AN ABSTRACT FOR THE THESIS OF

Kelley Tuel in Master of Science in Biology presented on March 27, 2013 by Emporia State University.

Title: Development and Field Trial of a Molecular Biology Teaching Kit Utilizing *Halobacterium* sp. NRC-1.

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A teaching kit entitled *The Genotype-Phenotype Connection* was created around a novel mutant of *Halobacterium* sp. NRC-1 called KBT-1. The activities in the kit recapitulate the isolation and characterization of KBT-1 and were optimized for use in typical high school biology classrooms. Beginning with observations about the organism on the petri plate and forming conclusions from a gel electrophoresis image, students experience the relationship between genotype and phenotype while exploring molecular biology hands-on. To determine if this kit was an effective teaching tool, I performed a field trial with 13 different sections of AP Biology in fall 2012 consisting of 9 teachers and 192 students. Overall, teachers and students had an overall positive response to *The Genotype-Phenotype Connection* kit. The kit has been published by Carolina Biological Supply Company and is now available for mainstream teacher purchase.

**Key words:** AP Biology, hands-on molecular biology, high school laboratory, Archaea, halophile, *Halobacterium* sp. NRC-1

# Development and Field Trial of a Molecular Biology Teaching Kit

## Utilizing *Halobacterium* sp. NRC-1

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A thesis presented to
The Department of Biological Sciences
EMPORIA STATE UNIVERSITY

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The teachers who participated in kit field trial. I have much gratitude for this group of 9 people. They agreed to participate before knowing what changes the College Board would make to the AP curriculum for fall 2013. They used the kit with great scheduling flexibility and creativity, even as molecular biology now falls in the 2$^{nd}$ semester under the new AP guidelines. They and their students offered valuable feedback to improve the kit.

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# Preface

When I was teaching high school biology from 1998-2003, I faced the dilemma of how to incorporate molecular and microbiology into my classroom. Hands-on molecular biology seemed inapproachable unless I ordered expensive kits but even then, they often included DNA samples sent in a tube precluding students from actually seeing or understanding the organism from which the DNA was extracted. I wanted to teach my students laboratory methods and microbiology/molecular concepts using a safe, living microorganism but did not know where to start. My desire to involve students with microbiology and molecular biology labs led me to investigate the potential of a halophilic Archaean, *Halobacterium* sp. NRC-1, as a graduate project at Emporia State University.

This thesis is organized around three chapters. The first chapter is an introduction to attributes of *Halobacterium* sp. NRC-1 that make it an ideal model organism to use in high school laboratories. Chapter two contains the results of characterization and optimization experiments performed in the development of a teaching kit for Carolina Biological Supply Company. Finally, chapter three details a field trial of the teaching kit and the feedback from the teachers and students who tested it.

This paper is formatted in the manner required by the American Biology Teacher publication.

# Table of Contents

Acknowledgements ..... ii
Preface ..... iii
List of Tables ..... vii
List of Figures ..... viiii
Chapter 1: Call for a Classroom Microbe ..... 1
Laboratory Activities Should Engage Students ..... 2
Potential Constraints to Hands-On Activities Involving Microbes ..... 3
A Model Classroom Organism: *Halobacterium* sp. NRC-1 ..... 6
*Halobacterium* sp. NRC-1 Addresses the Issues of Cost and Contamination ..... 6
*Halobacterium* sp. NRC-1 Addresses the Archaean Domain ..... 7
Chapter 2: Genetics of *Halobacterium* sp. NRC-1 Gas Vesicles, Basis for Design of a Teaching Kit ..... 11
Gas Vesicles of *Halobacterium* sp. NRC-1 ..... 11
Development of the Teaching Kit ..... 14
Materials and Methods ..... 14
PCR of *gvpA* region for pNRC100 ..... 19
Identification, Isolation and Characterization of *gvp* Mutants ..... 19
Adapting protocols for use in high schools ..... 25
Pilot Study and Further Refinements ..... 27
Gel Electrophoresis Protocol ..... 30
Discussion ..... 30
Chapter 3: Field Trial of *The Genotype-Phenotype Connection* Kit ..... 35
Materials and Methods ..... 36
Results ..... 37
Teacher and School Participants ..... 37
Equipment Needs of Field Trial Teachers ..... 39
Success with Kit Protocols ..... 40
Teacher Perspectives ..... 42
Breakdown of Concepts Presented in the Kit ..... 44

Teacher Perspectives of Student Guide ..... 44
Analysis of Student Pre/Post Test Scores ..... 46
Student Perspectives of the Kit Experience ..... 52
Discussion ..... 58
References ..... 62
Appendix A: Kit Manual ..... A1
Appendix B: Solicitation Email to Potential Field Trial Teachers ..... A55
Appendix C: Online Screening Survey for Potential Field Trial Teachers ..... A58
Appendix D: Sample of Individualized Suggested Field Trial "Lesson Plan" ..... A64
Appendix E: Student Field Trial Pre/Post-Tests ..... A67
Appendix F: Teacher Post-kit Evaluation ..... A71
Appendix G: Informed Consent Document ..... A76
Appendix H: Carolina Biological Copyright Permissions ..... A78
Appendix I: HaloWeb Copyright Permissions ..... A80

# List of Tables

Table 1. Primer sequences for each primer set.

Table 2. Percentage of student respondents for various components of the teaching.

# List of Figures

Figure 1. Gas vesicle protein gene region from pNRC100.

Figure 2. *Halobacterium* NRC-1 colonies of *Vac*+ and *Vac*-.

Figure 3. PCR amplicons using three different primer sets.

Figure 4. A sectored BSYC plate containing five possible *gvp*A mutants.

Figure 5. PCR amplicons of a wild type and two mutants.

Figure 6. A comparison of the gas vesicle gene regions of pNRC100 for sp. NRC-1, KBT-1 and KBT-2.

Figure 7. Modified "Extremely Easy DNA Extraction" samples (lanes 2-4).

Figure 8. PCR of crude lysates prepared by lysing a single colony.

Figure 9. 1% agarose gel with PCR products that were in the thermal cycler for approximately 55 minutes.

Figure 10. 1 kb DNA ladder used to quickly estimate size of dna fragments on a 0.8% agarose gel.

Figure 11. Example classroom gels provided by field trial teachers.

Figure 12. Teacher satisfaction with topics as presented in the kit.

Figure 13. Average student pre/post-test scores for matching vocabulary questions.

Figure 14. Comparison of average student pre/post-test multiple choice scores.

Figure 15. Comparison of average student short essay scores.

# Chapter 1
# Call for a Classroom Microbe

"While the 20th century was shaped largely by spectacular breakthroughs in the fields of physics and chemistry, the 21st century will belong to the biological sciences" (Rifkin, 1998).

With an explosion of discoveries in recent years, new fields of research have evolved under the umbrella term of "molecular biology" (NRC, 2012, p. 11). To stay relevant on the topics of science, technology and health, secondary education teachers and students must be educated in the emerging sub-disciplines of molecular biology such as *bioinformatics, synthetic biology, molecular medicine* or *biomaterials engineering* (NRC, 2012, p. 39-64). Veteran teachers are unlikely to have had training in these sub-disciplines and consequently are unlikely to effectively incorporate these topics into the curriculum.

Organizations that encourage the advancement of science education advocate for incorporation of these emerging fields of molecular biology by encompassing them in testimonials and recommendations of pedagogical changes needed to drive biology education forward. In the book, *A New Biology for the 21st Century*, the National Research Council (NRC) relays recommendations to weave together emerging technology, societal needs and governmental resources. The NRC refers to this as the National New Biology Initiative (NRC, 2012, p. 87-89).

The most recent draft of the Next Generation Science Standards (NGSS), developed through a collaborative effort between NRC, The National Science Teachers Association (NSTA) and the American Association for the Advancement of Science

(AAAS), paves the way for inclusion of the new molecular biology sub-disciplines in curriculum reform (Next, 2013). For example, one objective within the Core Idea of Engineering, Technology and Applications of Science states, "Links among engineering, technology, science, and society in the Engineering, Technology, and Applications of Science" should be addressed (A Framework, 2012, p. 3-8; Next, 2013). The technological applications of molecular concepts can address this objective.

Concepts encompassed by molecular biology can be difficult for students to comprehend due their abstract nature and technical vocabulary. Comprehension can also be a challenge due to the varying levels of genetic concepts which are often segmented when they are typically presented to students. A study conducted by Duncan and Reiser found that students were not connecting the individual concepts to the larger picture of genetics (2007, p. 953-954). In another study, Lewis and Kattman present an interrelated or scaffolding approach to help students make the necessary connections (2004, p. 196). The book, *America's Lab Report: Investigations in High School Science*, the NRC also advocates for use of "integrated instructional units" in science classrooms. A key component of this approach is to enhance the traditional means of teaching (lecture, reading the text, classroom discussion, etc.) with laboratory investigative experiences (Singer et al., 2006, p. 4-5).

## Laboratory Activities Should Engage Students

Designed appropriately to engage students in their learning, laboratory investigative experiences hold the potential to aid enthusiasm for and mastery of course objectives. In addition, proper design can improve students' reasoning skills in science (Singer et al., 2006, p. 2-3). Educational research clearly demonstrates that engaging

students actively in the process of learning fosters critical thinking and problem solving (Taraban et al., 2007, p. 961).

A student-centered laboratory activity where students can be *engaged* in the inquiry process through questioning, protocol design, data collection and analysis is the most obvious way to involve the students in a science class (Marbach-Ad et al., 2010, p. 3; Yip, 2010, p. 485). Characteristics of student-centered labs include a direct means for students to collect data, apply scientific principles to analyze and interpret data and demonstrate learning though conclusions. This style of lab is in stark contrast to the traditional style "cookbook" labs, which are so named because there is a set protocol to follow without alteration. The end result in these types of labs typically has one successful solution and lacks any opportunity for student exploration (Singer et al., 2006, p. 123-124).

The use of living organisms in laboratory settings is another way to improve student engagement. With their fast doubling time and simple preparation, bacteria offer the potential for efficient open ended experiments. Bacterial cultures also offer the benefit of being inexpensive and can be applied to many concepts covered in a typical biology classroom (Johnson, 1958, p. 41; Peterson, 1973, p. 396). Because of their ability to be easily manipulated in the lab they have easily accessible online genomic data, microbes such as bacteria are an attractive model for use in laboratory activities designed for teaching molecular biology.

## Potential Constraints to Hands-On Activities Involving Microbes

Microbes are an attractive research model to efficiently incorporate molecular biology laboratory activities into the classroom but they are not often utilized at the high

school level (Yip, 2010, p. 485). The cost of the reagents and supplies required to work with bacteria combined with the threat of possible contamination with pathogens are restrictions that make hands-on labs difficult. Teacher experience with bacteria could also be a limiting factor to classroom use. Although there are not any formal guidelines in individual Kansas school districts, general safety recommendations are in place (Schrock, 2013, pers. comm.).

The materials and equipment needed for molecular biology can be costly, thereby limiting hands-on experiments. For schools to work with bacteria or other microbes, they may require the use of an autoclave to sterilize equipment and safely dispose of live cultures. However, the smallest portable industrial autoclave costs around $250 plus shipping (Amazon, 2013). Nearly all experimental work involving DNA requires the use of polymerase chain reactions (PCR). Thermal cyclers required for PCR cost $2,600 or more. Standard equipment for biology classrooms such as micropipettes ($125 each), microcentrifuges ($180 each) and gel electrophoresis chambers with power supply ($620 each) are extremely costly (MidSci, 2012). In addition to equipment, consumables and reagents required for experimentation with microbes are often costly as well.

To fund their science classes, schools usually budget a specific amount of money for the science department. Often, this amount is divided by the number of science teachers within the department regardless of the number of laboratory activities each teacher actually performs, the class size or number of sections taught. An informal poll of Advanced Placement (AP) biology teachers who participated in the field trial (Chapter 3) revealed yearly budgets that ranged from $285 to $500 per teacher. Each of these teachers taught two or more classes, often with more than one section per subject! Some

of these teachers were able to supplement their budgets by successfully competing for within-district grants or learned how to stretch their budgets by finding creative ways to save money. Clearly, the costs associated with doing molecular biology laboratories or the burden of writing grants to supplement insufficient budgets lessens the likelihood that effective hands-on instruction will take place in high school classrooms.

In addition to cost, safety is a classroom issue. Both The National Association of Biology Teachers (NABT) and The National Science Teachers Association (NSTA) have position statements on the role of safety in the laboratory. They state that appropriate safety measures should be in place for all labs, including those that use microbes (NABT, 2012; NSTA, 2012, p. 1-6). In their position statement on laboratory safety, the NSTA declares science teachers "must act as a reasonably prudent person would in providing and maintaining a safe learning environment for their students." (NSTA, 2012, p. 1). To provide the safest possible environment, teachers need to be educated on appropriate microbes to use and conduct the techniques themselves before teaching them in laboratories. Statements from authoritative sources such as these are judicious but carry the unintentional consequence of thwarting teachers' desires to incorporate molecular biology into their curriculum.

Teaching safety and aseptic technique using microbes can be an effective way for students to experience guidelines common in research laboratories (Horn, 1993, p. 1). However, mistakes in the lab can be dangerous and contamination of cultures or media costly. The fear of contamination when using bacteria or other microbes in a classroom faces teachers on two fronts. First, fear of the microbe being pathogenic (or even the perceived threat of pathogenicity) could limit teachers' use in the classroom (Johnson,

1958, p. 41). Secondly, the growth media used for most safe microbes are often the same as that used to grow pathogenic organisms creating a situation where teachers or students could unknowingly culture and perhaps spread truly dangerous pathogens. These possibilities, however remote, are risks that most teachers are not willing to take with their students.

## A Model Classroom Organism: *Halobacterium* sp. NRC-1

*Halobacterium* sp. NRC-1 is a microbe representative of the third domain of life, Archaea. It is a laboratory strain of *Halobacterium halobium* and one of the first organisms to be completely sequenced (DasSarma and DasSarma, 2006, p. 6). Similar to a bacterium, this organism offers ideal conditions for use in student labs by combining properties that are typical of most microbes (motility, chemotaxis, mutation by strong ultraviolet light, etc.). However, unlike bacteria, *Halobacterium* sp. NRC-1 is unique in that it can be used as a microbial model organism without the usual risk factors (DasSarma and DasSarma, 2005, p. 4).

## *Halobacterium* sp. NRC-1 Addresses the Issues of Cost and Contamination

It is relatively inexpensive to culture *Halobacterium* sp. NRC-1. A test tube of this culture is approximately $10 from Carolina Biological Supply (Halobacterium sp. tubes, 2013). The media can be easily made in the classroom in about an hour; it is comprised of common chemicals, such as Magnesium Sulfate, Sodium Citrate and Potassium Chloride, most of which are probably already found on most science department shelves. Salt, Casamino Acids, Yeast Extract and the other media components are reasonably priced. Even ordering every media component of BSYC, the

cost is less than $23 per liter, therefore, cost is not a restriction in using this microbe. Because of the extreme salinity required by *Halobacterium* sp. NRC-1, less equipment is required; an autoclave and sterile equipment are not necessary.

*Halobacterium* sp. NRC-1 avoids the potential for contamination of student experiments because of the halophilic conditions required by this organism. The 4.3 M NaCl BSYC media (Broth of Salt, Yeast and Casamino Acids) required when culturing *Halobacterium* in a classroom is approximately ten times the salinity of the ocean. Because of this extreme salinity, non-halophilic microorganisms are unable to grow in these conditions thus contamination issues are avoided (DasSarma and DasSarma, 2005, p. 4).

*Halobacterium* sp. NRC-1 is considered a "safe microbe" for laboratory use. The American Type Culture Collection (ATCC) classifies NRC-1 as a Biosafety Level 1 (BSL-1) organism. Materials classified at BSL-1 "are not known to cause disease in healthy adult humans" (American, 2013). In fact, *Halobacterium* will lyse if the salinity dips below 1.0-2.0 M NaCl (DasSarma and DasSarma, 2005, p. 4). Since this is a far higher concentration than any place on the human body (saline is ~0.15 M), *Halobacterium* does not pose a threat to students.

### *Halobacterium* sp. NRC-1 Addresses the Archaean Domain

Until recent molecular evidence indicated otherwise, Archaea were grouped with bacteria as under the term prokaryotes and were known as members of the kingdom Monera. The former taxonomic classification challenged in 1977 when Carl Woese and colleagues applied RNA sequencing data to describe cellular relationships (Postlewait and Hopson, 2009, p. 340; Woese and Fox, 1977, p. 5088-5089). It was not until 1990

when Woese used the term “three-domains” that the concept became widespread (Woese et al., 1990, p. 4578-4579; Postlewait and Hopson, 2009, p. 340). Domains are a higher grouping than kingdoms in the previous taxonomic classification system. The three-domain system consists of Bacteria (true bacteria), Eukaryota (cells with a nucleus) and Archaea (formally known as Archaebacteria) (DasSarma, 1995, p. 4).

Archaea do share many physical attributes to bacteria such as cellular size and ribosomal subunits size (Evans, 1983, p.141). They also share numerous genes, especially those that are metabolic in nature, that are not shared with Eukaryota (Woese, 2000, p. 8393). However similar Archaea and Bacteria might appear morphologically, they contain significant enough differences to each be classified into their own domain (Doolittle, 1992, p. 6). Molecular evidence revealed these organisms have unique cell membranes, cell walls and small-subunit ribosomal RNA (rRNA) sequence (Doolittle, 1992, p. 6; Kates, 1992, p. 51; Peirce, 1999, p. 132).

Archaean cell membranes are comprised of waxy molecules instead of the true fat molecules found in eukaryotes and bacteria (Flannery, 1997, p. 371; Evans, 1983, p. 141). Extremely high temperatures, such as those environments in which many Archaea thrive, are known to denature molecules. The Archaea that flourish under such conditions contribute part of their success to these waxy membranes that do not melt as easily as the fat containing membranes would (Flannery, 1997, p. 371).

However, it is the unique rRNA sequence that is most commonly used to define the three domains and distinguish Archaea (Roberts et al., 2008, p. 13953-13954). Ribosomal RNA is a universal molecule found in all living organisms. It universally has the same function: to facilitate translation of mRNA into proteins. Ribosomal RNA

comprises both subunit structures of the ribosome in the cell (small subunit and large subunit). The sequence of rRNA rarely mutates, thus is easy to use to determine relatedness of organisms (Woese, 2000, p. 8392).

Teachers often explain that Archaea, such as *Halobacterium* sp. NRC-1, are the "strange microbes" that they will never encounter. Many Archaea can live in environments where normal life forms cannot live and are therefore are called extremophiles. Euryarchaeota is the largest division within Archaea. Euryarchaeota has three main subgroups: the methanogens, thermoacidophiles and halophiles (Woese et al., 1990, p. 4578-4579).

The methanogens are Archaea which convert carbon dioxide ($CO_2$) into methane in a process to yield energy (Evans, 1983, p. 139-140). They are anaerobic and are found in hydrothermal vents, swampland, and even animal digestive tracts (Evans, 1983, p. 139). Some methanogens, such as *Methanococcus jannaschil,* can survive in hydrothermal vents at the depths of the ocean where the atmospheric pressure reaches up to 200 atm and temperatures range from 48-94°C (Flannery, 1997, p. 370).

Thermoacidophiles are the second group within Euryarchaeota. They flourish in acidic environments with high temperatures such as sulfur springs and smoldering coal tailings (waste from coal mining). In these conditions, acidity levels commonly reach a pH of 2 or less (Evans, 1983, p. 139). Thermoacidophiles have even been found in sulfur rich volcanic lava flows with a pH of less than 1 (Flannery, 1997, p. 371).

Because of their extremophilic lifestyle, the methanogens and thermoacidophiles require conditions that are difficult to maintain in a laboratory setting. However, halophiles are easy to cultivate and observe. Halophiles (salt-loving Archaea),

commonly inhabit very salty lakes like the Great Salt Lake in Utah or the Dead Sea in the Middle East. Excitingly, these halophilic Archaea are relatively easy to culture in the classroom. Not only can students have an authentic example of an Archaea microbe to discover evolution and explore taxonomy, but *Halobacterium* can be used in student inquiry labs to teach the topics of safety and sterility, microbiology, cytology, molecular genetics and bioinformatics. This microorganism can be used to teach the importance of microbes in general, serving as a unifying organism to weave together concepts in cell morphology, genetics and biotechnology (Evans, 1983, p. 142).

# Chapter 2
# Genetics of *Halobacterium* sp. NRC-1 Gas Vesicles: Basis for Design of a Teaching Kit

*Halobacterium* sp. NRC-1 has a completely sequenced and well-studied genome (Ng. et al., 2000, p. 12176-12177). It contains three replicons, one of which is the large chromosome that is approximately 2 Mb in size. This microbe also contains two "mini-chromosomes" that contain many essential genes and are much larger than typical bacterial plasmids. These mini-chromosomes are named pNRC100 (191 kb) and pNRC200 (365 kb) (Kennedy et al., 2001, p. 1641; Ng et al., 1998, p. 1131-1132).

## Gas Vesicles of *Halobacterium* sp. NRC-1

Fourteen percent of the entire *Halobacterium* genome consists of DNA that is duplicated elsewhere in the genome. The duplications occur in the form of transposons, inverted repeats and regions found on both mini-chromosomes (Kennedy et al., 2001, p. 1641). Insertion sequences (or IS elements) are small segments of DNA that move within the genome. They can range anywhere from 521 bp (ISH2) to 1402 bp (ISH8) in size (Fileé et al., 2007, p. 126-127). It is the IS elements that play a role in altering the genotype of the gas vesicle gene region within *Halobacterium* sp. NRC-1.

The *Halobacterium* cells use organelle-like structures called gas vesicles to control buoyancy in their natural environment (such as the Great Salt Lake or Dead Sea). Buoyancy offers a means for this Archaean to receive more sunlight and oxygen found higher in the water column, used for aerobic respiration and/or phototropism. Without gas vesicles, the *Halobacterium* cells sink.

Gas vesicles are constructed from multiple gene products. The *gvp* (gas vesicle protein) gene cluster is found on the mini-chromosome, pNRC100 (Figure 1). It consists of two divergent operons (one operon running rightward, one leftwards) and includes 12-13 genes (nearly 21,000 bp) (DasSarma et al., 1994, p. 7647). Three of the *gvp* genes (*gvpACN*) undergo transcription in the rightward direction while the remaining ten (*gvpDEFGHIJKLM*) undergo transcription in the leftward direction (Halladay et al., 1993, p. 684). The *gvpACN* operon produces the primary structural protein, GvpA, and a minor protein, GvpC, found in the membrane of gas vesicles (DasSarma et al., 1988, p. 6861; Halladay et. al, 1993, p. 684). The leftward-transcribed genes are involved in gas vesicle assembly (DasSarma, et al., 1994, p. 7649; Shukla, 2004, p. 3185).

A spontaneous mutation rate of approximately 1% of *Halobacterium* sp. NRC-1 cells has been documented to occur in the *gvp* gene cluster because of active IS elements (DasSarma et al., 1988, p. 6861). These mutants lack gas vesicles due to an IS element disruption, which is thought to occur in either the *gvp*A gene or the *gvpACN* promoter region (Halladay et. al, 1993, p. 688 and 690; DasSarma et al., 1988, 6861). When the genes involved in making the gas vesicles are disrupted by an IS element, the cells lose their ability to produce the vesicles and are referred to as *Vac*- colonies (DasSarma and Arora, 1997, p. 3-4; DasSarma et al., 1994, p. 7646-7647). Colonies with gas vesicles intact are referred to as *Vac*+ colonies.

The gas vesicles of *Halobacterium* sp. NRC-1 cause refraction of light (bending of light) within the cells to make them appear pink and opaque due to the presence of red carotenoids in the plasma membrane (DasSarma, 1995, p. 4). However, when gas vesicles do not form, the light is refracted differently and the cells appear red and

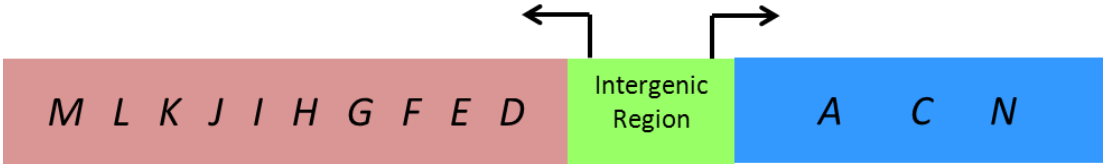


Figure 1. Gas vesicle protein gene region from pNRC100. Note the two divergent operons (*red and blue*) and the IS elements that are shown downstream of the gvp gene region. Other IS elements are found upstream in pNRC100 as well. Image based on graphic from HaloWeb and used with permission (Halobacterium sp. NRC-1, 2013).

translucent (DasSarma, 1989, p. 65; DasSarma and Arora, 1997, p. 2-3; DasSarma et. al, 1994, p. 7646). Figure 2 demonstrates the physical difference between the two types of colonies.

## Development of the Teaching Kit

The high mutability within the *gvp* gene cluster and the easily discernible phenotypic differences of the colonies makes it relatively easy to find mutant colonies that have disrupted *gvp* genes (*Vac*-) simply by color. These features served as the basis for the development of a set of classroom activities in which students utilize molecular biology techniques to relate genotype to phenotype.

To develop the molecular biology activities, I first had to isolate and characterize a *Vac*- mutant using PCR and DNA sequencing. Then, high school friendly techniques were developed that could effectively lead students through the activities of colony morphology observations, DNA extraction, PCR, gel electrophoresis and data analysis. This kit also includes a comprehensive teacher and student instruction guide (Appendix A).

## Materials and Methods

### Bacteria and Media

*Halobacterium* sp. NRC-1 was obtained from Carolina Biological Supply Company (Burlington, NC). It was cultured in liquid Broth of Salt, Yeast and Casamino Acids (BSYC) media or on BSYC containing 2.0% agar on plates at 37°C. When

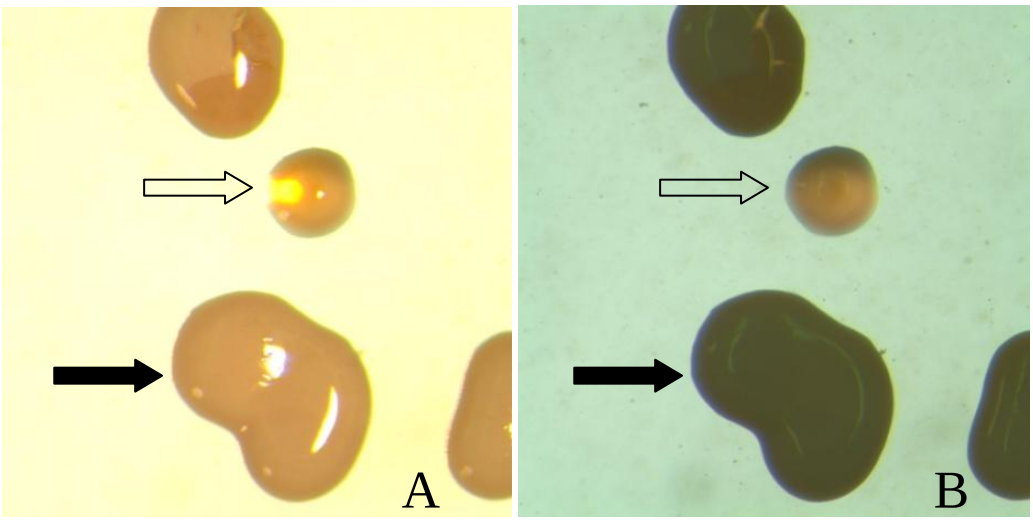


Figure 2. *Halobacterium* NRC-1 colonies of *Vac*+ (closed arrows) and *Vac*- (open arrows). The photomicrograph in panel A was photographed with top lighting to illustrate the pink (closed arrow) and red (open arrow) of *Vac*+ and *Vac*- phenotypes. Panel B is the same field of view lit from underneath to demonstrate the opacity of *Vac*+ colonies and the translucence of *Vac*- mutants.

cultured on solid media they were incubated inside resealable plastic bags. Growth typically took 4 days in liquid cultures or 7-10 days on plates.

## Primer Design

Three primer sets were designed to amplify the *gvp*A gene region of *Halobacterium* sp. NRC-1, pNRC100. The first primer set was designed by using "Primer-BLAST Primer designing tool" on the intergenic region between *gvpD* and *gvpA* of *Halobacterium* pNRC100 (National, 2011). These primers were predicted to produce a 1792 bp amplicon. The remaining two primer sets were designed using the NCBI nucleotide database for *Halobacterium* sp. NRC-1, plasmid pNRC100 (NCBI, 2011). BLAST of potential primer sequences was executed using 20 bp increments from the *gvpD-gvpA* intergenic region for the forward primers; *gvpC* and *gvpN* regions were used for the reverse primers (NCBI, 2011). These final two primer sets were determined to have the potential to amplify 1529 bp (primer set 2) and 721 bp (primer set 3). All primers were ordered from IDT (Integrated DNA Technologies, San Jose, CA). Primer sequences are shown in Table 1.

