

Laboratory Activities to Support Student Understanding of the Molecular Mechanisms of Mutation & Natural Selection

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ABSTRACT

The molecular basis of evolution is an important and challenging concept for students to understand. In a previous article, we provided some of the scientific background necessary to teach this topic. This article features a series of laboratory activities demonstrating that molecular events can alter the genomes of organisms. These activities are most appropriate for undergraduate students in Honors Biology, Genetics, or Molecular Biology courses. Student laboratory instructions are included to allow students to conduct the activities, make observations, interpret the results, and draw conclusions.

Key Words: Molecular evolution; mutation; natural selection; conserved DNA sequences.

The exercises provided here are designed to help students visualize how molecular evolution occurs by acquainting students with the basic principles of variable and conserved DNA sequences. These activities also provide living examples of organisms that illustrate the roles that mutation and selection have played in their evolution. Prerequisites for the students are a basic understanding of DNA structure, replication, transcription, translation, and mutation. A recommended classroom discussion and lab activity sequence are shown in Table 1, student instruction sheets are included in Appendix 1, and Appendix 2 is the teachers' key for student instructions.

○ TASK 1

Classroom Discussion of Molecular Evolution Concepts

The activities described in this article should be prefaced with a classroom discussion of basic molecular evolution concepts. In a previous article, we provided some of the scientific background necessary to discuss the classification of genomic sequences, mutations, and the

effects of selective pressure on DNA sequence variability. Discussing these topics will help students understand the basis for the activity results they will observe.

○ TASK 2

Lab Preparation: The Principles of PCR & Electrophoresis

Because polymerase chain reaction (PCR) and electrophoresis are powerful tools that allow researchers to produce copies of selected regions of DNA and visualize them, instructors should discuss the basic components and steps involved (Table 2). Background information for discussing these techniques, as well as simple activities to enhance student understanding, are available in a file of Supplemental Materials at <http://www.buildingthepride.com/faculty/trhubler/>.

○ TASK 3 (Activity 1)

Variability of Nonfunctional DNA Sequences

Data from the Human Genome Project and similar sequencing projects have allowed researchers to compare the genomes of a variety of organisms. When DNA sequences of organisms are compared, the sequences located in nonfunctional regions of the genome tend to exhibit considerable variability among organisms. Activity 1 demonstrates the tendency for nonfunctional DNA sequences to exhibit variation, even in closely related organisms. In this activity, students first use PCR to amplify a nonfunctional DNA sequence from two closely related primate species and gel electrophoresis to visualize the PCR products. Next, students utilize the BLAST tool available from the National Center for Biotechnology website (<http://www.ncbi.nlm.nih.gov>) to investigate sequence variability between the two species and to identify a mobile genetic element that introduces changes in primate DNA sequences.

The molecular basis of evolution is an important and challenging concept for students to understand.

Table 1. Summary of activities.

Task Order ^a	Task	Topics	Time Allotment ^a & Description
1	Class discussion	Genome organization, mutations, selection	Discuss: sequence classification (noncoding, coding, nonfunctional, functional), mutations, selection, nonfunctional sequence variability, functional sequence conservation.
2	Lab preparation	Principles of PCR and electrophoresis	Lab (2–3 hours). Discuss PCR and electrophoresis. Pour agarose gels and set up PCR reactions for Lab Activities 1 and 2. Completed PCR cycling reactions and agarose gels may be refrigerated until the next lab period.
3	Activity 1	Variability of nonfunctional DNA sequences	Lab (2–3 hours). Discuss Alu elements and the BLAST tool while performing electrophoresis. Review variable nonfunctional sequences. Stain gels, interpret results. Perform BLAST analyses. Students complete Lab Worksheet.
4	Activity 2	Conservation of functional DNA sequences	Lab (2–3 hours). Discuss New World and Old World primate evolutionary relationships during electrophoresis. Review conserved functional sequences. Stain gels, interpret results. Perform BLAST analyses. Students complete Lab Worksheet.
5	Reinforcement	Icelfish as an example of molecular evolution	View the video about icelfish evolution. Assign the recommended article about icelfish evolution for reading.

^aInstructors may need to adapt this to their available class and laboratory time.

Table 2. Materials needed for Tasks 3 and 4.

