Read the absorbance values on a spectrophotometer and graph results

**Aim:** How do we use a micro-pipette and dispense varying micro-liter amounts of a solution?

**1st 5' (do now)** Students work on a small sample set of problems involving conversions between micro-liters (µL) and other volumes.

**A** 1 l = ____ ml **B** 10 ml = ____ µl **C** 1,000,000 µl = ____ l

**D** 0.01 l = ____ µl **E** 150 ml = ____ µl

**2nd 25' micro-pipette practice procedure**

Materials: 10x 20-200 µL micro-pipette (carolina, cat#214627), 1-200 µl pipette tips (fisher scientific, 02-707-419), white absorbent paper, metric rulers, graphing paper

I. Review 20-200 micro-liter (µl) pipette parts (students follow along)

"mouth" at the bottom of the pipette and is where the tip is affixed

"window" where you see the volume readings (ie. 20.0 = 20 µl, 120.0 = 120 µl)

"wheel" above the window and is turned to the left or right to adjust the volume of liquid to be transferred

"plunger" at the top of pipette and moves up (to aspirate a liquid) and down (to expel a liquid)

"tip" (not part of pipette) it's affixed onto the mouth of the pipette and discarded after one use

II. Transferring varying volumes of a red dye solution (students follow along)

Adjust micro-pipette wheel to the desired volume (displayed in the window).

Affix the pipette's tip onto the "mouth" of the pipette.

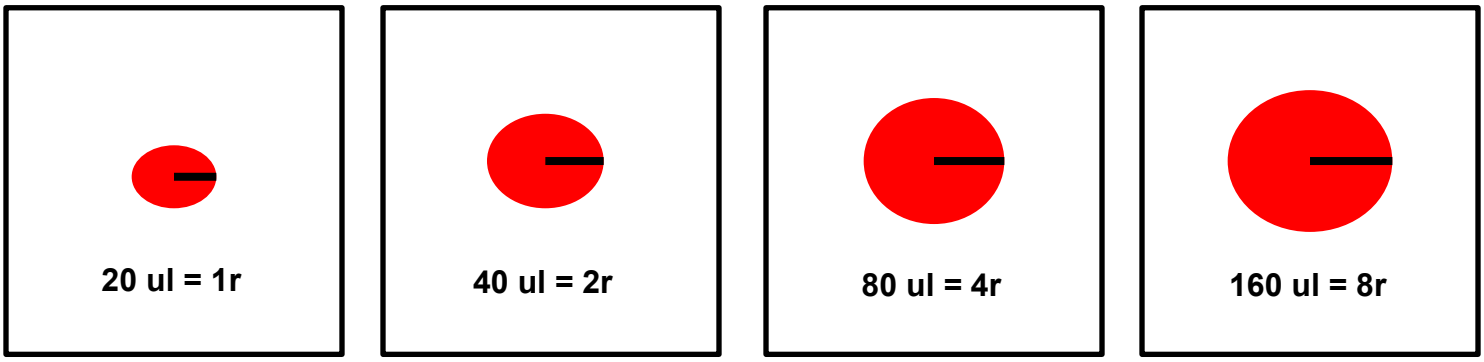
Grasp the micro-pipette with the thumb placed over the plunger.

Push down on the plunger until you feel resistance and stop; hold plunger at that position.

Place the tip just below the surface of a test tube's solution and release plunger to aspirate (Observe the solution within tip).

Place tip onto target area and push plunger down to expel solution (Discarding the tip is not necessary for this practice).

Students pipette increasing amounts of red dye (20 µl, 40 µl, 80 µl, 160 µl) into four separate areas onto a sheet of absorbent paper.




**Theoretically the radius (r) should increase proportionally with the increasing volumes of red dye you dispense.**

Students determine your pipetting accuracy by measuring area of the dye on the paper which should be directly proportional to the volume they are pipetting (circle's area = 3.14 x radius$^2$).

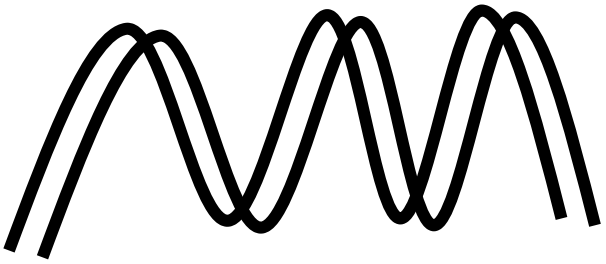
Students graph results (horizontal-axis "amounts of red dye" vs vertical-axis "area of dye"). *Slope should be close to 1.0 (change in rise divided by change in run).

**3rd 11'** Students share out graphing results (slope should equal one anywhere along line) and how it reflects the accuracy of their pipetting. Students further share out reasons explaining why their graph was not perfect (ie. solution got stuck in tip, plunger went beyond point of resistance, area of dye on paper was not perfectly shaped as a circle).

**Homework:** complete homework on the polymerase chain reaction process.

# Polymerase Chain Reaction Amplification of DNA

**1** Draw & Describe what happens to this DNA molecule (see below) at each of the thermocycling temperatures 95°C, 55°C, 72°C.



| | DRAW | DESCRIBE |
|---|---|---|
| **95°C:** | | |
| **55°C:** | | |
| **72°C:** | | |

**2A** How many molecules of DNA will you have after 2 cycles? **2B** After 3 cycles?

**3** Compare and Contrast the pcr reaction to what occurs naturally within the cell.

**DAY 3: "PERFORMING A POLYMERASE CHAIN REACTION" LESSON (41 min)**
***Note:** *After gel electrophoresis,* students are expected to submit a final patient diagnosis and prescription report; at that time, the teacher will reveal to them the actual diagnosis for their assigned patient.*

**Objectives - students will be able to …**

Explain the purpose of the polymerase chain reaction (pcr) and it's many applications.

Explain the relevance of the three thermocycling temperatures (98$^{o}$C, 65$^{o}$C, 72$^{o}$C).

Discuss the factors involved in determining a thermocycling-annealing temperature.

Perform micropipetting and thermocycling of a pcr amplification.

**Aim:** How do we perform a polymerase chain reaction?

**1$^{st}$ 5' (do now)** Students explain the relevance of the three thermocycling temperatures (98$^{o}$C, 65$^{o}$C, 72$^{o}$C) within their team.

**2$^{nd}$ 25' performing polymerase chain reaction (see next page for protocol)**

**3$^{rd}$ 11'** students take an open-ended response quiz

students work on worksheet on gel electrophoresis analysis

**Homework:** Students work on 5 point summary for teacher-assigned reading "16S ribosomal RNA gene" background information and explains why this region was targeted (sequence variability correlates with different bacterial species). (*reference: https://en.wikipedia.org/wiki/16S_ribosomal_RNA)*

Students think of ways this approach to identifying bacteria could be used in research (clarify taxonomy within bacteria domain; determine purity of water or various foods; better treat infected animals or plants).

**PCR amplify 630 bp amplicon of 16S rDNA (students perform)**

PCR & Thermocycling protocols: https://www.thermofisher.com/order/catalog/product/K0171

Materials: thermocycler (thermofisher scientific-Applied Biosystems), PCR mix (thermofisher scientific), 2-20 µl and 20-200 µl micro-pipettors (carolina), 1-200 µl micro-pipette filter tips (thermofisher scientific), PCR primers (invitrogen cat# 10336022-254528-C10, 10336022-254528-D07)

16S Amplicon PCR Forward Primer = 5'TCGTCGGCAGCGTCAGATGT GTATAAGAGACAG CCTACGGGNGGCWGCAG

16S Amplicon PCR Reverse Primer = 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC

**Procedure:**

Student teams pipette components along with a sample of their fictional patient's bodily fluid (10 µl of actual bacterial genomic DNA or water). Each team perform 3 pcr amplifications in separate 0.2 ml pcr-tubes ("patient's bodily fluid" is experimental tube (#5, 8 are positive); "positive control" contains bacterial 16S rRNA gene; "negative control" does not contains bacterial 16S rRNA gene). Note: keep all reagents on ice.

| | |
|---|---|
| 2x PCR Master Mix (w/ enzyme) | 25 µL |
| Forward primer | _____ (0.1-1.0 µM) |
| Reverse primer | _____ (0.1-1.0 µM) |
| **Patient fluid (DNA isolated)** | **2 µL (10 pg - 1 µg)** |
| Water (nuclease-free) | _____ (bring up to 50 µL total volume) |

***Note:** The blanks will vary in volume depending on the concentration of the primers and DNA isolated from the patient's bodily fluids; your teacher will provide you with this information.*

**Thermocycling (teacher performs)**

Initial denaturation 95°C - 3 min

**25 cycles x [**Denature 95 °C - 30 sec, Anneal 55°C - 30 sec, Extend 72 °C - 30 sec**]**

Final extension 72 °C - 5 min

Store 4 °C - overnight

***Note:** Patient profiles #5, 8 will be given 10 µL of bacterial genomic DNA and get a positive result (630 bp amplicon) for the pcr-amplification. Students given patient profiles #1-4, 6, 7, 8 will be given 10 µL of water and get a negative result but will see the 630 bp amplicon in the positive control test tube.*

**DAY 3: QUIZ "PCR amplification"**
**STUDENT NAME:**_____

**1.** Why do biologists perform polymerase chain reaction (pcr)?

**2.** Explain what happens to a targeted DNA molecule at these three temperature settings during a typical pcr reaction.

98 $^{o}$C:

65 $^{o}$C:

72 $^{o}$C:

**3.** Discuss one application of the pcr process in biology.

**DAY 4: "GEL ELECTROPHORESIS" LESSON (82 min – two periods)**
**Objectives - students will be able to …**

Explain the three factors that determine how fast and in which direction a molecule will migrate during gel electrophoresis.

Provide an analysis of their pcr amplification results.

Make suggestions to problem solve the pcr amplification process if their positive control fails.

**Aim:** How do we interpret a gel electrophoresis result?

**1st 10'** Students micro-pipette the loading buffer into their three test tubes. (Note: Teacher prepares the molecular weight standard.) Then, place it into the agarose gel wells and place the gel into the buffer-containing electrophoresis box and turn on the voltage.

**2nd 30'** Students explain the three factors that determine how fast and in which direction a molecule will migrate during gel electrophoresis.

**3rd 15'** Administer a formative diagnostic in the form of a short-essay response quiz on the molecular biology techniques – PCR.

**4th 27'** Student teams analyze results (630 bp 16S rRNA amplicon in positive control indicates experiment worked; 630 bp 16S rRNA amplicon in experimental indicates bacterial infection; no 630 bp 16S rRNA amplicon in experimental indicates a different problem for patient).