## DNA Purification

DNA was obtained from individual colonies, lawn or broth cultures using a commercially available DNA isolation kit (DNeasy, Qiagen. Germantown, MD), or by lysis followed by ethanol precipitation. For lysis/ethanol precipitation, the procedure known as "Extremely Easy DNA Extraction" (Carolina Biological Supply Company)

Table 1. Primer sequences for each primer set. Sequences are written in standard 5' to 3' notation.

| Primer Set | Fwd Sequence | Rev Sequence |
|---|---|---|
| 1 | agtctgtgggcgggtgagct | acggctctgtgatcgcccct |
| 2 | tacttcttctccagtcgatggc | gtcatgttttatcatccggc |
| 3 | ccatacacatccttatgtgatg | tgccgatatcttgtttgtct |

was followed. Briefly, a lawn of bacteria on a petri plate was lysed with 5 mL water and the lysate collected into a glass test tube. DNA was precipitated by adding 4 mL of chilled 95% ethanol. After incubation on ice for 20 minutes, the DNA could be spooled on a wooden spooling stick. Then, it was transferred to a microcentrifuge tube and centrifuged for 1 min at 2,200 x g. After removing the DNA and allowing the pellet to dry by evaporation for 15 minutes, it was resuspended in TE buffer (Tris and EDTA, pH=7.5). DNA yield and purity was determined by absorbance at 260 nm using a spectrophotometer.

Polymerase Chain Reaction (PCR) and Gel Electrophoresis

PCR was performed using purified DNA samples or from crude lysates of a single colony. PCR reactions were performed in 50 μL with 5 μL template, 0.1 μL of 100 μM forward and reverse primers, 19.8 μL $dH_2O$, 25 μL of Taq Master Mix Kit (item 201443, Qiagen, Alameda, CA) per reaction. PCR conditions for testing the primers were 3 min initial denaturation at 94$^{o}$C, 1 min denaturation at 94$^{o}$C, 1 min annealing at 60$^{o}$C and 3 min extension at 72$^{o}$C with 5 min final extension. After 30 cycles, PCR products were visualized by electrophoresis in Tris-Borate-EDTA (TBE) buffer on a 0.8% agarose gel for 75 min containing 5 μg/mL ethidium bromide in the gel and running buffer. When protocols were optimized for high school use, gels were post-stained with CarolinaBLU™ or GelGreen™ instead of ethidium bromide.

DNA sequencing

PCR products were purified using a Qiaquick PCR cleanup kit (Qiagen). They were sequenced at the University of Arkansas Medical Center in both directions using the same primers used for PCR.

## Results

### PCR of *gvpA* region for pNRC100

Three primer pairs were designed to amplify a region of the *Halobacterium* sp. NRC-1 genome containing *gvpA*. The primers were tested using template DNA prepared from three different wild-type colonies using Qiagen DNeasy kit. All 3 primer sets produced a single band of the expected size as determined by gel electrophoresis (Figure 3).

### Identification, Isolation and Characterization of *gvp* Mutants

Having the primers and PCR conditions validated, the next step was to identify and isolate *Vac*- mutants with IS elements within the amplified regions. Seven day old liquid cultures of *Halobacterium* sp. NRC-1 were serially diluted to 1:10,000,000 and spread plated on BSYC agar. After 10 days of growth at 37°C, mutants were isolated by subculturing red colonies. Red, translucent mutants (*Vac*-) were readily available. Fifty-four mutant colonies were found and transferred via sterile inoculating loop onto sectored BSYC plates for subculture (Figure 4). Thirty-five of these isolates grew out as pink, opaque colonies with the remaining 19 isolates continuing to grow out as red.

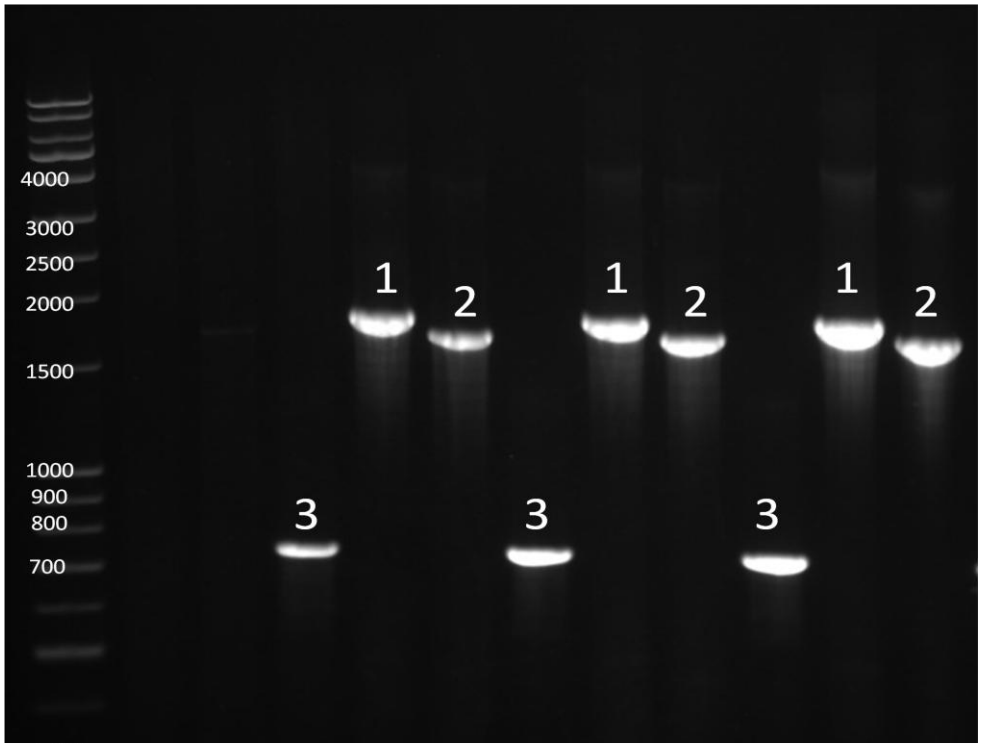


Figure 3. PCR amplicons using three different primer sets. Each primer set is numbered (1,2 and 3) and was used to amplified wild-type *Halobacterium* sp. NRC-1.

Figure 4. A sectored BSYC plate containing five possible *gvp*A mutants. All sectors were inoculated with a red colony. On this plate in particular, 4 of 5 sectors resulted in pink outgrowths. Only sector #13 shows a pure culture of red colony morphology.

The 19 red mutants of NRC-1 were tested to determine the presence of an IS element within the *gvpA* gene. DNA was extracted using Qiagen DNA-easy extraction kits and PCR was performed on each using primer set 1. PCR products were run on a 1% TBE agarose gel alongside PCR products from wild type samples. Of the 19 mutants tested, only two were found to produce a PCR amplicon different from the wild type. One contained an estimated extra 520 bp and the other contained an extra 1400 bp. These were named KBT-1 (Kansas Biology Teacher) and KBT-2 respectively (Figure 5).

Sequencing of KBT-1 and KBT-2 was done in both directions using the same primers utilized in PCR. All four sequencing reactions produced reads of approximately 880 bp. The sequences were assembled using a sequence editor (HaloWeb) to determine the reverse-complement of the sequences that were obtained using the reverse primer. A BLAST of each sequence was done against the *Halobacterium* sp. NRC-1 and were found to align with the genome locus containing *gvpA* to which the primers were designed.

Analysis of the sequences revealed KBT-1 contained 533 bp inserted immediately after position 129 in the *gvp*D/*gvp*A intergenic region. Using ntBLAST (NCBI), it was determined that the inserted DNA in KBT-1 matched an IS element called ISH2. Analysis of the assembled sequence of KBT-2 revealed it aligned for 73 bp with NRC-1 beginning at position 210 in the *gvpD/gvpA* intergenic region. Using ntBLAST data (NCBI), it was determined that a portion of KBT-2 matched the first 119 bp of both ISH8 and ISH11 (Figure 6).

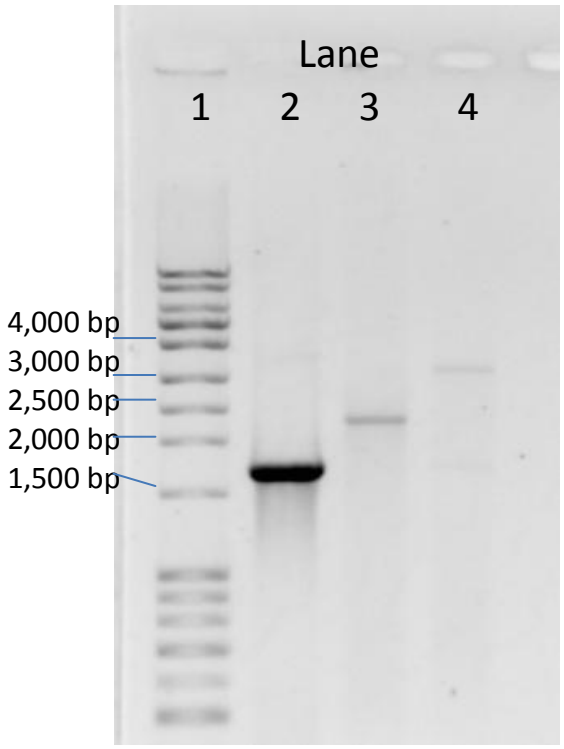


Figure 5. PCR amplicons of a wild type (lane 2) and Two Mutants (lanes 3 and 4). Primer set 1 was used on all samples. Lane 2 (NRC-1) has a band of approximately 1750 bp, the band in lane 3 is approximately 2300 bp (KBT-1) and the band in lane 4 is approximately 3200 bp (KBT-2).

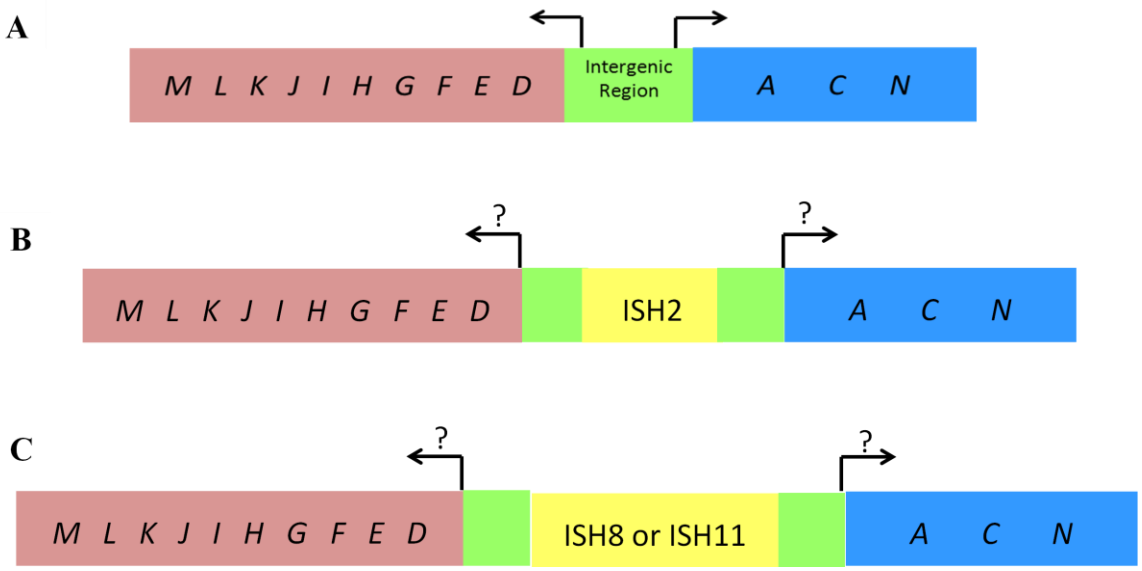


Figure 6. A comparison of the gas vesicle gene regions of pNRC100 for sp. NRC-1, KBT-1 and KBT-2. Panel A shows the *Vac*+ genes of sp. NRC-1 (wild type). Panel B shows the IS element, ISH2, which incorporated into the intergenic region between *gvp*D and *gvp*A of KBT-1 (*Vac*-). Panel C shows the same region for KBT-2 which also had ISH8/ISH11 insert into the intergenic region. Images not to scale.

## Adapting protocols for use in high schools

In order to develop a teaching kit for high school classrooms, it was essential to develop an easy and inexpensive DNA extraction protocol and to validate the DNA obtained was amenable to PCR. This is not a trivial set of experiments. Commercial kits are optimized to limit the effects of DNases and there are shear forces in pipetting and mixing, all of which limit the quality of DNA obtained. In addition, any contaminants left behind from simple DNA protocols make quantification difficult and potentially hinder the notoriously finicky PCR reactions.

The first method tested was based on a published protocol by the Carolina Biological Supply Company called "Extremely Easy DNA Extraction." Three plates containing wild type *Halobacterium* NRC-1 were cultured for 7 days at 37°C to create a lawn of growth. DNA was extracted from the lawn by lysis in water and subsequent precipitation with ethanol. This procedure produced a large amount of DNA. It was found to be 1.15 A260/A280 and with average yield 137.8 ng/μL and determined by spectrophotometer suitable for PCR if diluted to 12-30 ng/μL with TE buffer. However, because of the high mutability of *Halobacterium* sp. NRC-1, even DNA fragments from wild type (pink) pure cultures contained multiple amplicons (Figure 7).

Although the modified "Extremely Easy DNA Extraction" method provided an inexpensive way for high school students to extract *Halobacterium* DNA, it was not expected that high school laboratories would be equipped with a sensitive enough spectrophotometer to determine the appropriate amount of DNA for PCR. Furthermore, the multiple bands from a lawn would be problematic for interpreting results. In order to eliminate the measuring and diluting of DNA samples, PCR was tested using raw

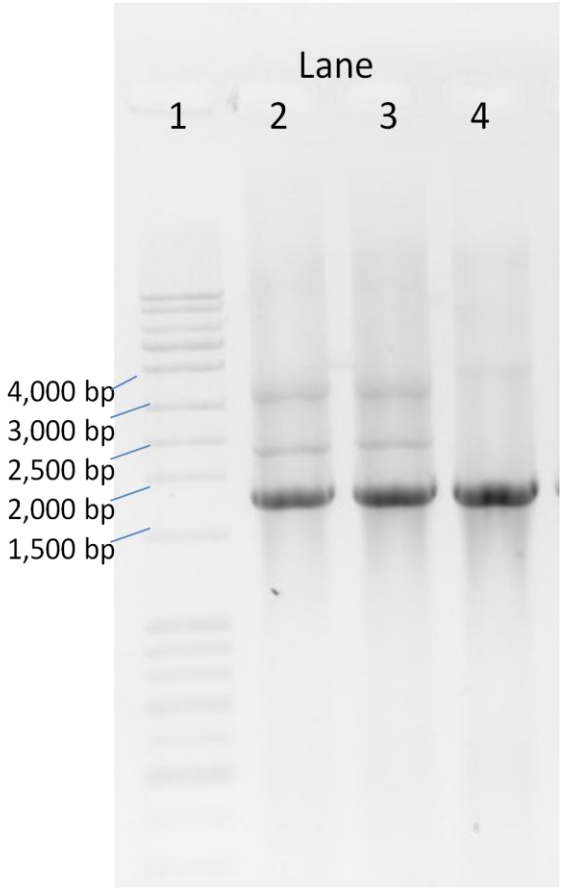


Figure 7. Modified "Extremely Easy DNA Extraction" samples (lanes 2-4). These samples were amplified by PCR then run on 1% TBE agarose gels. The samples in lanes 2-3 were each from plates containing 3 or 4 pure red cultures which shows the expected multiple bands. However, lane 4 sample was extracted from a lawn of pure wild type cells (pink) with the anticipated band at 1800 bp. An additional band is faintly visible at approx. 3800 bp. Although pure cultures would typically have only one band, the spontaneous mutation rate of approx. 1% in *Halobacterium* sp. NRC-1 means that additional bands are not unlikely.

cell lysate from a single colony which was lysed 500 μL water. As shown in Figure 8, these samples are suitable for PCR although there was some variation in the intensity of bands.

## Pilot Study and Further Refinements

In the spring 2011, the protocols we had developed up to this point were field tested by biotechnology students at Shawnee Mission West High School in Overland Park, KS, instructed by Mrs. Brenda Bott. It was immediately determined that the 3 ½ hour PCR protocol we had been using would be unacceptable in a classroom setting because it would preclude using the thermocycler in back-to-back 50 minute classes. Therefore, the times were adjusted to 3 min initial denaturation at 94$^{o}$C, 15 sec denaturation at 94$^{o}$C, 15 sec annealing at 60$^{o}$C and 45 sec extension at 72$^{o}$C, with 30 cycles then 5 min final extension at 72$^{o}$C, allowing the PCR to be completed in about 55 minutes (Figure 9).

Mrs. Bott also suggested we simplify the steps for PCR preparation of master mix and dispensing of aliquots into PCR tubes by suggesting the use of Taq Ready-To-Go™ PCR Beads. These retail for approximately $1.00 per reaction (GE Healthcare). Each bead consists of Taq polymerase, buffers, dNTPs and stabilizers needed for PCR reactions. The bead comes in a 0.2 ml PCR tube. Addition of primers and a DNA template were the only remaining requirements for a 25 μL reaction. To further streamline the process of preparing PCR reactions, a "primer mix" was created consisting of 0.5 μL forward primer, 0.5 μL reverse primer and 4 μL Dimethyl Sulfoxide (DMSO).

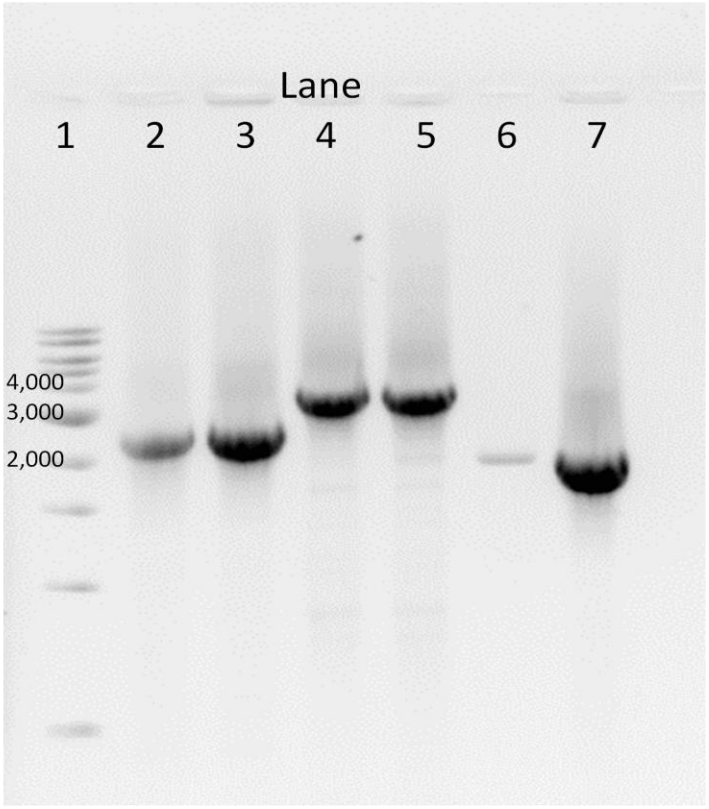


Figure 8. PCR of crude lysates prepared by lysing a single colony. Each colony was lysed in 500uL water followed by microcentrifugation. Lanes 2 - 3 contain amplicons from KBT-1. Lanes 4 – 5 contain amplified DNA from KBT-2. Lanes 6 - 7 contain amplicons from NRC-1. Photo by J. Hund.

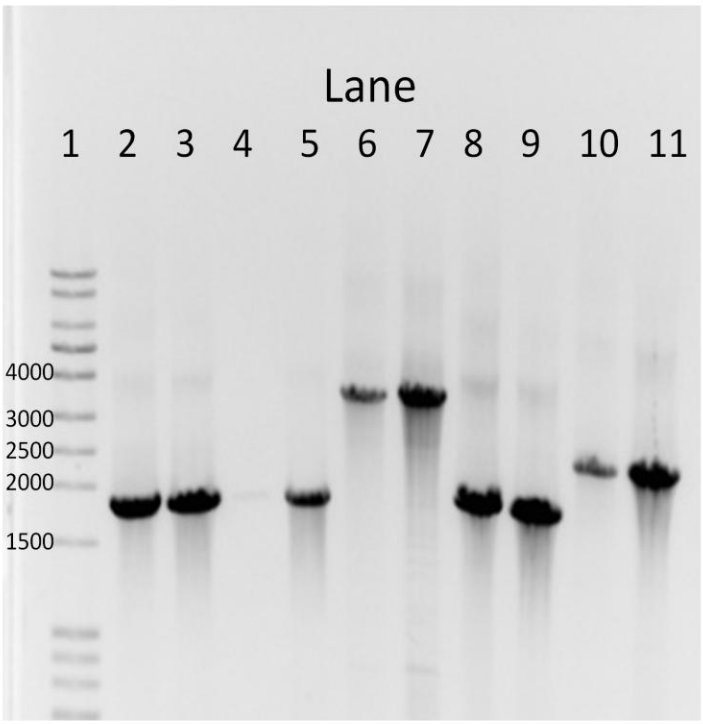


Figure 9. 1% agarose gel with PCR products that were in the thermal cycler for approximately 55 minutes. Lanes 2-5 and 8-9 contain amplicons from NRC-1. Lanes 6-7 contain amplicons from KBT-2. Lanes 10-11 contain amplicons from KBT-1.

These changes greatly reduced the number of pipetting steps thereby decreased PCR setup time without compromising quality of PCR reactions (data not shown).

## Gel Electrophoresis Protocol

The next step was to determine the ideal agarose gel percentage for gel electrophoresis. The DNA fragments for NRC-1 were 1792 bp and the fragments for KBT-1 were 2325 bp. Various gels were run with 0.8% - 1.2% TBE agarose to determine which had the best band separation with bands of this size in the time limits imposed by a high school class. It was determined that 0.8% gels provided good band separation when run at 115 V for 70 min (Figure 10).

A DNA ladder for gel electrophoresis was needed to allow the accurate measurement of the sizes of the bands with sufficient resolution to distinguish a wild type (1792 bp) from KBT-1 mutants (2325 bp). A DNA ladder with bands from 10,000 – 500 bp (1 kb DNA ladder, New England Biolabs) had bands in increments of 1,000 bp and allowed easy visualization of the fragment size that was either larger or smaller than 2,000 bp (Figure 10).

## Discussion

Our results found that 35 of 54 isolated red mutants resulted in pink wild type colonies upon sub-culture. This is likely either from contamination during isolation from the other colonies on the plate or due to them being revertants (not true *Vac*- colonies). Of the 19 red mutants that remained red during sub-culture, 17 were observed by gel electrophoresis to have the same amplicon size as did the *Vac*+ controls. Although they

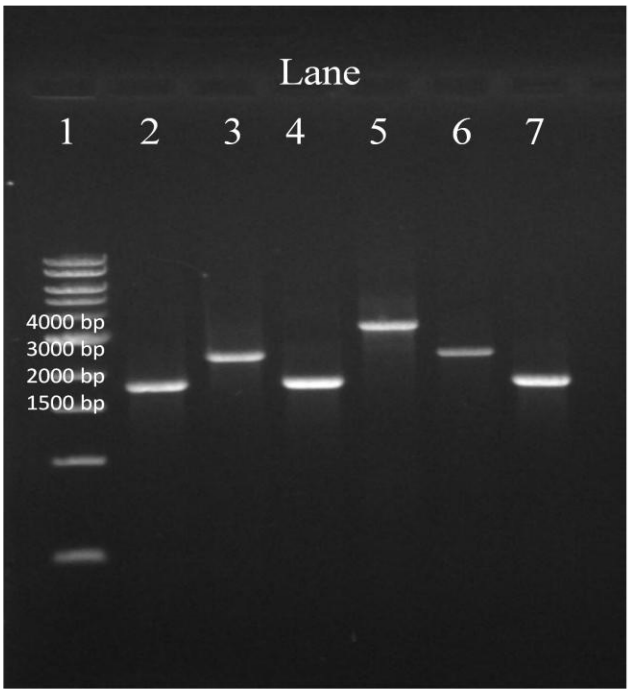


Figure 10. 1 kb DNA ladder used to quickly estimate size of DNA fragments on a 0.8% agarose gel. Lane 1 is the DNA ladder. Lanes 2, 4 and 7 contain amplicons from NRC-1 (1792 bp). Lanes 3 and 6 contain amplicons from KBT-1 (2325 bp) while lane 5 contains KBT-2 amplicons (approx. 3200 bp).

could have contained an IS element which caused a disruption to the *gvp* gene region, they did not contain an IS element within the amplified primer region.

Two of the isolated red mutants had amplicons which produced larger bands (approximately 2300 bp and 3200 bp) than the *Vac+* controls (1792 bp). We named the mutants KBT-1 and KBT-2, respectively. KBT-1 was determined to have an IS element, ISH2, that had inserted just before the *gvpA* gene start. ISH2 contained 521 bp followed by 12 bp direct repeat. Downstream of the direct repeat, the sequence of KBT-1 matched NRC-1 and continued uninterrupted for approximately 200 bp downstream. After this, there was incomplete sequence data for approximately 550 bp which was reconstructed using the NRC-1 sequence. KBT-1 was determined by sequence data to contain 2325 bp in the area amplified between primers. This was consistent with evaluations of gel electrophoresis amplicon size.

ISH2 is typically 521 bp long with up to 19 bp inverted repeats on each. When the insertion occurs, a sequence at the insertion site is duplicated from the beginning of the insertion site and duplicated at the end of the IS element (Filée et al., 2007, p. 126). This is called a direct repeat and was found to be 12 bp long in KBT-1. Thus, the entire insertion was 533 bp, making the entire amplicon 2325 bp.

The other isolated mutant, KBT-2, was found to align 73 bp with NRC-1 beginning 210 bp after the start of the *gvpD/gvpA* intergenic region. After those 73 bp, there were 113 bp that partially matched with both ISH8 and ISH11. Following the partial IS element insertion, KBT-2 aligned with NRC-1 for the final 684 bp in the amplified region. However, the construction of the sequence data is incomplete as it did not contain approximately 3200 bp that was shown on multiple gels. It is possible that

KBT-2 does contain either or both complete ISH8 (1402 bp) or ISH11 (1068 bp) sequences which would be consistent with the estimated 3200 bp. Alternatively, KBT-2 could contain partial sequences of either ISH8 or ISH11 or other IS elements which, when combined, would be approximately 3200 bp (Filée et al., 2007, p. 126). However, without complete sequence data, it is unknown. Both KBT-1 and KBT-2 sequences are diagramed in Figure 6.

We have reported that single colonies of *Halobacterium* lysed in water followed by crude purification were able to successfully undergo PCR. Using water as the only reagent and a microcentrifuge as the only equipment required for DNA extraction made the protocol inexpensive and simple for high school use. Single colony extractions were necessary to ensure DNA is extracted from a single pure culture. Because of the mutation rate of *Halobacterium* sp. NRC-1, extraction from a petri plate lawn of NRC-1 would not yield pure culture results, as Figure 7, lane 4 shows. In addition, it would be more costly and time consuming for teachers to produce enough entire lawns for each student to have one plate from which to extract DNA. Single colony extractions using water and microcentrifuge made the process cheaper and easier.

Following the suggestions of the school that piloted the kit, we altered the PCR kit protocols. Taq Ready-To-Go™ PCR Beads were used to simplify PCR preparation. Students would only need to add DNA template to the PCR tubes which already contain the Taq PCR bead. The thermal cycler times were also adjusted to run within a traditional class period. Both changes allowed for a much more simplified way for students to create a 25 μL PCR reaction.

The aforementioned materials and protocols were optimized for the teaching kit, *The Genotype-Phenotype Connection: Basic Molecular Biology and Bioinformatic Skills,* now available through Carolina Biological Supply Company (Burlington, North Carolina). Each protocol was written into a different lab activity found within the kit. Students choose a colony to perform DNA extraction, either KBT-1 or NRC-1. They then work through the activities of PCR, gel electrophoresis and data analysis to relate the concept of genotype to phenotype of *Halobacterium.* With the assistance of the DasSarma lab (Baltimore, MD), we also developed an extensive teacher's manual (Appendix A).

# Chapter 3:
# Field Trial of
# The Genotype-Phenotype Connection Kit

Engaging students in the process of learning will improve critical thinking and problem solving; therefore it is important to involve students in their learning (Taraban et al., 2007, p. 961). A student-centered laboratory activity where students can be *engaged* in the inquiry process through questioning, protocol design, data collection and analysis is the most obvious way to involve the students in a science class (Marbach-Ad et al., 2010, p. 3; Yip, 2010, p. 485). Yet, it has traditionally been difficult to apply these ideals when teaching molecular biology topics to high school students (Duncan et al., 2007, p. 938-939; Stieff, 2011, p. 1138).

Molecular biology concepts of DNA, transposable elements, bioinformatics, cell lysis, PCR, genotype-phenotype relationships and gel electrophoresis are the subjects of the hands-on laboratory teaching kit we designed for high school students called *The Genotype-Phenotype Connection.* This kit weaves the technological applications of the discipline of molecular biology with exploration of a living organism, *Halobacterium* sp. NRC-1. The kit uses a hands-on, engaging approach to introduce students to concepts and laboratory procedures they may not have experienced before such as Archaea, bioinformatics, polymerase chain reaction (PCR), IS elements and gel electrophoresis. To evaluate the kit as a teaching tool, we developed the following research questions with teachers and students in mind:

- Do teachers find this kit to be an effective molecular genetics & bioinformatics teaching tool?
- What suggestions would teachers have for improving it?
- What are students' reactions to laboratory exposure of these concepts?

