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Water in your neighbourhood: a model for implementing a semester-long course-based undergraduate research project in introductory biology

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ABSTRACT
Undergraduate biology education has changed over the past decade, incorporating an iterative and evidence-based approach. Many educational assessments have confirmed the effectiveness of integrating authentic research and open-ended inquiry into introductory biology courses, demonstrating a significant positive impact on students’ learning and attitude towards STEM majors. Despite these findings, only a handful of Biology instructors in 2-year colleges adopt this approach, and when adopted, most activities constitute a small fraction of these courses. Finding a feasible, sustainable, semester-long, and cost-effective strategy to incorporate authentic research in the curriculum which promotes integrated understanding of science and addresses socio-scientific issues, is a big challenge for both instructors and administrators at 2-year colleges. Here we present a unique model incorporating a semester-long authentic research-based set of laboratories in introductory Biology through which students investigate the water quality in areas close to their communities. Our approach is fully aligned with all five core components of an effective Course-based Undergraduate Research Experience according to CUREnet. The laboratories were developed to be fully incorporated into the teaching curriculum. Each laboratory was designed to ensure students acquire the knowledge and skills set out in the course syllabus through a process of discovery designed to promote students’ engagement.

KEYWORDS
Research in classroom; undergraduate biology; inquiry-based laboratories; science and society; semester-long project

Introduction
General Biology courses are usually taken by students in the first semester of their college education, so what they learn, and experience, can have an important impact on their future performance in upper-level courses, as well as on their perceptions of the significance of science in their lives. Data from our community college shows many students do not complete the first-semester Biology course, or if they do, a large number decide to change to a non-science major (Office of Institutional Research & Assessment Division of Information Technology, 2017). A wide variety of factors cause...
attrition, including financial problems, a lack of academic preparedness for college due to poor K-12 education (National Center for Educational Statistics, 2015), unclear ideas about what courses to take, and once in the course, a lack of interest in a subject that seems unrelated to students’ lives and realities (for a complete review see: US Department of Education, 2013). The feeling of alienation is particularly significant for colleges where student populations are largely from groups that are underrepresented in science. A study by Jackson, Galvez, Landa, Buonora, and Thoman (2016), found that understanding how science is connected to social problems and goals is more important in retaining underrepresented students in science fields than for students who are well represented and, for underrepresented students, highlighting the connection between the course material and its applicability to helping the community should begin as early as the freshman year.

For more than a decade, educators around the world have actively practiced different approaches intended to increase students’ interests and motivation to study science subjects, especially Biology. At the 2009 AAAS conference, Bruce Alberts (AAAS, 2009) noted that science students should acquire the knowledge and understanding necessary to interpret the natural world scientifically, comprehend the process leading to scientific knowledge, and participate in scientific practices. Several studies show that implementing active learning strategies in science courses improves students’ performance and conceptual understanding (Brame, 2016; Freeman et al., 2014; Mumtaz & Latif, 2017). When compared to labs where results of experiments are known ahead of time, incorporating research into the laboratory curriculum constitutes an active-learning pedagogy. Although documentation reporting the impact of CURE experiences in two-year community colleges is scant, especially when compared with data reporting the positive effects of Undergraduate Research Internships on student interest and retention in STEM (Auchincloss et al., 2014), preliminary assessments indicate that undergraduate students participating in CUREs report greater self-confidence in performing research tasks, interest in future biological research and more positive attitudes towards science (Brownell, Kloster, Fukami, & Shavelson, 2012). More recently, Jordan et al. (2014), assessed the effects of implementing a Research Course in Phage Discovery and Genomics for First-Year Undergraduate Students, in over 73 institutions and a total of 4,800 students. They concluded that this experience stimulates students’ interest in science, positively influences academic achievement, and enhances persistence in science, technology, engineering, and mathematics (STEM) disciplines (Jordan et al., 2014). More generally, undergraduate research promotes the development of skills such as the ability to work in teams, solve problems and communicate effectively, skills which are necessary in any job (Hart Research Associates, 2015).

In his review and analysis of innovative teaching practices meant to increase student learning, Wood (2009), uses Froyd’s rating system to evaluate the effectiveness and feasibility of these practices based on their (a) practicality of implementation, and (b) evidence for efficacy (Froyd, 2008). Although many studies show that providing undergraduate students opportunities to participate in scientific research is an effective practice in teaching STEM courses (point b), the practicality of introducing research into the curriculum to reach a broader number of students can be a much more difficult task (point a). In fact, fewer than 20% of courses in 2-year colleges adopt this approach, and most authentic research activities implemented in introductory science courses
constitute a small fraction of the course, usually due to limited availability of time and resources (Spell, Guinan, Miller, & Beck, 2014). Presented here is a complete body of work, designed to carry out a semester-long research project which is fully integrated into the laboratory learning objectives and activities.

The first-semester majors Biology course at LaGuardia Community College consists of lectures and laboratories introducing the molecular and cellular basis of life, including processes of DNA replication, transcription and translation, as well as cell metabolism, followed by cell division, genetics, and evolution. Traditionally, each one of these topics is illustrated in the laboratories, using hands-on exercises with predetermined expected outcomes, and little continuity between labs. In contrast, our approach is rooted in active learning pedagogies, encouraging the development of critical thinking, research, and communication skills by implementing a semester-long research project on the water quality of the Long Island Sound. These laboratories are relevant to most of the topics in an introductory Biology course and utilise the context of a major problem in the local community.

Each lab involves investigating different characteristics of a water sample, enabling students to generate research questions, understand how to define research methods, and gather and discover new information, while maintaining a sequential thread connecting their discoveries to have a full body of work by the end of the semester. This innovative approach to incorporating research into the curriculum means students learn by experiencing how discovery is built from previous knowledge, is ongoing, and can be considered from different angles. As students build their own knowledge, their inquiry, data collection and analysis skills are reinforced as they progress throughout the semester. The reporting of results involves developing students reading and writing skills, particularly reading and writing scientific reports.

By choosing the analysis of water quality in waterways close to students’ communities as a topic for the project, students can directly relate what they discover and learn about the water to what is occurring in their communities regarding policies around water conservation, waste management, and other aspects of environmental protection policies. As mentioned above, according to the National Center for Educational Statistics (2015), poor quality of K-12 education affects student success and retention. In order to try and mitigate this problem and ensure all students had high-school level background knowledge, including basic vocabulary and mathematical skills required to complete the activities for each lab, we implemented a “flipped class” modality, where students were introduced to these elementary concepts and assigned homework before each lab. Here we present our approach and considerations behind our design of pedagogical materials, aimed to reinforce students’ (1) mastery of the subject matter; (2) integrative understanding of the process of scientific inquiry, including the continuity, complexity, and ambiguity of empirical work; (3) various skills for good observation, data collection and data analysis used in scientific inquiry; (4) ability to analyse and problem solving; (5) use peer-reviewed literature; (6) ability to work effectively in a team; (7) basic written and oral competencies to communicate findings; and, (8) understanding of the role of scientific discovery in civic society. Overall, students that participated in sections where we implemented this semester-long project, had a higher passing rate (100% vs 78.5%) and a higher retention rate (92.5 vs 77.35) compared to students that attended regular sections according to data provided by the
Pedagogical design

General considerations

Providing undergraduate students opportunities to engage in research is considered one of the “High impact” practices shown to result in educational benefits, especially to underserved populations (Kuh, 2008). Introducing “research-based” laboratories has become a popular practice in recent years, yet there is no established formula or method for the implementation of such labs beyond the idea of students engaging, at some level, in the discovery process (Weaver, Russell, & Wink, 2008). The design of new laboratories focused on a research project, with an inquiry-based format, shifts students’ and instructors’ lab practices from a defined, “recipe” like format, to experiences that require dealing with uncertainty, greater decision-making, and collaborative group work (Lopatto, 2010). Importantly, both students and instructors participate in a process of discovery without knowing ahead of time what the results of their experiments will show. When outlining the overall-curricular design strategy, we ensured students had elementary basic knowledge before embarking in more complex problem solving, provided a logical and consistent sequence of information for all new labs, clear instructions outlining materials and methods, and instructions for data collection and analysis, including questions to assess understanding in each laboratory handout. This careful approach helped reduce sources of confusion. Table 1 provides a summary of the various resources and the competencies they are geared to fulfil, which were
Table 1. Resources developed for the implementation of a semester-long research project which is fully integrated into the curriculum for a first-year biology course.

| Type                        | Purpose                                                                 | Competency                                      |
|-----------------------------|-------------------------------------------------------------------------|-------------------------------------------------|
| Laboratory handouts         | - Present expected learning objectives and outcomes                      | - Mastery of subject matter                      |
|                             | - Provide relevant background                                           | - Technical and analytical skills               |
|                             | - Provide guides for laboratory activities                              | - Develop observation and critical thinking skills|
|                             | - Provide guides for data collection and organisation                   | - Problem solving                                |
|                             | - Provide means for ongoing assessment                                  | - Team work                                      |
|                             |                                                                         | - Science and society                            |
|                             |                                                                         | - Mastery of subject matter                      |
|                             |                                                                         | - Independent learning                           |
| Flipped Learning:           |                                                                         |                                                 |
| Pre-lab homework            | - Review fundamental concepts and background before each lab             |                                                 |
|                             | - Establish a “level playing field” for all students                    |                                                 |
|                             | - Familiarise students with the scientific method                       |                                                 |
|                             | - Allow more time for in-lab discussion of new concepts/ideas           |                                                 |
| Assignment guides           | - Provide list of resources with examples of scientific literature     | - Mastery of subject matter                      |
|                             | - Provide background information which helps students generate their own questions | - Independent learning                           |
|                             | - Build presentation of project results by scaffolding writing assignments |                                                 |
|                             | - Provide detailed guides, with requirements and objectives for written assignments |                                                 |
|                             | - Provide rubrics for each assignment                                   |                                                 |
| Oral/digital or poster      | - Provide guidelines outlining required elements found in a digital or a poster when presenting results from a scientific study | - Develop oral and scientific communication skills|
| presentation guide          | - Provide opportunity to present scientific results to peers            | - Team work                                      |
|                             | - Promote group work;                                                   |                                                 |
|                             | - Provide students with self and peer evaluation tools                  |                                                 |

developed for the implementation of this research project (all resources are available in the appendices and links); Table 2 provides a list of modified labs with summaries of activities and expected learning outcomes. In a standard semester of 12 weeks, students meet for labs once a week for 3 h. All work is completed during the labs and each lab is designed to be completed within the 3 h except for lab 8 which is done over two-lab periods; the last week in the semester is dedicated to oral and poster presentations.

Enhance mastery of the subject matter

To ensure students came to the labs with a baseline knowledge of the subject matter, we created presentations with explanations and embedded links for videos illustrating the different-basic concepts, followed by questions which were assigned as homework. These presentations were given a week before each lab, and students were expected to complete and upload the answers before coming to the lab. Having completed the homework, students had the necessary background knowledge to delve into their work at the beginning of each lab. The setting up of experiments and collection of data required an understanding of the concepts and processes beyond simple memorisation; to interpret their results, students needed to apply their knowledge, making learning a part of the problem solving which accompanies scientific inquiry.
| Laboratory | Summary of activities | Expected learning outcomes Students will be able to: |
|------------|-----------------------|-----------------------------------------------------|
| 1. Measurement | Use of metric system to measure parameters relating to water at time of collection (depth, temperature, volume, mass) | • Identify the different units of measurement in the metric system to document different properties of matter.  
• Perform conversions using metric system units.  
• Demonstrate skills in documenting data and keeping records using proper metric units. |
| 2. Microscopy | Use of microscope  
Specimen preparation for observation (cheek cell, onion cell).  
Calibration of ocular micrometres; measurement of vacuoles from epidermal onion cells placed in tap water and their sample water | • Use a microscope to view and measure microscopic samples.  
• Explain the importance of multiple sampling in obtaining significant measurements.  
• Describe differences between animal and plants cells. |
| 3. Organic molecules | Isolation of bacterial DNA from water sample for future metagenomic analysis based on 16s DNA sequencing | • Demonstrate an understanding of the nature and chemical composition of different cell structures when separating the DNA from the rest of the cell components.  
• Describe the different properties of macromolecules and their behaviours in different solutes.  
• Employ new technologies in molecular biology and demonstrate an understanding of their reach in the exploration of living organisms  
• Describe the contribution of different scientists in developing new technologies in Biology. |
| 4. Diffusion and Osmosis | Determination of salinity of water sample, directly and indirectly. Determine changes in mass of dialysis bags filled with different solutions after immersion in distilled water.  
Microscopic measurement of effects of solute concentration on cells. | • Learn how to determine water salinity; relate salinity to tonicity, osmosis, diffusion, membrane permeability and osmoregulation.  
• Observe and measure the effect of placing living cells in hypotonic, hypertonic and isotonic solutions.  
• Generate a standard curve and use it to determine the unknown salt concentrations  
• Use Excel for performing statistical analysis and drawing graphs. |
| 5. Cell Respiration and Fermentation | Use microbiological tests to determine presence of (facultative) anaerobic and aerobic bacteria.  
Measure (by titration) dissolved oxygen in water samples. | • Describe the processes of cell respiration and fermentation, as well as the differences in ATP production of aerobic and anaerobic respiration.  
• Predict possible effects of aerobic bacteria on dissolved oxygen and on eutrophication of waterbodies. |