Plasmids: May be requested from corresponding author ^a
Primers ^a
Variable forward: 5'-AGTTCCTCTCTACCTTGACC-3'
Variable reverse: 5'-GCCCTACTCTTGCATTAATGC-3'
CG forward: 5'-GCACCAAGGATGGAGATG-3'
CG reverse: 5'-GCGGATTGAGAAGCCTTTA-3'
DNA ladder ^a
GoTaq Green PCR Master Mix ^a
Nuclease-free water ^a
PCR tubes, ^a appropriate for thermal cycler
Pipettors and tips ^a
Agarose gels (1% agarose in pH = 8 Tris acetate/EDTA electrophoresis buffer) ^{a,b}
Electrophoresis system: Power supply, electrophoresis cell, gel tray, comb, and gel caster ^c
Thermal cycler ^d
Methylene blue staining solution ^a

The following safety precautions should be observed:

^aWear gloves.

^bWear eye protection when pouring hot solutions.

^cHigh voltage; disconnect power before opening chambers.

^dThermal cycler components reach high temperatures during PCR cycling.

Suggested vendors are listed in a file of Supplemental Materials at <http://www.buildingthepride.com/faculty/trhubler/>.

For each of the PCR reactions in Activity 1, the template DNA is provided in the form of plasmid DNA containing an intergenic region from owl monkey (om) or squirrel monkey (sm), two species of Platyrrhines, or New World monkeys. These plasmids

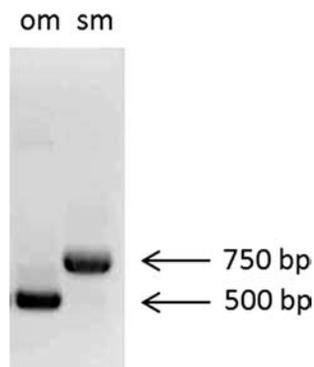


Figure 1. Results of PCR amplification of a variable region from two species of New World primates: owl monkey (om, *Aotus trivirgatus*) and squirrel monkey (sm, *Saimiri boliviensis*).

were developed in our research laboratories, specifically for use in Activity 1. Instructions for setting up the PCR reactions are included in Appendix 1. Figure 1 shows that PCR, gel electrophoresis, and staining reveal a 500-bp product from the owl monkey sample, whereas the squirrel monkey sample contains a 750-bp product. These data demonstrate that nonfunctional regions contain sequences that can differ, even among closely related organisms (two New World monkeys).

Next, students will use the BLAST tool to determine the reason for the difference in size of the PCR products. DNA sequences for the PCR products were either generated in our labs (owl monkey) or obtained from the Roos lab (squirrel monkey) (Osterholz et al., 2008). These DNA sequences are provided below and are available electronically in Supplemental Materials. When students compare the nucleotide sequences of these nonfunctional DNA regions, they should observe that the size difference in the PCR products results from an insertion into the squirrel monkey sequence.

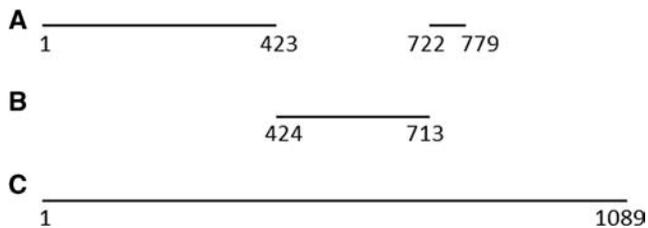


Figure 2. Schematic of the results of BLAST alignment of DNA regions from squirrel monkey (sm, *Saimiri boliviensis*), owl monkey (om, *Aotus trivirgatus*), and human (h, *Homo sapiens*). (A) BLAST alignment of the sm and om variable regions. (B) BLAST alignment of the sm variable region and an Alu element database. (C) BLAST alignment of the sm, om, and human chorionic gonadotropin (CG) genes. The numbers below each alignment indicate the position in the sm DNA sequence.

Specifically, the two sequences align except in a region toward one end of the squirrel monkey sequence. This gap in alignment represents a region that is present in the squirrel monkey sequence but absent in the owl monkey sequence (Figure 2A).