- Are the laboratory activities of the kit effective at teaching molecular biology concepts to AP biology students?

## Materials and Methods

Potential field testing teachers were identified from the Kansas State Department of Education (KSDE) database, which was compiled through statewide submissions by principals the previous fall (Kansas, 2011). We sent an e-mail soliciting participation in the study to 41 Advanced Placement (AP) Biology teachers (Appendix B). Teachers were asked to complete an online screening survey (Appendix C). The survey was conducted through Zoomerang and posted online through the Information Technology Services department at Emporia State University. The survey collected logistical and demographic information about the teacher and school so that selection of the field study participants could be made. Ten teachers were invited to participate in the field study, nine of which accepted the invitation. Three teachers taught two or more sections of AP Biology, resulting in a total of 13 sections of AP biology classes committing to the study. To control variability between classrooms, teachers were provided detailed instructions and timelines for completing the activities. This included students reading the background, completing the pre-labs, laboratory activities and the student follow-up questions. A lesson plan suggestion for the week was provided to teachers to help them with pacing of the kit components (Appendix D). To help identify variables in delivery of kit materials teachers were asked to report any portions of the kit they were unable to complete.

Student learning gains were assessed using short pre/post tests (Appendix E). These tests were timed, allowing 12 minutes for completion. Although 206 students participated in the kit field trial, 14 students' scores were omitted because they were

either missing the pre-test or post-test. Therefore, 192 students completed the pre/post assessment of the field trial (n=192).

Scores for the objective portion of the student tests were tabulated by entering each students responses into an Excel spreadsheet (Microsoft Office). One-tailed t-tests were used to determine if student post-test scores held higher student achievement than the pre-test scores. Results were tabulated for each question independently and considered statistically significant if $p<0.05$. The student short answer responses were evaluated by two independent evaluators using a rubric that distinguished three levels of responses; partially correct answers = 0.5 points, correct answers = 1.0 points and upper level answers = 1.5 points. Wrong or blank answers did not receive a score. Scores from the two evaluators were averaged; agreement on point values between the two evaluators was 97%.

Qualitative assessment was also performed. Teachers completed a post-kit evaluation of various aspects of the kit and additional comments were solicited (Appendix F). Students were also asked to provide general comments regarding their experience with the kit. These comments were sorted by topic (background, lab activities, etc.). Both teacher and student responses were tabulated in Excel.

## Results

### Teacher and School Participants

Teachers and students completed an informed consent document (Appendix G). All participating students (n=206) and teachers (n=9) filled out the informed consent document prior to participating in the field trial.

The nine teacher participants provided facts about their teaching credentials and experience with the topics of the teaching kit in the initial screening survey (Appendix C). Combined, these teachers represented 176 years of teaching experience (median = 18 yr). They had a combined 76 years teaching AP Biology (median = 3 yr). This was the first year for one participant to teach AP whereas another teacher indicated he had been teaching AP for 50 years. This group also had a wide range of education and teaching subject licenses. Nearly half (44%) of participants had a Bachelor of Science or Bachelor of Arts degree, whereas the others (54%) had either a Master of Science or Master of Arts graduate degree. All of the teachers indicated that they were licensed in Biology with 56% also licensed in General Science. One third of participants had additional teaching license in chemistry. One teacher indicated licensure in Botany while another in Psychology.

The schools represented in this field trial are primarily public high schools. However, one school (accounting for 4% of the participating students) was a private, non-religious affiliated school. The sizes of schools ranged from 525 total student enrollment to 2500 students, with median enrollment 1400 students.

There were striking similarities in the choice of textbooks among the schools participating in this study. Eight of nine (89%) had adopted the textbook, *Biology*, written by Neil Campbell and Jane Reece, published by Pearson. The remaining school had adopted the book *Biology*, written by Sylvia Mader and published by McGraw-Hill.

Because various high schools had the potential of offering different programs within the Advanced Placement class, teachers were asked to describe their AP biology classes. Seven of the schools (78%) participating in this study described their class as AP

Biology only. Two schools (22%) explained their class as AP Biology/College Now combination. College Now is a program offered through local community colleges or universities that allow high school students to receive college credit for completion of courses at their home high school. Both of the AP/College Now classes were offered by larger schools with a mean school size of 1550 students. These classes comprised 4 of the 13 class sections participating in this field trial, with 84 total students and mean class size of 21 students.

Additionally, teachers were asked to identify the origin of their most effective training in molecular biology techniques. Three of the teachers (33%) answered that they learned at specialty clinics for molecular biology labs. Two of those teachers specifically added that the specialty clinic was offered through Cold Springs Harbor (Cold Springs Harbor Laboratories in Cold Springs Harbor, NY). Other teachers indicated that they most effectively learned laboratory techniques through undergraduate coursework (22%) or AP Biology training workshops (22%). One teacher (11%) found the most effective way he learned molecular biology laboratory techniques was through a molecular biology teaching course offered by his nearby university. The final teacher participant indicated he learned molecular biology techniques from a variety of sources including graduate coursework and local, district or national workshops.

## Equipment Needs of Field Trial Teachers

Prior to the field trial, teachers were given a list of equipment required to complete the kit activities. The equipment that schools lacked was loaned to them by Emporia State University Department of Biology. Three of the participating schools (33%) either had a thermal cycler in their building or access to one through their district;

six schools needed to borrow a thermal cycler (67% participants). Two of the nine schools needed to borrow gel electrophoresis chambers and power supplies (22%). Three schools (33%) did not have access to micropipettes. Two schools (22%) borrowed a microcentrifuge, however one of those schools could have secured a microcentrifuge through a local community college if the high school would not have been able to borrow through ESU.

## Success with Kit Protocols

Following the timeline we provided, teachers guided their students through the background reading, pre-labs, kit activities and discussion. The entire process took approximately six classroom hours to complete. Success with the kit protocols was indicated by the presence of visible bands on a gel containing PCR products from various colonies. Five schools completed the kit procedures without significant problems as evidenced by production of analyzable gels (Figure 11A). Two schools produced gels but had difficulty in doing so. Two schools were unable to yield gels where students could see bands for analysis (Figure 11B).

Two of the seven schools that had measureable bands on the gels did encounter some challenges. One teacher ran out of time so he had his lab assistant combine the reagents for the PCR reactions instead of the students doing this step. With nearly 50 reactions to prepare, the lab assistant left the cell lysate (extracted DNA) sitting at room temperature longer than recommended. Therefore, the last 8 PCR reactions that the lab assistant set up were unsuccessful (results not shown). The second school that encountered a challenge was the one that requested GelGreen™ stain instead of Carolina BLU™ stain. GelGreen™ requires ultraviolet light to see the bands. This teacher used

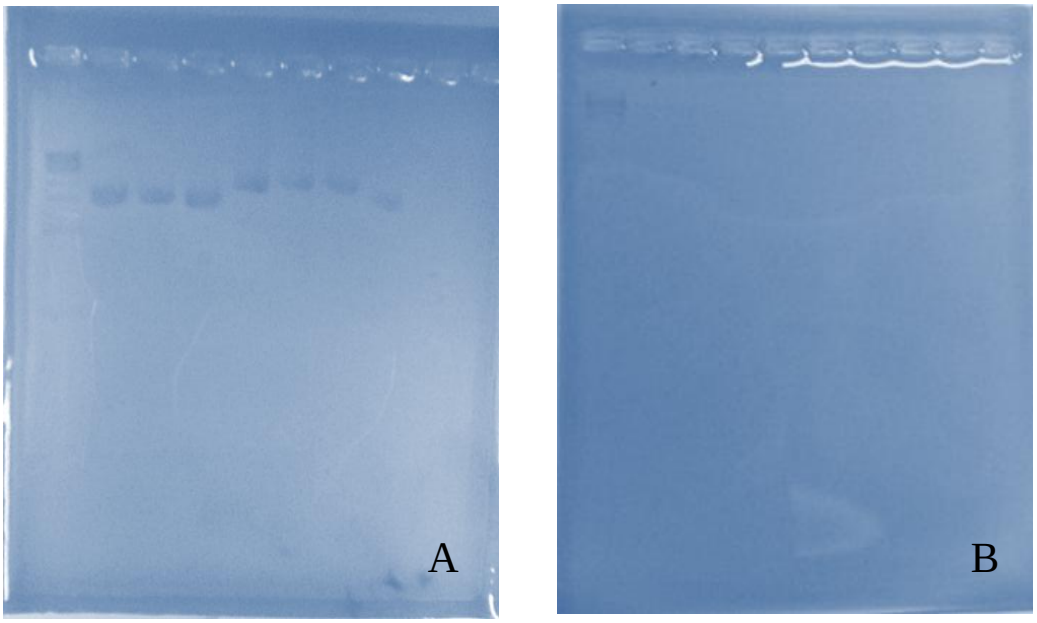


Figure 11. Example classroom gels provided by field trial teachers. Panel A is a typical classroom gel with favorable results. The ladder is lane 1; wild type DNA fragments (*Halobacterium* sp. NRC-1) are loaded in lanes 2, 3, 4 and 8. Mutant (KBT-1) fragments are in lanes 5, 6 and 7. Figure B is a gel with slight movement of the DNA ladder but no movement of the DNA fragments (barely visible in each lane).

an ultraviolet aquarium light but found that the aquarium light was not intense enough to see the bands, therefore the gel results could not be determined. However, this teacher had CarolinaBLU™ on hand and after staining with it, the bands showed up faintly and could be used in student analysis.

Out of the nine schools doing the field trial, two schools (22%) did not have any DNA bands appearing on their gels (Figure 13B). It was not immediately clear why one of the two schools had unfavorable results. The teacher was confident she and her students followed all of the instructions within the kit. In addition, she was certain her students kept the materials on ice when directed to do so, working briskly but carefully so nucleases did not degrade the DNA. She allowed the gel to run for 75 minutes, yet the material the students loaded into their wells did not migrate as would be expected.

A second school produced gels that the students could not analyze. Upon interviewing the teacher, it was determined the students in this class loaded the gels on a Friday afternoon and left the gel at room temperature over the weekend. Leaving the gels overnight before running or staining is not recommended in the kit directions.

## Teacher Perspectives

### Overall Post-Kit Evaluation

At the conclusion of the kit field trial, teachers completed an evaluation of the kit, rating various aspects of the kit (Appendix F). Most questions, as described below, were rated on a Likert scale from 5 through 1. Teachers rated their overall satisfaction with the concepts presented in the kit (question 1) with an average of 4.1 (mostly satisfied). When asked if the topics in this kit helped the teacher meet the course objectives (question 3),

teachers scored the kit an average of 4.3 on a 5.0 point scale with no individuals scoring the kit below 4.

Teachers rated the entire kit an average of 4.3 (agree) when asked if the kit information and activities within the kit were at an appropriate level for the AP Biology class (question 4). Teachers found that the information provided in the teacher guide was clearly stated and contained all necessary information (question 6) with an average 4.2 rating (agree). When asked if the time requirement for the various lab sections (as listed in the kit teacher guide) was reasonably accurate (question 8), teachers rated the timeline with 4.2 points. One teacher commented, "the timeline worked." Another teacher that scored this question as 4 (agree) commented that PCR preparation and gel loading took more time than stated. One teacher mentioned that information about gel storage if the schedule did not allow staining directly after running of the gel would have been helpful.

Teachers were asked about their comfort level with preparing the materials for the lab (such as diluting the TBE buffer, pouring and running a gel, etc.) given the information provided in the teacher instructions (question 7). Teachers rated their comfort level of lab preparation with an average of 4.4 points (somewhat comfortable). One teacher mentioned he had his students do the lab preparation and they followed right along. Another teacher delegated the teacher preparation to a biotechnology class within in his school. He reported that they had no problems with the preparation. Two teachers mentioned they felt more comfortable because they could call me to ask questions about any preparation issues in which they had uncertainty.

## Breakdown of Concepts Presented in the Kit

The major topics addressed in the kit are: Bioinformatics, Insertion Elements, Microbiology, PCR and electrophoresis. The kit covers all of these topics both as "wet lab," and conceptually through the background reading and student guide questions. An objective of this field trial was to determine if teachers would find this kit to be an effective teaching tool of these topics. Presumably, teachers would find the kit to be more effective at teaching concepts than their previous methods, particularly if the previous methods did not include a "wet lab" component. Teacher satisfaction with each topic as presented in the kit is shown in Figure 12.

## Teacher Perspectives of Student Guide

Since the primary student interaction with the kit was through the "student guide" it was of interest to ascertain teachers' impressions. Teachers rated the student guide by answering two questions in the post-kit evaluation questionnaire. The first question asked if the teacher found the instructions/protocols provided in the student guide to be clearly stated and containing all necessary information (question 9). Teachers scored this question with an average of 4.4 points (agree). One teacher commented that the "instructions for the students were well-written." Another teacher commented that visual aids would facilitate the instructions. Another teacher mentioned that he would have liked more directions and for it to be clearer where the lab was going. He also mentioned that a crime scene investigation (CSI) approach would be interesting. Another teacher commented that this was an "excellent student guide."

The second question pertaining to the student guide asked the teachers if "the descriptive information in the kit (background and within each activity) was sufficient for

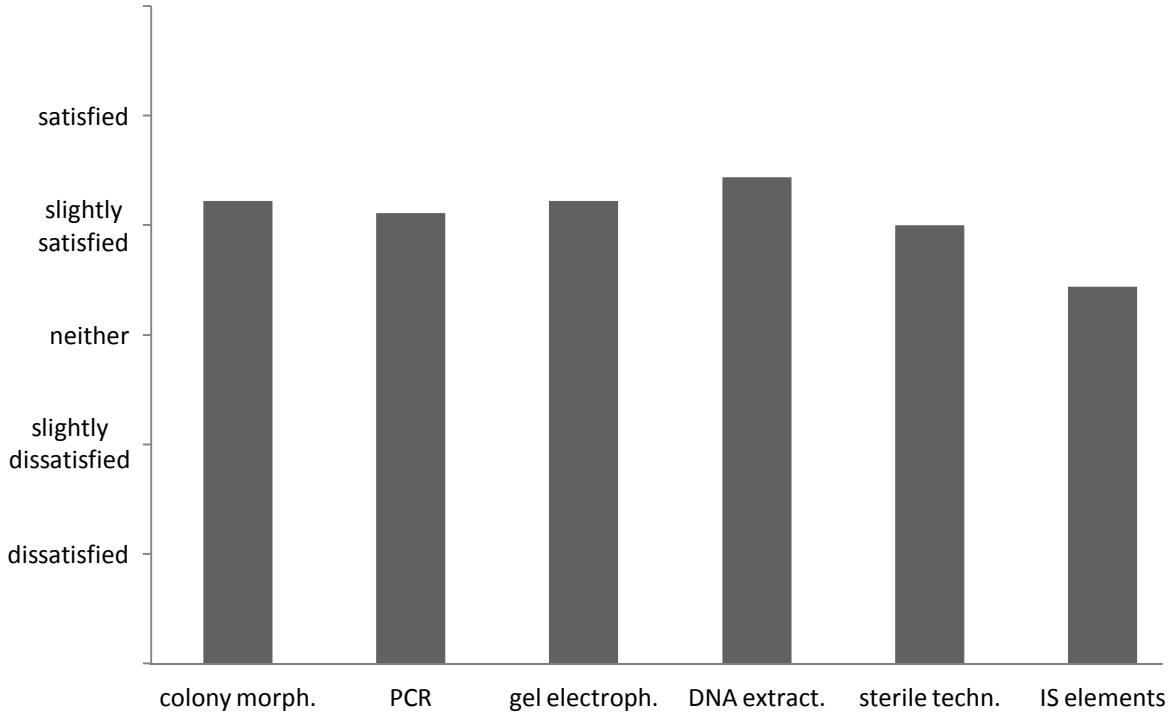


Figure 12. Teacher satisfaction with topics as presented in the kit. scores were rated on a Likert scale of 1-5 with n=9 teachers.

students to answer the student analysis questions" (question 12). Teachers scored this question with an average of 4.2 points (agree). One teacher commented that his students struggled with some questions, but were able to answer most of them. Another teacher observed that the information to answer the questions was embedded in the background for the most part but teacher instruction was needed to clarify the concepts.

## Analysis of Student Pre/Post Test Scores

To determine student knowledge and understanding of concepts, students completed a short test before and after using the kit (Appendix E). The pre/post-tests were divided into two portions: objective (multiple choice/matching) and short answer.

### Objective Questions

On the objective portion of the tests, the first question entailed matching 5 PCR-related vocabulary words with their meanings (question 1). A comparison of pre-test compared to post-test scores for each student participant showed that students improved their overall vocabulary scores by an average of 10% after completing the kit activities. Two vocabulary words, however, scored lower on the post-test than the pre-test. Students scored *lower* on average for the words "nucleotide" (-10%) and "DNA polymerase," (-6%). However, these differences were not statistically significant.

On the other hand, students scored *higher* on average for three words. For the word "DNA", students scored an average of 6% higher on the post-test and for the word "primers," students scored 8% higher. However, these differences were not statistically significant at $p<0.05$. On the post-test, students scored higher for the word "buffer" students scored 15% higher which was found to be statistically significant (Figure 13).

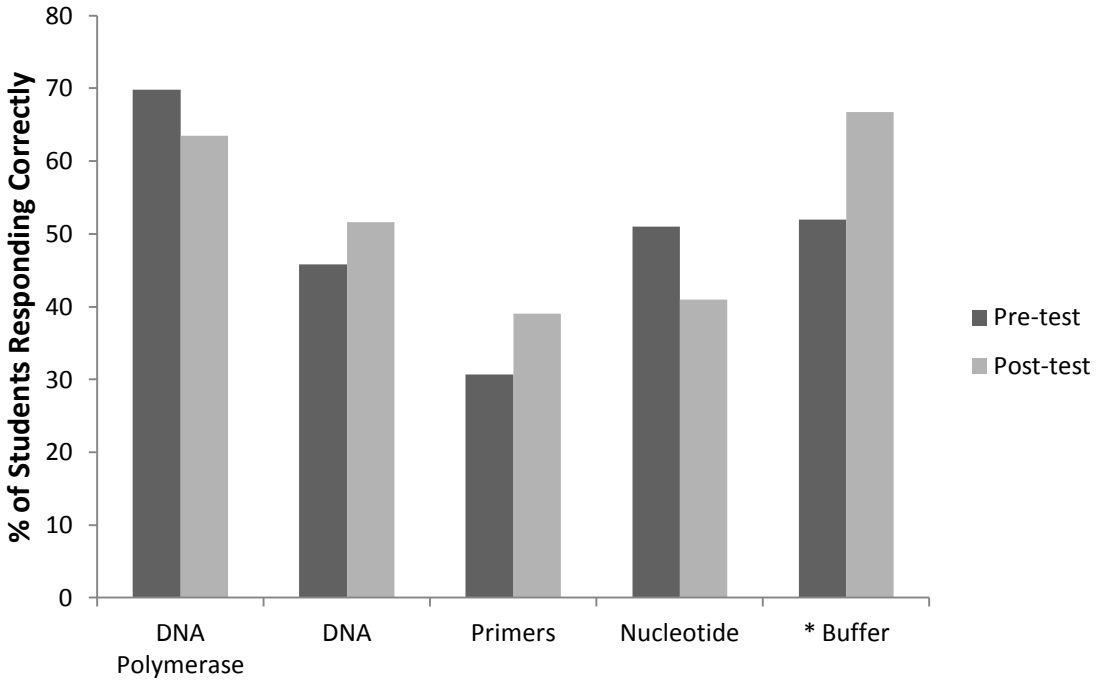


Figure 13. Average student pre/post-test scores for matching vocabulary questions. Only the word indicated by asterisk, "buffer," was found to be statistically significant. Score differences between pre-test and post-test were not found to be statistically significant at $p<0.05$ for the other vocabulary words.

The remainder of the objective portion of the pre and post-tests was comprised of 7 multiple choice questions. A comparison of student individual pre versus post-test scores indicated an average 35% overall increase in correct multiple choice answers after the students were exposed to the kit (Figure 14). Paired 1-tailed t-tests comparing each students' pre-test score to his/her post-test score were completed for each of the 7 questions. Each question had statistically significant student achievement for the multiple choice post-test at $p<0.05$. Question 2 addressed students' knowledge of the fundamentals of PCR. Question 3 assessed student understanding of band migration during gel electrophoresis. Question 4 had students choose what would happen if a cell was placed in a solution that was very salty (hypotonic solution). Question 5 asked the students to choose appropriate primers from a diagram to amplify a gene region known to contain IS elements. Question 6 also referred to the diagram from Question 5. Students were asked to explain gel electrophoresis results that yielded bands the same size, even though the mutant was known to contain an IS element. Question 7 described a case study of a patient that suffered physiological effects of an IS element in his/her genome. Question 8 asked students to identify characteristics of the Archaea Domain.

Short Essay Questions

The second portion of the pre and post tests included 3 short answer questions. Question 9 was in 2 parts: students were asked to A) briefly explain one cause of a gene disruption and B) explain the effects of that gene disruption. It became apparent during the scoring of the tests that some students confused the cause with the effects. Because it was more important to consider the students' knowledge not their ability to read the

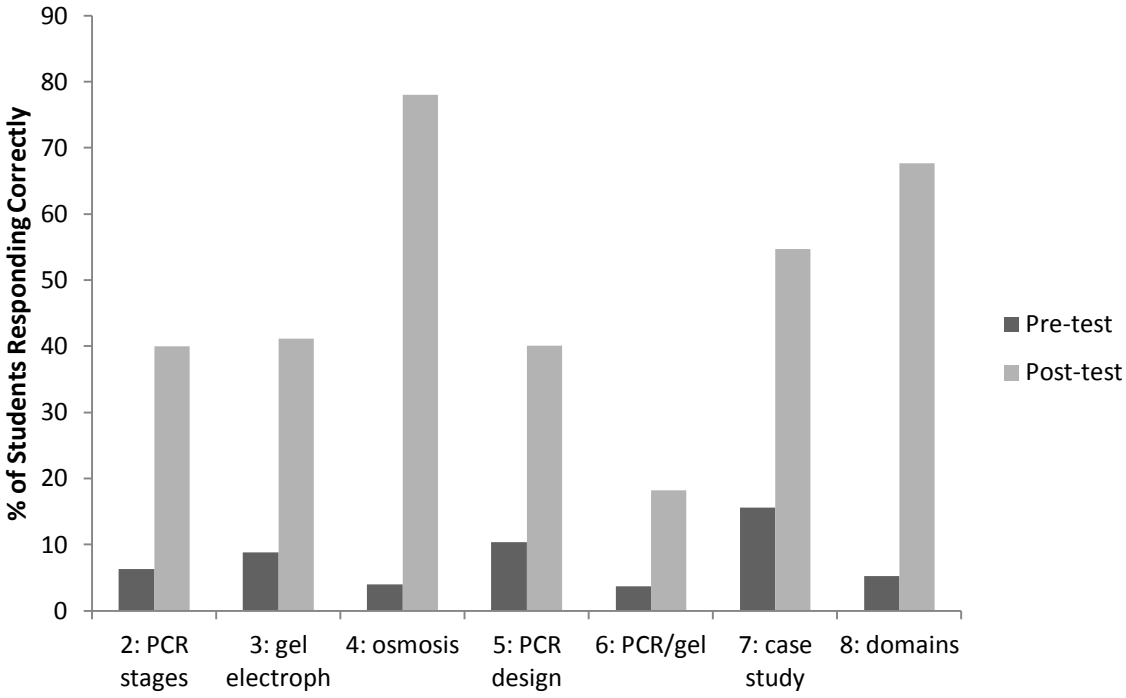


Figure 14. Comparison of average student pre/post-test multiple choice scores. The post-test scores were found to be statistically significant for each of the 7 questions.

question carefully, both A and B were considered before a score for each was rendered. Question 10 asked students to consider an evolutionary advantage of a genome that has IS elements (transfer of knowledge).

The student short answer responses were evaluated by two independent evaluators using a rubric that distinguished three levels of responses; partially correct answers = 0.5 points, clearly correct answers = 1.0 points and upper level answers = 1.5 points. Wrong or blank answers did not receive a score. Scores from the two evaluators were averaged. Comparing the pre-test to the post-test, approximately one-third of students went from 0 points (wrong or blank answer) on each essay question to receiving points for some form of correct answer (partially correct, clearly correct or upper level answer). There were no students (pre or post) that responded with an upper level answer on question 9A. Very few students had an upper level answer on the other questions. Two students had answered with upper level understanding on 9B on the pre-test, compared to 9 on the post-test. Zero students had an upper level understanding on question 10 on the pre-test, with 2 students demonstrating upper level understanding on the post-test.

Students improved their overall essay score on the post-test with an average of 22% (Figure 15). Student achievement for each short essay question on the post-test was found to be statistically significant at $p<0.05$. Question 9A asked students to describe a "cause of gene disruption." Question 9B had students explain the "effect of the gene disruption written in 9A". Question 10 asked students to describe "an evolutionary advantage " of a genome that has transposons. This question was not specific to IS elements but remained general with the term "transposon."

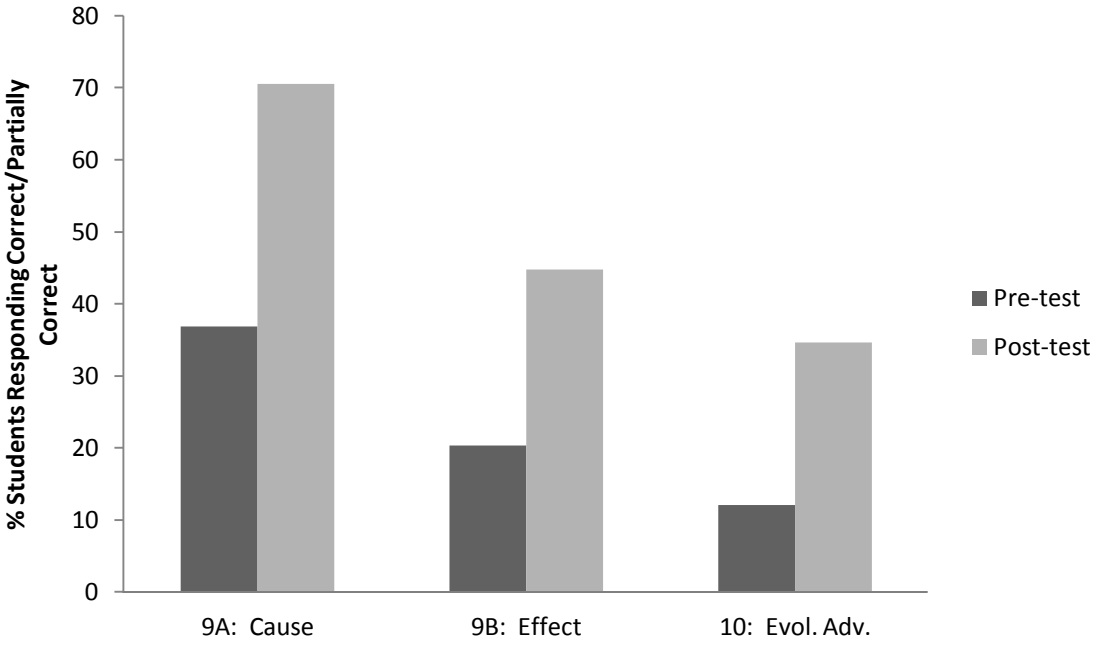


Figure 15. Comparison of average student short essay scores. Student achievement for each short essay question on the post-test was found to be statistically significant.

## Student Perspectives of the Kit Experience

Following the 12-minute post-test objective and short answer questions, students were given additional time to provide open-ended comments about the kit. They were asked to "please give me specific feedback regarding *The Genotype – Phenotype Connection: Molecular Genetics and Basic Bioinformatics Skills* kit." The only additional prompt offered was "I would love to have your thoughts about the various lab activities, the pre-labs, the background information and the Student Analysis and Follow-up Questions (good or bad)." Only ten percent of students either chose to leave this section blank or wrote "no comment" while 90% responded with remarks (173 students). Because they were given the opportunity to write as many comments as they wished, the total number of comments exceeds the number of student respondents. A summary of student remarks is found in Table 2.

### Overall Impressions

There were 232 general remarks regarding this lab. Seventy-seven percent of those comments were positive. Some of the positive comments included two students that mentioned that they found the instructions in the kit clear and detailed so the labs were easy to accomplish. Another student commented that this lab was "super fun", while another mentioned that he/she thought it "was neat being able to do higher level biotech labs." One student stated that he/she really enjoyed all the steps of the lab and another student stated he/she was glad to do this lab because "it was good to do something different, I have never done anything like this before." Other positive comments included mention of the step by step instructions with information about why each step was done was nice and another that stated he/she learned a lot about the

Table 2. Percentage of Student Respondents for Various Components of the Teaching Kit. The overall responses (n=387) could be broken down into general comments or various subtopics topics.

| Topic | Positive Comments | Negative Comments | Student Respondents |
|---|---|---|---|
| General Kit Comments | 77% | 23% | n=232 |
| Hands-On Component | 53% | 47% | n=34 |
| Background Reading | 61% | 39% | n=49 |
| Pre-labs | 36% | 64% | n=25 |
| Student Data Analysis | 83% | 17% | n=6 |
| Follow-up Questions | 54% | 46% | n=41 |

interaction between genotype and phenotype from this lab. One student commented that he/she "definitely learned a lot from this lab, potentially more than any other lab I've done in a science class."