**Homework:** Students continue to work on patient profile report.

**Load PCR samples (students perform)**

1. Pipette 5 µl 6x gel loading dye into 1.5 ml micro-centrifuge tube.

2. Add 25 µl from PCR tube #1 into same tube.

3. Pipette 30 µl of this mixture into the corresponding well of the submerged gel.
(Repeat these steps for PCR tubes #2 and #3)

4. Pipette the already prepared 30 µl Lambda DNA x EcoRI/HindIII molecular weight markers into the next well.

**Electrophorese & Visualize (teacher performs)**

1. Place lid onto gel box, plug leads into box and power supply.

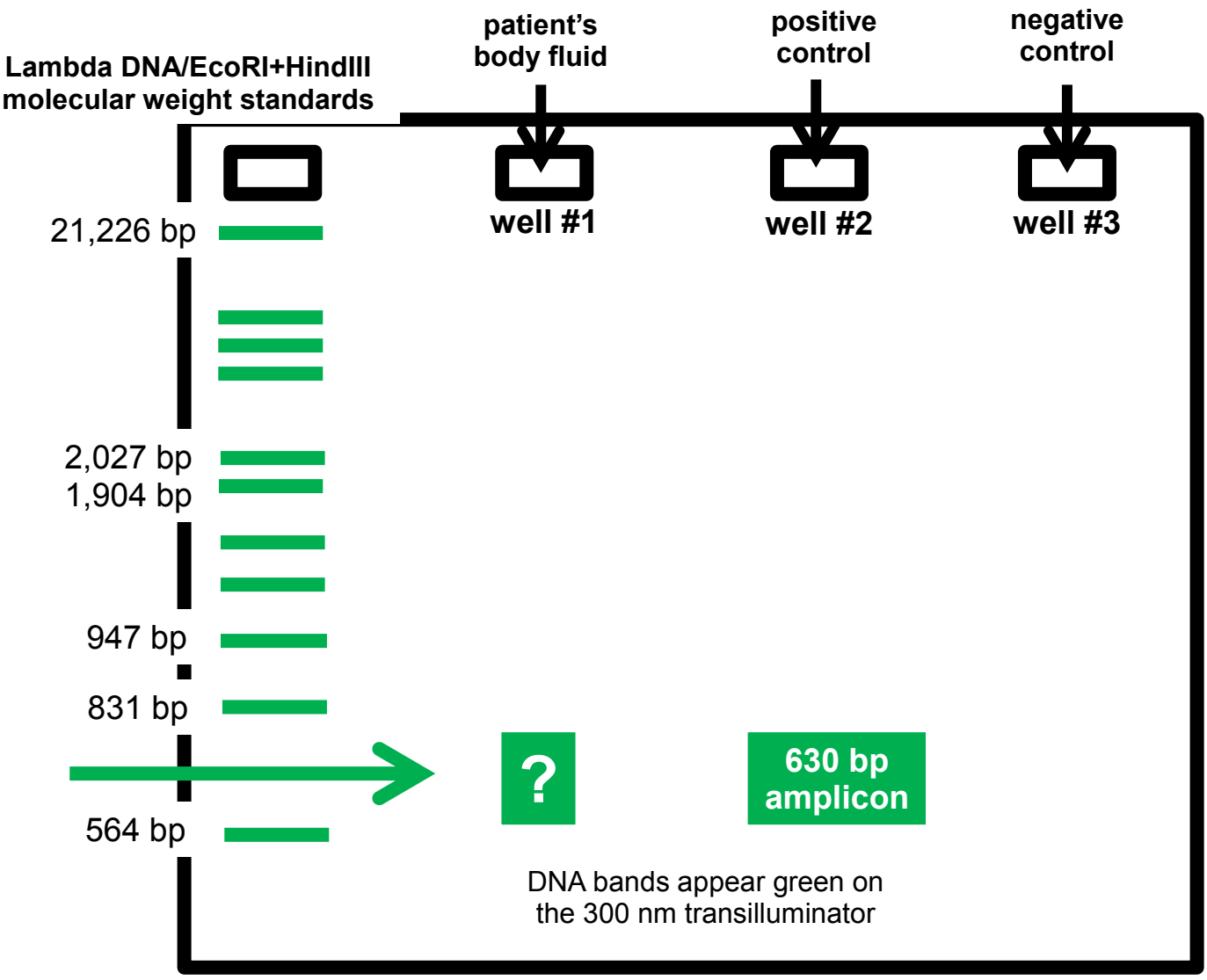
2. Turn on voltage to 125 V – 30 min.

3. After run is complete, place gel onto 300 nm transilluminator.

4. Place transparent protective lid over gel.

5. Turn on transilluminator to visualize.

6. Photograph gel results.


Lambda DNA/EcoRI+HindIII
molecular weight standards
patient's
body fluid
positive
control
negative
control
well #1
well #2
well #3
21,226 bp
2,027 bp
1,904 bp
947 bp
831 bp
564 bp
?
630 bp
amplicon
DNA bands appear green on
the 300 nm transilluminator

**DAY 4: "GEL ELECTROPHORESIS"**
**STUDENT NAME:_____**

**1.** Discuss the <u>three</u> ways that gel electrophoresis separates out a mixture of molecules.

1st way:

2nd way:

3rd way:

**2.** Describe your results today; did your patient test positive for the bacterial 630 bp 16S rRNA gene?

**3.** Regardless of your results, what might be one reason why a pcr amplification process did not work?

**DAY 5: "FIGHTING ANTIBIOTIC-RESISTANT BACTERIA" LESSON (41 min)**
***Note:** After gel electrophoresis, students are given restriction endonuclease mapping of plasmid that carries beta-lactamase to determine if Haemophilus influenza is resistant.*

**Objectives - students will be able to …**
Explain how bacteria have evolved the ability to resist antibiotics (penicillin) via beta-lactamase gene on its plasmid.

Describe specificity of beta-lactamase enzyme on only penicillin and no other antibiotics.

**Aim:** How have bacteria evolved the ability to resist antibiotics (penicillin)?

**1st 15'** Handout the same patient profile to each student team. The profile contains information on patient's symptoms and the lab report; it's confirmed to be bacterial (*Haemophilus influenza*), normally responsive to penicillin, but not this time!

**2nd 26'** Students perform an endonuclease digestion (EcoRI and HindIII) on isolated plasmid from this bacterium in order to determine if the plasmid is carrying the penicillin-resistant gene, beta-lactamase.

**Homework:** Students review properties of enzymes and specifically restriction endonucleases from assigned textbook chapters or online tutorials.

**REFERENCES *Haemophilus influenza*:**
1. "Signs and Symptoms". Centers for Disease Control and Prevention (CDC).

2. Puri J; Talwar V; Juneja M; Agarwal KN; et al. (1999). "Prevalence of antimicrobial resistance among respiratory isolates of Haemophilus influenzae". Indian Pediatr. 36 (10): 1029–32.

3. Kuhnert, P; Christensen, H, eds. (2008). Pasteurellaceae: Biology, Genomics and Molecular Aspects. Caister Academic Press.

**Restriction endonuclease digestion (students perform)**
Reference: http://www.thermofisher.com/us/en/home.html

Materials: EcoRI (thermofisher scientific, cat# ER0275, 10 U/µl),
HindIII (thermofisher scientific, cat# ER0505, 10 U/µl), 10x Buffer R (thermofisher scientific, cat# BR5), 1-20 µl & 20-200 µl micro-pipettes,1-200 µl pipette tips, 1.5 ml micro-centrifuge tubes (fisher scientific)

**Procedure:** Student teams perform three digests (see below)

tube #1 = plasmid x EcoRI (yields 4,539 bp linearized DNA fragment)
tube #2 = plasmid x HindIII (yields 4,539 bp linearized DNA fragment)
tube #3 = plasmid x EcoRI, HindIII (yields 2635 bp and 1904 bp linearized fragments)
**tube #4 = undigested plasmid (yields nick and supercoiled forms of 4,539 bp DNA)*

*One unit is defined as the amount of enzyme required to digest 1 µg lambda DNA in 1 hour at 37°C in 50 µl of recommended reaction buffer.*

**EcoRI** G^AATTC sites
nuclease-free water 12 µl
DNA (0.5-1 µg/µl) 5 µl
10X Buffer (EcoRI) 2 µl
10U/µl EcoRI 1 µl

37°C for 20 min to digest
65°C for 20 min to inactivate
Store at -20°C

**HindIII** A^AGCTT sites
nuclease-free water 12 µl
DNA (0.5-1 µg/µl) 5 µl
10X Buffer (HindIII) 2 µl
10U/µl HindIII 1 µl

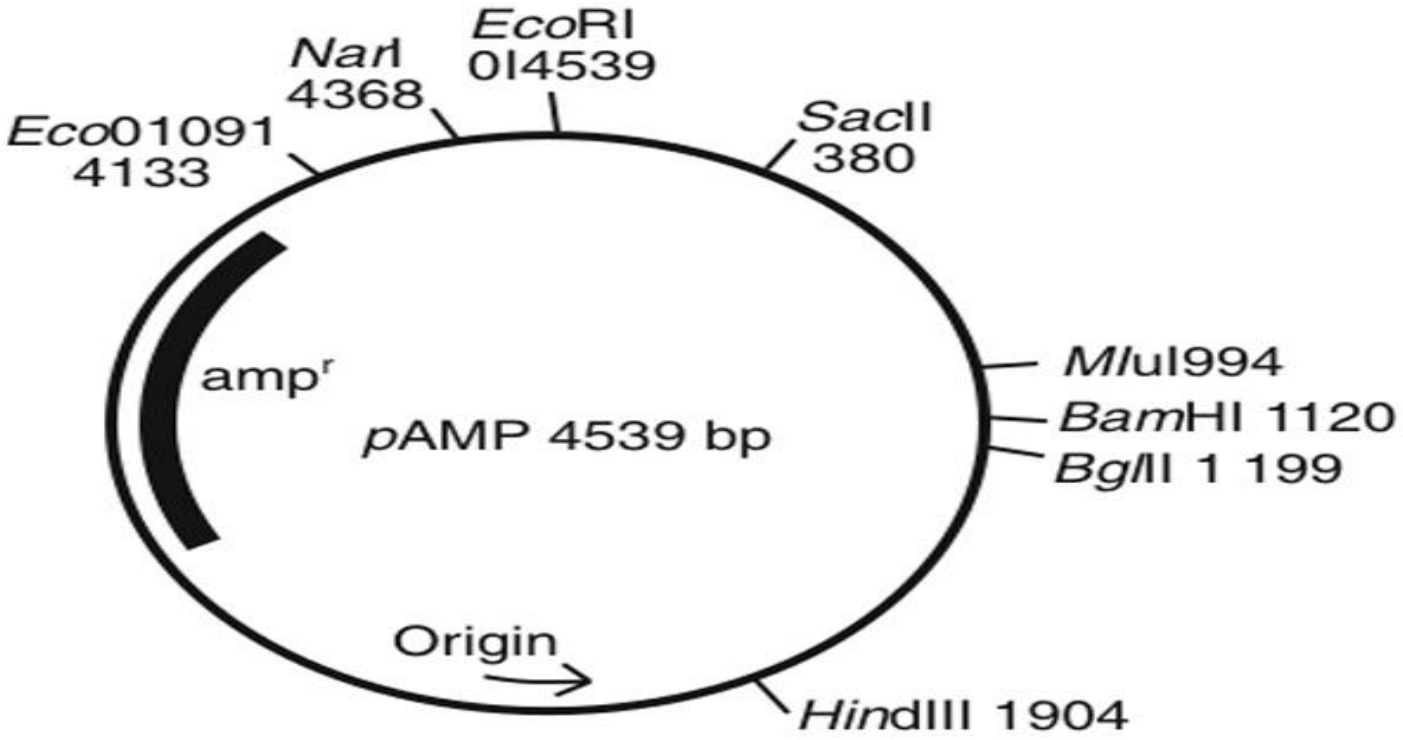
37°C for 20 min to digest
65°C for 20 min to inactivate
Store at -20°C

**EcoRI, HindIII** A^AGCTT sites
nuclease-free water 11 µl
DNA (0.5-1 µg/µl) 5 µl
10X Buffer R 2 µl
10U/µl EcoRI 1 µl
10U/µl HindIII 1 µl

37°C for 20 min to digest
65°C for 20 min to inactivate
Store at -20°C

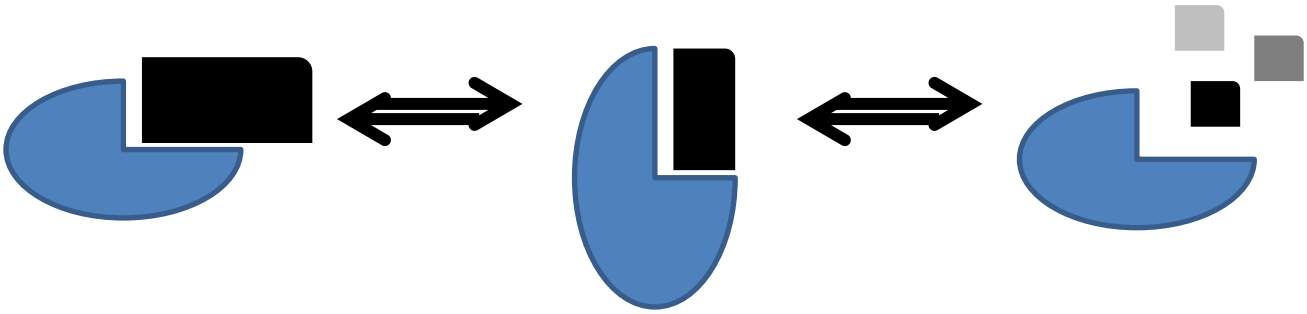
**DAY 5: FIGHTING ANTIBIOTIC-RESISTANT BACTERIA**
**Student Name: _____**

**RESTRICTION ENDONUCLEASE MAP OF AMPICILLIN-CARRYING PLASMID**



**Homework "Restriction Endonucleases"**

**Student Name:** _____



1. Describe 4 generic features associated with an enzyme. (See above diagram for help).

**1** **3**

**2** **4**

2. Discuss 2 specific features associated with a restriction endonuclease.

**1**

**2**

3. What purpose do restriction endonucleases serve the bacterium?

4. Discuss 2 applications of restriction enzymes in the laboratory.

**1**

**2**

**DAY 6: "GEL ELECTROPHORESIS" LESSON (82 min – two periods)**
***Note:** *<u>*After gel electrophoresis,*</u>* students are given restriction endonuclease mapping of plasmid that carries beta-lactamase to determine if Haemophilus influenza is resistant.*

**Objectives - students will be able to …**
Explain how bacteria have evolved the ability to resist antibiotics (penicillin) via beta-lactamase gene on its plasmid.