Squirrel monkey variable region (Alu element bold and underlined)

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agttcctctc taccttgtac ctgttccaga cccccggcct
aggcctggac actaaggaaa ttcttactaa acaaatgctt
gccagctca tccgtccctc actcttctct acctctcacc
ttgattcccc agaggaggag gggaaagtga aagagaactg
tcgagaacag ctgtcattta cccgggactt gctatgggcc
agggacttta cagacagcat cttgtctaag tttgacatca
tcccatgaag tggatcttac tattatcccc atttaacaaa
tgagaaatct gaggcattgg aaagttaagt gacttgtcca
agctcacata atgaagtagt ggtaccaggc agaactggct
atataatctg tgggaccagc tgcaaaatga aaatgtgggg
cctctgttaa aaaactatta atcggccggg cgcggtggct
caagcctgta atcccagcac tttgggaggc cgaggtgggt
ggatcacaag gtcgagagat cgagaccatc ctgggtcaaca
tggtgaaacc cgtctctac taaaaataca aaaagttagc
tgggcgtggt ggtgcatgcc tgtaatccca gctactcagg
aggctgaggc aggagaattg cctgagccca ggaggcggag
gttgcggtga gccgagatcg cgccattgca ctccagcctg
ggaacaaga gcaaaactcc gtctcaaaaa aaaaaaaaaa
aaaaaaaaaacta ttaatcattt caagaccagg acagaagagc
attaatgcaa gagttagggc

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Owl monkey variable region

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tagttcctct ctaccttgta cctgtcccag acccccggcc
taggcctgga cactgaggag attcttacta acaaatgct
tgcccagctc atcctcccct cactcttctc tacctctcac
cttgattccc cagaggagga gggaaagggg gaggggaggg
gaagtggnnn gagaattgac gagaacagct gtcatttagc
cgggacttgc tatgggccag ggactttann nacagegctt
tgtctaagct tgacatcacc ccatgaagtg gatcttactg
ttatccccat ttaacaaatg agaaatctga ggcattggaa
agttaagtga cttgtccaag ctcacataac caagtagtgt
accaggcaga actggctata taatttgtgg gaccagcgc
aaaatgaaaa tgtggggcct ctgttaaaaa accattaatc
atctcaagac caggacaaaag agcattaatg caagagttagg
gcta

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Finally, students will use the BLAST tool to compare the squirrel monkey sequence to a database of currently known “Alu sequences,” a type of mobile genetic element. As diagrammed in Figure 2B, this will allow them to identify the inserted region in the squirrel monkey DNA as an Alu element. Mobile genetic elements are DNA sequences that can be inserted into new locations in the genome through the action of specific enzymes. Alu elements are a common type of mobile genetic element found in the human genome (Batzer & Deininger, 2002). Insertion of Alu sequences has several impacts on genomes. Not only do they produce genetic variation, they may also disrupt regulatory regions and coding regions (Schmitz, 2012). These data demonstrate that one source of genomic variability is the insertion of DNA sequences via mobile genetic elements.

“Student Instructions for Activity 1: Variability of Nonfunctional DNA Sequences” in Appendix 1 provides guidance for students to perform the activity. BLAST results showing actual sequence alignments and additional information about Alu elements as important mechanisms of molecular evolution are provided in a file of Supplemental Materials at <http://www.buildingthepride.com/faculty/trhubler/>. Some important points to discuss with students are that Alu elements (1) are mobile, (2) are inserted >1 million times in the human genome, (3) contribute to a dynamic expansion of primate genomes, and (4) are known to cause human diseases such as hemophilia and breast cancer, as a result of disrupting gene coding sequences (Batzer & Deininger, 2002; Kramerov & Vassetzky, 2005).

○ TASK 4 (Activity 2)

Conservation of Functional DNA Sequences

The sequencing and comparison of genomes from a variety of organisms have revealed that although genomes are constantly changing, some regions exhibit remarkable similarity among organisms. As a result of comparative genomics, it is now understood that DNA sequences that perform important cellular functions tend to be similar, or conserved. Lab Activity 2 is intended to help students visualize conservation of functional DNA sequences. PCR is used to demonstrate that the DNA sequence of an important gene is conserved among two groups of primates: Catarrhines and Platyrrhines.