Of the general comments regarding the kit, 23% of them were negative. Some of the negative comments included "the general message was clear, just getting the information in some areas wasn't understandable" and "confusing at first." One student mentioned that he/she did what the sheet said but “wasn't really sure what I was doing." Another student had a similar remark with "we'd do something and I wouldn't get why." One student commented that he/she found the lab to be too hard and that it expected way too much. There was a specific comment regarding the gel electrophoresis procedure where the student said that more explanation of how gel electrophoresis works would have been helpful in understanding.

Hands-On Approach

Thirty-four students commented specifically on the hands-on aspect of doing this lab. Eighteen of those students mentioned a lab activity or equipment with which they enjoyed working. Some students were general, mentioning that the hands-on component of this lab was "really cool." Others were more specific such as one student who stated that he/she "loved making our own experiment" by choosing which colony to extract DNA from. Another student concurred by commenting, "I really liked being able to pick my own colony, it made me feel like a scientist." Four students mentioned they liked doing PCR, with one student commenting that now that he/she has seen it, it makes more sense than just reading it. Other students mentioned that they especially enjoyed working

with bacteria, using micropipettes, doing gel electrophoresis and working with DNA. Two students (who were in the class that the teacher had the lab assistant do the PCR reactions for the class) mentioned that they felt they would have learned more and understood how PCR worked if they would have been able to do it themselves.

Sixteen of the students that specifically mentioned the hands-on aspect of this lab did so with a negative comment. One person mentioned that they did not like working with the (halo)bacteria while another one stated "bacteria=smelly." One student stated that it was difficult to load the DNA into the wells for gel electrophoresis because his/her hand shook. Another student stated that he/she would rather have the lab explained instead of doing it hands-on. Curiously, 11 of the 16 students who mentioned they did not enjoy the hands-on component of the lab were all from the same school and had nearly the same comments regarding the micro scale. These students had comments such as, "if the amounts were larger, it would be easier to get results" or, "it was difficult to tell if we even had anything."

Student Impressions about the Background Information

All of the students participating in the field trial were assigned the background reading (either as homework or in-class). There were 49 student comments regarding background information. Sixty-one percent of students who specifically commented on the background information found it helpful. Some of the comments from students who found reading of the background to be a positive experience included one student who wrote that he/she could have referred back to the background to solidify the concepts. One student stated that the "background information helped a lot" and another described

the background as "solid, helpful and clear." Another student found it informative and well-organized.

Thirty-nine percent of students who commented on the background did not like it. One student stated that he/she found some of the background information irrelevant to the experiment. Another mentioned that he/she had to read through the background several times to fully understand it. One student stated that the background was "confusing at times" while another student commented that there was too much background information to absorb all at once. Because it could be construed positively or negatively, the 3 student comments that asked for *more* background information were omitted from the background percentages.

Pre-lab Comments

There were three pre-labs found within the kit. The first pre-lab had the students explore bioinformatics of *gvp*A online. The second pre-lab entailed students watching a PCR tutorial online. Pre-lab 3 was a video for students to view loading a gel for electrophoresis. Twenty-five students commented on the pre-labs. Over a third of those students liked the pre-labs (36%) while nearly two-thirds (64%) did not find them helpful. One student who liked the pre-labs mentioned that he/she found them to be very informative. Some of the comments from students who did not like the pre-labs were concise with "pointless" or "nothing learned." Three of the students that did not like the pre-labs specifically mentioned pre-lab 1 (bioinformatics) as being "way too confusing" or "wasn't sure how to connect pre-lab to the lab."

## Student Analysis Comments

There were only 6 comments regarding the student analysis portion of the lab. This was the portion of the kit that required individuals to report results to the rest of the class. Results were a calculation of his/her band size with a discussion of the size related to the original color of colony that the student chose. Five (83%) of the 6 were positive comments with 1 (17%) being negative. One student commented that the student analysis was fun while another wrote that the "student analysis was easy after the lab was done." The negative comment was a student that stated that the "student analysis was done as a class, I'd prefer to do it by myself." However, the nature of the student analysis required sharing information by the class; therefore this would have been impossible.

## Comments about Follow-up Questions

The student comments regarding the follow-up questions were polarized. There were 41 comments with 54% of them positive (22 comments) and 46% negative (19 comments). Some of the positive comments included "the questions were helpful in retaining information" and "the questions helped with understanding the lab." Three students stated that the questions helped highlight/test what they had learned. Negative comments regarding the follow-up questions included "I didn't enjoy the questions compared to the rest of the lab" and that there were a few "stupid/non-covered questions." Four students specifically mentioned that they found the questions confusing. A summary of all student written comments is shown in Table 2.

## Discussion

### Overall Results

Gel electrophoresis images were the end result of the kit laboratory activities and served as a measure of "success" using the kit materials and instructions. Students analyzed these gels to determine if their amplified DNA fragment had a potential IS element. Eleven out of the thirteen classes had gel results that they could use for analysis. The failure of two teachers to get results suggests a certain level of technical expertise is required to complete the experiments successfully. Although teachers agreed the information provided in the teacher guide was clearly stated and contained all necessary information, one teacher used the incorrect voltage setting on the electrophoresis equipment and the other allowed loaded gels to sit for several days. Two other teachers had some technical issues that likely would have produced unreadable gels had the issues not been resolved through communication with me. We conclude that the kit worked without incident for five of the nine teachers. Perhaps it is not surprising that the successful teachers were the most experienced and had received the most training in molecular biology techniques.

### Did teachers find the kit to be an effective teaching tool?

The teacher response to the kit was overall very positive. They were "mostly satisfied" with the concepts presented in the kit. The responses on the evaluation questionnaire indicated that teachers found the kit to be effective teaching the concepts. Teachers indicated the kit activities met their course objectives. Even with teachers "mostly satisfied" and finding that the kit did help them meet their course objectives, the teachers offered some constructive ideas for improvement.

A few teachers offered suggestions for improving the kit. One teacher specifically stated that pre-lab 1 (bioinformatics) was too confusing so it should be altered. One teacher had commented that the PCR preparation and gel electrophoresis both took too much time to load and run. This comment could be attributed to this teacher's inexperience as it was the first time the teacher had ever run either PCR or gel electrophoresis.

## Student Experience with Kit Concepts

Most students (77%) who provided comments about the lab were positive in their experience to performing these activities in a laboratory setting. However, 16 students commented specifically that the hands-on component of this lab was not enjoyable. Eleven of the 16 students were from the same school. These 11 students mentioned the small scale of the labs made it difficult to perform the steps and know if there was anything happening. Because these comments were similar and from the same school, there was likely teacher bias that influenced the 11 students comments. The teacher at this school was inexperienced with molecular techniques and had never used a micropipette, PCR or gel electrophoresis.

## Student Comprehension of Concepts Presented by Kit

When comparing the pre-test results to the post-test results, it was determined that the kit helped students learn the concepts. Their short answer, multiple choice and short essay scores all showed statistically significant improvement after completing the kit

activities. However, the matching vocabulary section did not have a significant difference in student achievement.

## Closing Remarks

By contributing to this study, students and teachers had the opportunity to participate as subjects in an authentic biology research project. They experienced a safe, user-friendly microbe that exhibits properties that allow for learning microbiology, molecular genetics and bioinformatics. Using the *Genotype-Phenotype Connection* Kit, students and teachers gained insight into development of kits that are often used in AP Biology classroom laboratories.

All 9 of the teacher participants were appreciative of the opportunity to use technology or a microbe that they had not incorporated into their AP Biology classrooms before. One teacher commented that without the complementary kit, his class would not have been able to do any DNA or molecular biology wet labs this year due to budget issues. Another teacher continued to use *Halobacterium* sp. NRC-1 in his classroom throughout the rest of the school year.

As evidenced by the student comments, the teacher has the ability to make this or any lab experience a positive one for the students by gauging student reactions and responding appropriately. A teacher versed in molecular lab techniques can enhance the materials found within the kit by encouraging students to trust the protocols, for example that there is DNA present even if it is not seen. An experienced teacher also has the skills to prepare the materials and run the equipment adequately. Checking for understanding

as the lab progresses will ensure students are not frustrated with these new concepts and are beginning to form connections to the interrelatedness to the rest of the curriculum.

*The Genotype-Phenotype Connection* kit offered a means for students to experience molecular biology and microbiology hands-on. Students used technology to amplify a region of DNA from either a red or pink colony *Halobacterium* sp. NRC-1. Beginning with observations about the organism on the petri plate and completing lab protocols until completion at gel electrophoresis image, students were able to experience the process of the relationship between genotype and phenotype. This experience improves student learning of the concepts of colony morphology, cell lysis, DNA extraction, PCR, gel electrophoresis and bioinformatics. By using tools for learning such as this kit, the students will be better prepared for the new fields of biological research.

"I think the biggest innovations of the twenty-first century will be the intersection of biology and technology. A new era is beginning, just like the digital one..."
- Steve Jobs, cofounder of Apple, Inc.
(Walter, 2013)

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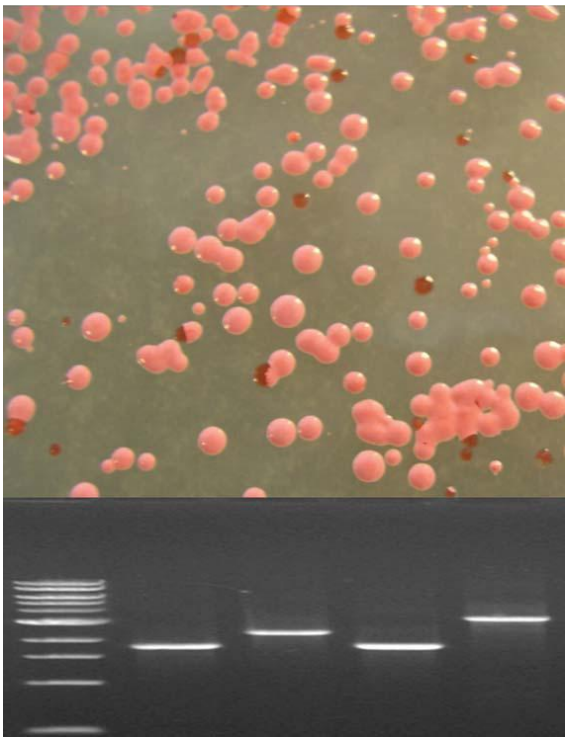
## Appendix A: Kit Manual



# The Genotype-Phenotype Connection: Basic Molecular Genetics and Bioinformatics Skills

**A Teacher's Manual and Student Guide**

**By Kelley Tuel, Dr. Shiladitya DasSarma, Dr. Tim Burnett and Priya DasSarma**

# Table of Contents

**Teacher's Manual**


Overview .....A4

Objectives .....A4

Content Standards .....A5

Time Requirements .....A5

Materials .....A6

Storage.....A7

Safety .....A7

Background .....A7

Detailed Teacher Preparation .....A8

Instructor notes.....A10

Answers to Questions in the Student Guide .....A13

Extension Activities .....A19

References & Resources .....A21

Related Products .....A22


**Student Guide*** *Photocopy the Student Guide as needed for use in your classroom.


Background .....A23

Procedure .....A29

Pre-Lab for Activity 1 .....A29

Activity 1- Halobacteria Exploration and Lysis .....A31

Pre-Lab for Activity 2 .....A33

Activity 2 - PCR .....A33

Pre-lab for Activity 3 .....A34

Activity 3 - Gel Electrophoresis .....A34

Activity 4- Data Comparison and Analysis .....A35

Student Analysis and Follow-up Questions .....A37

**Appendices**

I. Primer locations in *gvp*A1 gene region .....A43
II. Glossary .....A45
III. Troubleshooting .....A48
IV. Sample class gel image .....A49
V. Class Data Chart.....A50
VI. Staining with GelGreen™ .....A51
VII. Staining with *Carolina*BLU™ .....A52

## Overview

Using the model microbe *Halobacterium* sp. NRC-1, students perform DNA extractions, PCR (polymerase chain reaction), and gel electrophoresis. This activity is designed in inquiry format to allow students to observe and experience the Achaean and extreme halophile (salt-loving) microorganism, *Halobacterium* sp. NRC-1. Students will also work with a stable mutant derivative, *Halobacterium* sp. KBT-1, which was identified by a Kansas Biology Teacher (hence the name, KBT). The brightly colored microbial colonies and the organism's extreme lifestyle will foster student interest and inspire inquiry. As part of these exercises, students will formulate questions and hypotheses to explain observed differences in colony phenotypes. To detect genetic differences responsible for the different phenotypes, students will use PCR to analyze DNA from either a mutant (KBT-1), a wild-type (NRC-1), or a sectored colony. To draw conclusions regarding the link between the molecular differences they detect and the phenotypes observed, students will pool their data. They will then demonstrate their comprehension of the concepts by working through questions in the student guide.

Since individual laboratory experience is essential to student learning and comprehension, this kit was designed for 25 individual students to each complete all parts of the lab, though the activities can be performed in small groups as well.

The Background Information section can be used by the teacher to either introduce or review subtopics. Depending on the level of your students, you may want to omit sections of the background or, with advanced students, have them individually research subtopics. The lab has been divided into four related sections that can be done on consecutive days, or several days apart.

### Objectives

Students will

- Learn and practice sterile (aseptic) technique
- Study colony phenotypes
- Learn and practice pipetting techniques
- Extract DNA
- Perform polymerase chain reaction
- Perform agarose gel electrophoresis
- Analyze and draw conclusions from collected data
- Correlate genotype with phenotype
- Use basic bioinformatics tools

### Content Standards:

This kit is appropriate for students from high school to college and meets the following National Science Content Standards:

**Grades 9-12**

***Life Science***

- The cell
- Molecular basis of heredity

***Science as inquiry***

- Abilities necessary to do scientific inquiry

***Science and Technology***

- Abilities of technological design (if extension activities are done)
- Understandings about science and technology

**AP Biology® Objectives:**
Subject matter found within this kit can be applied to many areas covered in an AP Biology® course, either to introduce or reinforce topics. The subject matter addresses the following AP Biology concepts:

Big Idea #1: The process of evolution drives the diversity and unity of life.
Essential knowledge 1.A.1: Natural selection is a major mechanism of evolution. Essential knowledge 1.A.2: Natural selection acts on phenotypic variation in a population.
Essential knowledge 1.B.1: Organisms share many conserved core processes and features that evolved and are widely distributed among organisms today.

Big Idea #3: Living systems store, retrieve, transmit, and respond to information essential to life processes.
Essential knowledge 3.A.1: DNA, and in some cases RNA, is the primary sources of heritable information.
Essential knowledge 3.C.1: Changes in genotype can result in changes in phenotype.
Essential knowledge 3.C.2: Biological systems have multiple processes that increase genetic variation.

Big Idea #4: Biological systems interact, and these systems and their interactions possess complex properties.
Essential knowledge 4.A.1: The subcomponents of biological molecules and their sequence determine the properties of that molecule.

## Time Requirements

| | Teacher prep prior to activity | Teacher prep on day of activity | Classroom time |
|---|---|---|---|
| **Activity 1: *Halobacterium* exploration & cell lysis** | Copy student guide for each student (30' minutes). | Set up student work area (~1 hour.) | ~40 minutes |
| **Activity 2: PCR** | Program thermal cycler: ~10 minutes the first time you program it | Aliquot Primer Mix as needed for student stations: ~5 minutes | ~30 minutes<br><br>Thermal cycler running time 1-2 hours for most machines: |
| **Activity 3: Gel electrophoresis** | | Prepare buffer and pour gels (45-60 minutes).<br><br>Aliquot DNA marker as needed for student stations. | Load gels ~30 minutes<br><br>Run gels 45'-1hr hours<br><br>Staining Gels: Process varies depending on stain used. (30' to overnight) |
| **Activity 4: Analysis** | **Obtain semilog paper graph paper (can be printed** | Photograph gels: 20 minutes | ~1 hour |

## Materials

The materials in this kit are supplied for the use with the educational exercises described in this kit only. For the success of this lab, it is critical that your students conduct their exercises using safe and correct lab practices. Carolina Biological Supply Company disclaims all responsibility for other uses of these materials.

*Included in the kit:*

teacher's Manual with reproducible Student Background & Student Guide (with follow-up questions)
3 plates with colonies of *Halobacterium* sp. NRC-1 (wild type)
2 plates with colonies of *Halobacterium* sp. KBT-1 (mutant)
25 PCR tubes containing Ready-To-Go Beads™, 0.2 mL capacity
1 vial Primer/Loading Dye Mix
1 30 uL vial 1 kb ladder/DNA marker (Kits with GelGreen™)

***OR***

4 30 uL vial 1 kb ladder/DNA marker (Kits with *Carolina*BLU)

*Kits with electrophoresis material also contain:*

2 g agarose
1 50mL of 20X TBE buffer in a bottle
1 250mL bottle *Carolina*BLU ™ Final Stain (only in kits with *Carolina*BLU ™)
1 7mL vial *Carolina*BLU ™ Gel and Buffer Stain (only in kits with *Carolina*BLU ™)
1 100 uL vial GelGreen™ (only in kits with Gel Green)
1 tube 1.5 mL 5 M NaCl (only in kits with Gel Green)
4 staining trays
4 pairs of disposable gloves

*Needed, but not supplied:*

pipettes (2 uL – 500 uL capacity) with sterile tips. Alternatively, if adjustable pipettes are not available, fixed volume pipetting-devices for accurately pipetting 4, 10, 20, 100 and 500 uL may be used.
permanent fine-tip markers
microscope or hand lens (optional)
microcentrifuge with adaptors for holding 0.2-mL tubes*
ice (in beakers or plastic cups per student)
vortex (optional)
thermal cycler
power supplies
gel electrophoresis chamber(s) (to accommodate enough gels for each student to load 1 lane plus extra lanes per gel for the DNA ladder and to reload misloaded samples)
gel tray(s)
gel comb(s)
a microwave or a heatable stirplate and stirbar or a boiling waterbath
UV trans-illuminator, fluorescent white light box, or blue light box
UV safety goggles or blue light safety goggles or screen
camera to capture gel images
bleach solution (10%) or autoclave
gloves
lab coats or aprons (based on school policy)

*Adaptors for 0.2 mL tubes can be made by removing the lids from 1.5 mL and 0.5 mL microcentrifuge tubes, and nesting the 0.5 mL tube inside the 1.5 mL tube in the centrifuge.

## Storage

Upon delivery, store petri plates with *Halobacterium* colonies in heavy-duty sealed plastic bags or sealed plastic containers in the refrigerator, where they can be stored for a couple months. **It is critical that the bags or containers are sealed well, since loss of moisture leads to formation of salt crystals in the agar.**

The primer/loading dye mix and DNA marker should be stored in a freezer. Everything else can be stored at room temperature.

GelGreen™ should be stored in the dark

## Safety

Always ensure that each student understands and adheres to safe laboratory practices when performing any activity in the classroom or laboratory. Demonstrate the protocol for correctly using the instruments and materials necessary to complete the activities, and emphasize the importance of proper usage.

The American Type Culture Collection (ATCC) classifies *Halobacterium* sp. NRC-1 as a BioSafety Level 1(BSL-1) organism. Materials classified at BSL-1 "are not known to cause disease in healthy adult humans". *Halobacterium* sp. NRC-1 and KBT-1 do not survive outside of their extreme environment and few if any other organisms can survive the high salt of the media. These organisms are destroyed by exposure to the low salt concentrations found in the human body, tap water and even sea water. Even so, we always recommend treating microbes as though they could be pathogenic. We recommend the use of personal protective equipment (gloves, goggles, and lab apron or coat) when working in a biology or chemistry laboratory setting.

There is an odor associated with the media and cultures. The odor can easily be washed out of any clothing the cultures or media may be spilled on, but lab coats or aprons can prevent spills from getting on student clothing.

We suggest that you follow standard good laboratory microbiology laboratory practice and autoclave labware (or soak it in a 10 % bleach solution) before disposal.

## Background

Refer to the Student Guide to obtain the relevant background material.

To learn more about *Halobacterium* sp. NRC-1 and about molecular genetics in general, familiarize yourself with the websites HaloEd and MolGenT prior to these activities.

 HaloEd: http://halo4.umbi.umd.edu/~haloed/ or access from a search engine by searching for "the HaloEd Project." HaloEd was created to give educators, students and the general public insight into Halophiles and their environment as well as give teaching tips and more.

 HaloWeb: http://halo4.umbi.umd.edu or access from a search engine by searching for "HaloWeb". HaloWeb was created to facilitate viewing and understanding the genome of *Halobacterium* sp. NRC-1 and related organisms.

 MolGenT: http://halo4.umbi.umd.edu/~haloed/MOLGENT/index.htm or access from a search engine by searching for "MolGenT". The Molecular Genetics Tutor at MolGenT is particularly helpful for students to strengthen their understanding and visualize the vital concepts required for this activity.

## Pre-labs

The "Pre-labs" are recommended exercises to be completed prior to each laboratory activity. They review concepts necessary for a more complete understanding and learning experience. You may want to enhance your students' background knowledge by working through the pre-lab exercises either in class or as homework prior to each lab activity.

# Detailed Teacher Preparation

1. **Photocopy** "Student Guide" for each student.
2. **Program the thermal cycler** with the following times and temperatures:

   Step I. 3 minutes at 94°C
   Step II. 15 seconds at 94°C
   Step III. 15 seconds at 60°C
   Step IV. 45 seconds at 72°C
   Step V. Return to Step II – Step IV, repeating 30 times
   Step VI. 5 minutes at 72°C
   Step VI. Infinitely held at 4°C (Note: some machines do not have this capacity and you may have to be present at the end of the cycle to transfer the samples to a refrigerator or freezer.)

   **Note:** If you are using a machine that employs multiple blocks held at different temperatures in place of the more common single block that cycles between the different temperatures please use the program below.

   Step I. 3 minutes at 94°C
   Step II. 15 seconds at 94°C
   Step III. 15 seconds at 60°C
   Step IV. 45 seconds at 72°C
   Step V. Return to Step II – Step IV, repeating 30 times
   Step VI. 5 minutes at 72°C
   Step VI. Infinitely held at 4°C

   The PCR reaction will take approximately 1 hour or more to run. The time it takes will vary with the machine.

3. **Prepare 0.8% gel(s) and running buffer** according to the volumes specified for your type of gel chambers. If you are using Carolina Blu to stain your gels, please refer to Appendix VII when pouring the gel and making the running buffer. You will add the stain to both (when pouring the gel and to running buffer prior to gel electrophoresis).

**The directions below are written for making 4 gels for medium-sized gel chambers (the gels are approximately 9cm X 9cm).** Modify these instructions to suit the size of your gel chambers. The number of gels you require depends on the number of student samples and the number of wells you have per gel. For each gel reserve a lane for the1 kb ladder (the

molecular weight marker) and plan to have an empty lane to allow students to re-load in a different lane if they make a mistake in loading the first time.

**Preparation of 1X TBE buffer from the 20X TBE stock (provided)**
Determine the number of gels and chambers required for your class.

A. Determine the volume of buffer you need. Typically, you will need approximately 300-500 mL of 1X TBE buffer per gel chamber.

B. To make 2.0 liters of 1X TBE: combine 100 mL 20X TBE with 1900 mL of distilled or deionized water.

**Preparation of agarose gels.**
Prepare the 0.8% agarose solution at least 40 minutes prior to running the gel, as it takes about 15-30 minutes at room temperature for the gel to set. You can also make gels a day ahead and store them covered with TBE buffer in the electrophoresis chambers until you are ready to use them. Review the CarolinaBLU™ staining protocol in appendix VII to see if you want to add Carolina*BLU* Gel stain.to gel.

A. Again, assuming you are making 4 gels, add 2 g of agarose to a 500ml flask.
B. Add 250 mL 1X TBE buffer.
C. Dissolve the agarose using one of the methods described below. **Be aware that the time it takes to dissolve the agarose will vary greatly depending upon the volume prepared and the equipment used.**

**Microwave:** Heat the agarose and 1X TBE in a microwave until no particulate matter can be seen in the solution or stuck to the bottom of the flask after a gentle swirl of the hot flask (~10-30 seconds.) To prevent boiling over during heating, watch carefully and swirl the flask at the first sign of boiling and periodically thereafter.

**Hot Plate:** The agarose can also be dissolved using a hot plate. If possible, use a stirring hot plate that will enable you to stir using a magnetic stir bar. Otherwise, swirl the solution frequently to prevent boiling over and scalding, and to help disperse the agarose. Heat until no particulate matter can be seen in suspension or stuck to the bottom of the flask.

**Boiling Water Bath:** A boiling water bath can also be used. Make sure the water in the water bath is at least level with the agarose solution in the flask. Heat until no particulate matter can be seen in solution or stuck to the bottom of the flask. This usually takes 5-10 minutes. Allow additional time for the water bath to come to boiling.

D. On a level surface, pour an appropriate volume of the solution into each of the gel trays and add combs with the appropriate number of teeth.
E. Once the gels have set, place the gel tray into the electrophoresis chamber filled with TBE and gently pull the combs from the gels.

4. **Staining of Gels**

See Appendix VI and VII for staining protocols for Gel Green and Carolina*BLU*™.

# Instructor Notes

**Activity 1**

1. To make the activity more efficient, steps 1.2-1.3 can be done while students wait their turn for the various *Halobacterium* plates.

2. Students do not need to take much *Halobacterium* to get good results. As long as the quantity they take is visible and is even evenly dispersed in the water, they should get adequate results. If they take too much *Halobacterium*, their lane on the gel may appear smeared and overloaded.

3. Tube #1 can be thrown in the trash.

4. Cell lysates should be frozen (–20°C) in a freezer. It is advisable to store in a constant temperature freezer instead of a frost-free freezer. If the lysate is left at room temperature too long, the enzymes (nucleases) in the lysate will lyse the nucleic acids (see 1.11).

5. You may stop the lab for the day at the end of this activity or continue with the exercise.

**Pre-Lab Activity 2**

If you assign this Pre-Lab Activity as homework, you may want to lead a class discussion the following day to reinforce the concepts addressed in the tutorial.

**Activity 2**

1. Prior to class, aliquot the vial of Primer Mix into several other microcentrifuge tubes to avoid bottlenecks in the classroom.

2. Students should write their initials on both the hinge or lip of the tube lid **and** the side of the tube. (Writing only on the side risks having the writing rub off.) The students are instructed to retain their cell lysate tube on ice (see above) in case you would like to do additional reactions using the same lysate. You may also store the lysates in the freezer for later use by the students or other classes; otherwise, it may be thrown in the trash. Note: Because of the presence of nucleases even if it is kept frozen, the DNA in the lysate will, with time, not produce as much PCR product as it did originally.

3. See Appendix I for primer annealing locations.

**Pre-Lab Activity 3**

We recommend watching the suggested video as a class. If you assign it as homework, you may want to lead a class discussion the following day to reinforce what students watched in the video. Ask questions to make sure that your students understand such as

- the specifics about loading the specific pipettes that your class will use.
- what happens if the bottom of the well is punctured.
- the importance of using a new pipette tip for loading each lane.

**Activity 3**

1. Dilute the 20X TBE buffer to 1X and pour the gels, allowing them to solidify at least 15–30 minutes prior to class.

2. To avoid bottlenecks in the classroom, prior to class, aliquot the DNA marker into several microcentrifuge tubes.

3. Note that students should load 5 µL of their PCR reaction and 5 µL of the DNA ladder if the gels are being stained with GelGreen™ or a stain with similar sensitivity (e.g., ethidium bromide), and 10 µL of their PCR reaction and 25 µL of the DNA ladder if the gels are being stained with *Carolina*BLU™ or a similar stain.

4. **If more than one gel is required per class period**, distinguish the different gels to avoid confusion during analysis by loading the 1 kb ladder in different lanes on each gel and/or by removing specific corner(s) of each gel. Make sure that students record the distinguishing characteristic of the gel on which their sample is loaded.

5. As a confirmation of student records, the teacher should use the following chart to record students' gel lanes. Insert student name or initials and colony type in the appropriate box. Also indicate which lane the 1 kb ladder is loaded in.

| | Lane 1 | Lane 2 | Lane 3 | Lane 4 | Lane 5 | Lane 6 | Lane 7 | Lane 8 | Lane 9 | Lane 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Gel 1 | | | | | | | | | | |
| Gel 2 | | | | | | | | | | |
| Gel 3 | | | | | | | | | | |
| Gel 4 | | | | | | | | | | |

6. Refer to the manufacturer's instructions for your gel chamber for the voltage to use and the approximate time the gel should be run. For example, when using TBE buffer with a 0.8% gel, in a standard Carolina gel chamber (item 213641 or 213654), run the gel(s) at 135 volts for 45–60 minutes.

7. The gels need to be stained right way. Decide if the students will stain the gels, or if you will do it. Instruct them appropriately. See the instructions for *Carolina*BLU™ or GelGreen™ stain in the Appendix.