(Continued)
**Table 2. (Continued).**

| Laboratory                        | Summary of activities                                                                                                                                                                                                 | Expected learning outcomes Students will be able to:                                                                 |
|-----------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|
| 6. Photosynthesis                 | Measure dissolved CO₂ in water samples directly and by extrapolating value from a standard curve generated by measuring the rate of photosynthesis as a function of bicarbonate concentration.                         | • Describe the process of photosynthesis as well as the effects of CO₂ concentration on the rate of photosynthesis.  |
|                                   |                                                                                                                                                                                                                      | • Describe the effects of atmospheric CO₂ concentration on water acidification and its consequences on aquatic ecosystems. |
|                                   |                                                                                                                                                                                                                      | • Demonstrate an understanding of climate change and the contribution of human activities to climate change              |
| 7. Mitosis and Meiosis            | Grow onion roots in tap water, sample water and water with salinity equivalent to sample water. Fix, stain, and observe the root tips from different samples; identify stages of mitosis.                                      | • Describe the process of cell division and the distribution of chromosomes at different stages.                        |
|                                   |                                                                                                                                                                                                                      | • Predict the effect of tonicity in cell functions, including cell division, and record the impact of changes in salinity on freshwater plants. |
|                                   |                                                                                                                                                                                                                      | • Predict the impact of water contaminants on cell division and reproductive functions.                                  |
| 8. Evolution and metagenomics     | Relate ocean acidification to increase in atmospheric carbon dioxide due to use of fossil fuels. Relate acidification to selection and loss of species diversity. Compare amounts and distribution of bacteria taxa using the data obtained from the metagenomic analysis (sequencing data obtained from the DNA purified in lab 3). Investigate phylogenetic relationships of bacterial species using BLAST. | • Describe the process of selection and give examples of its effects on species diversity.                            |
|                                   |                                                                                                                                                                                                                      | • Describe the principles behind metagenomic analysis.                                                                 |
|                                   |                                                                                                                                                                                                                      | • Use raw data from the metagenomic analysis to illustrate the biodiversity of the microbiome in the water sample. Describe possibilities of using such data to understand the effect of pollution and climate change over time. |
|                                   |                                                                                                                                                                                                                      | • Describe how phylogenetic trees can be used to understand evolutionary relationships.                              |
Develop an integrative understanding of the process of scientific inquiry, including the continuity, complexity, and ambiguity of empirical work

Although each lab was designed to determine different characteristics of the water samples, we purposely chose labs with overlaps for the parameters studied. This strategy ensured students could make connections between their findings from previous labs and the aspect investigated in the following lab, illustrating the continuity and interdependence of the process of scientific inquiry. For example, students measured the salinity of their samples during the diffusion and osmosis lab (week 4); later, in both the photosynthesis (week 6) and the cell respiration (week 7) labs, when measuring dissolved oxygen and carbon dioxide in their water samples, they became aware of the effect of salinity on the amount of dissolved gases in water.

Before each lab, as part of homework assigned in the “flipped presentations”, students were asked to make a flow chart describing the question(s) or problem(s) they would be looking into, then state their hypothesis and describe the experimental design, including control and experimental groups, as well as independent and dependent variables for their experiments.

Develop various skills for data collection and data analysis used in scientific inquiry

For the collection of data, in every lab, students were required to do multiple readings of their measurements and compare their own results with those of their peers. For their research project, and incorporated into the lab activities, students were required to measure mass, volume, length, and other properties of matter in every lab throughout the semester. They quickly learned that to obtain reliable data, their measurements needed to be precise and accurate. For example, in the mitosis and meiosis lab (7), students are required to determine the % of cells in different stages of mitosis in the root-tip slides they prepare for each treatment. After obtaining their data they compare their results with those of their peers and discuss any discrepancies they may find. In addition, several labs (4, 6, 7) require students to produce standard curves to measure an unknown.

Develop the ability to theorise, analyse, and problem solve

In the new format, the labs were designed to guide students on their road to discovery. The information and data gathered by each group were novel, and we, as mentors, provided guidance for their research without having exact knowledge of what their findings would demonstrate, despite other descriptions of characteristics of similar bodies of water. Instead of the usual tasks found in traditional labs, where students record data and complete a lab report which then results in a grade, students performing the research project labs are required to take an active role while setting up their trials, brainstorm questions, elaborate hypothesis, and think of explanations when experiments fail to provide meaningful information, and importantly, reconcile results from experiments in previous labs with the new data.

Develop the ability to work effectively as part of a team

Students worked collectively, in groups of three or four, established at the beginning of the semester. Students were encouraged to develop skills, including interacting, listening and understanding others’ points of view, delegating and sharing tasks, communicating ideas, both in written and oral form, and engaging in discussion. Each student
was given a guide to assess his/her own, as well as their peers contributions and ability to work as a team (see under “EVALUATION SHEET” https://academicworks.cuny.edu/lg_oers/8/).

**Develop written and oral competencies to communicate findings; use peer-reviewed literature**

We developed a sequence of assignments requiring students to read peer-reviewed papers and reports, write summaries from their readings, and eventually write their own papers reporting their findings (see “Assignments 1, 2, 3, and 4 https://academicworks.cuny.edu/lg_oers/8/). The assignments were designed to encourage students to read the articles critically, learn how to find reliable sources and extract any information useful for their investigation, including coming up with a hypothesis for each one of their experiments in the laboratory sessions. The scaffolded nature of the assignments allowed students to build the background for their final report and presentation. This approach helps students improve their writing skills and develop content knowledge gradually. Students were introduced to the proper structure of a research paper by providing examples and having students write a summary of a paper or report. A second assignment required students to write their own introduction and methods and materials sections. Finally, with the feedback they were given, they wrote their full paper which included abstract, introduction, methods and materials, results, discussion, and conclusions. Students were provided detailed rubrics (https://academicworks.cuny.edu/cgi/viewcontent.cgi?filename=0&article=1015&context=lg_oers&type=additional) and also received constructive feedback for each written assignment. Based on their written reports, students were asked to make an oral presentation using either digital media or a poster.

Following in-class presentations, groups were selected to make oral and/or poster presentations in the LaGuardia college-wide Research Symposium, as well as in State and Country-wide meetings (details provided following conclusions).

**Understand the connection between science and society**

Students were asked questions about the significance of their findings in relation to the use of water by the surrounding communities, including the effect of industries, urban development, recreation, and other human activities. For example, when studying cell respiration (Lab 5) and photosynthesis (Lab 6), students measured dissolved oxygen and dissolved carbon dioxide, as well as the pH, of their water samples, compared their measurements to the standards published by the Environmental Protection Agency (EPA), and documented differences found for different bodies of water. Later in the semester (Lab 8), we presented videos illustrating the process of ocean acidification and its effects on marine life, diversity and selection, as well as the relationship between acidification and use of fossil fuels. Each video was followed by activities which required students to answer questions and discuss the importance of understanding the science behind changes in the environment which have direct effects on the community’s well-being.

**Limitations and suggestions for future implementation**

We ran this pilot over three semesters (a total of seven sections). Although we used the same sites for the collection of different samples, we were unable to collect all the water
required for the entire semester on 1 day. This meant that samples used for later labs were collected at later dates, introducing one more variable to be considered when characterising and comparing different waterbodies. Although most of the labs do not require added equipment or supplies, support by the college’s administration for the implementation of a CURE is essential. We participated in the Authentic Research Experience in Microbiology (AREM) project, an NSF-funded project involving several colleges from the City University of New York – details of this project can be found at http://arem.cuny.edu/sites/about/-and were provided with kits for DNA purification performed in the Organic Molecules lab.

Conclusions

Incorporating research experiences into first-year Biology has usually meant modification of one lab, activities outside class and lab sessions, or some combination of the two. Here we provide a full set of laboratory activities and supporting material which allows first-year Biology instructors to implement a semester-long research project to determine water quality of a body of water close to the community. Importantly, the laboratory activities, designed to build students critical thinking, inquiry and problem solving and communication skills, are fully integrated into the existing curriculum.

Lessons learned: examples of students’ comments on experience

“As we explored the quality of two water bodies in New York, all the while learning important biological principles, I was able to understand how different properties of water impact not only human life, but marine life and ecosystem welfare as well. It was a significant introduction to research that inspired me to look for further research opportunities while continuing my studies in sciences.”

“Both teams also agree that they learned a lot about how dissolved CO₂, pH, and temperature can affect a water body’s aquatic life by affecting the water body itself. None of us realised how dependent each of these factors were on each other. We’re glad to have a better understanding of the important factors and issues that affect our local water bodies.”

“Doing this study has shown us the holistic approach scientific research can take when looking into environmental evaluations, and how the results of these multifaceted experiments come together to not only show a bigger picture but can be used to improve community awareness about the environment as a whole.”

Titles: students’ presentations at meetings

Makwana, S and Jankowski, S. Impact of water quality on bacterial diversity in the Long Island sound revealed by metagenomics. CCURY Fall 2017 Colloquium on Nov30th-Dec1st, in Austin, Texas

Siera Martinez. Tetrahymena as a Bioindicator of Water quality in East River. Honours research Symposium, LaGuardia Community College, 14 December 2017

Diana Ilyayeva, Edmark Nyantaki. Comparison of water pollution in East River and Fushing Meadow lake. Honours research Symposium, LaGuardia Community College, 14 December 2017
Marta Candia Franco, Baniya, Yatri, Makhmudova, Nodira, Bhandari Binaya.

Effect of Dissolved O2, CO2, pH, Hardness and Temperature on Aquatic Life, East River Estuary, NYC. Honours research Symposium, LaGuardia Community College, 17 December 2018

Pierce, M and Vimos, L. Can metagenomic analysis give us clues into what’s contaminating the East River? SUNY/CUNY Undergraduate Research Conference (SURC). Farmingdale State College, 26 April 2019.

Soler, S. and Wilson. A Comparative Study on the Effect of Water Quality and Bacterial Growth in an Estuary. SUNY/CUNY Undergraduate Research Conference (SURC). Farmingdale State College, 26 April 2019.

Note
1. Students’ comments were transcribed with their authorisation.

Notes on contributors

Ana-Lucía Fuentes is an Associate Professor in the Department of Natural Science at LaGuardia Community College of the City University of New York. She obtained her PhD from the University of British Columbia, Canada, and worked as an investigator and educator, initially at the Agriculture Canada Research Station in Vancouver and then at Douglas College, New Westminster, British Columbia. Her research interests in the field of education relate to finding best practices to democratize education in science, specially in providing opportunities for first-generation-to-college students to engage in research and share experiences with the larger community. She is also involved in research on the role of the brain phagocytes, microglia, in the development of Alzheimer’s Disease.

Maria Entezari is a Professor in the Department of Natural Science at LaGuardia Community College of the City University of New York. She obtained her PhD from the Tehran University of Medical Sciences, Iran, and worked as an associate researcher, at the Feinstein Institute for Medical Research Research in NY and Postdoc at Pharmaceutical Sciences Department, St. John’s University, NY. Her pedagogical research interests relate to exploring how students learn science through a variety of methods such as project-based learning, community-based learning which also cultivate students’ motivation for social action in the context of science education. Her scientific research interests involved in research on the role of the oxidative stress on microglia malfunctions in the development of Alzheimer’s Disease.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Appendix: Students interested in publishing data compiled over several semesters are now working on compiling results of metagenomic analysis of water samples together with Physical-chemical data and meteorological data corresponding to the dates of sampling. The graph below was created using the data collected from surface water from the East River in NYC.

Lab 1. The Metric System and Measurement

OBJECTIVES: During this lab, you will be measuring length, volume, temperature, and mass of different objects. You will become familiar with the use of different devices to measure
these properties and determine the accuracy and precision of the instruments and your measurements. You will learn the units and conversions in the metric system, as well as practice proper recording of data. For the rest of the semester, you will be applying these skills and knowledge when measuring the physical and chemical properties of your water sample.

**SPECIFIC LEARNING OBJECTIVES:**
- Measure the dimensions of regular geometric shapes using a metric ruler and calculate the areas of each shape.
- Measure the volume of a water sample using a beaker, a graduated cylinder and a graduated pipette, and compare the precision of the measuring devices.
- Measure the mass of a vessel using a triple-beam balance and determine the mass of a powder by calculating the difference.
- Measure the temperature of water and the change in temperature after heating the same water for 3, 5 and 8 min.
- Record results using significant figures and correct units.

**Background:** Before coming to the lab, go over the background lecture recording and/or video. Make sure you have a clear understanding of the following words/concepts:

Measurement  
Units in the metric system  
Data  
Uncertainty  
Precision  
Accuracy  
Significant figures  
Meniscus  
Kilo, Hecto, Deca, deci, centi, milli, micro, nano

**Materials**  
Sugar  
Ice water  
Boiling water  
Small plastic scoops or spoons  
Metric rulers  
Geometric shape cut outs  
Large test tubes  
Bottles for water samples (glass or plastic)  
100-ml beaker with volume markers  
250-ml beaker  
100-ml graduated cylinder  
10-ml pipette  
Pipette pump  
Double-beam balance  
500-ml beakers  
Thermometer  
Hot plate

**LAB ACTIVITIES:** Every student must do all the calculations, record all the results and draw the graphs during the lab activity. Your instructor will write her/his initials once you have completed recording your results.
I. Measuring length and width of different geometric shapes

Procedure:

1. Obtain two different geometric shape cut outs from your instructor.
2. Using the metric ruler provided in your workspace, measure the length and width of each one of the shapes. Record your measurements in the table below. Make sure you use significant figures, so in the example below, the ruler markings are every 0.1 cm. The correct reading is 4.58 cm, the two-first digits, 4.5 are known exactly, the 8 is uncertain. When measuring, you record all the digits that are known exactly, plus the first one that is uncertain.