Describe specificity of the beta-lactamase enzyme on only penicillin and not other antibiotics

predict where digested plasmid DNA will migrate during gel electrophoresis.

**Aim:** How do we interpret a gel electrophoresis result?

**1st 30'** Students micro-pipette the loading buffer into the three test tubes of digested plasmid (test tube #4 is undigested plasmid, test tube #5 is molecular weight marker) and then into the agarose gel's wells. Teacher places gel into the buffer-containing electrophoresis box and turns on the voltage

**2nd 30'** Administer a formative diagnostic, restriction endonuclease worksheet

Students continue to work on final patient diagnosis report

**3rd 22'** Students visual electrophoresis results with aid of teacher

students analyze electrophoresis results with use of plasmid's restriction endonuclease map (provided by teacher), to determine if *Haemophilus influenza* is penicillin resistant.

**Homework:** Students continue to analyze gel electrophoresis results

Students continue to work on final patient diagnosis report

**Load plasmid digested samples (students perform)**

1. Pipette 4 µl 6x gel loading dye into 1.5 ml micro-centrifuge tube.

2. Add 20 µl from digest test tube #2 into same tube.

3. Pipette 24 µl of this mixture into the corresponding well of the submerged agarose gel. (repeat these steps for PCR tubes #3, #4)

4. Pipette the already prepared 24 µl Lambda DNA x EcoRI/HindIII molecular weight markers into a nearby well.

5. Pipette the already prepared 24 µl undigested plasmid into a second nearby well.

**Electrophorese & Visualize (teacher performs)**

1. Place lid onto gel box, plug leads into box and power supply.

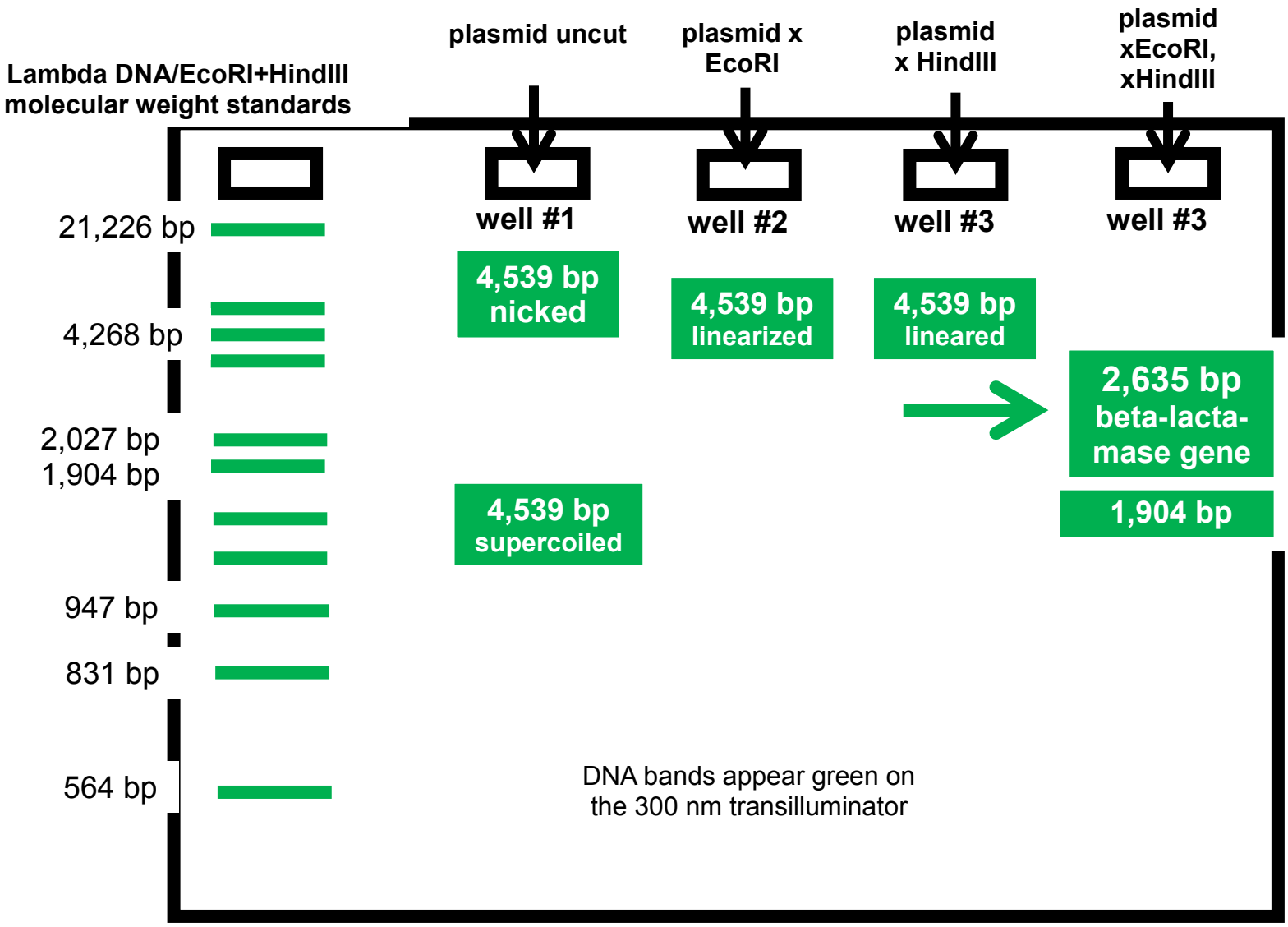
2. Turn on voltage to 125 V for 30 min.

3. After run is complete, place gel onto 300 nm transilluminator.

4. Place transparent protective lid over gel.

5. Turn on transilluminator to visualize.

6. Photograph gel results.


Lambda DNA/EcoRI+HindIII
molecular weight standards
plasmid uncut
plasmid x
EcoRI
plasmid
x HindIII
plasmid
xEcoRI,
xHindIII
well #1
well #2
well #3
well #3
21,226 bp
4,268 bp
2,027 bp
1,904 bp
947 bp
831 bp
564 bp
4,539 bp
nicked
4,539 bp
linearized
4,539 bp
lineared
2,635 bp
beta-lacta-
mase gene
1,904 bp
4,539 bp
supercoiled
DNA bands appear green on
the 300 nm transilluminator

**DAY 7: "FIGHTING ANTIBIOTIC-RESISTANT BACTERIA" LESSON (41 min)**
***Note:** *<u>After gel electrophoresis,</u>* students are given restriction endonuclease mapping of plasmid that carries beta-lactamase to determine if Haemophilus influenza is resistant.*

**Objectives - students will be able to …**
Explain how bacteria have evolved the ability to resist antibiotics (penicillin) via beta-lactamase gene on its plasmid.

Describe specificity of the beta-lactamase enzyme on only penicillin and not other antibiotics.

Explain why penicillin is no longer effective against most bacterial species after excessive use for too many years.

Discuss other means of eliminating bacteria from the host (such as kanamycin, vaccine, or a yeast-probiotic).

**Aim:** How have bacteria evolved the ability to resist antibiotics (penicillin)?

**1st 11' (do now)** Students discuss and share out the basic elements of evolution (random mutation, natural selection favoring new phenotype, survive and reproduce).

**2nd 30' A** Demonstrate the results of inoculating the MM294/pAMP E. coli slant culture into three different test tubes of media alone (expect growth as a negative control), with penicillin (expect growth because ampicillin is related to penicillin via beta-lactam ring), with kanamycin (expect <u>no</u> growth because beta-lactamase can<u>not</u> block kanamycin's ability to inhibit protein synthesis via binding to the ribosomal 30S subunit).

**B** Review with students how penicillin-resistant bacteria (beta-lactamase hydrolyzes penicillin's beta-lactam ring open).

**C** Students share out ways ampicillin resistance spreads throughout a population of bacteria (sexual reproduction – share plasmid carrying the beta-lactamase gene).

**Homework:** Students work on "antibiotic-resistant evolving bacteria" worksheet.

Students continue to work on completing patient's final report on patient's diagnosis and prescription.

**Living Environment - "He's Back"**

**Team's Name:** _____

**Patient's name:** Hyme Styl Syk

**What is your major complaint?** fever, very weak, and ache all over

**How long have you had this condition?** on and off for months

**Have you experienced this condition in the past?** for a while now

**What do you think caused this condition?** It's from last winter

**Dr. Areal Kwak (primary physician) notes:** Mr. Syk is 78 years old and is a regular at my office, coming in for a variety of reasons a few times a year. I often prescribed a low dose of penicillin for him, and it's always successful. This time though it appears that he is only getting worse. I hesitate to give him a larger dose because of the increased risk of adverse side effects but I don't what else to do.

**Disease Buster Report:** Patient tested positive for 16S rRNA, so the illness is definitely bacterial and apparently penicillin resistant.

**Your Next Step:** Characterize this new and scary bacteria by isolating its plasmid DNA. (Your teacher has performed this step.) Then, perform a restriction endonuclease digest with Eco RI & HinDIII mapping to identify the beta-lactamase gene. If beta-lactamase gene is not released. (See restriction enzyme map for calculated size.) Inform Dr. Kwah to go ahead with the higher dose of penicillin. If though the gene is resistant, you're going to have to prescribe a different antibiotic altogether.

**REFERENCES *Haemophilus influenza*:**

1. "Signs and Symptoms". Centers for Disease Control and Prevention (CDC).

2. Puri J; Talwar V; Juneja M; Agarwal KN; et al. (1999). "Prevalence of antimicrobial resistance among respiratory isolates of Haemophilus influenzae". Indian Pediatr. 36 (10): 1029–32.

3. Kuhnert, P; Christensen, H, eds. (2008). Pasteurellaceae: Biology, Genomics and Molecular Aspects. Caister Academic Press.

## Disease Busters' laboratory report

**Background:** Penicillin inhibits formation of peptidoglycan cross-links in bacterial cell wall via binding to the four-membered β-lactam ring of penicillin to the enzyme DD-transpeptidase. As a result the bacteria cannot maintain a wall and the increasing osmotic pressure eventually leads to cell death. (While the number of penicillin-resistant bacteria is increasing, penicillin can still be used to treat a wide range of infections caused by certain susceptible bacteria, including Streptococci, Staphylococci, Clostridium, and Listeria genera.)

Haemophilus influenzae is a gram-negative, pathogenic bacterium. This species was the first free-living organism to have its entire genome sequenced. It is opportunistic in that it will only cause harm to the host under a certain scenario such as a viral infection or reduced immune function. It infects the upper-respiratory tract initiating flu-like symptoms such as a low-grade fever, coughing, shortness of breath and fatigue. It typically responds favorably to penicillin!