**Activity 4**

1. Appendix IV includes a picture of an ideal gel. Should your students fail to get results, you may photocopy the picture from Appendix IV to provide students with data to analyze.

2. Lead a class discussion asking students to point out the banding pattern in their lane and describe the colony they isolated. Collect the class data using the chart in Appendix V or some other method and make it available to the whole class for either individual or group analysis.

# Answers to Student Questions

## From Activity 4

4.1. Calculate the approximate number of base pairs (bp) in your DNA fragment(s).

**See the student guide for the full question. Using the provided primers, the expected sizes of the bands generated from *Halobacterium* sp. NRC-1 (wild-type)and *Halobacterium* sp. KBT-1 are 1792 bp, and 2325 bp, respectively.**

## Student Analysis and Follow-up Questions

1. You observed the possible phenotypes of Halobacterium on the petri plates.

a. Describe each of the phenotypes observed.

**The possible phenotypes on the plates are 1) Pink and opaque, 2) Red and translucent, and 3) Sectored (mix of both within one colony).**

b. What do you propose was the source or cause of the different phenotypes?

**Pink colonies have gas vesicles. Red colonies do not. The red colonies have mutations in the gas vesicle gene cluster region. The sectored phenotype results from a phenotype-altering mutation occurring in the gas vesicle gene cluster region of one or more of the daughter cells early in the growth of the colony. Subsequent daughter cells of the mutant pass on the red phenotype.**

**Note: From the Background reading, students should understand that the gas vesicle gene cluster of the red colonies may contain an insertion sequence (also referred to as a transposon or transposable genetic element) that disrupted a gene responsible for gas vesicle production. When gas vesicles are present, as they are in the wild-type (pink) colonies, light is bent as it moves from the denser cytoplasm, through the hollow gas vesicles and then back out again into the denser cytoplasm. This bending of light, a process known as refraction, changes the colonies' appearance (or phenotype) from the inherent red color (without gas vesicles) to pink.**

2. From your background reading, which unique properties of *Halobacterium*, an extreme halophile, allow the cells to be lysed with the simple addition of water?

***Halobacterium* requires a high salt content (a minimum of 2.5 M NaCl). When the microbes are placed in water, the water acts as a hypertonic solution, floods into the *Halobacterium* cells and causes the cells to lyse. The cells also need the high salt concentration for their membrane stability.**

3. You lysed your cells in water.

a. Which cellular components would you expect to find in your lysate?

**You would expect to find nucleic acids (DNA and RNA), proteins, lipids, and other cellular components.**

b. Why was the cell lysate centrifuged?

**This crudely separates the insoluble cellular debris from the other soluble cellular components, including the nucleic acids (DNA and RNA).**

c. What purpose was served by removing 100 uL of supernatant and transferring it to another tube?

**The supernatant contains the nucleic acids. Moving the supernatant to another tube helps ensure that the unwanted cellular components spun to the bottom of the tube are not added to the PCR reaction along with the DNA. These unwanted cellular components include proteins such as nucleases, which are enzymes that break down DNA and RNA, as well as other cellular components that may inhibit the PCR reaction.**

4. What are the necessary components in the PCR reactions? List the components and the purpose of each.

**• PCR bead (contains Taq polymerase, the nucleotides necessary for synthesizing new DNA, and the salts and buffer needed for creating the appropriate conditions for Taq polymerase.)**
**• DNA template (provides the starting material from which more DNA is made).**
**• primer mix (provides both the forward and reverse primers needed to specifically target DNA replication to a specific sequence and to provide the start point for the polymerase to begin DNA synthesis).**

5. You mixed the PCR bead, primers and cell lysate sample thoroughly before placing the reaction in the thermal cycler. Why was thorough mixing necessary?

**It is necessary to mix the PCR beads, primers and template so that all of the needed reaction components are evenly distributed in the reaction; i.e., the mixture must be homogenous. Even mixing ensures that the primers, template DNA, and polymerase are in close enough proximity for the reaction to proceed.**

6. A polymerase chain reaction goes through several cycles of temperature changes. List each step of a cycle, and describe what occurs during each step.

**Denaturation: the DNA strands are heated so that they separate from one another.**

**Annealing: the primers anneal or attach to their complementary region on the template DNA.**

**Extension: the polymerase extends the sequence out from the primer sequence, thus replicating the targeted sequence of the DNA in your sample.**

7. Why did you place your nucleic acid extract and PCR reagents on ice until the PCR reaction was set up and placed in the thermal cycler?

**The main concern is that any remaining nucleases, naturally occurring cellular enzymes present in the cell lysate containing the template DNA, could break down the DNA template. Keeping the reaction on ice until it is placed in the thermal cycler also prevents the polymerase from starting to synthesize DNA before the primer has annealed in the correct location.**

8. What are two purposes served by the loading dye?

**a. to weigh down the sample (your PCR reaction) so it will sink to the bottom of the well and not float up into the buffer when it is loaded on the gel.**

**b. to provide a visual aid (the color) to make it easier to load the sample and to track how far the molecules on the gel have run. Being able to track how far the molecules on the gel have run helps you to predict where fragments of a particular size are running on the gel so that you know when to turn off the power to the gel chamber.**

9. You loaded the PCR samples into the wells of the gel electrophoresis chamber.

a. Are the wells (and thus your DNA) nearer to the positive or negative electrode?

**The wells are closer to the negative (black) electrode.**

b. Is DNA positively or negatively charged, and how does its charge impact how the gel chamber is set up?

**Because DNA is negatively charged (due to the negatively charged phosphate group in each nucleotide), it MUST be loaded on the side of the gel nearest to the negative electrode. Since like charges repel and opposite charges attract, the DNA will travel away from the negative electrode, through the gel, toward the positive electrode.**

10. How many bands appeared in your lane? _____ What are the bands made of?

**Answers will vary. Most likely the students will see one band, but they may see two (if the template DNA came from a sectored colony). Sometimes students will see none. No bands would result from a deletion or major reorganization of the PCR priming sites, or from student error. The bands consist of the DNA that was amplified.**

11. Look at the table with class results to determine if your band(s) ran a different distance into the gel than some of your classmate's bands or if you had a different number of bands than others. Think about the observations you originally made about the colony types. Can you find a correlation between the size and number of bands you and your classmates observed in each lane and the phenotypes of the colonies used to generate those bands? Using the class data, explain any correlation or lack of correlation you see.

**If students extracted DNA from a pink colony from the wild-type *Halobacterium* sp. NRC-1 plate, they are most likely to have a single 1792-bp band.**

**If they extracted DNA from a red colony from the *Halobacterium* sp. KBT-1 plate, they are most likely to have a single 2325-bp band. This band confirms that the colony is KBT-1. Banding patterns different from 2325- or 1792-bp indicate a novel mutant.**

**If they had extracted DNA from a red colony from the wild-type plate is likely to be a novel mutant that may or may not have bands at 1792 or 2325 bp depending on the nature of the mutation in the *gvp* gene cluster. The mutation could be an IS element insertion, a deletion, or a single or multiple nucleotide change in the *gvp* gene cluster.**

**If they had extracted DNA from a sectored colony (if found, it would most likely originate on the wild-type plate), they may see one or two bands—a 1792-bp band (from the wild-type portion of the colony) and, if the mutation in the red portion of the colony is in a region amplified by the primer, a second band as well.**

12. Your gel band gives you information about the genotype of the colony you isolated. Based upon your observations regarding the phenotype and genotype of your colony, what genotype (strain) of *Halobacterium* do you hypothesize you isolated (*Halobacterium* sp. NRC-1, KBT-1, or another)? Identify whether your colony was wild type (wt) or mutant (mut). What makes you conclude that?

**See the answer to question 11.**

# Challenge Questions (Answers)

**13. Write a well constructed paragraph explaining how an IS element could cause a colony of *Halobacterium* sp. NRC-1 to appear red.**

*Students should cover the concepts included in the following paragraph:*
*An IS element could disrupt a gvp gene, so one of the proteins required to make the gas vesicles was not made correctly. Without this protein, gas vesicles were unable to form. The absence of the gas vesicles changes how light interacts with the colony, causing the appearance of the colony to change from pink to red.*

**14. It is possible to occasionally find a red colony (mutant) that yields a band that appears to be the same size and sequence as the wild-type (*Halobacterium* sp. NRC-1) band. Explain how this could occur.**

*The detection of mutations – single nucleotide, deletion, and/or IS elements– by PCR amplification is limited by the primers (forward and reverse) used. If an IS element inserted, or another mutation occurred, outside of the area amplified by the primers used in this lab, but within the gvp gene cluster, the amplified DNA fragment would have a wild type sequence like NRC-1, even though the colony assayed was red.*

**15. The DNA sequence in Figure 5 shows the location of some of the genome region you just amplified using PCR. The region shown contains the gene sequence for *gvp*A1. (hint: if you have trouble with questions 15a-15d, review pre-lab #1.)**

**a. Why are some letters capitalized?**
*In DNA notation letters are usually capitalized to indicate that these nucleotides are coding regions and will be translated into an amino acid sequence.*

**b. Label the gene start codon (usually ATG).**
*See label on figure 5 below.*

**c. Label the gene stop codon (usually TAA, TGA or TAG).**
*Stop codon for gvpA1 is TGA. See label on figure 5 below.*

**d. How did you pick your start and stop codon, given that were multiple codons like them in the sequence?**
*These were the first start codon and last stop codon of the amino acid coding region, indicated by the capital letters, so they were the most likely to be functional (i.e. actually used as a start and stop codon).*


Figure 5. A portion of the wild type *Halobacterium* sp. NRC-1 *gvp*A1 gene region sequence amplified using the primers in this kit.

**16. Translate the capitalized sequence for *gvp*A1 (from question 15, Figure 5) into an amino acid sequence. Use the sequence editor function on HaloWeb to help you do this.**

*Be aware that some students may remember from Prelab exercise 1 that they can get the amino acid sequence directly from the HaloWeb site without having to use the sequence editor.*

*Go to HaloWeb and click the "search" tab. Search for gvpA1, get the sequence, copy and paste into the sequence editor window. Students should enter in the top strand sequence (all in capital letters) for gvpA1 from the start to stop only. Sequence editor gives the following amino acid sequence (in one letter abbreviations) after clicking "translate it":*

M A Q P D S S R N P D R R G A G R R R L G G H L P P L R R R N R Q D R T S R T Y R R R R G G T R G L

M = methionine, A=Alanine, Q=Glutamine, etc.

**17. When the *gvp*A1 gene region was amplified from KBT-1 and sequenced, the sequence in Figure 6 was obtained. How does the KBT-1 sequence shown here differ from the NRC-1 sequence used for question 15 (Figure 5)?**

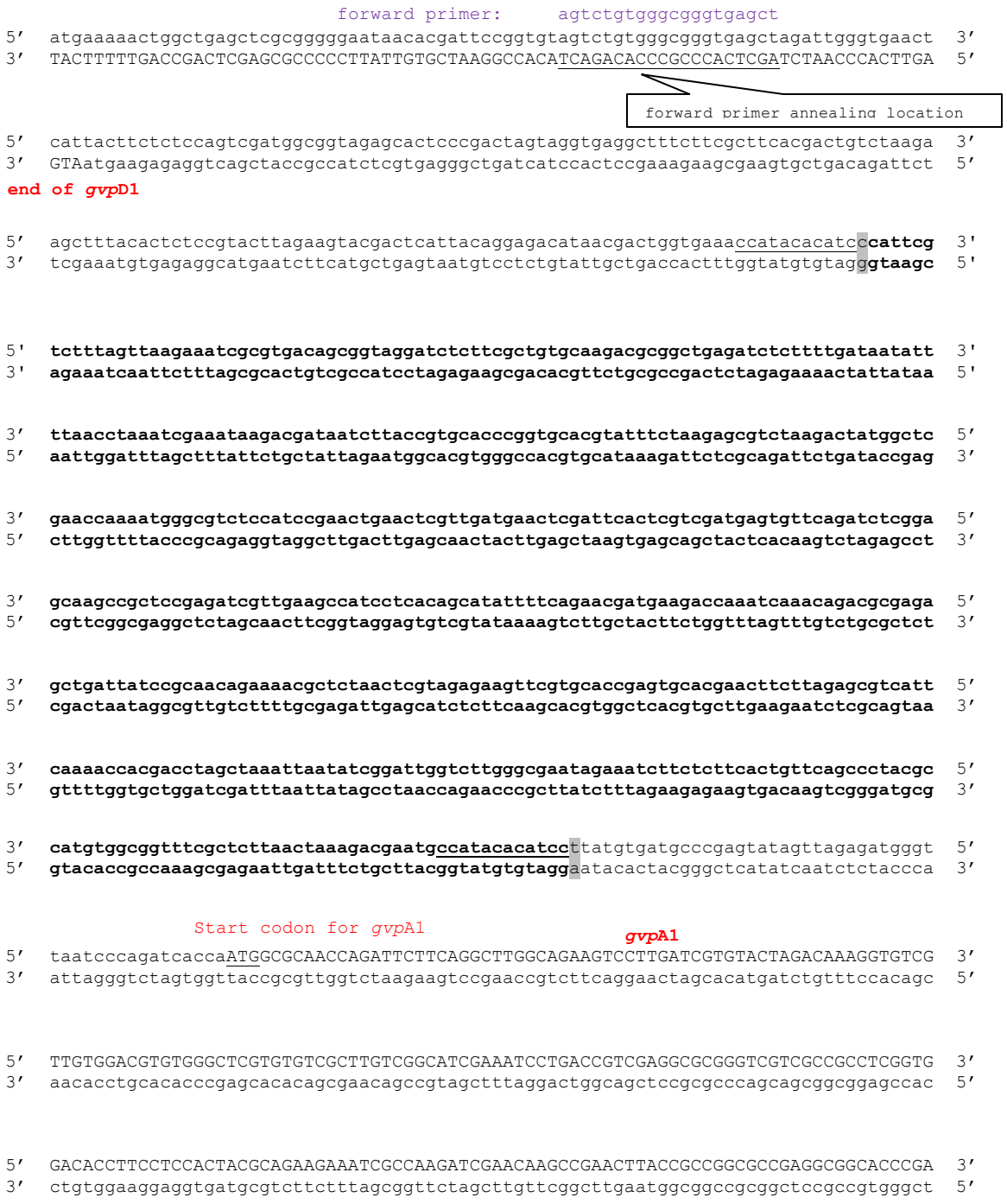



**Figure 6.** *Halobacterium* sp. KBT-1 sequence of gvpA1 gene region. The boldfaced region indicates an IS sequence. The underlined sequence on either side of the insertion element are the direct repeats. The direct repeat result from the insertion element copying the 12 nucleotides located just before the insertion site and repeating these nucleotides at the end of the IS as it inserts.

## Optional Extension Activities

1. Use bioinformatics tools to design your own primers for amplifying the gas vesicle protein genes. Do this in theory or have a commercial lab produce your primers so you can try them out! To design a primer to amplify a sequence, do the following:

a. Looking at the top strand only, select about 20 bases before (5') and after (3') the region of interest and highlight them. Note that by convention the top strand is depicted 5' to 3' from left to right. In addition, if the gene you are working with is on the top strand, the top strand is referred to as the coding, or sense strand, and the bottom strand is referred to as the non-coding or anti-sense strand.

b. If the gene is located on the top strand you should name the primer that is 5' of the sequence you want to amplify, the forward primer (i.e. *gvp*A1 forward). Because DNA replication proceeds 5' to 3', the sequence of the primer 5' to the gene will have a sequence that is identical to a sequence in the coding strand and the reverse compliment of a sequence in the non-coding strand.

c. To design the reverse primer (e.g. *gvp*A1Reverse), paste the 3' sequences you highlighted on the coding strand(the upper strand) into the sequence editor and click on "reverse compliment" and then "Do it." Remember that the sequence editor can be found on the left sidebar on the NRC-1 HaloWeb page (http://halo4.umbi.umd.edu/cgi-bin/haloweb/nrc1.pl).

d. The output sequence in the lower window will be the reverse complimentary sequence to the 3' end sequence that you entered. This is the sequence you use to order the reverse primer e. It is standard when placing primer orders to write the sequence 5' to 3'.

e. In the laboratory, the following guidelines are followed to design a primer that will work well:

i. The primer should have a relatively low GC content (<60%) because the GC bond is harder to break than the AT bond.

ii. The primer should not have a run of bases of the same kind – especially Gs or Cs (e.g. GGGG).

iii. There should not be any predicted hairpin turns (usually the website where you order from can provide information regarding this) – especially not at the 3' end.

iv. Most importantly: the primer sequence should be unique—not found anywhere else in the genome—check for this by BLASTing the desired primer sequence using the HaloWeb page (the BLAST tool can be found on the left sidebar; see "BLAST NRC-1 genome"). The primer sequence should only have homology to the region you are targeting. If it is not unique to the region, then you will have to choose another set of bases farther 5' or 3' from the region you are interested in.

2. Experiment with how gas vesicles from *Halobacterium* sp. NRC-1 respond to pressure. First, obtain a liquid culture of *Halobacterium* sp. NRC-1. Wearing safety goggles and lab aprons, fill two microcentrifuge tubes to the brim with a stationary phase (pink) liquid culture of *Halobacterium* sp. NRC-1. Quickly snap the cap shut on one of them and observe. What happens to the color of the culture in the closed tube? What do you think happened to the gas vesicles? You can look at the cells under the phase microscope and see the difference in their appearance. Also, you can let the two tubes sit for a few weeks on the bench and then see what happens.

3. There is an abundance of topics that can be researched involving *Halobacterium*. For instance, what do this organism have to do with potential life on the planet Mars? How is *Halobacterium* metabolism different from that of other organisms? Research these or other aspects of *Halobacterium* that may have sparked your interest from the background reading.

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**Web Sites**

*At the time of this printing, the Web sites given below were active.*

- http://halo4.umbi.umd.edu/~haloed/
- http://halo4.umbi.umd.edu /~haloed/MOLGENT/index.htm
- http://www.ncbi.nlm.nih.gov/
- http://www.nobelprize.org/
- http://www.the-aps.org/education/k12 curric/pdf/mcclintock.pdf

## Related Products

The following is a list of related items available from Carolina Biological Supply Company. For more information, please refer to the most recent *Carolina™ Science* catalog, call toll free 800-334-5551, or visit our Web site at **www.carolina.com**.

154771 Basic Microbiology Skills Kit, Part 1
154772 Basic Microbiology Skills Kit Part 2
154776 Antibiotics in Action Kit
154773 Extremely Easy DNA Extraction Kit
154770 Introduction to Life in an Extreme Environment Kit
821449 Halobacterium Broth, 5-mL tubes, Pack of 10
154777 Culture Packet of Brine Salt Inclusions
154800 Halobacterium sp. NRC-1, Tube
154801 Halobacterium sp. NRC-1, Plate

Name:_____ **Student Guide**
Period:_____

# Background

**1. Nucleic Acids**

Deoxyribonucleic Acid (DNA) is a polymer of nucleotide subunits. Each **nucleotide** contains a 5-carbon sugar (deoxyribose), a negatively charged phosphate group, and a **nitrogenous base**. Because of the way the nucleotides that form the DNA molecule are linked together, one end of the DNA molecule ends in a phosphate and the other in a hydroxyl group. The end with the phosphate group is always referred to as the 5' end, and the end with the hydroxyl group as the 3' end. The 3' and 5' designations derive from how the carbons in an organic molecule are described; they are described as either 1', 2', 3', etc., depending upon their position in the molecule. The hydroxyl group at the 3' end of a DNA molecule is attached to the 3' carbon on the deoxyribose sugar. The phosphate group at the 5' end is attached to the 5' carbon on the deoxyribose sugar. This 5' and 3' notation is important when studying the replication of DNA and designing **primers** for molecular biology experiments. New nucleotides are always added to the end with the hydroxyl group. Thus, a single-stranded piece of DNA (such as a primer) can be extended only from the 3' end (5' to 3').

When the nucleotides are joined together to form a polymer strand of DNA, the sugar and phosphate groups link in an alternating pattern to form a sugar–phosphate backbone. DNA is a double-stranded structure in which the two strands of DNA are joined together, in an **antiparallel** arrangement, meaning that, one strand runs in the 5' to 3' direction, while the other runs in the 3' to 5' direction. The nitrogenous bases are complementary to each other, with adenine (A) pairing with thymine (T) and guanine (G) pairing with cytosine (C).

For the sake of simplicity, the DNA **double helix** is often depicted in a simplified two-dimensional form (like a ladder or a railroad track). DNA is actually three-dimensional and helical (like a twisted ladder) with 10.5 base pairs per turn as described by Francis Crick, James D. Watson and Maurice Wilkins who won a Nobel prize in 1962 for the discovery of the DNA double helix. (Watson and Crick, 1953) Their discovery was made possible by X-ray diffraction images created by Rosalind E. Franklin.

During the process of transcription, DNA is used as a template to create strands of messenger **ribonucleic acid (mRNA)**. Like DNA, RNA is a polymer composed of four base types, except that in RNA, uracil is used in place of thymine. In addition, unlike DNA, RNA is single stranded and the nitrogenous bases are attached to ribose rather than deoxyribose. During translation, mRNA is used to code for the amino acids that form proteins. This process is carried out by the ribosome which reads the **mRNA** nucleotides in groups of three. Each group of three nucleotides (a triplet) is called a **codon** and designates which amino acid will be added to the growing polypeptide chain. This process was deciphered by Robert W. Holley, H. Gobind Khorana and Marshall W. Nirenberg and secured them a Nobel Prize in 1968 (Khorana, 1965).

## 2. Genotype, Phenotype

The central dogma of molecular genetics is that information is transferred from DNA to mRNA to protein. The **genotype** of an organism is the nucleotide sequence of its DNA. The nucleotide sequence dictates, through transcription and translation, which proteins are produced. The proteins carry out many of the specialized functions of the cell and thus determine the organism's **phenotype**.

This laboratory exercise uses the microorganism *Halobacterium*, and focuses on how the cell's genotype, with respect to the gas vesicle protein (*gvp*) gene cluster, dictates whether the cells produce intracellular gas-filled vesicles (GVs) or not. Depending on the DNA sequence of the *gvp* genes, the colonies of cells appear pink (wild-type colonies of *Halobacterium* sp. NRC-1), red (mutant derivatives of *Halobacterium* sp. NRC-1 such as KBT-1) or sectored (a mixture of sectors of pink and red cells within one colony). The presence or absence of the vesicles and the resulting color is a colony's phenotype.

## 3. Colony Phenotype

Each cell landing on a surface of an agar plate gives rise to a **colony** by exponentially dividing into "identical" daughter cells (**clones)** via binary fission. Colony morphology, the visual appearance of colonies, has long been used by microbiologists to determine the nature of organisms that they observe. Details they look for include:

A) the perimeter of the colony: is it smooth, wavy, rough or uneven?
B) the surface of the colony: is it glossy, matte, smooth or rough?
C) the height of the colony: is it raised or flat?
D) the color of the colony
E) opacity or transparency of the colony

## 4. Haloarchaea

Haloarchaea are evolutionary relics thought to be ancient life forms that may have existed since early earth. They are classified as members of the **Archaea**, prokaryotes that constitute one of the three **domains** of life (the others are the **Eukaryotes** and **Eubacteria).** You may sometimes see the Archaea referred to by their original name, the Archaebacteria, but the Archaea are no longer considered "bacteria" at all. Many Archaea grow in unusual environments, and are extremophiles (extreme-loving microbes). For example, heat-loving thermophiles from which enzymes used in PCR are isolated, live at extremely high temperatures. You will be working with a **halophile** (halo=salt, phile=loving).

Halophilic microbes, called 'Haloarchaea', include, ***Halobacterium*, sp. NRC-1** (sp. meaning "species.") These microbes require extremely high salt concentrations in order to maintain their membrane stability, and grow in media with NaCl concentrations up to 5.2 M (saturation). Their ability to grow in environments with high salt concentrations as well as other mechanisms for handling harsh environments (i.e. those with toxic metals and high levels of radiation) allows these organisms to persist in surprising places. They can sometimes even be isolated from table and rock salt! Few other organisms would survive such salty, harsh conditions! However the

unique adaptations required to survive these extreme conditions makes them incapable of surviving in what humans consider "normal" environments. *Halobacterium* sp. NRC-1 **lyses** (bursts open) when salinity levels around it are lowered to less than about 2 M NaCl. For comparison, sea water has a total salinity of 0.6 M, while human blood contains only 0.14 M NaCl.

***Halobacterium* sp. NRC-1** has been well studied for decades, and was one of the first microbes to have its genome sequenced (Ng et al, 2000). The DasSarma laboratory led the sequencing effort and created the HaloWeb genome website (http://halo4.umbi.umd.edu/) as a repository of information on the sequence of *Halobacterium* sp. NRC-1's genome. At HaloWeb anyone can explore the whole genome of *Halobacterium* – from gene locations, to gene sequences, to protein sequences and more. The Web site also includes access to other, more recently sequenced, haloarchaeal genomes (SL DasSarma et al, 2010.)

## 5. Gas Vesicles

When *Halobacterium* sp. NRC-1 cultures are observed on an agar plate, most colonies appear pink (pink is the "wild-type" phenotype). However, red or **sectored colonies** may also be found. A sectored colony looks like a red or pink pie with one or more slivers of the other color in it. The red color is the inherent color of the cells (see cover image). However, the wild-type cells produce a type of organelle called a gas vesicle (GV), that refracts light, changing the red translucent appearance of the colonies or liquid cultures to an opaque pink (see cover image). Refraction is the bending of light as it moves through materials of different density. In this case, the materials the light travels through are the cytoplasm and the hollow gas vesicles of the cell.

GVs allow the cells to float to the surface of their briny liquid environments (like the Great Salt Lake in Utah) in order to access light and oxygen. Access to plenty of light and oxygen allows the organisms to more efficiently utilize the mechanisms they use to generate energy. In addition to using respiration, these extraordinary microbes also use a process called **phototrophy** to obtain energy directly from sunlight. The process is analogous to, but distinct from, photosynthesis in plants (chloroplasts are not utilized). In addition, *Halobacterium* can respire anaerobically using chemicals other than oxygen, though the process is not as energy-efficient as aerobic respiration. The GVs allow *Halobacterium* to float to the top of its liquid environment, where oxygen for aerobic respiration as well as sunlight for phototrophy are more plentiful.

GVs are very unique in nature, as they are an organelle that is a membrane-like structure made solely of protein and filled only with ambient gas – hence the name "gas vesicle". The 13 genes that code for gas vesicle proteins or *GVP*s (the P standing for proteins) are organized into two, divergent operons (operons are sets of genes which are expressed together; divergent means transcription from the operons goes in opposite, or divergent, directions) (DasSarma, et. al., 1994; DasSarma and Arora, 1997).

## 6. Transposable/Mobile Genetic Elements

In 1983, at age 81, Barbara McClintock received a Nobel Prize for her work in genetics discovering transposable elements (McClintock, 1961; Fedoroff, 1984). Transposable elements are segments of DNA that may relocate to other areas of a genome with the transposon usually

showing little, if any specificity as to where in the genome it integrates. Some transposable elements actually make a copy of themselves and the copy then inserts into a different part of the genome, while the original transposable element stays in place.

You have transposable genetic elements in your genome. More than 10% (by mass) of your human genome can be attributed to transposable elements! These relocating segments of DNA include the Alu gene family of mobile elements (Cook et al, 2011). In prokaryotic microorganisms, there is a specific class of transposable elements known as Insertion Sequence (IS) elements.

When a transposon integrates into a vital region of the genome, the effect of this mutation may be observed phenotypically. For example, in the case of *Halobacterium* sp. NRC-1, disruption of critical areas in any of the genes coding for gas vesicle proteins can cause a change in color of the organism (approximately 1% of NRC-1 colonies spontaneously show evidence of this kind of genotypic/phenotypic change.)

Research into the apparent ease with which *Halobacterium* could change from a pink to a red phenotype led to the discovery of transposable insertion sequences in *Halobacterium*. It was found that the insertion sequences commonly inserted at certain "hot spots," such as in the gas vesicle gene region. These insertion sequences were named Insertion Sequences in Haloarchaea (ISH) (DasSarma et al 1987, Jones et al 1989, DasSarma 1989).

**7. PCR**

Polymerase Chain Reaction (PCR) is the primary way scientists can amplify (make copies of genetic sequences for molecular cloning, sequencing, and other molecular analysis. Forensics, medical diagnostics, and environmental testing laboratories use PCR for DNA fingerprinting a species identification. The method uses primers and the enzyme DNA polymerase (a heat stab DNA polymerase, called Taq polymerase, is used) along with a series of temperature changes amplify DNA.

The primers used in PCR are short segments of DNA (around 20 bp) that are designed to ampli specific genetic sequence. Two primers are used: a forward primer and a reverse primer. The fo primer is composed of sequence from the 5' end of the coding strand of the sequence to be ampl and the reverse primer is the reverse complement of the 3' end of the coding strand *Note: By convention the top strand is depicted 5' to 3' from left to right. If the gene you are working with the top strand, the top strand is referred to as the coding strand. If the gene were on the bottom strand, the bottom strand would be referred to as the coding strand.* Using the primers as a start point, the DNA polymerase synthesizes DNA 5' to 3'. After multiple rounds of amplification, th polymerase chain reactions results in amplification only of the DNA that is between the forward reverse primers. See Figure 1.