3. Using the values you obtained, calculate the area of the geometric shapes. You calculate the area of a square or a rectangle, by multiplying width X length. Record your results in the following table and don’t forget the units!

If multiplying or dividing measured values, the result should be reported with the lowest number of significant figures used in the calculation.
For example 4.58 cm X 2.53 cm = 11.59 cm$^2$ (omit the other decimals you obtain when you perform the multiplication).

II. Measuring the Volume of your Water Sample

Procedure:

1. Obtain your water sample from your instructor.
2. Pour your water sample into the 100-ml beaker.
3. Measure the volume of water in the beaker and record this measurement in the table below. Remember to write the numerical value and the units, as well as the significant figures.
4. Carefully transfer the water in the beaker into the 100-ml graduated cylinder.
5. Measure the volume of water in the 100 ml graduated and record your measurement in the table below. Remember to write the numerical value and the units, as well as the significant figures.
6. Do the measured values have the same number of significant figures? Explain your answer (why yes or why no)
7. Empty the water of your graduated cylinder into the beaker, and then pour enough water back into the graduated cylinder to measure 10 ml; pour the rest of the water in the beaker into the sink and dry the beaker.
8. Pour the 10 ml of water you measured with the graduated cylinder, back into the beaker. Using a 10-ml pipette measure the volume of water in the beaker. Write the volume you obtained in the table below. Remember to write the numerical value and the units, as well as the significant figures.

| Vessel               | Volume measured |
|----------------------|-----------------|
| 100-ml graduated cylinder |                |
| 10-ml pipette        |                 |

III. Measuring Mass

Procedure:

1. Use a double-beam balance to obtain the mass of a 250-mL beaker. Record the mass in the table below. Remember to write the numerical value and the units, as well as the significant figures.
2. Remove the beaker from the balance and add three scoops of sugar to the beaker.
3. Obtain the new-combined mass of the beaker and the sugar. Record this new mass in the table below. Remember to write the numerical value and the units, as well as the significant figures.
4. Use your two measurements to determine the mass of the sugar and record it in the table below. Remember to write the numerical value and the units, as well as the significant figures.

| Sample                      | Mass                   |
|-----------------------------|------------------------|
| 250-ml beaker               |                        |
| 250-beaker + 3-scoops of sugar |                    |
| Sugar                       |                        |

IV. Measuring Temperature

Procedure:

1. Obtain a thermometer from your instructor. Before making your measurements, examine the markings on the thermometer.
2. Obtain three 500-ml beakers.
3. Fill one beaker with tap water
4. Half fill another beaker with tap water, then add ice to fill beaker.
5. Obtain hot water from your instructor. ***Handle the beaker with insulating gloves***
6. Measure the temperature in each one of the beakers and record your results below. Remember to write the numerical value and the units, as well as the significant figures.

| Sample | Temperature |
|--------|-------------|
| Tap water |            |
| Hot water |           |
| Ice water |            |

7. Compare your results of each measurement with those of your other group members. Are the measurements close in value? What does this tell you about the precision of your measurements as a group?
Lab 2. Microscopy

Abstract: This lab will give the student brief explanations of the basic principles by which microscopes work as well as some hands-on-experience with the use of the compound microscope, preparation and staining of wet mounts and measurements of microscopic structures. Students will learn units and conversions using the metric system.

Objectives: By the end of this lab, the student should be familiar with:

1. Basics of microscope use.
2. The properties of light that are important in understanding how microscopes work.
3. The markings on the individual-objective lenses and their meanings.
4. Preparation of wet mounts, plant and animal cells.
5. Using stains to improve contrast.
6. Methods for determining field diameter, object size, drawing and photo-magnification.
7. The meaning of resolution and its relationship to magnification.

Equipment:
Compound microscope
Unit conversion table (in manual) Plastic (clear) ruler
Stage Micrometre slide
Plain slides
Coverslips
Ocular micrometre
Toothpicks or cotton swabs

Reagents:
Methylene blue stain Fresh white onions Lugol’s stain

Introduction

The microscope is an indispensable tool in the study of cells. Anton van Leeuwenhoek (1632–1723) first observed protozoans using simple microscopes with a single lens to magnify the image. Today, compound microscopes have a two-lens system that achieves much greater magnification with greater resolution.

The objective of this lab is for you to become familiar with the use of compound microscope and to review its parts while learning slide preparation techniques, measuring techniques, and observing different types of cells.

The compound microscope you will be using in this lab is shown in Figure A1. Before you begin working with the microscope, you should be familiar with all its parts.

1. The illuminator is built into the base of the microscope and the light that is produced here comes from a high-intensity bulb. The size of the illuminated field produced can be regulated by the field diaphragm. Each objective lens will require different sizes of illuminated fields to work optimally.
2. Light then passes to the condenser, which consists of an adjustable system of lenses that focus the light on the specimen. The condenser/iris diaphragm controls the diameter of the light beam entering the condenser. Both the field diaphragm and the iris diaphragm must be centred and adjusted in order to get the optimum illumination that will allow the best resolution for each objective lens and the ocular lenses. When light isn’t centred, there will be scattering.
3. The objective lens produces an enlarged and inverted projection of the object, while the ocular lenses produce the final image that is further enlarged and still inverted.
Magnification and resolving power:

- Microscopes vary in magnification and resolving power.
- Magnification: the ratio of an object’s image to its real size.
- Resolving power: is a measure of image clarity.

When you magnify a picture, you lose resolution
Images can be magnified but that does not increase the level of detail that can be observed. Detail observed depends on the resolving power of a microscope. The limit of resolution is the minimum distance two points can be separated and still viewed as two-separate points.

For our eyes, two points that are closer than 0.19 mm, look like a single point:

Two points that are 0.09 mm apart, we see as a single point

Two points that are 0.3 mm apart, we see as two separate points

For microscopes, the resolving power is limited by the medium through which light travels (air or oil) and the quality of the lenses.

Lenses in microscopes are converging lenses (thicker in the middle). Lenses cause the light to refract (bend); since converging lenses are curved on both sides, the light rays converge at one point, the focal point.

It is very important to have all the lenses aligned, and that the rays of light are falling on the lenses instead of scattering.
For a good explanation of how converging lenses work, watch: https://www.youtube.com/watch?v=R-uMcngNsSk

Questions: Before going on to the next section, answer the following questions.

**Have your instructor review your answers.**
1. In your own words, explain what is meant by the resolving power of a lens.
2. How does the resolving power of the 10X objective compare to the resolving power of the 40X objective?
3. Draw a diagram of light rays going from a source and through a converging (convex) lens. Label the focal point and briefly describe what happens to light as it goes through the lens.

**Procedures Part I**

Follow the instructions below and during the experiment record your data in the tables and space given to you for this purpose. Write all the information during the lab.

The following are the different sections you will be completing during the first period of this lab session (approx. 1 h):

1. Familiarisation with the Microscope Components.
2. Measurement of field diameter for 10X and 40X objective lenses and calibration of the ocular micrometre.

**Familiarisation with Microscope Components:**

1. Familiarise yourself with your microscope by identifying the components labelled in Figure A1.
2. Note the magnification of each objective and the ocular on your microscope and compute the total magnification with each objective and enter the values in Table A1.

**Table A1. Magnification.**

| Objectives        | Ocular lens | Total magnification |
|-------------------|-------------|---------------------|
| 10X low power     |             |                     |
| 40X high dry      |             |                     |
| 100X oil-immersion|             |                     |

**Measurements**

1. **Determine the field diameter of your field of view: use metric system**

To estimate the size of the structures you will be viewing, you can measure the diameter of your field of view for the 10X lens using a transparent ruler.

You must use the metric system. You will be expected to know all the conversion factors in the metric system.
1. Place a transparent plastic ruler on the stage and focus, using the 10X objective, on the lines on the ruler:

![Example of what the ruler will look like under the microscope.]

2. Once you have focused the ruler, count how many divisions can be seen from one edge to the opposite edge (the diameter of the field). Each small division represents 1 mm. How many millimetre is the field diameter when using the 10X objective?

3. Convert the total length in mm into micrometre

2. **Determine the size of cells in a cross section of a stem**

1. Examine a prepared slide from a cross section of a plant stem. Use your 10X objective.
2. Choose an area of the stem where you can clearly see the cell walls, and which will allow you to count how many cells fill one line across the diameter of your field of view; chose an area where all cells are roughly the same size.
3. Count how many cells across the field of view.
4. Calculate the average diameter of each cell. To do this, remember that you determined the diameter of your entire field of view, so:

   \[
   \text{Average size of one cell} = \frac{\text{total diameter of the field of view (\(\mu m\))}}{\text{total number of cells}}
   \]

   Show your computations below:

**Calibration of the Ocular Micrometre:**

A stage micrometre is used to calibrate an ocular micrometre found in your eyepiece. The ocular micrometre will allow you to measure specimens when using different objectives. The ocular micrometre has no units attached to it and therefore needs to be calibrated by its user using the stage micrometre of a known length.

The stage micrometre looks like a microscope slide but has a standard scale etched into it. The total length of the scale is usually 1 mm. The smallest divisions are 0.01 mm in length (*Figure A2).*
To Calibrate the Ocular Micrometre

1. Place the stage micrometre on the stage and using your 10X objective and focus on the stage micrometre using the fine-adjustment knob.
2. The two scales (ocular and stage) should appear to be superimposed or slightly below one another (Figure A3).
3. Move the stage micrometre to match up its left end with the left end of the ocular micrometre (i.e. the zeros on both scales should be aligned). If necessary, rotate the eyepiece to line-up the micrometres.
4. Scan for a marking on the stage micrometre slide that aligns perfectly with a marking on the ocular micrometre (Figure A3). Measure the distance from zero to the markings that line up on the stage micrometre. Use this value to calculate the length of one unit (the smallest subdivisions) in your ocular micrometre when using the particular-objective lens. You must repeat this process for the 40X objective.

X stage micrometre: number of mm
Y eyepiece graticule: number of intervals
Calculation: $X/Y = \text{distance covered by 1 subdivision in the ocular micrometre when using the particular lens used to make this measurement.}$

Figure A2. Stage micrometre size and divisions
The example of the steps for calibrating your eyepiece micrometre:
http://inside107and109.blogspot.com/2017/09/measuring-your-zebrafish-embryo.html

1. Align the eyepiece micrometre (arbitrary scale) with the stage micrometre (known scale).
2. Count how many divisions on the eyepiece micrometre correspond to a set number of stage micrometre divisions.

**TIP:** if you forget which scale is which, rotate your eyepiece a little – the eyepiece micrometre is the one that rotates
So in this example for every 10 stage micrometre divisions there are 21-eyepiece micrometre divisions.
3. Calculate how big one-eyepiece division is.

This is where you need to know how long every small division on your stage micrometre is. Check the size of your micrometre – If your micrometre is 1 mm long in total, then every set of 10-small divisions is actually 0.1 mm long (the 1 mm is divided up into 10-equal parts, each of which is 0.1 mm long), and so every small division is 0.01 mm long.

So, if 21-eyepiece micrometre divisions correspond to 0.1 mm on the stage micrometre then 1-eyepiece micrometre division must correspond to \( \frac{0.1}{21} = 0.0048 \text{ mm} \)
Now you can measure your sample:

Once you have gone through all of the above steps, you can measure your specimen using the eyepiece micrometre, and convert the number of eyepiece micrometre divisions you have counted to an actual length:

So, if I am looking at some cheek cells, I can see that the length of one cheek cell is 16 units long on the eyepiece micrometre. I now know that one-eyepiece micrometre division is 0.0048 mm long, so this cell must be $16 \times 0.0048 = 0.0768$ mm long.

5. Now calibrate the 40X objective using the above steps.
   Enter these values in Table A2.

**Table A2. Calibration of an ocular micrometre.**

| Objectives  | Total length of scale on ocular micrometre (µm) | µm/smallest division on ocular micrometre |
|-------------|-----------------------------------------------|------------------------------------------|
| 10X low power |                                              |                                          |
| 40X high dry |                                              |                                          |

**Procedures Part II**

Follow the instructions below and during the experiment, draw what you are observing in the spaces provided; you must record all the information requested in this lab handout. Your instructor will check your drawings and calculations during the lab, make sure you show your work before leaving the lab.

**Preparation of Wet Mounts**

Onion Epidermis Cheek cell

- A. Drawing and calculating your drawing magnification:
- B. Drawings of what you observe should be done in pencil.
- C. Each drawing should be properly labelled.
- D. You must record the size of each object you are observing to be able to calculate your drawing magnification.
**Preparation of Wet Mounts, drawings and calculating magnification**

In a wet mount, a piece of tissue is placed on a clean slide with a drop of water, stain or reagent. A coverslip is gently lowered on the preparation. In this lab section, you will be preparing two different wet mounts: onion epidermis and elodea.