Catarrhines include Old World monkeys such as macaques and baboons, as well as chimpanzees, apes, and humans; they are found primarily in Africa and southern Asia, with the exception of humans, which are widespread. Platyrrhines include the New World monkeys, such as squirrel monkeys and owl monkeys, that inhabit Central and South America. Catarrhines and Platyrrhines diverged from a common ancestor >35 million years ago (Goodman et al., 1998; Schrago & Russo, 2003; Perelman et al., 2011). Although Catarrhines and Platyrrhines have evolved genetic and physiological differences over millions of years in geographic isolation (Müller et al., 2004; Westberry et al., 2006; Ward & Vallender, 2012), many functional DNA sequences, including genes coding for peptide hormones, have been conserved.

In Activity 2, students will (1) use PCR to amplify the chorionic gonadotropin (CG) gene from three primate species, (2) analyze the PCR products by electrophoresis, and (3) perform sequence comparisons to demonstrate that functional DNA sequences are conserved. CG is a peptide hormone made in primates by placental cells

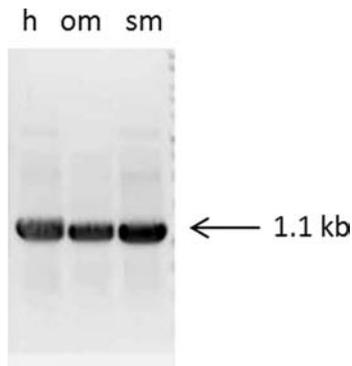


Figure 3. Results of PCR amplification of the chorionic gonadotropin gene from human (h, *Homo sapiens*), owl monkey (om, *Aotus trivirgatus*), and squirrel monkey (sm, *Saimiri boliviensis*).

Table 3. Comparison of the nucleotide similarity of selected genes

Genes compared ^a	Percent Identical Nucleotides
h CG vs om CG	83
h CG vs. sm CG	80
sm CG vs. om CG	92
h CG vs. h Growth Hormone	No similarity
h CG vs. h Oxytocin	No similarity

^aAbbreviations: h = human, om = owl monkey, sm = squirrel monkey, and CG = chorionic gonadotropin.

during the first 10 weeks of pregnancy. It supports the implantation and development of the fetus (Hanson et al., 1971). CG therefore represents a critical gene for reproductive success in primates.

Plasmids containing the CG gene from humans, owl monkeys, or squirrel monkeys were developed in our research laboratories for use as the template for PCR. Instructions for setting up the PCR reactions are included in Appendix 1. Figure 3 shows that PCR, electrophoresis, and staining reveal a 1.1-kb PCR product in each sample lane.

Next, students will determine the similarity between Catarrhine and Platyrrhine CG gene sequences using the BLAST tool. The DNA sequences for BLAST analyses are available electronically in a file of Supplemental Materials at <http://www.buildingthepride.com/faculty/trhubler/> or can be obtained from NCBI (<http://www.ncbi.nlm.nih.gov>) using the following accession numbers: human CG (X00265.1), squirrel monkey CG (GU117708.1), owl monkey CG (JN613228), human growth hormone (E00140.1), and human oxytocin (M11186.1). The squirrel monkey and owl monkey CG sequences were generated in our research labs (Vasauskas et al., 2010). DNA sequences for growth hormone and oxytocin are employed as examples of unrelated genes. Figure 2C shows graphically that the squirrel monkey, owl monkey, and human DNA sequences align throughout the entire length of the PCR products. The BLAST results will also display the percent identical nucleotides and should agree with the information in Table 3. These data (1) provide students with a

method for quantitative assessment of sequence similarity, (2) demonstrate that functional DNA sequences are similar among organisms, (3) reveal higher sequence conservation among Platyrrhines than between Catarrhines and Platyrrhines, and (4) indicate the degree of similarity that is observed in conserved DNA sequences. We do not expect 100% identity, because neutral mutations contribute to variability. “Student Instructions for Activity 2: Conservation of Functional DNA Sequences” in Appendix 1 provides guidance for students in performing the activity.