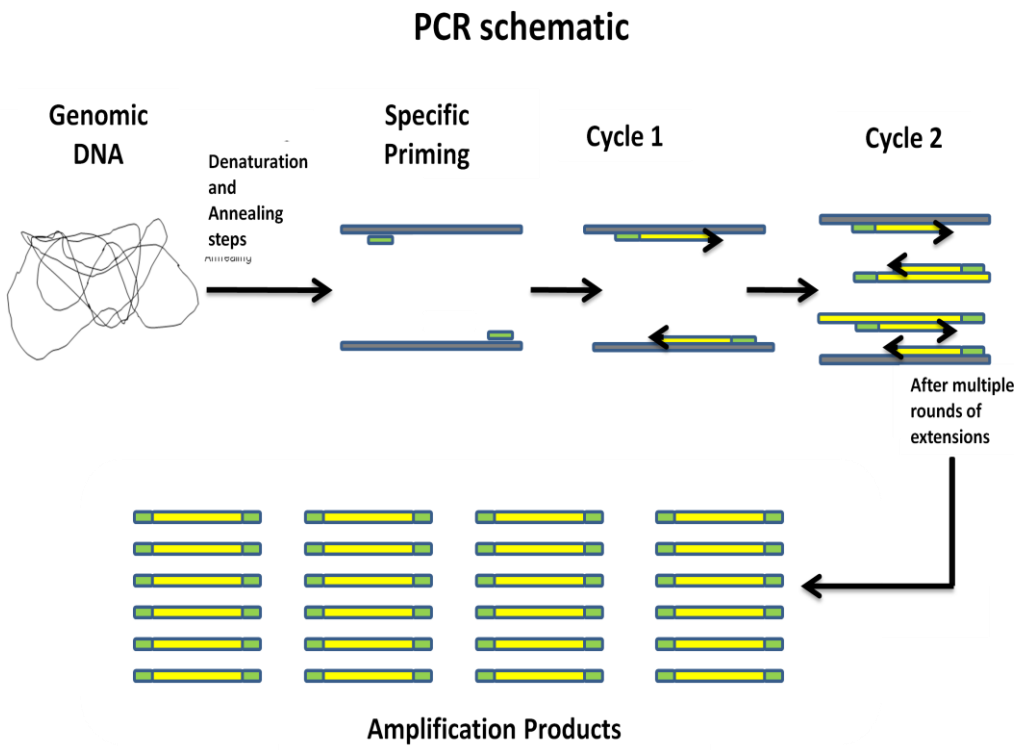


Figure 1. PCR schematic outlining the process of PCR amplification of a desired region of DNA located between the two primers. (Image adapted from MolGenT).

PCR involves a series of temperature changes applied to denature double-stranded DNA bonds, to anneal primers, and to then replicate DNA. This series of temperature change steps is repeated multiple times in a cyclical fashion.

Details of the steps of PCR can be described as follows (these steps are also demonstrated in MolGenT at http://halo4.umbi.umd.edu/~haloed/MOLGENT/d03.htm):

1. The denaturation step: The double-stranded DNA used as the template is denatured by heating to a high temperature, almost always 94°C. Separating the doublestranded DNA allows the primers to bind.
2. The annealing step: The temperature is reduced to allow the primers to anneal to their complimentary sequences on the denatured single strands of DNA.
3. The extension step: The temperature is brought up to 72°C and DNA polymerase extends out new strands of DNA using each primer as a starting point and the single strands of DNA as the templates. The series of steps are repeated, and the number of new DNA strands doubles after each round. Going through 30 rounds can produce, from a single double stranded molecule, over a billion copies of the desired DNA fragment ($2^{30}$ = 1,073,741,824).

For each set of primers, one needs to optimize the temperatures for the annealing step. The annealing temperature will vary depending on several parameters, including the GC content and length of the primers used.

## 8. Electrophoresis

**Electrophoresis** is a process by which DNA molecules can be separated by size. During electrophoresis, DNA is drawn through an agarose gel matrix by an electric current. Agarose is a highly purified seaweed extract that forms a gel when heated in water and cooled. The agarose is dissolved in buffer and heated. When the solution is poured into the gel tray, it solidifies, forming a matrix through which the DNA is run. Because DNA has an overall negative charge (due to the negatively charged phosphate groups in its backbone) the DNA, which was placed into the wells in the end of the gel closest to the negative electrode, moves through the gel toward the positive electrode of the gel chamber. DNA fragments move through the gel matrix at different rates depending on their size. Larger fragments take longer than shorter fragments to move through the gel, so larger fragments are seen closer to the wells than smaller fragments. Fragments of the same size will form discrete bands since they pass through the matrix at the same rate.

The gel is made with and immersed in a salt buffer to provide the ions necessary to produce the electric current that runs through the gel. In this exercise we will use TBE (containing Tris, Boric Acid, EDTA).

To make the gel loading process go more smoothly in the classroom, **loading dye** will be added directly to the reaction mix prior to PCR. There is sugar (in this case, sucrose) in the loading dye to make the DNA sample more dense than the buffer, so it will sink to the bottom of the well. The loading dye used in this lab also contains a dye called Cresol Red (which, unlike most other dyes, does not inhibit the action of Taq polymerase). On a 0.8% gel, cresol red runs through the gel at approximately the same rate as a 1.5 kB DNA fragment. Watching the progress of the dye helps you track where your DNA fragments should be running on the gel, so you know when to turn off the power supply. A **DNA ladder**, or another set of DNA markers, containing DNA fragments of known size, is loaded into one lane of a gel. Comparing the amplified PCR fragments to the fragments of known size in the marker allows you to determine the approximate size of the amplified fragments.

## 9. Bioinformatics

**Bioinformatics** is a branch of biology that combines molecular biology data with computer databases that hold information from scientists worldwide. Bioinformatics came about after the amount of data generated by biological experiments and data collection became so overwhelming that biologists needed help analyzing, organizing, and archiving the information. For example, the *Halobacterium* sp. NRC-1 **genome** is about 2.5 million base pairs in size, and codes for about 2500 proteins! Imagine organizing the data that generated this entire sequence and analyzing it without the aid of computer programs and programmers to write the code to organize and interpret the data.

This lab will use bioinformatics to evaluate information regarding the gas vesicle gene region. Bioinformatics tools such as those found on HaloWeb (http://halo4.umbi.umd.edu/) will help you explore the genotype of the red and pink colonies of *Halobacterium* sp. NRC-1.

**Name:_____** **Student Guide**
**Period: _____**

# The Genotype-Phenotype Connection: Basic Molecular Genetics and Bioinformatics Skills

Following these instructions, you will use the model microbe, *Halobacterium*, to perform four related activities: 1) Determination of Colony Phenotype and Cell Lysis, 2) Polymerase Chain Reactions (PCR), 3) Gel Electrophoresis, and 4) Data Comparison and Analysis. In the analysis, you and your classmates will compare data. It is your careful observations of the *Halobacterium* colony phenotypes at the beginning of this lab that will provide clues in deciphering your PCR/gel electrophoresis results at the conclusion.

## Pre-Lab for Activity 1

*HaloWeb is a haloarchaeal-specific bioinformatics resource tool that has been publically available for over a decade, and be can used it to explore the genomes of several Haloarchaea, including Halobacterium sp NRC-1.*

Use HaloWeb to explore *Halobacterium* gas vesicle genes.

1. Go to the HaloWeb gateway page (http://halo4.umbi.umd.edu or access from a search engine, by searching for "HaloWeb").
2. Click on "*Halobacterium* sp. NRC-1" to go to the individual genome web page.
3. Click on the "search" tab to search the genome.
4. In Search, "Keyword in", the "both" button is already preselected for you.
5. Type in "*gvp*A1" into the annotation search box, in order to search for the *gvp*A1 gene related to gas vesicle assembly.
6. Click on the search button on the end of the page or hit return.
7. Select "*gvp*A1", one of the gas vesicle protein genes that is integral in the formation of gas vesicles. You may choose *gvp*A1 for either replicon (plasmid).
8. Now you are on the gene web page. Here you can explore all aspects related to the gene and the regions around it. Try out some of the options listed on the page:
   - "Get data for this gene" gives the DNA sequence of *gvp*A1 and also the amino acid sequence.
   - Either "BLAST" option could be chosen if you had a DNA/RNA or protein sequence you wanted to compare to the *gvp*A1 nucleotide or amino acid sequence to find similarities or differences between the sequences.
9. You can expand or shrink the region you want to see on the gene map that is at the bottom of the page – for example, use the dropdown menu "Number of genes in this neighborhood" and select 23 genes for the region you want to see. Scroll down to see all 23 genes in the *gvp*A1 gene region. Just below the chart of surrounding genes, scroll down to look at the gene map. Notice that there are other *gvp* genes surrounding *gvp*A1. Notice the location of *gvp*D1. Also note that there are insertion elements (ISH8-1, ISH3-2, etc. just downstream of *gvp*A1. You

may wish to select fewer than 23 genes or more than 23 genes in the area around *gvp*A1 to get a different view.

10. Now look at the nucleotide and consequent amino acid sequences for however many genes you have chosen in the neighborhood of *gvp*A1 by clicking on the "submit" button at the bottom of the screen. **Note:** your computer must be able to open a popup for this function, so be sure to check if pop-ups are enabled if at first you do not get a sequence.

11. Look at the DNA sequence that has come up in the pop-up window. You will notice that some of the bases are capitalized, and some are not. Bases that are transcribed into RNA are capitalized; untranscribed areas, known as intergenic regions, are shown in lower case.

12. Go back to the gene webpage and "select top strand only" at the bottom of the screen, then hit "submit" as before. Now you will get a single-stranded version of the gene region.

13. You can also see if there are other, similar regions in the genome. Copy the whole or partial sequence and then click on the "BLAST NRC-1 Genome" link on the main page.

The BLAST function on the HaloWeb site is carried out at the NCBI (National Center for Biotechnology Information). A BLAST allows you to compare a DNA sequence you are curious about to the sequences already in the genome databases. The homologous (similar) regions between the sequence you submit and any sequences in the database are identified and scores indicating the probability that they are truly homologous are assigned to them.

14. Paste in your sequence into the "Enter Query Sequence" box towards the bottom of the page, followed by clicking on "Begin Search" button near the bottom of the page. Your sequence is the query sequence (referenced below)

15. Click on "View report" and you will see the NCBI results.

- "Graphic summary" shows you how similar the sequence you copied is to other sequences within *Halobacterium* sp. NRC-1.
- The "Descriptions" chart includes a query coverage which tells you what percentage of your query sequence is found elsewhere in NRC-1.
- "Alignments" gives you a base pair alignment of your query to the subject (*Halobacterium* sp. NRC-1.) Vertical lines between the two sequences indicate exact matches. Spaces between the vertical lines mean there was not an exact match. Dashes tell you that there was a misalignment and space was added to get the sequences to align from that point forward.

# Activity 1 – Halobacteria Exploration and Cell Lysis

**Materials (per student unless otherwise noted)**
2 sterile microcentrifuge tubes, 1.5-mL capacity
permanent fine-tip marker
5 petri plates with *Halobacterium* culture(s), shared among the class
1 micropipet for measuring 100 uL and 500 uL (per student or student group) with sterile tips
sterile distilled water
vortexer (not essential)
microcentrifuge
dissecting scope or hand lens (not essential)
gloves

**Laboratory Procedure**
1.1 Set up a clean, sterile work area as your teacher instructs.
1.2 Obtain your microcentrifuge tubes. With permanent insoluble marker, label the tubes with your initials and each of the following: "#1" and "#2 extract." Set the tube labeled "#2 extract" aside for later use.
1.3 Add 500 uL of sterile distilled water to your microcentrifuge tube labeled "#1."
1.4 Observe a petri plate of *Halobacterium* colonies by holding the plates up to a light. To make more detailed observations you can view them using a dissecting microscope or a hand lens if they are available.
1.5 Describe at least two different-looking colonies. Include color and colony morphology (do they have raised/irregular borders, are they raised or flat, are they matte or glossy, etc?)

| **Colony description (color, etc.):** | **Colony morphology observations:** |
|---|---|

1.6 While observing the various *Halobacterium* colonies, select one colony from which to extract DNA. Use a permanent marker on the bottom outside of the petri plate to circle your colony and mark it with your initials. Your colony information will be compared with that of your classmates. It is the compilation of all the data that will allow you to form an analysis of the genotype of your colony.

1.7 Observe your colony with a hand lens or stereomicroscope if available. Use varying amounts of light on the scope or hold the plate up to the light for thorough examination. **It is critical to accurately record your observations regarding your colony.** Record information about your colony here:

| **Individual colony appearance and observations:** | **Diagram of YOUR colony, noting color**: |
|---|---|

1.8 Using a micropipet with a sterile tip (or a disposable sterile plastic micropipet), pick up your colony from the surface of the agar using a swiping motion (be careful not to stab the agar.) Depending upon the size of the colony you may not need to take all of it. As long as you have a visible amount of material on your tip you should have enough. Make sure not to touch your pipette tip to any other colony or surface.

1.9 Put the pipette tip (containing the colony) into your tube labeled #1. Pipet up and down at least 10 times in the distilled water to remove your colony from the tip. Continue this action until you no longer see your colony on the tip. You may see the colony fragments in the water. Make the solution homogeneous.

1.10 Securely close the cap of your microcentrifuge tube.

1.11 Allow your microcentrifuge tube to sit undisturbed on the lab bench for 5 minutes. This will ensure that the cells from your colony are all lysed. You now have "cell lysate" in your tube. If you let the cell lysate stand too long, your DNA may be degraded by cellular enzymes called DNases.

1.12 Vortex (or tip side to side) until mixed.

1.13 Spin your microcentrifuge tube containing the cell lysate in a microcentrifuge at maximum speed for 1 minute to pellet the insoluble cellular fragments (the pellet may not always be visible.) The water soluble components of the cell, including the DNA will be in the supernatant (liquid above the pellet).

1.14 Transfer 100 uL of the supernatant into your other, unused tube labeled "#2 extract". To avoid disturbing the material at the bottom of the tube, when removing your sample pipette carefully from the very top of the sample.

1.15 Follow your teacher's instructions for storage of tube"#2 extract" and disposal of tube #1.

1.16 Answer questions 1-3 in "Student Analysis and Follow-up Questions" at the end of this packet.

# Pre-Lab for Activity 2

Go to the MolGenT (Molecular Genetics Tutor) website to watch the unit on PCR:
http://halo4.umbi.umd.edu/~haloed/MOLGENT/index.htm
NOTE: this activity requires that you have QuickTime installed on your computer in order to view the units.

1. Go to Part D: Recombinant DNA and Society.
2. Click on the PCR amplification tutorial link (on the left menu) and push "play".
   This narrated animation demonstrates how PCR works.

# Activity 2 – PCR

**Materials (per student unless otherwise noted)**

Student microcentrifuge tube from Activity 1 containing nucleic acid extract (labeled #2 extract)
1 PCR tube containing Ready-To-Go Bead™, 0.2-mL capacity
Permanent fine-tip insoluble marker
Micropipet (4- to 20-uL capacity) with sterile tips
Ice (in beaker or plastic cup)
1 vial Primer Mix, shared among several students

**Procedure**

2.1 Set up a clean work area as your teacher instructs.

2.2 Collect your individual supplies. Notice that the PCR microcentrifuge tube contains a white bead. The bead contains the reagents that are necessary for synthesizing the new DNA, including a DNA polymerase called Taq polymerase, dNTP's, and buffer components.

2.3 Keep your microcentrifuge tube (#2 extract) containing the cell lysate from Activity 1 on ice. It should remain on ice while you are working.

2.4 Label your PCR tube (containing the bead) with your initials as your teacher instructs. You will be adding reagents to ***this*** tube from here on. Work with this tube on ice as much as possible until you place it in the thermal cycler.

2.5 Obtain Primer/Loading Dye Mix from your teacher. It contains a forward primer and reverse primer as well as gel electrophoresis loading dye (purple-pink color). The loading dye is added now to make your later steps easier. Pipet 20 uL of the Primer Mix into your PCR tube

2.6 Allow the tube with the PCR bead and primer to sit on ice for 1-2 minutes.

2.7 With a new sterile tip (or new microcapillary pipette), add 4 uL lysate from the microcentrifuge tube (#2 extract) from Activity 1 to the liquid in the bottom of the PCR tube and gently pipette up and down several times to mix the contents.

2.8 Follow your teacher's instructions for placement of your PCR tube into the thermal cycler.

2.9 Answer questions 4–8 in "Student Analysis and Follow-up Questions" at the end of this packet.

# Pre-Lab for Activity 3

Watch the Carolina Video (5 minutes, 46 seconds) explaining how to load a gel. Find Teacher Resources on www.carolina.com. In the educational videos, select "Biotechnology," and in that category, click on "Loading a Gel for Electrophoresis."

Determine which type of pipette you will be using (micropipette, fixed volume pipette, microcapillary, etc.).

# Activity 3 – Gel Electrophoresis

**Materials per student**

your PCR tube from Activity 2 (it has already undergone PCR)
ice (in beaker or plastic cup)
micropipette (for pipetting 10 uL) with sterile tips or 1 disposable microcapillary pipette
stain
staining tray
gloves

**To Be Shared**

gel electrophoresis chamber
1X TBE running buffer
agarose gel poured and ready
1 vial 1 kb DNA ladder
white light or *BLU*e light

**Procedure**

3.1 Obtain your *individual* materials. Keep your PCR tube on ice when possible during the following steps.

3.2 Your teacher or another student should pipette 5.0 uL (if you are using GelGreen™ or a stain of similar sensitivity) or 25 uL (if you are using *Carolina*BLU™ or a stain of similar sensitivity) of the DNA ladder into the appropriate lane.

3.3 Following your teacher's instructions, carefully pipette your PCR sample into your assigned gel well. Load 10 µL if you are staining with *Carolina*BLU. Load 5 µL if you are using Gel Green.

3.4 Because each student is evaluating a different colony, **it is important to know which lane in the gel belongs to you.** Record the gel and the lane number in which your sample is loaded.

**Gel (if more than one gel ran during your class period):**

**I pipetted my sample into Lane #:**

3.5 Run your gel according your instructor's directions. Before staining, measure the distance from the front edge of the well to the front edge of the dye front and record here.

_____cm

3.6 Follow your teacher's instructions for staining your gel. Staining makes the DNA visible.

3.7 Answer questions 9-11 in "Student Analysis and Follow-up Questions" at the end of this packet.

Figure 4

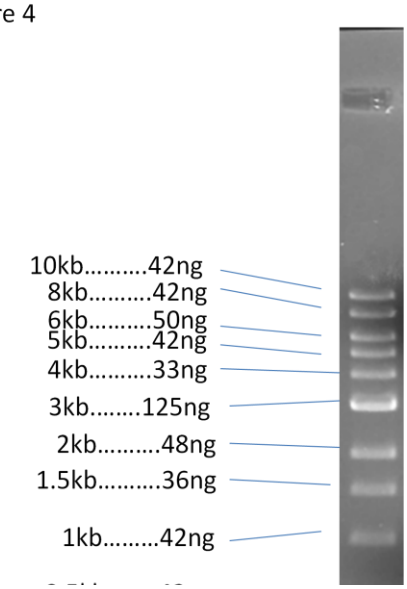


Figure 4. Labeled DNA marker

# Activity 4 – Data Comparison and Analysis

**Materials (per student unless otherwise noted)**
Photos of various gels (or gels themselves)

**Procedure**
4.1 Calculate the approximate size [in base pairs (bp)] of your DNA fragment(s).

a. You will interpolate the molecular weights of your PCR products by using a standard curve created using the 1 kb ladder. For this exercise use either a photo of the gel or a representation created by placing a piece of acetate over the gel and tracing around the location of each band. The size of the DNA bands in the ladder are given in figure 4.

b. Using your own gel, a photograph, or a representation of the gel traced on acetate, carefully measure the distance (in mm) each DNA band in the DNA ladder traveled from the

well. Also measure the distance traveled by the bands in the lane loaded with your sample. Measure from the front edge of the well to the front edge of each band. Enter the distances into the table.

| DNA band | 0.5 kb | 1.0 kb | 1.5 kb | 2.0 kb | 3.0 kb | 4.0 kb | 5.0 kb | 6.0 kb | 8.0 kb | 10.0 kb | PCR product | PCR product |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Distance traveled | | | | | | | | | | | | |
| Rf | | | | | | | | | | | | |

c. Calculate the $R_f$ for each fragment. $R_f$ is calculated by dividing the distance traveled by a given band by the distance traveled by the dye front (measured in step 3.5), and is used when calculating molecular weight.

d. Set up semilog graph paper with $R_f$ as the x-(arithmetic) axis and the base-pair length as the y- (logarithmic) axis. Then, plot the $R_f$ versus the base-pair length for each of the DNA bands in the marker.

f. Connect the data points with a best-fit line. This best fit line is the standard curve.

g. Locate on the x-axis the $R_f$ for one of the DNA bands in the lane loaded with your sample. Using a ruler, draw a vertical line from this point to its intersection with the standard curve (the best fit line.)

h. Extend a horizontal line from this point on the standard curve to the y-axis. This gives the base-pair size of this band.

i. Repeat steps g and h for each band in the lane loaded with your sample and write the answers below.

4.2 Share the observations you wrote down regarding the morphology of your colony (while it was still on the agar plate) with your class. While your classmates are looking at the gel (or photo), indicate the location of your lane and describe the size of the band in that lane.

4.3 Record the class data in a chart so that it can be easily observed. Your teacher may provide you with a chart to use.

4.4 Answer questions 11 and 12 in "Student Questions" at the end of this packet. Your teacher may also assign some or all of the challenge questions, questions 13-18.

**Name:_____** **Student Guide**
**Period: _____**

# Student Analysis and Follow-up Questions

**(hint: if you are stumped on any question, go back and re-read the background section)**

1. You observed the possible phenotypes of *Halobacterium* on the petri plate.
   a. Describe each of the phenotypes observed.

   b. What do you propose was the source/cause of the different phenotypes?

2. From your background reading, which unique properties of *Halobacterium*, an extreme halophile, allows the cells to be lysed with the simple addition of water?

3. You lysed your cells in water.
   **a.** Which cellular components would you expect to find in your lysate?

   **b.** Why was the cell lysate centrifuged?

   **c.** What purpose was served by removing 100 uL of supernatant and transferring it to another tube?

4. What are the necessary components in the PCR reactions? List the components and the purpose of each.

5. You mixed the PCR bead, primers, and lysate sample thoroughly before placing the reaction in the thermal cycler. Why was thorough mixing necessary?

6. A Polymerase Chain Reaction goes through several cycles of temperature changes. List each step of a cycle, and describe what occurs during each step.

7. Why did you place your nucleic acid extract and PCR reagents on ice until the PCR reaction was set up and placed in the thermal cycler?

8. What are 2 purposes served by the loading dye?

9. You loaded the PCR samples into the wells of the gel electrophoresis chamber.
   a. Are the wells (and thus your DNA) nearer to the positive or negative electrode?

   b. Is DNA positively or negatively charged, and how does its charge impact how the gel chamber is set up?

10. How many bands appeared in your lane? _____ What are the band(s) made of?

11. Look at the table with class results to determine if your band(s) ran a different distance into the gel than some of your classmate's bands or if you had a different number of bands than others. Think about the observations you originally made about the colony types. Can you find a correlation between the size and number of bands you and your classmates observed in each lane and the phenotypes of the colonies used to generate those bands? Using the class data, explain any correlation or lack of correlation you see.

12. Your gel band gives you information about the genotype of the colony you isolated. Based upon your observations regarding the phenotype and genotype of your colony, what genotype (strain) of *Halobacterium* do you hypothesize you isolated (*Halobacterium* sp. NRC-1, KBT-1, or another)? Identify whether your colony was wild type (wt) or mutant (mut). What makes you conclude that?

**Challenge Questions**

13. Write a well constructed paragraph explaining how an IS element could cause a red colony of *Halobacterium* sp. NRC-1 to appear red.

14. It is possible to occasionally find a red colony (mutant) that yields a band that appears to be the same size and sequence as the wild-type (*Halobacterium* sp. NRC-1) band. Explain how this could occur.

15. The DNA sequence in Figure 5 (on the following page) shows the location of some of the genome region you just amplified using PCR. The region shown contains the gene sequence for ***gvp*A1**. (hint: if you have trouble with questions 15a-15d, review pre-lab #1.)

a. Why are some letters capitalized?

b. Label the gene start codon (usually ATG).

c. Label the gene stop codon (usually TAA, TGA or TAG).

d. How did you pick your start and stop codon, given that were multiple codons like them in the sequence?


Figure 5. A portion of the wild type *Halobacterium* sp. NRC-1 *gvp*A1 gene region sequence amplified by PCR

16. Translate the entire **capitalized sequence** for *gvp*A1 (from question 15, Figure 5) into an amino acid sequence. Use the sequence editor function on HaloWeb (or your textbook) to help you do this.

17. When the *gvp*A1 gene region was amplified from KBT-1 and sequenced, the sequence in Figure 6 (on the following page) was obtained. How does the KBT-1 sequence shown (figure 6) differ from the NRC-1 sequence used for question 15 (Figure 5)?

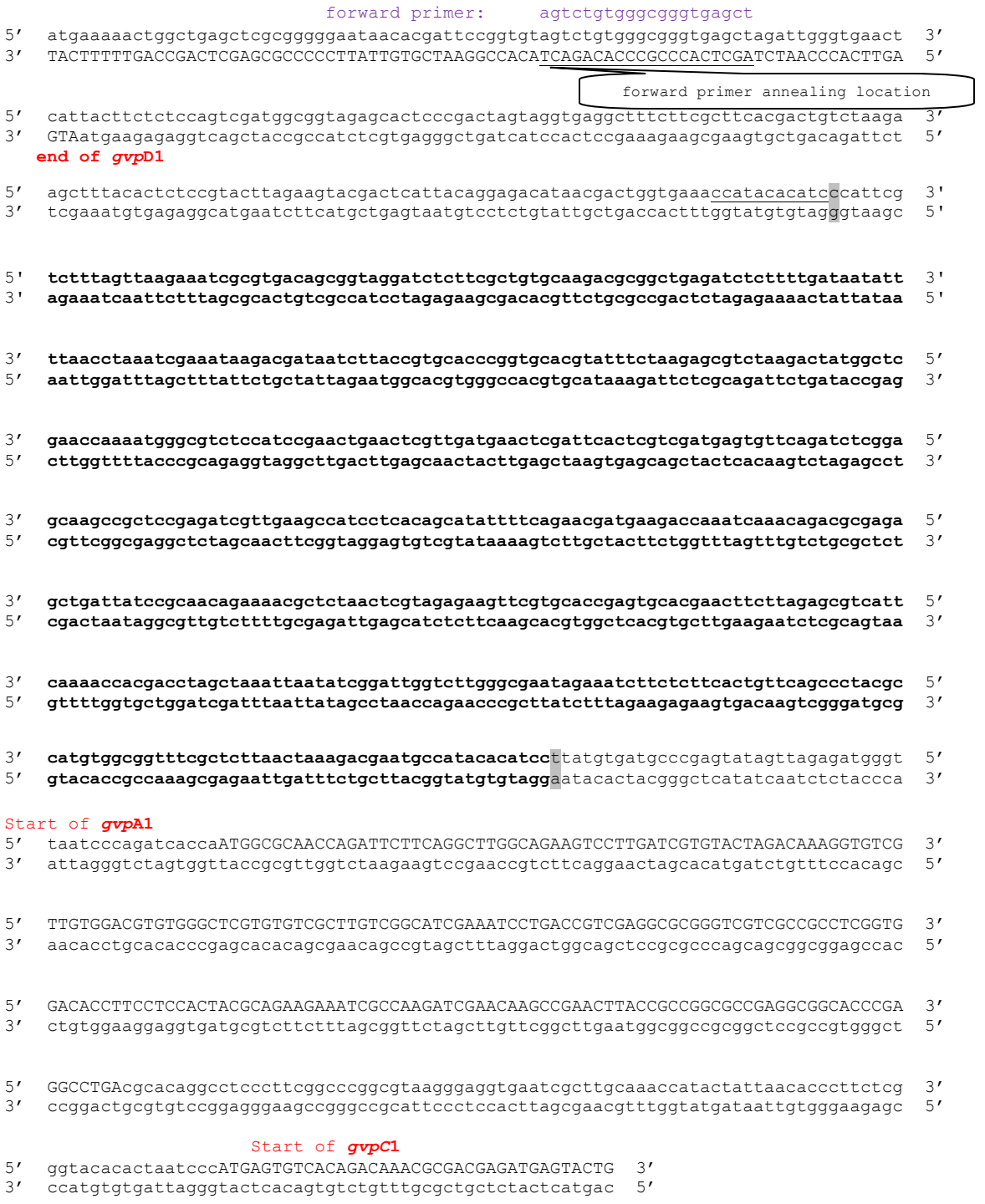


**Figure 6.** *Halobacterium* sp. KBT-1 sequence of *gvp*A1 gene region. The area between the brackets indicates an IS sequence. Shaded nucleotides indicate the nucleotides between which the IS sequence inserted. The underlined sequence on either side of the insertion element are the direct repeats. The direct repeats result from the insertion element copying the 12 nucleotides located just before the insertion site and repeating these nucleotides at the end of the IS as it inserts.