**1.** Discuss the gel electrophoresis results of your digests below…
**Tube #1** (plasmid x EcoRI) =

**Tube #2** (plasmid x HindIII) =

**Tube #3** (plasmid x EcoRI, HindIII) =

**Tube #4** (undigested plasmid) =

**2.** Based on the results, what will you prescribe for Mr. Syk?

**3.** How do you think future scientists will battle antibiotic-resistant bacteria?

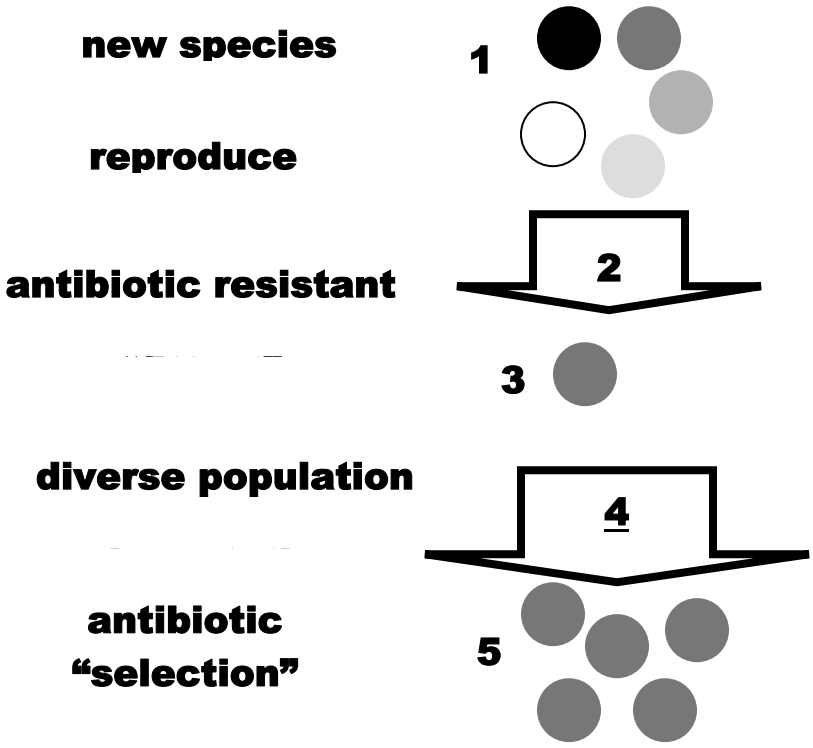
**DIAGNOSIS REPORT RUBRIC**
**on your 2nd patient's illness**

| GRADE/ CATEGORY | 100-90 | 90-80 | 80-70 | 70-60 |
|---|---|---|---|---|
| **2nd PATIENT DIAGNOSIS REPORT (100 pts)**<br>answers formatted in arial font, 12 size, 1.5 spacing<br><br>factual answers, with references, for Q# 1-7 and 11, 12<br><br>creative (somewhat feasible) answers for Q# 8-10 and 13-15<br><br>followed protocol when performing the restriction endonuclease digests<br><br>got the expected digests results (uncut, EcoRI, HindIII,EcoRI/HindIII)<br><br>accuracy of your final diagnosis | detailed, accurate & comprehensive answers (5 pts)<br><br>diagnosis was reasonable and well supported | content is not comprehensive (missing 1-2 responses)<br><br>not all supportive points for diagnosis were reasonable | content is not comprehensive (missing 3-4 responses)<br><br>diagnosis was inaccurate and not well founded | content is not comprehensive (missing $\geq$ 5 responses)<br><br>both diagnosis and arguments were very poor<br><br>plagiarism; poor grammar or spelling |

**Student Name:** _____

**"Antibiotic-resistant evolving bacteria" worksheet**

Connect 5 terms below to describe this evolving bacteria
(one circle represents $10^3$ individuals).




**Directional selection** describes a population's individuals moving toward a phenotype reflective of a new and favored allele; discuss two more examples of this phenomenon (Cannot use the one above involving bacteria).

**1**

## 2

**Your teacher demonstrated to you how the penicillin-resistant bacteria, *Haemophilus influenza,* responded to the three test tubes of media treated with varying antibiotics.**

Give reasons for the results described below…

**3.** Test tube #1 Luria Broth alone = bacterial growth

**4.** Test tube #2 Luria Broth + ampicillin = bacterial growth

**5.** Test tube #3 Luria Broth + tetracyclin = no bacterial growth

**6.** Discuss one way a bacterium can become resistant to an antibiotic.

**7.** Why wasn't the beta-lactamase effective with the tetracycline?

**8.** Discuss two general ways man battle an antibiotic-resistant bacterium.

**DAY 8: "CONFERENCE: PATIENT'S FINAL DIAGNOSIS" LESSON (41 min)**
***Note:** Students are given a grading rubric for the poster presentation portion of this project.*

**Objectives - students will be able to …**
Orally present information regarding their patient (initial and final diagnoses and prescription).

Summarize new information, from their classmates, about how the inflammation is triggered from bacterial and viral infections, as well as toxic poisoning and cancer that they learned.

Communicate information to their classmates both verbally and with the use of their poster.

**Aim:** How do we effectively communicate information about infection to others?

**1st 1'** Students arrange seats in semi-circle formation and set up their posters.

**2nd 30' – first 15'** At least one student from the team visits at least five posters and writes down a summary of information (disease's microbe, symptoms, prescription).

**- second 15'** Other students from the teams visit at least five posters and writes down a summary of information (disease's microbe, symptoms, prescription).

**3rd 10'** Students share out what they learned from the applications of the molecular biology techniques to diagnose and treat a patient's illness.

Students submit their doctor's report and poster along with the written summaries from the conference.

# RESEARCH CONFERENCE (POSTER & PRESENTATION) RUBRIC

**on your patient's illness** *(teacher will reveal to you patient's actual diagnosis)*

| GRADE/ CATEGORY | 100-90 | 90-80 | 80-70 | 70-60 |
|---|---|---|---|---|
| **POSTER (75 pts)**<br>*teacher provides poster board*<br><br>**TITLE (5 pts)**<br>A) name of illness B) picture of inflammatory response C) date, period#, team's names | all information is present | missing 1 item of information | missing 2 items of information | missing title page |
| **BIBLIOGRAPHY (5 pts)**<br>(5 references) | ≥5 current and reputable publications | 3-4 current and reputable publications | 1-2 current and reputable publications<br><br>but also blogs | not use enough references to support findings<br><br>but also blogs |
| **CONTENT (65 pts)**<br>(1) mechanism of infection<br>(2) inflammatory response (changes in blood cell count)<br>(3) host's symptoms<br>(4) diagnosis technique<br>(5) treatment | detailed, accurate & comprehensive information (5 pts)<br><br>major points are addressed and well supported | content is not comprehensive (missing 1-2 pts)<br><br>not all major points were addressed | content is not comprehensive (missing ≥ 3 pts)<br><br>few major points were addressed | content is not comprehensive (missing ≥ 4 pts)<br><br>few major points are addressed<br><br>plagiarism; poor grammar or spelling |
| **5 min PRESENTATION (25)**<br>loud & clear voices, good eye contact, accurate information, creative poster (colors, big print, engaging pictures) | loud, clear voices with eye contact;<br><br>accurate information and entertaining | weak delivery with little eye contact<br><br>inaccurate information but entertaining | poor delivery no eye contact<br><br>inaccurate information and not entertaining | <5' and poor delivery no eye contact<br><br>inaccurate or little information and not entertaining |

**STUDENT SECTION**

## I. Rationale

### Introduction

This laboratory-based lesson is aimed at reviewing some of the more salient concepts in immunology, such as the various types of environmental factors that can induce the inflammatory effect, the mechanisms underlying inflammation and the power of biotechnology (polymerase chain reaction and gel electrophoresis) in determining the cause of the inflammation.

Your body's immune system is designed to protect you from all kinds of things from the environment including microbes such as pathogenic bacteria or viruses, toxins, and even the onset of cancer. Symptoms such as sneezing, coughing, or fever are some of the common events associated with inflammation; they are your body's means of eliminating the environmental threat. Fever, induced by a bacterium's lipopolysaccharide or an activated leukocyte's interleukin-1 followed by prostaglandin-E2 enhances motility, phagocytosis or proliferation of various innate and adaptive white blood cells. Swelling further aids in our battle against infection. Here, a macrophage's released vasoactive amine (i.e. histamine) induces the vasodilation and permeability of blood vessels, enabling antigen presenting cells to enter the site of infection from circulation and phagocytose the pathogen. Some of the more predominant white blood cells (leukocytes), from the body's second line of defense, include macrophages, dendritic cells, Thelper- and Tregulatory-cells. Their job is to eliminate the pathogen and at the same time induce clonal expansion of specific B- and T-cells, members of the third and final line of defense. As complex and effective as these defense mechanisms are, they fail many times to eliminate the enemy, which is why we depend heavily on scientists and their research to design new and more effective antibiotics, vaccines, or other therapeutics to aid us in recovering from environmental threats.

In a follow up case, students will read about another patient who is infected with a bacterium, who normally responds to penicillin, but for an unknown reason is not this time and is getting worse. Here, the students will perform a restriction endonuclease digestion (EcoRI and HindIII) on the bacteria's plasmid DNA. If during the gel electrophoresis a 2635 bp band is released (see plasmid's restriction enzyme map.) It will confirm that this pathogen has evolved penicillin resistance, in the form of beta-lactamase (if not, it just may be that the penicillin was bad.) The band will appear, and students will be asked to suggest alternate antibiotic treatments. The next day, the teacher will display test tubes of cultures from a fictional laboratory where bacterial growth is only apparent in the penicillin-treated broth but not the other test tubes carrying different antibiotics such as kanamycin. After viewing this phenomenon, students will work on a worksheet that prompts thinking and discussion about evolution of penicillin resistance at the level of the species as well as on a molecular level.

**Overview**
In the next few days, your team (2-4 students) will be representing the "Disease Busters," a very promising diagnostic research center comprised of medical professionals.

**The first day,** you will receive a troubling report from a physician, Dr. Kwak. The report will provide you with valuable information regarding the patient's physical description and symptoms along with Dr. Kwak's observational notes. In addition, you will have access to a complete blood profile of the patient that was generated by your technicians. In light of this information, you will make an initial diagnosis. You will hold off prescribing a treatment for your new patient because there just is not enough information to be 100% certain of the illness.

**The second day,** your team will confirm if Dr. Kwak's original diagnosis, a bacterial infection, is accurate by performing a polymerase chain reaction (pcr) and gel electrophoresis analysis. The first day of this laboratory exercise is to practice your micro-pipetting skills with the use of a 20-200 µl pipette and red-colored dye. After pipetting various amounts of the dye onto a sheet of paper, you will prepare a graph of the data (horizontal-axis "amount of red dye" vs vertical-axis "area of dye") to determine your pipetting accuracy. If the calculated slope, for the graphed data, is close to 1.0 (change in rise divided by change in run) your team will move on to perform actual pcr reactions on a sample of your patient's body fluid!

**The third day,** you will perform a pcr-amplification of the targeted 16S ribosomal RNA gene, a commonly used marker for the presence of bacteria. The results, 630 base pair (bp) amplicon, will not tell you what species of bacteria you are dealing with. If your test results are negative you will have to research other possibilities (virus, poison, cancer, or allergen) before prescribing a treatment for your patient.

**The fourth day,** you will perform gel electrophoresis of your pcr results from day three. There will be four lanes of samples (a molecular weight marker, patient's body fluid, a negative control from a healthy person and DNA from 16S rRNA genomic DNA as the positive control). You will determine if the cause of inflammation that the patient results from a bacterial infection.

**The fifth day,** you will receive a second patient report. Mr. Syk is ill with *Haemophilus influenza* which is usually cleared up with a light dose of penicillin but not this time! You will perform a restriction endonuclease digestions on its plasmid DNA in order to release the beta-lactamase gene, if present.

**The sixth day,** your team will perform a gel electrophoresis of the digested plasmid DNA you prepared on day five. Compare your gel's results to a restriction enzyme map of the plasmid in order to determine if this normally penicillin-vulnerable pathogen has really evolved a resistance or it's just a matter of prescribing a heavier dose of penicillin.

**The seventh day,** you will observe samples of bacterial culture *(Haemophilus influenza)* grown in media containing various antibiotics. Discuss results and how it exemplifies the mechanics of evolution and what future implications it may hold for the fight against pathogenic bacteria.

**The eighth day,** all classroom teams will hold a celebratory research poster conference, where there will an exchange of new information and ideas about the patients' illnesses and the treatments that were prescribed.

**II. Materials**

Students will be working in teams of 4 people. You must read the background and protocols ahead of time. Everyone must be properly, attired in an apron, goggles, and gloves.