○ TASK 5 (Reinforcement)

Icelfish as an Example of Molecular Evolution

After observing examples of variable and conserved sequences, we suggest that teachers emphasize the mutually important roles of mutation and selection by providing a living example of the effect of beneficial mutations on organisms. Icelfish are believed to have evolved from a population of temperate-environment fish. The fish were exposed, over time, to a significant drop in water temperature due to changes brought about by continental drift. Because of mutations, some fish produced a protein that defended them against freezing temperatures. Fish with this increased hardiness survived and passed the new trait and the gene that controlled it to their offspring. This is a classic example of natural selection.

To reinforce how molecular changes contribute to survival and diversity, we recommend a short video about icelfish that is found on the DVD titled *The Making of the Fittest*. It is available at no charge from the Howard Hughes Medical Institute (<http://www.hhmi.org>) and contains high-quality videos on natural selection in fish, rock pocket mice, and humans. Additionally, we recommend the story “In Cold Blood: The Tale of the Icelfish” for students with some knowledge of genetics (Carroll, 2009). In the story, Sean Carroll provides a vivid historical account of the discovery of the icelfish and its evolutionary implications. A question sheet to help students read the article is available in a file of Supplemental Materials (<http://www.buildingthepride.com/faculty/trhubler/>). These examples elucidate a definitive relationship between mutations, selection, and evolution.

○ Summary

To conceptualize the process of molecular evolution, students need to understand mechanisms that contribute to the dynamic nature of genomes and the effect that natural selection has on sequence conservation. Our instructional series includes classroom discussion of basic molecular-evolution concepts followed by two lab activities. The activities use analyses of variable and conserved DNA sequences to demonstrate how selective pressure affects the persistence of mutations in populations. For reinforcement of the process by which evolution occurs, the molecular evidence recorded in the genome of the icelfish is used to explain how mutation followed by natural selection produces changes in organisms.

○ Acknowledgments

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Appendix 1

Student Instructions for Activity 1: Variability of Nonfunctional DNA Sequences

PCR of Nonfunctional DNA Sequences

This activity will be used to illustrate that nonfunctional DNA sequences are variable, even in closely related organisms. PCR will be performed to show that an intergenic nonfunctional region exhibits variability in two species of New World monkeys: squirrel monkeys and owl monkeys. DNA sequences for the variable region will be used to identify the nature of the variation.

Plasmid DNA containing the variable region from owl monkeys or squirrel monkeys is used as the template for PCR. The components needed for a 25- μ L reaction in a 200- μ L PCR tube and the PCR conditions are listed below (Table A1). Following PCR, samples are loaded onto 1% agarose gels and electrophoresed at 100 V for 55–60 minutes. A DNA ladder should be loaded into a separate well to estimate PCR product size. The gels are stained with methylene blue gel stain according to the manufacturer's instructions. PCR products are visualized by placing the gel on a white illuminated surface.

Investigating the Reason for the Difference in Size of the PCR Products

The DNA sequence for the squirrel monkey PCR product will be provided electronically. The insertion of a DNA sequence into the squirrel monkey variable region can be detected by accessing the BLAST page (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and following these steps. Select “Align” under Specialized BLAST. Copy and paste the squirrel monkey sequence into the query box and the owl monkey sequence into the subject box. Select “somewhat similar sequences” under Program Selection. At the bottom of the page select “BLAST.” Next, the insertion can be identified as an Alu element, a type of mobile DNA sequence, by following these steps at the BLAST page. Select “nucleotide blast.” Copy and paste the squirrel monkey sequence into the query box. In the second box, select the database “Human Alu repeat elements” from the dropdown list. At the bottom of the page select “BLAST.”

Table A1. PCR setup.

PCR Components	
Component	Volume (μL)
DNA template (dilute to 10 ng/μL)	1.0
Forward primer (10 μM) 5'-AGTTCCTCTCTACCTTGACC-3'	1.0
Reverse primer (10 μM) 5'-GCCCTACTCTTGACATTAATGC-3'	1.0
GoTaq Green Master Mix	12.5
Nuclease-free water	9.5

PCR Conditions		Record of PCR Observations	
Stage	Conditions	Sample	PCR Product Size
Initial denaturation	94°C, 1 minute	1. Owl monkey	
		2. Squirrel monkey	
Cycling (30 times):	Denaturation		
	Annealing		
	Elongation		
Final extension	72°C, 5 minutes		

BLAST Interpretations

1. Sketch the results of the BLAST of sm and om variable regions. Approximately how many nucleotides are inserted into the squirrel monkey region? Indicate this on your sketch.
2. Sketch the results of the BLAST of the sm variable region and the database of Alu sequences. How does this compare to the sketch above?