**A. Onion Epidermis: Observing the structure of plant cells**

1. Add a small drop of water to the centre of a clean slide.
2. Take a fresh piece of onion and remove a layer.
3. Using forceps strip a small piece of epidermis from the concave surface of a layer and place it on the drop of water, being careful that it does not fold over on itself. Add a drop of water and a coverslip.
4. Use the 5X (scanning) objective to find your specimen and focus using the coarse adjustment. Without touching the adjustment knobs, rotate the revolving nosepiece and place the 10X objective over your slide.
5. Examine the cells with the 10X and the 40X objectives; use the fine adjustment to focus.
6. Slowly close the condenser diaphragm while looking at your slide.

   a. What do you observe when you close the condenser diaphragm?
   b. How does the opening of the condenser diaphragm affect the contrast?

7. Add one or two drops of Haemalum acid solution at one edge of the coverslip and draw it through by touching a piece of paper towel to the opposite edge of the coverslip. Wait 10 min for the onion skin to stain.
8. Examine the stained cells with the 10X and 40X objectives, varying the opening of the iris diaphragm until the nucleus is clearly visible.
9. Using the ocular micrometre and the 40X objective, measure the length and width of four cells and record it in the table below, use µm as units (use the table where you recorded the calibration for the markings on the ocular micrometre when using the 40X objective).

   Calculate the average length and width of the onion cells and record it in the table below.

| Cell | Width | Length |
|------|-------|--------|
| 1    |       |        |
| 2    |       |        |
| 3    |       |        |
| 4    |       |        |
| Average |       |        |

10. In the space provided, draw one typical onion epidermal cell, labelling the visible cell structures. Drawings should be done in pencil and should reflect what you see when looking at the structures under the microscope. Every drawing done should have the following information:

   Name of organism (*Allium cepa* in this case) Cell type
   Stain or technique used for the preparation
   Plate/Drawing magnification (size of your drawing of object)/(actual size of object)

**Onion Epidermis Drawing**

Stain:__________
Actual Size:__________
Plate/Drawing Magnification:__________ (size of your drawing of object)/(actual size of the cell)
Questions:

1. How does using a stain change the visibility of the structures? Why?
2. What features can you see under the microscope which allow you to characterise these cells as eukaryotic cells?
3. Plant cells are surrounded by a plasma membrane and they also have cell walls. What are the major components of plasma membranes? What is the major component of plant cell walls?

B. Cheek epidermal cell: Observing the structure of animal cells

1. Put a drop of methylene blue stain on a slide.
2. Gently (lightly) scrape the inside of your cheek with the flat side of a toothpick.
3. Stir the end of the toothpick in the stain and throw the toothpick away.
4. Place a coverslip onto the slide; if you have excess stain, use a small piece of paper towel to draw the excess stain.
5. Use the 5X (scanning) objective to find the area with cells and focus. You probably will not see the cells at this power.
6. Switch to the low power (10X objective) by turning the revolving nosepiece. You should be able to see the cells although they will appear as small purplish little “clouds”. Any large objects you see are probably crystals from the stain, not cells.
7. Once you think you have located a cell, switch to high power (40X) and refocus using ONLY the fine adjustment.
8. Using your ocular micrometre measures the diameter of the cells; you will need to record the size of the cells in order to calculate your drawing magnification.
9. In the space provided, draw one typical onion epidermal cell, labelling the visible cell structures. Drawings should be done in pencil and should reflect what you see when looking at the structures under the microscope. Every drawing done should have the following information:

   Type of organisms:
   Cell type:
   Stain or technique used for the preparation

   Plate/Drawing magnification (size of your drawing of object)/(actual size of object)

Cheek cell drawing

Stain:__________
Actual Size:__________
Plate/Drawing Magnification:__________ (size of your drawing of object)/(actual size of the cell)

Question:

1. What differences could you see, using a microscope, between the onion cells and the cheek cells? – compare structures and size-
Lab 3. Organic Molecules-DNA ISOLATION

OBJECTIVES:
During this lab, you will be isolating DNA as one type of organic molecule in the collected water samples.

SPECIFIC LEARNING OBJECTIVES:

- Understand the meaning of water pollution
- Understand the source of organic materials in water.
- Understand the correlation between the number of microorganisms in water and the levels of organic materials in water
- Understand the steps of DNA isolation from water samples.
- Understand how the properties of lipids, polysaccharides and proteins present in cells, are used to eliminate these organic molecules and obtain purified DNA.

Introduction:
Deoxyribonucleic acid (DNA) isolation is an extraction process of DNA from various sources. There are different methods to isolate DNA which are dependent on the source, age, and size of the sample. In spite of particular differences, these methods aim to separate DNA present in the nucleus, or nucleoid (in the case of prokaryotes), of the cell, from other cellular components. Isolation of DNA is needed for genetic analysis, which is used for scientific, medical, or forensic purposes. Scientists use DNA in a number of applications, such as introduction of foreign DNA into cells (to make transgenic organisms) or for diagnostic purposes.

Presence of proteins, lipids, polysaccharides and some other organic or inorganic compounds in the DNA preparation can interfere with DNA analysis methods. Sources for DNA isolation are very diverse. Basically, it can be isolated from any living or dead organism. In this lab, we will be isolating DNA from the microorganisms that are in the water samples.

Microbial Metagenomics
Metagenomics is the study of genetic material recovered directly from environmental samples. Previously, microbial genome sequencing and genomics relied upon first culturing the microbial clones, however, much of the microbial biodiversity was missed when using this procedure. Instead, we will be using the “PCR or Shotgun” method, which will allow us to identify a large number of bacterial populations, which cannot be cultured in the lab using traditional microbiological techniques.

Make sure you have a clear understanding of the following concepts after reviewing the provided videos and reading materials:

Carbon-based molecules:
Polymer
Monomer
Hydrolysis reaction
Dehydration synthesis reaction
Monosaccharide
Polysaccharide
Triglyceride
Phospholipids
Cell membrane
Hydrophilic
Hydrophobic
Protein denaturation
Protein structures: Primary, secondary, tertiary and quaternary
DNA
RNA
Nucleotide
Metagenomic

MATERIALS:
Test tubes
Eppendorf tubes
Pipette
Pipettor
Tips
Centrifuge
Vortex
Filter
Portable Vacuum
Mobio Waterpower kit
Test tube rack
Water bath 65°C

LAB ACTIVITIES: Each group will work together to conduct this activity. Every student must record, in their notebook, the results obtained by the group. Your instructor will write her initials once you have completed recording your observations.

*Isolating DNA from Water Sample by MoBio WaterPower® Kit*

Before you begin the purification process, examine all the tubes and solutions on your lab bench and make sure you have everything you need in your tube rack.

Label all your tubes with your group number

1. Filter water samples using a reusable or disposable filter funnel attached to a vacuum source.
The PowerWater® DNA Isolation Kit starts with the filtration of a water sample onto a filter membrane.
The membrane is then added to our special 5-ml bead beating tube containing a unique bead mix.

2. Insert the filter into the 5 ml PowerWater® Bead Tube:
   Loosely rolling and inserting the filter membrane into the PowerWater® Bead Tube allows for efficient bead beating and homogenisation in proceeding steps.
The following videos illustrate these procedures:
https://www.youtube.com/watch?v=KUT6nKJPj4s
https://www.youtube.com/watch?v=v4ggmR1b0pU

3. Add 1 ml of Solution PW1 to the PowerWater® Bead Tube:
   Solution PW1 must be warmed to dissolve precipitates prior to use. Solution PW1 should be used while still warm.
   *What’s happening:* Solution PW1 is a strong lysing reagent that includes a detergent to help break cell walls and will remove non-DNA organic and inorganic material. It is also part of the patented Inhibitor Removal Technology® (IRT). When cold, this solution will form a white precipitate in the bottle. Heating to 55°C will dissolve the components without harm. Solution PW1 should be used while it is still warm.

4. Secure the PowerWater® Bead Tube horizontally to a Vortex Adapter. Vortex at maximum speed for 5 minutes.
   *What’s happening:* The mechanical action of bead beating will break apart the surface of the filter membrane that contains trapped cells and aids in cell lysis. Use of the vortex adapter will
maximise homogenisation by holding the tubes equal distance and angle from the centre of rotation.

5. Centrifuge the tubes ≤ 4000 x g for 1 minute at room temperature.  
The speed will depend on the capability of your centrifuge.  
*What’s happening:* The debris and beads will precipitate, and the supernatant will contain DNA.

6. Transfer the supernatant to a clean 2 ml Collection Tube (provided).  
Draw up the supernatant using a 1-ml pipette tip by placing it down into the beads. Placing the pipette tip down into the beads is required. Pipette more than once to ensure removal of all supernatant. Any carryover of beads will not affect subsequent steps. Expect to recover between 600 and 650 µl of supernatant depending on the type of filter membrane used.  
*What’s happening:* The supernatant is separated and removed from the filter membrane and beads at this step.

7. Centrifuge at 13,000 x g for 1 minute.  
Transfer the supernatant to a clean 2-ml Collection Tube (provided). Make sure to not remove the pellet.  
*What’s happening:* Any remaining beads, proteins, and cell debris are removed at this step. This step is important for removal of any remaining contaminating non-DNA organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

8. Add 200 µl of Solution PW2 and vortex briefly to mix. Incubate at 4°C for 5 minutes.  
*What’s happening:* Solution PW2 is another part of the patented Inhibitor Removal Technology® (IRT) and is a second reagent to remove additional non-DNA organic and inorganic material including humic acid, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

9. Centrifuge the tubes at 13,000 x g for 1 minute.  
After centrifuging, carefully transfer the supernatant to a clean 2-ml Collection Tube (provided). Make sure not to remove the pellet.  
*What’s happening:* The pellet at this point contains additional non-DNA organic and inorganic material. For best DNA yields and quality, avoid transferring any of the pellet.
10. Add 650 µl of Solution PW3 and vortex briefly to mix. 
   Note: Check Solution PW3 for precipitation prior to use. Warm if necessary. Solution PW3 can be used while still warm.
   *What's happening*: Solution PW3 is a high concentration salt solution. Since DNA binds tightly to silica at high salt concentrations this will adjust the DNA solution salt concentration to allow binding of the DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the spin filter.
   High concentration of salt helps to neutralise the negative charge on the nucleic acid backbone, causing the DNA to become less hydrophilic and fall out of solution.

11. Load 650 µl of the mixed solution prepared in step 10 onto a Spin Filter and centrifuge at 13,000 x g for 1 minute.
   The spin filter is a small silica filter basket which is in one of the tubes provided to you. After you centrifuge, discard the flow through (filtrate). To discard the filtrate, you will need to carefully take the filter basket out of the tube, then discard the fluid in the tube. Once you have discarded the filtrate, place the filter in the tube, and add the rest of the mixed solution prepared in step 10 (don’t add more than 650 µl, if you need to, repeat until all the supernatant has been loaded onto the Spin Filter).
   Once you have filtered all your solution through the filter, place the Spin Filter basket into a clean 2-ml Collection Tube (provided).
   *What’s happening*: Due to the high concentration of salt in solution PW3, it is important to place the Spin Filter basket into a clean 2-ml Collection Tube to aid in the subsequent wash steps and improve the DNA purity and yield.

12. Add 650 µl of Solution PW4 and centrifuge at 13,000 x g for 1 minute.
   Shake to mix Solution PW4 before use.
   *What’s happening*: Solution PW4 is an alcohol-based wash solution used to further clean the DNA that is bound to the silica filter membrane in the Spin Filter. This wash solution removes residual salt and other contaminants while allowing the DNA to stay bound to the silica membrane.

13. Discard the flow through by carefully removing the Spin Filter, decanting the filtrate, then placing the Spin filter back into the tube. Then add 650 µl of Solution PW5 and centrifuge at 13,000 x g for 1 minute.
   *What’s happening*: Solution PW5 ensures complete removal of Solution PW4 which will result in higher DNA purity and yield.

14. Discard the flow through and centrifuge again at 13,000 x g for 2 minutes to remove residual wash.
   *What’s happening*: The second spin removes residual Solution PW5 (ethanol). It is critical to remove all traces of wash solution because the ethanol in Solution PW5 can interfere with many downstream DNA applications such as PCR, restriction digests, and gel electrophoresis.

15. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).

16. Add 100 µl of Solution PW6 to the centre of the white filter membrane.
   *What's happening*: Placing Solution PW6 (sterile elution buffer) in the centre of the small-white membrane will make sure the entire membrane is wetted. This will result in a more efficient and complete release of the DNA from the silica Spin Filter membrane. As Solution PW6 passes through the silica membrane, the DNA that was bound in the presence of high salt is selectively released by Solution PW6 (10 mM Tris) which lacks salt.
17. Centrifuge at 13,000 x g for 1 minute. The DNA will now be in the filtrate, do not discard the filtrate. Discard the Spin Filter basket. The DNA is now ready for any downstream application. No further steps are required. We recommend storing the DNA frozen (−20°C to −80°C).

Questions:

1. Where is DNA found in a Eukaryotic cell? Where is DNA found in a prokaryotic cell?
2. To isolate DNA from a cell, which macromolecules need to be eliminated?
3. What are the components of the cell membrane? Draw a diagram showing the arrangement of these molecules in the cell membrane.
4. Given the composition of the cell membrane you just described, what methods can be used to lyse (or disrupt) the cell membrane?
5. After the cells are lysed, what procedure can be used to remove the lipids from the rest of the components?
6. Why is using a high concentration of salt important for isolating DNA?
7. What does ethanol do in the DNA isolation procedure?