**III. Procedure**

**DAY 1: REVIEWING THE IMMUNE SYSTEM AND DIAGNOSING PATIENT'S SYMPTOMS**

**I. The first day, you will take a quiz** that reviews your body's inflammatory effects that follows an infection or injury to the first line of defense.

**II. In class today, your teacher will give you a troubling patient's report** from a physician, Dr. Areal Kwak (See "Initial Diagnosis Report" on next page.). The content will provide you with valuable information regarding a patient's physical description and symptoms. In addition, you will have access to a complete blood profile of the patient that was generated by your "Disease Busters" team of technicians. In light of this information, make an initial diagnosis. Do not commit to prescribing a treatment just yet because there just is not enough information to be 100% certain of the illness. If you recall, inflammatory-associated symptoms could be the result of a bacterial-based infection or something else such as a virus, toxin, allergen, or possibly cancer.

**III. Tonight's homework assignments** (see attachments.) include the completion of the "Inflammation Review" worksheet and the "Initial Diagnosis Report."

**DAY 1: REVIEWING THE IMMUNE SYSTEM AND DIAGNOSING PATIENT'S SYMPTOMS**
**STUDENT NAME:**_____

# I. Initial Diagnosis Report

**Cite evidence** from referring doctor, patient's comments or journal articles for all responses
**Suggested References:** Biol 1406 tutorial, NCBI, HHMI, Biotechniques, Science Direct

**1.** Regarding the patient's complaints and Dr. Kwak's notes, what is your diagnosis?

**2.** Why did Dr. Kwak's prescribe penicillin? (Describe the mechanism of action.)

**3.** Do you agree with Dr. Kwak's decision? If not, what would you have prescribed?

**4.** What do expect to "see" in a blood sample of someone experiencing an infection? Discuss the events along with the cells and molecules associated with an inflammatory response?

**(Q# 5-7) Define these terms and what they indicate to a physician when out of the normal range.**

**5.** Platelets (definition and diagnosis if low)

**6.** Hematocrit & Red blood cells & Hemoglobin (definition and diagnosis if low)

**7.** Granulocytes – Neutrophils, Monocytes, Eosinophils, Basophils
(definition and diagnosis if high)

**8.** What technique(s) do you think the Disease Busters technicians used to isolate and identify these individual blood cell types?

**9.** In addition to attempting to PCR amplify the bacterial 16S rRNA gene (variable genomic regions 3 and 4), what other ways could you identify this microbe?

**10.** In reviewing all of the report, including patient remarks, Dr. Kwak's notes and any other data provided, what do you think is wrong with this patient?

## REFERENCE DATA FOR PHYSICIANS ONLY!

### Normal Ranges of Blood Cell Counts for Healthy Adults and Children

(https://www.lls.org/managing-your-cancer/lab-and-imaging-tests/understanding-blood-counts)

| | Red Cells per µl blood | White Cells per µl blood | Platelets per µl blood | Hematocrit[1] % of blood composed of Red cells | Hemoglobin[1] g/dl |
|---|---|---|---|---|---|
| **Men** | 4.7 to 6.1 million | 5,000 to 10,000 | 150,000 to 400,000 | 42 to 52 | 14 to 18 |
| **Women[2]** | 4.2 to 5.4 million | 4,500 to 11,000 | 150,000 to 400,000 | 37 to 47 | 12 to 16 |
| **Children[3]** | 4.0 to 5.5 million | 5,000 to 10,000 | 150,000 to 400,000 | 32 to 44 | 9.5 to 15.5 |

[1]The ratio of hematocrit to hemoglobin is about 3 to 1.
[2]Normal ranges for women who are pregnant differ from these ranges.
[3]These ranges are for children from infancy to adolescence.

### White Cell Differential

Differential count, sometimes referred to as a "diff," is a breakdown of the different types of white cells. A white cell (WBC) differential also checks whether white cells appear normal. The five types of white cells and the approximate percentage they make up in the blood are:

- Neutrophils (55% to 70%)
- Band neutrophils (0% to 3%)
- Lymphocytes (20% to 40%)
- Monocytes (2% to 8%)
- Eosinophils (1% to 4%)
- Basophils (0.5% to 1%)

Until children are more than 4 years old, they have a higher percentage of lymphocytes in their blood than adults do.

### How Blood Cancers Affect Blood Counts

Blood cancers can affect blood cell counts in a number of ways, either lowering or increasing measurements. If you're currently receiving cancer treatment such as chemotherapy, drug therapy or radiation, your blood counts will be affected. Blood counts usually return to normal after treatment is complete.

## REFERENCE DATA FOR PHYSICIANS ONLY!

### Should You Keep Track of Your Blood Counts?

Some people want to know the results of their blood count tests so they can take preventive measures to protect their health or to what's causing their symptoms. For example:

- If you have anemia as a result of low red cell counts, you'll understand why you have low energy levels or are unable to carry out everyday tasks.
- If you have low white cell counts and develop a fever, you'll know to contact your doctor promptly.
- If your platelet counts are too low, you can bleed or bruise easily, so you may choose to avoid activities that have a risk of injury.

### Noncancerous Conditions

About 5 percent of healthy people will have test results outside of the "normal" range. If one or more of your blood cell counts is higher or lower than normal, your doctor will try to find out why. Many noncancerous conditions can contribute to low or high blood cell counts, such as those in the table below.

| | Red Cells | White Cells | Platelets |
|---|---|---|---|
| **High counts** | • Smoking<br>• Carbon monoxide exposure<br>• Chronic lung disease<br>• Kidney disease<br>• Certain forms of heart disease<br>• Alcoholism<br>• Liver disease<br>• Conditions that affect the body's fluid level | • Infection<br>• Inflammation<br>• Severe physical or emotional stress (such as fever, injury or surgery)<br>• Burns<br>• Kidney failure<br>• Lupus<br>• Rheumatoid arthritis<br>• Malnutrition, thyroid problems<br>• Certain medicines | • Bleeding<br>• Mild to moderate iron deficiency<br>• Problems with bone marrow function |
| **Low counts** | • Anemia from too little iron, folic acid or vitamin B12<br>• Bleeding<br>• Inflammatory bowel disease<br>• Other diseases that might cause malnutrition<br>• Certain drugs | • Infection<br>• Chemotherapy and other medicines<br>• Malaria<br>• Alcoholism<br>• AIDS<br>• Lupus<br>• Enlarged spleen | • Pregnancy<br>• Idiopathic thrombocytopenic purpura<br>• Thrombotic thrombocytopenic purpura<br>• Hemolytic uremic syndrome<br>• Autoimmune diseases |

**REFERENCE DATA FOR PHYSICIANS ONLY!**

**Normal Ranges of White Blood Cell Counts for Healthy Adults**

(http://clinicalgate.com/pediatric-and-geriatric-hematology/)

| | TOTAL LEUKOCYTES | | NEUTROPHILS† | | | LYMPHOCYTES | | | MONOCYTES | | EOSINOPHILS | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | Mean | Range | Mean | Range | % | Mean | Range | % | Mean | % | Mean | % |
| Birth | –‡ | – | 4.0 | 2.0-6.0 | – | 4.2 | 2.0-7.3 | – | 0.6 | – | 0.1 | – |
| 12 hr | – | – | 11.0 | 7.8-14.5 | – | 4.2 | 2.0-7.3 | – | 0.6 | – | 0.1 | – |
| 24 hr | – | – | 9.0 | 7.0-12.0 | – | 4.2 | 2.0-7.3 | – | 0.6 | – | 0.1 | – |
| 1-4 wk | – | – | 3.6 | 1.8-5.4 | – | 5.6 | 2.9-9.1 | – | 0.7 | – | 0.2 | – |
| 6 mo | 11.9 | 6.0-17.5 | 3.8 | 1.0-8.5 | 32 | 7.3 | 4.0-13.5 | 61 | 0.6 | 5 | 0.3 | 3 |
| 1 yr | 11.4 | 6.0-17.5 | 3.5 | 1.5-8.5 | 31 | 7.0 | 4.0-10.5 | 61 | 0.6 | 5 | 0.3 | 3 |
| 2 yr | 10.6 | 6.0-17.0 | 3.5 | 1.5-8.5 | 33 | 6.3 | 3.0-9.5 | 59 | 0.5 | 5 | 0.3 | 3 |
| 4 yr | 9.1 | 5.5-15.5 | 3.8 | 1.5-8.5 | 42 | 4.5 | 2.0-8.0 | 50 | 0.5 | 5 | 0.3 | 3 |
| 6 yr | 8.5 | 5.0-14.5 | 4.3 | 1.5-8.0 | 51 | 3.5 | 1.5-7.0 | 42 | 0.4 | 5 | 0.2 | 3 |
| 8 yr | 8.3 | 4.5-13.5 | 4.4 | 1.5-8.0 | 53 | 3.3 | 1.5-6.8 | 39 | 0.4 | 4 | 0.2 | 2 |
| 10 yr | 8.1 | 4.5-13.5 | 4.4 | 1.8-8.0 | 54 | 3.1 | 1.5-6.5 | 38 | 0.4 | 4 | 0.2 | 2 |
| 16 yr | 7.8 | 4.5-13.0 | 4.4 | 1.8-8.0 | 57 | 2.8 | 1.2-5.2 | 35 | 0.4 | 5 | 0.2 | 3 |
| 21 yr | 7.4 | 4.5-11.0 | 4.4 | 1.8-7.7 | 59 | 2.5 | 1.0-4.8 | 34 | 0.3 | 4 | 0.2 | 3 |

**DAY 1: REVIEWING THE IMMUNE SYSTEM AND DIAGNOSING PATIENT'S SYMPTOMS**
**STUDENT NAME:**_____
**INFLAMMATION REVIEW WORKSHEET**

Lesson #1 – Homework – Discuss within the "boxes" names and events associated with our immune response to this pathogenic infection (Use textbook or other sources to complete the diagram below)

**DAY 2: PRACTICING MICROPIPETTING**
**STUDENT NAME:**_____

**The second day,** your team will confirm if Dr. Kwak's original diagnosis, a bacterial infection, is accurate by performing a polymerase chain reaction (pcr) and gel electrophoresis analysis. In order to ensure this experiment is performed correctly and the results are accurate you will practice the micro-pipetting technique, using a 20-200 µl pipette and red-colored dye.

After pipetting various amounts of the dye onto a sheet of paper, you will prepare a graph of the data (horizontal-axis "amount of red dye" vs vertical-axis "area of dye") to determine your pipetting accuracy. If the calculated slope, for the graphed data, is close to 1.0 (change in rise divided by change in run) your team will move on to perform actual pcr reactions on a sample of your patient's body fluid!

**Work on a small sample set of problems involving conversions between micro-liters (µl) and other volumes.**

**A** 1 l = ____ ml **B** 10 ml = ____ µl **C** 1,000,000 µl = ____ l

**D** 0.01 l = ____ µl **E** 150 ml = ____ µl

**I. Review 20-200 micro-liter (µl) pipette parts**
**(students follow along with your teacher)**

"mouth" at the bottom of the pipette and is where the tip is affixed

"window" where you see the volume readings (ie. 20.0 = 20 µl, 120.0 = 120 µl)

"wheel" above the window and is turned to the left or right to adjust the volume of liquid to be transferred

"plunger" at the top of pipette and moves up (to aspirate a liquid) and down (to expel a liquid)

"tip" (not part of pipette) it's affixed onto the mouth of the pipette and discarded after one use

**II. Transfer varying volumes of a red dye solution**
**(students follow along with your teacher)**

1. Adjust micro-pipette wheel to the desired volume (displayed in the window)

2. Affix the pipette's tip onto the "mouth" of the pipette

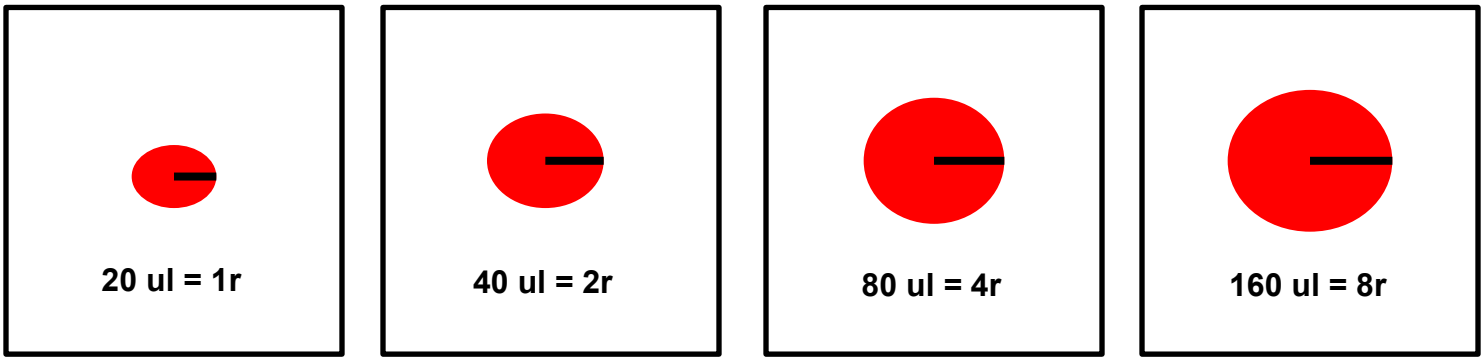
3. Grasp the micro-pipette with the thumb placed over the plunger

4. Push down on the plunger until you feel resistance and stop; hold plunger at that position

5. Place the tip just below the surface of a test tube's solution and release plunger to aspirate. (Observe the solution within tip.)