18. On the HaloWeb genomes page (halo4.umbi.umd.edu), click on *Halobacterium* NRC-1, then on the Resources link. Click on the Insertion Sequence elements (IS elements). Based on the size difference between the sequences listed for questions 16 and 18 (Figures 5 and 6), which IS element do you predict may have inserted? Note that the length of the IS element as shown in the chart does not include either direct repeat.

# Appendix I

## Primer locations in the *gvp*A1-*gvp*N1 gene region

```
5′  GCTCCGTGATATATCTGATCTGGTTGCAGCAATCGACGACTTCCAAGAGGAATTCATCGCCGTGCAGGACGCATTTGACA  3′
3′  cgaggcactatatagactagaccaacgtcgttagctgctgaaggttctccttaagtagcggcacgtcctgcgtaaactgt  5′


5′  ACTACGCTGGTGACTTCGATGCGGAGATCGACCAGCTCCACGCTGCCATCGCTGACCAGCACGACAGCTTCGACGCTACC  3′
3′  tgatgcgaccactgaagctacgcctctagctggtcgaggtgcgacggtagcgactggtcgtgctgtcgaagctgcgatgg  5′


5′  GCGGACGCCTTCGCAGAGTACCGAGATGAGTTCTATCGCATAGAGGTGGAAGCACTGCTTGAGGCGATCAACGACTTCCA  3′
3′  cgcctgcggaagcgtctcatggctctactcaagatagcgtatctccaccttcgtgacgaactccgctagttgctgaaggt  5′


5′  GCAGGACATCGGTGACTTCCGAGCGGAGTTTGAAACGACTGAGGACGCGTTCGTTGCCTTCGCCCGTGACTTCTAgatgg  3′
3′  cgtcctgtagccactgaaggctcgcctcaaactttgctgactcctgcgcaagcaacggaagcgggcactgaagataccgg  5′


5′  ACGAGATCACGGCCGAGGAAGGCGCCGCCGAAGCGGAAGCCGAACCCGTCGAGGCTGACGCGGACGTCGAAGCGGAAGCA  3′
3′  tgctctagtgccggctccttccgcggcggcttcgccttcggcttgggcagctccgactgcgcctgcagcttcgccttcgt  5′


5′  GAAGTCTCTCCAGACGAAGCTGGCGGAGAATCCGCCGGTACCGAGGAAGAAGAGACAGAGCCGGCCGAGGTGGAAACAGC  3′
3′  cttcagagaggtctgcttcgaccgcctcttaggcggccatggctccttcttctctgtctcggccggctccacctttgtcg  5′


5′  GGCTCCAGAAGTAGAGGGGAGTCCTGCGGACACGGCAGACGAAGCGGAAGATACGGAAGCAGAGGAGGAGACAGAGGAAG  3′
3′  ccgaggtcttcatctcccctcaggacgcctgtgccgtctgcttcgccttctatgccttcgtctcctcctctgtctccttc  5′


5′  AGGCACCGGAAGACATGGTGCAGTGCCGGGTGTGCGGCGAATACTATCAGGCCATCACGGAGCCCCATCTCCAGACCCAT  3′
3′  Tccgtggccttctgtaccacgtcacggcccacacgccgcttatgatagtccggtagtgcctcggggtagaggtctgggta  5′


                                                                    gvpN1
5′  GATATGACGATTCAGGAGTACCGCGACGAGTACGGTGAGGATGTCCCCCTTCGGCCGGATGATAAAACATGAcgaacgag  3′
3′  ctatactgctaagtcctcatggcgctgctcatgccactcctacaggggggaagccggcctactattttgtactgcttgctc  5′
                                                  reverse primer annealing location

5′  tcccgtaaacgcaaggtacgagggtcgcagatccgctcctcacgcggcgacaagaaacaggggcgatcacagagccgtga  3′
3′  agggcatttgcgttccatgctcccagcgtctaggcgaggagtgcgccgctgttctttgtccccgctagtgtctcggcact  5′
                                        Reverse Primer:  5'acggctctgtgatcgcccct 3'


5′  tgataaggagatcgagcgtctcgagaggcagaacgacgctcgtggccaggagtcgtctacccacgtcgacgaggg…
3′  actattcctctagctcgcagagctctccgtcttgctgcgagcaccggtcctcagcagatgggtgcagctgctccc…
```

# Appendix II
## Glossary

**Amino Acid (AA):** monomer unit of proteins. Amino acids have the same basic structure with the exception of a "R group" that is variable. The R group gives each amino acid a different chemical property.

**Amplify: with respect to PCR -** the process of making more copies of a specific segment of DNA.

**Annealing:** attachment by base pairing of one strand of nucleotides to its complementary strand (such as a DNA primer base pairing with the complementary DNA template).

**Antiparallel:** a term used to describe DNA strands that run in opposite directions in a double-stranded molecule.

**Archaea:** a classification (or Domain) of prokaryotic life distinct from the Eubacteria (commonly known as bacteria) and higher organisms (Eukaryotes). This domain includes many organisms from extreme environments, e.g. extreme halophiles (organisms that grow in high-salt environments); methanogens (anaerobes that produce methane); and thermophiles (organisms that thrive in high-temperature environments).

**Bioinformatics:** The use of computers and related tools to store, manage, and analyze biological data. The use of a computer in this way is especially common in molecular biology, genetics, and genomics.

**Clones:** organisms that are genetically identical.

**Codon:** a group of three nucleotides in a DNA or RNA polynucleotide chain that, depending upon their sequence, serves as either a start or stop signal for the translation of a protein, or as a code for a specific amino acid to be added to the growing polypeptide during translation.

**Colony:** a visible clonal population of microbial cells on an agar or other surface, resulting from many generations of cell division of a founder (mother) cell.

**Complementary:** in DNA, nucleotide sequences which can be joined by base pairing, to form double-stranded DNA.

**Denature:** to separate double-stranded DNA into two single strands, usually by heating or exposure to alkaline conditions.

**Deoxyribonucleic acid (DNA):** a chemical substance found in all cells, consisting of two polynucleotide chains forming a double-helix with Watson and Crick pairing of the nucleotide bases. DNA functions as the hereditary or genetic material of the cell. It contains genetic information stored as triplet codons.

**DNA polymerase:** an enzyme, found in all cells, which functions in the replication and repair of DNA.

**Domain:** one of the three broadest categories of life forms in current biological taxonomy: Eukaryotes, Eubacteria, and Archaea.

**Double helix:** the structure of DNA, in which two chains are wrapped around each other like the sides of a twisted ladder.

**DNA ladder:** also called a DNA Marker. A sample of DNA containing multiple DNA fragments most of which increase in size in regular increments. For example, a ladder may have fragments of the following sizes: 500 bp, 1000 bp, 1500 bp, etc. When the fragments are separated using gel electrophoresis, they create a banding pattern that resembles a ladder and can be used for measuring the size and quantity of other DNA fragments on the gel.

**Electrophoresis:** The process by which molecules are separated by size or charge by being drawn through a matrix by an electric current (e.g. DNA molecules are often separated by size by drawing them through a matrix formed by agarose).

**Eubacteria:** the Domain of "true" bacteria. Eubacteria are prokaryotic, single celled microbes and may have a cell wall made of peptidoglycan.

**Eukaryotes:** the Domain also referred to as "Eukarya." These cells have nucleic acid surrounded by a nuclear membrane, and membrane-bound organelles. The eukaryotic organisms are often, but not always multi-celled.

**Forward primer:** a short sequence of DNA designed to amplify a specific genome region. The forward primer is a composed of sequence from the 5' end of the coding strand of the sequence you want to amplify. You need both a forward and a **reverse primer** to amplify the template DNA.

**Gas vesicles (GVs):** organelles found in many microorganisms, including the Archaeon, *Halobacterium* sp. NRC-1. They consist of a rigid protein structure, filled with ambient gas. These vesicles give buoyancy to this organism, allowing it to float in the hypersaline aquatic environment in which it is found and thus giving it access to more oxygen and sunlight.

**Gel:** for the purposes of this lab**,** the word refers to the agarose gel used in gel electrophoresis. See also **electrophoresis**.

**Genome:** all of the genetic information contained in one cell.

**Genotype:** the genetic makeup of an organism, either in total or in terms of one or a few sets of genes.

***Halobacterium* sp. NRC-1:** an extremely halophilic microorganism (an Archaeon) found in extremely salty environments all over the world. It is used as a model organism for research and teaching.

**Halophile:** an organism that requires salt concentrations above that found in seawater for optimal growth. Those that require nearly saturating salinity are called "extreme halophiles."

**Insertion sequences (IS elements):** also see **transposable element**. DNA sequences that insert into the genome; also known as "jumping genes." When IS elements insert into a new location of the genome, they cause mutations that can bring about phenotypic change.

**Insertion Sequences** in **Haloarchaea** (**ISH**): Insertion sequences in Haloarchaea, (first identified in *Halobacterium)* that commonly insert at certain "hot spots," such as in the gas vesicle gene region.**Loading dye:** pigments and sugars (e.g. sucrose, ficoll, glycerol, etc.) mixed and added to a DNA sample before it is run on a gel. The pigment makes it easier to see the sample while you are loading and helps you judge the location of the DNA fragments on the gel. The sugar adds density to the sample so it sinks to the bottom of the well.

**Lysate:** the solution/mixture of cell components that results after cells are broken open (lysed).

**Lyse:** to cause the breaking open of a cell so that that the contents are released and the cell dies (the breaking open itself is referred to as "lysing."

**mRNA:** messenger RNA is a polynucleotide chain of ribonucleotides transcribed from DNA and, through the process of translation (by ribosomes), used to make proteins.

**Mutation:** a change in the genetic code. A mutation can result from the addition or removal of one or more nucleotides into the DNA sequence. These types of mutations are called insertion or deletion mutations respectively. Mutations can also occur if one or more base pairs in the DNA sequence are changed to a different base pair(s).

**Nitrogenous base:** one of three components that makes up a nucleotide. The nitrogenous base is called such because it contains nitrogen. A DNA nucleotide can contain any of four different

nitrogenous bases: adenine (A), thymine (T), guanine (G) or cytosine (C). Adenine will pair with thymine and guanine will pair with cytosine. In RNA, uracil is found in place of thymine.

**Nucleotide:** any one of the building blocks of DNA (deoxyribonuclotides) or RNA (ribonucleotides). A nucleotide is composed of a phosphate group, a sugar, and a nitrogen base.

**Operon:** a group of linked genes or segments of DNA that are transcribed together and often regulated together.

**Phenotype:** observable characteristic or trait of an organism, e.g., brown, blue, or green eyes in people, or colony color or opacity in microorganisms.

**Phototrophy:** an energy-generating pathway similar to, but distinct from photosynthesis that uses sunlight and the protein, bacteriorhodopsin. The pathway allows an organism to use sunlight to produce energy.

**Polymer:** A chain of monomer units that link together, usually through dehydration synthesis, to make a longer strand. Proteins, carbohydrates, DNA, and RNA are all polymers.

**Polynucleotide:** a chain of nucleotides forming a strand of DNA or RNA.

**Polymerase Chain Reaction (PCR)**: a process for amplifying a specific segment of DNA from a few copies of that DNA. PCR is a powerful tool which simplifies and, in many cases, makes possible, the manipulation and analysis of specific sequences of DNA.

**Precipitate:** to cause a dissolved material to come out of a solution or suspension often so that it can be physically collected at the bottom of a vessel.

**Primer:** in DNA replication, a short RNA or RNA/DNA fragment which serves to prime DNA synthesis. DNA polymerase attaches the first nucleotide in DNA synthesis to the primer. In PCR, an artificially made DNA fragment (oligonucleotide) is used as a primer for DNA synthesis. See also **forward primer** and **reverse primer.**

**Protein:** a biomolecule made of chains of amino acids. Peptide bonds link the amino acids in the chain. Proteins can function as enzymes, structural molecules, and signal molecules and can have other functions as well.

**Reverse primer:** is the reverse complement of the 3' end of the coding strand to be amplified by PCR. See also **forward primer**.

**Ribonucleic Acid (RNA):** a chemical substance found in all cells, which is synthesized by using parts of the DNA sequence in the cell as a template. RNA is synthesized in a process called transcription. One type of RNA, mRNA, is used as a template in translation; other types of RNA serve other functions.

**Sectored colony:** an occasionally observed colony that contains slivers of microbes with different phenotypes. The sectors are often reminiscent of slices of a pie.

**Transcription:** the process of DNA being used as a template to form a new RNA molecule with a sequence complimentary to the original DNA template. The process is carried out by RNA polymerase.

**Translation:** the process in which messenger RNA (mRNA) is used to code for the synthesis of an amino acid chain. This process is carried out by the ribosome which reads the mRNA nucleotides in groups of three. Each group of three nucleotides is called a codon and designates which amino acid is to be added to the growing polypeptide chain. Different codons code for different amino acids.

**Transposable element:** segment of DNA that can either relocate to other areas of a genome, or copy and insert the copy into other areas of a genome.

# Appendix III
## Troubleshooting

**A student had a band that is larger or smaller than KBT-1 or NRC-1.** *For examples of this see Figure 8, lanes 7, 9, and 10 below. Also see the answer to student question 11. If a lane is overloaded it can also alter the apparent mobility of DNA bands in the sample.*

**The bands are so faint, they are difficult to see.** *This could be caused by students having very little DNA extracted and thus very little amplified. It may also be a result of the student not getting enough of their sample into the well of the gel.*

**The bands are very bright and there is a great deal of smearing visible**. *This is likely caused by loading too much DNA in the lane. Either too much template DNA was added to the PCR reaction, or too much DNA was loaded into the lane. Students may end up with too much template DNA if they used too much halobacterium for their DNA isolation.*

**Some of my students do not have any band(s) in their gel lane.** *All of the following are possible explanations for this occurring:*

*1. Students made a pipetting error when they were setting up their PCR reaction.*

*2. When the students removed the supernatant (in step 1.14) during the DNA purification, they remove part of the "pellet" at the bottom of the tube. The pellet contains enzymes (nucleases) that can degrade the DNA and other cellular components that could inhibit the PCR reaction. Nuclease activity can especially be a problem if the sample is not chilled sufficiently. Chilling slows the activity of the enzyme.*

*3. There was a loading error.*

**Other possible problems as illustrated in figure 8:**

* Lanes 2 and 3 show bands amplified from NCR-1 and KBT-1 respectively.

I Lanes 4, 6, 8 appear empty. Suggesting that either the student did not successfully load the lane, or that their PCR reaction did not work.

* Lane 11 is empty, but the two bands in the adjacent lane 10 suggest the possibility that lane 10 is double loaded and one student loaded the wrong lane. Two bands may also be seen if the student who loaded lane 10 picked a sectored colony.

* In lane 12, there is a faint band above the main band, the most likely causes of this are a student picking and amplifying DNA from a sectored colony in which cells with a red phenotype are not numerous, or contamination of the PCR reaction.

* Lanes 5, 7, 9, 10, and to a certain extent, lane 12 all have too much DNA loaded in them. Overloading allows you to see the smearing and the background bands that you see in the majority of these lanes. It also causes smearing of predominant bands.

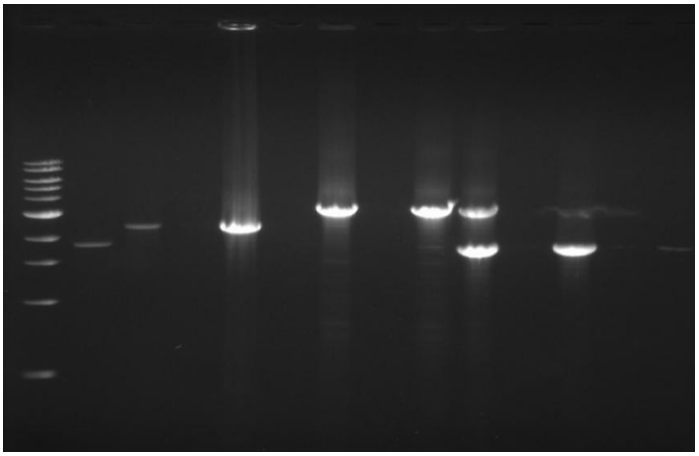

Figure 8. Shows some possible student mistakes that can occur in the classroom.
*Photo by Jolene Hund, Emporia State University.*

# Appendix IV
## Sample Class Gel Image

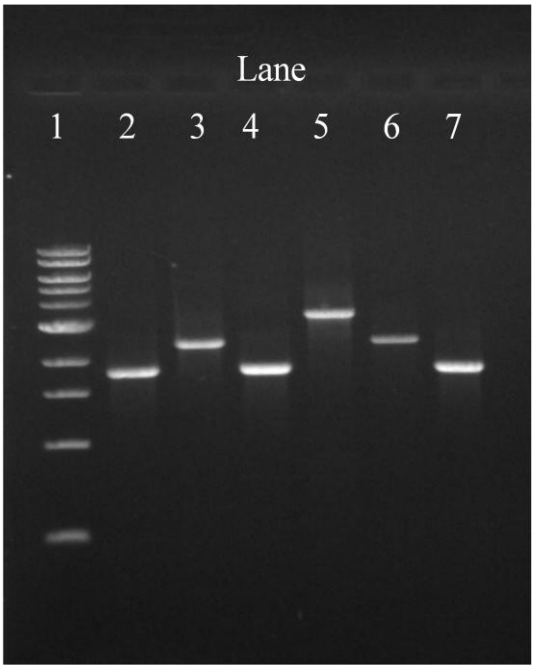


Figure 9. A sample class gel image with DNA marker, *Halobacterium* sp. NRC-1, KBT-1 and unknown mutant.

*PCR products for sample class gel provided by Shawnee Mission West Biotechnology Students, Overland Park, KS.*

**Lane 1: 1 kb DNA ladder (DNA marker)**
**Lane 2: *Halobacterium* sp. NRC-1**
**Lane 3: *Halobacterium* sp. KBT-1**
**Lane 4: *Halobacterium* sp. NRC-1**
**Lane 5: an unknown red mutant containing an insertion sequence larger than KBT-1 yet still found within the region amplified by the primers in this kit.**
**Lane 6: *Halobacterium* sp. KBT-1**
**Lane 7: *Halobacterium* sp. NRC-1**

# Appendix V
## Class Data Chart

| Student name: | Colony color: | Approximate size of PCR band(s) in base pairs amplified in your sample: |
|---|---|---|
| | | |
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# Appendix VI
## Staining With GelGreen™

**Note:** View and photograph gels stained with Gel Green within an hour or two of staining. If left in solution longer, the bands begin to diffuse and are not as sharp. If bands are faint, diffusion can make them even more difficult or impossible to see.

### Storage and Handling:
Store the 10,000X aqueous solution in the dark at room temperature. The 3X working solutions should also be stored in the dark at room temperature and is good for at least one year. If precipitation of the dye occurs, heat the solution in a 45–55°C water bath for two minutes and/or vortex.

### Safety Information:
While studies by the manufacturer and independent labs have shown GelGreen™ to be nonmutagenic, noncytotoxic, and unable to cross the cell membrane barrier, as is prudent when using any chemical in the classroom, it is suggested that caution be used and gloves and any other required personal protective equipment be worn when handling this stain. The appropriate orange filter or orange safety goggles should be kept between the viewer's eyes and blue light when viewing the gel.

### Staining Protocols:
1. To make the needed volume of 3X staining solution, dilute the GelGreen™ 10,000X stock solution ~3,300 times in 0.1 M NaCl.

Mix: 15 uL GelGreen 10,000X stock solution
5 mL 1 M NaCl
45 mL water

2. Place the gel in a suitable polypropylene container or staining tray and add just enough of the 3X staining solution to cover the gel.

3. Soak the gel for 30 minutes with constant, gentle agitation, if possible. If constant, gentle agitation is not possible, agitate every 5-10 minutes by hand.

### Viewing and Photographing the Gel
1. Because GelGreen has a UV absorption peak between 250 nm and 300 nm and a stronger adsorption peak around 500 nm, it can be visualized with either a 254-nm UV transilluminator or a "blue light" transilluminator.

2. Photograph the gel using any suitable imaging equipment. Use a long path green filter such as a SYBR** filter or GelStar** filter for photography. *Note: Some "blue light" transilluminators incorporate filters appropriate for photography.* The stain's peak emission occurs between approximately 500 and 550 nm.

GelGreen and its uses are covered by pending US and international patents.
**SYBR is a registered trademark of Molecular Probes Inc. and GelStar is a registered trademark of FMC.

# Appendix VII
# Staining with CarolinaBLU™

Adding CarolinaBLU™ to the gel and buffer allows you to faintly see many of the bands in the gel while the gel is running. This can be instructive for the student, as they can actually see the bands move through the gel. However, be aware, **you will still have to use the final stain** to see all of the bands clearly.

Stain can be added using the dropper bottle. Alternatively, if a calibrated pipette is available, the lid of the dropper bottle can be removed for quicker addition of larger volumes.

While CarolinaBLU™ is not toxic, we recommend that students wear gloves to prevent staining of skin.

**Step 1: Addition of CarolinaBLU™ to Agarose**

The dropper bottle provided delivers 40 uL/drop.

A. In a beaker, prepare the agarose solution required. Cool the molten agarose to about 60°C by placing the beaker in a 60°C water bath or allowing it to stand at room temperature for several minutes. Swirl the beaker occasionally so that the agarose will cool evenly throughout. The quantity of stain to add to the agarose and the buffer depends on the voltage used for electrophoresis. At voltages less than 50, a slightly lower concentration is used than at voltages greater than 50. Refer to the following chart:

| Voltage | Agarose Volume | Stain Volume * |
|---|---|---|
| 30 mL | | 40 uL |
| 50 mL | | 70 uL (1 drop) |
| 400 mL | | 533 uL(8 drops) |
| >50 volts | 50 mL | 70 uL (1 drop) |
| | 400 mL | 640 uL(9 drops) |

B. After you add the stain to the agarose, swirl to mix and then pour the gel immediately. Gels can be prepared 1 day ahead of the lab day, if necessary. Gels stored longer than 1 day tend to fade and lose their ability to stain bands during electrophoresis. Store the gels covered (in a plastic bag or plastic container with a lid) with a small amount of buffer (leave the masking tape or dam in place).

Alternatively, store them covered with buffer in the gel box. **Do not use more stain than recommended in your gel**. This leads to precipitation of the DNA in the wells and/or aggregation of DNA to form artificial bands in the gel.

**Step 2: Addition of CarolinaBLU™ to Running Buffer**

Because you added stain to the gel, you must also add stain to the buffer. Use the chart below for addition of the stain to 1xTBE electrophoresis buffer:

| Electrophoresis Voltage | Buffer Volume | Stain Volume |
|---|---|---|
| <50 volts | 500 mL<br>2.6 L | 500 uL (7 drops)<br>2.6 mL (37 drops) |
| >50 volts | 500 mL<br>2.6 L | 960 uL (14 drops)<br>l5 mL (71 drops) |

**Step 3: Running the gel**

Load the DNA samples and apply current. Amount of time is dependent on type of gel electrophoresis apparatus and voltage.

Following electrophoresis, remove the gel from the box. The DNA bands are best visualized when viewed against a white background or, even better, on a light box.

**Step 4: Staining the gel with CarolinaBLU™ Final Stain**
*As previously mentioned, CarolinaBLU (as CarolinaBLU Gel and Buffer Stain) can be added to the gel and buffer in order to visualize some of the larger bands while the gel is running. However, you will still have to do a final stain of the gel with CarolinaBLU Final Stain.*

Place the gel in a staining tray. Cover the gel with the final CarolinaBLU stain (you do not need to dilute it) and allow it to sit for 20–25 minutes. Agitate the tray gently, if possible. If you allow the gel to stain for more than an hour the gel will be difficult to destain.

**Step 5: Destaining with deionized water**

A. Pour the stain back into the bottle. The stain can be reused up to eight times. Cover the gel with **deionized water to destain**. (Tap water contains chloride ions, which can partially remove the stain from the DNA bands. **Therefore, tap water yields inferior results**). Occasionally, gently agitate the gel. Change the water 3–4 times over the course of 30–40 minutes. The gel can be left at room temperature in a little water to destain fully.

**B.** Once sufficiently destained, the gel can be removed and covered in plastic wrap or a plastic storage bag, or it can be left in the staining tray and covered with plastic wrap. The gel can be stored for 8 weeks in a refrigerator with no significant loss of staining.

**Step 6: Viewing and Photographing Gels with CarolinaBLU™**

Transillumination, in which light passes up through the gel, gives superior viewing of gels stained with CarolinaBLU™. White light tranilluminators are available from Carolina. Alternatively, a fluorescent light box for viewing slides and negatives provides ideal illumination for blue-stained gels. An overhead projector may also be used. Cover the surface of the light box or projector with plastic wrap to keep liquid off the apparatus. An instant or digital camera can be used to photograph the gels

# Appendix B: Solicitation Email to Potential Field Trial Teachers

Hello,

I am a graduate student at Emporia State University, working on my master's degree in biology. I taught biology and AP biology for a number of years before taking sabbatical. As part of my thesis research, my advisor and I developed a new lab that would be appropriate for use in AP Biology classes. We are looking for AP teachers to test this lab and give us feedback. This lab has been formatted as a kit that utilizes *Halobacterium* sp. NRC-1 which is an Archaean and extreme halophile, making it ideal to use in high schools.

We call the kit, "**The Genotype-Phenotype Connection: Basic Molecular Genetics and Bioinformatics Skills."** It covers the following concepts as a hands-on lab that takes the students through the process from organism to gel electrophoresis image analysis:

- Archaeon lifestyles
- DNA structure (review)
- Gas vesicle organelles (structure and function)
- Colony morphology
- Phenotype/Genotype connection
- Bioinformatics
- Transposons (Insertion Elements)
- Gel electrophoresis
- Polymerase Chain Reaction (PCR)/primers

The development of the kit was the first part of my research. Part 2 involves AP Biology teachers conducting a field trial of the kit.

We are in need of AP Bio teachers to try this kit out with their students **Fall 2012** (with all components completed by **October 10, 2012**). I realize that with the changing AP Framework, this does not come at a very convenient time, however if you are willing (*and chosen to participate after filling out the eligibility survey – see attached*), this kit will be provided free of charge to you for use this fall with your students. I would also provide my email and cell phone to you if you require any "technical support" while preparing and using the kit.

I am excited to have teachers try this kit because it takes students from the entire organism (a colony on a petri dish) through the process of DNA extraction, all the way to PCR and gel electrophoresis. Students will be able to link the gel image (genotype) to the phenotype of the colony they selected. I found that while teaching AP Biology, so many kits were compartmentalized into their specific topic and the idea of the "whole organism" was lost.

If you are interested, please check out the attachment for more details as well as a link to the online eligibility survey. It would be great to have you participate!

Thank you!
Kelley Tuel
MS Biology Degree Candidate
Emporia State University

attachment: field trial details and timeline

# Field Trial Details and Timeline

If chosen to participate based on your online eligibility survey (link below), you would receive the kit materials and agree to follow the kit directions to facilitate it with your students, completed by October 10, 2012.

Time line:

**Spring 2012**

April – Teacher eligibility online survey completed (10 minutes)
May – Teachers notified if they were selected to be in test group. Project information sheet given to teachers to share with their administration/district.

**Summer 2012**

August – Participating teachers contacted to set up kit shipping date and confirm study participation. "Informed consent document" emailed to teachers for student and teacher to fill out.

**Fall 2012** (At your own pace, completing prior to Oct. 10)

Before lab:

1. Teachers complete class logistical survey prior to kit administration (<10 minutes).
2. Confirm that all students have completed the "informed consent document" prior to participation and that your administration/district knows of your participation.

During lab:

Teachers facilitate kit pre-labs, homework and labs (based on ~50 minute class periods, can be combined for block periods):

Day 1:

Pre-test of concepts by taking multiple choice pre-test (10 minutes). Begin "background reading" found within the kit and discussion (it's a great review!) and assign any reading not completed as student homework.

Day 2:

Complete "pre-lab 1" (as class or in computer lab) with discussion of bioinformatics and time for students to explore website attributes.

Day 3:

Determination of Colony Phenotype and Cell Lysis/DNA Extraction. Student homework: complete "pre-lab 2" (PCR tutorial online, 10 minutes or less).

Day 4:

PCR activity (teacher aliquots material prior to class) (~25 minutes, plus ~55 minutes thermal cycler running time). Optional homework: depending on student experience, you may assign "pre-lab 3" (how to load gels).

Day 5:

Gel Electrophoresis activity (teacher dilutes buffer and pours gel prior to class). *Time for additional class work while gel running.*

Day 6:

Data Comparison and Analysis (~25 minutes).

After lab completed:

1. Teachers complete kit evaluation and write additional comments/suggestions (~15 minutes)
2. Students complete multiple choice "post-test" of concepts with additional comments (15 minutes)
3. Teacher mails back class logistical survey, teacher evaluation and student "pre/post tests" in postage pre-paid envelope.