Watch the following videos and read the following article to answer questions 8 and 9
https://www.youtube.com/watch?v=2mx6KHMPfVw
https://www.youtube.com/watch?v=ifrHogDujXw Climate change
https://www.youtube.com/watch?v=h198sZXP7tU water pollution documentary
https://www.youtube.com/watch?v=GNGKsubYJ9U Water Pollution

8. What information, in this case regarding your water sample, does a metagenomic analysis provide?

9. How can we use the result of metagenomic suites to understand climate change?

Lab 4. Diffusion and Osmosis: Passive movement of molecules in biological systems

OBJECTIVES:
During this lab, you will be determining the salinity of the collected water samples. You will also compare the effect of the solute concentration of your samples with that of solutions of known solute concentrations on plant cells.
Note: You will be incorporating the results obtained during this lab to your final research reports and ppt. presentations.

SPECIFIC LEARNING OBJECTIVES:
- Understand the concept of kinetic energy of molecules and Brownian movement
- Understand the effect of solute concentration and temperature on the diffusion of solutes across a selectively permeable membrane
- Understand the effect of solute concentration on osmosis
- Observe the effect of placing living cells in hypotonic, hypertonic and isotonic solutions
- Determine salinity of water sample
- Understand how to generate a standard curve in order to determine the concentration of an unknown sample

Background: Before coming to the lab, go over the background PowerPoint slides and videos posted for this lab. Complete the exercises embedded in the pre-lab PowerPoint and videos and make sure you hand these into your instructor following the instructions given to you. Make sure you have a clear understanding of the following concepts:
Concentration of a solute in a solution
Kinetic energy  
Diffusion  
Osmosis  
Selectively permeable membrane  
Plasma membrane  
Plant vacuoles  
Tonicity: hypertonic, isotonic and hypotonic solutions

**Materials**

- NaCl solutions (0.1 M; 0.2 M; 0.3 M; 0.4 M; 0.5 M; 0.6 M) – 50 ml each/group  
- 8–10 pieces of 15 cm long, 2.5-cm diameter dialysis tubing, pre-soaked in distilled water  
- String to tie dialysis tubing  
- 8 cups/250 ml beakers  
- distilled water  
- funnel  
- Balance  
- 25-ml graduated cylinder  
- 10-ml pipettes  
- Markers  
- Microscope  
- Ocular micrometre  
- Micrometre slide  
- Fresh red onions  
- Hot plate  
- 4 Large watch glasses  
- Small spatula  
- Weighing boats  
- Eight 250-ml beakers

**LAB ACTIVITIES:** Every student must do all the calculations, record all the results and draw the graphs during the lab activity. Your instructor will write her/his initials once you have completed recording your results.

**I. Determining the salinity of your water sample:**

Total Dissolved Salts (TDS) is measured by evaporating a known volume of water to dryness, then weighing the solid residue remaining*.

*([http://agriculture.vic.gov.au/agriculture/farm-management/soil-and-water/salinity/measuring-the-salinity-of-water](http://agriculture.vic.gov.au/agriculture/farm-management/soil-and-water/salinity/measuring-the-salinity-of-water))

**Procedure:**

1. Using your balance, weigh a clean, dry watch glass. Record the mass in the table below.  
2. Using a 5 or 10-ml pipette, take 10 ml of your water sample and place it in the clean and dry watch glass.  
3. Carefully place the watch glass on the hot plate and turn the dial to position 2 or 3 (don’t heat the plate too much or too quickly).  
4. Leave the watch glass until all the water has evaporated. (Proceed to the next section of the lab while you wait for the water to evaporate).  
5. Let the watch glass cool; weigh the watch glass with its contents and record the value in the table below.  
6. Calculate the total mass of dissolved salt in the watch glass by subtracting the mass of the clean-watch glass from the mass of the watch glass + salt.  
7. Record the total-dissolved salt by calculating the amount in mg/l. Remember you started with 10 ml of your water sample and the TDS is the amount of salt in 1 l.
Determining approximate solute concentration of water samples using changes in mass of dialysis bags with known concentrations of NaCl.

Note: Each group will work together to conduct this activity. Every student must record, in their manual, the results obtained by the group, and draw a graph as instructed below.

In this exercise, you will need to prepare six dialysis bags with different solutions of known NaCl concentration, as well as one dialysis bag with your water sample and one with distilled water. Each one of the bags needs to be weighed BEFORE placing it in the beaker with distilled water. Follow the instructions carefully; if you have any questions, ask your instructor.

The table below gives you the amount of NaCl you would need to weigh (as in example), to prepare 100 ml each of the following six solutions (the solutions have been prepared ahead of time, you can fill out the table while you wait for the dialysis):

| NaCl concentration | Amount to weigh for 100 ml |
|--------------------|---------------------------|
| 0.1 M              | 584.4 mg                  |
| 0.2 M              |                           |
| 0.3 M              |                           |
| 0.4 M              |                           |
| 0.5 M              |                           |
| 0.6 M              |                           |

Example of how to calculate the amount NaCl needed to prepare solutions of different concentrations:

For NaCl, 1 MV = 58.44 g/l

so 0.1 MV = 5.844 g/l; you only want to prepare 100 ml, not 1 l, which is 1,000 ml.

5.844 g = 1000 ml

X = 0.5844 g

which is = 584.4 mg

Procedure:

1. Prepare eight 100 or 250-ml beakers; label one as “distilled water”, another as “water sample”, and the rest with each one of the NaCl concentrations on the table above.
2. After labelling your beakers, add DISTILLED WATER TO ALL OF THEM, about three-fourths full. Set them aside.

Filling dialysis bags:
You will need eight dialysis bags; you will fill each bag with one of the NaCl solution (so six bags), one bag with distilled water, and another bag with your sample.

After following the instructions below, you will have a setup which looks like the picture:

3. Remove the dialysis tubing from the distilled water and tie-off one end using the string or clamp provided.
4. To open the other end of the tubing, rub it between two fingers. While one person holds the tubing, another person can fill the bag with one solution; if necessary, use a funnel to make sure it doesn’t spill. Do not overfill the tubing, leave some space for expansion, but no air.
5. Tie the other end of the tubing; you can use a clamp, string, or carefully tie the dialysis bag itself. If you are using string, wet the string completely before using.
6. Before going on to the next step, dip the filled bag in distilled water, then gently blot the bag dry on paper towel. This will decrease the error in the measurements you will make in step 9.
7. While two members of the group continue filling the rest of the tubing, another student should proceed to weigh and record the mass of each one of the filled tubes in the table below.
8. Once all eight tubes have been filled and weighed, place them in the distilled water in the labelled beakers. Record the time.
9. After 45 min, take the bags out of the beakers, gently blot the bags dry, and weigh each one. Record the mass in the table below.

**NOTE: AT THIS POINT, YOU MAY TAKE PICTURES OF YOUR SET-UP IN ORDER TO INCLUDE THEM IN YOUR FINAL PRESENTATION.**

10. In the space below (record all your calculations), calculate the % change in mass in the bags, for the different NaCl concentrations.

| Bag content | Initial mass | Mass after 45 min | Mass difference | % change in mass |
|-------------|--------------|-------------------|-----------------|-----------------|
| Distilled water |              |                   |                 |                 |
| Water sample |              |                   |                 |                 |
| 0.1 M NaCl   |              |                   |                 |                 |
| 0.2 M NaCl   |              |                   |                 |                 |
| 0.3 M NaCl   |              |                   |                 |                 |
| 0.4 M NaCl   |              |                   |                 |                 |
| 0.5 M NaCl   |              |                   |                 |                 |
| 0.6 M NaCl   |              |                   |                 |                 |

**Example:** To calculate the % change in mass, you have the initial mass, for example, 27 g, and a final mass, for example, 27.5 g.
1. Calculate the mass difference: 27.5 g – 27 g = 0.5 g (record this value in the table above)
2. Calculate the % change this difference represents:

\[
27 \text{ g (initial mass)} = 100 \%
0.5 \text{ g (mass difference)} = X\%
0.5 \text{ g (100%)} = 1.85\%
\]

27 g

YOUR CALCULATIONS: Make sure you record all your calculations!
11. Use the graph paper provided in the next page to graph the % change in mass for the distilled water and the six bags with different known NaCl concentrations. This is your standard curve which will allow you to find out the salt concentration of your water sample. *Why are you asked to calculate the % of change, not just the change in mass?*

12. Calculate the % change in mass for the bag with the water sample, extrapolate the value on the graph, find the corresponding NaCl concentration and record it:

> Salt concentration of water sample based on change in mass and extrapolated from the standard curve you created:__________

13. How does the mass of the bags change as the molarity of the solutions in the bags change? Explain why this change occurs:

II. **Effect of extracellular solute concentration on vacuoles of plant cells**

> **Note:** To complete this section of the lab, you must have calibrated your ocular micrometer on your microscope. Each student must prepare their own wet mount, examine, measure, and record, each one of the preparations.

1. Prepare three watch glasses with solutions which are hypotonic (distilled water), hypertonic (20% NaCl), and your water sample. Make sure you label each sample.
2. Using tweezers, peel a single layer of the onion, take the coloured layer. Place a few strips in each one of the solutions and leave them for a few minutes.
3. Make a wet mount, start with the hypotonic solution. Observe under your microscope (follow the guidelines in the lab microscopy unit), and focus under the 10X, then the high dry (40X) objective. Measure the length of at least five vacuoles. The vacuoles are visible, as the purple-pigment colours the water inside them.
4. Proceed to make wet mounts of the hypertonic solution and make measurements of the length of at least five vacuoles. Finally, do the same for the onion strips in your water sample.

Examples of onion cells in a hypotonic solution
Example of onion cells in a hypertonic solution

You can take pictures of your cells as these will allow you to illustrate the results in your assignments and final poster.

5. Calculate the average length of the vacuoles for each one of the solutions and recorded in the table below

| Measurements | Length vacuoles hypothonic solution | Length vacuoles hypertonic solution | Length vacuoles water sample |
|--------------|------------------------------------|------------------------------------|-----------------------------|
| 1            |                                    |                                    |                             |
| 2            |                                    |                                    |                             |
| 3            |                                    |                                    |                             |
| 4            |                                    |                                    |                             |
| 5            |                                    |                                    |                             |

6. How does the tonicity of the extracellular fluid affect the size of the vacuoles? What process is illustrated by this effect, explain.

7. How do the vacuoles of the cells placed in your water sample compare with the vacuoles of cells placed in the hypotonic and hypertonic solutions?

8. What prevents the onion cells, which were placed in a hypotonic solution, from bursting?

9. Based on your findings, predict possible effects that changes in the salinity of waterbodies may have on unicellular organisms.

**Lab 5. CELLULAR RESPIRATION FERMENTATION**

**OBJECTIVES:**

1. In this lab, you will investigate how glucose concentration affects the rate of fermentation in yeast, a single-celled eukaryote which is capable of alcoholic fermentation.

2. During this lab you will also investigate the presence or absence of facultative anaerobic bacteria in your water samples, particularly, you will determine whether your water presents faecal contamination, indicated by the release of acid and gas into the media, due to fermentation.
3. Finally, you will measure the amount of dissolved oxygen in your water samples and determine its quality, by comparing your results with the Environmental Protection Agency (EPA) standards.

**SPECIFIC LEARNING OBJECTIVES:**
- Describe the process of alcoholic fermentation in yeast and determine the effect of concentration of glucose on the rate of fermentation
- Understand the differences between anaerobic fermentation and aerobic cell respiration.
- Using your water samples, determine the presence of bacteria that are facultative anaerobes, particularly *E. Coli* and enterococci bacteria, which ferment sugars producing acid as well as gas.
- Determine the amount of oxygen in your water samples and compare it to the EPA standards for water designated for different uses.

**Background:**
Most present day unicellular and multicellular organisms have enzymes that allow cells to harvest chemical energy in organic molecules, such as glucose (Figure A4), and use that energy to make ATP. **Heterotrophs**, for example, animals, need to ingest those organic molecules, while **autotrophs**, such as plants, are able to harvest the energy from light, in the process of photosynthesis, and “fix” carbon dioxide to synthesise their own organic molecules.

Cellular respiration is a controlled process where the energy of glucose is released in a series of steps, with orderly chemical reactions that allow the high-energy electrons in glucose, to be picked up and used to make ATP (illustration below). The first step, **glycolysis**, does not require oxygen to take place, it is an **anaerobic process**. The resulting molecule, **pyruvate**, still holds a lot of chemical energy in it. This chemical energy is harvested in two steps which are oxygen-dependent or **aerobic**, the **citric-acid cycle** and the **electron transport chain and oxidative phosphorylation**.

Aerobic Cellular Respiration:
During the early history of life on Earth, there was little oxygen in the atmosphere. Scientists believe that glycolysis in early bacteria evolved as what we know today as fermentation, an anaerobic method to break down glucose to harvest chemical energy. Fermentation therefore provides a mechanism by which some cells can oxidise organic material and generate ATP without using oxygen; **fermentation allows for the oxidation of NADH to NAD\(^+\), which is necessary for glycolysis to go forward.**

Fermentation:

![Fermentation Diagram](image)

Today there are relatively few environments on our planet where oxygen is absent. Natural locations such as swamps and human-made structures, such as sewage treatment plants, harbour organisms that are incapable of growing in the presence of oxygen (obligate anaerobes). There are also unicellular organisms that have evolved a method for surviving in the presence of oxygen while retaining the ability to thrive and reproduce in the absence of oxygen. These organisms are called facultative anaerobes, and include the yeasts and many species of bacteria. Facultative anaerobes carry out fermentation when oxygen is absent and aerobic cellular respiration when oxygen is present.