6. Place tip onto target area and push plunger down to expel solution. (Discarding the tip is not necessary for this practice.)

7. Pipette increasing amounts of red dye (20 µl, 40 µl, 80 µl, 160 µl) into four separate areas onto a sheet of absorbent paper




**The radius (r) should increase proportionally with the increasing volumes of red dye you dispense.**

8. Determine your pipetting accuracy by measuring area of the dye on the paper which should be directly proportional to the volume they are pipetting (circle's area = 3.14 x radius$^2$).

9. Graph results (horizontal-axis "amounts of red dye" vs vertical-axis "area of dye").
***Note:** Slope should be close to 1.0 (change in rise divided by change in run).*

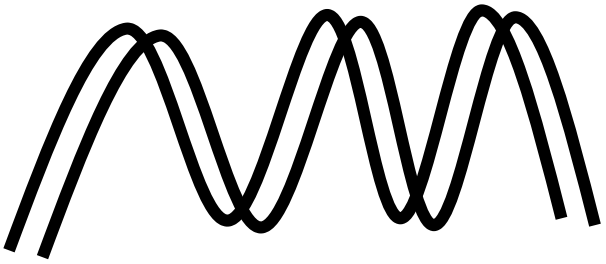
10. Share out graphing results (slope should equal one anywhere along line) and how it reflects the accuracy of their pipetting. Students further share out reasons explaining why their graph was not perfect (ie. solution got stuck in tip, plunger went beyond point of resistance, area of dye on paper was not perfectly shaped as a circle).

**III. Tonight's homework assignment** involves describing the polymerase chain reaction process. (see the worksheet on the next page.)

**DAY 2: PRACTICING MICROPIPETTING**
**STUDENT NAME:**_____

**POLYMERASE CHAIN REACTION AMPLIFICATION WORKSHEET**

**1** Draw & Describe what happens to this DNA molecule (see below) at each of the thermocycling temperatures 95°C, 55°C, 72°C.



| | **DRAW** | **DESCRIBE** |
|---|---|---|
| **95°C:** | | |
| **55°C:** | | |
| **72°C:** | | |

**2A** How many molecules of DNA will you have after 2 cycles? **2B** After 3 cycles?

**3** Compare and Contrast the pcr reaction to what occurs naturally within the cell.

**DAY 3: PERFORMING A POLYMERASE CHAIN REACTION (PCR)**
**The third day,** you will perform a polymerase chain reaction (pcr) amplification of the 16S rRNA gene to confirm that the infection is bacterial based before prescribing a treatment. The resulting 630 base pair (bp) amplicon is a well-documented marker for the presence of bacteria.

**I.** Students explain relevance of the three thermocycling temperatures (98$^{o}$C, 65$^{o}$C, 72$^{o}$C).

**II. Procedure:**
**PCR-Amplification**
Pipette components along with a sample of their fictional patient's bodily fluid (10 µl of actual bacterial genomic DNA or water). Each team performs 3 pcr amplifications in separate 0.2 ml pcr-tubes ("patient's bodily fluid" is the experimental tube; "positive control" contains bacterial 16S rRNA gene; "negative control" does not contains bacterial 16S rRNA gene).

**"Patient's Bodily Fluid" 0.2 ml Tube**

| | |
|---|---|
| 2x PCR Master Mix (w/ enzyme) | 25 µl |
| Forward primer | _____ (0.1-1.0 µM) |
| Reverse primer | _____ (0.1-1.0 µM) |
| **Patient fluid (DNA isolated)** | **2 µl (10 pg - 1 µg)** |
| Water (nuclease-free) | _____ (bring up to 50 µL total volume) |

***Notes: A)** Keep all reagents on ice. **B)** All 3 reactions involving either the patient's body fluid, negative control, or positive control) will use the same amount DNA, water and primers.*

**Thermocycling (teacher performs)**
Initial denaturation 95$^{o}$C - 3 min

**25 cycles x [**Denature 95$^{o}$C - 30 sec, Anneal 55$^{o}$C - 30 sec, Extend 72$^{o}$C - 30 sec**]**

Final extension 72$^{o}$C - 5 min

Store 4$^{o}$C - overnight

**II.** Your teacher will administer an open-ended response quiz on the pcr-amplification process.

**III.** Students complete a worksheet on gel electrophoresis analysis

**IV. Tonight's homework assignments involve A)** working on a 5 point summary from an assigned reading "16S ribosomal RNA gene" background information, **B)** explaining why this region was targeted, and **C)** thinking of one way this bacteria species-identifying approach, could be used in research.

**DAY 4: GEL ELECTROPHORESIS OF PCR RESULTS (82 minutes – two periods)**
**The fourth day,** you will perform gel electrophoresis of your pcr-amplification results in order to determine if the cause of your patient's symptoms is a result of a bacterial infection or something else. Each team loads 4 lanes of samples (a molecular weight marker, the patient's body fluid, a negative control from a healthy person and DNA from 16S rRNA genomic DNA as the positive control).

***Note: A)** Be sure to use a fresh, unused tip for each pipetting step. **B)** At the end of today your team will submit a patient report (see attached page), complete with final diagnosis and prescription. Only at this time then will your teacher reveal your patient's illness!*

**I. Procedure:**
**A) Preparing the 4 samples (1.5 ml micro-centrifuge tubes)**
**Tube #1 Molecular weight marker**
1. Add 1 µl (0.5 µg) Lambda DNA x EcoRI/HindIII molecular weight marker.
2. Add 5 µl of 6X DNA loading dye.
3. Add 24 µl $H_2O$.
4. Incubate at 65°C for 5 min and then ice for 3 min.

**Tubes #2, 3, 4 Samples (patient's body fluid, negative control, and positive control)**
1. Add 25 µl **sample** (patient's body fluid, negative control, positive control).
2. Add 5 µl of 6X DNA loading dye.
3. Keep on ice.

**B) Loading the four tubes of samples into the gel wells (See diagram on next page.)**
Pipette 30 µl from each tube into the corresponding well of the submerged gel.
**Lane 1:** molecular weight marker.
**Lane 2:** patient's body fluid.
**Lane 3:** negative control.
**Lane 4:** positive control.

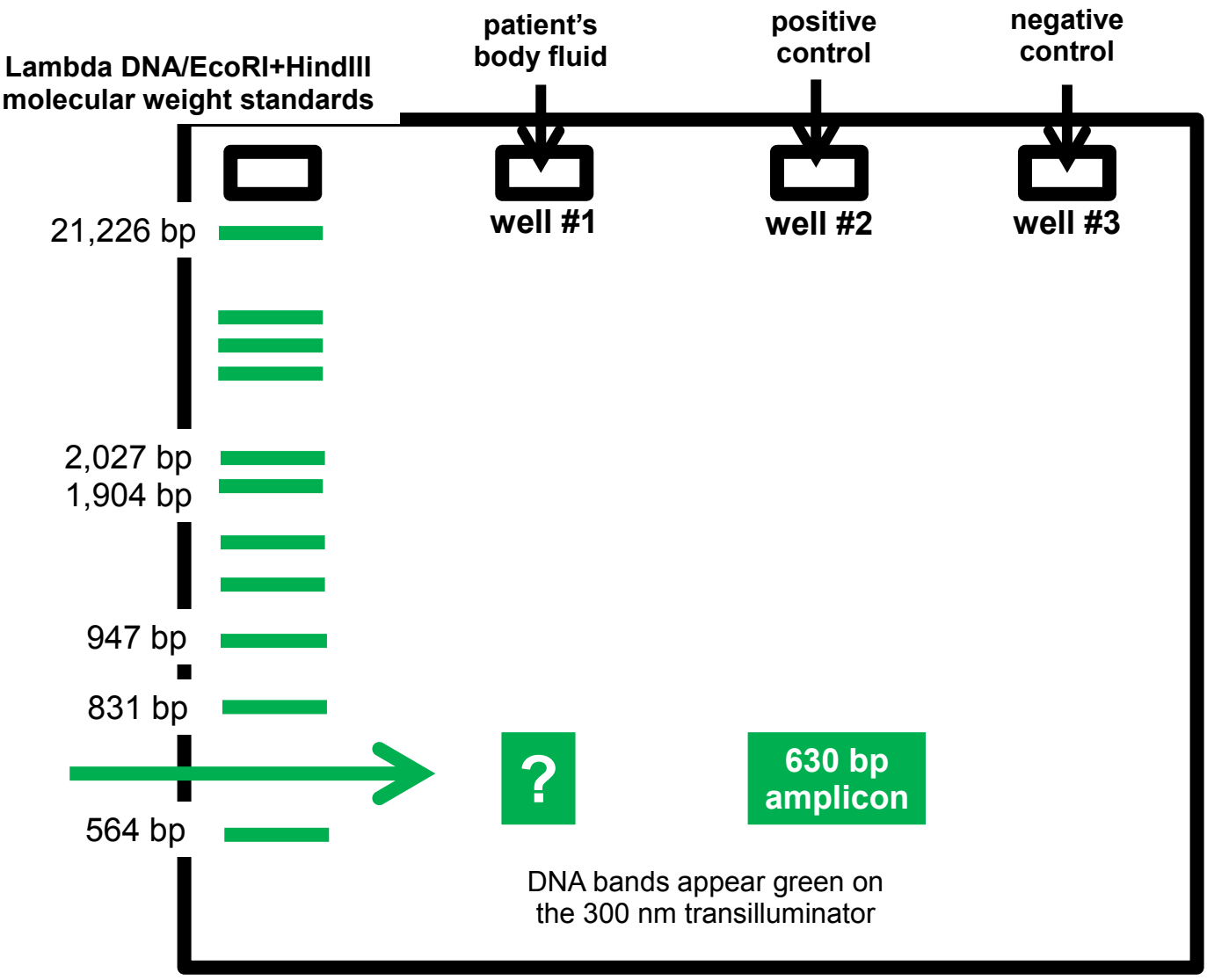
**C) Electrophorese and Visualize the PCR results**
1. Place lid onto gel box, plug leads into box and power supply.
2. Turn on voltage to 125 V – 30 min.
3. After run is complete, place gel onto 300 nm transilluminator.
4. Place transparent protective lid over gel.
5. Turn on transilluminator to visualize.
6. Photograph gel results with digital camera.

**II. Today's class activity involves** completing the "GEL ELECTROPHORESIS OF PCR RESULTS" worksheet. (see attachment.)

**III. Tonight's homework assignment involves** completing the "Final Diagnosis and Prescription Report." (see attachment.)