Concluding Questions

1. Owl monkeys and squirrel monkeys are closely related primates of the parvorder Platyrrhini (New World monkeys). What do your observations of PCR products tell you about DNA sequences in nonfunctional regions of closely related organisms?
2. In this example, what is the form of genetic variability (substitution, insertion, or deletion) that occurs in a nonfunctional region of the genome?
3. Based on lecture discussions of mutation and natural selection, describe in your own words why mutations in nonfunctional regions may persist over many generations and lead to high variability in these regions.

Student Instructions for Activity 2: Conservation of Functional DNA Sequences

Students who complete this activity will use PCR to begin to understand conservation of functional DNA sequences among two groups of primates: Catarrhines and Platyrrhines. Catarrhines include Old World monkeys such as baboons and rhesus macaques as well as chimpanzees, apes, and humans. Catarrhines primarily inhabit Africa and southern Asia, with the exception of humans, whose distribution is widespread. By contrast, Platyrrhines such as squirrel monkeys, marmosets, and owl monkeys inhabit Central and South America. Catarrhines and Platyrrhines evolved independently from a common ancestor >35 mya. Still, functional DNA sequences have been conserved. The sequences of the PCR products will be provided electronically for evaluation of the similarity in the DNA sequences.

In this activity, the size and DNA sequence of the primate chorionic gonadotropin (CG) gene will be compared. CG is a peptide hormone made in primates by placental cells during the first 10 weeks of pregnancy. It supports the implantation and development of the fetus. CG therefore represents a critical gene for reproductive success in primates.

Plasmid DNA containing the CG gene from humans, owl monkeys, or squirrel monkeys will be used as the template for PCR. The PCR components and settings are indicated below (Table A2). PCR products are fractionated and visualized using methylene blue stain as in Lab Activity 1.

Table A2. PCR setup.

PCR Components	
Component	Volume (μL)
DNA template (dilute to 10 ng/μL)	1.0
Forward primer (10 μM) 5'-GCACCAAGGATGGAGATG-3'	1.0
Reverse primer (10 μM) 5'-GCGGATTGAGAAGCCTTTA-3'	1.0
GoTaq Green Master Mix	12.5
Nuclease-free water	9.5

PCR Conditions		Record of PCR observations	
Stage	Conditions	Sample	PCR Product Size
Initial denaturation	94°C, 1 minute	1. Owl monkey	
		2. Squirrel monkey	
		3. Human	
Cycling (30 times):			
Denaturation	94°C, 1 minute		
Annealing	54°C, 30 seconds		
Elongation	72°C, 90 seconds		
Final extension	72°C, 5 minutes		

Determining the Similarity between Catarrhine & Platyrrhine CG Gene Sequences

DNA sequences for the PCR products and for the unrelated genes, growth hormone and oxytocin, will be used for sequence comparisons. On the BLAST web page (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>), select “align two sequences.” Copy and paste the human sequence into the query box. Copy and paste the squirrel monkey sequence into the subject box. At the bottom of the page select “BLAST.” Results will list the percent identical nucleotides. Repeat this to compare the other sequences.

BLAST Interpretations

Genes Compared	Percent Identical Nucleotides
1. hCG vs. om CG	
2. hCG vs. sm CG	
3. sm vs. om CG	
4. hCG vs. h growth hormone	
5. h CG vs. h oxytocin	

Concluding Questions

1. What do these results suggest about the similarity of functional DNA sequences?
2. Why do you think the sequence similarity is higher among Platyrrhines?
3. Why do you think 100% sequence similarity is not observed in the functional sequences?
4. Based on lecture discussions of mutation and natural selection, describe in your own words why conserved sequences persist in functional regions of genomes.

APPENDIX 2: Teachers' Key for Student Instructions

Activity 1: Variability of Nonfunctional DNA Sequences

PCR Observations

1. 500 bp
2. 750 bp

BLAST Interpretation

1. The sketch should show that the sequences align, except that there is a gap toward one end of the sm sequence, representing a region that is NOT found in the om sequence (250 bp).
2. The sketch should show that the region missing in the sm sequence in the first sketch is the same region that aligns with an Alu sequence, thus identifying it as similar to an Alu sequence.