One consequence of pollution of bodies of water by storm water, sewage and industrial or agricultural waste dumping, is eutrophication. In this process, nitrates, phosphates and other nutrients released into the water, promote the growth of algae. In still waters, the algae “bloom”, forming a layer, like a carpet, over the water surface. Underwater plants die because of lack of light; the “blooming” algae also die. The organic matter resulting from this die-off is used as a substrate for bacteria to get nutrients. Bacteria use up the oxygen in the water, as they use the organic molecules as source of energy, thus promoting “hypoxia” -low amounts of oxygen, and “anoxia” -no oxygen. Since there is no oxygen in the water, all aerobic organisms start dying off; their cells can no longer produce ATP in the mitochondrion, and fermentation does not supply nearly enough energy for their survival.
LAB ACTIVITIES: Each group will work together to conduct the activities below. Every student must do all the calculations, record all the results and draw the graphs during the lab activity. Your instructor will write her initials once you have completed recording your results.

Yeast are one class of organisms that carry out alcohol fermentation. There are 60 genera and over 500 species of yeast, the most important of which are members of the fungal division Ascomycota. The Ascomycete *Saccharomyces cerevisiae* has been used for millennia to make bread, wine and beer. Different strains of *S. cerevisiae* have been developed for different uses.

In alcoholic fermentation, glucose is first broken down to pyruvate in glycolysis. Pyruvate is then decarboxylated (i.e. a carboxyl group is removed) to acetaldehyde, which in turn is converted to ethanol.

*Figure A4. Chemical structure of glucose*
The formula for converting glucose into ethanol is:

\[
C_6H_{12}O_6 + 2 \text{ADP} + 2 P_i \rightarrow 2 C_2H_5OH + 2 \text{CO}_2 + 2 \text{ATP}
\]

| Glucose | Inorganic | Ethanol | Carbon | Dioxide |
|---------|-----------|---------|--------|---------|
| Phosphate |           |         |        |         |

EVERY INDIVIDUAL STUDENT MUST FILL OUT THE AREAS REQUIRING INFORMATION AS WELL AS RECORD ALL THE DATA.

SECTION A: ALCOHOL FERMENTATION IN YEAST

PROCEDURE: Label the four-falcon tubes with the yeast solutions and water

Falcon tube #1: _____water + yeast____________
Falcon tube #2: _____5% glucose + yeast____________
Falcon tube #3: _____7.5% glucose + yeast__________
Falcon tube #4 _____7.5% glucose + water__________

Propose a hypothesis for the following: What do you predict with respect to comparative fermentation rates for each of the three yeast solutions.

Hypothesis:

_________________________________________________________________
_________________________________________________________________

1. If it is not already done, mark each ml line on the cuvette with a permanent marker so the lines are visible when the yeast solutions are added (Figure A5(a)).
2. Add 5 mL each from the labelled dispensers (#1 water, #2 5% glucose, #3 7.5% glucose, #4 7.5% glucose your chosen solutions recorded above) into the appropriate falcon tube (Figure A5(a)).
3. Stir the prepared yeast solution (which is in the 37°C water bath), then carefully pour your yeast solution into each falcon to the very brim. Remember each line represents 1 ml.
4. Cap the tubes so that you feel them snap closed, cover the cap with your thumb and vigorously invert the cuvette 3 times to mix the yeast and the added solution.
5. Take the cap off and place 50-ml falcon tube over each 15-ml tube (Figure A5(b)).
6. Invert the falcon tubes, as seen in the picture. Place them in the holder and record the initial volume displaced (this would be time 0).
7. Begin measuring the amount of liquid displaced every 5 min for 30 min, and then every 10 min for another 30 min and write your results in Table A3.
8. Plot the ml displaced (y-axis) for each condition vs. time (x-axis) on a piece of graph paper. Title it and label each axis.
RESULTS:

Table A3. Volume of liquid displaced by fermentation in water, and chosen solutions.

| Time (min.) | ml liquid displaced | #1: | #2: | #3: | #4 |
|-------------|---------------------|-----|-----|-----|-----|
| 0           |                     |     |     |     |     |
| 5           |                     |     |     |     |     |
| 10          |                     |     |     |     |     |
| 15          |                     |     |     |     |     |
| 20          |                     |     |     |     |     |
| 25          |                     |     |     |     |     |
| 30          |                     |     |     |     |     |
| 40          |                     |     |     |     |     |
| 50          |                     |     |     |     |     |
| 60          |                     |     |     |     |     |

QUESTIONS:

1. Graph the results for the different solutions, place the CO₂ evolved on the Y-axis and the time (minutes) on the X-axis.
2. Calculate the average fermentation rate for each one of the solutions (ml CO₂ evolved/ min.) The ml CO₂ evolved per minute can be calculated by examining the amount of fluid displaced per minute. [(final – initial)/time elapsed]
3. Compare the fermentation rates of the three yeast solutions.
a) Was there a difference in the fermentation rates of your solutions? Is this what you expected? Explain.
b) Did you expect any fermentation to occur in sample #1? Sample #4? Why or why not?

SECTION B: DETERMINING PRESENCE OF FACULTATIVE ANAEROBIC BACTERIA IN YOUR WATER SAMPLE

MATERIALS

Test tube rack
70% ethanol for disinfecting
2 Lauryl Tryptose Broth fermenting tubes
2 lactose broth with Durham tube
TSA-agar plates pre-inoculated with water from different sites

Answer the following questions:

1. What is the purpose of the uninoculated control tubes used in this test?
2. What is the purpose of adding mineral oil to the test tube after performing the inoculation?
3. All bacteria known as “enterics” are facultative anaerobes, which means they have both respiratory and fermentative enzymes. What colour results would you expect in O-F media inoculated with enterics, in sealed and unsealed tubes? Explain

Determining presence of coliform bacteria in your water samples

The intestinal tract of animals, including humans, harbour bacteria which are essential in maintaining health. On the other hand, many human pathogens (disease-producing agents) are also found in the intestines of humans who have been infected; these pathogens are released in the faeces, together with other faecal components.

In this exercise, you will be testing for the presence of coliform bacteria, which are a group of bacteria found in faecal matter. Coliforms are facultative anaerobes, and can ferment sugars, producing both carbon dioxide (gas) and acids.

You will be using lauryl tryptose broth (LSB) fermentation tubes which have special media that only sustains growth of coliforms (it inhibits other types of bacterial growth), and allows you to determine water contamination with faecal coliforms, which ferment lactose with gas production.

Recording results from LSB tubes previously inoculated with 1 ml of your water samples

1. Retrieve two LSB tubes inoculated, by your instructor: one was inoculated with your water sample, the other was inoculated with tap water. These tubes were left incubating at 37°C for 48 h, to allow for bacterial growth.
2. To record your results in the table below, you must check for two parameters:

   - Turbidity of media: if the media appears opaque and diffracts light, this means bacteria grew.

| Area of Collection/date of inoculation | Turbidity (not turbid -, less turbid+, turbid ++, very turbid ++) | Interpretation of results |
|---------------------------------------|-------------------------------------------------|--------------------------|

EDUCATION INQUIRY
Answer the following questions:

1. If you were to trace the origin of the atoms found in the CO$_2$ released during fermentation, where would you find them (in what compound)?
2. How much ATP is produced when one molecule of glucose is fermented?
3. When a water sample tests positive for coliform bacteria, what can be said about the original source of these types of bacteria (where are these bacteria normally found, and what is a reasonable explanation of how they got into the waterbody you are researching)

When glucose is depleted, cells can break down other carbohydrates as well as fat, and use them as a source of energy. To do this, **cells need the right enzymes to catalyse the breakdown reactions**.

In this section of the lab, you will be recording the results of culturing bacteria from your water sample, in broth with lactose, which is a disaccharide.

The broth has **phenol red**, which is a **pH indicator**. At neutral pH, the broth is red, it turns yellow when the broth becomes acidic.

Inside the tube, you will also observe another small tube called Durham tube. When gas is produced, bubbles will appear in the tube.

Gas presence or absence in Durham tube: if bacteria produce gas during fermentation, you will see a bubble in the glass tube inside the media.

**Recording results from Broth with lactose and methyl-red tubes previously inoculated with your water samples**

Read the background given in part III and fill-out the table with your observations.

| Area of Collection/date of inoculation | Colour of broth | Gas Production (yes/no) | Interpretation of results |
|---------------------------------------|-----------------|--------------------------|---------------------------|

Answer the following questions:

1. What is the purpose of adding phenol red to the broth in the tubes?
SECTION C. Determining the amount of dissolved oxygen in your sample

Materials: LaMotte oxygen titration kit
    Glass bottles with water samples

Aquatic animals, including vertebrates and invertebrates, as well as many protists and bacteria, require oxygen to live. In fact, all aerobic organisms, including plants, require oxygen for cellular respiration. Oxygen from the atmosphere dissolves in water, until it reaches saturation. The dissolved oxygen will diffuse throughout the water, depending on how much the water is aerated (rivers and oceans with currents are more aerated than still ponds).

Today you will be measuring the amount of dissolved oxygen in your water sample with a test kit (LaMotte) that uses the azide modification of the Winkler method for determining dissolved oxygen.

Collection of your water sample

1. The water sample you will be testing was collected by your instructor by submerging the glass bottle in the water and immediately sealing it with the cap. Since oxygen in the air will dissolve in water, it is important to ensure the sample is not aerated after collection.
2. After collecting the water, your instructor added two-chemical reagents that will “fix” the oxygen present in the water. This will allow you to titrate the amount of dissolved oxygen in your water and compare it to standards set for different uses, by the Environmental Protection Agency (EPA).

Titration
    Follow the procedure describe below. Before beginning your titration, make sure you have gone through the steps and have all the reagents and material you need. The procedure below is provided by LaMotte.
TEST PROCEDURE
PART 3 – THE TITRATION

1. Fill the titration tube to the 20 mL line with the fixed sample. Cap the tube.

2. Depress plunger of the Titrator.

3. Insert the Titrator into the plug in the top of the Sodium Thiosulfate, 0.025N titrating solution.

4. Invert the bottle and slowly withdraw the plunger until the large ring on the plunger is opposite the zero (0) line on the scale.

   **NOTE:**
   If small air bubbles appear in the Titrator barrel, expel them by partially filling the barrel and pumping the titration solution back into the reagent container. Repeat until bubble disappears.

5. Turn the bottle upright and remove the Titrator.

   **NOTE:**
   If the sample is a very pale yellow, go to Step 9.

6. Insert the tip of the Titrator into the opening of the titration tube cap.

7. Slowly depress the plunger to dispense the titrating solution until the yellow-brown color changes to a very pale yellow. Gently swirl the tube during the titration to mix the contents.
8. Carefully remove the Titrator and cap. Do not to disturb the Titrator plunger.

9. Add 8 drops of Starch Indicator Solution (4170WT). The sample should turn blue.

10. Cap the titration tube. Insert the tip of the Titrator into the opening of the titration tube cap.

11. Continue titrating until the blue color disappears and the solution becomes colorless.

12. Read the test result directly from the scale where the large ring on the Titrator meets the Titrator barrel. Record as ppm Dissolved Oxygen. Each minor division on the Titrator scale equals 0.2 ppm.

**NOTE:**
If the plunger ring reaches the bottom line on the scale (10 ppm) before the endpoint color change occurs, refill the Titrator and continue the titration. Include the value of the original amount of reagent dispensed (10 ppm) when recording the test result.

**NOTE:**
When testing is complete, discard titrating solution in Titrator. Rinse Titrator and titration tube thoroughly. DO NOT remove plunger or adapter tip.
Record your results in the table below:

| Sample Name and collection date/time | Oxygen saturation (in ppm) | Levels considered appropriate for survival of most aquatic species | Levels considered stressful for most species | Levels considered fatal for most species |
|--------------------------------------|---------------------------|---------------------------------------------------------------|---------------------------------------------|-----------------------------------------|

Answer the following questions:

1. How does oxygen go into cells, give the name of the process and explain the factors that affect the movement of oxygen into a cell.
2. What metabolic process directly requires oxygen in order to go forward?
3. What is the exact role of oxygen in this process?
4. Illustrate your answer for question 2 by making a drawing to explain how oxygen is involved in this process – where in the cell and how does this process occur.

Notes:

Lab 6. Photosynthesis

OBJECTIVES:
To learn the factors that affect the net rate of photosynthesis such as different lighting conditions (light, dark), and CO₂ concentrations. To measure the CO₂ concentrations of the water samples by looking at the net rate of photosynthesis in these samples and by direct titration.

SPECIFIC LEARNING OBJECTIVES:

- Understand that photosynthesis is a measurable process.
- Understand factors affecting photosynthesis.
- Understand the correlation between cell respiration and photosynthesis.
- Understand the correlation between organic materials and the rate of photosynthesis.
- Learn to measure the concentration of CO₂ in the water samples by using the standard curve and titration assay.

BACKGROUND:
Photosynthesis is a process that converts carbon dioxide into sugars such as glucose using energy from the sun. When light is absorbed by pigments in a leaf, the energy absorbed is used to incorporate the carbon dioxide into organic molecules in a process called carbon fixation.