***Note:** The grading rubric and references of information, that will help you complete the final diagnosis report, are attached.*

**DAY 4: LOADING DIAGRAM OF "GEL ELECTROPHORESIS OF PCR RESULTS"**



**DAY 4: GEL ELECTROPHORESIS OF PCR RESULTS WORKSHEET**
**STUDENT NAME:**_____

**1.** Discuss the <u>three</u> ways that gel electrophoresis separates out a mixture of molecules.

**1st way:**

**2nd way:**

**3rd way:**

**2.** Describe your results; did your patient test positive for the bacterial 630 bp 16S rRNA gene?

**3.** Regardless of your results, what might be one reason why a pcr amplification process did not work?

**DAY 4: GEL ELECTROPHORESIS OF PCR RESULTS WORKSHEET**
**STUDENT NAME:**_____

## II. Final Diagnosis and Prescription Report (post PCR analysis of 16S rRNA gene)

**Cite evidence** from referring doctor, patient's comments or journal articles for all responses
**Suggested References:** Biol 1406 tutorial, NCBI, HHMI, Biotechniques, Science Direct

**11.** What were your PCR 16S rRNA gene test results?

Positive control:

Experimental (patient's fluid):

Negative control:

**12.** What would you conclude if the positive control did not work?

**13.** In addition to PCR amplifying the 16S rRNA gene's variable regions 3 and 4 (genomic) what other ways could you detect this microbe?

**14.** If the PCR results are positive for a bacterium, how would you identify the species? (Describe a technique.)

**15.** Based on the information available, including the PCR test results, what is your final diagnosis and what treatment(s) would you prescribe?

**DIAGNOSIS REPORT RUBRIC**
**on your 1st patient's illness**

| GRADE/ CATEGORY | 100-90 | 90-80 | 80-70 | 70-60 |
|---|---|---|---|---|
| **1st PATIENT DIAGNOSIS REPORT (100 pts)**<br>answers formatted in arial font, 12 size, 1.5 spacing<br><br>Factual answers, with references, for Q# 1-7 and 11, 12<br><br>Creative (somewhat feasible) answers for Q# 8-10 and 13-15<br><br>Followed protocol when performing the pcr-amplification<br><br>Got the expected results (at least for the negative & positive controls)<br><br>Accuracy of your final diagnosis | detailed, accurate & comprehensive answers (5 pts)<br><br>diagnosis was reasonable and well supported | content is not comprehensive (missing 1-2 responses)<br><br>not all supportive points for diagnosis were reasonable | content is not comprehensive (missing 3-4 responses)<br><br>diagnosis was inaccurate and not well founded | content is not comprehensive (missing $\geq$ 5 responses)<br><br>both diagnosis and arguments were very poor<br><br>plagiarism; poor grammar or spelling |

**DAY 5: FIGHTING ANTIBIOTIC-RESISTANT BACTERIA**

**The fifth day,** your will receive a second patient report from Dr. Areal Kwak. Mr. Syk's illness (*Haemophilus influenza*) is usually cleared up with a light dose of penicillin; not this time though! Your team will perform a number of restriction endonuclease digestions on its plasmid DNA in order to and determine if the plasmid is carrying the penicillin-resistant gene, beta-lactamase.

**I. Procedure: Perform 3 restriction digests on plasmid DNA from resistant bacteria.**
Tube #1 = plasmid x EcoRI (yields 4,539 bp linearized DNA fragment)
Tube #2 = plasmid x HindIII (yields 4,539 bp linearized DNA fragment)
Tube #3 = plasmid x EcoRI, HindIII (yields 2635 bp and 1904 bp linearized fragments)

***Note: A)** All reactions are in 20 µl final volume. **B)** One unit is defined as the amount of enzyme required to digest 1 µg lambda DNA in 1 hour at 37°C in 50 µl of recommended reaction buffer. **C)** See next page for restriction endonuclease map of the ampicillin-carrying plasmid. **D)** Tube #4 = undigested plasmid (yields nick and supercoiled forms of 4,539 bp DNA.*

**EcoRI** G^AATTC sites
nuclease-free water 12 µl
DNA (0.5-1 µg/µl) 5 µl
10X Buffer (EcoRI) 2 µl
10U/µl EcoRI 1 µl

**HindIII** A^AGCTT sites
nuclease-free water 12 µl
DNA (0.5-1 µg/µl) 5 µl
10X Buffer (HindIII) 2 µl
10U/µl HindIII 1 µl

**EcoRI, HindIII** A^AGCTT sites
nuclease-free water 11 µl
DNA (0.5-1 µg/µl) 5 µl
10X Buffer R 2 µl
10U/µl EcoRI 1 µl
10U/µl HindIII 1 µl

**Incubate 30 min at 37°C for all digest reactions**
**Incubate 30 min at 65°C to terminate reactions**
**Store at -20°C**

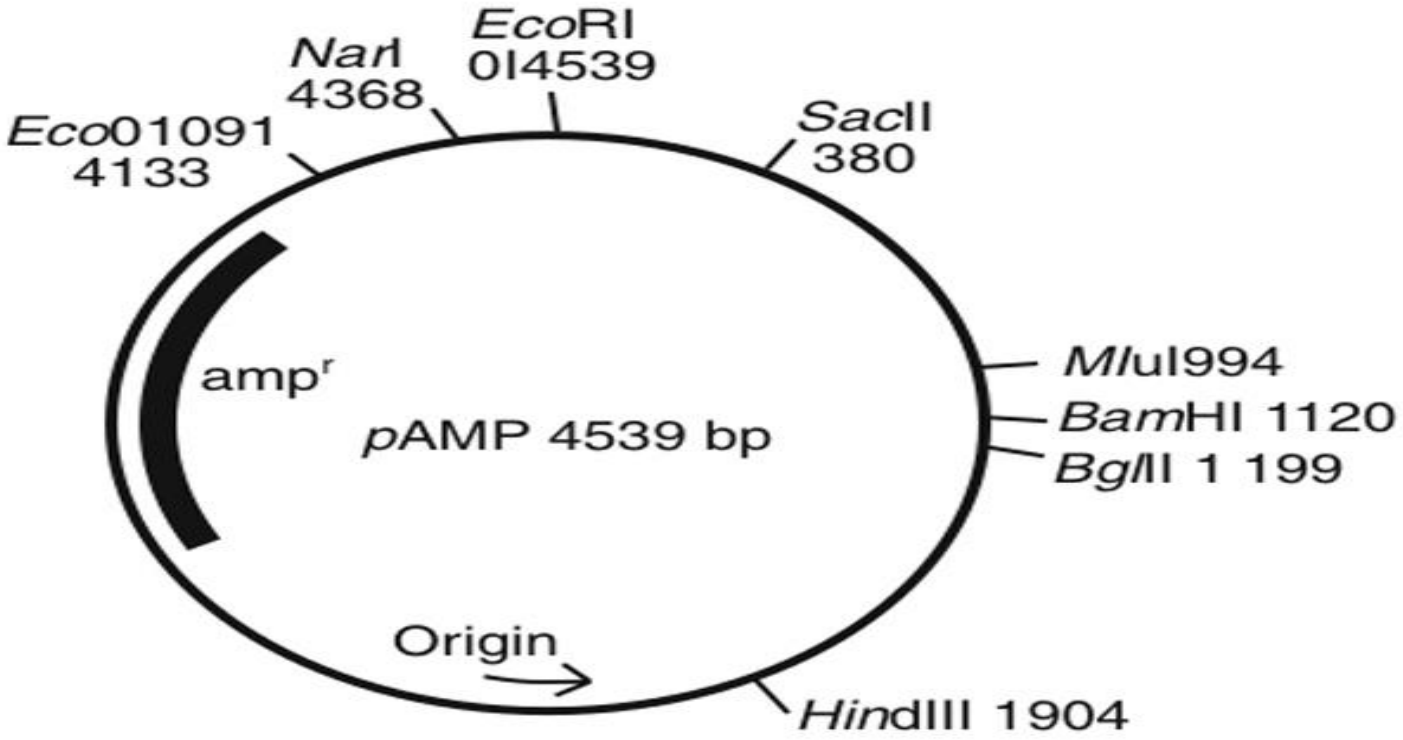
**II. Today's class activity involves** completing the "RESTRICTION ENDONUCLEASE REVIEW" worksheet (See below.).

**III. Tonight's homework assignment involves** completing the "Final Diagnosis and Prescription Report." (see below.)

**REFERENCES *Haemophilus influenza*:**
1. "Signs and Symptoms". Centers for Disease Control and Prevention (CDC).
2. Puri J; Talwar V; Juneja M; Agarwal KN; et al. (1999). "Prevalence of antimicrobial resistance among respiratory isolates of Haemophilus influenzae". Indian Pediatr. 36 (10): 1029–32.
3. Kuhnert, P; Christensen, H, eds. (2008). Pasteurellaceae: Biology, Genomics and Molecular Aspects. Caister Academic Press.

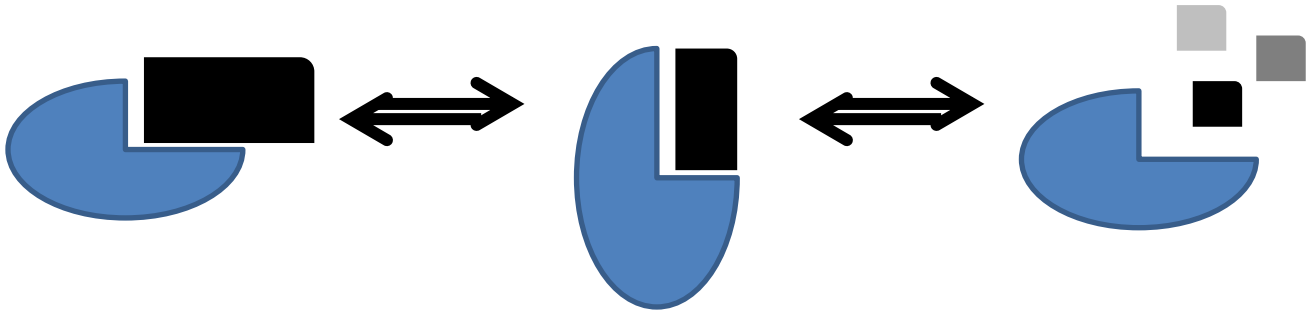
**DAY 5: FIGHTING ANTIBIOTIC-RESISTANT BACTERIA**
**Student Name: _____**

**RESTRICTION ENDONUCLEASE MAP OF AMPICILLIN-CARRYING PLASMID**



**DAY 5: FIGHTING ANTIBIOTIC-RESISTANT BACTERIA**
**Student Name:** _____

**"RESTRICTION ENDONUCLEASE REVIEW" WORKSHEET**



**1.** Describe 4 generic features associated with an enzyme (See above diagram for help).

**A)**

**C)**

**B)**

**D)**

**2.** Discuss 2 specific features associated with a restriction endonuclease.

**A)**

**B)**

**3.** What purpose do restriction endonucleases serve the bacterium?

**4.** Discuss 2 applications of restriction enzymes in the laboratory.

**A)**

**B)**

**DAY 6: GEL ELECTROPHORESIS" LESSON (82 min – two periods)**
**The sixth day (a double period),** your team will perform a gel electrophoresis with the digested plasmid DNA you prepared on the fifth day. You need to compare your gel's results to a restriction enzyme map of the plasmid (teacher provided) in order to determine if this normally penicillin-vulnerable pathogen has really evolved a resistance or it's just a matter of prescribing a heavier dose of penicillin.

**I. Procedure:**
**A) Preparing the 5 samples (1.5 ml micro-centrifuge tubes)**
**Tube #1 Molecular weight marker**
1. Add 1 µl (0.5 µg) Lambda DNA x EcoRI/HindIII molecular weight marker.
2. Add 5 µl of 6X DNA loading dye.
3. Add 24 µl $H_2O$.
4. Incubate at 65°C for 5 min and then ice for 3 min.

**Tubes #2, 3, 4, 5 (undigested plasmid; plasmid x EcoRI; x HindIII; x EcoRI/HindIII)**
1. Add 20 µl **samples** into separate tubes.
2. Add 5 µl of 6X DNA loading dye.
3. Keep on ice.

**B) Loading the four tubes of samples into the gel wells (see diagram on next page.)**
Pipette 30 µl from each tube into the corresponding well of the submerged gel.
**Lane 1:** molecular weight marker.
**Lane 2:** undigested plasmid.
**Lane 3:** plasmid x EcoRI.
**Lane 4:** plasmid x HindIII.
**Lane 5:** plasmid x EcoRI/HindIII.