Depending on the number of teachers that respond, not everyone will be able to participate in the study. However, if you agree to the timeline and are interested in being in the pool of teachers participating in this study (selection of teacher participants will be made depending on amount of returned surveys and responses to get an appropriate test group), **please click on the following link to fill out an anonymous eligibility survey**. Your email will be the only personal information required at this time so that I can contact you to inform you if you are selected or not.

http://www.zoomerang.com/Survey/WEB22FECYD9U9S

Please complete the survey by May 5 (Cinco de Mayo!) so all surveys can be evaluated and I inform participants before the end of the school year.

I am very excited about this research and would be thrilled to have you participate. Thank you in advance! If you have any further questions about what your participation would entail, please feel free to contact me.

Thank you!
Kelley Tuel
MS Biology Degree Candidate
Emporia State University
kelley@tuel.com

# Appendix C: Online Screening Survey for Potential Field Trial Teachers

***Note that formatting was different when this document appeared online.***

By filling out this eligibility survey, you agree that (if selected) you will follow the instructions in the kit for preparation, instruction and administration of pre- and post-evaluations as detailed in the email.

1. Are you willing and able to have your AP Biology class(es) do a field trial of the kit (covering concepts of molecular biology as previously described) sometime between August – October 10, 2012?

- ○ Yes [Skip to 3]
- ○ No [Skip to 3]
- ○ I am unsure at this time [Skip to 2]

My participation would depend upon

2. The Advanced Placement class you teach is best described as

- ○ AP biology
- ○ AP bio/College Now
- ○ AP bio/IBC
- ○ College preparatory/taking the AP exam is optional
- ○ Other, please specify

3. Approximately how many AP Biology students do you anticipate teaching in fall 2012?

4. Including the current school year, how many years have you been teaching AP Biology?

5. Including the current school year, how many years have you been a teacher?

6. What is your highest degree?

- ○ B.S./B.A.
- ○ M.S./M.A.
- ○ Ed.S.
- ○ Ph.D./Ed.D.

7. What textbook does your AP Biology class use?

- Title _____
- Description (example: green/7th edition) _____
- Author _____
- Publisher _____

8. As it reads on your teaching certificate, what are you certified to teach? (check all that apply)

- ☐ Agriculture
- ☐ Biology
- ☐ Chemistry
- ☐ Earth and Space Science
- ☐ General Science
- ☐ Enter an answer
- ☐ Other, please specify

9. How many students attend your school?

10. Your school is best described as

- ○ Public high school
- ○ Private, secular (non-religious)
- ○ Private, religion affiliated
- ○ Enter an answer
- ○ Other, please specify

11. Does your school have access to a thermocycler (“PCR Machine”) for your use?

- ○ Yes
- ○ No (answering no does not automatically exclude you from the trial. We may have one you could borrow)

12. Your training in molecular biology laboratory techniques has most effectively come from

- ❍ Undergraduate coursework
- ❍ Graduate coursework
- ❍ Local/district workshops/training
- ❍ State workshops/training (KATS, KABT, etc.)
- ❍ Laboratory experience in industry
- ❍ AP Biology Training workshops
- ❍ Specialty clinics for molecular biology labs
- ❍ Other (please specify):

  _____

The following questions refer to your AP Biology classroom only:

13. How do you cover the topic of Bioinformatics in your AP Biology classroom (BLAST, sequence alignments done on the computer, using NCBI for primer design, etc.)? Check all that apply.

- ❑ I do not cover
- ❑ Computer analysis with wet lab
- ❑ As a simulated lab or on computer
- ❑ In lecture/conceptually

14. How do you cover the topic of Transposons in your AP Biology classroom (Insertion Sequences, Insertion Elements, Transposable Genetic Elements, etc.)? Check all that apply.

- ❑ I do not cover
- ❑ As a wet lab
- ❑ As a simulated lab
- ❑ In lecture/conceptually

15. How do you cover the topic of PCR (Polymerase Chain Reaction) in your AP Biology classroom? Check all that apply.

- ❑ I do not cover
- ❑ As a wet lab
- ❑ As a simulated lab
- ❑ In lecture/conceptually

16. How do you cover the topic of Gel Electrophoresis in your AP Biology classroom? Check all that apply.

- ❑ I do not cover
- ❑ As a wet lab
- ❑ As a simulated lab
- ❑ In lecture/conceptually

17. How do you cover the topic of Microbiology Techniques (aseptic technique, plating, etc.) in your AP Biology classroom? Check all that apply.

- ❑ I do not cover
- ❑ As a wet lab
- ❑ As a simulated lab
- ❑ In lecture/conceptually

18. How do you cover the topic of Archaeon lifestyles (Archaea domain) in your AP Biology classroom? Check all that apply.

- ❑ I do not cover
- ❑ As a wet lab
- ❑ As a simulated lab
- ❑ In lecture/conceptually

19. Rank your students' difficulty grasping the following concepts:

| | Great difficulty | Moderate difficulty | Some difficulty | Slight difficulty | No difficulty | N / A |
|---|---|---|---|---|---|---|
| Transposons (Insertion sequences/IS elements/Transposable genetic elements, etc.) | ❍ | ❍ | ❍ | ❍ | ❍ | ❍ |
| PCR/primers (annealing, extension 5' to 3', etc.) | ❍ | ❍ | ❍ | ❍ | ❍ | ❍ |
| Bioinformatics (sequence alignment using NCBI, BLAST, etc.) | ❍ | ❍ | ❍ | ❍ | ❍ | ❍ |
| Archaea and domains of life | ❍ | ❍ | ❍ | ❍ | ❍ | ❍ |

20. Rate your comfort level teaching these biology laboratory skills:

| | Very uncomfortable | Slightly uncomfortable | Neutral | Somewhat comfortable | Very comfortable |
|---|---|---|---|---|---|
| Colony morphology | ❍ | ❍ | ❍ | ❍ | ❍ |
| PCR | ❍ | ❍ | ❍ | ❍ | ❍ |
| Gel electrophoresis | ❍ | ❍ | ❍ | ❍ | ❍ |
| DNA extraction | ❍ | ❍ | ❍ | ❍ | ❍ |
| Microbiology technique | ❍ | ❍ | ❍ | ❍ | ❍ |
| Insertion sequences/IS elements | ❍ | ❍ | ❍ | ❍ | ❍ |
| Enter an answer | ❍ | ❍ | ❍ | ❍ | ❍ |

21. Rate your satisfaction with labs you have previously used that cover these techniques:

| | Very uncomfortable | Slightly uncomfortable | Neutral | Somewhat comfortable | Very comfortable |
|---|---|---|---|---|---|
| Colony morphology | ❍ | ❍ | ❍ | ❍ | ❍ |
| PCR/primers (annealing, extension 5' to 3', etc.) | ❍ | ❍ | ❍ | ❍ | ❍ |
| Gel electrophoresis | ❍ | ❍ | ❍ | ❍ | ❍ |
| DNA extraction | ❍ | ❍ | ❍ | ❍ | ❍ |
| Microbiology technique | ❍ | ❍ | ❍ | ❍ | ❍ |
| Insertion sequences/IS elements | ❍ | ❍ | ❍ | ❍ | ❍ |

22. What email address is best to contact you for correspondence pertaining to this kit?

Note: I will only use this email for contact concerning the field trial of the kit. I respect your need for confidentiality. As the researcher, I will know which teacher/school the information is coming from (so that I can correlate all the data to that teacher/school and so I can ship you the kit). However, all analyses will be written confidentially, referring to teacher/school by number. I am the only one that will know which teacher/school participated and your responses.

Email address:

Thank you for your participation!

I will contact you mid-May (at the email address you provided) to inform you if you are selected to be in the field trial participation group.

Kelley Tuel
kel@tuel.us

# Appendix D: Sample of Individualized Suggested Field Trial "Lesson Plan"

***This school has 51 minute class periods 4 days a week and a double/block period 1 day a week.***

**Teacher instructions:**

Copy PRIOR TO day 1 (for each student):

- Student background information (in student section of manual)
- Student section of manual: protocols, etc.. This includes pre-labs, activities & student questions.
  **Items in grey can be completed as homework assignment OR in class.**

**Suggested Lesson Plans:**

Day 1:

1. Pre-test of concepts by (10-12 minutes).
   Make sure you pre-test BEFORE they read the "background" from the student section in the teacher's manual.
2. Homework: "Background"
   assign reading student homework with discussion next time.

Day 2:

1. Have "Background" discussion. ~10-15 minutes. I like to use pink balloons to explain the gas vesicle concepts. I'll bring some for you when I bring the Thermal Cycler.
2. "Pre-lab 1" ~30 minutes
   (as class on big computer screen or on student laptops) with discussion of bioinformatics. I would only do steps 1-12 of Pre-lab 1 in class. However, if they are really into it and got that far quickly, feel free to give them time to continue all of the pre-lab.
3. Homework: if you didn't get all the way through the pre-lab, assign it as homework (since your kids are brighter than most, I'd love their feedback if they understood how to do steps 13-15. They'll need to repeat a little bit of the previous steps to get the sequence on their home computers, but I think your kids will be able to do this just fine.

Day 3 (double class period):

1. "Activity 1: Determination of Colony Phenotype and Cell Lysis/DNA Extraction".
   Allow ~35 minutes for this. On Activity 1, procedure 1.5, notice the red colonies are translucent, the pink colonies are opaque.
   Keep the extract (which will be your DNA template for PCR) on ice until needed for "Activity 2".
2. "Activity 2: PCR" (~20 minutes for students to put reactants into PCR tubes). Have students load their samples in the thermal cycler AND you start it right after they have loaded their samples.
   Thermal cycler will run for 53 minutes-65 minutes (depends on heating up between cycle time).
   When program is complete, place samples into the fridge until tomorrow.
3. Students prep the buffer & gels for tomorrow: Your kids are so good, you could have them dilute the buffer and make/pour the gels! If you want to, you could do it instead of them Wednesday prior to class.
4. Student homework (or do in class if you don't want them to do the buffer/gel prep): complete "pre-lab 2" (PCR tutorial online, 10 minutes or less). That way, they know what is going in the thermal cycler while they are gone!
5. Optional homework, depending on student experience: complete "pre-lab 3" (how to load gels, 5-10 minutes).

Day 4:

1. "Activity 3: Gel Electrophoresis". Hopefully, you have some gel chambers that are more efficient than when I was there – they used to take overnight!! UGH!
2. Time for additional class work (moving on to another chapter, working on kit student analysis questions, etc.) while gel running.
3. If they haven't seen a gel run before, it's kind of neat to take a peak every 10 min or so to see the progress.
4. If gel only takes an hour (newer gel chambers than when I was there), put gels into gel trays and cover with buffer. Slide gel tray (with buffer & gel) into Ziploc and seal. Store in fridge overnight.

Day 5:

1. Gel staining and destaining.
2. If you have a digital camera, take a picture! – if you can, please send me the digital image!!!
3. "Activity 4: Data Comparison and Analysis". Depending on how long the staining takes, you may or may not get to the first questions of this (graphing).
4. Homework idea?? If you get the gel stained & photographed, it would be nice to email them a digital copy of the gel (or print out a hard copy for each student), so they could go home and attempt to graph, determining the approximate bp of their fragment. You can choose to have them do this on Excel or with pencil on semi-log paper. It would be a good way to see how good they are at graphing on their own!

Day 6:

1. Complete "Activity 4: Data Comparison and Analysis".
2. Discuss results and wrap-up.
3. EITHER today OR Monday: "Post-test" for students, last 15-18 minutes of class (12 minutes timed for post-test, additional 3+ minutes for their open-ended comments).

# Appendix E: Student Field Trial PRE and POST-Tests

**Directions: Please place the letter of the correct answer on the line provide. Write all letters in CAPITAL letters. You will have 12 minutes to complete the "test". Because this field trial is interested in which answer you choose, do not guess. If you do not know an answer, select the option "I do not know" (if that is a choice).**

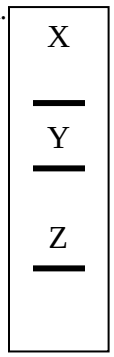
1. Match the following components of PCR with their function. Write your answer in capital letters on the lines provided.

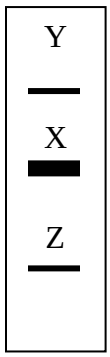
| Component: | Function: |
|---|---|
| ____DNA | A. provides appropriate reaction conditions |
| ____DNA polymerase | B. define the 3' and 5' end of the replicon |
| ____primers | C. enzyme that carries out extension of DNA |
| ____nucleotide | D. the "raw material" added to create a new DNA molecule |
| ____buffer | E. template for PCR |

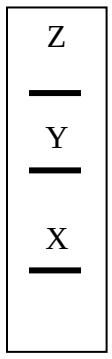
2. ____ A Polymerase Chain Reaction goes through several cycles of temperature changes. What occurs during the annealing stage?
   A. DNA strands are heated and separate from one another.
   B. the primers attach to the targeted DNA.
   C. the targeted sequence of the DNA in your sample is replicated.
   D. All of the above are correct.
   E. None of the above are correct.
   F. I do not know.

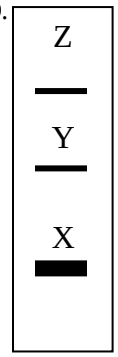
3. ____ Which of the following gel electrophoresis images would correctly show the band labeled "X" as the larger DNA fragment?

*Negative electrode is positioned on top of each image.*

A. 


B. 


C. 


D. 


E. I do not know

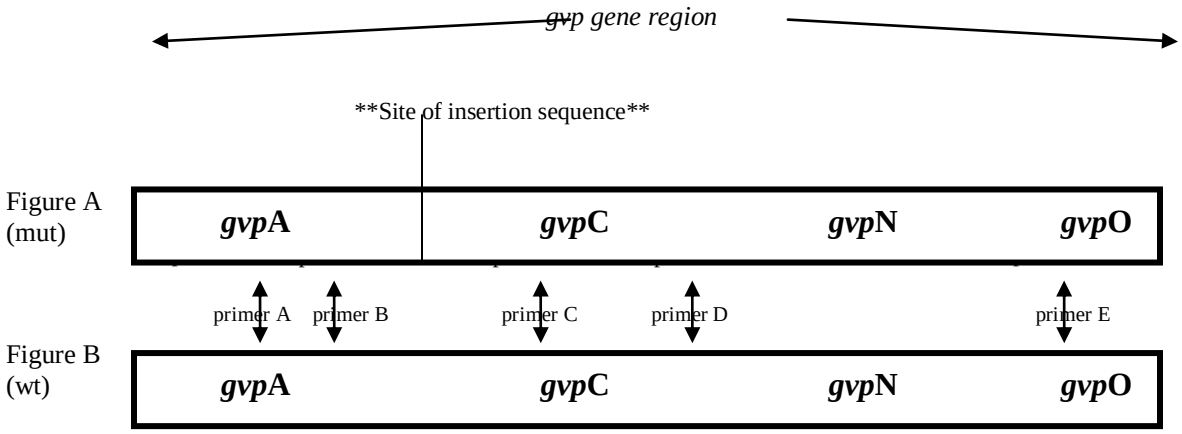
*Positive electrode is positioned at the bottom of each image.*

4. ____ A cell is placed into a solution. The solution contains less salt than the salt found in the cytoplasm of the cell. Which of the following will occur?

A. The solution will absorb the salt ("wick" the salt out) from the cell's cytoplasm.
B. The cell will absorb water from the solution causing it to swell and potentially lyse.
C. The solution will absorb water from the cell causing it to shrink and potentially be hypertonic.
D. None of the above are correct.
E. I do not know.

**Use the information below to answer questions 5-6.**

A *Halobacterium* mutant (mut) contains an insertion sequence within the gas vesicle protein (*gvp*) gene region (figure A). The *Halobacterium* wild type (wt) does not contain the insertion sequence within the *gvp* gene region (figure B). Refer to the figures below when answering questions 5-6.




5. ____ Design a PCR reaction:
Which primer combination would result in different sizes of amplified fragments from the mut & wt DNA?
A. primers A and B.
B. primers B and C.
C. primers C and D.
D. primers D and E.
E. I do not know.

6. ____ After performing PCR on a wild type and a suspected mutant, a gel is run using gel electrophoresis. The gel reveals that the mutant (mut) lane has a band that migrates the identical distance as the wild type (wt) band.

What is the best conclusion?
A. The mutant and wild type organisms have identical genes, so the mutant is not really a mutant at all.
B. The primers attached to the mutant DNA but not the wild type DNA, making the mutant band "catch up" to the wild type band.
C. Primers C and D may have been used.
D. Only primer B may have been used.
E. I do not know.

7. ____ Neurofibromatosis (NF) is a human autosomal dominant disorder. Patients with NF develop numerous small tumors on the skin and within the body caused by a defect in neurofibromin. The neurofibromin gene of an affected patient is approximately 6000 base pairs longer than the normal neurofibromin gene. Additionally, the mutant gene appears to be out of reading frame because the amino acid sequence only matches the normal protein for a short stretch at the beginning of the sequence. Which conclusion explains the cause of the longer gene of NF affected patients?

A. Additional telomeres
B. A polygenic trait
C. A defect in polymerase during transcription
D. An insertion sequence
E. I do not know.

8. ____ Which of the following statements is unique to the domain Archaea?

A. Often lives in extreme environments. Single-cellular organisms.
B. Lives in aqueous environments. Multi-cellular or single celled organisms.
C. Contains cell membranes and nuclear envelope. Multi-cellular or single celled organisms.
D. Can contain cell walls or cell membranes but not both. Does not have a true nucleus.
E. I do not know.

9. **Short Answer.**

a) Briefly explain one cause of a gene disruption.

_____

b) Explain the effects of that gene disruption.

_____

_____

10. **Short Answer.**

What might be an evolutionary advantage of a genome that contains transposable elements?

_____

_____

_____

# POST-Test Additional Questions

Please give me specific feedback regarding "The Genotype – Phenotype Connection: Molecular Genetics and Basic Bioinformatics Skills" kit.

I would love to have your thoughts about the various lab activities, the pre-labs, the background information and the Student Analysis and Follow-up Questions (good or bad):

- _____
- _____
- _____
- _____

*Thank you for your participation!*

# Appendix F: Teacher Post-kit Evaluation

Please complete the following questions at the conclusion of all lab components with your students. *Please remember that all of your responses will be reported anonymously.* Any comments you make will help me interpret your experience.

1. Rate your satisfaction with this kit as a teaching tool for ***each of*** the following concepts ***as they were presented in the kit***:

5=strongly satisfied 4=mostly satisfied 3=neither satisfied nor dissatisfied 2=slightly dissatisfied 1= strongly dissatisfied

| | | | | | |
|---|---|---|---|---|---|
| Colony morphology | 5 | 4 | 3 | 2 | 1 |
| PCR | 5 | 4 | 3 | 2 | 1 |
| Gel electrophoresis | 5 | 4 | 3 | 2 | 1 |
| DNA extraction | 5 | 4 | 3 | 2 | 1 |
| Sterile technique | 5 | 4 | 3 | 2 | 1 |
| Insertion sequences/IS elements | 5 | 4 | 3 | 2 | 1 |

Comments:

2. During this field trial, you were given a "timeline" of what kit sections to cover. Please indicate the level you were able to execute each section.

5=covered it as kit directions indicated 3=skimmed or touched on as a group 1=did not cover

| | | | |
|---|---|---|---|
| Background reading | 5 | 3 | 1 |
| Pre-lab 1: Bioinformatics | 5 | 3 | 1 |
| Activity 1: Determination of colony phenotype | 5 | 3 | 1 |
| Activity 1: Cell lysis/DNA extraction | 5 | 3 | 1 |
| Pre-lab 2: PCR tutorial | 5 | 3 | 1 |
| Activity 2: PCR | 5 | 3 | 1 |
| Pre-lab 3: how to load gels | 5 | 3 | 1 |
| Activity 3: Gel electrophoresis | 5 | 3 | 1 |
| Activity 4: Data comparison & analysis | 5 | 3 | 1 |

Comments:

3. The topics covered in this kit helped you meet your course objectives.

___Strongly agree
___Agree
___Disagree
___Strongly disagree
___No opinion

Comments:

4. The kit information and activities were presented to the students at an appropriate level.

___Strongly agree
___Agree
___Disagree
___Strongly disagree
___No opinion

Comments:

5. When you received the kit materials, they were packaged so that it was clear how to store supplies until use.

___Strongly agree
___Agree
___Disagree
___Strongly disagree
___No opinion

Comments:

6. The information provided in the *teacher guide* was clearly stated, containing all necessary information.

___Strongly agree
___Agree
___Disagree
___Strongly disagree
___No opinion

Comments:
*Would you make any changes to the teacher guide information? If so, what changes would you make?*

7. After reading the teacher instructions (teacher guide), how comfortable were you to prepare the lab materials (pour gels, aliquot primer mix, stain the gels, etc.) for your students?

___Very comfortable
___Somewhat comfortable
___Neutral
___Slightly uncomfortable
___Very uncomfortable
___None of the above. I did not read the teacher guide.

Comments:

8. The time requirement for the various lab sections (as listed in the teacher guide) was reasonably accurate.

___Strongly agree
___Agree
___Disagree
___Strongly disagree
___No opinion

Comments:

9. The instructions/protocols provided in the *student guide* was clearly stated, containing all necessary information.

___Strongly agree
___Agree
___Disagree
___Strongly disagree
___No opinion

Comments:

10. Rate your students' difficulty grasping these techniques ***<u>as presented in the kit</u>*:**

| 5=great difficulty | 4=moderate difficulty | 3=some difficulty | | 2=slight difficulty | 1= no difficulty |
|---|---|---|---|---|---|
| Colony morphology | 5 | 4 | 3 | 2 | 1 |
| PCR | 5 | 4 | 3 | 2 | 1 |
| Gel electrophoresis | 5 | 4 | 3 | 2 | 1 |
| DNA extraction | 5 | 4 | 3 | 2 | 1 |
| Sterile technique | 5 | 4 | 3 | 2 | 1 |
| Insertion sequences/IS elements | 5 | 4 | 3 | 2 | 1 |

Comments:

11. How did you use the *student analysis questions* (check all that apply)?

___Students completed individually, assigned as homework or self-check.

___Assigned for small groups/partners to discuss and work on.

___Not assigned formally, but used for class discussion.

___Used to occupy students while they were waiting to load gel, etc.

___As test questions following the lab.

___I assigned all of them EXCEPT the Challenge Questions.

___I did not use any of them.

___I omitted a few of the regular level (non-challenge) questions. *Please state which were omitted and your reasons:*

Comments:

12. The descriptive information in the kit (background and within each activity) was sufficient for students to answer the *student analysis questions*.

___Strongly agree
___Agree
___Disagree
___Strongly disagree
___No opinion

Comments:

13. Please briefly answer to following questions to help me define your experience with the "Genotype-Phenotype Connection" kit:

a) What portion(s) of the lab went well?

b) Did anything go wrong? Explain.

c) Where there any substitutions or alterations you had to make? If so, why?

d) What would you change about this lab?

e) Describe any additional information or section(s) that you think are needed to aid in:
- classroom management
- materials management
- facilitating of the lab activities
- other

*Thank you for your participation in this field study!*

# Appendix G: Informed Consent Document

For field trial of "Genotype-Phenotype Connection" teaching kit

Your student's AP biology classroom has been chosen to participate in a field trial of a newly developed teaching kit to enhance their laboratory experience. Not only will your student experience cutting edge laboratory techniques in their AP classroom, but they will also be participating in real science as subjects in a research project for a thesis at Emporia State University.

***Purpose of study***

To have AP biology classrooms experience and evaluate the "Genotype-Phenotype Connection" molecular biology laboratory kit.

***Description of participation***

All of the laboratory activities within the kit (colony exploration, DNA extraction, Polymerase Chain Reaction, gel electrophoresis and analysis) will be facilitated by your student's AP Biology teacher as per the instructions within the kit. This will be done as part of their classroom experience and, other than a possible homework assignment, will not extend beyond classroom time.

The students will use the safe microbe *Halobacterium* sp. NRC-1 which is similar to bacteria, but technically a halophilic Achaean (it is only viable when grown with extremely salty conditions). They will view colony differences of the wild type and mutant varieties on a Petri dish. Students will then extract DNA from their chosen colony and perform PCR and gel electrophoresis. The resulting gel image will be compared to the data collected by classmates to determine the genetics involved in their colony. Bioinformatics (computer gene analysis of *Halobacterium*) will also be utilized.

Your student will take a "pre-test" evaluating their knowledge of biology laboratory skills and concepts prior to experiencing the kit. Later, your student will fill out a "post-test" evaluating their knowledge upon completion of the kit with their class, along with the opportunity to provide feedback of their experience.

***Participation risk***

*Halobacterium* sp. NRC-1 is deemed safe to use with human subjects. The American Type Culture Collection (ATCC) classifies NRC-1 as a BioSafety Level 1(BSL-1) organism. All biological components are sent to us by Carolina Biological Supply Company.

***Benefit of study***

Students will use a microbe, *Halobacterium* sp. NRC-1 in their classroom. By following procedures within the kit, they will make the connection between the physical appearances of *Halobacterium* sp. NRC-1 (phenotype) to the gel electrophoresis image analysis (genotype). Most other kits at this level do not provide the students the opportunity to experience the intact organism, allowing students to do all the procedures, finishing with gel electrophoresis.

***Confidentiality***

The names and schools of student participants will be kept confidential when reporting results of this field trial in a thesis at Emporia State University and to Carolina Biological Supply.

Questions regarding this project may be directed to your student's AP Biology teacher.

----------*Please detach and return the portion below to class*----------

*"I have read the above statement and have been fully advised of the procedures to be used in this project. I have been given sufficient opportunity to ask any questions I had concerning the procedures and possible risks involved. I understand the potential risks involved and I assume them voluntarily."*

_____ _____

Student Date

_____ _____

Parent or Guardian Date

# Appendix H: Carolina Biological Copyright Permissions

CAROLINA BIOLOGICAL SUPPLY COMPANY
2700 YORK ROAD

BURLINGTON, NC 27215

January 11, 2013

Kelley Tuel
15669 S. Rene St.
Olathe, KS 66062

Dear Ms. Tuel,

Conditional Copyright Permission
Carolina Biological Supply Company has received your request to reprint information found in several of our products from Carolina Biological Supply Company in a number of publications, specifically as a part of your thesis and also in an article being submitted to The American Biology Teacher.

It is our understanding that you will be submitting manuscripts for an article which will explain uses for Halobacterium sp. NRC-1 to The American Biology Teacher, published by the NABT. You would like to include the following:

> BSYC media protocol, PCR temperature and settings, modified large scale DNA extraction protocol from Carolina's "Extremely Easy DNA Extraction", and small scale DNA extraction protocol from "Genotype-Phenotype" kit.

You will also include the above information in your thesis, as well as a reprint of the "Genotype-Phenotype" kit manual as an appendix in the thesis. You would like permission to reprint all or portions of the above material.

Lastly, you would also like to include a portion of the material as a reprint for a Science Pioneers presentation that you are likely to do in February.

As you are asking to reproduce this content in printed form in a not-for-profit publication, in support of these efforts to help teachers and students, Carolina by this letter grants a royalty-free permission on the following conditions:

The information may be used only in the above indicated used. If you with to use this information in any other form or in any other publication, then you will request Carolina's permission for those uses at a later date.

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Please acknowledge your acceptance of this permission and agreement to the above by returning an e-mail with this e-mail attached stating your agreement to the terms.

Yours very truly,

CAROLINA BIOLOGICAL SUPPLY COMPANY

Roger E. Phillips, Jr.

Director of Anatomical Operations & Senior Scientist

# Appendix I: HaloWeb Copyright Permissions

Hi Ms Tuel,

Nonprofit usage of HaloWeb is fine, as long as the source is attributed, i.e.:
Graphic generated using HaloWeb <http://halo4.umbi.umd.edu>

Full Citation:
DasSarma SL, Capes MD, DasSarma P, DasSarma S. HaloWeb: the haloarchaeal genomes database. Saline Systems. 2010;6:12.

Regards,

Satyajit DasSarma
HaloWebMaster

On 7 March 2013 17:05, Kelley Tuel wrote:

Hello, Satyajit,

I have been working with Priya DasSarma on the *Genotype-Phenotype Connection* lab manual that we published, along with Dr. Shil DasSarma and Dr. Tim Burnett. We will be submitting an article to the journal, The American Biology Teacher next month. We would like to use a graphic from HaloWeb in the article that we will submit next month. The graphic shows the gene map of the *gvpA1* region. May we have your permission to use it for publication in ABT (a non-profit publication)?

In addition, I have written my Master's thesis about *Halobacterium* sp. NRC-1 and would like to use the same graphic in my thesis. The thesis is through Emporia State University in Emporia, KS. May I have your permission to use the same gene map graphic for publication in my thesis as well?

I have enjoyed using the HaloWeb - very user friendly!

Thank you,
Kelley Tuel

I, Kelley Tuel, hereby submit this thesis to Emporia State University as partial fulfillment of the requirements for an advanced degree. I agree that the Library of the University may make it available to use in accordance with its regulations governing materials of this type. I further agree that quoting, photocopying, digitizing or other reproduction of this document is allowed for private study, scholarship (including teaching) and research purposes of a nonprofit nature. No copying which involves potential financial gain will be allowed without written permission of the author. I also agree to permit the Graduate School at Emporia State University to digitize and place this thesis in the ESU institutional repository.

_____

Signature of Author

_____

Date

_____

Title of Thesis

_____

Signature of Graduate School Staff

_____

Date Received