The process of photosynthesis can be expressed by the following word equation and chemical equation.

\[
\text{Carbon dioxide + Water} \rightarrow \text{Glucose + Oxygen}
\]

\[
6\text{CO}_2 + 6\text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2
\]

In this lab, you will be using leaf disks, to assay the net rate of photosynthesis under various lighting conditions. Leaf disks normally float, however if you remove the air from the air spaces, the overall density of the leaf disk increases and the leaf disk sinks. When sodium bicarbonate is added to the water, the bicarbonate ion acts as a carbon source for photosynthesis. When the disks are exposed to sufficient light, photosynthesis proceeds, and oxygen is released into the interior of the leaf, which changes its buoyancy causing the disk to rise. As a result, the rate at which the disks rise is indirectly proportional to the net rate of photosynthesis.
TERMINOLOGY:
Make sure you have a clear understanding of the following concepts after reviewing the provided videos and reading materials:

ADP
ATP
Autotroph
Chloroplast
Chlorophyll
Calvin Cycle
Light reaction
Oxidation
Reduction

MATERIALS:
- Sodium Bicarbonate (baking soda) solutions (0, 1.25%, 2.5%, 3.25%, 4.5%, 5.25%, 6.75%).
- Liquid Soap
- Syringe (20 ml or larger)
- Leaf (baby spinach from grocery store)
- Hole Punch (or plastic straw)
- Timer
- Light Source
- Stir sticks
- Plastic cups or 200-ml beakers (should be clear)/10 cups per group
- Graduate Cylinder (100 mL)
- Tin Foil to cover cups

-LaMotte* Carbon Dioxide and Alkalinity test kits for salt water

LAB ACTIVITIES: Each group will work together to conduct this activity. Every student must record, in their notebook, the results obtained by the group. Your instructor will write her initials once you have completed recording your observations.

Before starting the experiment answer the following questions based on your background knowledge that you acquired through the lecture or the posted videos on Blackboard about photosynthesis.

Which condition light/dark will produce the fastest rate of photosynthesis? State your hypothesis in the following form: If .... then .... because ...

Which concentrations of CO₂ (high/low) will produce the fastest rate of photosynthesis? State your hypothesis in the form: If .... then .... because ......

Which temperature 0°C, 37°C or 95°C will produce the fastest rate of photosynthesis? State your hypothesis in the form: if ... ... then ... ... ... ... ... because ... ...

1. EFFECT OF CARBON DIOXIDE CONCENTRATION ON THE RATE OF PHOTOSYNTHESIS

PROCEDURE:

1. Obtain 8-clear cups, label seven of them according to the following amounts of baking soda: 0, 1.25%, 2.5%, 3.25%, 4.5%, 5.25%, 6.75%. Label the seventh cup with the name of your water sample.
2. Add approximately 100 ml of each one of the solutions of baking soda into the labelled cups.
3. Add approximately 100 ml of your water sample to the cup labelled with the name of your water sample.

4. Add 1 drop of **liquid soap** to each beaker of bicarbonate solution and to your water sample. Make sure not to add too much soap to your solution to prevent making bubbles.

5. Hole punch at least 40-leaf disks, from fresh spinach leaves; choose areas that are uniform in texture and thickness, avoiding major leaf veins (at least 5 for each trial, you can use more disks if you wish, but use the same number of disks for each solution).

6. Remove the plunger of the syringe and place five-leaf disks in the syringe barrel.

7. Replace the plunger being careful not to crush the leaf disks. Push on the plunger until only a small volume of air and leaf disk remain in the barrel.

8. Draw a small volume (about 5 mL) of the sodium bicarbonate solution into the syringe. Start with the lowest concentrations of bicarbonate solution which is 2.5 g/100 ml, after you have added all the bicarbonate solutions, rinse the syringe and repeat the procedure with your water sample.

9. Invert the syringe and tap the syringe to suspend the leaf disks in the solution.

10. Push the plunger removing as much air as possible from the syringe.

11. Hold a finger over the syringe opening and draw back on the plunger to create a vacuum. Hold this for 10 s while swirling the syringe to further suspend the leaf disks in solution.

12. Let-off the vacuum and repeat steps 8–10, if needed, 2–3 more times until all leaf disks sink. If the leaf disks do not sink, add one more drop of soap to the bicarbonate solution and repeat the vacuum steps.

13. Pour the disks and solution back into the labelled cup for each concentration of bicarbonate solution and water sample.

14. Once all your cups are ready with the disks, put them under the lamp; record the time (**this will be time 0**).

15. Record the number of floating disks at the end of each time period (every 5 min) in the table in your notebook. Keep time for 30 min. Gently swirl the cup with a stir stick to dislodge any disks that are stuck to each other or on the side of the cups.

16. For the dark trial, obtain 1 cup, label it “dark 7.5%” and add 100 ml of the 7.5% bicarbonate solution. Wrap the beaker with tin foil to cover the plastic cup when not taking observations.

17. **Record your data in Table A4.**

18. Graph a **standard curve** for different concentrations of bicarbonate under light conditions. Make sure to label the X (bicarbonate concentration) and Y-axis (rate of photosynthesis measured in # of disks floating/unit of time) with the appropriate units. Use this graph to estimate the CO$_2$ concentration in the water sample.

**NOTES:**

**Table A4.** The net rate of photosynthesis at different concentrations of bicarbonate solutions in light and dark conditions.

| Number of disks floating under light or dark conditions | Bicarbonate Concentrations (for samples under light) | Water Sample | Dark (6.25%) | Notes |
|--------------------------------------------------------|-----------------------------------------------------|--------------|--------------|-------|
| Time (minute)                                          | 0 1.25% 2.5% 3.25% 4.5% 5.25% 6.75%               |              |              |       |
| 0                                                      | 5                                                  | 10           | 15           | 20    | 25    | 30    |
II. The Effect of Temperature on The Rate of Photosynthesis:
1. Obtain three-clear cups, label them according to the different temperatures.
2. Obtain solutions that contain 6.5% baking soda from the ice bucket, 37°C and 95°C water bath and add 100 ml of these solutions to each cup.
3. Prepare five-leaf discs for each trial as you did for the previous experiment. Once all your cups are ready with the disks, put them under the lamp; record the time (this will be time 0).
4. Record the number of floating disks at the end of each time period (every 5 min) in the table in your notebook. Keep time for 30 min. Gently swirl the cup with a stir stick to dislodge any disks that are stuck to each other or on the side of the cups.
5. Record your data in Table A5.

| Time (Minute) | 0°C | 37°C | 95°C |
|---------------|-----|------|------|
| 0             |     |      |      |
| 5             |     |      |      |
| 10            |     |      |      |
| 15            |     |      |      |
| 20            |     |      |      |
| 25            |     |      |      |
| 30            |     |      |      |
QUESTIONs:

1. What was the purpose of adding the baking soda in this experiment?
2. How do different concentrations of bicarbonate affect the rate of photosynthesis? Why?
3. Which trial resulted in all the leaf disks floating the fastest? Explain why you think this happened.
4. Explain the process that caused the leaf disks to rise.
5. How does light affect the rate of photosynthesis? Why?
6. How does temperature affect the rate of photosynthesis?
7. If the leaf disks were boiled, what kind of result would you expect? Hint: think about the enzymes that assist this reaction.

II. DIRECT MEASUREMENT OF AMOUNT OF DISSOLVED CARBON DIOXIDE AND ALKALINITY IN WATER SAMPLES

Measure the concentration of CO₂ in the water sample, with the kits provided, by using the titration assay. Follow the instructions provided by the manufacturer (see below).

**Record your data here:**
**Water sample NAME _________________**
Temperature: ________________
PH _____________

You need this data for your final presentation.

**CARBON DIOXIDE (LaMotte Titration kit instructions)**

NOTE: Read Direct Reading Titrator Manual before performing test.

Procedure

1. Fill test tube to 20-mL line with sample water. For best results, test on freshly obtained sample, and avoid splashing or prolonged contact with air.
2. Add 2 drops of *Phenolphthalein Indicator, 1%. If sample turns red, no free carbon dioxide is present. If sample is colourless, continue to Step 3.
3. Fill Direct Reading Titrator with *Carbon Dioxide Reagent B. Insert Titrator in centre hole of test tube cap.
4. While gently swirling tube, slowly depress plunger to titrate sample until colour changes to a faint pink which persists for 30 s. Read test result where the plunger tip meets the titrator scale. Record results as ppm Carbon Dioxide.

**MEASUREMENT OF ALKALINITY IN WATER SAMPLES:**

Alkalinity refers to the amount of bicarbonates, carbonates and hydroxides present in water. More specifically alkalinity is a measure of the buffering capacity of the water or the ability of water to resist changes in pH.  
RANGE: 0 – 200+ ppm as Calcium Carbonate (CaCO₃)

**ALKALINITY (La Motte Kit Instructions)**

1. Fill the test tube to 5-mL line with sample water.
2. Add one BCG-MR Indicator Tablet. Cap and shake until dissolved. A blue-green colour will develop.
3. Fill the Direct Reading Titrator with *Alkalinity Titration Reagent B. Insert Titrator into the centre hole of the test tube cap.

4. While gently swirling the tube, slowly depress plunger to titrate until the blue-green colour changes to pink. Read the test result directly from the scale where the large ring on the Titrator meets the Titrator barrel.

5. Record results as Total Alkalinity in ppm (CaCO₃). Each minor division on Titrator scale = 4 ppm Total Alkalinity as CaCO₃. EXAMPLE: Plunger tip is three-minor divisions below line 140. The test result is 140 plus (3 divisions x 4) equals 152 ppm.

6. If the plunger tip reaches the bottom line on the titrator scale (200 ppm) before the endpoint colour change occurs, refill the titrator and continue titration. When recording the test result, be sure to include the value of the original amount of reagent dispensed (200 ppm).

Lab 7. Mitosis and Meiosis

OBJECTIVES:

During this lab you will prepare two slides from root tips grown in your water sample, in water + NaCl, and in tap water, to recognise and describe the stages of mitosis. You will keep a tally of the number of cells in each stage of mitosis and compare your results from onions grown in tap water, tap water + NaCl, and your water sample.

After watching a video and discussing information provided by your instructor, you will learn about the mechanisms behind the carcinogenic nature of certain water pollutants, and how they relate to mitosis.

Using drawings and models, you will learn about the function of meiosis and its importance in sexual reproduction.

SPECIFIC LEARNING OBJECTIVES

1. Become familiar with the process involved in preparing and staining tissue to observe chromosomes in cells undergoing mitosis.
2. Recognise, describe and draw the different stages of mitosis.
3. Understand, in general terms, how carcinogens affect the cell cycle to promote mitosis.
4. Identify and draw the phases of meiosis.

BACKGROUND: Refer to slides provided

Make sure you have a clear understanding of the following concepts after reviewing the provided videos and reading materials:

Mitosis
Meiosis
Cell cycle
Cytokinesis
Chromosome
Chromatid
Centromere
Mitotic and meiotic spindle
Prophase
Metaphase
Anaphase
Telophase
cancer
MATERIALS

Microscope
Onion-tip prepared slides
Lens paper
Lens cleaning solution
Test tube (13 × 100 mm)
fixative (9 part 45% acetic acid and 1 part 1N HCL)
watch glass
onion
scissors
razor blade
metal spatula
aceto-orcein stain
microscope slides
cover slips.

LAB ACTIVITIES: Every student must do all the calculations, record all the results and complete drawings during the lab activity. Your instructor will write her initials once you have completed recording your results.

I. PREPARATION OF ROOT TIPS

Adapted from: Babich, H., Segall, M.A. and Fox, K.D. (1997). The Allium Test – A Simple, Eukaryote Geneotoxicity Assay. The American Biology Teacher. 59, 580–583.

A week before the lab, students placed onions in beakers to allow roots to grow, left – onion placed in tap water, right – onion placed in East river water. A control placed in water with dissolved NaCl equivalent to the previously determined salinity for the water sample is not shown in the picture.

1. Before starting the onion tip preparation, observe and record the growth of roots in regular water and East River water. You may take pictures of each sample.
Record:

Tap water (length and number of roots): ___________ _______________
Tap water + NaCl (length and number of roots): ___________ _______________
East River Water (length and number of roots): ___________ _______________

Harvesting and Fixing the Root Tips:

1. Obtain test tube, pair of scissors, watch glass, and fixative (9 parts 45% acetic acid and 1 part HCl).
2. Add fixative to a small test tube, fill about 3/4
3. Cut off two root tips, each approximately 1 cm long.
4. Place the root tips into the test tube of fixative and incubate at 50°C for 6 min. Then, dump heated fixative and tips into watch glass.

Staining the Cells:

5. Take root tips, one at a time, out of the watch glass and place each one in the middle of a microscope slide. See Figure A6
6. Cut all excess from the root tips except for 2 mm at the very tip of the root (end that was not cut from the plant). See Figure A7
7. Place two drops of aceto-orcein or aceto-orcein stain on top of the 2-mm root tip.
8. Let stain soak into root tip for 2 min. See Figure A8

9. Squash the root tip, on each slide, pressing straight down so as not to overlap the cells. See Figure A9

10. Place a coverslip flat upon the root tip, making certain **not to move the cover slip horizontally.**

11. Press the cover slip gently with a pencil eraser, again only straight down without moving the cover slip.

**Figure A8.** Use a small dropper to add one or two drops of aceto-orcein stain over the root tip and let it stand for 2 minutes.