**C) Electrophorese and Visualize the restricted plasmid DNA results**
1. Place lid onto gel box, plug leads into box and power supply.
2. Turn on voltage to 125 V – 30 min.
3. After run is complete, place gel onto 300 nm transilluminator.
4. Place transparent protective lid over gel.
5. Turn on transilluminator to visualize.
6. Photograph gel results with digital camera.

**II. Today's class activity involves** completing the "HE'S BACK" worksheet.
(See attachment.)

**III. Tonight's homework assignment involves** completing the "HE'S BACK" worksheet.
(See attachment.)

**DAY 6: GEL ELECTROPHORESIS" LESSON (82 min – two periods)**
**"HE'S BACK" WORKSHEET**
**Team's Name:** _____

**Patient's name:** Hyme Styl Syk

**What is your major complaint?** fever, very weak, and ache all over

**How long have you had this condition?** on and off for months

**Have you experienced this condition in the past?** for a while now

**What do you think caused this condition?** It's from last winter

**Dr. Areal Kwak (primary physician) notes:** Mr. Syk is 78 years old and is a regular at my office, coming in for a variety of reasons a few times a year. I often prescribed a low dose of penicillin for him, and it's always successful. This time though it appears that he is only getting worse. I hesitate to give him a larger dose because of the increased risk of adverse side effects but I don't what else to do.

**Disease Buster Report:** Patient tested positive for 16S rRNA, so the illness is definitely bacterial and apparently penicillin resistant.

**Your Next Step:** Characterize this new and scary bacteria by isolating its plasmid DNA. (Your teacher has performed this step.) Then, perform a restriction endonuclease digest with Eco RI & HinDIII mapping to identify the beta-lactamase gene. If beta-lactamase gene is not released. (See restriction enzyme map for calculated size.) Inform Dr. Kwah to go ahead with the higher dose of penicillin. If though the gene is resistant, you're going to have to prescribe a different antibiotic altogether.

**REFERENCES *Haemophilus influenza*:**
1. "Signs and Symptoms". Centers for Disease Control and Prevention (CDC).
2. Puri J; Talwar V; Juneja M; Agarwal KN; et al. (1999). "Prevalence of antimicrobial resistance among respiratory isolates of Haemophilus influenzae". Indian Pediatr. 36 (10): 1029–32.
3. Kuhnert, P; Christensen, H, eds. (2008). Pasteurellaceae: Biology, Genomics and Molecular Aspects. Caister Academic Press

# Disease Busters' laboratory report

**Background:** Penicillin inhibits formation of peptidoglycan cross-links in bacterial cell wall via binding to the four-membered β-lactam ring of penicillin to the enzyme DD-transpeptidase. As a result, the bacteria cannot maintain a wall, and the increasing osmotic pressure eventually leads to cell death. (While the number of penicillin-resistant bacteria is increasing, penicillin can still be used to treat a wide range of infections caused by certain susceptible bacteria, including Streptococci, Staphylococci, Clostridium, and Listeria genera.)

Haemophilus influenzae is a gram-negative, pathogenic bacterium. This species was the first free-living organism to have its entire genome sequenced. It is opportunistic in that it will only cause harm to the host under a certain scenario, such as a viral infection or reduced immune function. It infects the upper-respiratory tract initiating flu-like symptoms such as a low-grade fever, coughing, shortness of breath and fatigue. It typically responds favorably to penicillin!

**1.** Discuss the gel electrophoresis results of your digests below…

**Tube #1** (plasmid x EcoRI) =

**Tube #2** (plasmid x HindIII) =

**Tube #3** (plasmid x EcoRI, HindIII) =

**Tube #4** (undigested plasmid) =

**2.** Based on the results, what will you prescribe for Mr. Syk?

**3.** How do you think future scientists will battle antibiotic-resistant bacteria?

**DIAGNOSIS REPORT RUBRIC**
**on your 2nd patient's illness**

| GRADE/ CATEGORY | 100-90 | 90-80 | 80-70 | 70-60 |
|---|---|---|---|---|
| **2nd PATIENT DIAGNOSIS REPORT (100 pts)**<br><br>Answers formatted in arial font, 12 size, 1.5 spacing<br><br>Factual answers, with references, for Q# 1-7 and 11, 12<br><br>Creative (somewhat feasible) answers for Q# 8-10 and 13-15<br><br>Followed protocol when performing the restriction endonuclease digests<br><br>Got the expected digests results (uncut, EcoRI, HindIII,EcoRI/HindIII)<br><br>Accuracy of your final diagnosis | detailed, accurate & comprehensive answers (5 pts)<br><br>diagnosis was reasonable and well supported | content is not comprehensive (missing 1-2 responses)<br><br>not all supportive points for diagnosis were reasonable | content is not comprehensive (missing 3-4 responses)<br><br>diagnosis was inaccurate and not well founded | content is not comprehensive (missing $\geq$ 5 responses)<br><br>both diagnosis and arguments were very poor<br><br>plagiarism; poor grammar or spelling |

**DAY 7: "FIGHTING ANTIBIOTIC-RESISTANT BACTERIA" LESSON (41 min)**
**The seventh day,** your teacher will bring in samples of media containing various antibiotics that were inoculated with a bacterial culture of *Haemophilus influenza*. You will discuss the results of this experiment, how it exemplifies the mechanics of evolution, and what future implications it may hold for the fight against pathogenic bacteria.

**I. Today's class activities involve…**

**A)** Observe the inoculation experiments your teacher performed yesterday. Complete the "ANTIBIOTIC-RESISTANT EVOLVING BACTERIA" WORKSHEET #1 worksheet (see next page) with your observations and explanation of the phenomena!

**B)** Students share out ways ampicillin resistance spreads throughout a population of bacteria (sexual reproduction – share plasmid carrying the beta-lactamase gene).

**D)** Discuss and share out the basic elements of evolution (i.e. random mutation, natural selection favoring new phenotype, survive and reproduce).

**III. Tonight's homework assignment involves…**

**A)** Completing "ANTIBIOTIC-RESISTANT EVOLVING BACTERIA" WORKSHEET #2 (See next page.)

**B)** Completing the "He's Back" worksheet. (See attachment.)

**C)** Completing your patient's "Final Diagnosis and Prescription Report." (See attachment.)

**DAY 7: "FIGHTING ANTIBIOTIC-RESISTANT BACTERIA" LESSON (41 min)**

## "ANTIBIOTIC-RESISTANT EVOLVING BACTERIA" WORKSHEET #1

**Name:** _____

**Document your observations and explains the results in the following categories…**

**1.** Tube of Luria Broth alone + bacteria:

**2.** Tube of Luria Broth + ampicillin + bacteria:

**3.** Tube of Luria Broth + tetracycline + bacteria:

**4.** Discuss one way a bacterium can become resistant to an antibiotic.

**5.** Why wasn't the beta-lactamase effective with the tetracycline?

**6.** Discuss two general ways man battle an antibiotic-resistant bacterium.

**A)**

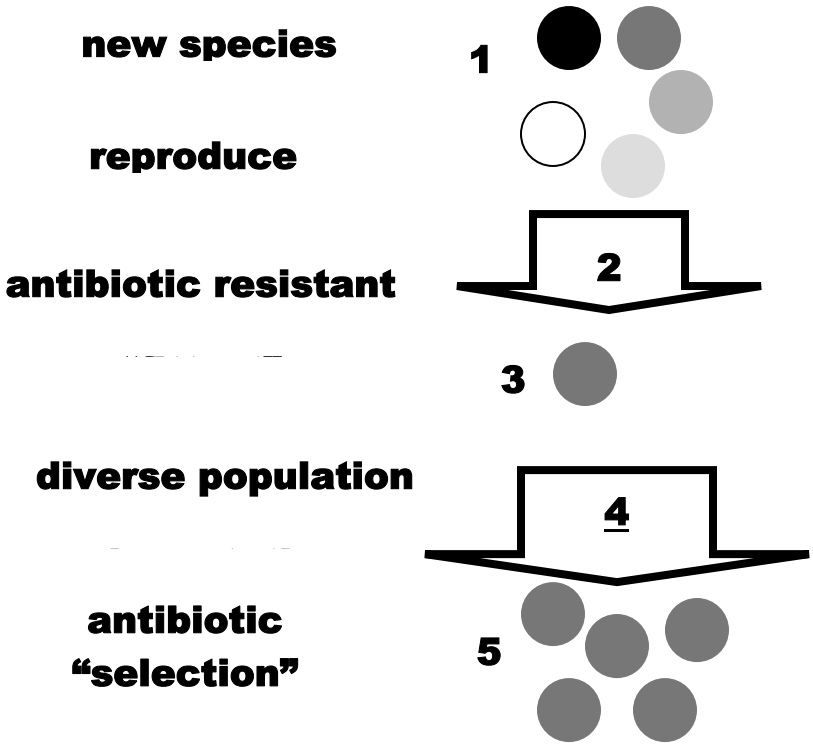
**B)**

**DAY 7: "FIGHTING ANTIBIOTIC-RESISTANT BACTERIA" LESSON (41 min)**

## "ANTIBIOTIC-RESISTANT EVOLVING BACTERIA" WORKSHEET #2

**Name:** _____

Connect 5 terms below to describe this evolving bacteria
(one circle represents $10^3$ individuals).




**Directional selection** describes a population's individuals moving toward a phenotype reflective of a new and favored allele; discuss two more examples of this phenomenon (You cannot use the one above involving bacteria).

**1**

**2**

## DAY 8: "PATIENT'S FINAL DIAGNOSIS" CONFERENCE

***Note:*** *Students are given a grading rubric for the poster presentation portion of this project.*

**The eighth day,** your team along with the other classroom teams will hold a celebratory research poster conference, where there will an exchange of new information and ideas about your patients' illnesses and the treatments that were prescribed.

### I. Today's class activities involve…

**A)** Students arrange seats in semi-circle formation and set up their posters.

**B) First 15 minutes:** Two students from the teams visit three posters and write a summary of information (disease's microbe, symptoms, prescription).

**C) Second 15 minutes:** Two other students from the teams visit three posters and write a summary of information (disease's microbe, symptoms, prescription).

**D)** Students share out what they learned from the applications of the molecular biology techniques to diagnose and treat a patient's illness.

**E)** Students submit their initial and final reports and poster along with the written summaries from the conference.

# RESEARCH CONFERENCE (POSTER & PRESENTATION) RUBRIC

**on your patient's illness** *(teacher will reveal your patient's actual diagnosis)*

| GRADE/ CATEGORY | 100-90 | 90-80 | 80-70 | 70-60 |
|---|---|---|---|---|
| **POSTER (75 pts)**<br>*teacher provides poster board*<br><br>**TITLE (5 pts)**<br>A) name of illness B) picture of inflammatory response C) date, period#, team's names | all information is present | missing 1 item of information | missing 2 items of information | missing title page |
| **BIBLIOGRAPHY (5 pts)**<br>(5 references) | ≥5 current and reputable publications | 3-4 current and reputable publications | 1-2 current and reputable publications<br><br>but also blogs | not use enough references to support findings<br><br>but also blogs |
| **CONTENT (65 pts)**<br>(1) mechanism of infection<br>(2) inflammatory response (changes in blood cell count)<br>(3) host's symptoms<br>(4) diagnosis technique<br>(5) treatment | detailed, accurate & comprehensive information (5 pts)<br><br>major points are addressed and well supported | content is not comprehensive (missing 1-2 pts)<br><br>not all major points were addressed | content is not comprehensive (missing ≥ 3 pts)<br><br>few major points were addressed | content is not comprehensive (missing ≥ 4 pts)<br><br>few major points are addressed<br><br>plagiarism; poor grammar or spelling |
| **5 min PRESENTATION (25)**<br>loud & clear voices, good eye contact, accurate information, creative poster (colors, big print, engaging pictures) | loud, clear voices with eye contact;<br><br>accurate information and entertaining | weak delivery with little eye contact<br><br>inaccurate information but entertaining | poor delivery no eye contact<br><br>inaccurate information and not entertaining | <5' and poor delivery no eye contact<br><br>inaccurate or little information and not entertaining |