Conclusions

1. The sequences can be variable, even among closely related organisms.
2. Alu insertion
3. Mutations in nonfunctional regions have no effect on organism survival and are not selected out (organisms are not weakened or do not die).

Activity 2: Conservation of Functional DNA Sequences

PCR Observations

1. 1100 bp
2. 1100 bp
3. 1100 bp

BLAST Interpretation

1. 83%
2. 80%
3. 92%
4. No similarity
5. No similarity

Conclusions

1. Functional sequences remain similar among organisms.
2. Organismal relationships are based on morphological and physiological characteristics. These characteristics result from the use of DNA sequences that are used to produce proteins. Platyrrhines are more closely related to one another than Platyrrhines are to Catarrhines; thus, their DNA sequences are expected to be more similar.
3. Neutral and beneficial mutations persist because they either have no effect or provide an advantage to the organism, respectively.
4. Harmful mutations in functional sequences weaken organisms, reduce their reproductive capacity, and/or cause them to die. Therefore, harmful mutations are not likely to be passed to offspring. Neutral mutations in functional sequences contribute to some variability. Beneficial mutations in functional regions confer an advantage and may increase life span and/or reproduction. If reproduction is enhanced, more offspring harbor the beneficial mutation and it is passed to future generations. Therefore, as a result of removal of harmful mutations and transmission of beneficial mutations, functional sequences remain similar (conserved) from one generation to the next.

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- Social and ethical implications of biology and how to teach such issues, genetic engineering, energy, pollution, agriculture, population, health care, nutrition, sexuality, and gender, and drugs
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- Other timely and relevant and interesting content like discussions of the role of the Next Generation Science Standards in biology teaching, considerations of the history of biology with implications for the classroom, considerations of the continuum of biology instruction from K-12 to post-secondary teaching environments, contributions that consider the likely/ideal future of science and biology instruction.

Research on Learning (up to 4000 words) includes reports of original research on innovative teaching strategies, learning methods, or curriculum comparisons. Studies should be based on sound research questions, hypotheses, discussion of an appropriate design and procedures, data and analysis, discussion on study limitations, and recommendations for improved learning.

Inquiry and Investigations (up to 3000 words) is the section of *ABT* that features discussion of innovative and engaging laboratory and field-based strategies. Strategies in this section should be original, focused at a particular grade/age level of student, with all necessary instructions, materials list, worksheets and assessment tools, practical, related to either a particular program such as AP and/or linked to standards like NGSS. The most appropriate contributions in this category are laboratory experiences that engage students in inquiry.

Tips, Tricks and Techniques (up to 1500 words but may be much shorter) replaces the How-To-Do-It and Quick Fix articles. This section features a range of suggestions useful for teachers including laboratory, field and classroom activities, motivational strategies to assist students in learning specific concept, modifications of traditional activities, new ways to prepare some aspect of laboratory instruction, etc.

Submission Guidelines

All manuscripts must be submitted online at <http://mc.manuscriptcentral.com/ucpress-abt>

- Authors will be asked to register the first time they enter the site. After receiving a password, authors can proceed to upload their manuscripts through a step-by-step process. Assistance is always available in the "Author Help" link found in the menu on the left side of the page. Additional assistance is available from the Managing Editor (managingeditor@nabt.org).
- Manuscripts must be submitted as Word or WordPerfect files.
- Format manuscripts for 8.5 × 11-inch paper, 12-point font, double-spaced throughout, including tables, figure legends, and references.
- Please place figures (including photos) and tables where they are first cited in the text along with appropriate labels. Make sure to include figure and table citations in the text as it is not always obvious where they should be placed. At the time of initial submission, figures, tables and images should be low resolution so that the final file size remains manageable.
- If your article is accepted, we will require that figures be submitted as individual figure files in higher resolution form. See below for file format and resolution requirements.
- **NOTE:** Authors should be aware that color is rarely used within the journal so all artwork, figures, tables, etc. must be legible in black and white. If color is important to understanding your figures, please consider alternative ways of conveying the information.
- Authors are encouraged to submit multimedia files. Acceptable file formats include MP3, AVI, MOV, WMV, and FLV.