**Figure A9.** Use a small spatula or the back of the forceps to squash the tip before placing a cover slip.
12. Soak-up any extra stain that may have accumulated around the coverslip with a paper towel, make sure not to move the coverslip.

13. Observe and record steps of cell division under microscope (at 400x) and number of cells in each stage of mitosis in the table provided below.

1. What is a distinguishing visible feature of each stage of mitosis?
   - Prophase:
   - Metaphase:
   - Anaphase:
   - Telophase:

**Comparison of number of cells in different stages of mitosis**

2. Using your microscope, scan the cells in your root-tip, count the number of cells in each stage and record the results of your cell counts in the table. When scanning the slide, start on one end and zig-zag from one side to the other while counting. This will ensure that you count cells only once and then calculate percentages.

|                     | Tap water | Percent of total # of cells | Water sample | Percent of total # of cells | Water + NaCl | Percent of total # of cells |
|---------------------|-----------|----------------------------|--------------|-----------------------------|--------------|----------------------------|
| Prophase            |           |                            |              |                             |              |                            |
| Metaphase           |           |                            |              |                             |              |                            |
| Anaphase            |           |                            |              |                             |              |                            |
| Telophase           |           |                            |              |                             |              |                            |
| Total               |           |                            |              |                             |              |                            |
3. Find and draw a cell showing each stage of mitosis. Your drawing should be in pencil and should reflect what you see under the microscope. You may also take pictures of your cells.

Prophase

Metaphase

Anaphase

Telophase

Questions:
1. Could you observe any differences between the roots (as far as growth) of onions grown in tap water and onions grown in East River water, if any, what do you think would be the simplest explanation for the cause of these differences – check your control sample results-?
2. Are there any differences between onions grown in tap water or in East River water as far as the relative number of cells in different stages of mitosis? If yes, hypothesise on why you found these differences.

MITOSIS AND CANCER

In this section, we will be going over data and watching some video clips from the film “Erin Brokovich” (2000), which dramatised a true case of ground water contamination. In 1996, the Pacific Gas & Electric Co., in the town of Hinkley, California, was found guilty of contaminating the groundwater with the carcinogen chromium-6.

Cancerous cells divide (undergo mitosis) uncontrollably -non-stop-, due to mutations (changes in their DNA), that interfere with the regulation of the cell cycle. Chemicals that induce mutations leading to cancer are called carcinogens.
Normal cells have mechanisms to detect damaged DNA. When mutations occur that affect the DNA coding for the proteins that are involved in that detection, mutant cells replicate. Those with mutations that result in uncontrolled division are cancerous cells.
Questions

1. Consider a hypothetical situation where your community is suffering from a high incidence of lung cancer due to smoking. You are asked to explain, in 10 min or less, what cancer is and why smoking causes cancer, to a group of 10-year-old children. What would you tell them?

MEIOSIS

After using models to go through the stages of meiosis I, draw each stage of meiosis I, starting with a diploid cell with four chromosomes, in the spaces below.

After using models to go through the stages of meiosis II, draw each stage of meiosis II (start with only one of the cells resulting from meiosis I) starting with a diploid cell with four chromosomes, in the spaces below.

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A. L. FUENTES AND M. ENTEZARI
Lab 8. Evolution and Metagenomics

OBJECTIVES:
In this lab, you will learn the major causes for ocean acidification and how this acidification is affecting the food webs and, consequently, the entire ecosystem. You will also learn how DNA sequence comparisons can be used to construct phylogenetic trees and cladograms, enabling scientists to establish evolutionary relationships, including common ancestry.

SPECIFIC OBJECTIVES
- After watching two videos relating to ocean acidification, answer questions related to this phenomenon.
- Build a phylogenetic tree and understand how phylogenetic trees can be built using physical characteristics, and more recently, DNA sequences.
- Using BLAST analysis, determine the identity of bacteria in water samples by comparing sequences of DNA coding for 16s RNA.

I OCEAN ACIDIFICATION
BACKGROUND: During the lab, you will be watching two videos on ocean acidification

After watching the videos on ocean acidification, answer the following questions:
Questions on video “Demystifying ocean acidification”:
https://www.youtube.com/watch?v=GL7qJYKzcsk

1. What percentage of the CO₂ released into the atmosphere is dissolved in the ocean?
2. Using the chemical equation to illustrate this phenomenon, explain why when CO₂ combines with H₂O, this leads to acidification.
3. How does ocean acidification lead to diminishing numbers of crustaceans (like crabs), as well as some foraminifera in the ocean?
4. How would you predict increased levels of CO₂ in the ocean would affect aquatic plants?

QUESTIONS TO BE ANSWERED AFTER WATCHING VIDEO
http://apps.seattletimes.com/reports/sea-change/2013/sep/11/pacific-ocean-perilous-turn-overview/

1. Name three commercially important organisms that are already impacted by ocean acidification. (0–4:10 min)
2. Carbon dioxide seeps as “natural laboratories” to study the potential impacts of ocean acidification on coral ecosystems 50 to 100 years into the future (i.e. Papua New Guinea). Explain how coral communities compare at a “normal” versus seep site in regard to: (4:10–6:20 min)
   • How does coral diversity differ?
   • Where are corals, where are algae dominating?
   • Where do you find a higher number and diversity of associated reef organisms (i.e. fish)?
3. Ocean acidification and fish. Give three-specific examples of how fish species (i.e. clown fish, pollock) may be directly (e.g. physiologically) or indirectly (food web shifts) affected by ocean acidification. Explain! (6:20–7:30 min)

II. DNA AND EVOLUTION

1. **Phylogenetic trees and cladograms**

How we use DNA sequences to study evolution
Creating phylogenetic trees from DNA sequences:
http://www.pbs.org/wgbh/nova/labs/videos/#evolution
http://www.pbs.org/wgbh/nova/labs/video_popup/6/38/

We will be using the slides and videos from the HHMI unit on Creating phylogenetic trees using DNA. Your instructor will stop to give you time and answer the questions below as you advance through the topic.
http://media.hhmi.org/biointeractive/click/Phylogenetic_Trees/05-vid.html

Questions:
1. How are DNA sequences used to deduce evolutionary relationships?
2. What is one advantage of building phylogenetic trees using DNA comparisons rather than anatomical features?
3. Watch the video clip on slide 3 and then draw a simple tree illustrating the evolutionary relationships between gorillas, chimpanzees, humans, and orangutans.
4. What are two common types of mutations?
5. Watch the short animation on slide 6 and describe a SNP. Watch the short animation on slide 7 and describe an indel.
6. Watch the short animation on slide 10 and explain what is meant by “aligning” DNA sequences.
7. Define a branch point (also called a node) on a phylogenetic tree and describe what it represents.
III. IDENTIFICATION OF BACTERIA USING SEQUENCE ALIGNMENT

2. BLAST Analysis of 16S rRNA Gene Sequence [from Robert Kranz, Kathleen Weston-Hafer, and Eric Richards. (2006) Identifying Unknown Bacteria Using Biochemical and Molecular Methods. Washington University in Saint Louis.]

BLAST (Basic Local Alignment Search Tool) is a web-based program that is able to align your search sequence to thousands of different sequences in a database (that you choose) and show you a list of the top matches.

This program can search through a database of thousands of entries in under a minute. (The time will be longer if there are many users using the search program at the same time.)

For this lab, you will use a database that contains all the bacterial sequences that have been published. BLAST performs its alignment by matching up each position of your search sequence to each position of the sequences in the database.

When the results are given, the most similar sequence is the first result listed.

This program can be accessed at: http://www.ncbi.nlm.nih.gov/BLAST

Procedure:

1. Examine the sequence from the 16s rRNA gene of one of the unknown bacteria from your water sample. How many bases are represented in your sequence?_________________

EAST River Deep – 34th ST
ATTGAA CGCTG GCGTAGGTTA AACAC ATGCAAG TCGAGCGGAAAAGC ACTCTAG CTGTTAAGGC CGTCGAACGG CGGACGGGCG AGTTGCTA AGCTACCCG CCTTACG CGG

EAST River Surface – 34th st
GATGAA CGCTG GCGTAGGTTA AACAC ATGCAAG TCGAGCGGAAAAGC ACTCTAG CTGTTAAGGC CGTCGAACGG CGGACGGGCG AGTTGCTA AGCTACCCG CCTTACG CGG

East River Surface LOC
GATGAA CGCTG GCGTAGGTTA AACAC ATGCAAG TCGAGCGGAAAAGC ACTCTAG CTGTTAAGGC CGTCGAACGG CGGACGGGCG AGTTGCTA AGCTACCCG CCTTACG CGG
GGCGCAAGCCTGAAACCAGCCCATGCGCGTGACGATGACGGTCCTATGGATTGAAA-
CTGCTTTTGTACAGGAAGAAACACTCCCTCGTGAGGGAGCTTGACGGTACTGTAAGA-
ATAAGGATCGGCTAATCCGTCGAGCCCGCGCCCGTGAAATAC

East River Surface 34th sample 19073
AGTGAACGCCTGGCCTGGTGTGATAAGACATGCAAGTCGAACGAGATTATACTGATAGA-
AGCTTCGGTGGAAAGATGTATATGAGAGTGGCAAACGGGTGCGTAACACGTGAACAA-
TCTGCCCTAAAGATCGGAATAGCTTGGGGAAACTCAAATTAATGCCTCGGTAGTGAC-
GTAACACTCATGTTGATAGCTGCATCAAATGCGCTCAAGTCCTAGGAGAAGGAGGAT-
TCCTGAGTGTGGTAGGATGTGGAAGGAACTTAACTAGAAGGTTCTAGCGGGAACGGA-
TGGAGAGAATCTGCTGATGACGGACGCCGGCAACAGAGAGAGAGAGAGAGAGAGAG-
AGTGAAGGCCCTTCCGTCGGAACCTTCTGTCCTCGGCGCCGCGCCGCGCCCGTGAGG-
ATGAAGGCCCTTCCGTCGGAACCTTCTGTCCTCGGCGCCGCGCCGCGCCGCGCCG-
CGCAAGCCTGAAACCAGCCCATGCGCGTGACGATGACGGTCCTATGGATTGAAA-
CTGCTTTTGTACAGGAAGAAACACTCCCTCGTGAGGGAGCTTGACGGTACTGTAAGA-
ATAAGGATCGGCTAATCCGTCGAGCCCGCGCCCGTGAAATAC

East River surface LOC sequence 18938
AACGAACGCTGGCCTGGTGTGATAAGACATGCAAGTCGAGCGGAACTTTTTTGGTCG-
CAATACTAGAAGATTCTACCCGCGGCAACCGGGTGCGAATAGCTCGGCGGCGCCG-
CTCCTGAGTGTGGTAGGATGTGGAAGGAACTTAACTAGAAGGTTCTAGCGGGAACGGA-
TGGAGAGAATCTGCTGATGACGGACGCCGGCAACAGAGAGAGAGAGAGAGAGAGAG-
AGTGAAGGCCCTTCCGTCGGAACCTTCTGTCCTCGGCGCCGCGCCGCGCCGCGCCG-
CGCAAGCCTGAAACCAGCCCATGCGCGTGACGATGACGGTCCTATGGATTGAAA-
CTGCTTTTGTACAGGAAGAAACACTCCCTCGTGAGGGAGCTTGACGGTACTGTAAGA-
ATAAGGATCGGCTAATCCGTCGAGCCCGCGCCCGTGAAATAC

2. Submit your sequence to the NCBI database using these directions and answer the following questions:

   a) How many matches (“hits”) did you obtain from the search?
   b) What is the name of the organism with the best match to your sequence?
   c) How good is the match between your sequence and the top match (score)?
   d) Why is sequencing the 16S rRNA gene a useful way to discriminate among bacteria? Clearly
      explain your answer
   e) You can retrieve other information about the bacteria you have identified, by clicking on
      one of the options below.

Other reports: Search Summary [Taxonomy reports] [Distance tree of results]
Taxonomy reports: Will give you the names and classifications of the bacteria with the highest scores or relatedness, for example:

| Organism                          | Blast Name     | Score | Number of Hits | Description                                    |
|-----------------------------------|----------------|-------|----------------|-----------------------------------------------|
| Bacteria                          | Bacteria       | 106   | 4              |                                               |
| .Gemmatimonadaceae                | Bacteria       | 4     | 3              |                                               |
| .Gemmatimonas                     | Bacteria       | 3     | 2              |                                               |
| ... Gemmatimonas aurantiaca T-27  | Bacteria       | 643   | 1              | Gemmatimonas aurantiaca T-27 hits             |
| ... Gemmatimonas phototrophica    | Bacteria       | 632   | 1              | Gemmatimonas phototrophica hits               |
| .Gemmatirosa kalamazooensis       | Bacteria       | 604   | 1              | Gemmatirosa kalamazooensis hits               |

Once you have the name of the bacterium, you can search elsewhere to find other characteristics of that bacterium (this bacterium, for example, is an obligate aerobe)

Distance tree of results: will give you a cladogram showing the evolutionary relationship of your bacterium, compared to other bacteria derived from a common ancestor:
Which bacteria is more closely related to your bacteria (in yellow), firmicutes or Solirubrobacter? Explain why you chose one or another.