Editorial Procedures

- Communications will be directed to only the first author of multiple-authored articles.
- At least three individuals who have expertise in the respective content area will review each article.
- Although the editors attempt to make decisions on articles as soon as possible after receipt, this process can take six to eight months with the actual date of publication to follow. Authors will be emailed editorial decisions as soon as they are available.
- Accepted manuscripts will be forwarded to the Copy Editor for editing. This process may involve making changes in style and content. However, the author is ultimately responsible for scientific and technical accuracy. Page proofs will be sent to authors for final review before publication at which time, only minor changes can be made.

Writing and Style Guidelines

The *Chicago Manual of Style, 14th Edition* is to be used in regards to questions of punctuation, abbreviation, and style. List all references in alphabetical order on a separate page at the end of the manuscript. References must be complete and in *ABT* style. Please review a past issue for examples. Use first person and a friendly tone whenever appropriate. Use concise words to emphasize your point rather than capitalization, underlining, italics, or boldface. Use the SI (metric) system for all weights and measures.

NOTE: If all authors are not members of NABT, there will be page charges of \$100 per journal page to be paid before publication.

Guidelines for Preparing Figure Artwork

General requirements

- When your article is accepted, we will require that figures be submitted as individual figure files in higher resolution format. See below for file format and resolution requirements.
- **NOTE:** Authors should be aware that color is rarely used within the journal so all artwork, figures, tables, etc. must be legible in black and white. If color is important to understanding your figures, please consider alternative ways of conveying the information.

Halftone (photographic) figures

Digital files must meet the following guidelines:

- Minimum resolution of 300 DPI, though 600 DPI is preferred.
- Acceptable file formats are TIFF and JPEG.
- Set to one-column (3.5" wide) or two-column size (7" wide).
- If figure originates from a web site, please include the URL in the figure caption. Please note that screen captures of figures from a website are normally too low in resolution for use.

Line art figures

- Minimum resolution of 600 DPI, though 1200 DPI is preferred.
- Acceptable file formats are TIFF, BMP, and EPS.
- Set to one-column (3.5" wide) or two-column size (7" wide).

**If you have any questions, contact
Mark Penrose at managingeditor@nabt.org.**

Several times a year the *ABT* has issues that focus on a specific area of biology education. Future focus issues are published in most issues. The editors highly encourage potential authors to consider writing their manuscripts to align with the future focus topics.

Thank you for your interest in *The American Biology Teacher*. We look forward to seeing your manuscripts soon.

William McComas, Editor-in-Chief
ABTEditor@nabt.org

Mark Penrose, Managing Editor
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Requirements for Submitting Cover Photographs for *The American Biology Teacher*

Submissions of cover photographs from NABT members are strongly encouraged. Covers are selected based on the quality of the image, originality, overall composition, and overall interest to life science educators. *ABT* has high standards for cover image requirements and it is important for potential photographers to understand that the size of the cover image generally precludes images taken with cell phones, point-and-shoot camera and even some older model digital SLR cameras.

Please follow the requirements listed below.

1. E-mail possible cover images for review to Assistant Editor, Kathleen Westrich at kmwestrich@yahoo.com.
2. Choose images with a vertical subject orientation and a good story to tell.
3. Avoid cropping the subject too tightly. It is best to provide an area of background around the subject.
4. Include a brief description of the image, details of the shot (i.e., circumstances, time of day, location, type of camera, camera settings, etc.), and biographical information in your e-mail message.
5. Include your name, home and e-mail address, and phone numbers where you can be reached.
6. Please ensure that the image meets the minimum standards for publication listed below and has not been edited or enhanced in any way. The digital file must meet the minimum resolution of 300 pixels per inch (PPI)—preferred is 400 PPI—and at a size of 8.5 x 11.25". We accept TIFF or JPEG images only.
7. For exceptional images, the editors will also accept sharp, clear, color 35 mm slides. Submit only the original; duplicates will not be accepted. Be sure to clearly label your slides with your name and contact information in ink. Contact Assistant Editor Kathy Westrich beforehand to discuss the possibility of submitting a 35mm slide or other non-digital format for consideration as an *ABT* cover.